PROLONGED RELEASE FORMULATIONS FOR INDOMETHACIN

BY

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PROLONGED RELEASE FORMULATIONS

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT.

Gastro-intestinal side effects may interrupt essential therapy with indomethacin, a non-steroidal anti-inflammatory drug. Formulation of this drug into sustained release form may reduce some of these side effects by avoiding contact of drug crystals with gastro-intestinal mucosa, at high concentrations as may happen with immediate release dosage forms. Indomethacin sustained release microparticles were prepared from pectin-gelatin or alginate-gelatin complex coacervates, under controlled pH and temperature conditions. Coacervation resulted from ionic complex formation between the positively charged gelatin and the negatively charged pectin or alginate. Some batches were prepared by a combination of spherical agglomeration using carnauba wax and coacervation. Prolonged release of upto 12 h has been achieved with pectin or alginate to gelatin ratio of 1:2 and drug to hydrocolloid complex ratio 1:2 (33% w/w). Drug release in pH 6.2 phosphate buffer was analyzed according to different release kinetic models. Data fit showed correlation in the following order: spherical matrix > first order ≥ square root of time > zero order ≥ planar matrix > cube root law. Incorporation of other materials like carnauba wax offered prospects for achieving zero-order drug release. They also improved particle morphology, size distribution and flow properties. Aging of the microparticles did not significantly affect drug release. The preparation method was compatible with the form of IMC used, except in formulations containing Aquateric ^{R}.

Key Words: Indomethacin, Sustained Release, Microparticles, Coacervation, Release Kinetics.

To Mmonie, Engie, Dobie and Lary.

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CHAPTER 1. INTRODUCTION.

1.1. INDOMETHACIN.

1.1.1. Chemistry and physical properties.

Indomethacin (IMC), 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (Figure 1), a white to brownish yellow, odourless, crystalline powder, is one of the most potent non-steroidal anti-inflammatory drugs (NSAIDS). Four polymorphic forms of indomethacin have been reported with melting points ranging from 133°C to 162°C [Form I (160°C to 161°C); Form II (154°C); Form III (148°C to 149°C); and Form IV (133°C to 134°C)]. Indomethacin with a pK_a of 4.5 is practically insoluble in water (0.40 mg/100 ml and 0.52 mg/100 ml, Forms I and II at 25°C, respectively) and highly acidic aqueous solutions, but it is soluble in phosphate buffer solutions of pH 6.2 (11 mg/100 ml to 16 mg/100 ml, Forms I and II at 25°C, respectively). Indomethacin shows poor stability in more alkaline solutions. It is soluble in ethanol (1 in 50), acetone, chloroform (1 in 30) and in ether (1 in 40 to 45) (1,2).

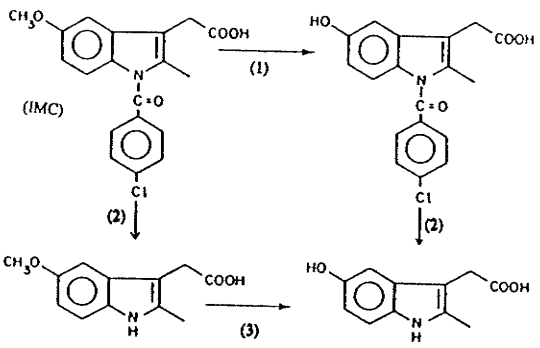


Figure 1. A schematic representation of the chemical structure of IMC ($C_{19}H_{16}ClNO_4$ = 357 . 8 molecular weight) and main metabolic pathways: (1) *O*-demethylation; (2) *N*-deacylation; and (3) combination of *O*-demethylation and *N*-deacylation.

1.1.2. Pharmacokinetics.

Following oral administration indomethacin is rapidly absorbed from the gastrointestinal tract. Peak plasma levels are obtained within 2 hours of dosing. The terminal plasma half-life (2.6 - 11.2 hours) varies between individuals and within the same individuals if tested at different times. In neonates the half-life grows to a range of 15 to 30 hours ((**3,4**). Concomitant food intake slows the time for maximum plasma levels, decreases peak levels and increases the mean residence time (MRT) (**5**). At least 90% of the absorbed drug is plasma protein bound. It is distributed into the synovial fluid, excreted in breast milk and it crosses the placenta (**3,6**).

Indomethacin is metabolised to *O*-desmethyl-, *N*-deschlorobenzoyl-and *O*-desmethyl-*N*-deschlorobenzoylindomethacin (Figure 1), all of which are without anti-inflammatory effects. The drug and its metabolites are glucuronide conjugated and undergo entero-hepatic recycling (**3,4**). The cholesterol reducing agent cholestyramine interferes with the entero-hepatic circulation, and thereby reduces the plasma concentrations of NSAIDS, which undergo entero-hepatic recycling including indomethacin (**7**). Indomethacin and its metabolites are eliminated through the kidneys in urine and to a lesser extent, in the faeces as a result of biliary excretion (**8**). However, impaired renal function does not have any pronounced effect on plasma concentrations of indomethacin. Instead, there is an increase in the conjugated drug and metabolites eliminated in the faeces (**4**). It may be possible that some acyl glucuronidation occurs in the kidney. This speculation is supported by the reduction of indomethacin acyl glucuronidation by 50% and the almost complete inhibition of the production of *O*-desmethylindomethacin when IMC is administered concurrently with probenecid. Probenecid is a competetive substrate to glucuronidation in the kidneys (**7**).

The extent of indomethacin biliary excretion (6.4% biliary *vs* 32.22% urinary) has been shown to be higher than that of other non-steroidal anti-inflammatory drugs like ibuprofen (0.82% biliary *vs* 50.42% urinary) (**8**).

1.1.3. Pharmacological action, uses and administration.

Indomethacin is used for its analgesic, anti-inflammatory and antipyretic effects. The analgesic effects are observed only in pain resulting from inflammation (**9**). Its mechanism of action is by inhibiting cyclooxygenase or prostaglandin synthetase. This mechanism is shared with other acidic anti-inflammatory drugs like aspirin, ibuprofen and diflunisal (**9,10**).

Indications for indomethacin include the symptomatic relief of conditions like osteoarthritis, bursitis, tendonitis, ankylosing spondylitis, rheumatoid arthritis and acute gouty arthritis. Although indomethacin is primarily used for musculoskeletal and joint disorders it is also used for the relief of mild to moderate pain of dysmenorrhoea. The doses employed vary according to the condition and possibly, the individual. In general, it is given in initial doses of 25 mg three time daily with meals. These may be increased, as necessary, to 50 mg daily up to 150 mg to 200 mg daily in divided doses. It has also been used by injection as the sodium salt for closing the patent ductus arteriosus in neonates (**4**).

1.1.4. Side effects and precautions.

The use of indomethacin has not been without untoward adverse effects. The most common of these include the central nervous system: headache and dizziness and gastro-intestinal disturbances: nausea, abdominal pain, diarrhoea and gastritis. Gastric and

intestinal ulceration may also occur. Other effects include hypersensitivity reactions, circulatory and blood disorders, electrolyte imbalance, psychiatric disturbances, peripheral neuropathy, blurred vision, skin reactions such as pruritus, rashes and urticaria, stomatitis, hepatitis, jaundice and renal failure (3,4).

It has been hypothesized that the physicochemical properties of NSAIDS responsible for pharmacokinetic characteristics are also responsible for the anti-inflammatory effect and the resulting untoward effects (10). This hypothesis was based on observations that:

(i) early rapid absorption in the gastro-intestinal tract, especially in the stomach was related to gastro-duodenal irritation or even ulceration; and

(ii) extensive entero-hepatic recycling had an association with ileal and jejunal ulcerations and perforations.

In fact, some studies have previously reported the likelihood of prostaglandin inhibition involvement in indomethacin induced gastric mucosal injury (11). Indomethacin at therapeutic doses increased basal and secretagogue gastric acid secretion in correlation with the significant inhibition of prostaglandin synthesis. The ability of indomethacin to increase gastric motility, enhance vascular permeability and increase intestinal were demonstrated to precede mucosal lesion formation along the gastro-intestinal tract (12).

The ability of indomethacin to undergo entero-hepatic recycling has been implicated in this drug's gastro-intestinal toxicity. That is, the increased intestinal permeability preceding ulceration is tri-factorial. First it is due to the local effect of the drug as it undergoes absorption, followed by the subsequent systemic effect, and later, the local re-exposure to the non-metabolized drug excreted in the bile (13).

1.1.5. Dosage forms and rationale for sustained release indomethacin (IMC-SR). Indomethacin is available in a variety of dosage forms: capsules, tablets, oral suspension, sterile lyophilised powder for reconstitution and suppositories. Examples of these include: Indocid and Imbrilon (25 mg and 50 mg) capsules for immediate release; Indocid R and Indolar SR (75 mg) capsules for sustained release; Indomax, Slo-Indo, Rheumacin SR (75 mg) modified release capsules; Indomod (25 mg and 75 mg) modified release capsules with enteric coated pellets; Flexin-25 Continus (25 mg), Flexin-LS Continus (50 mg) and Flexin Continus (75 mg) as controlled release tablets; Indocid Suspension (25 mg/ml); Indocid PDA (indomethacin sodium trihydrate, 1 mg/vial) powder for reconstitution; and macrogol based suppositories containing 100 mg of IMC.

Many old drugs have recently been marketed in new formulations as sustained release preparations. Most of these have achieved a modified *in vivo* drug release profile of the drug. That is, any one or more of the following properties have been modified: lag time for onset of drug absorption, rate and extent of absorption (**14**).

Indomethacin is a potent NSAID with analgesic and antipyretic effects. It is used in many musculo-skeletal and joint disorders. However, adverse reactions which include gastrointestinal disturbances may inhibit therapy with this drug (**4**). Gastro-intestinal ulcers in rats following a single toxic dose have been reported (**15,16**).

Formulation of indomethacin as an oral sustained release preparation has been hypothesized to have potential to reduce or prevent untoward local reactions within the upper gastro-intestinal tract; to control the delivery pattern of the drug relative to a desired action; to increase the extent of absorption and to prolong the duration of the action following administration (**10,14**).

In clinical settings sustained release indomethacin preparations were reported to

be better tolerated than immediate release forms among individuals prone to gastro-intestinal and centrally mediated side effects (17). Patients receiving indomethacin sustained release preparation for rheumatoid arthritis had a superior side effect profile, reduced abdominal and epigastric pain, compared to those on an immediate release formulation (18). Other reports however, have stated similarities in side effects profiles of controlled release preparations compared to immediate release ones when used in treatment of osteoarthritis (19,20), bursitis, tendinitis (21), ankylosing spondylitis (22) or other painful and inflammatory conditions (23).

The incidence of indomethacin-induced ulcers is related to the initial systemic concentration of indomethacin (24), and ulceration severity decreased, according to dosage form, in the following order: intravenous indomethacin > oral indomethacin suspension > oral indomethacin in an amphoteric gel (25). A nanocapsules preparation of indomethacin (poly[*d-l*-lactide] and poly[isobutyl-cyanoacrylate]) protected against jejunal ulceration by preventing direct contact of indomethacin with mucosal surfaces (26). In addition, drug absorption from indomethacin sustained release formulations was found to be more uniform, prolonged and reliably reproducible when compared to immediate release preparations. There was no effect on the absolute bioavailability of the drug (27,28).

There has been some controversy concerning the rationale for indomethacin sustained release formulation (29,30). The SR preparations in general, improve patient compliance as a result of less frequent dosing (30,31). Some studies have proposed microencapsulation to provide protection towards ulceration, but others have shown no benefit (24,32). It is speculated that the anti-ulcer effects of alginate have contributed to the reduction in stomach irritation resulting from administering an IMC alginate dispersion formulation to dogs (33).

1.2. FACTORS INFLUENCING THE DESIGN OF SUSTAINED RELEASE SYSTEMS.

A number of factors have to be taken into consideration when designing oral SR systems. These have been reviewed at length by previous authors (**34-36**). They are classified into gastro-intestinal tract characteristics, including anatomic and physiologic properties and drug characteristics or physicochemical properties. Others include disease state, the patient and factors affecting the pharmacokinetic and pharmacodynamic properties of the drug.

1.2.1. Gastro-intestinal tract characteristics.

The anatomical and physiological features of the gastro-intestinal (GI) tract form a basis for the design of pharmaceutical dosage forms and, in this context oral SR preparations. These features include surface area and length, the chemical environment, gastric emptying, intestinal motility, specific absorption sites and metabolic degradation, in the GI mucosa and eventually in the liver. Some of these features are summarized in Table 1. For simplicity, the GI tract will be discussed mainly as three joining portions namely, the stomach, the small intestines, duodenum, jejunum and ileum, and the large intestine, the colon.

The stomach has a major function of storing, mixing and grinding food and other stomach contents. During fasting, the pH in the stomach ranges from 1 - 3 and the contents are mainly mucus and acid comprising about 50 ml of the so-called residual volume (**37**). For acid labile drugs, this is a very harsh environment. However, postprandially the pH may increase from pH 3 to 5. Along the mucosal lining the pH is slightly alkaline to protect the lining of the stomach (**38**). Due to a relatively small surface area, 0.1 - 0.2 m^2, a thick mucous layer coating, the lack microvilli, and a

relatively quick transit time for most drugs, the stomach offers insignificant absorption for most drugs (**36**).

The stomach is joined to the duodenal region of the small intestine through the pylorus. The contents of the proximal duodenum result from secretions from various other areas of the digestive system. These include duodenal secretions like bicarbonate and mucus and gall-bladder contents like bile and pancreatic juices including lipolytic, proteolytic and carbohydrate digesting enzymes. The pH in this region ranges from 4.6 - 6.0. It increases to pH of 7.5 - 8.0 in the jejunum and ileum. The intestinal region has a highly viscous surface area and is tubular with a length of about 3 m. The large surface area of up to 4500 m^2 is due to the presence of villi which are small finger-like formations of the mucosa (**36**).

Like the stomach, the colon does not have villi and hence has a less surface area when compared to the small intestine. It is joined to the small intestines through the ileo-caecal valve. The contents of the colon consist of normal resident flora and mucus neutralizing acids produced by bacteria (**38**).

Table 1. Properties of the gastro-intestinal tract.

(Modified from (37)

Property		GI tract Region			
		GI tract	Stomach[b]	Small intestine	Large intestine
Surface area (m^2)		200	0.1-0.2	100 (4500)[c]	0.5-1.0
Length(m)		-	-	3.0	1.5
Transit time	Fluid[a]	-	50 min	2-6 h	2-6 h
	Solid	-	8 h[d]	4-9 h[e]	3 h - 3 days
pH	Fasting	-	1-3	5-8	5-7
	Fed state	-	3-5	-	-

[a] Isotonic saline solution, 500 ml, was ingested.
[b] Residual volume = 50 ml.
[c] Taking intestinal microvilli into account.
[d] Solid food, 50 g, was ingested.
[e] Food first appeared in the caecum after 4 h and all the indigestible material entered the large intestine within 9 h.

1.2.1.1. Gastro-intestinal tract dynamics.

The rate of gastric emptying and intestinal motility are the primary determinants of the residence of SR dosage forms in the GI tract. The stomach, at least both the human and canine, shows different patterns of reactivity during fasting and fed states. Many factors determine gastric emptying and these include volume, composition, pH, temperature, caloric value, osmolality and viscosity of the stomach contents, the endocrine as well as the autonomic activity, disease states and the effect of many drugs (**36**).

During the fasting state, GI motility is made up of four distinct phases: Phase I is the period of minimum contractile activity with a duration of about 1 hour; Phase II which lasts for about 30 minutes is the period of some irregular, on-and-off contractions; Phase III lasting for about 5 - 15 minutes is the period of maximum, regular and migratory contractions; and finally Phase IV which has intermittent or no contractions is the period of transition from Phase III to the resting state of Phase I. In the fed state there is an interruption of the interdigestive motility cycle described above by continuous contractions induced by the gastric contents, depending on the volume and caloric content (**36,38**). It is therefore logical that sustained release dosage forms be made to resist the clearing effects of Phase III in order to prolong the GI residence time. This could be achieved with multiparticulate dosage forms composed of pellets of less than 2 mm diameter and with density much less or much greater than that of the stomach contents.

1.2.1.2. Gastro-intestinal tract transit.

The transit time of particles through the GI tract is a function particle size, physical state of the dosage form, volume and the feeding state. Particles smaller than 1 mm diameter and fluids are emptied from the stomach at the same rate, during the fed state, while

particles of 2 mm or greater diameter are kept to be cleared by the next Phase III of the interdigestive migratory complex (**36-38**). Recent reports have questioned the role of size in gastric emptying (**39**). There seems to be strong evidence to suggest a threshold density of about 2.4 - 2.6 g cm^{-3} above which gastric emptying is delayed. Emptying of volumes less than 100 ml may be delayed whereas those greater than 150 ml are immediately emptied, probably due to stimulation of gastric motility (**40**).

The presence of food in the stomach could delay gastric clearance of larger particles for up to 6 h compared to 2 h in the fasting state (**41-42**). However, transport through the small intestines does not seem to be size dependent, and it is independent of the feeding status. Transit time in the intestines is about 3 to 4 h.

The use of multiparticulate dosage forms (of density within the specified threshold) could therefore offer an advantage of delayed stomach emptying while not affecting intestinal transit. The delay in gastric emptying would offset the relatively short GI transit which hinders attempts to design once a day dosage forms.

Coupe et al (**43**) studied the correlation between gastric emptying of a pellet dosage form and GI motility. They observed that while food emptying correlated with GI motility, the emptying of pellets was delayed in the majority, 6 out of 8 volunteers, of the subjects studied. Inter-subject variation was found to be quite significant. This implied that drugs not well absorbed in the distal part of the small intestine might not be suitable for sustained release formulation without manipulation of the residence time. Not withstanding that GI transit may be 3 - 6 h, SR dosage forms intended for once or twice a day dosing may be achieved by slowing down gastric emptying time.

Multiparticulates and a non-disintegrating single unit dosage form would take on average about 4 or 5 h to reach the colon, subject to interpatient variability (**43**). The

proportion of the drug, theophylline, not released in either the stomach or the small intestines was released and taken up within the colon, signifying its role in drug absorption for certain drugs (**41**). In another investigation using SR diltiazem in dogs, Murata and Noda (**45**) observed that 60% of the administered tablet reached the colon intact. Dosage forms remaining intact beyond the ileum could be targeted for colonic delivery. The high colon content resident flora is responsible for azoreduction and enzymatic cleavage processes which may be exploited for colon targeted drug delivery (**46**). However, due to its high water absorbing capacity, the colon contents remain highly viscous and poorly mixed, which may affect the rate of drug absorption (**36**).

1.2.2. Drug properties.

Intrinsic drug properties to be considered in the design of SR dosage forms may be classified into two subgroups - physicochemical and biological. These properties influence drug behaviour within the delivery system and also the drug and the delivery system behaviour in the body. Unlike conventional immediate release dosage forms where drug absorption is the bioavailability rate limiting step, in SR dosage forms drug release is the rate limiting step. That is, the release rate constant(s) are much smaller than the absorption rate constants (**35**).

The physicochemical properties of the drug include, dose size and safety index, aqueous solubility, partition coefficient and molecular size, pKa and charge, drug stability, and protein binding.

1.2.2.1. Dose size and safety index.

The size of an oral solid dosage form is limited by the ease with which it would be

swallowed. Drugs available in conventional doses of more than 500 mg would result in large SR dosage units which may not be easily ingested. If the oral route is preferred a liquid dosage form could be an alternative, otherwise alternte routes of administration should be considered.

Drugs with a narrow therapeutic/safety index require careful dose and blood concentration titration to avoid toxicity while maintaining effectiveness. The SR formulation may be useful in providing careful control over the amount of drug released (34). However, dose dumping remains to be a potential problem of single unit SR dosage forms which usually contain as much as three times an equivalence of a single therapeutic dose (47).

1.2.2.2. Aqueous solubility.

In order for absorption to commence the drug has to be in solution. Drugs with very low aqueous solubility have dissolution rate limited absorption which may result in inherent sustained release properties. Oral bioavailability of such drugs may be hindered due to limited GI transit time unless solubility is modified (35). Furosemide and griseofulvin are good examples of drugs with variable oral bioavailability attributed to poor solubility (48,49). Some drugs have high solubility in areas where absorption is poor. In these cases, a SR preparation designed to be retained in the area of highest solubility would produce poor bioavailability.

Aqueous solubility dictates the choice of drug release mechanism. Highly soluble drugs tend to leak out of aqueous carrier systems, resulting in poor drug loading efficiency. Poor solubility may result in otherwise low aqueous concentrations necessary to drive diffusion in diffusion dependent SR preparations (35).

Poorly soluble drugs intended for SR formulation have been dispersed in, or in some cases, co-precipitated with hydrophilic substances to enhance their solubility and dissolution, resulting in improved absorption. Hasegawa et al (**50**) dispersed nifedipine in hydoxypropylmethylcellulose phthalate and methacrylic acid-methacrylic acid methyl ester copolymer resulting in improved dissolution and bioavailability. Mefenamic acid and indomethacin dispersions in egg albumin and low molecular weight alginate, respectively, also improved dissolution of these drugs, respectively (**33,51**).

In summary, a proper balance between aqueous solubility and lipophilicity has to be reached before SR dosage form formulation can be considered.

1.2.2.3. Partition coefficient and molecular size.

Partition coefficient and molecular size are essential for the drug to diffuse through the coating polymeric membrane or matrix pores and for permeation through cell lipid membranes (**38**). Drugs with an extremely high degree of lipid solubility, high partition coefficient of log $P > 3$ readily penetrate the cell membranes but would be trapped in the lipid membrane. On the other hand drugs with extremely low partition coefficients of log $P < -1$ or with high water solubility will not be able to penetrate the membranes effectively. However, the relationship between partition coefficient and GI absorption seems to exist from log $P \geq -1$ to log $P \leq 3$. Outside these limits partition independent absorption mechanisms tend to prevail, resulting in slow absorption. Furthermore, similar log P values do not necessarily reflect similar absorption rates.

Molecular size also influences drug permeation through membranes. The ability of molecules to diffuse through membranes is shown to be a function of either molecular volume or molecular weight according to the following equation,

$$\text{Log}D = -s_V \log V + kV = -s_M \log M + kM \quad (0)$$

where D is the diffusivity, V is molecular volume, M is molecular weight and s_V, s_M, k_V and k_M are constants in a specific medium (**36**).

1.2.2.4. Charge and pKa.

The nonionized form of a drug crosses the lipid membranes more readily than the ionized form (**52**). This relates the rate of absorption directly to the concentration of the unionized form of the drug and the pH of the medium. The environmental pH of the GI tract ranges from 1 - 8 (Table 1). Generally, drugs with pK_a values ranging from 5 to 7 are more readily absorbed than acidic drugs with even higher pK_a values. Basic drugs with pKa values less than 5 and acidic drugs with pK_a values higher than 7.5 show pH independent absorption. Tammara et al (**53**), observed greater extent of absorption for indomethacin morpholinoalkyl esters with pK_a values in the range of 6.89 to 8.92 and increased aqueous solubility, compared to indomethacin alone with pK_a of 4.5.

1.2.2.5. Stability.

The extent to which a drug is stable in the environment to which it will be exposed after administration should be considered prior to SR design. Drugs that are unstable in the stomach may benefit from enteric coating protection against gastric contents or chemical modification. Erythromycin-propionate-*N*-acetylcysteinate was found to be more stable in the GI tract and produced higher and more sustained plasma levels compared to erythromycin stearate (**54**). However, drugs metabolized or hydrolysed in the intestines could be adversely affected by SR dosage which prolongs the exposure of the drug to that

environment. Examples of such drugs include propantheline which is metabolised in the distal parts of the intestine and nitroglycerin which undergoes saturable first pass metabolism (**35**).

Stability in the upper GI tract is a prerequisite for prodrugs designed for enzymatic or microbial cleavage at or near the target site which may be the colon (**55,56**). Recent advances in peptide drugs technology have shown that some, *e.g.* metkephamid are not metabolized in the colon (**56**). Therefore, protection from the upper GI tract brush border enzymes would improve their bioavailability.

1.3. SR SYSTEM DESIGN AND DRUG RELEASE KINETICS.

Most SR systems depend on dissolution or bioerosion, diffusion or a combination of dissolution and diffusion to release the drug slowly in the gastrointestinal environment. Given the parameters discussed earlier, by modifying the pharmacokinetics of the drug, the formulator can approximate the dosage required for an amount of drug to be released. It should be noted however, that not all preparations show correlation between *in vitro* dissolution and *in vivo* bioavailability. Therefore, *in vitro* dissolution studies should be followed with *in vivo* bioavailability tests in acceptable subjects.

The terms microcapsule and microsphere, monolithic or matrix, are used to describe the structure of the particles without categorising the particles according to release characteristics. In this review the particles will be primarily categorised according to whether drug release is dissolution or bioerosion, or diffusion rate limited. The overlap between drug release properties of microcapsules and microspheres is recognized (**34,38**).

1.3.1. Dissolution-rate-limited SR.

In dissolution-rate-limited SR systems the drug release rate is dependent on the dissolution of the polymer. The majority of the dissolution dependent SR preparations fall into two main categories: (1) encapsulation dissolution dependent SR system, and (2) matrix dissolution dependent SR system. Figure 2. (a) and (b) is a representation of dissolution dependent drug release showing a drug core coated with a polymeric membrane (a) and drug-polymer matrix (b). In both cases the release of the drug is governed by polymer dissolution or erosion. Some drugs however, have inherent delayed dissolution rates, for example digoxin and griseofulvin. Others, including ferrous sulphate, react with gastrointestinal contents to form slow dissolution end products. In such cases drug release becomes drug dissolution rate limited (**59**).

For drugs with high aqueous solubility, it is theoretically possible to coat them with erodible polymeric materials or waxes to delay the release rate. Potassium chloride-wax matrix is an example.

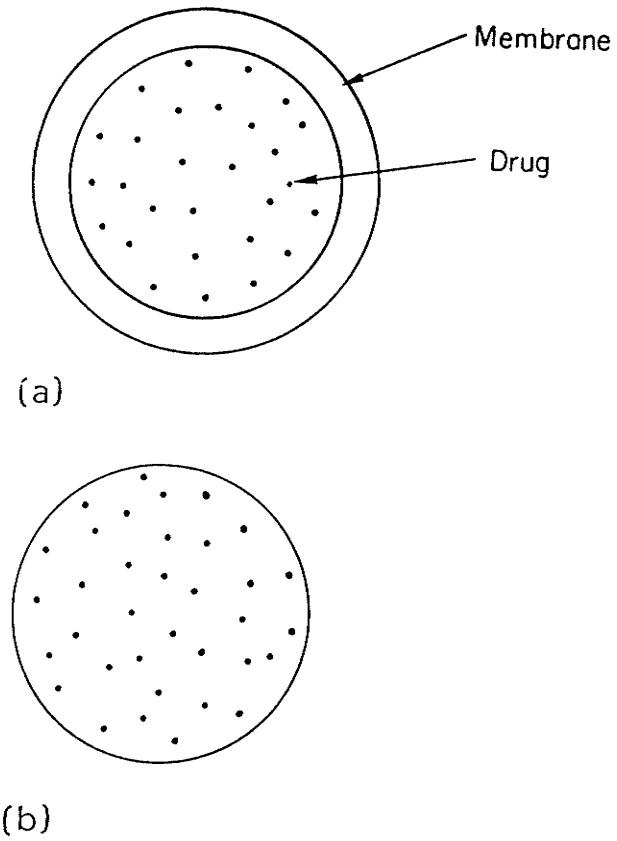


Figure 2. Schematic drawings of dissolution rate limited SR systems: (a) encapsulated system (membrane dissolution dependent drug release); and (b) matrix system (matrix erosion/dissolution dependent drug release). Ref. (59)

Drug release kinetics from dissolution rate limited systems is described by Fick's law of diffusion, which describes the rate of diffusion from an undisturbed solute surface through a film formed around the solute and into the medium. It is given by the following equation;

$$J = -D\left(\frac{dC}{dx}\right) \quad (1)$$

or

$$J = \frac{1}{A}\left(\frac{dM}{dt}\right) \quad (2)$$

where J (mol/cm^2.sec) is the flux (release); D is the diffusion coefficient (cm^2/sec); $\frac{dC}{dx}$ is the concentration gradient from the solute surface to the medium over distance x (mol/cm^3/cm); A is the diffusion area (cm^2); and $\frac{dM}{dt}$ is the flow rate of the material across the diffusion area (mol/sec) at time t (sec). For a linear concentration gradient across a diffusion layer of thickness, h (cm), then

$$\frac{dC}{dx} = \frac{C_s - C_b}{h} \quad (3)$$

where C_s and C_b are the concentration at the solute surface and in the medium, respectively. Therefore,

$$\frac{dM}{dt} = -\frac{DA}{h}(C_b - C_s) = k_i A(C_s - C_b) \quad (4)$$

where k_i is the intrinsic dissolution constant (sec $^{-1}$). Thus, the dissolution rate is determined by the surface area, intrinsic dissolution, diffusion coefficient, diffusion layer thickness and the concentration gradient (**34**). Integration of Equation (4) from time t to

∞ gives

$$\frac{M_t}{M_\infty} = k_m t^{\,n} \qquad (5)$$

where M_t and M_∞ (mol) are the amount of drug released at time t and ∞, respectively; k_m is an empirical constant incorporating structural and geometric characteristics of the system (sec $^{-n}$); and n is the exponent characterising drug diffusion. Equation (5) approximates Fickian drug release when n is equal to 0.5 or release rate is proportional to $t^{\frac{1}{2}}$. For some systems such as membrane-type, diffusion controlled systems or swelling polymer systems, n equals 1 resulting in zero order release kinetics (**60**)

The surface area may not remain constant in systems where drug release could be a result of chemical reactions, cleavage from a prodrug backbone, hydrolysis or biodegradation. For such systems the following release equation has been proposed:

$$\frac{dM_t}{dt} = k_e A_e \qquad (6)$$

which on integration becomes:

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_e t}{C_{io} r}\right)^n \qquad (7)$$

where k_e is the erosion kinetic constant (mol/cm^3·sec); A_e is the area of erosion (cm^2); C_{io} is the initial drug concentration (mol/cm^3); and r is the initial radius (cm). It has been observed that for a sphere of radius of r, n equals 3; for a cylinder of radius r, n equals 2; and for a slab with a thickness of $2r$, n equals 1 (**60**).

For spherical coated or non-coated particles, with dissolution dependent release kinetics, under an assumption of sink conditions, the change in surface area may be weight related. In this case Equation (4) is integrated to give the cube root dissolution equation given by:

$$W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}} = \left(\frac{W_0}{d_0}\right)^{\frac{1}{3}}\left(\frac{2k_i C_s}{\rho}\right)t = k't \qquad (8)$$

where W_0 and W_t are the weight (mg)of the particle or drug at time zero and at time t, respectively; d_0 is the initial diameter (cm); ρ is the density of the particle; and k' is the cube root dissolution rate constant (**34,61**).

1.3.1.1. Encapsulated dissolution dependent SR.

Drug release from an encapsulated dissolution dependent SR system is modulated by either erosion or dissolution of the polymeric membrane followed by diffusion of the drug to the bulk medium. As shown in Figure 1 (a), a drug can be coated with a slow dissolution polymeric material or wax to give a microcapsule. The solubility of the coating material in GI juices determines the site of delivery of the drug. The drug becomes available for absorption once the polymeric material has dissolved. The release rate depends on the thickness and the dissolution rate of the polymeric material (**34**).

Stomach irritation due to drugs like potassium chloride and aspirin has been minimized by coating or microencapsulation of the drug particles with materials such as ethyl cellulose, acrylics, and gelatin complexes (**62**). Initial coating of the drug with polymers or waxes insoluble in water and acid solution such as cellulose acetate phthalate and carnauba wax before microencapsulation offers some protection from uncontrolled release in aqueous environments for excessively water soluble drugs (**63**).

1.3.1.2. Matrix dissolution dependent SR.

In matrix dissolution dependent systems, microsphere or monolithic type, the dissolution of the polymer controls the release of the drug. The mechanism by which the polymer is eroded may be subject to influence by the changes in the GI tract environment through which the dosage form is transported. The physicochemical properties of the drug are equally important in determining the release pattern of the drug. The release rate of a drug with poor solubility in higher pH would benefit from a matrix polymer with solubility increasing with pH (**14**).

In the encapsulated dissolution dependent SR systems phase separation is the commonly employed method of preparation. Matrix systems are either aqueous dispersions of drug in a slow dissolution polymer/wax or a product of wax-drug congealed melt compressed into tablets (**59**). The hardness, hydrophobicity and porosity of the tablet or particle are essential in controlling the release rate. Drug release from a product of spray-congealed microcapsules compressed into a tablet was influenced by erosion, solubilization and leaching of the drug (**59**).

The mechanism of release from matrix dissolution dependent systems is not well characterised probably due to compounding factors like the method of preparation, physicochemical properties of the drugs studied and polymers utilized, the dissolution media employed and the formulations studied (**59**). The release mechanism of diphenhydramine from a polymer-wax matrix dispersion was found to follow square root of time release (Equation 20) (**64**). Zero-order release (Equation 5) was observed following a spherical agglomeration technique (**65**).

The square root of time release mechanism has been derived from an equation describing drug release kinetics from ointment bases by Higuchi (see ***Matrix diffusion dependent SR***), based on Fick's first law shown in Equation (1) (**66**).

1.3.2. Diffusion dependent SR.

In diffusion dependent systems drug release is a function of drug dissolution and diffusion through a polymeric membrane which remains fairly intact in the process. Two types have been described: (1) reservoir diffusion dependent systems; and (2) matrix diffusion dependent systems.

1.3.2.1. Reservoir diffusion dependent systems.

Drug release from a reservoir device or microcapsule is a three step process involving: (1) aqueous medium penetration through the polymeric membrane to the drug core; (2) drug core dissolution; and (3) diffusion of the drug solution along a concentration gradient into the bulk aqueous medium (**34,58**). Ideally, step 1 is quick enough not to influence the release rate. However, step 2 is essential for determining the release rate limit. That is, the release rate of a poorly soluble drug may be dissolution rate limited. In such a case Equation (8) would be used to describe the release kinetics. In most cases step 3 predominates. The three step process was used by Madan et al (**67**) to explain clofibrate release from simple gelatin coacervation microcapsules. Figure 3 shows a schematic representation of a diffusion dependent SR system of a drug completely encapsulated in an insoluble membrane.

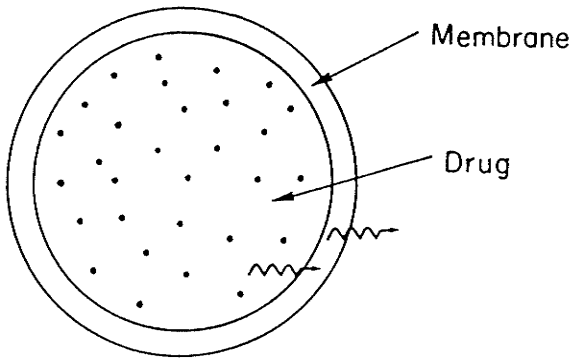


Figure 3. Schematic drawing of a diffusion dependent SR system representing a drug encapsulated in an insoluble semi-permeable membrane (**59**).

Under steady state conditions the diffusion is described by Fick's first law given in Equation (1), and the drug concentration is in equilibrium on either side of the membrane, subject to the partition coefficient effects, as follows:

$$K = \frac{C_{ms}}{C_s} \; at \; x = 0 \tag{9}$$

and

$$K = \frac{C_{mb}}{C_b} \; at \; x = h \tag{10}$$

where K is the partition coefficient, C_{ms} and C_{mb} are the drug concentrations inside the surfaces of the membrane, above the inner surface and below the outer surface, respectively, C_s and C_b are the concentration at the surface of the core and in the bulk medium adjacent to the membrane, respectively, and h is the membrane thickness.

Equation (1) can be integrated between the two concentration levels, assuming D and K to be constant, to give:

$$J = \frac{D(C_{ms} - C_{mb})}{h} \tag{11}$$

The concentration in the membrane surfaces is not easy to determine therefore, Equation (11) can be written as

$$J = \frac{DKC_h}{h} \tag{12}$$

where C_h is the concentration gradient across the membrane. Since the drug diffuses

through an insoluble membrane of an effective surface area A at a flow rate of $\frac{dM}{dt}$, then Equation (12) can rewritten as

$$\frac{dM}{dt} = \frac{ADKC_h}{h} \qquad (13)$$

Equation (13) applies to a slab, hence the release rate from a spherical device of a relatively thick wall, with an external radius of r_e and an internal radius of r_i would be given by

$$\frac{dM}{dt} = \frac{2\pi(r_i^2 + r_e^2)DKC_h}{(r_e - r_i)} \qquad (14)$$

Some parameters are important in attempting to maintain constant drug release rate:

Membrane thickness - The effect of membrane thickness may be predicted directly from Equations (13) and (14). Release rate is indirectly proportional to thickness if other parameters are held constant. Madan (**68**) classically demonstrated that increasing the thickness of the coating material on clofibrate microcapsules decreased the release rate quite significantly. Also observed was the variation in release kinetics involved. That is, thinner walled microcapsules followed square root of time release mechanism, while thick walled microcapsules followed zero-order release kinetics.

Swelling of the membrane, upon exposure to dissolution medium, could change the effective thickness and the unit area or even the diffusion coefficient or change in pore size, resulting in drug release variations (**34**).

Microcapsule hardness - The effect of hardening on microcapsule drug release was studied by Madan et al (**67**). While not affecting the release kinetics, hardening was also

inversely proportional to drug release rate of clofibrate from microcapsules. A similar observation was made by Nixon (**69**) after studying compression force on chlorthiazide tablets produced from microcapsules of complex gelatin-acacia coacervation.

Polymer ratio in the membrane - Suryakusuma and Jun (**70**) studied the release rate of indomethacin from polymeric membranes of varying concentrations of ethylcellulose. The release rate decreased as polymer concentration increased. When polyvinyl pyrrolidine (PVP) was introduced in the formulation, the release rate increased with increasing concentrations of PVP. Thus PVP acted as a wetting agent and thereby hastening step 2. The solubilising effects of PVP have been demonstrated elsewhere (**71**).

Concentration of the drug in the core - Drug saturation concentration, C_s, does not remain constant for extended periods of time due to the depletion of the drug from the core. The dilution by imbibed water eventually results in an exponential fall in release rate or first-order release kinetics. The change in concentration could also be enhanced by the presence of pores in the reservoir which affect diffusion characteristics of the system (see ***Matrix diffusion dependent SR***).

The exponential decline in the amount of drug inside the microcapsule or microparticle is decribed by the first order release kinetics (**72**). For a microcapsule of volume V_m, containing total drug amount of M_0, the amount of drug released into a bulk medium of volume V_b, is M_t, and the amount of drug remaining in the microcapsule is M_m, then Equation (14) may be re-written to show the change in amount of drug in the microcapsule at time t as follows:

$$\frac{dM_m}{dt} = -M_0 k e^{-kt} \qquad (15)$$

which on integration becomes

$$M_m = M_0 e^{-kt} \tag{16}$$

and similarly, amount in the medium increases exponentially

$$M_t = M_0(1 - e^{-kt}) \tag{17}$$

where k represents

$$2\pi(r_i^2 + r_e^2)\frac{DK}{(r_e - r_i)V_m}$$

a function of membrane thickness, diffusion and partition coefficients and the volume of the microcapsule.

In an ideal situation where the unit area, membrane thickness, diffusion coefficient, partition coefficient and the concentration (saturation) remain constant, then a zero-order release rate is observed (**73**). However, this often holds only for a fraction of the release duration (**32,61,68,73**). An initial burst preceded the zero-order pattern in most cases. This was possibly due to some drug adhering to the microcapsule surface and/or ill-formed microcapsules. A combination of techniques, polymerization followed by phase separation for microcapsule production minimized the burst effect (**70**).

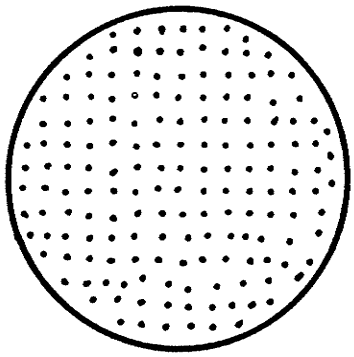


Figure 4. A schematic drawing of a diffusion dependent SR system. A solid drug is dispersed in an insoluble polymer matrix (59).

1.3.2.2. Matrix diffusion dependent SR.

In this system the drug is not concentrated in the core, rather it is uniformly blended and/or dissolved in an insoluble polymeric material to form a matrix. Therefore, for the drug to be released from this system it has to undergo dissolution and diffuse through the inert polymer matrix following a three step process described in the previous sub-section. Figure 4 shows a diagrammatic representation of this system.

Drug release from a matrix system has been described by Higuchi (**66,74**), based on the following assumptions: the drug is uniformly dispersed in the system; the drug concentration in the matrix, C_{io} , is greater than the drug solubility in the matrix, C_s ; the average distance of diffusion, h, is greater than drug particles average diameter; perfect sink conditions exist or drug concentration at the interface of the matrix and the bulk medium is always zero; the diffusion coefficient stays constant; and the drug does not interact with the matrix. Two models have been suggested which are applicable mainly to planar systems:

$$M_t = A[\, D_s C_s (2C_{io} - C_s) t \,]^{\frac{1}{2}} \qquad (18)$$

and

$$M_t = A[D_a \frac{\epsilon}{\tau} C_a (C_{io} - \epsilon C - a) t \,]^{\frac{1}{2}} \qquad (19)$$

where ϵ and τ are the porosity ($\frac{\text{cm}^3}{\text{cm}^3}$) and tortuosity of a porous matrix, D_s and D_a are the diffusion coefficients of the drug in the matrix and the bulk aqueous medium (cm^3/sec) and C_{io} , C_a and C_s are the drug concentration in the matrix and the drug solubilities in the bulk aqueous medium and in the matrix, respectively (mol/cm^3).

For data processing Equation 18 is usually reduced to

$$M_t = kt^{\frac{1}{2}} \tag{20}$$

where a plot of amount of drug released against square root of time is linear with a slope of k.

Equation (19) is applicable to matrix systems with identifiable porosity (**60**). That is if the pores are more than 150 Å, then it should be assumed that diffusion through the aqueous bulk medium predominates, since the drug in the matrix channels through the medium filled pores. However, where the pores measure less than 100 Å and the system is hydrophillic, then the swelling of the matrix in the aqueous medium corrects for the porosity and tortuosity. If it is suspected that the drug might partition into the polymer pore walls, interfering with diffusion, then the partition coefficient should be incorporated into the diffusion coefficient (D_{eff}), an effective diffusion coefficient) by the following relationship:

$$D_{eff} = D_a K_p K_r \tag{21}$$

where K_p is the partition coefficient, for pore wall adsorption and K_r is a restriction coefficient given by

$$K_r = \left(1 - \frac{r_s}{r_p}\right)^2 \tag{22}$$

and r_s and r_p are the drug solute radius and the pore radius, respectively (**60**).

In an attempt to account for drug release from spherical matrices, Higuchi (**74**) developed the following general equation:

$$1 - 3\left(\frac{R'}{R_0}\right)^2 + 2\left(\frac{R'}{R_0}\right)^3 = \frac{6DC_s}{C_{io}R_0^2}t \qquad (23)$$

where R' and R_0 are the radii of the whole sphere and the portion not yet extracted, respectively. Further work by other investigators, produced the following useful relationship describing drug release rate from spherical matrices (**58**):

$$\frac{3}{2}(1 - (1 - F)^{\frac{2}{3}}) - F = \left(3\frac{DC_s}{C_{io}R_0^2}\right)t \qquad (24)$$

where

$$F = \frac{M_t}{M_\infty}$$

Jun and Lai (**75**) found a linear relationship describing the release of nitrofurantoin from albumin microspheres after plotting

$$\frac{3}{2}(1 - (1 - F)^{\frac{2}{3}} - F$$

against time.

Benita et al (**76**) reported first-order release kinetics of indomethacin from microspheres prepared from a combination of ethyl cellulose and polyethylene glycol (PEG). The microspheres obtained displayed some porosity, due to PEG solubility which resulted in the observed release pattern, *i.e.* drug release via pores instead of diffusion through intact polymeric material.

Leucuta (**77**) investigated the release of pilocarpine from either gelatin or albumin

microspheres using various release kinetic models. A biphasic release pattern was observed for all the models studied: first order kinetics; planar matrix release; square root of time kinetics; spherical matrix kinetics; and zero-order release kinetics.

The multi- or biphasic release kinetics have been reported for microparticles made from biodegradable polymeric materials such as gelatin, albumin and poly(D,L-lactide) (77-78). Biphasic release kinetics is an indication of a fast initial phase followed by a slow phase. The following biexponential equation has been applied to describe the release pattern:

$$M_m = Ae^{-\alpha t} + Be^{-\beta t} \tag{25}$$

or

$$M_t = M_\infty - (Ae^{-\alpha t} + Be^{-\beta t}) \tag{26}$$

where A and B are constants, and α and β are first order release rate constants for the initial and terminal phases, respectively. The polymer type, polymer swelling characteristics and the distribution of the drug in the microparticle determine the release profile of the drug (79-80).

1.3.3. Combined dissolution and diffusion dependent SR.

This borderline system is a result of attempts to improve drug release from reservoir and/or matrix type devices. It is not surprising therefore that much has been covered in the previous sections. Figure 5 shows a schematic representation of this type of system (59). The drug is encapsulated in an insoluble polymeric membrane like ethyl cellulose

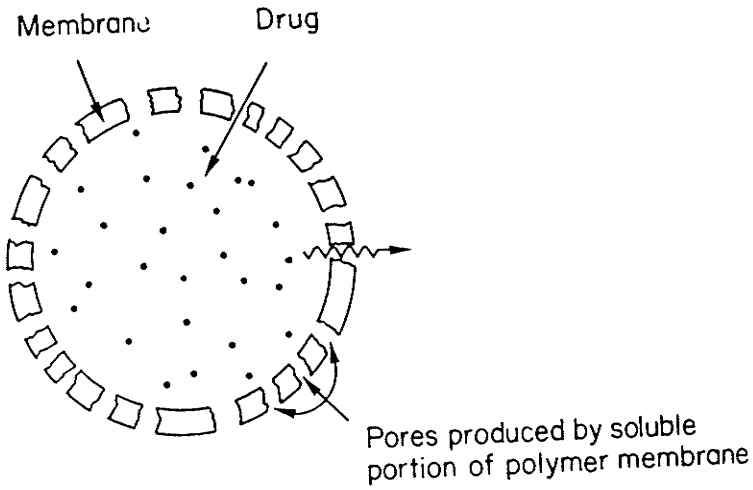


Figure 5. A schematic representation of a combined dissolution and diffusion SR system showing pores within an insoluble polymeric membrane (59).

which has been mixed with a soluble material like polyethylene glycol (PEG) or PVP (**70,76**). The dissolution of the soluble fractions would create pores through which the drug is released, over and above diffusion across an intact membrane. In this system the release pattern is given by

$$\frac{dM_t}{dt} = \frac{AD_a\epsilon(C_s - C_b)}{\tau h} \qquad (27)$$

where C_s is the drug concentration in the core, D_a is the diffusion coefficient in the bulk aqueous medium, and others were explained earlier. If the partition coefficient is significant then the effective diffusion through the pores, D_{eff} would be applied as in Equation (21) (**59,60**).

1.3.4. pH-Independent SR systems.

Orally administered drugs are exposed to wide pH variations from administration time to absorption ranging through, neutral in the mouth, acidic in stomach and neutral to basic in the intestines. Some of these conditions have been summarized in Table 1. The pH values in the GI tract may vary due to several factors including: ingestion of food; drug intake; aging; and disease states (**46**). These could result in alteration of drug bioavailability. Most drugs are either weak acids or weak bases, therefore the release from sustained release preparations would be influenced by the pH in specific sites in the GI tract. Unlike immediate release preparations, SR forms release the drug over extended periods of time in the GI tract, hence they become more prone to the changing physiological conditions of the GI tract.

A number of reports have shown the dependency of drug release from sustained release preparations (**59**). Papaverine hydrochloride SR preparation showed preferential

release in areas of low pH in the GI tract, stomach region, whereas free drug was precipitated at intestinal pH (**81**).

Some reports have shown promising results of oral SR preparations of drugs with high solubility in acidic media such as papaverine and dipyridamole (**81-82**). The systems are usually prepared by blending the drug, a buffering agent, *e.g.* citric acid and some suitable granulating excipient like PVP to produce granules which could be coated with an insoluble polymeric material such as ethyl cellulose and filled into capsules. Alternatively, the granules may be tableted prior to coating.

Kohri, Yatabe et al (**81**) have suggested an equation that describes the drug release mechanism from a pH independent controlled release tablet derived from Equation (5), such that

$$M_t = k_1 t^{n} \qquad (28)$$

where k_1 is a constant derived from the initial weight and structural and geometric characteristic of the SR system. It was demonstrated that the initial weight of the device did not influence the drug, papaverine release profile. The release rate was dependent on the drug content ratio in the device, the surface area and diffusion characteristics.

1.4. MICROENCAPSULATION: CONSIDERATIONS, USES AND METHODS.

Microencapsulation has emerged as one of the most commonly used methods for producing sustained release products (**72,83**). Besides sustained release, reasons for microencapsulation are quite diverse (attention will focus on pharmaceutical use only).

1.4.1. Microencapsulation considerations.

Prior to microencapsulation some factors need to be considered. These involve: route of

administration, polymer selection, solvent selection and core preparation. All these are interdependent with the method of microencapsulation (**83**).

1.4.2.1. Route of administration.

Not all methods of microencapsulation are applicable to the production of dosage forms for all the available routes of administration. Size of particles produced is one such limiting factor for some phase separation techniques and spray coating methods. This limits these methods to the production of oral dosage forms. Polymerization and multiple emulsion solvent evaporation methods may be suitable for parenteral, and inhalation products judging by the reduced size particles, nanoparticles produced.

Other requirements like sterility of parenteral and eye preparations also limit the generalized use of various techniques. Some polymers may not be able to withstand certain methods of sterilization (thermal, chemical or radiation). Aseptic technique may be required in the early stages of preparation using sterile raw materials. The type of dosage form (solid or liquid) may reduce the methods to select from. Reduced stability of some microencapsulated preparations in suspension form may only limit their use to solid preparations. Furthermore, some microcapsules would not withstand compression pressures during tableting.

1.4.1.2. Polymer selection.

A wide variety of polymers is available, including synthetic biodegradable polymers and modified natural products like polysaccharides, gums, proteins, fats and waxes (**83**). Not all are suited for every microencapsulation process. Conditions existing at different routes of administration also limit the use of some polymers. In general polymers should be non-toxic, non-allergenic and non-irritating. Polymer selection may be determined by

solubility, pH sensitivity and ionization, water permeability, hydrophilicity and erodability in the environment of drug release. These properties are usually important for determining the drug release mechanism. For oral dosage forms enteric properties, solubility only at intestinal pH, might be essential. Depending on the method of preparation, *e.g.* pan or air suspension coating, film forming capacity might be a necessity. Glass transition temperature (T_g) is important for the integrity of the film formed.

1.4.1.3. Solvent selection.

Almost all microencapsulation processes (except melt-congealing technique) require solvents in the process (**72**). The selection of solvents is determined by process safety (fire and explosion), toxicity (carcinogenicity), economic and environmental aspects (energy costs), as well as polymer and drug solubilities. The use of chlorinated hydrocarbons and aromatics has met resistance due to carcinogenicity potential. Other organic solvents may be explosive under certain conditions and are generally highly inflammable.

While aqueous solvents are highly desirable being environmentally safe and non-toxic, they have limited applicability due to solubility limitations and evaporation costs. Stability problems of some drugs and polymers in an aqueous environment is also a major obstacle towards the use of aqueous solvents (*e.g.* acetyl salicylic acid).

Solvent selection requires an understanding of the possible interactions between the solvent and polymer (**83**). Film formation is a result of polymer chain entanglement. The entanglement may be tight or loose depending on the chemical composition of the polymer, attraction to solvent molecules and temperature. A good solvent loosens the chain and allows the polymer to expand, but a poor solvent allows for chain coiling and

incomplete exposure of the polymer to the solvent. Encapsulation processes like phase separation, spray coating and solvent evaporation may produce particles of varying polymer permeability and film structure due to solvent effects.

1.4.1.4. Core preparation.

Depending on the method of microencapsulation used and the drug under encapsulation, drugs may be encapsulated in their original forms of existence or may require modification of some sort (**72,83**). Core preparation is an attempt to improve size distribution, shape, surface morphology and uniformity and to limit solubility, where not desirable, in polymer solvent and other characteristics like swelling. To that end therefore, cores may be products of other microencapsulation processes and other physical interventions. Some commonly employed core preparation techniques include: non-pareil seeds, spherical crystallization, extrusion-spheronization, polymerization (**70**), droplet extrusion and disperse-phase congealing (**84-85**) and solvent evaporation/removal (**86**). Most of these techniques are discussed under appropriate microencapsulation headings below. Only the ones not primarily classified under microencapsulation are discussed here.

Non-pareil seeds or pellets have been utilized in core preparation. The pellets, usually sucrose, are moistened and rendered adhesive with a binder solution like 10% PVP in ethanol or hydroxypropyl methylcellulose (HPMC) before successive layers of drug are applied to desired drug load. In one example non-pareil seeds were coated with bermoprofen mixed with lactose and starch and successively sprayed with a solution of HPMC. After drying, the granules were spray coated with a film solution of ethyl cellulose and HPMC (**87**).

Spherical crystallization is a process which is acclaimed to combine crystallization

and spheronization in a single step

(88-90). The technique involves suspending or dissolving the drug in a solvent mixture, *e.g.* acetone:*n*-hexane. A small amount of an immiscible liquid, the so-called a bridging liquid like ammonia water or distilled water is then added drop-wise. The drug crystals collect as spherical agglomerates. The procedure is then followed by an appropriate microencapsulation technique.

1.4.1.5. Additives.

Additives in the form of plasticizers, surfactants or emulsifiers, anti-foaming agents and anti-tack agents are not uncommon in microencapsulation processes (72,83). The requirements for additives are similar to those for polymers and solvents with respect to safety and toxicity. Plasticizers are required to improve polymer chain mobility by being self-interposed between the solvent and the polymer. This helps prevent cracks on the coating at the point of solvent removal.

The plasticizer must be compatible with the polymer. That is, polar plasticizers like ethers, esters and hydroxy compounds, *e.g.* polyethylene glycol, glycerol, triacetin, dibutyl phthalate should be used with polar polymers such as cellulose derivatives and polyacrylate esters. Non-polar plasticizers like hydrocarbons are suited for use with hydrocarbon chain polymers.

Plasticizers affect the glass transition temperature (T_g) of the polymer. Incompatibility can result in the plasticizer undergoing phase separation and affecting the integrity of the film. Polar plasticizers can leach out during drug release leading to increases in release rates.

Surfactants, anionic, cationic and non-ionic, are often required to improve wetting in techniques like polymerization and solvent evaporation. Emulsifiers are often sought

to stabilize emulsions and prevent aggregation and coalescence (**91**). However, drug encapsulation can be negatively affected by the use of either emulsifiers or surfactants (**92-93**). The use of a cationic surfactant in a simple coacervation process of gelatin, which positively charged, resulted in incomplete drug encapsulation. Also, emulsifiers may promote drug solubilization on the microsphere surface, which appear as crystals after drying.

Other additives may also complicate the microencapsulation process. Anti-tack agents such as fatty acids, *e.g.* stearic acid are used to reduce adhesion and friction during coating, in the coating pan. However, excessive amounts may render the core too slippery and difficult to coat (**72**).

Aggregation is a concern during microencapsulation. Adherents like talc and mineral silicates have been used in non-solvent addition and temperature modification phase separation techniques (**83**). These adhere to the polymeric wall and help prevent aggregation. However the large quantities required prohibit their use in parenteral preparations. Process modifiers like polyisobutylene, a non-walling polymer, have been used in low concentrations to prevent aggregation. The mechanism was thought to involve sterical stabilization and viscosity modification (**83,94**). Polyethylene and butyl rubber have also been used.

1.4.2. Uses of microencapsulation.

Stability of drugs in the GI environment is essential for a specific dose to be absorbed. Some drugs have been microencapsulated to protect the drug core material from the environmental conditions. A classical example has been the stability enhancement of vitamin A palmitate and some fat-soluble vitamins after microencapsulation. Presently, this concept is being pursued, with promising results, to stabilize peptide drugs, *e.g.*

human calcitonin and insulin in polyisobutylcyanoacrylate nanocapsules, against protease enzymes of the GI tract (**95-96**).

Microencapsulation has been used to improve the palatability of those drugs with unpleasant organoleptic properties (**97**). Among some of these drugs are acetaminophen, aspirin, cephalosporins, penicillins and vitamins. Various coating materials have been employed which include acrylics, cellulose acetate phthalate, ethyl cellulose, wax, gelatin-polymer complexes and other cellulose derivatives. Recently, Sjogvist et al (**98**), reported using a taste-masking microencapsulation technique to produce an oral palatable suspension of remoxipride, a D2-dopamine receptor antagonist. Similarly, Ueda et al (**89-90**) described a technique that combined spherical agglomeration and phase separation to mask the bitter taste of enoxacin using Eudragit RS.

Many orally administered drugs produce gastric irritation to some extent. In some instances the gastric irritation is of central mediation, but in others it is associated with local gastric mucosal damage (**10,16**). Microencapsulation may be used to reduce direct local contact of drug particles with gastric mucosa by coating the drug with water insoluble polymeric materials like ethyl cellulose, gelatin complexes, lactides and some acrylics (**26**). Enteric polymers like hydroxypropyl methyl cellulose phthalate and cellulose acetate phthalate are also effective in protecting gastric mucosa by preventing drug release in the stomach (**88**). The gastric irritation effects of potassium chloride were decreased following administration of a microencapsulated formulation when compared to a wax-matrix preparation (**97**).

The production of biocompatible cells has been widely studied utilizing microencapsulation techniques (**98**). Bioartificial organ systems are a result of microencapsulating live cells like islets of Langerhans to provide an alternate treatment for diabetes, and hepatocytes for the treatment of fulminant hepatic failure. The inert

nontoxic polymers, *e.g.* alginate-polylysine-alginate membrane, lack antigenicity and therefore minimize the potential problem of host immunological rejection of the cells compared to whole organ transplant. In addition, the polymer matrix also serves as a source of nutrients to support the cells. Microencapsulation of many viable mammalian cells and tissues has been successful (**99**).

Drug targeting studies have benefited greatly from microencapsulation applications (**100**). In this case, the drug-carrier-homing device combination ensures that the drug is released at the target cells or tissue and remains there over an extended period. Albumin, gelatin, starch, poly(alkyl cyanoacrylate) microspheres or microcapsules have been prepared for antitumor targeting of drugs like actinomycin-D, 5-fluorouracil and doxorubicin.

Microencapsulation has been used to improve the dry handling of liquid drugs or solids with poor flow properties (**101-102**). Co-precipitation of ibuprofen with some Eudragit polymers resulted in improved flow and tableting characteristics, and gelatin-acacia coacervation produced free flowing microparticles containing peanut-oil, olive-oil and fish-oil. Lin and Ayres have shown the possibility of using alginate beads as drug

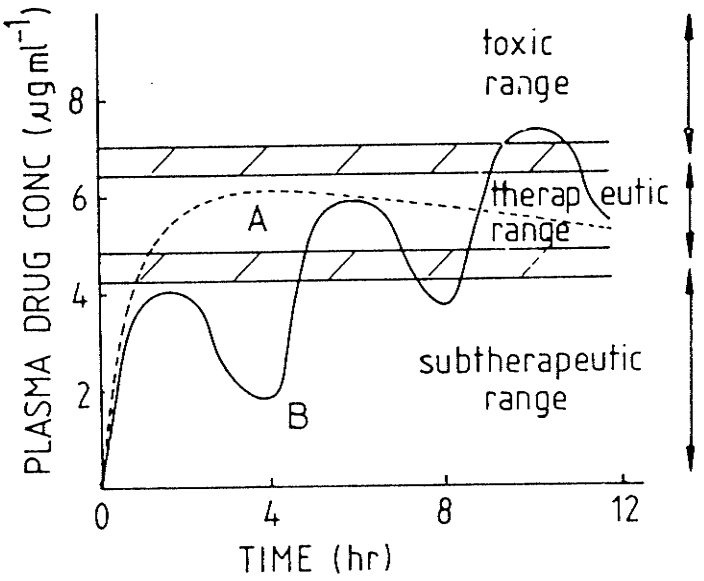


Figure 6. An idealized plasma drug concentration against time curve for an SR product (A) compared to an immediate release dosage form (B) (72).

carriers for further enteric coating (**85**).

The use of microencapsulation for imparting sustained release properties to drugs has long been recognized (**72**). The drug is encapsulated in a polymeric membrane to form a microcapsule or entrapped in a polymer matrix to form a microsphere, depending on the method of preparation. The aim is to produce a dosage form capable of releasing the drug slowly over time in the GI tract or other site of absorption. As a result the *in vivo* dissolution profile is enhanced (**50,71**); oral bioavailability is improved (**49,70,82**); uniform and prolonged plasma levels are obtained (**17**); and patient compliance and tolerance may be improved due to reduced dosing frequency (**17**).

Figure 6 shows an idealized plasma drug concentration-time curves obtained after administration of an SR microencapsulated preparation (A) compared to a conventional immediate release dosage form (B). SR system gives prolonged and uniform plasma drug levels within a respective therapeutic range.

1.4.3. Methods and processes of microencapsulation.

A variety of microencapsualtion processes have been undertaken (**72,82-99,101-103**). Some common processes include: coacervation/phase separation, interfacial polymerization, solvent removal, droplet extrusion, disperse-phase congealing, spray drying and congealing, pan coating, air suspension coating, extrusion/spheronization and others like multiorifice centrifugal process, electrostatic microencapsulation and liposomal encapsulation. Overlaps between some techniques are inevitable since similar or the same polymer systems are used in most situations.

1.4.3.1 Coacervation/phase separation process.

Coacervation/phase separation process of microencapsulation, perhaps the most widely used and the oldest technique, is the deposition of the polymer out of solution onto the core material induced by changes in temperature, or addition of a non-solvent or desolvating agent or a complexing agent. The system may involve a single polymer, simple coacervation or several polymers, complex coacervation. Either aqueous or non-aqueous methods may be employed depending on the physical properties of the core material and the polymer(s) selected. Therefore, careful selection of the core material, the solvent and the polymer are essential for successful microencapsulation.

Drug encapsulation through this process is a result of a three stage transition **(104)**:

(1) Phase separation of the polymer solution to form the primary wall made up of the coarcevate, polymer rich phase, the liquid phase and polymer-lean liquid phase, and the core material, solid or liquid phase.

(2) Formation of a polymeric membrane by the deposition of the polymer-rich microdroplets on the core material surface.

(3) Hardening and shrinkage of the polymeric membrane by an appropriate method: temperature change; solvent removal; or chemical reaction. Figure 7 shows schematic representation of the three stages described.

The three stages outlined above do not necessarily occur consecutively. Therefore, there is always a great chance of overlap of stages which leads to aggregation. Consequently, microcapsules of variable morphology may be formed if conditions are not carefully controlled.

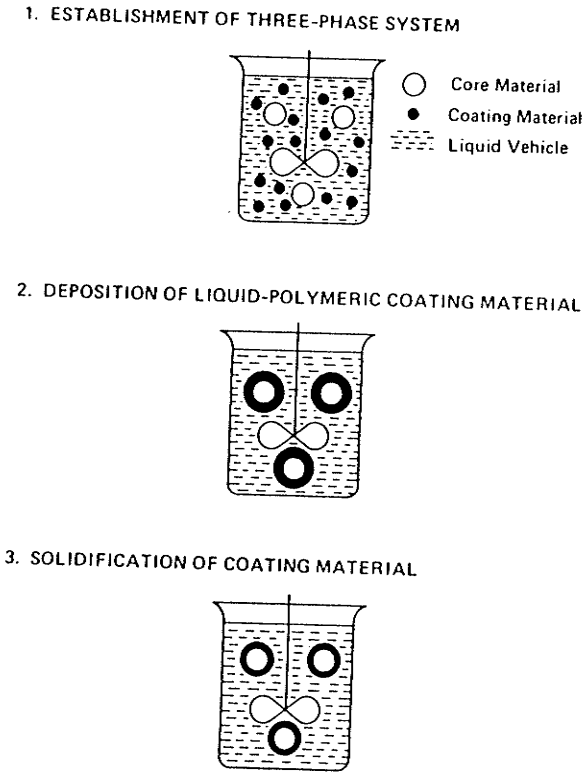


Figure 7. Schematic illustration of the phase separation/coacervation microencapsulation process (**105**).

Figure 8 shows some of the possible mono- and multi-cored microcapsule morphologies. Collision between uncoated core particles or between droplets of polymer-rich liquid could result in coalescence of the droplets. Therefore, the microcapsules could be monocore or multiple core units (A) as a result of light impact on unhardened liquid walls. As the stability of the wall increases, some particles may adhere to each other at the points of impact to form aggregates (B). Further deposits of the polymer onto the stabilized wall could occur, resulting in laminations and variations in porosity and density (C). A new wall could also be deposited around the aggregated particles (D). The occurrences in (C) and (D) contribute towards the increase in wall thickness.

Polymer droplets could adhere to the microcapsule surface as accretions which gelled before fusing into a continuous membrane (E). Empty or cored polymer droplets could act as binders to form other aggregated particles (F). Different physical states of the polymer exist during the three stage transition. Therefore, rapid rigidization of adhered polymer droplets could result in failure to form a continuous wall (G) or matrix formation by empty polymer droplets entrapping free core material (H).

Table 2 shows a summary of some of the most common coacervation/phase separation processes, the polymers used and the types of drugs that could be encapsulated.

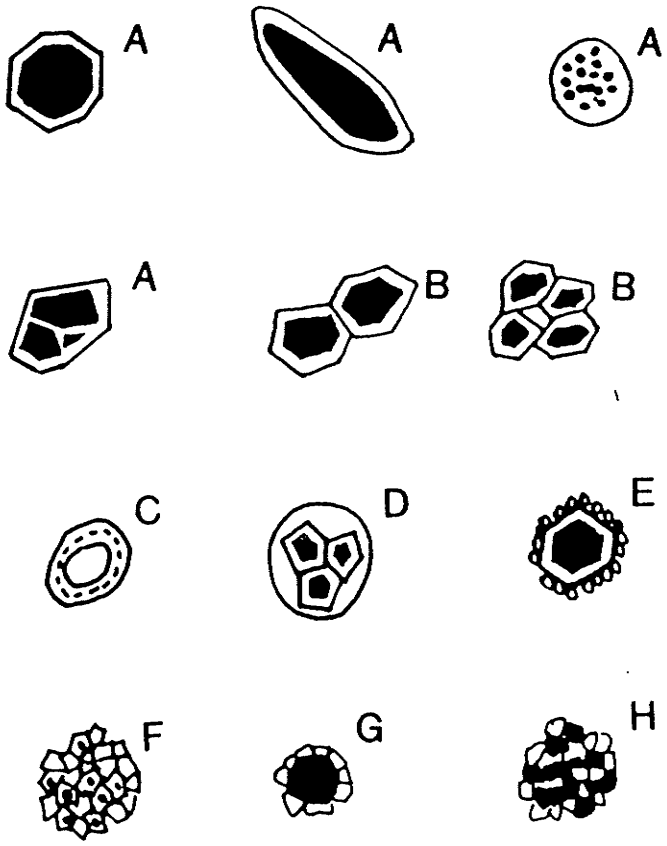


Figure 8. A schematic representation of possible microencapsulation product morphologies A to H. Explanation can be found in the text (83).

Table 2. Coacervation/phase separation methods and typical polymers employed.

Coating material (and phase inducer)	Method of preparation	Drug types	Ref.
1. Gelatin, simple coacervation (desolvating agent)	Oil or solid was dispersed in gelatin solution at 50 °C. Add Ethanol or sodium sulphate was added to coacervate. Temperature was reduced to gel the polymer (agitate). Particles were washed with water, hardened with formaldehyde or glutaraldehyde and dried.	Water insoluble but stable drugs, oils or water soluble drugs coated in wax.	72, 102.
2. Gelatin/ acacia, complex coacervation (pH, ionic complexation)	Oil phase was added to 10% gelatin (-ve) solution to form o/w emulsion. 10% acacia (-ve) solution was added at elevated temperature (agitate). pH was lowered to 4 - 4.5 (gelatin becomes +ve). Ionic reaction causes coacervation. Cooling gels the polymers.	Same as above and oil soluble drugs.	72, 102, 106.
3. Gelatin/ pectin complex coacervation (pH, ionic complexation)	Gelatin and pectin were dispersed individually in water at 45 °C and pH of each was adjusted to 10. The colloids were mixed and agitated while decreasing pH to 5 or highest pH in which the drug is stable. Drug was dispersed. pH lowered to coacervation (3.5 - 3.8). The mixture was cooled, hardened with formaldehyde, filtered, washed (with water and isopropanol) and allowed to dry.	Same as (2) above.	107, 108.
4. Albumin/ acacia complex coacervation (pH, ionic complexation)	Deionized bovine serum albumin and acacia were mixed at 40 °C and agitated. pH was adjusted to 3.9. The microgel particles were washed with water at 6 °C and dried under nitrogen or freeze-dried.	Relatively unstable drugs, biological agents and cells.	109.

Coating material (and phase inducer)	Method of preparation	Drug types	Ref.
5. Ethyl cellulose (EC) (temperature and incompatible polymer)	EC and polyethylene were added to cyclohexane. The drug was dispersed in the mixture and agitated while raising the temperature to 80 °C to dissolve the polymers. The mixture was slowly cooled (to reduce free interfacial energy) to obtain microcapsules. Particles were separated and washed with pure solvent.	Water soluble and sensitive drugs.	105.
6. Acrylate and methacrylate polymers and copolymers, (temperature)	Eudragit and drug were dissolved in a solvent (acetone) then diluted with water. The mixture was cooled to 10 °C, agitating gently. Particles filtered, dried and milled to size.	Water stable but insoluble drugs.	101.
7. Cellulose acetate phthalate or cellulose acetate trimellitate (desolvating agent and pH)	5% polymer aqueous bicarbonate solution was added to deionized water. Drug was dispersed with, agitation. 20% aqueous solution of sodium sulphate was added slowly for coacervation. pH was lowered to 4.0 with addition of citric acid to solidify microcapsules, then separated, wash and dried.	Water stable drugs.	97.
8. Poly(DL-lactic acid), (desolvating agent and non-solvent)	A water soluble polymer (PVA) was added to a saturated electrolyte solution ($MgCl_2$). Drug and PLA were dissolved in acetone. Salt solution was added to drug-polymer solution to obtain a liquid-liquid two phase system under agitation. Pure non-solvent (water) was added to remove acetone and form particles which were filtered (microfiltration) and freeze-dried.	Lipophilic drugs.	110.

1.4.3.2. Interfacial polymerization process.

Interfacial polymerization or polycondensation is the formation of a polymer, to encapsulate the disperse phase, by the reaction of usually two monomers at the interface between two immiscible liquid phases. Many polymers have been investigated for pharmaceutical use. Most are candidates for parenteral applications because they are biodegradable and produce particles in the nanometre size range (**83**). These include: polyamides, polysilanes, silicone rubbers, copolymers of maleic anhydride and polyvinyl derivatives, polylactic and polyacrylic acids and polyacrylamides. Donbrow (**83**) has highlighted at least four possible modes and sites of polymerization within the disperse system. Polymerization can take place:

(1) Within the liquid droplets in which the drug is dispersed (disperse phase), and it is induced by thermal or radiation or chemical reactions, *e.g.* starch, polyacryldextran or polyacrylamide. The external liquid phase in this case serves only as a vehicle.

(2) Within the external medium in which the drug is dispersed as a liquid or solid. The polymer diffuses to the interface, solidifies and gets deposited onto the drug surface.

(3) At the interface *in situ*, as a result of either spontaneous reaction, *e.g.* $\propto$-cyanoacrylate monomers or as a result of contact between the monomer from one phase and the inducing agent or catalyst from another. The polymer is deposited onto the drug in the internal disperse phase.

(4) At the interface *in situ*, due to chemical reaction between monomers diffusing from either phase and forming the polymer at the interface. The polymer is then deposited onto the drug in the internal phase.

The structure of the particles formed under polymerization tends to vary according to the mode and site. Matrix formations (micro- or nanospheres) are more likely to result from (1) and (2) above, while (3) and (4) tend to produce micro- or nanocapsules (**83**).

Table 3 summarizes examples of some of the polymerization techniques categorized above by mode and site occurrence. A wide variety of substances can be encapsulated, including solid and liquid substances, artificial cells containing enzymes, detoxicants and haemoglobin and peptide drugs. However, some problems may be encountered due to high partitioning into the organic phase for some highly lipophilic compounds (**111**). Some drugs are unstable in slightly alkaline conditions that exist inside the microcapsule. Quaternary ammonium compounds were found to interfere with the nylon formation reaction, resulting in poor to no encapsulation (**111**). Ammoury et al (**78**) reduced the partitioning of indomethacin by the addition of albumin to the external phase of poly[D,L-lactide] and phopholipids dissolved in acetone.

Polymerization usually occurs at the internal-external phase interface. Therefore addition of emulsifiers and surfactants, while necessary for stabilizing an emulsion, may interfere with polymerization (**92**). Removal of unreacted monomer, initiator or other stabilizers may be problem for the polymerization technique, particularly, when very small particles are produced (**72**).

Table 3. Examples of interfacial polymerization processes: polymers, mode and site of formation.

Polymer (monomers example). Mode and site of polymerization.	Preparation procedure.	Ref.
1. Polyacrylamide (*e.g.* *N,N'*-methylenebisacrylamide). Conforms to (1) above for mode and site.	Acrylamide, drug (calcitonin), bis(2-ethylhexyl) sodium sulphosuccinate were emulsified and polyoxyethylene 4 lauryl ether in *n*-hexane. Irradiation was used for polymerization. Ethanol was added to precipitate polyacrylamide. Particles were washed and lyophilized.	**96.**
2.(a). Polycyanoacrylate (*e.g.* isobutylcyanoacrylate monomer). (b). Polylactic acid (*e.g.* poly(D,L-lactide). Conform to (3) above for mode and site.	Either the cyanoacrylate monomer or preformed polylactic acid was dissolved together with drug in a mixture of benzyl benzoate, phospholipids and acetone. the mixture injected slowly into an aqueous phase containing poloxamer. The polymer was formed by anionic polymerization at contact with hydroxyl ions. Acetone and water were removed under reduced pressure.	**78, 96.**
3. Poly(N^{α},N^{ε}-L-lysinediylterephthaloyl) (*e.g.* L-lysine and terephthaloyl dichloride). Conforms to (4) above for mode and site.	Drug (hemolysate), L-lysine and sodium carbonate were dissolved in water, then slowly added to terephthaloyl chloride solution in cyclohexane, chloroform, and tetraethylammonium chloride. Interfacial tension was decreased by applying electrical potential between the syringe needle (anode) and a platinum wire (cathode) located in the organic phase. Particles were formed.	**112.**

Polymer (monomers example). Mode and site of polymerization.	**Preparation procedure.**	**Ref.**
4. Polyamide or nylon (*e.g.* hexamethylenediamine and sebacyl chloride). Conforms to (4) above for mode and site.	In 30 ml aqueous 0.4 *M* hexamethylenediamine the drug was dispersed. The mixture was emulsified in 300 ml organic phase (cyclohexane and chloroform). Slowly sebacyl chloride added to polymerize nylon at the interface. The mixture was cooled in ice bath. Particles were separated and allowed to dry.	111.

1.4.3.3. Solvent evaporation process.

Solvent evaporation, as the name implies, is a process by which microencapsulation is effected by evaporating or removing the low boiling point solvent in which the polymer is dissolved and the drug is either dissolved or dispersed (**72,91,113**). Initially, the drug is dissolved or dispersed in an organic solution of the polymer. The mixture is then emulsified in a continuous aqueous phase containing a surfactant or hydrophillic polymer as an oil-water (o/w) emulsion stabilizer. Solvent evaporation/removal is effected by low pressure or heat or addition of a liquid miscible with both the solvent and the continuous phase. Particles produced may be small enough for parenteral use.

Variations of this technique include continuous, interrupted and complex emulsion (**113**). Both the continuous and interrupted systems are limited to the bi-phasic emulsion systems of various combination, water-oil, oil-water or oil-oil. The continuous system differs from the interrupted system in that solvent removal in the former continues until the solid particles are harvested. In the latter however, as particles begin to form the continuous phase is replaced with pure water, or pure continuous phase before all the solvent is removed. Aqueous phase replacement helps reduce the formation of free crystals of the active ingredient on the surface of the particles or in the aqueous phase. Crystal formation has a negative effect on the encapsulation efficiency (**91-92**).

The complex or multiple emulsion system is based on a tri-phasic emulsion, (water-oil)-water or (oil-water)-oil. Water-oil-water emulsion has been suggested for the immobilization and encapsulation of hydrophillic drugs. The stability of the primary emulsion is important for achieving high drug encapsulation (**91**). The type (hydrophile-lipophile balance) and concentration of the surfactant are important for the morphology, microcapsule, multivesicular or microsphere of particles produced by stabilizing the primary emulsion system. It was observed that as the concentration of the surfactant was

increased the morphology changed from a reservoir type to a matrix type (**114**).

Watts et al (**92**), have reviewed various polymers used in this technique. These include poly(D,L-lactic-co-glycolic acid), poly(ß-hydroxybutyrate), polycarbonates, acrylic polymers, ethyl cellulose and other cellulose derivatives and polyanhydrides. Table 4 shows some examples of solvent evaporation technique applications corresponding to the continuous, interrupted or complex emulsion systems. Most of the particles obtained by this method are microspheres. To produce reservoir type, Göpferich et al (**86**), performed a series of multiple or complex emulsion solvent evaporation techniques to microencapsulate microspheres. The procedure suppressed the initial burst effect from the microparticles when applied to suspended rather than dried microspheres.

Solvent evaporation and coprecipitation techniques have not been considered independently although the latter usually does not involve the emulsion formation step. Also, almost always the drug is dissolved in the polymer solvent. Therefore, coprecipitation usually produces larger particles which require further milling and screening to desired size (**101**).

Table 4. Examples of solvent evaporation process, polymer type, method variation and type of particles produced.

Polymer type and method variation	Preparation procedure and type particle formed	Ref.
1.Ethyl cellulose (EC). Continuous solvent removal.	EC was dissolved in methylene chloride. The drug (ibuprofen) was added. The polymer phase was added to aqueous methylcellulose solution. Agitation was continued until all the methylene chloride had evaporated. Microspheres were obtained.	115.
2. Poly(L-lactic acid). Interrupted solvent removal.	The polymer, drug (griseofulvin) and phospholipids were dissolved in chloroform. Aqueous polyvinyl alcohol solution was added. Agitation was continued to allow for solvent removal. The continuous phase was then replaced with sodium chloride solution. Microspheres were obtained.	116.
3.Poly(D,L-lactide). Complex emulsion and continuous solvent removal.	Aqueous bovine serum albumin solution was emulsified into the polymer solution in methylene chloride with or without a surfactant. The primary emulsion was then poured into an aqueous solution of polyvinyl alcohol with agitation and at 0 °C. Temperature was increased to 20 °C to remove the solvent. Particles of various morphologies were formed.	115.
4.Ethyl cellulose (EC) and methyl cellulose. Complex emulsion and interrupted solvent removal (MC).	MC was dissolved in warm water and the drug (indomethacin) was dispersed in the solution. The dispersion was added to a solution of EC in ethyl acetate. The primary emulsion was re-emulsified in an aqueous medium with or without a surfactant. Particles were formed upon solvent evaporation under agitation at 5 °C. The continuous phase was replaced with fresh aqueous medium and evaporation continued. Microspheres were obtained.	117.

Polymer type and method variation	Preparation procedure and type particle formed	Ref.
5. Gelatin. Continuous solvent removal (dehydration).	Drug (pilocarpine) was added to gelatin solution at 55 °C. The mixture was added to sunflower oil containing Span 80 at 55 °C and stirred. The emulsion was cooled to 5 °C. Solvent removal was effected through addition of isopropanol. The microspheres formed were hardened under formaldehyde vapour.	77.

1.4.3.4. Droplet extrusion, spray drying and congealing processes.

Probably the simplest of all the microencapsulation techniques, droplet extrusion is a process by which the core material in the form of liquid, melt or solution is passed through a nozzle to produce microdroplets (**72,83**). The microdroplets are dropped into an agitated suitable medium to effect rigidization. Hardening is effected by chemical reaction, temperature change or solvent removal. The coating material may be taken up from the medium or incorporated into the core droplet. The size of the particles depends on the nozzle diameter and the jet velocity.

A variety of polymers have been used, alginates, pectinates, acrylates and cellulose derivatives to produce microspheres containing drugs ranging from water soluble drugs to insoluble drugs and biological cells. Lim and Moss (**99**) microencapsulated living cells, hepatoma and pancreatic islets, using sodium alginate saline solution. The cells were suspended in the alginate solution and then extruded through a capillary tube and dropped into a beaker of calcium chloride. This method was preferred over other microencapsulation techniques because neither organic solvents nor heat were utilized.

Bodmeier et al (**84**) reported the use of this technique to entrap drug-containing microparticles. The ethyl cellulose microspheres containing propranolol were prepared by the solvent evaporation process, then dispersed in either aqueous chitosan or sodium alginate solutions. The dispersion was then dropped into either tripolyphosphate or calcium chloride to form the gel beads. The chitosan system released the microparticles under gastric conditions, while the alginate released the microparticles under intestinal conditions. The final drug release depended on the ethyl cellulose drug release characteristics. In another investigation Bodmeier and Wang (**118**) microencapsulated ibuprofen, theophylline, guafenesin or pseudoephedrine HCl dispersed or dissolved in aqueous solution of sodium alginate and added to dispersions of ethyl cellulose or acrylic

co-polymers. The sodium alginate-polymer-drug mixture was dropped into a solution of calcium chloride to produce gelled particles with SR properties. Polk *et al* (119), extruded a sodium alginate solution into a mixture of calcium chloride and chitosan to encapsulate bovine serum albumin. Polyelectrolyte complex beads for albumin delayed-release were produced.

The effects of drug loading, calcium concentration and gelling time on the characteristics of drug encapsulation and release by calcium alginate gel beads were studied extensively by Ostberg et al (120-121). Drug release decreased with increase in calcium concentration, alginate concentration and gelling time. However, high calcium concentration and longer gelling time could reduce final drug loading.

Drugs have also been encapsulated in hydrophobic material like molten waxes such as beeswax, paraffin wax, cetostearyl alcohol, glyceryl tristearate and carnauba wax dispersed, extruded or spray congealed into a cool air stream or cool aqueous environment (72). Sjöqvist et al (98) encapsulated remoxipride, a D_2-dopamine receptor antagonist, by melting the drug and wax at about 85 °C. The molten mixture was sprayed through cool air. The droplets solidified into microspheres as a taste masking procedure. Al-Kassas et al (122), prepared sustained release ibuprofen microspheres by dispersing the drug in molten cetostearyl alcohol and then adding the mixture to a heated, acidic aqueous solution. While continuing the agitation, the temperature was lowered, resulting in the formation of microspheres. It was observed that an increase in cooling time or a decrease in stirring rate tend to result in prolonged of drug release.

Spray drying is similar to spray congealing except that in the former the drug is dispersed in a coating material solution not in a melt. The dispersion is then sprayed into a heated air stream which supplies the latent heat of vaporization for the removal of the solvent. Hydrophilic polymers like, acacia, polyvinylpyrrolidine, gelatin, polyvinyl

alcohol, sodium carboxymethylcellulose and other cellulose derivatives have been used as coating materials with this technique (**72**).

Another closely related technique was first suggested by Kawashima et al (**65**) as an alternative to spray-congealing. Drugs such as sulphanilamide or sulphamethoxazole were surface treated with palmitic acid to render them highly hydrophobic. The surface treated drug was then dispersed in water with agitation. The temperature of the system was increased to enable the wax, white beeswax to melt. As the system was cooled to room temperature, the molten wax collected into spherical agglomerates containing the drug. The agglomerates displayed prolonged release characteristics.

1.4.3.5. Pan coating process.

Pan coating is quite a common undertaking in the pharmaceutical industry (**72,103**). This is a technique which has evolved over time for multiple purposes such as elegance by film and colour coating, taste-masking by sugar coating, enteric coating of tablets and pellets and microencapsulation of cores of at least 500 μm diameter. The ease of large scale production is an advantage of this technique. Also the production of multiple layers offers an opportunity to regulate the drug release profiles. However, the technique requires artistic skill and experience of the operator.

Coating is applied onto non-pareil seeds or core material described under ***Core Preparation*** in a coating pan fitted with pneumatic atomizer to supply the polymer solution or dispersion, a hot air supplier to provide latent heat of vaporization for the polymer solvent, and an exhaust system to remove vaporized solvent.

A variety of coating pans have been developed over time. Although achieving the same results, they differ in shape, mounting, ventilation and coating material supply such as immersion tube. Some common pans include the conventional pan, side-vented Accela-

Cota, Strunck immersion tube and Glatt immersion sword. The Accela-Cota type of equipment are sealed and therefore minimize solvent escape into the operating area. Appendix 1.1 shows a schematic diagram of a Strunck immersion tube coating pan.

1.4.3.6. Air suspension coating process.

Air suspension or fluid-bed coating is another process readily available for large scale production (**72,103**). It offers some advantages over the pan method. Coatings can be applied on cores of irregular shape, size and surface morphology. A wider variety of polymers can be used without creating tack problems. Since less coating is lost to the pan walls, less polymer can be used and coating can be applied rapidly. The coating process takes place in a closed system which minimizes environmental contamination. Appendix 1.2 (A and B) shows schematic diagrams of Wurster-type coating apparatus (A) and a close-in of the coating chamber (B). The process depends on controllable parameters such as inlet and exhaust air flow and temperature, spray rate and pressure. Therefore, it does not require the same artistic skill as the pan coating technique. However, explosion and fire are more likely to occur with this method.

As illustrated in Appendix 1.2 (B), the core material is placed in the coating partition and fluidized by the air directed upwards through the air distribution plate. The coating material is applied through the nozzle in the direction of the air to wet the fluidized particles. The temperature of the air is controlled in order to effect immediate drying of the particles as they travel upwards and fall into the annular space and back to be subjected to another coating cycle in a continuous pattern.

Not all fluid-bed apparatus are equipped with spray nozzles located at the bottom, spraying upwards, *e.g.* Wurster. Some have side-wall nozzles pointing downwards at an angle, *e.g.* Freund Flow-coater. In others the spray nozzle is placed at the top, *e.g.*

Aeromatic. Others have upward angular spray systems, *e.g.* Hüttlin Kugelcoater.

Various drugs such as potassium chloride, isosorbide dinitrate and acetyl salicylic acid have been coated using this technique for different reasons, enteric coating, SR and taste-masking (**123**).

1.4.3.7. Extrusion and spheronization process.

Extrusion/spheronization is a process in which wet granulation, drug and excipients is extruded into cylindrical segments. The cylindrical material is then broken down and rolled into solid spheres. Appendix 1.3 (A and B) shows a schematic representation of the extrusion/spheronization process (A) and the types of extruders, both screw and gravity (B) (**103,124**).

The process can be used to prepare SR matrix spheres or pellets for further coating. Tapia et al (**125**) demonstrated SR of diclofenac sodium from matrix spheres formed by extruding the drug with chitosan. Yuen et al (**42**), used the technique to prepare core material containing theophylline for further coating, fluid-bed coating with a mixture of methyl cellulose and ethyl cellulose to impart SR properties.

1.4.3.8 Other techniques used in microencapsulation.

Various other methods of microencapsulation have been investigated with varying degrees of application in the pharmaceutical industry.

(1) Multi-orifice centrifugal process: A schematic diagram of a multi-orifice centrifugal head is shown in Appendix 1.4. This process has been used in a large scale pilot production of wax microencapsulated preparations (**72**). The coating solution or melt is filled into the grooves above and below the orifices around the cylindrical head and

overflows the weirs to form a membrane across the orifices. The core material solution is then fed onto the inner rotating disk located concentrically inside the head, at the level of the orifices. As it rotates the droplets of the core material force their way through the polymers-lined orifices to exit as encapsulated particles.

(2) Electrostatic encapsulation process: In this process the oppositely charged coating polymer and core material, as a solution or suspension, are atomized into a closed chamber. Upon contact, the opposite charges attract and result in core encapsulation. The encapsulated drug is then cooled and collected into aerosol containers. The coating material should be of a lower surface tension than the core material for encapsulation to occur. Electrostatic process apparatus is diagrammatically represented in Appendix 1.5 (72).

(3) Liposomal encapsulation: Liposomes are vesicles consisting of parallel phospholipid bilayers enclosing an aqueous phase (**72,100,126**). Figure 9 shows a schematic representation of multilamellar, small unilamellar and large unilamellar liposomes. Water soluble drugs can be encapsulated inside the aqueous phase, whereas the lipophilic drugs can incorporated into the phospholipid bilayer (see Figure 9 for illustration). In general, multilamellar vesicles have a low encapsulation efficiency of polar drugs. Liposomes are prepared from phospholipids derived from both natural and sysnthetic sources of which

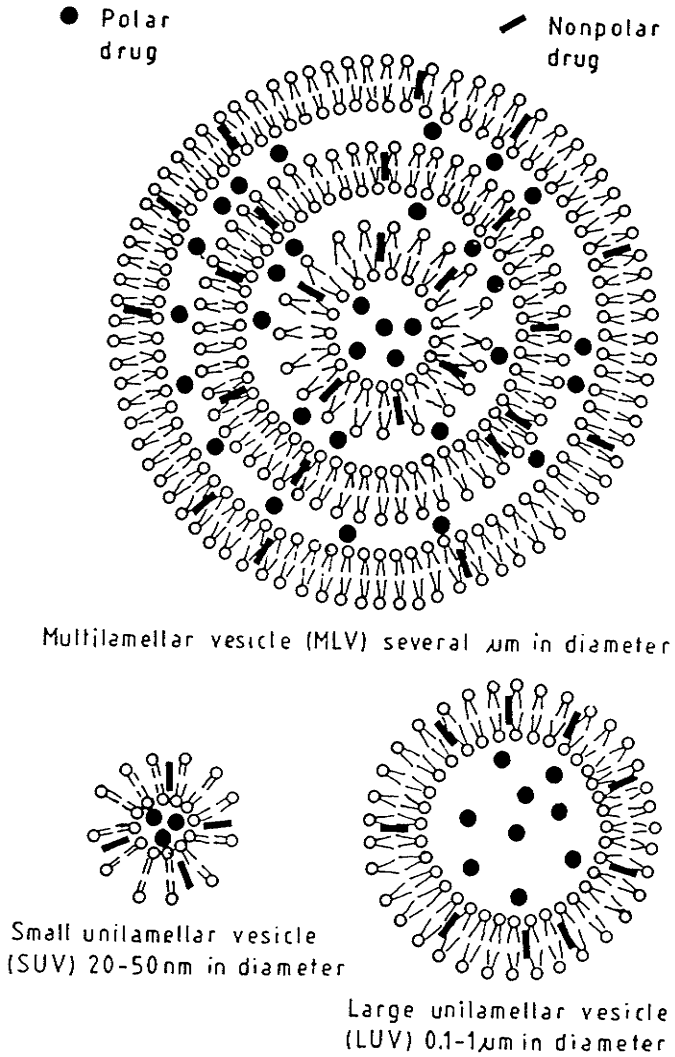


Figure 9. Schematic drawings of the common types liposomes and the positioning of encapsulated drugs (72).

phosphatidylcholine is the most common. Cholesterol has been incorporated to increase the liposome stability in plasma.

Various preparation methods have been used to produce the different types, as shown in Figure 9. Multilamellar (MLV) liposomes form spontaneously when a dry phospholipid film is hydrated. Extrusion through polycarbonate filters produces liposomes of a relatively uniform size. Ultrasonication also reduces the size but renders the bilayers unilamellar. Reverse phase evaporation preparation technique usually produces large unilamellar liposomes with higher encapsulation efficiency of polar drugs. A new preparation technique which does not utilize organic solvents has been suggested (127).

The preferential uptake of liposomes by the mononuclear phagocytic system (MPS) into the liver, spleen and bone marrow has made liposomes good candidates for drug targeting. Steric stabilization of the lipid bilayers with sialic acid derivatives and polyethylene glycol has produced liposomes with the ability to evade the MPS and hence remain in circulation for prolonged periods (128). High concentrations in infected and inflammed tissues and tumour cells has led to numerous investigations of liposomes as carriers for antitumor drugs (doxorubicin), antimalarials (primaquine), immunomodulators (lymphokines), antibacterials (streptomycin) and antifungals (amphotericin B).

1.5. OBJECTIVES.

The main purpose of this study was to produce an SR IMC formulation with no significant gastric release using phase separation/coacervation microencapsulation technique. Secondly, to demonstrate that oppositely charged hydrocolloids such as pectin or sodium alginate and gelatin could be used for the production of SR microparticles with better handling properties compared to IMC alone. To that end an attempt was made to:

- improve flow properties of IMC;

- reduce IMC release in gastric conditions;
- improve IMC dissolution hence the bioavailability; and
- extend the duration of IMC release.

The above modifications would result in:

- reduction in frequency of administration hence improve patient compliance;
- reduction in IMC induced GI irritation associated with local physical contact with IMC crystals; and
- improvement in patient tolerance.

The following *in vitro* studies have been carried out to meet the objectives mentioned above:

(1) particle morphology by microscopic analysis of different formulations to observe deposition of coating material;

(2) flow properties of particles compared to non-encapsulated IMC (angle of repose);

(3) in vitro release profiles at different pH levels simulating pH conditions in the gastrointestinal tract (stomach and small intestines);

(4) the effect of different selected polymers and additives on the release profile of the drug;

(5) the effect of polymer ratio on degree of encapsulation and drug release;

(6) the effect of drug load on degree of encapsulation and drug release;

(7) mechanism of drug release or release kinetics;

(8) dissolution stability of selected, or representative formulations at room temperature and a higher temperature (40° C);

(9) effect of encapsulation on the physical integrity of IMC by DSC analysis.

CHAPTER 2. EXPERIMENTAL.

2.1. MATERIALS AND EQUIPMENT.

2.1.2. Materials.

1. Gelatin Type A, "Pharmagel A". Ruger Chemical Co. Inc (Irvingham, New Jersey);
2. Citrus Pectin, N.F. Pharmaceutical Grade. S. B. Pennick and Co. (New York);
3. Sodium Alginate. BDH Chemicals Ltd (Poole, England);
4. Indomethacin was a gift from Biovail Research Corporation (Steinbach, Manitoba);
5. Aquateric^{R}. FMC Corporation (Newark, Delaware);
6. Carnauba wax, B.P. S. B. Pennick and Co. (New York);
7. Sodium lauryl sulphate, B.P. BDH Inc. (Toronto, Ontario);
8. Calcium chloride. BDH Chemicals Ltd (Poole, England);
9. Glycerin. Mallinckrodt Specialty Chemicals (Pointe-Claire, Quebec);
10. Potassium chloride. Mallinckrodt Specialty Chemicals (Paris, Kentucky);
11. Hydrochloric acid. Baxter Corporation (Toronto, Ontario);
12. Sodium phosphate (dibasic). Mallinckrodt Specialty Chemicals (Paris, Kentucky);
13. Sodium phosphate (monobasic). Mallinckrodt Speciality Chemicals (Paris, Kentucky);
14. Sodium hydroxide. Mallinckrodt Specialty Chemicals, (Pointe-Claire, Quebec);
15. Formaldehyde solution 37% (w/w). Fisher Scientific Company (Fair Lawn, New Jersey);
16. Methanol. Baxter Health Care Corporation (Muskegan, Michigan);
17. Isopropyl alcohol. Mallinckrodt Specialty Chemicals (Paris, Kentucky).
18. Phosphoric acid. Mallinckrodt Specialty Chemicals (Paris, Kentucky).

2.1.2. Equipment.

1. Corning hot plate (magnetic) stirrer. Corning Glass Works (Corning, New York);

2. Fisher Accumet^{R} pH meter, model 610A. Fisher Scientific Company (U.S.A.);

3. Olympus BH2-UMA high magnification optical microscope equipped with a Hitachi Model VK-150 video camera, a Mitsubishi video copy processor thermal paper printer and a Panasonic monitor. Medical and Scientific Company (Markham, Ontario);

4. Ultrasonic cleaner. Cole-Parmer Instrument Company (Chicago, Illinois);

5. Dissolution testing apparatus (USP apparatus 1). Vander Kamp 600. Van-kel Industries (New Jersey);

6. USP standard sieves sizes 16, 30 and 60;

7. Shimadzu UV-160 spectrophotometer. Shimadzu Corporation (Kyoto, Japan);

8. Differential scanning calorimeter 910 with a computer/analyzer system 9900. Dupont Instruments (Wellington, Delaware).

2.2. METHODOLOGY.

2.2.1. Preparation of the coacervate microparticles.

The method of preparation and recovery of the coacervation microparticles was a modification of the ionic complex coacervation method first described by McMullen *et al* (123-124). The microparticles were prepared from batches of varying proportions of either pectin or sodium alginate and gelatin type A containing varying amounts of indomethacin (Table 5). Batch sizes of 100 ml were prepared from initial solutions of 2% (w/v) concentration for each hydrocolloid.

First, the colloids (pectin, sodium alginate and gelatin) were individually dissolved in distilled water at about 60°C. Selected proportions of the individual colloids (Table 5)

were cooled to 45 °C and adjusted to a desired mixing pH of 10 with appropriate amounts of 1.0 *N* sodium hydroxide. The amount of sodium hydroxide required to adjust the pH was about 2.5 ml for pectin, about 1.5 ml for gelatin and 0.2 ml for sodium alginate. The temperature was maintained at about 45 °C. Either pectin or sodium alginate solution was mixed with gelatin solution. Continuous agitation was maintained using a magnetic stirrer.

The pH of the mixture was lowered quickly to about 5 with 0.5 *N* hydrochloric acid. At this point a desired amount of indomethacin was added. Sodium lauryl sulphate (0.02% (w/w)) was added to aid the dispersion of IMC. The pH was further lowered slowly until coacervation and formation of discrete particles was observed. Usually this occurred between pH 3.5 and pH 4.5. The amount of hydrochloric acid required to reach coacervation was found to be about 5.5 ml for alginate-gelatin and about 6.0 ml for pectin-gelatin. Agitation was maintained for a further 30 minutes while allowing the mixture to cool to room temperature.

The formed microparticles were hardened with 10 ml of 37% (w/w) formaldehyde. After 30 minutes a suitable stock solution of calcium chloride was added to give a final calcium chloride concentration of 1.5% (w/v). The total volume of the preparation came to 150 ml. This treatment rendered any unreacted pectin or alginate water insoluble. The end product was then left to stand for 15 hours.

The settled suspension was carefully decanted of the supernatant and the residue re-suspended in 10 ml glycerol and dispersed with 50 ml of an isopropyl alcohol and water mixture (1:2 (v/v)), under continuous agitation. The flocculated and partially dehydrated microparticles were filtered using a Buchner funnel and washed repeatedly with three 50 ml volumes of isopropyl alcohol in water.

After this process, the partially dehydrated microparticles were harvested into watch glasses to be oven dried at 37°C until there was no significant change in weight. The resulting dry granules were then sieved using standard sieves of sizes 16, 30 and 60 mesh. Two size categories (16/30 and 30/60) were later studied.

Formulation variations were prepared, which contained either Aquateric^{R} (hydrophilic enteric polymer) or carnauba wax (lipophilic erodible wax) as additives. Aquateric^{R} was added to the mixture at pH 10. The pH was then adjusted to pH 6 followed by addition of the drug and further pH decrease until coacervation. After coacervation the procedure was the same as outlined above.

In order to incorporate carnauba wax a procedure similar to the spherical agglomeration technique described by Kawashima *et al* (**65**) was adopted. The pH of the colloidal mixture was lowered from pH 10 to about pH 5. The wax was added and the temperature raised to about 95°C to melt the wax. The drug was then added with continued agitation as the system was allowed to cool to the working temperature about 45°C. The pH was lowered further until coacervation occurred. The system was then treated in the same manner as described earlier.

Table 5. Composition of the formulations prepared.

Formulation No.	Polymer[a] type	Polymer to polymer ratio	Drug to polymer ratio
1.	Pec-Gel	1:0	1:2
2.	Pec-Gel	1:1	1:2
3.	Pec-Gel	1:2	1:2
4.	Pec-Gel	1:2	1:1
5.	Pec-Gel	2:1	1:2
6.	Alg-Gel	1:0	1:2
7.	Alg-Gel	1:1	1:2
8.	Alg-Gel	1:2	1:2
9.	Alg-Gel	1:2	1:1
10.	Alg-Gel	2:1	1:2
11.	Pec-Gel-Aq	1:2:3	1:2
12.	Pec-Gel-Aq	2:4:3	1:3
13.	Pec-Gel-Aq	1:2:3	1:4
14.	Alg-Gel-Aq	1:2:3	1:2
15.	Alg-Gel-Aq	1:2:3	1:4
16.	Alg-Gel-Aq	2:4:3	1:3
17.	Pec-Gel-Cw	1:2:3	1:4
18.	Pec-Gel-Cw	1:2:3	3:8
19.	Pec-Gel-Cw	2:4:3	1:3
20.	Pec-Gel-Cw	2:4:3	1:2
21.	Alg-Gel-Cw	2:4:3	1:2
22.	Alg-Gel-Cw	1:2:3	3:8
23.	Alg-Gel-Cw	1:2:3	1:4
24.	Alg-Gel-Cw	1:2:3	1:3

[a] Pec. for pectin; Gel. for gelatin; Alg. for alginate; Aq. for Aquateric[R]; and Cw. for carnauba wax.

2.2.2. Yield determination.

Dried microparticles were weighed and the total yield determined from the total weight of individual ingredients as follows:

$$\%Yield = \frac{total\ weight\ of\ microparticles}{total\ weight\ of\ individual\ ingredients} \times 100$$

The microparticles were then categorised according to mesh size.

2.2.3. Angle of repose measurement.

The flow properties were determined from the angle of repose measurements according to the following tangential relationship:

$$Tan\,\theta = \frac{2 \times h}{D}$$

where θ is the maximum angle that could be obtained between the free standing microparticle heap surface and the horizontal plain, h is the heap height, and D is the diameter of the heap.

To obtain the above parameters, a convenient procedure was adopted. A funnel of neck diameter of 4 mm was fastened to a small stand at a fixed height of 2 cm above a cartesian graph paper (horizontal surface). About 2.0 g of microparticles was slowly poured down the funnel to form a free standing heap below. The height of the heap was accurately measured, and the circumference was traced on the gragh paper to determine the diameter. The procedure was repeated three times and average of each measurement was used to determine the angle of repose.

2.2.3. Microscopy.

The morphology of dried microparticles was studied using a high magnification optical microscope at 100X magnification. The micrographs were captured by a video camera mounted on the microscope and were printed through an attached copy processor thermal paper printer. Photographs of the bulk microparticles were also taken.

2.2.4. Microparticle drug content determination.

The weight, *M*, of the encapsulated drug in a sample of microparticles of weight, *W*, was estimated using a modification of the method described in USP 23 for determining content uniformity. Instead of using methanol and pH 7.5 phosphate buffer (Appendix 2.1.1.1) in a 1:1 ratio, a ratio of 2:1 was used to improve extraction efficiency. A known weight, *W*, of microparticles was triturated before being transferred to a 200 ml volumetric flask containing 100 ml of a mixture of methanol and pH 7.5 phosphate buffer (2:1 by volume). The flask was then placed in an Ultrasonic cleaner to sonicate the mixture until fully dispersed in the medium for 1 hour.

After sonication the volume was made up to the mark with methanol/buffer mixture. The dispersion was centrifuged and the supernatant collected for spectrophotometric analysis. The U.V. absorbance of the supernatant (appropriately diluted to correlate with the calibration curve) was determined at 318 nm. The concentration was determined using the following calibration regression curve (Appendix 2.1.2) derived from pure indomethacin:

$$y = 0.17415\,x + 0.0005$$

where x and y correspond to the drug concentration and absorbance, respectively. This procedure was performed in multiples of three to ensure uniformity of content and

reporducibility.

2.2.5. Microparticle degree of drug encapsulation.

The experimental drug load (D_e) was given by *M/W*. The theoretical drug load (D_t) could be determined from the drug-polymer or D:P ratio as shown in Table I. For example, a ratio of 1:2 suggests 0.33 of D_t. The percent encapsulation efficiency (*%E*) could then be determined by

$$\%E = \left(\frac{D_e}{D_t}\right)100 \qquad (31)$$

2.2.6. Dissolution testing procedure.

Drug release patterns were studied using USP Apparatus 1, (6 vessel Vander Kamp 600) at a rotational speed of 75 rpm and mass of microparticles equivalent to 75 mg of IMC placed in 900 ml of an appropriate medium at 37.0 ± 0.1° C, equilibrated for two hours prior to use. For the first two hours the medium used was pH 2.0 KCl-HCl buffer (Appendix 2.1.1.2.). Then the microparticles were transfered into pH 6.2 phosphate buffer medium (Appendix 2.1.1.3.) for the following 12 hours of the test. The pH 6.2 medium was selected in accordance with USP 23 *Drug Release Test 2* for Indomethacin Extended-release. The microparticles containing 75 mg equivalence of indomethacin were filled into either size 0 or 00 plain gelatin capsules and placed into the baskets. The baskets were covered with a nylon cloth of less than 200 mesh to protect the microparticles during the transfer and then attached to the spindles. The spindles were centered and adjusted to a suitable height using centering and height standardizing devices provided with the equipment. Samples of 5 ml were drawn at pre-determined

intervals: at 15 minutes, 30 minutes, 1 hour and 2 hours, then transfered to pH 6.2 buffer where sampling was the same for the following 2 hours. Sampling then continued every hour up to 6 hours, every 2 hours up to 10 hours and after 4 hours at the 14 th hour of the test. After each sample was removed, the lost amount of medium was replaced with the same volume of fresh buffer initially warmed up to the required temperature. The U.V. absorbance at 318 nm was determined and concentration estimated from the calibration curve (Appendix 2.1.2.1.) for each sample. All tests were done in triplicate and the mean values and standard deviations were reported.

2.2.7. Effect of aging on dissolution.

Formulations 3, 8, 20, and 21 were selected for studies on the effect of aging on dissolution and drug release from the microparticles after storage at different conditions. To ensure enough material for testing, six batches of each formulation were pooled and blended well. Half of the microparticles were stored in a drawer at room conditions (23.2 $\pm$ 0.1°C and 21% RH) and others were kept in an oven at 40.0 $\pm$ 0.1°C and 15% RH. The oven was equilibrated at 40.0 $\pm$ 0.1°C for three days prior to use. Test samples were withdrawn at monthly (30 days) intervals for drug release testing as described under **Dissolution testing procedure.**

2.2.8. Procedure to determine polymorphic changes in indomethacin following microencapsulation.

Differential scanning calorimetry analysis was conducted on Formulations 3, 8, 12, 15, 17, 21, pure IMC, pectin, gelatin, sodium alginate, carnauba wax and Aquateric^{R}, to determine the polymorphic behaviour of indomethacin due the method of

microencapsulation and the polymers used. A mass of microparticles containing IMC equivalence of 0.75 mg was used. A Du Pont Instruments differential scanning calorimeter (model 910) attached to a computer/thermal analyser (model 9900) was used. The equipment had been calibrated using indium prior to use. The heater was set to ramp up to 25°C and remained isothermal for 0.01 minutes before continuing the heating process at the rate of 10°C/min up to 300°C. The DSC sample pan equipped with two ports was loaded with two sealed microcups, one containing the sample and the other as an empty control. The heating process was carried out under nitrogen gas environment to prevent inadvertant oxidation of sample material being analysed. Two samples were analysed for each formulation. The heat flow from the samples was analysed for the presence of IMC melting endotherms corresponding to melting points of different polymorphic forms: Form I (160°C to 161°C); Form II (154°C); Form III (148°C to 149°C); and Form IV (133°C to 134°C).

CHAPTER 3. RESULTS AND DISCUSSION.

Complex ionic coacervation/phase separation has been studied widely using gelatin (cationic) and acacia (anionic) hydrocolloids (**72,102,106**). The process is a result of gelatin acquiring a positive charge below the isoelectric point when the solution pH is altered, to form an ionic complex with the negatively charged acacia. However, the use of some colloids such as pectin has been limited due to inadequate complex formation and phase separation as a result of insufficient pH change in the system when the reaction was carried out at isohydric conditions (**107-108**). Pectin-gelatin coacervation was achieved after mixing the colloids with pH previously increased to effect a high degree of pectin ionization. Upon decreasing the pH of the mixture the amphoteric gelatin acquired a positive charge to form a complex with the pectin.

There have been no reports demonstrating the use of alginate in ionic complex coacervation/phase separation microencapsulation. Alginate has been used widely for the preparation of gel beads resulting from gellation in the presence of calcium chloride. Colloids like polylysine and chitosan, which carry positively charged amide groups, have been used with alginate as re-enforcing agents during bead formation. The process of bead formation usually involved droplet extrusion or dispersion congealing techniques and not phase separation/coacervation (**119-121,132**).

Pectin is primarily composed of α-D-galactopyranosyluronic acid units partially esterified with methanol. These units are joined in a $^{-4}C_1$ configuration with (1 → 4) linkages. The treatment with sodium hydroxide de-esterifies the molecule to produce ionizable sodium pectate (**130-131**). Alginate is a polyuronide composed of β-D-mannuronic (M) acid and α-L-guluronic (G) acid units in $^{-4}C_1$ and $^{-1}C_4$ configurations,

respectively. High gelling properties have been observed with high alginate G content (120-121,132). Pectin and alginate are known to form water insoluble matrices in the presence of multi-valent ions like Ca^{2+}, Mg^{2+} and Al^{3+}. At high pH, the carboxylic acid groups in either pectin or alginate are ionized and can react to form complexes with the positively charged gelatin, below the isoelectric point.

McMullen *et al* (107-108), have encapsulated drugs like sulfamerazine from complex ionic coacervation of pectin and gelatin (type A). Polk *et al* (119), exploited the complex ionic relations between chitosan, a polysaccharide derived from chitin and alginate to produce controlled release microcapsules of albumin. The present work demonstrates the encapsulation of indomethacin with either pectin-gelatin or sodium alginate-gelatin complex coacervation under controlled pH conditions. This method was selected for IMC because it is insoluble in water. With a pKa of 4.5, IMC is not expected to be affected by the processing at pH range of 2.5 to 6.0, for short periods of time involved in the process. Also the method of preparation is free of toxic organic solvents.

3.1. YIELD AND ENCAPSULATION EFFICIENCY.

Coacervation was observed at pH 3.60 for most of the formulations. Microparticles were recovered from all Formulations except 1 and 6 which showed irregular lump formation due to lack of gelatin necessary for complex formation and coacervation. Formulations with higher alginate proportions like Formulation 7 and 10 only formed high turbidity at pH 3.6. Distinct particle formation was not observed with these formulations until the pH was lowered to 2.5 and 2.0, respectively. This could have been due to precipitation of excess alginate in the formulation.

Table 6 summarizes the microencapsulation results in terms of yield, drug load

encapsulation effeciency and flow propertiees. A high yield of 94% - 100%, measured as a proportion of ingredients used recovered in microparticle form, was achieved by this encapsulation method. McMullen *et al* (**107-108**) reported lower yield of about 70%. The calcium chloride cross-linking step could have contributed to the high yield by further precipitating the pectin or alginate that remained non-reacted.

A high degree (86%-99%) of encapsulation was reached as shown in Table 6. Due to remarkable encapsulation effeciency the experimental drug load for each formulation was predictable from the theoretical drug load. There was no clear trend to predict encapsulation efficiency, but to some extent high pectin or alginate concentration gave some of the highest encapsulation efficiencies. This may be explained by the availability of free pectin or alginate (not complexed to gelatin) which is precipitated in the presence of calcium chloride and thereby forming drug containing matrix.

Table 6. Tabulation of microparticle drug encapsulation, experimental drug load and flow properties.

Formulation No.	Yield (%)	Theoretical drug load (%)	Experimental drug load (%)	Encapsulation effeciency (%)	Angle of repose (°)
1	-	-	-	-	-
2	97	33	31.0	94	28.6
3	96	33	29.7	90	29.1
4	98	50	48.0	96	29.6
5	100	33	32.7	99	27.6
6	-	-	-	-	-
7	98	33	31.4	95	27.2
8	95	33	28.4	86	28.6
9	100	50	49.5	99	28.8
10	99	33	32.6	98	26.1
11	96	33	29.6	90	30.7
12	98	25	23.5	94	30.4
13	96	20	18.0	90	30.4
14	99	33	32.0	97	30.6
15	95	20	18.2	91	30.3
16	98	25	23.5	94	30.4
17	98	25	24.0	96	31.1
18	99	27	26.1	97	31.3
19	97	25	23.7	95	32.0
20	94	33	29.1	88	31.2
21	100	33	32.0	97	31.3
22	100	27	25.9	96	30.2
23	98	20	18.8	94	30.1
24	100	25	-	-	20.0

3.2. MICROPARTICLE MORPHOLOGY.

Two types of microparticles produced are shown in Figures 10 and 11 (A and B). The particles without carnauba wax (Figure 11 (A)) exist as aggregates of small spherical microparticles. This represents the two stages of the preparation process, *i.e.* coacervation and calcium chloride cross-linking. With carnauba wax (Figure 11 (B)) the size of the individual microparticles is considerabley increased, and therefore aggregation is minimized. The microparticles exist as microspheres made up of primarily carnauba wax and a thin surface coating, presumably made up of the coacervate. Close examination shows lack of uniformity in the surface coating. The microparticles containing Aquateric were similar to those in Figure 11 (A).

The existence of multiple morphology microparticles has been introduced earlier in the text as shown in Figure 8. The smaller particles could be mono-cored or multi-cored (Figure 8 (A - D) microparticles and aggregates resulting from collisions during polymer deposition step of microencapsulation (Figure 7 step 2). The larger particles could be aggregates, as illustrated in Figure 8 (F), whose formation was aided by the hardening and cross-linking steps of the preparation process (addition of formaldehyde and calcium chloride). The addition of carnauba wax improved microparticle shape as shown in Figure 11 (B). Reduction in aggregation could be due to viscosity reduction, which renders newly formed particles to circulate at a higher speed and thus minimizing contact and collision (**83,94**).

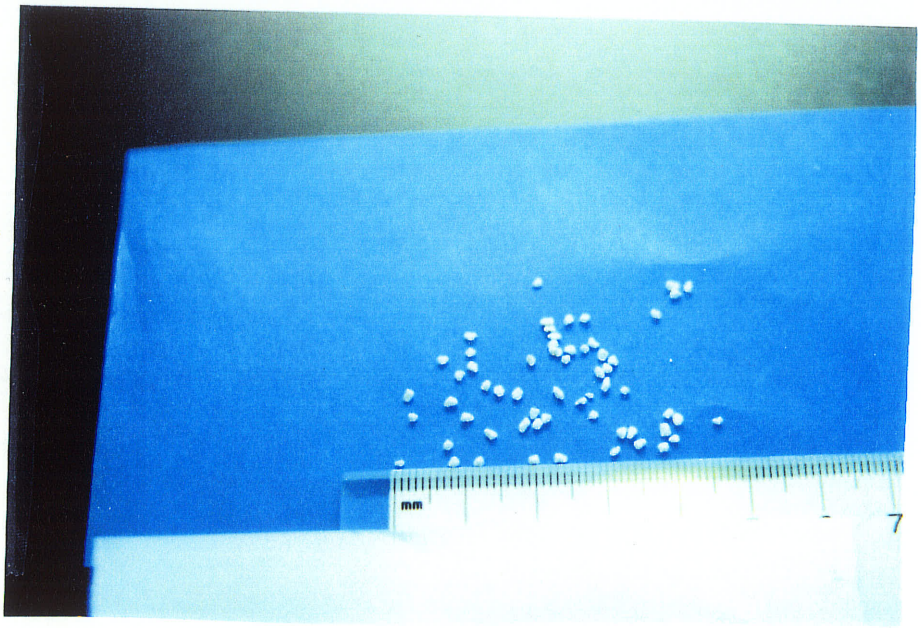
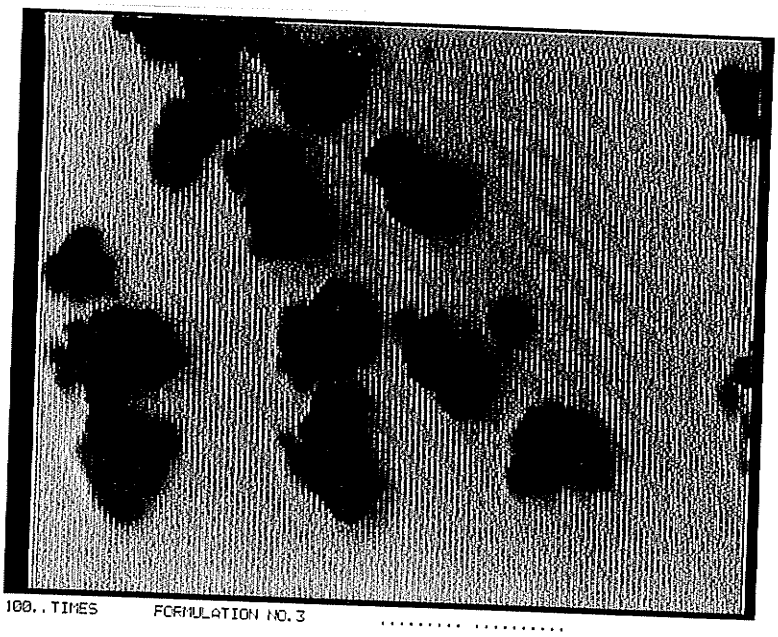


Figure 10. A photogragh of size 16/30 microparticles.

A.



B.

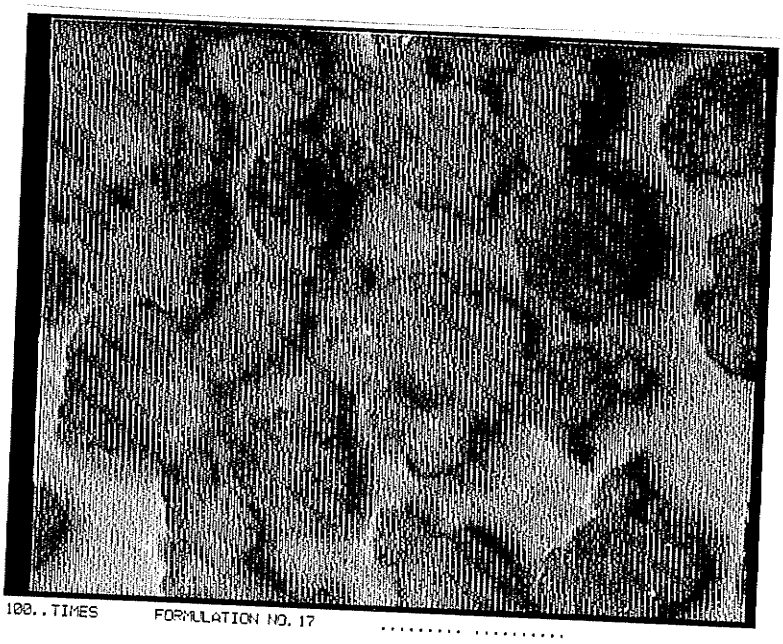


Figure 11. (A) photomicrogragh of microparticles without additives; and (B) photomicrogragh of microparticles containing carnauba wax.

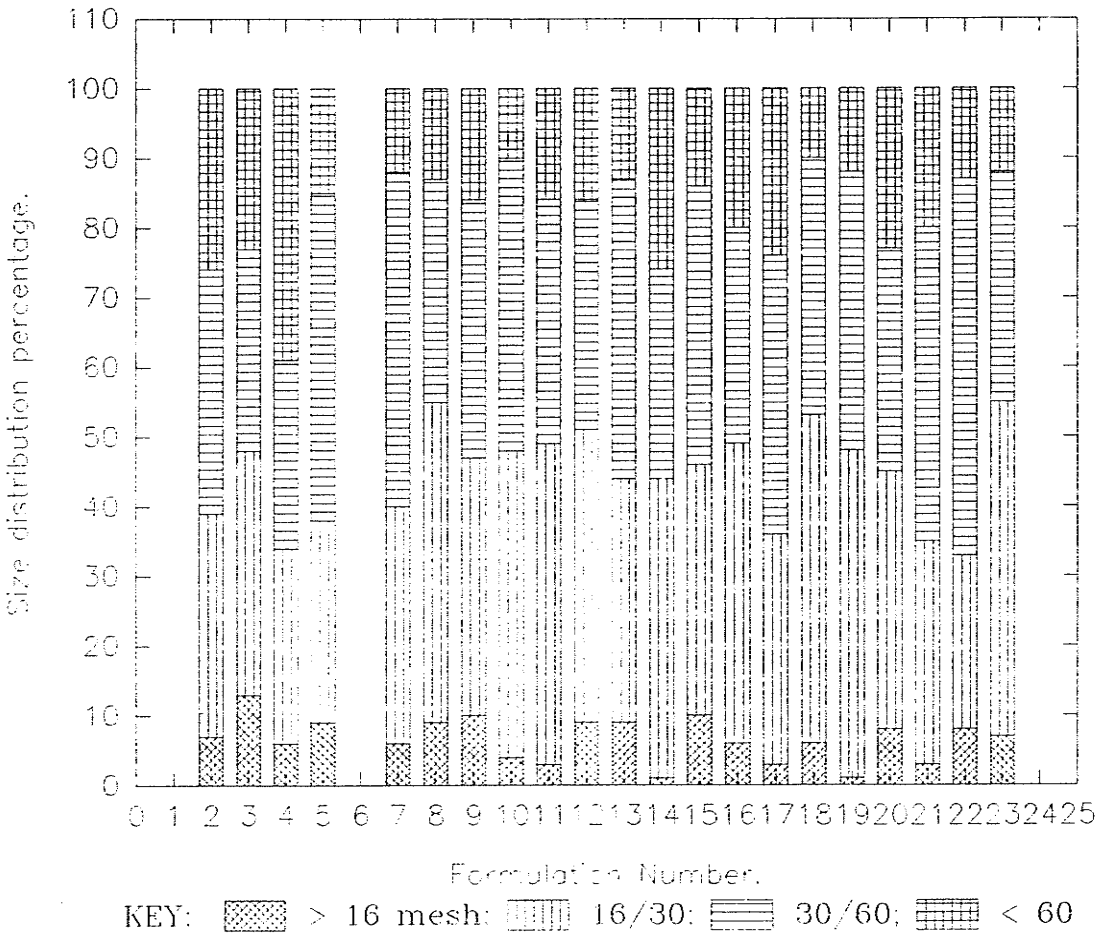


Figure 12. Stacked bar chart illustrating size distribution of microparticles from various formulations studied.

3.3. MICROPARTICLE SIZE DISTRIBUTION AND FLOW PROPERTIES.

The bulk of microparticles ranged from 30/60 mesh to 16/30 mesh although particles as fine as < 60 mesh and those as large as > 16 mesh were also seen. Indomethacin powder alone was much smaller than 100 mesh. Figure 12 shows the size distribution of the microparticles from the various formulations prepared. The larger particles were possibly a result of aggregation of smaller particles and by doing so entrapping the finer particles in between. Hence the larger particles were not as spherical and as free-flowing as the smaller particles. Since aggregation occured during hardening and crosslinking stages, changing stirring speed reduced aggregation without affecting polymer deposition at the coacervation step. Incorporation of additives like Aquateric^{R} or carnauba wax to the formulation also considerably changed the size distribution of the microparticles by reducing aggregation without the need for altering stirring speed.

The majority of the formulations prepared showed good flow properties (Table 6) compared to IMC with practically no flow. Formulation 24 produced needle shaped aggregates resulting in poor flow properties (20 ° angle of repose), hence further studies were not conducted with this formulation.

In Formulations 2 and 5, where pectin concentration was relatively high (1:1 and 2:1, pectin:gelatin), the apparent viscosity of the coacervate dispersion (before hardening step) was visibly higher than when lesser concentration was used (1:2, pectin:gelatin). Microparticle recovery was also relatively more difficult (during filtering) due to very fine particles produced. The size distribution in these formulations demonstrated a slightly higher proportion of 30/60 and < 60 mesh microparticles. This observation was also made by McMullen *et al* (**107,108**). The increase in the alginate proportion did not seem to have any effect on the microparticle size (Figure 12).

Drug concentration in pectin formulations seemed to have an effect on particle size distribution. Formulation 4 with 50% drug load (theoretical) prodcued a high proportion of microparticles in the micro-fine size range (< 60 mesh). This was not as evident among the alginate preparations.

3.4. *IN VITRO* DISSOLUTION AND DRUG RELEASE.

In vitro drug release was studied in buffer at simulated gastroinstestinal pH. The physical properties of the drug and the structure of the microparticle determine the drug release profile. Extremely high aqueous solubility or extremely low solubility may hinder the bioavalability of the drug **(35,48,49)**. Although *in vitro* dissolution studies may not always predict the behaviour of the drug *in vivo*, the release kinetics observed from dissolution studies offer the basis for understanding the absorption behaviour of the drug. Indomethacin is well absorbed throughout the GI tract and has a pH dependent aqueous solubility (less than 0.52 mg/ml in water and acidic aqueous soultions and 11 mg/ml in pH 6.2 phosphate buffer). Delaying the release of the drug may decrease the amount of drug absorbed in the upper GI tract and thus reduce entero-hepatic recycling of the drug **(10)**. This would help prevent untoward drug induced duodenal ulceration. Encapsulation of IMC in hydrophilic material would also improve the wetting of the drug and enhance its water solubility. Reducing the amount of drug in crystal form in contact with the GI tract at any particular time during transition due to improved wetting and solubility has been cited as a possible mechanism of preventing ulceration in rats **(33,50,51)**.

The dissolution of IMC from microparticles was found to be more enhanced and prolonged when compared to pure IMC powder. Figure 13 - 18 show the dissolution profiles for microparticles of various formulations and sizes. The formulation numbers,

polymer to polymer and drug to polymer or D:P ratios are shown, where applicable in each figure. The release in pH 2.0 medium was almost negligible in all cases. This could be attributed to both the poor solubility of IMC in acidic media and limited diffusion due to lack of polymer swelling in the low pH environment. Although all formulations showed delay in drug release compared to IMC alone, some were more pronounced than the others. IMC alone also showed no release in the acid medium and after buffer change at 2 h, showed a steep initial release of about 59% in the first 1 h in pH 6.2 medium, but, the dissolution rate dropped and showed a plateau to reach a final value of 73% at the end of 14 h. However, IMC release from size 16/30 microparticles (Figures 14-15 and 17-18) proceeded gradually to reach values of at least 77% at 14 h. Microparticles in the size 30/60 category (Figures 13, 15, 16 and 18) did not show profiles much different from pure IMC during the first 1 hour in pH 6.2 medium. However, the release then became more gradual until at least 92.5% was released at the end of 14 h. The difference observed between the drug release from the microparticles of different sizes could be explained by the microscopic observation discussed earlier in the text. That is, aggregation of the particles produced a membrane-like effect which increased the difusion path length in larger particles, and thereby slowing the drug release rate. (Differences in release profiles are discussed in detail under appropriate headings below).

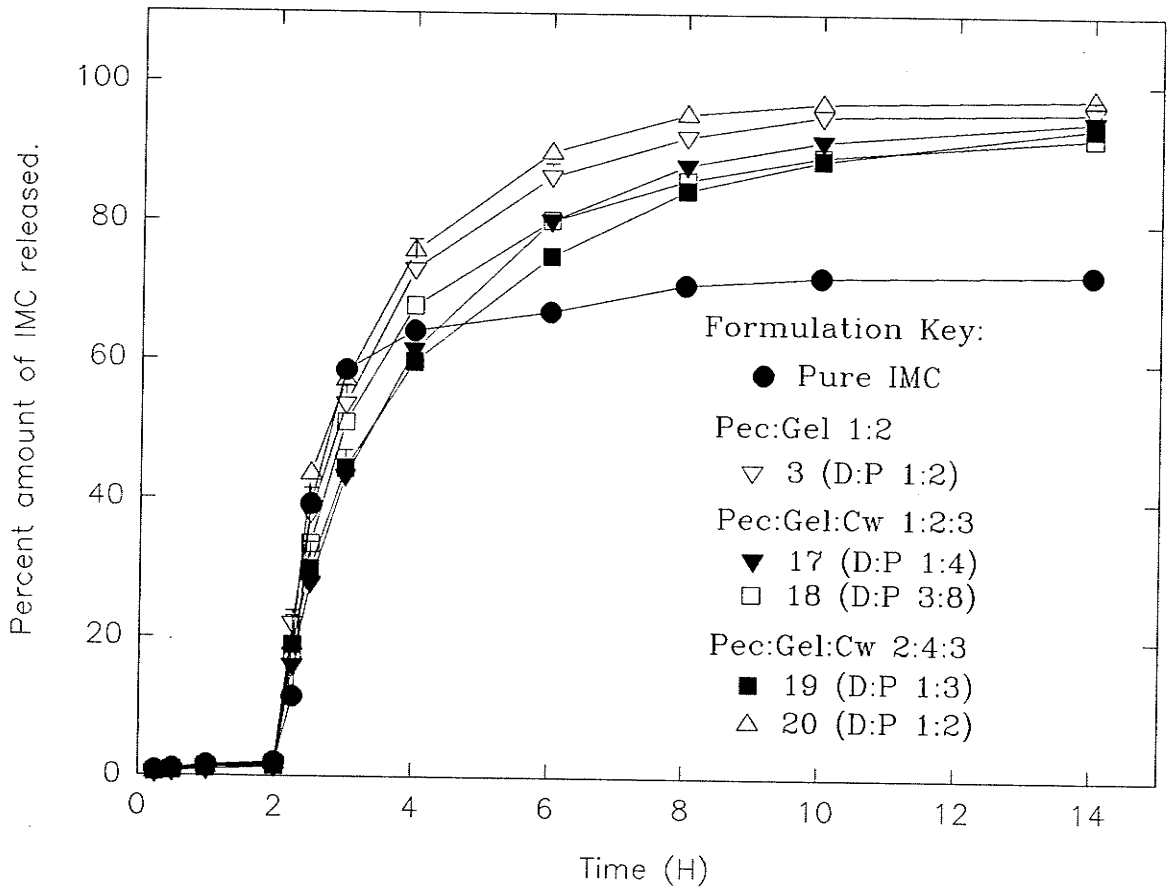


Figure 13. Cumulative percentage of IMC released from pectin and pectin-carnauba wax preparations of the 30/60 size category.

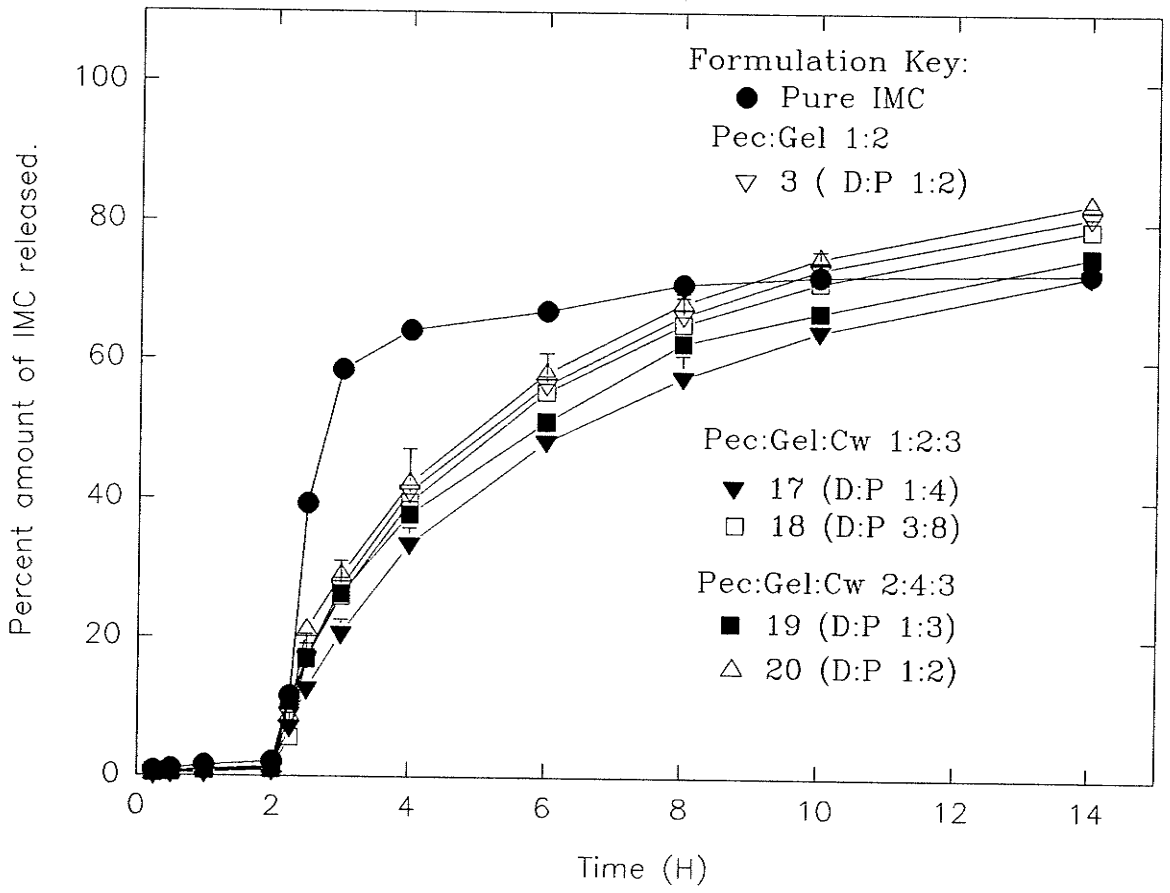


Figure 14. Cumulative percentage of IMC released from pectin and pectin-carnauba wax preparations of the 16/30 size category.

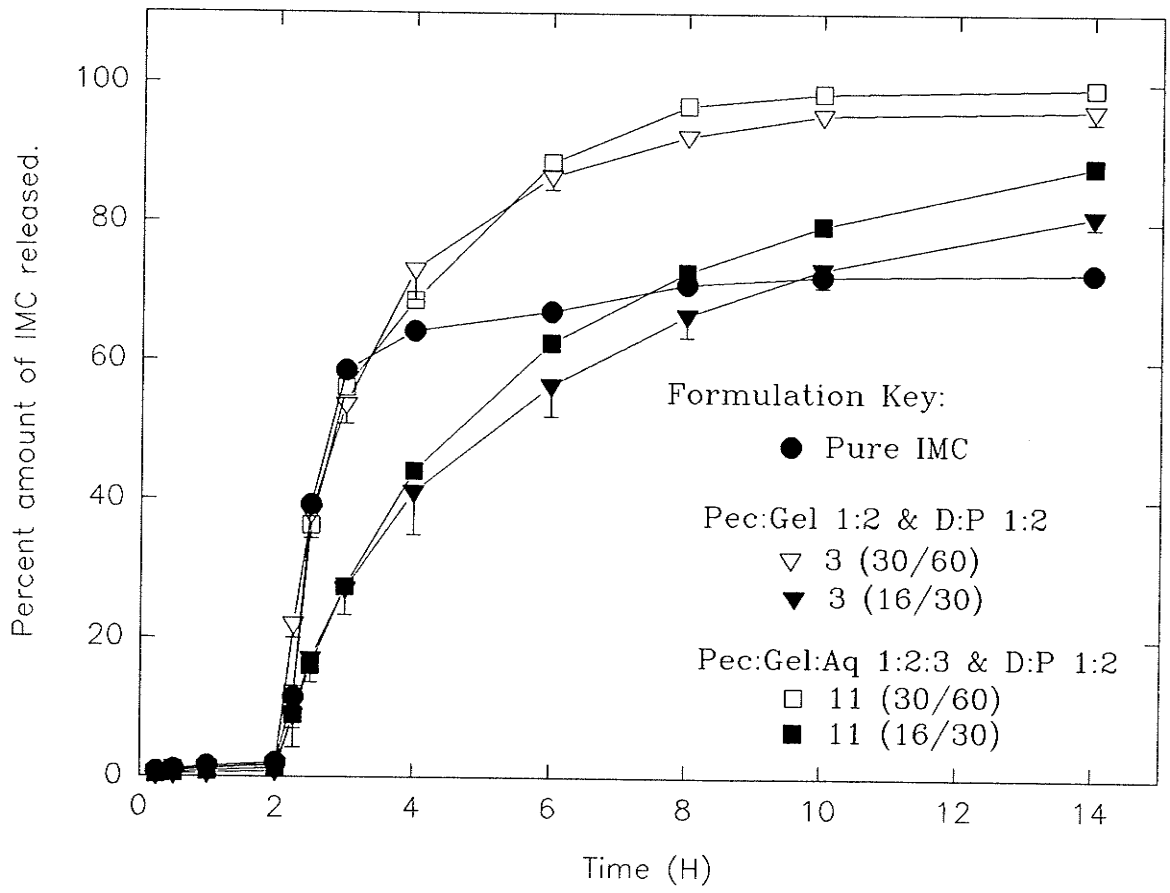


Figure 15. Cumulative percentage of IMC released from pectin- Aquateric[R] preparations in both the 30/60 and 16/30 size categories. Pure IMC and Formulation 3 are included for comparison.

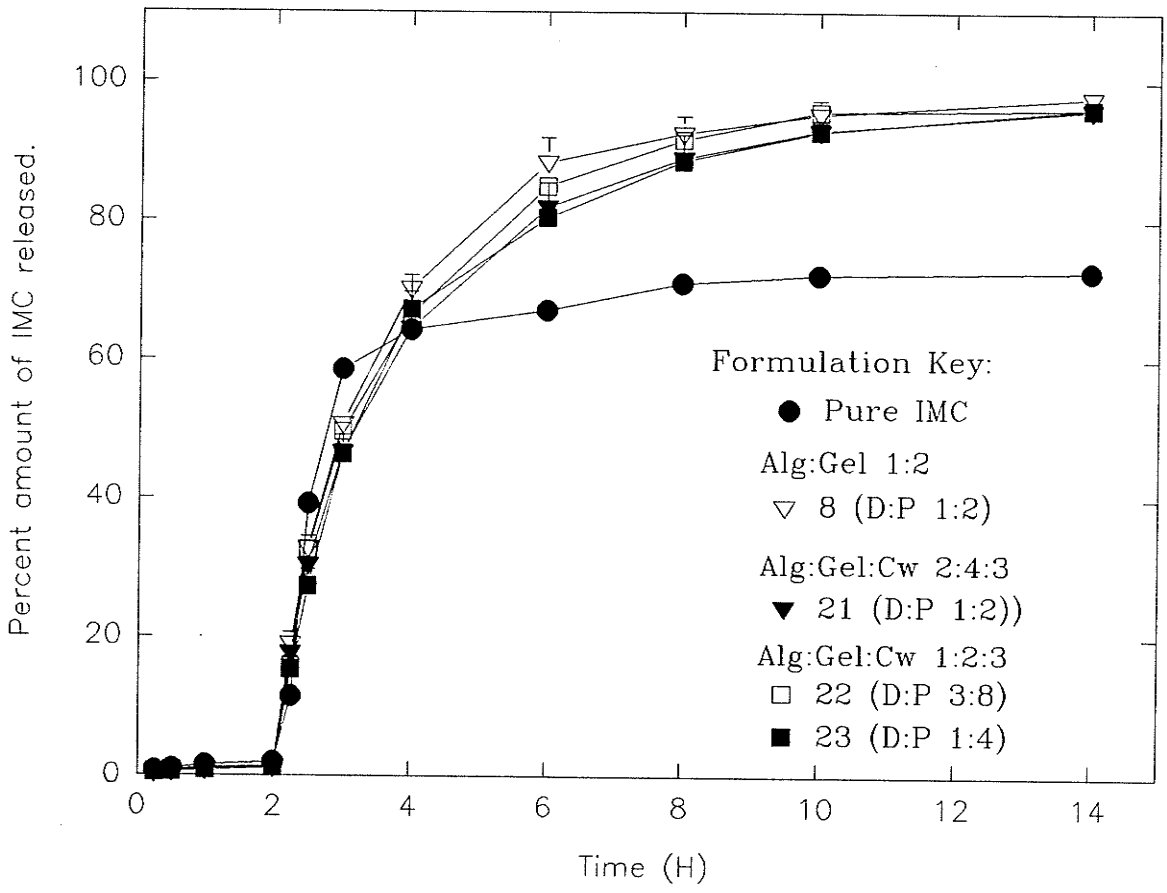


Figure 16. Cumulative percentage amount of IMC released from alginate and alginate-carnauba wax preparations in the 30/60 size category.

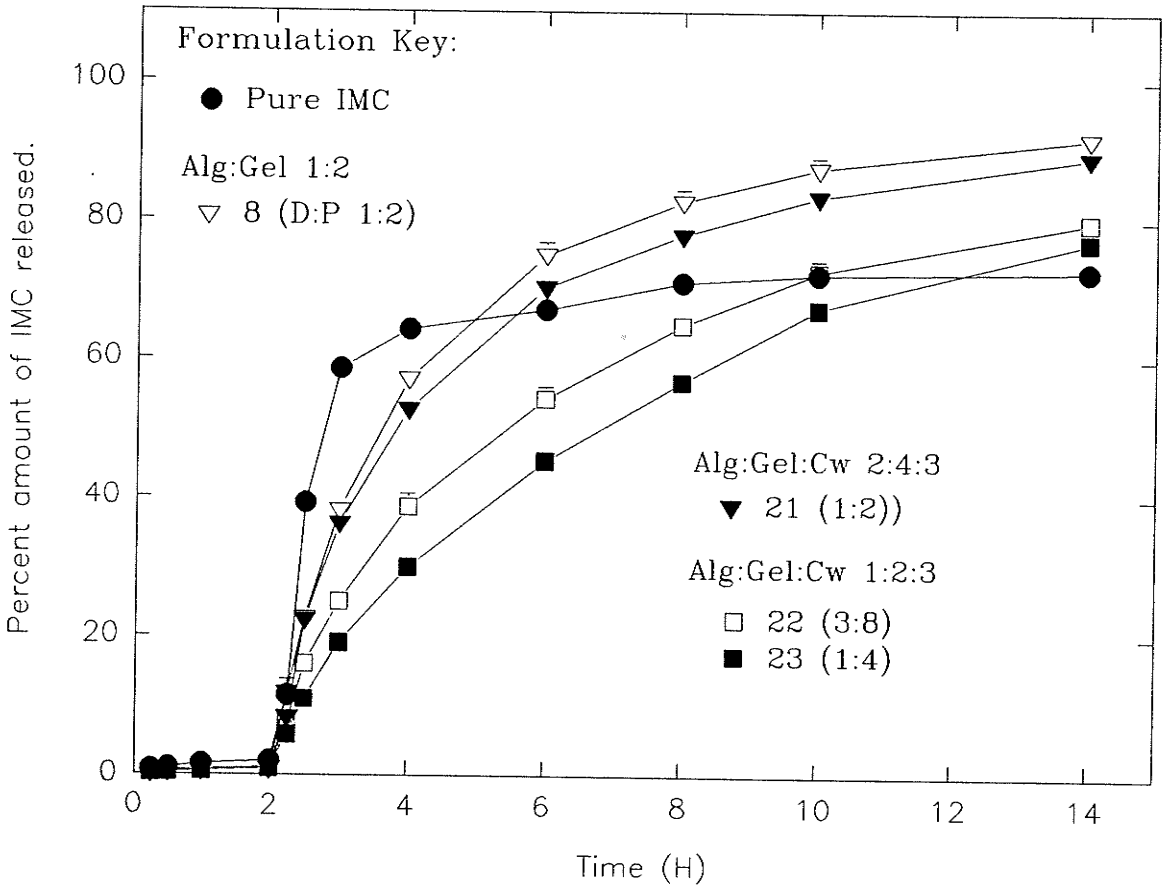


Figure 17. Cumulative percentage amount of IMC released from alginate and alginate-carnauba wax preparations in the 16/30 size category.

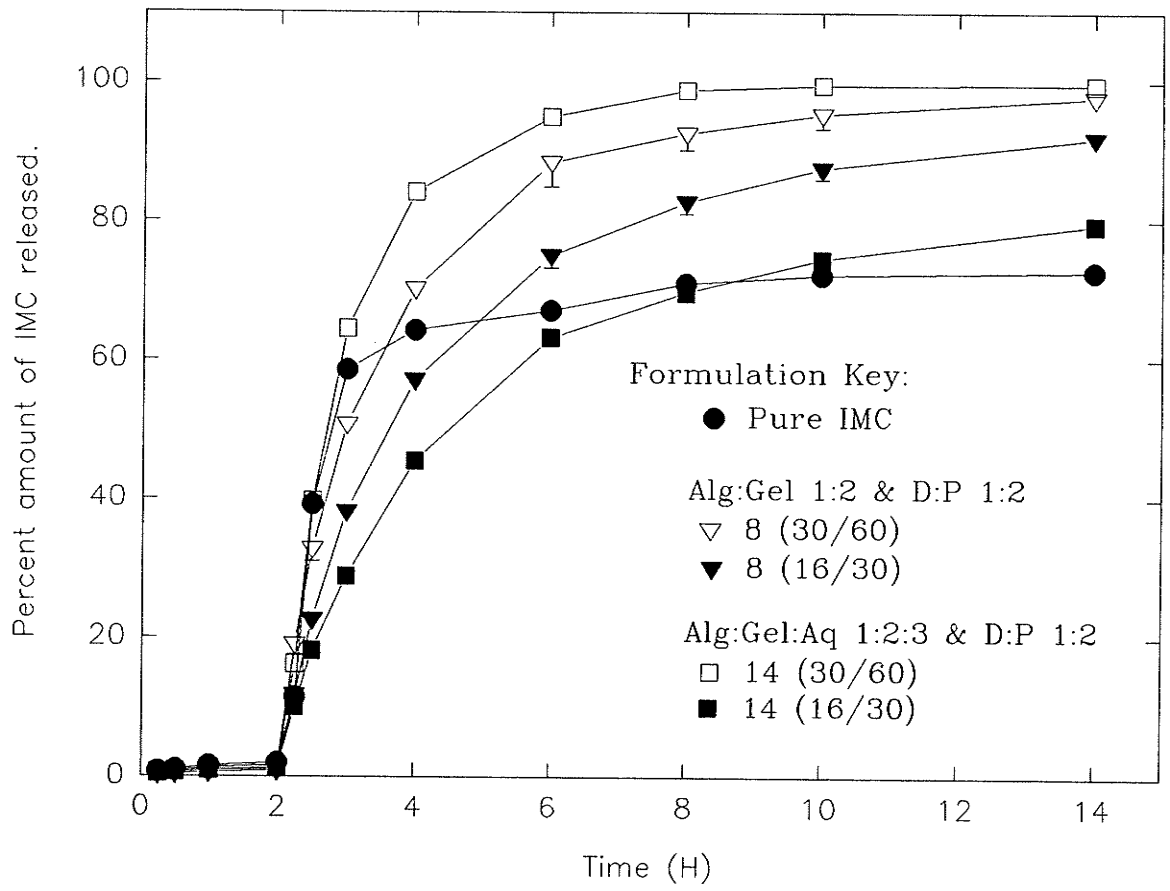


Figure 18. Cumulative percentage amount IMC released from alginate-Aquateric[R] preparations in both the 30/60 and 16/30 size categories. Pure IMC and Formulation 8 are included for comparison.

3.4.1. Statistical methods and data analysis.

Drug release from microparticles was compared to pure IMC and analysed for statistical significance in differences observed at three selected points from the drug release against time plots (3 hours, 6 hours and 14 hours). Comparisons were carried out to isolate differences (if any) in drug release due to particle size, additives, polymer concentration differences, and type of polymer. To achieve this analysis of variance (One-Way-ANOVA) was utilized to test the null hypotheses that there were no differences between drug release from formulations produced and pure IMC powder, and that there were no differences between various formulations themselves. Where differences were detected (*i.e.* null hypothesis rejected), demonstrated by a significantly large F values compared to the critical value at the test p level, further paired comparisons were carried out employing Student-Newman-Keuls (SNK) multiple comparison procedure (**133**). The comparisons were carried out at 95 % confidence level ($\propto$ level of 0.05). Data analysis and graphics were made possible by the use of a personal computer equiped with a SigmaPlot Scientific Graphic System, Version 5.00 software.

Analysis of variance validity was based on some very important assumptions. First, it was assumed that the data were of equal variance within each group or that there were no confounding factors that might have acted as sources of variation within the group. Secondly, it was assumed that the data were independent and normally distributed (**134**).

The following were definitions of some abbreviations from the ANOVA output:

N - sample size of a treatment group;

Std Dev - standard deviation of data within a treatment group;

SEM - Standard error of the mean;

DF - Degrees of freedom;

SS - Sum of squares of the groups means;

MS - Mean squares;

F - The test statistic (ratio of pooled variance to variance estimate);

p - Probability that the noted differences may be due to chance at a certain confidence level, $\propto$.

There was a statistically significant difference ($p < 0.001$) between drug release from pure IMC and most of the formulations produced (Tables 7 to 24). Multiple comparison (SNK) results are shown in Appendix 3. IMC is insoluble in acidic aqueous solutions, therefore there were no differences in drug release in pH 2 medium. Release in acid was significantly low compared to that from pH 6.2 medium (Figures 13 and 14). Pure IMC released rapidly for the first 3 hours then started to plateau. This could be attributed to poor wetting and hence limited dissolution of pure IMC (**33**). Some IMC powder was found floating on the surface of the dissolution medium at the end of the test. In some studies, IMC poor hydration phenomenon has led investigators to incorporate wetting agents to the dissolution medium for the dissolution tests (**70,135**). The effect of wetting agents on the dissolution of IMC and other insoluble drugs has been demonstrated (**50-51,135**). Encapsulating IMC in pectin or alginate and gelatin complex coacervates improved the hydration of the drug while prolonging the extent of release. Pectin and alginate have shown solubilizing properties (**33,131**).

Table 7. ANOVA results summary comparing drug release after 3 hours of dissolution testing of pectin containing formulations.

Group	N
Pure IMC 3h	3
3(30/60)	3
3(16/30)	3
17(30/60) 3h	3
17(16/30) 3h	3
18(30/60) 3h	3
18(16/30) 3h	3
19(30/60) 3h	3
19(16/30) 3h	3
20(30/60) 3h	3
20(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
3(30/60)	40.189	2.0700	1.1951
3(16/30)	20.345	2.8701	1.6571
17(30/60) 3h	32.351	0.4505	0.2601
17(16/30) 3h	15.383	1.4999	0.8660
18(30/60) 3h	38.243	0.2067	0.1193
18(16/30) 3h	19.363	1.0721	0.6190
19(30/60) 3h	33.264	1.9911	1.1495
19(16/30) 3h	19.638	1.7548	1.0131
20(30/60) 3h	42.842	0.3144	0.1815
20(16/30) 3h	21.688	0.2939	0.1697

Source of Variation	DF	SS	MS	F	P
Between Treatments	10	3423.5608	342.3561	157.073	< 0.001
Residual	22	47.9512	2.1796		
Total	32	3471.5119			

Table 8. ANOVA results summary comparing drug release after 6 hours of dissolution testing of pectin containing formulations.

Group	N
Pure IMC	3
3(30/60) 6h	3
3(16/30) 6h	3
17(30/60) 6h	3
17(16/30) 6h	3
18(30/60) 6h	3
18(16/30) 6h	3
19(30/60) 6h	3
19(16/30) 6h	3
20(30/60) 6h	3
20(16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC	50.571	0.2935	0.1695
3(30/60) 6h	64.892	1.4483	0.8362
3(16/30) 6h	42.308	3.3945	1.9598
17(30/60) 6h	60.103	0.8632	0.4984
17(16/30) 6h	36.158	1.9729	1.1390
18(30/60) 6h	60.155	0.1863	0.1076
18(16/30) 6h	41.516	1.6280	0.9399
19(30/60) 6h	56.279	0.1863	0.1076
19(16/30) 6h	38.329	0.9891	0.5711
20(30/60) 6h	67.545	0.3227	0.1863
20(16/30) 6h	43.566	0.1367	0.0789

Source of Variation	DF	SS	MS	F	P
Between Treatments	10	3789.1363	378.9136	188.050	< 0.001
Residual	22	44.3292	2.0150		
Total	32	3833.4655			

Table 9. ANOVA results summary comparing drug release after 14 hours of dissolution testing of pectin containing formulations.

Group	N
Pure IMC 14h	3
3(30/60) 14h	3
3(16/30) 14h	3
17(30/60) 14h	3
17(16/30) 14h	3
18(30/60) 14h	3
18(16/30) 14h	3
19(30/60) 14h	3
19(16/30) 14h	3
20(30/60) 14h	3
20(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
3(30/60) 14h	72.110	1.0353	0.5977
3(16/30) 14h	60.672	1.2349	0.7130
17(30/60) 14h	71.163	0.3902	0.2253
17(16/30) 14h	54.332	0.6940	0.4007
18(30/60) 14h	69.371	0.0597	0.0345
18(16/30) 14h	59.294	1.2255	0.7075
19(30/60) 14h	70.422	0.2601	0.1502
19(16/30) 14h	56.348	0.0298	0.0172
20(30/60) 14h	73.557	0.0597	0.0345
20(16/30) 14h	62.067	0.1790	0.1034

Source of Variation	DF	SS	MS	F	P
Between Treatments	10	1670.0176	167.0018	379.453	< 0.001
Residual	22	9.6825	0.4401		
Total	32	1679.7001			

Table 10. ANOVA results summary comparing drug release after 3 hours of dissolution testing of alginate containing formulations.

Group	N
Pure IMC 3h	3
8(30/60) 3h	3
8(16/30) 3h	3
21(30/60) 3h	3
21(16/30) 3h	3
22(30/60) 3h	3
22(16/30) 3h	3
23(30/60) 3h	3
23(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
8(30/60) 3h	37.984	0.9473	0.5469
8(16/30) 3h	28.596	0.7566	0.4368
21(30/60) 3h	34.935	0.6953	0.4014
21(16/30) 3h	27.132	0.0517	0.0298
22(30/60) 3h	37.175	0.5477	0.3162
22(16/30) 3h	18.708	1.0087	0.5824
23(30/60) 3h	34.780	1.9995	1.1544
23(16/30) 3h	14.281	0.1957	0.1130

Source of Variation	DF	SS	MS	F	P
Between Treatments	8	2200.7616	275.0952	313.817	< 0.001
Residual	18	15.7790	0.8766		
Total	26	2216.5406			

Table 11. ANOVA results summary comparing drug release after 6 hours of dissolution testing of alginate containing formulations.

Group	N
Pure IMC 6h	3
8(30/60) 6h	3
8(16/30) 6h	3
21(30/60) 6h	3
21(16/30) 6h	3
22(30/60) 6h	3
22(16/30) 6h	3
23(30/60) 6h	3
23(16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC 6h	50.267	0.8012	0.4626
8(30/60) 6h	66.270	2.6394	1.5238
8(16/30) 6h	56.227	1.2910	0.7453
21(30/60) 6h	61.464	0.2652	0.1531
21(16/30) 6h	52.679	0.5526	0.3190
22(30/60) 6h	63.669	0.3101	0.1790
22(16/30) 6h	40.672	1.2306	0.7105
23(30/60) 6h	60.293	3.6958	2.1338
23(16/30) 6h	33.953	0.1550	0.0895

Source of Variation	DF	SS	MS	F	P
Between Treatments	8	2818.4704	352.3088	127.115	< 0.001
Residual	18	49.8884	2.7716		
Total	26	2868.3588			

Table 12. ANOVA results summary comparing drug release after 14 hours of dissolution testing of alginate containing formulations.

Group	N
Pure IMC 14h	3
8(30/60) 14h	3
8(16/30) 14h	3
21(30/60) 14h	3
21(16/30) 14h	3
22(30/60) 14h	3
22(16/30) 14h	3
23(30/60) 14h	3
23(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
8(30/60) 14h	73.316	0.7241	0.4181
8(16/30) 14h	68.941	0.7752	0.4476
21(30/60) 14h	72.041	0.5755	0.3323
21(16/30) 14h	66.891	1.1052	0.6381
22(30/60) 14h	72.093	0.1034	0.0597
22(16/30) 14h	59.879	0.4303	0.2484
23(30/60) 14h	72.127	0.5990	0.3458
23(16/30) 14h	57.761	0.3115	0.1799

Source of Variation	DF	SS	MS	F	P
Between Treatments	8	1226.9866	153.3733	414.465	< 0.001
Residual	18	6.6509	0.3701		
Total	26	1233.6476			

3.4.2. Effect of particle size on drug release.

The morphology and size of microparticles can influence the rate and extent of drug release. Figures 13 to 18 and Tables 7 to 12 (ANOVA results) show the differences in release profiles of microparticles in two size categories (30/60 and 16/30). Regardless of formulation studied smaller particles (30/60) showed higher release rates ($p < 0.05$) than larger ones (16/30). The difference is believed to be due to microparticle surface area variation between small and large aggregated particles. That is, the particle surface area increases as size decreases allowing greater exposure to the dissolution medium. The effect of particle size on drug release from products of miroencapsulation has been well documented (117,122). Nevertheless, all sizes showed sustained drug release compared to indomethacin alone. Unlike larger particles the smaller ones were more likely to release the drug completely during the specified testing period of 14 hours. The SNK multiple comparison ($p < 0.05$) results showed that smaller particles were more likely to release the drug at rate not different from that seen with pure IMC, particularly during the first three hours of the test. This could be due to initial burst and defects on the smaller particles (32).

3.4.3. Effect of initial drug load and polymer ratio on release.

Different initial drug concentrations (drug load) were used to study the effect of drug loading on drug release. The drug loads ranged from 20% to 50% (w/w), expressed as drug to polymer ratio or theoretical drug load. Figures 14 and 17 illustrate the effect of drug loading on drug release to some extent. Formulation 17, with the experimental drug load of 23% showed the release rate was considerably lower than that of Formulation 20 with 32% experimental drug load. Similarly, Formulation 23 (19% experimental drug

load) had lower drug release compared to Formulation 21 (32% drug experimental load). The effect of drug load on drug release was reported by previous investigators (**69-70,117,135**). In one of these studies, it was found that indomethacin diffusivity from a polymeric matrix increased with increase in initial drug concentration, hence the increase in release rate (**135**). In this study, it was observed that not only the rate differed but the extent as well. ANOVA results are summarized and shown in Tables 7 to 12.

The effect of change in colloid ratio on drug encapsulation and release was studied. The batches were prepared in combination ratios of either pectin or sodium alginate to gelatin 1:0, 1:1 and 1:2 prepared from 2% (w/v) stock solution of each. Figures 19 and 20 show drug release from formulations in which pectin to gelatin and alginate to gelatin ratios have been modified, respectively. The 1:0 ratio (Formulations 1 and 6 without gelatin) resulted in lumps which were difficult to dry and had poor content uniformity. For easy handling, formulations with polymer to polymer ratio of 1:2 were studied. Formulations with higher pectin or alginate content produced highly viscous suspensions, difficult to filter. This was due to the presence of excess gelling polymer which formed thickened suspension on addition of calcium chloride. ANOVA results summarized in Tables 13 to 18 show the significance in the differences of some of these formulations. It was observed that as the pectin or alginate was increased, up to 1:1 ratio to gelatin (Formulations 2 and 7), the release rate decreased (Figures 19 and 20). However, there was no difference between release rates at the 1:2 and 2:1 (Formulation 8 and 10) alginate to gelatin ratios (Figure 20), and Formulation 5 (2:1, pectin:gelatin) in Figure 19 showed even higher release rate than Formulation 3 (1:2, pectin:gelatin). Higher pectin or alginate concentration probably interferes with the formation of the "egg-box" dimers configuration observed in gellation. This would result in weak complexation due to shielding of ionic-bonding sites by unreacted pectin or alginate.

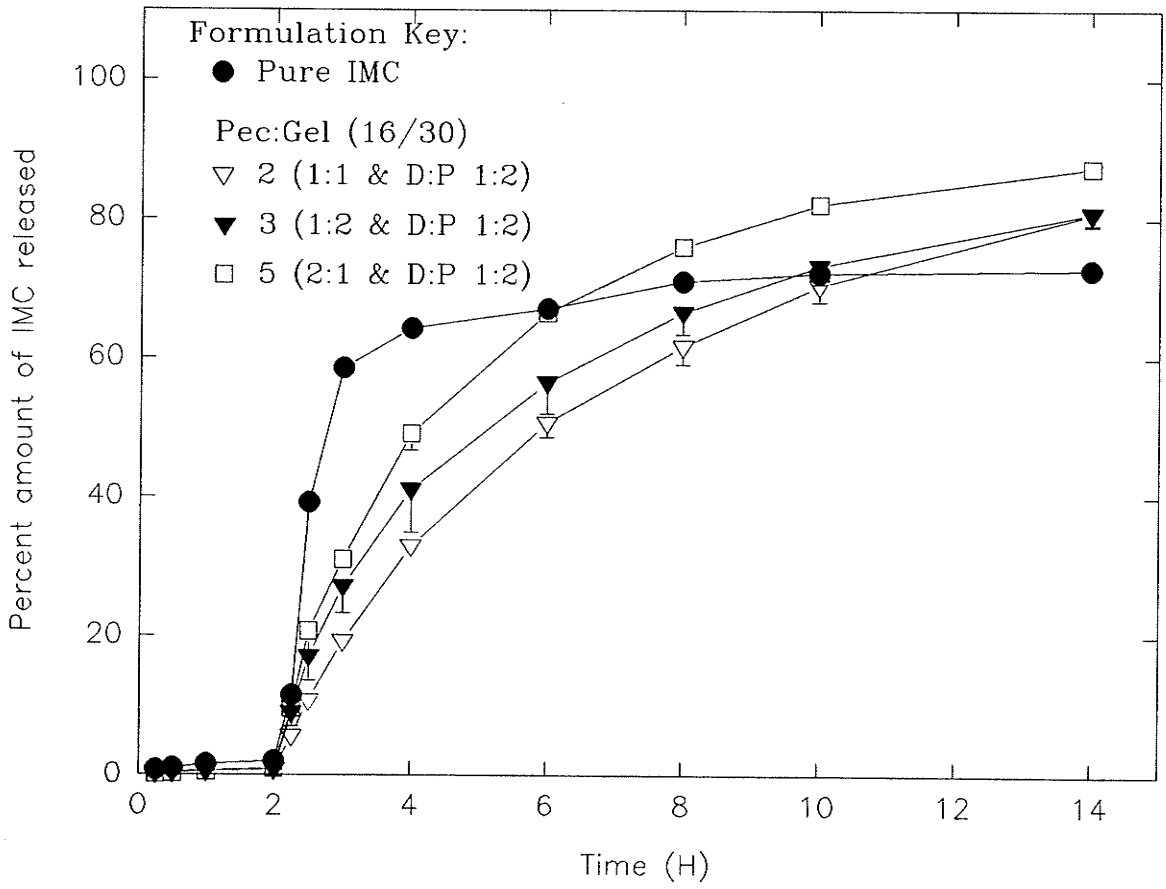


Figure 19. Drug release profile of formulations containing differing pectin to gelatin ratios.

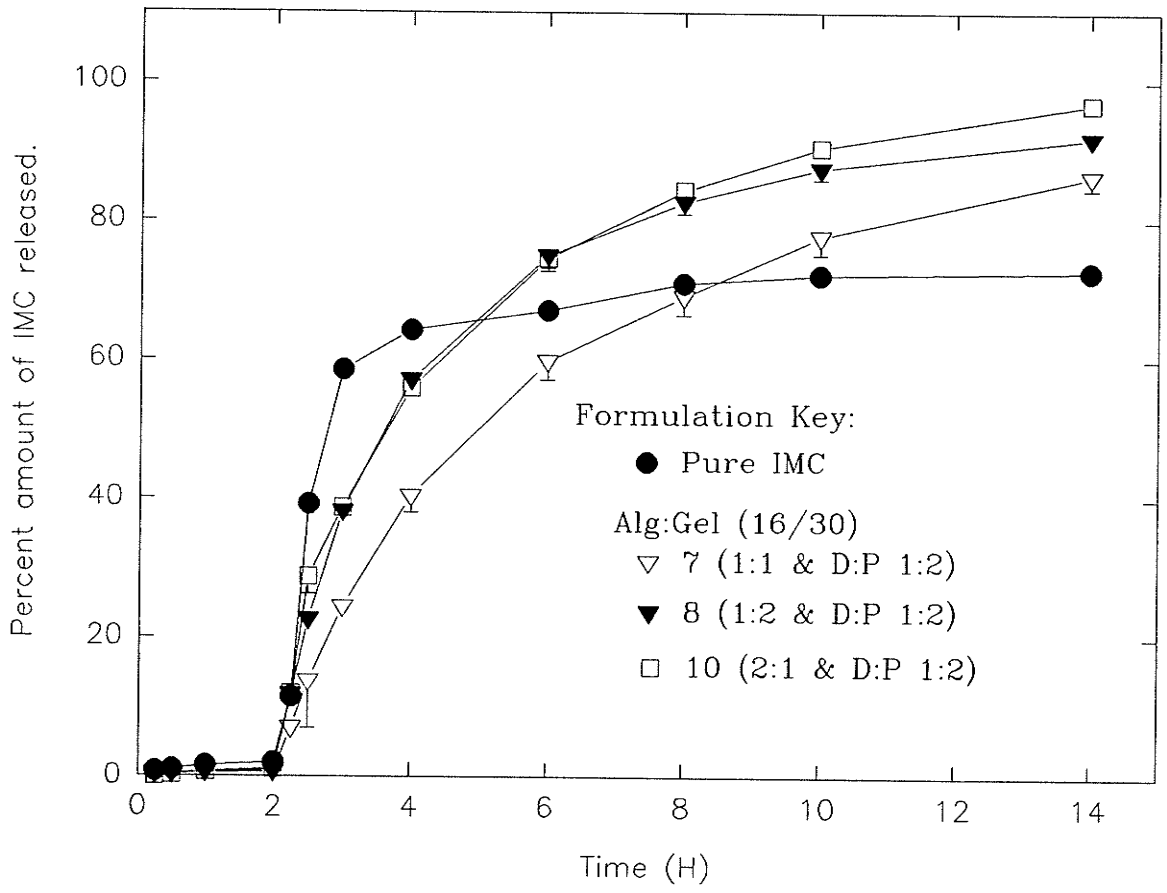


Figure 20. Drug release profile of formulations containing differing alginate to gelatin ratios.

Table 13. ANOVA results summary comparing drug release after 3 hours of dissolution testing in formulations of varying pectin content.

Group	N
Pure IMC 3h	3
2(16/30) 3h	3
3(16/30) 3h	3
5(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
2(16/30) 3h	20.345	2.8701	1.6571
3(16/30) 3h	23.239	0.7775	0.4489
5(16/30) 3h	14.401	0.7054	0.4073

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	1477.6309	492.5436	198.621	< 0.001
Residual	8	19.8386	2.4798		
Total	11	1497.4694			

Table 14. ANOVA results summary comparing drug release after 6 hours of dissolution testing in formulations of varying pectin content.

Group	N
Pure IMC 6h	3
2(16/30) 6h	3
3(16/30) 6h	3
5(16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC 6h	50.267	0.8012	0.4626
2(16/30) 6h	37.950	1.5141	0.8742
3(16/30) 6h	42.308	3.3945	1.9598
5(16/30) 6h	49.854	0.8290	0.4786

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	324.6286	108.2095	28.581	< 0.001
Residual	8	30.2884	3.7861		
Total	11	354.9170			

Table 15. ANOVA results summary comparing drug release after 14 hours of dissolution testing in formulations of varying pectin content.

Group	N
Pure IMC 14h	3
2(16/30) 14h	3
3(16/30) 14h	3
5(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
2(16/30) 14h	60.603	1.2704	0.7335
3(16/30) 14h	60.672	1.2349	0.7130
5(16/30) 14h	65.564	0.8791	0.5075

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	182.7621	60.9207	62.280	< 0.001
Residual	8	7.8254	0.9782		
Total	11	190.5875			

Table 16. ANOVA results summary comparing drug release after 3 hours of dissolution testing in formulations of varying alginate content.

Group	N
Pure IMC 3h	3
7(16/30) 3h	3
8(16/30) 3h	3
10(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
7(16/30) 3h	18.260	1.1232	0.6485
8(16/30) 3h	28.596	0.7566	0.4368
10(16/30) 3h	29.009	0.9534	0.5504

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	999.9085	333.3028	401.274	< 0.001
Residual	8	6.6449	0.8306		
Total	11	1006.5534			

Table 17. ANOVA results summary comparing drug release after 6 hours of dissolution testing in formulations of varying alginate content.

Group	N
Pure IMC 6h	3
7(16/30) 6h	3
8(16/30) 6h	3
10)16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC 6h	50.267	0.8012	0.4626
7(16/30) 6h	44.789	2.0051	1.1576
8(16/30) 6h	56.227	1.2910	0.7453
10)16/30) 6h	55.952	1.3006	0.7509

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	265.0295	88.3432	44.059	< 0.001
Residual	8	16.0407	2.0051		
Total	11	281.0702			

Table 18. ANOVA results summary comparing drug release after 14 hours of dissolution testing in formulations of varying alginate content.

Group	N
Pure IMC 14h	3
7(16/30) 14h	3
8(16/30) 14h	3
10(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
7(16/30) 14h	64.806	1.3673	0.7894
8(16/30) 14h	68.941	0.7752	0.4476
10(16/30) 14h	72.627	0.9999	0.5773

Source of Variation	DF	SS	MS	F	P
Between Treatments	3	547.7687	182.5896	210.410	< 0.001
Residual	8	6.9422	0.8678		
Total	11	554.7109			

3.4.4. Effect of additives on drug release.

Hydrophilic or lipophilic polymers, in varying combinations, were incorporated into the formulation and studied with respect to microparticle morphology and size (discussed under Section 3.1.) and drug release.

Drug release was significantly modified by the incorporation of coating materials like Aquateric^{R} or carnauba wax while amounts of other materials and the drug remained unchanged. As shown in Figures 13 to 18, there was an indication that these materials, especially carnauba wax, in various proportions produced additional suppression of drug release (Formulations 17 and 23). However, when the amount of initial drug load was increased (Formulations 18 and 22), the release also increased. There was a significant difference (SNK, $p < 0.05$) in drug release between Formulation 3 and 20 (pectin) or between Formulation 8 and 21 (alginate) at the polymer to drug ratio of 1:2. Tables 19 to 21 show ANOVA results for the test of differences in Aquateeric^{R} containing preparations. In pectin formulations (Figure 15) the release profiles were virtually superimposable for the first 3 hours but after that Aquateric^{R} showed higher rate and extent of drug release. The effect of modifying the proportion of the additives in the formulations was over-shadowed by the resulting change in polymer to drug ratio.

Table 19. ANOVA results summary comparing drug release after 3 hours of dissolution testing pectin-Aquateric[R] formulations.

Group	N
Pure IMC 3h	3
3(30/60) 3h	3
3(16/30) 3h	3
11(30/60) 3h	3
11(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
3(30/60) 3h	40.189	2.0700	1.1951
3(16/30) 3h	20.345	2.8701	1.6571
11(30/60) 3h	42.050	0.1076	0.0621
11(16/30) 3h	20.500	0.1815	0.1048

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	1702.5928	425.6482	161.887	< 0.001
Residual	10	26.2929	2.6293		
Total	14	1728.8858			

Table 20. ANOVA results summary comparing drug release after 6 hours of dissolution testing of pectin-Aquateric[R] formulations.

Group	N
Pure IMC 6h	3
3(30/60) 6h	3
3(16/30) 6h	3
11(30/60) 6h	3
11(16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC 6h	50.267	0.8012	0.4626
3(30/60) 6h	64.892	1.4483	0.8362
3(16/30) 6h	42.308	3.3945	1.9598
11(30/60) 6h	66.374	0.0789	0.0456
11(16/30) 6h	46.891	0.2846	0.1643

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	1418.4607	354.6152	123.566	< 0.001
Residual	10	28.6984	2.8698		
Total	14	1447.1591			

Table 21. ANOVA results summary comparing drug release after 14 hours of dissolution testing of pectin-Aquateric[R] formulations.

Group	N
Pure IMC 14h	3
3(30/60) 14h	3
3(16/30) 14h	3
11(30/60) 14h	3
11(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
3(30/60) 14h	72.110	1.0353	0.5977
3(16/30) 14h	60.672	1.2349	0.7130
11(30/60) 14h	74.505	0.1076	0.0621
11(16/30) 14h	66.081	0.1492	0.0861

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	804.4663	201.1166	382.117	< 0.001
Residual	10	5.2632	0.5263		
Total	14	809.7295			

Table 22. ANOVA results summary comparing drug release after 3 hours of dissolution testing of alginate-Aquateric[R] formulations.

Group	N
Pure IMC 3h	3
8(30/60) 3h	3
8(16/30) 3h	3
14(30/60) 3h	3
14(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
8(30/60) 3h	37.984	0.9473	0.5469
8(16/30) 3h	28.596	0.7566	0.4368
14(30/60) 3h	48.338	0.3666	0.2117
14(16/30) 3h	21.637	0.3441	0.1987

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	1437.8328	359.4582	780.680	< 0.001
Residual	10	4.6044	0.4604		
Total	14	1442.4372			

Table 23. ANOVA results summary comparing drug release after 6 hours of dissolution testing of alginate-Aquateric[R] formulations.

Group	N
Pure IMC 6h	3
8(30/60) 6h	3
8(16/30) 6h	3
14(30/60) 6h	3
14(16/30) 6h	3

Group	Mean	Std Dev	SEM
Pure IMC 6h	50.181	0.7161	0.4134
8(30/60) 6h	66.270	2.6394	1.5238
8(16/30) 6h	56.227	1.2910	0.7453
14(30/60) 6h	71.318	0.2685	0.1550
14(16/30) 6h	47.356	0.1492	0.0861

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	1268.9419	317.2355	171.664	< 0.001
Residual	10	18.4800	1.8480		
Total	14	1287.4219			

Table 24. ANOVA results summary comparing drug release after 14 hours of dissolution testing of alginate-Aquateric[R] formulations.

Group	N
Pure IMC 14h	3
8(30/60) 14h	3
8(16/30) 14h	3
14(30/60) 14h	3
14(16/30) 14h	3

Group	Mean	Std Dev	SEM
Pure IMC 14h	54.556	0.0298	0.0172
8(30/60) 14h	73.316	0.7241	0.4181
8(16/30) 14h	68.941	0.7752	0.4476
14(30/60) 14h	74.625	0.0895	0.0517
14(16/30) 14h	59.535	4.2462e-007	2.4515e-007

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	927.3973	231.8493	> 1000.0	< 0.001
Residual	10	2.2684	0.2268		
Total	14	929.6656			

3.4.5. Effect of polymer type on drug release.

A comparison of pectin and alginate containing microparticles was performed. Release profiles shown in Figures 18 and 19 were analysed for variance (ANOVA) and then compared pair-wise (SNK). The comparisons were done at three dissolution testing time points for formulations with corresponding polymer to polymer and polymer to drug ratios. Tables 25 to 30 summarize ANOVA output for the comparisons carried out (SNK results are in Appendix 3). Overall, there were significant differences ($p < 0.001$) between pectin and alginate formulations to conclude the existence of different rate and extent of release from the two systems. Formulation 8 (alginate) showed faster release rate and a greater extent of release compared to Formualation 3 (pectin). However, pair-wise comparison showed no difference between Formulation 18 (pectin-carnauba wax) and 22 (alginate carnauba wax) ($p < 0.05$). Formulations 17 and 23 showed no difference in release up to 8 hours, but differed towards the end of the test. This indicated the higher extent of release from alginate preparations.

Table 25.ANOVA results summary for drug release after 3 hours of dissolution testing comparing pectin formulations to those of alginate.

Group	N
Pure IMC 3h	3
3(16/30)	3
17(16/30) 3h	3
18(16/30) 3h	3
19(16/30) 3h	3
20(16/30) 3h	3
8(16/30) 3h	3
21(16/30) 3h	3
22(16/30) 3h	3
23(16/30) 3h	3

Group	Mean	Std Dev	SEM
Pure IMC 3h	43.876	0.7613	0.4395
3(16/30)	20.345	2.8701	1.6571
17(16/30) 3h	15.383	1.4999	0.8660
18(16/30) 3h	19.363	1.0721	0.6190
19(16/30) 3h	19.638	1.7548	1.0131
20(16/30) 3h	21.688	0.2939	0.1697
8(16/30) 3h	28.596	0.7566	0.4368
21(16/30) 3h	27.132	0.0517	0.0298
22(16/30) 3h	18.708	1.0087	0.5824
23(16/30) 3h	14.281	0.1957	0.1130

Source of Variation	DF	SS	MS	F	P
Between Treatments	9	2009.5787	223.2865	131.246	< 0.001
Residual	20	34.0257	1.7013		
Total	29	2043.6044			

Table 26. ANOVA results summary for drug release after 6 hours of dissolution testing comparing pectin formulations to those of alginate.

Group	N
3(16/30) 6h	3
17(16/30) 6h	3
18(16/30) 6h	3
19(16/30) 6h	3
20(16/30) 6h	3
8(16/30) 6h	3
21(16/30) 6h	3
22(16/30) 6h	3
23(16/30) 6h	3

Group	Mean	Std Dev	SEM
3(16/30) 6h	42.308	3.3945	1.9598
17(16/30) 6h	36.158	1.9729	1.1390
18(16/30) 6h	41.516	1.6280	0.9399
19(16/30) 6h	38.329	0.9891	0.5711
20(16/30) 6h	43.566	0.1367	0.0789
8(16/30) 6h	56.227	1.2910	0.7453
21(16/30) 6h	52.679	0.5526	0.3190
22(16/30) 6h	40.672	1.2306	0.7105
23(16/30) 6h	33.953	0.1550	0.0895

Source of Variation	DF	SS	MS	F	P
Between Treatments	8	1281.7364	160.2171	63.881	< 0.001
Residual	18	45.1451	2.5081		
Total	26	1326.8815			

Table 27. ANOVA results summary for drug release after 14 hours of dissolution testing comparing pectin formulations to those of alginate.

Group	N
3(16/30) 14h	3
17(16/30) 14h	3
18(16/30) 14h	3
19(16/30) 14h	3
20(16/30) 14h	3
8(16/30) 14h	3
21(16/30) 14h	3
22(16/30) 14h	3
23(16/30) 14h	3

Group	Mean	Std Dev	SEM
3(16/30) 14h	60.672	1.2349	0.7130
17(16/30) 14h	54.332	0.6940	0.4007
18(16/30) 14h	59.294	1.2255	0.7075
19(16/30) 14h	56.348	0.0298	0.0172
20(16/30) 14h	62.067	0.1790	0.1034
8(16/30) 14h	68.941	0.7752	0.4476
21(16/30) 14h	66.891	1.1052	0.6381
22(16/30) 14h	59.879	0.4303	0.2484
23(16/30) 14h	57.761	0.3115	0.1799

Source of Variation	DF	SS	MS	F	P
Between Treatments	8	536.6281	67.0785	106.926	< 0.001
Residual	18	11.2921	0.6273		
Total	26	547.9201			

3.5. MICROPARTICLE DRUG RELEASE KINETICS.

Most SR systems depend on drug or polymer dissolution or bioerosion, drug diffusion or a combination of dissolution and diffusion to slowly release the drug in the gastrointestinal environment (**34,38**). A variety of drug release mechanisms have been reviewed at length in previous sections (Section 1.4). Data from dissolution studies in pH 6.2 medium was graphically analysed according to the various drug release kinetic models with the intention of identifying the best fitting model to describe IMC release from the microparticles. The effect of formulation modifications on the extent to which a particular model holds was also briefly investigated. Release kinetics were studied in formulations prepared from pectin-gelatin and alginate-gelatin with or without carnauba wax.

Figures 21 to 35 show the release of IMC according to different release models; zero order, planar matrix, first order, square root of time, cube root law and spherical matrix release models. Tables 28 to 34 summarize the linear correlation results for the individual curves. The highest correlation coefficients (r) recorded indicate that the microparticles released the drug according to the spherical matrix release model, as high as $r > 0.9900$ for some formulations.

Zero order release model: The linear correlation values from ploting the fraction of IMC released against time are summarized in Table 28. In both the pectin and alginate formulations, the larger particles showed higher zero order correlation than the smaller ones. Alginate formulations containing carnauba wax displayed fairly high zero order correlation ($r > 0.9300$) than those without carnauba wax. In pectin formulations there was no apparent difference between preparations with carnauba wax and those without. However, most formulations showed poor correlation with the zero order release model.

For zero order release to prevail, the membrane thickness (diffusion path) shoud remain constant and the concentration difference should be such that the saturation concentration is maintained at the microparticle surface. Equation 14 shows the relationship between these factors and drug release. It is therefore logical that smaller particles with a greater surface area could be subjected to a faster depletion of the core contents, which leads to an exponential release mechanism (**34,61,67-70**). Although zero order release is always aimed at when designing SR dosage forms, it is conceivable that other factors intrinsic to the polymers and the drug may render the release non-zero order.

Zero order results when drug release is proportional to time. When n is equal to 1 (in Equation 5), then the following relationship which describes zero order release kinetics is obtained:

$$\frac{M_t}{M_\infty} = k_m t \qquad (29)$$

Ploting percent amount released against time would give a straight line with slope k_m, representing the release constant, and a y intercept of zero, representing amount of drug released at time zero.

Table 28. Correlation values according to the zero order release model plot (Equation 29).

Formulation.		Correlation values.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC	-	0.0349	0.6817	0.3537
Pectin.				
3	30/60	0.0563	0.8250	0.3460
	16/30	0.0598	0.9305	0.0917
17.	30/60	0.0637	0.8677	0.2326
	16/30	0.0556	0.9432	0.0495
18.	30/60	0.0557	0.8352	0.3015
	16/30	0.0596	0.9253	0.0777
19.	30/60	0.0602	0.8893	0.2450
	16/30	0.0540	0.9381	0.0978
20.	30/60	0.0562	0.8022	0.3709
	16/30	0.0599	0.9292	0.1085
Alginate.				
8.0	30/60	0.0610	0.8337	0.3037
	16/30	0.0648	0.8778	0.1800
21.	30/60	0.0621	0.8618	0.2626
	16/30	0.0637	0.8875	0.1538
22.	30/60	0.0621	0.8409	0.2794
	16/30	0.0605	0.9359	0.0719
23.	30/60	0.0634	0.8538	0.2487
	16/30	0.0613	0.9641	0.0088

Planar matrix release model: The correlation coefficients (Table 29) obtained with the planar matrix release model were similar to those obtained with the zero order model. Figures 21 to 23 show the relatively poor linear correlation obtained from applying the planar matrix model to IMC release data. According to Equation 5, Fickian drug release is observed when $n = 0.5$ and zero order release is observed as n approaches 1 (**60**). Taking the natural logarithm on both sides of Equation 5 gives:

$$\ln \left(\frac{M_t}{M_\infty}\right) = \ln \; k_m + (n) \ln \; t \qquad (30)$$

Ploting the natural logarithm of the percent of drug released against the natural logarithm of time shouldl give a straight line with slope n, and y-intercept corresponding to the drug release rate k_m. In Table 29 the slope values ($0.5 < n < 1$ and $n > 1$) show that drug release involved non-Fickian mechanism. The y-axis intercept which corresponds to the rate of drug release, k_m, would show the differences between release rates observed from various formulations. However, relatively fast drug release within the first 4 to 6 hours followed by a slower phase has resulted in poor linear correlation.This may be reflection of the possible effect of aggregation of the microparticles (Figure 8) which results in a heterogenous drug distribution within the matrix leading to non-constant drug release rate. This model could be useful in determining the drug release rate constants of the formulations where zero order applicatons are limited or impaired. The formulations with n values close to 1, like Formulations 3, 17, 19, 20, 22 and 23 in the 16/30 size category, were also found to approximate zero order release as shown in Table 28.

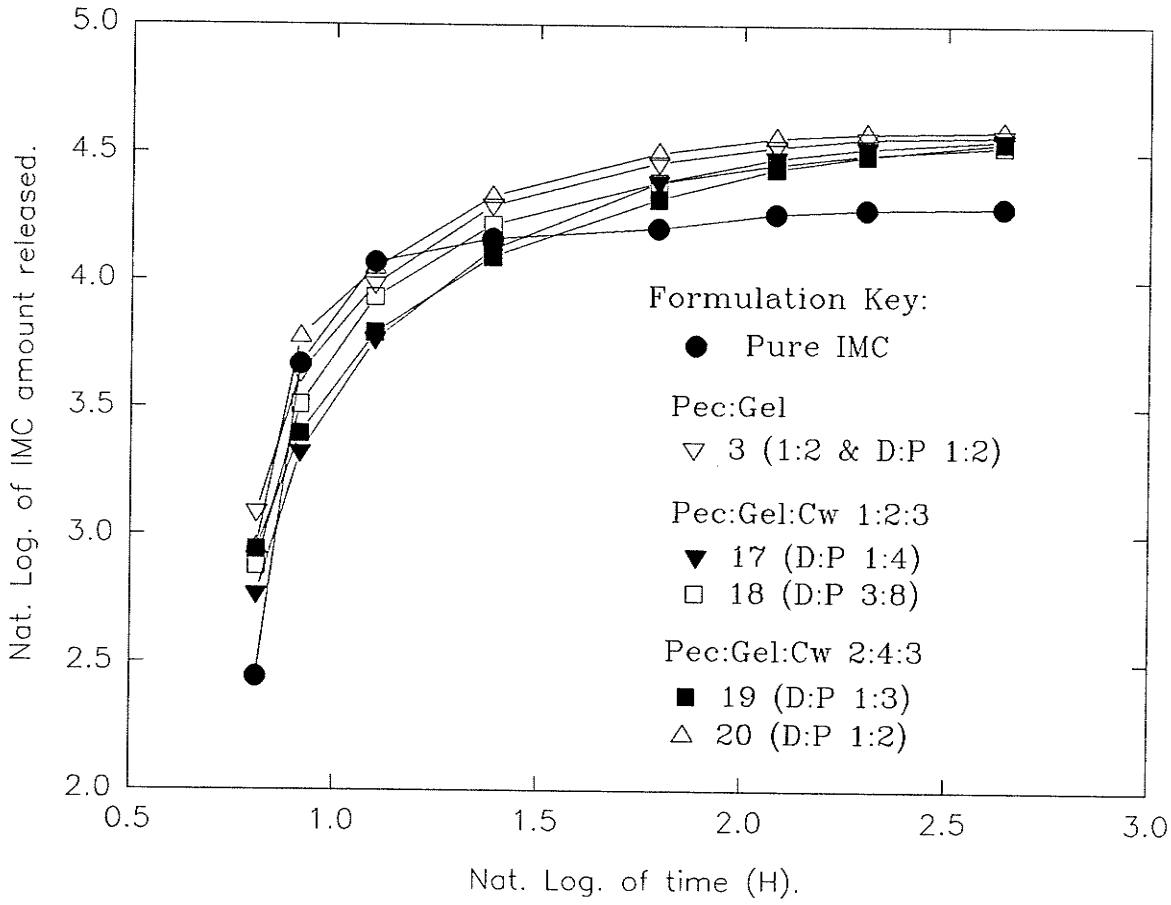


Figure 21. Plot of the natural logarithm of IMC amount released against the natural logarithm of time (planar matrix model) - size 30/60 pectin formulations.

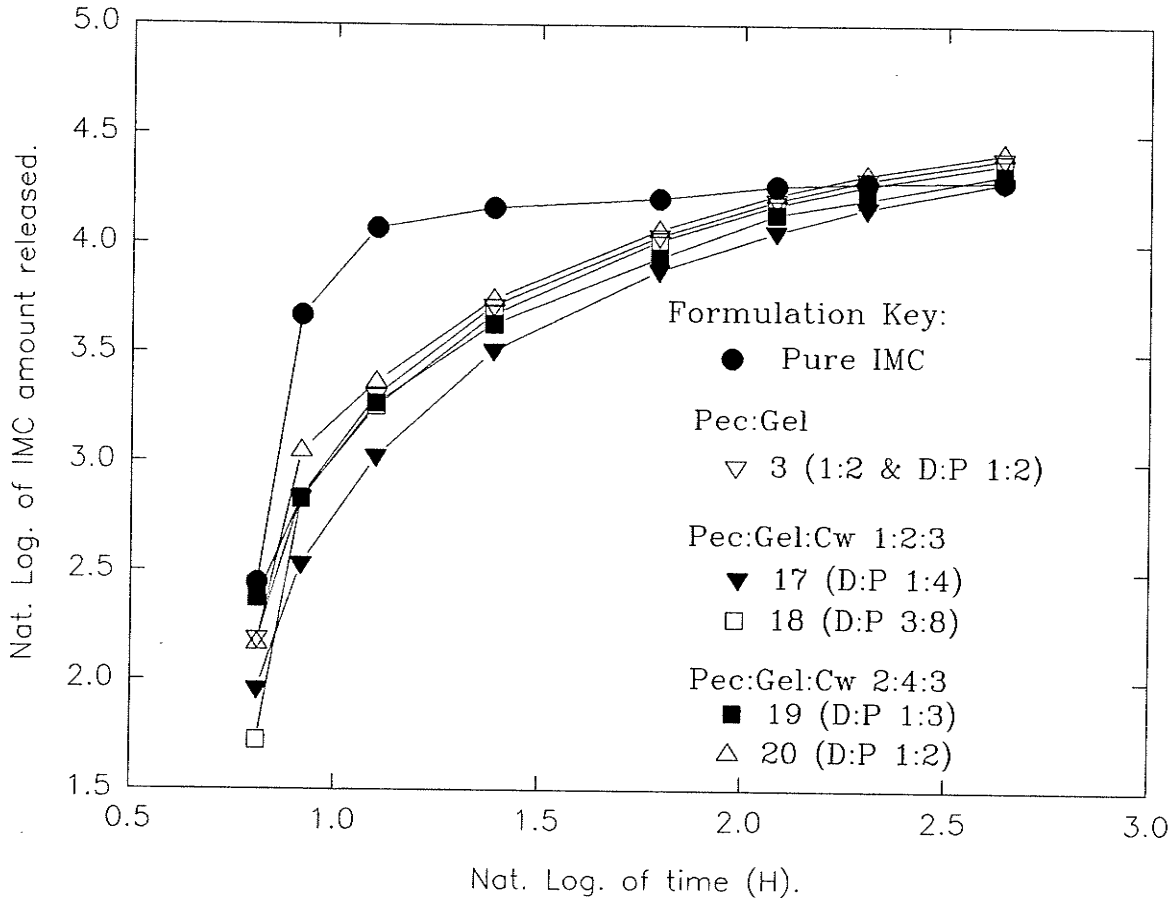


Figure 22. Plot of the natural logarithm of IMC amount released against the natural logarithm of time (planar matrix model) - size 16/30 pectin formulations.

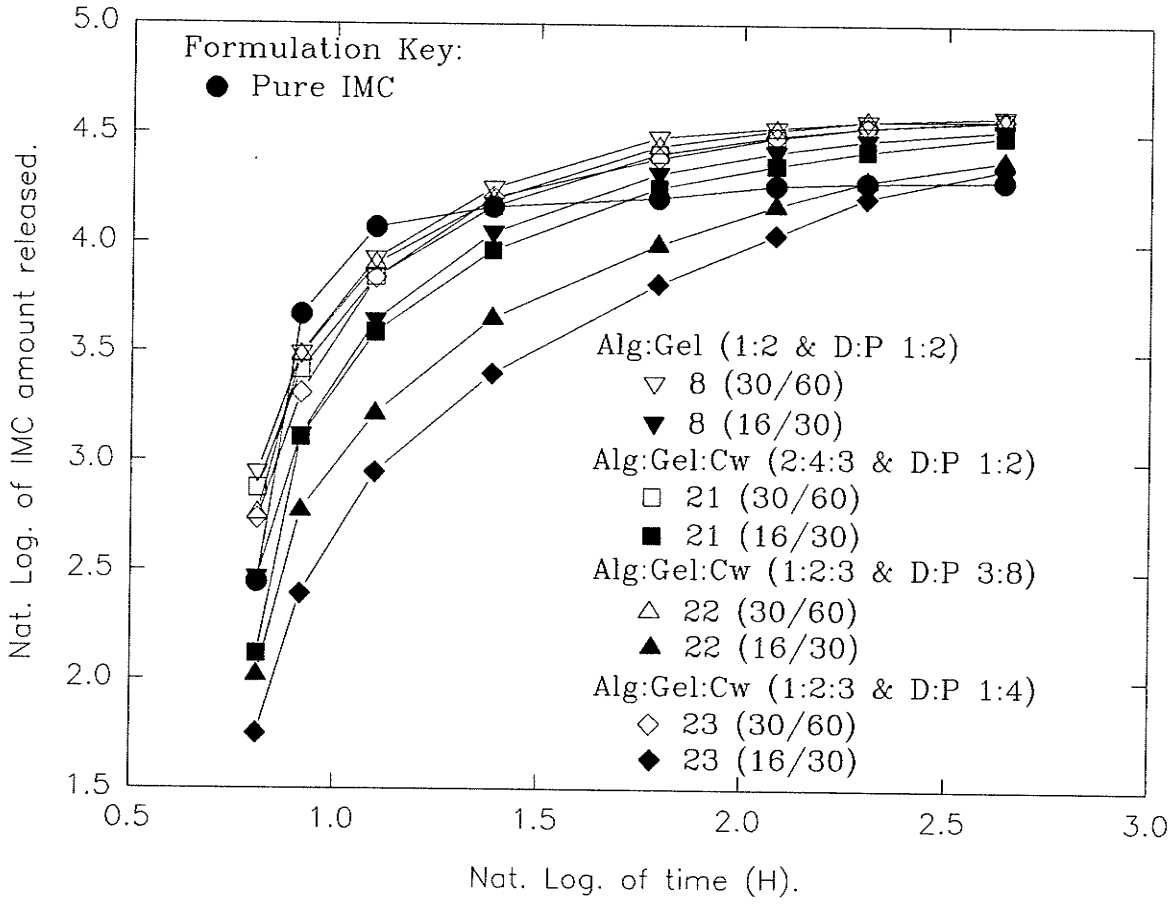


Figure 23. Plot of the natural logarithm of IMC amount released against the natural logarithm of time (planar matrix model) - alginate formulations.

Table 29. Linear correlation values according to the planar matrix release model (Equation 30).

Formulation.		Correlation values.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC	-	0.6405	0.6885	2.8793
Pectin.				
3.	30/60	0.6850	0.8651	3.0223
	16/30	1.0684	0.9207	1.8793
17.	30/60	0.8618	0.8924	2.5850
	16/30	1.1654	0.9347	1.5257
18.	30/60	0.7409	0.8557	2.8431
	16/30	1.1809	0.8838	1.6159
19.	30/60	0.7796	0.9101	2.7315
	16/30	0.9786	0.9402	1.9925
20.	30/60	0.6931	0.8220	3.0364
	16/30	1.0315	0.9046	1.9871
Alginate.				
8.	30/60	0.7714	0.8711	2.8393
	16/30	0.9728	0.8870	2.2828
21.	30/60	0.8067	0.8879	2.7223
	16/30	1.0516	0.8642	2.0745
22.	30/60	0.8206	0.8598	2.7171
	16/30	1.1313	0.9190	1.7195
23.	30/60	0.8621	0.8733	2.6028
	16/30	1.2917	0.9425	1.2581

Cube root law release model: The cube root law drug release model (Equation 8) is a modification of Equation 4 which accounts for change in particle area in relation to weight of spherical microparticles (**34,61**). Therefore, this model is closely related to the zero order release model. That is, the microparticle system should display a constant diffusion path length and perfect sink conditions should be maintained throughout the dissolution process. Relatively poor linear correlation was obtained with this model as shown in Figures 24 to 26 and Table 30. This model has better application when the drug release is suspected to be solubility dependent, as in a drug with poor solubility. The solubility of IMC in pH 6.2 medium was therefore not a rate limiting step in drug release.

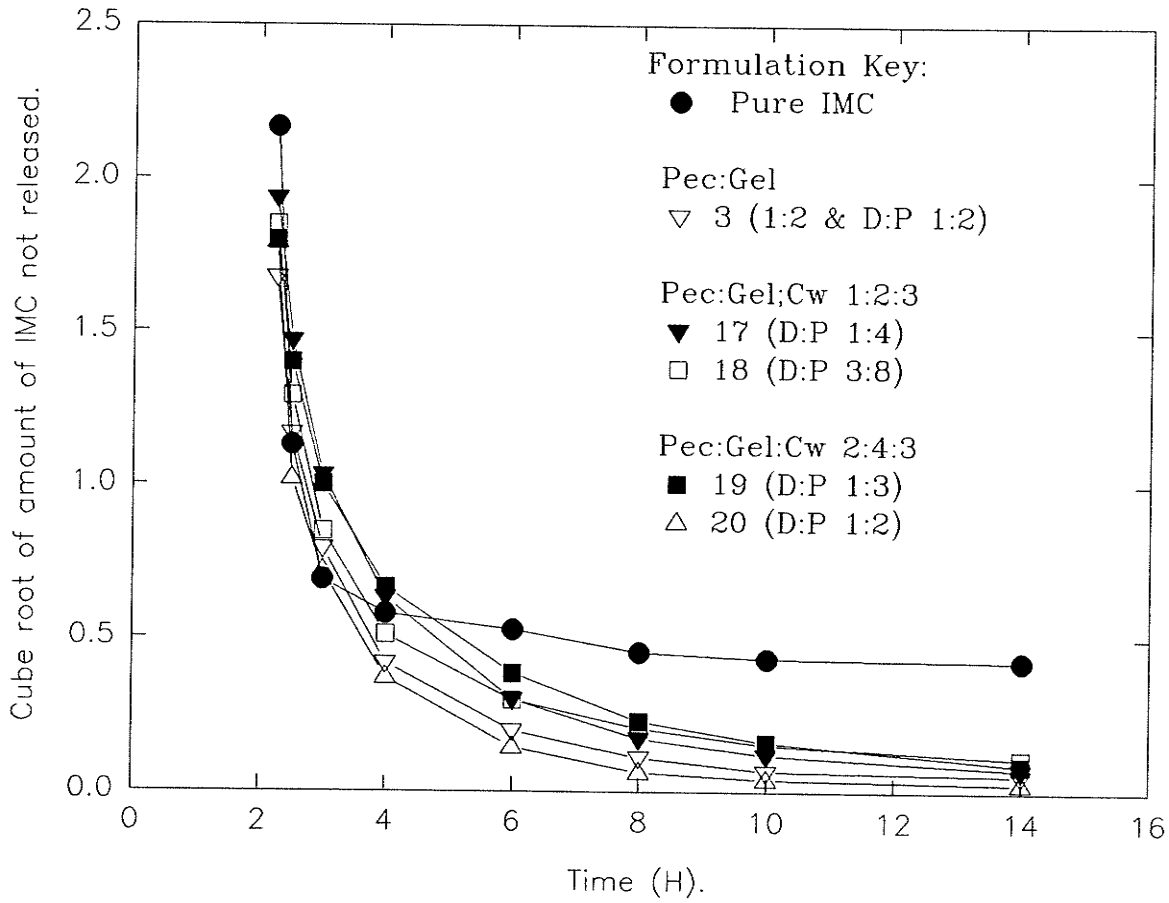


Figure 24. Plot of the cube root of the IMC amount not released against time (cube root law release model) - size 30/60 pectiin formulations.

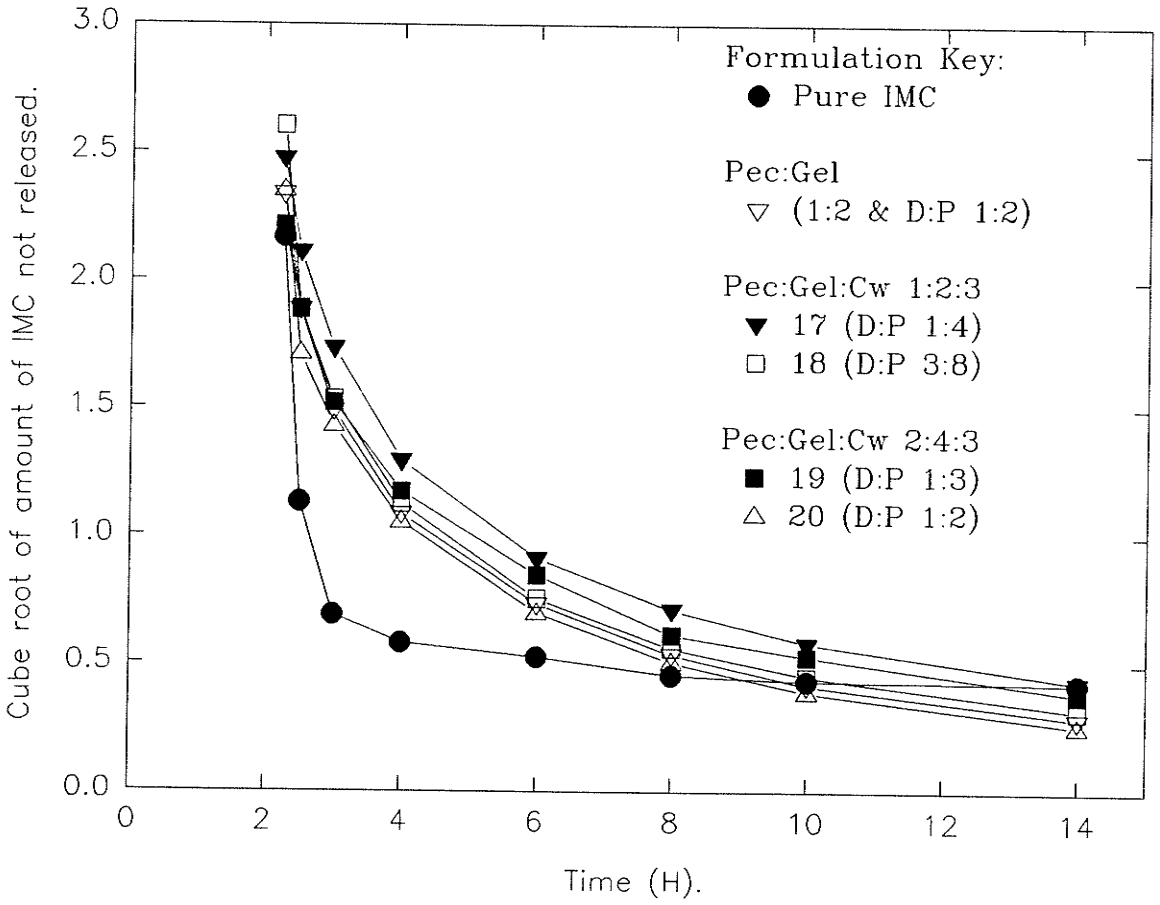


Figure 25. Plot of the cube root of the IMC amount not released against time (cube root law release model) - size 16/30 pectin formulations.

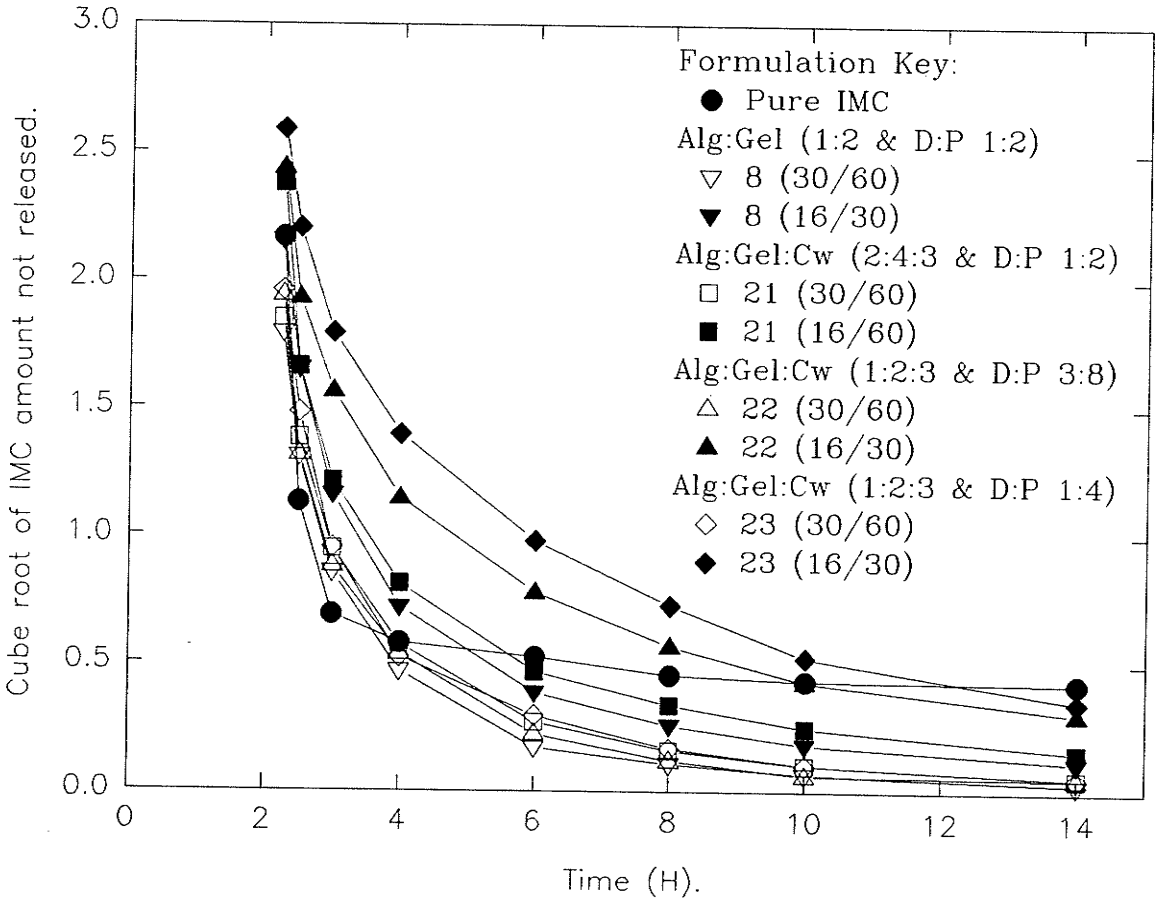


Figure 26. Plot of the cube root of the IMC amount not released against time (cube root law release model) - alginate formulations.

Table 30. Linear correlation values according to the cube root law release model (Equation 8).

Formulation.		Correlation values.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC	-	-0.0868	0.6079	1.3404
Pectin.				
3.	30/60	-0.1109	0.7751	1.2496
	16/30	-0.1526	0.8648	2.0480
17.	30/60	-0.1349	0.8126	1.5553
	16/30	-0.1585	0.8802	2.2646
18.	30/60	-0.1161	0.7745	1.3801
	16/30	-0.1607	0.8415	2.1522
19.	30/60	-0.1254	0.8359	1.4950
	16/30	-0.1415	0.8823	2.0248
20.	30/60	-0.1105	0.7395	1.2092
	16/30	-0.1496	0.8567	1.9791
Alginate.				
8.	30/60	-0.1230	0.7824	1.3651
	16/30	-0.1466	0.8139	1.7398
21.	30/60	-0.1282	0.8070	1.4651
	16/30	-0.1517	0.8069	1.8526
22.	30/60	-0.1284	0.7780	1.4403
	16/30	-0.1593	0.8676	2.1344
23.	30/60	-0.1339	0.7930	1.5248
	16/30	-0.1751	0.9000	2.4086

Firt order drug release model: First order drug release, as shown in Equation 17, represents an exponential increase of the amount of the drug in the release medium. This release mechanism is a result of continuous change in saturation concentration (non-sink conditions) and change in diffusion path due to core depletion (**61,72**). All formulations displayed good correlation ($r > 0.9500$) with this model except for Formulation 21 which displayed a terminal phase much slower than the fast initial phase (Figures 27 to 29). It was not uncommon to find small particles with higher correlation than their larger counter-parts, as in Formulations 8 and 19. This was probably due to increased surface area in smaller particles. The curves obtained have a faster initial phase followed by a slower terminal phase, indicative of a biexponential release mechanism described by Equation 26. Each of the phases displays a release rate proportional to the experimental drug load of the microspheres (**58**). In Figure 28, Formulations 3 and 20 with 30 % and 29% experimental drug load, respectively exhibited a fast initial phase. In contrast, Formulations 17 and 18 showed a relatively slower initial phase. This phenomenon has important implications in drug dosing to ensure adequate therapeutic plasma levels. A fast release rate may produce toxic plasma levels for a drug with a narrow therapeutic index but may also be necessary for drugs that undergo first pass metabolism.

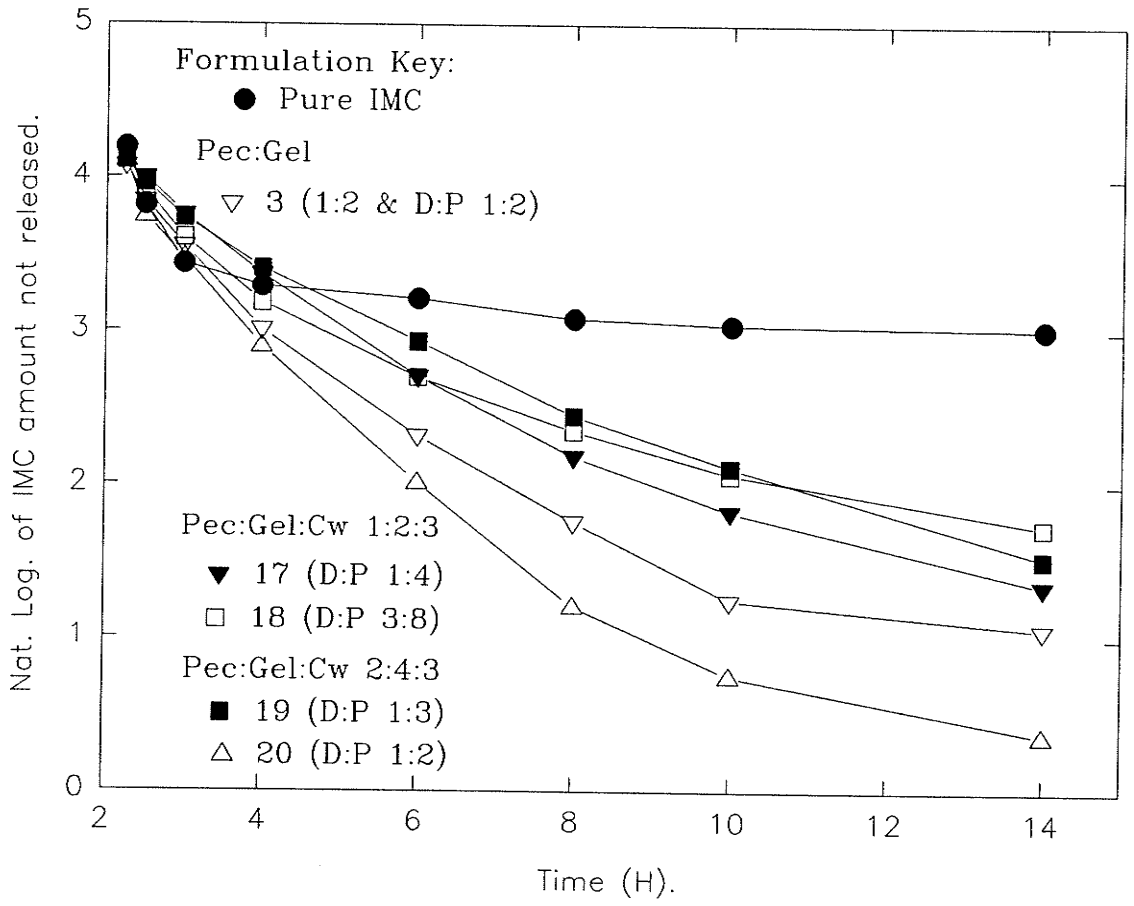


Figure 27. Plot of the natural logarithm of IMC amount not released against time (first order release model) - size 30/60 pectin formulations.

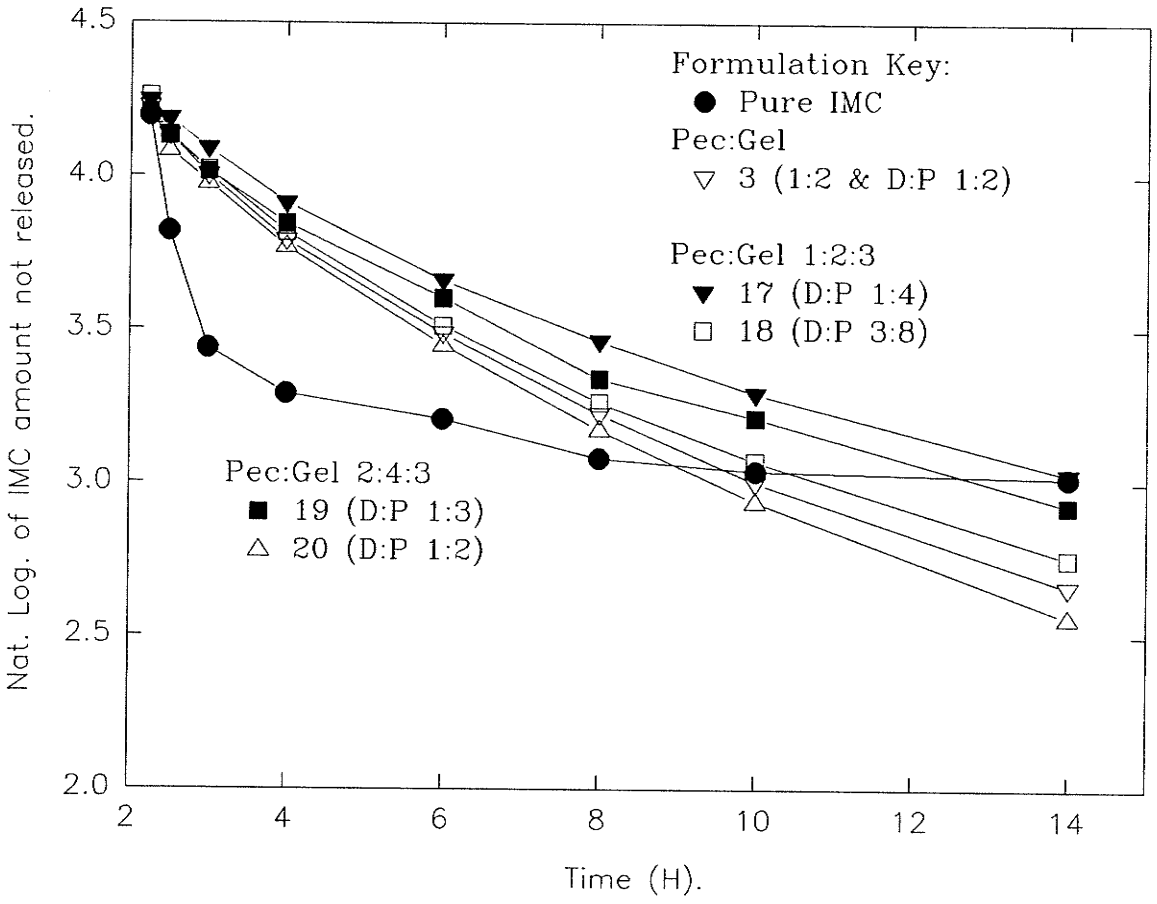


Figure 28 Plot of the natural logarithm of IMC amount not released against time (first order release model) - size 16/30 pectin formulations.

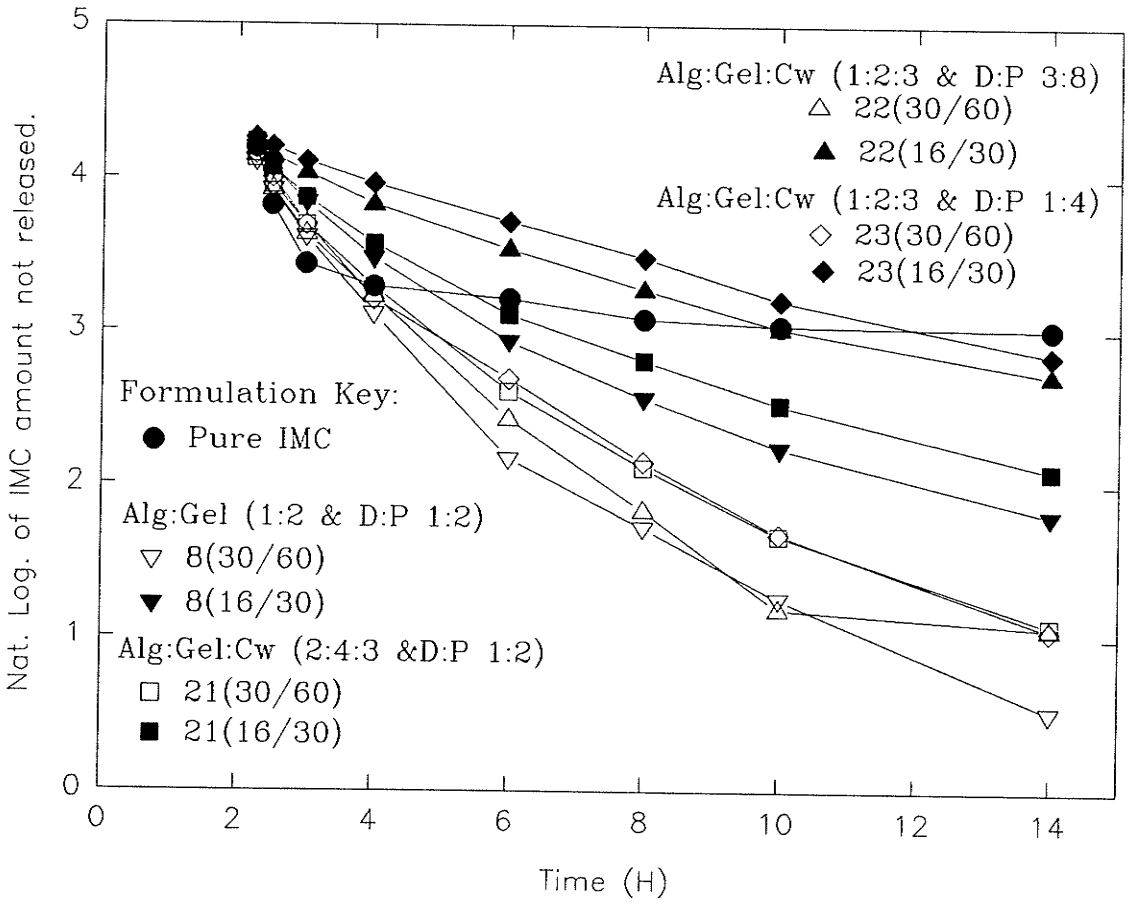


Figure 29. Plot of the natural logarithm of IMC amount not released against time (first order release model) - alginate formulations .

Table 31. Linear correlation values according to first order release model plot (Equation 17).

Formulation.		Correlation parameters.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC		-0.0770	0.7683	3.8649
Pectin.				
3	30/60	-0.2680	0.9508	4.2718
	16/30	-0.1332	0.9841	4.3933
17	30/60	-0.2456	0.9737	4.4403
	16/30	-0.1046	0.9803	4.3841
18	30/60	-0.2012	0.9523	4.2081
	16/30	-0.1268	0.9802	4.3917
19	30/60	-0.2202	0.9857	4.3968
	16/30	-0.1093	0.9810	4.3386
20	30/60	-0.3289	0.9576	4.3640
	16/30	-0.1390	0.9862	4.3909
Alginate.				
8	30/60	-0.3092	0.9747	4.4737
	16/30	-0.2065	0.9736	4.4220
21	30/60	-0.2628	0.9147	4.4493
	16/30	-0.1800	0.9794	4.4026
22	30/60	-0.2795	0.9561	4.4191
	16/30	-0.1308	0.9853	4.4121
23	30/60	-0.2648	0.9809	4.4740
	16/30	-0.1210	0.9959	4.4744

Square root of time drug release model: The square root of time model (Equation 20) is based on the original models suggested by Higuchi (**66,74**) to describe drug release from matrix systems. Larger particles showed higher correlation than smaller ones. Even then the correlation values were only marginally high among the alginate formulations (Figures 30 to 32 and Table 32). The possible existence of different morphologies of particles (Figure 8) may contribute to the low correlation with this model. That is, although there is high correlation to the spherical matrix model, which suggests matrix type of microparticles, this could be due to cross-linking and aggregation. The poor correlation shown by smaller particles suggests a non-matrix type release mechanism. Furthermore, there could be some defects on the microparticle surface like cracks which alter the diffusion coefficient of the drug across the membrane (**60**). The aggregation of smaller particles into larger ones might have decreased the effect of these defects. Alginate formulations containing carnauba wax displayed higher correlation than those without. The presence of carnauba wax might have reduced the porosity of the matrix and stabilize drug diffusion parameters. A similar observation was made for pectin formulations, except in situations where the drug load was high (Formulation 20).

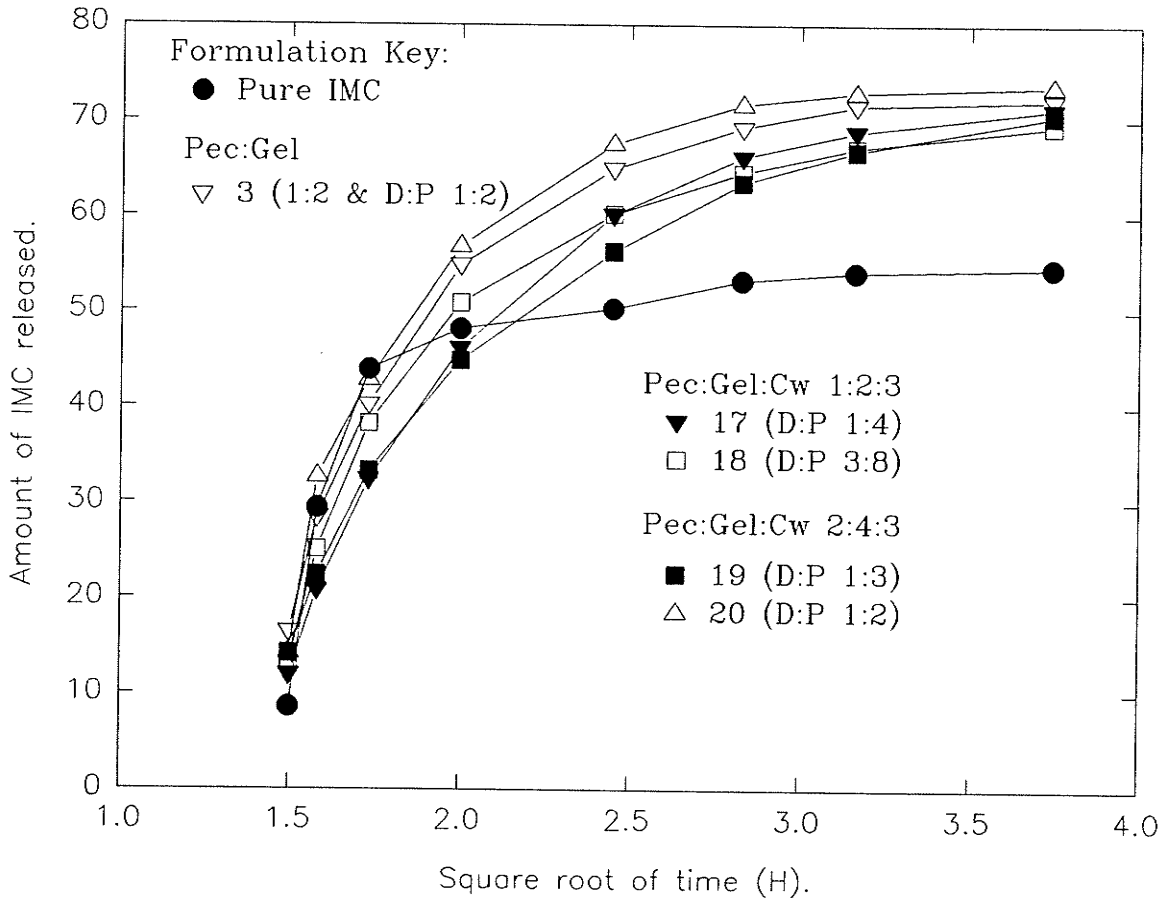


Figure 30. Plot of the IMC amount released against the square root of time (square root of time release model) - size 30/60 pectin formulations.

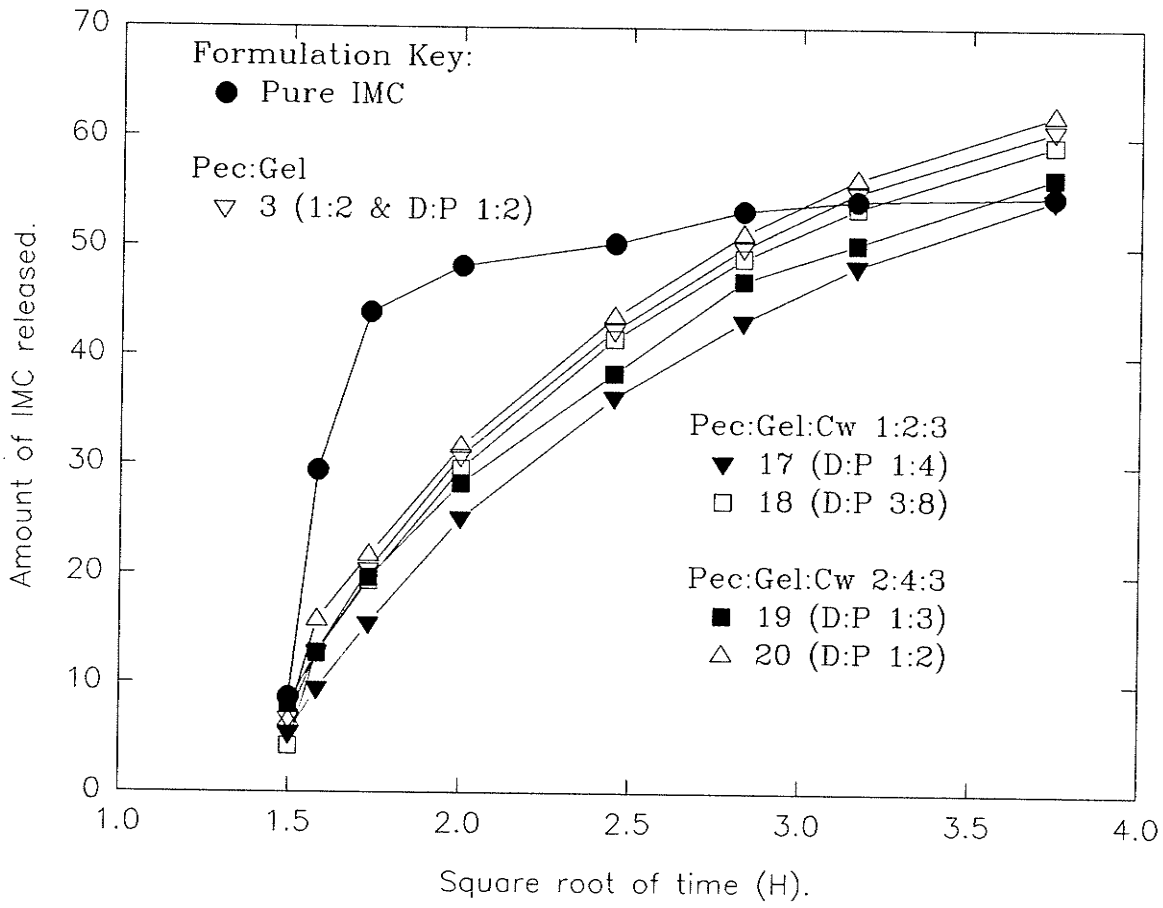


Figure 31. Plot of the IMC amount released against the square root of time (square root of time release model) - size 16/30 pectin formulations.

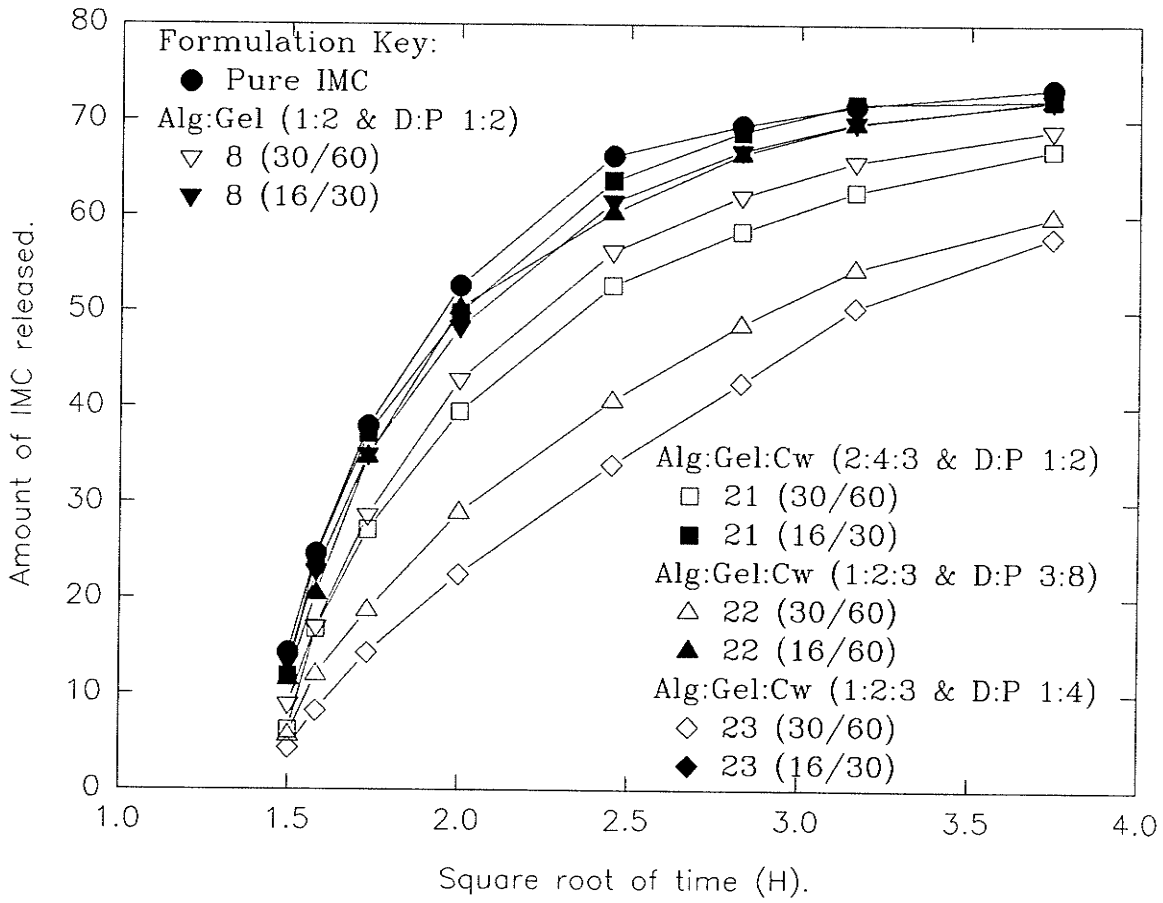


Figure 32. Plot of the IMC amount released against the square root of time (square root of time release model) - alginate formulations.

Table 32. Linear correlation values according to the square root of time release model plot (Equation 20).

Formulation.		Correlation values.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC	-	14.655	0.7430	7.9877
Pectin.				
3.	30/60	23.235	0.8833	-2.9711
	16/30	23.985	0.9671	-22.082
17.	30/60	26.056	0.9197	-14.699
	16/30	22.193	0.9761	-23.082
18.	30/60	22.895	0.8903	-5.7755
	16/30	23.926	0.9630	-23.180
19.	30/60	24.434	0.9810	-11.571
	16/30	21.603	0.9724	-18.756
20.	30/60	23.312	0.8623	-1.3067
	16/30	24.002	0.9657	-20.923
Alginate.				
8.	30/60	25.164	0.8911	-8.5012
	16/30	26.422	0.9275	-18.994
21.	30/60	25.423	0.9145	-11.683
	16/30	25.871	0.9342	-20.173
22.	30/60	25.565	0.8972	-10.770
	16/30	24.214	0.9710	-23.880
23.	30/60	25.956	0.9069	-13.425
	16/30	24.237	0.9889	-28.317

Spherical matrix drug release model: The spherical matrix drug release model (Equation 24) eliminated the need to determine the particle radius required in the Higuchi model shown in Equation 23 (**58**). This release model has been used to describe drug release from biodegradable microspheres (**74,77**). The highest correlation coefficients (*r* up to 0.9970) were obtained with this model, as shown in Figures 33 to 35 and Table 33. The lower values obtained from smaller particles were probably due to existence of more than one release phase. As observed with the first order phase, this model also demonstrated the presence of a fast initial release phase and a slow terminal release phase (Figures 33 and 35). The relatively poor correlation displayed by small particles suggests that the matrix behaviour shown by larger particles is due to aggregation. Small particles probably formed slab-like aggregates from which drug release would not be fully described by this model. Correlation with this model strongly suggests the lack of a hydrodynamic diffusion layer at the surface of the particles, as it would be observed for reservoir type systems. Therefore, drug release from the microparticles was influenced by the particle morphology, porosity and other matrix properties resultiing from aggregation and drug distribution in the matrix.

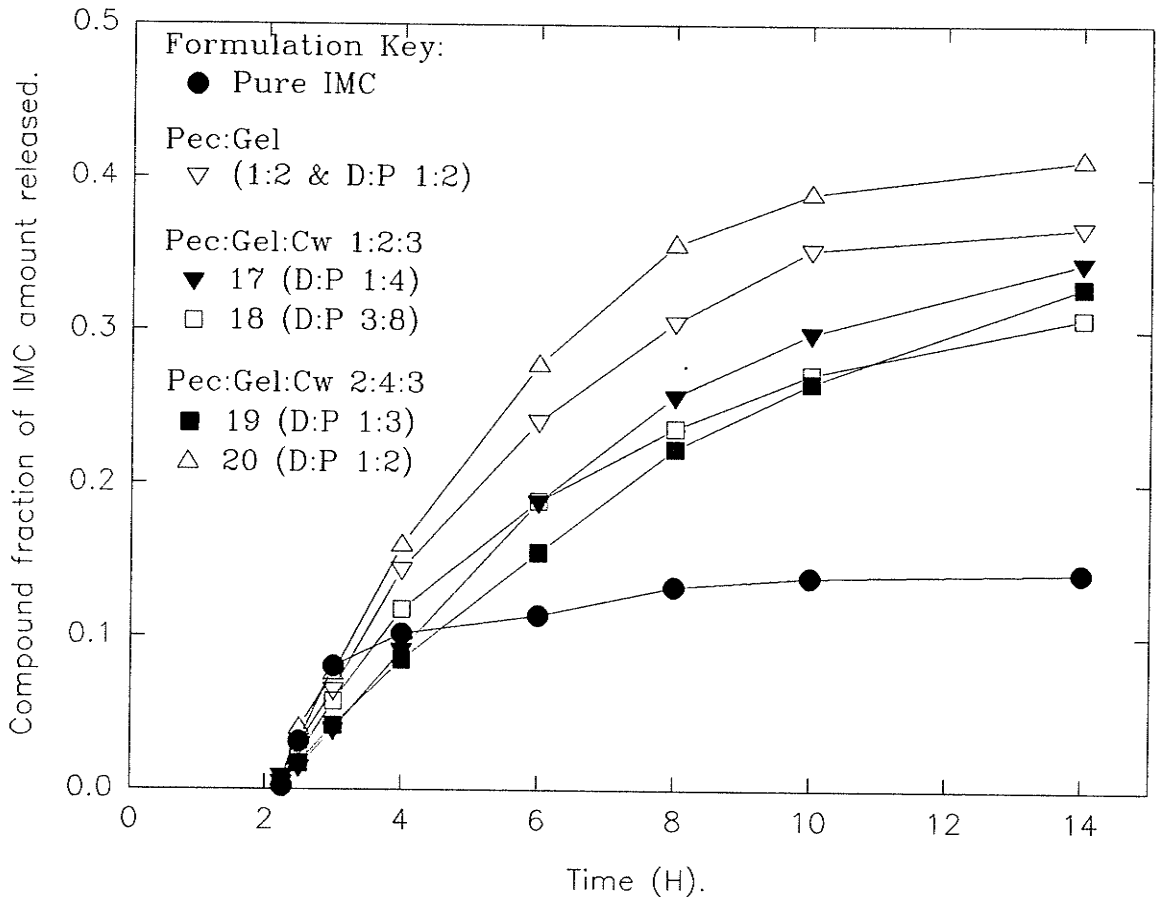


Figure 33. Plot of a compound fraction of IMC amount released against time (spherical release model plot) - size 30/60 pectin formulations.

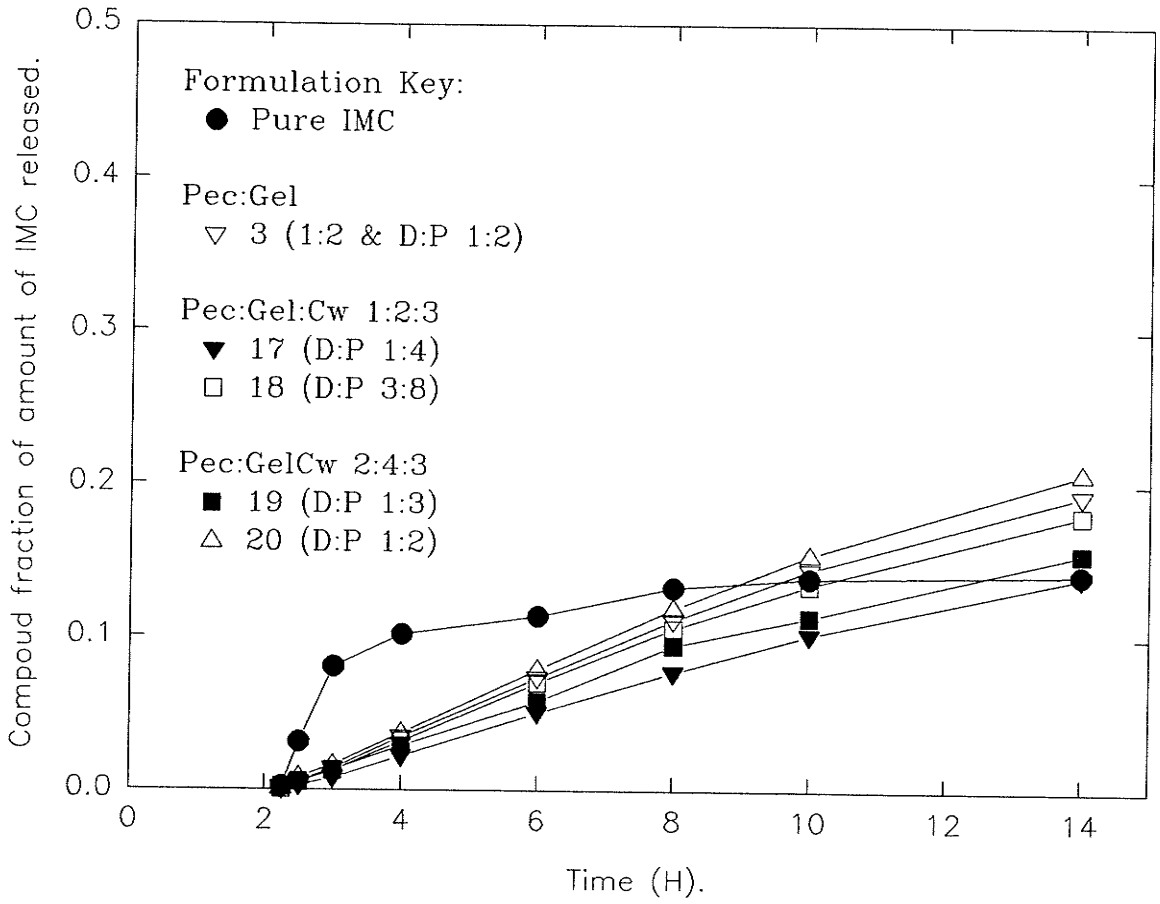


Figure 34. Plot of a compound fraction of IMC amount released against time (spherical release model plot) - size 16/30 pectin formulations.

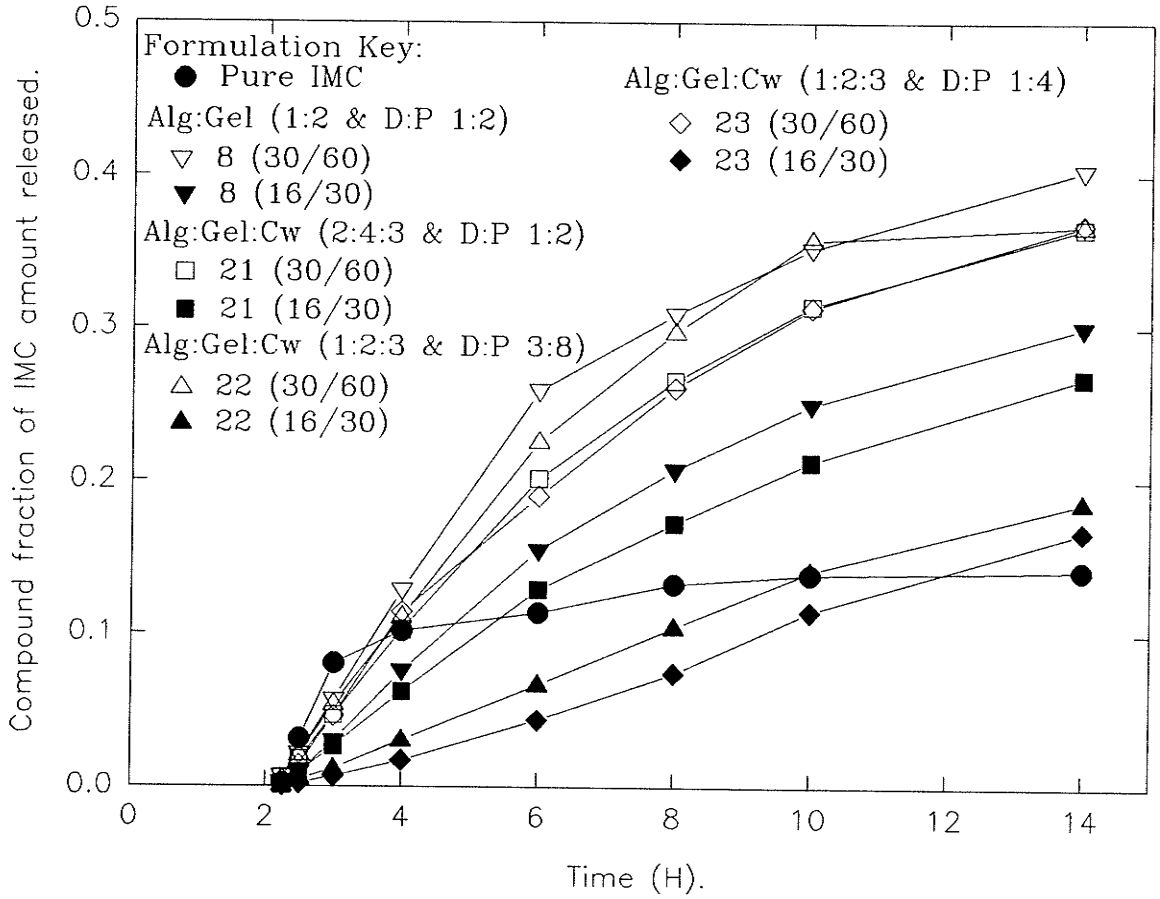


Figure 35. Plot of a compound fraction of IMC amount released against time (spherical release model plot) - alginate formulations.

Table 33. Linear correlation values according to the spherical matrix release model (Equation 24).

Formulation.		Correlation values.		
Type and No.	Size.	Slope.	r.	Intercept.
IMC	-	0.0100	0.8105	0.0307
Pectin.				
3.	30/60	0.0326	0.9336	-0.0139
	16/30	0.0170	0.9954	-0.0333
17.	30/60	0.0311	0.9650	-0.0388
	16/30	0.0123	0.9973	-0.0263
18.	30/60	0.0264	0.9498	-0.0136
	16/30	0.0159	0.9946	-0.0311
19.	30/60	0.0286	0.9820	-0.0373
	16/30	0.0135	0.9944	-0.0252
20.	30/60	0.0364	0.9276	-0.0118
	16/30	0.0181	0.9960	-0.0346
Alginate.				
8.	30/60	0.0357	0.9484	-0.0360
	16/30	0.0269	0.9747	-0.0388
21.	30/60	0.0325	0.9669	-0.0372
	16/30	0.0236	0.9835	-0.0368
22.	30/60	0.0339	0.9435	-0.0313
	16/30	0.0165	0.9956	-0.0342
23.	30/60	0.0325	0.9700	-0.0384
	16/30	0.0145	0.9971	-0.0371

3.6. EFFECT OF MICROPARTICLE AGING ON DISSOLUTION.

During aging important dosage form physicochemical charateristics may undergo changes that could affect the bioavailability of the drug. Dissolution rate is one of these important physicochemical characteristics. The extent to which a preparation maintains the dissolution characteristics within specified limits from time of manufacture until expiration date defines the dissolution stabilty of the drug product (**136**). Storage conditions, manufacturing processes, formulation variation and packaging, are among several factors that influence dissolution stability.

The effect of aging on microparticle dissolution was studied at both room conditions (23.2 ± 0.1°C and 21% RH) and in an oven (40.0 ± 0.1°C and 15% RH). Figures 36 - 39 show the IMC release profiles comparing drug release before and after exposure to storage conditions described above. Tables 34 - 37 summarize ANOVA and multiple comparison results for the amount of drug release after 6 h of dissolution testing, for the formulations studied. the alginate Formulation 8 was not significantly affected by the storage conditions in the given time. Formulation 3 and 17 (pectin) showed a shift in the dissolution curve over time. Drug release decreased over the 60 days of storage at the stated conditions. The shift could be an indication of either loss of entrapped moisture or a curing effect phenomenon which is not uncommon in SR preparations (**136**). However, Formulation 17 (pectin-carnauba wax), after 30 days at room conditions did not show a significantly different dissolution profile. Drug release from Formulation 21 (alginate-carnauba) showed an initial increase over the 30 day storage period followed by a decrease noted after 60 days. The changes observed in the drug release profiles necessitate that a curing step to be included in the preparation process to stabilize the formulations.

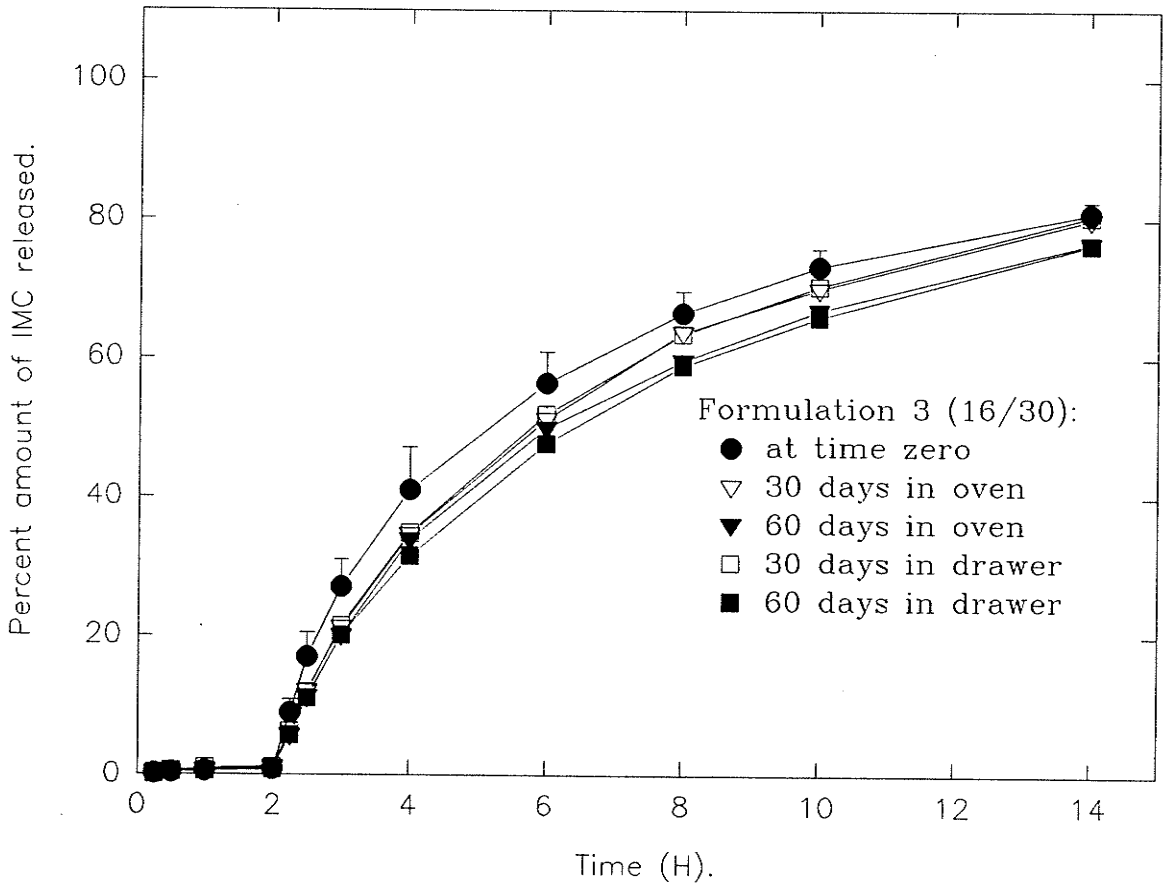


Figure 36. Drug release profiles from Formulation 3 at different storage conditions over time.

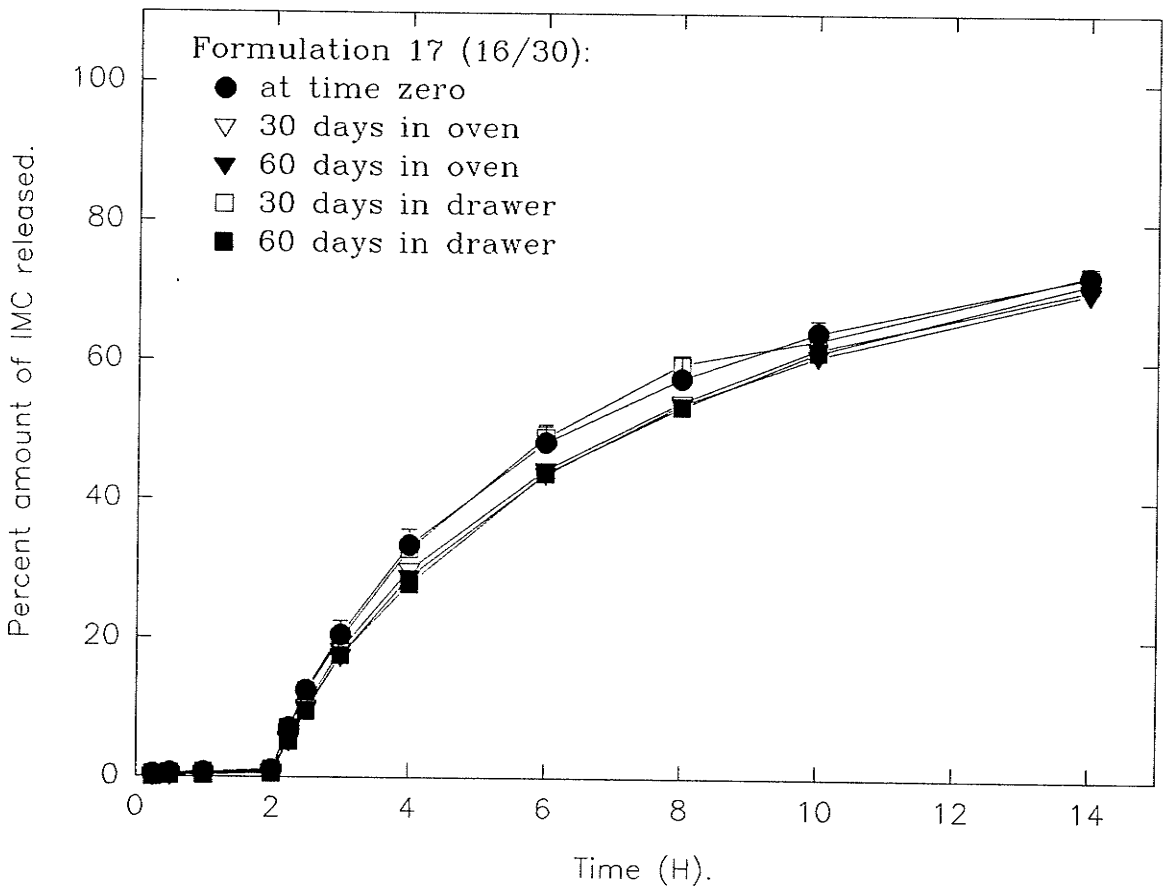


Figure 37. Drug release profiles from Formulation 17 at different storage conditions over time.

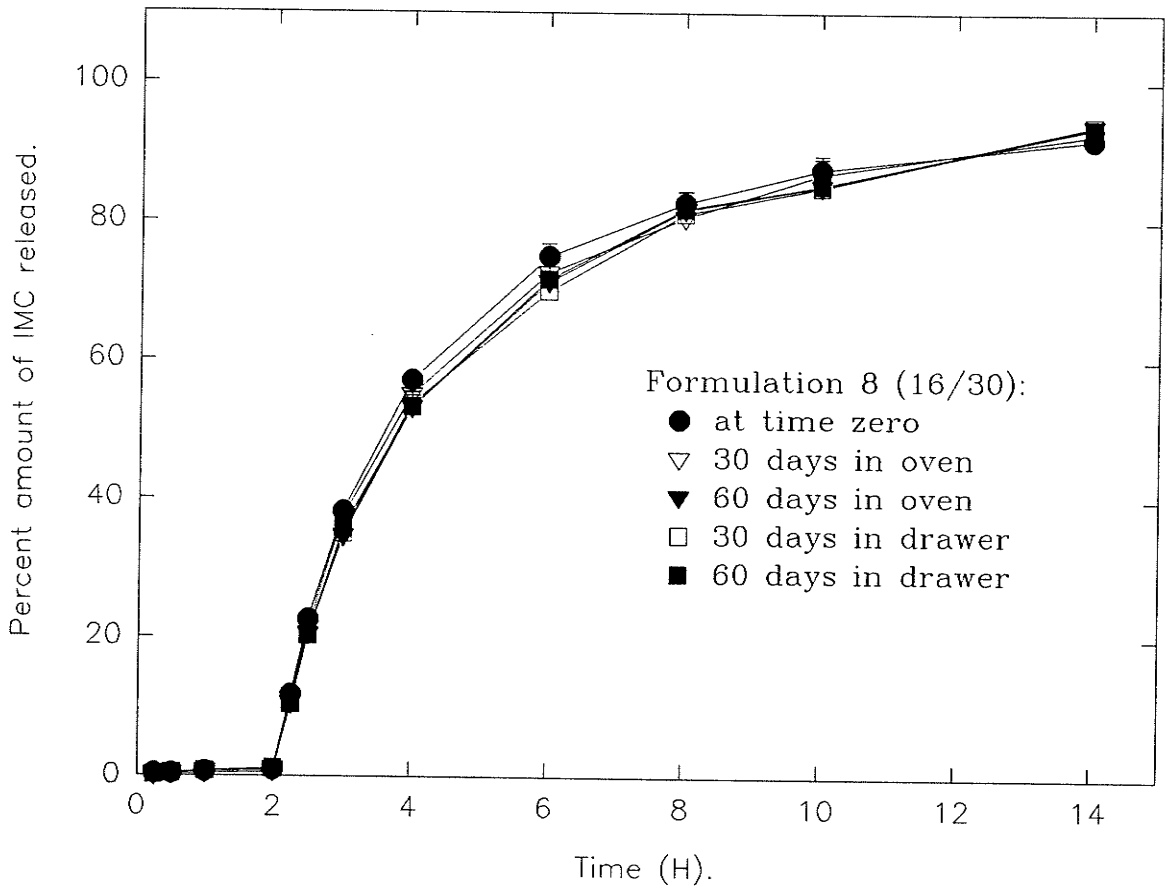


Figure 38. Drug release profiles from Formulation 8 at different storage conditions over time.

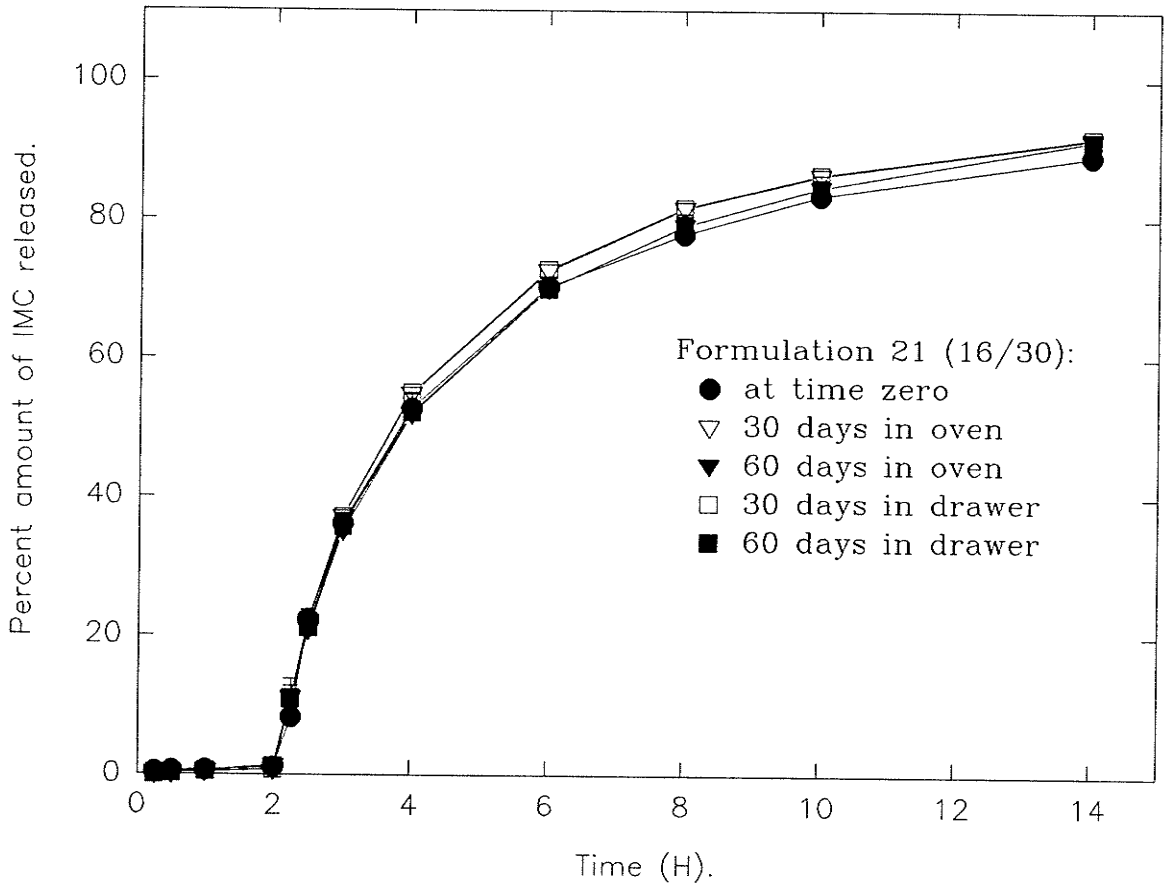


Figure 39. Drug release profiles from Formulation 21 at different storage conditions over time.

Table 34. Summary of ANOVA results comparing drug release after 6 hours from Formulation 3 at different storage conditions over time.

Group	N
3 6h	3
S3 6h	3
SS3 6h	3
R3 6h	3
RR3 6h	3

Group	Mean	Std Dev	SEM
3 6h	42.308	3.3945	1.9598
S3 6h	38.415	1.4620	0.8441
SS3 6h	37.433	0.5477	0.3162
R3 6h	38.915	0.7803	0.4505
RR3 6h	35.797	0.2846	0.1643

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	69.3039	17.3260	5.913	0.010
Residual	10	29.3002	2.9300		
Total	14	98.6041			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.010). To isolate which group(s) differ from the others, use a multiple comparison procedure

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
3 6h vs RR3 6h	6.512	5	6.589	Yes
3 6h vs SS3 6h	4.875	4	4.933	Yes
3 6h vs S3 6h	3.893	3	3.939	Yes
3 6h vs R3 6h	3.394	2	3.434	Yes
R3 6h vs RR3 6h	3.118	4	3.155	No
R3 6h vs SS3 6h	1.481		Do not test	
R3 6h vs S3 6h	0.500		Do not test	
S3 6h vs RR3 6h	2.618		Do not test	
S3 6h vs SS3 6h	0.982		Do not test	
SS3 6h vs RR3 6h	1.637		Do not test	

Table 35. Summary of ANOVA results comparing drug release after 6 hours from Formulation 17 at different storage conditions over time.

Group	N
17 6h	3
S17 6h	3
SS17 6h	3
R17 6h	3
RR17 6h	3

Group	Mean	Std Dev	SEM
17 6h	36.158	1.9729	1.1390
S17 6h	33.247	0.2152	0.1242
SS17 6h	32.748	0.1492	0.0861
R17 6h	36.744	1.0939	0.6315
RR17 6h	32.868	0.6093	0.3518

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	44.9464	11.2366	10.162	0.002
Residual	10	11.0570	1.1057		
Total	14	56.0034			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002). To isolate which group(s) differ from the others, use a multiple comparison procedure

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
R17 6h vs SS17 6h	3.997	5	6.583	Yes
R17 6h vs RR17 6h	3.876	4	6.384	Yes
R17 6h vs S17 6h	3.497	3	5.760	Yes
R17 6h vs 17 6h	0.586	2	0.965	No
17 6h vs SS17 6h	3.411	4	5.618	Yes
17 6h vs RR17 6h	3.290	3	5.420	Yes
17 6h vs S17 6h	2.911	2	4.795	Yes
S17 6h vs SS17 6h	0.500	3	0.823	No
S17 6h vs RR17 6h	0.379		Do not test	
RR17 6h vs SS17 6h	0.121		Do not test	

Table 36. Summary of ANOVA results comparing drug release after 6 hours from Formulation 8 at different storage conditions over time.

Group	N
8 6h	3
S8 6h	3
SS8 6h	3
R8 6h	3
RR8 6h	3

Group	Mean	Std Dev	SEM
8 6h	56.227	1.2910	0.7453
S8 6h	54.419	1.6921	0.9769
SS8 6h	53.437	1.9638	1.1338
R8 6h	52.403	1.3546	0.7821
RR8 6h	53.730	1.3696	0.7907

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	24.2033	6.0508	2.501	0.109
Residual	10	24.1937	2.4194		
Total	14	48.3970			

The differences in the mean values among the treatment groups are not great enough to exclude the possiblility that the difference is just due to random sampling variability; there is not a statistically significant difference (P = 0.109).

Table 37. Summary of ANOVA results comparing drug release after 6 hours from Formulation 21 at different storage conditions over time.

Group	N
21 6h	3
S21 6h	3
SS21 6h	3
R21 6h	3
RR21 6h	3

Group	Mean	Std Dev	SEM
21 6h	52.679	0.5526	0.3190
S21 6h	54.401	0.2089	0.1206
SS21 6h	53.643	0.7453	0.4303
R21 6h	54.505	0.0789	0.0456
RR21 6h	53.127	0.3144	0.1815

Source of Variation	DF	SS	MS	F	P
Between Treatments	4	7.5309	1.8827	9.325	0.002
Residual	10	2.0191	0.2019		
Total	14	9.5500			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002). To isolate which group(s) differ from the others, use a multiple comparison procedure

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
R21 6h vs 21 6h	1.826	5	7.039	Yes
R21 6h vs RR21 6h	1.378	4	5.312	Yes
R21 6h vs SS21 6h	0.861	3	3.320	No
R21 6h vs S21 6h	0.103		Do not test	
S21 6h vs 21 6h	1.723	4	6.640	Yes
S21 6h vs RR21 6h	1.275	3	4.914	Yes
S21 6h vs SS21 6h	0.758		Do not test	
SS21 6h vs 21 6h	0.965	3	3.718	No
SS21 6h vs RR21 6h	0.517		Do not test	
RR21 6h vs 21 6h	0.448		Do not test	

3.7. EFFECT OF THE MICROENCAPSULATION PROCESS ON IMC POLYMORPHISM.

Indomethacin exhibits polymorphism (1-2). At least four of IMC polymorphs have been documented, with melting points ranging from 133°C to 162°C. The solubility of IMC varies according to the predominant form. Form I and Form II have solubilities of 11 mg/ml and 16 mg/ml in pH 6.2 phosphate buffer, respectively. The differences could influence dissolution rates in *in vitro* studies.

Differential scanning calorimetric (DSC) analysis was carried out on various formulations produced. The DSC thermograms for pure IMC, carnauba wax and Aquateric^R and Formulations 3, 8, 12, 15, 17 and 21 are shown in Figures 40 - 42, and thermograms for pure gelatin, pectin and alginate are shown in Figure 43. Table 38 summurizes DSC data obtained.

The IMC form used in the experiment showed melting endothermic peak at 160.2°C and transition energy (enthalpy) of 130.5 J/g, confirming that it was Form I. As shown in Table 38, the melting endotherms for IMC in all samples were lower than that for pure IMC. The low melting points from the various formulations could be related to the increased dissolution of IMC observed in Figures 13 - 18. This leads to speculatiion that IMC Form II could have been formed during microencapsulation. The lowest melting endotherm of 145.7°C, was reported for Formulation 15 containing alginate and Aquateric^R. This formulation also showed the highest drug release rate among the alginate preparations (Figure 17). The diffuse peaks in Formulations 12 and 15, when compared to a sharp peak in pure IMC, could be indicating the existence of more than one form of IMC.

Table 38. DSC data for IMC from various formulations.

Formulation No.	Onset Temperature, °C	Peak, °C	Transition energy, J/g (+ or -)[a]
IMC	158.6	160.2	130.5(-)
3	154.8	157.7	122.5(-)
8	154.5	157.5	120.3(-)
12	147.0	152.7	121.9(-)
15	135.8	145.7	129.3(-)
17	154.5	157.5	118.5(-)
21	153.9	157.2	116.9(-)

[a]Values adjusted to the amount of IMC in the sample.

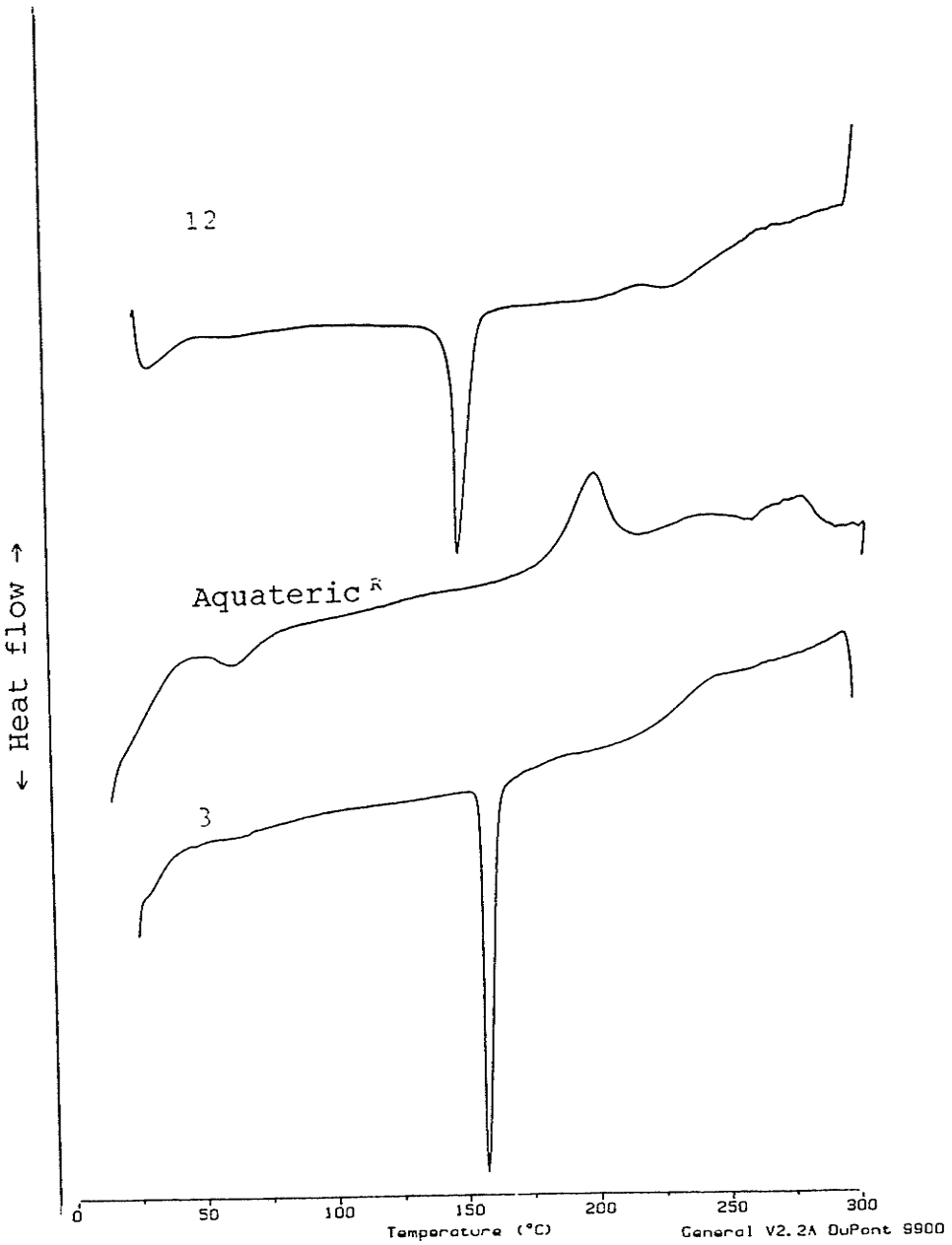


Figure 40. DSC thermograms for formulations 3, 12 and pure Aquateric[R].

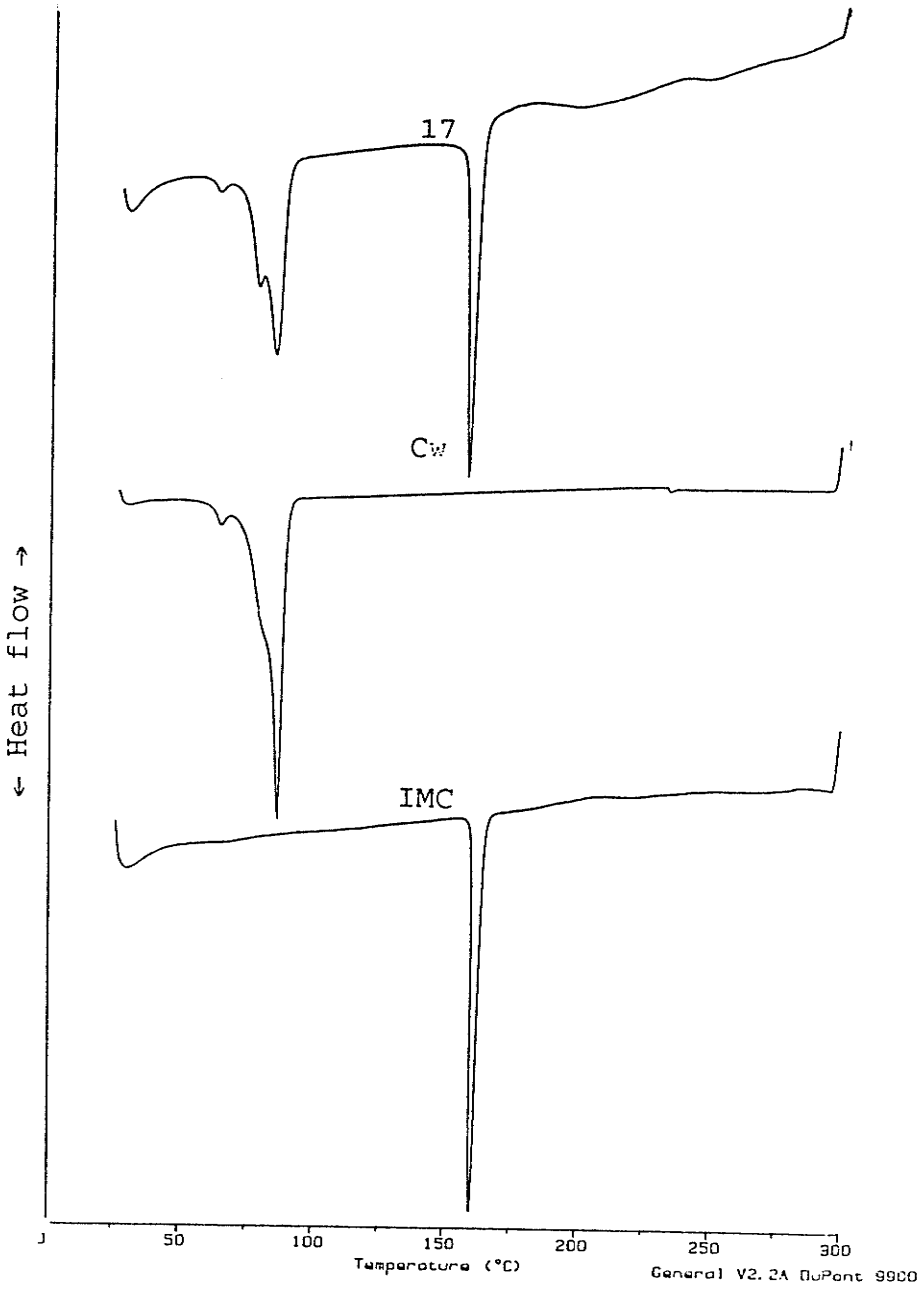


Figure 41. DSC thermograms for pure IMC, Formulation 17 and pure carnauba wax (Cw).

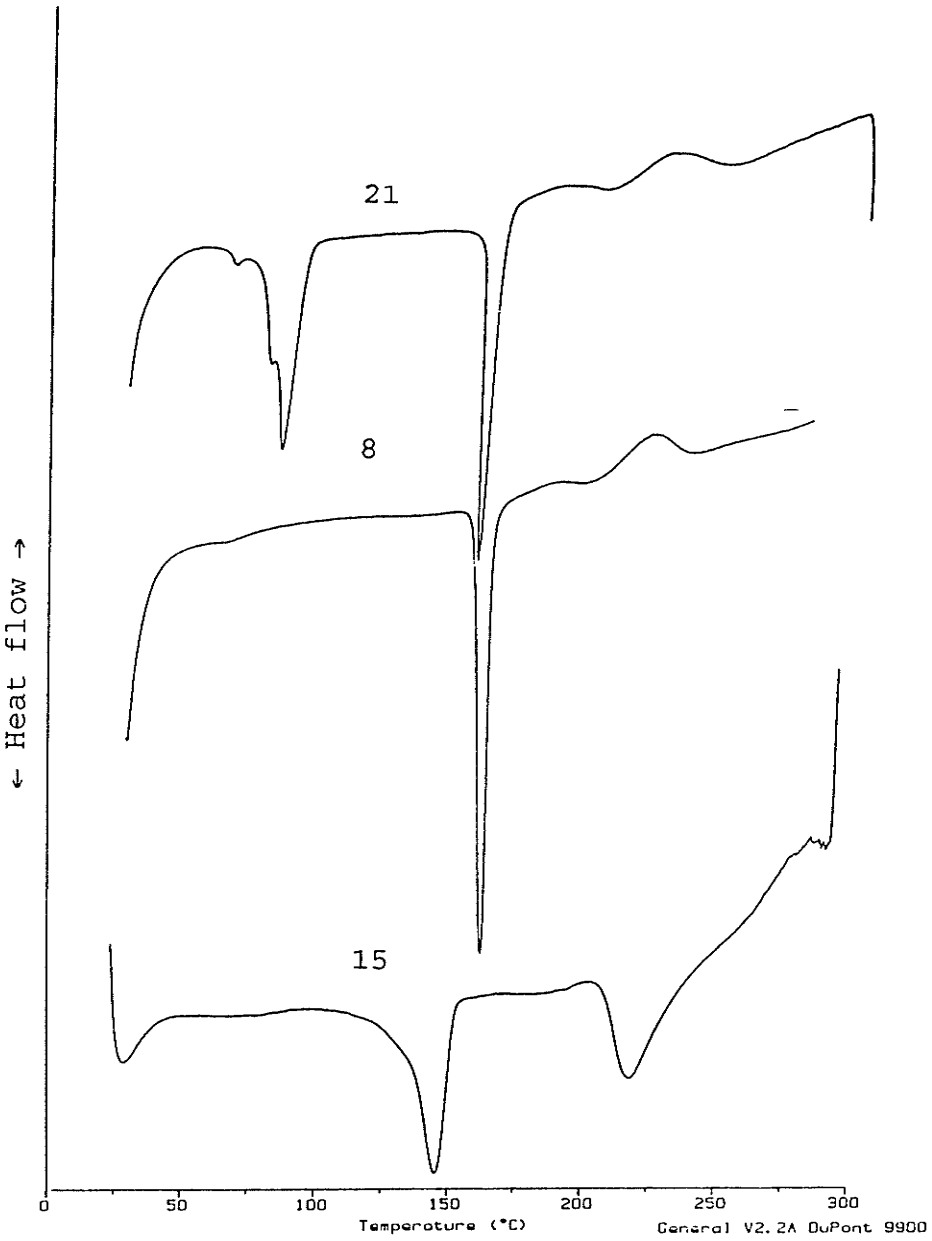


Figure 42. DSC thermograms for Formulations 8, 15 and 21.

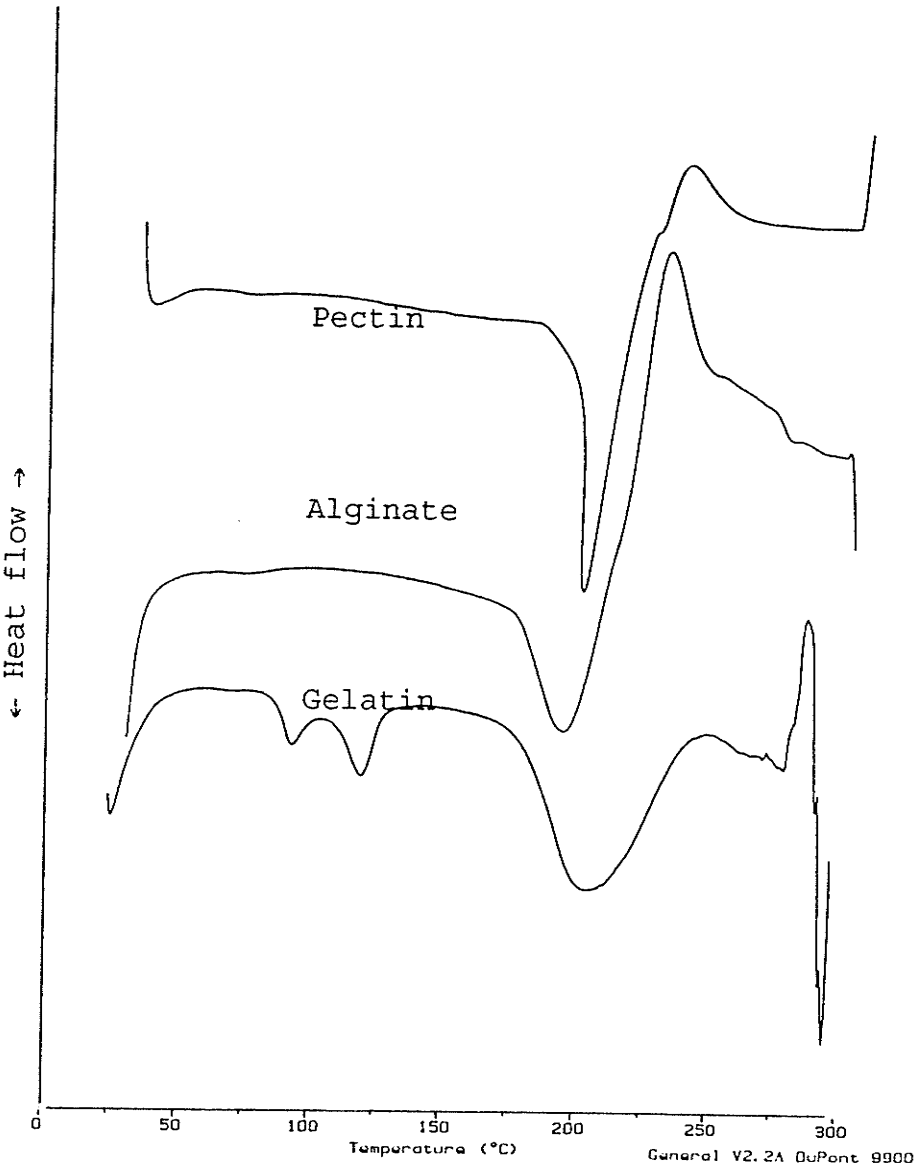


Figure 43. DSC thermograms for pure gelatin, pectin and alginate.

CHAPTER 4. CONCLUSION.

Indomethacin microparticles demonstrating sustained release characteristics, up to at least 12 hours, have been prepared from the ionic complex coarcevation of pectin and gelatin or alginate and gelatin. The microparticles formed demonstrated good size distribution and superior flow and handling properties for direct capsule filling, when compared to IMC powder. The drug load, ratio of the individual hydrocolloids, the drug to hydrocolloid ratio and microparticle size affected the release properties substantially. The polymer to polymer ratio or hydrocolloid ratio of 1:2 pectin or alginate to gelatin was found to be easier to handle during production of the microparticles when compared to ratios of 1:1 or 2:1 which were difficult to filter.

A combination of spherical agglomeration and coacervation/phase separation improved the microparticle physical and drug release properties. The incorporation of carnauba wax prior to coacervation resulted in spherical agglomeration of the drug, reduced forces of aggregation and improved microparticle size, morphology and flow. Spherical agglomeration also resulted in improved drug release profiles from the microparticles, particularly those produced from alginate. Slower release was achieved upon controlling the amount of the wax or lipophilic phase in the formulation, as in Formulation 17, Pec:Gel:Cw 1:2:3, D:P 1:4 and size 16/30. However, the addition of a hydrophilic polymer like Aquateric^{R} did not improve the drug release profile, although it reduced microparticle aggregation.

Microparticles in the size range of 30/60 showed close to complete drug release when compared to IMC alone or microparticles in the 16/30 size range. However, the larger microparticles, in the 16/30 size range demonstrated a substantial delay in drug

release when compared to the smaller microparticles. Combining the two sizes in suitable proportions could be one way of delivering a desired dose of the drug over a specified dosing schedule.

The drug release rate was shown to be influenced by the drug load. The slowest release rate was obtained from microparticles with drug to polymer ratio (D:P) of 1:4, as demonstrated by Formulations 17 and 23, whereas microparticles with D:P ratio of 1:2 released the drug at higher rates. This finding is important for producing an SR product of desirable drug release rate without exceeding dose size limits for oral solid dosage forms.

The drug release mechanism was also affected by particle size, within the same formulation, particle aggregation and drug load. However, the solubility of IMC in the dissolution medium did not influence the release mechanism. Drug release from pectin or alginate and gelatin complex coacervate microparticles showed correlation with different kinetic models in the following decreasing order: spherical matrix > first order ≥ square root of time > zero order ≥ planar matrix > cube root law. A non-Fickian diffusion process was responsible for the release kinetics. The high correlation of small particles with the first order release model compared to the spherical release model demonstrated that small microparticles existed in aggregates of a slab configuration, whereas the larger particles approximated a spherical configuration. Although drug release depends to some extent on the structure (microcapsule *vs* microsphere) of the microparticles, the structure and drug distribution within the microparticles could not be deduced from the release mechanism. Aggregation of microparticles and porosity influenced the drug release characteristics. However, the existence of a combination of the fast initial phase and the slow terminal release could be an indication of a change in

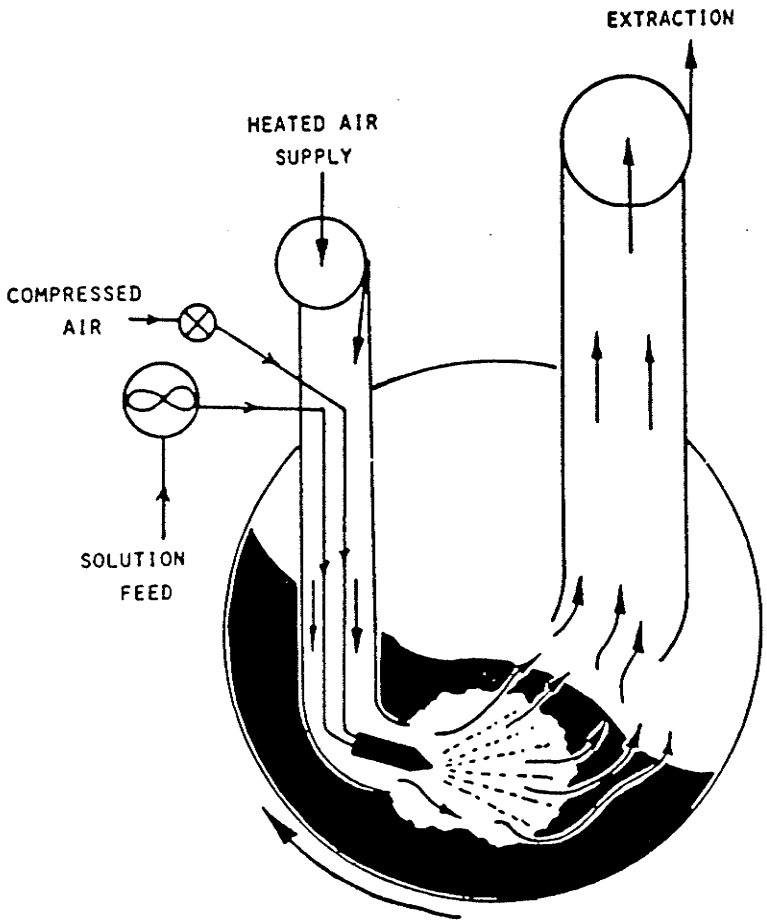
drug distribution within the microparticle which affects the diffusion path length and the concentration gradient.

Aging for up to 60 days at room temperature and at 40°C did not drastically affect the drug release profiles from the microparticles. However, there was an indication that the microparticles might require longer times and higher temperatures for curing during drying before drug release could stabilize.

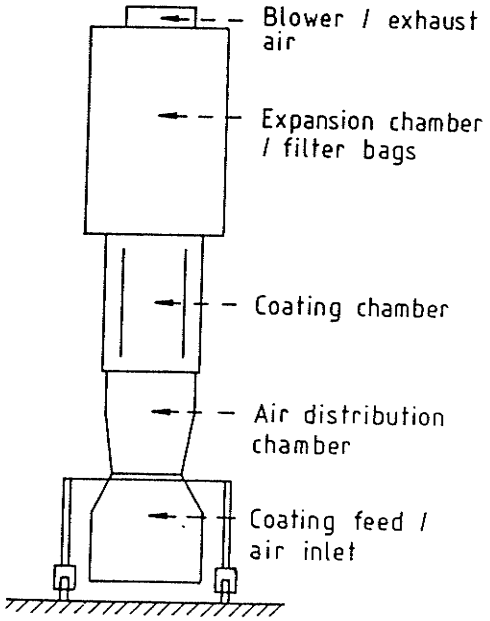
Differential scanning calorimetry analysis of the microparticles revealed only a small shift in the melting endotherm of IMC after microencapsulation in all formulations not containing Aquateric^{R}. This could indicate minor or lack of interaction between the drug and the polymers used, although the formation of Form II IMC could not be ruled out. However, the significant shift observed in the presence of Aquateric^{R} could indicate an incompatibility between IMC and this polymer.

The rate of release of IMC from the microparticles was affected by the polymer to polymer ratio, drug to polymer ratio and microparticle size. Selection of the correct ratio and particle size distribution wolud permit the production of a formulation that would promote the optimal rate of release for an IMC-SR formulation for up to 12 hours. The results of these studies provide the guidelines for the selection of the optimal ratios.

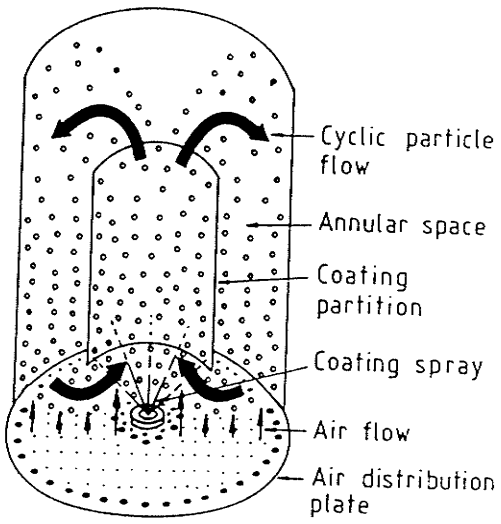
APPENDIX 1.



Appendix 1.1. Schematic representation of a Strunck immersion tube coating pan illustrating polymer solution feed, air supply and exhaustion relative to the material being coated (103).

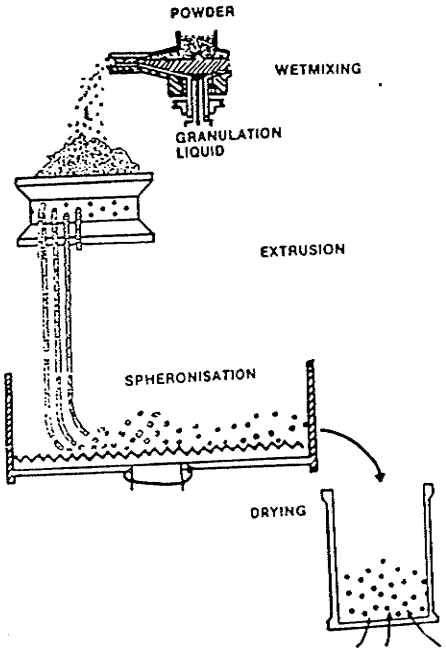


A.

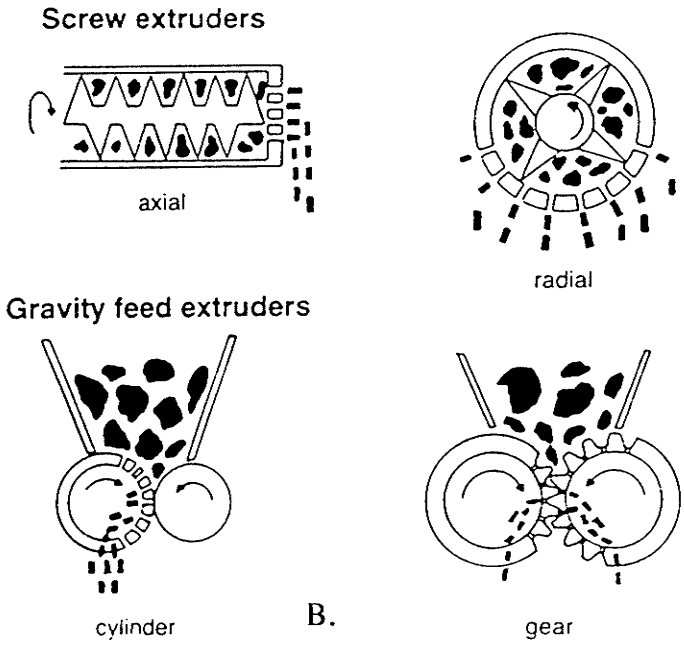


B.

Appendix 1.2. (A) An outline drawing of a Wurster-type fluid-bed apparatus; and (B) An enlarged portion of the coating chamber of a Wurster-type fluid-bed coating apparatus showing direction of air, coating polymer and core material during coating (72).

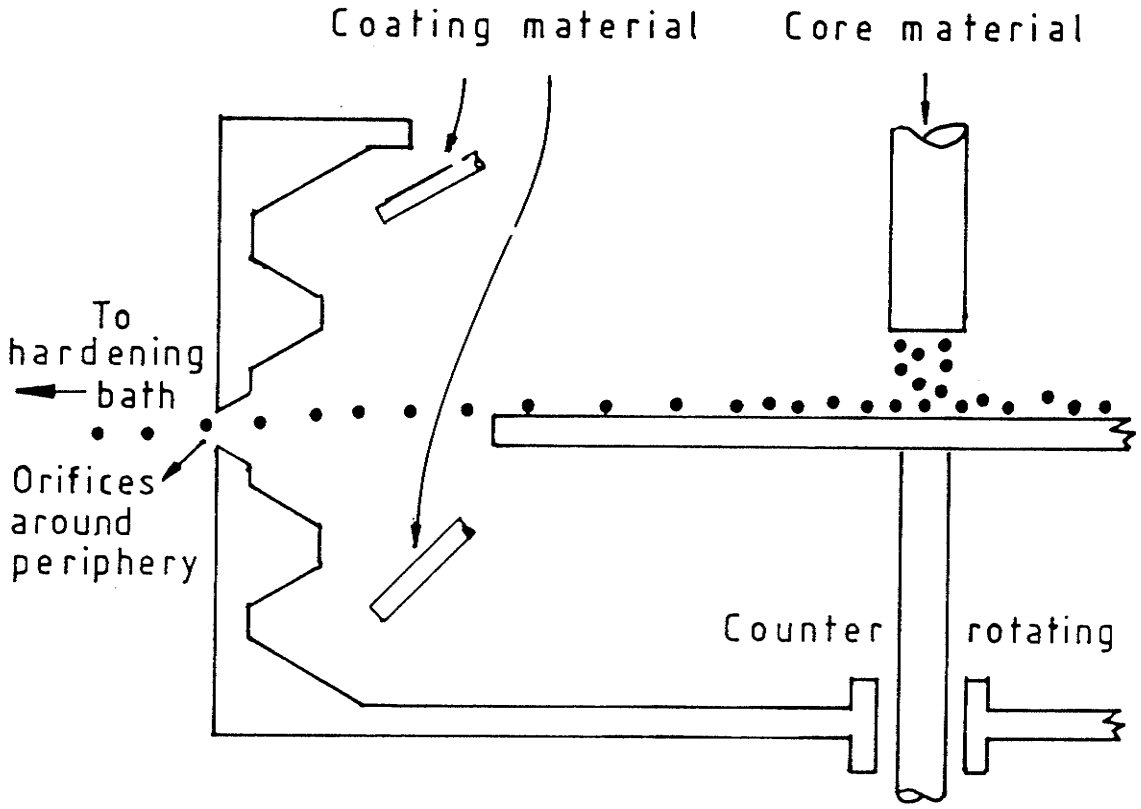


A.

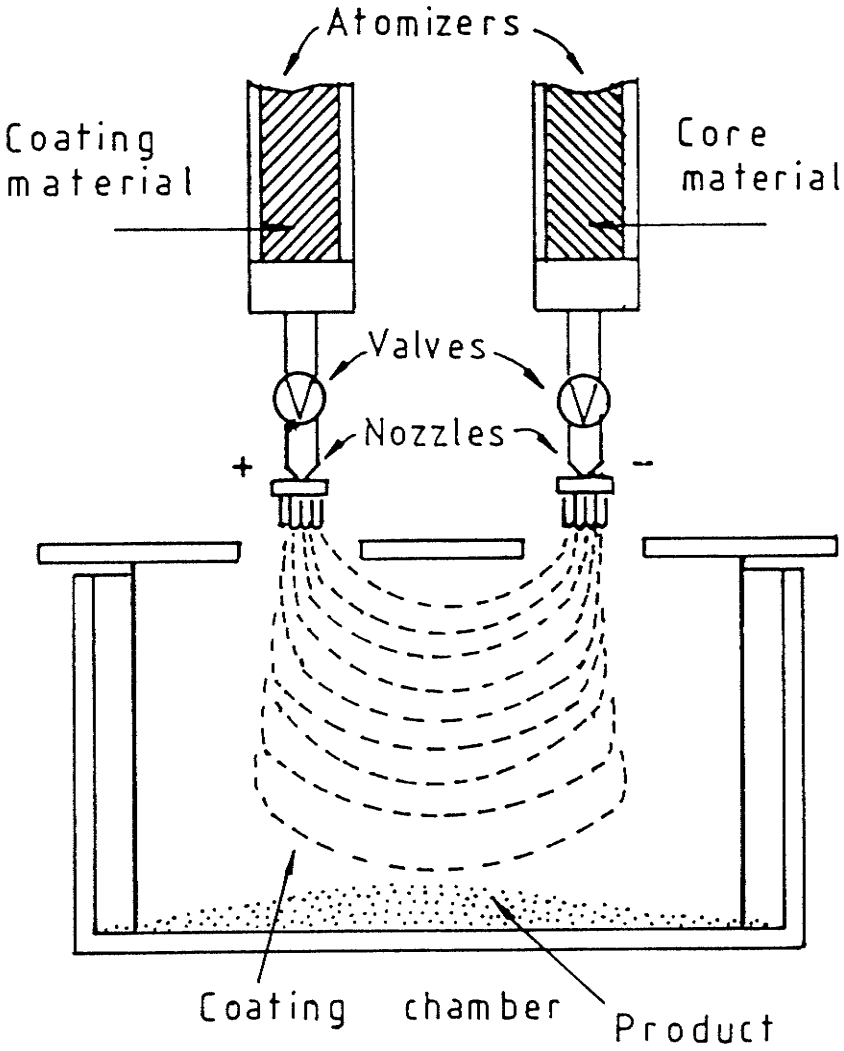


B.

Appendix 1.3. (A) A schematic representation of an extrusion/spheronization process showing main stages in the process (103); (B) Illustration of the types of extruders (screw and gravity types) (124).



Appendix 1.4. A multiorifice centrifugal coating apparatus schematic representation showing the positioning of the rotating disc relative to the orifices (72).



Appendix 1.5. A schematic diagram of an electrostatic microencapsulation equipment illustrating the atomization of the oppositely charged coating material and core material (72).

APPENDIX 2.

Appendix 2.1. PREPARATION OF BUFFER SOLUTIONS.

Appendix 2.1.1. pH 7.5 phosphate buffer.

The buffer was prepared by dissolving 17.42 g of dibasic potassium phosphate in 800 ml distilled water then adjusting the pH to 7.5 with phosphoric acid and adjusting the volume to 1 litre with distilled water. The pH was checked and adjusted as necessary with phosphoric acid or a solution of dibasic potassium phosphate.

Appendix 2.1.2. pH 2.0 HCl/KCl buffer.

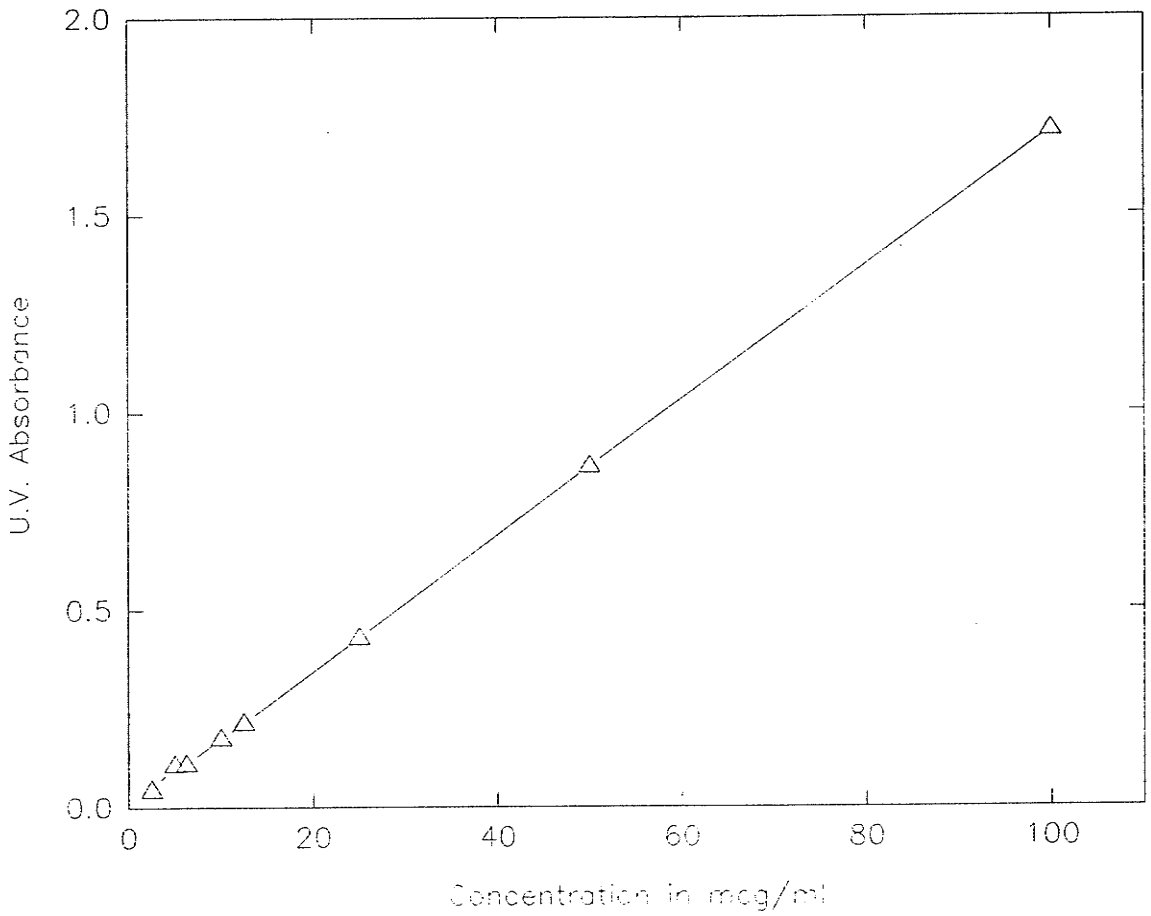
Acid buffer was prepared in 20 litre batches to meet the demand of the multiple dissolution tests performed. For each batch 74.55 g of potassium chloride was accurately weighed and then dissolved in a 1 litre distilled water. One thousand three hundred millilitres of 0.2 *M* hydrochloric acid was diluted with distilled water to make 2 litres. The solutions were then transferred to a 20 litre container and diluted to volume with 17 litres of distilled water. The mixture was stirred until pH stabilized before being adjusted, if necessary, with either 1 M HCL or 1 M sodium hydroxide to pH 2.0.

Appendix 2.1.3. pH 6.2 phosphate buffer.

To prepare pH 6.2 phosphate buffer 136.10 mg (enough to make 20 litres of buffer) of monobasic potassium phosphate was dissolved in 1 litre of distilled water and mixed with 810 ml of 0.2 *M* sodium hydroxide in a 2 litre volumetric flask. Distilled water was added to make up to volume. The mixture was then transferred to a 20 litre container and diluted to volume with 18 litres distilled water. Stirring was maintained until the pH stabilized before being adjusted as necessary.

Appendix 2.2. INDOMETHACIN ABSORBANCE/CONCENTRATION CALIBRATION CURVE.

Indomethacin was dried for 2 hours at 100 °C and about 25 mg was weighed. The IMC amount was dissolved in 100 ml of previously filtered pH 6.2 phosphate buffer in methanol mixture (1:1). The solution was diluted with the same mixture to give concentrations of 5, 6.25, 10, 12.5, 15, 20, 25, 50 and 100 μg/ml. A Beer's plot of absorbance (ABS) against concentration was prepared from which a regression curve was produced (Appendix 2.1.2.1). The curve gave a correlation coefficient of 0.99992 for a concentration range giving absorbance of $0.090 \leq ABS \leq 1.700$.



Appendix 2.2.1. A Beer's plot calibration curve for indomethacin dissolved in methanol and pH 6.2 phosphate buffer mixture (1:1). U.V. absorbance against concentration.

APPENDIX 3. MULTIPLE COMPARISON RESULTS (SNK).

Appendix 3.1. SNK results summary comparing drug release after 3 hours of dissolution testing of pectin containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 3h vs 17(16/30)`3h	28.493	11	33.428	Yes
Pure IMC 3h vs 18(16/30) 3h	24.513	10	28.759	Yes
Pure IMC 3h vs 19(16/30) 3h	24.238	9	28.436	Yes
Pure IMC 3h vs 3(16/30)	23.531	8	27.607	Yes
Pure IMC 3h vs 20(16/30) 3h	22.188	7	26.031	Yes
Pure IMC 3h vs 17(30/60) 3h	11.525	6	13.521	Yes
Pure IMC 3h vs 19(30/60) 3h	10.612	5	12.449	Yes
Pure IMC 3h vs 18(30/60) 3h	5.633	4	6.609	Yes
Pure IMC 3h vs 3(30/60)	3.686	3	4.325	Yes
Pure IMC 3h vs 20(30/60) 3h	1.034	2	1.213	No
20(30/60) 3h vs 17(16/30) 3h	27.459	10	32.215	Yes
20(30/60) 3h vs 18(16/30) 3h	23.480	9	27.546	Yes
20(30/60) 3h vs 19(16/30) 3h	23.204	8	27.223	Yes
20(30/60) 3h vs 3(16/30)	22.498	7	26.394	Yes
20(30/60) 3h vs 20(16/30) 3h	21.154	6	24.818	Yes
Comparison	Diff of Mean	p	q	P < 0.05
20(30/60) 3h vs 17(30/60) 3h	10.491	5	12.308	Yes
20(30/60) 3h vs 19(30/60) 3h	9.578	4	11.237	Yes
20(30/60) 3h vs 18(30/60) 3h	4.599	3	5.396	Yes
20(30/60) 3h vs 3(30/60)	2.653	2	3.112	Yes
3(30/60) vs 17(16/30) 3h	24.806	9	29.103	Yes
3(30/60) vs 18(16/30) 3h	20.827	8	24.434	Yes
3(30/60) vs 19(16/30) 3h	20.551	7	24.111	Yes
3(30/60) vs 3(16/30)	19.845	6	23.282	Yes
3(30/60) vs 20(16/30) 3h	18.501	5	21.706	Yes
3(30/60) vs 17(30/60) 3h	7.838	4	9.196	Yes
3(30/60) vs 19(30/60) 3h	6.925	3	8.124	Yes
3(30/60) vs 18(30/60) 3h	1.947	2	2.284	No

(Appendix 3.1. continued).

18(30/60) 3h vs 17(16/30) 3h	22.860	8	26.819	Yes
18(30/60) 3h vs 18(16/30) 3h	18.880	7	22.150	Yes
18(30/60) 3h vs 19(16/30) 3h	18.605	6	21.827	Yes
Comparison	Diff of Mean	p	q	P < 0.05
18(30/60) 3h vs 3(16/30)	17.898	5	20.998	Yes
18(30/60) 3h vs 20(16/30) 3h	16.555	4	19.422	Yes
18(30/60) 3h vs 17(30/60) 3h	5.891	3	6.912	Yes
18(30/60) 3h vs 19(30/60) 3h	4.978	2	5.841	Yes
19(30/60) 3h vs 17(16/30) 3h	17.881	7	20.978	Yes
19(30/60) 3h vs 18(16/30) 3h	13.902	6	16.310	Yes
19(30/60) 3h vs 19(16/30) 3h	13.626	5	15.986	Yes
19(30/60) 3h vs 3(16/30)	12.920	4	15.158	Yes
19(30/60) 3h vs 20(16/30) 3h	11.576	3	13.581	Yes
19(30/60) 3h vs 17(30/60) 3h	0.913	2	1.071	No
17(30/60) 3h vs 17(16/30) 3h	16.968	6	19.907	Yes
17(30/60) 3h vs 18(16/30) 3h	12.989	5	15.238	Yes
17(30/60) 3h vs 19(16/30) 3h	12.713	4	14.915	Yes
17(30/60) 3h vs 3(16/30)	12.007	3	14.086	Yes
17(30/60) 3h vs 20(16/30) 3h	10.663	2	12.510	Yes
Comparison	Diff of Mean	p	q	P < 0.05
20(16/30) 3h vs 17(16/30) 3h	6.305	5	7.397	Yes
20(16/30) 3h vs 18(16/30) 3h	2.326	4	2.728	No
20(16/30) 3h vs 19(16/30) 3h	2.050		Do not test	
20(16/30) 3h vs 3(16/30)	1.344		Do not test	
3(16/30) vs 17(16/30) 3h	4.961	4	5.821	Yes
3(16/30) vs 18(16/30) 3h	0.982		Do not test	
3(16/30) vs 19(16/30) 3h	0.706		Do not test	
19(16/30) 3h vs 17(16/30) 3h	4.255	3	4.992	Yes
19(16/30) 3h vs 18(16/30) 3h	0.276		Do not test	
18(16/30) 3h vs 17(16/30) 3h	3.979	2	4.669	Yes

Appendix 3.2. SNK results summary comparing drug release after 6 hours of dissolution testing of pectin containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
20(30/60) 6h vs 17(16/30) 6h	31.387	11	38.298	Yes
20(30/60) 6h vs 19(16/30) 6h	29.216	10	35.649	Yes
20(30/60) 6h vs 18(16/30) 6h	26.029	9	31.761	Yes
20(30/60) 6h vs 3(16/30) 6h	25.237	8	30.794	Yes
20(30/60) 6h vs 20(16/30) 6h	23.979	7	29.259	Yes
20(30/60) 6h vs Pure IMC	16.974	6	20.711	Yes
20(30/60) 6h vs 19(30/60) 6h	11.266	5	13.747	Yes
20(30/60) 6h vs 17(30/60) 6h	7.442	4	9.080	Yes
20(30/60) 6h vs 18(30/60) 6h	7.390	3	9.017	Yes
20(30/60) 6h vs 3(30/60) 6h	2.653	2	3.237	Yes
3(30/60) 6h vs 17(16/30) 6h	28.734	10	35.061	Yes
3(30/60) 6h vs 19(16/30) 6h	26.563	9	32.412	Yes
3(30/60) 6h vs 18(16/30) 6h	23.376	8	28.524	Yes
3(30/60) 6h vs 3(16/30) 6h	22.584	7	27.557	Yes
3(30/60) 6h vs 20(16/30) 6h	21.326	6	26.022	Yes
Comparison	Diff of Mean	p	q	P < 0.05
3(30/60) 6h vs Pure IMC	14.321	5	17.474	Yes
3(30/60) 6h vs 19(30/60) 6h	8.613	4	10.510	Yes
3(30/60) 6h vs 17(30/60) 6h	4.789	3	5.843	Yes
3(30/60) 6h vs 18(30/60) 6h	4.737	2	5.780	Yes
18(30/60) 6h vs 17(16/30) 6h	23.997	9	29.280	Yes
18(30/60) 6h vs 19(16/30) 6h	21.826	8	26.632	Yes
18(30/60) 6h vs 18(16/30) 6h	18.639	7	22.743	Yes
18(30/60) 6h vs 3(16/30) 6h	17.847	6	21.776	Yes
18(30/60) 6h vs 20(16/30) 6h	16.589	5	20.242	Yes
18(30/60) 6h vs Pure IMC	9.584	4	11.694	Yes
18(30/60) 6h vs 19(30/60) 6h	3.876	3	4.729	Yes
18(30/60) 6h vs 17(30/60) 6h	0.052	2	0.063	No

(Appendix 3.2. continued).

17(30/60) 6h vs 17(16/30) 6h	23.945	8	29.217	Yes
17(30/60) 6h vs 19(16/30) 6h	21.774	7	26.569	Yes
17(30/60) 6h vs 18(16/30) 6h	18.587	6	22.680	Yes
Comparison	Diff of Mean	p	q	P < 0.05
17(30/60) 6h vs 3(16/30) 6h	17.795	5	21.713	Yes
17(30/60) 6h vs 20(16/30) 6h	16.537	4	20.179	Yes
17(30/60) 6h vs Pure IMC	9.532	3	11.631	Yes
17(30/60) 6h vs 19(30/60) 6h	3.824	2	4.666	Yes
19(30/60) 6h vs 17(16/30) 6h	20.121	7	24.551	Yes
19(30/60) 6h vs 19(16/30) 6h	17.950	6	21.902	Yes
19(30/60) 6h vs 18(16/30) 6h	14.763	5	18.014	Yes
19(30/60) 6h vs 3(16/30) 6h	13.971	4	17.047	Yes
19(30/60) 6h vs 20(16/30) 6h	12.713	3	15.512	Yes
19(30/60) 6h vs Pure IMC	5.708	2	6.964	Yes
Pure IMC vs 17(16/30) 6h	14.413	6	17.586	Yes
Pure IMC vs 19(16/30) 6h	12.242	5	14.938	Yes
Pure IMC vs 18(16/30) 6h	9.055	4	11.049	Yes
Pure IMC vs 3(16/30) 6h	8.263	3	10.082	Yes
Pure IMC vs 20(16/30) 6h	7.005	2	8.548	Yes
Comparison	Diff of Mean	p	q	P < 0.05
20(16/30) 6h vs 17(16/30) 6h	7.407	5	9.038	Yes
20(16/30) 6h vs 19(16/30) 6h	5.237	4	6.390	Yes
20(16/30) 6h vs 18(16/30) 6h	2.050	3	2.501	No
20(16/30) 6h vs 3(16/30) 6h	1.258		Do not test	
3(16/30) 6h vs 17(16/30) 6h	6.150	4	7.504	Yes
3(16/30) 6h vs 19(16/30) 6h	3.979	3	4.856	Yes
3(16/30) 6h vs 18(16/30) 6h	0.792		Do not test	
18(16/30) 6h vs 17(16/30) 6h	5.357	3	6.537	Yes
18(16/30) 6h vs 19(16/30) 6h	3.187	2	3.889	Yes
19(16/30) 6h vs 17(16/30) 6h	2.171	2	2.648	No

Appendix 3.3. SNK results summary comparing drug release after 14 hours of dissolution testing of pectin containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
20(30/60) 14h vs 17(16/30) 14h	19.225	11	50.193	Yes
20(30/60) 14h vs Pure IMC 14h	19.001	10	49.608	Yes
20(30/60) 14h vs 19(16/30) 14h	17.209	9	44.931	Yes
20(30/60) 14h vs 18(16/30) 14h	14.264	8	37.240	Yes
20(30/60) 14h vs 3(16/30) 14h	12.885	7	33.642	Yes
20(30/60) 14h vs 20(16/30) 14h	11.490	6	29.999	Yes
20(30/60) 14h vs 18(30/60) 14h	4.186	5	10.929	Yes
20(30/60) 14h vs 19(30/60) 14h	3.135	4	8.186	Yes
20(30/60) 14h vs 17(30/60) 14h	2.394	3	6.252	Yes
20(30/60) 14h vs 3(30/60) 14h	1.447	2	3.778	Yes
3(30/60) 14h vs 17(16/30) 14h	17.778	10	46.415	Yes
3(30/60) 14h vs Pure IMC 14h	17.554	9	45.830	Yes
3(30/60) 14h vs 19(16/30) 14h	15.762	8	41.153	Yes
3(30/60) 14h vs 18(16/30) 14h	12.817	7	33.462	Yes
3(30/60) 14h vs 3(16/30) 14h	11.438	6	29.864	Yes
Comparison	Diff of Mean	p	q	P < 0.05
3(30/60) 14h vs 20(16/30) 14h	10.043	5	26.221	Yes
3(30/60) 14h vs 18(30/60) 14h	2.739	4	7.151	Yes
3(30/60) 14h vs 19(30/60) 14h	1.688	3	4.408	Yes
3(30/60) 14h vs 17(30/60) 14h	0.947	2	2.474	No
17(30/60) 14h vs 17(16/30) 14h	16.830	9	43.941	Yes
17(30/60) 14h vs Pure IMC 14h	16.606	8	43.356	Yes
17(30/60) 14h vs 19(16/30) 14h	14.815	7	38.679	Yes
17(30/60) 14h vs 18(16/30) 14h	11.869	6	30.988	Yes
17(30/60) 14h vs 3(16/30) 14h	10.491	5	27.390	Yes
17(30/60) 14h vs 20(16/30) 14h	9.096	4	23.747	Yes
17(30/60) 14h vs 18(30/60) 14h	1.792	3	4.677	Yes
17(30/60) 14h vs 19(30/60) 14h	0.741	2	1.934	No
19(30/60) 14h vs 17(16/30) 14h	16.090	8	42.007	Yes
19(30/60) 14h vs Pure IMC 14h	15.866	7	41.423	Yes
19(30/60) 14h vs 19(16/30) 14h	14.074	6	36.745	Yes

(Appendix 3.3. continued).

Comparison	Diff of Mean	p	q	P < 0.05
19(30/60) 14h vs 18(16/30) 14h	11.128	5	29.054	Yes
19(30/60) 14h vs 3(16/30) 14h	9.750	4	25.456	Yes
19(30/60) 14h vs 20(16/30) 14h	8.355	3	21.813	Yes
19(30/60) 14h vs 18(30/60) 14h	1.051	2	2.744	No
18(30/60) 14h vs 17(16/30) 14h	15.039	7	39.264	Yes
18(30/60) 14h vs Pure IMC 14h	14.815	6	38.679	Yes
18(30/60) 14h vs 19(16/30) 14h	13.023	5	34.002	Yes
18(30/60) 14h vs 18(16/30) 14h	10.078	4	26.311	Yes
18(30/60) 14h vs 3(16/30) 14h	8.699	3	22.713	Yes
18(30/60) 14h vs 20(16/30) 14h	7.304	2	19.070	Yes
20(16/30) 14h vs 17(16/30) 14h	7.735	6	20.194	Yes
20(16/30) 14h vs Pure IMC 14h	7.511	5	19.609	Yes
20(16/30) 14h vs 19(16/30) 14h	5.719	4	14.932	Yes
20(16/30) 14h vs 18(16/30) 14h	2.773	3	7.241	Yes
20(16/30) 14h vs 3(16/30) 14h	1.395	2	3.643	Yes
Comparison	Diff of Mean	p	q	P < 0.05
3(16/30) 14h vs 17(16/30) 14h	6.339	5	16.551	Yes
3(16/30) 14h vs Pure IMC 14h	6.115	4	15.966	Yes
3(16/30) 14h vs 19(16/30) 14h	4.324	3	11.289	Yes
3(16/30) 14h vs 18(16/30) 14h	1.378	2	3.598	Yes
18(16/30) 14h vs 17(16/30) 14h	4.961	4	12.953	Yes
18(16/30) 14h vs Pure IMC 14h	4.737	3	12.368	Yes
18(16/30) 14h vs 19(16/30) 14h	2.946	2	7.691	Yes
19(16/30) 14h vs 17(16/30) 14h	2.016	3	5.262	Yes
19(16/30) 14h vs Pure IMC 14h	1.792	2	4.677	Yes
Pure IMC 14h vs 17(16/30) 14h	0.224	2	0.585	No

Appendix 3.4. SNK results summary comparing drug release after 3 hours of dissolution testing of alginate containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 3h vs 23(16/30) 3h	29.595	9	54.749	Yes
Pure IMC 3h vs 22(16/30) 3h	25.168	8	46.559	Yes
Pure IMC 3h vs 21(16/30) 3h	16.744	7	30.976	Yes
Pure IMC 3h vs 8(16/30) 3h	15.280	6	28.267	Yes
Pure IMC 3h vs 23(30/60) 3h	9.096	5	16.826	Yes
Pure IMC 3h vs 21(30/60) 3h	8.941	4	16.540	Yes
Pure IMC 3h vs 22(30/60) 3h	6.701	3	12.397	Yes
Pure IMC 3h vs 8(30/60) 3h	5.891	2	10.899	Yes
8(30/60) 3h vs 23(16/30) 3h	23.704	8	43.850	Yes
8(30/60) 3h vs 22(16/30) 3h	19.276	7	35.660	Yes
8(30/60) 3h vs 21(16/30) 3h	10.853	6	20.077	Yes
8(30/60) 3h vs 8(16/30) 3h	9.388	5	17.368	Yes
8(30/60) 3h vs 23(30/60) 3h	3.204	4	5.927	Yes
8(30/60) 3h vs 21(30/60) 3h	3.049	3	5.641	Yes
8(30/60) 3h vs 22(30/60) 3h	0.810	2	1.498	No
Comparison	Diff of Mean	p	q	P < 0.05
22(30/60) 3h vs 23(16/30) 3h	22.894	7	42.353	Yes
22(30/60) 3h vs 22(16/30) 3h	18.467	6	34.163	Yes
22(30/60) 3h vs 21(16/30) 3h	10.043	5	18.579	Yes
22(30/60) 3h vs 8(16/30) 3h	8.579	4	15.870	Yes
22(30/60) 3h vs 23(30/60) 3h	2.394	3	4.430	Yes
22(30/60) 3h vs 21(30/60) 3h	2.239	2	4.143	Yes
21(30/60) 3h vs 23(16/30) 3h	20.655	6	38.210	Yes
21(30/60) 3h vs 22(16/30) 3h	16.227	5	30.020	Yes
21(30/60) 3h vs 21(16/30) 3h	7.804	4	14.436	Yes
21(30/60) 3h vs 8(16/30) 3h	6.339	3	11.727	Yes
21(30/60) 3h vs 23(30/60) 3h	0.155	2	0.287	No
23(30/60) 3h vs 23(16/30) 3h	20.500	5	37.923	Yes

(Appendix 3.4. continued).

Comparison	Diff of Mean	p	q	p < 0.05
23(30/60) 3h vs 22(16/30) 3h	16.072	4	29.733	Yes
23(30/60) 3h vs 21(16/30) 3h	7.649	3	14.149	Yes
23(30/60) 3h vs 8(16/30) 3h	6.184	2	11.441	Yes
8(16/30) 3h vs 23(16/30) 3h	14.315	4	26.482	Yes
8(16/30) 3h vs 22(16/30) 3h	9.888	3	18.292	Yes
8(16/30) 3h vs 21(16/30) 3h	1.464	2	2.709	No
21(16/30) 3h vs 23(16/30) 3h	12.851	3	23.774	Yes
21(16/30) 3h vs 22(16/30) 3h	8.424	2	15.583	Yes
22(16/30) 3h vs 23(16/30) 3h	4.427	2	8.190	Yes

Appendix 3.5. SNK results summary comparing drug release after 6 hours of dissolution testing of alginate containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(30/60) 6h vs 23(16/30) 6h	32.317	9	33.622	Yes
8(30/60) 6h vs 22(16/30) 6h	25.599	8	26.633	Yes
8(30/60) 6h vs Pure IMC 6h	16.003	7	16.650	Yes
8(30/60) 6h vs 21(16/30) 6h	13.592	6	14.141	Yes
8(30/60) 6h vs 8(16/30) 6h	10.043	5	10.449	Yes
8(30/60) 6h vs 23(30/60) 6h	5.978	4	6.219	Yes
8(30/60) 6h vs 21(30/60) 6h	4.806	3	5.000	Yes
8(30/60) 6h vs 22(30/60) 6h	2.601	2	2.706	No
22(30/60) 6h vs 23(16/30) 6h	29.716	8	30.916	Yes
22(30/60) 6h vs 22(16/30) 6h	22.997	7	23.926	Yes
22(30/60) 6h vs Pure IMC 6h	13.402	6	13.944	Yes
22(30/60) 6h vs 21(16/30) 6h	10.991	5	11.434	Yes
22(30/60) 6h vs 8(16/30) 6h	7.442	4	7.742	Yes
22(30/60) 6h vs 23(30/60) 6h	3.376	3	3.513	No
22(30/60) 6h vs 21(30/60) 6h	2.205	Do not test		

Comparison	Diff of Mean	p	q	P < 0.05
21(30/60) 6h vs 23(16/30) 6h	27.511	7	28.622	Yes
21(30/60) 6h vs 22(16/30) 6h	20.792	6	21.632	Yes
21(30/60) 6h vs Pure IMC 6h	11.197	5	11.650	Yes
21(30/60) 6h vs 21(16/30) 6h	8.786	4	9.140	Yes
21(30/60) 6h vs 8(16/30) 6h	5.237	3	5.448	Yes
21(30/60) 6h vs 23(30/60) 6h	1.171	Do not test		
23(30/60) 6h vs 23(16/30) 6h	26.339	6	27.403	Yes
23(30/60) 6h vs 22(16/30) 6h	19.621	5	20.414	Yes
23(30/60) 6h vs Pure IMC 6h	10.026	4	10.431	Yes
23(30/60) 6h vs 21(16/30) 6h	7.614	3	7.922	Yes
23(30/60) 6h vs 8(16/30) 6h	4.065	2	4.230	Yes
8(16/30) 6h vs 23(16/30) 6h	22.274	5	23.174	Yes

(Appendix 3.5. continued).

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 6h vs 22(16/30) 6h	15.556	4	16.184	Yes
8(16/30) 6h vs Pure IMC 6h	5.960	3	6.201	Yes
8(16/30) 6h vs 21(16/30) 6h	3.549	2	3.692	Yes
21(16/30) 6h vs 23(16/30) 6h	18.725	4	19.482	Yes
21(16/30) 6h vs 22(16/30) 6h	12.007	3	12.492	Yes
21(16/30) 6h vs Pure IMC 6h	2.412	2	2.509	No
Pure IMC 6h vs 23(16/30) 6h	16.314	3	16.972	Yes
Pure IMC 6h vs 22(16/30) 6h	9.595	2	9.983	Yes
22(16/30) 6h vs 23(16/30) 6h	6.718	2	6.990	Yes

Appendix 3.6. SNK results summary comparing drug release after 14 hours of dissolution testing of alginate containing formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(30/60) 14h vs Pure IMC 14h	18.760	9	53.414	Yes
8(30/60) 14h vs 23(16/30) 14h	15.556	8	44.291	Yes
8(30/60) 14h vs 22(16/30) 14h	13.437	7	38.258	Yes
8(30/60) 14h vs 21(16/30) 14h	6.425	6	18.295	Yes
8(30/60) 14h vs 8(16/30) 14h	4.376	5	12.458	Yes
8(30/60) 14h vs 21(30/60) 14h	1.275	4	3.630	No
8(30/60) 14h vs 22(30/60) 14h	1.223		Do not test	
8(30/60) 14h vs 23(30/60) 14h	1.189		Do not test	
23(30/60) 14h vs Pure IMC 14h	17.571	8	50.030	Yes
23(30/60) 14h vs 23(16/30) 14h	14.367	7	40.907	Yes
23(30/60) 14h vs 22(16/30) 14h	12.248	6	34.874	Yes
23(30/60) 14h vs 21(16/30) 14h	5.237	5	14.911	Yes
23(30/60) 14h vs 8(16/30) 14h	3.187	4	9.074	Yes
23(30/60) 14h vs 21(30/60) 14h	0.086		Do not test	
23(30/60) 14h vs 22(30/60) 14h	0.034		Do not test	
Comparison	Diff of Mean	p	q	P < 0.05
22(30/60) 14h vs Pure IMC 14h	17.537	7	49.932	Yes
22(30/60) 14h vs 23(16/30) 14h	14.332	6	40.809	Yes
22(30/60) 14h vs 22(16/30) 14h	12.214	5	34.776	Yes
22(30/60) 14h vs 21(16/30) 14h	5.202	4	14.813	Yes
22(30/60) 14h vs 8(16/30) 14h	3.152	3	8.976	Yes
22(30/60) 14h vs 21(30/60) 14h	0.052		Do not test	
21(30/60) 14h vs Pure IMC 14h	17.485	6	49.784	Yes
21(30/60) 14h vs 23(16/30) 14h	14.281	5	40.661	Yes
21(30/60) 14h vs 22(16/30) 14h	12.162	4	34.628	Yes
21(30/60) 14h vs 21(16/30) 14h	5.151	3	14.666	Yes
21(30/60) 14h vs 8(16/30) 14h	3.101	2	8.829	Yes
8(16/30) 14h vs Pure IMC 14h	14.384	5	40.956	Yes

(Appendix 3.6. continued).

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 14h vs 23(16/30) 14h	11.180	4	31.833	Yes
8(16/30) 14h vs 22(16/30) 14h	9.061	3	25.800	Yes
8(16/30) 14h vs 21(16/30) 14h	2.050	2	5.837	Yes
21(16/30) 14h vs Pure IMC 14h	12.334	4	35.119	Yes
21(16/30) 14h vs 23(16/30) 14h	9.130	3	25.996	Yes
21(16/30) 14h vs 22(16/30) 14h	7.011	2	19.963	Yes
22(16/30) 14h vs Pure IMC 14h	5.323	3	15.156	Yes
22(16/30) 14h vs 23(16/30) 14h	2.119	2	6.033	Yes
23(16/30) 14h vs Pure IMC 14h	3.204	2	9.123	Yes

Appendix 3.7. SNK results summary comparing drug release after 3, 6 and 14 hours of dissolution testing in formulations of varying pectin content.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 3h vs 5(16/30) 3h	29.475	4	32.419	Yes
Pure IMC 3h vs 2(16/30) 3h	23.531	3	25.882	Yes
Pure IMC 3h vs 3(16/30) 3h	20.637	2	22.699	Yes
3(16/30) 3h vs 5(16/30) 3h	8.837	3	9.720	Yes
3(16/30) 3h vs 2(16/30) 3h	2.894	2	3.183	No
2(16/30) 3h vs 5(16/30) 3h	5.943	2	6.537	Yes

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 6h vs 2(16/30) 6h	12.317	4	10.964	Yes
Pure IMC 6h vs 3(16/30) 6h	7.959	3	7.084	Yes
Pure IMC 6h vs 5(16/30) 6h	0.413	2	0.368	No
5(16/30) 6h vs 2(16/30) 6h	11.904	3	10.596	Yes
5(16/30) 6h vs 3(16/30) 6h	7.545	2	6.716	Yes
3(16/30) 6h vs 2(16/30) 6h	4.358	2	3.880	Yes

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
5(16/30) 14h vs Pure IMC 14h	11.008	4	19.278	Yes
5(16/30) 14h vs 2(16/30) 14h	4.961	3	8.688	Yes
5(16/30) 14h vs 3(16/30) 14h	4.892	2	8.568	Yes
3(16/30) 14h vs Pure IMC 14h	6.115	3	10.710	Yes
3(16/30) 14h vs 2(16/30) 14h	0.069	2	0.121	No
2(16/30) 14h vs Pure IMC 14h	6.047	2	10.589	Yes

Appendix 3.8. SNK results summary comparing drug release after 3, 6, and 14 hours of dissolution testing in formulations of varying alginate content.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 3h vs 7(16/30) 3h	25.616	4	48.682	Yes
Pure IMC 3h vs 8(16/30) 3h	15.280	3	29.039	Yes
Pure IMC 3h vs 10(16/30) 3h	14.866	2	28.253	Yes
10(16/30) 3h vs 7(16/30) 3h	10.749	3	20.429	Yes
10(16/30) 3h vs 8(16/30) 3h	0.413	2	0.786	No
8(16/30) 3h vs 7(16/30) 3h	10.336	2	19.643	Yes

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 6h vs 7(16/30) 6h	11.438	4	13.991	Yes
8(16/30) 6h vs Pure IMC 6h	5.960	3	7.291	Yes
8(16/30) 6h vs 10)16/30) 6h	0.276	2	0.337	No
10)16/30) 6h vs 7(16/30) 6h	11.163	3	13.654	Yes
10)16/30) 6h vs Pure IMC 6h	5.685	2	6.954	Yes
Pure IMC 6h vs 7(16/30) 6h	5.478	2	6.701	Yes

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
10(16/30) 14h vs Pure IMC 14h	18.071	4	33.599	Yes
10(16/30) 14h vs 7(16/30) 14h	7.821	3	14.542	Yes
10(16/30) 14h vs 8(16/30) 14h	3.686	2	6.854	Yes
8(16/30) 14h vs Pure IMC 14h	14.384	3	26.745	Yes
8(16/30) 14h vs 7(16/30) 14h	4.134	2	7.687	Yes
7(16/30) 14h vs Pure IMC 14h	10.250	2	19.058	Yes

Appendix 3.9. SNK results summary comparing drug release after 3, 6 and 14 hours of dissolution testing pectin-Aquateric® formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
Pure IMC 3h vs 3(16/30) 3h	23.531	5	25.136	Yes
Pure IMC 3h vs 11(16/30) 3h	23.376	4	24.970	Yes
Pure IMC 3h vs 3(30/60) 3h	3.686	3	3.938	Yes
Pure IMC 3h vs 11(30/60) 3h	1.826	2	1.950	No
11(30/60) 3h vs 3(16/30) 3h	21.705	4	23.185	Yes
11(30/60) 3h vs 11(16/30) 3h	21.550	3	23.020	Yes
11(30/60) 3h vs 3(30/60) 3h	1.860	2	1.987	No
3(30/60) 3h vs 3(16/30) 3h	19.845	3	21.198	Yes
3(30/60) 3h vs 11(16/30) 3h	19.690	2	21.032	Yes
11(16/30) 3h vs 3(16/30) 3h	0.155	2	0.166	No

Comparison	Diff of Mean	p	q	P < 0.05
11(30/60) 6h vs 3(16/30) 6h	24.065	5	24.605	Yes
11(30/60) 6h vs 11(16/30) 6h	19.483	4	19.920	Yes
11(30/60) 6h vs Pure IMC 6h	16.107	3	16.468	Yes
11(30/60) 6h vs 3(30/60) 6h	1.481	2	1.515	No
3(30/60) 6h vs 3(16/30) 6h	22.584	4	23.090	Yes
3(30/60) 6h vs 11(16/30) 6h	18.002	3	18.405	Yes
3(30/60) 6h vs Pure IMC 6h	14.625	2	14.953	Yes
Pure IMC 6h vs 3(16/30) 6h	7.959	3	8.137	Yes
Pure IMC 6h vs 11(16/30) 6h	3.376	2	3.452	Yes
11(16/30) 6h vs 3(16/30) 6h	4.582	2	4.685	Yes

Comparison	Diff of Mean	p	q	P < 0.05
11(30/60) 14h vs Pure IMC 14h	19.948	5	47.626	Yes
11(30/60) 14h vs 3(16/30) 14h	13.833	4	33.025	Yes
11(30/60) 14h vs 11(16/30) 14h	8.424	3	20.111	Yes
11(30/60) 14h vs 3(30/60) 14h	2.394	2	5.717	Yes
3(30/60) 14h vs Pure IMC 14h	17.554	4	41.909	Yes
3(30/60) 14h vs 3(16/30) 14h	11.438	3	27.309	Yes
3(30/60) 14h vs 11(16/30) 14h	6.029	2	14.395	Yes
11(16/30) 14h vs Pure IMC 14h	11.525	3	27.514	Yes
11(16/30) 14h vs 3(16/30) 14h	5.409	2	12.914	Yes
3(16/30) 14h vs Pure IMC 14h	6.115	2	14.600	Yes

Appendix 3.10. SNK results summary comparing drug release after 3, 6 and 14 hours of dissolution testing of alginate-Aquateric[R] formulations.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
14(30/60) 3h vs 14(16/30) 3h	26.701	5	68.156	Yes
14(30/60) 3h vs 8(16/30) 3h	19.742	4	50.391	Yes
14(30/60) 3h vs 8(30/60) 3h	10.353	3	26.427	Yes
14(30/60) 3h vs Pure IMC 3h	4.462	2	11.389	Yes
Pure IMC 3h vs 14(16/30) 3h	22.239	4	56.767	Yes
Pure IMC 3h vs 8(16/30) 3h	15.280	3	39.003	Yes
Pure IMC 3h vs 8(30/60) 3h	5.891	2	15.038	Yes
8(30/60) 3h vs 14(16/30) 3h	16.348	3	41.729	Yes
8(30/60) 3h vs 8(16/30) 3h	9.388	2	23.964	Yes
8(16/30) 3h vs 14(16/30) 3h	6.960	2	17.764	Yes

Comparison	Diff of Mean	p	q	P < 0.05
14(30/60) 6h vs 14(16/30) 6h	23.962	5	30.531	Yes
14(30/60) 6h vs Pure IMC 6h	21.137	4	26.931	Yes
14(30/60) 6h vs 8(16/30) 6h	15.090	3	19.227	Yes
14(30/60) 6h vs 8(30/60) 6h	5.047	2	6.431	Yes
8(30/60) 6h vs 14(16/30) 6h	18.915	4	24.100	Yes
8(30/60) 6h vs Pure IMC 6h	16.090	3	20.500	Yes
8(30/60) 6h vs 8(16/30) 6h	10.043	2	12.796	Yes
8(16/30) 6h vs 14(16/30) 6h	8.872	3	11.304	Yes
8(16/30) 6h vs Pure IMC 6h	6.047	2	7.704	Yes
Pure IMC 6h vs 14(16/30) 6h	2.825	2	3.600	Yes

Comparison	Diff of Mean	p	q	P < 0.05
14(30/60) 14h vs Pure IMC 14h	20.069	5	72.984	Yes
14(30/60) 14h vs 14(16/30) 14h	15.090	4	54.879	Yes
14(30/60) 14h vs 8(16/30) 14h	5.685	3	20.674	Yes
14(30/60) 14h vs 8(30/60) 14h	1.309	2	4.761	Yes
8(30/60) 14h vs Pure IMC 14h	18.760	4	68.223	Yes
8(30/60) 14h vs 14(16/30) 14h	13.781	3	50.118	Yes
8(30/60) 14h vs 8(16/30) 14h	4.376	2	15.912	Yes
8(16/30) 14h vs Pure IMC 14h	14.384	3	52.310	Yes
8(16/30) 14h vs 14(16/30) 14h	9.406	2	34.205	Yes
14(16/30) 14h vs Pure IMC 14h	4.978	2	18.105	Yes

Appendix 3.11. SNK results summary for drug release after 3 hours of dissolution testing comparing pectin formulations to those of alginate.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 3h vs 23(16/30) 3h	14.315	9	19.009	Yes
8(16/30) 3h vs 17(16/30) 3h	13.213	8	17.545	Yes
8(16/30) 3h vs 22(16/30) 3h	9.888	7	13.131	Yes
8(16/30) 3h vs 18(16/30) 3h	9.233	6	12.261	Yes
8(16/30) 3h vs 19(16/30) 3h	8.958	5	11.895	Yes
8(16/30) 3h vs 3(16/30)	8.252	4	10.957	Yes
8(16/30) 3h vs 20(16/30) 3h	6.908	3	9.173	Yes
8(16/30) 3h vs 21(16/30) 3h	1.464	2	1.944	No
21(16/30) 3h vs 23(16/30) 3h	12.851	8	17.065	Yes
21(16/30) 3h vs 17(16/30) 3h	11.748	7	15.601	Yes
21(16/30) 3h vs 22(16/30) 3h	8.424	6	11.186	Yes
21(16/30) 3h vs 18(16/30) 3h	7.769	5	10.317	Yes
21(16/30) 3h vs 19(16/30) 3h	7.494	4	9.951	Yes
21(16/30) 3h vs 3(16/30)	6.787	3	9.013	Yes
21(16/30) 3h vs 20(16/30) 3h	5.444	2	7.229	Yes
20(16/30) 3h vs 23(16/30) 3h	7.407	7	9.836	Yes
20(16/30) 3h vs 17(16/30) 3h	6.305	6	8.372	Yes
20(16/30) 3h vs 22(16/30) 3h	2.980	5	3.957	No
20(16/30) 3h vs 18(16/30) 3h	2.326		Do not test	
20(16/30) 3h vs 19(16/30) 3h	2.050		Do not test	
20(16/30) 3h vs 3(16/30)	1.344		Do not test	
3(16/30) vs 23(16/30) 3h	6.064	6	8.052	Yes
3(16/30) vs 17(16/30) 3h	4.961	5	6.588	Yes
3(16/30) vs 22(16/30) 3h	1.637		Do not test	
3(16/30) vs 18(16/30) 3h	0.982		Do not test	
3(16/30) vs 19(16/30) 3h	0.706		Do not test	
19(16/30) 3h vs 23(16/30) 3h	5.357	5	7.114	Yes
19(16/30) 3h vs 17(16/30) 3h	4.255	4	5.650	Yes
19(16/30) 3h vs 22(16/30) 3h				

(Appendix 3.11. continued).

19(16/30) 3h vs 18(16/30) 3h	0.930		Do not test	
18(16/30) 3h vs 23(16/30) 3h	0.276		Do not test	
18(16/30) 3h vs 17(16/30) 3h	5.082	4	6.748	Yes
18(16/30) 3h vs 22(16/30) 3h	3.979	3	5.284	Yes
22(16/30) 3h vs 23(16/30) 3h	0.655		Do not test	
22(16/30) 3h vs 17(16/30) 3h	4.427	3	5.879	Yes
17(16/30) 3h vs 23(16/30) 3h	3.325	2	4.415	Yes
	1.102	2	1.464	No

Appendix 3.12. SNK results summary for drug release after 6 hours of dissolution testing comparing pectin formulations to those of alginate.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 6h vs 23(16/30) 6h	22.274	9	24.361	Yes
8(16/30) 6h vs 17(16/30) 6h	20.069	8	21.949	Yes
8(16/30) 6h vs 19(16/30) 6h	17.898	7	19.575	Yes
8(16/30) 6h vs 22(16/30) 6h	15.556	6	17.013	Yes
8(16/30) 6h vs 18(16/30) 6h	14.711	5	16.090	Yes
8(16/30) 6h vs 3(16/30) 6h	13.919	4	15.223	Yes
8(16/30) 6h vs 20(16/30) 6h	12.661	3	13.848	Yes
8(16/30) 6h vs 21(16/30) 6h	3.549	2	3.881	Yes
21(16/30) 6h vs 23(16/30) 6h	18.725	8	20.479	Yes
21(16/30) 6h vs 17(16/30) 6h	16.520	7	18.068	Yes
21(16/30) 6h vs 19(16/30) 6h	14.350	6	15.694	Yes
21(16/30) 6h vs 22(16/30) 6h	12.007	5	13.132	Yes
21(16/30) 6h vs 18(16/30) 6h	11.163	4	12.209	Yes
21(16/30) 6h vs 3(16/30) 6h	10.370	3	11.342	Yes
21(16/30) 6h vs 20(16/30) 6h	9.113	2	9.967	Yes
Comparison	Diff of Mean	p	q	P < 0.05
20(16/30) 6h vs 23(16/30) 6h	9.612	7	10.513	Yes
20(16/30) 6h vs 17(16/30) 6h	7.407	6	8.101	Yes

(Appendix 3.12. continued).

20(16/30) 6h vs 19(16/30) 6h	5.237	5	5.727	Yes
20(16/30) 6h vs 22(16/30) 6h	2.894	4	3.165	No
20(16/30) 6h vs 18(16/30) 6h	2.050		Do not test	
20(16/30) 6h vs 3(16/30) 6h	1.258		Do not test	
3(16/30) 6h vs 23(16/30) 6h	8.355	6	9.138	Yes
3(16/30) 6h vs 17(16/30) 6h	6.150	5	6.726	Yes
3(16/30) 6h vs 19(16/30) 6h	3.979	4	4.352	Yes
3(16/30) 6h vs 22(16/30) 6h	1.637		Do not test	
3(16/30) 6h vs 18(16/30) 6h	0.792		Do not test	
18(16/30) 6h vs 23(16/30) 6h	7.562	5	8.271	Yes
18(16/30) 6h vs 17(16/30) 6h	5.357	4	5.859	Yes
18(16/30) 6h vs 19(16/30) 6h	3.187	3	3.485	No
18(16/30) 6h vs 22(16/30) 6h	0.844		Do not test	
Comparison	Diff of Mean	p	q	P < 0.05
22(16/30) 6h vs 23(16/30) 6h	6.718	4	7.348	Yes
22(16/30) 6h vs 17(16/30) 6h	4.513	3	4.936	Yes
22(16/30) 6h vs 19(16/30) 6h	2.343		Do not test	
19(16/30) 6h vs 23(16/30) 6h	4.376	3	4.785	Yes
19(16/30) 6h vs 17(16/30) 6h	2.171	2	2.374	No
17(16/30) 6h vs 23(16/30) 6h	2.205	2	2.412	No

Appendix 3.13. SNK results summary for drug release after 14 hours of dissolution testing comparing pectin formulations to those of alginate.

All Pairwise Multiple Comparisons: (Student-Newman-Keuls method)

Comparison	Diff of Mean	p	q	P < 0.05
8(16/30) 14h vs 17(16/30) 14h	14.608	9	31.945	Yes
8(16/30) 14h vs 19(16/30) 14h	12.593	8	27.538	Yes
8(16/30) 14h vs 23(16/30) 14h	11.180	7	24.449	Yes
8(16/30) 14h vs 18(16/30) 14h	9.647	6	21.096	Yes
8(16/30) 14h vs 22(16/30) 14h	9.061	5	19.815	Yes
8(16/30) 14h vs 3(16/30) 14h	8.269	4	18.082	Yes
8(16/30) 14h vs 20(16/30) 14h	6.873	3	15.031	Yes
8(16/30) 14h vs 21(16/30) 14h	2.050	2	4.483	Yes

(Appendix 3.13. continued).

21(16/30) 14h vs 17(16/30) 14h	12.558	8	27.462	Yes
21(16/30) 14h vs 19(16/30) 14h	10.543	7	23.055	Yes
21(16/30) 14h vs 23(16/30) 14h	9.130	6	19.966	Yes
21(16/30) 14h vs 18(16/30) 14h	7.597	5	16.613	Yes
21(16/30) 14h vs 22(16/30) 14h	7.011	4	15.332	Yes
21(16/30) 14h vs 3(16/30) 14h	6.219	3	13.599	Yes
21(16/30) 14h vs 20(16/30) 14h	4.823	2	10.548	Yes
Comparison	Diff of Mean	p	q	P < 0.05
20(16/30) 14h vs 17(16/30) 14h	7.735	7	16.914	Yes
20(16/30) 14h vs 19(16/30) 14h	5.719	6	12.507	Yes
20(16/30) 14h vs 23(16/30) 14h	4.307	5	9.418	Yes
20(16/30) 14h vs 18(16/30) 14h	2.773	4	6.065	Yes
20(16/30) 14h vs 22(16/30) 14h	2.188	3	4.784	Yes
20(16/30) 14h vs 3(16/30) 14h	1.395	2	3.051	Yes
3(16/30) 14h vs 17(16/30) 14h	6.339	6	13.863	Yes
3(16/30) 14h vs 19(16/30) 14h	4.324	5	9.455	Yes
3(16/30) 14h vs 23(16/30) 14h	2.911	4	6.366	Yes
3(16/30) 14h vs 18(16/30) 14h	1.378	3	3.014	No
3(16/30) 14h vs 22(16/30) 14h	0.792		Do not test	
22(16/30) 14h vs 17(16/30) 14h	5.547	5	12.130	Yes
22(16/30) 14h vs 19(16/30) 14h	3.531	4	7.723	Yes
22(16/30) 14h vs 23(16/30) 14h	2.119	3	4.634	Yes
22(16/30) 14h vs 18(16/30) 14h	0.586		Do not test	
Comparison	Diff of Mean	p	q	P < 0.05
18(16/30) 14h vs 17(16/30) 14h	4.961	4	10.849	Yes
18(16/30) 14h vs 19(16/30) 14h	2.946	3	6.442	Yes
18(16/30) 14h vs 23(16/30) 14h	1.533	2	3.353	Yes
23(16/30) 14h vs 17(16/30) 14h	3.428	3	7.497	Yes
23(16/30) 14h vs 19(16/30) 14h	1.413	2	3.089	Yes
19(16/30) 14h vs 17(16/30) 14h	2.016	2	4.408	Yes

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