Development and evaluation of a novel pharmaceutical dose form for the induction of emesis in canines

Dennis Donald Cote

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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by

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Acknowledgements:

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Abstract:

Ingestion of toxic materials continues to be a problem with both humans and canines. Treatment procedures such as emesis have now fallen out of favor largely due to well designed studies in humans which have shown that they are ineffective therapeutically and probably provide no benefit in terms of patient outcome. In veterinary medicine emesis is still used therapeutically in dogs as emesis is somewhat more useful in this species due to differences between human and canine intoxications primarily in terms of the nature of the intoxicant and the quantity consumed. For induction of emesis in dogs, apomorphine is the most appropriate drug but it is generally not used since it has a range of potentially serious dose-related adverse-effects, wide patient variability in terms of response and at present can only be administered by injection. Since the drug can be absorbed by the ocular route, a controlled-release product in the form of an ocular insert was designed, developed and tested. After placement in the eye, apomorphine would be released in a controlled fashion and absorption would occurr until emesis is achieved. Further drug absorption would be stopped by removal of the insert so the minimal amount of drug required for emesis would be administered and the occurance of dose-related adverse effects may be avoided or reduced. In this project, a suitable insert was designed, a method of fabrication developed and the mechanism of release and shelf-life of the product determined. In a clinical trial the insert was shown to induce emesis with an efficacy similar to intravenously administered apomorphine but with a much lower incidence of adverse effects. Although the insert performed well clinically there were some short-comings with the product and several suggestions regarding product improvement are presented.

List of Abbreviations:

7-OH-DPAT	7-hydroxy-dipropylaminotetralin
AACT	American Academy of Clinical Toxicology
AAPCC	American Association of Poison Control Centres
α	Separation factor (Chromatography)
AcCN	Acetonitrile
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ASPCA	American Society for the Prevention of Cruelty to Animals
ATCC	American Type Culture Collection
AUC	Area under the curve
BCCA	Bicinchoninic acid
bFGF	Basic fibroblast growth factor
BP	British Pharmacopeia
C_{18}	Octadecyl silane
cAMP	Cyclic adenosine monophosphate
cfu	Colony-forming unit
C_{Max}	Maximum serum concentration
CMC	Carboxymethylcellulose
CSF	Cerebrospinal fluid
DCA	Direct current amperometry
D_p	Diffusion coefficient
DSC	Differential scanning calorimetry
DSS	Dodecyl sodium sulfate
E_a	Energy of activation

EAPCCT	European Association of Poisons Centers and Clinical Toxicologists
EC	Electrochemical
ECD	Electrochemical detector (Chromatography)
ED	Erectile dysfunction
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
ε	Extinction coefficient (UV spectroscopy)
FDA	US Food and Drug Administration
Flu	Fluorescence
FTIR	Fourier-transform infrared
GI	Gastrointestinal
GLC	Gas-liquid chromatography
HBL	Hydrophilic bandage lens
HPLC	High-pressure liquid chromatography
HPMC	Hydroxypropyl methylcellulose
IM	Intramuscular
IP	Intraperitoneal
IR	Infrared
IV	Intravenous
k'	Capacity factor (Chromatography)
MDMA	3,4-Methylenedioxymethamphetamine
MLR	Multiple linear regression
MS	Mass spectrometer
N	Number of column plates (Chromatography)

NAPCC	National Animal Poison Control Centre
NAPINet	National Animal Poison Information Network
NF	National Formulary
NMR	Nuclear magnetic resonance
PC	Paper chromatography
PD	Parkinson's Disease
PDA	Photodiode array
PEEK	Polyethylethylketone
PEO	Polyethylene oxide
PO	Orally
psig	Pounds per square inch (gauge)
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
r^2	Coefficient of determination
R_s	Resolution (Chromatography)
RSD	Relative standard deviation
SC	Subcutaneous
SPE	Solid-phase extraction
T	Tailing factor (Chromatography)
t_{90}	Time to 90% of original concentration
t_a	Column dead time (Chromatography)
TAPP-Br	2'-bromo-1,3-di-(p-aminophenoxy)-bis-2,2-(p-(amidinophenoxymethyl)propane
TESS	Toxic Exposure Surveillance System
TLC	Thin-layer chromatography

T_{Max}	Time to maximum serum concentration
TMCS	Trimethyl chlorosilane
TNBS	Trinitrobenzene sulfonic acid
USP	United States Pharmacopeia
WBI	Whole bowel irrigation

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Introduction:

Apomorphine is a very potent emetic agent which has fallen into disuse due to the fact that it is not effective orally and for some individuals, the recommended subcutaneous emetic dose of 0.1 mg/kg is associated with toxicity most notably respiratory depression and persistent emesis which may lead to acute circulatory collapse, coma and death [1, 2]. As well as having serious side-effects, apomorphine also shows a very high level of interpatient variability [1, 3-6]. In spite of these drawbacks, apomorphine is currently being investigated, or has recently been investigated, as a therapeutic agent in the treatment of Parkinson's disease, chorea, schizophrenia, alcoholism and erectile dysfunction [7-10].

A method of administration which allows a controlled rate of release could reduce the incidence of adverse effects which are dose-related and could provide a method of dealing with the interpatient variability seen with the drug. If emesis was the therapeutic end point and drug absorption could be stopped at that point, apomorphine could be used as an effective and safe emetic agent. There may also be a possibility that such a delivery system or a variation of it would be useful for administration of apomorphine in the treatment of some of the other diseases where it is being investigated as a therapeutic agent or adjunct.

Project proposal:

A possible way to avoid the toxicity and prolonged action of apomorphine after the desired emetic effect has been achieved would be to incorporate the drug into a small polymeric insert suitable for ophthalmic use. The insert would be placed into the conjunctival sac and release the drug in a controlled way. Once emesis has occurred, the insert which would serve as a drug reservoir can be removed or washed out of the eye. In this way the minimum dose required for emesis is given and the possibility of excess drug absorption occurring is reduced. This may also avoid the need to administer other drugs to counteract the adverse effects of apomorphine.

The ocular insert would require the following product attributes or specifications:

- be non-irritating to the conjunctival membranes
- be easy to apply and soft and adhesive enough to remain in place
- release most of the drug within a five to ten minute time window
- have a robust matrix to allow for easy removal when emesis has occurred
- have sufficient chemical stability to allow a reasonable shelf life
- be supplied as a sterile product

Project hypothesis:

The hypothesis for this project is: Apomorphine can be administered as a safe and effective emetic for use in canine patients through the ocular route using a polymeric delivery system.

Objectives:

The specific objectives of the proposed project are:

1. Devise a formulation which meets the product attributes or specifications
2. Characterize the final product in terms of drug release and drug stability
3. Conduct a clinical trial in canines to determine the efficacy and safety of the product

Section 1 - Overview of toxic oral ingestion

1.1 Acute poisoning in human medicine

In the United States many of the poison control centers have been collecting data in the Toxic Exposure Surveillance System (TESS) since 1983 and by the end of 1999 had accumulated more than 24 million human poison exposure cases [11]. In spite of the fact that another report points out that not all the poison control centers participate in this program and the reported values probably represent only about 26% of the actual totals, the data base represents the largest set of such data available [12]. A report issued in 1998 [11] shows that about 92% of the poisonings occurred at the home of the patient and that 40% of the cases were children under the age of 3 years and 15% were children between the ages of 3 and 6 years. Overall 87% of the cases were unintentional and of the 12% which had an intentional component 8% represented suicide attempts, 4% represented abuse/misuse of a substance and 1% were malicious. Although these are somewhat sobering numbers, there were only 775 deaths reported of the 24 million cases or about 3 for every 100,000 cases reported.

The poisons involved in the cases were reported in groups and Tables 1.1 and 1.2 provide summaries extracted from those reports:

Table 1.1 Intoxicants and frequency [11]
* Includes street drugs
** Topical, vaporous, venomous, unknown exposures

Intoxicant	% of Total Cases
Medications *	23.1
Cleaning materials	10.2
Plants	5.5
Alcohol/antifreeze	5.2
Foreign bodies	4.6
Insecticide/Pesticide	3.9
Hydrocarbons	3.0
Other **	44.5

Table 1.2 Incidence of death for selected categories [11]

Intoxicant	Death (%)
Medication	1.12
Street drugs	0.35
Alcohols/Glycols	0.18
Pesticides	0.02
Cleaning materials	0.01

The general initial management of poisoning consists of four basic elements:

1. Stabilization of patient and support of vital functions
2. Identification of intoxicating agent
3. Removal, neutralization or reversal of intoxicant effect
4. Block or reduce further absorption of intoxicant

The presentation of a poisoned patient in the emergency room of a hospital demands prompt action to address all four of these elements through an orderly and thoughtful process [13]. The general urgency of the situation often requires that each of these must be started

almost simultaneously. After an initial rapid assessment supportive care should be provided if it is needed, to ensure that respiratory, circulatory and central nervous system functions are stable and maintained. Blood and urine specimens should be taken for toxicological screening and relevant laboratory data. Two specific situations demand special attention: the unconscious patient and the convulsing patient [14].

Some protocols recommend that patients with an altered level of consciousness should be given an intravenous dose of naloxone, a narcotic antagonist, and that intravenous dextrose should be started but both of these procedures have received some criticism. It is now recommended that naloxone be reserved for patients with bradypnea and that 100 mg of thiamine be given before dextrose in patients where there is suspicion of alcoholism to avoid Wernicke's encephalopathy [13, 15]. Drug or toxin induced seizures may be difficult to control; seizures are often seen with overdoses of cocaine and tricyclic antidepressants and agents such as theophylline and pyrethroid insecticides cause seizures which are refractory to conventional anticonvulsant drugs. Benzodiazepine drugs such as diazepam or midazolam given intravenously may be useful in these cases as prolonged seizures may lead to serious morbidity or death due to severe lactic acidosis or hypoxia [14]. Identification of the intoxicant depends on questioning the patient, family members or attending friends, clinical assessment of signs and symptoms displayed by the patient and laboratory screening [13].

In some cases reversal or neutralization of the toxin may be accomplished by the use of specific antidotes; unfortunately there are relatively few intoxicants which have specific antidotes. Some examples of those which do are listed below in Table 1.3:

Table 1.3 Examples of specific antidotes [12-14, 16]

Intoxicant	Antidote
Acetaminophen	N-Acetylcysteine
Alcohols, glycols	4-Methylpyrazole
Anticoagulants	Vitamin K_1
Cyanide	Sodium nitrite/thiosulfate
Digitalis	Digoxin-specific antibody
Heavy metals	EDTA, penicillamine
Narcotics	Naloxone
Pesticides (organophosphate)	Atropine

Preventing further absorption of the intoxicant has been attempted by means of inducing emesis, gastric lavage and/or administration of activated charcoal. These procedures are collectively referred to as gastrointestinal decontamination and for the past decade have been a topic of some controversy.

Gastrointestinal decontamination

A number of studies have compared the efficacy of ipecac induced emesis, gastric lavage and activated charcoal administration as methods of reducing drug absorption. The outcomes of these studies have been conflicting but several have suggested that the administration of charcoal alone was as effective as any combination of emesis, lavage and charcoal [16]. The concept of gastrointestinal decontamination is largely based on intuition; removal of any unabsorbed toxin should benefit the patient and reduce increasing toxicity [17] but a number of controlled trials and a series of position papers published by the American Academy of Clinical Toxicology (AACT) and the European Association of Poisons Centers and Clinical Toxicologists (EAPCCT) have challenged this concept and suggested that routine gastrointestinal decontamination be abandoned except for specific situations [18-22]. The largest consideration appears to be time and if more than an hour has passed since the

ingestion and the patient is symptomatic, absorption has likely progressed to the point that the risks associated with gastrointestinal decontamination will outweigh any potential benefit with the possible exception of ingestion of controlled-release pharmaceuticals [17]. In spite of these recommendations and the evidence presented, a study of emergency room procedures in the United Kingdom showed that the routine use of gastrointestinal decontamination is still very common [23] and in terms of early treatment, support for the inclusion of an emetic such as ipecac syrup in home first-aid kits has been expressed [24].

Emesis

Generally emesis is not recommended if the intoxication is due to corrosive materials, petroleum distillates or if the patient is unconscious or convulsing. Corrosive materials will worsen oral and esophageal injury on emesis and aspiration is likely with low viscosity hydrocarbons or if the patient is unconscious or convulsing [12]. Emesis is possibly useful for substances not absorbed by charcoal (eg arsenic, ethylene glycol, alcohols, iron and lithium salts) or for solid controlled-release oral dose forms [17, 25]. Syrup of ipecac is the most commonly used emetic and is frequently a component in home first-aid kits. Ipecac appears to be most effective if it is used within 30 minutes of toxin ingestion [13]. Other agents which have been used to induce emesis include apomorphine, copper sulfate solution, fluid extract of ipecac and hydrogen peroxide [12]. It should be noted that ipecac contains two active alkaloids emetine and cephaline and the fluid extract contains about 2% of alkaloids (calculated as emetine) while ipecac syrup contains about 0.14% alkaloids; the emetic dose for adults is about 20-40 mg of alkaloids and for children 14-20 mg depending on age. [1] Emesis with ipecac syrup usually occurs 20 minutes after administration [12, 13, 16]. The position statement regarding Ipecac from AACT and EAPCCT concluded that ipecac should not be administered routinely in the management of poisoned patients. This was based on a review of published studies and an assessment which suggested that the amount of marker removed by ipecac was highly variable and diminished with time. Overall there was no evidence that

ipecac improved the clinical outcome of poisoned patients [18]. Human studies had demonstrated that 21-38% of the ingested drug or toxin was removed from the stomach if ipecac was administered one hour after toxin ingestion. It was also pointed out that the average arrival to an emergency room was between 2 and 3 hours post-ingestion [16, 18]. Ipecac syrup is generally a safe product when taken in the recommended dose but may produce lethargy, diarrhea, and persistent emesis. Since the time from administration to emesis with ipecac is about 30 minutes the use of activated charcoal and administration of oral antidotes such as N-acetylcysteine is delayed for this time and these therapies may be more beneficial than emesis [16, 25].

Gastric lavage

For this procedure, a large bore nasogastric tube (36-40F for adults) is positioned in the stomach and 200-300 mL aliquots of warm tap water or saline is introduced through the tube then withdrawn. This process is repeated until the return fluid is clear. For children a proportionally smaller tube and smaller aliquots are used. At this point a slurry of activated charcoal (50-100 g) is usually administered through the tube [13]. The AACT and EAPCCT position paper on gastric lavage concluded that gastric lavage should not be employed routinely in the management of a poisoned patient. The review and evaluation process led to the conclusion that the amount of marker removed by gastric lavage was highly variable and diminished with time and there was no certain evidence that routine use of gastric lavage improved clinical outcomes and that gastric lavage could in fact cause significant morbidity [19]. The study data reviewed by the committee was similar to those data seem for ipecac-induced emesis; about 32% of the ingested drug/toxin is removed from the stomach when lavage is performed one hour post ingestion [16]. Gastric lavage is the one method of gastrointestinal decontamination associated with significant morbidity and the rate of complications has been set at about 3% with the reported complications including aspiration pneumonia, esophageal injury, cardiac arrest and hypoxia [13, 26, 27].

Activated charcoal

Many drugs and chemicals are adsorbed onto charcoal and made unavailable for gastrointestinal absorption. Charcoal is an odorless, tasteless, fine black powder generally prepared by pyrolysis of cellulose. It is not absorbed by the gastrointestinal tract and is given in a dose of 1 g/kg. In acute poisoning dosing may be repeated every 2-6 hours when the intoxicant is known to undergo entero-hepatic circulation [1, 13]. Although charcoal is able to adsorb many compounds, agents such as alcohols, glycols, iron, lithium, heavy metals, and cyanide are not adsorbed [1, 13, 25]. Administration of charcoal may bind ipecac and orally administered antidotes such as N-acetylcysteine therefore charcoal should not be given until after the emetic effects of ipecac have been realized and not at all when an oral antidote is to be administered [13, 25]. The position paper prepared by the AACT and EAPCCT on activated charcoal suggested that single-dose activated charcoal not be given routinely in the management of poisoned patients; they reported that effectiveness decreased with time and the greatest benefit was when administration was within one hour of ingestion [20, 21]. Several studies showed that when a dose of at least 50 g was given within 30 minutes of ingestion, a mean decrease in drug absorption of 89% was found; 37% if administered one hour post ingestion [16].

Whole-bowel irrigation

The procedure of whole-bowel irrigation (WBI) involves the oral administration of a balanced electrolyte solution such as Golytely ™ usually containing polyethylene glycol, at a rate or 1-2 L/h until the rectal effluent is clear. Total wash-out of the gut is usually achieved in 3-4 hours [13]. The position statement of the AACT and EAPCCT on whole-bowel irrigation finds that WBI should not be used routinely in the management of poisoned patients as there is no conclusive evidence that this procedure will improve the clinical outcome for the patient. The procedure should be reserved for cases where the intoxicant is not adsorbed by charcoal or where the agent is in the form of a solid controlled-release pharmaceutical [22].

As a result of the AACT and EAPCCT position statements, current practice should not use gastrointestinal decontamination as a routine procedure. It would appear that charcoal administration as a single dose is beneficial if less than one hour has passed since the ingestion and multi-dose charcoal if the drug is known to undergo entero-hepatic circulation. Gastric lavage is not recommended although in some cases it may be useful if performed within one hour of ingestion if the potential benefit outweighs the risk associated with the procedure. Emesis is not recommended unless performed within less than one hour after ingestion and whole-bowel irrigation is not recommended unless the intoxicant is not adsorbed by charcoal or if solid oral controlled-release pharmaceuticals have been ingested. These recommendations have been verified by clinical trials which have been reported since the publication of the position statements and earlier trials which examined combinations of various gastrointestinal decontamination procedures [26, 28-33].

In the TESS report, of 24 million cases, about 5% were treated with gastrointestinal decontamination and Table 1.4 shows the method used and the incidence of use for this 5% of the cases [11].

Table 1.4 Incidence of GI decontamination methods [11]

Method	Incidence (%)
Charcoal (Single)	63.7
Lavage	20.4
Ipecac	12.1
Charcoal (Multi)	5.3
Other emetic	2.9
Whole-bowel	0.9

Choice of Emetics

Historically a number of chemical agents have been used to induce emesis including ammonium carbonate (2 g dose), copper sulfate (1% solution), zinc sulfate, mustard powder, sodium chloride and hydrogen peroxide [1, 34]. These agents acted primarily through irritation of the gastric mucosa and most fell into disuse in the 1950s when gastric lavage became the method of choice for evacuating the stomach of ingested poisons. Apomorphine as an emetic agent also fell into disuse around this time due to toxicity and increased use of gastric lavage [34]. Ipecac syrup is something of an exception and has continued to be used as an emetic likely due to its safety profile and the fact that it can be given by mouth making it suitable for use at home where most poisonings occur [12, 24]. Two studies attempted to compare the safety and efficacy of ipecac syrup and apomorphine [33, 35]. The first of these was an animal study conducted using dogs and compared the efficacy of gastric lavage, ipecac syrup, lobeline and apomorphine. The animals were given a test meal containing 5 g of barium sulfate and the different methods of gastrointestinal decontamination were assessed at time intervals of zero, thirty minutes and sixty minutes post ingestion. The amount of barium sulfate recovered was used as an index of efficacy. The study found that the efficacy of all the test procedures declined with time but apomorphine gave a significantly higher recovery of barium at the 30 minute test interval and overall appeared to show the best efficacy. Lobeline was effective in producing emesis but had a very high incidence of adverse effects including convulsions and respiratory paralysis. Over the 60 minute time period all of the emetic agents provided better recovery than gastric lavage although the differences at time zero were less apparent and ipecac syrup had the longest latency period. The authors concluded that apomorphine was the emetic agent of choice for induction of emesis. The authors recognized that having to administer apomorphine by injection was a drawback to use of this agent and ran a small trial to determine if apomorphine would be effective when administered as an eye-drop. The same dose was administered initially subcutaneously and later as an eye-drop and

their findings were that for all the parameters assessed, there was no significant difference between the two routes of administration [33]. This was the first study to demonstrate that apomorphine was absorbed by the ocular route.

The second study compared the efficacy of apomorphine and ipecac syrup in a series of 86 children brought to the emergency department of Johns Hopkins hospital. The patients were randomly assigned to receive either apomorphine 0.07 $mg \cdot kg^{-1}$ subcutaneously or ipecac syrup 15-30 mL orally if emesis was indicated. Their finding indicated that both drugs were effective in inducing emesis but apomorphine had a significantly shorter latency period and gave better gastric evacuation. Their conclusion was to recommend ipecac syrup which while it did not perform as well, had a lower profile of toxicity and since it could be given orally, was suitable for use in the home [35]. These studies indicated that apomorphine was an effective drug but since it had to be administered by injection, it was not convenient and once the dose was given, it could not be recovered if signs of toxicity appeared.

1.2 Acute poisoning in veterinary medicine

Although there are a number of parallels between humans and dogs in the nature of the intoxicants ingested, there are some notable differences. The nature of the intoxicants ingested by dogs include items such as garbage, carrion, and foreign objects such as batteries, balls and clothing which would be uncommon in human intoxications. In human medicine more than half of the accidental poisonings occur in children under the age of six years whereas with dogs there does not seem to be much of an age bias and the probability of exposure seems to quite consistent over the life-span of a dog [11]. Metabolic differences between canines and humans also are factors in intoxications; chocolate for example is relatively non-toxic to humans but presents the potential for a serious intoxication in dogs [36, 37] and with dogs the packaging materials holding the intoxicant are often consumed along with the intoxicant.

As has already been pointed out, the Toxic Exposure Surveillance System (TESS) provides a powerful tool for tracking and analyzing human exposures. Since 1993, however, the database began to include information on other species as well and these include cats, dogs, birds, cows, horses, rodents, and sheep [38]. In the 1993-94 report, 140,614 animal exposures were reported with 82% of the cases being dogs and 14% being cats. Of these reported cases 98% were of an acute nature and 97% of them involved exposure to a single agent. In 32% of the cases no specific treatment was administered; in 7% of the cases an emetic was administered and in 28% of the cases skin/eye irrigation or dilution of the stomach contents was performed. For dogs the rate of unintentional poisonings was reported as 94%, intentional as 0.45% and unknown as 5.6%. As with human cases most of the exposures (90%) occurred in the home and about 94% of the cases involved ingestion of the material. In terms of outcome, about 0.45% of the canine cases resulted in death directly attributed to the exposure. Cases where the ingested material was a foreign object not considered as a poison, for example clothing or rubber balls, are not included in the data base whereas foreign objects such as a batteries or paint which potentially could cause intoxication are included. The poisons were also reported in groups in a fashion similar to the report on human exposure and Table 1.5 and Table 1.6 show a summary for canines extracted from the report [38]:

Table 1.5 Intoxicants and frequency [38]
* Includes batteries, building materials, fireworks, mothballs
** Topical, vaporous, venomous, unknown exposures

Intoxicant	% of Total Cases
Medications	21.5
Street drugs	4.0
Cleaning materials	5.9
Plants	8.8
Alcohol/antifreeze	1.6
Foreign bodies *	7.3
Pesticides	8.6
Hydrocarbons	2.3
Food/Garbage	2.1
Other **	37.9

Table 1.6 Incidence of death for selected categories [38]

Intoxicant	Death (%)
Medication	0.07
Street drugs	0.01
Alcohols/Glycols	0.04
Pesticides	0.16
Cleaning materials	0.02

Although reporting programs such as TESS provide data which can be used to allow determination of trends and analysis of treatment outcomes and organizations such as the American Association of Poison Control Centers (AAPCC) provide consultation and treatment advice, the number of poison control centers in the US has been declining. The primary reason for this is a lack of adequate funding and efforts are being made to secure stable funding from industry and government [39]. The value of having a cooperative network

of data sharing for veterinary medicine has been recognized in Europe as well and in 1999 there was a call to establish such a network [40]. Regional services, usually based in veterinary colleges, established standards of care and began sharing information in the early 1990s using the National Animal Poison Information Network (NAPINet) [41, 42]. Incorporation of animal data into TESS may help to detect emerging environmental toxic hazards as well as providing more effective treatment for both human and animal patients.

1.2.1 Role of Emesis in Veterinary Medicine

In general terms, the approach to a poisoned patient either human or canine has the same four elements as previously outlined; stabilization and support of vital functions; identification of the intoxicating agent; removal, neutralization or reversal of the intoxicant effect; and blocking or reducing further absorption of the intoxicant [3, 4, 37]. As with human patients, the use of GI decontamination is usually restricted to specific situations and it has been pointed out that in parallel with human medicine, there is a general trend away from using gastric evacuation and that early administration of activated charcoal alone is now being used instead [3]. Although gastric lavage or whole bowel irrigation may be considered with canine patients in situations where gastric evacuation is indicated but administration of an emetic is contraindicated, these procedures may pose further risk to the patient. Since the conscious canine patient is unlikely to be cooperative, the use of an anesthetic agent is usually required and this may add further complications to the case and increased risk to the patient [3]. In veterinary medicine the primary agents which may be considered for induction of emesis include syrup of ipecac, 3% hydrogen peroxide, apomorphine and xylazine. Peroxide or syrup of ipecac may be available in the home and consideration should be given to having the owner administer one of these to the patient if there will be a delay in bringing the animal to the hospital. This may be a difficult decision and should only be considered if the ingestion has occurred within the past hour, the intoxicant is not caustic or petroleum-based and the patient is alert [4]. If the ingestion occurred more than two hours previously emesis will likely

be of no value and if the ingested material is caustic or petroleum-based, emesis may result in esophageal burns or aspiration pneumonitis respectively and these may pose life-threatening complications to the patient. In a clinical setting where the patient is under the supervision of a veterinarian, apomorphine would be the drug of choice for dogs and xylazine for cats [3, 4]. Hackett [4] discussed drugs which may be useful and his recommendations for dosing in dogs and cats are presented in Table 1.7. He also suggested that apomorphine could be given by preparing a solution from a soluble tablet and administering the solution drop-wise to the eye.

Table 1.7 Emetic drugs and doses [4]
* Contains 7% Ipecac
** Administered as a 10% suspension

Drug	Canine	Feline
Apomorphine	0.03 mg/kg IV	Not used
Xylazine	1.1-1.2 mg/kg IV	0.44 mg/kg IV or SC
Ipecac Syrup	1.0-2.5 mL/kg PO	3.3 mL/kg PO
Hydrogen peroxide 3%	1-2 mL/kg PO	1-2 mL/kg PO
Activated charcoal **	1-5 g/kg PO	1-5 g/kg PO

1.2.2 Canine intoxications

Medications

The data presented in Table 1.5 indicate that medications constitute about 20% of the canine poisonings presented in the 1993-1994 TESS report. In reviewing cases of ibuprofen, aspirin and acetaminophen toxicosis of dogs and cats, Villar et al pointed out that toxic exposure to these specific agents was associated with three general sets of circumstances. Most commonly the animal was able to gain access to the medication due to inappropriate storage by the pet owner, improper dosing of medication by the care-giver, or the inappropriate administration of medication by a well-intentioned owner based on their diagnosis rather than that of a veterinarian [43]. These sets of circumstances could be extrapolated to almost all cases of toxic exposure to medications or medicinal substances.

Cases of ibuprofen, asprin and acetaminophen toxicosis were reviewed by Villar et al [43]. In cases of ibuprofen ingestion in dogs, they reported that chronic dosing at 8 $mg \cdot kg^{-1} \cdot day^{-1}$ would cause gastric ulceration within 30 days but situations of single acute dosing up to 70 mg/kg did not usually lead to serious problems or sequalae. Single doses in excess of 70 $mg \cdot kg^{-1}$ usually resulted in some degree of gastric ulceration which seemed to be dose-related and doses in excess of 400 $mg \cdot kg^{-1}$ were potentially and often fatal in dogs. In terms of treatment for acute ibuprofen exposure, Villar recommended emesis or lavage only if the interval between exposure and treatment was short although no specific time window was given. The use of activated charcoal was not mentioned but the use of specific agents such as H_2-histamine receptor antagonists and misoprostol was recommended as these could potentially offer protection to the gastric mucosa from the action of the ibuprofen. Other supportive treatment included the use of IV fluids (120 $mL \cdot kg^{-1} \cdot day^{-1}$) to prevent decreased renal blood flow and hypotension, administration of IV sodium bicarbonate (1-3 $mmol \cdot kg^{-1}$) to promote ibuprofen excretion and administration of anticonvulsant drugs if needed. With aspirin intoxication, single acute doses exceeding 450 $mg \cdot kg^{-1}$ resulted in severe toxicosis and

treatment was essentially the same as for ibuprofen intoxication. For acetaminophen exposure Villar et al found that doses exceeding 500 $mg \cdot kg^{-1}$ were potentially life-threatening and doses between 200 and 500 $mg \cdot kg^{-1}$ would result in signs of hepatic damage and some degree of hepatic failure which was dose-related. For treatment the use of emesis or lavage was recommended if the interval between exposure and treatment is short but the primary treatment consisted of supportive measures and the administration of N-acetylcysteine at a dose of 140 $mg \cdot kg^{-1}$ followed by doses of 70 $mg \cdot kg^{-1}$ PO or IV four times a day for seven treatments if the acute dose of acetaminophen was equal to or less than 200 $mg \cdot kg^{-1}$. If the dose of acetaminophen exceeded 200 $mg \cdot kg^{-1}$, N-acetylcysteine was recommended at a dose of 280 $mg \cdot kg^{-1}$ initially followed by doses of 70 $mg \cdot kg^{-1}$ four times a day for three days. The N-acetylcysteine functions to replenish glutathione stores in the liver and provides sulfate to facilitate renal excretion of the acetaminophen via sulfonation.

Richardson [44] reviewed a series of reports regarding canine toxic exposures to the antiviral agent acyclovir. Over a fifty month period, he found reports of 105 cases of which 10 were classified as acute intoxications. In the cases reported, the major toxic effect of the ingestion was crystalluria-induced nephropathy. The treatment recommendations in this review included emesis of exposure was recent, administration of activated charcoal as charcoal has been shown to be effective in adsorbing acyclovir and finally aggressive hydration to minimize crystal formation in the renal tubules. Of the ten acute cases reported by Richardson, nine had consumed the oral capsule dose form and one had consumed a tube of a topical preparation.

Volmer [45] reported 17 cases of acute toxicity associated with the ingestion of cisapride, a drug formerly used to promote esophageal and gastric emptying. Symptoms of severe toxicity which included abdominal pain, ataxia and hyperthermia were seen with acute doses exceeding 18 $mg \cdot kg^{-1}$ and the usual exposure history involved the dog chewing and consuming a container of the owner's prescription medication. The treatment

recommendations included induction of emesis but only if done within 15 minutes of the ingestion, the administration of activated charcoal which was shown to be very effective in adsorbing the cisapride, and the administration of diazepam or a barbiturate if needed to reduce muscle fasciculations and rigidity.

A retrospective study was done using the National Animal Poison Control Center database looking for reported cases of 5-hydroxytryptophan intoxication [46]. The authors found ten reports within a ten year time-frame. 5-Hydroxytryptophan is sold in health food stores as a dietary supplement claimed to be useful in managing the symptoms of depression, chronic headaches, insomnia, and obesity. 5-Hydroxytryptophan is a precursor of serotonin and in dogs acute doses exceeding 25 $mg \cdot kg^{-1}$ produce severe effects. The symptoms include potentially life-threatening neurological, respiratory, GI, cardiovascular and coagulation disorders very consistent with severe serotonin excess. The treatment recommendations resulting from this review included emesis but only if this is done within 30 minutes of ingestion and administration of activated charcoal. Supportive treatment with anticonvulsant drugs and hydration are usually necessary. The authors considered the possible role of the serotonin antagonist cyproheptadine in treating these patients and concluded it may be of benefit to some patients. They recommended the rectal administration of cyproheptadine since oral administration was problematical with concurrent activated charcoal administration.

Isoniazid intoxication in dogs was also reviewed by Villar et al [47]. Isoniazid which is used for the treatment and prophylaxis of tuberculosis in both animals and humans has a low therapeutic index or narrow margin between a therapeutic dose and a dose which will produce symptoms of toxicity. Dogs seem to be especially sensitive to these toxic effects and a dose as low as a single 300 mg tablet may be lethal to a 6 kg dog. Villar reviewed reported cases of canine intoxications with isoniazid and assessed the various treatment regimens in terms of their outcome for the patient. In his treatment recommendations induction of emesis and administration of activated charcoal was only of value if done within one hour post

exposure since isoniazid is so rapidly and completely absorbed by the GI tract. The induction of emesis with these patients also seemed to trigger convulsions so the overall strategy of GI decontamination appears to be of very limited value in cases of isoniazid intoxication. The primary treatment recommended was the administration of pyridoxine in a dose equivalent to the amount of isoniazid ingested and the administration of anticonvulsants, specifically diazepam, to control the isoniazid-induced seizures.

Two further papers making treatment recommendations based on surveys of case reports of canine poisoning due to medications included one which dealt with hexachlorophene ingestion and one which dealt with terfenadine intoxication [48, 49]. Although neither of these drugs are available today, it is worth noting that both papers only recommended emesis induction if ingestion occurred within one hour of treatment. Both recommended the use of activated charcoal to reduce further drug absorption and the paper dealing with terfenadine recommended multiple dosing with activated charcoal since terfenadine and particularly its metabolites undergo enterohepatic cycling. Charcoal could bind with and thereby reduce the re-absorption of these materials.

Drugs of abuse

A report [50] reviewing three cases of marijuana ingestion by dogs recommended emesis induction as part of the treatment particularly if the marijuana was in an uncut form. Further treatment is symptomatic and includes maintaining cardiovascular function as well as restoring body temperature to normal since hypothermia seemed associated with the ingestion. The authors further suggested that doses as high as 3 g/kg were generally non-lethal to dogs but would produce severe and prolonged clinical symptoms of central nervous depression. Further to canine ingestion of illicit or street drugs was a report dealing with the special case of accidental ingestion which may occur with dogs used by police for drug searches [51]. Occasionally with the excitement of the search, the dogs may quickly ingest their findings

before the officer accompanying the animal can retrieve the drugs. Since the officer is on hand very shortly after the ingestion, if steps can be taken quickly to minimize the effects of the material, the chance of recovery for the dog is increased. The report dealt with several specific illicit drug materials and dealt with actions which could be taken by the police officers at the time of the ingestion. In the case of marijuana, induction of emesis was recommended and the author suggested dissolution of an apomorphine tablet in water, withdrawing the solution into a 3 mL syringe and administering this dropwise into the animals conjunctival sac. Following emesis, the eye would be rinsed with clear water. The authors mentioned the use of oral hydrogen peroxide 3% at a dose of 1 $mL \cdot kg^{-1}$ or ipecac syrup at a dose of 2.2 $mL \cdot kg^{-1}$ but suggested that neither of these was as effective as the use of apomorphine. The use of activated charcoal at a dose of 1-4 $g \cdot kg^{-1}$ following emesis was recommended and the authors also pointed out that if signs of intoxication had already occurred, emesis was not recommended but the use of activated charcoal was strongly suggested. If the ingested material was cocaine, the report suggested that absorption of cocaine from any membrane surface would be so rapid that emesis would be of very limited value but they strongly recommended that charcoal be given as soon as possible. Death from cocaine poisoning is generally due to cardiac arrest, respiratory arrest and hyperthermia so supportive treatment for these eventualities will be necessary. In the case of narcotic ingestion the use of emesis is not recommended if any signs of intoxication are present but the administration of charcoal is recommended for all cases as well as the administration of naloxone, a potent narcotic antagonist. The usual dose of naloxone is 0.01-0.02 $mg \cdot kg^{-1}$ given subcutaneously. In the case of amphetamine ingestion the authors suggested that as with cocaine, absorption is so rapid that the use of emesis to limit absorption is of little value but the administration of oral charcoal is essential. The use of sedatives will probably be useful but this must be done under the supervision of a veterinarian.

Pesticides

Various types of pesticides represented 8.6% of the cases reported in the survey of the NAPCC and showed the highest rate of mortality [38]. In a retrospective review of 21 cases of rodenticide poisonings in dogs by Shea and Couto [52] five of the cases were due to warfarin ingestion, nine to diphacinone or bromdifacoum and for the remaining nine the exact agent was not known. Warfarin and dicoumarol are classed as first-generation rodenticides while diphacinone and bromdifacoum are classed as second-generation; the second generation compounds have much longer half-lives than the first. The anticoagulant rodenticides act by inhibition of the enzyme vitamin K eopxide reductase which results in an inability of the body to produce clotting factors II, VII, IX and X. Vitamin K functions as a co-factor in the production of these clotting factors and the enzyme functions to regenerate the vitamin K. Without the action of the enzyme the existing body store of vitamin K is depleted and the patient becomes dependent on external sources of new vitamin K. Treatment therefore consists of vitamin K_1 administration. Of the 21 cases reviewed, those with first-generation rodenticide intoxication received a median dose of 2.7 $mg \cdot kg^{-1} \cdot day^{-1}$; those with second-generation rodenticide intoxication received a median dose of 5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 21-42 days while those in which the agent unknown received a median dose of 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 15-28 days. In this series of patients the overall survival rate was about 83% In terms of treatment the use of emetics was not mentioned and treatment focused on replacement of clotting factors by administration of blood products where indicated and treatment with vitamin K_1.

Metaldehyde is a compound used in gardens to control slug and snail infestations. It is often provided in the form of pellets made with bran and in this pelletized form they can be easily picked up and dogs have been reported to find them palatable [53]. There is no specific antidote to metaldehyde so treatment is supportive in cases of ingestion. Emesis or lavage is recommended if early after ingestion. The pellets are usually colored with a blue dye so the presence of the dye in the vomitus or washings may provide confirmation of metaldehyde

poisoning. Oral charcoal and hydration with intravenous fluids is recommended and diazepam is used to manage the seizures associated with metaldehyde ingestion. Death is usually due to liver failure or the result of severe convulsions.

An unusual case of herbicide intoxication was reported by Harrington et al [54] where a dog was found drinking from a pool of accidentally spilled phenoxy herbicide. There is no specific antidote to these agents and acute exposure produces nausea, vomiting, abdominal pain, and weakness; chronic exposure will likely adversely affect the liver, kidneys, GI tract and skeletal muscles. They are also hematotoxic and may cause aplastic anemia, agranulocytosis, neutropenia and thrombocytopenia. In the case reported, the dog survived with supportive treatment including intravenous fluids, oral charcoal and diazepam. Emesis was not recommended as the product contained petroleum distillates and emesis would carry a high risk of aspiration so in fact, metoclopramide was administered as an antiemetic.

Antifreeze

Ethylene glycol is a toxic substance commonly used as antifreeze for automotive engines which both dogs and cats find palatable. Khan et al [55] reviewed the ASPCA database over a 30 month period for incidents of ethylene glycol intoxication and reported 510 cases of exposure. These cases involved both dogs and cats but 82% of the cases involved dogs. In the canine cases, sex distribution was about equal but there was an age bias and adult animals accounted for the majority of the cases. Death or euthanasia was reported in 16% of the cases. The minimal lethal dose for dogs has been reported to be 6-7 $mL \cdot kg^{-1}$ and this dose level results in a mortality rate between 60-70%. The toxicity of ethylene glycol is due to the toxic metabolites formed in the body rather than the parent compound which has a toxicity profile similar to alcohol. Ethylene glycol is metabolized to form glycoaldehyde, glycolic acid, glyoxalic acid and oxalic acid. These metabolites cause a severe metabolic acidosis and acute renal failure which is usually the cause of death. In terms of treatment, ethanol has been used as an antidote since it competes with ethylene glycol for the enzyme alcohol dehydrogenase

and slows the metabolism of the glycol. Fomepazole or 4-methylpyrazole is a specific inhibitor of alcohol dehydrogenase and is now used as an antidote to ethylene glycol and methanol intoxication [56]. In the review by Khan et al, treatment recommendations included emesis, administration of either ethanol of fomepazole as an antidote and aggressive intravenous fluid therapy. The use of charcoal is not recommended since it is ineffective in adsorbing alcohols or glycols [55].

Plants

Plants or plant materials are often associated with canine poisonings and a number of these have been reported in the literature [57, 58]. Cycad plants grow naturally in tropical or subtropical regions around the world and are popular as ornamental or house plants. The toxic component in cycad palms is a glycoside cycasin which is metabolized to form methylazoxymethanol which causes gastrointestinal and liver damage. The cause of death is usually due to liver damage or blood loss from the GI tract. A review of the National Animal Poison Control Centre database by Albretsen et al [57] identified sixty cases of cycad intoxication in dogs over a ten year period. This review found that there were no differences in numbers between males and females; no breed specificity was seen and the age of the dogs ranged from 8 weeks to 11 years. In terms of treatment after reviewing these cases, Albretsen et al suggested that treatment should be aggressive and emesis is recommended followed by administration of activated charcoal. The patient should be hydrated with 5% dextrose intravenously and blood should be transfused if blood loss from the gastrointestinal tract is severe and seizures and tremors may be managed with diazepam. With active and supportive treatment, the recovery rate for the patient reviewed was about 68%.

Albretsen et al [58] also published an evaluation of castor bean toxicosis in dogs. The castor bean *Ricinus communis* is often used as an ornamental plant but it contains a glycoprotein ricin which is highly toxic and ingestion has caused death in many species including domestic livestock such as cattle and horses as well as dogs and cats. Ricin

concentration is highest in the seed or bean of the plant and since the bean must be broken or crushed to release the toxin, symptoms may be delayed. Over an eleven year period the authors found 98 NAPCC reported incidences of ingestion with 76% of the cases showing acute signs of toxicity. The major signs of toxicity are a severe gastrointestinal disturbance which leads to severe hemorrhagic gastroenteritis followed by hypotension and myocardial necrosis. Treatment is supportive and emesis may be of benefit if the seeds are still intact and treatment is early following ingestion. Although there is no specific antidote, the administration of charcoal is very beneficial since it effectively adsorbs the ricin.

Both people and dogs find chocolate products palatable but theobromine a methylxanthine derived alkaloid and a component in chocolate, is toxic to canines [36, 37]. Fatal poisonings with this alkaloid have been reported in pigs, fowl, horses and dogs and Sutton [36] reported a case of cocoa poisoning in a dog which had consumed a 250 g container of cocoa. Death was due to heart failure in conjunction with acute circulatory failure. Sutton recommended that treatment be supportive with emesis or lavage being indicated if treatment was initiated shortly after ingestion. There is no antidote to methylxanthine intoxication. Tremors or seizures may be managed with diazepam [37].

Ingestion and toxicosis with macadamia nuts was investigated using the ASPCA National Poison Centre database over a five year period. Twenty-nine cases were found and reviewed by Hansen et al [59]. The symptoms of toxicity included weakness particularly of the hind limbs and general nervous system depression. Although the toxic component of the nut and the mechanism of toxicity have not been identified, toxicity is associated with functional disruption the motor neurons, neuromuscular junctions or neurotransmitters. In this study all of the exposed dogs reviewed and a small series of four dogs experimentally exposed to macadamia nut intoxication survived and eventually recovered. The authors suggested that some cases may require observation and possibly supportive treatment, but for most cases active treatment is not required.

Ten cases of toxicity in dogs due to ingestion of grapes or raisins were identified in the ASPCA database by Gwaltney-Brant et al [60]. In all of the cases vomiting occurred within the first few hours following ingestion and symptoms of anorexia, diarrhea, lethargy and abdominal pain were present and continued for several days. Of the ten cases, only five patients survived; the cause of death for the five which died was renal failure. The causative component in the grapes or raisins responsible for the development of renal failure is not known. The treatment recommendations resulting from this review included induction of emesis if the ingestion was recent and emesis had not already occurred, administration of activated charcoal and intravenous fluids for a minimum of 48 hours along with clinical chemistry monitoring for signs of renal failure developing.

Toxicosis due to the ingestion of hops was reviewed by Duncan et al [61]. Hops are the flowers or cones of the female plant *Humulus lupulus* and are used in brewing beer to give beer the characteristic pungent odor and bitter taste. In the case reported, two dogs had access to about 900 mL of hops which had been discarded onto a compost pile after the homeowner had been making beer. One of the dogs became clinically affected about three hours after the exposure and died within six hours of the exposure. The second dog had signs of abdominal discomfort six hours after exposure and after admission to an emergency clinic had a rectal temperature of 39.7°C. Treatment consisted of induction of emesis and lavage where about 250 mL of hops material was recovered; administration of activated charcoal; intravenous administration of corticosteroid and intravenous hydration. The dog survived the exposure and recovered completely within a few days. A review of the NAPCC database found three other reports of hops ingestion and all of the cases showed the patients had body temperatures in excess of 40°C and a clinical assessment of malignant hyperthermia was made in all three cases. Malignant hyperthermia is a life-threatening disorder of skeletal muscle and may be initiated by triggers including stress, anesthetics both general and local, and depolarizing skeletal muscle relaxants. Of the five cases presented in this paper, four of the patients were

greyhounds suggesting some level of breed specificity. The treatment recommendations from this review include aggressive gastrointestinal decontamination with emesis and lavage followed by administration of activated charcoal, intravenous hydration and administration of dantrolene sodium. If necessary, cooling with ice-packs should also be considered.

An unusual case of ethanol poisoning was reported by Kammerer et al [62] where a dog had consumed a quantity of rotten apples. A literature review by the authors found that although ethanol poisoning in dogs is rare, it had been reported due to ingestion of sweet alcoholic beverages, house-hold products, garbage and fermented foods such as bread dough. Although in the case reported, the patient did not survive the exposure, the authors did present treatment recommendations. These included extensive supportive care with respiratory support if necessary and placement of an endotracheal tube to prevent aspiration since vomiting would be common. Hypoglycemia should be treated with intravenous dextrose but the author strongly recommended administration of thiamine prior to administering dextrose and careful monitoring of body temperature. Metabolic acidosis should also be corrected as it develops. Treatments not recommended included gastrointestinal decontamination and charcoal administration since neither appeared efficacious. The authors did not discuss administration of the alcohol dehydrogenase inhibitor 4-methylpyrazole.

Foreign objects

In human medicine, foreign body ingestion is more commonly seen with children than adults and in adult cases there is usually some underlying psychiatric disorder. In a study of adult admissions for foreign body ingestion over a ten year period at the University of Wisconsin Hospital, 75 admissions were identified [63]. This study excluded cases of esophageal foreign body lodgment and patients under the age of 18 years. The 75 admissions involved 22 male patients all of who were inmates from a local prison. A total of 256 foreign objects were ingested and the types of object included items such as light bulbs, typewriter keys, razor blades, spoons, pencils and nail clippers. The findings of the study suggested that

with conservative management most foreign bodies would eventually pass spontaneously with no serious consequences. Endoscopic retrieval had a high failure rate and was associated with complications such as esophageal tearing. The authors recommended that surgical intervention should be reserved for patients showing symptoms of an acute abdomen and that generally a conservative approach should be taken when dealing with ingested foreign bodies. The size, shape and number of the objects did not seem to be predictive factors regarding whether there would be problems with spontaneous passage.

In a study of foreign object ingestion by children [64], coins were the most common item ingested and of these, the majority was pennies. These authors also recommended conservative treatment and found that complications and problems were much less common when the patient was initially seem by their own physician in private practice. Emesis induction, particularly in cases of foreign object ingestion does not seem to be a common treatment modality possibly due to the danger of esophageal injury occurring. Emesis itself especially if forceful is associated with the risk of esophageal rupture or tearing. Mullen et al [65] described an unusual case where rupture of the esophagus occurred at two sites in the esophagus post-emesis and if at the time of surgical repair, the second site had not been detected the prognosis for the patient would have been grave. Esophageal rupture usually occurs at the gastro-esophageal junction and is associated with a high mortality rate. Without surgical intervention to repair the damage mortality has been placed at 50% within the first 24 hours and 90% at 48 hours.

Dogs have been known to ingest a wide range of foreign objects many of which are unpalatable and in some cases difficult to swallow. Stauffer and Swails [66] described a case where a dog was admitted to clinic for an apparent GI obstruction. During surgery, jewelry, a leather bracelet, a brassiere and a pair of panty-hose were all recovered from the ileum. None of the objects showed evidence of chewing and all appeared to have been swallowed intact. The authors speculated that consumption of these items had a psychological component in that

the home-life of the dog had been disrupted due to the family having on-going domestic difficulties.

Latimer et al [67] reported a case of a 4 month old dog admitted to a clinic with symptoms of anorexia, vomiting and salivation. A hematological examination showed a severe hemolytic anemia with spherocytosis suggestive of zinc intoxication. An x-ray examination showed the presence of four foreign bodies in the stomach which on removal by gastrotomy, were found to be pennies. After surgical removal of the pennies and supportive care which included blood transfusion, the patient recovered without complications. The authors recommendations for treating patients who have ingested metallic foreign bodies containing zinc include removal of the objects, whole blood transfusions if the packed cell volume is low or if there are signs of anemia, and fluid therapy to correct electrolyte imbalance due to vomiting and to improve renal function. As to removal of the foreign bodies the recommendation in the paper is to use endoscopy or gastrotomy although the author concedes that zinc nuts have been eliminated by spontaneous or induced emesis. In spite of this, emesis is still not recommended since it may not be effective as was seen in the case presented where the patient was admitted partly due to vomiting and the process of emesis exposes the patient to the risk of aspiration and the possibility of esophageal injury.

Other reported cases of ingested foreign bodies by dogs include a case of an 8 year old German shepherd which had swallowed a 20 cm wooden shiskabob skewer [68] and a dog which had a fishhook lodged in its esophagus [69]. In both of these cases treatment was by surgical intervention.

Another unusual case was reported by Polloch [70] where a canine patient was admitted to clinic for evaluation of intermittent regurgitation accompanied by persistent drooling and apprehension on movement of the head and neck. A radiological examination showed two small radiopaque objects at the thoracic inlet of the esophagus. A gastroscope was passed into the esophagus where a wooden stick was visualized and then gently extracted by

means of a forceps within the gastroscope. The stick which was recovered was about 30 cm long and the opaque objects seen on the x-ray film were two small stones located inside a hollow portion of the stick.

Ingestion of the small button or disc batteries commonly used in miniaturized electronic equipment and toys present a serious problem with children and pets particularly dogs. In a review of pediatric ingestions of these batteries the potential risk was discussed [71]. In terms of chemical composition, at the present time these batteries fall into four major groups; mercury/potassium hydroxide, manganese/potassium hydroxide, silver/potassium hydroxide, and manganese/lithium. The authors suggested, based on their review, that the amount of damage produced by the battery is determined by the site where the battery is lodged or located and the length of time it remains at the site and pointed out that larger batteries (20-23 mm) tended to become lodged in the esophagus while smaller batteries tended to pass into the stomach and then progress into the small intestine. Batteries lodged in the esophagus presented a higher risk of tissue damage since they are held in a localized area and there is little or no benefit from dilution of the chemical and electrical effects as would be provided by the gastrointestinal secretions in the more distal segments. Tissue damage from batteries is caused by leakage of the alkali in them causing direct caustic injury, absorption of the toxic heavy metals present in many batteries, pressure necrosis especially in the esophagus and finally electrical discharge which may cause mucosal burns. Contact between the tissues and the battery can cause the release of low voltage direct current and it has been suggested that this can liberate intracellular potassium and result in cellular death. The authors recommended conservative treatment for the smaller batteries but for those lodged or impacted in the esophagus, endoscopic retrieval be attempted to avoid esophageal erosion. A device essentially consisting of two tubes each with a ferrite magnet at the end corresponding to the diameter of the ingested battery has been described in the literature and may be less invasive than the use of an endoscope [72].

In a study designed to assess the effectiveness of different medication regimes in treating cases of button battery ingestion, Litovitz et al [73] assessed 64 ingestion episodes in 18 dogs over a four month period. Battery ingestion was accomplished by placing the battery on the posterior aspect of the tongue then allowing the dog to swallow. This procedure was used to prevent the animal from biting or puncturing the battery. The dogs were divided into study groups and different medication regimes of magnesium citrate, cimetidine and metoclopramide were used. In all of the 64 trials battery transit through the gastrointestinal tract was faster than in humans and no abnormal signs or symptoms related to the battery ingestion such as caustic injury or mercury toxicity were seen. In view of the fact that no symptoms appeared, no therapeutic advantage with any of the medications compared to control ingestions could be demonstrated. The authors did however suggest that use of these medications may be of benefit in patients with prolonged gastric retention.

In the previous study, care was taken to ensure that the batteries did not become lodged in the esophagus and since batteries lodged in the esophagus pose a potential risk in terms of esophageal erosion, a study was carried out to determine what early treatment prior to battery extraction might reduce this risk [74]. In this study batteries were surgically lodged in the esophagus and the study animals were administered bolus doses of 20 mL of tap water at time intervals of 15, 30, 60 and 90 minutes. Animals were sacrificed at these time intervals and the tissues examined for signs of damage. The authors postulated that oral administration of water might lessen the amount of tissue damage done by the alkali leaking out of the battery or alkali produced by the direct current discharge of the battery. Water at pH 7 was used since although acidified water would effectively neutralize any alkali produced, it would also enhance the flow of current and produce no net benefit. The outcome of the study showed that although the amount of electrical discharge from the batteries was reduced in the test animals treated, the amount of tissue damage was not reduced at and after the 30 minute test interval. The authors concluded that administration of water to the patient within 30 minutes of the

ingestion would reduce the extent of local alkali injury to the esophagus when a battery is lodged in the esophagus.

Garbage

Garbage and carrion ingestion by dogs may lead to poisoning by various toxins produced by microorganisms during the decomposition process. A report describing mycotoxin poisoning in a dog after ingestion of garbage identified two mycotoxins, penitren A and roquefortine, present in the washings following gastric lavage [75]. Treatment for this patient included gastric lavage and supportive therapy for hyperthermia and seizures. The author pointed out that although the patient was also treated with activated charcoal, the benefit and efficacy of charcoal in the treatment of this type of intoxication is not known and would merit further investigation.

Conclusions: Section 1

Ingestion of toxic materials continues to be a problem with both humans and canines as evidenced by databases which track these occurrences. Over the past years, the approach to treatment has changed and gastrointestinal decontamination procedures such as emesis, gastric lavage and whole bowel irrigation have now fallen out of favor largely due to well designed studies which have shown that in most cases, these procedures are ineffective and presumably have little or no effect on the patient outcomes while exposing the patient to increased risks such as aspiration of ejected material and trauma to the esophagus. Current treatment appears to be supportive in nature although the use of activated charcoal to adsorb toxic material and reduce further absorption remains common and when appropriate, antidotes may be used for some specific intoxication. In human medicine, emesis may be induced if the ingestion occurred shortly before treatment or if the intoxicant is in the form of a prolonged release pharmaceutical where there is some chance of recovering some of the material but these situations are apparently uncommon. In veterinary medicine, particularly with canine patients,

emesis may still be used therapeutically but generally this is the case only if the time between ingestion and treatment is short. There are some differences however, between human and canine intoxications which make emesis somewhat more useful in veterinary medicine and these have already been pointed out: the nature of the intoxicants ingested by dogs include items such as garbage, carrion, and foreign objects such as batteries, balls and clothing which would be uncommon in human intoxications and with canines, the quantities which may be consumed are surprisingly large. Metabolic differences between canines and humans also are factors in intoxications as in the case of chocolate which is very toxic to dogs and with dogs the packaging materials holding the intoxicant are often consumed along with the intoxicant. In some cases where the intoxicant is not known, emesis may be used as a diagnostic tool if the clinician believes the procedure poses a suitable risk-benefit balance; metaldehyde which contains a distinctive dye and cases where the packaging is ingested may offer some clue as to what the intoxicant was. In human medicine more than half of the accidental poisonings occur in children under the age of six years whereas with dogs there does not seem to be much of an age bias and the probability of exposure seems to quite consistent over the life-span of a dog so the incidence of these cases in veterinary medicine may be relatively higher.

For induction of emesis in dogs, apomorphine would be the drug of choice in that it is reliable and fast-acting but as will be seen, it also has a range of potentially serious adverse-effects and currently, the only practical route of administration is by injection and even so, there is no commercial product on the market.

The range of toxic materials ingested by canines is quite wide, occurrence is relatively frequent, emesis is indicated more often than in human cases and there is no easily available commercial apomorphine dose form so from this brief review, it would appear that there is some need for this drug and a dose form or drug delivery system which may reduce the incidence of adverse effects would be beneficial.

Section 2 - Preformulation

Introduction:

In order to formulate a safe and effective pharmaceutical product a number of physicochemical properties of the drug must be known. The gathering and assessment of these data are referred to as the preformulation process and experimentation required to obtain this information is referred to as preformulation studies [76, 77]. These intrinsic physical and chemical properties may dictate the type of dosage form which is feasible and the range and nature of excipients which may be required in the formulation; they may also dictate which excipients may have a deleterious effect on the active component [78].

For a new drug, preformulation data will have to be obtained experimentally whereas for an established drug much of the information may be obtained from the literature although some experimentation may still be required. Since apomorphine is an established drug as well as an official drug in the USP [79], a large amount of information relevant to preformulation may be obtained from the literature and standard reference texts such as The Merck Index [80].

Preformulation studies provide a basis for anticipating formulation problems and looking for possible ways to circumvent or avoid these problems. Often, for example, poor water solubility of the drug substance is a potential problem both in formulating the product and in the way a product may perform in the body. If a drug with poor water solubility is to be formulated as a parenteral product some strategy to enhance solubility will be required to allow preparation of a product with a suitable drug concentration; possible strategies in this case may involve use of a mixed solvent vehicle or selection of a suitable salt form [81]. In the situation where poor water solubility may result in poor bioavailability use of micronized drug may be an appropriate and satisfactory strategy. An appropriate preformulation process will identify these potential problems as well as identifying possible solutions to the problem which will not adversely affect the drug or dose form.

In a text on preformulation, Wells [82] listed a large number of physicochemical parameters which may require assessment in a preformulation program. A summary of these parameters is provided in Table 2.1. As well as the physicochemical properties of the drug, preformulation also includes a review of the pharmacology and pharmacokinetics of the active drug and a review of any pre-existing dose forms for the drug as this information may provide some insight into problems which may arise during clinical use of the dose form under development. With this in mind, the physiochemical properties, pharmacology, pharmacokinetics, indications and existing dose forms for apomorphine will be reviewed.

Table 2.1 Preformulation criteria from Wells [82]

Test	Specific	Function/Characterization
Spectroscopy	UV	Simple assay
	IR	Identification
Solubility	Phase solubility	Purity
	Aqueous	Intrinsic and pH effects
	pKa	Solubility, stability control
	Salts	Solubility, stability, hydroscopicity
	Solvents	Vehicle, solubility, stability
	Partition coefficient	Lipophilicity, absorption
	Dissolution	Bioavailability
Melting point	DSC	Polymorphism, compatibility
Assay development	UV, HPLC, TLC	QC, quantitation, stability
Stability	Hydrolysis, oxidation	Stabilization strategy
Microscopy	Micromeritics	Flow, dissolution, bioavailability
Bulk density		Tablet/capsule formulation
Flow property		Tablet/capsule production
Compression		Tablet formulation
Compatibility		Excipient selection

The specific parameters to be investigated would be dependent on both the nature of the active drug component and the nature of the dose form which is being developed. Essential to the preformulation process is a validated stability-indicating assay. The assay may require extensive development as in the case of a new drug or for established drugs an existing published method may be used as published or provide a basis or starting point for a modified method. If the drug is official, the USP assay method may be appropriate. In the case of apomorphine, the drug is both established and official so much of the required preformulation data are available in the literature and the USP. The official assay is, however, a non-aqueous titration and therefore probably lacks the specificity necessary to be considered stability-indicating. One of the first tasks in the preformulation process for this project will, therefore, be to develop a suitable assay or find and adapt a published method of analysis.

2. Apomorphine:

2.1 Chemistry:

Names

Apomorphine hydrochloride USP
6aβ-aporphine-10,11-diol hydrochloride hemihydrate

$C_{17}H_{18}ClNO_2$ (Apomorphine HCl)
CAS 41372-20-7 (Apomorphine hydrochloride hemihydrate) [79]

Structure

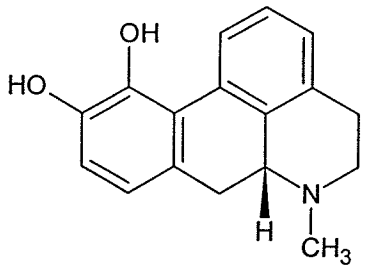


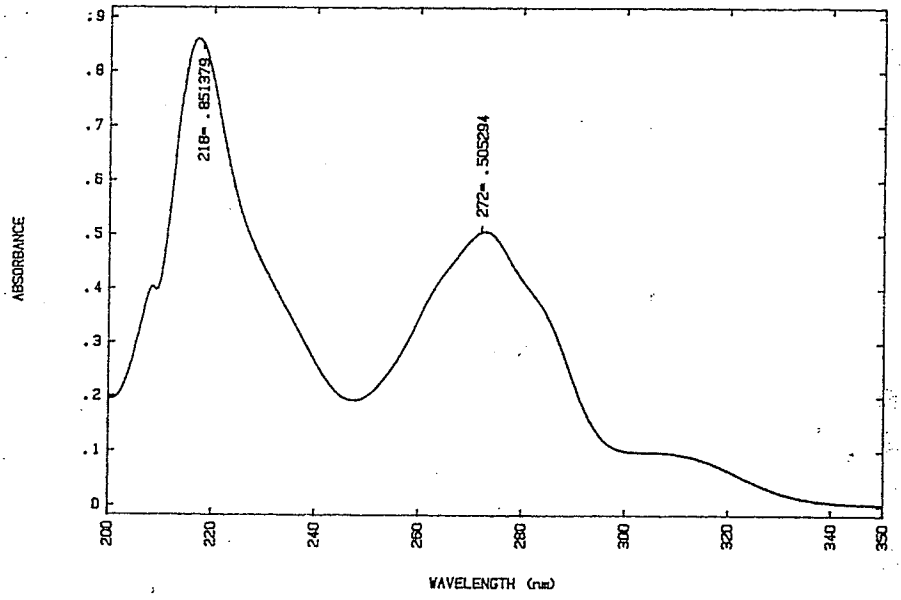
Figure 2.1 Apomorphine ($C_{17}H_{17}NO_2$)

The molecular weight of apomorphine is 267.32 and the elemental composition is: C 76.38%; H 6.41%; N 5.24%; O 11.97% [79, 80]. Apomorphine as the hydrochloride salt appears as white or grayish-white odorless glistening crystals which become green on exposure to light and air [79]. Apomorphine hydrochloride melts with decomposition between 225-236°C [79]. One gram of apomorphine HCl is soluble in 50 mL of water, 20 mL of water at 80°C and 50 mL of alcohol. It is practically insoluble in chloroform and very slightly soluble in ether. The free base is soluble in alcohol, acetone and chloroform and slightly soluble in water, benzene and ether. The pKa values for Apomorphine are 7.2 and 8.9 and a 1% solution in water has a pH of 4.5 to 5.5 [1, 79].

Ultraviolet (UV) spectrum

The UV absorption spectrum of apomorphine HCl 12 $ug \cdot mL^{-1}$ in 0.05 M hydrochloric acid was obtained over the wavelengths 200 to 350 nm using a Hewlet Packard diode array spectrophotometer. Absorption bands were observed at 218 and 272 nm with $\varepsilon = 13171.5$ at 272 nm and these are shown in Figure 2.2. Other UV maximum absorption data for apomorphine have been reported; in methanol 268 nm with $\varepsilon = 9592.17$; in aqueous acid 272 nm $\varepsilon = 19581.3$; and in 98% ethanol the free base shows absorption bands at 336 and 399 nm [80, 83].

Figure 2.2 UV spectrum of apomorphine in 0.05M HCl



2.2 Methods of Analysis

Titrimetric methods

Non-aqueous titrations are still used today for many compendial assays and an advantage to this method is that the measurement is direct rather than by comparison to a reference standard. Although useful for raw drug material, the method is much less useful in assaying finished products due to interference from excipients and the error involved in isolating the active ingredient from these interfering substances [79]. The USP assay for apomorphine hydrochloride is a non-aqueous titration using perchloric acid and crystal violet as indicator and the BP assay is similar but the end-point is determined potentiometrically [84]. The USP also contains a monograph for apomorphine hydrochloride tablets and the official assay for this finished product is a residual titration.

Spectrophotometric methods

A number of colorimetric methods have been described in the literature and these methods are based on the oxidation of apomorphine to colored quinone compounds which can

then be measured photometrically [85, 86]. A method was described by Kaul et al [85] which involved reacting apomorphine with 5% mercuric chloride in a buffer at pH 6. The mixture was heated at 70°C for 3-4 minutes, cooled to room temperature and extracted into isoamyl acetate. The oxidation product, which the authors identified as apomorphine orthoquinone, partitions into the ester; the isoamyl acetate is separated and the absorption is measured at 330 nm. The reaction product provides different colors in different solvents; the authors reported green in water, blue in methanol, ethanol and chloroform; pink in benzene and ether; and violet in isoamyl acetate. This method was modified for use in a stability study of apomorphine by Lundgren and Landersjo [86] where after acidifying the samples, they were extracted with ethyl acetate to remove any decomposition products, then running the reaction and again extracting with ethyl acetate. Although these authors did not provide validation data for the method, their results were supported by analysis using paper chromatography. A similar method to that described by Lundgren was used by Burkman [87] to develop a bioassay for apomorphine and comparison of their spectroscopic data to that of the bioassay showed good correlation. The bioassay was based on changes in intensity of pecking syndrome with changes in apomorphine dose with pigeons. Other method variations where the apomorphine was oxidized by potassium dichromate and hydrogen peroxide in acetic acid and a method where the apomorphine was treated with oxygen in an alkaline solution containing dioxane have been described [83]. Apomorphine shows an absorption band at 273 nm and this has been used as a means of measuring concentration; the BP uses this property of apomorphine as the basis for the assay of apomorphine injection and the USP uses UV absorption as the basis for the apomorphine tablet content uniformity test.

Apomorphine emits a bluish fluorescence under UV light with excitation at 270 nm and emission at 370 nm and this property of apomorphine was used as the basis for a method of measuring apomorphine in tissue reported by VanTyle and Burkman [88] where the apomorphine is extracted into ethyl acetate and quantitated by spectrofluorimetry.

Chromatographic methods

A thin-layer chromatographic (TLC) method for the identification of apomorphine is presented in a text by Clarke [83] where the stationary phase is silica gel-G 0.25 mm thick and the solvent system is methanol:ammonia (100:1.5). Apomorphine gives a spot which is visualized with acidified iodoplatinate spray and the R_f value for apomorphine is about 0.57. TLC is used in the USP as the method to identify ordinary impurities in apomorphine hydrochloride raw material. For this official test, the sample and standard are dissolved in methanol and the solvent system is n-butanol:water:formic acid (7:2:1). The chromatograms are visualized using a freshly prepared solution of 10% ferric chloride and 5% potassium ferricyanide (2:1) [79].

The issue of apomorphine stability was dealt with in a paper by Erhardt et al [89] describing TLC analysis of apomorphine analogues which may have use as potential anti-Parkinsonian prodrugs. During the course of their investigation, the authors confirmed that apomorphine orthoquinone was the main decomposition product of apomorphine oxidation, isolated the product and confirmed its structure with proton and ^{13}C NMR spectra. As to methods to avoid decomposition during metabolic studies and analysis, they found the most effective strategies were to incorporate either bisulfite or dithiothreitol into any aqueous media in contract with the drug substrates and to purge any organic solvents used with nitrogen prior to use.

A gas-liquid chromatographic (GLC) method for the quantitation of apomorphine in biological samples was reported by Baaske et al [90]. Although GLC methods have high sensitivity, they tend to be labor-intensive as they frequently require derivization of the analyte and for the past 20 years, use of this technology for drug analysis seems to have been largely replaced by high-pressure liquid chromatography [91, 92] although exceptions to this are the alcohol determination test (Method II) and the test for residual solvents presented in the USP [79].

From the late 1970s high-pressure liquid chromatography (HPLC) has become the method of choice for drug analysis both in biological systems and pharmaceutics and analytical systems, both quantitative and qualitative, using spectroscopy and chromatography; paper, column thin- layer and gas; have all largely been replaced with HPLC [93]. The primary reasons for this are that HPLC methodology is generally economical, fast, highly sensitive and specific with a minimum of sample preparation. The technology is also very flexible and methods have been developed to measure molecules of wide diversity ranging from large proteins to small inorganic ions. As well as quantitation, HPLC may be used to isolate or purify chemical entities. These points were presented in a review of recent trends in analytical technology by Rao and Nagaraju [91] and these authors presented data showing that in excess of 50% of the chromatographic techniques presented in the literature from 1995-2001 were HPLC.

A number of HPLC methods for the analysis of apomorphine particularly in biological samples have been published using a variety of column packing materials, solvent systems and detection methods. A number of these were reviewed to determine their suitability for this project; the published HPLC methods which were reviewed and the column type, mobile phase composition and method of detection are summarized in Table 2.2.

Table 2.2 Summary of HPLC method variables

Column	Mobile Phase	Detection	Additive	Ref
Phenyl	MeOH-AcCN-Phos Buffer (36:9:55) pH 3.25	UV $_{(273)}$	Na dodecyl sulfate	[94-96]
Phenyl	MeOH-Acetate Buffer (40:60) pH 3.25	FLU		[97]
Cyano	AcCN-Phos Buffer (11:89) pH 3.0	ECD		[98]
C_{18}	AcCN-Phos Buffer (30:70) pH 3.0	ECD	EDTA	[99]
C_{18}	AcCN-Phos Buffer (30:70) pH 3.3	FLU	Heptanesulfonic acid EDTA	[100, 101]
C_{18}	MeOH-Phos Buffer (35:65) pH 4.5	FLU	Hepatanesulfonic acid EDTA	[102]
C_{18}	AcCN-Phos Buffer (25:75) pH 3.0	ECD	Octanedisulfonic acid	[103]
C_{18}	AcCN-Phos Buffer (5:95) pH 3.5	UV $_{(279)}$	Tetrabutyl ammonium hydrogen sulfate	[104]
C_8	AcCN-Phos Buffer (15:85) pH 3.0	ECD		[105]
Cyano	AcCN-Phos Buffer (15:85) pH 3.0	ECD	EDTA	[106]

2.3 Pharmacology:

Mitchelson [107] reviewed the mechanisms and the neuronal pathways involved in emesis and in his review he described the following: Vomiting is initiated by the vomiting center located in the medulla which receives input from several sources to activate the reflex. The vomiting centre is not a discrete anatomical site, but represents inter-related neuronal networks. When vomiting is initiated by local irritation of the sensory receptors in the gastrointestinal tract, input to the vomiting centre is mediated through afferent neurons in the vagus and sympathetic nerves which provides a direct path to the vomiting centre. When vomiting is initiated from the vestibular apparatus in the inner ear, the input travels to the vestibular nucleus then through the cerebellum to the vomiting centre so this path would be used for emesis due to motion sickness. Initiation of vomiting due to input from the fauces travels from the fauces to the nucleus tractus solitarius and then on to the medullary vomiting centre. Vomiting may also be initiated by impulses from the higher centers, presumably in the cortex, and impulses induced by sensory input due to sights, sounds or smells have direct access to the vomiting centre. This may be the path used by the conditioned emesis seen in chemotherapy treatment.

The chemoreceptor trigger zone (CTZ) is a chemosensitive region in the area postrema on the upper surface of the medulla [107, 108]. This region lies outside of the blood-brain barrier so it does not have the protection afforded the vomiting centre and can be activated by toxins and drugs which do not cross the blood-brain barrier. Neurons pass from the CTZ to the vomiting centre so stimulation of the receptors in the CTZ can generate input to the vomiting centre. Wang and Borison [109] demonstrated that the vomiting center itself is not sensitive to emetic agents but serves to coordinate the reflex process. The vomiting centre, when stimulated by input, activates motor pathways which involve contraction of the diaphragm and abdominal muscles to increase intragastric pressure. Relaxation of the sphincter at the lower end of the esophagus allows the contents of the stomach to be expelled.

There is no contraction of the stomach but rather an inhibition of gastric motility preceding vomiting and this gastric relaxation may be mediated by dopamine [107, 108]. These effects are referred to as the motor correlates of vomiting. Emesis induced by apomorphine appears to be specifically due to stimulation of the D_2 receptor sites on the CTZ as the emetic action of apomorphine is inhibited by domperidone which is a potent dopamine D_2 receptor antagonist [110].

Lang and Marvig [110] studied the roles of cholinergic, dopaminergic, serotonergic and opioid receptors in the initiation of the gastrointestinal motor correlates of vomiting and attempted to identify the location of these receptors. The study was done with dogs and these authors studied three drugs: cholecystokinin, copper sulfate and apomorphine as emetic agents; each of these activate the motor correlates of vomiting by different neural pathways. Although cholecystokinin does not induce emesis, it does activate the gastrointestinal motor correlates [111]. Copper sulfate does cause vomiting and it acts by stimulating the receptors in the gut wall that in turn activate the vomiting center by afferent fibers of the vagus and splanchnic nerves [109]. Apomorphine also causes vomiting but does this by stimulation of the chemoreceptor trigger zone of the area postrema which in turn activates the vomiting centre but this is mediated through the vagus nerve only [111]. A number of drugs were investigated to determine their ability to block the effects of the three emetic agents. The drugs included cholinergic antagonists (atropine and hexamethonium), dopaminergic antagonists (domperidone, haloperidol and SCH 23390), serotonergic antagonists (methylsergide and cinanserin) and opioid agents (naloxone and fentanyl). For apomorphine, the authors found that the GI motor correlates were seen with doses from 2.1-3.5 $\mu g \cdot kg^{-1}$ and vomiting was seen with doses from 3.5-6.3 $\mu g \cdot kg^{-1}$. The latency period for onset of vomiting was 162 ± 18 seconds and although the latency of onset did not change with dose, the number of emetic episodes increased with dose. In their results, the authors found that the cholinergic antagonists atropine and hexamethonium did not prevent vomiting with apomorphine

suggesting that apomorphine-induced emesis is not mediated by cholinergic receptors. The dopamine D_2 antagonist domperidone, which does not cross the blood-brain barrier, blocked all the responses to apomorphine; the non-specific dopamine receptor antagonist haloperidol was also effective in preventing apomorphine-induced emesis but the D_1-specific antagonist SCH 23390 was not effective. These findings suggested that with apomorphine, emesis was due to activation of the peripheral D_2-receptors. The non-specific serotonergic agent methylsergide was effective in blocking apomorphine-induced emesis but the selective 5-HT_3 antagonist cinanserin was not. This suggested that 5-HT_2 receptors may be involved. The opioid agonist fentanyl was effective in preventing apomorphine-induced emesis and since fentanyl readily crosses the blood-brain barrier, the authors suggested that the central vomiting centre contained an opioid receptor which mediates inhibition of emesis. This was supported by their finding that naloxone, a non-specific opioid receptor antagonist, lowered the threshold dose, increased the frequency and prolonged the effect for apomorphine-induced emesis.

There are two families of dopamine receptors identified as D_1 and D_2. The D_1 family consists of D_1 and D_5 subtypes and the D_2 family consists of D_2, D_3 and D_4 receptor subtypes [112]. The receptors in the D_1 family show high adenylcyclase activity and are positive regulators of cyclic AMP when stimulated by agonists and their stimulation produces vasodilation, natriuresis and generally increases motor activity. The receptors in the D_2 family show low adenylcyclase activity when stimulated by agonists and their stimulation causes a reduction in noradrenalin levels with consequent vasodilation, a decrease in heart rate and a reduction of motor function [113]. Dopamine receptors are found peripherally and centrally. Centrally, the D_1 receptors appear to be involved with incentive learning, D_2 receptors with Parkinson's disease, D_3 with behaviors including yawning, and penile erection while D_4 receptors are involved with neuropsychiatric illness [112]. There is also some evidence that D_2 and D_3-receptors may interact and modulate attention span [114] and research involving erectile dysfunction offers some evidence that a combination of D_3 and D_4 receptors may be

involved in penile erection [115]. Peripherally, dopamine receptors are principally involved in the regulation of the cardiovascular and renal systems and emesis induced by apomorphine appears to be specifically due to stimulation of the D_2 receptor sites on the CTZ as the emetic action of apomorphine is inhibited by domperidone which is a potent dopamine D_2 receptor antagonist which is not able to cross the blood-brain barrier [112]. There has been some suggestion that D_3 receptors may be involved in canine emesis since 7-OH-DPAT, a potent dopamine D_3-receptor agonist induces emesis in the dog [116].

In a radioligand study of apomorphine, Hsieh et al [115] showed that apomorphine binds in a non-specific fashion with all of the dopamine receptors and also with the μ- and κ-opioid receptors. The binding affinity they found for these receptors are listed in Table 2.3:

Table 2.3 Apomorphine affinity determined using the Cheng-Prusoff equation [115, 117]

Receptor	K_i (nM)
D_1	101
D_2	32
D_3	26
D_4	2.6
D_5	10
μ	2000
K	5000

Dopaminergic ligands can distinguish between the D_1 and D_2 families but most of them do not clearly differentiate between members of the subfamilies; Hsieh's data show that domperidone, a dopamine antagonist only binds to D_2 receptors but cannot be used to distinguish between D_2, D_3 or D_4 receptors [115].

Dissociation constants for a variety of dopamine receptor agonists and antagonists are presented in Table 2.4. Genetically-modified animals where a particular receptor is absent will

also provide a powerful tool to help in determining the selectivity of a compound for a particular receptor [118].

Table 2.4 Dissociation constants (Ki) for ligands at dopamine receptors [112, 118-120].

Ligand	K_i Values (nM)				
	D_1-Like		D_2-Like		
	D_1	D_5	D_2	D_3	D_4
Agonists					
Apomorphine	101	10	32	26	2.6
Bromocriptine	440	450	8	5	290
Cabergoline	1460		1	1.3	
Dopamine	1	1	7	4	30
Fenoldopam	0.8	0.6	3		150
Lisuride	51		1	1	
Pergolide	45		23	0.86	
Pramipexole	10,000		500	0.97	
Quinpirole	1900		4.8	24	30
Ropinerole	10,000		3500	35	
SKF38393	1	0.5	150	5000	1000
Antagonists					
Butaclamol	2	15	0.75	7	45
Chlorpromazine	90	130	3	4	35
Clozapine	170	330	230	170	21
Domperidone			0.3	9.5	
Haloperidol	80	100	1.2	7	2.3
Nemonapride			0.06	0.3	0.15
Olanzapine			45		27
Raclopride	18000		1.8	3.5	2400
Remoxipride			300	1600	2800
Risperidone			5	6.7	7
SCH-23390	0.2	0.3	1100	800	3000
Spiperone	350	3500	0.06	0.6	0.08
Sulpiride	19,000	29,000	15	13	1000

2.3.1 Indications:

Acute poisonings

Apomorphine HCl is a potent emetic which may be used to induce vomiting in the early management of oral poisoning or to expel foreign bodies from the stomach [35, 121]. If there are no contraindications apomorphine emesis may be indicated in cases of oral poisoning although its use in cases where the intoxicant has CNS-depressant activity may be problematical since this may be potentiated by apomorphine. Where the poisoning is due to petroleum distillates (paint thinner, gasoline, kerosene, and cleaning fluids) emesis or lavage is contraindicated due to an increased risk of aspiration which is possibly the most life-threatening complication of the exposure [25]. Apomorphine is effective in evacuating the contents of the upper GI tract only and the patient should be observed for signs of increasing intoxication due to on-going absorption [35]. Administration of apomorphine should not preclude other measures used in the emergency treatment of poisoning such as the administration of activated charcoal [121]. In the management of most acute oral poisonings, apomorphine would be considered preferable to gastric lavage [33].

Apomorphine induces emesis by direct stimulation of the medullary chemoreceptor trigger zone when the medullary centers are responsive and since movement intensifies and recumbency reduces the emetic effect of the drug, it had been suggested that the vestibular centers may also be involved [2]. Apomorphine depresses medullary centers controlling respiration and vasomotor tone resulting in respiratory depression, hypotension, and sedation; apomorphine also stimulates salivation [2,122]. It has also been reported that stimulation of dopamine receptors by apomorphine results in a rise in serum growth hormone (human) and a decrease in prolactin levels [123]. As was discussed earlier in Section 1, the therapeutic benefit of emesis in cases of intoxication is questionable.

Parkinson's disease

Parkinson's disease is a progressive degenerative disease of the central nervous system characterized by symptoms of akinesia, tremor, rigidity and a generally flexed posture; the etiology is unknown but histologically cell loss is seen in the aggregates of melanin containing nerve cells in the brainstem along with distinctive eosinophilic intracytoplasmic inclusions known as Levi bodies [124]. From a biochemical perspective, there is a reduction in the concentration of dopamine in the basal ganglia due to loss of cells and the resulting reduction of dopamine leads to an imbalance where dopaminergic influence is reduced and cholinergic influence is increased [125]. Symptomatic treatment is to correct this imbalance so therapy may be directed to using drugs able to increase dopamine levels in the central nervous system, using drugs with dopaminergic agonist activity or by moderating cholinergic effects with drugs having anticholinergic activity [126, 127]. Levodopa is the most effective anti-Parkinsonian drug for most patients and in a patient with newly diagnosed disease the initial response to levodopa is often dramatic and positive. Although dopamine is unable to cross the blood-brain barrier, levodopa which is a dopamine precursor is able to cross but passage is regulated by a saturable transport system which is specific for aromatic and branched chain amino acids. Since there is competition for the transport system serum levels of levodopa must be kept high which requires high doses of levodopa; the dynamics are further complicated by the fact that levodpoa is metabolized outside of the central nervous system which also pushes the dosing requirement upward [127]. As the disease progresses the CNS requirement for dopamine increases again putting upward pressure on dosing. With increasing dose the amount of dopamine outside the CNS increases and adverse effects from increasing levels of peripheral dopamine such as nausea, vomiting, postural hypotension and cardiac arrhythmias begin to appear [128]. The use of peripheral decarboxyase inhibitors such as carbidopa and bensarazide reduce the amount of levodopa which is metabolized to peripheral dopamine and effectively increase the amount of levodopa available to enter the CNS [129]. Unfortunately

with time therapy becomes a compromise between side effects and therapeutic benefit [126, 128].

Patients with Parkinsonism who initially respond well to therapy with levodopa may begin to experience fluctuations in motor response after long-term use. Although L-dopa therapy controls the symptoms of the disease, it does not affect the underlying pathological process which continues to progress. The clinical features of these fluctuations can be described by four phenomena as outlined by Coleman [130] and in many cases these fluctuations may be relieved through the use of direct acting dopamine agonists such as apomorphine. This therapeutic approach is referred to as continuous dopaminergic stimulation [130-132]. Apomorphine has been given subcutaneously in increasing dosage as a diagnostic test to distinguish patients with idiopathic Parkinsonism from those with other central nervous system degenerative diseases such as supranuclear palsy and multiple system atrophy but it can also be used therapeutically [133]. Apomorphine is a dopamine agonist which is effective at both the D_1- and D_2-receptors and although considered ineffective by the oral route, subcutaneous injections provide an effective but short-lived anti-Parkinsonian effect [134]. The use of apomorphine has been limited due to the vomiting response it produces but the development of domperidone, a peripheral dopamine antagonist used as an anti-emetic has allowed the use of apomorphine in the treatment of Parkinson's disease to be reconsidered [10]. In order to determine the therapeutic window for treatment of Parkinson's disease with apomorphine, a study was designed by van Laar et al [5] to characterize the pharmacodynamics of apomorphine in Parkinson's disease with the goal being to use these data as a tool to assist in developing an appropriate dosing regime for apomorphine. In this study while the authors found a direct relationship between serum levels and beneficial effect, they also found that there was considerable interpatient variability in the response to apomorphine.

Due to the wide inter-patient variability and the observation that the motor effects of apomorphine do not correlate well with serum levels, a study was undertaken by Przedborsk et al [6] to examine the relationship between serum and ventricular cerebral-spinal fluid (CSF) apomorphine levels. From this study, the authors found that apomorphine readily and rapidly crosses the blood-brain barrier. In order to determine if development of tolerance to apomorphine developed with continuing use, Gancher et al [135] conducted a study where apomorphine was administered to eight patients with Parkinson's disease. The results of this study suggested that tolerance to the effects of apomorphine did develop and that a period of time without apomorphine administration was necessary to restore and optimize the dopaminergic action of apomorphine.

In several reviews of apomorphine use in managing the motor fluctuations seen in late-stage Parkinson's disease, apomorphine was generally considered to be effective and perhaps underutilized [134, 136]. A ten year retrospective audit of apomorphine use in Parkinson's disease was presented by Tyne et al [137] and this study concluded that subcutaneous apomorphine was easy for patients to use, was well tolerated and had a low incidence of side-effects. A somewhat more subdued but similarly positive view was expressed by Bowron [138] who discussed the importance of planning, patient education and patient counseling in achieving successful treatment using subcutaneous apomorphine.

Erectile dysfunction

Patients receiving apomorphine for the treatment of Parkinson's motor fluctuations reported frequent penile erections and these erections are believed to be caused through stimulation of central D_2 type dopamine receptors [8, 139]. In a review of oral pharmacotherapeutic options to treat erectile dysfunction, Vitezic and Pelcic [140] suggested that stimulation of the centrally located D_1- and D_2-dopaminergic receptors by apomorphine sends signals down the spinal cord to the sacral parasympathetic nucleus which in turn activates the sacral nerves supplying the penis and leads to an erection. The medial pre-optic

area plays a role in integrating the sexual input and dopamine agonists and the D_2-dopaminergic agonists in particular appear to be involved. Since the erectogenic effects of dopamine agonists is blocked by centrally acting but not by peripheral acting dopamine antagonists, central stimulation appears to be responsible for the erectile response. This also allows the use of peripheral antagonists such as domperidone to be used to control the nausea, vomiting and hypotension associated with the peripheral actions of apomorphine without compromising the erectogenic effect [141]. At present, there are no commercial dose forms of apomorphine suitable for treatment of erectile dysfunction but because of the market potential of such a product, work is being done which will be discussed in the section regarding apomorphine dosage forms.

Other

It has been shown that patients subject to migraine headaches have a sensitivity to dopaminergic agonists which is manifested in the form of yawning; this fact was investigated as a potential tool for the diagnosis of migraine where the number of yawns per hour was counted [142]. An interesting and possibly related observation was that patients receiving apomorphine showed increased lacrimation. Further to this work, patient assessment using videotaping to record and measure frequency and duration of yawning was presented as a tool for the study and diagnosis of migraine headache [143].

Initial research reported that apomorphine exhibited anti-psychotic activity and may be of benefit in the treatment of schizophrenia [7]. A postulated mechanism was stimulation of presynaptic autoregulatory inhibitory dopamine receptors by apomorphine. Subsequent investigations however, established that apomorphine had no anti-psychotic properties and was of no value in the management or treatment of schizophrenia [144, 145].

2.3.2 Adverse effects:

CNS depression and in particular respiratory depression are the major adverse effects of apomorphine; slow, shallow and irregular breathing may be seen even with subemetic doses of apomorphine [35].The CNS and respiratory depression may be reversed by an opiate antagonist such as naloxone [146]. Excessive doses of apomorphine may exert a depressant effect on the heart and cause bradycardia which may be treated with atropine. Salivation and hypotension may also be seen following apomorphine administration. Many patients experience euphoria, restlessness, tremor, bradycardia, and irregular and shallow respiration; mild myoclonic jerks, head bobbing and dyskinesia may also be seen [2].

Respiratory depression

Montastruc et al [147] used a controlled trial to investigate the effect of naloxone, haloperidol and yohimbine on the respiratory depression elicited by apomorphine; naloxone represented an opiate antagonist, haloperidol a non-specific dopamine receptor antagonist and yohimbine to represent an α_2-adrenoceptor antagonist. In this study yohimbine was shown to be ineffective and while naloxone and haloperidol were effective, naloxone showed fewer side-effects. The authors concluded that the respiratory depression seen with apomorphine involved both dopamine and opioid receptors and that the respiratory depression induced by apomorphine could be treated with naloxone.

Hypotension

Apomorphine was reported to cause a hypotensive response mediated by dopamine receptors and Ramirez and Enero [148] investigated this in anesthetized rats. The objective of this study was to determine whether the hypotensive effect seen with apomorphine was due to interaction with central or peripheral dopamine receptors. The findings of this study were that in the control animals there was a dose-dependent fall in blood pressure with apomorphine and this effect was blocked by the dopamine receptor antagonists pimozide and haloperidol. Atropine and hexamethonium had only a modest effect and only at the higher apomorphine

dosages where apomorphine induces a bradycardia which could be reversed by these agents. The authors concluded that in rats, the hypotensive effect of apomorphine was due to dopamine receptor agonism and that the central receptors were primarily involved. To further clarify whether the apomorphine-related hypotension was due to stimulation of central or peripheral dopamine receptors, De Meyer et al [149] administered apomorphine to anesthetized cats by the intravenous and intravertebral routes. Although apomorphine administration by both routes produced hypotension, the hypotension produced by the intravenous route was significantly greater than that achieved by the intravertebral route for an equivalent dose and these authors concluded that the peripheral rather than the central dopamine receptors played a more significant role in inducing apomorphine-related hypotension.

Pellissier and Demenge [150] studied the effect of dopamine receptor antagonists with specificity for D_1 or D_2 receptors on the hypotensive effect of apomorphine. From their findings, the authors concluded that the hypotension seen after apomorphine administration was due to activation of the D_1 and/or D_2 receptors but that the bradycardia was due to activation of the D_2 receptors.

Montestruc et al [146] conducted a controlled trial to determine if the opioid antagonist naloxone would prevent or reverse the hypotension and vomiting induced by apomorphine. This study was conducted using dogs and the authors concluded that neither the hypotensive nor changes in heart rate associated with apomorphine was mediated through the opioid receptors. Apomorphine-induced emesis was however, prolonged and intensified by administration of naloxone.

In a study of the hemodynamic effects of apomorphine in dogs, Nakayama et al [151] monitored cardiovascular responses in anesthetized dogs after administration of an emetic dose of apomorphine. From this study the reduction in blood pressure was attributed to vasodilation produced by the D_1 and D_2 agonist effects of apomorphine and the authors

suggested that the drop in blood pressure was more likely due to the stimulation of the D_2 receptors which results in inhibition of norepinephrine release and subsequent vasodilation.

Cardiac effects (Bradycardia and tachycardia)

In their study of apomorphine-induced hypertension, Ramirez and Enero [148] also examined the changes in heart-rate after administration of apomorphine to anesthetized rats. The authors noted that after administration of intravenous apomorphine there was a marked bradycardia as well as hypotension and the degree of bradycardia appeared to be dose-related. Pre-treatment of the rats with atropine prevented bradycardia but had no effect on the hypotension while administration of the dopamine agonist pimozide prevented both hypotension and bradycardia. This study suggested that the bradycardia seem with apomorphine was due to stimulation of dopamine receptors and that it had a vagal component. Montastruc et al [152] investigated the cardiovascular effects of apomorphine in anesthetized normotensive dogs and in adrenal demedullated dogs. From their findings, the authors concluded that the tachycardia seen was due to apomorphine acting on dopaminergic presynaptic inhibitory receptors located on the vagal nerve endings.

Since both bradycardia and tachycardia had been observed in subjects receiving apomorphine, Paalzow and Paalzow [153] examined the relationship between serum levels of apomorphine and the effect on heart rate. This study was done using awake, unrestrained rats and apomorphine was administered at a dose of 2 $mg \cdot kg^{-1}$ intravenously as a reference then subcutaneously at dose levels of 0.05, 0.01 and 5 $mg \cdot kg^{-1}$. The authors found the low dose of apomorphine produced bradycardia, the high dose produced tachycardia followed by a brief period of bradycardia and the moderate dose produced bradycardia. The findings of this paper suggested that apomorphine influences heart-rate in two opposing ways depending on the concentration at the site of effect. Through analysis of their blood concentration-heart rate data using curve fitting with the Hill equation [154], the authors were able to divide the dose-response curve into two components: 'pure' bradycardia and 'pure' tachycardia. They

suggested the possibility that two opposing dopamine functional systems are involved in the high and low concentration related effects on heart rate. The involvement of dopamine receptors in the heart rate effects seen with apomorphine was further studied by Pellissier and Demenge [150]. They looked specifically at the bradycardia induced by apomorphine and their conclusion from this work was that the bradycardia seen with apomorphine is due to stimulation of the D_2 receptors alone.

Since nutrition may affect receptor binding [155], Bredberg and Paalzow [156] studied the effect of malnutrition on apomorphine-induced heart rate changes. The authors concluded that their data provided evidence that two receptors were involved in the heart rate effects seen and that their findings were consistent with the theory that some dopamine receptors decreased in number with malnutrition and in this case those were the receptors responsible for induction of tachycardia [155]. In earlier studies [153, 156], the effect of apomorphine on heart rate was modeled with a composite sigmoid E_{max} equation with one term representing bradycardia and the other tachycardia. Bredberg and Paalzow postulated that since stimulation of D_1 receptor increased cAMP activity and stimulation of D_2 receptors decrease this activity or have no effect on cAMP, it is possible that stimulation of these receptors will produce opposite effects [157]. They suggested that the idea of two separate receptor interactions could be demonstrated by blocking one of the two effects by a specific antagonist so they selected supiride which is a selective D_2 antagonist for study. Rats were administered either saline of supiride as an infusion and after 30 minutes an apomorphine infusion was started. Several steady-state serum levels were investigated and heart rate was monitored over the course of each experiment. In the subjects receiving supiride and apomorphine, no bradycardia was seen and the concentration-response curve for tachycardia was shifted to the left compared to the control group but the maximum values were not changed. The authors suggested that this finding demonstrated that the bradycardia seen with

apomorphine is due to D_2 receptor stimulation and that the tachycardia seen with apomorphine is due to the stimulation of another receptor.

The biphasic nature of apomorphine on heart rate where lower doses were associated with bradycardia and higher with tachycardia was also discussed by Nakayama et al [151] in their study of the hemodynamic effects of apomorphine in dogs. They suggested that at the lower doses, bradycardia resulted from a direct effect on the SA node whereas at higher doses tachycardia predominates due to vagal inhibition. The possible explanation by Paalzow and Bredberg is more likely and is further supported by the fact that tachycardia induction has been reported with a number of dopamine D_1 agonists including dihydrexidine which is the first full dopamine D_1 receptor agonist discovered to date [158-161].

Nausea and vomiting

Since protracted vomiting is occasionally seen when apomorphine is used to induce emesis, Keith et al [162] conducted a series of experiments to determine what agents might be useful to manage this adverse effect. In their results, the authors found that emesis was prevented by the dopamine antagonist haloperidol and a droperidol-fentanyl combination. Their study demonstrated that apomorphine-induced emesis was due to dopaminergic stimulation and that naloxone was ineffective in preventing emesis due to apomorphine.

In order to further clarify the antiemetic specificity of dopamine agonists, Niemegeers [163] conducted a study investigating twelve potential antagonists of apomorphine-induced emesis. His study used dogs although he also used rats in part of the study in order to compare the neuroleptic activity of the agents between the two species. One object of the study was to determine whether the benefit of the antiemetic effect would be offset by neurological adverse effects as measured by the animals' ability to pass through a maze. The data from the studies revealed that all twelve of the compounds tested were effective as antiemetic agents but there were differences in terms of potency, time of onset and duration of action. In terms of neuroleptic activity, nine of the twelve compounds exhibited central dopamine receptor

blocking at doses 4-6 times the antiemetic dose; of the remaining three, domperidone was found to be a very effective antiemetic agent for blocking apomorphine-induced emesis and it was essentially free of neurological adverse effects at doses well in excess of that needed for the antiemetic effect. The twelve agents and the ratio of the dose where neurological adverse effects occurred as a function of the antiemetic dose (dose factor) are given in Table 2.5

Table 2.5 Dopamine agonists and dose factor (dose inducing neurological adverse effects as a function of dose preventing apomorphine-induced emesis) [163].

Compound	Dose factor
Domperidone	318
Metopimazine	197
Pimozide	18.2
Droperidol	6.58
Metoclopramide	6.25
Thiethylperazine	5.94
Chlorpromazine	5.87
Haloperidol	5.53
Prochlorperazine	5.29
Bromopride	4.84
Clebopride	4.83
Benzquinamide	4.01

Blancquaert et al [164] studied the hypothesis that δ- and μ-opioid receptors mediate the emetic and antiemetic effect of opioids respectively. In this study, the authors selected opioid compounds with specificity for one of the opioid receptors (μ, δ and κ) and studied their emetic activity as well as their antiemetic effect against vomiting induced by apomorphine. Their study demonstrated that antiemetic activity was shown by both μ- and κ-receptor agonists but not by δ-receptor agonists; the μ agonists fentanyl, methadone and

morphine block emesis due to intravenous apomorphine and the κ agonists bremazocine and etylketocyclzocine prevented the emetic response to apomorphine.

Scherkl et al [165] noted that with apomorphine administration there was a delay in onset of emesis after intramuscular administration compared to subcutaneous administration and in order to explain this, they studied the pharmacokinetic parameters found after intravenous, intramuscular and subcutaneous administration of apomorphine in dogs. Their study consisted of three parts: in the first, apomorphine was administered at two dose levels 0.04 and 0.1 $mg \cdot kg^{-1}$ using the intramuscular and subcutaneous routes; efficacy of emesis-induction was determined by time of onset and frequency of vomiting; in the second part 0.1 $mg \cdot kg^{-1}$ was given by the three routes and timed blood samples were collected for analysis and in the third part they examined the effect of pretreating animals with intravenous naloxone 10 minutes before challenge with 0.1 $mg \cdot kg^{-1}$ of apomorphine given intravenously. In the first part, they found that the time to onset of emesis was faster with subcutaneous administration compared to intramuscular administration. In the second part of the study, they found that the onset to emesis with the intravenous route was about 1.6 minutes; emesis was induced in all the subjects and the mean number of episodes was 2.1. The serum levels at onset of emesis were about 51 $ng \cdot mL^{-1}$ and at the end of vomiting 42 $ng \cdot mL^{-1}$. For the subcutaneous route, emesis occurred after about 3.7 minutes with an average of 5.9 episodes of vomiting. The serum levels at onset of emesis were about 12 $ng \cdot mL^{-1}$ and at the end of vomiting 27 $ng \cdot mL^{-1}$. For the intramuscular route, emesis occurred after about 4.5 minutes with an average of 5.4 episodes of vomiting. The serum levels at onset of emesis were about 21 $ng \cdot mL^{-1}$ and at the end of vomiting 31 $ng \cdot mL^{-1}$. In the third part of the study where the subjects were pretreated with naloxone before intravenous apomorphine administration, emesis was induced in all the subjects after about 1.1 minutes which was the same as in the non-pretreated subjects but the number of episode increased to 9.2 and emesis was prolonged to about 20 minutes. In this study wide variability in response was seen with emesis occurring with serum levels anywhere

between 8 to 25 $ng \cdot mL^{-1}$.The authors explained their finding by pointing out that apomorphine induces emesis by stimulation of dopamine D_2-receptors in the chemoreceptor trigger zone. Once apomorphine crosses the blood-brain barrier, however, it is able to interact with the opioid μ-receptors within the vomiting center of the brain which suppresses vomiting. The rapid and high serum levels achieved by the intravenous route means that the drug is able to cross the blood-brain barrier faster and effectively blocks emesis by stimulation of the μ-receptors. Naloxone is a potent μ-receptor antagonist and would prevent interaction of these receptors with apomorphine so vomiting would continue even when the apomorphine has crossed the blood-brain barrier. The experimental evidence from the trial with naloxone supported this and the trials with apomorphine alone showed that with increasing serum levels the emetic action of apomorphine becomes self-limiting. The authors point out that if emesis does not occur with a dose of apomorphine, further doses will not help and that administration of naloxone will not reverse the emetic effect of apomorphine. In their conclusion, the authors suggest that the somewhat lower serum levels and slower rate of absorption achieved with subcutaneous administration may be a more effective way of emesis induction than the intravenous or intramuscular routes.

Another study to determine whether some of the effects of apomorphine were due to interaction with opioid receptors was conducted by Bonuccelli et al [166]. The study population consisted of patients with Parkinson's disease and in their findings the authors showed that naloxone was able to significantly reduce yawning, sleepiness, nausea, retching and vomiting induced by the acute administration of apomorphine but naloxone did not modify the motor effects of the apomorphine. From this work, the author suggested that there was some involvement of opiate receptors in the side-effects seen with apomorphine but not in the motor effects which were not changed with the administration of naloxone.

Barnes et al [167] investigated the ability of fentanyl to prevent apomorphine induced emesis. Their study was conducted using ferrets as subjects and the authors showed that

pretreatment of the subjects with fentanyl before a challenge dose of apomorphine abolished the emetic response to apomorphine whereas the emetic response to apomorphine was enhanced by pretreatment with naloxone. When the animals were pretreated with both naloxone and fentanyl, the emetic activity of apomorphine was the same as animals pretreated with saline. The authors concluded apomorphine induces emesis through dopamine receptors rather than opioid receptors and that the anti-emetic effect of apomorphine is due to interaction with the opioid μ-receptors.

In their study to determine whether naloxone was effective in reversing apomorphine-induced hypotension, Montastruc et al [146] also investigated whether naloxone was effective in reversing or preventing apomorphine-induced emesis in dogs. This work was discussed earlier under the section dealing with hypotension and this work was in part to verify or refute the earlier findings of Bonuccelli [166] who reported that naloxone partly counter-acted some of the adverse effects seen with apomorphine administration. In this study, Montastruc examined the effect of a challenge dose of intravenous apomorphine on blood pressure, heart rate and emesis. The subjects were in two groups, one awake and one anesthetized and each of these groups were further divided into a control group pretreated with IV saline and a group pretreated with naloxone. In terms of heart rate and hypotension, there were no significant difference among all four groups; all showed a decrease in blood pressure which was maximal after 5 minutes and remained significant during 10 minutes; all showed a biphasic change in heart-rate, a bradycardia during the first minute followed by a tachycardia which was maximal after 5 minutes and lasted 15 minutes. In terms of emetic activity however, the group which was conscious and pretreated with naloxone differed significantly from the other three in showing an increase in both duration and number of emetic episodes. The time to emesis was not different among the four groups. The authors concluded that naloxone was of no value in preventing or reversing the hypotension or changes in heart-rate associated with apomorphine and that naloxone enhanced the emetic effect of apomorphine in conscious dogs.

Sedation

Gessa et al [168] investigated the biphasic behavior that apomorphine has on rats; low doses producing sedation and high doses inducing hyperactivity. In their study, rats were pretreated with either SCH 23390 at a dose of 1 $mg \cdot kg^{-1}$ or saline by the intraperitoneal route. After 30 minutes either placebo, apomorphine 0.05 $mg \cdot kg^{-1}$ (low dose) or apomorphine 1 $mg \cdot kg^{-1}$ (high dose) was administered by the subcutaneous route. The animals were monitored for activity by means of photocells placed in their containment boxes and EEG activity was recorded. SCH 23390 is a specific D_1-receptor antagonist which antagonizes the motor stimulation produced by apomorphine but has no effect on the drowsiness induced by apomorphine; this is unlike haloperidol (non-specific D_1- and D_2-antagonist) and sulpiride (selective D_2 antagonist) which do antagonize the drowsiness [169]. Pretreatment with SCH 23390 led to a significant decrease in motor activity and the animals were cataleptic for about 30 minutes after administration; SCH 23390 did not induce any changes in the EEG. A few minutes after administration of the low dose apomorphine, the animals showed EEG recordings consistent with sleep (synchronization or the presence of slow waves) and they presented signs of drowsiness including lying on their side, closed eyelids, and head tucked under the body. Locomotor activity was reduced by about 60% from normal. With the low dose apomorphine there was no significant difference in the EEG or locomotor findings between the SCH 23390 and saline pretreated animals. In the control animals, administration of high dose apomorphine produced marked stereotyped behavior characterized by continuous gnawing with a burst of locomotor activity. The EEG was characteristic of arousal and no slow waves or synchronization was seen. In the SCH 23390 pretreated animals, high dose apomorphine produced profound behavioral sedation and EEG patterns of slow activity consistent with those seen in the low-dose apomorphine treated animals. The authors further investigated the effect of the low dose apomorphine by examining the effect of sulpiride a specific D_2-receptor antagonist. One group of animals was treated with SCH 23390, saline and

apomorphine while the test group was treated with SCH 23390, sulpiride and apomorphine. In this experiment sulpiride prevented the EEG and behavioral changes induced by the low dose apomorphine. Starr and Starr [170] investigated the effects of apomorphine and D_1 and D_2 antagonists on moving and grooming behaviors in mice. They found that D_2 receptors seemed to be primarily involved in the mechanisms of locomotion but that there seemed to be a connection between D_1 and D_2 receptors as antagonism of D_1 receptors led to some of the effects seen with antagonism of D_2 receptors; they also showed that low doses of non-specific antagonists like haloperidol are able to prevent the drowsiness induced by apomorphine even though the antagonists themselves are able to induce drowsiness at higher doses.

Biphasic dose responses

The observations that apomorphine and dopamine or dopamine agonists in general, induce quite different responses with high and low doses for a number of effects including locomotion, blood pressure and heart rate were reviewed by Calabrese [171]. In this review, he points out that although there are distinct activities associated with D_1 and D_2 receptors, there is a growing body of evidence that there are functional interactions between the two receptors. Saller and Salama [172] demonstrated that low doses of haloperidol sufficient to block D_2 but not D_1 receptors, would potentiate the effects of SKF 38393 a D_1 agonist suggesting that D_1 receptor activity might be enhanced by D_2 blockade. Two other studies showed that in some cases a synergy was achieved when both D_1 and D_2 receptors were stimulated by agonists; in a study comparing the effects of the D_1 agonist SKF 81297, the D_2 agonist quinpirole and the two together, Rsukin et al [173] showed that the firing rates for basal ganglia neurons were increased by SKF 81297 and unchanged by quinpirole but dramatically increased when both agents were administered. A similar study by Waszczak et al [174] also showed that the effects of dopamine receptor stimulation are not necessarily due to separate and competing effects, but rather to interactions between receptor types leading to complex variable effects. Although the concept of the stimulatory and inhibitory effects of

dopamine agonists being mediated by two receptors having opposing actions and different affinities for the respective ligands is attractive, this is likely an oversimplification. The processes become more complex when interactions between quite different receptor systems are considered and there is evidence that many dopamine receptor ligands cross-react with opioid receptors [175] and adrenergic receptors. [176]

2.4 Routes of Administration and Pharmacokinetics

The oral absorption of apomorphine is erratic and incomplete [177] but administration of the drug by subcutaneous or intramuscular injection results in good absorption. The pharmacokinetics of apomorphine following intravenous and subcutaneous administration was studied by Gancher et al [105] and results of this study indicated that onset of action is very rapid and duration of action is dose-dependent. Generally the maximum duration of action even with large parenteral doses is about three hours. Pharmacokinetic studies in animals [178-180] showed the drug is rapidly cleared from the plasma with a distribution half-life of 5 minutes and an elimination half-life of 47 minutes. Apomorphine equilibrates rapidly between blood and tissue compartments and brain concentrations are about eight times serum concentrations at equilibrium [179]. In animals apomorphine is rapidly metabolized in the liver to the glucuronide conjugate and is excreted in the urine; a very small amount of the drug is excreted unchanged [178, 180].

In a study primarily designed to investigate the pharmacokinetics and pharmacodynamics of apomorphine, van derGeest et al [103] also considered metabolism in humans. Ten patients were given an intravenous infusion of apomorphine at a dose of 30 $\mu g \cdot kg^{-1}$ over 15 minutes. Serum samples were taken at timed intervals for analysis of apomorphine and urine was collected for analysis of apomorphine metabolites. Analysis of the collected samples was done by HPLC using an enantioselective or chiral column which allowed analysis of both the R- and S-enantiomers of apomorphine as the authors wished to

determine whether the administered R-apomorphine could be converted into the S-enantiomer in the body. In terms of the pharmacokinetics, the study data provided a mean clearance value of about 40 $mL \cdot min^{-1} \cdot kg^{-1}$, a volume of distribution of 1.6 $L \cdot kg^{-1}$ and a mean terminal half-life value of about 41 minutes. About 99% of the administered dose was found to be protein-bound and the pharmacodynamic portion of the study found the onset of antiparkinsonian activity was very rapid with symptom improvement occurring within 3-9 minutes after the infusion was started. Analysis of the urine for metabolites showed that conjugated metabolites of apomorphine appeared rapidly in the urine and that the sulfated conjugate accounted for 3.8% of the administered dose while the glucuronated conjugate accounted for 6%; only about 0.3% of the dose appeared in the urine as unchanged apomorphine. There was also no evidence that enantiomeric interconversion occurred between the S- and R-enantiomers. Since the mean total clearance of apomorphine of 40 $mL \cdot min^{-1} \cdot kg^{-1}$ exceeds hepatic blood flow, the authors concluded that apomorphine must be partly eliminated by extra-hepatic metabolism. The study also demonstrated that there is considerable intersubject variation in the pharmacokinetic parameters studied.

Traditional routes of administration and their associated dosage forms are quite diverse but often the nature of the dose form and the route of administration are restricted by the physicochemical nature of the drug or its pharmacological and pharmacokinetic properties. Other factors which may limit the route or nature of the dose form include various patient-related factors and factors associated with the disease being treated [181].

In the case of apomorphine, an early trial investigating the absorption and clinical efficacy of apomorphine when given by different routes of administration was conducted by Gancher et al [177]. The routes of administration examined included sublingual (SL), oral (PO), intravenous (IV), subcutaneous (SC) and topical. Data analysis from the trial indicated that bioavailability as a percentage of the administered dose was 17% for the sublingual route, 1.7% for the oral route and no drug could be detected from the topical route (apomorphine

dispersed in Eucerin). The authors concluded that sublingual administration could be used as an alternative to the parenteral route but that a product specifically formulated for sublingual use rather than the tablet triturates used in the trial could result in better clinical application.

In the case of veterinary application of apomorphine as an emetic agent, a trial was designed by Harrison et al [182] to investigate apomorphine efficacy when administered by various routes. The routes of administration studied were intramuscular, oral, ocular, sublingual and rectal and the study subjects were dogs. The object of the trial was to determine the optimal route for apomorphine administration to induce emesis. In this study the different routes were compared with intramuscular administration in terms of required dosage, time to emesis and number of emetic occurrences induced. In the study, five dogs were given apomorphine by each route of administration being studied using doses ranging from 0.07 to 0.46 $mg \cdot kg^{-1}$ but the dose given intramuscularly was standardized to 0.07 $mg \cdot kg^{-1}$ to serve as a reference point. Oral administration was accomplished by administration of an apomorphine solution using a syringe inserted at the angle of the upper and lower jaws with the dog's head elevated; for ocular administration the drug solution was placed in the conjunctival sac of the lower lid with gentle traction applied to prevent loss of the solution; sublingual administration was accomplished by saturating a cotton pad with the solution, wrapping it in a single layer of gauze and securing it beneath the tongue; and rectal administration was accomplished by passing a 13-gage cannula 8 cm long into the rectum, administering the solution with a syringe and flushing the cannula with tap water. The data generated from the study showed a consistent response to intramuscular administration with emesis occurring at a mean time of 4.6 minutes. Oral administration at the lower doses was inconsistent in producing emesis but at a dose of 0.26 $mg \cdot kg^{-1}$ emesis was induced in all the subjects with a mean time to emesis of 6.8 minutes. Ocular administration of apomorphine solution gave results similar to oral administration with emesis occurring in all subjects at a dose of 0.26 $mg \cdot kg^{-1}$ and the mean time to emesis was 7.0 minutes. The results with sublingual administration were erratic and

inconsistent. The authors suggested that this was in part due to loss of apomorphine as a result of drooling by the subjects rather than a failure of the drug to be absorbed by the buccal membranes. The rectal route was ineffective in inducing emesis at all the dose levels studied. The authors concluded that the oral or ocular route of administration provided an alternative to parenteral administration and these routes were more convenient.

Oral

Drug administration via the oral route has been reviewed by DeMario and Ratain [183] and Sood and Panchagnula [184] and these reviews discussed oral administration in general terms as to advantages and shortcomings.

In the case of apomorphine, oral absorption is poor and bioavailability has been estimated at less than 4 % [177] and this poor bioavailability has been associated with a very rapid and nearly complete first-pass hepatic metabolism [178, 185]. In an early study of oral apomorphine bioavailability in rats, Campbell et al [186] demonstrated that tissue levels of drug were not detected in intact rats but in rats after portacaval venous anastomosis, tissue levels comparable to those obtained with subcutaneous administration were seen which suggested a strong first-pass hepatic metabolism. Apomorphine has also been reported as having an extremely bitter taste which may make the oral route more prone to patient non-compliance particularly since an oral dose of 750-1500 mg is needed to provide the same clinical response as a dose of 1-4 mg given parenterally [177, 186]. Although chemical modification of apomorphine to provide a molecule more suited to oral administration has been studied, to date no analogue suitable for use as a prodrug has been found [187, 188].

Sublingual/buccal

The sublingual route and transmucosal drug delivery was reviewed and discussed by Manganaro [189] and in another review Shojaei [190] discussed the buccal mucosa as a route for systemic drug delivery. Absorption of drugs via the oral sublingual or buccal membranes is a variation of the oral route of administration and the advantages offered by this route include

rapid absorption of the drug generally without the lag phase normally seen with the oral route and first-pass metabolism is avoided. There are, however, significant limitations on the size of dose which may be given and not all drugs are well absorbed by this route. The study by Harrison et al [182] of different routes of administration for apomorphine-induced emesis suggested that in dogs, the sublingual route provided erratic results.

The use of sublingual apomorphine for the treatment of Parkinson's disease provides a somewhat longer duration of action than that which is seen with subcutaneous administration but the sublingual route also has a longer latent period prior to the onset of action [134, 191]. In a study by Durif et al [191] the clinical efficacy of sublingual apomorphine in the treatment of idiopathic Parkinson's disease was studied. A number of pharmacokinetic parameters were determined from the serum levels including C_{max}, T_{max}, AUC, elimination half-life, volume of distribution and total clearance. The authors pointed out that the pharmacokinetic parameters reported showed a very wide variability and this was attributed to intersubject variability. The authors suggested that some of this variability could be attributed to the patients swallowing part of the dose and that a dose form better designed and formulated for sublingual use could overcome or reduce this problem although they did point out that intersubject variability was also seen with subcutaneous administration.

Sublingual administration of apomorphine has been used successfully as a tool to aid in the study and possibly in the diagnosis of migraine headache. In a double-blind placebo-controlled study it was demonstrated that patients prone to migraine headaches showed a higher frequency of yawning than control patients [143]. The study described an assessment method involving the videotaping of patients under controlled conditions after a sublingual dose of 0.25 mg of apomorphine and measuring the frequency and duration of yawning.

In another study assessing sublingual administration of apomorphine as an alternative route to subcutaneous administration, Montastruc et al [192] studied six patients with idiopathic Parkinson's disease in order to compare the pharmacokinetic and pharmacodynamic

parameters of these routes of administration. Data analysis from the study showed that the magnitude of the motor response was similar for both routes of administration although the mean onset of action was slower with the sublingual route (30 minutes) than the subcutaneous route (10 minutes). The pharmacokinetic parameters for the two routes showed an essentially identical AUC and C_{max} with values of 1000 ± 174 ng·mL^{-1}·minute^{-1} and about 31 ng·mL^{-1} respectively. The T_{max} values showed a statistically significant difference with the value being 43 ± 6 minutes for the sublingual route and 23 ± 6 minutes for the subcutaneous route. The side-effect profiles for each route were similar with sedation, yawning and nausea occurring with both routes and local irritation was reported in the mouth with sublingual administration and at the site of injection with the subcutaneous route. This study was important as it established that a 3 mg subcutaneous dose was approximately bioequivalent to a 30 mg sublingual dose, confirmed that both routes were clinically effective and that the sublingual route offered an alternative to subcutaneous administration. The only significant difference in the clinical application of the two routes was the longer delay to onset of action with sublingual administration.

In an effort to shorten the latency period with sublingual apomorphine, van Laar et al [193] investigated the effects of incorporating ascorbic acid into a sublingual formulation of apomorphine. They suggested that the ascorbic acid would lower the pH of the saliva, enhance apomorphine solubility and possibly by this, enhance the rate of absorption thus shortening the time to onset of action. In order to determine whether this world be effective, they studied the pharmacokinetics and clinical efficacy of apomorphine sublingual tablets with and without ascorbic acid. For the trial, 13 patients were studied and the two sublingual formulations investigated contained either 10 mg of apomorphine or 10 mg of apomorphine combined with 250 mg of ascorbic acid. No other details of the formulations were presented. A subcutaneous dose was used as a reference for comparison of the sublingual dose forms. In assessing the data from the sublingual doses, the authors noted wide interpatient variability in clinical

response and the calculated bioavailability values were about 18% for apomorphine alone and about 6% for apomorphine combined with ascorbic acid. Although the addition of ascorbic acid appeared to reduce the bioavailability of apomorphine the reduction was not statistically significant. In terms of explaining why the addition of ascorbic acid did not improve the efficacy of sublingual apomorphine, the authors suggested that an inherent problem with this route is that the bitter taste of apomorphine induces salivation and subsequent swallowing of a portion of the dose which is then lost to first-pass metabolism. The presence of ascorbic acid which also has a tart taste may promote even more salivation and swallowing which would effectively negate any absorption benefits due to acidification of the saliva. In this study the authors saw few adverse effects and better patient acceptance with sublingual as compared to subcutaneous administration but low bioavailability and a longer period of latency were disadvantages to this route of administration.

A dose-ranging study and evaluation for efficacy and tolerability of another sublingual formulation was presented by Onodo et al [194]. This study was conducted with Parkinson's disease patients and the authors compared two dose levels of apomorphine, 20 and 40 mg using a placebo-controlled cross-over design. The study enrolled 10 subjects and the adverse effects noted included nausea (n = 1), hypotension (n = 1) and disagreeable taste associated with the product. (n = 8). Although this trial was done to assess an improved product formulation, no information regarding the formulation was provided.

The treatment of erectile dysfunction with apomorphine has led to further studies into the potential for sublingual administration possibly due to anticipation that this route may have better patient acceptance than other routes and the fact that efficacy for this condition seems to be associated with serum levels of 2 $ng \cdot mL^{-1}$ or less of apomorphine which is much lower than the levels of 4 $ng \cdot mL^{-1}$ (1.4-10.7 $ng \cdot mL^{-1}$) required for treatment of PD or 8-25 $ng \cdot mL^{-1}$ required for emesis induction [165, 195, 196]. In an effort to deal with the problem of interpatient variability seen with apomorphine, Dula et al [141] designed a study to assess the

efficacy of apomorphine in treating ED where a sublingual formulation which allowed gradual and controlled drug release was combined with a dose-optimization regimen. In the study which covered an 8-week span, 569 patients were randomized to four groups: one group used a fixed dose of 5 mg, one used a fixed dose of 6 mg, one was a placebo group and the last was the dose-optimization group where the patients began with a 2 mg dose and then were allowed to increased or decreased the dose as needed for a four-week period and then remain at that dose for the next four weeks. Efficacy was assessed through the use of questionnaires completed by both partners. The results of the study showed that efficacy was significantly lower in the placebo group; that adverse effects, primarily nausea and vomiting, were highest in the fixed-dose groups but that incidence of adverse effects was reduced in the second 4-week period for all groups studied and that there was a positive correlation between dosage and the incidence of adverse effects. Overall, the dose-optimization group showed the lowest incidence of adverse effects and the authors concluded that sublingual administration of apomorphine was effective and safe in the treatment of ED and that the use of a controlled dosage form with the opportunity for dose-optimization reduced the incidence of nausea without compromising the clinical effectiveness of the therapy. Although the controlled-release aspects of the sublingual dose form described in this study were alluded to, no specifics regarding either the formulation or the rate of release were discussed. This controlled-release aspect was also mentioned in two reviews of sublingual apomorphine administration in the treatment of ED but again no formulation specifics were provided [197, 198].

Rectal

Rectal drug administration was reviewed by deBoer et al [199] and the pharmacokinetic aspects of this route discussed; Bergogne-Berezin and Bryskier [200] reviewed suppository dose forms of antibiotics and discussed the clinical application of the route of administration. The rectal route may provide an alternative to oral administration for

some drugs and the usual dose forms consist of suppositories or retention enemas. This route may be useful in dealing with situations where a patient is unable to take oral medications due to nausea and vomiting or a patient who is comatose or having seizures. Drug absorption occurs through the rectal mucosa but there is some level of variability in absorption between subjects and the serum levels which can be achieved through this route may be limited. Apart from the disadvantage of variable and limited absorption is a lack of patient acceptance for this route of administration largely due to the inconvenience associated with it [185]. In his study of apomorphine-induced emesis in dogs comparing different routes of administration, Harrison et al [182] found the rectal route ineffective at the doses studied.

In a clinical trial to assess the rectal absorption of apomorphine from suppositories using different formulations, van Laar et al [201] enrolled five patients with idiopathic Parkinson's disease and the study was conducted using a randomized cross-over design where the patients each received one of the preparations in the morning on five consecutive days. The suppositories assessed had a base consisting of either Witepsol-H or glycerol-gelatin and each was prepared with a drug load of 25 or 50 mg of apomorphine. A 1% solution of apomorphine in saline provided a standard to which the suppositories could be compared. Blood samples were taken at timed intervals and patient assessment for timed walking, tremor and dyskinesia were done at the time of blood sampling. Time-serum concentration curves were constructed and these showed wide inter-patient variability and bioavailability which varied between 18.2 to 40.2%. The adverse effects reported in this study were mild and during the 25 experiments conducted sleepiness was reported with 52% of the subjects and yawning was seen in 32% of the subjects. One subject experienced an episode of hypotension and no irritation of the rectal mucosa was seen. From this study the authors stated that the efficacy of rectally administered apomorphine was equal to that seen with subcutaneous and intranasal administration but had the advantage of a much longer duration of effect with the drug effect lasting up to 2.5 hours. Based on their findings, the authors claimed that the Witepsol based

formulations performed better than the glycerol-gelatin ones and that this product could have clinical utility.

Parenteral

Parenteral administration of drugs was reviewed by Tuttle [202] and although the review was largely concerning intramuscular administration, other parenteral routes were discussed as well and the following is abstracted from his review. In general any route other than enteral or topical could be considered to be parenteral although parenteral usually implies administration by means of a needle or cannula penetrating through the skin. This is a common route of administration when the oral route is not possible or is inadequate. In terms of a route of administration, it has a number of advantages. Apart from providing rapid and predictable blood levels, the parenteral route bypasses the GI tract and therefore avoids many of the problems which may be associated with the oral route. It is useful for drugs which would be unstable in the gastrointestinal fluids or which are not absorbed by the GI mucosa; avoids first-pass metabolism and allows administration of drugs which would be irritating to the GI tract either due to the nature of the drug itself or due to the size of dose required to provide the desired serum levels and subsequent desired therapeutic effect. There are a number of significant disadvantages to the parenteral route of administration: since the product bypasses the protective barrier provided by the skin, the dose form and all the ancillary equipment used in its administration must be sterile and pyrogen free. This adds to the cost of providing the drug by this route and also adds the potential risk of sepsis or infection. Once administered parenterally, the drug is irrevocable so medication errors and adverse effects become apparent very quickly and may be more difficult to manage since the dose form cannot be retrieved and steps to avoid absorption are essentially after the fact. The parenteral route is also associated with some pain or discomfort and the possibility of irritation at the site of administration so this combined with the inconvenience associated with parenteral administration may in some cases lead to non-compliance with some patients.

The most common parenteral routes of administration include intravenous (IV), intramuscular (IM) and subcutaneous (SC) [203]. Generally, the intravenous route provides very rapid achievement of serum levels while there is a variable delay by the intramuscular and subcutaneous routes. In some cases it is difficult to achieve adequate drug levels at the site of action so the drug may be injected more directly to the site of action. Examples of these include intra-articular administration where the drug is injected directly into a joint and intrathecal administration where the drug is injected directly into the cerebral-spinal fluid to achieve high drug concentrations in the central nervous system. The central nervous system is protected by the blood-brain barrier which may restrict passage of the drug so the intrathecal route provides a mechanism to bypass this protective barrier.

Apomorphine administration by the intravenous route has been investigated in a number of pharmacokinetic studies and a number of clinical trials primarily for the treatment of Parkinson's disease. An early study by Smith et al [204] examined plasma levels of apomorphine in mice and rats after intravenous, intraperitoneal (IP) and oral dosing. In this study tritium-labeled apomorphine was used and drug levels were determined by measuring the radioactivity of the samples after extraction with ethyl acetate. After dosing with 6.6 $mg \cdot kg^{-1}$, the IV route gave an area under the curve (AUC) of 76.4 $\mu g \cdot min/mL$ with a C_{max} of 4.7 $\mu g \cdot mL^{-1}$ and a T_{max} of 3 minutes. The values obtained from the same dose given by the IP route were essentially identical but the T_{max} was 7.5 minutes. The oral dose resulted in a C_{max} which was about 10% of the value obtained by the IV and IP routes.

The peripheral pharmacokinetics of apomorphine were investigated by Nicolle et al [205] in a clinical study using apomorphine administered by intermittent subcutaneous injections, continuous subcutaneous infusion and IV bolus injection. Twenty patients with Parkinson's disease were enrolled in the study. The patients had been pre-treated with domperidone so adverse effects from the apomorphine were minimal but the authors did note that many of the patients treated with SC infusions developed nodules the site of the injection.

The data from this study demonstrated that the SC route of administration had very good absolute bioavailability but they also found that there was a very high level of inter-subject variability in apomorphine serum levels for both the IV and SC routes of administration. The pharmacokinetic values for apomorphine found in this study were consistent with the study of IV apomorphine pharmacokinetics conducted by van der Geest et al [103] where they reported a mean clearance of 40 ± 15 $mL \cdot min^{-1} \cdot kg^{-1}$, a mean volume of distribution of 1.6 ± 0.5 $L \cdot kg^{-1}$ and a mean terminal half-life of 41 ± 13 minutes.

Stocchi et al [206] proposed the use of an implanted vascular device (Port-a-Cath™) in conjunction with a programmable pump which was proposed as an alternative for PD patients who had shown good success with subcutaneous apomorphine administration but were unable to continue subcutaneous treatment due to severe skin reactions. The authors reported good control of the Parkinson's disease symptoms for all the patients, virtually no complications with sepsis or thrombosis and that the patients who had been on a mean subcutaneous dose of 6.18 ± 1.73 $mg \cdot h^{-1}$ were able to lower their dose to 4.25 ± 0.84 $mg \cdot h^{-1}$ with the continuous intravenous administration system. These results were somewhat different from the findings of a study by Manson et al [207] where the authors investigated IV administration as an alternative to SC administration. As with the previous trial, the patients studied had used SC infusions with good success in controlling the PD symptoms but had developed skin lesions, primarily nodules, and were experiencing fluctuations in their symptom control. It was suspected that the nodules were affecting apomorphine absorption and the resulting variations in serum levels were responsible for the fluctuations in symptom control. The authors suggested that IV administration might be a way of stabilizing serum levels and providing better control of symptoms. The results of the trial showed that overall, the patients were able to reduce the amount of oral anti-Parkinsonian medication they were taking and the average 'off' periods experienced by the patients was reduced from 5.4 hours to 0.5 hours daily which led to a better quality of life. During the trial there were, however, some

longer term problems noted with IV infusion of apomorphine. Three cases of intravascular thrombotic complications occurred during the trial and these were of sufficient severity to require surgical intervention. A number of the cannula used for administration, including all those where the thrombotic complications occurred, showed signs of a crystalline deposit at the tip and it was proposed that the crystalline material was composed of apomorphine degradation products and that this could have acted as a nidus for thrombus aggregation. From the data generated by the trial the authors concluded that IV administration was a viable alternative to SC administration and that this route had the possibility of providing better symptom control than SC administration, but pointed out that further work was required to provide a formulation of apomorphine where the problem of crystal deposition and build-up could be avoided.

The results of a small clinical trial where subcutaneous apomorphine was used as an emetic were presented by deCastro [121]; this trial was conducted in 1978 at a point in time where emesis induction was still being used as a primary treatment modality in cases of acute oral intoxications. DeCastro reported the results of an uncontrolled trial involving 20 children admitted to an emergency room after ingestion of different toxic products. Apomorphine was administered at a dose of 0.07 $mg \cdot kg^{-1}$ subcutaneously in order to induce emesis and 11 of the patients were also treated with activated charcoal. Of the patients, 19 of the 20 vomited within 12 minutes of administration of the drug and the mean time to emesis was 7.1 minutes with a range of 3-12 minutes. The child where emesis induction failed had ingested chlorpromazine and the authors suggested that this was the result of the anti-emetic action of the chlorpromazine; antagonism of some of the effects of apomorphine by chlorinated phenothiazines had previously been demonstrated in altered pecking behavior of pigeons [208]. All of the patients showed signs of lethargy after administration of the apomorphine and this was reversed with administration of naloxone at a dose of 0.02 $mg \cdot kg^{-1}$ and four of the patients showed signs of mild respiratory depression. Based on the results of this trial the

authors recommended the use of apomorphine over syrup of ipecac as an emetic based on the prompt action, low incidence of severe adverse effects and the fact that activated charcoal could be successfully administered concurrently with apomorphine.

Although used to induce emesis, subcutaneous administration of apomorphine has been investigated primarily as a route of administration for the treatment of Parkinson's disease [9, 10]. A significant adverse effect of subcutaneously administered apomorphine which has consistently appeared and been noted in these trials has been the local irritation caused by apomorphine. This is manifested as a painful nodule at the site of injection and a histological study by Acland et al [209] showed these nodules to be an eosinophilic panniculitis.

Intranasal

Nasal administration of drugs for systemic effects has been reviewed by Turker et al [210] and some of the problems and possibilities of this route of administration were reviewed by Illum [211, 212] and earlier by Jones et al [213]. The physicochemical nature of drugs which have been successfully delivered by this route was reviewed by McMartin et al [214] and the role of pharmaceutical excipients in nasal delivery was discussed by Behl et al [215]. Intranasal administration may be for local effect and to provide symptomatic relief from the congestion associated with allergic rhinitis or head-colds or for some drugs it provides a route of administration for systemic effect. Administration of drugs for systemic effects by the intranasal route has some potential advantages primarily due to the fact that this route by-passes the GI tract so issues of drug loss through gut metabolism or drug instability are avoided as well as hepatic first-pass effects. For drugs which are absorbed through this route, the rate and extent of absorption and subsequent serum concentration-time curves are comparable to administration of the drug by the intravenous route. There are some disadvantages associated with this route, largely associated with the physiological conditions in the nose and may lead to variability in the rate and extent of drug absorption. Factors such

as the quantity of mucus and speed of mucus flow, pH and viscosity all can affect absorption and these may change with conditions such as seasonal allergy or the presence of a head-cold. At this time, there are a number of commercial products available which are administered by the nasal route for systemic effect: for the treatment of migraine headache there are Sumatriptan (GalaxoSmithKline), Zolmitriptan (Astra-Zenneca) and Ergotamine (Novartis). As well as these lipophilic and low molecular weight molecules, there are several peptide products: Calcitonin (Novartis), Desmopressin (Ferring) and Busevelin (Aventis) [211, 213].

In the review by Illum [211], the author makes a number of points; the intranasal route is useful for drugs where a rapid onset of action is required and the only other alternative route is by injection. Lipophilic drugs are generally well absorbed from the nasal cavity and although there is a large surface area with an extensive blood supply, low molecular weight (<1000 daltons) more polar molecules usually show a bioavailability of less than 10% and bioavailability of peptides such as calcitonin and insulin is usually less than 1%. The two major factors limiting nasal absorption involve permeability of the drug molecule and the movement of the nasal mucus. The nasal membrane has low permeability for polar drugs and high molecular weight molecules. Drugs may cross the nasal membrane by either a transcellular route where the molecules pass through the cells of the membrane or by a paracellular route where the molecules pass through the junctions between the cells of the membrane. Generally, the large peptide molecules use the transcellular route and polar drugs with a molecular weight less than 1000 daltons use the paracellular route [214].

Since the nasal route of administration would be more convenient for patients with advanced Parkinson's disease, an open clinical trial was conducted by vanLaar et al [216] to determine the efficacy of this route of administration using apomorphine administered as a metered nasal spray. Assessment of the patients by tapping and walking tests showed that intranasally administered apomorphine appeared to work as well as subcutaneously

administered apomorphine but that a higher dose was required by the intranasal route. A follow-up to the previous study was conducted by Sam et al [217]. Patients who had responded well to subcutaneous apomorphine were selected and for one 'off' episode were administered subcutaneous apomorphine and for another, intranasal apomorphine administered via a metered aerosol. Blood samples were collected at timed intervals over a two hour period and the serum levels of apomorphine determined by HPLC. This study showed that apomorphine was rapidly absorbed by both routes with T_{max} for the nasal route being 23 minutes and 18 minutes for the subcutaneous route. The authors determined that the bioavailability of the nasal solution was about 45% of that found for the subcutaneous route.

A double-blind, placebo controlled cross-over study of intranasal apomorphine was conducted by Dewey et al [218]. The patients enrolled in this study had advanced Parkinson's disease and used an apomorphine spray consisting of a solution of apomorphine 10 $mg \cdot mL^{-1}$ delivered by a metered aerosol which delivered 1 mg per spray. The outcome of the trial demonstrated that intranasal administration of apomorphine was effective in treating the symptoms of Parkinson's disease but the high incidence of severe nasal irritation was a major drawback. Apart from nasal irritation, the use of aqueous solutions for nasal administration had the disadvantage of a short therapeutic half-life due to rapid and unsustained absorption, drainage from the site of absorption and rapid clearance due to ciliary beating. The lack of chemical stability of the solution was also problematical. Ugwoke [219] postulated than some of these issues could be resolved through the use of a powdered product using a mucoadhesive carrier. Ugwoke suggested that with this type of system, the powdered material would absorb water from the site of deposition on the mucosa which would lead to adhesion followed by swelling and gel formation. Adhesion would increase the drug concentration gradient across the epithelium and the residence time in the nasal cavity and release would be prolonged due to diffusion through the gel layer. The powdered formulations studied included carbopol 971P,

polycarbophil and lactose. The subjects for this study were six rabbits and the apomorphine dose for the lactose carrier and subcutaneous administration was 0.3 $mg \cdot kg^{-1}$; for the polymers the dose was 0.6 $mg \cdot kg^{-1}$. The results from this study showed that the powdered dose forms all gave bioavailability values which were not different from the subcutaneous route (100%) while the nasal solution bioavailability was about 71%. The study therefore showed that the effect of the polymer carriers was to prolong the action of the drug and this was reflected by the derived pharmacokinetic values where the half-life of the polymeric systems was longer.

A follow-up study on the use of carbopol-based powdered delivery systems was also published by Ugwoke [220]. This work addressed the nasal toxicity of delivery system in rabbits. Carbopol alone, carbopol with apomorphine, glucose alone and glucose with apomorphine were evaluated in terms of nasal ciliary beat frequency and mucosal inflammation. They found that all of the combinations caused some degree of irritation but that the carbopol and carbopol with apomorphine caused a much more severe inflammatory response which tended to increase with duration of treatment. From this work they concluded that carbopol was not a satisfactory polymeric carrier for nasal delivery. A third study was conducted by Uwoke [221] this time using carboxymethylcellulose (CMC) as a polymeric carrier. In this study, a lyophilized powder of apomorphine and CMC was compared to biodegradable starch microspheres and a lactose/apomorphine mixture using subcutaneous apomorphine as a standard and basis of comparison. The study data showed that the release of apomorphine from lactose, the microspheres and the subcutaneous route was rapid but not sustained. Relative to the subcutaneous route, the bioavailability of the lactose and CMC was about 85% while the microspheres and CMC gave values approaching 100%. The authors concluded that the intranasal administration of apomorphine with CMC provided a rapid initial absorption and serum levels were maintained for a longer period of time than that seen with subcutaneous administration. This type of delivery system could potentially provide

therapeutic levels of drug with a reduced frequency of dosing and could be a useful method for rescue of Parkinson's disease patients experiencing off periods.

An extremely interesting and possibly very useful observation was reviewed by Illum [212]. She noted that for some time it has been known that some agents had the ability to pass directly from the nasal mucosa into the central nervous system by way of the olfactory region and this was a significant site for entry of viruses into the brain [222]. In a review of this type of transport Mathison et al [223] described some of the factors influencing the direct transport of solutes to the CSF and brain. Although the amount of material which can be transported in this way is very small relative to the amount applied, there may be great potential for a delivery system which could deliver apomorphine directly to the central nervous system and avoid the peripheral dopamine receptors which are responsible for the undesired effects of nausea and vomiting in treating Parkinson's disease patients with apomorphine.

Transdermal

Transdermal absorption of apomorphine was investigated by Gancher et al [177] in a series of experiments to compare different routes of administration. In this trial apomorphine was dispersed in a petroleum-based ointment base. Blood samples were collected at timed intervals over 60 minutes and analyzed using HPLC for apomorphine content. No apomorphine was detected in any of the samples and the authors concluded that apomorphine is not absorbed by this route.

An in vitro study was conducted by Peira et al [224] using octanoic acid as a penetrating enhancer. The product was prepared as a thickened micro-emulsion containing 3.9% apomorphine and absorption across hairless mouse skin was studied using a diffusion cell. The authors reported that the drug crossed the skin in an apparent zero order fashion and approximately 15% of the drug was released into the receptor chamber after eight hours. The authors concluded that transdermal absorption facilitated by a penetrating enhancer like

octanoic acid could provide an effective dose form for apomorphine delivery if the rate of release could be increased.

Durif [106] conducted a study comparing apomorphine absorption after percutaneous and subcutaneous administration in rabbits. The transdermal product consisted of apomorphine dissolved in hydroxypropylmethylcellulose gel at a concentration of 10 $mg \cdot g^{-1}$ with ascorbic acid added as an antioxidant. The authors found that absorption did occur across rabbit skin and although the time to peak concentration (T_{max}) was not significantly different for the two routes, the peak concentrations (C_{max}) and areas under the curve (AUC) were different with the values for the transdermal route being significantly smaller. The bioavailability of the transdermal product compared to the subcutaneous product was about 35%

The possibility that effective transdermal delivery of apomorphine could be achieved using an iontophoretic system was investigated by a collaborative group from The Netherlands and a series of papers describing their findings were published [196,225-229]. With this technology, the transport of a drug across the skin is facilitated by means of a small electrical current. The results of their studies demonstrated that precise control of apomorphine flux could be achieved by varying the current density and in this way a specific serum concentration of apomorphine could be maintained. They also demonstrated that when no current was applied, no drug was delivered which was in agreement with the earlier findings of Gancher that passive diffusion of apomorphine does not occur across human skin [177]. Overall, these studies have shown that in vivo delivery of apomorphine can be achieved and controlled using transdermal iontophoresis but the serum levels which can be achieved are only to the order of 2.5 $ng \cdot mL^{-1}$. The serum levels needed for treatment of Parkinson's disease are between 1.4-10.7 $ng \cdot mL^{-1}$; for ED 2.0 $ng \cdot mL^{-1}$ and for emesis 8-25 $ng \cdot mL^{-1}$ [165, 196].

Ocular

In a review of ophthalmic drug delivery systems, LeBourlais et al [230] stated that eye drops represent about 90% of all ophthalmic dosage forms and most of these are used for local effects in the eye. Ludwig [231] reviewed ocular drug delivery and discussed formulation strategies which have been used to increase local bioavailability in the eye, most of which were designed to enhance bioavailability by increasing contact time with the ocular tissues and included viscous solutions, particulate systems, semi-solid systems and solid systems. Other strategies which have been investigated for enhancing bioavailability include reduced drop size [232, 233], vehicle composition [234] and the use of absorption promoters [235].

Systemic adverse effects resulting from topical ocular treatment was reviewed by Labetoulle et al [235] and earlier by LeBourlais et al [230]. In these reviews the authors stated that only about 1-5% of an applied dose actually penetrates the ocular tissues so relatively high concentrations of drug need to be used in the dose form. This poor bioavailability is largely due to the efficient lacrimal drainage system of the eye and the complex and relatively impermeable corneal barrier. The cornea consists of three tissue layers; the epithelium, inner stroma and endothelium. The epithelium has a lipophilic cell layer consisting of closely-packed cells which functions as an effective barrier to ion transport and diffusion of large molecules via the paracellular route. The stroma beneath the epithelium is a hydrophilic cellular layer which resists the passage of lipophilic drugs so essentially passage of hydrophilic drugs is hindered by the epithelium and passage of lipophilic drugs is hindered by the stroma and endothelium. This structure provides a very effective barrier to prevent the passage of highly hydrophilic or hydrophobic agents into the eye [237, 238]. A further hindrance to absorption is the lack of vascularization in the tissues of the eye [231, 239]. In contrast, the conjunctival membrane lining the inner surfaces of the eyelids and over the sclera up to the cornea is very vascular and has a large surface area making it an effective site for

systemic drug absorption. Another effective site for absorption is the lacrimal drainage system which is contiguous with the nasal membranes. Tears are secreted by the lacrimal gland, spread over the ocular surface by the eyelids during blinking and drained through a collection system consisting of the canaliculi, the lacrimal sac and the nasolacrimal duct which drains into the inferior nasal passage [231]. The basal tear flow is approximately 1.2 $\mu L \cdot min^{-1}$ so after topical administration of an ophthalmic solution the contact time is only 1-2 minutes due to blinking which pushes the fluid towards the lacrimal drainage system. Salminen [240] suggested that absorption occurred during conjunctival and nasal mucosal contact but not during passage through the lacrimal drainage system; this was based on observations that in some individuals early and then later plasma peaks were seen with the administration of timolol eye drops. In order to minimize systemic absorption a number of strategies have been used including reduction of drop size in solutions [232], increasing the viscosity of eye drop formulations [241], use of polymeric inserts [242, 243], use of lipophilic prodrugs [244] and even application of pressure to block the puncta and reduce lacrimal drainage [240]. Frangie [245] reviewed the clinical pharmacokinetic of various topical ophthalmic delivery systems and identified a number of barriers and factors affecting local bioavailability. In his review he points out that the eye is protected by a series of complex defence mechanisms which are important in protecting the delicate components of the eye from the external environment; most of these defence mechanisms are however, also very effective in preventing ocular absorption of topical medication. Intraocular absorption will be primarily dependent on the amount of drug presented to the absorbing surface and the length of contact or residence time that the drug is in contact with this surface. The normal volume of tears contained in the ocular adnexa is usually about 6 μL and a drop of ophthalmic solution will usually have a volume ranging from 50 to 100 μL depending on the physical nature of the solution and size of the nozzle aperture used to apply the solution. This relatively large volume of applied drug

will overflow the capacity of the adnexa and there will be a large increase in drainage through the lachrymal drainage system or even overflow over the lids [233]. A related effect which reduces the residence time of the drug in the eye is induced lacrimation due to irritation by the product which results in a reflex increase in tear production which rapidly removes the drug from the intended site of absorption. Various strategies to alter the product and reduce its irritation potential are commonly used; buffering to physiological pH, maintaining isotonicity, the use of micronized drug particles for suspension formulations, and the use of viscosity-increasing agents are a few of these strategies [246]. There is also a problem with non-productive absorption through non-specific sequestering of the drug by binding to protein components present in the precorneal film [247, 248].

Unwanted systemic absorption of some ophthalmic products designed and intended for local effect has led to development of strategies to prevent this systemic absorption but has also led to interest in using the ophthalmic route as a portal for systemic effects [241, 249]. In a review by Salminen [240] the systemic absorption of topically applied ocular drugs was discussed. In the eye, drugs are often applied for local effects and the therapeutic effect occurs when the drug penetrates the inner and outer parts of the globe and periocular tissues. The products applied to the eye come in a variety of forms including solution, suspensions, gels and ointments and the drug load of these products is usually between 0.1 to 10%. Depending on the condition being treated, the duration of therapy may range form a one-time only as in the case of diagnostic applications, to a few days as in the case of seasonal rhinitis irritation, to a few weeks as in the case of corneal injury being treated with steroids or antibiotics to years as in the case of glaucoma therapy with pilocarpine. The local bioavailability of tends to be low with many of these products and this is due to a number of factors. In her review, Salminen [240] discussed the systemic absorption after topical ocular application of timolol, atropine, cyclopentolate, scopolamine, phenylephrine and betamethasone. She suggested

possible sites for systemic absorption are the conjunctival membranes, the tear outflow channels and the nasal cavity; it appears that the nasolacrimal duct acts more as a drug depot rather than an absorbing surface. Using lachrymal scintillography systemic absorption of timolol still occurred when the tear outflow was blocked by punctual occlusion but in her conclusion of the review, the author suggested that the larger part of systemic absorption likely occurred via the nasal mucosa.

In the case of apomorphine, ocular absorption has been demonstrated in the dog and this type of delivery system may be useful as a means of providing controlled delivery of the drug [4, 33, 182].

Conclusions: Section 2

In this section various aspects of apomorphine which may be relevant to developing a dose form were reviewed and this is a process known as preformulation. The physiochemical properties of apomorphine were examined and it is likely that product stability will be an issue as apomorphine appears to undergo auto-oxidation quite easily and the dose form and packaging will need to protect the drug from this type of degradation. Also since the drug will be prone to degradation, stability studies will be important and a stability-indicating method of analysis will be required for these studies. Since HPLC is the analytical method most likely to suit this requirement, published methods were reviewed and although a number are available, most were designed to measure apomorphine in biological fluids and although none seemed especially suitable for pharmaceutical use in terms of providing a rapid, simple and stability-indicating method, one of them may be suitable or at least provide a starting point for development. UV spectroscopy may also be of value in developing and evaluating a dose form since apomorphine has a strong absorbance band at about 272 nm.

The pharmacology and pharmacokinetics of apomorphine are complex and most of the routes of administration have problems associated with them. Extensive first-pass metabolism is an issue which precludes the use of the oral route; local irritation is a problem with the parenteral, sublingual and nasal routes; transdermal absorption appears to be poor and with the rectal route, absorption appears to be erratic. Although not well studied, absorption via the ocular route appears promising for use in dogs. The pharmacodynamics of apomorphine also appear problematical in that apomorphine distributes quickly into the CNS and at that site is able to inhibit emesis through interaction with the opioid μ-receptors so the dose form will need to deliver drug rapidly enough to allow peripheral serum levels to reach therapeutic levels before central suppression of emesis can occur. This is further complicated by the fact that wide variability in response among patients is seen with emesis occurring somewhere between serum levels of 8 to 25 $ng \cdot mL^{-1}$ in dogs. The weight range seen in canines also presents a problem in term of dosing; weights may range from 1 to 100 kg so selection of the drug load in the dose form will present a problem in that the load appropriate for a larger patient may present a definite hazard to a smaller patient. The adverse effects seen with apomorphine are dose-related and in many cases biphasic so control of absorption rate from the dose form would be useful; if only sufficient drug is absorbed to induce emesis and then absorption is stopped, some of the adverse effects may be avoided.

From this preformulation work-up, it appears that a controlled-release dose form with an intermediate drug load applied via the ocular route could be designed to have the appropriate attributes; the dose form would need to release the drug at a rate sufficient to raise the peripheral serum levels to that required for emesis before distribution into the CNS suppressed emesis and the dose form would have to maintain integrity to allow easy removal from the eye and prevent excess drug absorption.

Section 3 - Method Development

Introduction:

For the purposes of this project, suitable methods for the analysis of apomorphine and gelatin will be required; work on the formulation design was being done concurrently and it was known at this time that the hydrogel matrix for the insert would be composed of gelatin with glycerin as a plasticizer. In this section, high-pressure liquid chromatography (HPLC) and UV-spectroscopy will be investigated as possible analytical methods and the methods developed will be validated to verify their suitability.

3.1. Apomorphine analysis

Overview:

An analytical method to measure apomorphine is required for this project which will:

- provide an assay method for quality control purposes
- allow stability and compatibility studies to be carried out on the raw material
- allow stability studies to be carried out on the finished product
- allow studies characterizing the rate of release from the product to be conducted

The assay method which would probably be most appropriate would be HPLC since this technology has the potential to separate an analyte from excipients and breakdown products and is capable of quantitative determinations at the same time [250]. This ability to separate components of a mixture is characteristic of any chromatographic process and depending on the specific type of process being used, paper (PC), column (LC), thin-layer (TLC), gas (GLC) or high-pressure liquid chromatography (HPLC), there are a number of variables which can be manipulated to achieve satisfactory separations for a specific purpose. In general terms some of these variables are common to all chromatographic processes since all of the methodologies are based on the relative affinity of the analyte to some type of stationary material. Most of

these variables are, however, unique to the type of chromatographic technique being used and the nature of the separation process which is involved [93, 251, 252].

In HPLC, separation is achieved by dissolving the analyte into a solvent (mobile phase) which flows through the column packing material (stationary phase) and as the analyte moves through the column an equilibrium is established between the mobile and stationary phases for the analyte. This equilibrium is dependent on the relative affinity the analyte has for each of the phases and as the process continues, separation is gradually achieved. With HPLC there are several distinct modes of separation and these may be classed as reverse phase (RP), normal phase (NP), ion exchange and size exclusion; each of these has application to specific groups of analytes [253]. For analysis of drugs with molecular weights below 1000 daltons, reverse phase HPLC is widely used [93, 254].

For both reverse phase and normal phase separations, the separation process is dependent on a combination of adsorption and partition. In his textbook on HPLC method development, Snyder [255] provides an overview of the mechanisms involved in separation (pg 235-270) and the mechanisms involved in reverse-phase systems was reviewed by Neue et al [252] for ionizable analytes. In normal phase chromatography the stationary phase tends to be very polar with the mobile phase being relatively non-polar; in reverse phase chromatography the stationary phase is relatively non-polar with a polar mobile phase being used. Since with reverse phase chromatography the sample or analyte may be dissolved in an aqueous medium, this often makes sample preparation and handling easier when working with biological samples or water soluble drugs. The mechanism of separation in normal and reverse phase systems is different with normal phase separations being primarily due to displacement while reverse phase separations are primarily due to analyte polarity. In normal phase separations, analyte and mobile phase molecules compete for active sites on the stationary phase; initially the sites are occupied by mobile phase molecules and analyte molecules must

displace these to gain access to the active sites therefore retention is related to the relative affinities of the stationary phase for analyte and mobile phase molecules. The use of a more polar mobile phase will increase competition for the stationary phase and result in reduced analyte retention. In reverse phase separations, retention is related to the polarity of the analyte with more polar molecules eluting first. Competition in this case is based on the relative affinity of the analyte for the mobile and stationary phases and in this case use of a more non-polar mobile phase will result in reduced analyte retention [252, 255].

The quality of a chromatographic separation may be assessed by examination of four basic chromatographic parameters: capacity factor (k'), column plate number (N), separation factor (α) and tailing factor (T). Definitions and the method of calculation for these parameters are detailed in the USP [79] (pg 2380-2392) in the general section <621> Chromatography.

In a given chromatographic system the retention time or time from injection of the sample until the peak elutes from the column is characteristic of a compound but not unique. Retention time then can be used as a component in an identity profile but is insufficient on its own to establish identity. Quantification of the analyte may be achieved by measuring the peak height or area and comparing it to the response for a standard containing a known concentration of analyte [253]. The capacity factor (k') is usually a more useful measure of retention than retention time which can change with variations in column length and mobile phase flow rate whereas k' remains constant [256]. Capacity factor is calculated using equation 3.1 where t is the retention time of the analyte measured from time of injection to time of elution of peak maximum and t_a is the retention time of the sample solvent or a non-retained component; the value for t_a is also referred to as column dead time [257].

$$k' = \frac{t}{t_a} - 1 \qquad \text{Equation 3.1}$$

As long as the mobile and stationary phases are not changed, the value for k' will remain constant and changes in flow rate and column length will have no effect on k'. This may be useful in method development since for a given system, changing the strength of the mobile phase in a reverse phase system will lead to a predictable change in capacity factor. Larger values of k' are associated with shorter broader peaks and for most separations values between 0.5 and 10 are acceptable [258].

The column plate number (N) is essentially a measure of peak narrowness and is often used to determine whether a used column is still functioning properly since this parameter is one of the manufacturers' specifications for a new column [253, 257]. The column plate number is calculated from equation 3.2 where t is the retention time of the analyte and $W_{h/2}$ is the peak width at half-height which usually can be obtained directly from electronic integrators.

$$N = 5.54\left(\frac{t}{W_{h/2}}\right)^2 \qquad \text{Equation 3.2}$$

Larger values of N suggest narrower peaks and generally better separations but as been mentioned, the value of N can be affected by a number of operating variables such as the particle diameter of the stationary phase which is inversely related to N. Changing to a column where the particle size is half of the original will effectively double the value of N. Also increasing the column length will increase the value of N in proportion to the length [257]. Some characteristics of the mobile phase will affect the value of N as well; increasing the viscosity of the mobile phase will reduce N and within limits, increasing temperature will increase N usually due to a reduction in mobile phase viscosity at higher temperatures [259, 260]. The use of temperature to modify selectivity in reversed-phase HPLC has been reviewed in some depth by Dolan [261].

The relative retention or separation factor (α) is essentially the ratio of capacity factors for peaks of two compounds which need to be resolved from each other [253, 256]. This factor is used as part of the system suitability testing presented in the USP but also is important in stability studies where breakdown products need to be separated from the parent compound or in finished product studies where the active component needs to be separated from product excipients. The separation factor can be calculated from equation 3.3 where the terms have already been defined:

$$\alpha = \frac{t_2 - t_a}{t_1 - t_a} \qquad \text{Equation 3.3}$$

The value of α is a function of both stationary and mobile phase composition so changes in either mobile phase or stationary phase will affect the value of α. Manipulating the value of α by changing any of these variables may allow resolution of any closely eluting peaks [256, 262].

The tailing factor (T) is a measure of peak symmetry with a value of unity representing a perfectly symmetrical peak. The tailing factor may be calculated from equation 3.4 where $W_{0.05}$ is the width of the peak at 5% of its height and f is the distance from the peak maximum to the leading edge of the peak with the distance being measured at 5% of the peak height. These measurements are illustrated and specified in the USP [79] in general chapter <621> Chromatography (pg 2380-2392).

$$T = \frac{W_{0.05}}{2f} \qquad \text{Equation 3.4}$$

An asymmetrical peak is said to be tailing and inspection of a peak with a value of T larger than 1, will show asymmetry on the downside of the peak. Tailing results in poor resolution of adjacent peaks with the trailing edge of a peak running into the leading edge of the next peak. This is especially undesirable in quantitative situations where peak area is being used as the

index of response [263-265]. With reverse phase separations, tailing usually suggests there is more than one mechanism of retention taking place and with bonded phase columns it often is due to the analyte interacting with free silanol groups remaining after the bonding process; the use of a modifier such as triethylamine in the mobile phase may compete for these free silanol groups and reduce the tailing [262]. For most analytical work a tailing factor in the range 0.9-1.2 is generally satisfactory.

The primary goal in an HPLC assay is the adequate separation of the analyte from any other components present in the sample and a chromatogram from an adequate system will show well shaped symmetrical peaks sufficiently separated to allow the space between peaks to return to baseline [266, 267]. The basic parameters of capacity factor (k'), plate number (N) and separation factor (α) are all components in resolution and equation 3.5 shows how they contribute to overall resolution (R_s) [253, 257].

$$R_s = 0.25 \left(\sqrt{N}\right)\left(\frac{k'}{1+k'}\right)(\alpha-1) \qquad \text{Equation 3.5}$$

Manipulation of capacity factor k' to increase resolution is practical only if the current value of k' is below 2 [256]. Examining the relationship between resolution and k' as shown in equation 3.5, it can be seen that as k' is increased, there is an incrementally smaller return in the value of R_s; at k' values of 10 or higher further increases have very little effect on increasing resolution and actually will have a detrimental effect on the assay by increasing analysis time and broadening peak width and reducing peak height. Generally the greater the capacity factor the greater the capacity of the column and the longer a peak will be retained and the longer a peak is retained the broader it will become which may lead to detection and quantitation difficulties [268-270]. Adjusting capacity factor by changing the strength of the mobile phase is probably the first and easiest change which should be made if resolution is

inadequate but for a given system there will be an optimum value of k' and that value will probably be between 2 and 6 [256, 258, 271].

Overall resolution has a direct square root dependence on plate number so an increase in plate number will lead to increased resolution but because it is a square root relationship, a four-fold increase in N is necessary to double resolution. It has been shown that the value of N may be increased by reducing the particle size of the column packing, increasing the length of the column or reducing the viscosity of the mobile phase either by increasing column operating temperature or changing the mobile phase composition [256, 257, 272]. In practical terms, increasing resolution by manipulation of plate number is useful if a short column (50-100 mm) or a column with large particle packing (10 μm) is being used; in these cases, replacement of the column with a longer one or one with smaller packing particle size will benefit resolution. Apart from the above situation, increasing plate number is of limited value in attempting to increase resolution.

Manipulation of the separation factor (α) is the most effective way to provide an increase in resolution and separation factor will be changed with component changes in either the mobile or stationary phases [256, 273]; changing the primary solvent in the mobile phase to a solvent of different strength is the easiest and most practical strategy to effect a change in α although changing to a different stationary phase may be necessary [274].

Column Selection

The initial packing materials used in HPLC were pellicular in nature where the packing usually consisted of small glass or ceramic beads and later beads of non-porous silica. These were covered with a layer of porous material which was chromatographically active and the layer of material and the glass or silica bead gave a particle with a diameter of 30-45 μm in diameter. Currently, particle size is usually in the 3-10 μm diameter range therefore these early packings had a much smaller total surface area of the chromatographically active

material or stationary phase than what is generally in use today [257]. As was discussed earlier, capacity is a descriptive term applied to columns used in chromatography and may be defined as the ability of the column to retain the analyte [273]. Capacity was increased with the use of totally porous particles composed of microspheres which over time have evolved into three sizes; diameters of 10, 5 and 3 μm and the correspondingly larger surface areas have provided increased capacity. The support material most often used is silica and initially these silica particles were quite irregular in shape but advances in silica processing technology has allowed for the development of spherical particulate silica [275]. This advancement allowed the development of bonded phase packings where the spherical silica particles serve as a support for the stationary phase.

In a review of the chemical and thermal stability of stationary phases, Claessens and vanStraten [276] discussed the nature, problems and future trends in the stationary phases used in reverse-phase HPLC. The most widely used packing are totally porous microspheres which are available in a variety of diameters, pore sizes and total surface area and while smaller particles tend to give faster separations, they have decreasing sample load characteristics [277]. Silica is the most common support as it has a number of advantages over other materials: it has high mechanical strength; can be chemically modified with bonded phases; is compatible with both aqueous and organic solvents and does not swell with solvent changes. Its major disadvantage is that it becomes soluble at pH values in excess of 7.5 although silica particles prepared by aggregation of silica sols (solgel type) as opposed to particles prepared by precipitation of soluble silicates (silgel) may tolerate pH values as high as 9.0 [278, 279]. In their review, Claessens and vanStraten also discussed polymer-coated phases on inorganic substrates such as silica and zirconia and totally polymer substrates. Porous polymers may be used as stationary phase material with the most common type being polystyrene derivatives. Like silica they are available as pellicular and totally porous particles with a range of pore

sizes. These are able to withstand pH environments from 1 to 13 and are useful for protein separations. They generally have lower column efficiency and slower separations than silica-based packings and are limited as to the number of functional groups which can be bonded to them [277, 279]. Other materials such as graphitized carbon, alumina and zirconia are available for use a support material but these usually have very specific applications rather than general use [280].

The stationary phase is bonded to the silica by means of a silica-oxygen silica-carbon covalent bonding system. This is achieved by the use of chlorosilane derivatives containing the desired functional group [276]. The USP [79] (pg 2380-2392) lists a number of bonded phase packings with specific functional groups to be used in various official assays and tests. They are designated numerically and are prefixed by the letter L and the phase, support and particle size are specified. The listing for example for a C_{18} column is 'L1 - Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 μm in diameter.' Some of the functional groups bonded to the silica are quite bulky, for example C_{18}, and steric hindrance may prevent bonding to many of the surface hydroxyl groups on the silica which would leave these polar groups available to interact with the analyte; if the analyte is polar it will interact with these groups as well as the intended functional groups and tailing of the analyte peak may result [275, 276]. This problem is dealt with by use of a procedure referred to as capping. In this process, the material after treatment with the organic silane carrying the desired functional group is then treated with a much smaller and therefore less sterically hindered organic group such as trimethyl chlorosilane (TMCS). The TMCS is able to react with these remaining hydroxyl groups and by capping them, prevents them from interacting with the analyte [278].

Although the USP specifies the column packing or stationary phase for a given assay, the same designated column prepared by different manufacturers may show different retentive

and selective properties so some adjustment of mobile phase may be required. This was reviewed by Felinger et al [281] and these variations are usually the reason for system suitability testing which may be specified in the individual monograph. Although the columns may perform differently between manufacturers, each of the individual manufacturers columns are quite consistent with little variation in performance characteristics. Variation from manufacturer to manufacturer may be due to differences in particle size distribution which affects area and pore volume, the amount of bonded phase applied and the degree of capping. Bonded phase packings are stable to most organic solvents and to aqueous systems where the pH is between 2.5 and 7.5 but at pH values greater than 7.5 the silica will begin to dissolve and column life is significantly reduced [276, 278, 282].

A review of trends in HPLC column usage from 1984 to 1991 was presented by Majors [254]. A variety of column configurations and materials are available but the most common are straight lengths of stainless steel tubing with highly polished interior surfaces and compression end fittings. Glass and glass-stainless steel combinations have also been used but experience has not demonstrated any particular advantage to these. Rigid polymer (PEEK) columns and radial compressed columns have been used in an effort to reduce cost but these have not been widely accepted. Columns come in a wide variety of lengths and diameters suited to particular applications. For analytical applications a column with a 4.6 or 3 mm internal diameter and packing of particle size of 3-10 μm is common; microbore columns with internal diameters of 1-2.1 mm provide high sample mass detection sensitivity and are useful when the HPLC is interfaced to a mass spectrometer which requires a small solvent input volume. Columns with internal diameters of 8 to 50 mm are used for preparative applications where the object is to collect chromatographically pure material. The length of the column may vary from 30 to 250 mm but are generally available commercially in lengths of 50, 100 and 250 mm. Trends in HPLC column usage from 1984-1991 was presented by Majors [254].

The stationary phase in a column will degrade with use so column stability is an important consideration and column condition should be assessed periodically during its life [257]. Column stability was also reviewed by Kirkland et al [278]; longer chain alkyl bonded-phase packings such as C_{18} and C_8 tend to be relatively stable since stability tends to increase with increasing chain length. Degradation is usually due to loss of the bonded phase material by hydrolysis of silyl bond holding the functional group to the support so the rate of column degradation is also related to the type of silica support used and composition of mobile phase being used as to pH, organic modifiers and type of buffer system being used. Factors such as increased temperature, lower pH and highly aqueous mobile phases will accelerate the reaction rate for this hydrolysis. Sterically protected functional groups such as diisopropyl may be used to improve stationary phase stability for low pH applications. Column assessment may be done by periodically running system suitability test and determining the number of theoretical plates in the column (N) since this value declines in a deteriorating column.

Mobile phase selection

In reverse-phase HPLC, the three solvents most often used in the mobile phase are acetonitrile, methanol and tetrahydrofuran in combination with an aqueous component; these components are usually designated as %A (aqueous) and %B (organic modifier) [271]. Retention of an analyte is dependent on the polarity of the analyte, strength of the mobile phase, column type and temperature so there are a number of variables which can be manipulated to obtain the desired level of resolution [256, 271]. The primary mechanism of retention is related to the interaction of analyte with the mobile phase and the stationary phase but these interactions are complex and not yet fully understood [283]. The level of resolution achieved is a summation of all of these factors [284] but the precise molecular mechanisms involved are not well understood [285]. Since the mobile phase contains an aqueous component, there will be some level of repulsion of the analyte from the mobile phase based

on hydrophobic interaction and this has been discussed in terms of n-octanol-water partition coefficient (P) and relating this to a plot of log k' as a function of volume fraction of organic modifier [286, 287]. This hydrophobic repulsion will have an inverse relationship with analyte polarity so as analyte polarity increases, the amount of hydrophobic repulsion will decrease and this will result in more polar analytes being eluted first as they will tend to remain associated with the mobile phase and more non-polar analytes eluting later since they are repulsed from the mobile phase and are able to interact with the relatively non-polar stationary phase [283].

Mobile phases are often referred to in terms of strength. This relates to the polarity of the mobile phase and since relatively non-polar mobile phases give short retention times, they are considered strong mobile phases. In a review of retention mechanism, Gritti and Guiochon [283] discuss organic modifiers in terms of their relative strength and suggest that acetonitrile is stronger than methanol and that they also have quite different physicochemical properties. Methanol interacts with water through hydrogen-bonding whereas acetonitrile has little interaction with water and tends to self-associate and this leads to quite different separation characteristics when one or the other is used as organic modifier on the same column. Outinen et al [288] also discussed organic modifier strength and assigned relative values to them; tetrahydrofuran 4.5, acetonitrile 3.2 and methanol 2.6. As the polarity of the mobile phase is increased, usually by increasing the water content, there is more repulsion of the analyte from the mobile phase, more opportunity to interact with the stationary phase and a resulting longer retention time. As polarity of the mobile phase increases, the strength of the mobile phase declines with a mobile phase composed of only water being the weakest. For a given analyte, changing the strength of the mobile phase by altering the volume fraction of the aqueous component in the mobile phase provides a very accessible tool to effect changes in retention time. There is also a level of predictability to this tool; since mobile phase polarity controls

retention time in a given system, there actually is a linear relationship between log k' and the volume fraction of organic solvent in the mobile phase. This allows some prediction of what the resulting retention time will be with a specific change in the mobile phase [271].

Although the primary mechanisms of retention involved in reverse-phase HPLC involve partition and relative polarity, there usually is some element of adsorption especially when the support for the stationary phase is silica. Even after bonding of the stationary phase and end-capping has been performed on the column, some free silanol groups may still be present in the column and these can interact with the analyte through adsorption and have an effect on retention [276]. This phenomenon is often responsible for tailing of the analyte peak and tailing may have a significant detrimental effect when the chromatogram is being used for quantitative purposes [273]. In many cases, this problem of free silanol groups may be dealt with by including a masking or suppressing agent in the mobile phase [262, 267]; a common masking agent is triethylamine which is able to interact with the silanol groups and effectively block them from interacting with the analyte. This will correct the problem of peak tailing if the cause is interaction with free silanol groups.

In their review of retention mechanisms in reversed-phase HPLC, Gritti and Guiochon [283] discussed separations of ionizable analytes and the role of buffers to fix the pH and ionic strength of the mobile phase. They pointed out that because the solubility of the ionic species is higher in aqueous mobile phases than that of the corresponding neutral species, it is less strongly retained and pH can be used to control the retention of compounds with acidic and basic forms. For these compounds, when the retention factor is plotted as a function of pH, the retention factors vary sharply around the pKa (± 2 pH units) and outside this pH domain, the retention of ionizable compounds is nearly independent of pH and depends only on the nature of the buffer used when the ionic form produces an ion-pair with one of the buffer ions. Lewis et al [289, 290] demonstrated that computer simulations based on a theoretical model of

ionization was able to predict accurately both retention and resolution for acidic and basic solutes as a function of pH. Their findings showed that ionized analytes will interact more strongly with the mobile phase than non-ionized species so selecting a mobile phase pH where the analyte is essentially unionized will increase retention time. Vervoort et al [93] pointed out that when using buffers in the mobile phase, pH will change after addition of the organic modifier since the pKa values of the acids used to prepare the buffer changes with solvent composition. In their work, these authors presented an algorithm to allow calculation of the pH of an aqueous-organic modifier mobile phase. In another report Vervoort et al [268] discussed buffer selection in terms of the components used and their solubility in mixtures of aqueous and organic solvents and the use of volatile components to provide compatibility with mass spectrometer detection systems.

At present, computer simulations have become a valuable tool for development and optimization of HPLC methods and although providing only estimates they significantly reduce the time required for these activities [289-292].

Detector selection

Once separation of the analyte has taken place on the HPLC column, the eluted material passes through a detector which is able to respond to the quantity of material passing through and by this provide a means of quantitation [253]. In order to provide an effective means of quantitation, the detector must have adequate sensitivity, show a linear relationship between concentration and response, and have suitable accuracy and precision [293]. At present there is no universal detector which is capable of providing a consistent and quantitative response for any given analyte although the use of refractive index does approach this attribute of universality since refractive index is a physicochemical property of all chemical compounds. Refractive index has been used to measure propylene carbonate [294] and acetylcholine [295] in pharmaceuticals as these materials show little UV absorbance

above 200 nm. Limitations of sensitivity and issues of specificity with mobile phase components however, limit the practical use of this type of detector [296]. Generally some specific physicochemical property of the analyte will dictate which type of detector of modality of detection is most appropriate for a given analyte.

Probably the most widely used detector is the UV detector since most compounds show some level of absorbance and although UV absorption generally shows a high level of sensitivity, there are some significant limitations to its use as a detection method [297-300]. The composition of the mobile phase must be taken into account as components of the mobile phase may absorb UV radiation and some analytes exhibit very weak or no response to UV detection [299, 301]. UV detectors are available with a variety of detection diodes; the original detectors had a fixed wavelength and used a photomultiplier cell to measure transmitted light; the detection wavelength was usually 240 nm which was an effort to provide a compromise between selectivity and sensitivity. The next type of detector had a variable wavelength and the operator is able to select detection at wavelengths from 200 to 800 nm. These detectors also have the ability to perform a UV scan over the entire range of wavelength when the peak is caught in the detection cell by stopping the pump and this attribute may be used to help in identification of the analyte and has some, but limited value in ascertaining peak homogeneity [302]. The most significant advancement in UV detectors was the development of the diode array detector which is able to allow simultaneous collection of chromatographic data at different wavelengths during a single analysis [303]. This ability allows the UV spectrum from 200-800 nm to be obtained for each single peak which may be an aide in peak identification and again may be used as partial evidence of peak homogeneity. Peak purity or homogeneity may also be evaluated in cases where the UV spectra of co-eluting peaks differ significantly. The ratio of absorbances collected at two different wavelengths will be a constant for a given compound. The use and value of diode array detectors for determination

of peak homogeneity or purity was illustrated by Chan and Carr [304] using a series of benzodiazepine drugs.

Since the response of a UV detector to a peak depends on the extinction coefficient (ε) of the compound, sensitivity for compounds with low values of ε and those with higher values may give absorbance values several magnitudes of order different from each other even though the analytes may be present in the same sample and be of the same order of molar concentration. This lack of relationship of detector response or peak magnitude to relative concentration of each analyte prevents the use of UV detection as being viewed as a potential universal means of detection [302, 305].

With a fluorescence detector, detection is based on the native fluorescence of the analyte or a fluorescent derivative of the analyte. General strategies, methods and selection of appropriate fluorescent derivatives were reviewed by Krull et al [306]. Generally, sensitivity of a fluorescence detector is about three magnitudes of order greater than a UV detector; this increased sensitivity was shown by Huck and Bonn [300] who compared the sensitivities of UV, fluorescence and mass spectroscopic detectors to measure flavonoids and VanderHoorn et al [307] who compared fluorescence detection to electrochemical detection in the measurement of catecholamines. Enhanced selectivity using fluorescence compared to UV detection was discussed by Kutlan et al [308] in work involving the analysis of amines. A description of how a fluorescence detector was presented by Marzo [309]; light from a source lamp passes through a filter to provide monochromatic light of a wavelength suitable to provide excitation of the analyte molecules as they pass through the flow cell. The wavelength of the excitation or emission light may be controlled by either a filter (fixed wavelength) or a grating (variable wavelength). The excited molecules emit light (fluoresce) of a higher wavelength and this light passes through a filter positioned perpendicular to the incident or

excitation light path. The emitted light passes through the second filter and is measured by a photomultiplier cell or diode.

Marzo [309] also provided an overview of the instrumentation used in electrochemical detection; the electrochemical (EC) detector is usually based on the principle of direct-current amperometry (DCA) and these detectors have sensitivities comparable to fluorescence detectors. The mode of detection is based on current being generated at a working electrode. The working electrode is held at a fixed potential and as analyte passes the fixed electrode, it is oxidized increasing the background current. The detector may be tuned to a specific analyte by adjusting the fixed potential of the working electrode. Most working electrodes are made of glassy carbon while the reference electrodes are composed of Ag/AgCl and the auxiliary electrode is usually stainless steel. Two limitations or disadvantages of EC detectors are that they require a conductive medium to function so the mobile phase must include an aqueous component thus generally precluding normal-phase separation and the surface of the electrodes become contaminated with use and require frequent regeneration [307]. The use of EC detection in pharmaceutical analyses was investigated by Musch et al [297] comparing UV and EC detection for 72 different drugs and in their conclusion, they suggested that the increased sensitivity of ECD and its broad application range made this a valuable detection method for HPLC applications.

A number of sophisticated detection systems have been developed primarily to aid in characterizing compounds in chromatographic eluents. These detectors include Raman wavelength [310] and a number of 'hyphenated' systems. These hyphenated systems include nuclear magnetic resonance (NMR), Fourier-transform infra-red (FTIR) and mass spectroscopic (MS) and the mechanics and application of these detectors have been reviewed by Wilson [311]. A discussion of the use of LC-MS combined with diode-array detection for establishing peak purity has been presented by Lincoln et al [312].

Before the development of these hyphenated systems, Ostojic [313] proposed the use of multiple detectors in HPLC. Although his goal was primarily to provide a method of peak overlap deconvolution [314], his idea also was used by Castledine et al [315], Gergely et al [298] and Carter et al [302] to provide a means of assuring peak purity. Meras et al [316] used this technique with UV and fluorescence detectors in series as a means of identifying the components in a mixture of quinolonic and cinolonic antibiotics. At present, this approach of using multiple detection systems still provides an alternative to the more sophisticated and expensive hyphenated methods for establishing peak purity.

Quantitation

The major advantage to HPLC over many other modes of analysis is the ability to separate and quantitate analytes from complex matrices with efficiency and minimal sample preparation. Quantitation is achieved by measuring the detector response to the analyte and comparing this value to a standard preparation of the analyte where the concentration is known and detector response is determined by measuring peak height or peak area through the use of integrators or computer-managed data-handling systems [317]. The response value obtained from the detector must be related to a concentration value and so a process of calibration is necessary. Errors due to peak sensing and random noise were discussed by Chleser and Cram [318] and errors associated with asymmetric curves have been investigated by Grubner [319]. The most common methods of calibration are the use of external standards, internal standard, or method of standard addition. The USP methods generally use external standards where the analyte is compared to USP reference standard material. The method of external standards uses a series of standard solutions with known concentrations prepared to be within the range of the assay. Each solution is analyzed and the responses obtained are plotted against the concentration values; solutions of unknown concentration can then be run and the response obtained may be compared to the values of the standard solutions and a

concentration value is obtained. The concentration versus response plot for the standard solutions should be linear with an intercept approaching zero and in this situation a single standard may be used in future assays provided the concentrations of the test and standard are similar to each other; this process is commonly seen in official USP assays. The error associated with the method of external standards was discussed by Schepers et al [320] in a study of HPLC precision over wide concentration ranges. An internal standard is a compound different from the analyte, its breakdown products and compounds which may be present as contaminants in the analyte. The internal standard should also have a number of attributes; it should be well resolved from the analyte or any peak associated with the analyte; it should have a detector response similar to the analyte; it should behave in a similar way to the analyte in any sample clean-up procedures; and it should be stable and available in a pure form. The method of using internal standards involves preparing a standard curve or calibration plot with varying concentrations of analyte but including a fixed concentration of internal standard. The ratio of response to the analyte and response to the internal standard is plotted against the concentration of analyte. This plot may then be used to determine the concentration of analyte in a sample run with a known concentration of internal standard. This method is useful to compensate for changes in injection volume due to instrumental variations and for losses during sample clean-up procedures. It is most useful when the internal standard is added to the sample prior to clean-up as it then can compensate for losses and variability in analyte recovery. The errors associated with HPLC analysis after sample clean-up procedures were discussed by Snyder et al [321] and a method for analysis of an antihypertensive drug in serum using solid-phase extraction (SPE) and quantitation using an internal standard was described and discussed by Ferreiros et al [322]. The method of standard addition is useful when the analyte is already inherently present in the sample. This situation is most often seen when the sample matrix is biological as for example serum, and it is desirable to prepare the

standards in a serum matrix. If the analyte is a substance inherently present in blood, there will be a variable amount of the substance in the blank serum used to prepare the standards. In this type of situation, a plot of response to concentration should be linear over the range of the assay but the calibration plot will not pass through the origin. The y-intercept of the line will represent the amount of analyte which was inherently present in the sample. The method of standard addition as it applies to the analysis of pharmaceutical products where unknown excipients or degradation products are present was investigated by Youssef [323].

Method development plan

A number of published HPLC methods for apomorphine determination have already reviewed in this project and these should be assessed as to whether any of these would either provide a suitable method as published or provide a basis for a modified method which would meet the project requirements. If none of these methods prove satisfactory and it is necessary to devise a totally new method, a structured plan must be devised for method development. The initial step in the plan would again involve reviewing the physicochemical properties of the analyte. Information regarding dissociation constant (pKa) and partition coefficients is useful if a sample clean-up step is anticipated or required and liquid/liquid extraction techniques are to be used; this and data on the chromatographic properties of the analyte may be useful if solid-phase extraction (SPE) will be used for sample clean-up. This information will also be useful for column and mobile phase selection. Information regarding UV absorbance, fluorescence, refractive index, and redoxy potentials will be useful to determine possible means of detection and ultimately selection of the detector. Solubility data may be useful in column selection and design of mobile phase composition.

Although there will necessarily be considerable iterative trial and error involved in the process of method development, this may be minimized by using a systematic approach. Methods of development using computer controlled equipment, equipment specifically

designed for method development and/or computer simulation procedures may be very useful [250, 291, 324, 325].

Experimental 3.1 – Apomorphine analysis - HPLC

In order to provide quality assurance of the product and study the release of drug from the developed ocular insert, a pharmaceutically suitable method of analysis was required. The general attributes of the method should include suitable accuracy, precision and linearity with an appropriate range; the method should be stability-indicating and finally the method should be robust and as simple as possible. A review of analytical methods for apomorphine has already been presented and the method most likely to meet the above criteria would be high pressure liquid chromatography (HPLC). Table 2.2 (previously presented) summarizes the HPLC methods reviewed and from this table it is apparent that a variety of column packings, mobile phases and detection methods have been used in the HPLC analysis of apomorphine. All of the methods with the exception of that presented by Priston and Sewell [102] were developed and validated for analysis of apomorphine in biological fluids rather than pharmaceutical products. In spite of this, these methods could serve as a starting point for the development of a method suitable for pharmaceutical analyses. To this end, a series of experiments were conducted as a method development process. The initial experiments were to screen the published methods for suitability in a pharmaceutical application and the subsequent experiments were to validate the developed method and finally to challenge the method by assessing it's performance as an assay method, a method for conducting content uniformity testing and a method suitable for studying the release of apomorphine from a polymeric matrix.

Equipment and materials:

The equipment and materials used for the method development and validation are as follows: The chromatographic system used was manufactured by Knauer (Berlin, Germany) and consisted of a Wellchrom K-501 pump, a Wellchrom K-2501 variable wavelength UV detector, a Basic-Marathon autosampler type 816 and a Wellchrom HPLC Interface box. A Waters (Milford, MA, USA) model 420 fluorescence detector and a Bioanalytical Systems Inc (Lafayette, IN, USA) model LC-4B electrochemical detector were used in tandem with the Knauer system via the HPLC Interface box. The operating, data acquisition and data analysis software used was Eurochrome 2000® (Knauer, Germany). The HPLC columns used were Cyano 4.6 x 150 mm particle size 4 µm (Jones, Hengoed, UK), C_{18} 4.6 x 150 mm particle size 5 µm (Supelco Canada, Mississauga, ON), C_8 4.6 x 150 mm particle size 4 µm (Jones) and Phenyl 4.6 x 150 mm particle size 4 µm (Jones). All chromatography was performed at ambient temperature.

Apomorphine HCl was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade phosphoric acid and acetonitrile as well as analytical grade hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific (Nepean, ON, Canada). Gelatin USP, glycerol USP and ascorbic acid USP were purchased from Spectrum Chemical (Gardenia, CA, USA). Hydrogen peroxide USP (3%) was purchased from Pure Standard Products (Edmonton, AB, Canada) De-ionized water was obtained using an Aqua-Summa II reverse-osmosis system (Culligan, Toronto, ON, Canada). All glassware used was Class A (Fisher Scientific, Nepean, ON, Canada) and where required, dilutions were done using a 1000 µL adjustable volume pipette (Hamilton Company, Reno, NV, USA). A model AE-50 analytical balance (Mettler, Hightstown, NJ, USA) was used for weighing. Filtration of mobile phase was accomplished using 0.2 µm membranes (Pall, Gelman Laboratories, East Hills, NY, USA).

Standard solutions of apomorphine HCl in 0.05 M HCl were prepared, stored in glass at 5° C and discarded after seven days. Reference standard apomorphine was obtained from USP Convention, Rockville, MD, USA). Statistical analysis and graphical presentations were done using SigmaStat ® (SPSS Inc Chicago, IL, USA), Excel 2000® (Microsoft) and SigmaPlot 2000® (SPSS Inc Chicago, IL, USA).

All statistical analysis was done at the 95% confidence level unless otherwise indicated and data are presented as mean ± standard deviation unless otherwise indicated.

Experiment 3.1.1 - Column Selection:

Introduction:

One or more of the stationary phases used in these methods will provide an acceptable level of separation and resolution using a simple mobile phase. This hypothesis will be tested by preparing a simple mobile phase and analyzing a standard solution of apomorphine using each of the different columns in turn. The quality of the separation will be evaluated by measurement of retention time, peak area, resolution, tailing factor, capacity and number of plates.

Purpose:

The purpose of this experiment was to select a column for the assay. This was to be done using a screening process to assess the separation efficacy of the different stationary phases used in the reviewed HPLC methods using a simple mixture of acetonitrile and phosphoric acid run under isocratic conditions.

Study design and method:

A mobile phase consisting of acetonitrile:phosphoric acid 0.03M (500:500) was prepared, filtered through a 0.2 μm nylon membrane and degassed under vacuum. A standard solution containing apomorphine 50 $\mu g \cdot mL^{-1}$ and 5-fluorouracil 40 $\mu g \cdot mL^{-1}$ was prepared and

20 µL of this solution was injected onto the column being evaluated. A preliminary screening showed that 5-fluorouracil was poorly retained on all of the stationary phases being assessed so it was used as a marker to calculate resolution. Six replicate injections were done using the C_{18} column initially then repeated using the phenyl column, the cyano column and finally using the C_8 column in turn. The flow rate was 1.0 $mL \cdot min^{-1}$ and detection was accomplished using the variable wavelength UV detector at 272 nm. Calculations for chromatographic parameters were done using USP methods as previously described. The recommended values for these parameters are: capacity should be 1.5 or larger; resolution should be 2.0 or larger; tailing factor should be 2.0 or smaller and the theoretical number of plates should be 2000 or larger [293].

Results and discussion:

The data generated from this experiment are presented in Table 3.1. Since under the conditions of the experiment, apomorphine was not retained on the phenyl column, these data are not presented.

Table 3.1 Column performance for apomorphine under isocratic conditions with acetonitrile: phosphoric acid 0.03M (500:500).

Parameter	C18	Cyano	C8
Retention (min)	4.32 ± 0.06	5.24 ± 0.07	6.95 ± 0.04
Peak area (mAU*min)	54.24 ± 0.38	55.05 ± 1.06	51.83 ± 0.38
Resolution (R)	3.23 ± 0.14	7.70 ± 0.14	8.88 ± 0.03
Tailing Factor (T)	2.25 ± 0.12	1.78 ± 0.09	1.98 ± 0.04
Capacity (k')	1.16 ± 0.03	1.62 ± 0.03	2.48 ± 0.02
Plates (n)	1583 ± 33	2068 ± 39	1165 ± 15
Values as Mean ± std deviation n = 6			

Since the trial using the Phenyl stationary phase showed no retention of apomorphine under the conditions of the experiment, none of the derived values could be calculated. The remaining stationary phases assessed all showed retention and reasonable potential to be suitable for pharmaceutical analysis.

In assessing the retention times for the different columns, analysis of variance (ANOVA) indicated that the times differed significantly ($p < 0.001$). The cyano column provided an intermediate value of about 5.2 minutes which was towards the centre of the desired window of 10 minutes but generally, all of the columns provided retention times which would be acceptable for the method. The peak areas provided by the sample also differed significantly for the three columns ($p < 0.001$) and the column providing the greatest response value for the sample was the cyano column. In terms of tailing factor, ANOVA suggested that the values differed significantly among the columns ($p < 0.001$) with the cyano column providing the lowest and therefore best, value. The theoretical number of plates varied significantly among the columns ($p < 0.001$) and only the cyano column provided a value in the recommended range. The capacity factor was significantly different ($p < 0.001$) among the columns and the C_8 was the only column to provide a value above the recommended threshold. For the final parameter of resolution, again the difference among the columns was significant ($p < 0.001$) and although all of the columns gave values in the recommended range, the value obtained for the C_{18} column was about half that of the other two columns.

On a comparative basis the C_{18} column demonstrated a tailing factor greater than 2 and the lowest value for resolution. Although these values could likely be improved by modification of the mobile phase, the present value for the tailing factor suggests there may be possible problems with quantitation variability. The relatively low resolution suggests there may be problems due to interference from apomorphine break-down products or excipients used in formulating the product and this interference could compromise the stability-indicating

aspect of the assay. The C_8 column demonstrated the longest retention time and the lowest number of theoretical plates. This longer retention time may result in an analysis time in excess of ten minutes and reduced efficiency when a large number of samples have to be processed. The cyano column gave acceptable values for all the parameters assessed except for capacity and on a comparative basis none of the values were the least acceptable for a given parameter.

Conclusions:

Although all of the stationary phases except for the phenyl gave acceptable values, the cyano column performed best in that it never showed the poorest value for any one of the parameters tested. On this basis, the cyano column was selected for further evaluation.

Experiment 3.1.2 – Mobile phase optimization

Introduction:

Variation in the concentration of organic modifier in the mobile phase will affect the nature of the chromatographic separation. Examination of the chromatograms obtained from mobile phases with increasing concentrations of organic modifier will allow empirical prediction of the effect of changing the ratio of aqueous phase to organic modifier and allow estimation of the ratio giving an optimal separation. The quality of the separation will be evaluated by measurement of retention time, peak area, tailing factor, capacity and number of plates.

Purpose:

The purpose of this experiment was to assess the effect of increasing concentrations of organic modifier in the mobile phase and determine the optimum concentration for the assay. This experiment will also provide an index of the ruggedness of the assay in terms of minor variation in the composition of the mobile phase.

Study design and method:

Mobile phases with varying concentration of organic modifier were prepared and used to assay a standard preparation of apomorphine dissolved in 0.05M HCl. The composition of the mobile phases were acetonitrile 40%, 45%, 50%, 55% and 60% with the balance being made up with phosphoric acid 0.03M. A cyano column was used with a flow rate of 1.0 $mL \cdot min^{-1}$ and detection was by UV at 272 nm. The concentration of the standard solution was 40.9 $\mu g \cdot mL^{-1}$ and the volume injected was 20 µL. For each mobile phase six replicates of the standard were injected. The area and retention time for the apomorphine peak of each assay were recorded and the derived values were calculated using the USP methods as previously described.

Results and discussion:

The data generated from this experiment are presented in Table 3.2.

Table 3.2 Mobile phase optimization

Acetonitrile (%)	RT (min)	Peak Area	Tailing factor	Plates	Capacity
40	4.68 ± 0.03	37.33 ± 1.11	1.68 ± 0.09	3208 ± 150	1.34 ± 0.02
45	4.98 ± 0.06	36.79 ± 0.92	1.72 ± 0.16	3123 ± 163	1.49 ± 0.03
50	5.27 ± 0.06	37.17 ± 0.85	1.56 ± 0.11	3937 ± 179	1.64 ± 0.03
55	5.49 ± 0.03	37.52 ± 0.23	1.73 ± 0.08	2339 ± 67	1.75 ± 0.02
60	5.52 ± 0.03	35.04 ± 0.32	1.88 ± 0.07	2342 ± 17	1.76 ± 0.01
Values as Mean ± Std deviation n = 6					

Varying the amount of organic modifier in the mobile phase between 40 – 60% primarily effected a change in the retention time of the apomorphine peak. Assessing the data through ANOVA showed that the differences in retention time among the variations were significantly different ($p < 0.001$) although the difference between the values obtained with 55 and 60% acetonitrile were not ($p = 0.272$). Generally, the retention time increased with increasing concentration of acetonitrile but over the range of concentration change examined,

the relationship between concentration and retention time was not linear and as the concentration was increased, the magnitude of effect on retention time decreased. Over the range studied, the retention time remained towards the centre of the desired 10 minute time window so in terms of retention time, concentrations of organic modifier from 40 to 60% had no great effect on the overall performance of the analysis. Over the concentration range examined, the peak areas obtained differed significantly ($p < 0.001$) but assessing the values for the concentration range between 45 and 55 no significant difference was seen using ANOVA ($p = 0.264$). In terms of tailing factor, ANOVA suggested a significant difference in the values for all the concentrations studied ($p < 0.001$) and examination of a plot of tailing factor as a function of acetonitrile concentration suggested that the tailing factor was minimal around the 50% concentration. The number of theoretical plates showed a similar pattern to tailing factor with the values being obtained over the concentration range being different ($p < 0.001$) but a plot suggesting that the value showed a maximum around 50%. Generally, capacity factor increased with increasing concentration of acetonitrile and as expected, was not linear with the magnitude of effect decreasing with increasing concentration. Also as expected, ANOVA showed a significant difference among the values obtained for the different concentration ($p < 0.001$). At acetonitrile concentrations below about 45%, the value for capacity factor was below the recommended value of 1.5 suggesting that at these concentrations of acetonitrile the method may have difficulties with quantitation if any degradation products or excipients have retention times near the apomorphine peak.

Conclusions:

Assessing the effects of variations in the composition of the mobile phase suggested that the overall best performance was achieved with a mixture of acetonitrile: phosphoric acid 0.03M (500:500). Since ANOVA suggested that there were no significant difference in the performance parameters for acetonitrile concentrations between 45 and 55%, the method was

quite robust and able to tolerate variation of several percent in terms of mobile phase composition.

Experiment 3.1.3 - Detector selection

Introduction:

A variety of different detection systems including ultraviolet, fluorescence and electrochemical detection have been used in the analysis of apomorphine. Each of these detectors have different levels of advantage in terms of and sensitivity and the hypothesis for this experiment is that one of them will have a range of acceptable sensitivity more suited to the needs of this project than the other detectors.

Purpose:

The HPLC methods for measurement of apomorphine which had been previously reviewed used three different types of detectors. Since these methods were developed for measuring apomorphine in biological fluids, a primary attribute of the detector used would have been the ability to measure small amounts of apomorphine quantitatively. For a pharmaceutical application however, this attribute would be of much less importance and for the purpose of this project the ability of the detection system to measure apomorphine over a fairly wide range of concentration would be a much more important attribute. The purpose of this experiment was to examine the concentration ranges of apomorphine which can be measured using ultraviolet (UV), fluorescence (Flu) and electrochemical (ECD) detection. A mobile phase consisting of acetonitrile: phosphoric acid 0.03M (500:500) was used at a flow rate of 1.0 $mL \cdot min^{-1}$ and separation was done using a cyano column. The selection of these conditions were based on the results of the previous method development experiments,

Study design and method:

The HPLC method as it had developed to this point was used and the linearity, range, accuracy, precision and limit of quantitation of the method using three different detection

systems were determined. The mobile phase consisted of acetonitrile: phosphoric acid 0.03M (500:500) and a cyano column was used. Detection was accomplished using the variable wavelength ultraviolet detector, the fluorescence detector and the electrochemical detector connected in series or tandem by means of short lengths of narrow bore tubing. The specifics for each of the instruments used for the experiment are detailed in the equipment and materials section previously presented. UV detection was at 272 nm and fluorescence detection was done with an excitation wavelength of 338 nm and an emission wavelength of 425 nm. Electrochemical detection was done using a glassy carbon working electrode with an Ag/AgCl reference electrode and a preliminary experiment was performed to determine the optimal applied voltage for the detection of apomorphine. This was accomplished by preparing a standard solution of apomorphine at a concentration of about 15 $\mu g \cdot mL^{-1}$ and injecting this into the chromatograph under the conditions of the experiment already described. A series of analyses were conducted with the applied voltage being set to progressively higher values. The response in terms of peak area was also determined using UV detection at 272 nm to serve as a control. Each trial was repeated in triplicate and the response in terms of peak area was compared at each voltage level; three trials were conducted. Once the optimum applied voltage for the electrochemical detector was determined, this voltage was used. In order to determine the linearity of concentration to response using each of the detectors a series of standard solutions were prepared and analyzed with response being quantified by peak area. The standard solutions were prepared to cover concentration ranges of approximately 1 to 10 $\mu g \cdot mL^{-1}$, 5 to 50 $\mu g \cdot mL^{-1}$ and 2.5 to 125 $\mu g \cdot mL^{-1}$. Four separate trials were conducted and two replicate injections of each sample were injected; each concentration range consisted of 10 samples.

The accuracy and precision of each detection system was evaluated by preparing standard solutions covering a range of approximately 125 to 5 $\mu g \cdot mL^{-1}$ and performing twelve

replicate injections of each solution using each of the detectors. For this experiment, an arbitrary acceptance level of 2% was used for accuracy and a RSD value of 4% for precision. A discussion of these selected values and literature recommendations will be presented in the method validation section.

A final experiment was done to determine whether the detector response from UV and fluorescence detection was degraded as the sample passed from one detector to the next while they were connected in series. Degradation of the response could occur from diffusion or spreading of the sample due to turbulence along the tubing or in the detector or from residual effects of the sample having been exposed to UV energy. In order to assess whether response degradation was a factor, a standard solution of apomorphine at a concentration of about 30 $\mu g \cdot mL^{-1}$ was prepared and injected into the chromatograph with the detectors connected in series (UV, fluorescence, electrochemical) then the sample was injected into the chromatograph with each detector in turn attached separately. The peak area, tailing factor and number of apparent plates were compared for each detector when run in series or alone. Four trials were made for each configuration and tailing factors and apparent number of plates were determined using the USP calculation methods.

Results and discussion:

Prior to conducting these experiments, the operating parameters of the electrochemical detector needed to be established for apomorphine measurement. In order to determine the optimum applied voltage for detection of apomorphine with the electrochemical detector a solution containing about 15 $\mu g \cdot mL^{-1}$ was injected into the system and peak areas found for a range of applied voltages from 0.6 to 1.2 v. The results of this trial are presented in Table 3.3 and UV detection was used as a control.

Table 3.3 Electrochemical detector: Applied voltage versus response

Applied voltage (v)	UV Peak Area (mAU*min)	ECD Peak Area (mV*min)
0.6	15.60 ± 0.26	319.3 ± 2.5
0.7	15.53 ± 0.04	890.8 ± 41.0
0.8	15.52 ± 0.04	1122 ± 64
0.9	15.59 ± 0.06	1226 ± 86
1.0	15.56 ± 0.04	1384 ± 4
1.1	15.66 ± 0.04	1175 ± 91
1.2	15.74 ± 0.13	1254 ± 111
Values as Mean ± Std deviation		n = 3

From these data, it was determined that an applied voltage of 1.0 v would provide a maximal peak response so this setting was used for all the analyses using electrochemical detection.

UV Detection:

Standard solutions of apomorphine were analyzed using UV detection with solution concentration ranges of 1 to 10 $\mu g \cdot mL^{-1}$, 5 to 50 $\mu g \cdot mL^{-1}$ and 12.5 to 125 $\mu g \cdot mL^{-1}$; four trials of each concentration range were run, each range series consisted of 10 dilutions and each dilution was run in duplicate. Values smaller than about 4 $\mu g \cdot mL^{-1}$ gave no response under the conditions of the experiment and a graphical presentation of all the data points where a response was seen are shown in Figure 3.1 below. These data indicated that response as a function of concentration was linear over a range of about 5 to 100 $\mu g \cdot mL^{-1}$ and the results of regression analysis over this range are presented in Table 3.4.

Figure 3.1 Plot of peak area versus concentration using UV detection. (n = 224)

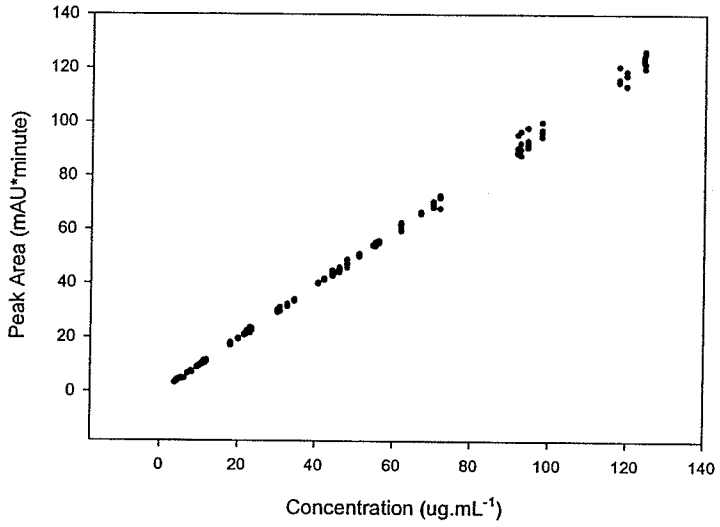


Table 3.4 Regression analysis of apomorphine concentration as a function of response using UV detection: Range 5 to 100 $\mu g \cdot mL^{-1}$.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Observations (n)	36	32	40	40
Slope (m)	0.9847	0.9836	0.9813	0.9802
Intercept (b)	0.5452	0.2256	0.5217	0.3037
Coefficient of determination (r2)	0.9999	0.9999	0.9999	0.9998

Standard solutions over a range of 125 to 5 $\mu g \cdot mL^{-1}$ were prepared and twelve replicate injections were done to determine accuracy and precision. The data from this experiment are presented in Table 3.5.

Table 3.5 Accuracy and precision – UV detection. Peak area in mAu*min.

	Precision		Accuracy	
Conc (μg mL^{-1})	**Mean Peak Area**	**Peak Area RSD**	**% Theoretical**	**Std dev**
124.0	123.24	0.52	101.0	0.53
118.0	117.21	2.01	100.9	2.03
104.5	102.66	0.34	99.72	0.34
83.60	82.49	0.62	100.1	0.63
73.15	72.11	0.60	99.87	0.60
62.70	61.95	0.24	100.0	0.24
52.25	51.98	0.56	100.6	0.57
41.80	41.73	0.39	100.7	0.40
31.35	31.53	0.63	101.1	0.65
20.90	20.93	0.96	99.95	0.98
10.45	10.48	1.95	97.96	2.00
5.225	5.22	2.78	93.81	2.82
n = 12				

Overall, the data suggest that the method using UV detection shows satisfactory linearity over a range from 5 to 100 $\mu g \cdot mL^{-1}$. The data for accuracy and precision are presented in Table 3.6 and suitable accuracy was seen for the concentration range from 20 to 125 $\mu g \cdot mL^{-1}$ where the measured values deviated from theoretical by under 2%; suitable precision was shown over the concentration range of 5 to 125 $\mu g \cdot mL^{-1}$ where the relative standard deviation of the peak areas was smaller than 4%.

Fluorescence detection:

Standard solutions of apomorphine were analyzed using fluorescence detection with solution concentration ranges of 1-10 $\mu g \cdot mL^{-1}$, 5-50 $\mu g \cdot mL^{-1}$ and 12.5-125 $\mu g \cdot mL^{-1}$; four trials of each concentration range were run, each range series consisted of 10 dilutions and each dilution was run in duplicate. A graphical presentation of all the data points is shown in Figure 3.2 below. These data indicated that response as a function of concentration was linear over a range of about 5 to 70 $\mu g \cdot mL^{-1}$ and the results of regression analysis over this range are presented in Table 3.6.

Figure 3.2 Plot of peak area versus concentration using fluorescence detection. (n= 240)

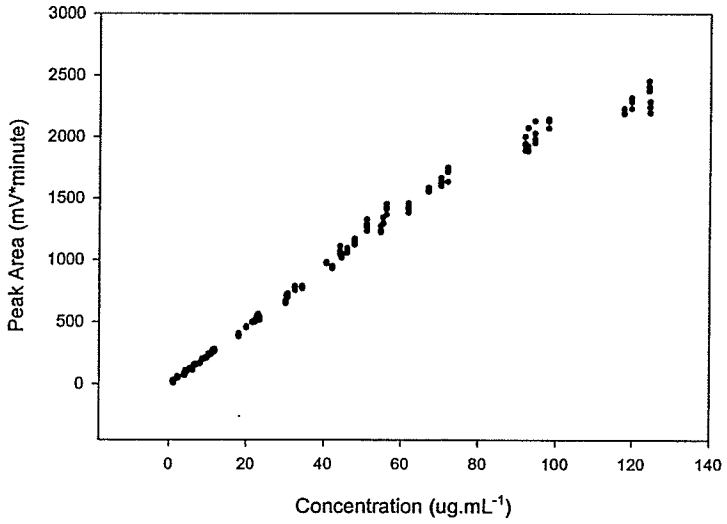


Table 3.6 Regression analysis of apomorphine concentration as a function of response using fluorescence detection: Range 1 to 60 $\mu g\ mL^{-1}$.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Observations (n)	24	28	28	24
Slope (m)	22.21	20.73	21.64	21.04
Intercept (b)	5.30	7.09	11.56	-0.31
r2	0.9999	0.9997	0.9998	0.9999

Standard solutions over a range of 125 to 5 $\mu g \cdot mL^{-1}$ were prepared and twelve replicate injections were done to determine accuracy and precision. The data from this experiment are presented in Table 3.7.

Table 3.7 Accuracy and precision – Fluorescence detection. Peak area in mV*min.

	Precision		Accuracy	
Conc ($\mu g\ mL^{-1}$)	**Mean Peak Area**	**Peak Area RSD**	**% Theoretical**	**Std dev**
124.0	2333	4	89.11	3.71
118.0	2233	2	89.58	1.58
104.5	1940	1	87.77	0.78
83.60	1721	1	97.21	0.75
73.15	1558	2	100.4	1.72
62.70	1375	5	103.3	4.71
52.25	1123	3	100.9	3.24
41.80	903.7	3.2	101.1	3.33
31.35	681.2	3.1	100.9	3.25
20.90	453.8	3.8	99.43	3.88
10.45	226.7	4.9	95.13	5.04
5.23	114.3	6.1	87.55	6.34
			n = 12	

Overall, the data suggest that the method using fluorescence detection shows satisfactory linearity over a range from 1 to 70 $\mu g \cdot mL^{-1}$. The data for accuracy and precision are presented in Table 3.7 and suitable accuracy was seen for the concentration range from 20 to 70 $\mu g \cdot mL^{-1}$ where the measured values deviated from theoretical by about 2%. Acceptable precision was shown over the concentration range of 20 to 125 $\mu g \cdot mL^{-1}$ where the relative standard deviation

of the peak areas was smaller than 4% although a value in excess of 4% was seen at the concentration level around 60 $\mu g \cdot mL^{-1}$.

Electrochemical detection:

Standard solutions of apomorphine were analyzed using electrochemical detection with solution concentration ranges of 1-10 $\mu g \cdot mL^{-1}$, 5-50 $\mu g \cdot mL^{-1}$ and 12.5-125 $\mu g \cdot mL^{-1}$; four trials of each concentration range were run, each range series consisted of 10 dilutions and each dilution was run in duplicate. A graphical presentation of all the data points is shown in Figure 3.3 below. These data suggested that response as a function of concentration was linear over a range of about 1 to 50 $\mu g \cdot mL^{-1}$ and the results of regression analysis over this range are presented in Table 3.8.

Figure 3.3 Plot of peak area versus concentration using electrochemical detection. (n = 200)

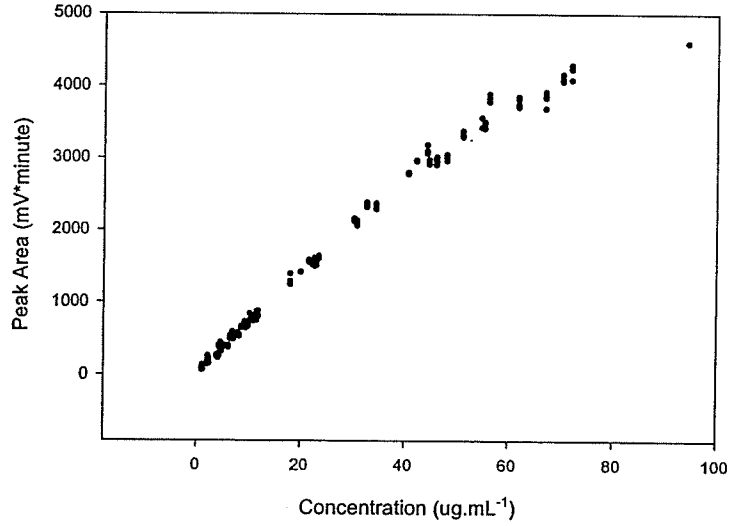


Table 3.8 Regression analysis of apomorphine concentration as a function of response using electrochemical detection: Range 1 to 50 $\mu g\ mL^{-1}$.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Observations (n)	20	24	20	24
Slope (m)	75.63	74.20	72.58	68.31
Intercept (b)	51.85	58.97	76.92	80.11
r2	0.9994	0.9989	0.9995	0.9980

Table 3.9 Accuracy and precision – electrochemical detector: peak area in mV*min. Concentrations of 80 $\mu g \cdot mL^{-1}$ and higher exceeded the capacity of the detector to show a response.

	Precision		Accuracy	
Conc ($\mu g\ mL^{-1}$)	Mean Peak Area	Peak Area RSD	% Theoretical	Std dev
83.60	-	-	-	-
73.15	4088	15	77.32	11.72
62.70	4297	2	94.96	2.05
52.25	3683	4	97.28	3.96
41.80	3106	4	100.0	2.95
31.35	2392	3	99.10	1.85
20.90	1614	3	101.8	2.97
10.45	814.0	3.3	98.36	3.67
5.23	419.6	5.7	92.85	6.45
n = 12				

The data for accuracy and precision for electrochemical detection are presented in Table 3.9 and suitable accuracy was seen for the concentration range from 20 to 40 $\mu g \cdot mL^{-1}$ where the measured values deviated from theoretical by less than 2%; adequate precision was shown over the concentration range of 10 to 60 $\mu g \cdot mL^{-1}$ where the relative standard deviation of the peak areas was less than 4%.

Limit of quantitation:

The limits of quantitation for the three detection systems was calculated from regression analysis of the data presented in Tables 3.6, 3.8 and 3.10 using equation 3.6:

$$LOQ = 10(SD/S) \qquad \text{Equation 3.6}$$

where S is the slope of the regression line and SD is the standard deviation of the y-intercepts of the regression lines or the residual standard deviation of the regression lines [293]. The results of these calculations are presented in Table 3.10 and the limits of quantitation found were 4.87, 3.85 and 2.00 $\mu g \cdot mL^{-1}$ for UV, fluorescence and electrochemical detection respectively.

Table 3.10 Limit of quantitation for detection methods; LOQ from equation 3.6.

	Detector		
Parameter	**UV**	**Fluorescence**	**Electrochemical**
Slope (m)	0.9820 ± 0.0018	21.56 ± 0.86	73.29 ± 2.16
Intercept (b)	0.1348 ± 0.4779	3.314 ± 8.298	57.46 ± 14.66
r^2	1.000	0.9999	0.9990
Range ($\mu g \cdot mL^{-1}$)	5 - 80	5 – 60	5 – 40
LOQ ($\mu g \cdot mL^{-1}$)	4.87 ± 0.01	3.85 ± 0.15	2.00 ± 0.06
Values as Mean ± Std deviation n = 12			

Simultaneous UV, fluorescence and electrochemical detection:

In order to determine whether the detectors could be connected and used in tandem for sample analysis, apomorphine standards were run using the detectors individually and then connected in series. The order in which the detectors were run was UV, fluorescence then electrochemical. Representative chromatograms of a sample are presented in Figure 3.4; the difference in retention times is due to the sample having to pass from one detector to the next. The results of this test are presented in Table 3.11 and statistical analysis of the data (t-test) indicated that there were no significant differences in the peak areas found when a given detector was used alone or in tandem; UV detector p = 0.339, fluorescence detector p = 0.781 and electrochemical detector p = 0.290.

Figure 3.4 Representative chromatograms using the different detectors: A = UV, B = Flu, C = ECD.

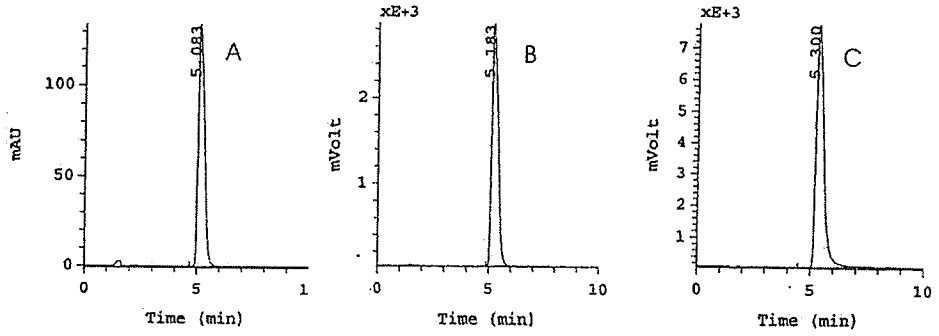


Table 3.11 Comparison of peak response with detectors used singly and in tandem.

	Ultraviolet		Fluorescence		Electrochemical	
	Tandem	**Alone**	**Tandem**	**Alone**	**Tandem**	**Alone**
Peak area	31.62 ± 0.21	31.51 ± 0.08	721.8 ± 33.8	716.8 ± 2.7	2438 ± 68	2386 ± 11
T Factor	1.90 ± 0.09	1.88 ± 0.07	1.70 ± 0.02	1.78 ± 0.07	1.87 ± 0.03	2.02 ± 0.05
Plates	1415 ± 19	1426 ± 20	1429 ± 19	1429 ± 8	1174 ± 14	1188 ± 9
Peak area	p = 0.339		p = 0.781		p = 0.290	
Values as Mean ± Std dev			n = 4			

Conclusions:

UV, fluorescence and electrochemical detectors were evaluated for the assay system and under the conditions of the experiment, UV detection provided the largest range with acceptable linearity, accuracy and precision. This range was from 5 to 125 $\mu g \cdot mL^{-1}$ for UV detection while that for fluorescence detection was 20 to 70 $\mu g \cdot mL^{-1}$ and for electrochemical detection 10 to 50 $\mu g \cdot mL^{-1}$. The detectors performed satisfactorily when connected in series with the electrochemical detector being the last in the series. Based on the range of detection, UV detection will be used for the assay system but the use of simultaneous fluorescence and electrochemical detection as a means of monitoring peak purity will be investigated for sample analysis in stability studies.

Although the C_{18}, C_8 and cyano columns all performed satisfactorily, the cyano column was marginally better in that it comparatively never gave the poorest value for the parameters investigated: retention time, peak area, resolution, tailing factor, capacity and theoretical number of plates. The mobile phase in conjunction with the cyano column gave adequate separations at acetonitrile concentrations exceeding 45% and a concentration of around 50% seemed to provide the best resolution. In terms of detection, the UV detector provided the largest range with acceptable linearity, accuracy and precision. A comparison of the peak areas obtained with different detectors both connected and independent showed no significant difference; the sequencing of the detectors was UV, fluorescence and finally electrochemical. Using UV detection as the primary detection system but simultaneously running either or both fluorescence and electrochemical detection may offer a convenient means for monitoring peak purity [298, 326].

Based on the results of the experiments used for method development, the following HPLC method for apomorphine was proposed:

The mobile phase consisted of a suitably degassed and filtered mixture of acetonitrile and phosphoric acid 0.03M (500:500). The standard preparation consisted of an accurately weighed quantity of USP Apomorphine hydrochloride RS dissolved in 0.05M hydrochloric acid to obtain a solution having a known concentration between 20 and 80 $\mu g \cdot mL^{-1}$. The chromatographic system consisted of a liquid chromatograph equipped with a 272-nm detector and a 4.2-mm x 25-cm column that contains packing L10 (Cyano). The flow rate was about 1.0 mL per minute. For the assay procedure, equal volumes (about 20 μL) of suitably prepared standard preparation and assay sample were injected into the instrument the chromatograms were recorded and the responses for the major peaks were measured.

3.2 Method Validation:

Overview:

In the areas of pharmaceutics and biopharmaceutics a great deal of research in product development and evaluation is at some point based on the analysis of the drug material being studied. Once a method has been developed for a particular analysis, it is necessary to demonstrate that the method will be capable of providing reliable data for the application being considered. If, for example a stability study is being undertaken it is important that the method is capable of distinguishing the active drug from breakdown products and the excipient which may be present in the dose form [250]; if a bioavailability study is being undertaken the method must be capable of quantitatively measuring the active drug at the expected concentration range and distinguishing it from the biological material it is in and from metabolites which may be formed [327]. From this, it is apparent that any method must be evaluated and challenged to ensure it is capable of generating reliable data for the study being undertaken and this process is referred to as method validation.

Although the specific requirements of the method will vary with the intended application, a number of general requirements must be met regardless of the method being considered. For pharmaceutical and biopharmaceutical applications the Unites States Pharmacopeia (USP) provides method validation criteria in a section of the General Chapters and is applicable to any methodology [79]. Other useful sources of validation criteria have been published [328-330] through various federal regulating bodies such as the Health Protection Branch (Canada) and the US Food and Drug Administration (FDA). Some journals, such as the Journal of Chromatography, have published validation requirements needed before a method will be published in their journal [331] and a method published by Gross et al [332] provides an excellent example which meets their criteria. Using the USP as a reference point, a number of primary validation criteria are detailed for the evaluation of method reliability and overall performance and these are selectivity, accuracy, precision, linearity, range and sensitivity and robustness.

Selectivity

Selectivity or specificity refers to the ability of the method to detect and quantify the analyte in the presence of excipients, degradation products and metabolites; for an HPLC method there should be no peaks in the chromatogram which will interfere with the analyte or internal standard if one is used. [79, 293] In the course of conducting a stability study the method must be able to distinguish the drug of interest from any breakdown products and excipients present in the product. If the mechanism of decomposition is known and the degradation products are available, they should be chromatographed using the method and the retention time window for the active drug examined to ensure that none of the degradation products have retention times which put them into this window [250]. A range on concentrations of reference standard for the drug should be chromatographed in the presence

of these degradation products and the peak areas obtained should be compared to the areas for the reference standards run alone to ensure that the compounds have no effect on quantitation of the standards. If authentic samples of degradation products are not available, a procedure of forced degradation could be used [333]. In forced degradation, the drug is exposed to severe conditions which will result in degradation and the product of this process is used as described in place of the authentic samples. Forced degradation is usually designed to mimic the decomposition of the drug as it would occur in the samples to be studied; if decomposition is by oxidation, heat and an alkaline pH in the presence of oxygen may be suitable; if decomposition is by hydrolysis application of heat and a buffer at an appropriate pH may be suitable. An example of experimental and protocol design for forced degradation is detailed in a validation study of an HPLC method by Bakshi et al [334] for the analysis of three alpha-adrenergic blocking agents.

Analysis of a drug in a biological sample presents a challenge in that the composition of the matrix will be variable. The usual biological medium is serum and the analyte should be examined in the presence of blank sera from at least six different sources [327, 335]. Since factors such as subject food intake, use of medications and smoking may affect selectivity, blank sera from a number of sources are examined and frequently pooled serum from a blood bank is included in the samples. Dadgar et al [335] have made a number of recommendations regarding drug metabolites; potential interference from metabolites must also be considered and if authentic samples of metabolites are not available, a small pilot study could be conducted and the serum samples from this used; blood is drawn from the subjects and the sera are examined for peaks which are not present in the pre-dose sample. The drug being studied will show increasing concentrations in the samples until t_{max} (time to maximum serum concentration) is reached and decline in subsequent samples. Metabolites will follow a similar pattern but on a different time-frame. If a pilot study is not feasible, Dadgar et al suggest it

may be possible to obtain similar samples by incubating the drug in the presence of liver homogenate and sampling over time.

Accuracy

Accuracy of a method is the closeness of the test result obtained by the method to the true value [293, 336]. Accuracy may be determined by replicate injections of reference standard or in some cases by comparison of the results obtained by the method to those of a second well characterized method for which the accuracy has been defined [79]. Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range; this usually represents three replicate injections of concentrations at the high, low and middle areas of the range [293, 331]. The generally accepted criteria for acceptance of accuracy is that the values should not exceed a relative standard deviation (RSD) of 15% for analysis of analyte in biological material and 3-5% for analysis in drug dose forms [79, 327].

Precision

Precision refers to the degree of agreement among individual test results when the method is applied repeatedly to multiple replicates of a homogeneous sample. Precision is usually expressed as the relative standard deviation of a series of measurements [107, 293] and precision of a method is often determined in conjunction with the assessment of accuracy. Precision testing is done by using a minimum of nine determinations covering the range of the assay and usually involves three replications of three concentrations although for some finished product assays a minimum of six replicates at 100% of the test concentration is acceptable [79, 337]. An on-going process of validation was recommended by Tamisier-Karolak et al [337] and they suggested that during the course of any study involving an analytical method, samples should be run regularly to determine precision in order to detect

transient problems with the method or more subtle problems such as deterioration of the column with age and use.

Linearity, Range and Sensitivity

Linearity refers to the ability of a method to generate test results or responses which are directly proportional to the concentration of the analyte within a given range; the range is the interval between the upper and lower levels of analyte which have been shown to have suitable levels of accuracy and precision [79, 293]. Linearity should be established using a minimum of five concentrations within the range which will be required for the study being conducted. In an assay for the drug content of raw material or finished product for example, the range required will be 80-120% of the test concentration [293]; for a bioavailability study the range would be from zero to the C_{max} value anticipated [336] and for a stability study the range would be from the initial concentration to about 20% of the initial concentration [250]. Regression analysis can be used to test the relationship between assay response and concentration and although some analytical procedures may require non-linear calibration and data transformation, it is conventional to use a linear model and univariate regression [335, 336]. The term sensitivity is often confused with limit of detection or limit of quantitation but Tamisier-Karolak et al [337] demonstrated it is actually is a property of the slope of the regression line and sensitivity is in fact the smallest concentration difference that can be quantified with sufficient precision.

Robustness

Robustness is a measure of the ability of the method to remain unaffected by small but deliberate variations in method parameters and provides an indication of the reliability and durability of the assay whereas the term ruggedness refers more to an assessment of the reproducibility of test results obtained by the analysis of the same samples by different

laboratories where conditions such as analyst, equipment and materials would vary [79, 293]. The most common test of robustness with HPLC involves changes in the composition and/or pH of the mobile phase as these are variables subject to change with new batches of mobile phase being prepared [335]. Chromatograms run under conditions where the mobile phase components or pH differ should be compared as to retention time, peak character and quantitation.

Experimental 3.2 – Method validation

This block of experiments was done to validate the proposed HPLC method for apomorphine analysis. Briefly, the method used a cyano column (25 x 0.25 cm) with a mobile phase consisting of acetonitrile: phosphoric acid 30mM (500:500), a flow rate of 1.0 $mL \cdot min^{-1}$ and a sample volume of 20 µL. Detection was by UV absorbance at 272 nm. These validation experiments addressed linearity and range; accuracy and precision; specificity in terms of forced degradation, peak purity and excipient interference; and ruggedness in terms of mobile phase composition, flow rate and detection wavelength. Validation of all these parameters has been recommended in the literature [293].

Experiment 3.2.1 – Linearity and range:

Purpose:

This experiment was conducted in order to validate that the detector response showed a linear relationship with apomorphine concentration and that the detector response can be used to quantitate apomorphine concentration.

Study design and method:

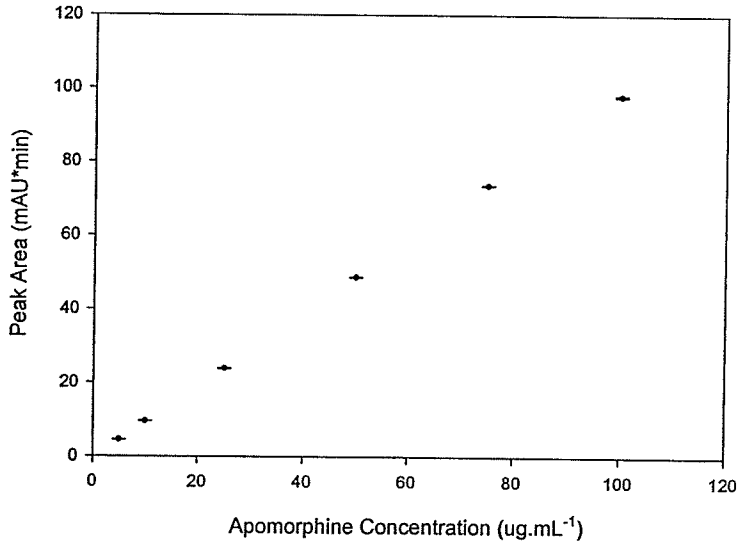
In order to determine the linearity and range of the method, calibration curves were prepared with apomorphine concentrations ranging from 100 to 5 $\mu g \cdot mL^{-1}$ using 0.05M HCl to prepare the appropriate concentrations from a stock solution of apomorphine. The proposed

HPLC method using UV detection was used as described in the previous section; four trials were conducted and three replicate injections were done for each sample. The data generated for each curve were analyzed by linear regression of the peak area as a function of apomorphine concentration. Concentration values for the accuracy and precision studies were determined using the regression coefficients obtained from these data.

Results and discussion:

The data from this experiment are presentated is given in Figure 3.5. Peak area as a function of concentration for all the samples ranging from 100 to 5 $\mu g\ mL^{-1}$ showed very good linearity as determined by linear regression. The regression values found were: slope 0.984, y-intercept -0.493 and the coefficient of determination (r^2) was 1.000.

Figure 3.5 Linearity of apomorphine peak area as a function of concentration with detection by UV absorbance at 272 nm. The data points are mean values and the error bars are standard deviations. (n = 4)



Experiment 3.2.2 – Accuracy and precision:

Purpose:

This experiment was conducted in order to validate that the accuracy and precision of the analysis is acceptable over the range of linearity determined from the previous experiment.

Study design and method:

The accuracy and precision of the method was evaluated by preparing apomorphine solutions over the concentration range of 100 to 5 $\mu g\ mL^{-1}$ and conducting 12 replicates for each concentration. Accuracy was considered to have been met if the mean recovery was within 100 ± 2% at each concentration level assessed and precision was determined by calculating the relative standard deviation (RSD) for peak area at each concentration level. Various levels of acceptance have been suggested depending on the proposed application of the method and values of 1% and 3% have been suggested [293, 330]. For the proposed use of this method, an acceptance level of RSD <3% will be used.

Results and discussion:

The data from this experiment are presented in Table 3.12.

Table 3.12 Precision and % accuracy of apomorphine determination using UV detection at 272 nm. *Precision taken as RSD of peak area.

Conc ($\mu g \cdot mL^{-1}$)	Peak Area	Conc (Calc)	Accuracy (%)	Precision *
100	97.88 ± 0.20	99.93 ± 0.21	99.93	0.21
75	73.52 ± 0.11	75.19 ± 0.12	100.3	0.15
50	48.69 ± 0.08	49.97 ± 0.08	99.94	0.16
25	23.83 ± 0.10	24.71 ± 0.10	98.84	0.40
10	9.53 ± 0.04	10.18 ± 0.04	101.8	0.38
5	4.45 ± 0.04	5.02 ± 0.04	100.4	0.83
Values as Mean ± Std deviation			n = 12	

Experiment 3.2.3 – Specificity:

Purpose:

Validation of method specificity was assessed in three ways: forced degradation, peak purity and excipient compatibility. The goal of these experiments is to demonstrate that the degradation or breakdown products of apomorphine do not interfere with the analysis of the parent compound, that the peak identified as apomorphine does not contain any other product and finally that the excipient materials which may possibly be present in the analytical sample do not interfere with the analysis of the apomorphine.

3.2.3.1. Forced degradation:

Study design and method:

A solution of apomorphine containing about 250 $\mu g \cdot mL^{-1}$ was prepared and 10-mL aliquots of this solution were mixed with either 20 mL of 0.1 M HCl, 0.1 M NaOH or 3% hydrogen peroxide. The solutions were stored at 70° C for four hours and then 1.0-mL samples were removed, diluted with an equal volume of 0.5 M HCl and analyzed for apomorphine content using the proposed HPLC method.

Results and discussion:

After four hours the samples incubated at 70° C with 0.1 M HCl showed no change. The samples incubated with 0.1 M NaOH showed 84.44 % decomposition and the presence of peaks at retention times 0.27, 0.375 and 0.466 minutes relative to apomorphine. The samples incubated with peroxide showed 88.74% decomposition and the presence of peaks with retention times 0.31 and 0.365 minutes relative to apomorphine. The new peaks which possibly represented apomorphine degradation products all eluted well before the apomorphine peak.

3.2.3.2. Peak purity:

Study design and method:

A sample of apomorphine was decomposed by taking 10 mL of apomorphine at a concentration of 250 μg mL^{-1} and mixing it with 20 mL of 0.05 M phosphate buffer (pH 8.0). This solution was stored at 30° C and at timed intervals 1.0-mL aliquots were removed, diluted with 1.0-mL of 0.05M HCl and analyzed for apomorphine using the proposed HPLC method but with detection using both UV and fluorescence.

Peak purity for the assay system was determined using a Waters 996 PDA detector. Samples from the alkaline decomposition studies (time 0.5, 4, 10 and 14 hours) and two standards were analyzed using this photo diode array detector and data were collected over the range of 210 to 400 nm. One of the standards was used to create a library entry for apomorphine and the other samples compared to this using the Waters software package.

Results and discussion:

Timed samples of an apomorphine solution stressed by storage at 30°C and pH 8.0 were taken and analyzed using both UV and fluorescence detection. Three separate trials were run with four replicates of each sample. The data from this experiment are presented in Table 3.13 as well as the p values obtained by comparing the data from the two detectors at each time interval using a t-test. All of the samples except that at 14 hours showed no significant difference suggesting that the peaks obtained for the analysis were pure and there was no interference in the analysis from apomorphine degradation products. The samples taken at time 20 and 24 hours had an apomorphine content below the limit of quantitation for the UV detector. The samples collected at times 0.5, 3 and 14 hours were also assessed for peak purity using a diode array UV detector. For each of the samples analyzed, the software showed a positive match for the standard apomorphine entered into the library. These data are presented

in Table 3.14 and indicate that the method was able to separate apomorphine from its decomposition products and that the method was stability-indicating.

Table 3.13 Peak purity assessment with UV and fluorescence detection.

	Conc ($\mu g \cdot mL^{-1}$)		
Sample	**UV**	**Fluorescence**	**p**
0 h	45.15 ± 0.12	44.47 ± 1.28	0.08
0.5 h	38.40 ± 0.35	38.10 ± 1.33	0.453
1 h	35.75 ± 0.33	35.55 ± 0.49	0.346
1.5 h	34.36 ± 0.54	34.04 ± 0.70	0.252
2 h	32.65 ± 0.54	31.51 ± 2.68	0.165
2.5 h	30.75 ± 0.17	30.53 ± 2.31	0.746
3 h	29.20 ± 0.58	28.92 ± 0.59	0.310
4 h	25.98 ± 0.51	25.51 ± 0.45	0.053
6 h	20.33 ± 0.18	20.64 ± 0.56	0.079
10	12.73 ± 0.01	12.53 ± 0.22	0.111
14	7.91 ± 0.04	7.47 ± 0.32	<0.001
20	-	3.11 ± 0.08	-
24	-	2.15 ± 0.14	-
Values as Mean ± Std deviation			n = 12

Table 3.14 Peak purity assessment with diode-array detection (Waters 996 PDA).

Sample	Conc ($\mu g\ mL^{-1}$)	Angle	Threshold	Match Angle	Match Threshold	Library
0.5 h	39.63	0.093	0.34	0.101	1.06	Apo
3 h	24.64	0.135	0.325	0.143	1.063	Apo
10 h	9.98	0.478	0.422	0.165	1.118	Apo
12 h	7.50	0.425	0.405	0.382	1.117	Apo
Std	52.2	0.131	0.41	0.086	1.085	Apo

3.2.3.3. Excipient interference/compatibility:

Study design and method:

In order to determine whether excipients which could possibly be used in the formulation of an ophthalmic insert were compatible with apomorphine and whether these excipients would affect the assay system, a range of excipient types, plasticizers and antioxidants were mixed with a standard solution of apomorphine and analyzed. The concentration of each excipient was what might be expected to be present in an analytical sample and the concentration of apomorphine in each sample was 50 $\mu g \cdot mL^{-1}$. The matrix materials selected were gelatin, hydroxypropylmethylcellulose (HPMC), polyvinylpyrrolidone (PVP), and polyvinyl alcohol (PVA); the plasticizers selected were glycerin, propylene glycol and triethyl citrate and the antioxidants were sodium metabisulfite and ascorbic acid. The samples were prepared then left at room temperature overnight and analyzed in triplicate 24 hours later.

Results and discussion:

The results of this experiment are shown in Table 3.15 and for each of the potential excipients, the recovery of apomorphine was to the order of 100% suggesting that under the conditions of the experiment, there were no compatibility issues between apomorphine and each of these excipients. The data also indicate that none of the excipients interfered with the analysis of apomorphine. Analysis of variance of apomorphine recovery in the presence of each excipient and a control showed no significant difference when assessed using ANOVA. ($p = 0.093$).

Table 3.15 Excipient compatibilities and interference with assay.

Excipient	Excipient Conc	% Apomorphine Recovered
Gelatin	260 μg mL^{-1}	100.1 ± 0.2
Glycerin	150 μg mL^{-1}	99.81 ± 0.35
Ascorbic acid	4 μg mL^{-1}	99.61 ± 1.06
Sodium bisulfite	4 μg mL^{-1}	100.5 ± 0.2
PVP-K30	270 μg mL^{-1}	100.8 ± 0.4
HPMC	260 μg mL^{-1}	100.5 ± 0.7
PVA	260 μg mL^{-1}	99.79 ± 0.08
Propylene glycol	160 μg mL^{-1}	99.75 ± 0.17
Triethyl citrate	160 μg mL^{-1}	100.1 ± 0.3
Values as Mean ± Std deviation		n = 3

Conclusions:

The experiments in the series 3.2.3 demonstrated that the method has suitable specificity. Break-down products induced by forced degradation using heat combined with acid, base or peroxide showed no new peaks present in the immediate vicinity of the apomorphine peak suggesting that the method was stability-indicating [250]. Peak purity was assessed using a photo-diode array (PDA) UV detector and a comparison of the results obtained using UV and fluorescence detection. The results of the trial with the PDA detector indicated that there were no differences among the apomorphine standard and the apomorphine peaks in the presence of degradation products as assessed by comparing UV absorbances over a range of 210 to 400 nm. The results obtained by comparing the values derived from UV and fluorescence detection showed no statistical difference except for the sample take at time 14 hours. No obvious reason for this discrepancy could be found but this observation suggested that care should be taken in interpreting analysis when the level of

apomorphine degradation exceeds 85% of the original amount. The screening experiment with apomorphine and possible excipients which may be used in the fabrication of the inserts demonstrated that the substances tested did not interfere with the assay of apomorphine and suggested that there were no compatibility issues between them and apomorphine.

Experiment 3.2.4 – Ruggedness:

Purpose:

These experiments were conducted in order to validate that the developed method was able to withstand small variations in mobile phase composition, detector wavelength and flow rates without a loss in accuracy and/or precision.

Study design and method:

The effect of mobile phase variability on the method was investigated by varying the amount of acetonitrile present in the mobile phase over a range from 40 to 60% in increments of 5%; five mobile phases were prepared and used to analyze a solution containing apomorphine at a concentration of about 38 $\mu g \cdot mL^{-1}$ and six trials were run for each mobile phase variation. In order to determine the effect of variations in the wavelength used for detection, a sample of apomorphine containing about 33 $\mu g \cdot mL^{-1}$ was prepared and analyzed using detection wavelengths of 270, 272 and 274 nm; four trials were run for each wavelength. In order to determine the effect of variations in the flow rate, a sample of apomorphine containing about 40 $\mu g \cdot mL^{-1}$ was prepared and measured using flow rates of 0.9, 1.0 and 1.1 $mL \cdot min^{-1}$; four trials were run at each flow rate. Retention time, peak area, tailing factor, number of plates, capacity and resolution were determined for each chromatogram and quantitation was accomplished through linear regression using a standard curve prepared using acetonitrile and 0.03M phosphoric acid (500:500) as mobile phase with detection at 272 nm and a flow rate of 1.0 $mL \cdot min^{-1}$. Since the method had been shown to be linear over the

concentration range used in these experiments, only the peak area data are shown; the quantitation data are not presented.

3.2.4.1. Mobile phase variation

Results and discussion:

The chromatographic data obtained from the experiment where the content of organic modifier (acetonitrile) present in the mobile phase was varied, are presented in Table 3.16.

Table 3.16 Chromatographic effects of variation in the content of acetonitrile (ACN) present in the mobile phase.

CAN (%)	RT (min)	Peak Area	Tailing factor	Plates	Capacity
40	4.68 ± 0.03	37.33 ± 1.11	1.68 ± 0.09	2209 ± 150	1.34 ± 0.01
45	4.98 ± 0.06	36.79 ± 0.92	1.72 ± 0.16	2124 ± 163	1.49 ± 0.03
50	5.27 ± 0.06	37.17 ± 0.85	1.56 ± 0.11	2938 ± 179	1.64 ± 0.03
55	5.49 ± 0.03	37.52 ± 0.23	1.73 ± 0.08	2340 ± 67	1.75 ± 0.02
60	5.52 ± 0.03	35.04 ± 0.32	1.88 ± 0.07	2342 ± 17	1.76 ± 0.01
Values as Mean ± Std deviation n = 6					

Over the acetonitrile concentration range 40-60% ANOVA suggested significant differences in the UV detector response expressed as peak area ($p < 0.001$) however over the more limited range of 45-55% acetonitrile, no significant difference was found ($p = 0.264$).

3.2.4.2. Detector wavelength variation

Results and discussion:

The data for this experiment are presented in Table 3.17. Over the detection wavelength from 270 to 274 nm there was no significant difference in the values obtained for peak area ($p = 0.310$) and quantitation of the peak areas using the mean regression coefficients from the linearity studies also showed no significant difference ($p = 0.310$).

Table 3.17 Effect of detector wavelength variation.

Wavelength (nm)	RT (min)	Peak Area	Tailing factor	Plates	Capacity
270	6.07 ± 0.10	33.73 ± 0.70	1.66 ± 0.26	2213 ± 144	2.03 ± 0.05
272	5.93 ± 0.11	33.70 ± 0.74	1.94 ± 0.18	2167 ± 63	1.97 ± 0.06
274	6.03 ± 0.06	32.98 ± 0.76	2.06 ± 0.22	2238 ± 104	2.01 ± 0.03
Values as Mean ± Std deviation n=4					

3.2.4.3. Flow rate variation

Results and discussion:

The data from this experiment are presented in Table 3.18. Peak areas and the concentration values derived from the peak areas showed no significant difference in value for the flow rate ranging from 0.9 to 1.1 $mL \cdot min^{-1}$ ($p = 0.184$)

Table 3.18 Effect of flow rate variation.

Flow ($mL \cdot min^{-1}$)	RT (min)	Peak Area	Tailing factor	Plates	Capacity
0.9	6.26 ± 0.07	40.57 ± 1.55	1.97 ± 0.11	2299 ± 80	2.13 ± 0.03
1.0	5.95 ± 0.07	38.19 ± 2.40	1.85 ± 0.27	2273± 90	1.99 ± 0.02
1.1	5.55 ± 0.15	38.26 ± 1.58	2.17 ± 0.52	2087 ± 50	1.78 ± 0.07
Values as Mean ± Std deviation n = 4					

Conclusions:

The results of the experimental series 3.2.4 demonstrated that the proposed method showed suitable ruggedness. Although the detector response was affected by variation in the concentration of organic modifier over a range of 40-60%, it was not affected over a concentration range of 45-55% so small variations in the composition of the mobile phase will have little effect on the outcome of the analysis. The method was unaffected by variation in

the detector wavelength over a range of 270-274 nm and flow rate variation over a range of 0.9 to 1.1 $mL \cdot min^{-1}$.

Validation of the proposed HPLC method for apomorphine analysis was conducted through a series of experiments (Experimental 3.2). These experiments examined the linearity and range of the method and it was determined that the method showed good linearity over a range of 5-100 $\mu g \cdot mL^{-1}$ with a coefficient of determination (r^2) of 1.000. The accuracy and precision of the method were determined over this range and it was shown that the method had a mean accuracy of 100.2 ± 0.96%.

The specificity of the method was investigated through forced degradation using heat stress with acid, base and peroxide and following these treatments, no degradation product, as evidenced by a new peak, was found near the retention time of apomorphine. Specificity was also investigated using peak purity and it was shown using PDA detection that the apomorphine peak in degraded samples did not differ from the standard apomorphine peak when the absorbance of the peaks were measured over a range of 210 to 400 nm and compared. Further evidence of peak purity was provided by comparing the analytical values obtained from UV and fluorescence detection of a series of timed samples of apomorphine withdrawn from a standard solution heated in alkaline buffer to 90% decomposition of the original concentration. These experiments and results suggested that the proposed method was stability-indicating. It was further shown that the analysis of apomorphine was not affected by the presence of a series of excipients which potentially could be used in the fabrication of the inserts.

That the method was robust was demonstrated in experiments which showed that, within limits, quantitation of apomorphine was not significantly affected by small variations in mobile phase composition, detection wavelength or flow rate. These investigations

demonstrated that the HPLC method developed for apomorphine analysis in ophthalmic inserts is appropriate and acceptable.

3.3. Gelatin analysis

Overview:

Studies regarding formulation and product fabrication were being run concurrently with the method development work and at this point in time, it had been decided that gelatin provided the most suitable polymer for use in the apomorphine ocular inserts.

Gelatin is a protein polymer derived from naturally occurring collagen. Two types of gelatin are used pharmaceutically; type A which is derived from acid pre-treated collagen and is cationic with an isoelectric point between pH 7-9 and type B which is derived from alkali-treated collagen and is anionic with an isoelectric point between pH 5-6 [338-340]. Gelatin is useful as a delivery matrix for drugs due to its biocompatibility, biodegradability, lack of antigenicity and the potential it has for cross-linking which allows for flexibility in adjusting the rate of swelling and subsequent drug release [340-342]. Gelatin and a number of other hydrogels may release drug through both swelling and erosion [341, 343].

Since part of this project will study the release kinetics of the apomorphine inserts, a method of measuring the rate and extent of gelatin dissolution may provide some insights into the release mechanisms and provide an index of the extent of drug release due to erosion of polymer relaxation. In a review of colorimetric assay methods for protein quantitation Sapan et al [344] discussed four commonly used methods; the Biuret, Lowry, Coomassie blue dye-binding and the bicinchoninic acid methods. Of these, the Coomassie blue and bicinchoninic acid methods appeared to be best suited to this application so preliminary experiments were conducted with these methods to determine their suitability for measuring gelatin in solution.

A preliminary experiment was conducted and the Coomassie brilliant blue or Bradford assay was assessed for quantitation of gelatin in solution. Because of its ease and simplicity, this method is used for staining proteins after separation using electrophoresis on polyacrylamide gels as well as for proteins in solution [345, 346]. The assay is based on the binding of the dye to the protein which forms a dye-protein complex with absorbance between 595-600 nm. The reaction is run in an acidic environment using hydrochloric, perchloric or phosphoric acid in ethanol [344, 347, 348]. A number of refinements to the original assay have been suggested in terms of the best acid to use [349] and Lopez et al [350] suggested the use of a surfactant, dodecyl sodium sulfate (DSS) for proteins where the dye-protein complex was insoluble. Marshall and Williams [351] reported that the Bradford method was relatively free of interference from drugs which might be in the samples.

Standard solutions of gelatin in water were prepared over a concentration range of 30 to 240 μg·mL-1 and 1 mL of standard and 5 mL of the dye reagent [348] were mixed. After mixing, the solutions immediately became turbid and absorbance values could not be determined. An attempt to keep the complex in solution through the use of DSS was later tried unsuccessfully so further evaluation of this method for gelatin quantitation was abandoned.

A similar preliminary experiment was conducted to assess the suitability of the bicinchoninic acid method for gelatin quantitation during release studies of the apomorphine ocular inserts. The bicinchoninic acid (BCCA) assay for protein uses BCCA to detect cuprous ions generated from cupric ions by reaction with protein under alkaline conditions [344]. Wiechelman et al [352] studied the reaction in an effort to identify the functional groups on the protein molecule which reduced the cupric ions. In their study they found that cystein, cystine, tryptophan, tyrosine and the peptide bond residues were able to reduce cupric to cuprous ions which in turn formed a colored complex with the bicinchoninic acid. They also found that a number of other reducing substances were able to do this and interfere with the

assay. The authors provided a list of substances which interfered and they also attempted to compensate for the interference by including them in the reagent blank but were unsuccessful in this effort. In a further attempt to deal with interfering substances, Shihabi and Dyer [353] examined interference from glucose, ascorbic acid and uric acid and claimed that the inclusion of borate ions in the form of a buffer reduced the interference from these substances. Brown et al [354] also addressed this problem and recommended precipitation of the protein using deoxycholate and trichloroacetic acid, removing the supernatant and re-dissolving the protein with the aid of dodecyl sodium sulfate (DSS). Interference in the assay from drugs was investigated by Marshall and Williams [355]. They examined interference from chlorpromazine, ampicillin, penicillin-G and acetaminophen and then attempted to compensate for the interference by including an appropriate amount of the drug in the reagent blank. In agreement with the findings of Wiechelman et al [352], this effort was unsuccessful. A refinement to the precipitation method was described by Brown et al [354] as they found that some of the interfering substance was carried over in the precipitate and that this was a problem when small amounts of protein were being measured. In their work, they found that washing the precipitate with 1N hydrochloric acid prior to re-dissolving the protein eliminated this problem.

Standard solutions of gelatin in water were prepared over a concentration range of 30 to 240 μg·mL-1 and assayed using the BCCA method. Excellent results were obtained for the standards with good linearity, accuracy and precision but when samples including apomorphine were analyzed very large absorbance values were obtained; this was not unexpected since apomorphine undergoes oxidative degradation and would function as a reducing substance in the assay. An attempt to use the protein precipitation technique was unsuccessful as the gelatin would not precipitate under the conditions used. Since this analysis would be done on samples which were also analyzed for apomorphine content, subtraction of

the absorbance which would be due to the apomorphine was attempted and this also was unsuccessful as relative standard deviations in excess of 20% were seen with triplicate trials of samples containing known amounts of apomorphine and gelatin.

Since neither of the colorimetric assays for gelatin was appropriate and earlier work in this study showed that apomorphine release from the inserts could be done using a flow cell and direct UV spectroscopy, a series of experiments were conducted to determine if gelatin could be quantitated using direct UV spectroscopy and whether it was possible to simultaneously determine both gelatin and apomorphine using a flow cell and direct UV absorbance.

Experimental 3.3 – Gelatin Analysis

In order to characterize and study the release of apomorphine from the inserts, analytical methods to measure both apomorphine and gelatin will be required. An efficient way to conduct these dissolution studies would be to measure both apomorphine and gelatin simultaneously using a UV spectrophotometer equipped with a flow cell. In order to determine whether this method would be feasible, a number of experiments were performed.
Each insert consists of the following components:

Apomorphine HCl USP	2.0 mg
Gelatin NF	12.4 mg
Glycerin USP	8.3 mg
Ascorbic acid USP	0.2 mg
Sodium metabisulfite NF	0.2 mg

It was anticipated that for the dissolution studies, one insert would be dissolved in 200 to 500-mL of solvent. An earlier study conducted during the development of the HPLC method for apomorphine had demonstrated that the concentrations of ascorbic acid and sodium bisulfite which would result from this would show no appreciable UV absorption over the

wavelengths of 200 to 300 nm whereas the glycerin, gelatin and apomorphine would show absorption over these wavelengths. The anticipated maximum concentrations of each component using dissolution volumes of 100, 250 and 500 mL are presented in Table 3.19.

Table 3.19 Maximum concentrations of components dissolved in 100, 250 and 500 mL of dissolution medium.

	Maximum Concentration in Dissolution Medium		
Component	**100 mL**	**250 mL**	**500 mL**
Apomorphine	20 $\mu g \cdot mL^{-1}$	8 $\mu g \cdot mL^{-1}$	4 $\mu g \cdot mL^{-1}$
Gelatin	124 $\mu g \cdot mL^{-1}$	49.6 $\mu g \cdot mL^{-1}$	24.8 $\mu g \cdot mL^{-1}$
Glycerin	83 $\mu g \cdot mL^{-1}$	33.2 $\mu g \cdot mL^{-1}$	16.6 $\mu g \cdot mL^{-1}$
Ascorbic acid	2 $\mu g \cdot mL^{-1}$	0.8 $\mu g \cdot mL^{-1}$	0.4 $\mu g \cdot mL^{-1}$
Sodium metabisulfite	2 $\mu g \cdot mL^{-1}$	0.8 $\mu g \cdot mL^{-1}$	0.4 $\mu g \cdot mL^{-1}$

Experiment 3.3.1 – UV Characterization

Purpose:

The purpose of this experiment was characterization of UV absorption for apomorphine, gelatin and glycerin to determine whether direct UV absorbance can be used to quantitate gelatin in solution.

Study design and method:

In order to assess whether the main components of the inserts could be determined using UV spectroscopy, solutions of apomorphine 7 $\mu g \cdot mL^{-1}$, gelatin 35 $\mu g \cdot mL^{-1}$and glycerin 90 $\mu g \cdot mL^{-1}$ were prepared and the UV spectra of each recorded using a diode-array spectrophotometer over the range 200 to 350 nm for apomorphine and gelatin; 200 to 300 nm in the case of glycerin since it showed little UV absorption. The solvent used for all these studies was 0.05 M HCl.

Standard solutions of gelatin at about 470 $\mu g \cdot mL^{-1}$, apomorphine at about 80 $\mu g \cdot mL^{-1}$ and glycerin at about 450 $\mu g \cdot mL^{-1}$ were prepared and a series of dilutions done with each. The absorption of each solution was determined at 210, 220 and 272 nm and three trials were conducted. The linearity of each wavelength was calculated using linear regression analysis.

Due to the heterogeneous nature of gelatin in terms of chain length [356] and possible variation in the distribution of amino acid residues present [338, 339], the spectral character of gelatin from different suppliers and different lot numbers was assessed. Two different lots of gelatin from Spectrum Pharmaceuticals were assessed as well as samples from Sigma Chemicals and Medisca Pharmaceuticals. All of the materials were labeled as meeting NF specifications for gelatin. Stock solutions of each gelatin sample were prepared at a concentration of about 75 $\mu g \cdot mL^{-1}$ and these were diluted to provide further solutions with concentrations of about 60, 45, 30 and 15 $\mu g \cdot mL^{-1}$. The solutions were all prepared in triplicate with 0.05M HCl as solvent and the absorbances of each solution were recorded at 210, 220 and 272 nm and the data at 220 and 272 nm were analyzed using linear regression to determine the slope and intercept values for each sample. Spectral scans from 200 to 300 nm were also recorded using representative samples at a concentration of 45 $\mu g \cdot mL^{-1}$.

Results and discussion:

The UV spectra for gelatin, apomorphine and glycerin are presented in Figures 3.6, 3.7 and 3.8 respectively, Gelatin showed a maximum band of absorbance at 204 nm, apomorphine at 208 and 272 nm and glycerin at 202 nm.

Figure 3.6 UV spectrum of gelatin 35 $\mu g \cdot mL^{-1}$.

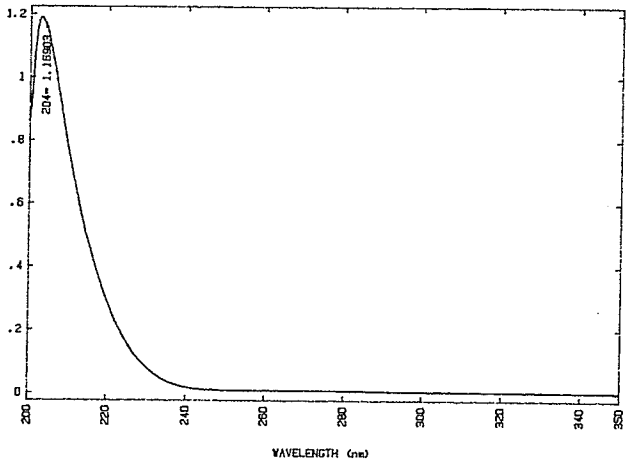


Figure 3.7 UV spectrum of apomorphine HCl 7 $\mu g \cdot mL^{-1}$

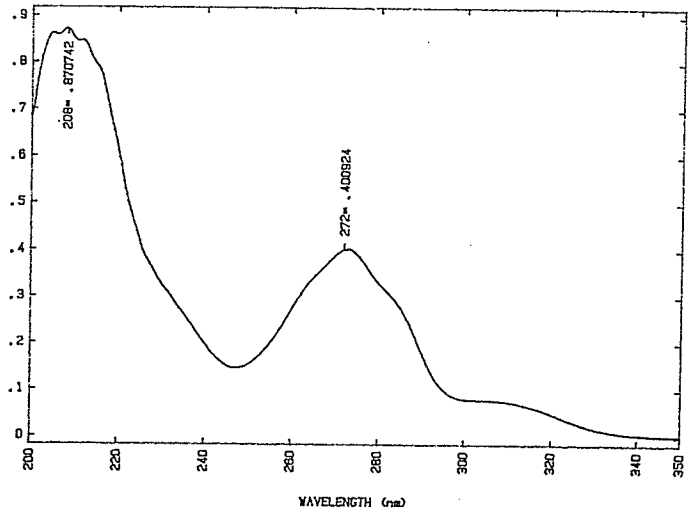
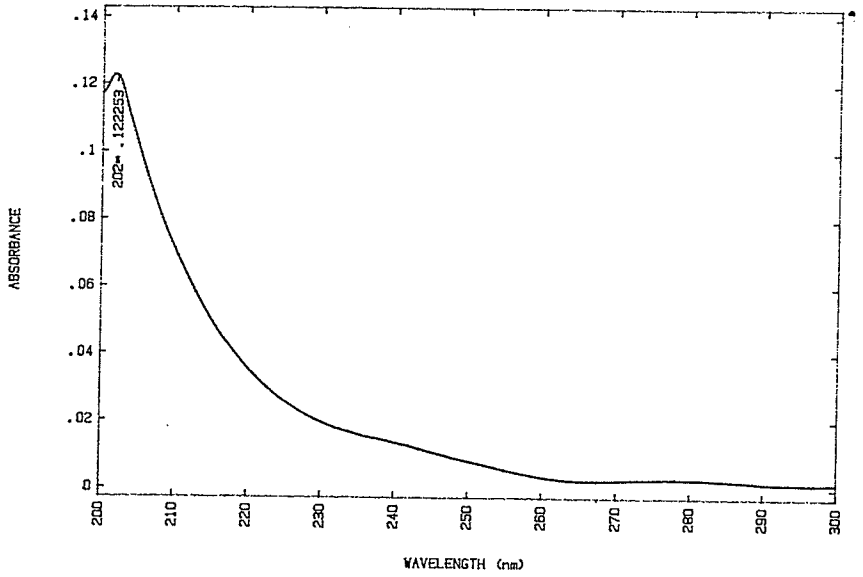


Figure 3.8 UV spectrum of glycerin 90 $\mu g \cdot mL^{-1}$



A series of dilutions of gelatin, apomorphine and glycerin were prepared and the absorbances of each were measured at 210, 220 and 272 nm. Three trials were conducted and these data were analyzed using linear regression. These data and the results of regression analysis are presented in Tables 3.20, 3.21 and 3.22 respectively.

Table 3.20 Linearity for gelatin at 210, 220 and 272 nm.

Conc ($\mu g \cdot mL^{-1}$)	$Abs_{(210)}$	$Abs_{(220)}$	$Abs_{(272)}$
77.44	1.645 ± 0.012	0.6885 ± 0.0072	0.0183 ± 0.0023
58.08	1.708 ± 0.009	0.5251 ± 0.0059	0.0168 ± 0.0041
38.72	0.9226 ± 0.0173	0.3570 ± 0.0089	0.0103 ± 0.0017
19.36	0.4637 ± 0.0153	0.1782 ± 0.0102	0.0089 ± 0.0104
Intercept	0.1027	0.0125	0.0049
Slope	0.0203	0.0088	0.0002
r^2	0.9937	0.9983	0.3912
Values : Mean ± Std dev n = 3			

Table 3.21 Linearity of apomorphine HCl at 210, 220 and 272 nm.

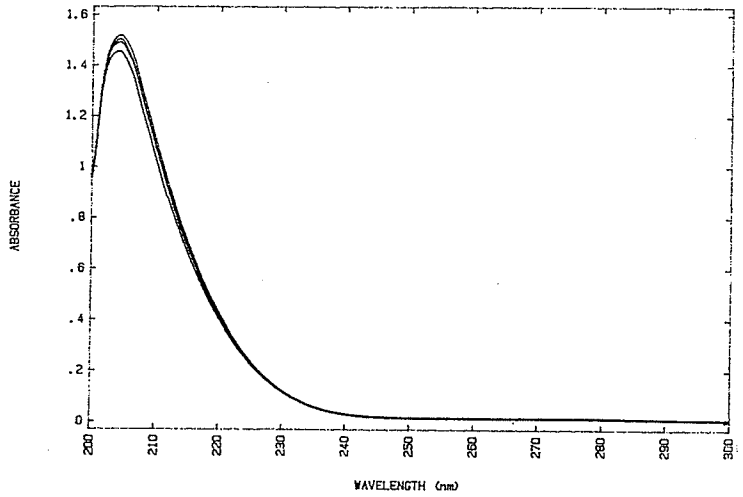
Conc ($\mu g \cdot mL^{-1}$)	$Abs_{(210)}$	$Abs_{(220)}$	$Abs_{(272)}$
12.88	1.464 ± 0.006	1.058 ± 0.008	0.7167 ± 0.0041
9.66	1.116 ± 0.004	0.8010 ± 0.0036	0.5376 ± 0.0019
6.44	0.7648 ± 0.0143	0.5481 ± 0.0079	0.3609 ± 0.0025
3.22	0.4053 ± 0.0242	0.2893 ± 0.0154	0.1856 ± 0.0069
Intercept	0.0555	0.0344	0.0077
Slope	0.1096	0.0795	0.0550
r^2	0.9990	0.9992	0.9997
Values ; Mean ± Std dev n = 3			

Table 3.22 Linearity of glycerin at 210, 220 and 272 nm.

Conc ($\mu g \cdot mL^{-1}$)	$Abs_{(210)}$	$Abs_{(220)}$	$Abs_{(272)}$
76.10	0.0671 ± 0.0002	0.0310 ± 0.0002	-0.0020 ± 0.0002
38.05	0.0311 ± 0.0003	0.0195 ± 0.0003	0.0053 ± 0.0003
19.03	0.0152 ± 0.0003	0.0093 ± 0.0002	0.0017 ± 0.0002
9.51	0.0127 ± 0.0001	0.0088 ± 0.0002	0.0028 ± 0.0001
Intercept	0.0047	0.0062	0.0040
Slope	0.0008	0.0003	0.0006
r^2	0.9705	0.9557	0.4271
Values : Mean ± Std dev n = 3			

Due to the heterogeneous nature of gelatin [338, 339], material from different sources may have different spectral characteristics. To determine whether this was a problem two samples of different lots from Spectrum Pharmaceuticals and samples of material from Sigma Chemical and Medisca Pharmaceuticals were used to prepare a series of dilutions. Representative UV scans from the four samples are shown in Figure 3.9:

Figure 3.9 Spectral scans of gelatin samples from four different sources (Spectrum Pharmaceuticals, Sigma Chemical and Medisca Pharmaceuticals) at concentration levels of about 45 $\mu g \cdot mL^{-1}$.



The absorbance values for the dilutions prepared from the four sources of gelatin were measured at 210, 220 and 272 nm and these data are presented in Tables 3.23, 3.24 and 3.25 for the respective wavelengths. Three trials were conducted.

Table 3.23 UV absorbance at 210 nm for gelatin samples from different sources.

	Absorbance (λ = 210 nm)			
Conc ($\mu g \cdot mL^{-1}$)	**Spectrum A**	**Spectrum B**	**Sigma**	**Medisca**
75	1.643 ± 0.013	1.643 ± 0.004	1.646 ± 0.010	1.621 ± 0.006
60	1.364 ± 0.012	1.385 ± 0.002	1.398 ± 0.004	1.368 ± 0.006
45	1.086 ± 0.007	1.088 ± 0.013	1.106 ± 0.018	1.066 ± 0.016
30	0.7440 ± 0.0074	0.7571 ± 0.0112	0.7761 ± 0.0167	0.7400 ± 0.0141
15	0.3871 ± 0.0031	0.4057 ± 0.0159	0.4014 ± 0.0082	0.3750 ± 0.0026
Slope	0.0209	0.0207	0.0207	0.0208
Intercept	0.105	0.125	0.132	0.0979
r^2	0.996	0.996	0.993	0.995
Values : Mean ± Std dev			n = 3	

Table 3.24 UV absorbance at 220 nm for gelatin samples from different sources.

	Absorbance (λ = 220 nm)			
Conc (μg·mL^{-1}) *	**Spectrum A**	**Spectrum B**	**Sigma**	**Medisca**
75	0.6999 ± 0.0053	0.6929 ± 0.0018	0.6921 ± 0.0070	0.6869 ± 0.0037
60	0.5497 ± 0.0070	0.5589 ± 0.0038	0.5650 ± 0.0058	0.5558 ± 0.0054
45	0.4278 ± 0.0038	0.4247 ± 0.0073	0.4293 ± 0.0087	0.4180 ± 0.0079
30	0.2828 ± 0.0036	0.2881 ± 0.0071	0.2968 ± 0.0097	0.2845 ± 0.0060
15	0.1430 ± 0.0001	0.1627 ± 0.0234	0.1521 ± 0.0033	0.1502 ± 0.0087
Slope	0.00920	0.00887	0.00899	0.00896
Intercept	0.00644	0.0261	0.0226	0.0157
r^2	0.999	0.997	0.999	0.999
Values : Mean ± Std dev		n = 3		

Table 3.25 UV absorbance at 272 nm for gelatin samples from different sources.

	Absorbance (λ = 272 nm)			
Conc (μg·mL^{-1}) *	**Spectrum A**	**Spectrum B**	**Sigma**	**Medisca**
75	0.0300 ± 0.0013	0.0253 ± 0.0002	0.0241 ± 0.0020	0.0319 ± 0.0009
60	0.0226 ± 0.0020	0.0216 ± 0.0018	0.0234 ± 0.0034	0.0277 ± 0.0015
45	0.0211 ± 0.0013	0.0160 ± 0.0023	0.0152 ± 0.0020	0.0182 ± 0.0017
30	0.0093 ± 0.0004	0.0100 ± 0.0019	0.0126 ± 0.0031	0.0137 ± 0.0007
15	0.0068 ± 0.0006	0.0082 ± 0.0026	0.0052 ± 0.0015	0.0062 ± 0.0024
Slope	0.000399	0.000305	0.000325	0.000436
Intercept	-0.00000777	0.00247	0.00146	-0.0000914
r^2	0.935	0.924	0.878	0.969
Values : Mean ± Std dev		n=3		

The data obtained from the UV measurements of the different gelatin samples appeared to be similar for each sample and the absorbance data for each concentration and wavelength presented in Tables 3.23, 3.24 and 3.25 were assessed for difference using ANOVA. The p-values obtained are presented in Table 3.26.

Table 3.26 p-values from ANOVA of UV absorbance for gelatin samples from different sources. *Significant difference.

Conc ($\mu g \cdot mL^{-1}$)	210 nm	220 nm	272 nm
75	0.034*	0.064	<0.001*
60	0.001*	0.056	0.050*
45	0.051	0.295	0.014*
30	0.033*	0.141	0.055
15	0.013*	0.353	0.358

The concentration and wavelength where significant differences were found among the gelatin samples are identified with an asterisk in Table 3.26 and for all the concentrations evaluated at 220 nm, no difference was found among the different samples. These data indicate that UV absorbance at 220 nm should be useful for quantitation of gelatin but because differences were seen at other wavelengths which may be used in the quantitation calculation for inserts, it would be appropriate to use standards composed of gelatin from the same source as that used to fabricate the inserts being studied.

In summary, these experiments demonstrated that apomorphine shows absorbance peaks at 208 and 272 nm while gelatin shows an absorbance peak only at 204 nm but measurable absorbance at 220 nm. Glycerin shows absorbance at 202 nm but generally the absorbance is quite weak. At any of the anticipated concentrations in a dissolution/release study, neither sodium metabisulfite nor ascorbic acid showed any absorbance over a range of 200 to 300 nm. The absorbance and linear regression data presented in Tables 3.20 and 3.21 are presented graphically in Figures 3.10 and 3.11. The data demonstrate that over a range of about 3 to 12 $\mu g \cdot mL^{-1}$ apomorphine shows a linear relationship between absorbance and concentration at wavelengths of 220 and 272 nm while gelatin shows linearity at 220 nm over a range of 20 to 80 $\mu g \cdot mL^{-1}$ and little absorbance at 272 nm.

Figure 3.10 Plots of apomorphine absorbances at 210, 220 and 272 nm as a function of concentration. (Data from Table 3.21).

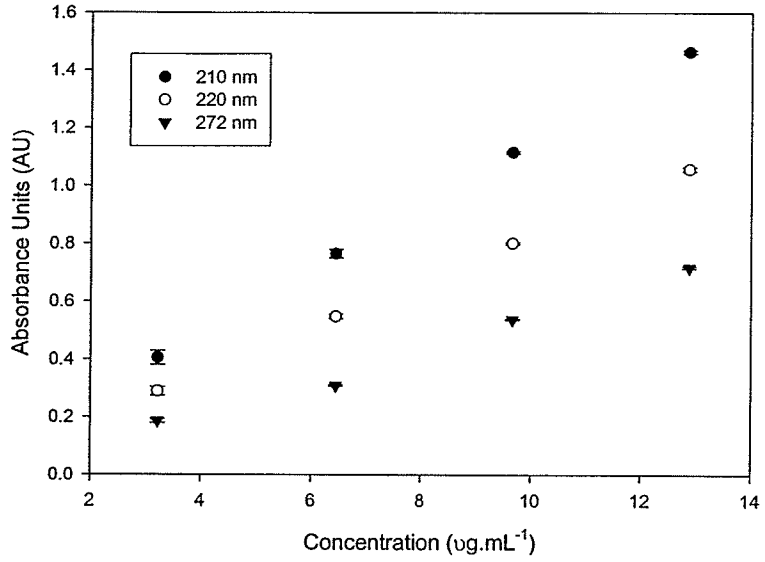
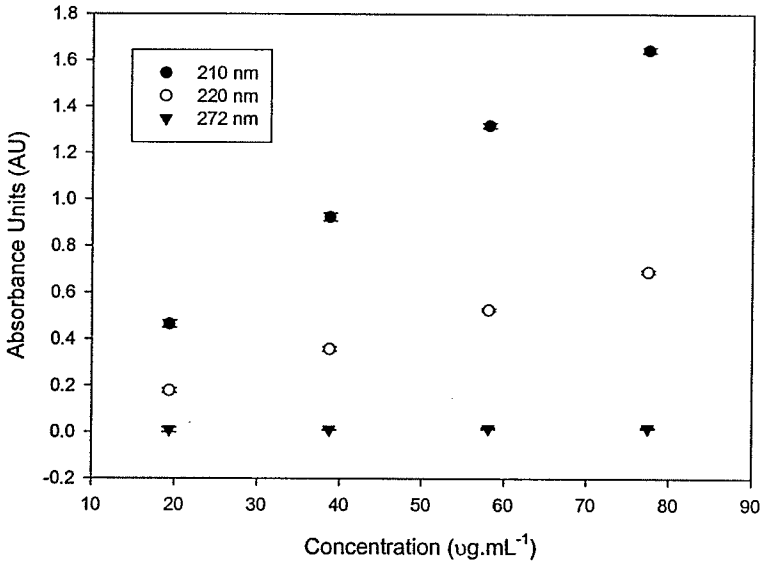


Figure 3.11 Plots of gelatin absorbances at 210, 220 and 272 nm as a function of concentration. (Data from Table 3.20).



Based on these findings, it may be possible to conduct dissolution/release studies of the proposed ocular inserts and simultaneously determine apomorphine and gelatin concentrations by measuring UV absorbances at 220 and 272 nm. One insert dissolving in 300 to 350 mL of

dissolution medium will provide concentrations of apomorphine and gelatin within the linear ranges at 220 and 272 nm and the other components of the inserts should not interfere with these measurements although in the case of glycerin, this will need to be verified.

Experiment 3.3.2 – Simultaneous determination of apomorphine and gelatin

Purpose:

To establish that apomorphine and gelatin can be measured simultaneously using UV spectroscopy

Study design and method:

A series of solutions containing varying amounts of apomorphine, gelatin and glycerin were prepared and the absorbance of each solution was measured at 220 and 272 nm. The solvent used in all of these experiments was 0.05M HCl and the concentration ranges studied were consistent with one insert being dissolved in 350 mL of dissolution medium. Glycerin was included to demonstrate that it did not interfere with the analysis.

The study design for these experiments was a full factorial design with three factors at two levels and the design matrices are shown in Table 3.27. Each experiment was run in triplicate and the order of analysis for each sample was done in a random fashion.

Table 3.27 Full factorial design with three factors at two levels to evaluate UV assay for apomorphine and gelatin.

Tube	Apomorphine	Gelatin	Glycerin
1	+	+	+
2	+	+	-
3	+	-	+
4	+	-	-
5	-	+	+
6	-	+	-
7	-	-	+
8	-	-	-

Level	Apomorphine	Gelatin	Glycerin
+	5.61 $\mu g \cdot mL^{-1}$	41.5 $\mu g \cdot mL^{-1}$	21.0 $\mu g \cdot mL^{-1}$
-	1.96 $\mu g \cdot mL^{-1}$	14.5 $\mu g \cdot mL^{-1}$	7.37 $\mu g \cdot mL^{-1}$

In order to measure the apomorphine and gelatin concentrations of the samples, standard curves of both apomorphine and gelatin were prepared at wavelengths of 220 and 272 nm. The range of concentration for apomorphine was from about 7 to 1.5 $\mu g \cdot mL^{-1}$ and for gelatin about 80 to 20 $\mu g \cdot mL^{-1}$. These data were analyzed using linear regression and the slope and intercept for each data set were calculated and used to quantitate the apomorphine and gelatin.

Quantitation was achieved by setting up the following equation:

$$\mathbf{x} = \mathbf{A}^{-1}(\mathbf{b}\text{-}\mathbf{i})$$

where **x** is a vector for the unknown concentration values, **A** is a matrix of the slope values, **i** is a matrix of the intercept values and **b** is result vector which contains the observed absorbance values at 220 and 272 nm respectively. The matrix was solved using the MINVERSE(array) and MMULT(array:array) functions in Excel. The calculated values for

apomorphine and gelatin were compared to the known values and expressed as % recovered. These data were analyzed using ANOVA comparing the concentrations of apomorphine and gelatin found in each sample to the theoretical amount.

Results and discussion:

The standard curves for apomorphine and gelatin are presented in Tables 3.28 and 3.29 respectively; the coefficients from regression analysis were used for quantitation of the apomorphine and gelatin as described earlier.

Table 3.28 Apomorphine standard curve.

Conc ($\mu g \cdot mL^{-1}$)	$Abs_{(220)}$	$Abs_{(272)}$
7.01	0.5664 ± 0.0050	0.3790 ± 0.0018
5.61	0.4472 ± 0.0050	0.2993 ± 0.0018
4.21	0.3386 ± 0.0049	0.2246 ± 0.0021
2.81	0.2158 ± 0.0005	0.1430 ± 0.0003
1.40	0.1057 ± 0.0017	0.0681 ± 0.0012
Intercept	-0.0111	-0.0106
Slope	0.0822	0.0555
r^2	0.9993	0.9997
Mean ± Std dev		n = 3

Table 3.29 Gelatin standard curve.

Conc ($\mu g \cdot mL^{-1}$)	$Abs_{(220)}$	$Abs_{(272)}$
77.44	0.6885 ± 0.0072	0.0183 ± 0.0023
58.08	0.5251 ± 0.0059	0.0168 ± 0.0041
38.72	0.3570 ± 0.0089	0.0103 ± 0.0017
19.36	0.1782 ± 0.0102	0.0089 ± 0.0104
Intercept	0.0125	0.0049
Slope	0.0088	0.0002
r^2	0.9883	0.3912
Mean ± Std dev		n = 3

The absorbance values and calculated concentration values are presented in Table 3.30.

Table 3.30 Measured values for apomorphine and gelatin in the presence of glycerin.

Conc ($\mu g \cdot mL^{-1}$) Actual			Absorbance		Conc ($\mu g \cdot mL^{-1}$) Found	
Apo	**Gel**	**Gly**	**220 nm**	**272 nm**	**Apomorphine**	**Gelatin**
5.61	41.50	21.00	0.8476 ± 0.0107	0.3202 ± 0.0068	5.75 ± 0.13	43.85 ± 0.43
5.61	41.50	7.35	0.8126 ± 0.0086	0.3124 ± 0.0037	5.62 ± 0.07	41.06 ± 0.40
5.61	14.53	21.00	0.5807 ± 0.0082	0.3049 ± 0.0042	5.61 ± 0.08	14.80 ± 0.25
5.61	14.53	7.35	0.5812 ± 0.0019	0.3062 ± 0.0011	5.64 ± 0.02	14.63 ± 0.07
1.96	41.50	21.00	0.5181 ± 0.0034	0.1092 ± 0.0020	1.96 ± 0.04	41.84 ± 0.09
1.96	41.50	7.35	0.5115 ± 0.0042	0.1063 ± 0.0025	1.91 ± 0.05	41.55 ± 0.05
1.96	14.53	21.00	0.2850 ± 0.0035	0.1043 ± 0.0021	2.00 ± 0.04	14.97 ± 0.26
1.96	14.53	7.35	0.2861 ± 0.0093	0.1048 ± 0.0036	2.01 ± 0.07	15.02 ± 0.55
Mean ± Std dev				n = 3		

The concentration data from Table 3.30 expressed as a % of found over theoretical are presented in Table 3.31:

Table 3.31 % measured/actual of apomorphine and gelatin in the presence of glycerin.

Conc ($\mu g \cdot mL^{-1}$) Actual			% Found/Actual	
Apomorphine	**Gelatin**	**Glycerin**	**Apomorphine (%)**	**Gelatin (%)**
5.61	41.50	21.00	102.6 ± 2.2	105.7 ± 1.1
5.61	41.50	7.35	100.2 ± 1.2	98.94 ± 0.96
5.61	14.35	21.00	100.1 ± 1.3	101.4 ± 2.0
5.61	14.35	7.35	100.5 ± 0.3	100.7 ± 0.5
1.96	41.50	21.00	100.0 ± 1.8	100.8 ± 0.2
1.96	41.50	7.35	97.62 ± 2.30	100.1 ± 0.1
1.96	14.53	21.00	102.0 ± 1.8	103.1 ± 1.8
1.96	14.53	7.35	102.4 ± 3.3	103.4 ± 3.8
Mean ± Std dev			n = 3	

The data from Table 3.31 indicate that apomorphine and gelatin can be measured with accuracy within about 3% over the range studied and that the presence of glycerin in the concentrations examined, did not pose a problem for quantitation. In all of the combinations studied, the overall apomorphine recovery was 100.6 ± 2.1% and gelatin recovery was 101.3 ± 1.9% of the theoretical amount. The recovery of apomorphine and gelatin for each of the combinations were assessed using ANOVA. There were no significant differences in the recovery rate for either apomorphine ($p = 0.109$) or gelatin ($p = 0.123$) in any of the combinations studied. Using the regression data from the standards and applying equation 3.6, the limits of detection were estimated at 0.8 $\mu g \cdot mL^{-1}$ and 3.8 $\mu g \cdot mL^{-1}$ for apomorphine and gelatin respectively. The effective ranges for the assay were therefore from 1-5 $\mu g \cdot mL^{-1}$ for apomorphine and from 5-40 $\mu g \cdot mL^{-1}$ for gelatin.

Conclusion:

The results of these experiments indicated that both apomorphine and gelatin could be measured simultaneously using direct UV spectroscopy. This method provides suitable accuracy and precision for the concentration ranges investigated and would be suitable for dissolution/release studies of the proposed apomorphine insert provided that the studies are conducted using about 350 mL of dissolution medium. Under these conditions, there is no interference from any of the other components which might be present in the inserts.

Conclusions: Section 3

Published HPLC analytical methods for apomorphine were reviewed and a series of preliminary trials were run using several of the methods; these trials showed that the methods investigated had problems with precision which was possibly due to asymmetrical peaks due to tailing which was most noticeable with the C_{18} column. It should be noted that the majority of these methods underwent modification in later studies suggesting that perhaps some improvement to the method was required. A systematic set of experiments were conducted to determine the best-performing column, mobile phase and detection system and this resulted in the development of a simple isocratic HPLC method which appeared to be suitable for apomorphine analysis in a gelatin-based ocular insert. The HPLC method was validated using the USP criteria and it was shown that the HPLC method met the validation criteria as outlined in the corresponding experimental sections.

For gelatin analysis, the usual colorimetric assays for protein were unsuccessful either due to solubility problems or severe interference from the apomorphine content of the insets. Direct UV spectroscopy however, worked well and it was possible to simultaneously measure apomorphine and gelatin under conditions expected for dissolution studies.

Section 4 – Insert fabrication

Overview:

Ocular inserts have a number of potential advantages over other dose forms as a system for delivering medication either locally to the eye or systemically using the eye as an entry point. Bonferoni et al [357] discussed precorneal loss due to lacrimal flow and blinking and demonstrated that these problems could be reduced through the use of a solid carageenan-gelatin delivery system. Friedrich et al [358] discussed the pharmacokinetic difference between ocular inserts and eye drops and showed significantly improved bioavailability with solid ocular inserts. Lee et al [359] used a gelatin-based insert to deliver phenylephrine and tropicamide in order to induce mydriasis prior to ophthalmoscopic examination and these authors showed improved efficacy and reduced adverse effects, particularly local irritation, with the insert compared to traditional drop formulations. A number of ocular inserts have been described and these include inserts containing cellulose derivatives for treatment of 'dry eye' or keratoconjunctivitis sicca [360, 361], inserts for delivery of local anesthetics [362] and inserts for prolonged release of antibiotics [363-368]. The use of pilocarpine for the treatment of glaucoma and the high frequency of dosing required has led to a number of polymeric delivery systems and devices for this drug [238, 369-371]. Gurtler and Gurny [372] reviewed the literature for ocular inserts and reported a listing of those for which patents had been granted; most of these designed for local effect and all showed improved bioavailability of the agents being delivered. They defined an ophthalmic insert as being a sterile product with a solid or semi-solid consistency in a size and shape suitable for ocular application. These inserts can be used for topical or systemic therapy and the purpose of this dose form is to increase the contact time between the device and the ocular issues to ensure a sustained release and subsequent therapeutic effect. The devices designed to produce systemic effect usually used absorbable gelatin as a carrier. The authors divided the ocular inserts into two general

groups; insoluble and soluble and in the insoluble groups described diffusional, osmotic and contact lens systems. A diffusional system consisted of a central reservoir of drug enclosed by a semi-permeable membrane where the solvent system in the reservoir was glycerin, propylene glycol or an oil mixture and the membrane was composed of polycarbonate, polyvinyl or polyamine derivatives. Osmotic systems were composed of a central part with two components; the drug is surrounded by polymer dispersed in an osmotic solute and the entire device enclosed by a semi-permeable membrane. The matrix polymer is usually based on an ethylene vinyl ester, the osmotic solute may be sodium chloride, calcium lactate or a phosphate salt and the enclosing membrane is usually composed of a cellulose acetate derivative although little information on the composition of the contact lens systems was presented as these are usually proprietary. Drug loading is achieved through soaking the device in a solution of the drug followed by a drying process. The soluble group consisted of inserts made from natural materials or synthetic polymers. The natural materials such as collagen were loaded with drug again through a soaking/drying process. Synthetic polymers used are generally cellulose derivatives containing plasticizers such as polyethylene glycol, propylene glycol or glycerin and these may be coated with an enteric polymer such as cellulose acetate phthalate. The last in the soluble group is described as bioerodible and the matrix material is usually cross-linked gelatin or polyester or polycarbonate derivatives. In this review the authors pointed out that although there are numerous advantages with these delivery systems and many have been patented, very few have gained commercial acceptance. The authors attributed this to cost, difficulty or discomfort in use and a general reluctance for prescribers and patients to accept an unfamiliar dosage form. The authors were optimistic regarding the future and felt that due to the evolution of drugs through biotechnology where the best route would be parenteral, an ophthalmic route might be an option more acceptable to patients.

Although an ocular insert has been used as a means of preventing or reducing systemic absorption of a drug [359], there are situations where ocular delivery for systemic effect is desirable and some of these situations have been discussed by Chiou [373]. For some drugs oral administration is not feasible and the primary route of administration is parenteral. In order to avoid the discomfort and inconvenience associated with this route, the ocular route may provide an attractive alternative. In his discussion, Chiou claimed that ocular delivery could be as precise and rapid as parenteral administration, first-pass metabolism could be avoided and that the ocular tissues are much less prone to the development of immunological reactions [373, 374]. Ocular delivery of insulin has been studied [375] and while it is possible to deliver insulin by this route, a drop formulation produces low therapeutic efficiency in terms of a short duration of action and low bioavailability. The efficacy of the ocular route for insulin delivery has been significantly improved by providing insulin in a gelatin matrix [374, 376]. In formulating a drug for systemic effect via the ocular route a number of factors need to be considered including finding a release rate giving an appropriate concentration-time profile, compatibility of the drug and matrix with ocular tissues and the release of drug must be in a consistent and reproducible pattern. The ocular delivery of systemic insulin was reviewed by Lee et al [376]. They discussed the use of insulin solutions which although absorbed by the eye, generally gave low bioavailability values. Although the bioavailability of insulin could be increased through the use of absorption enhancers, the increase was not large; some of the enhancers discussed included surfactants such as benzalkonium chloride, Brij-78 and cholate. In a study of systemic insulin delivery to dogs by the ocular route, Morgan and Huntzicker [377] assessed a number of enhancers. In this study, insulin was applied as an eye drop and insulin alone was compared to insulin with enhancer. Although the data showed that absorption was somewhat erratic, they did demonstrate that insulin could be absorbed systemically by dogs using the ocular route and several of the enhancers were able to increase

the bioavailability of insulin. The use of an ocular insert composed of absorbable gelatin sponge for insulin delivery was examined by Lee et al and then the use of this device with absorption enhancers was compared to the use of insulin solution [378, 379]. Although they showed that the insulin bioavailability was improved by the use of the insert and enhancer combination, they later published a paper recommending the use of an insert without enhancer [380]. At the conclusion of the review, Lee suggested that for ocular insulin delivery better success in improving bioavailability was achieved through the mechanism of increasing contact time by the use of an insert than through the use of absorption enhancers. Lee later described the use of absorbable gelatin sponge material as a matrix to deliver a mixture of phenylephrine and tropicamide for local use in the eye [359]. A study of the release kinetics for several ophthalmic drugs from an erodible ocular insert was presented by DiColo and Zambito [381] and the description and characterization of a carrageenan-gelatin ocular insert was described by Bonferoni et al [357] suggesting that this type of delivery system is receiving attention as a means of delivering a variety of drugs for both local and systemic effects. Apart from insulin, ocular delivery for systemic effect has been investigated for calcitonin [382] and this route of administration has potential for polypeptide drugs [373].

In a review of the pharmacokinetics of ophthalmic delivery systems, Frangie [245] also considered newer systems and discussed hydrophilic bandage lenses, collagen shields and ocular inserts. Hydrophilic bandage lenses (HBLs) are essentially soft contact lenses which are worn on an extended basis for therapeutic purposes and they have been used for the treatment of corneal lacerations and persistent epithelial defects [383, 384]. They have also been used as a non-erodible vehicle for drug delivery and an example of this involved the prolonged therapeutic effect of pilocarpine when a lens was soaked in pilocarpine solution prior to insertion [385]. Other studies along these lines of loading the lens with drug by soaking prior to use, have shown some problems; release is generally non-zero and a pulse-dose type of

release is seen. The author also reported that there is also some risk to the patient including corneal edema, uveitis and most seriously, infectious kerititis; this type of delivery system seems to have largely abandoned today.

Collagen shields made of porcine scleral collagen which hydrolyses slowly on contact with the cornea have been used as an erodible controlled-release device. Drug loading is usually accomplished by soaking the material in a solution of the drug and allowing it to dry. In a study of Pseudomonas kerititis in a rabbit model by Hobden [386], drug release was prolonged and the shields were well tolerated by the animals. In this study kerititis was induced in the rabbits by an intrastromal injection of Pseudomonas aeruginosa and the subjects treated with either a topical 4% tobramycin drop applied every 30 minutes or by placement of a collagen shield rehydrated in 4% tobramycin. There was no difference between the two groups in terms of colony-forming unit reduction but the treatment with the collagen shield was more convenient and exposed the patient to much less drug. In a placebo-controlled trial using the same model, Clinch et al [387] showed that application of a treated shield which was supplemented by additional tobramycin in the form of a drop was more effective in reducing the number of viable organisms than either the shield alone, the drops alone or the saline placebo. In his review [245], Frangie suggested that the pulse-dose type of release from collagen shields was still problematical.

Ocular inserts may be erodible or non-erodible and an example of a non-erodible device which went to market in the USA is the Ocusert® which consists of a pilocarpine layer surrounded by a double wall of polymer membrane sealed peripherally. The insert is placed into the ocular cul-de-sac and drug is released across the polymer membrane in a zero-order fashion. An example of an erodible system is Lacrisert™ which is a small rod of a cellulose derivative which is placed into the ocular cul-de-sac and although it contains no active drug, it is used to augment a defective precorneal tear film. These devices were discussed in the

review by Frangie [245]. Disadvantages with these systems are difficulty in insertion and the patient has to be able to accommodate a feeling of having a foreign body in the eye.

Experimental 4.1 – Insert fabrication

Introduction:

For this project, rapid but controlled release is desired and a matrix type of delivery system would be appropriate. Only two reports could be found where apomorphine was delivered through a matrix system. In one report, Ugwoke et al [388] formed gelatin microspheres containing apomorphine to be studied in the treatment of Parkinsonism. In the second, Raasch et al [104] developed and conducted release studies, both in vitro and in vivo using rats, of an insert containing apomorphine in an ethylene vinyl acetate polymer which was also to be used in the treatment of Parkinson's disease. Both of these systems were designed for long-term release of apomorphine with the microsphere system to be used as for nasal administration while the system by Raasch et al was designed as a subcutaneous implant system.

In the specifications for the apomorphine ophthalmic inserts, it was determined that the insert would possess a number of qualities and these attributes included:

-be non-irritating to the conjunctival membranes

-be easy to apply and soft and adhesive enough to remain in place

-release most of the drug within a five to ten minute time window

-have a robust matrix to allow for easy removal when emesis has occurred

-have sufficient chemical stability to allow a reasonable shelf life

-insert must be able to be sterilized

From a review of polymeric delivery systems used in the eye and in view of the above specifications a number of experiments were planned to determine what materials would

provide a suitable matrix for the insert, which if any added substances might be required and a suitable fabrication procedure which would allow for the preparation of sterile inserts.

Several problematical issues which could arise were anticipated. These included finding a matrix combination which would allow for rapid but controlled release while maintaining sufficient product integrity to allow for easy removal from the eye after the therapeutic end-point has been reached. The in-vitro release character will be related to the in-vivo response but this relationship will likely be complex and may require formulation adjustments depending on the outcome of preliminary clinical trials. The inherent instability of apomorphine to oxidative degradation will likely require the addition of added substances such as an antioxidant or buffer system but the total solute load of the insert will be limited since ocular irritation will be a factor; mild irritation will be necessary to ensure adequate tear flow to provide solvent to allow drug release but excessive tearing will result in the drug being washed away before absorption can occur. Many of the polymers used for ocular delivery are affected by exposure to heat so providing a sterile product may present some challenges. The packaging system for the inserts must protect the inserts yet be convenient for the clinician to use.

Formulation design, by its very nature, requires some element of trial and error so in view of the problematic issues raised, the design of these experiments should provide sufficient information that formulation adjustments can be made in a fashion that the outcome of the adjustment can be predicted. This suggests that wherever possible a factorial design should be used as in some cases it may be possible to develop a mathematical model to predict outcomes of formulation changes in a quantitative manner. In cases where the outcome cannot be objectively quantified and modeling is therefore not possible, this type of study design will still provide some level of insight into how the formulation change will affect product performance.

Based on the above review of polymers and plasticizers which have been used in the eye, gelatin, polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC) were selected as candidate polymers and glycerin and triethyl citrate were selected as plasticizers. A number of arbitrary decisions were made; a circular insert about 6 mm in diameter and 1 mm in thickness would be an appropriate size for insertion into the conjunctival sac and the method of preparation would be casting of a solution to form a film. The dimensions were thought to provide an insert which was large enough and suitably robust for the clinician to handle and place into the eye yet small enough to fit easily into the eye. Punches with a 6 mm diameter are also readily available and could be sterilized by either dry heat of autoclaving. Casting from solution was selected as the fabrication method since this technique would most likely provide suitable content uniformity of the inserts and having the materials in the form of a solution could allow sterilization by membrane filtration. Sterile disposable Petri dishes with a diameter of 100-mm were selected as being an acceptable container for casting the inserts; the plates are uniform, sterile and readily available.

Although there is a great deal of inter-patient variability, the emetic dose selected for apomorphine in canines was 0.1 $mg \cdot kg^{-1}$ [165] so the drug load per insert was set at 2 mg. Since the drug release will be controlled, this drug load would be appropriate for patients with weights ranging from 5 to 20 kg although larger patients may require an insert in each eye and very small patients may require only ½ of an insert. Due to difficulties in proving a reliable way of distinguishing one insert from another and the high potential of the drug for toxicity, the idea of inserts with varying drug loads was discarded for the present and only inserts containing 2 mg of apomorphine were used for this project.

Preliminary studies suggested that for the polymers being investigated, a load of about 5 g cast into a 100-mm Petri dish would provide a film with a thickness of about 1 mm after curing. The film can then be removed from the dish and cut using a 6 mm punch. In order to

provide a drug load of 2 mg per insert, the ratio of the cured matrix from the Petri dish area to insert area was calculated and used to calculate the quantity of apomorphine required for the batch. The average diameter of the cured matrix material from the Petri dish was found to be 91 mm therefore the area was 6500 mm^2 while the area of each insert was 28.3 mm^2 so the required load of apomorphine for each batch would be 460 mg in order to provide inserts with a content of 2 mg of apomorphine.

Equipment and materials:

The equipment and materials used in this series of experiments was: a chromatographic system manufactured by Knauer (Berlin, Germany) which consisted of a Wellchrom K-501 pump, a Wellchrom K-2501 variable wavelength UV detector, a Basic-Marathon autosampler type 816 and a Wellchrom HPLC Interface box. A Waters (Milford, MA, USA) model 420 fluorescence detector and a Bioanalytical Systems Inc (Lafayette, IN, USA) model LC-4B electrochemical detector were used in tandem with the Knauer system via the HPLC Interface box. The operating, data acquisition and data analysis software used was Eurochrome 2000® (Knauer, Berlin, Germany). The cyano HPLC column used was purchased from Jones Inc (Hengoed, UK) and all chromatography was performed at ambient temperature.

The strip-packaging equipment was manufactured by Cadet Packaging (Toronto, ON, Canada) and the blister packaging materials were obtained from PharmaSystems (Toronto, ON, Canada). The Roche friabulator used was produced by Erweka (Frankfurt, Germany).

Apomorphine HCl, triethyl citrate, hydroxypropylmethylcellulose, polyvinylpyrrolidone, and polyvinyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade phosphoric acid and acetonitrile as well as analytical grade hydrochloric acid, sodium hydroxide and trypticase soy broth and agar were purchased from Fisher Scientific (Nepean, ON, Canada). Gelatin USP, glycerol USP and ascorbic acid USP

were purchased from Spectrum Chemical (Gardenia, CA, USA); gelatin was also purchased from Sigma-Aldrich (St. Louis, MO, USA). De-ionized water was obtained using an Aqua-Summa II reverse-osmosis system (Culligan Toronto, ON, Canada). All glassware used was Class A (Fisher Scientific, Nepean, ON, Canada) and where required, dilutions were done using a 1000 μL adjustable volume pipette (Hamilton Company, Reno, NV, USA). A model AE-50 analytical balance (Mettler, Hightstown, NJ, USA) was used for weighing. Filtration of mobile phase was accomplished using 0.2 μm membranes (Pall, Gelman Laboratories, East Hills, NY, USA). Plastic Petri dishes were purchased from Fisher Scientific (Nepean, ON). Bacterial cultures (ATCC) were obtained from Christope Technologies (Lake Charles, LA, USA).

Water baths were purchased from VWR International (Sheldon Manufacturing Inc, Cornelius, OR, USA) and a biological containment cabinet manufactured by Baker Co (Sanford, ME, USA) was used.

Standard solutions of apomorphine HCl in 0.05 M HCl were prepared, stored in glass at 5° C and discarded after seven days. Statistical analysis and graphical presentations were done using SigmaStat ® (SPSS Inc Chicago, IL, USA), Excel 2000® (Microsoft) and SigmaPlot 2000® (SPSS Inc Chicago, IL, USA).

All statistical analysis was done at the 95% confidence level unless otherwise indicated and data are presented as mean ± standard deviation unless otherwise indicated.

Experiment 4.1.1 – Polymer and plasticizer selection:

Purpose:

A series of experiments were done in order to screen various combinations of polymer and plasticizer for suitability as matrix material for the ocular insert. The indexes of suitability are governed by the six criteria outlined at the beginning of this section as product attributes.

Study design and method:

Polymers selected for evaluation were gelatin, polyvinylpyrrolidone and hydroxypropylmethylcellulose and plasticizers selected were glycerin and triethyl citrate. Preliminary screening of polymer-plasticizer combinations suggested that films could be formed using one part of glycerin to one and one half parts polymer and one part of triethyl citrate to nine parts of polymer so these ratios were used as the mid-point and three levels of concentration were examined for each polymer-plasticizer combination. The matrix formulations evaluated are presented in Table 4.1.

Casting of a thick film from a solution was selected as the method of preparation as this method provided a high likelihood of homogeneity of the product in terms of polymer, plasticizer, drug and any added substances which may be required. Since all of the materials used were water soluble, water was used as the solvent with a total solute load of 5 g. Solutions were cast in 100-mm Petri dishes and the films were formed and cured by placing them in a level class 'A' biological containment cabinet with a vertical air flow of 24 $m \cdot min^{-1}$. A film was usually formed after 24 hours at which time the film could be pulled from the Petri dish and cured for 48 hours in a container over anhydrous silica gel. Inserts of 6-mm diameter could be cut using a circular punch and these could then be evaluated for suitability.

Table 4.1 Matrix formulations. PVP = polyvinylpyrrolidone and HPMC = hydroxypropylmethylcellulose.

Formulation	Polymer		Plasticizer	
1	Gelatin	4.75 g	Ethyl citrate	0.25 g
2	Gelatin	4.50 g	Ethyl citrate	0.50 g
3	Gelatin	4.00 g	Ethyl citrate	1.00 g
4	Gelatin	3.50 g	Glycerin	1.50 g
5	Gelatin	3.00 g	Glycerin	2.00 g
6	Gelatin	2.50 g	Glycerin	2.50 g
7	PVP	4.75 g	Ethyl citrate	0.25 g
8	PVP	4.50 g	Ethyl citrate	0.50 g
9	PVP	4.00 g	Ethyl citrate	1.00 g
10	PVP	3.50 g	Glycerin	1.50 g
11	PVP	3.00 g	Glycerin	2.00 g
12	PVP	2.50 g	Glycerin	2.50 g
13	HPMC	4.75 g	Ethyl citrate	0.25 g
14	HPMC	4.50 g	Ethyl citrate	0.50 g
15	HPMC	4.00 g	Ethyl citrate	1.00 g
16	HPMC	3.50 g	Glycerin	1.50 g
17	HPMC	3.00 g	Glycerin	2.00 g
18	HPMC	2.50 g	Glycerin	2.50 g

The films and discs resulting from the experiment were evaluated by grading the attributes of flexibility, clarity, tackiness and integrity after soaking in water (35°C) for 10 minutes. In terms of flexibility, the ideal film would be flexible enough to conform to the curvature of the eye but rigid enough to be handled and easily inserted into the conjunctival sac. This attribute was graded with a score from 1-5 with the higher score being given for a

good balance between rigidity and flexibility. The attribute of clarity was considered to be of lesser importance and was graded with a score from 1-3. Clarity in the film was desirable from an esthetic perspective but also would allow detection if one of the components of the product came out of solution during the casting or curing process which could lead to problems with content uniformity. Tackiness was another attribute where balance was important and it was graded with a score from 1-5. The film required sufficient tackiness to adhere to the eye tissue but not so tacky as to adhere to the surface of the packaging material and be difficult to use. Integrity after soaking in water was considered very important since one of the goals of the delivery system was to allow removal of the drug reservoir after the therapeutic end point of emesis was achieved. In order to allow easy removal at this point, it would be important that the device was intact and still contained what would be excess drug for the treatment. This attribute was scored from 1-6.

Results and discussion:

The polymer and plasticizer combinations were prepared and cast as described under study design and methods. After curing, the resulting films were graded as described and a total score assigned to each formulation. These data are presented in Table 4.2

Table 4.2 Attribute scores for matrix formulations

Formulation	Flexibility	Clarity	Tackiness	Integrity	Total
1	1	1	5	4	11
2	1	1	5	4	11
3	1	1	5	4	11
4	4	3	5	5	17
5	5	3	5	6	19
6	5	3	4	5	17
7	1	1	4	3	9
8	1	1	4	3	9
9	1	1	4	3	9
10	5	3	2	4	14
11	5	3	2	4	14
12	5	3	2	4	14
13	4	3	4	4	15
14	3	2	4	4	13
15	2	1	4	4	11
16	3	2	5	4	14
17	4	2	5	4	15
18	5	2	5	3	15
Range	**1 – 5**	**1 – 3**	**1 – 5**	**1 – 6**	**4 - 19**

The subjective overall assessment was that formulations 1 to 3 were somewhat brittle and opaque; formulations 4 to 6 gave acceptable films; formulations 7 to 9 were somewhat brittle; formulations 10 to 12 were quite tacky; formulations 13 to 15 were quite brittle and formulations 16 to 18 gave acceptable films. The variables of polymer, plasticizer and level of

plasticizer were coded as shown in Table 4.3 and these data analyzed using multiple linear regression (MLR).

Table 4.3 Factorial design with coded values

Formulation	b1	b2	B3	b11	b12	b13	b23	b22	B33	b123	y
1	1	1	1	1	1	1	1	1	1	1	11
2	1	1	0	1	1	0	0	1	0	0	11
3	1	1	-1	1	1	-1	-1	1	1	-1	11
4	1	-1	1	1	-1	1	-1	1	1	-1	17
5	1	-1	0	1	-1	0	0	1	0	0	19
6	1	-1	-1	1	-1	-1	1	1	1	1	17
7	0	1	1	0	0	0	1	1	1	0	9
8	0	1	0	0	0	0	0	1	0	0	9
9	0	1	-1	0	0	0	-1	1	1	0	9
10	0	-1	1	0	0	0	-1	1	1	0	14
11	0	-1	0	0	0	0	1	1	1	0	14
12	0	-1	-1	0	0	0	1	1	1	0	14
13	-1	1	1	1	-1	-1	1	1	1	-1	15
14	-1	1	0	1	-1	0	0	1	0	0	13
15	-1	1	-1	1	-1	1	-1	1	1	1	11
16	-1	-1	1	1	1	-1	-1	1	1	1	14
17	-1	-1	0	1	1	0	0	1	0	0	15
18	-1	-1	-1	1	1	1	1	1	1	-1	15

		Value		
Factor	**Code**	+1	0	-1
Polymer	b1	Gelatin	PVP	HPMC
Plasticizer	b2	Ethyl citrate	-	Glycerin
Plasticizer level	b3	High	Intermediate	Low
Response score	y	-	-	-

The results of the MLR analysis are presented in Table 4.4 and from these data it can be seen that the selection of plasticizer (b2) in this model is very important with ethyl citrate having a negative effect on the overall score of the formulation. The interaction between the polymer and the plasticizer (b12) is also important with the combination of gelatin and

glycerin having the most positive effect. The level of plasticizer present (b3) within the limits examined does not appear to be an important factor nor do the interactions between polymer, plasticizer and plasticizer level (b123).

Table 4.4 Results of MLR of coded formulation factors.

Factor	Code	Coefficient	P
Polymer	b1	0.250	0.637
Plasticizer	b2	-2.222	<0.001
Plasticizer level	b3	0.250	0.637
Polymer/Plasticizer	b12	-1.250	0.035
Polymer/Plasticizer level	b13	-0.375	0.564
Plasticizer/plasticizer level	b23	0.417	0.436
Polymer/Plasticizer/plasticizer level	b123	-0.625	0.344

Based on these data, the combination of gelatin and glycerin provide the closest fit to the desired insert attributes and although not a significant factor within the range studied, glycerin at an intermediate concentration was selected.

The selection of gelatin as the matrix material is further supported by other work done using gelatin as a matrix material. Young et al [341] recently reviewed the biomedical application of gelatin. Gelatin is a polymer derived from natural collagen and is useful as a matrix material because it is biodegradable and has a high level of physiological compatibility. Also it can be easily modified by cross-linking so that as a material it can have a wide range of physicochemical properties in terms of solubility and swelling behavior [389, 390]. Gelatin has been used in applications involving tissue engineering [341]. A variety of growth factors known to enhance bone formation have been placed in modified gelatin microspheres and although these factors when injected as a solution are short-lived and disappear rapidly from

the site of injection, the microspheres remain in place, slowly release the factors over time and result in bone regeneration. This was demonstrated by Yamada et al [391] and later by Hing et al [392] using a rabbit model of a skull bone defect. Delivery of basic fibroblast growth factor (bFGF) has the ability to promote angiogenesis and this property was investigated by Sakakibara et al [393] where gelatin microspheres loaded with bFGF induced coronary collateral growth and this technique may be of use in patients where coronary angioplasty or bypass surgery is not possible. In the area of gene therapy, viral vectors are associated with a high level of efficiency but there is the possibility of immunological or toxic responses to the vectors themselves [341] so gelatin hydrogels have been investigated as a way to improve gene delivery efficiency by allowing for the controlled release of the plasmid DNA and increasing the probability of transfection at the site of delivery; this was investigated and demonstrated by Kaspar et al [394]. Overall, controlled release using gelatin matrices may help increase the therapeutic efficacy of biomolecules which would otherwise be short-lived if injected directly. By protecting peptides or DNA and providing a reservoir for continued release over time, gelatin-based matrices may aid in keeping the agent localized and biologically active [341].

Gelatin matrices have been investigated as delivery systems for a variety of drugs where prolonged localized release is desirable. Examples would be the use of antibiotics to treat bone infections or the use of antineoplastic agents to treat solid tumors; these applications may be considered an elementary type of tissue targeting [395, 396].

Gelatin, particularly when formulated into microspheres, has been used to deliver insulin [397], ketoprofen [398], interferon [399] and the antibiotics gentamicin [400] and cefadroxil [401]. For treatment of solid tumors where again high prolonged localized drug levels are desired, gelatin-based dose forms for methotrexate [402, 403], cisplatin [404, 405], carboplatin [406], doxorubicin [405, 407, 408] and 5-fluorouracil [409, 410] have been

studied. The use and preparation of gelatin-based hydrogels for drug carrier matrices was discussed by Einerson et al [342].

Two different types of gelatin can be produced depending on the method used to pre-treat the collagen; pretreatment with alkali hydrolyses the amide groups of asparagine and glutamine producing free carboxylic acid functional groups whereas pretreatment with acid does not [341]. The result is that alkali-treated collagen yields gelatin with a larger number of free carboxylic acid groups making it more negatively charged and lowering the isoelectric point compared to acid treated collagen. This allows for flexibility in terms of enabling polyion complexation of the gelatin matrix with either positively or negatively charged drugs. Acidic gelatin should be used for basic proteins or drugs while basic gelatin should be used for acidic agents [403].

An important advantage of gelatin as a drug carrier is that the solubility and swelling behavior can be modified by crosslinking. Chemical crosslinking agents are of two types; non-zero length and zero length. Non-zero length agents are bifunctional and work by bridging free carboxylic acid or amine residues between adjacent gelatin molecules and examples of these are aldehydes, polyepoxides and isocyanates [411]. Zero length agents activate carboxylic acid groups to react directly with amine groups on adjacent gelatin molecules without introducing molecules between the groups and examples of these agents are acyl azides and water-soluble carbodiimides [341, 411]. Ofner and Bubnis [389] developed an analytical method for determining the extent of crosslinking by measuring the number of free amino residues present in the gelatin using reaction with trinitrobenzene sulfonic acid (TNBS) and subsequent colorimetric measurement. In their study which described this method, gelatin was crosslinked with gluteraldehyde or a water-soluble carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and the authors demonstrated that the release rate of a drug could be slowed by crosslinking gelatin and the more extensive the crosslinking, the slower the release. Welz and

Ofner [390] studied release from crosslinked gelatin matrices using chlorpromazine as a model drug. Their findings suggested that release from a gelatin matrix was primarily by a diffusional process and that the rate of swelling and subsequent drug release could be controlled by the extent of crosslinking. Young et al [341] in their review of gelatin as a delivery vehicle pointed out that the extent of crosslinking could be increased, within limits, by either using higher proportions of crosslinking agent or longer exposure times.

A number of reports have expressed concern regarding possible toxicities resulting from residual crosslinking agent being present in the final matrix material and a number of these investigated the use of less toxic agents or alternatives to chemical crosslinking. The use of native and oxidized mono- and disaccharides as crosslinking agents was investigated by Cortesi et al [412]. In their study, both microspheres and matrix disks were prepared using gelatin combined with either native or oxidized sugars; glucose, fructose and sucrose were used and oxidation was accomplished by reaction with m-periodate. The authors proposed that crosslinking would occur with the native sugars glucose and fructose through reaction of the aldehydes group of the sugars with amino residues on the gelatin. Oxidized sugars which are dialdehydes would react in a similar fashion but might be more efficient in forming bridges between gelatin molecules. They assessed crosslinking through differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) measurement and showed that crosslinking did occur and that the oxidized sugars were more efficient. A study investigating the use of D,L-glyceraldehyde as a crosslinking agent was conducted by Vandelli et al [413]. They studied the effect of varying concentrations of glyceraldehyde and varying reaction times and showed that both increased concentration and exposure time resulted in more extensive crosslinking; this was measured by use of the TNBS method [389] and release studies of a model drug. The drug they investigated was clonidine and administration by subcutaneous injection to hypertensive rats showed a prolonged effect

compared to a control solution of clonidine. Cortesi et al [414] also investigated the use of dextran as a crosslinking agent. In this study, the authors oxidized dextran with m-periodate to produce the active crosslinking agent and used the 2'-bromo derivative of 1,3 di-(p-aminophenoxy)-bis-2,2-(p-aminophenoxymethyl)propane (TAPP-Br), an experimental antineoplastic agent, as a model drug. The in vitro results of this study demonstrated that crosslinking did occur and the release of TAPP-Br could be modified through this process. Vandelli et al [415] investigated thermal crosslinking of gelatin using microwave radiation; earlier work had demonstrated that gelatin crosslinking could be achieved by heat and pressure stressing of gelatin [390] although there was some disagreement as to whether this process actually resulted in crosslinking [416, 417]. In Vandelli's study, the authors showed that exposure of gelatin to microwave radiation to produce a temperature of 220°C for a 10 minute period resulted in significant crosslinking as demonstrated by the release of diclofenac which was used as a model drug. From these studies, a number of methods are available to crosslink gelatin and provide material which is capable of being fabricated into a matrix dose form with varying rates of drug release.

Conclusion:

In terms of a preliminary formulation, the following was further investigated:

Apomorphine HCl USP	0.46 g
Gelatin NF	2.80 g
Glycerin USP	2.05 g

The above was dissolved in a suitable aqueous medium and cast in 100 mm sterile Petri dish. After curing each 10 mm circular insert will carry a drug load of 2 mg.

Experiment 4.1.2 – Addition of antioxidant

Introduction:

For the initial casting of inserts using the gelatin and glycerin matrix with apomorphine included, the gelatin and glycerin were weighed into a 100-mL beaker, dispersed with about 35 mL of water and gently heated until the gelatin dissolved. The apomorphine was weighed and dissolved in about 15 mL of water with the aid of gentle heating and when dissolution was complete, the solution was added to the gelatin and glycerin, gently mixed avoiding air entrainment and the solution poured into a 100-mm disposable Petri dish. This mixture was placed in a level class 'A' biological containment cabinet (Baker) with a vertical air flow of about 24 $m \cdot min^{-1}$ and allowed to cure. It was noted that the film formed had a distinct green discoloration suggesting that some decomposition of the apomorphine had occurred during the casting and curing process.

Apomorphine is subject to oxidative degradation to form an inactive quinone [418]. A number of formulation strategies are used to protect drugs from this type of degradation and include protection from exposure to light, excluding oxygen from the final packaging, including antioxidants in the formulation and formulating the product at an acidic pH [419]. Since both ascorbic acid and sodium bisulfite had been shown to function as effective antioxidants for products containing apomorphine and both of these substances have been used in ophthalmic products, these were assessed as potential added substances to extend the shelf-life of the product [86]. A number of authors have suggested that visible discoloration, usually green, of apomorphine may be present even when only 0.1% of the drug has decomposed [420] and that the relationship between the intensity of discoloration and amount of drug decomposed is unclear [86, 421, 422].

In the case of the apomorphine inserts, the discoloration detracted from the pharmaceutical elegance of the product so a trial was conducted to determine whether the

discoloration could be prevented by the inclusion of an antioxidant and sodium metabisulfite and ascorbic acid were selected for evaluation.

Purpose:

The purpose of this experiment was to determine whether the addition of sodium metabisulfite and/or ascorbic acid would prevent discoloration of the inserts during the casting and curing process. The effect of the antioxidants on the degradation rate of apomorphine in the inserts at 80°C was also investigated.

Study design and method:

Four sets of apomorphine inserts were prepared by the casting process already described. One set of inserts were cast from a solution containing both ascorbic acid and sodium metabisulfite, one containing ascorbic acid alone, one containing sodium metabisulfite alone and one containing neither ascorbic acid nor sodium metabisulfite. A concentration of 0.1% of the final casting solution volume was used for both agents; the formulations are presented in Table 4.5. For each formulation, samples consisting of four inserts were weighed and placed into 5-mL type 1 glass vials and polymeric closures applied. Four trials of each formulation were conducted. The vials were than placed into a water bath maintained at 80 ± 0.1°C and samples of each formulation were withdrawn at three day intervals and stored at -20°C until analysis. After all the samples were collected, each was analyzed for apomorphine content using the developed HPLC method. Peak purity was monitored by comparison of the data obtained by UV detection to that obtained from the fluorescence and electrochemical detectors. Data analysis consisted of determining degradation apparent rate constants for each formulation.

Table 4.5 Formulations evaluated. The component quantities were dissolved in about 50 mL of purified water with heating, cast into 100-mm Petri dishes and cured for 48 hours.

		Formulation			
Component	**Quantity (g)**	A	B	C	D
Apomorphine	0.460	+	+	+	+
Gelatin	2.85	+	+	+	+
Glycerin	1.95	+	+	+	+
Sod metabisulfite	0.050	+	-	+	-
Ascorbic acid	0.050	+	+	-	-

Results and discussion:

Films cast with both ascorbic acid and sodium metabisulfite added to the apomorphine casting solution showed no discoloration while films cast from solutions where only ascorbic acid was added showed a pink discoloration and films cast with only metabisulfite added showed a faint blue-grey discoloration. The films cast from solutions with neither ascorbic acid nor sodium metabisulfite were distinctly green in color and from this it appeared that including sodium metabisulfite and ascorbic acid each in concentrations of 0.1% to the casting solution prevented the development of discoloration in the inserts during the casting and curing process.

The apomorphine present in the samples expressed as % of the labeled content (2 mg) is presented in Table 4.6 and these data were analyzed using regression analysis and assuming a first-order degradation process; apparent first-order rate constants and t_{90} values were calculated from this analysis.

Table 4.6 % of Apomorphine remaining after storage at 80°C for formulations A to D.

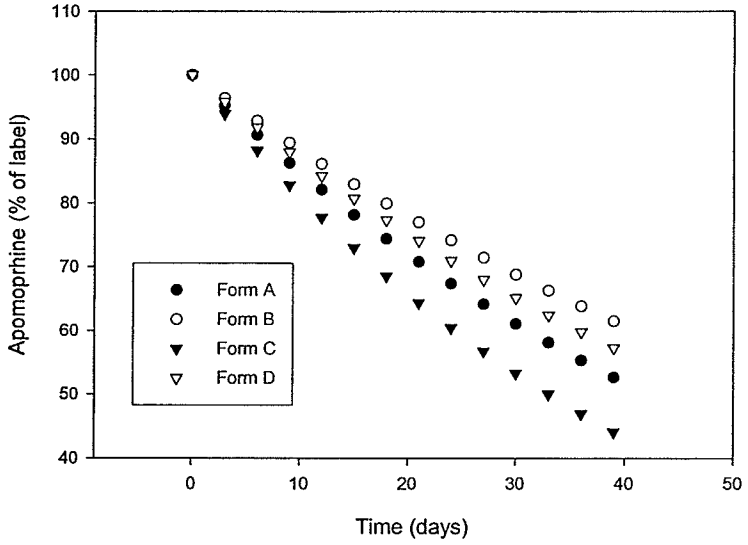
Day	Formulation A	Formulation B	Formulation C	Formulation D
0	99.24 ± 1.88	100.5 ± 2.6	91.72 ± 1.38	103.0 ± 2.9
3	84.92 ± 1.07	89.24 ± 0.42	88.19 ± 1.08	92.29 ± 5.02
6	88.49 ± 0.44	87.84 ± 1.11	76.38 ± 1.18	105.2 ± 5.8
9	83.78 ± 1.96	100.4 ± 1.06	75.97 ± 0.81	85.79 ± 3.89
12	74.72 ± 1.94	81.31 ± 1.01	71.91 ± 0.83	78.94 ± 6.29
15	67.36 ± 1.20	88.68 ± 0.92	78.10 ± 0.96	84.09 ± 3.89
18	71.33 ± 1.07	73.00 ± 1.98	69.80 ± 0.68	78.41 ± 3.51
21	71.03 ± 2.10	80.24 ± 1.23	50.91 ± 0.64	70.36 ± 3.49
24	62.28 ± 1.44	71.90 ± 1.02	57.29 ± 1.09	74.97 ± 4.24
27	63.22 ± 1.21	85.61 ± 1.47	51.51 ± 1.50	68.07 ± 2.99
30	57.14 ± 1.48	64.98 ± 0.78	44.04 ± 1.06	58.81 ± 2.82
33	63.29 ± 1.67	63.54 ± 0.48	56.50 ± 1.72	66.02 ± 2.94
36	61.64 ± 0.85	61.15 ± 0.83	53.62 ± 1.67	60.23 ± 2.36
39	49.33 ± 1.37	60.55 ± 1.34	34.22 ± 0.77	55.28 ± 2.78
Values: Mean ± std dev			n = 4	

The results of the regression analysis and the derived values for the apparent rate constants and the shelf life expressed as t_{90} are presented in Table 4.7.

Table 4.7 Curve fitting of data from Table 4.6 to an apparent first-order process using linear regression. (80°C)

Formulation	Slope	Intercept	r^2	k (day^{-1})	t_{90} (days)
A	-0.00715	1.976	0.946	1.65×10^{-2}	6.4
B	-0.00542	1.987	0.924	1.25×10^{-2}	8.4
C	-0.00912	1.968	0.848	2.10×10^{-2}	5.0
D	-0.00621	1.996	0.930	1.43×10^{-2}	7.4

Figure 4.1 Decomposition of apomorphine in formulations A-D at 80°C following first-order regression model.



A plot of the theoretical degradation pattern for each formulation using the results of the regression analysis are shown in Figure 4.1. From this plot it appears that the presence of bisulfite has a negative effect on the stability of the apomorphine whereas ascorbic acid enhances the stability of the product. The raw data presented in Table 4.6 show the standard deviations found in the analysis of formulation D where neither ascorbic acid nor sodium metabisulfite were added, were larger than those seen with the other formulations and the reason for this is unclear. In order to determine whether this finding can be accounted for by weight variation among the inserts, the weights of all the inserts used in the study were compared. For each formulation 225 inserts were prepared and used; the mean weight and standard deviation for each formulation were 22.6 ± 2.1 for formulation A, 22.2 ± 2.3 for formulation B, 22.9 ± 2.1 for formulation C and 22.8 ± 2.6 for formulation D. Comparison of the weights of each formulation using ANOVA showed that there was no significant difference in the insert weights among the four formulations ($p = 0.461$) so weight variability was not a factor. These data are shown in Table 4.8.

Table 4.8 Sample weights (mg) of four inserts for each formulation with mean ± standard deviation weight of an individual insert. Analysis of variance (ANOVA) of the individual insert weights for each formulation showed no significant difference ($p = 0.461$).

	Total Mass (mg) of Four Inserts for Each Formulation			
Vial	**A**	**B**	**C**	**D**
1	90.1	83.6	88.4	93.1
2	90.3	80.9	81.0	96.5
3	95.6	83.8	87.6	83.6
4	86.5	81.9	91.9	83.8
5	85.7	84.0	90.5	92.6
6	83.3	79.0	87.6	85.7
7	90.6	95.7	90.2	87.5
8	89.8	88.6	80.5	89.8
9	85.5	103.8	103.8	84.2
10	88.0	94.9	102.4	82.5
11	90.0	93.4	100.0	100.1
12	101.8	91.0	88.9	102.0
13	97.9	93.9	96.0	102.8
Insert weight (mg)	22.6 ± 2.1	22.2 ± 2.3	22.9 ± 2.1	22.8 ± 2.6

The findings of this experiment suggest that there is an interaction between bisulfite and apomorphine and in their studies of apomorphine stability, Lundgren and Landersjo [86] noted a rapid but slight decline in apomorphine concentration when it was mixed with bisulfite and heated. Subsequent examination of the solution using paper chromatography showed the presence of a yellow spot which had not been present before and they suggested that this spot might be the product of a reaction between apomorphine and bisulfite. A number of studies have indicated that bisulfite may react with some drugs and in these cases its use as an

antioxidant may be inappropriate. In an early study Higuchi and Schroeter [423] examined the reactivity of bisulfite with a number of drugs and in their findings, the authors demonstrated that drugs with a hydroxybenzyl alcohol grouping reacted with bisulfite to form corresponding sulfonic acid derivatives. Yeh and Lach [424] studied the stability of morphine solutions and showed that morphine interacts with bisulfite to form a stable sulfonic acid analog. From that study the authors suggested that compounds with an alpha-beta unsaturated functional group with a strong electron–attracting group on the alpha position will react with bisulfite. Bonevski et al [425] studied epinephrine oxidation in the presence of sodium metabisulfite and compared this with epinephrine in the presence of cysteine. They reported that in slightly alkaline solutions of epinephrine low concentrations of bisulfite were ineffective in stabilizing the solution which was consistent with the findings of a similar study by Hajratwala [426]. They suggested that cysteine might be a more effective antioxidant than bisulfite for stabilizing solutions of epinephrine. Cho et al [427] investigated the interaction between bisulfite and prostaglandins E_2 and A_2; prostaglandin E_2 readily undergoes dehydration to produce prostaglandin A_2 which in turn isomerizes to prostaglandin B_2. The authors postulated that bisulfite would form an addition complex with prostaglandin E_2 at the C-9 carbonyl group and could stabilize the molecule from dehydration. The results of this study showed that bisulfite had a higher affinity for the C10-11 unsaturated bond of prostaglandin A_2 and bisulfite effectively accelerated the degradation of prostaglandin E_2. Enever et al [428] demonstrated that bisulfite interacted with amitriptyline since addition of bisulfite to a solution of amitriptyline resulted in an immediate fall in drug concentration and a subsequent acceleration of the decomposition rate. The authors suggested that the bisulfite reacted at the double bond in the molecule. Work by Brustugun et al [429-431] demonstrated that the presence of sodium bisulfite had a negative effect on the photostability of epinephrine and a series of related sympathomimetic compounds.

From this experiment, it appears that bisulfite and apomorphine interact and since the use of bisulfite as an antioxidant for apomorphine has been recommended in a number of studies [86, 100-102, 432], it would seem that this interaction merits further study.

Conclusion:

In this experiment, the addition of sodium metabisulfite to the inserts appeared to result in the immediate loss of a small amount of apomorphine but this loss appeared to be reduced when bisulfite and ascorbic acid were used together. Although including sodium metabisulfite and ascorbic acid each in concentrations of 0.1% to the casting solution prevented the development of discoloration in the inserts during the casting and curing process, the residual sodium metabisulfite left in the inserts from the casting and curing process may not have a beneficial effect on the shelf-life of the product although this may be offset by the apparent stabilizing effect of the residual ascorbic acid. There may be some advantage in retaining these excipients in the formulation apart from prevention of early discoloration of the insert; it should be noted that all the formulations darkened with time and this was noticeable after about six months including one without apomorphine. Since tears would be necessary to allow release of the drug, a low level irritation would be desirable whereas excessive tearing would cause the released drug to be washed away before drug absorption could take place. The residual ascorbic acid and sodium metabisulfite left in the inserts from the casting and curing process could cause minor irritation and tearing which would be desirable provided the tearing is not excessive. This would be detected in the clinical trials of the product and if there is a problem with irritation, the addition of these substances during the casting and curing process will need to be reassessed and an alternative found. There may also be some benefit in having the bisulfite present as it has been shown to have some antimicrobial activity and could function as an antimicrobial preservative in the product [433, 434]. In the meantime, the inclusion of sodium metabisulfite and ascorbic acid in the

formulation to prevent discoloration and induce tearing may be useful so it appears appropriate to include both bisulfite and ascorbic acid in the formulation.

Experiment 4.1.3 – Packaging system

Introduction:

The packaging used for the inserts should protect the product and enhance the shelf-life. Since the product will be provided as a sterile product, protecting the inserts from microbiological contamination is important. The packaging must also allow for ease of use by the clinician and since the dose required for an individual patient will vary, a unit of use packaging would be appropriate. Two basic unit dose systems were assessed. The first was a commercial strip packaging system (Cadet Packaging, Toronto, ON) where each insert is placed into a flat pouch which is heat-sealed. One side of the package consists of a plastic film and the other a plasticized laminate of paper and metal foil which supports direct labeling for product identification. The second system consists of a pre-formed plastic well (25 x 10 x 10 mm) into which the insert is placed and then covered with a paper label which has adhesive on one side and supports printed information on the other (PharmaSystems, Toronto, ON).

The basic packed units of either type would be packaged in multiples of 4 or 5 into an opaque white polyethylene plastic container with a screw cap. The size used was 50-mm in diameter and 100-mm in height as 5 units of both packaging types could be contained in it with sufficient room to include a silica-gel desiccant. It had been observed that the inserts were somewhat hydroscopic and became soft and difficult to handle after being exposed to relative humidity levels exceeding 30% so if protection from room humidity was necessary, it could be achieved through the use of plastic screw-top containers and a desiccant. Since the plastic was opaque, the product would also be protected from exposure to light although preliminary experiments suggested the product was not adversely affected by light. The plastic

jars were robust and break-resistant yet very light in weight so were suitable for both shipping and storage.

Purpose:

The purpose of this investigation was to find an acceptable packaging system for the inserts.

Study design and method:

A set of inserts containing apomorphine was prepared and ten were packaged using the plastic film and paper-foil laminate system. A further sample of ten inserts was packaged using the plastic wells and adhesive labels. To assess the durability of the two packaging systems, five samples of each packaging system were individually placed into a Roche friabulator and run at 25 rpm for fifteen minutes which provided a total of 375 drops from a 10-cm height over the test procedure for each sample. After testing, each of the units was visually inspected for damage or any break in the integrity of the packaging.

Samples of both packaging types were sterilized using ethylene oxide. The sterilizing conditions used were 37 °C, relative humidity 35%, an ethylene oxide concentration of 550 $mg \cdot L^{-1}$ and exposure time was about 5 hours. After 48 hours of ventilation/aeration to allow dissipation of the residual ethylene oxide, the condition of the samples was assessed.

Results and discussion:

Both packaging systems were acceptable in terms of visual evaluation; both provided a robust system which appeared to be well-sealed to protect the inserts and no sample showed any visible damage or break in integrity after being run in a friabulator for fifteen minutes at 25 rpm. Although both systems were about equally labor-intensive requiring about 25 seconds of time per unit packaged, the plastic and foil laminate system would be much more suited to automation since the packaging material is provided as a continuous feed from a roll and labeling is done in line with the packaging and sealing. For this trial, the system with the rigid plastic wells required that the labels be prepared separately and in advance and the prepared

inserts must be hand placed into the wells and sealed. Although automation is certainly possible, this system may require more development than the plastic and foil laminate system.

Terminal sterilization with ethylene oxide was a problem with both packaging systems. After sterilization the inserts in both systems were badly deformed and showed strong adherence to the packaging material making them unusable. Although ionizing radiation might provide a satisfactory method of terminal sterilization, the resources necessary to assess this were not available so this could not be investigated and another method of providing a sterile product was required.

Conclusion:

Both packaging systems investigated were acceptable in appearance, labeling and durability however the plastic film-foil laminate system presented a problem in providing a sterile dose form as the inserts would be in direct contact with the equipment and there appeared to be no practical method to sterilize the equipment. Since the attempt at terminal sterilization using ethylene oxide was not successful for either packaging type, it would not be possible to use the laminate system and maintain the sterility of the product. The plastic blisters and labels withstood the gas sterilization process with no apparent degradation and could be sterilized using this method. If the inserts were sterilized using a different process, they could be aseptically packaged into ethylene oxide sterilized blisters and labels and although this is not a terminal sterilization process, it could be acceptable. In terms of a suitable packaging system for the apomorphine inserts, the plastic blisters provided the best option and was the system selected.

Experiment 4.1.4 - Humidity effects

Introduction:

During the casting process, it was noted that the inserts were affected by humidity conditions in that they became soft and tacky in response to high levels of relative humidity. If this happened to the finished product, there would be a possibility the inserts would adhere to the packaging materials and be difficult for the clinician to use.

Purpose:

The purpose of this investigation was to assess the effects of exposure of the inserts to high humidity conditions of storage.

Study design and method:

Six inserts were individually placed into the plastic blister packaging wells with the weights recorded. The inserts and wells were placed into a desiccator with the lower portion containing a slush of water and sodium chloride. Care was taken that no crystals protruded above the surface of the slush but that there were undissolved crystals within the slush. The temperature of the entire apparatus was maintained at 22°C by placing it in an incubator and these conditions provided a relative humidity of 75.6 % as measured by a humidity probe (Omega Engineering Inc). At time intervals over a three hour period, the desiccator was removed from the incubator and each of the inserts and corresponding plastic wells were removed and weighed. A final weighing was done after 24 hours of exposure of the inserts to the 75.6% relative humidity. A plastic blister well without an insert was included as a control.

Results and discussion:

The inserts showed a time-related increase in weight with exposure to high humidity conditions and as the weight increased, the inserts became soft and adhered to the plastic packaging materials. There was no appreciable weight change with the control shell without an insert. A slight distortion of the inserts occurred and they appeared to have swollen during

24 hours of exposure. Following over-night storage in a desiccator charged with dried silica gel, the original shape returned but the inserts remained adhered to the plastic packaging. Although they could be removed with some picking effort, this was an awkward process which would not be acceptable in a clinical use setting. The data from this experiment are presented in Table 4.9 where the weights of the plastic wells, the inserts at time zero and the combined inserts and wells are shown. The net change in weight for the inserts at each time interval is shown in Table 4.10. There was no change in the weight of the control empty well over the 24 hours the system was monitored.

Table 4.9 Weight changes of inserts and plastic wells after exposure to 75.6% relative humidity.

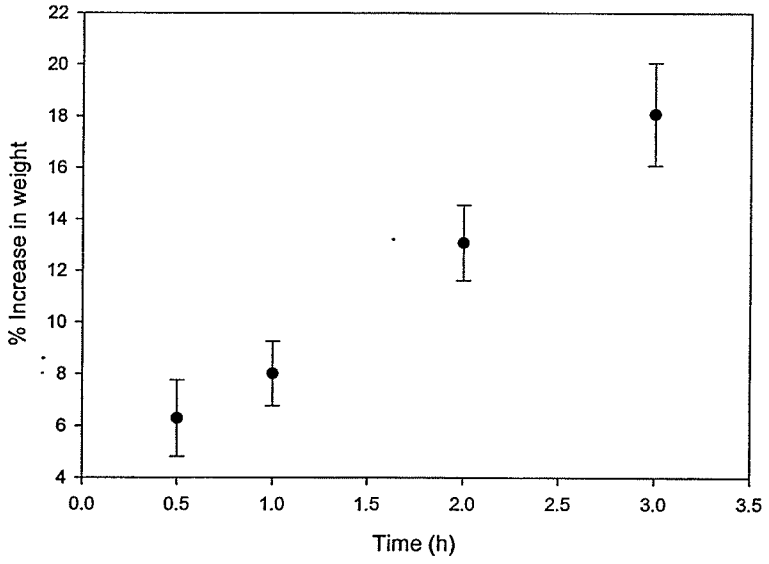
Sample	Well (mg)	Insert (mg)	0 h	0.5 h	1 h	2 h	3 h	24 h
1	395.6	24.1	419.7	420.8	421.3	422.4	423.6	427.1
2	390.3	24.7	415.0	416.8	417.1	418.6	419.6	422.8
3	365.9	24.0	389.9	391.1	391.4	392.8	393.7	397.1
4	393.1	24.6	417.7	419.7	419.9	421.3	422.9	425.6
5	386.9	24.5	411.4	413.2	413.5	414.8	416.1	418.7
6	425.6	24.0	449.6	450.9	451.8	452.5	453.8	457.2
7	378.9	-	378.8	378.9	379.0	378.9	378.9	378.8

The weight change in terms of % weight gained was calculated by ((Wc-Wi)/Wi) · 100 where Wc is the current weight and Wi is the initial weight. The % weight gain is shown in Table 4.10 and the weight gain over the three hour interval is graphically depicted in Figure 4.2.

Table 4.10 Insert weight gain expressed as % of the original weight.

Sample	0.5 h	1 h	2 h	3 h	24 h
1	4.56	6.64	11.20	16.18	30.71
2	7.29	8.50	14.58	18.62	31.58
3	5.00	6.25	12.08	15.83	30.00
4	8.13	8.94	14.63	21.14	32.12
5	7.35	8.57	13.88	19.18	29.80
6	5.42	9.17	12.08	17.50	31.67
Mean	6.29	8.01	13.08	18.08	30.98
Std dev	1.477	1.244	1.469	1.993	0.954

Figure 4.2 Plot of moisture uptake by inserts stored at 75.6% relative humidity. The means are percentage increase in weight and the error bars are the standard deviations.



Conclusion:

The data from this experiment show that the inserts rapidly pick up moisture and it was observed that after three hours, the inserts were soft and adhered to the plastic packaging material. These observations suggest that the inserts will require some protection from atmospheric moisture and an appropriate strategy for achieving this may be through use of screw-caped plastic containers and the inclusion of a desiccant packet inside the container.

Experiment 4.1.5 – Raw material bio-burden

Introduction:

It is generally accepted that pharmaceutical products designed for ophthalmic use be sterile and some of the pharmaceutical attributes of ophthalmic solutions, specifically solutions used to treat ocular hypertension, was discussed by Novack and Evans [435]. In order to determine the suitability of the materials to be used in fabricating the apomorphine ophthalmic inserts and to obtain some insight into appropriate sterilization procedures which might be used, the bio-burden or number of microorganisms present in the raw material was investigated.

Purpose:

The purpose of this investigation was to determine the bio-burden present in the materials used to prepare the inserts.

Study design and method:

Gelatin and glycerin were investigated as these would represent the most likely source of microorganisms which could be present in the finished inserts. Although there is no official requirement for microbial limits for glycerin, there are for gelatin and the USP/NF [79] requirement for gelatin are that the total bacterial count or number of colony-forming units (cfu) does not exceed 1000 cfu/g and that tests for Salmonella and Escherichia coli are

negative. Two lots of glycerin (Spectrum Chemical) and three lots of gelatin (Spectrum Chemical, Sigma Chemical and Medisca Pharmaceuticals) were tested using a procedure based on the USP Microbial limit test [79] page 2249. Briefly, 10 g of material were weighed out, dispersed in sterile Trypticase-soy broth and made up to 100 mL using sterile glassware. For the gelatin samples, the broth was warmed to 50°C prior to dispersing the sample and the gelatin allowed time to swell and dissolve. From this prepared solution 1.0 mL was placed into each of two sterile disposable Petri dishes using a sterile pipette and about 25 mL of sterile Trypticase-soy agar medium was added to each dish, gently mixed, allowed to cool and set and then incubated at 35°C for 72 hours. Three trials using samples of each material were run. After incubation, the number of colonies appearing in the agar was counted and that value multiplied by 10 to express the value as cfu/g.

Results and discussion:

All of the samples of glycerin tested showed no colonies growing after incubation and could therefore be considered as containing less than 10 microorganisms per g of material. Colonies were seen on most of the gelatin samples and the values are shown in Table 4.11:

Table 4.11 Colony counts (cfu) for gelatin samples. Values are cfu/g.

	cfu per g		
Trial	**Spectrum**	**Sigma**	**Medisca**
1	60	-	100
	60	40	80
2	110	70	70
	90	70	80
3	40	80	60
	-	110	110

Since the limit test specifies no more than 1000 colonies per g, all of the tested samples met the requirements of the test and it can be seen that the bio-burden for these lots of gelatin would be low and within the limits specified by the USP.

Conclusion:

The gelatin tested had rather low aerobic microbial counts and these lots will not need any preliminary clean-up or aggressive methods of sterilization; the usual methods and conditions for sterilization of a pharmaceutical should be quite adequate.

Experiment 4.1.6 – Sterilization procedure

Introduction:

Although a process of terminal sterilization for the product would be desirable, any sterilization process requiring either heat or moisture had a marked deleterious effect on the finished product as was seen with the trial using ethylene oxide sterilization. An alternative would be to sterilize the components prior to casting and then to aseptically fabricate the inserts and aseptically package them into sterile packing materials. Since sterilization of the strip packing equipment was not feasible, aseptic processing using this packaging system could not be done so the packaging system with the rigid plastic wells was selected for further investigation. A preliminary test had shown that the plastic wells and label component can be sterilized using ethylene oxide and a method of preparing sterile inserts was required.

Purpose:

The purpose of this investigation was to find an acceptable procedure for providing sterile inserts.

Study design and method:

Appropriate labeling of the backing material was done using a dot-matrix printer and the prepared labels and corresponding plastic wells were separately packaged into overwraps

suitable for ethylene oxide sterilization and sterilized using conditions previously described as those had no deleterious effect on the packaging materials. Since a preliminary trial of autoclaving the casting solution was unsuccessful as the process caused severe discoloration of the solution, sterilization of the gelatin, glycerin and some of the water using autoclaving and sterilization of the apomorphine, ascorbic acid and sodium metabisulfite dissolved in the remainder of the water using membrane filtration was considered. The two sterile solutions could be aseptically mixed and cast into sterile Petri dishes then cured and dried in the sterile environment of a containment cabinet.

The appropriate amounts of glycerin and gelatin were dissolved with gentle heating in about 40 mL of water for injection and placed into a 50-mL type one glass vial and a polymeric closure affixed. The vial was then placed in an autoclave and steam-sterilized at 121°C (15 psig) for 15 minutes. The appropriate amounts of apomorphine, sodium metabisulfite and ascorbic acid were dissolved with gentle heating in about 10 mL of water for injection and allowed to cool. After sterilization, the gelatin-glycerin solution was maintained at 45°C to prevent setting and the apomorphine solution was drawn up into a 10 mL syringe and a sterile disposable membrane (0.2 μm) and sterile vented needle were affixed. The apomorphine solution was then sterile-filtered into the gelatin-glycerin solution and the solution remaining in the filter housing rinsed through with an additional 3 mL of water for injection. The solutions were then gently mixed, the vial closure removed and the solution was poured into a sterile Petri dish and allowed to set and cure. After 48 hours of curing, a satisfactory film had formed and this was removed from the Petri dish and inserts cut from the film using a punch which had been sterilized by autoclaving. The inserts were then packaged into the sterilized plastic wells and the sterilized label-backing applied. All the procedures were done aseptically using accepted standards of practice and all the procedures were done in a biological containment cabinet with a vertical air flow of 24 meters per minute.

After storage for a week under ambient conditions, five of the inserts were tested for sterility. This was done using sterile Trypticase soy broth and positive, negative and main positive controls were simultaneously run with the samples. The test organisms used in the positive controls were Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 66333) and Pseudomonas aeruginosa (ATCC 27853).

Results and discussion:

The procedure outlined where two solutions were prepared and sterilized separately was workable and a sterile casting solution was obtained. The sterile casting solution was allowed to set and cure in the sterile environment of a biological containment cabinet using a discarded balance with a leveling bubble as a horizontal platform; the balance was carefully wiped down with 70% ethanol to sanitize it prior to placing it in the cabinet. The cast film was removed from the Petri dish aseptically and inserts were cut using a sterile punch then the inserts were packaged into the sterile plastic blister wells and sealed with the sterilized labels.

Seven days after preparation, sterility tests were run on five of the inserts and none of the samples tested showed growth in Trypticase soy broth after seven days incubation at 35°C. Positive controls showed growth and the negative controls showed no growth.

Conclusions: Section 4

A suitable matrix for the inserts was developed and a base of gelatin and glycerin was selected with sodium metabisulfite and ascorbic acid added to prevent degradation of the apomorphine during the casting and drying process. A suitable packaging system was selected and a desiccant was included in the packaging to protect the insert from moisture; experimental work demonstrated that the inserts were somewhat hydroscopic and deteriorated in the presence of high humidity; a procedure for preparing sterile inserts was also developed. The final product appeared to meet the appropriate initial criteria established for the inserts and this final product was used for further investigations and evaluation.

Section 5: Product evaluation

5.1 Release kinetics and mechanism

Overview:

In an extensive review of swellable matrices for controlled drug release, Colombo et al [436] defined swelling-controlled systems. They described them as those where the drug is essentially immobile when the polymer is in its glassy or dry state but relatively mobile when the polymer is wet and in its rubbery state. When a matrix containing a swellable polymer in the glassy state comes into contact with a solvent, there is a change from the glassy to the rubbery state as the solvent penetrates the matrix and this change is associated with swelling of the matrix [437-439]. The original polymer chains are in an unperturbed state but as they absorb the solvent they expand to a new solvated state and become rubbery. Colombo et al [436] attributed this to a lowering of the glass transitional temperature. There is usually a visual band or front between the glassy and rubbery regions and polymer chain relaxation behind the front leads to swelling and creates osmotic stress which leads to diffusion of drug imbedded in the matrix [440]. The diffusional behavior of the drug is characterized by the formation a gel layer which forms on the matrix surface as the polymer undergoes transition from the glassy to the rubbery state. As solvent continues to penetrate into the glassy matrix, drug is dissolved and polymer chains are disentangled. The disentangled chains form a gel layer around the matrix and this layer acts as a barrier which controls water penetration inward and drug diffusion outward. Close to the penetrating solvent front the polymer chains are strongly entangled and the gel layer is quite resistant to water movement inwards and drug movement outwards. Farther out from the solvent front the gel layer becomes progressively hydrated and the chains become less entangled and eventually may be dissolved into the surrounding medium [437, 439].

Colombo et al [436] identified the thickness of the gel layer, gel strength and rate of hydration as variables which can modulate the swelling behavior and subsequent release of the drug. Gel strength is determined by polymer concentration, viscosity and the physicochemical nature of the rubbery polymer. Polymer-polymer and polymer-solvent interactions determine the structure of the gel network and rate of erosion.

Swelling behavior can be described by several front positions where the term front indicates the position in the matrix where the physical conditions change [441]. Colombo et al [442] described three distinct fronts and designated these as swelling, erosion and diffusional fronts. A swelling front can be identified where the rubbery and glassy regions meet and this front separates the rubbery and glassy regions of the swelling matrix. An erosion front can be identified which separates the swelling matrix from the solvent and the region between these two fronts form the diffusion gradient and the diffusion front is located at some point within this diffusion gradient. The diffusion front separates the dissolved drug from the undissolved drug and its nature is affected by both the water solubility of the drug and the drug load or quantity of drug in the matrix. In the case of erosion, drug solubility is not a major factor in determining the release kinetics but when release is associated with swelling, drug solubility becomes an important factor [439]. The distance from the swelling front to the diffusion front is associated with drug solubility and generally the higher the drug solubility, the closer the diffusion front is to the swelling front [442]. In the review by Colombo et al [436] review, the authors point out that the overall mechanism of drug release from the matrix involves solvent penetration, matrix swelling, dissolution of the drug, diffusion of the drug and erosion at the outer surface of the gel layer.

The gel layer thickness is measured as the distance between the erosion and swelling fronts and there is a time-dependent relationship between the front movements and gel layer thickness [443]. Initially the erosion front moves outward due to the swelling of the matrix

then begins to move inwards as the matrix dissolves. The swelling front moves inwards as water penetrates the matrix and if the polymer has sufficient solubility a point may come where the erosion and swelling fronts move at the same rate or move in parallel and gel layer thickness remains constant for a period of time [437]. During this time while the gel layer thickness remains constant, the rate of drug release also remains constant. This phenomenon is very dependent on the physicochemical nature of the polymer and erosion is the primary mechanism determining the kinetics of drug release. The position of the diffusion front or boundary between dissolved and undissolved drug is relatively unimportant in this situation so drug solubility is not a major factor in determining the release kinetics [443].

In the case of a swellable matrix delivery system, activation is initiated by water and release rate is controlled by interactions between water, polymer and drug [442]. In this case drug solubility becomes an important variable in determining the release kinetics as the rate of drug release is dependent on the drug gradient in the gel layer [443]. The concentration of drug and the thickness of the gel layer govern the movement of drug or drug flux. The drug concentrations in the gel are functions of drug load in the polymer and drug solubility in the penetrating solvent while gel layer thickness is a function of solvent penetration, chain disentanglement and mass transfer of polymer and drug in the solvent.

Gel thickness dynamics show three distinct phases: thickness increases when solvent penetration is the fastest process; remains constant when disentanglement and solvent penetration occur at similar rates and finally gel thickness decreases when the polymer has disentangled and undergone the glassy-rubbery transition [444]. The release kinetics seen reflect these phases: Fickian to non-Fickian as swelling is initiated; constant or zero-order as swelling and disentanglement occur simultaneously and finally first-order when the entire mass of polymer has disentangled and erosion is the only active process taking place [445].

In the case of a swellable matrix, the central element of the release process is the formation of a gel layer around the matrix in response to solvent penetration and the factors governing gel layer formation and subsequent release are solvent penetration, polymer swelling, drug dissolution and subsequent diffusion through the gel layer and finally matrix erosion [445]. For the most part, drug release is controlled by drug diffusion through the gel layer which is also simultaneously undergoing dissolution or erosion.

Ju et al [446] examined the effect of polymer concentration and molecular weight on what they described as the diffusion coefficient (D_p) across the thickness of the gel layer. They theorized that in a swelling matrix the degree of polymer chain entanglement decreases from the central glassy core to the outside of the gel layer and eventually entanglement becomes so weak that it no longer holds the polymer chains together and polymer dissolution takes place.

In a study to investigate the factors which influence swelling behavior in polymers, three model matrix materials were examined by Vlachou et al [437]. The materials studied were hydroxypropylmethylcellulose (HPMC), polyethylene oxide (PEO) and sodium alginate. Although this study was primarily an examination of these polymers in solid oral dose forms, the authors also investigated films of these materials cast from an aqueous medium and then cut into 10-mm discs.

Drug release from a matrix is often characterized as a function of the square root of time but this is usually applicable only when the mechanism of release is governed by diffusion. Swellable systems however, also have a component of release through erosion and as a result the relaxation or dissolution of the polymer matrix must be taken into account. An empirical equation which was developed by Ritger and Peppas [447] is often used to describe the kinetics of drug release where the fraction of drug released is related to time raised to an exponent (Equation 5.1):

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{Equation 5.1}$$

Where M_t is the amount of drug released at time t, M_∞ is the amount of drug released at time infinity, k is the kinetics constant and n is the diffusional exponent. This equation was further refined by Peppas and Sahlin [448] in an effort to account for release due to polymer relaxation (Equation 5.2):

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \qquad \text{Equation 5.2}$$

Where k_1 is the diffusional rate constant, k_2 is the relaxational rate constant and m is the diffusional exponent. The relative contributions of drug diffusion, polymer relaxation and matrix erosion may be controlled or tailored through the use of modified polymers or mixed polymers [449]. These equations have been used to describe drug release from swellable matrices in a number of studies [437, 446]. In their paper Peppas and Sahlin further showed that the ratio of relaxational over Fickian contribution can be calculated from Equation 5.3:

$$\frac{R}{F} = \frac{k_2}{k_1} t^m \qquad \text{Equation 5.3}$$

Measurement of release-rate of the active component from a matrix type delivery system is achieved by placing the device into a dissolution medium of defined composition at a defined temperature and measuring the amount of drug present in the dissolution medium at timed intervals [343, 450-452]. These data are then fitted to various models which have been developed to explain the mechanisms involved in the release; a number of these models have been reviewed [451, 453, 454]. Costa and Lobo [453] reviewed a number of models used to characterize release profiles and relate these to the mechanisms involved in the release. They first discussed models following zero-order kinetics which can be used to describe the release from some transdermal systems, matrix tablets, coated dose forms and osmotic systems; this

release pattern is the ideal for achieving prolonged pharmaceutical activity as the release rate can be designed to match the rate of removal from the body. These models are described by the Equation 5.4:

$$Q_t = Q_0 + k_0 t \qquad \text{Equation 5.4}$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in solution and k_0 is the zero-order rate constant. Costa and Lobo next considered models following first-order kinetics which follow the general form shown in Equation 5.5:

$$\log Q_t = \log Q_0 + \frac{k_1 t}{2.303} \qquad \text{Equation 5.5}$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in solution and k_1 is the first-order rate constant. For this equation, a plot of log released drug as a function of time will be linear and generally describes the release of soluble drugs from porous materials. An improved but empirical equation for apparent first-order release was proposed by Weibull and adapted by Langenbucher [455] to apply to release from pharmaceutical dose forms. This model took the form described by Equation 5.6:

$$\log[-\ln(1-(Q_t/Q_\infty))] = b(\log t - \log a) \qquad \text{Equation 5.6}$$

Where b is a shape parameter and a is the scale parameter; the curve shape may be exponential ($b = 1$), sigmoidal ($b > 1$) or parabolic ($b < 1$). The scale parameter is estimated from 1/a at time $t = 1$. From this, a plot of log drug dissolved as a function of log time will be linear. A refinement of this treatment was introduced by Higuchi [456] and this model was designed to describe the release of drugs from solid or semi-solid matrices where the matrix behaved as a diffusion medium. The equation describing the release was:

$$Q_t = k\sqrt{t} \qquad \text{Equation 5.7}$$

A plot of the fraction of drug released (Q) as a function of the square root of time was shown to be linear. Other models where the changing surface area of the dose form was considered were also discussed; these included the Hixson-Crowell cube-root, Baker-Lonsdale spherical and the Hopfenberg geometrical shape models [457]. Overall, Costa and Lobo suggested that the more empirical models were the most satisfactory with the general zero-order, Higuchi and Peppas models being the most useful.

Measurement of release of the active component is usually performed in a defined medium at a specified composition, pH, temperature and volume. A specified apparatus is used and samples are withdrawn at timed intervals. These samples are subsequently analyzed for drug content and the sample volume withdrawn is replaced with an equal volume of fresh medium to maintain a constant volume [458]. Since this traditional method is labor-intensive, a number of automated systems have been developed [459, 460]. The error associated with sample volume replacement is avoided with one of these systems where analysis is done using a diode-array spectrophotometer and the analytical sample is returned to the testing vessel [460]; this system allows multi-component analysis and accounts for excipient interference by the use of multivariate analysis at multiple wavelengths usually from 200-400 nm. A variation of this type of system claimed to be effective even in the presence of insoluble excipients such as titanium dioxide [452]. A more recent variation of this technology used fiber-optic probes combined with a software package for data capture, recording and analysis. This system was described by Johansson et al [461] and was used to measure release of test samples of prednisolone and a tablet containing two active components which was under development.

Polymer matrix swelling has been assessed and quantified using a variety of techniques which can be placed into three general categories; gravimetric, dimensional and volumetric. A gravimetric procedure is illustrated in a paper by Vlachou et al [437]; in this paper the swelling properties of a variety of polymeric matrices were investigated and

swelling was measured by removal of specimens from the dissolution bath at timed intervals, wiping them gently with a tissue to remove surface water and weighing them. This technique was also used by Klech and Li [441] but the samples were returned to the bath after each weighing. Dimensional measurements have been done using microscopy and/or photography and methods have been presented by Gao and Meury [462] and Martini et al [410]. The use of dyes and fixing the matrix between two sheets of rigid acrylic plastic then allowing hydration to occur has been reported by a number of authors [440, 444, 463] and this method has been used to allow visualization and measurement of the diffusion, swelling and erosion fronts [463]. Zuleger et al [440] used a method where the matrix material was fixed to the bottom of a flat-bottomed test tube, dissolution medium added to the tube and the height of the swelling matrix was recorded as a function of time and Karatus and Baykara [464] used a micrometer with an electric current running through it to measure the thickness of swelling matrices. An example of a volumetric approach was presented by Ofner and Bubnis [389] where the swollen matrix was placed into a pycnometer which was then filled with toluene. The equilibrium swelling volume (Vs) was than calculated from:

$$Vs = \frac{Wmt - Wt}{\rho t} \qquad \text{Equation 5.8}$$

Where Wmt is the weight of the swollen matrix and the toluene, Wt is the weight of toluene alone and ρt is the density of toluene.

The nature and composition of the polymer, drug to be delivered and any added excipients will be important factors in determining the nature and rate of release from a matrix dose form [365, 381, 400, 403, 437, 450, 456, 465-468] but other parameters such as shape and geometry of the matrix may affect release kinetics [457, 469]. The effects of the

dissolution media pH have been studied and in the case of gelatin, pH has very little effect on either swelling or release [441, 470].

The effects of temperature on the swelling and subsequent drug release from gelatin matrices were studied by Klech and Pari [471]. These authors suggested that drug release from a gelatin matrix was determined by solvent penetration and polymer swelling and that these two variables were interdependent and could be described by rate equations. Both processes were also governed by activation energies and consequently would be affected by temperature changes. They postulated that when a polymer is in a rubbery state or above its glass transition temperature during solvent penetration, mass transport or release would obey Fick's law but when polymer matrix is in an amorphous glassy state, non-Fickian release would be seen. They suggested that non-Fickian transport would be the more highly activated process since the solvent must penetrate the glassy polymer and at the point of contact increases chain mobility and induces mechanical relaxation. In contrast, Fickian transport would require less activation because the polymer would be a rubbery solid in which the chains would be already mobile and able to react immediately to solvent perturbations. In their study, the swelling and penetration fronts were measured in gelatin microspheres suspended in distilled water using a microscope. Temperatures were maintained using a stage with a flow-through jacket and circulating water from a heating bath; the temperatures studied ranged from 15 to 40°C. Although they found that the penetration front rate constant increased with temperature, the rate of swelling was less affected and that overall, the temperature effects were not of the magnitude the authors expected. A further study by Klech et al [441] investigated the effect of drug load on the swelling kinetics of gelatin matrices and found a much larger effect from drug load when a hydrophilic drug was incorporated into the matrix; as drug load increased, the rate of release and penetration from movement increased. They also examined temperature

effects with this system and again the rate of release and penetration increased with temperature but not to the extent the authors had predicted.

The effect of temperature is important for the apomorphine ocular system being investigated as there may be variations in the temperature of the eye which could affect drug release. Ocular surface and conjunctival temperatures have been investigated by a number of authors [472-475]. The normal temperature of the conjunctival membranes in humans has been reported to be 34.9 ± 1.4°C [475] and 34.1 ± 0.6°C [476] with the corneal surface temperature being somewhat lower and there is evidence that the temperature of the eye tissues may be affected by a change in the core body temperature [474]. Comparable temperatures have been reported for rabbits and beagle dogs [477]. Intoxication with an agent like imipramine [478] can cause significant hypothermia and so a drop in the ocular tissue temperature could be expected in these cases while a drug like 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) [479] may cause hyperthermia and a corresponding increase in ocular temperature could be expected. For this reason it would be important to ensure that the release kinetics for the apomorphine insert remain appropriate over a temperature range consistent with what might be expected in the ocular tissues.

Experimental 5.1 - Release Studies

Overview:

In order to study the kinetics of drug release from the inserts, profiles of insert swelling, drug dissolution and matrix dissolution were investigated over a series of temperatures. Matrix swelling was determined using a gravimetric method to assess weight change as a function of time while apomorphine and matrix dissolution were monitored by measuring changes in apomorphine and gelatin concentrations as a function of time. Apomorphine and gelatin concentrations were measured using the spectroscopic method for

binary mixtures previously validated in the section on gelatin analysis. The temperature range to be studied was from 28°C to 36°C at 4°C intervals.

Equipment and materials:

The equipment and materials used in this block of experiments is as follows: the chromatographic system used was manufactured by Knauer (Berlin, Germany) and consisted of a Wellchrom K-501 pump, a Wellchrom K-2501 variable wavelength UV detector, a Basic-Marathon autosampler type 816 and a Wellchrom HPLC Interface box. A Waters (Milford, MA, USA) model 420 fluorescence detector and a Bioanalytical Systems Inc (Lafayette, IN, USA) model LC-4B electrochemical detector were used in tandem with the Knauer system via the HPLC Interface box. The operating, data acquisition and data analysis software used was Eurochrome 2000® (Knauer, Berlin, Germany). The HPLC column used was a cyano 4.6 x 150 mm particle size 4 μm (Jones, Hengoed, UK). All chromatography was performed at ambient temperature. A model 8451A diode-array spectrophotometer was used (Hewlett-Packard, Houston, TX, USA), quartz-silica cells (Fisher Scientific, Nepean, ON, Canada) and a peristaltic pump (Cole-Palmer, Vernon Hills, IL, USA) and a model 1390 rotational shaker (Barnstead Lab-line, Melrose Park, IL, USA) were used for the release studies; thermostated water baths model 1226 from Sheldon Manufacturing (Bristol, CT, USA) were used for temperature control.

Apomorphine HCl was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade phosphoric acid and acetonitrile as well as analytical grade hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific (Nepean, ON, Canada). Gelatin USP, glycerol USP and ascorbic acid USP were purchased from Spectrum Chemical (Gardenia, CA, USA). De-ionized water was obtained using an Aqua-Summa II reverse-osmosis system (Culligan, Toronto, ON, Canada). All glassware used was Class A (Fisher Scientific, Nepean, ON, Canada) and where required, dilutions were done using a 1000 μL adjustable volume

pipette (Hamilton Company, Reno, NV, USA). A model AE-50 analytical balance (Mettler, Hightstown, NJ, USA) was used for weighing. Filtration of mobile phase was accomplished using 0.2 μm membranes (Pall, Gelman Laboratories, East Hills, NY, USA).
Standard solutions of apomorphine HCl in 0.05 M HCl were prepared, stored in glass at 5° C and discarded after seven days. Reference standard apomorphine was obtained from USP Convention, Rockville, MD, USA). Statistical analysis and graphical presentations were done using SigmaStat ® (SPSS Inc Chicago, IL, USA), Excel 2000® (Microsoft) and SigmaPlot 2000® (SPSS Inc Chicago, IL, USA).

All statistical analysis was done at the 95% confidence level unless otherwise indicated and data are presented as mean ± standard deviation unless otherwise indicated.

Experiment 5.1.1 – Release/swelling

Purpose:

The purpose of these experiments was to characterize the release mechanism of apomorphine from the ocular inserts, determine whether the release rate would be suitable for the intended purpose of the inserts and investigate the effects of temperature on the release of apomorphine.

Preliminary studies:

A series of preliminary experiments were conducted to determine the most satisfactory procedures for measuring the release of apomorphine from the inserts, the dissolution profile of the gelatin and the swelling behavior of the inserts. Initially the official USP basket method was attempted to study release and dissolution but it was found that very inconsistent results were obtained and this was attributed to attrition of the swelling inserts due to mechanical abrasion by the mesh of the basket. The inserts became delicate as they became swollen and with time the inserts were observed to fragment in the basket. Attempts with the USP paddle

method and with a magnetic stirring bar demonstrated a similar problem in that the inserts began to fragment as they collided with the paddle or the stirring bar. An attempt using a 500 mL Erlenmeyer flask agitated at about 30 rpm using a rotary shaker platform gave consistent release results and there was no visible fragmentation of the inserts so this procedure was selected for the study.

A similar problem also related to the fragility of the swelling inserts was encountered with the swelling studies. As previously described in the overview section, the swelling behavior of matrix drug delivery has been studied and quantified using a variety of techniques including dimensional measurement, volumetric measurement or gravimetric measurement of the swelling matrix. In this present case dimensional measurement was attempted by removing the swelling insert from the dissolution medium at timed intervals and measuring the thickness and height at each interval. Initially, measurement was attempted using a caliper but it was found that the swelling insert became rather fragile as swelling progressed and the handling caused the insert to break apart making this process impractical. An attempt was made to photograph the swelling insert at timed intervals using a digital camera and to measure the dimensions from the photographs but it was still necessary to remove the insert from the dissolution medium and again the fragile nature of the swelling insert was problematical. A volumetric measurement was also attempted where the insert was retrieved from the dissolution medium, placed into a 10-mL pycnometer and the pycnometer made to volume with toluene. Knowing the density of the toluene and the weight of the filled pycnometer allowed calculation of the volume of the swelling insert. Although this technique seemed to work well during the initial stages of swelling, the insert again became damaged through the retrieval process and the handling and introduction of a reasonably intact insert into the pycnometer was not always possible. The method which was finally used was a gravimetric procedure where a small basket was made using 16 gauge aluminum mesh; a weighed insert

was placed into the basket and the loaded basket placed into the dissolution medium. At timed intervals, the basket was removed, blotted once with a tissue to remove excess dissolution solution and weighed. This process could be accomplished in about 12 seconds and the basket was again placed back into the dissolution medium. The changes in weight were attributed to the process of swelling and erosion. A series of time retrievals of a basket without an insert showed that the weight of the basket varied somewhat but when the blotting with tissue was supplemented by briefly exposing the basket to a gentle jet of compressed air, the weight remained quite constant indicating that this procedure satisfactorily removed the excess dissolution medium; this also demonstrated that the medium had no adverse effect on the basket insofar as weight changes were concerned.

Study design and method:

Apomorphine and gelatin dissolution

A spectroscopic method was used to determine the dissolution profiles of gelatin and apomorphine simultaneously using a diode-array spectrophotometer. This methodology was described and validated in a previous section. For the procedure, an insert was placed into approximately 375 mL of HCl 0.05M in a 500 mL Erlenmeyer flask held at the temperature being studied. A flow-through quartz cell was used and the bulk solution was circulated through the cell at a rate of 80 mL/min using a peristaltic pump. Absorption measurements were taken at three to five-minute intervals and the absorption at wavelengths 220 and 272 nm was recorded. Quantitation of the apomorphine and gelatin was done by using the matrix algebra procedure previously described and standard curves for apomorphine and gelatin were run for each block of experiments to obtain the respective slope and intercept values required for the calculations. Temperature control of the bulk solution was managed by keeping the solvent reservoir in a water bath (± 0.5°C) and narrow bore polyethylene tubing was used to circulate the bulk fluid from the reservoir to the pump, through the flow-through cell and back

into the reservoir flask. The reservoir flask in the water bath was agitated by means of a reciprocating shaker set at 30 rpm and four trials at each temperature were conducted. The concentrations at time infinity were determined by warming the dissolution medium to 50°C until the entire insert had dissolved, allowing it to cool back to the temperature under study and then measuring the apomorphine and gelatin concentrations.

Swelling

A gravimetric method was used to determine the matrix swelling profile of the inserts. For this procedure, a cage made from aluminum mesh (14 gauge) with an approximate weight of 0.5 g and a volume of about 5 cm^3 was used. The cage was weighed, the weight recorded and an insert was placed into it. The assembled apparatus was again weighed, the weight recorded and the cage was placed into a beaker of solvent held at specified temperatures in a water bath. The solvent used was 0.05M HCl to avoid decomposition of the apomorphine. The time was noted and recorded and at appropriate time intervals the cage and insert were carefully removed from the beaker allowing the solution to drain out. Excess moisture was removed by a gentle jet of compressed air followed by gentle wiping with tissue. The dried cage and swollen insert were weighed, the weight recorded and immediately placed back into the beaker of solvent. This process was continued until no visible portion of the insert remained. At this time the cage was rinsed with hot distilled water, allowed to dry thoroughly and re-weighed. The average value of the cage weights before and after the sampling period was deducted from the recorded weights with the insert and the difference taken as the weight of the swollen insert. Quantitation of the swelling was expressed as the ratio of the swollen weight (W_t) at time equals t over the initial weight of the insert (W_i).

Results and discussion:

Individual ocular inserts were placed into 375 mL of dissolution medium (0.05M HCl) as described in study design and methods above and the absorbances at 220 and 227 nm

monitored. Apomorphine concentrations were calculated as described and the amount released (M_t) at each time interval was expressed as a fraction of the total amount released (M_∞) at time infinity. Temperatures of 36, 32 and 28°C were studied and four trials were conducted at each temperature. Data from these trials are presented in Table 5.1 with the sampling intervals being 3, 5 and 10 minutes for 36, 32 and 28°C respectively. Data points were collected until the ratio M_t/ M_∞ was approaching unity for each sample. The data presented in Table 5.1 for each temperature level up to a release of about 90% were fit to Equation 5.1 as described by Ritger and Peppas [447] using non-linear regression.

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{Equation 5.1}$$

This equation was discussed and the terms defined in the overview for this section. In the regression analysis, the data for the quantity released were weighted using the inverse of the standard deviation of the four trials.

Table 5.1 Quantity of apomorphine released as a ratio of the quantity dissolved at time t (M_t) over the total quantity present in the insert (M_∞). Temperatures of 36, 32 and 28°C.

36°C		32°C		28°C	
Time (min)	**M_t/M_∞**	**Time (min)**	**M_t/M_∞**	**Time (min)**	**M_t/M_∞**
3	0.153 ± 0.027	5	0.159 ± 0.024	10	0.273 ± 0.054
6	0.341 ± 0.036	10	0.258 ± 0.048	20	0.403 ± 0.071
9	0.509 ± 0.031	15	0.341 ± 0.069	30	0.496 ± 0.082
12	0.653 ± 0.046	20	0.407 ± 0.083	40	0.570 ± 0.091
15	0.793 ± 0.029	25	0.477 ± 0.082	50	0.628 ± 0.098
18	0.890 ± 0.042	30	0.535 ± 0.088	60	0.676 ± 0.103
21	0.957 ± 0.042	35	0.589 ± 0.094	70	0.716 ± 0.110
24	0.988 ± 0.028	40	0.642 ± 0.090	80	0.749 ± 0.111
27	0.996 ± 0.012	45	0.698 ± 0.084	90	0.778 ± 0.110
30	1.000 ± 0.004	50	0.751 ± 0.097	100	0.813 ± 0.086
33	1.001 ± 0.001	55	0.815 ± 0.122	110	0.841 ± 0.073
36	1.001 ± 0.002	60	0.855 ± 0.120	120	0.861 ± 0.064
39	1.002 ± 0.001	65	0.895 ± 0.101	130	0.878 ± 0.056
42	0.999 ± 0.001	70	0.930 ± 0.086	140	0.894 ± 0.052
45	1.000 ± 0.001	75	0.934 ± 0.074	150	0.906 ± 0.046
48	0.999 ± 0.002	80	0.959 ± 0.057	160	0.918 ± 0.042
		85	0.969 ± 0.042	180	0.938 ± 0.034
		90	0.977 ± 0.031	200	0.951 ± 0.032
		100	0.988 ± 0.015	220	0.974 ± 0.005
		120	1.000 ± 0.002		
		140	1.002 ± 0.004		
Mean ± Std dev			n = 4		

The results of the non-linear curve fit to Equation 5.1 for the three temperatures are presented in Table 5.2.

Table 5.2 Coefficients k and n from non-linear regression of data from Table 5.1 fit to Equation 5.1.

Temp (°C)	k	n	r^2
36	0.0774	0.842	0.988
32	0.0581	0.652	0.998
28	0.142	0.370	0.982

These data indicated a complex pattern of apomorphine release where both diffusion and relaxation or dissolution of the gelatin matrix is involved. Values of n around 0.42 to 0.5 suggest a Fickian or diffusional process while higher values suggest a relaxational process [447, 480]. Plots of the observed values and values fit to Equation 5.1 are shown in Figures 5.1, 5.2 and 5.3 for temperature 36, 32 and 28°C respectively.

Figure 5.1 Apomorphine release at 36°C; observed values and values fit to Equation 5.1. Error bars are standard deviations of found values. (n = 4)

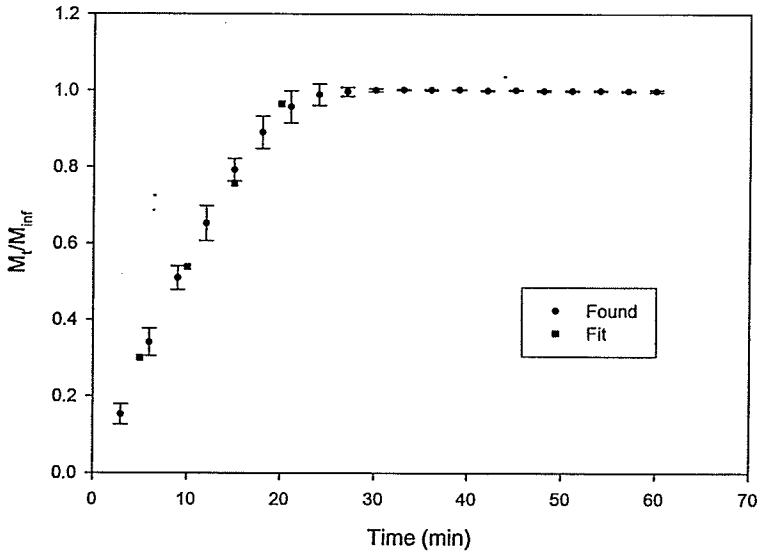


Figure 5.2 Apomorphine release at 32°C; observed values and values fit to Equation 5.1. Error bars are standard deviations of found values. (n = 4)

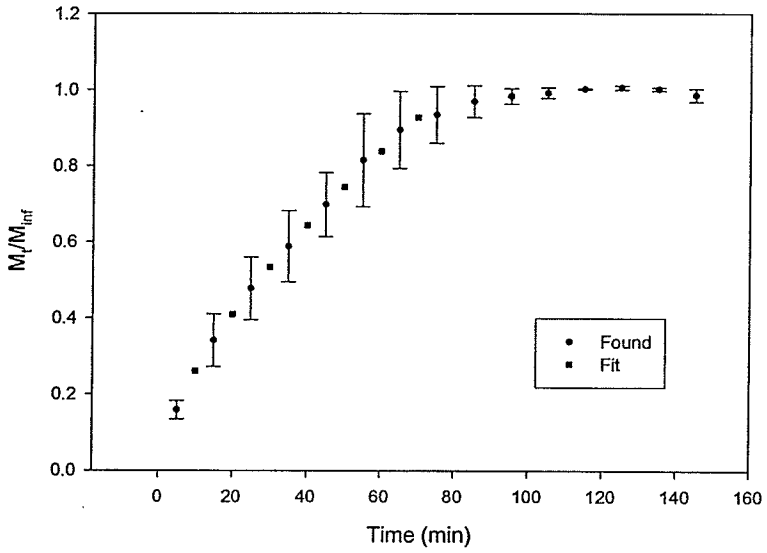
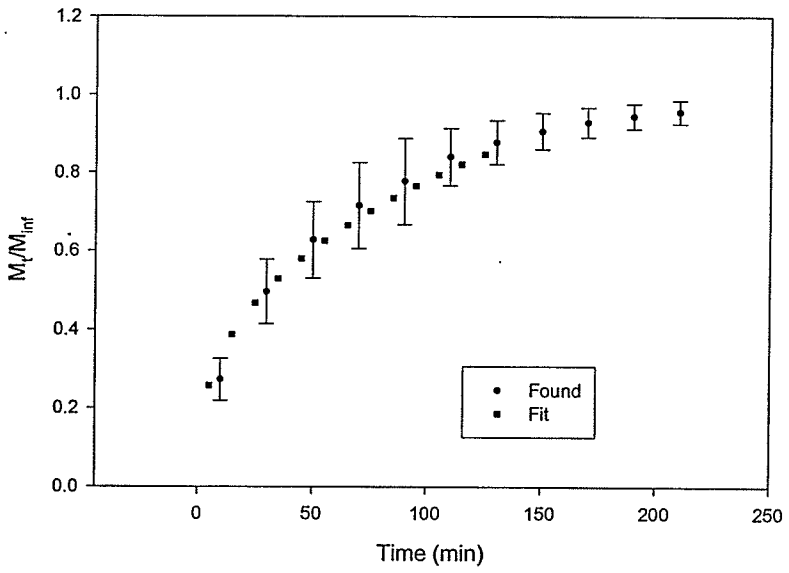


Figure 5.3 Apomorphine release at 28°C; observed values and values fit to Equation 5.1. Error bars are standard deviations of found values. (n = 4)



The plots of apomorphine fraction released as a function of time for the different temperatures studied showed that temperature had an effect on the rate of release. The time for half of the apomorphine to be released can be calculated by substituting the coefficients shown in Table

5.2 back into Equation 5.1. Half the apomorphine was released in 9.2 minutes at 36°C, 27.2 minutes at 32°C and 30.0 minutes at 28°C. These results are consistent with work by Liu et al [481] where theophylline release from gelatin, agar and κ-carageenan matrices was investigated at different temperatures and work by Gil et al [482] studying the properties of gelatin/silk fibroin matrices.

As already described in the overview to this section, Equation 5.2 was presented by Peppas and Sahlin [448] as a model for determining the relative contributions of diffusion and erosion to drug release from a swellable matrix.

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \qquad \text{Equation 5.2}$$

The data presented in Table 5.1 up to a release of about 90% were fit to this equation using non-linear regression and the dependent variable was weighted using the inverse of the standard deviation for the four trials. The results of this analysis and the coefficients found are presented in Table 5.3 below:

Table 5.3 Coefficients k_1, k_2 and m from non-linear regression of data from Table 5.1 fit to Equation 5.2.

Temp (°C)	k_1	k_2	m	r^2
36	0.0447	5.06×10^{-4}	1.180	1.000
32	0.0501	3.63×10^{-4}	0.725	0.999
28	0.0778	1.59×10^{-3}	0.591	1.000

The coefficients determined from the regression analysis were used to generate data showing the fraction of apomorphine expressed as a percentage, released through diffusion and erosion respectively as a function of time for each temperature. This was done using Equation 5.3 as previously described in the overview to this section [448]:

$$\frac{R}{F} = \frac{k_2}{k_1} t^m \qquad \text{Equation 5.3}$$

where R/F is the ratio of relaxational over Fickian contribution. From the release rate measurements and derived contributions of erosion and diffusion, 50% of the insert content of apomorphine was released in 9.2 minutes at 36°C and 13.4% of this was released through relaxation; 50% of the insert content of apomorphine was released in 27.2 minutes at 32°C and 7.4% of this was released through relaxation and 50% of the insert content of apomorphine was released in 30 minutes at 28°C and 2.0% of this was released through relaxation.

Overall, apomorphine release from the inserts appears to be dependent on both diffusional and relaxational processes with erosion predominating at temperatures where the gelatin is soluble. The mean temperature in the conjunctival sac is 34°C so both release processes should be involved but in vivo studies will be required to determine whether release is rapid enough with insert integrity being maintained during clinical use. In the specifications set out for the inserts, a balance is required where release is prompt but controlled and the integrity of the insert is maintained to allow its removal when the therapeutic end point of emesis is achieved.

The swelling properties of the ocular inserts were studied as described under study design and method. For these studies a mesh basket was used and quantitation was gravimetric. These experiments were also conducted at temperature of 36, 32 and 28°C and four trials were run at each temperature. The data from this study are presented in Table 5.4.

Table 5.4 Weight of ocular inserts after immersion in dissolution medium at temperatures of 36, 32 and 28°C.

36°C		32°C		28°C	
Time (min)	**Weight (mg)**	**Time (min)**	**Weight (mg)**	**Time (min)**	**Weight (mg)**
0	24.7 ± 0.7	0	24.1 ± 0.6	0	24.7 ± 0.4
3	34.2 ± 3.2	5	67.0 ± 2.6	5	60.3 ± 8.0
6	29.8 ± 4.4	10	71.8 ± 5.9	10	67.2 ± 10.1
9	24.6 ± 1.8	15	56.6 ± 8.6	15	80.3 ± 13.6
12	16.6 ± 4.2	20	49.8 ± 9.2	20	89.4 ± 16.1
15	13.0 ± 4.2	25	40.0 ± 7.9	25	94.9 ± 22.1
18	7.35 ± 3.8	30	39.5 ± 8.5	30	110.0 ± 22.7
21	5.05 ± 0.2	35	36.5 ± 13.2	35	110.9 ± 24.1
24	-	40	30.8 ± 11.5	40	116.5 ± 27.2
27	-	45	24.6 ± 6.2	45	120.7 ± 23.7
		50	15.4 ± 6.8	50	121.8 ± 24.1
		55	-	55	129.3 ± 22.3
		60	-	60	126.0 ± 19.2
				65	137.1 ± 20.5
				70	131.1 ± 14.4
				75	125.7 ± 14.2
				80	127.0 ± 17.2
				85	133.4 ± 15.2
				90	129.7 ± 23.6
Mean ± Std dev			n = 4		

The raw data in Table 5.5 were converted to a ratio of swollen weight (W_t) to initial weight (W_i) for each temperature and these data are presented in Table 5.5.

Table 5.5 Ratio of swollen weight (W_t) to initial weight (W_i) for ocular inserts after immersion in dissolution medium at temperatures of 36, 32 and 28°C.

36°C		32°C		28°C	
Time (min)	**W_t/W_i**	**Time (min)**	**W_t/W_i**	**Time (min)**	**W_t/W_i**
0	1	0	1	0	1
3	1.38 ± 0.09	5	2.78 ± 0.14	5	2.44 ± 0.30
6	1.21 ± 0.16	10	3.00 ± 1.15	10	2.72 ± 0.37
9	1.00 ± 0.10	15	2.35 ± 0.38	15	3.25 ± 0.50
12	0.67 ± 0.16	20	2.06 ± 0.35	20	3.62 ± 0.59
15	0.52 ± 0.16	25	1.66 ± 0.31	25	3.84 ± 0.84
18	0.30 ± 0.15	30	1.62 ± 0.33	30	4.45 ± 0.87
21	0.20 ± 0.01	35	1.49 ± 0.52	35	4.49 ± 0.91
24	-	40	1.26 ± 0.46	40	4.71 ± 2.05
27	-	45	1.01 ± 0.24	45	4.88 ± 0.89
		50	0.63 ± 0.27	50	4.93 ± 0.91
		55	-	55	5.23 ± 0.86
		60	-	60	5.10 ± 0.72
				65	5.56 ± 0.82
				70	5.31 ± 0.56
				75	5.09 ± 0.58
				80	5.15 ± 0.71
				85	5.40 ± 0.62
				90	5.26 ± 0.99
Mean ± Std dev				n = 4	

The swelling behavior of the ocular inserts is shown graphically in Figures 5.4, 5.5 and 5.6 at temperatures of 36, 32 and 28°C respectively.

Figure 5.4 Insert swelling at 36°C expressed as a ratio of current weight to initial weight; values lower than unity represent a net loss in the weight of the insert. Error bars are standard deviation. (n = 4)

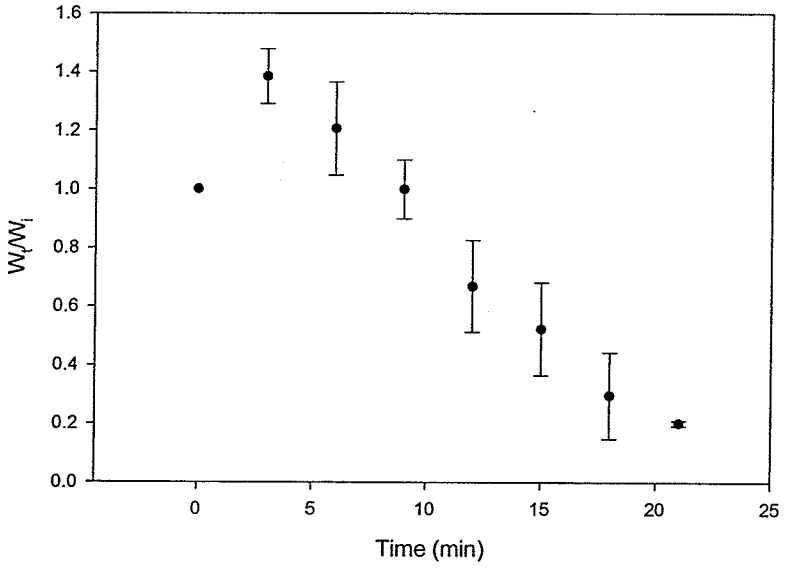


Figure 5.5 Insert swelling at 32°C expressed as a ratio of current weight to initial weight. Error bars are standard deviation. (n = 4)

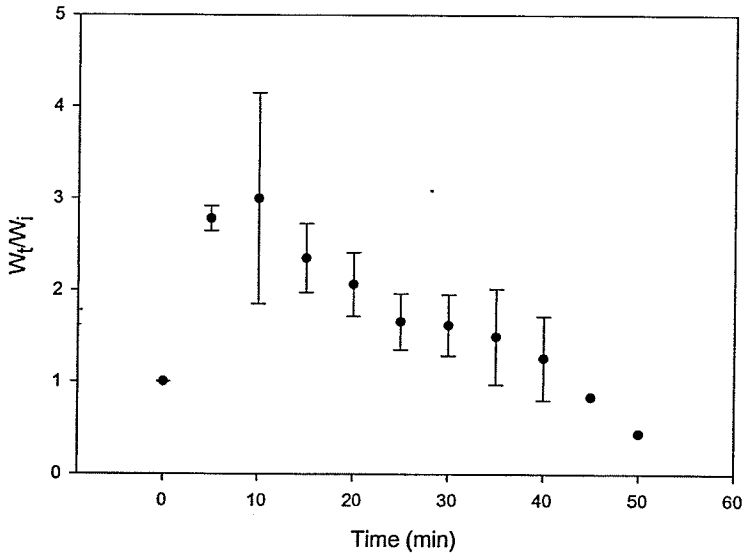
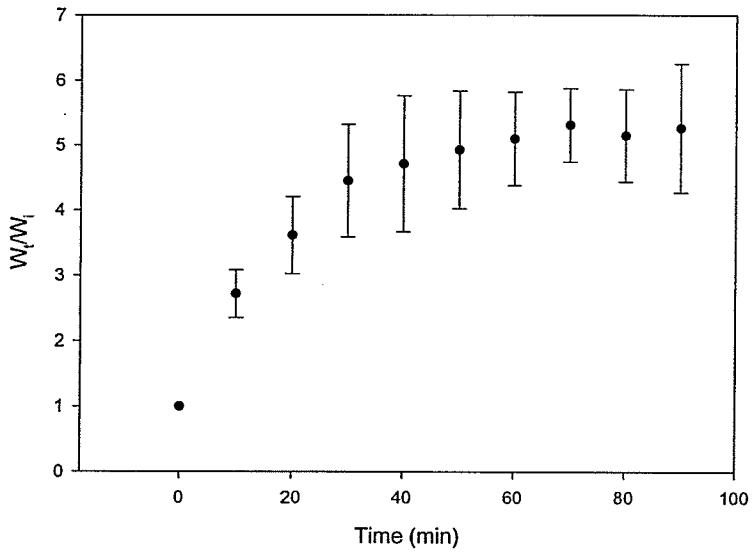


Figure 5.6 Insert swelling at 28°C expressed as a ratio of current weight to initial weight. Error bars are standard deviation. (n = 4)



Ofner and Schott [438] studied the swelling behavior of gelatin strips at 20 and 25°C and found that swelling seemed to follow a second-order process and developed an empirical model to describe the process using Equation 5.9:

$$\frac{t}{W} = A + Bt \qquad \text{Equation 5.9}$$

Where t is time, W is weight and both A and B are constants. Rearrangement and differentiation of this equation provided Equation 5.10:

$$\frac{dW}{dt} = \frac{A}{(A+Bt)^2} \qquad \text{Equation 5.10}$$

These equations were used to build models of the insert swelling as a function of time and the models were compared to the data points which were found experimentally. These data are shown graphically in Figures 5.7, 5.8 and 5.9.

Figure 5.7 Observed swelling insert mean weights compared to prediction by model using Equations 5.9 and 5.10; temperature 36°C.

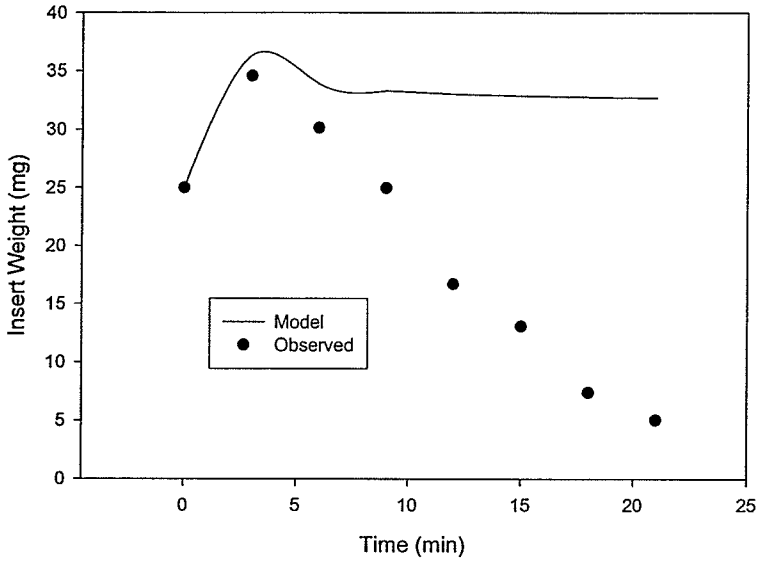


Figure 5.8 Observed swelling insert mean weights compared to prediction by model using Equations 5.9 and 5.10; temperature 32°C.

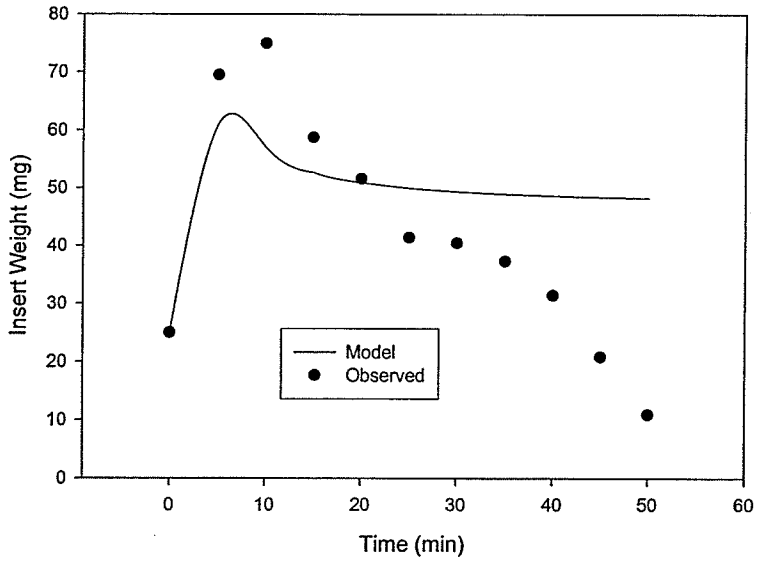
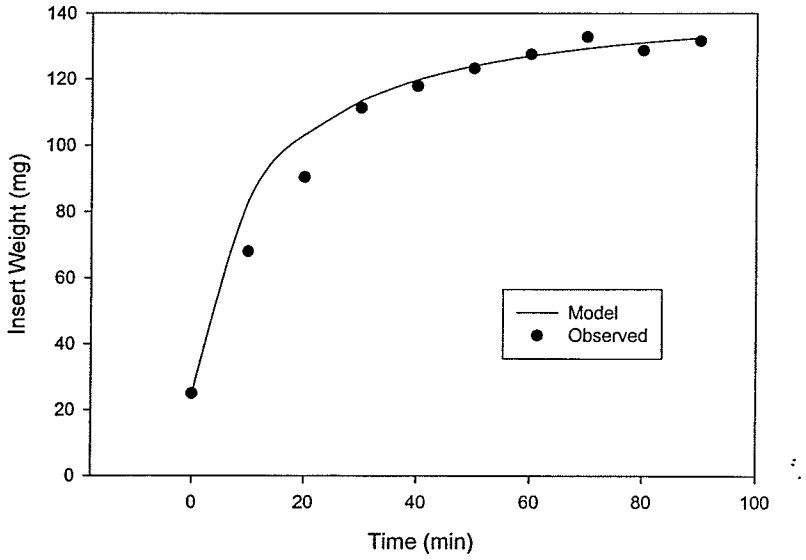


Figure 5.9 Observed swelling insert mean weights compared to prediction by model using Equations 5.9 and 5.10; temperature 28°C.



In Figure 5.7 and 5.8 at temperatures 36 and 32°C respectively, the observed data points can be seen to deviate from the swelling model and it is apparent that matrix dissolution is occurring in both since the insert weight falls below the starting weight after about 10 minutes for the samples at 36°C and 45 minutes for the sample at 32°C. At 36°C the amount of swelling was relatively small with the insert showing a maximum weight gain of about 10 mg in the first few minutes followed by a rapid loss of weight; these findings suggest that swelling is not a major mechanism for drug release at this temperature. The process at 32°C appears quite different; the model underestimates the amount of swelling and the observed data points show the inserts gaining about 50 mg of weight within about ten minutes followed by weight loss so it appears that swelling contributes to drug release during the early phases but relaxation or erosion becomes the more important after about 10 minutes. At 28°C the model and observed data points appear to agree and it appears that apomorphine release at this temperature is almost entirely due to swelling with relaxation or erosion playing a very minor role.

Both apomorphine and gelatin in the dissolution media were measured and the values for gelatin dissolution are presented in Table 5.6 and Figures 5.10 to 5.12.

Table 5.6 Quantity of gelatin dissolved as a ratio of the quantity dissolved at time t (M_t) over the total quantity present in the insert (M_∞) at temperatures of 36, 32 and 28°C.

36°C		32°C		28°C	
Time (min)	**M_t/M_∞**	**Time (min)**	**M_t/M_∞**	**Time (min)**	**M_t/M_∞**
3	0.07 ± 0.03	5	0.03 ± 0.01	10	0.01 ± 0.01
6	0.26 ± 0.05	10	0.08 ± 0.07	20	0.02 ± 0.01
9	0.44 ± 0.05	15	0.13 ± 0.11	30	0.02 ± 0.01
12	0.60 ± 0.06	20	0.19 ± 0.14	40	0.03 ± 0.01
15	0.77 ± 0.02	25	0.24 ± 0.16	50	0.03 ± 0.01
18	0.88 ± 0.04	30	0.29 ± 0.18	60	0.04 ± 0.01
21	0.95 ± 0.04	35	0.34 ± 0.18	70	0.04 ± 0.01
24	0.97 ± 0.03	40	0.40 ± 0.19	80	0.05 ± 0.01
27	0.98 ± 0.02	45	0.47 ± 0.18	90	0.05 ± 0.01
30	1.01 ± 0.02	50	0.54 ± 0.19	100	0.06 ± 0.01
33	1.00 ± 0.01	55	0.62 ± 0.22	110	0.06 ± 0.01
36	1.00 ± 0.01	60	0.66 ± 0.18	120	0.07 ± 0.02
39	0.99 ± 0.01	65	0.75 ± 0.19	130	0.07 ± 0.02
42	1.00 ± 0.01	70	0.81 ± 0.18	140	0.08 ± 0.01
45	1.00 ± 0.01	75	0.81 ± 0.17	150	0.10 ± 0.01
48	1.00 ± 0.01	80	0.88 ± 0.16	160	0.11 ± 0.01
		85	0.91 ± 0.13	180	0.15 ± 0.03
		90	0.91 ± 0.09	200	0.19 ± 0.04
		100	0.95 ± 0.06	220	0.24 ± 0.08
		120	1.02 ± 0.01		
		140	0.97 ± 0.01		
Mean ± Std dev				n = 4	

Figure 5.10 Gelatin dissolved at 36°C expressed as a ratio of amount dissolved at time = t (M_t) to total amount (M_{inf}). Error bars are standard deviation. (n = 4)

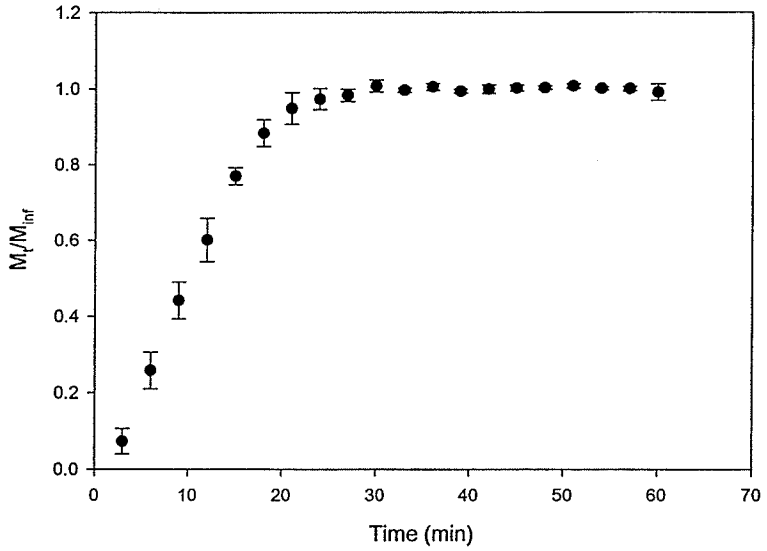


Figure 5.11 Gelatin dissolved at 32°C expressed as a ratio of amount dissolved at time = t (M_t) to total amount (M_{inf}). Error bars are standard deviation. (n = 4)

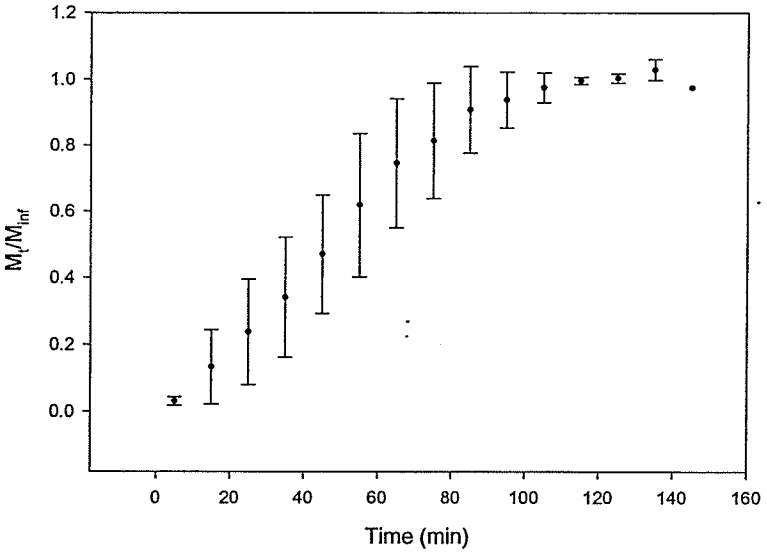
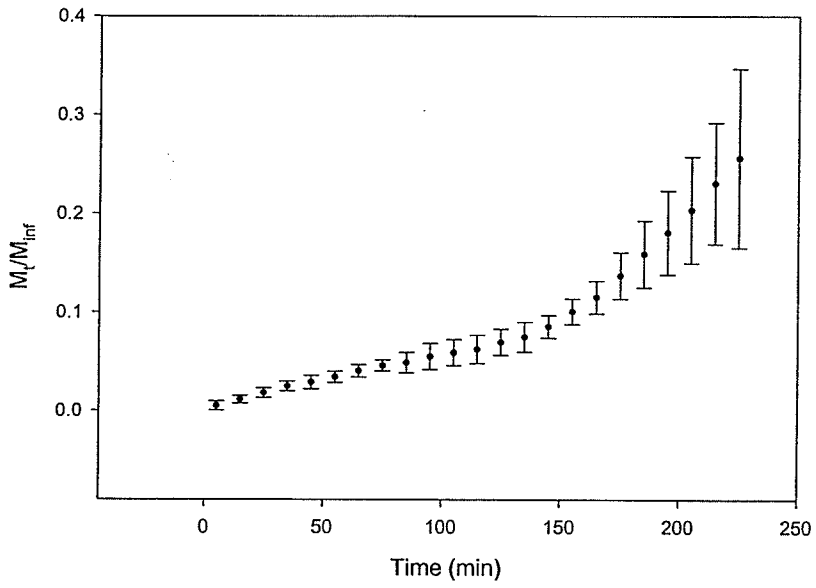


Figure 5.12 Gelatin dissolved at 28°C expressed as a ratio of amount dissolved at time = t (M_t) to total amount (M_{inf}). Error bars are standard deviation. (n = 4)



The data regarding gelatin dissolution suggest that temperature effects are important with increasing temperature resulting in faster release and a larger contribution of erosion to release. These interactions may be seen in Figure 5.13 to 5.15 where apomorphine and gelatin dissolution is superimposed on swelling behavior; the values for swelling are adjusted to show net weight changes and the scale is reduced by a factor of 10 for better clarity.

Figure 5.13 Fraction of apomorphine released and gelatin dissolved as a function of time. Swelling values adjusted to show relative change in weight as a function of time; temperature 36°C.

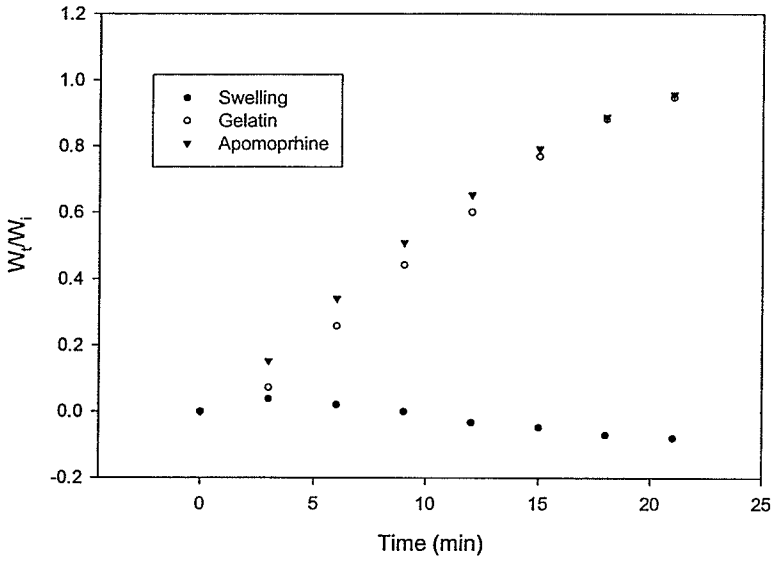


Figure 5.14 Fraction of apomorphine released and gelatin dissolved as a function of time. Swelling values adjusted to show relative change in weight as a function of time; temperature 32°C.

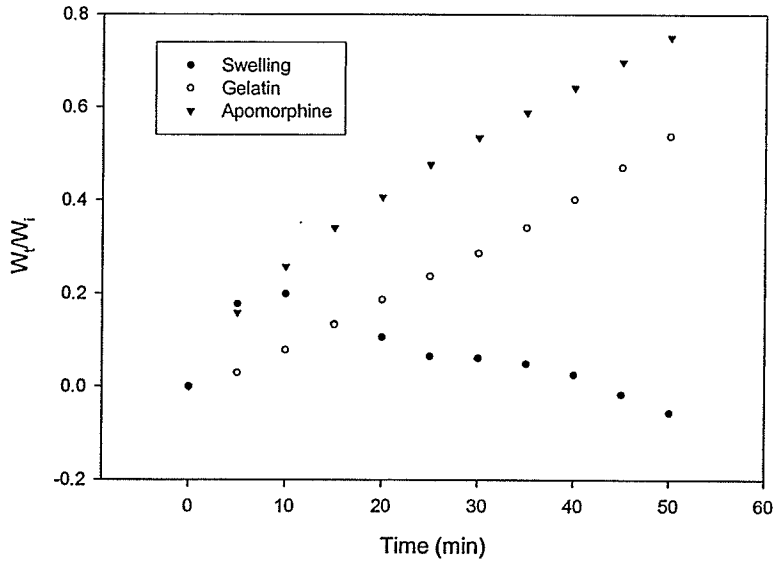
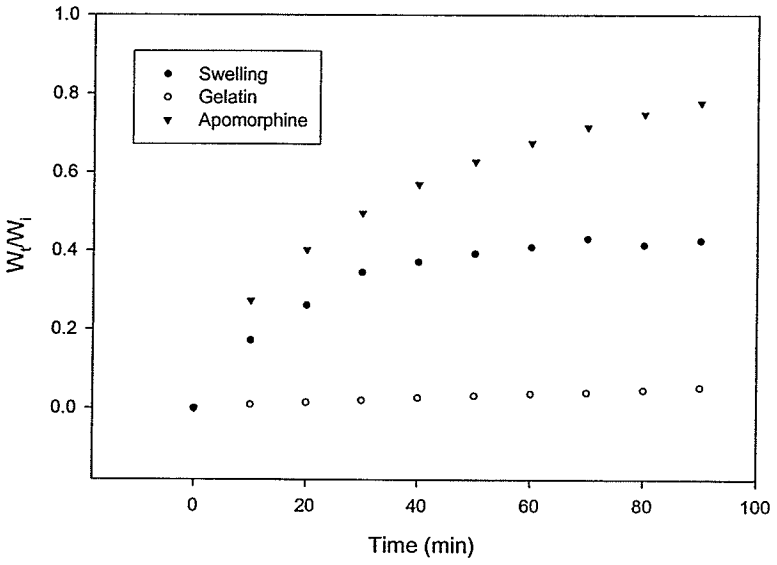


Figure 5.15 Fraction of apomorphine released and gelatin dissolved as a function of time. Swelling values adjusted to show relative change in weight as a function of time; temperature 28°C.



In the composite figures above, it can be seen that at 36°C the dissolution of apomorphine and gelatin are virtually superimposed suggesting that the apomorphine release is almost entirely due to relaxation. Swelling at this temperature is a minor factor and as was already shown, release is quite rapid. The inserts retained their integrity for about 20 minutes but after this time became semi-solid and easy removal from the eye would likely be problematical. The plots at 32°C show that both swelling and erosion are involved in drug release with swelling probably being the primary mechanism during the first 15 minutes and insert integrity was maintained for about 50 minutes. At 28°C, release was almost entirely through the process of swelling and release was relatively slow; insert integrity at this temperature was maintained throughout the entire time of the study.

Conclusions:

Since, as discussed in the overview to this section, the anticipated temperature of the conjunctival sac would be about 34°C, release of the apomorphine should follow a pattern

intermediate to the data presented for the experiments conducted at 36 and 32°C. In the initial product specifications a release time of about 15 minutes was required and the insert should maintain its integrity over this time to allow removal from the eye. The present formulation appears to meet these requirements and would be appropriate to use initially for a clinical trial. If the release of apomorphine is found to be too rapid and/or the integrity of the insert is inadequate during the early part of a clinical trial, the product could be re-formulated using cross-linked gelatin and this should shift the product performance more towards the attributes seen with the existing formulation at 28°C. If the release is found to be too slow during clinical use, it will probably be necessary to use a different polymeric matrix.

5.2 Stability studies

Overview:

In 1962 Garrett [483] published a review in which he outlined how the scientifically sound principles of chemical kinetics could be used to predict and quantify drug stability under a set of defined conditions. Although the review was primarily concerned with systems where the drug of interest was in solution and degradation was by an apparent first-order process, it laid the foundations for an analytical approach which could be used for other systems. In the review, Garrett first dealt with the concept of changes in drug concentration as a function of time and described the kinetic equations which could be used to calculate the apparent rate constants and how these could be used to determine the time where a 5 or 10% loss of the initial drug concentration occurred. The author dealt with pH and catalysis effects and provided examples where a pH-rate constant plot could be generated. He further demonstrated how plots of log (k) as a function of pH could provide a useful visual tool for determining the pH where the product would show optimal stability. The kinetic effects of dielectric constants and ionic strengths were then discussed and various mathematical models

were provided to assess these variables and use them in a predictive way and the final variable affecting stability to be discussed was temperature. Garrett reviewed the Arrhenius equation and showed how it could be used to predict stability and shelf-life at temperatures other than those used experimentally. The models he provided described the foundations of accelerated stability studies where experimental data from elevated temperatures could be used to predict shelf-life at lower temperatures. In the final section of the review, Garrett pointed out that although pharmaceutical formulations could be complex in their composition and although the exact mechanism involved in the degradation of the drug may not be known, the models presented generally apply and provide a useful basis for estimation of shelf-life. The models also provided a tool to permit a rational approach to assessing strategies which could increase product stability. This review was important as apart from providing useful tools, it also marked the point in time where sound scientific principles were applied to predicting drug product stability.

The accelerated stability approach which evolved from Garret's work was a two-step process where samples were held at various elevated temperatures and then sampled at timed intervals for chemical analysis. From these data a rate order was assigned and appropriate rate constants were calculated using linear regression analysis. For apparent zero-order processes drug content was plotted as a function of time since this is a linear relationship whereas for apparent first- and second-order reactions, it was necessary to transform the drug content values to achieve a linear relationship with time in order to perform the regression analysis. For first- and second-order processes this was done by plotting log concentration or inverse concentration values as functions of time respectively. The linearized equations for zero-, first- and second-order process are shown in Equations 5.11, 5.12 and 5.13 respectively; C_t is concentration at time t, C_0 is the initial concentration, t is time and k is the rate constant. The log value of the rate constant was then plotted as a function of the inverse of temperature in

absolute degrees which is the Arrhenius equation as shown in equation 5.14 where E_a is the activation energy, R is the universal gas constant, A is a pre-exponential function and T is temperature in absolute degrees.

$$C_t = C_0 - kt \qquad \text{Equation 5.11}$$

$$\ln C_t = \ln C_0 - kt \qquad \text{Equation 5.12}$$

$$\frac{1}{C_t} - \frac{1}{C_0} = kt \qquad \text{Equation 5.13}$$

$$\ln k = \ln A - \frac{E_a}{RT} \qquad \text{Equation 5.14}$$

The data generated from the linear regression of the Arrhenius equation allows the calculation of the rate constant at room temperature and from this, the t_{90} or shelf-life can be calculated.

The classical approach outlined by Garrett is not without some problems. The Arrhenius treatment of the data uses the mean rate constants obtained at the elevated temperatures and the errors associated with the analytical measurement of the drug and calculation of the rate-constants is not included. Furthermore, the error associated with the extrapolation process frequently lead to such wide confidence intervals for the predicted shelf-life that this information may be of limited value.

In an early publication Tootill [484] expressed concern about the error associated with accelerated stability studies. In this publication Tootill summarized the overall process proposed by Garret and pointed out that that there will be error associated with the analytical method used and that no use of this experimental error is used in fitting the data to the Arrhenius equation or in the final estimate of the expiration date. He further pointed out that situations may arise where the assumed linearity of the Arrhenius relationship may not be true. To deal with these short-coming, he recommended that the study be done at three temperatures selected so that their reciprocal values are in an arithmetic progression. If the Arrhenius

relationship is linear, the respective slopes will therefore be in a geometric progression and this could provide an independent check that the use of the Arrhenius equation is appropriate. He further recommended that the sampling times be selected so that about 50% of the initial drug concentration is present at the time of the second or the final sampling for each temperature trial. Toothill then presented a method of statistical analysis incorporating analysis of variance (ANOVA) which allowed the error associated with fitting the data to the Arrhenius equation and the subsequent extrapolated expiration date to be estimated.

Carstensen and Su [485] addressed some of the problems associated with fitting measured kinetic data to the Arrhenius equation. They suggested that when the degradation of the drug was less than 10% of the initial amount, zero- and first-order processes are indistinguishable and statistical methods are facilitated by the use of zero-order kinetics. Using diazepam injection as a model product, they demonstrated that application of accelerated stability methods make a number of assumptions: under the conditions of the reaction, only one degradation pathway is significant; that the Arrhenius equation is obeyed over the temperature interval being investigated and finally that the order of the reaction is known. Using the model of diazepam hydrolysis, the authors recommended that at least four points should be used to obtain reasonable extrapolation values from the Arrhenius equation and they further recommended that the best general procedure is to perform the experiment three times and estimate the limits on the extrapolated figure by the average and standard deviations of the three values obtained.

Munguia et al [486] investigated several statistical methods to determine whether the use of the Arrhenius equation could be justified in assessing an accelerated stability study data set. The authors noted that the method proposed by Garrett makes the assumption that the errors associated with the calculation of rate constants are distributed with equal variance and that these errors are independent of temperature. They proposed that if there is evidence that

the error is not independent of temperature, then the use of weighted linear regression might be more appropriate or another option might be the use of nonlinear regression of rate-constant as a function of time. In their conclusion, the authors recommended that a test for variance equality or homogeneity be done to confirm that application of the Arrhenius equation to the data would be appropriate.

Prior to the previous study, Bentley [487] also addressed the issue of error magnitude associated with the use of the Arrhenius equation to extrapolate and determine the rate constant at room temperature. In that paper, Bentley outlined a method for prediction of product stability using a weighted least-squares technique as well as statistical tests to determine the validity of applying the Arrhenius equation. Weighted least-squares reflect the behavior of the random errors in the model and works by incorporating extra non-negative constants or weights associated with each data point into the fitting criteria. The technique has the ability to handle regression situations where the data points are of varying quality and ensures that each data point has an appropriate level of influence on the final parameter estimates. If there are replicates in the data, which is usually the case in accelerated stability studies, the weight used for each data point would normally be the inverse of the variance although Bentley suggested the use of the inverse of the square of the width of its confidence interval. He also recommended the use of an F-statistic which is calculated by dividing the Arrhenius variance estimate by the combined variance. A significantly large F ratio would indicate that the Arrhenius relationship does not hold for that case. Bentley suggested that the use of non-weighted regression could force the Arrhenius plot through the lower temperature values giving more importance to these than should be warranted.

Further to the recommendations of Munguia et al [486], Arambasic and Jatic-Slavkovic [488] investigated the decomposition of diclofenac sodium injection at temperatures of 30, 40, 50 and 60°C and compared the use of linear and nonlinear regression in fitting the

experimental data to the Arrhenius equation. In the case of these data, a test for consistent variance failed ($p < 0.001$) and a more reliable fit for the data was observed using nonlinear regression with the data being fitted to a third-order polynomial.

In a recent review by Waterman and Adami [489], a number of factors which could cause the Arrhenius relationship to deviate from linearity were discussed. Among these situations or factors the authors included phase transitions which could occur with temperature changes. In this case the solubility of the drug or reactive species in a solvent or excipient could change and affect reaction rate; examples of this involved suspension dosage forms where increasing temperature caused increased dissolution of the drug and situations where degradation involves reaction with molecular oxygen and the amount of oxygen dissolved in the reaction medium decreases with increasing temperature. They also cited pH change as a factor which could cause changes in the reaction rate when the drug degrades through more than one pathway and change in pH may favor one reaction over another. The authors pointed out that pH can change with temperature even in buffered systems and this effect could lead to a discontinuity or deviation in the linearity of the Arrhenius relationship. A third factor discussed was in the case of complex reaction mechanisms where temperature may affect the equilibrium between reactants or reaction rate may begin to slow as a result of accumulation of degradation product which may be in a reversible reaction and which may have different solubility at different temperatures.

A number of variations on the classical approach proposed by Garrett have been investigated including using a plot of log shelf-life as a function of temperature [490], a modified transformation of the Arrhenius plot [491], nonlinear regression analysis of drug content as a function of time [492]and a method described as initial-rate analysis [493].

An investigation into whether a useful linear relationship existed between shelf-life (t_{90}) and the reciprocal of temperature in absolute degrees was conducted by Amirjahed [490].

In this study the author proposed that this relationship was valid and that it was not affected by the order of the reaction involved in the degradation. This work established that there was a reproducible linear relationship between log t_{90} and the reciprocal of absolute temperature and most importantly, that this was independent of the order of the reaction involved. The author cautions that this method is appropriate only when the Arrhenius relationship holds so this should be tested and can be done using the F-ratio test as was previously described by Bentley [487]. A further advantage of the method of data handling is that since it is independent of reaction order, sample analysis need not be carried beyond the point at which the quantity of drug remaining becomes less than 80% of the labeled amount.

Nash [491] presented a method for estimating shelf-life from accelerated stability studies using a modified Arrhenius plot where the log of the rate constant is plotted as a function of the inverse absolute temperature minus the inverse of the storage temperature. He suggested that this treatment of the Arrhenius equation allowed convergence the y-intercept of a linear plot to occur at log k for room temperature. Nash claimed that this method would be useful for preformulation studies in cases where experimental data would be limited due to low availability of test material which would be problematical for other methods. He claimed that even with limited data, this method would produce meaningful data with as little as two potency-time points at two temperatures. The outline of a computer program which could be used to this end was presented as part of the publication.

A more direct treatment of accelerated stability data using nonlinear regression was proposed by King et al [492]. The method which they proposed made the assumptions that the energy of activation is not a function of temperature and therefore the Arrhenius relationship is linear and that room temperature is 25°C (298°K). The authors rearranged the Arrhenius equation to deal with two temperatures, one of which is room temperature, and derived the following equation, the terms of which have already been defined:

$$k = k_{298} \exp\left(\frac{E_a}{R298}\right) \exp\left(\frac{-E_a}{RT}\right) \qquad \text{Equation 5.15}$$

The relationships for shelf-life and rate constant for zero- and first-order reactions are shown in Equations 5.16 and 5.17 respectively:

$$t_{90} = \frac{0.1}{k_0} \qquad \text{Equation 5.16}$$

$$t_{90} = \frac{0.1054}{k_1} \qquad \text{Equation 5.17}$$

Substitution of Equation 5.15 into the appropriate rate equation 5.11 or 5.12 and application of Equation 5.16 or Equation 5.17 provided Equation 5.18 and Equation 5.19 for zero- and first-order reactions respectively. These equations may now be used for nonlinear regression analysis to provide a direct estimate of E_a and t_{90} for a zero- and first-order respectively. Time (t) and temperature (T) are the two independent variables and concentration (C) is the dependent variable with E_a and t_{90} being the unknown values.

$$C = C_0 \left(1\text{-}t\left(\frac{0.1}{t_{90}}\right)\exp\left(\left(\frac{E_a}{R}\right)\left(\frac{1}{298} - \frac{1}{T}\right)\right)\right) \qquad \text{Equation 5.18}$$

$$C = C_0 \exp\left(-t\left(\frac{0.1054}{t_{90}}\right) \exp\left(\left(\frac{E_a}{R}\right)\left(\frac{1}{298} - \frac{1}{T}\right)\right)\right) \qquad \text{Equation 5.19}$$

In the experimental section of their study, the authors generated theoretical data sets using the appropriate rate equation for each order and a range of elevated temperatures.

For each data set they generated a series of random numbers using a normal distribution with a mean of zero and standard deviations of 2.5, 5.0 and 7.5% and these values were added to the

'errorless' data sets to simulate noise and provide the sets of simulated raw data. These data sets were analyzed using both the proposed nonlinear model and the classical method proposed by Garrett and the calculated values for E_a and t_{90} were compared to the theoretical values. Specific parameters which were examined included the influence of data noise, influence of different theoretical values of E_a and t_{90} and the influence of different reaction orders on the final estimates. Their findings suggested that with the simulated data, all three levels of noise provided values of E_a and t_{90} which were within 3% of the theoretical values, that different theoretical values of E_a and t_{90} had no apparent effect on the final estimates and that all three orders of reaction (zero-, first- and second-) were accommodated by the model. Comparison of the classical and nonlinear methods gave similar results for the mean values but the 95% confidence limits from the nonlinear method were much smaller and more symmetrical. The authors concluded that the nonlinear treatment of accelerated stability data provided a direct estimate of shelf-life using the parameters of concentration, temperature and time and that it provided a somewhat better statistical profile in terms of precision than the classical method. The use of nonlinear analysis of stability data was extended by Some et al [494] to include pH in order to generate pH-rate constant profiles.

Taylor and Shivji [493] reviewed drug stability testing methodologies and recommended the use of an initial-rate approach. The initial-rate method measures the appearance of degradation product and uses these data to calculate rate constants and order of reaction. The authors did point out that there were limitations with this approach and in some cases application was not possible. The method was predicated on there being only a single degradation product formed, that the nature of the degradation product was known and that the material was available in a pure form to allow its use as a standard and the method could only be used if these criteria were met. The advantages of this method are that shelf-life can be estimated, limits for degradation product could be established relative to the shelf-life and

reaction mechanism could be studied all from a single set of experiments. This initial-rate approach was recently used by Darrington and Jiao [495] in a study of the stability of an experimental macrolide antibiotic.

As an alternative to the classical method described by Garrett, the use of nonisothermal methodologies has been proposed [496, 497]. In this procedure, the storage temperature is varied and samples are withdrawn and analyzed for drug content at timed intervals. Provided that sufficient drug decomposes during the experiment, it is possible to calculate activation energy, reaction rates at any temperature and stability predictions [498]. Only one experiment is conducted with this method as opposed to the minimum four, each at different temperatures, required by the classical isothermal method so nonisothermal methodology should reduce both the time and analytical work involved in conducting the study. A number of heating rate schemes have been investigated including the original by Rogers which was varying the log inverse of temperature with time [496, 497], the inverse of temperature with time [499], adjusting the rate of temperature change according to the analytical findings during the experiment [498, 500] and increasing temperature in consecutive equal steps [501]. In the method described by Zoglio et al [502] where nonisothermal and isothermal techniques are combined, the nonisothermal procedure is followed until degradation is rapid enough to proceed at a convenient isothermal rate for sufficient half-lives so that reaction order can be determined unambiguously. Some recent variations of the nonisothermal method have been suggested by Zhan et al [503]; in one study they investigated a procedure where the rate of heating was increased by 2-4 times for every increase of 10°C and found results comparable to other heating regimens were obtained in much less time. Another improvement suggested and investigated by this group was the use of a rise in temperature to a maximum followed by cooling in progressive steps similar to that

used in the heating program [504]. They claimed that this process of heating followed by cooling allowed a better determination of reaction order.

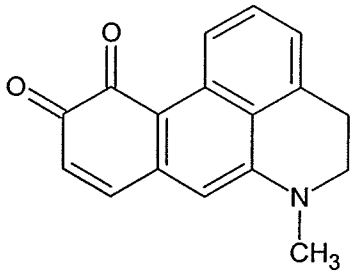
All of the studies reviewed which used the nonisothermal approach were done with solutions of the drug and all reported acceptable results. With solid state forms however, the method appears to have some limitations. Ellstrom and Nyqvist [505] investigated the application of nonisothermal methodology to drugs in the solid state. Curve fitting to a polynomial where the first derivative of the found equation is equal to the slope of the tangent of the curve was the accepted way of calculation [506-508] but these authors elected to determine the slope of three consecutive equidistant points and claimed that the this slope would be parallel to the tangent and would therefore approximate the degradation rate constant for the middle point. Using this method the rate constant was calculated for various temperatures and the energy of activation was calculated using these values and the Arrhenius equation. In their discussion, the authors pointed out that the nonisothermal data could be obtained with about half the number of samples required by the isothermal method and the time required to run the isothermal method was much longer. The authors concluded that nonisothermal stability testing in the solid state would be appropriate for substances with a simple degradation profile and if the method is applicable to the substrate, it offers savings in time, material and effort.

In an early study of apomorphine decomposition, Kaul et al [421] allowed a solution of apomorphine to stand at room temperature for several days. At this time, the solution had turned a dark bluish-green color and a dark-colored deposit was present on the sides of the flask. The material was extracted with chloroform, washed with 1N hydrochloric acid and the chloroform removed under reduced pressure. The isolated material had a melting point of 195°C and elemental analysis gave a negative test for nitrogen. Spectral analysis in the IR range showed no absorbance around 2500 cm^{-1}, a wavelength where an amino group would

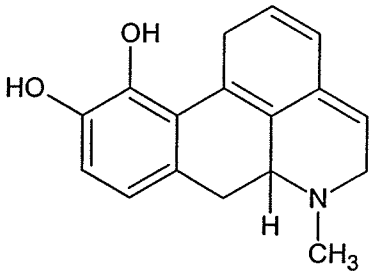
absorb. Largely on the basis of an apparent lack of nitrogen in the isolated product, the authors postulated that it was possibly an 8-substituted phenanthrene-3,4-dione resulting from cleavage of the heteroatom-containing ring of the apomorphine. As to the lack of nitrogen in the product, the authors suggested it was eliminated as methylamine.

More recently, the mechanism involved in the oxidative degradation of apomorphine was investigated by Cheng et al [509]. These authors used cyclic voltammetry with a Nujol-carbon paste electrode for their analysis and based on their data, proposed that apomorphine oxidized through a two-step process and that the final product of this was oxo-apomorphine (Figure 5.16). Isolation of the breakdown product was accomplished by washing the blue-green material off of the electrode with chloroform followed by recrystallization from hot benzene. The resulting material gave an identical UV spectrum in isoamyl acetate as oxo-apomorphine and elemental analysis was consistent with oxo-apomorphine.

Figure 5.16 Chemical structures of apomorphine and its degradation product oxo-apomorphine.



Oxo-apomorphine



Apomorphine

One of the first studies of apomorphine degradation was conducted by Burkman [422]. In this study, buffered and unbuffered solutions of apomorphine were incubated at 30°C. Samples were withdrawn at timed intervals and analyzed for apomorphine content and biological activity. The chemical analysis was accomplished using a spectroscopic method [85] and the biological activity was determined using pecking activity in pigeons [87, 208]. The biological activity measurements were based on earlier work by Burkman where he had shown that there was a quantitative relationship between the dose of apomorphine administered to pigeons and the frequency of a pecking response; this bioassay was later refined to include an electronic counter to monitor and record pecking frequency [510]. In this study, Burkman found that the unbuffered solutions (pH 5.5) retained 75% of their biological activity after sixty days while the solutions buffered to pH 6.0 were biologically inert after 16 days. He noted that samples which were extremely discolored still showed biological activity. The samples with 75% of their original activity were dark green with a black precipitate on the walls of the storage container. In order to determine whether the degradation products showed biological activity, the material was extracted into chloroform, the chloroform removed by evaporation and the residue tested using the pigeon assay; no activity was noted with this material. The results of the spectroscopic analysis was comparable to the quantitation calculated using the biological activity measurements. After reviewing the results Burkman also concluded that the intense discoloration seen in aged or stressed apomorphine samples could be seen when only minute amounts of apomorphine had decomposed and that degree or intensity of color was not a good index of the extent of the decomposition.

Further to the findings of the previous study, Burkman [420] conducted another investigation to study the kinetics of apomorphine degradation. In this study apomorphine solutions were buffered to pH values between 5.2 and 6.8 at increments of 0.2 pH units. Solutions were stored at 30 and 40°C and samples were withdrawn from the sample flasks at

timed intervals. The apomorphine concentrations were determined using a spectroscopic method [85] and rate constants (k) were calculated for each set of pH and temperature conditions. From these data the heat of activation (ΔH_a) was calculated. Curve fitting of the data suggested that the reaction proceeded by an apparent first-order process so a first-order model was used for the data analysis. The findings of this study showed that the first visually distinct discoloration of the solutions started when 0.1- 1.2% of the apomorphine had decomposed. The heat of activation for the process was calculated to be 12.2 $Kcal \cdot mol^{-1}$ and the rate of reaction increased with increasing pH in an exponential fashion.

In a more recent study Lundgren and Landersjo [86] investigated the decomposition kinetics of apomorphine solutions in an effort to assess various stabilization strategies. They studied the reaction at elevated temperatures and over a pH range of 2.85 to 7.05 under constant oxygen tension. In this study apomorphine concentration was measured using a colorimetric assay similar to that used by Burkman. The authors assumed the decomposition reaction followed an apparent first-order process and experiments were run at 50, 60, 70 and 80°C, rate constants were calculated for each temperature and then using an Arrhenius plot the energy of activation (E_a) was calculated. They found an apparent energy of activation of 14.8 . $Kcal \cdot mol^{-1}$ at pH 3.78. In agreement with Burkman, they noted that pronounced discoloration of the solutions occurred even when only a small amount of the apomorphine had decomposed. The stabilization strategies investigated included removal of atmospheric oxygen by purging the solution and final container with nitrogen, adjustment of pH and inclusion of the antioxidant sodium metabisulfite. While investigating these strategies, the authors noted that solutions containing bisulfite with the apomorphine showed a distinct yellow discoloration after they were heated and suggested the possibility of a reaction between apomorphine and bisulfite under these conditions. In their conclusions from this study, the authors suggested that a stable parenteral product could be prepared by buffering the

apomorphine solution to a pH around 3.0, including sodium metabisulfite at a final concentration around 0.1%, replacing the air in the container headspace with nitrogen and using filtration as the method of sterilization in order to avoid application of excessive heat.

Wilcox et al [432] studied the effects of ascorbic acid and sodium bisulfite as stabilizers of apomorphine solutions. Earlier work by this group had suggested that there was a correlation between circulating levels of apomorphine and stereotyped cage climbing and hypothermia in mice [511, 512]. In this study they investigated whether ascorbate or bisulfite would stabilize apomorphine solutions without interfering with cage climbing or hypothermia. This was of interest to them as they were proposing that this pharmacological model be used as part of an evaluation process to assess apomorphine prodrugs and the inclusion of ascorbic acid and/or sodium bisulfite might provide a means of stabilizing future formulations [512, 513]. In their results, the authors reported that both antioxidants were effective in delaying apomorphine degradation compared to the aqueous controls with higher levels being more effective than lower levels. They also showed that refrigeration was an effective strategy in stabilizing the apomorphine solutions. Neither antioxidant system influenced the biological response measurements of cage climbing or hypothermia induced by apomorphine. The outcome of these studies from the perspective of stabilizing the product suggested that both the strategies of including and antioxidant and storage at reduced temperature would be effective in extending the shelf-life of an apomorphine-containing product.

Experimental 5.2 - Stability studies

Introduction:

A series of experiments were done in order to characterize the stability of the inserts and to establish an approximate expiration date for the product. The traditional methodology for this involves stressing the product by storage at a series of elevated temperatures and sampling at selected time intervals. These data will allow the calculation of an apparent rate constant for each temperature and this information can then be used to construct an Arrhenius plot. The rate constant for an extrapolated temperature can then be determined from the Arrhenius plot and an approximate shelf life estimated. The following series of experiments were done to achieve this goal and the previously developed stability-indicating HPLC assay was used for apomorphine determinations.

Equipment and materials:

The equipment and materials used in this block of experiments is as follows: the chromatographic system used was manufactured by Knauer (Berlin, Germany) and consisted of a Wellchrom K-501 pump, a Wellchrom K-2501 variable wavelength UV detector, a Basic-Marathon autosampler type 816 and a Wellchrom HPLC Interface box. A Waters (Milford, MA, USA) model 420 fluorescence detector and a Bioanalytical Systems Inc (Lafayette, IN, USA) model LC-4B electrochemical detector were used in tandem with the Knauer system via the HPLC Interface box. The operating, data acquisition and data analysis software used was Eurochrome 2000® (Knauer, Berlin, Germany). The HPLC column used was cyano 4.6 x 150 mm particle size 4 μm (Jones, Hengoed, UK) and all chromatography was performed at ambient temperature. Thermostated water baths (model 1226) from Sheldon Manufacturing (Bristol, CT, USA) were used for temperature control.

Apomorphine HCl was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade phosphoric acid and acetonitrile as well as analytical grade hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific (Nepean, ON, Canada). Gelatin USP, glycerol USP and ascorbic acid USP were purchased from Spectrum Chemical (Gardenia, CA, USA). De-ionized water was obtained using an Aqua-Summa II reverse-osmosis system (Culligan, Toronto, ON, Canada). All glassware used was Class A (Fisher Scientific, Nepean, ON, Canada) and where required, dilutions were done using a 1000 μL adjustable volume pipette (Hamilton Company, Reno, NV, USA). A model AE-50 analytical balance (Mettler, Hightstown, NJ, USA) was used for weighing. Filtration of mobile phase was accomplished using 0.2 μm membranes (Pall, Gelman Laboratories, East Hills, NY, USA).

Standard solutions of apomorphine HCl in 0.05 M HCl were prepared, stored in glass at 5° C and discarded after seven days. Reference standard apomorphine was obtained from USP Convention, Rockville, MD, USA). Statistical analysis and graphical presentations were done using SigmaStat ® (SPSS Inc Chicago, IL, USA), Excel 2000® (Microsoft) and SigmaPlot 2000® (SPSS Inc Chicago, IL, USA).

All statistical analysis was done at the 95% confidence level unless otherwise indicated and data are presented as mean ± standard deviation unless otherwise indicated.

Experiment 5.2.1 - Preliminary

Purpose:

The purpose of this preliminary experiment was range-finding to determine a set of appropriate temperatures for conducting the accelerated stability study and to estimate appropriate sampling times for each temperature. In order to have a measure of data quality for these experiments, two other factors were considered. Firstly monitoring peak purity to ensure that no degradation products affected the analysis and secondly to determine whether weight variation among the inserts was a variable affecting data quality.

Study design and method:

A set of apomorphine inserts were prepared as described under Fabrication and thirty-two inserts from this lot were individually weighed and the weights recorded. Each insert was placed into a 20-mL type I glass vials and sealed using polymeric septa and aluminum closures. The individual vials were coded for identity, the weights of the inserts were recorded and the vials were placed into a water bath maintained at 86 ± 0.2°C. Four sample vials were removed at 48-hour intervals and held at -20°C until analysis.

When all samples were collected, the insert in each vial was dissolved in about 15 mL of 0.1 M HCl with the aid of gentle heating and the solution made to volume in a 25-mL volumetric flask using 0.05 M HCl. Samples of each solution were further diluted 1:1 with 0.05 M HCl and analyzed for apomorphine content using the developed HPLC method. For the analysis UV detection was used for apomorphine quantitation with simultaneous fluorescence and electrochemical detection used to monitor peak purity; the detectors were connected in series as previously described and data collected from all three. The samples were coded and analyzed in a randomized order and a set of standards were analyzed at the beginning and end of each set of samples. Data handling involved quantitation of peak

response from each detection system by fitting these data to linear equations derived from regression analysis of the standards. Apomorphine content of the inserts was calculated from the concentration values and these were examined to determine whether normalization to the mean weight of the inserts was necessary to account for weight variation among the samples. The insert content was expressed as a percentage of the labeled drug content (2 mg) and the resulting % content of apomorphine remaining in the inserts was tabled as a function of time and these data were analyzed to characterize the decomposition kinetics.

Results and discussion:

Data from this preliminary study are presented in Table 5.7

Table 5.7 Sample weights for each time interval and insert content as % of the label amount remaining after storage at 86°C.

Time (days)	Weights (mg)	Content (% of label)
0	22.1 ± 0.3	96.04 ± 2.05
2	22.9 ± 0.9	95.86 ± 1.13
4	22.0 ± 2.1	91.16 ± 1.23
6	23.2 ± 0.7	82.61 ± 3.70
8	22.1 ± 0.8	82.07 ± 2.73
10	23.1 ± 1.0	78.40 ± 1.19
12	22.8 ± 0.9	77.02 ± 0.19
14	23.0 ± 0.2	75.89 ± 0.75
Values Mean ± std dev n = 4		

Since the purpose of this preliminary study was primarily range finding in order to have a basis for planning and designing the accelerated stability studies, one of the first objectives was to establish methods for assessing data quality in terms of peak purity and whether insert weight variation was affecting the data. Since three very different detection systems were

used, peak purity could be demonstrated by comparison of the apomorphine concentration determined from each detector at each sampling time. The amount of apomorphine in the samples calculated using the different detection systems and expressed as percentage of the label amount are presented in Table 5.8 and the level of significance was determined using ANOVA.

Table 5.8 Quantity of apomorphine remaining as % of label amount calculated from responses of UV, fluorescence (Flu) and electrochemical (ECD) detectors.

	Apomorphine Content (% of label)			
Time (d)	**UV**	**Flu**	**ECD**	**P**
0	96.04 ± 2.05	96.63 ± 4.16	98.27 ± 4.23	0.677
2	95.86 ± 1.13	95.73 ± 1.25	95.99 ± 2.52	0.977
4	91.16 ± 1.23	89.69 ± 1.45	89.64 ± 1.71	0.301
6	82.61 ± 3.70	83.18 ± 3.34	84.25 ± 4.71	0.839
8	82.07 ± 2.73	82.84 ± 2.61	80.79 ± 3.34	0.505
10	78.40 ± 1.19	77.64 ± 1.37	79.71 ± 3.61	0.473
12	77.02 ± 0.19	75.51 ± 0.70	76.81 ± 1.07	0.380
14	75.89 ± 0.75	74.53 ± 0.46	73.02 ± 2.07	0.051
Values: Mean ± Std dev			n = 4	

The inserts used for this range-finding study were prepared by casting from a solution as described under the fabrication section and it was assumed that the drug was distributed in a homogeneous fashion throughout the matrix material and that there would be a direct relationship between insert weight and drug content. For this preliminary trial, each insert was weighed and the 32 inserts used in the study had a mean mass of 22.6 mg with a standard deviation of 1.0; a test for normality (Kolmogorov-Smirnov) gave a distribution value of 0.076 ($p > 0.200$) which indicates that the data match the pattern expected if the data were

drawn from a population with a normal distribution. To test the assumption that there was a direct relationship between the sample weight and apomorphine content, the weights and apomorphine content of the samples for time zero were examined; a plot of drug content as a function of insert weight showed a linear relationship and regression analysis of these data showed a coefficient of determination (r^2) of 0.833 indicating a direct relationship between weight and drug content. In the future stability studies to be undertaken, weight variability among the inserts might exert a significant effect on the resulting data so it may be necessary to deal with this by normalizing each sample to the mean insert mass of the samples being studied. The analytical data obtained in this experiment before and after normalizing to the mean sample weight were compared using a t-test. This test indicated that there was no significant difference between the adjusted and unadjusted values ($p = 0.757$) so for this preliminary trial, no adjustment would be necessary for weight variation. For the future stability studies however, it would be appropriate to check the data for each trial to ensure that weight variation was not affecting the data.

Under the conditions of time and temperature used in this preliminary study, the drug content of the samples only decomposed by about 25% so it is unlikely that an apparent reaction order could be assigned to the process and since accelerated stability studies provide only an approximation of shelf-life, the literature is somewhat divided as to the importance of assigning an apparent order to the process. Nonetheless, the data generated in this study were assessed to see if there was some indication as to order or if order would in fact be important in assigning a shelf-life (t_{90}). The % amount, log % amount and inverse % amount of apomorphine remaining was plotted as a function of time to determine whether an apparent zero-, first- or second-order process respectively could be assigned to the decomposition process. These data were analyzed using linear regression and the coefficients for slope and intercept as well as the coefficient of determination (r^2) were determined. These data and the

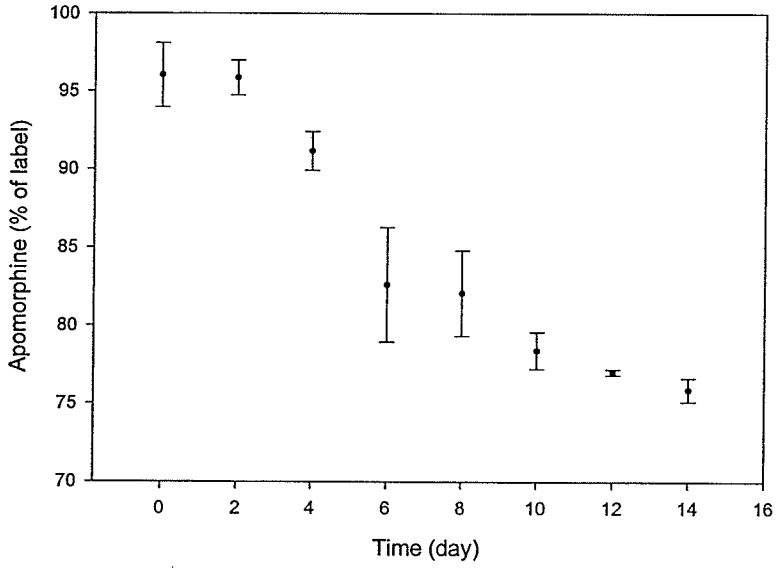
results of the analysis are presented in Table 5.9 and a plot of % apomorphine remaining as a function of time is presented in Figure 5.17.

Product shelf-life is often defined as the time at which the content falls to 90% of the labeled quantity and this is defined as the t_{90} value. Using the apparent zero- and first- order models of the data, the respective t_{90} values were calculated from the regression coefficients and are presented in Table 5.9. In this preliminary trial the apomorphine content had only dropped by about 25% of the original labeled amount and as was anticipated, there were insufficient data to assign a rate-order to the decomposition model. Since the goal is to assign an approximate shelf-life and this is identified as the time at which the drug content declines by 10% from the original amount, normally an apparent zero- or first-order process is accepted as a working model [86, 420].

Table 5.9 The amount of apomorphine remaining with these data fit to an apparent zero- and first-order model respectively.

Time (d)	% Remaining	log % Remaining
0	96.04 ± 2.05	1.982 ± 0.009
2	95.86 ± 1.13	1.982 ± 0.005
4	91.16 ± 1.23	1.960 ± 0.006
6	82.61 ± 3.67	1.917 ± 0.019
8	82.07 ± 2.73	1.914 ± 0.014
10	78.40 ± 1.19	1.896 ± 0.006
12	77.02 ± 0.19	1.887 ± 0.001
14	75.89 ± 0.75	1.880 ± 0.004
Slope	-1.600	-0.00813
y-Intercept	95.97	1.983
r^2	0.867	0.873
K	1.600 d	0.0187 d^{-1}
t_{90} **(d)**	6.25	5.63
Values: Mean ± Std deviation		n = 4

Figure 5.17 The amount of apomorphine remaining in each insert as % of the labeled content. The error bars represent the standard deviation and n = 4. The storage temperature was 86°C.



A very empirical and approximate method of determining temperature effects on shelf-life is seen with the Q_{10} test [514]. The equation used in this estimation is:

$$t_{90}(T_2)=\frac{t_{90}(T_1)}{Q_{10}^{(\Delta T/10)}} \qquad \text{Equation 5.20}$$

Where $t_{90}(T_2)$ is the estimated shelf-life for temperature T_2, $t_{90}(T_1)$ is the given shelf-life at T_1, ΔT is the temperature difference between T_1 and T_2 and Q_{10} is an integer value of 2, 3 or 4 depending on how conservative an estimate is desired.

Using $Q_{10} = 2$ and an apparent first-order rate constant (k) of 0.0187 d^{-1} for 86°C, estimated t_{90} values and the derived rate constants can be calculated for a series of temperatures as shown in Table 5.10.

Table 5.10 Estimated shelf-lives and derived first-order rate constants calculated using the Q_{10} method for apomorphine inserts stored over a range of temperatures.

Temperature (°C)	t_{90} (d)	k (d^{-1})
86	5.6	1.88×10^{-2}
80	8.5	1.24×10^{-2}
70	17.0	6.21×10^{-3}
60	34.0	3.10×10^{-3}
50	67.9	1.55×10^{-3}
40	135.8	7.76×10^{-4}
30	271.6	3.88×10^{-4}

From these preliminary estimated values, the accelerated stability study should be conducted over the temperature range of 80 to 50°C at 10°C intervals and sampling should be done at intervals of 3, 7, 14 and 21 days respectively. The duration of the experiment should be approximately 40, 90, 180 and 270 days for the respective storage temperatures as this will allow about 50% degradation for all the samples.

Based on the data obtained from this preliminary study, quantitation of the apomorphine samples could be done using UV detection and peak purity could be monitored by statistically comparing the data obtained from UV, fluorescence and electrochemical detection. Although in this trial weight variation among the inserts was not a significant factor affecting quantitation, this should be checked in each subsequent trial. Insufficient data were collected during this trial to assign a reaction order to the degradation so a longer collection period should be used in the subsequent trials however these data are not inconsistent with an apparent first-order reaction and this is supported in the literature [86, 420] so for the accelerated stability study an apparent first-order process was assumed.

Experiment 5.2.2 – Accelerated stability study

Purpose:

The purpose of these experiments was to perform an accelerated stability study to determine a set of apparent rate constants over a range of temperatures and by using an Arrhenius plot, estimate a shelf-life for the product at room temperature.

Study design and method:

Apomorphine inserts were prepared as described in the fabrication section, individually weighed and the weights recorded. Each insert was placed into a 20-mL type I glass vial and sealed using polymeric septa and aluminum closures. The individual vials were coded for identity then divided into four groups of 56 vials. Each group of vials was placed into a water bath and one bath was maintained at 80 ± 0.2°C, one at 70 ± 0.1°C, one at 60 ± 0.1°C and the last at 50 ± 0.1°C. Four sample vials were removed at the timed intervals shown in Table 5.11 and held at -20°C until analysis.

Table 5.11 Storage temperatures and sampling frequency for each set of 56 vials.

Temperature (°C)	Sampling Interval (days)
80	3
70	7
60	14
50	21

When all the samples were collected, the insert in each vial was dissolved in about 15 mL of 0.1 M HCl with the aid of gentle heating and the solution made to volume in a 25-mL volumetric flask using 0.05 M HCl. Samples of each solution were further diluted 1:1 with 0.05 M HCl and analyzed for apomorphine content using the developed HPLC method. For the analysis UV detection was used for apomorphine quantitation and fluorescence and

electrochemical detection were used to monitor peak purity; the detectors were connected in series as previously described. The prepared samples were all coded and run in a randomized order and a set of standards were run at the beginning and end of each set of samples.

Data handling involved quantitation of peak response from each detection system by fitting these data to linear equations derived from regression analysis of the standards. These data were examined to confirm peak purity and the apomorphine content of the inserts was calculated from the concentration values calculated from UV detection. The data were examined as previously described to determine whether normalization to the mean weight of the inserts was necessary to account for weight variation among the samples. The insert content was expressed as a percentage of the labeled drug content (2 mg) and the resulting % content of apomorphine remaining in the inserts was tabled as a function of time and these data were analyzed to characterize the decomposition kinetics.

Results and discussion:

The data from the accelerated stability study are presented in Table 5.12:

Table 5.12 Overall data for four trials at each temperature level; the time and % of label content is presented.

80°C		70°C		60°C		50°C	
Day	**% Content**	**Day**	**% Content**	**Day**	**% Content**	**Day**	**% Content**
0	100.2 ± 2.5	0	102.3 ± 2.3	0	101.4 ± 2.9	0	100.4 ± 1.5
3	95.21 ± 1.79	7	98.57 ± 2.50	14	99.38 ± 3.58	21	101.7 ± 5.4
6	90.95 ± 4.48	14	98.96 ± 7.71	28	100.9 ± 4.7	42	101.4 ± 2.4
9	87.90 ± 6.49	21	91.16 ± 3.03	42	89.98 ± 1.85	63	99.85 ± 5.58
12	84.97 ± 2.63	28	86.22 ± 3.66	56	86.78 ± 3.69	84	95.54 ± 2.90
15	74.18 ± 2.74	35	81.46 ± 5.74	70	87.45 ± 3.93	105	93.74 ± 5.18
18	68.74 ± 5.96	42	78.61 ± 0.28	84	78.67 ± 2.83	126	95.07 ± 9.89
21	72.33 ± 1.79	49	79.57 ± 2.07	98	77.44 ± 1.92	147	79.25 ± 1.28
24	63.61 ± 2.50	56	70.99 ± 6.74	112	63.57 ± 2.32	168	78.47 ± 3.52
27	61.64 ± 1.47	63	68.20 ± 4.72	126	66.73 ± 7.20	189	72.94 ± 1.98
30	72.66 ± 4.21	70	76.12 ± 3.92	140	64.68 ± 3.96	210	69.98 ± 7.49
33	61.85 ± 3.32	77	60.85 ± 0.89	154	61.01 ± 4.57	231	60.29 ± 4.74
36	57.39 ± 6.75	84	65.30 ± 4.58	168	48.37 ± 3.59	252	64.23 ± 2.66
39	55.36 ± 1.56	91	65.31 ± 3.88	182	48.76 ± 4.26	273	62.71 ± 6.04
Values Mean ± std dev n = 4							

The quality of the data presented in Table 5.12 was assessed in the same way it was for the preliminary trial: The data for each temperature trial was checked for peak purity by comparing the responses for each of the three detectors at each time interval and in order to determine whether weight variation among the inserts used affected the content calculation, the labeled content calculations were compared to those with the results normalized to the

mean insert weight; these data were compared using a paired t-test. The data from the experiment where the samples were held at 80°C are presented in Table 5.13.

Table 5.13 Overall data for four trials using samples stored at 80°C; content found from UV, fluorescence and electrochemical detection and is not adjusted for weight variation in the insert samples.

		Content (% of label)			
Time (days)	**Weights (mg)**	**UV**	**Flu**	**ECD**	**P**
0	26.2 ± 0.4	100.2 ± 2.5	100.9 ± 7.4	99.91 ± 2.65	0.946
3	26.1 ± 0.6	95.21 ± 1.79	94.90 ± 5.32	94.58 ± 3.56	0.780
6	24.2 ± 1.5	90.95 ± 4.48	89.89 ± 5.19	91.05 ± 7.07	0.874
9	24.1 ± 2.2	87.90 ± 6.49	86.51 ± 3.08	88.20 ± 9.84	0.886
12	24.7 ± 0.9	84.97 ± 2.63	83.95 ± 4.84	85.21 ± 3.58	0.770
15	23.5 ± 0.9	74.18 ± 2.74	73.74 ± 5.28	74.18 ± 3.40	0.888
18	24.4 ± 2.5	68.74 ± 5.96	68.14 ± 2.60	68.41 ± 8.85	0.939
21	24.6 ± 1.2	72.33 ± 1.79	71.58 ± 4.03	72.39 ± 2.02	0.784
24	25.7 ± 1.1	63.61 ± 2.50	62.33 ± 4.58	65.14 ± 4.55	0.604
27	25.1 ± 1.0	61.64 ± 1.47	60.88 ± 3.06	61.35 ± 3.29	0.831
30	25.1 ± 1.6	72.66 ± 4.21	71.30 ± 6.09	73.03 ± 3.77	0.782
33	23.1 ± 1.7	61.85 ± 3.32	60.96 ± 3.53	61.67 ± 4.45	0.780
36	24.1 ± 2.9	57.39 ± 6.75	56.56 ± 6.89	57.21 ± 7.39	0.931
39	24.9 ± 0.9	55.36 ± 1.60	54.61 ± 2.86	55.11 ± 3.66	0.673
Values Mean ± std dev n = 4					

The check for peak purity using ANOVA to compare the data obtained from each of the detectors at each time interval was acceptable ($p>0.05$). The mean weight and standard deviation of the inserts used for this experiment was 24.7 ± 1.6 mg and the quantity remaining in the inserts expressed as % of label amount when compared to values calculated with insert

weight normalized to the mean weight using a paired t-test showed no significant difference ($p = 0.109$) indicating that weight variation was not affecting the analytical data for the samples maintained at 80°C.

The data from the experiment where the samples were held at 70°C are presented in Table 5.14.

Table 5.14 Overall data for four trials using samples stored at 70°C; content calculated from UV, fluorescence and electrochemical detection and is not adjusted for weight variation in the insert samples.

		Content (% of label)			
Time (days)	**Weights (mg)**	**UV**	**Flu**	**ECD**	**p**
0	26.2 ± 0.3	102.3 ± 2.3	102.4 ± 2.5	102.8 ± 2.1	0.806
7	24.5 ± 0.7	98.57 ± 2.50	97.75 ± 1.89	98.95 ± 4.15	0.784
14	24.1 ± 1.7	98.96 ± 7.71	98.04 ± 7.67	99.54 ± 8.84	0.958
21	25.9 ± 0.5	91.16 ± 3.03	89.85 ± 3.46	92.59 ± 3.39	0.553
28	25.6 ± 1.3	86.22 ± 3.66	84.82 ± 3.30	87.98 ± 6.21	0.505
35	23.8 ± 1.9	81.46 ± 5.74	80.48 ± 6.37	81.94 ± 6.40	0.925
42	24.0 ± 0.3	78.61 ± 0.28	77.39 ± 2.34	79.90 ± 2.21	0.265
49	23.8 ± 0.9	79.57 ± 2.07	77.86 ± 2.52	80.67 ± 3.78	0.360
56	24.0 ± 2.5	70.99 ± 6.74	70.52 ± 6.96	71.10 ± 8.18	0.986
63	25.4 ± 0.8	68.20 ± 4.72	65.55 ± 1.71	72.49 ± 12.0	0.372
70	25.6 ± 1.3	76.12 ± 3.92	74.45 ± 3.51	77.46 ± 6.28	0.470
77	24.5 ± 0.8	60.85 ± 0.89	60.08 ± 1.16	61.57 ± 2.77	0.544
84	24.9 ± 1.5	65.30 ± 4.58	64.95 ± 5.67	65.27 ± 4.44	0.977
91	25.2 ± 1.1	65.31 ± 3.88	64.65 ± 3.45	65.15 ± 5.87	0.891
Values Mean ± std dev n = 4					

The check for peak purity using ANOVA to compare the data obtained from each of the detectors at each time interval was acceptable ($p>0.05$). The mean weight and standard

deviation of the inserts used for this experiment was 24.8 ± 1.4 mg and the quantity remaining in the inserts expressed as % of label amount when compared to values calculated with insert weight normalized to the mean weight using a paired t-test showed no significant difference (p = 0.213) indicating that weight variation was not affecting the analytical data for the samples maintained at 70°C.

Table 5.15 Overall data for four trials using samples stored at 60°C. The % of label content based on concentration found from UV, fluorescence and electrochemical detection and is not adjusted for weight variation in the insert samples.

		Content (% of label)			
Time (days)	**Weights (mg)**	**UV**	**Flu**	**ECD**	**p**
0	26.2 ± 0.4	101.4 ± 2.9	99.93 ± 2.36	103.2 ± 4.3	0.392
14	25.2 ± 0.6	99.38 ± 3.58	99.05 ± 3.75	98.33 ± 3.85	0.754
28	23.2 ± 1.1	100.9 ± 4.7	99.53 ± 5.16	100.3 ± 5.4	0.647
42	25.0 ± 0.5	89.98 ± 1.85	88.01 ± 1.85	89.49 ± 2.03	0.070
56	24.9 ± 1.0	86.78 ± 3.68	84.45 ± 3.73	86.39 ± 5.68	0.274
70	24.5 ± 1.1	87.45 ± 3.93	85.07 ± 3.42	86.90 ± 4.79	0.245
84	23.9 ± 1.2	78.67 ± 2.83	76.32 ± 2.38	79.14 ± 5.88	0.341
98	25.4 ± 0.4	77.44 ± 1.92	75.55 ± 1.71	76.79 ± 4.16	0.178
112	26.2 ± 0.2	63.57 ± 2.32	63.46 ± 1.31	62.25 ± 5.26	0.488
126	24.0 ± 2.4	66.73 ± 7.20	66.43 ± 6.95	65.91 ± 8.53	0.931
140	24.9 ± 1.3	64.68 ± 3.96	65.43 ± 2.88	62.28 ± 7.27	0.468
154	24.9 ± 1.6	61.01 ± 4.57	62.08 ± 3.85	62.08 ± 3.85	0.635
168	24.4 ± 1.9	48.37 ± 3.59	49.25 ± 2.48	46.50 ± 6.23	0.555
182	25.4 ± 0.8	48.76 ± 4.26	51.37 ± 0.63	43.47 ± 11.9	0.228
Values Mean ± std dev n = 4					

The data from the experiment where the samples were held at 60°C are presented in Table 5.15. The check for peak purity using ANOVA to compare the data obtained from each

of the detectors at each time interval was acceptable ($p>0.05$). The mean weight and standard deviation of the inserts used for this experiment was 24.9 ± 1.3 mg and the quantity remaining in the inserts expressed as % of label amount when compared to values calculated with insert weight normalized to the mean weight using a paired t-test showed no significant difference ($p = 0.077$) indicating that weight variation was not affecting the analytical data for the samples maintained at 60°C.

Table 5.16 Overall data for four trials using samples stored at 50°C. The % of label content based on concentration found from UV, fluorescence and electrochemical detection and is not adjusted for weight variation in the insert samples.

		Content (% of label)			
Time (days)	**Weights (mg)**	**UV**	**Flu**	**ECD**	**p**
0	24.9 ± 1.6	100.4 ± 1.5	98.48 ± 4.47	100.3 ± 3.4	0.260
21	24.9 ± 1.2	101.7 ± 5.4	101.1 ± 5.9	102.1 ± 6.5	0.969
42	25.6 ± 0.2	101.4 ± 2.4	101.0 ± 3.5	100.8 ± 2.4	0.663
63	24.2 ± 1.1	99.85 ± 5.58	99.01 ± 6.65	99.39 ± 5.19	0.859
84	25.2 ± 0.5	95.54 ± 2.90	93.29 ± 2.09	96.40 ± 6.00	0.375
105	23.0 ± 1.4	93.74 ± 5.18	90.98 ± 5.98	94.48 ± 3.81	0.459
126	23.9 ± 2.0	95.07 ± 9.89	92.01 ± 9.92	95.44 ± 10.4	0.731
147	26.2 ± 0.4	79.25 ± 1.28	74.64 ± 2.39	81.79 ± 1.39	0.053
168	24.5 ± 1.6	78.47 ± 3.52	73.53 ± 3.31	80.89 ± 3.93	0.487
189	26.0 ± 0.6	72.94 ± 1.98	68.99 ± 1.79	74.80 ± 2.66	0.448
210	24.6 ± 2.2	69.98 ± 7.49	67.46 ± 6.86	69.72 ± 10.5	0.664
231	23.9 ± 2.1	60.29 ± 4.74	57.84 ± 4.23	62.12 ± 5.14	0.664
252	24.7 ± 1.0	64.23 ± 2.66	62.66 ± 3.08	65.63 ± 3.18	0.387
273	24.3 ± 2.3	62.71 ± 6.04	60.13 ± 6.19	66.03 ± 8.04	0.478
Values Mean ± std dev n = 4					

The data from the experiment where the samples were held at 50°C are presented in Table 5.16. The check for peak purity using ANOVA to compare the data obtained from each of the detectors at each time interval was acceptable ($p>0.05$). The mean weight and standard deviation of the inserts used for this experiment was 24.7 ± 1.5 mg and the quantity remaining in the inserts expressed as % of label amount when compared to values calculated with insert weight normalized to the mean weight using a paired t-test showed no significant difference ($p = 0.342$) indicating that weight variation was not affecting the analytical data for the samples maintained at 50°C.

Overall, the data from the accelerated study appears acceptable in that there was no interference from degradation products in the quantitation as demonstrated by peak purity evaluation and weight variation among the inserts did not significantly affect the quantitation of the samples. The data presented in Table 5.12 were therefore analyzed using the classical method of Garret to establish a shelf-life.

The content-time data from Table 5.12 are presented graphically in Figures 5.18 to 5.21:

Figure 5.18 Apomorphine content (% label) of inserts as a function of time during storage at 80°C. The error bars are standard deviations. (n = 4)

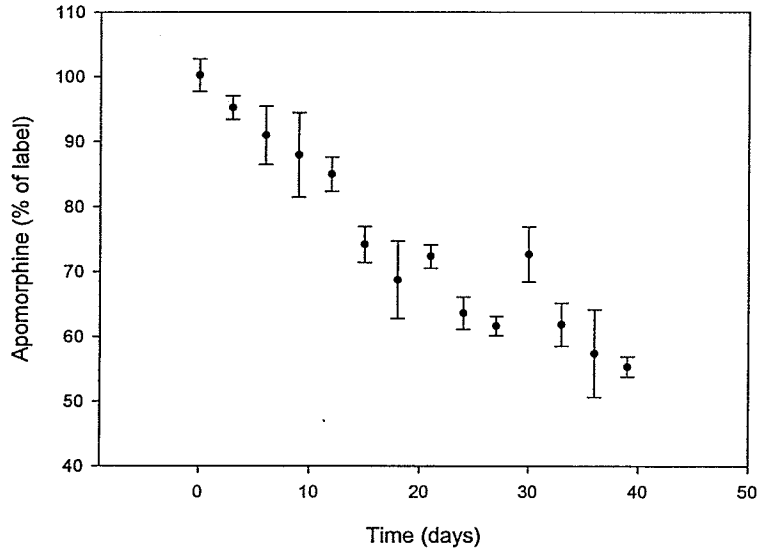


Figure 5.19 Apomorphine content (% label) of inserts as a function of time during storage at 70°C. The error bars are standard deviations. (n = 4)

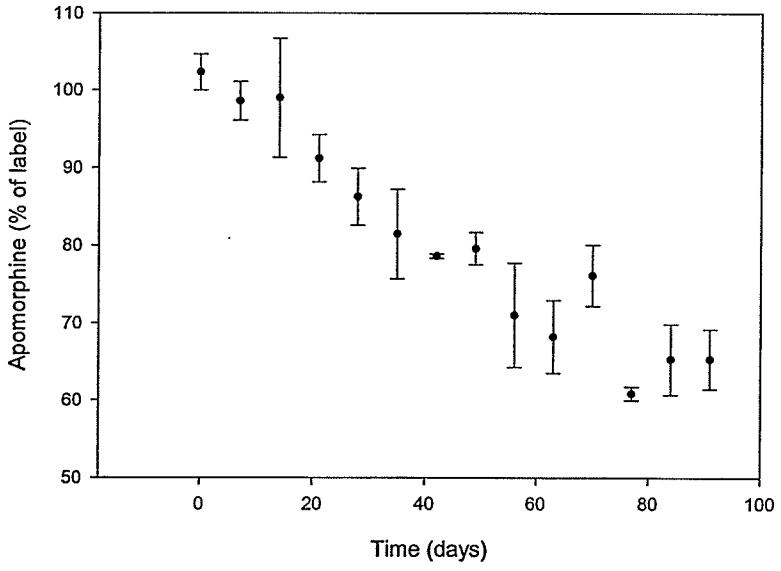


Figure 5.20 Apomorphine content (% label) of inserts as a function of time during storage at 60°C. The error bars are standard deviations. (n = 4)

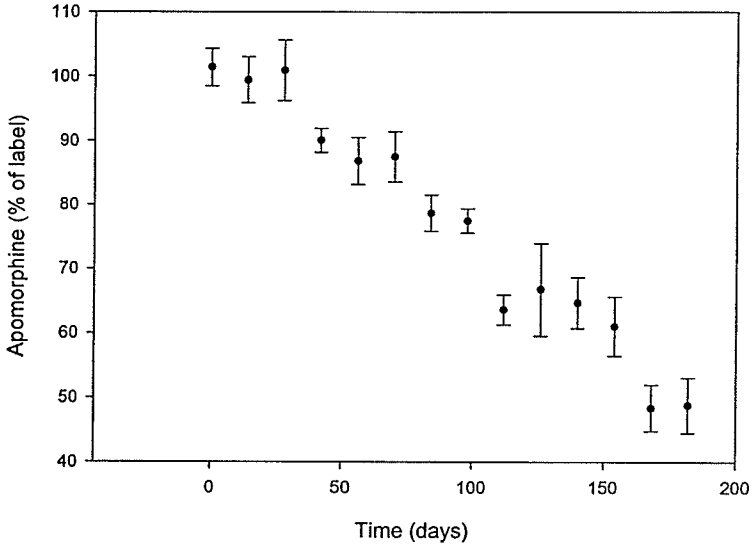
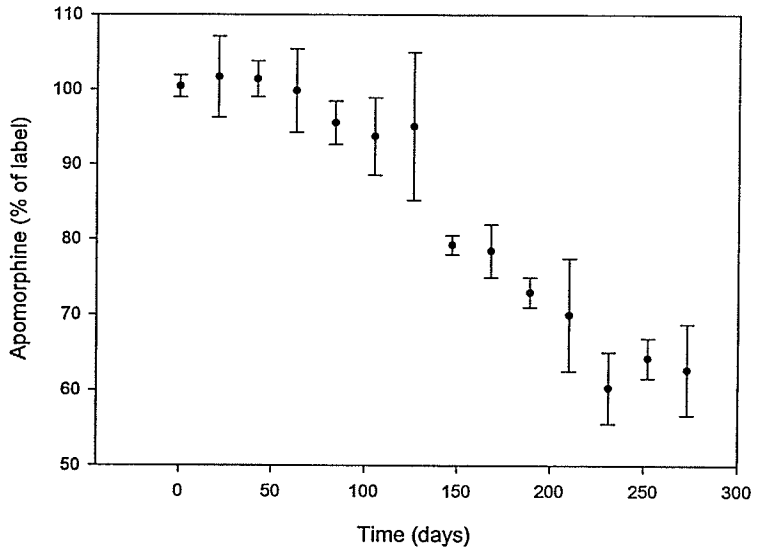


Figure 5.21 Apomorphine content (% label) of inserts as a function of time during storage at 50°C. The error bars are standard deviations. (n = 4)



The data presented in Table 5.12 and Figures 5.18 to 5.21 were analyzed using linear regression and assuming a first-order process. The log values of content as a function of time

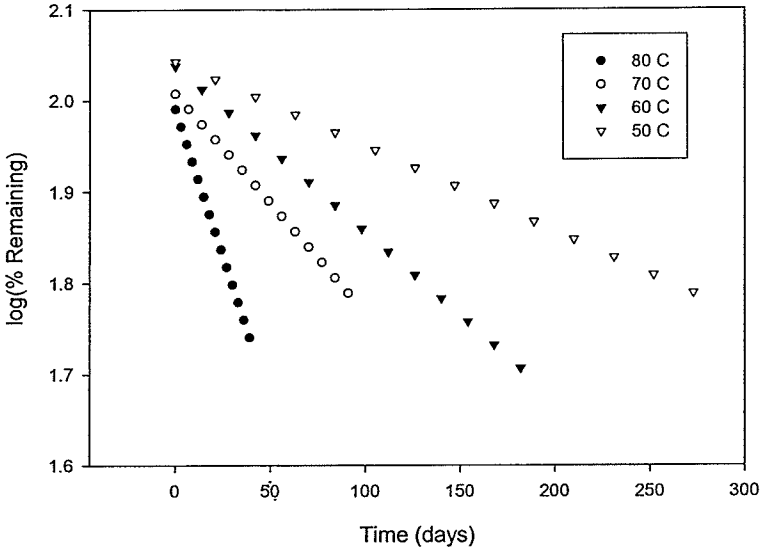
were examined for each temperature and a summary of these findings is presented in Table 5.17.

Table 5.17 Regression analysis of data from Table 5.12 fit to an apparent first-order model; log content as a function of time.

	80°C	70°C	60°C	50°C
Intercept	1.991	2.007	2.038	2.043
Slope	-6.41×10^{-3}	-2.40×10^{-3}	-1.82×10^{-3}	-9.31×10^{-4}
r^2	0.907	0.916	0.943	0.914

Although the results of linear regression assuming a first-order process showed reasonable linearity as evidenced by the coefficients of determination, visual inspection of the plots presented in Figure 5.18 to 5.21 suggests that a latent or lag period is present at the lower temperatures and this is supported by the respective values for the y-intercepts for the different temperatures. This latent period may be due to the presence of the residual metabisulfite and ascorbic acid which had been added to prevent discoloration of the inserts during the casting and curing processes and may serve to invalidate the accelerated stability findings. Plots of the regression lines for each temperature are presented in Figure 5.22 and a visual inspection of this plot shows a possible discrepancy between the lines for 70 and 60°C which could be due to the latent period and suggests that the accelerated stability model may underestimate the calculated shelf-life of the ocular inserts.

Figure 5.22 Linear regression plots of apomorphine remaining as a function of time assuming an apparent first-order process.



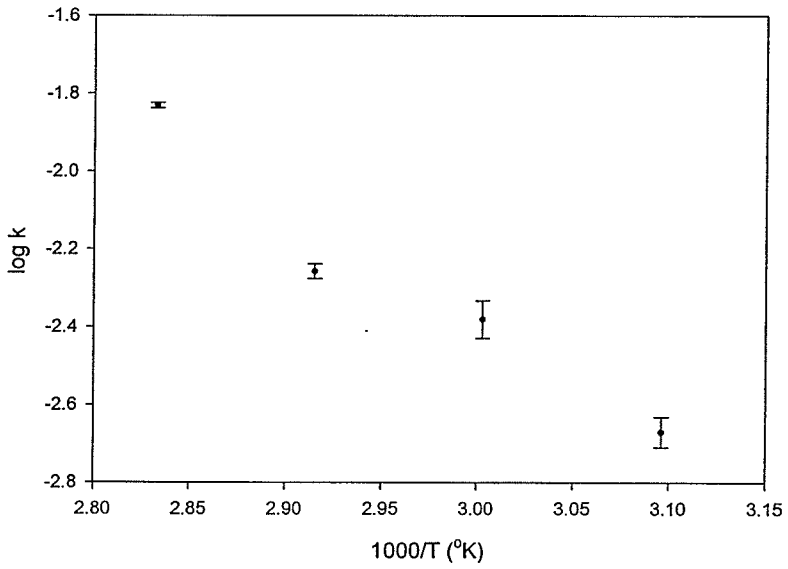
The data generated from the apomorphine degradation at different temperatures were analyzed using an Arrhenius plot where the log values of the rate constants are plotted as a function of the inverse of temperature in degrees Kelvin. These data are summarized in Table 5.18 and this relationship is then used to extrapolate and estimate the rate constant at room temperature which was taken as 20°C. The values for the rate constants were determined using Equation 5.12.

Table 5.18 Data used in preparing Arrhenius plot.

Temp (°C)	1/T (°K)	k (days^{-1})
80	2.833×10^{-3}	1.476×10^{-2}
70	2.916×10^{-3}	5.527×10^{-3}
60	3.003×10^{-3}	4.191×10^{-3}
50	3.096×10^{-3}	2.144×10^{-3}

An Arrhenius plot of the experimental data is shown in Figure 5.23 and regression analysis of this plot gave a slope of 3002.4, a Y-intercept of 6.608 and a coefficient of determination (r^2) of 0.9510.

Figure 5.23 Arrhenius plot of data from Table 5.23; temperature transformed by factor of 1000 for ease of reading and error bars are standard deviation of each trial for each temperature. (n = 4)



This information was then used to calculate through extrapolation an estimate of the rate constant (k) and shelf-life (t_{90}) for temperatures from 80 to 10°C and these calculated values are presented in Table 5.19; the shelf-life values were calculated using Equation 5.17. The energy of activation was calculated using Equation 5.14 and was found to be 13.75 Kcal·mol^{-1}.

Table 5.19 Calculated and extrapolated values for k and t_{90} over a temperature range of 80 to 10°C.

Temp (°C)	k (day^{-1})	t_{90} (days)
80	12.67×10^{-3}	8.3
70	7.16×10^{-3}	14.7
60	3.92×10^{-3}	26.8
50	2.06×10^{-3}	50.9
30	0.50×10^{-3}	208.6
25	0.34×10^{-3}	305.6
20	0.23×10^{-3}	453.7
10	0.10×10^{-3}	1042.7

The results of this accelerated stability study suggested that the shelf-life of the inserts stored at room temperature of 20 to 25°C is probably between 10 and 15 months. Based on these findings, a preliminary dating of one year from the time of preparation would not be inappropriate.

The data in Table 5.12 were also analyzed using the nonlinear method proposed by King et al [492] using Equation 5.19; non-linear regression was used and time and temperature were the independent variables with content as dependent variable and the content variable was weighted to the inverse of standard deviation for the four trials at each temperature investigated. The results of this analysis were quite different from those found using the classic method of Garret as the energy of activation (E_a) was calculated as 17.29 $Kcal \cdot mol^{-1}$ and t_{90} of 728 days at 25°C suggesting greater insert stability. This finding and the previous observations suggested that the Arrhenius relationship between rate constants and temperature may not be linear and therefore the accelerated stability model would not be valid. The previous observations included the appearance of a possible lag or latent period at the lower

temperatures which is noticeable in Figures 5.20 and 5.21; in Figure 5.22 showing the regression lines for the different temperatures where there is an apparent discontinuity between the two higher and lower temperatures and the Arrhenius plot in Figure 5.23 which shows a possible curvature although the coefficient of determination (r^2) is 0.9510 suggesting linearity.

Experiment 5.2.3 – 'In use' conditions

Purpose:

The purpose of this experiment was to obtain preliminary data regarding the real-time stability of the apomorphine ocular inserts. This experiment was range-finding to determine appropriate sampling times and also to serve as a comparison to the findings of the accelerated stability study.

Study design and method:

A set of apomorphine inserts was prepared in advance of conducting the accelerated stability testing. They were packaged in their final containers as described in the fabrication section; each insert was packaged in a plastic shell and five inserts were placed in the each polyethylene screw-cap jar along with a desiccator pack. Six of the packages were further protected from light by wrapping them in aluminum foil and of these, three were purged with nitrogen prior to applying the screw cap. Six packages were stored without foil wrapping and three of these were also purged with nitrogen prior to application of the cap. They were all stored at room temperature in normal lighting conditions on a shelf in the laboratory and samples were to be taken for analysis at intervals dictated by the accelerated stability study. The temperatures in the laboratory varied between 20 and 24°C over the course of a year. Analysis was done using the developed HPLC method and four inserts were analyzed from each group of samples.

Results and discussion:

Based on the data from the accelerated stability study, samples of the stored inserts were taken for analysis at time intervals of 540 days and then at 1530 days after preparation. The results of the analysis are shown in Table 5.20:

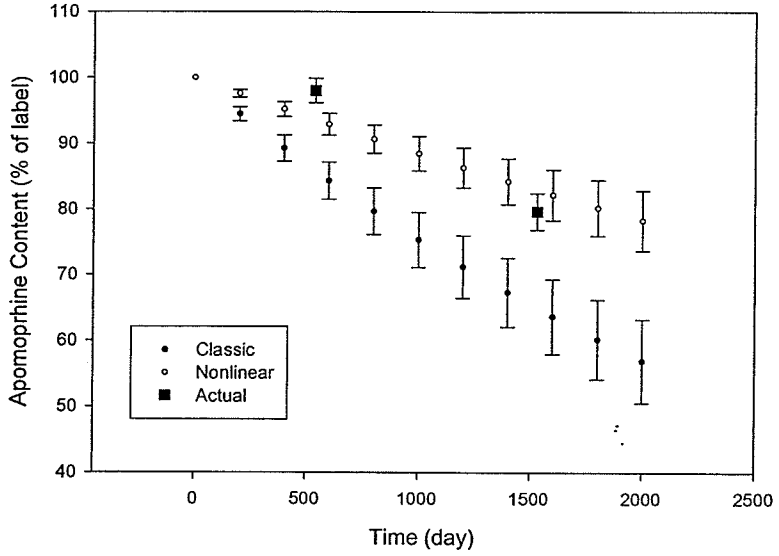
Table 5.20 Samples stored in final packaging at ambient temperature; Effects of foil and nitrogen purge.

Sample	540 days	1530 days
Foil/N_2 purge	98.26 ± 1.50	81.63 ± 3.05
Foil	97.89 ± 2.79	78.53 ± 2.18
N_2 Purge	98.12 ± 2.28	78.97 ± 1.25
No foil/no purge	97.68 ± 1.29	79.44 ± 4.07
Values Mean ± std dev n = 4		

The data presented in Table 5.20 for the sampling times at 540 and 1530 days after preparation were analyzed using ANOVA and the samples in the four groups at time 540 days showed no significant difference ($p = 0.979$) and those at time 1530 also showed no significant difference in content expressed as % of labeled amount ($p = 0.449$). These data suggest that additional protection from light and purging the final container with nitrogen prior to closure had no effect on the shelf-life. Overall, the content of the samples at day 540 was 97.99 ± 2.82% of the labeled amount and those from day 1530 was 79.64 ± 2.82% of the labeled amount.

Based on the accelerated study data, the theoretical content as a function of time using the data obtained from classical method of Garret and the nonlinear method of King were calculated and these curves are presented in Figure 5.24. The values found from the real-time study are superimposed on these plots:

Figure 5.24 Actual values of insert content under real storage conditions superimposed on theoretical plots of content based on classical and non-linear models. The upper error bar for the theoretical plots represents content under storage at 20°C and the lower at 25°C.



Based on the information presented in Figure 5.24, the inserts did not lend themselves well to accelerated stability testing and neither the classical nor the nonlinear models provided a picture consistent with the preliminary trial under normal storage conditions. It appears that there is a latent or lag period which is not evident at the higher temperatures and neither model was able to accommodate this and consequently underestimated the shelf life of the product; it is also possible that change of state was a factor since the inserts underwent a change of state going from a solid at lower temperatures to a semi-solid at higher temperatures. The preliminary real time assessment suggests that a latent period is present, likely due to the antioxidant activity of the residual metabisulfite and ascorbic acid and in-use storage conditions will be needed to establish an actual shelf-life. In these real time studies, more intensive sampling will be required over the time period of 800 to 1200 days as it appears that the latent period ends and the content drops to below 90% within this time window.

Conclusions:

Part of the product evaluation involves the assignment of an expiration date for the product so a preliminary accelerated study was conducted to establish parameters to be used in a formal accelerated study. The results of the accelerated stability study suggested that an accelerated model was likely not suitable for the ocular inserts since linearity of the Arrhenius relationship between rate constant and temperature was not linear and this was confirmed through a preliminary study of the product stability under conditions of use. Since the accelerated models appeared to underestimate the shelf-life and suggested suitable potency up to 10 to 15 months and stability was confirmed beyond this time in the preliminary study of in-use conditions, a tentative shelf-life of one year will be used for the product which will be investigated in the subsequent clinical trial. The assignment of a true shelf-life will require a future designed study under in-use conditions of storage.

5.3 Clinical Trial

Overview:

Historically emetic agents have been used to induce vomiting in cases of oral ingestion of poisons but their use for this purpose has declined dramatically over the past two decades. This was mainly due to the results of studies which have demonstrated that in most cases emesis lacks clinic efficacy as a therapeutic tool for humans [1, 18, 26, 29-33]. In veterinary medicine, however, there are situations where emesis is still indicated, particularly in canine patients [3, 4, 37]. Although there are a number of parallels between humans and dogs in the nature of the intoxicants ingested, there are some notable differences and situations which would be uncommon in human cases; the nature of the intoxicants ingested by dogs include items such as garbage and carrion, the quantity of material ingested is often very large and with dogs the packaging materials holding the intoxicant are often consumed along with the intoxicant. Ingestion of foreign objects such as balls and clothing also appear to be common with canine patients but would be unusual in human cases. In human medicine more than half of the accidental poisonings occur in children under the age of six years [11] whereas with dogs there does not seem to be much of an age bias and the probability of exposure seems to be quite consistent over the life-span of a dog [46,55,56,57]. Metabolic differences between canines and humans may also be a factor in intoxications. Chocolate for example is relatively non-toxic to humans but theobromine content of chocolate presents the potential for a serious intoxication in dogs [36, 37].

A number of chemicals have been used as emetic agents including ammonium carbonate, copper sulfate, zinc sulfate, mustard powder, sodium chloride and hydrogen peroxide; these act primarily through irritation of the gastric mucosa and have largely fallen into disuse [1, 12, 34]. In terms of emetic drugs, syrup of ipecac which contains the alkaloids emetine and cephaline has been a popular emetic because it is effective orally and has an

acceptable safety profile [12, 24]. With ipecac, there is a latent period or delay and emesis usually occurs after about 20 minutes [12, 13, 16]. Since ipecac is adsorbed by activated charcoal, these treatments cannot be used simultaneously [16, 25].

Apomorphine is a very potent emetic agent which has fallen into disuse largely due to the fact that it is not effective orally and for some individuals, the recommended parenteral emetic dose of 0.1-0.04 $mg \cdot kg^{-1}$ is associated with toxicity most notably respiratory depression and persistent emesis which may lead to acute circulatory collapse, coma and death [1,2]. As well as having a range of serious side effects, apomorphine also shows a very high level of interpatient variability [1, 3-6]. Apomorphine is a dopamine D_2 agonist able to interact with central dopamine D_2-receptors and acts at the level of the chemoreceptor trigger zone (CTZ) in the area postrema of the medulla [110, 111]. Although apomorphine induces emesis by interaction with the D_2-receptors in the chemoreceptor trigger zone, it also has an anti-emetic activity once it crosses the blood-brain barrier and interacts with the μ-opioid receptors in the centrally located vomiting centre. This can lead to suppression of emesis and is the reason why if an initial dose fails to induce emesis, a second dose is usually ineffective [165]. Routes of administration for apomorphine and corresponding traditional dose forms which have been investigated and reported in dogs include parenteral, sublingual, nasal, rectal and ocular [4, 33, 182]. For induction of emesis the most practical route has been parenteral and the drug is usually given at a dose of 0.08 $mg \cdot kg^{-1}$ intravenously. A method of administration which avoids injection and yet allows a controlled rate of administration could reduce the major disadvantages of this drug which are toxicity and variability of response. If emesis were the therapeutic end point and drug absorption could be abruptly stopped at that point, some of the adverse effects related to overdosage might be avoided and the toxicity associated with the inherent interpatient variability seen with apomorphine might be reduced through the use of this type of a controlled-release drug delivery system. Since the drug has been shown to be

absorbed systemically in dogs after ocular administration [4, 182], incorporation of the drug into a polymeric matrix and application of this device to the eye could result in controlled release and absorption of the apomorphine. When the therapeutic end-point of emesis is reached, the device could be removed from the eye thus removing the drug reservoir and stopping further absorption of the drug. Since the clinician would want a prompt response to the drug, sufficient release of the drug from the polymer matrix would have to be complete in 5-10 minutes.

In the initial specifications set out for apomorphine ocular inserts, the product would require the following attributes:

-be non-irritating to the conjunctival membranes

-be easy to apply and be soft and adhesive enough to remain in place

-release most of the drug within a fifteen minute time window

-maintain its integrity to allow easy removal when emesis has occurred

-have sufficient chemical stability to allow a reasonable shelf life

-insert must be able to be sterilized

An insert meeting the above criteria was formulated using a gelatin-glycerin matrix and in-vitro testing of the inserts showed that at 34°C which is the anticipated temperature of the conjunctival sac [475, 476], drug release was through both diffusion and erosion controlled mechanisms and an acceptable time window for release of 15 minutes was achieved. Stability testing demonstrated a shelf-life of at least one year and a method of fabrication resulting in the production of sterile inserts was established.

As part of the product evaluation, these inserts were made available to veterinary clinics interested in using and evaluating the inserts in canine patients. The dose of apomorphine recommended for induction of emesis using the ocular route was set at 0.1 $mg \cdot kg^{-1}$ and since the weights of the patients could range from 1 to 100 kg, the drug load or

amount of apomorphine in each insert was problematical. Although a series of inserts with different drug loads for different weight ranges might be appropriate, smaller patients would be put at risk if an insert containing an inappropriately high load were used so only inserts carrying 2 mg of apomorphine were used for this trial.

Experiment 5.3 - Clinical Trial

Purpose:

The purpose of this trial was to investigate the efficacy of the developed apomorphine ocular inserts in terms of emesis induction and to determine the nature and incidence of adverse effects when they were used under clinical conditions.

Materials:

The ocular inserts developed and described in earlier section were used for this trial; the inserts met the product specifications originally set out and were prepared and packaged as described in the section on product fabrication. In the interest of patient safety, only one strength of insert was used and it carried a drug load of 2 mg of apomorphine; since the recommended emetic dose by the ocular route is to be 0.1 $mg \cdot kg^{-1}$ and the anticipated patient weight range was from 1 to 100 kg, application of one insert would be appropriate for patients with weights ranging from 10 to 20 kg; smaller patients would require a portion of one insert and larger patients would require the application of multiple inserts.

In order to provide a basis of comparison and a control, a small series of patients were treated with apomorphine administered by the intravenous route. The drug was supplied as a single-use 2 mL vial containing 1 $mg \cdot mL^{-1}$ of apomorphine HCl. Sodium chloride was used as a tonicity adjuster, sodium metabisulfite 0.1% as an antioxidant and the solution was buffered to a pH of 5.5 using a phosphate buffer in water for injection. Sterilization was achieved by

filtration and the vial was sealed under an atmosphere of nitrogen. Samples were taken from each lot of the injectable product prepared and tested for apomorphine content and sterility.

Study design:

Inserts were supplied to the participating clinics along with an information sheet and case report questionnaire. The information sheet described the inserts as to their use, dosage, storage and handling. The questionnaire supplied for each insert recorded the breed and weight of the patient, the reason for use and the nature of the intoxication, the time to emesis, dose applied and adverse effects noted. For each case the ease of use and the usefulness of the product was also documented. A list of potential adverse effects including prolonged vomiting, tachycardia, excitation, respiratory depression, bradycardia, sedation, ocular irritation and other were on the questionnaire and the clinician was asked to assign a grade for each. Grades were subjective and ranged from 0 to 5 with 5 being severe and 0 being not present. The ease of use and usefulness for each case was also assigned a subjective grade from 0 to 5 with 5 representing very easy to use and very useful. Copies of the information sheet and questionnaire are presented as Figures 5.25 and 5.26. The same questionnaire was used for the patients receiving intravenous apomorphine. The completed forms were returned by fax or mail and for the first year of the study copies were also sent to the Bureau of Veterinary Drugs at Health Canada. As the completed forms were returned, the data were maintained in a database.

Figure 5.25 Apomorphine ocular insert information sheet.

APOMORPHINE OCULAR INSERTS
Pharmaceutics Research Lab
Tel: ( )
Email:

Apomorphine ocular inserts are a unique experimental delivery system for use in inducing emesis in canine patients. Each disc contains 2 mg of apomorphine HCl in a non-irritating, biocompatible polymer.

Administration:

The insert should be carefully removed from the protective wrapping by peeling the paper backing off of the plastic well. Using sterile blunt forceps, the insert is placed into the lower subconjunctival space. It is useful to wet the eye and/or the insert with saline or artificial tears for about 10 seconds prior to insertion as this additional moisture allows the insert to soften and conform to the shape of the eye more quickly. Release of the drug occurs as the polymer hydrates and drug release continues as the insert remains in the eye. Once the clinical effect of emesis is realized, the insert should be removed or flushed out without delay to avoid further absorption of the drug.

The recommended emetic dose is 0.1 mg/kg therefore some patients may require two inserts (one in each eye) while smaller patient may only require part of an insert. It is important that drug be administered as a single dose and the insert(s) removed promptly after emesis has occurred. Due to the toxic nature of the drug, the insert should be handled with care. Overdosage symptoms of respiratory depression can be treated with a narcotic antagonist such as naloxone, continuing emesis with metoclopramide and bradycardia may be treated with atropine. The purpose of this dose form is to allow drug absorption to occur at a controlled rate and once the therapeutic goal of emesis is achieved, to remove the drug reservoir, stop drug absorption and avoid overdosage. Some patients will be resistant to the emetic action of apomorphine and for these, oral hydrogen peroxide could be used rather than a second dose of apomorphine. This product should only be used in canine patients.

The product should be stored in a cool, dry area away from exposure to direct light.

Figure 5.26 Case report form.

CLINICAL USE REPORT

Apomorphine Ocular Inserts 2 mg

In order to assess the efficacy of this product please complete and return this form for each patient treated.

Expiration date: **Product Lot Number:**

Date: _____ **Breed:** _____

Patient weight: ____ kg **Time from administration to emesis:** _____min

Reason for Apomorphine use:

1. Poisoning _____
2. Foreign object _____
3. Other _____

Provide details of intoxication:

_____

_____

_____

Adverse effects: Please grade in order of severity on a scale from 0 to 5 with 5 being very severe

1. Prolonged vomiting _____
2. Tachycardia _____
3. Excitation _____
4. Respiratory depression _____
5. Bradycardia _____
6. Sedation _____
7. Ophth irritation _____
8. Other _____

Patient outcome:

Product assessment: Please grade acceptability on a scale of 0 to 5 with 5 being very easy/useful

1. Ease of use _____
2. Usefulness for this case _____

Dose: _____

Treatment with the inserts was considered a success if emesis occurred within 15 minutes after application. This was an arbitrary dividing line and selection of this time was based on the need for a prompt emetic response to the drug and the fact that emesis with ipecac usually occurs within 20 minutes after administration whereas apomorphine should provide a faster response [12, 16, 121]. A preliminary questioning of clinicians also revealed that their expectation for the product would be a response within this time window of 15 minutes and that beyond this time, consideration would be given to administering a supplemental dose of oral hydrogen peroxide. Since it was anticipated that some patients would experience emesis at a time later than the arbitrary 15 minutes, the numbers of patients experiencing no emesis were differentiated from those considered to be therapeutic failures.

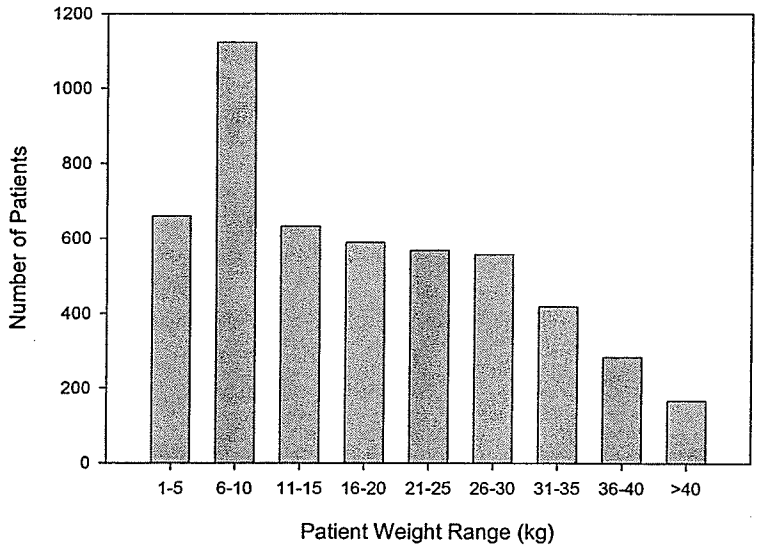
All data analysis was done at the 95% confidence level and where appropriate, values are presented as mean ± standard deviation. Since the frequency distributions for much of the data deviated from normality, the Mann-Whitney rank sum test was used to test for significant difference between two data sets and the Kruskal-Wallis one way ANOVA on ranks was used to test for significant difference between multiple data sets. If a multiple comparison was required with ANOVA, Dunn's method was used since the data sets usually had differing numbers of elements.

Results and discussion:

Case reports for patients receiving apomorphine in the form of an ocular insert were reviewed for a total of 5200 reports. Of these, 175 were discarded due to being incomplete and 24 were reports where the patients were felines so a total of 5001 reports were available for analysis. A total of 142 clinics participated in the trial and approximately 10,000 report forms were sent out so the rate of return for usable reports was about 51%.

In the study population patient weights ranged from 1 to 80 kg with a median weight of 16.0 kg (8 and 28 kg for 25 and 75% of the population respectively). A frequency distribution of the population weights is presented in Figure 5.27 where the weights were grouped at intervals of 5 kg.

Figure 5.27 Frequency distribution of weights for patients receiving apomorphine by the ocular route. (n = 5001)



Time to emesis which was the time interval from application of the insert until emesis occurred is presented in Figure 5.28 and the same data are presented as a cumulative distribution in Figure 5.29.

Figure 5.28 % of patients experiencing emesis as a function of time. (n = 5001)

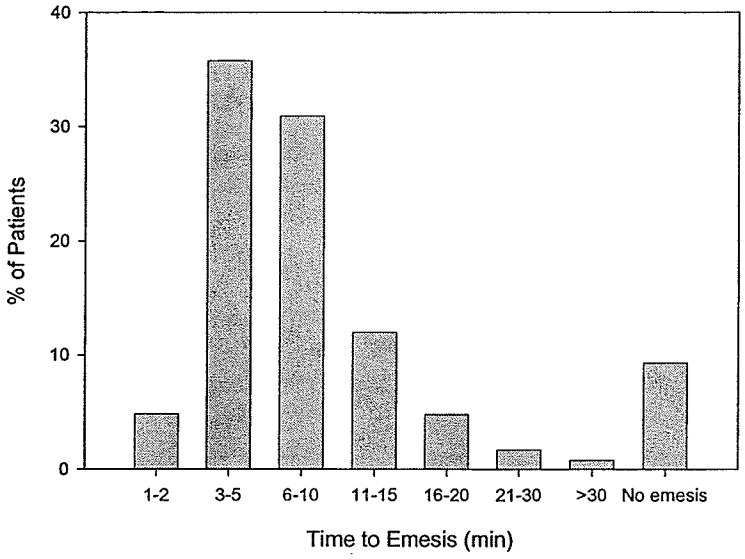
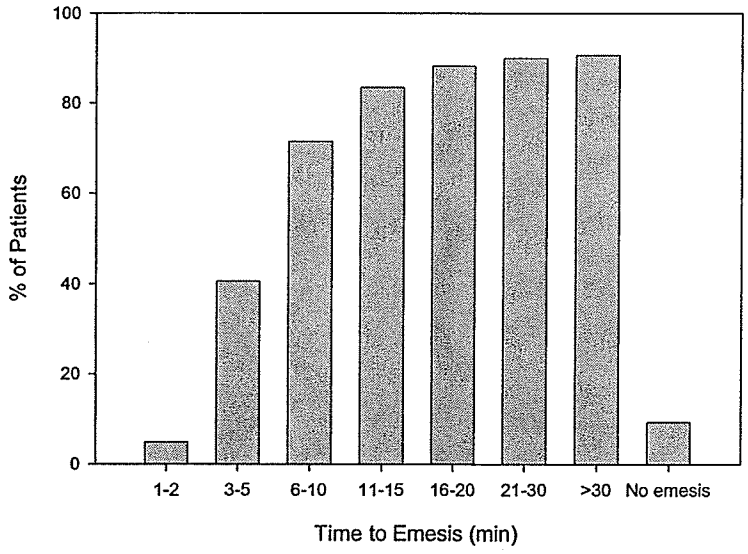


Figure 5.29 Cumulative % of patients experiencing emesis as a function of time. (n = 5001)



5.3.1 Efficacy

Using the 15 minute mark as the dividing point between success and failure with times greater than 15 being failures in inducing emesis, a comparison of body weight between the success and fail populations was done and these data are presented in Table 5.21. In the overall population, 83.5% of the patients had experienced emesis by the fifteen minute period and no emesis was experienced by 9.3% of the population.

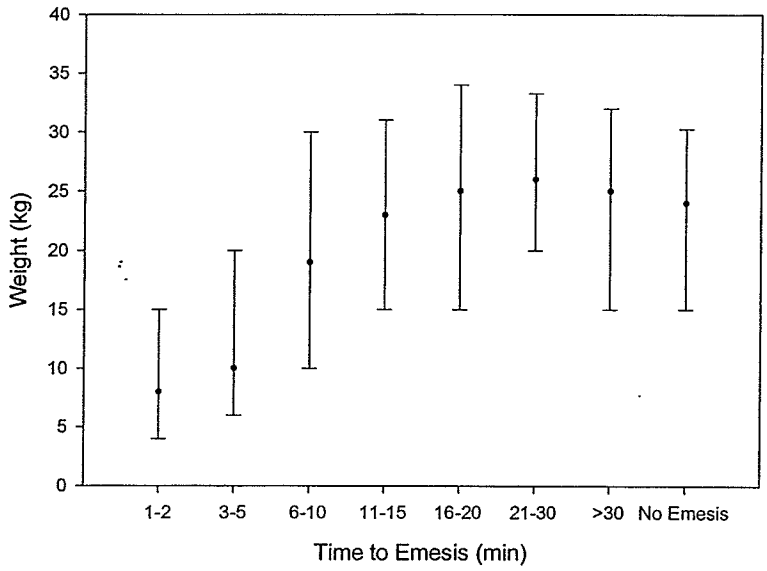
Table 5.21 Comparison of apomorphine inserts success and failure rates; a therapeutic failure was any case where the time to emesis was greater than 15 minutes.

	Success	**Failure**	**Difference**
Time to emesis (min)*	6.00	-	-
Median Weight (kg)	15	25	$p < 0.001$
% of Patients	83.5	16.5	-
N	4174	827	-
*Median value. 25%=5 min; 75%=10 min			

The data presented in Table 5.21 also suggest that more therapeutic failures were associated with the heavier patients in the study population; the weights of the success group were compared with the weights of the failure group using a rank sum test (Mann-Whitney) and the weights between the two groups were found to be significantly different ($p < 0.001$). This finding was somewhat unexpected since no difference was anticipated but this could be explained by the fact that the apomorphine release was time-dependent and if the release of drug was too slow, the arbitrary time of 15 minutes could pass before sufficient drug had been released to bring serum levels to those associated with emesis. Patient weight as a function of time to emesis was examined and these data are presented in Figure 5.30. From this plot it is

apparent that there is a bias towards heavier patients having a longer time to emesis and since the release of drug from the insert is time-dependent, this is not unexpected.

Figure 5.30 Patient weight as a function of time to emesis; median weight values are used and the lower and upper error bars are 25% and 75% of the population respectively. (n = 5001)



In order to explore this finding further, the patient population was organized into groups based on body weight and then the time to emesis profile for each group was prepared. These data are presented in Table 5.22 and clearly show that both therapeutic failures and instances where no emesis occurred increased with increasing patient weight.

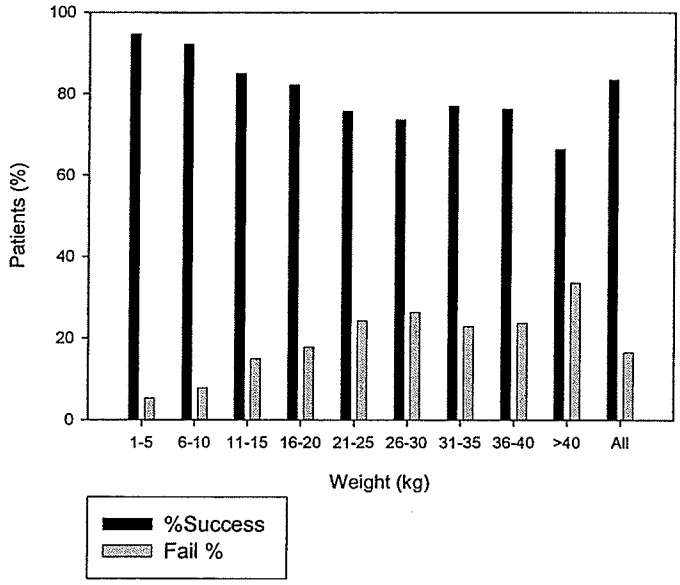
Table 5.22 Patients categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes. (n = 5001)

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	13.2	70.9	91.1	94.7	5.3	3.2	660
6-10	6.8	55.3	84.3	92.2	7.8	4.2	1123
11-15	3.5	40.8	76.0	85.0	15.0	9.3	633
16-20	2.7	37.8	68.6	82.0	18.0	11.2	590
21-25	2.3	25.0	57.5	75.8	24.2	13.9	569
26-30	2.2	22.9	57.0	73.7	26.3	13.8	558
31-35	1.4	24.2	60.5	77.0	23.0	12.7	418
36-40	3.5	22.3	59.7	76.3	23.7	11.3	283
>40	0	15.6	44.3	66.5	33.5	18.6	167
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Data comparing success and failure, where success is defined as emesis occurring within 15 minutes, are presented in Figure 5.31 and again the increase in failure rate with increasing weight can be seen. As already mentioned, this finding could reflect the fact that since drug release from the insert is time dependent, with increasing weight more time will be required for sufficient drug to be absorbed and serum levels rise to those associated with emesis. This is, however, further complicated by the wide patient variability in response related to serum levels which has been reported by a number of authors [5, 141, 191, 205]. Scherkl et al, in their study reported that in dogs emesis occurred with serum levels anywhere between 8 to 25 $ng \cdot mL^{-1}$ [165].

Since patients with a body weight in between 20 and 40 kg would receive a larger drug load (2 x 2 mg insets), their apomorphine serum levels should rise faster than the group from 1 to 20 kg and a corresponding drop in time to emesis and failure rate were expected but as can be seen in Figure 5.31, that did not seem to happen until the weight group of 31-35 kg.

Figure 5.31 Therapeutic success/failure as a % of patients for each weight group (n = 5001).

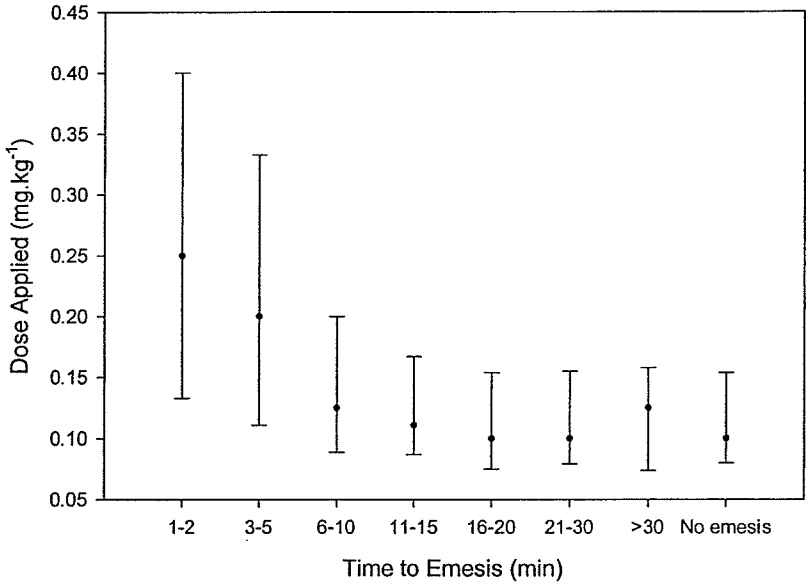


With the case reports received, 25 of them indicated that when two or more inserts were required, they were given in divided dose rather than a single dose. Generally these reports indicated that one insert was placed then if emesis did not occur, the second was placed about 15 minutes later. The stated reasons for giving the drug in divided dose appeared to be either an uncooperative patient or the attending clinician wishing to be conservative in the treatment and use as low a dose of apomorphine as possible. Apomorphine induces emesis by direct stimulation of the D_2-receptors in the medullary chemoreceptor zone [107] which is outside of the blood brain barrier [162] but apomorphine is also rapidly distributed from the serum across the blood brain barrier and into the central nervous system where it is able to interact with the μ-opioid receptors [6, 165]. Blancquaert et al clearly demonstrated that μ-

receptor agonists in the central nervous system have an ant-emetic activity and this has been supported by other studies [166, 167]. The net effect of this is that apomorphine-induced emesis may be self-limiting and administration of the second half of the dose will not likely induce emesis.

Although it would not be possible, other than for the 25 reports mentioned above, to determine how often the drug was administered in divided dose, the dose applied to the eye was reported in all the cases so the data pertaining to dose applied were examined. The dose applied in $mg \cdot kg^{-1}$ was examined as a function of time to emesis and these data are presented in Figure 5.32. As expected the higher the applied dose, the shorter the time to emesis except for the group where emesis occurred after 30 minutes. It is also apparent from these data, that a number of patients were under-dosed and had less than the recommended 0.1 $mg \cdot kg^{-1}$ applied suggesting that only one insert was used in patients who should have received two or more. The fact that some of these under-dosed patients still experienced emesis can be explained, at least in part, by the inter-patient variability of emetic response to apomorphine serum levels.

Figure 5.32 Dose applied as a function of time to emesis. Median values with the upper and lower error bars representing 75 and 25% of the population respectively. (n = 5001)



Of the total population, 1382 patients had less than the recommended dose of 0.1 $mg \cdot kg^{-1}$ applied and for these patients the median weight was 30 kg (25 and 35 kg for 25 and 75% of the population respectively), the median time to emesis was 10 minutes (5 and 15 minutes for 25 and 75% respectively) and the median dose applied was 0.0769 $mg \cdot kg^{-1}$ (0.0667 and 0.0857 $mg \cdot kg^{-1}$ for 25 and 75% respectively). In this group, the success rate was 73% and the failure rate was 27%. About 15% of the patients experienced no emesis. The patients were grouped according to the dosage applied and the success/failure rate as well as the number of patients experiencing no emesis determined. These data are presented in Table 5.23.

Table 5.23 Success/failure rates for population grouped according to dose applied. The values are number of patients in group with the respective percentages in parentheses. Success based on a time to emesis of 15 minutes or less.

Dose Applied ($mg \cdot kg^{-1}$)	Success	Failure	No Emesis	n
0.36-0.40	573 (93.5%)	40 (6.5%)	24 (3.9%)	613
0.31-0.35	199 (93.4%)	14 (6.6%)	10 (4.7%)	213
0.26-0.30	200 (88.5%)	26 (11.5%)	17 (7.5%)	226
0.21-0.25	361 (91.2%)	35 (8.8%)	22 (5.6%)	396
0.16-0.20	605 (87.1%)	90 (12.9%)	42 (6.0%)	695
0.11-0.15	826 (83.4%)	164 (16.6%)	91 (9.2%)	990
0.06-0.10	1267 (76.1%)	399 (23.9%)	231 (13.9%)	1666
< 0.06	143 (70.8%)	59 (29.2%)	28 (13.9%)	202

Although these data may suggest that the recommended dose of 0.1 $mg \cdot kg^{-1}$ is too low and should be increased, these values could be prejudiced due to the drug being given in divided dose. Serum samples collected at the time of emesis and analyzed for apomorphine content possibly could provide much more meaningful data but even this without corresponding CSF levels would have limited value. Assessment of dose applied as a function of weight is presented in Figure 5.33 and this shows a somewhat different picture than dose as a function of time to emesis. In this figure the recommended dose is 0.1 $mg \cdot kg^{-1}$ and since a fixed drug load of 2 mg per insert was used, the small patients in the weight range of 1-2 kg had a large excess of drug applied and as expected this declined to 0.1 $mg \cdot kg^{-1}$ for the group in the 16 to 20 kg range. The detail shown in Figure 5.34, however, clearly show that under dosing was very common for patients with weights above 20 kg and it is possible that for those who had the appropriate dose applied, some probably had so as a divided dose. These findings strongly indicate that the requirement of having to apply more than one insert will result in a high rate of non-compliance with a corresponding apparent drop in product

efficacy. A more appropriate presentation of the product would be to have two dosage strengths; a 2 mg insert for patients with a body weight of 20 kg or less and an insert with a higher drug load for patients with a body weight exceeding 20 kg.

Figure 5.33 Applied dose as a function of patient weight; median values presented and the upper and lower error bars representing 75 and 25% of the population respectively. (n = 5001)

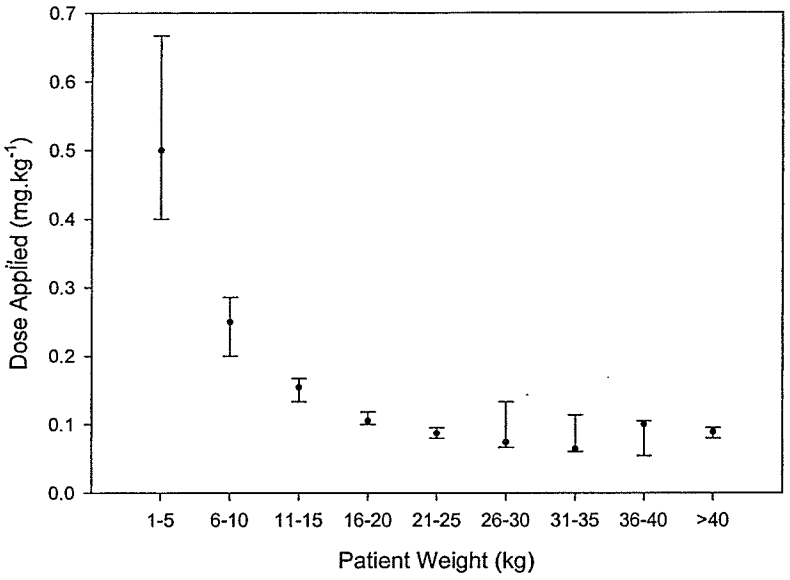
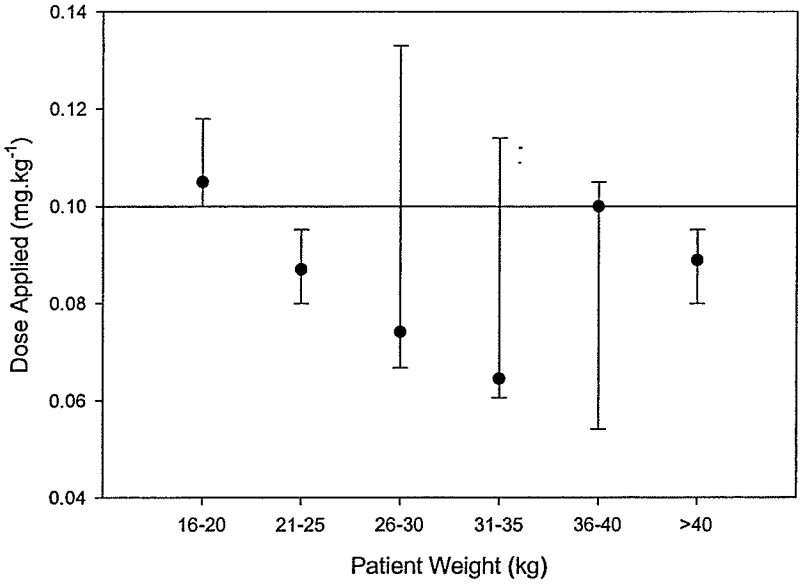


Figure 5.34 Detail of Figure 5.33 for patient weights greater than 16 kg with reference line at the recommended dose. Median values presented with the upper and lower error bars representing 75 and 25% of the population respectively.



Intravenous Administration

A smaller group of patients received apomorphine by the intravenous (IV) route (n = 32) at a dose of 0.03-0.04 $mg \cdot kg^{-1}$. In this study population patient weights ranged from 1 to 50 kg with a median weight of 18.0 kg (11.0 and 27 kg for 25 and 75% respectively). A frequency distribution of the population weights for this group is presented in Figure 5.35 where the weights were again grouped at intervals of 5 kg.

Weights for the small population treated with intravenous apomorphine were compared to those in the insert study population using a rank sum test (Mann-Whitney) and there was no significant difference in patient weights between the two groups ($p = 0.446$). The data from the group receiving intravenous apomorphine were further grouped as to success and failure with failure being considered as a time to emesis larger than 15 minutes. These data are presented in Table 5.24 and unlike the insert group, there was no significant difference in weight between the success and failure group ($p = 0.561$) as determined using a rank sum test (Mann-Whitney).

Figure 5.35 Frequency distribution of weight for patients receiving apomorphine by the intravenous route. (n = 32)

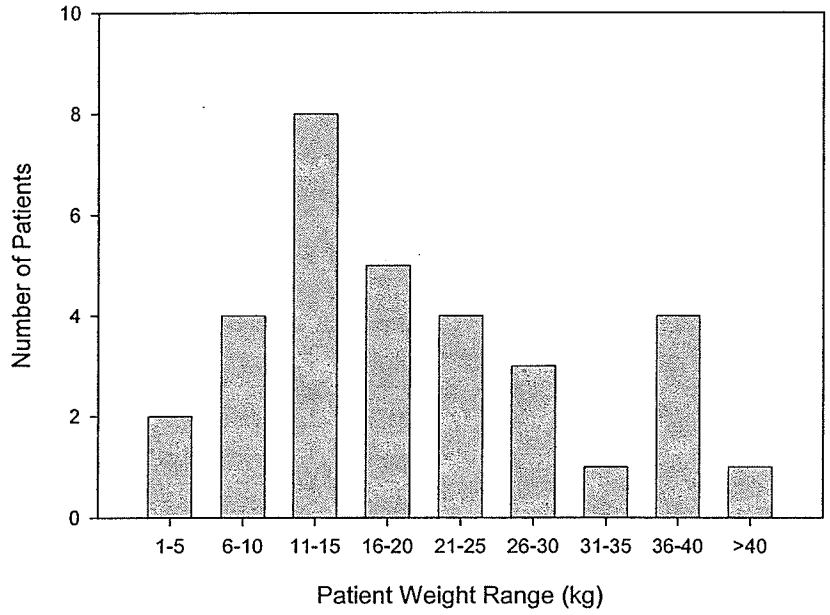


Table 5.24 Comparison of apomorphine IV success and failure rates; no emesis occurred with three patients.

	Success	**Failure**	**Difference**
Time to emesis (min)*	1.0	-	-
Median Weight (kg)	16	21	p = 0.561
% of Patients	90.6	9.4	-
n	29	3	-
* Median value. 25%=1 min; 75%=2 min			

Overall, the median time to emesis with the intravenous apomorphine was much shorter at 1 minute compared to the inserts where the median time to emesis was 6 minutes ($p < 0.001$ Mann-Whitney rank sum test). The success rate with the IV route was 90.6% and the ocular route 83.5%. There was no significant difference between the success rates (Chi square $p = 0.399$). If only the patients where there was assurance that the apomorphine was given as a single dose i.e. with a body weight of 20 kg or less are considered, the success rate with the

inserts increased to 87.1% (Chi square p = 0.743). In terms of patients showing no emesis at all, the rate with the IV route was 9.4% and with the ocular route 9.3%.

5.3.2 Safety - Adverse Effects

The adverse effects reported included prolonged vomiting, tachycardia, excitation, respiratory depression, bradycardia, sedation and ocular irritation. On the report forms supplied with the product, if any of these occurred the clinicians were asked to grade them on a subjective scale of 1 to 5 with a score of 1 being mild and 5 being severe. Clinicians were also asked to rate the ease of use of the inserts for each case with a score of 5 being very easy to use and 1 being very difficult. Patients in whom adverse effects were noted were grouped according to the adverse effect and the frequency and assigned severity scores are presented in Table 5.25.

Table 5.25 Frequency of adverse effects reported and clinicians reporting some level of difficulty using the inserts. The score is based on a subjective value assigned by the attending clinician on a scale of 0 to 5 with 5 being severe.

Category	Frequency (%)	Score*
Prolonged vomiting	2.3	2 (1-3)
Tachycardia	0.6	2 (1-2)
Excitation	0.4	2 (1-2)
Respiratory depression	0.6	2 (1-3)
Bradycardia	0.7	2 (1-3)
Sedation	11.1	2 (2-3)
Ocular irritation	16.2	3 (2-3)
Difficulty with use	6.3	3 (3-4)
* Median (25%-75%) n = 5001		

The incidence of adverse effects seen with the inserts was arranged according to body weight with the patients being placed into groups with weight ranges of 5 kg; these data are

presented in Table 5.26. Overall, there did not appear to be any particular association of a specific adverse effect with any of the weight groups although there may be a trend towards an increased incidence of prolonged vomiting and sedation in the patients with lower body weights. Even though patients with the lower body weights had proportionately a much larger dose of apomorphine applied, they did not show a substantial increased incidence of adverse effects suggesting that the rate of release of drug from the insert was acceptable and subsequent removal of the insert after emesis avoided overdosing.

The incidence of adverse effects was also examined in context of time to emesis and these data are presented in Table 5.27 where the patients were grouped according to time to emesis. Again there did not seem to be any particular association of a specific adverse effect with any of the time groups except again there may be a trend for the patients with shorter times to emesis to have an increasing incidence of prolonged vomiting and sedation.

Table 5.26 % Incidence of adverse effects and difficulties with insert use for patients in different weight groups.

	% of Patients experiencing adverse effects – Grouped by patient weight									
Wt (kg)	**1-5**	**6-10**	**11-15**	**16-20**	**21-25**	**26-30**	**31-35**	**36-40**	**>40**	**All**
Prolonged emesis	3.94	3.12	1.90	2.37	1.93	1.26	2.15	1.06	-	2.34
Tachycardia	0.61	0.62	0.47	1.02	-	0.54	1.20	0.71	-	0.60
Excitation	0.46	0.36	0.47	0.51	-	0.54	0.24	0.71	0.60	0.40
Resp depression	0.46	0.62	0.63	0.85	-	0.36	0.96	0.71	0.60	0.56
Bradycardia	0.76	0.89	0.79	0.68	0.53	0.54	0.24	0.71	-	0.66
Sedation	12.9	11.7	10.1	8.48	10.9	12.4	10.1	12.0	9.58	11.1
Irritation	14.7	16.6	15.6	17.1	15.6	17.4	18.2	15.9	13.2	16.2
Difficulty in use	7.58	6.50	6.00	6.27	5.27	5.38	5.98	7.42	5.84	6.26
n	660	1123	633	590	569	558	418	283	167	5001

Table 5.27 % Incidence of adverse-effects and difficulties with insert use for patients grouped by time to emesis.

	% of Patients experiencing adverse effects – Grouped by time to emesis							
Time (min)	**1-2**	**3-5**	**6-10**	**11-15**	**16-20**	**21-30**	**No emesis**	**All**
Prolonged emesis	3.31	3.08	2.78	1.34	1.26	-	-	2.34
Tachycardia	0.41	0.62	0.78	0.50	0.84	-	0.20	0.60
Excitation	-	0.39	0.32	0.67	1.26	-	0.20	0.40
Resp depression	-	0.50	0.84	0.67	0.42	-	0.20	0.56
Bradycardia	1.24	0.62	0.84	0.50	0.42	-	0.40	0.66
Sedation	9.09	10.8	12.8	10.5	13.0	14.1	6.94	11.1
Irritation	13.6	16.2	17.5	16.0	17.2	21.2	12.7	16.2
Difficulty in use	6.61	4.75	6.47	5.51	7.56	9.41	10.5	6.26
n	242	1788	1545	599	238	85	504	5001

In the patient group receiving apomorphine by the intravenous route the adverse reactions seen are presented in Table 5.28.

Table 5.28 Frequency of adverse effects reported for patients receiving apomorphine by the intravenous route. The score is based on a subjective value assigned by the attending clinician on a scale of 0 to 5 with 5 being severe.

Category	Frequency (%)	Score*
Prolonged vomiting	3.1	3 (3-3)
Tachycardia	15.6	2 (2-3)
Excitation	-	-
Respiratory depression	-	-
Bradycardia	-	-
Sedation	43.8	2 (1-2)
* Median (25%-75%) n = 32		

Prolonged vomiting occurred in 2.3% of the study subjects receiving apomorphine by the ocular route. Scherkl et al [165] had demonstrated that emesis induced by apomorphine was self-limiting in that as the drug crossed the blood-brain barrier it bound to the opioid μ-receptors in the vomiting centre of the brain and this interaction induces a strong antiemetic effect. This study, as well as others [146, 162] clearly show that narcotic antagonists such as naloxone will not block apomorphine-induced emesis and may in fact make it worse by displacing any apomorphine bound to opioid μ receptors. Protracted vomiting after apomorphine administration was studied by Keith et al [162] and they attributed this to individual biological variation in the time from dopamine receptor stimulation in the CTZ to suppression of emesis by stimulation of the opioid receptors in the brain. Niemgeers [163] examined twelve dopamine antagonists for efficacy and safety and although all worked well, he found domperidone to rank as best; metoclopramide overall ranked fifth. In the current

study there were 117 reported incidents of prolonged vomiting and of these 3 indicated that the attending clinician believed the problem severe enough to administer an antiemetic; in all three cases metoclopramide was used apparently with success. However, since the data collection sheet did not specifically ask if an antiemetic was administered, there may have been other instances. As already indicated, with the inserts there may be a trend for patients with smaller body weight and patients with a short time to emesis to be more subject to this adverse effect although this may simply be a reflection of patients with a higher sensitivity to apomorphine-induced emesis. In the group which received apomorphine by the IV route the frequency of prolonged vomiting was somewhat higher at 3.1% and the subjective grade of severity was also slightly higher with a median value of 3 as opposed to the insert group which was 2 on a scale of zero to five.

Apomorphine-induced hypotension was studied by Ramirez et al [148] and found in rats that a dose-dependent hypotension was caused by apomorphine and in their study concluded that the hypotensive response was due to dopamine receptor agonism and that the central receptors were primarily involved. In contrast, a study by De Meyer et al [149] indicated that the peripheral receptors were primarily involved. In a study by Pellissier and Demenge [150] the authors claimed that the hypotensive response was due to activation of the D_1 and/or D_2 receptors and in a final study using dogs, Nakayama et al [151] showed that the drop in blood pressure was not clinically significant and was likely due to stimulation of the peripheral D_2 receptors which results in inhibition of norepinephrine release and subsequent vasodilation. In the trial by Ramirez et al [148] the authors also studied changes in heart-rate after administration of apomorphine and found using rats, that after administration of IV apomorphine a dose-dependent bradycardia was seen; atropine prevented the bradycardia but had no effect on the hypotension whereas administration of the dopamine agonist pimozide prevented both the hypotension and the bradycardia and this suggested that the bradycardia

was associated with stimulation of dopamine receptors and also seemed to have a vagal component. Montastruc et al [152] investigated apomorphine in adrenal demedullated dogs and they observed tachycardia when high doses of apomorphine were administered. The tachycardia and hypotension were reversed with haloperidol. Atropine prevented the tachycardia but not the hypotension, domperidone intravenously prevented both but when it was given intracisternally it only prevented the tachycardia. From this rather complex picture, the authors concluded that the tachycardia was due to stimulation of peripheral dopamine receptors and specifically due to apomorphine acting on dopaminergic presynaptic inhibitory receptors likely located on the vagal nerve endings. They also showed that the tachycardia was not a reflex response to the hypotension induced by the apomorphine and Pellissier and Demenge [150] in their study showed that the hypotension seen with apomorphine was not due to bradycardia.

Paalzow et al [153] investigated the relationship of apomorphine serum concentration and the corresponding effect on heart rate in an attempt to explain the observations that both tachycardia and bradycardia had been reported with apomorphine administration. They showed that high serum levels of apomorphine were associated with tachycardia while low levels were associated with bradycardia and this suggested that two opposing dopamine functional systems were involved. The work by Pellissier and Demenge [150] indicated that stimulation of the peripheral D_2 receptors were responsible for the bradycardia. Other studies [158-161] now suggest stimulation of the D_1 receptors is responsible for the tachycardia. The cardiac effect seen may therefore depend on the relative affinities these receptors have for apomorphine, their effector potency and the concentration of apomorphine present. Atropine or dopamine antagonists such as haloperidol and domperidone may be used to correct the bradycardia and tachycardia seen with apomorphine [152].

The data showed that tachycardia appeared with a frequency of 0.6% with a median subjective severity score of 2 (25%=1: 75%=2) in the patients treated with the ocular insert while the patients who received apomorphine intravenously had a much higher frequency of 15.6% with a median severity score of 2 (25%=2: 75%=3). This finding suggested excessive serum levels in the IV group since Bredberg and Paalzow [156] had shown that tachycardia only developed in rats when the serum levels exceeded 70 $ng \cdot mL^{-1}$ while the study done by Scherkl et al [165] suggested serum levels inducing emesis range from 8 to 25 $ng \cdot mL^{-1}$. The finding suggested that the apomorphine inserts were able to deliver the drug until the emetic serum level for a particular patient was reached and the higher levels associated with tachycardia were avoided.

Bradycardia appeared with a frequency of 0.7% and a median severity score of 2 (25%=1: 75%=3) but was not seen in the population which received apomorphine intravenously. Since bradycardia is associated with lower serum levels of apomorphine, it was likely the levels in the IV group were above those associated with bradycardia and this is somewhat supported by the higher incidence of tachycardia seen in the group. There was no indication on any of the reports that medication was used to correct either bradycardia or tachycardia. Due to the potential difficulty of obtaining blood pressure data while treating a poisoned patient and the fact that the hypotension was not clinically important, apomorphine-induced hypotension was not studied during the trial.

Respiratory depression may occur with apomorphine administration and it may be successfully treated with an opioid antagonist such as naloxone [147]. In the study population respiratory depression occurred with a frequency of 0.6% and had a median severity score of 2 (25%=1: 75%=3). Respiratory depression was not reported in any of the patients receiving apomorphine intravenously.

Sedation seemed to be an issue with apomorphine whether it was delivered by the ocular insert or intravenously. In the ocular insert group of patients sedation occurred with a frequency of 11.1% and a median severity score of 2 (25%=2: 75%=3) while the IV group had a frequency of 43.8% with a median severity score of 2 (25%=1: 75%=2). The data presented in Tables 5.26 and 5.27 suggest a relationship between body weight and sedation with the incidence of sedation increasing in the smaller patients. There also may be a relationship between an increase in incidence of sedation with the longer the inserts are left in place as can be seen with the data where patients were grouped according to time to emesis. These observations would be consistent with the smaller patients having a proportionally larger dose applied and absorbing more drug than might be needed for emesis and consistent with the nature of the dose form where the longer it is in place, the more drug will be absorbed. These trends are not striking and are probably complicated by the wide inter-patient variability in response to apomorphine.

In terms of apomorphine-induced sedation, Gessa et al [168] noted that sedation occurred in rats with low apomorphine doses (0.05 $mg \cdot kg^{-1}$) and hyperactivity or excitation occurred with higher doses (1.0 $mg \cdot kg^{-1}$). Further work by Starr and Starr [170] suggested that the sedation was associated with stimulation of D_2 receptors while the cause of hyperactivity or excitation is unclear but appears to be associated with some type of interaction between D_1 and D_2 receptor stimulation. Although the concept of the stimulatory and inhibitory effects of dopamine agonists being mediated by two receptors having opposing actions and different affinities for the respective ligands is possible, this is likely an oversimplification [171-175].

In the insert group of patients, excitation or hyperactivity was reported with an incidence of 0.4% and a median severity rating of 2 (25%=1: 75%=2); no cases were reported with the group receiving apomorphine intravenously. Although there did not appear to be an association between excitation and the body weight groupings, there may be a trend in the

time to emesis grouping in that the incidence of excitation seems to increase with time to emesis. This would be consistent with more drug being absorbed the longer the insert is left in place.

Ocular irritation with the inserts was widely seen; virtually all of the reports indicated at least a low level of irritation as evidenced by inflammation and tearing but many of these rated the severity with a score of zero stating that it was not of clinical consequence. There are four possible explanations for the irritation seen; simple foreign body irritation is likely a factor [515]; the excipients particularly the residual ascorbic acid and sodium bisulfite and the apomorphine itself may produce a hypertonic microenvironment and cause subsequent irritation [516]; mechanical trauma resulting from the placement of the insert may be a factor [517] and lastly, there maybe some inherent irritation factor associated with apomorphine. A small amount of local irritation is desirable to ensure that there is sufficient tearing to allow swelling of the insert and subsequent drug release but the insert should not cause serious patient discomfort and certainly not cause tissue damage.

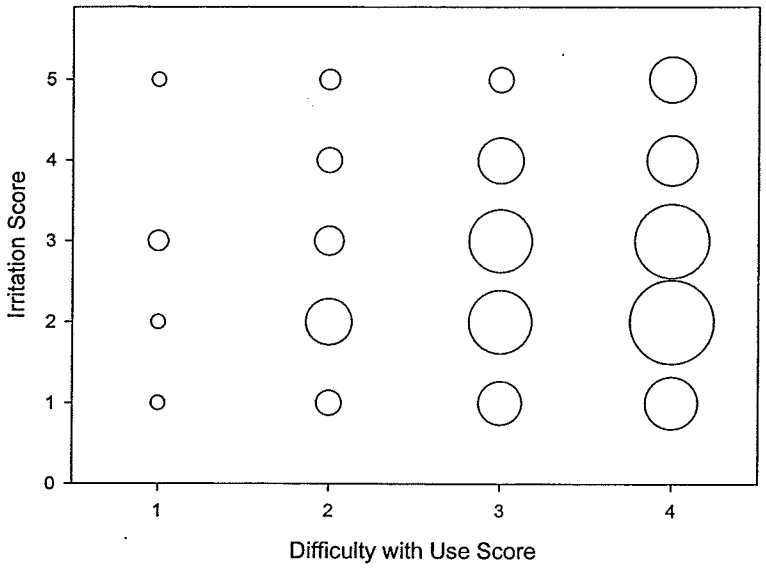
The frequency of ocular irritation was 16.2% with a median severity scale value of 3 (25%=2: 75%=3) and no association of irritation with any particular group could be seen with the patients grouped by weight or time to emesis. The majority of reports indicated that ocular irritation was transient and resolved quickly after removal of the insert but there were 24 cases which were assigned a severity score of 5 and in 6 of these cases the reports indicated corticosteroid eye drops were administered to successfully resolve the inflammation.

The report form requested a score between 0 and 5 for ease of use with a score of 5 indicating that the inserts were very easy to use; no scores of zero were returned. For analysis of the data, these values were transformed into scores of difficulty with use i.e. a reported score of 5 which indicated that the insert was very easy to use was converted to a 0 indicating that the insert was used with no difficulty and so on. Analysis of these data provided a

frequency of 6.3% with a median severity rating of 3 (25%=3: 75%=4) indicating that some degree of difficulty was found with using the inserts in just over 6% of the cases. Unfortunately the data collection form was not very specific with this question and some of the difficulties in use arose from the clinicians finding the insert awkward to place and maintain and some of the problems arose from the patients. Some patients actively resisted placement of the insert and others were successful in dislodging the insert once it had been placed leading to a rather discontinuous therapy. Due to the manner in which the question was phrased it was difficult to distinguish whether the difficulty in using the insert was related to the clinician or the patient. Since the scoring of the inserts seemed to become better with time, it is assumed that with experience the clinicians became quite skilled in using the inserts but the issues with some of the patients likely remained unchanged.

In terms of ocular irritation, some of the irritation could arise from trauma occurring during the placement of the insert and since this might be reflected in the score assigned to difficulty with use, these data were examined. There appeared to be no correlation between the levels of irritation as a function of the difficulty with use score; linear regression provided a coefficient of determination (r^2) of essentially zero and a scatter plot of the values appeared uniform over the entire plot. A bubble plot indicating the frequency of irritation at each level of difficulty with use, however, appeared to show a relationship. A bubble plot of these data is presented in Figure 5.36. Unfortunately the quality of the data from this trial is such that further analysis is not possible other than to say a relationship probably exists.

Figure 5.36 Relation of irritation score as a function of difficulty with use score; the size of the bubbles indicates the relative frequency of occurrence.



Apomorphine itself appears to have some property causing tissue irritation but the etiology remains unknown; sublingual administration of apomorphine has been reported to cause local irritation of the oral mucosa [192] and subcutaneous administration is associated with severe local irritation and the development of nodules at the site of injection [192, 205, 518]. In a histological study of these nodules, Acland [209] showed them to be an eosinophilic panniculitis which was severe enough to hinder drug absorption at that site. Chronic IV administration of apomorphine was investigated by Manson et al [207] and they found three of seven patients developed intravascular thrombotic complications severe enough to require surgical intervention. Although these authors claimed that crystal deposition with apomorphine degradation products was responsible, local irritation could have been a contributing factor. Dewey [218] investigated the use of an apomorphine nasal spray to treat off-on fluctuations in Parkinson's disease patients and found although the treatment worked well, a very high incidence of severe nasal irritation was a major drawback to this mode of

therapy. In a series of studies [225-227] investigating the iontophoretic transdermal absorption of apomorphine local skin irritation at the site of application was noted but it was unclear whether this was due to the apomorphine or electrical current applied. However, in a study investigating the rectal absorption of apomorphine [201] no irritation of the rectal mucosa was noted.

The frequency of adverse effects seen with the inserts and apomorphine given intravenously are summarized together in Table 5.29 and within the limits of this trial the inserts appeared to have been successful in reducing the overall frequency of adverse effects and specifically the tachycardia which would be associated with high serum levels of apomorphine.

Table 5.29 Comparison of adverse effect frequency for the ocular and intravenous routes. (From Tables 5.25 and 5.28)

	% Frequency	
Adverse effect	**Ocular**	**IV**
Prolonged vomiting	2.3	3.1
Tachycardia	0.6	15.6
Excitation	0.4	-
Respiratory depression	0.6	-
Bradycardia	0.7	-
Sedation	11.1	43.8
n	5001	32

Hydrogen peroxide supplementation

If emesis had not occurred 15 minutes after application of the insert, some clinicians elected to administer an oral dose of hydrogen peroxide to supplement the action of the apomorphine; in total, oral hydrogen peroxide was administered to 96 patients or 1.9% of the

total population. The median weight for this group of patients was 21.5 kg (16 and 32.5 kg for 25 and 75% respectively) and the weight distribution for this group was significantly different from the entire population ($p < 0.001$) where the median weight was 16 kg. Within this population, 40.6% of the patients were underdosed as less than 0.1 $mg.kg^{-1}$ of apomorphine was applied. Emesis occurred with 87 of the patients (90.6%) and the median time to emesis was 18 minutes (15 and 20 minutes for 25 and 75% respectively) so hydrogen peroxide supplementation was generally used for heavier patients, patients who were underdosed with apomorphine and at a time around 15 minutes after application of the insert. Because of the time frame, all of these patients were classified as therapeutic failures but only 9 patients (9.4%) actually experienced no emesis. These data suggest that oral hydrogen peroxide supplementation may be of value in cases where the emetic response to apomorphine is slow.

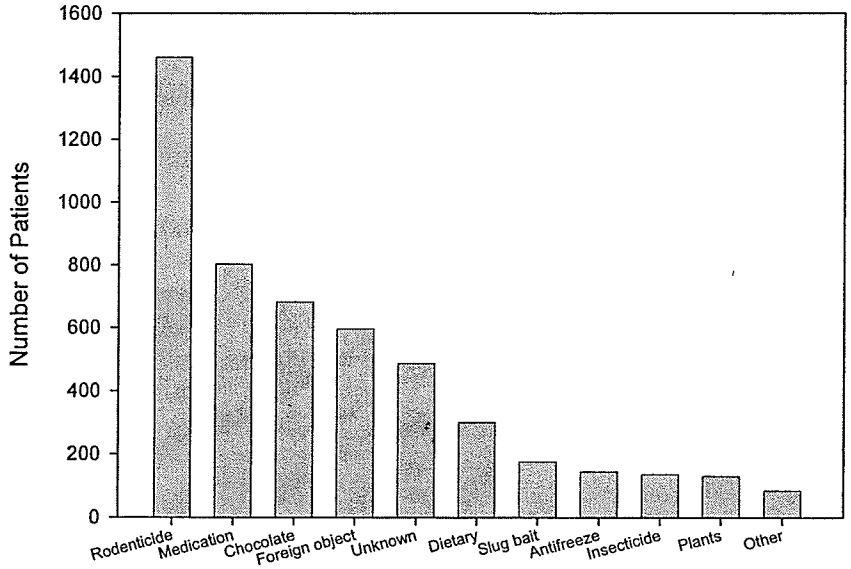
5.3.3 Intoxicants

The patients were also classified into groups based on the material ingested. The groupings were, in order of frequency, rodenticide, medication, chocolate, foreign object, unknown, dietary, slug bait, antifreeze, insecticide, plants and other. It is important to note that this is a rough grouping as in some of the cases there was only a suspicion that the material was ingested and no quantitative data to indicate how much if any was consumed and possibly absorbed. Although most of the classifications are self-explanatory, several of them require some clarification: In the classification Unknown the usual situation involved the patient presenting to the clinic with symptoms of intoxication but a causative agent could not be identified. The classification Plants included ingestion of houseplants, ornamental garden flowers and bulbs, tobacco products, mushrooms/toadstools and marijuana. Most of the cases in Dietary involved consumption of spoiled food, carrion, and compost but cases where the patient ate excessive amounts of pet or human food were also included into this classification. These cases are often referred to as dietary indiscretions. The group Other was used for

miscellaneous materials which did not fit into any of the categories; many of these cases involved the consumption of household chemicals particularly bone meal-based garden fertilizer and patients requiring pre-surgical emesis were also included in this group. The Foreign object category included non-food items and most commonly included articles of clothing and toys such as tennis balls.

In the majority of cases, classification assignments were based on the attending clinician's interpretation of the symptoms, findings in the ejected material and accounts of witnesses so these classifications are probably appropriate from a qualitative perspective. In a few cases it was likely that no intoxicant was consumed but emesis was induced and these patients were included in the appropriate classification. One such situation involved three canines who were found in the kitchen by the owner. On the floor were the remains of a recently-purchased container of 100 ibuprofen 200 mg tablets which had been chewed open and part of the plastic bottle and all of the tablets were missing. Although as it turned out, only one of the patients was the instigator, one a low level participant and one an innocent by-stander, all three were treated with apomorphine after arrival at the clinic and subsequently all three were placed into the Medication classification. In spite of these limitations, classifying the ingestions provides an approximation of the frequency of exposure of the patients to different intoxicants. The classifications and numbers of patients in each group are presented in Figure 5.37.

Figure 5.37 Classifications and frequencies for ingested materials. (n = 5001)



The patient population was arranged into groupings based on the nature of the intoxicant and the time to emesis profile, success/failure rate and % of patients showing no emesis were determined and these data are presented in Table 5.30. Inspection of these data suggests that the classifications Slug bait and Other stand out as having a higher incidence of therapeutic failure.

Table 5.30 Cumulative time to emesis, failure and % of patients showing no emesis as a function of toxin group. (n = 5001)

	Time to emesis - % of Patients (Cumulative)						
Toxin	1-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
Rodenticide	4.04	41.6	71.5	85.6	14.4	6.30	1461
Medication	6.73	45.5	75.8	86.2	13.8	7.10	803
Chocolate	6.45	46.5	76.3	86.7	13.4	6.89	682
Foreign object	2.85	34.9	69.6	82.6	17.4	8.39	596
Unknown	6.37	35.7	71.5	79.3	20.7	15.6	487
Dietary indiscretion	2.33	35.0	64.7	81.0	19.0	11.3	300
Slug bait	3.98	30.7	53.4	63.1	36.9	30.1	176
Antifreeze	4.83	42.8	69.7	84.8	15.2	11.7	145
Insecticide	6.62	39.7	76.5	86.0	14.0	7.35	136
Plant	3.85	48.5	75.4	82.3	17.7	8.46	130
Other	2.35	24.7	56.5	72.9	27.1	21.2	85
All	4.84	40.6	71.5	83.5	16.5	9.30	5001

The incidence of adverse effects for each intoxicant group are presented in Table 5.31. Values given are the numbers of patients experiencing a particular adverse effect expressed as a percentage of the number of patients in that group. Generally no particular adverse effect occurred with a higher frequency in any particular group although the patients exposed to insecticides may have experienced more episodes of tachycardia and excitation and a lower incidence of sedation. The group of patients who had been exposed to slug bait (metaldehyde), as well as appearing to have a higher failure rate, also appeared to have a lower incidence of adverse effects most notably a lower incidence of sedation. There may also be some

association of respiratory depression with ingestion of plant material as there was a somewhat higher incidence of this adverse effect seen within this group.

Table 5.31 % Incidence of adverse effects and difficulties with insert use overall and for patients in different intoxicant groups.

Toxin	Rodenticide	Medication	Chocolate	Foreign object	Unknown	Dietary	Slug bait	Antifreeze	Insecticide	Plant	Other	All
Prolonged emesis	2.53	2.62	3.08	2.35	2.05	1.33	-	0.69	3.68	2.31	1.18	2.34
Tachycardia	0.62	0.50	0.73	1.18	0.41	-	-	-	1.47	0.76	-	0.60
Excitation	0.62	0.25	0.59	0.34	0.21	-	-	-	0.74	0.76	-	0.40
Resp depression	0.62	0.50	0.73	0.50	0.41	0.67	-	0.69	-	1.54	-	0.56
Bradycardia	0.82	0.50	1.03	0.17	0.82	0.67	-	1.38	-	0.77	-	0.66
Sedation	11.4	11.2	11.7	13.8	8.62	12.7	3.4	8.97	7.35	14.6	8.24	11.1
Irritation	16.4	17.1	17.0	16.8	12.1	20.7	10.8	13.1	16.2	23.9	8.24	16.2
Difficulty in use	6.22	6.85	5.72	6.88	6.16	5.33	4.55	8.28	5.88	7.69	3.53	6.26
n	1773	803	682	596	487	300	176	145	136	130	85	5001

Rodenticide

In the grouping Rodenticide, 1461 patients were included and the success rate as defined as emesis within 15 minutes or less was 85.6%. No emesis was seen with 6.3% of the patients. The specific agent and the corresponding frequency where this agent was the intoxicant are listed in Table 5.32.

Table 5.32 Specific intoxicants in the classification Rodenticide. * Carcass represents cases where the patient consumed the remains of a possibly poisoned rodent.

Intoxicant	Frequency (%)
Not known	57.2
Warfarin	19.8
Bromodiolone	15.2
Strychnine	2.3
Carcass *	2.0
Chlorophacinone	1.8
Diphacinone	1.7
n = 1461	

The specific agent responsible for the intoxication was not specified in more than half of the cases; the usual reason for this was owner knowing it was a rodenticide but neglecting to bring the container or information along to the clinic or the container had been previously discarded. In several cases the patient had been seen consuming the carcass of a rodent which had possibly been poisoned. In almost all of the cases in this group, the patient was observed consuming the material. Twenty-one cases of rodenticide poisonings in dogs were reviewed by Shea [52] and treatment focused on administration of vitamin K_1. The use of an emetic agent was not specifically mentioned. Although not specifically a rodenticide, strychnine was included in this group and most of the cases reported were due to an incident where a person had deliberately left poisoned meat scattered throughout a neighborhood. In most of the cases reported, the quantity consumed by the patient, if any, was not known.

The weight distribution for the patients in this group are presented in Figure 5.38 and using the Mann-Whitney rank sum test there is no significant difference between the weight distribution of this group compared to the total population of 5001 ($p = 0.887$). The median

weight for this population was 17 kg (8 and 27 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.133 $mg \cdot kg^{-1}$ (0.095 and 0.250 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 384 out of 1461 or 26.3 % of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.33:

Figure 5.38 Weight distribution as % of total in the grouping Rodenticide. (n = 1461)

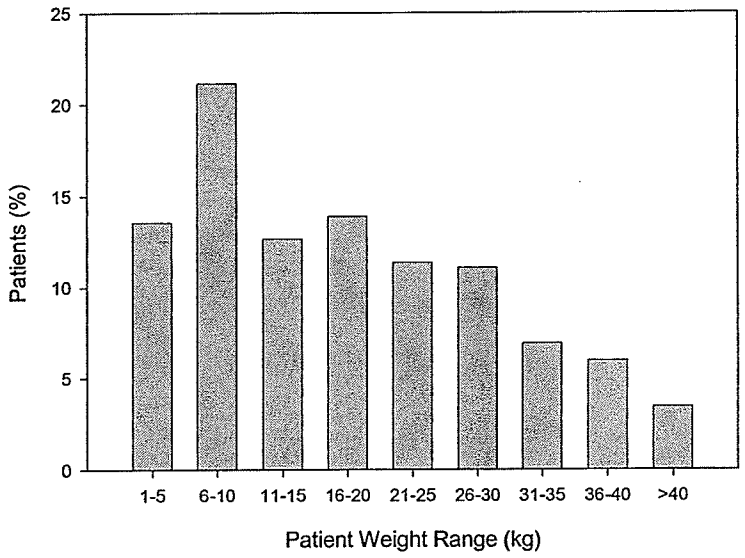


Table 5.33 Patients ingesting rodenticide: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	13.6	70.7	90.9	97.0	3.0	1.5	198
6-10	4.5	58.3	83.5	93.9	6.1	2.9	309
11-15	3.2	44.9	78.9	86.5	13.5	5.4	185
16-20	1.5	33.0	66.0	81.8	18.2	9.4	203
21-25	0.6	26.5	59.0	78.9	21.1	9.0	166
26-30	1.9	22.2	56.2	74.7	25.3	10.5	162
31-35	0	26.7	61.4	84.2	15.8	6.9	101
36-40	5.8	26.4	59.8	78.2	21.8	9.2	87
>40	0	14.0	46.0	74.0	26.0	8.0	50
Group	4.0	41.6	71.5	85.6	14.4	6.3	1461
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Medication

In the grouping Medication, 803 patients were included and the success rate as defined by emesis within 15 minutes or less was 86.2%. No emesis was seen with 7.1% of the patients. The specific agent and the corresponding frequency where this agent was the intoxicant are listed in Table 5.34.

Table 5.34 Specific intoxicants in the classification Medication.

Intoxicant	Frequency (%)
Acetaminophen	19.2
Miscellaneous	17.6
Ibuprofen	16.6
Benzodiazepines	5.5
Cardiac/antihypertensive	5.5
L-Thyroxin	5.2
Antihistamine/decongestant	4.0
Steroids	3.6
Antidepressant	3.4
Not specified	3.0
Barbiturates/sleeping	2.7
Vitamins	2.5
Other NSAIDs	2.3
CNS Stimulant	2.3
Nicotine gum	1.6
ASA	1.4
Ivermectin (Heartworm)	1.4
Antibiotics	1.2
Narcotics	1.1
n = 803	

A very wide range of medications was involved and in most cases the reports were in agreement with the findings of Villar et al [43] that toxic exposure to these agents were associated with three general sets of circumstances. Most commonly the animal was able to gain access to the medication due to improper storage by the pet owner, improper dosing of

medication by the care-giver or the inappropriate administration of medication by a well intentioned owner based on their diagnosis rather than that of a veterinarian. In the cases, ingestion of acetaminophen and ibuprofen accounted for 19.2 and 16.6% respectively of the cases. Exposure to these agents was reviewed by Villar et al [43] and emesis was recommended only if the interval between exposure and treatment was short but no specific times were suggested. In the case of acetaminophen intoxication he recommended supportive therapy and the use of N-acetylcysteine as an antidote; for ibuprofen the administration of misoprostol to protect the gastric mucosa was suggested.

Of the reports included in this group, only about 0.5% of the cases involved consumption of multiple drug products and only about 5.2% of the cases reported indicated consumption of the patients own medication. The majority of cases involved medication belonging to the pet owner or another family member.

The weight distribution for the patients in this group are presented in Figure 5.39 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median weight for the medication population was 13 kg (7 and 24 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.154 $mg \cdot kg^{-1}$ (0.100 and 0.286 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 170 out of 803 or 21.2 % of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.35:

Figure 5.39 Patient weight distribution for grouping Medication. (n = 803)

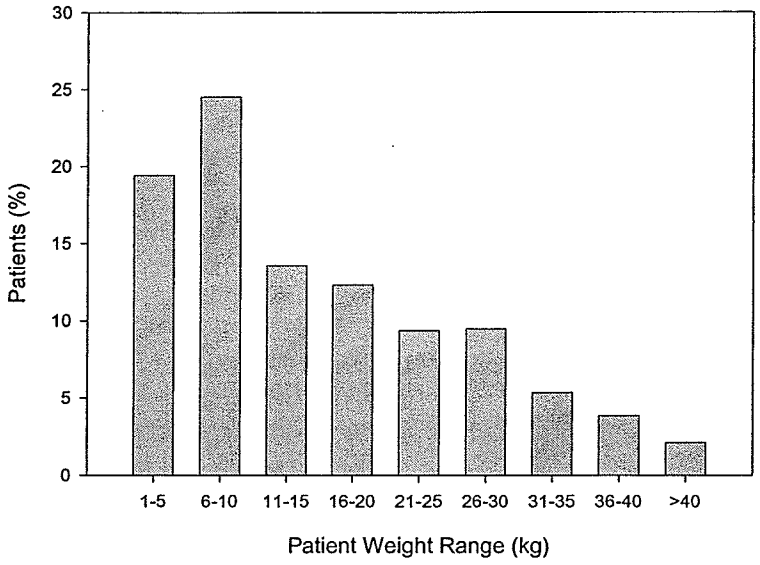


Table 5.35 Patients ingesting medication: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	10.9	71.8	91.0	94.2	5.8	4.5	156
6-10	10.7	53.3	82.2	90.4	9.6	3.6	197
11-15	7.3	39.5	78.9	89.9	10.1	4.0	99
16-20	3.0	49.5	76.8	89.9	10.1	4.0	99
21-25	2.7	29.3	65.3	76.0	24.0	1.3	75
26-30	1.3	26.3	59.2	79.0	21.0	7.9	76
31-35	2.3	16.3	51.2	69.8	30.2	11.6	43
36-40	3.2	16.1	61.3	74.2	25.8	6.5	31
>40	0	11.8	47.1	58.8	41.2	35.3	17
Group	6.7	45.5	75.8	86.2	13.8	7.1	803
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Chocolate

In the grouping Chocolate, 682 patients were included and the success rate as defined by emesis within 15 minutes or less was 86.7%. No emesis was seen with 7.0% of the patients. Very large quantities of chocolate were often consumed and quantities ranging from 500 to 1000 g were common even with rather small patients. Packing materials such as foil was usually consumed as well and several reports indicated that even portions of the cardboard boxes holding chocolates were consumed. Sutton [36] reported a case of cocoa ingestion and subsequent death in a canine and discussed treatment of chocolate intoxication. He reported toxicity was due to the theobromine content of chocolate and recommended supportive treatment for the heart and circulatory failure which may occur. Emesis was indicated if treatment was initiated shortly after ingestion.

The weight distribution for the patients in this group are presented in Figure 5.40 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median weight for the chocolate population was 12 kg (7 and 25 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.167 $mg \cdot kg^{-1}$ (0.100 and 0.286 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 143 out of 682 or 21.0 % of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.36:

Figure 5.40 Patient weight distribution for grouping Chocolate. (n = 682)

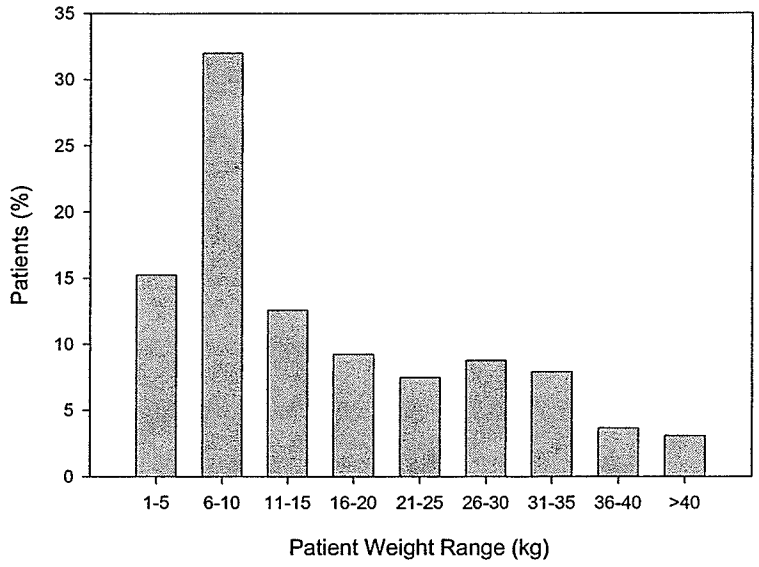


Table 5.36 Patients ingesting chocolate: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	8.7	74.0	94.2	96.2	3.8	1.0	104
6-10	10.1	61.0	89.0	93.1	6.9	2.3	218
11-15	3.5	40.7	69.8	81.4	18.6	11.6	86
16-20	3.2	46.0	76.2	87.3	12.7	9.5	63
21-25	3.9	19.6	54.9	86.3	13.7	5.9	51
26-30	5.0	23.3	55.0	76.7	23.3	13.3	60
31-35	3.7	25.9	66.7	74.1	25.9	18.5	54
36-40	4.0	12.0	56.0	76.0	24.0	12.0	25
>40	0	9.5	42.9	66.7	33.3	9.5	21
Group	6.5	46.5	76.2	86.7	13.3	7.0	682
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Foreign objects

In the grouping Foreign objects, 596 patients were included and the success rate as defined by emesis within 15 minutes or less was 82.6%. No emesis was seen with 8.6% of the patients. The specific objects and the corresponding frequency where this type of object was involved are listed in Table 5.37. Balls and clothing accounted for 90% of the ingestions but a very wide range of objects were involved; some of the more common objects are included in Table 5.37 but frequencies are not included as the values were well below 1%.

Table 5.37 Specific objects in the classification Foreign objects.

Intoxicant	Frequency (%)
Balls (tennis, golf etc)	46.0
Clothing (socks/underwear)	44.0
Batteries	< 1
Diapers	< 1
Gloves/mittens	< 1
Plastic bags	< 1
Styrofoam	< 1
n = 596	

Stauffer [66] speculated on some underlying psychological components which may be involved in the consumption of foreign objects and he described a case where a dog admitted for GI obstruction was examined and during surgery jewelry, a leather bracelet, a brassiere and a pair of pantyhose were recovered from the ileum of the patient. All were intact and showed no evidence of chewing. In the case reports received, one reported the recovery of a leather glove through emesis but before the ejected glove could be removed, the patient re-swallowed it.

The weight distribution for the patients in this group are presented in Figure 5.41 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median weight for this population was 20.5 kg (10 and 30 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.125 $mg \cdot kg^{-1}$ (0.083 and 0.20 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 211 out of 596 or 35.4 % of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.38:

Figure 5.41 Patient weight distributions for grouping Foreign objects. (n = 596)

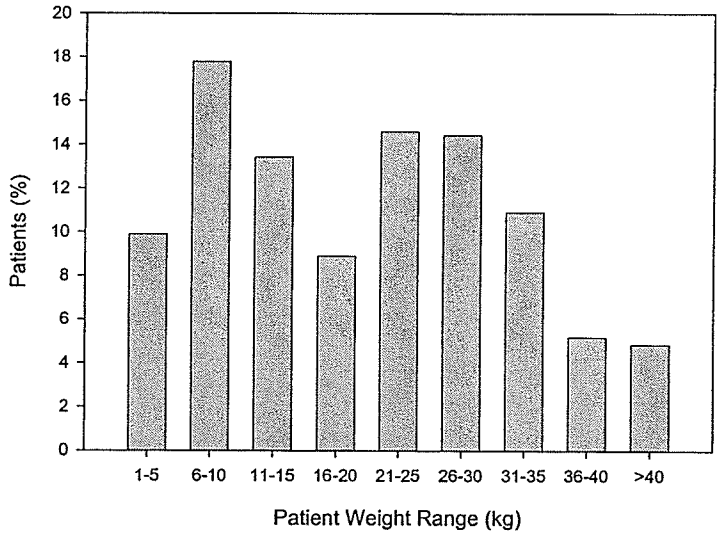


Table 5.38 Patients ingesting foreign objects: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	8.5	64.4	84.7	88.1	11.9	5.1	59
6-10	3.8	50.9	90.6	98.1	1.9	0.9	106
11-15	1.3	40.0	82.5	90.0	10.0	5.0	80
16-20	5.7	34.0	64.2	81.1	18.9	7.5	53
21-25	3.5	23.0	55.2	74.7	25.3	11.5	87
26-30	1.2	26.7	64.0	73.3	26.7	12.8	86
31-35	0	20.0	55.4	80.0	20.0	12.3	65
36-40	0	16.1	54.8	74.2	25.8	12.9	31
>40	0	17.2	44.8	62.1	37.9	20.7	29
Group	2.9	34.9	69.6	82.5	17.5	8.6	596
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Unknown

In the grouping Unknown, 487 patients were included and the success rate as defined by emesis within 15 minutes or less was 79.3%. No emesis was seen with 15.8% of the patients. This group represented patients who presented at the clinic with symptoms of intoxication but no specific toxin was ever identified. Generally treatment consisted of a physical exam with a blood workup and a flat film x-ray. If it was deemed safe, emesis was often used as a diagnostic tool in these cases with the hope that some clue as to the nature of the intoxicant could be found in the ejected material. In almost all of the cases if emesis did not help with the diagnosis, treatment was supportive and charcoal was administered.

The weight distribution for the patients in this group are presented in Figure 5.42 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p = 0.002$). The median weight for the population in this grouping was 20 kg (10 and 30 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.167 $mg \cdot kg^{-1}$ (0.100 and 0.286 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 155 out of 487 or 31.8% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.39:

Figure 5.42 Patient weight distributions for grouping Unknown. (n = 487)

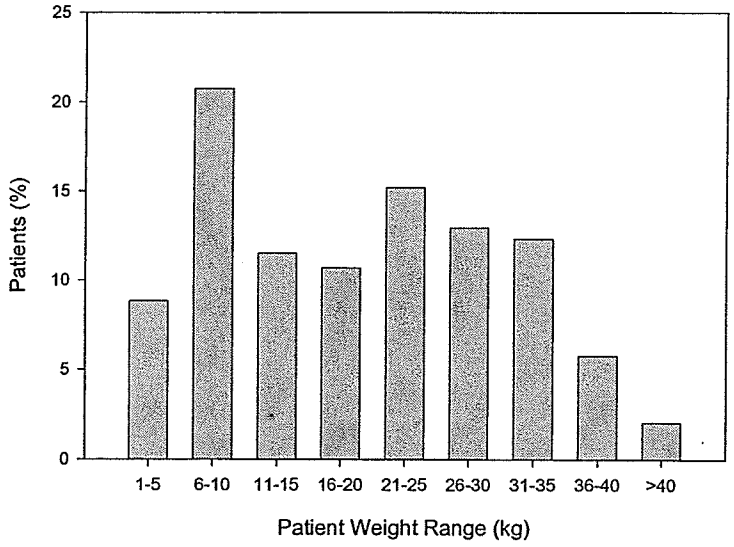


Table 5.39 Patients in group Unknown: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	27.9	67.4	93.0	97.7	2.3	2.3	43
6-10	8.9	56.4	87.1	91.1	8.9	7.9	101
11-15	1.8	30.4	67.9	71.4	28.6	26.8	56
16-20	3.9	38.5	76.9	82.7	17.3	13.5	52
21-25	1.3	26.7	66.7	76.0	24.0	16.0	75
26-30	3.2	16.1	61.3	74.2	25.8	21.0	62
31-35	5.0	26.7	63.3	70.0	30.0	20.0	60
36-40	3.6	17.9	50.0	67.9	32.1	21.4	28
>40	3.6	17.9	50.0	67.9	32.1	21.4	28
Group	6.4	35.7	71.5	79.3	20.7	15.8	487
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Dietary indiscretion

In the grouping Dietary indiscretion, 300 patients were included and the success rate as defined by emesis within 15 minutes or less was 81.0%. No emesis was seen with 11.7% of the patients. This group represented patients who had consumed very large quantities of food, compost or carrion. Consumption of large portions consisting of almost complete turkeys and beef roasts, entire bags of cat food, as many as eight entire raw chicken breasts and in one case an entire 1 kg box of fig Newton cookies were among some of the items reported. Toxicity to canines due to the production of mycotoxins in rotting food has been described [75] and Kammerer et al [62] described a case where death resulted from a dog consuming rotting apples.

The weight distribution for the patients in this group are presented in Figure 5.43 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median weight for the population in this grouping was 20.5 kg (10 and 32 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.111 $mg \cdot kg^{-1}$ (0.08 and 0.20 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 115 out of 300 or 38.3% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.40:

Figure 5.43 Patient weight distributions for grouping Dietary indiscretion. (n = 300)

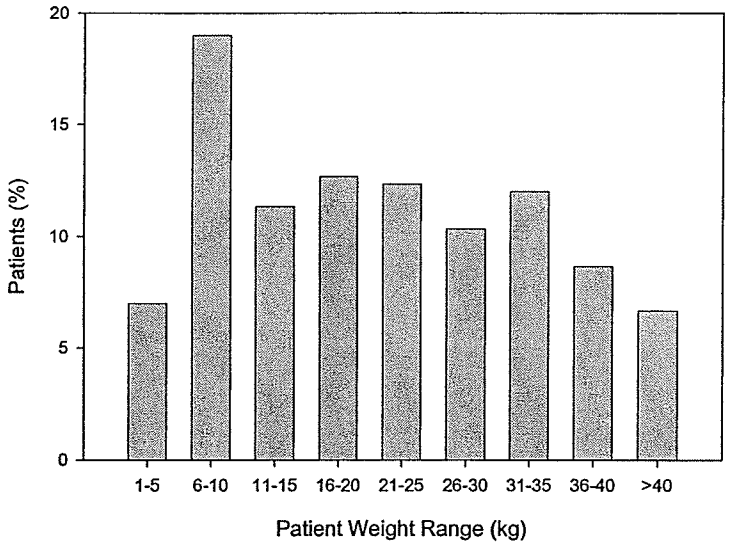


Table 5.40 Patients in dietary indiscretion group: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	19.1	71.4	90.5	95.2	4.8	0	21
6-10	1.8	42.1	77.2	93.0	7.0	3.5	57
11-15	0	29.4	73.5	82.4	17.6	11.8	34
16-20	0	34.2	63.2	84.2	15.8	13.2	38
21-25	5.4	29.7	51.4	70.3	29.7	24.3	37
26-30	0	25.8	38.7	51.6	48.4	32.3	31
31-35	0	27.8	69.4	86.1	13.9	2.8	36
36-40	0	38.5	65.4	88.5	11.5	0	26
>40	0	20.0	45.0	70.0	30.0	20.0	20
Group	2.3	35.0	64.7	81.0	19.0	11.7	300
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Slug bait

In the grouping Slug bait, 176 patients were included and the success rate as defined by emesis within 15 minutes or less was 63.1%. No emesis was seen with 30.7% of the patients. In all of the cases, the active ingredient in the bait was metaldehyde. Slug bait is usually provided in the form of pellets made with bran and as pellets, they can be easily picked up and dogs have been reported as finding them palatable [53]. The pellets are usually colored with a blue-green dye do the presence of the dye in the vomitus may provide confirmation of metaldehyde ingestion. There is no specific antidote to metaldehyde and in a

discussion of treatment options, Udall [53] suggests supportive treatment with oral charcoal, hydration and diazepam for control of the seizures associated with metaldehyde ingestion. Emesis is recommended if this is done shortly after ingestion and the usual cause of death with this intoxication is liver failure.

The weight distribution for the patients in this group are presented in Figure 5.44 and using the Mann-Whitney rank sum test there is no significant difference between the weight distribution of this group compared to the total population of 5001 ($p = 0.711$). The median weight for the population in this grouping was 15.5 kg (8 and 28 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.143 $mg \cdot kg^{-1}$ (0.095 and 0.25 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 47 out of 176 or 26.7 % of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.41:

Figure 5.44 Patient weight distributions for grouping Slug bait. (n = 176)

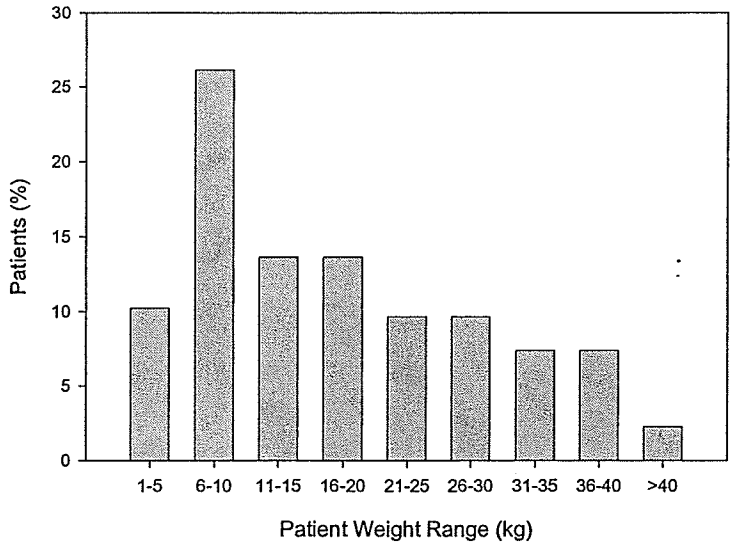


Table 5.41 Patients ingesting slug bait: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	5.6	61.1	88.9	88.9	11.1	11.1	18
6-10	4.3	39.1	65.2	73.9	26.1	26.1	46
11-15	4.2	45.8	62.5	70.8	29.2	20.8	24
16-20	0	12.5	29.2	37.5	62.5	50.0	24
21-25	5.9	11.8	29.4	52.9	47.1	41.2	17
26-30	5.9	11.8	47.1	64.7	35.3	35.3	17
31-35	0	38.5	53.9	53.9	46.1	23.1	13
36-40	7.7	7.7	38.5	53.8	46.2	30.8	13
>40	0	25.0	25.0	25.0	75.0	75.0	4
Group	3.8	30.7	53.4	63.1	36.9	30.7	176
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Antifreeze

In the grouping Antifreeze, 145 patients were included and the success rate as defined by emesis within 15 minutes or less was 84.8%. No emesis was seen with 11.7% of the patients. The specific agents reported and their frequency of occurrence are presented in Table 5.42. Although not specifically antifreeze, the few cases of alcoholic beverage ingestion were included in this group.

Table 5.42 Specific intoxicants in the classification Antifreeze. (n = 145)

Intoxicant	Frequency (%)
Ethylene glycol	69.0
Not specified	16.5
Methanol	11.7
Alcoholic beverages	2.1
Isopropanol	0.7
n = 145	

Three cases of alcoholic beverage consumption were reported; vodka, whiskey and wine. In the case of the wine ingestion, the patient knocked an opened 750 mL bottle of wine off a kitchen table, consumed the spilled content which was most of the bottle and also swallowed the plastic cork.

Ethylene glycol is a toxic substance commonly used as antifreeze in automotive engines and both dogs and cats find the substance palatable. In a review of 510 cases of ethylene glycol intoxication, Khan.et al [55] stated the minimal lethal dose for dogs was 6-7 $mL \cdot kg^{-1}$ and this dose was associated with a mortality rate of 60-70%. The toxicity of ethylene glycol is due to the formation of toxic metabolites rather than the parent compound and the metabolites include glycoaldehyde, glycolic acid, glyoxalic acid and oxalic acid. The metabolites cause a severe metabolic acidosis and acute renal failure which is usually the cause of death. In terms of treatment, ethanol has been used as an antidote since it competes with ethylene glycol for the enzyme alcohol dehydrogenase and slows metabolism of the ethylene glycol. Fomepazole or 4-methylpyrazole is a specific inhibitor of alcohol dehydrogenase and is now used as an antidote [56]. In the review by Khan, treatment recommendations included emesis, administration of either ethanol or fomepazole as antidote

and aggressive intravenous fluid therapy; he does not recommend the use of charcoal since it is ineffective in adsorbing alcohols or glycols.

The weight distribution for the patients in this group are presented in Figure 5.45 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median weight for the population in this grouping was 22 kg (11 and 32 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.118 $mg \cdot kg^{-1}$ (0.0866 and 0.2 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 44 out of 145 or 30.3% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.43:

Figure 5.45 Patient weight distributions for grouping Antifreeze. (n = 145)

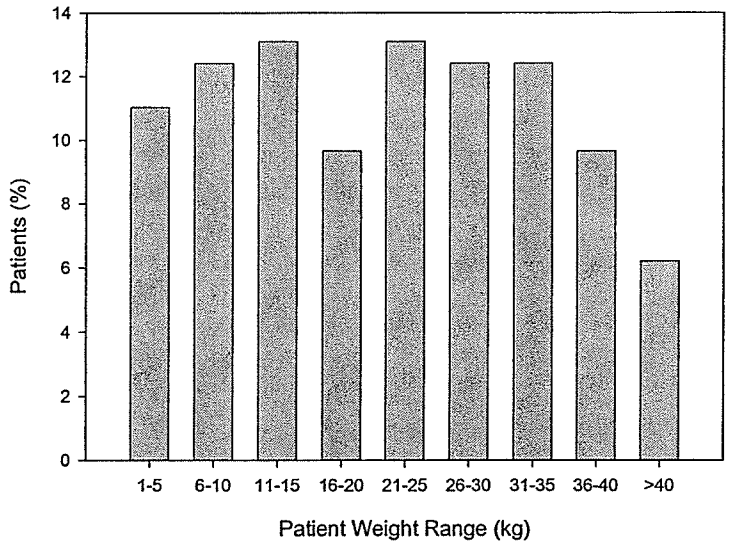


Table 5.43 Patients ingesting antifreeze: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	31.3	81.3	100	100	0	0	16
6-10	5.6	61.1	72.2	83.3	16.7	11.1	18
11-15	5.3	47.4	78.9	89.5	10.5	10.5	19
16-20	0	42.9	71.4	85.7	14.3	14.3	14
21-25	0	15.8	47.4	73.7	26.3	21.1	19
26-30	0	22.2	55.5	83.3	16.7	5.6	18
31-35	0	33.3	61.1	77.8	22.2	22.2	18
36-40	0	35.7	85.7	92.9	7.1	7.1	14
>40	0	55.6	55.6	77.8	22.2	11.1	9
Group	4.8	42.8	69.7	84.8	15.2	11.7	145
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Insecticide

In the grouping Insecticide, 136 patients were included and the success rate as defined by emesis within 15 minutes or less was 86.0%. No emesis was seen with 7.4% of the patients. The specific agents reported and the frequency of occurrence are presented in Table 5.44.

Table 5.44 Specific intoxicants in the classification Insecticide.

Intoxicant	Frequency (%)
Ant bait	52.2
Earwig bait	20.6
Not specified	12.5
Roach bait	8.8
Fly bait	5.9
n = 136	

The weight distribution for the patients in this group are presented in Figure 5.46 and using the Mann-Whitney rank sum test there is no significant difference between the weight distribution of this group compared to the total population of 5001 ($p = 0.055$). The median weight for the population in this grouping was 20 kg (10 and 29 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.111 $mg \cdot kg^{-1}$ (0.0833 and 0.2 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 46 out of 136 or 33.8% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.45:

Figure 5.46 Patient weight distributions for grouping Insecticide. (n = 136)

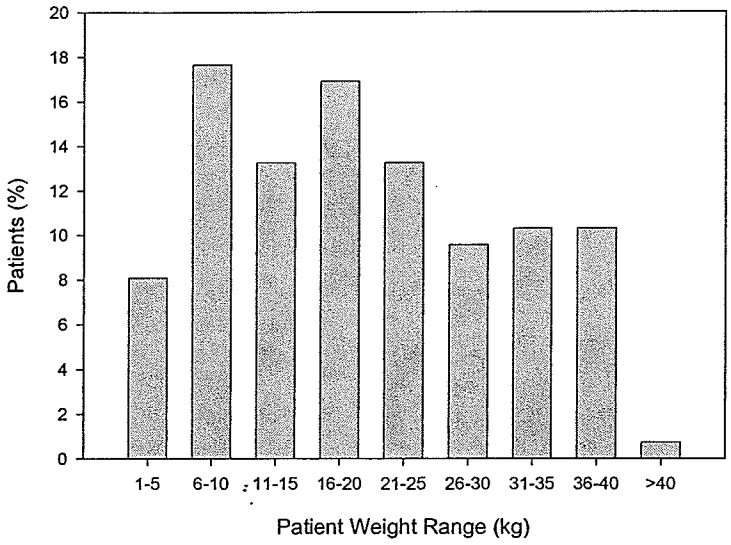


Table 5.45 Patients ingesting insecticide: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	18.2	63.6	90.9	90.9	9.1	9.1	11
6-10	8.3	62.5	91.7	100	0	0	24
11-15	5.6	44.4	72.2	94.4	5.6	5.6	18
16-20	8.7	39.1	87.0	91.3	8.7	8.7	23
21-25	5.6	22.2	50.0	55.6	44.4	22.2	18
26-30	0	38.5	76.9	84.6	15.4	0	13
31-35	0	7.1	64.3	92.9	7.1	7.1	14
36-40	7.1	35.7	71.4	71.4	28.6	7.1	14
>40	0	0	100	100	0	0	1
Group	6.6	39.7	76.5	86.0	14.0	7.4	136
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Plant

In the grouping Plant, 130 patients were included and the success rate as defined by emesis within 15 minutes or less was 82.3%. No emesis was seen with 9.2% of the patients. The specific agents reported and their frequency of occurrence are presented in Table 5.46.

Table 5.46 Specific intoxicants in the classification Plant.

Intoxicant	Frequency (%)
Marijuana	26.2
Mushroom/toadstool	19.2
Other	16.8
Not specified	10.0
Poinsettia	6.2
Cigarettes	6.2
'Magic' mushrooms	6.2
Dieffenbachia	4.6
Lilly	4.6
n = 130	

The patients included in this group were those who had consumed house or ornamental plants containing a toxic component or toadstools which may grow in lawns or gardens. Ingestion of plant forms of drugs of abuse such as marijuana and magic mushrooms were included in this group as well patients who had consumed cigarettes. The designation 'other' were mostly ingestions of ornamental plants which were identified while the patients in the classification 'not specified' were cases where the generic term houseplant was used and the specific plant was not identified.

The weight distribution for the patients in this group are presented in Figure 5.47 and using the Mann-Whitney rank sum test there is a significant difference between the weight

distribution of this group compared to the total population of 5001 ($p = 0.004$). The median weight for the population in this grouping was 12 kg (6 and 25 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.167 $mg \cdot kg^{-1}$ (0.1 and 0.333 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 32 out of 130 or 24.6% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.47:

Figure 5.47 Patient weight distributions for grouping Plant. (n = 130)

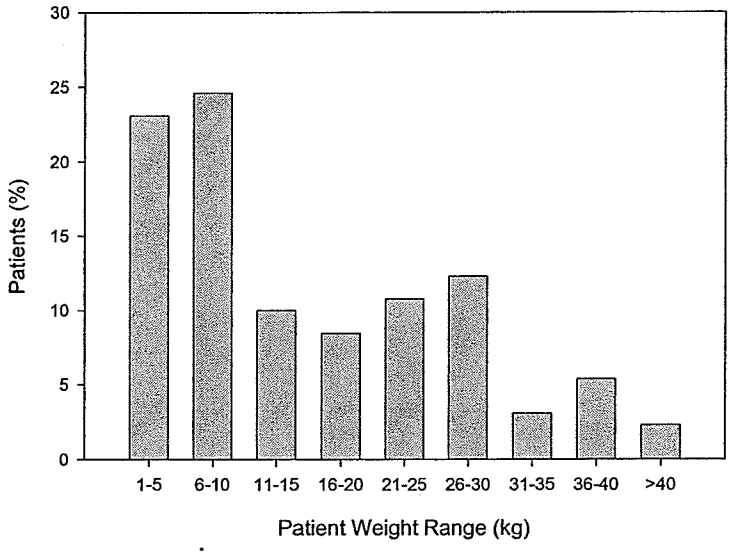


Table 5.47 Patients ingesting plants: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	13.3	73.3	86.7	86.7	13.3	10	30
6-10	0	56.3	90.6	93.8	6.2	0	32
11-15	0	38.5	61.5	76.9	23.1	15.4	13
16-20	0	54.5	72.7	90.9	9.1	9.1	11
21-25	0	35.7	57.1	64.3	35.7	14.3	14
26-30	6.3	31.3	56.3	62.5	37.5	12.5	16
31-35	0	50.0	75.0	100	0	0	4
36-40	0	0	85.7	85.7	14.3	14.3	7
>40	0	0	33.3	66.7	33.3	33.3	3
Group	3.9	48.5	75.4	82.3	17.7	9.2	130
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

Other

In the grouping Other, 85 patients were included and the success rate as defined by emesis within 15 minutes or less was 72.9%. No emesis was seen with 21.2% of the patients. The specific agents reported and their frequency of occurrence are presented in Table 5.48.

Table 5.48 Specific intoxicants in the classification Other.

Intoxicant	Frequency (%)
Pre-op	35.3
Fertilizer	27.1
Other	17.7
Household chemicals	8.2
Herbicide (2,4-D)	5.9
Matches	3.5
n = 85	

The classification 'other' was a miscellaneous group and included patients who were treated with apomorphine in order to empty the stomach prior to surgery, patients who had ingested bone-meal fertilizers and other garden chemicals and patients who had ingested matches. The sub-grouping 'other' in Table 5.48 was mostly cases where the patient had ingested chemicals used in the yard, garden or swimming pool and household chemicals were usually cleaning supplies. Herbicide ingestion was not common in the case reports received but in all of those which were submitted the clinician had determined that the material did not contain petroleum distillates [54].

The weight distribution for the patients in this group are presented in Figure 5.48 and using the Mann-Whitney rank sum test there is a significant difference between the weight distribution of this group compared to the total population of 5001 ($p < 0.001$). The median

weight for the population in this grouping, the median was 25 kg (11 and 30 kg for 25 and 75% of the population respectively). The median dose of apomorphine applied was 0.100 $mg \cdot kg^{-1}$ (0.0741 and 0.155 $mg \cdot kg^{-1}$ for 25 and 75% of the population respectively). In this grouping, 35 out of 85 or 41.2% of the patients were underdosed in that less than 0.1 $mg \cdot kg^{-1}$ of apomorphine was applied. The time to emesis for each weight group is presented in Table 5.49:

Figure 5.48 Patient weight distributions for grouping Other. (n = 85)

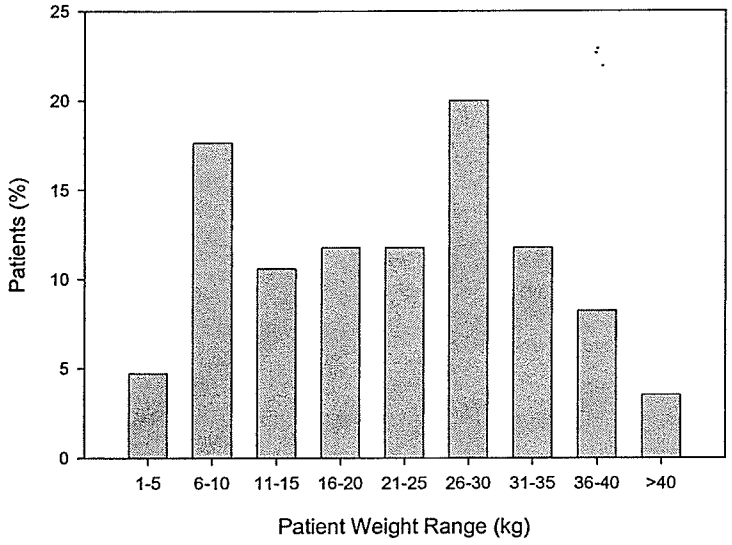


Table 5.49 Patients in group Other: categorized by weight and groups compared as to time to emesis, failure (time to emesis >15 minutes) and no emesis. The time to emesis is expressed as the % of patients responding within the given time interval. The column labeled 'Failure' is the % of patients where there was either no emesis or emesis occurred at a time greater than 15 minutes.

	Time to Emesis – Cumulative % of Patients						
Wt (kg)	0-2 min	3-5 min	6-10 min	11-15 min	Failure	No Emesis	n
1-5	25.0	100	100	100	0	0	4
6-10	0	40.0	73.3	80.0	20.0	6.7	15
11-15	0	55.6	100	100	0	0	9
16-20	9.1	27.3	36.4	45.5	54.5	45.5	11
21-25	0	10.0	40.0	90.0	10.0	10.0	10
26-30	0	5.9	41.2	70.6	29.4	29.4	17
31-35	0	0	44.4	44.4	55.6	33.3	9
36-40	0	14.3	42.9	71.4	28.6	28.6	7
>40	0	0	66.7	66.7	33.3	33.3	3
Group	2.4	24.7	56.5	72.9	27.1	21.2	85
All	4.8	40.6	71.5	83.5	16.5	9.3	5001

The different intoxicants along with patient weights and failure rates are presented in Table 5.50 and the incidence of failure is associated with heavier patient weights. In the whole population of 5001 cases, 27.6% of the patients had less than the recommended dose of 0.1 $mg \cdot kg^{-1}$ applied and the median weight for these patients was 30 kg (25 and 35 kg for 25 and 75% respectively) which was significantly different from the total population ($p < 0.001$) and suggests that there was a tendency for heavier patients to be underdosed. From this, it is likely that the groupings in Table 5.50 where the patient weights were significantly higher than the overall population, underdosing would contribute to increasing the failure rate and this can be

seen with the groupings Foreign object, Unknown, Dietary indiscretion, Antifreeze, Insecticide, and Other.

Table 5.50 Patient weights and failure rates grouped by intoxicant. *Significance of difference from total population. (Mann-Whitney rank sum test)

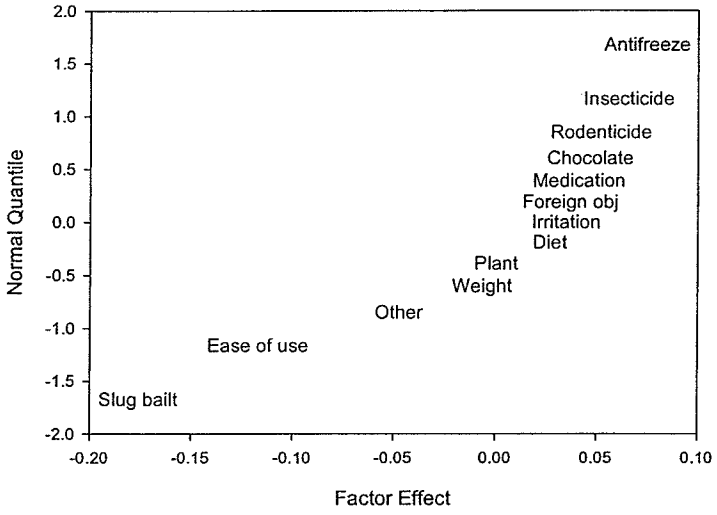
	Patient Weight (kg)						
Intoxicant Group	**25%**	**Median**	**75%**	**p ***	**% Failure**	**% No Emesis**	**n**
Rodenticide	8	17	25	0.887	14.4	6.30	1461
Medication	7	13	24	<0.001	13.8	7.10	803
Chocolate	7	12	25	<0.001	13.4	6.89	682
Foreign object	10	20.5	30	<0.001	17.4	8.39	596
Unknown	10	20	30	0.002	20.7	15.6	487
Dietary indiscretion	10	20.5	32	<0.001	19.0	11.3	300
Slug bait	8	15.5	28	0.711	36.9	30.1	176
Antifreeze	11	22	32	<0.001	15.2	11.7	145
Insecticide	10	20	29	0.055	14.0	7.35	136
Plant	6	12	25	0.004	17.7	8.46	130
Other	11	25	30	<0.001	27.1	21.2	85
All	8	16	28	-	16.5	9.30	5001

Factor analysis

In order to gain further insight into the effects of some of the treatment variables on the success or failure of emesis induction a factorial analysis was performed. The independent variables investigated included patient weight, intoxicant group, adverse effects and ease of use and the dependent variable was success as previously defined and this was coded as 1 for success and 0 for failure. The coded data were analyzed using multiple linear regression to obtain the coefficients for each factor and these values were used to construct the normal plot

presented in Figure 5.49. The coefficients showing significance ($p < 0.05$) included patient weight, slug bait and ease of use.

Figure 5.49 Normal plot of treatment variables on success rate of emesis induction within 15 minutes.



This analysis suggests that the probability of successful emesis induction is reduced with increasing patient weight, difficulty in using the inserts and if the patient ingested slug bait.

Conclusion:

In terms of successful emesis induction, the inserts performed in a satisfactory fashion and showed a success rate, as defined by emesis within 15 minutes, of 83.5% and only 9.3% of the patients did not experience emesis; the median time to emesis was 6 minutes. An association between body weight and higher failure rates was noted and this was attributed to heavier patients being underdosed or possibly having the drug administered in divided dose. If only patients with a body weight of 20 kg or less are considered, the success rate increases to 87.1% and the occurrence of no emesis remains unchanged at 9.3% which is comparable to intravenous administration where the success rate was 90.6% and 9.4% of the patients

experienced no emesis. The time to emesis with the IV route was very fast with the median time to emesis being 1 minute as opposed to the 6 minutes seen with the ocular route.

In terms of safety or incidence of adverse effects, no particular correlation was seen between occurrence of adverse effects and body weight, dose of apomorphine applied or intoxicant ingested. Although the range of adverse effects was wider with the ocular route, both the frequency and severity was lower than that seen in the IV group.

There was an association between failure rates and the degree of difficulty with use experienced by the clinician but this relationship was not clear and possibly involved the learning time required for the clinicians to become accustomed to using this different dose form and the incidence of uncooperative patients. Oral hydrogen peroxide supplementation was useful for inducing emesis in patients where the response to apomorphine was slow but almost half of these patients were underdosed with the apomorphine.

The groupings of intoxicants showed that there was a relationship between the probability of exposure and patient weight; larger patients had a higher exposure to the groups classed as Foreign object, Unknown, Antifreeze, Insecticide and Other and higher failure rates with these groupings could due to more patients being underdosed or receiving the apomorphine in divided dose. The grouping Slug bait however, had a patient weight distribution which was not different from the overall population ($p = 0.711$) yet the failure rate was very high at 36.9% and 30.7% of the patient experience no emesis. The cause for this is unclear but it appears that apomorphine may be unsuitable for induction of emesis when the patient has consumed metaldehyde.

Conclusions: Section 5

The ocular insert formulation was evaluated for release character, product stability and clinical efficacy and safety. In terms of drug release, a satisfactory rate was achieved with the current insert formulation and release was characterized as being through both diffusional and erosion mechanisms and the integrity of the insert was also maintained after swelling and drug release was complete. The selection of gelatin as a matrix material would allow some flexibility in modifying this through cross-linking but the clinical assessment suggested this would not be necessary. The accelerated stability study suggested a shelf-life of about one year but there was evidence that the data deviated from the Arrhenius model and a preliminary study under real storage conditions implied that a more accurate shelf-life might be to the order of two years. The inclusion of sodium metabisulfite and ascorbic acid may serve to increase shelf-life as evidenced by an apparent lag-time which was seen at the lower temperatures used in the accelerated study. The clinical trial demonstrated that the product had efficacy similar to apomorphine administered intravenously and that the incidence of adverse effects was lower with a lower level of severity. A serious shortcoming was the requirement that more than one insert needed to be applied for patients with body weights exceeding 20 kg and these patients were often underdosed or the drug may have been administered in divided dose. The drug performed poorly in patients who had ingested metaldehyde (slug bait) but gave satisfactory success rates with the other intoxicants.

Section 6: Project overview and recommendations:

A gelatin-based insert was designed to deliver apomorphine by the ocular route; a suitable method of fabrication and packaging was found and the product was shown to have a drug release profile and a shelf-life which were both acceptable. A clinical trial showed the product to have an efficacy similar to intravenously-administered apomorphine with a better safety profile in terms of adverse effects although a longer time was required for therapeutic effect. Overall, the original hypothesis that apomorphine could be administered effectively with fewer adverse effects by the ocular route was shown to be true.

The primary shortcoming of the product was the need for multiple inserts to be used in heavier patients. This requirement led to some degree of non-compliance by the clinicians in that these patients tended to be underdosed and there was documentation that with these cases, the drug was sometimes administered in divided dose. This is a problem which will need addressing and the obvious solution would be to supply a second product with a higher drug load for larger patients but it would be imperative that the two products be distinguishable from each other to avoid severe overdosing of small patients. Preliminary investigations have suggested that the same formulation with a 5 mg drug load can be prepared using the same matrix materials and method of fabrication and the pattern of release is similar between the two products. Another option which could be explored would be fabricating the insert as a rod with scoring to allow portions of it to be used depending on the patient weight. For this option, possible problems may be associated with the fabrication process, the ability of the product to maintain integrity for easy removal and changing the geometrical shape will likely affect the drug release profile [469, 519].

The inclusion of sodium metabisulfite and ascorbic acid to avoid discoloration of the product is also an aspect which could be revisited; the apparent interaction between apomorphine and bisulfite should be studied further and perhaps these excipients could be

excluded and replaced with a different strategy; the stability of the apomorphine standards in acidic solution suggests that perhaps discoloration could be avoided and shelf-life extended through the use of an acidic buffer, perhaps citrate, provided ocular irritation does not become a factor.

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