Microencapsulation of an Antiplatelet Agent

By

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82

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Faculty of Pharmacy

University of Manitoba

Winnipeg, Manitoba

ISBN 0-612-13247-1

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MICROENCAPSULATION OF AN ANTIPLATELET AGENT

BY

MTHIMKHULU KHOHLOKWANE

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

Dedicated to Skosie & Allan

ABSTRACT

A method has been developed for the preparation of sustained and enteric release acetylsalicylic acid (ASA) pellets by the phase separation microencapsulation technique. The phase separation coacervation of polymers was achieved by solvent alteration. The produced microcapsules were spheroidal units with the drug crystals evenly dispersed throughout the rate controlling polymer matrix. The matrix consisted of cellulose acetate hydrogen phthalate (CAP), and acrylate/methacrylate copolymer (Eudragit RSPM). Effects of drug loading on the release profiles and pellet micromeritic properties were evaluated on two mesh size (12/16 and 16/22) fractions. The effect of subsequent coating with (i) ethylcellulose, (ii) polyisobutylene, (iii) Aquateric^{R} dispersion on release kinetics were studied. The microcapsules were then filled in size 0 gelatin capsules and the *in-vitro* dissolution studies results indicated that the drug release kinetics vary from near zero order to square root of time release depending on the total polymer content. The level of drug deterioration products resulting from the formulation procedure was found to be comparable to the levels of deterioration products of the pure drug, and of two ASA commercial products.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Suresh Venkataram for his valuable guidance, firm support, overall supervision, and for the patience he exercised during the course of this work, and throughout my programme in the Faculty of Pharmacy. My heart felt gratitude are also extended to Mr. Alex Kamanga for his encouragement and trust that kept me going during the difficult times.

I would further like to express my appreciation and gratefulness to Lesotho Pharmaceutical Corporation management and directorship, as well as to CIDA for the opportunity, financial and other material support without which the commencement and completion of this work would not have been possible.

Finally I recognize and express my special thanks to my family for allowing me to engage in this endeavour, putting up with my divided attention, for their support, understanding and encouragement. All the other support I received from all my friends and from other angles is gratefully appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A LIST OF APPENDED FIGURES

CHAPTER 1

INTRODUCTION

1.0. THROMBOSIS

Blood platelets play a central role in all thromboembolic disorders (1). Thrombosis starts by circulating blood platelet aggregation in a damaged vessel, or adherence to arterial or venous walls to form a white thrombus or a red thrombus respectively. The white thrombus caused by the platelet nidus, and formed on the artery can reduce arterial blood flow to a detrimental extend causing hemodynamic compromise. White thrombus has also been suggested to exacerbate ischemia-induced myocardial injury or death (2). The red thrombus (a result of fibrin tail) on the other hand can be detached from the venous wall and travel as emboli to block the pulmonary arteries. Both of these conditions are strongly implicated in cardiovascular diseases. The two types of eicosanoid groups that are important in thrombogenesis are (a) thromboxane_A, produced via the arachidonic acid cascade pathway in the platelets, which in conjunction with secreted adenosine di-phosphate (ADP) and other prostaglandin endoperoxides induces thrombogenesis. The other (b) Prostacyclin, produced via the arachidonic acid metabolism in the vascular cells inhibits thrombogenesis.

1.0.1. TREATMENT

Blood coagulation disorders can be managed by:-

Anticoagulant drug therapy. Anticoagulants such as heparin, work by accelerating the antithrombin III activity, an endogenous compound that inhibits clotting factor protease by binding onto it to prevent coagulation. Anticoagulants can be administered intravenously or orally.

Fibrinolytic agents. These agents lyse thrombi by catalyzing the conversion of inactive plasminogen to active plasmin. Plasmin is an endogenous enzyme that hydrolyses fibrin into soluble products, and prevents blood clotting. An example of this class of agents is urokinase. Fibrinolytic agents are usually administered intravenously. One of the disadvantages of fibrinolytic therapy is its high cost.

Antithrombic drugs. They control the platelet function according to these three categories: (a) by controlling agents that are generated outside the platelet and interact with the platelet membrane receptors, and cause aggregation. (b) by controlling agents generated inside the platelet, and bind to membrane receptors and cause aggregation. (c) The third approach is by controlling agents that are generated within the platelet, and act inside the platelet to cause

blood clotting. Antithrombic, or platelet inhibitory drugs work by inhibiting prostaglandin metabolism, e.g. inhibiting thromboxane A_2 which is involved in the genesis of a blood clot, and by augmenting platelet cyclic adenosine-3',5'-monophosphate (cAMP) content. cAMP is an intracellular second messenger that mediates responses by mobilizing stored energy through the conversion of adenosine 5'-triphosphate, drugs that augment the intraplatelet concentrations of cAMP do not prolong the bleeding time (1). ASA has been used successfully in this approach. Studies are continuing to find alternative ways of effecting primary and secondary prevention of vascular death, or to retard the progression of tissue ischemia due to blood coagulation disorders using this group of drugs.

1.0.2. ACETYLSALICYLIC ACID

HISTORY. ASA belongs to a class of non-steroidal anti-inflammatory drugs, which are often used as analgesics. The history of ASA dates back to the times of Hippocrates 2400 years ago when salicylates were used for their analgesic effect by women in labour (extracting salicylates by chewing willow leaves), to the rediscovery of the white willow bark by Edward Stone circa 1757. Salicylic acid, which is responsible for the analgesic effects of ASA, was first isolated from the willow bark by Piria, and is discussed in

a number of reports (3,4), while the first synthetic production of salicylic acid was done by Gerland and is discussed in detail by Gibson (5). Today ASA is most commonly prepared by the acetylation of salicylic acid with acetic anhydride, in a mineral acid catalyzed reaction.

PHARMACOLOGICAL ACTION. ASA is used as a mild analgesic, antipyretic, and anti-inflammatory agent. The focus of this discussion will be on ASA as an antiplatelet agent. The value of ASA as an antithrombotic agent was not recognized until the early 1940's, making it the oldest of the antiplatelet drugs (6). In recent years ASA has gained attention for prophylactic use in the management of stroke and occlusive heart diseases (7-12). It exerts its antiplatelet properties by an irreversible acetylation of cyclooxygenase which is a key enzyme in the arachidonic acid metabolism. Acetylation of cyclooxygenase results in the inhibition of this enzyme and prevents if from catalyzing the synthesis of prostaglandins which speed up thrombogenesis and atherogenesis. There exists a controversy on whether or not ASA is as efficacious in males as it is in females. Some reports suggesting that ASA alleviate incidences of transient ischemic attacks and unstable angina in men have been documented (13), other reports suggest that the efficacy of ASA is not influenced by gender (14-17), and it can be used in women patients for prevention or reduction of sudden death

in female myocardial infarction survivors and in the treatment of pre-eclampsia. The analgesic effect of ASA has been attributed to salicylic acid resulting from the hydrolysis of ASA in the intestinal wall cells and in the liver. The entire ASA molecule on the other hand is required to acetylate and inhibit the platelet function by its acetyl group, to stop aggregation and clotting.

CHEMISTRY AND PHARMAKOKINETICS. ASA is a weak organic acid with a pK_a value of 3.5 while the pK_a value of Salicylic acid is 3.0. ASA and other salicylates are rapidly absorbed from the walls of the stomach and upper small intestines, reaching peak serum levels within 1-2 hours (1). Plasma half-life ($t_{1/2}$) of ASA is dependent on and increases with dose (13). At dosages of about 600 mg, ASA is eliminated by first-order kinetics and the plasma $t_{1/2}$ is 3-5 hours. At dosages of $\geq$ 4 g/day, the elimination assumes zero order kinetics, and plasma $t_{1/2}$ increases to 15 hours. Zapadniuk *et al.* (17) demonstrated that the elimination half-life of ASA varies with age at fixed dosages of 14 mg/kg. They reported half lifes of 12.2 hours in adult patients, 6.19 hours in the elderly patients, and 4.47 hours in young patients, making ASA an excellent candidate for a controlled release (CR) formulation for the elderly and young patient population groups.

PRODUCTS AND SIDE EFFECTS. The most commonly used acetylsalicylic acid dosage form is an uncoated tablet. Enteric coated and buffered aspirin tablet formulations were designed later in order to solve gastric intolerance problems associated with ASA (18-20). Undissolved ASA and non-ionized salicylates in the stomach cause irritation of gastric mucosa and lead to upper gastrointestinal hemorrhage (13). Ileal and jejunal ulceration and perforations, as well as hepatic and kidney damage are among the side effects associated with the use of ASA. Recently research efforts have concentrated on the design of multiparticulate ASA formulations (20-21) in order to combat the gastric injury complications in ASA therapy. Other side effects that are associated with high dosages of ASA include tinnitus, decreased hearing, vomiting, hyperpnea and respiratory alkalosis. These side effects appear to be dose related and may be reversed by reducing the dose.

1.1.ORAL CONTROLLED - RELEASE DRUG DELIVERY SYSTEMS

Conventional solid oral dosage forms deliver drug contents immediately upon contact with the GI tract fluids. The difference between sustained release and controlled release drug preparations is that sustained release refers to pharmaceutical dosage forms designed to retard the release of the drug into the GI fluids, resulting in the drug absorption

into the systemic circulation taking place over a longer period of time. The concept of controlled release (e.g. osmotic pressure-activated drug delivery) encompasses predictability and reproducibility of drug release kinetics by design in addition to just retarding the drug release. Controlled drug release products are designed to deliver an active principle of the drug product at a therapeutically effective rate and concentration to the desired absorption site for a duration of time, for an improved therapeutic benefits. The drug is delivered at therapeutic concentrations that are below toxic levels (**see Fig. 1**). The need for sustained release drug products was first discussed by Lipowski (**22**), and to date a wide variety of sustained and controlled release drug products are available for use in the management of a number of disease states. The different types of agents, excipient and approaches that are used to design oral sustained or oral controlled release delivery devices are discussed in the topics that follow, with a bias towards microencapsulation technique as a controlled delivery design.

1.1.1. TYPES OF SOLID ORAL CONTROLLED DRUG RELEASE DEVICES

A number of novel solid oral drug delivery devices have been designed that deliver drugs to their absorption sites in a

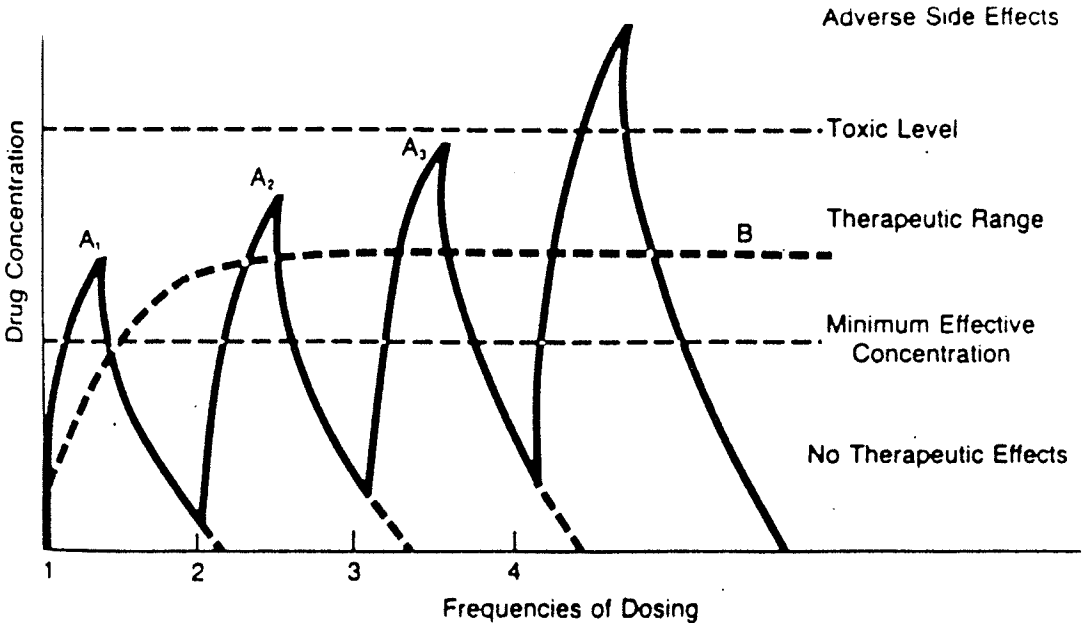


Figure 1. A pulsed drug release (A) and a controlled release (B) graph associated with a conventional and a controlled drug delivery systems respectively. (adapted from ref. 1)

controlled fashion after ingestion. The tablet dosage form is the most extensively used solid oral device because of its many inherent advantages over other types of solid dosage forms. Advantages of tablets combine the ease and low cost of manufacture, the precision of drug loading, further processability ease to improve the overall dosage quality, and a high patient compliance. Controlled release devices of other solid dosage forms such as granules (encapsulated) and spansules have been used to some extent as well. Multiparticulate devices such as microcapsules, pellets, microparticles and microspheres offer an alternative means of solid oral drug delivery systems. Microencapsulation technology has lately received considerable attention because of the enormous potential that microcapsules offer to overcome many formulation and delivery problems encountered in conventional dosage forms such as capsules, tablets and powders.

1.2. MICROENCAPSULATION

Bungenburg *et al.* (23) were the first researchers to publish work related to microencapsulation, working on the production of gelatin spheres. The technique of microencapsulation became useful in carbonless copying paper production, and later in photosensitive photographic paper production. The emergence of sustained-release pharmaceutical preparations in

the 1970's led to an adoption of microencapsulation technology into pharmaceutical formulations design. Today, microencapsulation is one of the most frequently used techniques in the manufacture of controlled and sustained-release drug delivery devices for various uses (24-27). Drug materials in their natural solid or liquid form may be microencapsulated as they are, or may be prepared in different ways prior to microencapsulation. The microencapsulation process refers to enclosing of drug materials in solid inert and impermeable or semi-permeable polymeric membranes, or wall formers to meet various clinical or even aesthetic objectives. A number of microencapsulation methods have been successfully employed (28-31) to prepare different types of microcapsules for various uses. The choice of microencapsulation technique to be employed to encapsulate drug materials can be strongly influenced by factors such as the physicochemical and pharmacological properties of the drug itself, the properties of the wall former/s and the intended use of the drug. Microcapsules are also referred to by other terms such as microspherules, spansules, coated granules, pellets or seeds. The differences between microcapsules and the conventional hard or soft gelatin capsules are size, a variety of coating materials that can be used in microencapsulation, the coating procedure, film or wall size, their unique release properties and their diversity of application in medicine. Sizes of

microcapsules range between 1 and 2000 µm (31, 32). Microcapsules that are smaller than 1 µm are referred to as nanoparticles, nanocapsules, microparticles or microspheres.

1.2.1. TYPES OF MICROCAPSULES

A wide variety of microcapsules have been prepared (33-35), yet it is difficult to appropriately describe microcapsules in terms of form, size, range, surface and interior quality. Microcapsule forms that have been described in various literature material indicate that different structural forms exist (**Fig. 2**), the most common type being the mononuclear spherical type, which refers to the form whereby a drug crystal forms a reservoir and is completely enveloped in a polymeric membrane to form a discrete unit. Microcapsules can also be described as matrix type, reservoir type and reservoir/matrix type to describe the way in which the drug is associated with the polymer.

RESERVOIR TYPE. This is the most common type of microcapsules. It consists of a drug core completely enclosed in a polymer membrane or wall (**see Fig. 3 A**). The core material can either be a pure drug, a mixture of the pure drug and excipient, or a microencapsulated drug (to

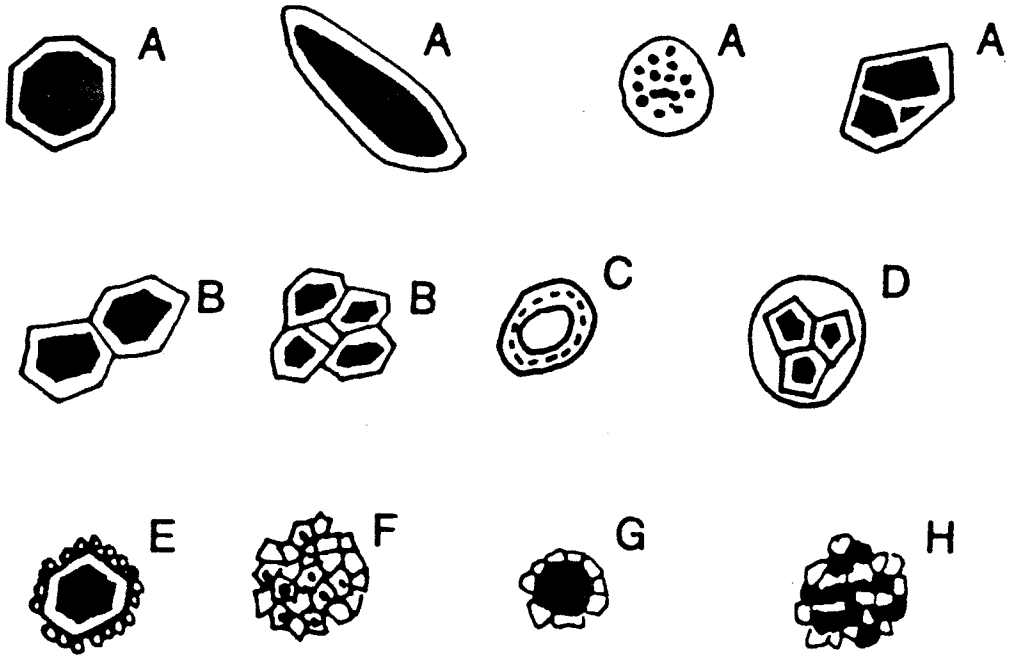


Figure 2. An illustration of microcapsule morphology variations, showing mononuclear and multinuclear shapes (A), fused mononuclear shapes (B), dual walled type (C), microencapsulated microcapsule (D), microencapsulated mononuclear with a spay dried finish (E), aggregated mononuclear microcapsule (F), a core material prepared by the spray drying method (G) and a granular matrix microcapsule (H). (adapted from ref. 33).

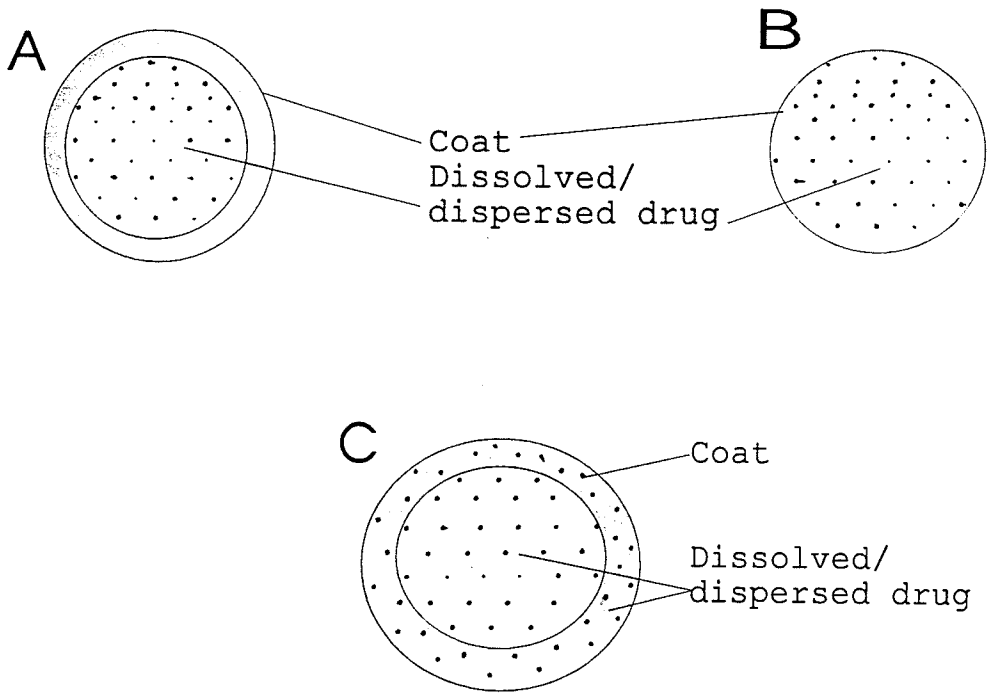


Figure 3. Schematic representation of microcapsules types, showing A reservoir, B monolithic (matrix) and C reservoir/monolithic type.

form a microencapsulated microcapsule **Fig. 2 C**).

MATRIX TYPE. These microcapsules consist of fine solid drug particles, or dissolved drug evenly dispersed throughout the polymeric matrix (**Fig. 3 B**). Drug release from such devices usually follows the Higuchi release model to be discussed under release kinetics.

RESERVOIR/MATRIX TYPE. This type of microcapsule can be manufactured by further coating the matrix type of microcapsule, with a solution containing the drug and the wall forming polymer.

1.3. DRUG RELEASE KINETICS

For a successful formulation of any solid oral controlled delivery system, factors that govern the drug release from the polymeric matrix should be clearly understood, in addition to understanding the physico chemical properties of the drug material. There is no drug release model that is specific to microcapsules. Drug release from microcapsules occurs by mass transfer (see equation 1) and can be controlled by manipulating a number of parameters to release the drug by any one or a combination of first order, (**see Fig. 4**) zero order, or a slow first order release kinetics (35-39).

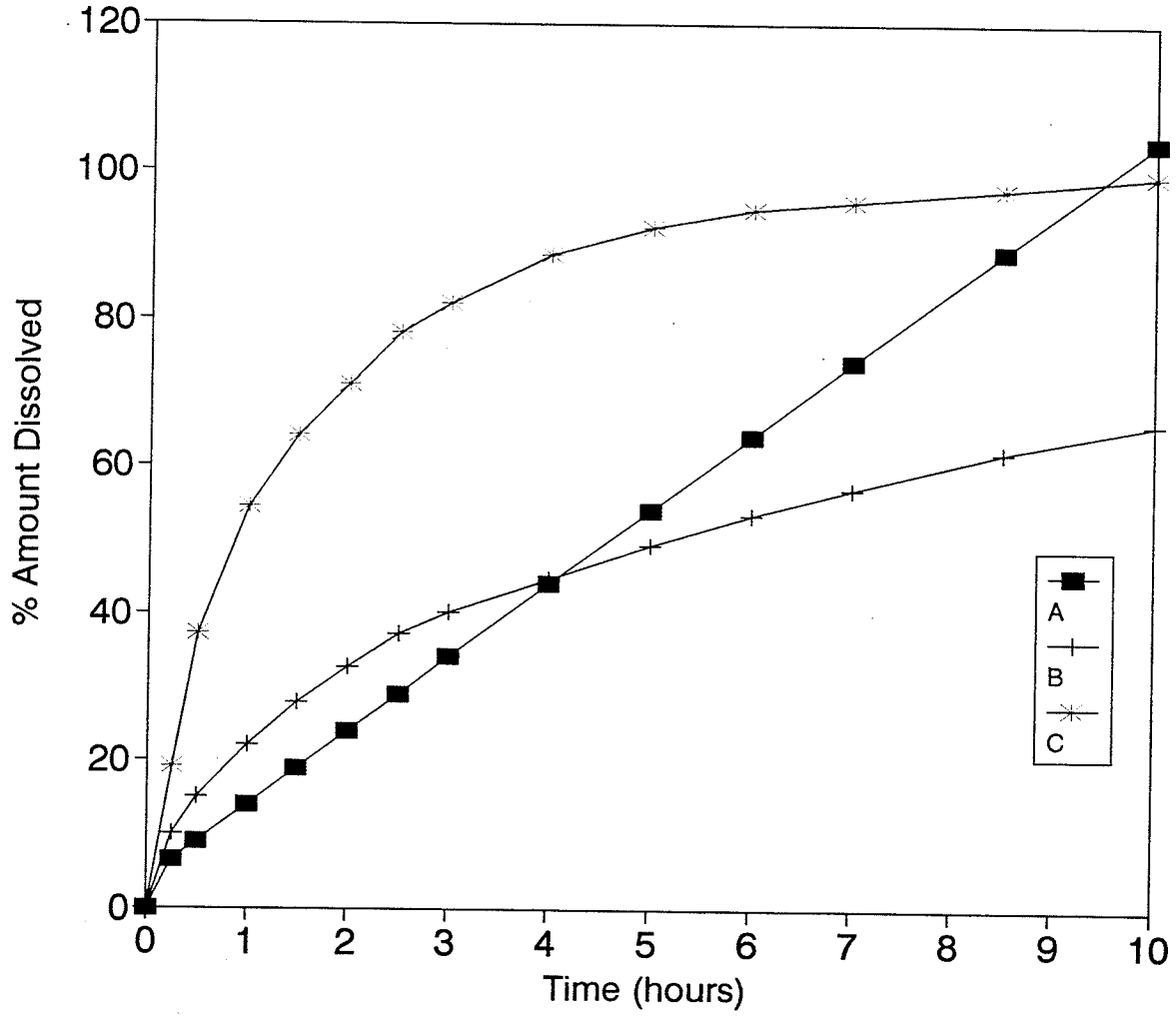


Figure 4. Graphs showing modes of drug release for a plot of M *vs.* t graphs for (A) zero order release (B) square root of time, and (C) first order release.

According to Fick's Law, the flux (J, mg/s) of material across a membrane (in the direction of declining concentration) is governed by the equation:

$$J = -D(dc/dx) \quad \text{.....Equation (1)}$$

Where:

D is the diffusion coefficient (cm^2/s), dc/dx the concentration gradient from the solid surface to the bulk solution (mg/cc/cm). The drug flux can be regulated by controlling the diffusion coefficient D.

A zero order drug release relates to the type of release where the amount of drug (M) released is constant and independent of the amount of drug remaining in the device. The following equation describes the zero order release.

$$dM/dt = K_0 \quad \text{.....Equation (2)}$$

Where K_0 is the zero order release rate constant expressed in units of mass/time. Integration of equation 2 yields the following equation

$$M = M_0 + K_0 t \quad \text{.....Equation (3)}$$

Where:

M_0 is the amount of drug at time t = 0. Based on this

equation, a plot of M versus t will yield a straight line graph with a slope = K_0, and y intercept = M_0.

When $M_0 = 0$ at $t = 0$, equation 2 can be re-written as follows

$$M = K_0 t^n \quad \text{.....Equation (4)}$$

taking logarithms on both sides yields the following equation

$$\log M = \log K_0 + (n) \log t \quad \text{..... Equation (5)}$$

where n = 1 for zero order release kinetics. A plot of log M *vs.* log t should give a straight line graph with a slope equal to 1 if the drug release follows zero order kinetics.

A drug release mechanism that occurs in the case of granular, porous matrices where the drug diffusibility from the matrix is the rate-determining factor has been discussed by Higuchi (**34**) and is represented by the following equation:

$$Q = \{D\epsilon / T(2A - \epsilon C_s) C_s t\}^{1/2} \quad \text{.....Equation (6)}$$

Where:

Q is the amount of drug released (mg/unit surface area), D is the drug diffusion coefficient, ϵ refers to the matrix porosity, T the matrix tortuosity, C_s is the solubility of

drug in the surrounding medium (g/ml), A is the drug concentration in the matrix (g/ml) and t the amount of time elapsed (hours). In order for these equations to hold, the following assumptions discussed by Gupta et al (**41**) have to apply:

- A pseudo-steady state is maintained during the release
- $A >> C_s$
- Drug particles are much smaller than those in the matrix
- The diffusion coefficient remains constant and
- No drug/matrix interaction exists

If all the parameters are held constant, the Higuchi's equation can be reduced to:-

$$Q = Kt^{1/2} \quad \text{.....Equation (7)}$$

Taking the logarithm of both sides of equation (6), the following equation results;

$$\log Q = \log K + 1/2 \log t \quad \text{.....Equation (8)}$$

Thus a plot of log Q *vs.* log t should yield a linear graph with a slope of 1/2.

K is a constant that takes into account the factors that constitute the characteristics of the polymer matrix, such as polymer porosity and tortuosity. The drug release control can be achieved by selecting polymers with a low overall **K** so that the drug diffusion rate can be better regulated.

If the amount of drug released from the device is proportional to the amount of drug (M) remaining in the device, then the release rate followed is first order (**Fig. 4 C**) and can be expressed by the following equation:

$$dM/dt = KM. \quad \text{.....Equation (9)}$$

Where:

K is the first order rate constant. Integrating equation (8) yields the following equation:

$$\log M = [(Kt)/2.303] + \log M_0 \quad \text{.....Equation (10)}$$

A graphical plot of logM *vs.* t will yield a straight line graph of slope = K/2.303 and a y intercept = $\log M_0$

1.4. POLYMERIC VEHICLES AND EROSION MECHANISMS

Two classes of polymers have been described by Ron and Langer (**39**) based on their degradation mechanisms, namely biodegradable and non-biodegradable. The release mechanisms of

controlled release preparations from polymeric vehicles are influenced by a combination of two or more of the following processes:

- dissolution, permeation, erosion, diffusion, osmosis, and ion-exchange.

Of these processes, the single most important factor is water permeation, since it dictates drug solubilization and diffusion rates across the inert matrix, membranes and hydrophillic gel barriers etc.

1.5. RATIONALE

From a clinical point of view, the rationale behind the design of drug formulations using the microencapsulation technique lies in the quest to improve drug therapy benefits through the improvement of therapeutic efficacy and safety of old and newly emerging drugs and to improve patient compliance (reduced daily doses). Re-designing conventional dosage forms into new microencapsulated controlled release dosage forms also provides inexpensive ways of re-patenting. The major criteria for the selection of a drug candidate for the design of sustained release system have been discussed by a number of researchers (**40, 41**) and are:

- short biological half-life (2-4 hours),
- narrow therapeutic index,
- efficient GI absorption,
- small daily dose,
- no first pass metabolism, and
- marketing benefits

Other characteristics of a good candidate for oral controlled release (CR) dosage forms include good aqueous solubility, because slow dissolving drugs have inherent slow release properties. Stability to a wide pH range, and to gastro-intestinal enzymes, and flora constitutes the physiologic considerations. Stability to a wide range of pH, GI enzymes and flora are important factors because an orally administered CR formulation (except for floating devices) will be exposed to luminal contents of the stomach with pH around 2, and the gastric flora and enzymes which are different from the conditions in the small intestines where the pH environment is more basic and different flora and enzymes are present.

1.6. DRUG RELEASE MECHANISMS OF CONTROLLED RELEASE DEVICES

In order for a device to pass as a controlled drug release (CR) system, it has to meet the *in-vivo* and *in-vitro* drug

release requirements which are predictability and reproducibility. The mechanism of drug release should be well defined and the release rate regulated by the normal physico-chemical principles. Some of the drug release mechanisms from polymeric vehicles are discussed.

1.6.1. NON-BIODEGRADABLE TYPES

STAGNANT LAYER CONTROLLED SYSTEMS. In this system, the core material is separated from the solution front by a porous or a hydrophobic matrix. The rate of drug dissolution into the surrounding medium depends on the solvent penetration of the matrix which is controlled by the matrix porosity, wettability, or the hydrophobic additives. An equation that describes the release rate in stagnant layer controlled systems can be obtained:

$$M_t/M_\infty = 1 - ((1 - K_0t)/C_0a)n) \quad \text{.....Equation (11)}$$

Where:

M_t is the amount of drug released at time t (mg), M_∞ is the total amount of drug released eventually (mg), K_0 is the zero order drug release rate constant, C_0 the initial drug concentration (mg/ml) while **'a'** refers to the half thickness of the device (cm), n is the shape factor, and n = 1 for a slab, 2 for a cylinder and 3 for a sphere.

According to equation (11) when designing a stagnant layer controlled system, dissolution control can be achieved by producing spherical shaped unit instead of slabs cylindrical or irregular shaped units, by increasing the size of the units or by a combination of the above mentioned factors.

DISSOLUTION CONTROL BY ENCAPSULATION. Drug particles are coated with a slowly dissolving material which releases the drug as it dissolves. The resulting product can either be compressed into tablets, or placed into gelatin capsules to make unit doses. If the drug is dispersed uniformly in the matrix, and the wall former does not swell, zero-order release kinetics can be achieved (**42, 43**). The equation that describes the drug release mechanism for dissolution controlled systems is as follows:

$$dM/dt = A\, dx/dt.f(c) \quad \text{.....Equation (12)}$$

Where:

dx/dt is the polymer dissolution rate (mg/h), A is the surface area, and f(c) the drug concentration in the matrix.

DIFFUSION CONTROL. Two categories of systems fall under this class. Reservoir type systems and matrix (or monolithic) type systems.

RESERVOIR CONTROLLED SYSTEMS. The solvent permeates the polymer wall to dissolve the drug, and the dissolved drug diffuses across the coating membrane or wall according to Fick's law. Equation (12) can be integrated to yield equation (13) that describes the drug release from the reservoir system, taking into consideration the diffusional path length, unit area and drug partition coefficient between solution and membrane:

$$dm/dt = ADK\ (c/L) \quad \text{.....Equation (13)}$$

Where:
dm/dt is the drug dissolution rate (mg/h), A is the surface area, K is the partition coefficient of the drug between solution and membrane, D is the diffusion coefficient, L the diffusional path length, and c the drug concentration.

Drug release rate control in this case can be achieved by increasing the diffusional path length of the drug (**see Appendix. 1 A**) or altering the surface area by varying the fraction of the soluble material in the wall former.

MATRIX CONTROLLED SYSTEMS. In these systems, the drug release control is achieved by controlling the rate at which the dissolution medium penetrates the matrix to solubilize the drug. Slow dissolving or insoluble polymers are used in

this method. The dissolution medium penetrates the polymer matrix to solubilize the drug. The dissolved drug then migrates down the concentration gradient across the polymer matrix into the dissolution medium, the diffusional path length increases continuously in this type of control as the drug is depleted. This diffusional path length increase results in a continuous change in the release rate of the dissolved drug due to the continuously changing path length. This condition results in slow first order drug release rates predominating. The major advantage of this device over the reservoir type is that the chances of drug dumping possibilities resulting from the device failure are reduced. The amount of drug released from the matrix is described by equation (7).

DIFFUSION AND DISSOLUTION-CONTROLLED SYSTEMS. These systems combine both diffusion and dissolution to modulate drug release. Polymers are used to envelope a drug core in partially soluble membranes, a combination of polymers which have different solubility rates can be used to coat the drug material. As the soluble part of the membrane dissolves, pores are created in the remaining coat, and the dissolution medium penetrates the core to dissolves the drug (**Fig. 3 B**). The rate of drug release from such systems can be described by the following equation:

$dm/dt = AD[(C_1 - C_2)/L]$.....Equation (14)

where:

L is the diffusion path length, C_1 is the drug concentration in the core, and C_2 the drug concentration in the dissolution medium. Drug release in this system can be controlled in the same way as in the case of reservoir device systems.

1.6.2. ION-EXCHANGE RESINS

Polymers that have ionizable groups are used to bind drugs at the ends of their strands. Basic drugs can be attached to acidic polymer ion exchangers, and acidic drugs to basic ion exchangers. When the device reaches the dissolution medium, the polymer strand will give up the ionizable drug in exchange to the competing ion. The equation below gives an illustration of the release of a basic primary amine drug from a cation ion-exchanger when surrounded by a dissolution medium containing a ionic compound (XY):

$(R\text{-}SO_3\text{-}H_3N^+\text{-}R) + (X^+Y^-) = (R\text{-}SO_3\text{-}H^+) + (H_3N^+\text{-}RY^-)$..Equation (15)

resin-drug complex resin active drug

Drug release control can be achieved by the alteration of the resin particles dimensions to slow down the drug molecule

diffusion through the resin particle structure. Other approaches include the alteration of the chemical composition of the resin particle, or coating of the drug-resin complex with polymers.

1.6.3. SLOW-DISSOLVING SALTS OR COMPLEXES

Chien (**43**) described a tannic acid/drug complex, made of a simple acid base reaction with a drug to form a drug salt or complex that has a low gastrointestinal fluid solubility. Polymers can be used to produce drug-polymer complex preparations where the polymer is not bound to the drug covalently, and the resulting complex releases the drug slowly due to equilibrium or due to the slow polymer degrading in the GI tract.

1.6.4. pH INDEPENDENT DEVICES

A drug formulation is prepared by blending an acidic or basic drug with a buffering agent, e.g. salts of citric acid (**see Appendix 2**) then granulating with excipient that are compatible with the drug and the buffering agents, and coated using suitable coating materials.

1.6.5. BIODEGRADABLE SYSTEMS

Drug release from biodegradable systems occur either by mechanical polymer matrix erosion or by hydrolytic cleavage of the polymer-drug bonds. Drug release mechanisms from biodegradable polymers occur by three mechanisms (**see Appendix 3**). The first mechanism (a) occurs if the drug is attached to the polymeric backbone by a labile bond which easily undergoes hydrolysis. Mechanism (b) occurs when the drug is in a core situated inside the polymer coat to form a reservoir-type of device. The third mechanism (c) occurs when the drug is homogeneously dispersed in the biodegradable polymer. These mechanisms can be used individually or in combination to provide multiple controlled drug release systems. The described erosion modes can either be homogeneous, heterogeneous or a combination of:

HOMOGENEOUS EROSION. If the drug is homogeneously dispersed throughout an erodible solid matrix (that is not porous), then the porosity and tortuosity terms can be eliminated from the equation, and ordinarily since $A \gg C_s$, equation (5) can be reduced to the following equation:

$$Q = (2ADC_st)^{1/2} \quad \text{.....Equation (16)}$$

HETEROGENEOUS EROSION. This describes the erosion method

where the drug release kinetics equals the polymer degradation rate. The equation that describes the drug depletion rate in a simple slab device is:

$$dM_t/dt = BC_oA \quad \text{.....Equation (17)}$$

Where:

M_t is the amount of drug released in time t (mg), B is the surface degradation rate (cm/s), C_o the drug concentration at t = 0, and A is the slab area (cm^2). The fraction of a drug released in a sphere is described by the following equation:

$$M_t/M_\infty = 1-[1-(t/t_\infty)]^3 \quad \text{.....Equation (18)}$$

Where:

M_∞ represents the amount of drug in the matrix.

1.7. DESIGN STRATEGY

The most important considerations in the design of any dosage form is safety and efficacy of the drug product. The formulation design is done while keeping in mind the needs of the physician, the patient and the cost of manufacturing. Within these confines, the physical, chemical and biological limitations of the drug have to be accommodated, and these dictate the route of administration of the drug. Microencapsulated controlled release formulations are not exempted from these rules. A number of *in-vivo* and *in-vitro*

considerations in the formulation of CR formulations have been discussed below:

1.7.1. *IN-VITRO* CONSIDERATIONS

The drug solubility, pK_a, solid state and solution stability (**39, 40**) should all be considered for the best clinical performance when formulating a drug product. The crystal habit, the particle size and morphology, of the drug are other factors for consideration because all these will affect the overall drug release. Some drug materials may require preparation before microencapsulation, or before processing into solid drug products. Selection of appropriate excipient that are compatible with the drug and processing method and conditions have to be made, so that the stability and bioavailability of the drug are maintained, and this topic will be covered later on in this thesis.

1.7.2. *IN-VIVO* CONSIDERATIONS

Among the *in-vivo* considerations, factors such as the effect of food, dosing time and dosing frequency, single or multiparticulate unit dose, on the *in-vivo* drug release have to be done, e.g. drugs that are known to produce gastric injury may be best formulated as multiparticulate dosage form for a rapid dispersal in the stomach. The *in-vitro/in-*

vivo correlation studies, clinical efficacy, inter- and intra- subject variability studies data are assessed and used in the optimization of the formulation design before the product can be accepted for clinical trials.

1.7.3. PHYSIOLOGICAL CONSIDERATIONS

The GI anatomy and physiology take part in the drug delivery and subsequent absorption of the drug, and has to be understood, and appropriate modifications done to the formulation. The disease states to be treated by the formulation, and the length of treatment time should all be considered in order to optimize the formulation performance and minimise adverse effects.

1.7.4. GRAPHIC INTERPRETATION OF THE *IN-VITRO* DISSOLUTION DATA

A plot of the cumulative fraction of drug dissolved or released D(t) from the drug product *vs.* time (t) in appropriate GI fluids, can often be correlated to the *in-vivo* release, thus *in-vitro* dissolution analysis forms an important basis for the analysis of controlled and sustained release systems.

1.8. METHODS OF MICROCAPSULE CORE PREPARATION

Depending on the physical and chemical properties drug materials in their natural occurring states such as liquid and solid state can be used to produce microcapsules as they are, or they may require preparation before microencapsulation. Most pre-microencapsulation core production methods (except size reduction) resemble and can be slightly modified to be used as microencapsulation techniques. Reasons for core preparation are many and are influenced by the following factors; the need to control the drug physical, pharmaceutical and pharmacokinetic properties in order to improve its handling during microencapsulation, as a coating optimization step, e.g. to improve the particle shape before coating, to narrow down the microcapsule size range, or to attain dual or multiple drug release control. The core preparation methods have a striking resemblance to the microencapsulation methods, even though the objectives of the two methods may differ. The purpose of the core preparation method is to manufacture an intermediate (or half product) for further coating processing. Microencapsulation on the other hand ends with a production of a finished product, that is ready for packaging, except in cases where the microcapsules were made for compressing into tablets. The core material can be prepared by the following methods;

Size reduction. Grinding or micronizing of drugs can be done in order to improve the encapsulation efficiency, to improve drug dispersal in the matrix, or for targeting purposes for microcapsules that are intended to be used in intravenous formulations. Drug materials that require size reduction before microencapsulation are usually those that crystallise as large, or irregular shaped units, and those that have irregular surface morphology especially.

Nonpareil seeds. Sucrose pellets (called nonpareils USP) can be used for loading the drug prior to coating. These pellets are loaded into the tablet coating pan, moistened with an adhesives such as 10% PVP in ethanol, and layers of powdered drug applied onto their surfaces. The production of core materials by nonpareil seeds can be achieved by the air suspension method in the same manner as in tablet coating, by spraying the seeds with layers of drug solution.

Extrusion spheronization. This technique is very economical because of the high speed and capacity that can be achieved. In this method, a plastic mass of the drug is formed by wetting a mixture of powdered drug and fillers such as microcrystalline cellulose (Avicel PH 101) with water, or a hydroalcoholic solution. This mass can then be extruded by forcing it through a series of holes in perforated screens, which compacts and sizes the product. The produced extrudate

can be spheronised and dried before use in microencapsulation.

Spherical crystallisation. This method is more commonly used as a microencapsulation technique, fine crystals of drug treated with a surface active agent and dispersed in a liquid are agglomerated by the addition of a small amount of a second liquid that can wet the drug, and is immiscible with the liquid dispersing the drug. The agglomerated material core size can be controlled by manipulating the stirring speeds, system temperature, and the content of the immiscible liquid. An example of the solvent system that is commonly used for this method is; water/ethanol mixture as the crystallization solvent, and chloroform as an immiscible liquid which promotes phase separation. The main disadvantage of this procedure is the low strength of the product which may not withstand pan or air suspension microencapsulation methods.

1.9. MICROENCAPSULATION TECHNIQUES

A number of microencapsulation techniques have been described by a number of researchers (**44-60**) for encapsulating a wide variety of drug substances. Some of the commonly used microencapsulation techniques include, phase separation, co-precipitation, spherical crystallisation, pan coating, air

suspension coating, interfacial poly-condensation, *in-situ* polymerization, solidifying in liquid, spray drying, and liposomal methods.

PHASE SEPARATION. Possibly one of the most widely used microencapsulation methods, the phase separation method involves first dissolving the polymer in a suitable solvent, then casting the solid (or dissolved) core into the polymer solution, usually containing a surfactant. The polymer can then be hardened by using any one or a combination of the following approaches; lowering the mixture temperature as in the case of waxy wall formers; addition of incompatible chemicals, phase separation or coacervation inducers, the addition of incompatible polymers or adding cross linking agents to harden the polymer (**Fig. 5 A**); Phase separation can also be induced by solvent alteration method, by adding polymer non-solvents (without dissolving the core) into the system to form units of a solid polymeric membrane or wall material enclosing the core. Other phase separation methods are achieved by temperature increase, pressure reduction, or solvent extraction as in the case of water-in-oil-in-water type of emulsions (**59**), containing an emulsion of core material dispersed in an aqueous solution of polyvinyl alcohol or gelatin (used as protective colloids). pH change has also been used to induce phase separation.

A

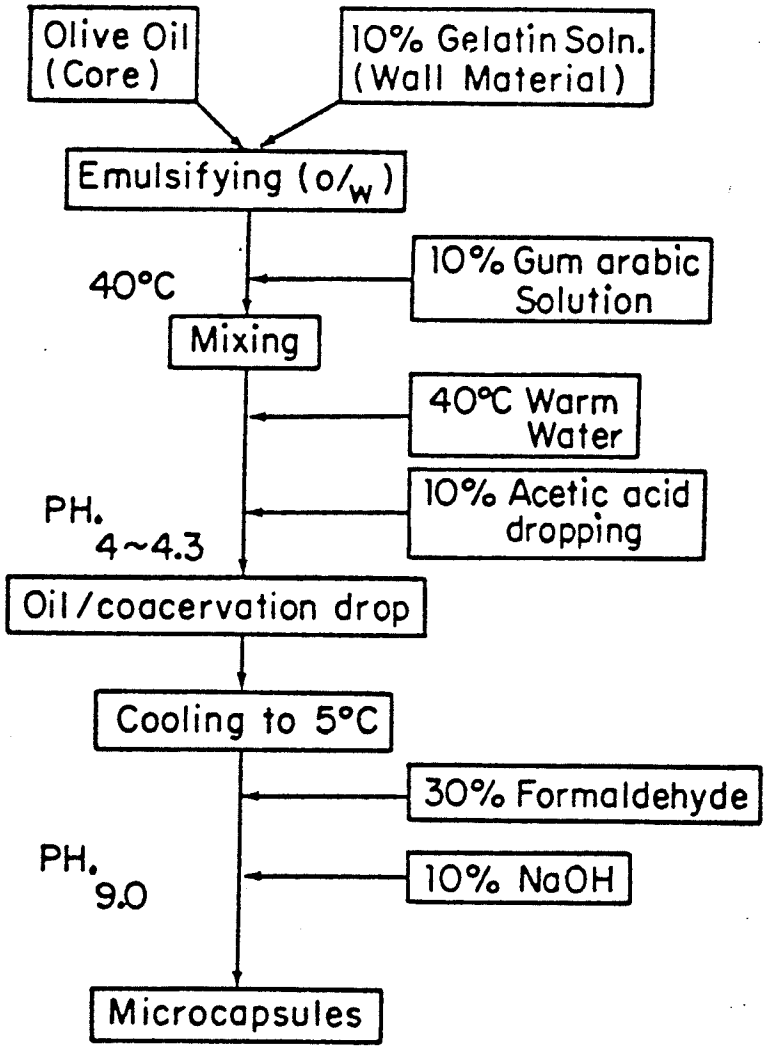


Figure 5 (A). Schematic flow diagrams illustrating microencapsulation procedure by phase separation method, using gelatin and formaldehyde as hardening agents.

B

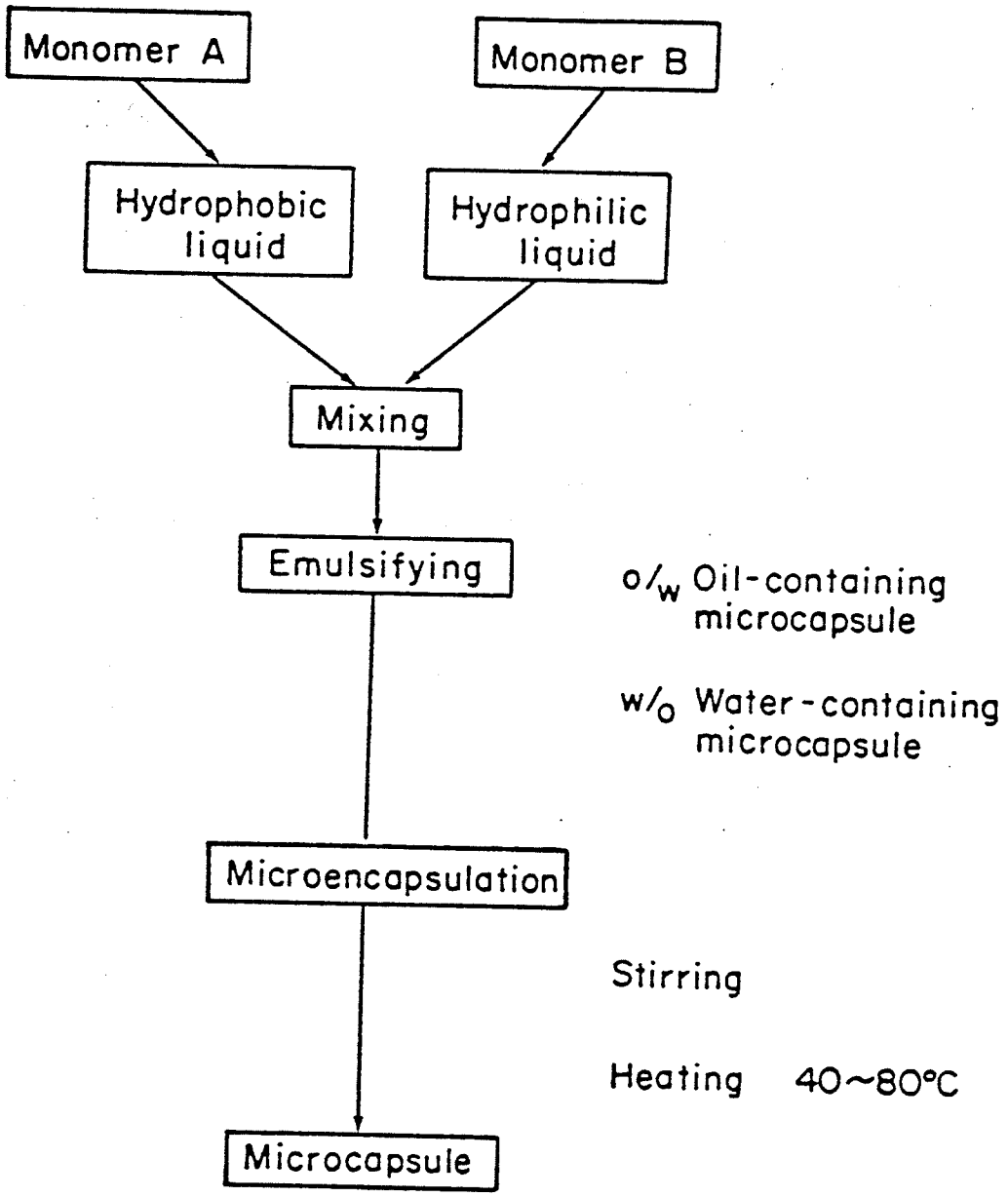


Figure 5 (B). Interfacial polymerization method by inducing polymerization of two polymer monomers by heating. (from ref.59)

CO-PRECIPITATION. The difference between this method and the phase separation method is that, in co-precipitation technique, after dissolving or mixing the core material in the polymer solution, the polymer solvent is gradually evaporated with stirring to produce aggregates of the core material coated with the polymer.

SPHERICAL CRYSTALLIZATION. This procedure is performed in a similar manner as explained under the core material preparation methods. Two approaches known as the quasi-emulsion solvent diffusion (QESD) and the solvent change (SC) discussed by Sano. (60) method are the most commonly used methods used in spherical crystallization technique. In the QESD method, an emulsifying agent e.g. 0.025% sucrose fatty acid ester (DK-F70) in an aqueous solution (forming the bulk of the solvent system) and water used as a crystallization agent, and a solubilizing agent e.g. dimethylformamide (DMF) used to dissolve the core material. The drug solution is stirred into the emulsifying agent, and the crystallization solvent added at controlled agitation and temperature conditions to form spherical agglomerates. In the SC method, the drug is dissolved in a suitable solvent, followed by the addition of the crystallization solvent. Finally a small amount of a bridging liquid e.g. a mixture of isopropyl acetate and ethanol is added, and the system maintained at suitable stirring and temperature conditions until

agglomeration is complete. This technique is usually employed in the design of the primary (drug crystals) and secondary (drug crystal agglomerates) particles with an improved solubility, compressibility and micromeritic properties.

PAN COATING AND DISH PELLETIZATION METHOD. This technique resembles the tablet pan coating method. In this method, it is important that the core material used is regular in shape and surface morphology, and the pan roughened so that the material being coated can roll instead of slipping in the pan. Previously prepared drug core material, i.e. nonpareil seeds, or drug/excipient mixture are loaded in the coating pan or pelletizer, and a solution of wall forming polymer e.g. 10% Rosin Hard Paraffin for the microencapsulation of aspirin (**20**) sprayed onto the core material placed in the rotating pan at speeds of about 20 - 30 revolution per minute (rpm). The procedure is continued until the required wall layer has been achieved; several different polymeric layers, and a final overcoat may be applied to improve the release properties and elegance of the product. A solvent that dissolves a polymer can also be used to spray the drug/polymer mixture loaded in the pan to form a drug/polymer matrix, and finally overcoated with an appropriate polymeric material. The major advantage of this method is its high capacity. One of the major disadvantages

encountered in this method is material attrition to processing equipment walls and to each other, and the control of the microcapsule size range.

AIR SUSPENSION COATING. This procedure is sometimes referred to as Wurster coating, specialized fluid bed coaters are used to coat core materials. Microencapsulation is done by loading the prepared core material, or solid drug crystals into the product vessel (**see Appendix 5**) and coating proceeds in a manner similar to tablet coating. The core material is fluidised inside the product vessel, an atomizer jet spraying the dissolved or dispersed polymer in a suitable solvent is situated at the bottom centre of the air distribution plate. As the core material flows in a vertical circular motion, it encounters the polymer solution which forms a uniform layer around the core material. The polymer solution is dried, and the core re-sprayed until required polymer layer has been achieved. The major advantage of this coating method is that the core material need not be regular in shape and surface morphology.

INTERFACIAL POLY-CONDENSATION. This technique which is similar to another technique called the *in-situ* polymerization, is used most commonly for microencapsulating liquid drug materials in solid wall polymers (**Fig. 5 B**). Two monomers capable of undergoing poly-condensation, or poly-

addition polymerization (one water soluble and the other oil soluble monomers) are brought with mixing into the liquid drug in an organic, aqueous or a mixture of organic and aqueous solvent in a vessel. A reactive component can be added into the mixture to start, or heating used to induce polymerization. The procedure is done in such a way that the hardening polymer chains completely envelope the liquid drug. Polyurea, and polyamide microcapsules are produced by this method.

IN-SITU POLYMERIZATION. The difference of this method and interfacial polymerization is that, in this method, one monomer (oil soluble) is dissolved only in the core material (in a liquid or a solid state), and this monomer is insoluble in the manufacturing vehicle, or the surrounding vehicle. When a polymerization catalyst is introduced, an insoluble polymeric coating forms around the core material (**Appendix 6**). The pharmaceutical applications of this method are limited due to the toxicity associated with residual monomers, catalysts and other additives such as polymerization termination agents contaminating the final product.

SOLIDIFICATION IN LIQUID. This process consists of dropping droplets of a polymer and drug solution in a hardening agent solution under stirring conditions, to form spherical

discrete units of a polymeric wall enclosing the core material which could be a dispersed drug, or microorganism (for their enzymes). A good example of this method is the hardening of sodium alginate, or pectinate solutions containing dissolved drug, by dropping the alginate/pectinate drug solution into calcium chloride solution. Soluble sodium salt solutions of alginate and pectinate form spherical insoluble calcium alginate or pectinate complexes when added to calcium chloride solutions.

SPRAY DRYING. Two methods can be used in this approach. In the first method, the wall material that melts at elevated temperatures when atomised and congeals at atmospheric temperatures is used. The core substance placed in the drying chamber (**Appendix 7**) is sprayed with the heated liquified wall former. After depositing on the core substances, the liquified wall former congeals and hardens around the core to form a microcapsule. In the second method, the core substance is dispersed in the liquified wall material. The dispersion is then sprayed into a chilled solvent, or sorptive particles to form microcapsules. This method has an advantage of automation ease over the other microencapsulation methods. The brief periods of time (5 to 30 seconds) of exposure of materials to elevated temperatures protects against thermal degradation of heat sensitive materials. This method thought useful for producing

microcapsule for taste masking purposes, is not well suited for manufacturing microcapsules for sustained or controlled drug release purposes, because of the porosity of the resulting microcapsules.

LIPOSOME ENCAPSULATION. For some time liposome technology was confined to the cosmetic industry. In recent years, liposome dosage forms have attracted growing attention in pharmaceutical dosage form design. Liposomes are uni- or multi-layered vesicles made by dispersing phospholipids in water. A simplified liposome procedure entails dissolving a phospholipid in an organic solvent, and evaporating the solvent to form a lipid film. A buffered aqueous phase suspending a drug is then mixed with the lipid film and shaken to form a suspension. The suspension is then centrifuged to collect the liposome pellet. The pellet contains the liposome vesicles with the drug entrapped within the vesicle layers. The pellet can be lyophilized and re-suspended in liquid as required. The use of liposomes for drug targeting is currently under study. They have also been reported to improve the penetration of drugs into the neoplastic cells.

1.10. REASONS FOR MICROENCAPSULATION

Microcapsules have found use in a diverse range of dosage

forms. They have been used in intravenous injection preparation, manufacture of solid and liquid oral controlled release dosage forms, ocular and nasal delivery systems to achieve a variety of clinical objectives. An outline of microcapsule uses is given below and discussed in depth in a number of reports.

DRUG TARGETING. Microcapsule size (61-64) is used for drug targeting, e.g. nanocapsules and microspheres in intravenous injections where nanocapsules are used to deliver drugs to the liver and spleen and microspheres to deliver the drug to the lungs. Liposomes can be used to target anti-tumour agents to tumour cells after being engulfed by macrophages.

SUSTAINED/CONTROLLED RELEASE DRUG DESIGN. Drugs with a short biological half-life e.g. ASA (65), and drugs with a narrow therapeutic index e.g. Lidocaine, are microencapsulated as sustained or controlled release pellets and compressed into tablets, or filled into hard gelatin capsules for improving their release properties to improve the efficacy, safety and patient compliance.

IMPROVEMENT OF PHYSICAL STABILITY. The polymeric layer in microencapsulated drugs serves to improve the chemical and physical stability of chemicals (66) e.g. ethylcellulose film coated Vitamins A and K by protecting the drug substance from

the environmental degradation.

IMPROVEMENT OF ORGANOLEPTIC PROPERTIES. Drug materials that have an objectionable odour (67) e.g. mebendazole, beclosamide or an unpleasant taste e.g. acetyl-p-amino-phenol and methionine, can be microencapsulated in order to overcome these undesirable properties, without affecting the pharmacological activity of the drug.

LIQUID-SOLID CONVERSION. Handling of potent drugs in their liquid state can be (68) difficult. This condition can be improved by microencapsulating such drugs, e.g. castor oil by enclosing them in a solid polymeric wall.

SEPARATION OF INCOMPATIBILITIES. Microcapsules with layers of incompatible materials separated by layers of inert materials (69-71) can be manufactured using the pan coating method, to keep the incompatible materials separated. e.g. ferrous fumarate and oxidising substances.

IMPROVEMENT OF FLOW PROPERTIES. Most microencapsulation methods yield spherical, or spheroidal units with improved flow properties. The flow of drug materials that have poor flow properties such as 1,2, dimethyl-3-hydroxy-pyrid-4-one (DMHP) can be improved through microencapsulation.

REDUCTION IN TOXICITY. Microencapsulating potentially harmful materials such as antibiotics (72), reduces the toxicity risk to factory workers who are exposed to the material. The side effects of some drug substances can also be reduced by microencapsulation e.g. microencapsulated ASA.

IMPROVEMENT OF SELECTIVITY AND ACTIVITY. Cytotoxic anti-cancer agents microencapsulated in liposomes help to improve the selectivity and activity of the agent as in the case of adriamycin.

ENTERIC COATING. Enteric coating is useful for controlled and localised drug release in the intestines, and for reduction of gastrointestinal side effects (73, 74). Drugs with a high gastric intolerance incidence exhibit reduced side effects when microencapsulated with enteric release polymers, e.g. ASA. A novel approach of particles-in-tablets (75) has been used in which individual erythromycin particles are enteric coated prior to tabletting, the incidence and severity of nausea associated with erythromycin therapy has been found to be reduced when this form of erythromycin product is given.

IMPROVEMENT OF AESTHETIC PROPERTIES. Thought of little pharmacological value, aesthetic properties of pharmaceutical products, play a role in the way in which a product is viewed

both by health professionals and patients alike. A microencapsulated and colour coated drug pellet can be filled in a transparent (cheaper) gelatin shell and still look as appealing as a product filled in a more expensive coloured shell.

AN INTERMEDIATE. This use of microcapsules has already been covered under the methods of core preparations.

TRAPPING OF METABOLITES. Microcapsules containing charcoal and enzymes such as urease are currently being investigated for their effectiveness in trapping metabolites for use as artificial cells.

1.11. SOME CONSIDERATIONS AND DIFFICULTIES IN MICROENCAPSULATION

Microencapsulation technology has been extensively studied and used to some success, but many challenging difficulties still remain to be resolved (**76, 77**). The most common microencapsulation problems are; the uneven deposition of coating material, microcapsule clumping, unsatisfactory or lack of reproducibility in drug release profiles and difficulties in scale-up procedures. Some of these problems can be overcome by a careful selection of the formulation excipient, the core preparation and microencapsulation method

which differ for different core materials. The properties of all the ingredients and adjuvants and their effects on the process and stability of the core should be examined and carefully considered before microencapsulation. In most cases, a slight alteration of either the material quantities, or the manufacturing conditions can result in a number of problems ranging from poor wall formation leading to poor drug release kinetics, or a complete failure to encapsulate the core.

1.11.1. CORE MATERIAL

Microcapsule core material can either be in the form of a solid or liquid. If the core material is a liquid, its polarity and the polarity of the polymer solvent should be taken into account when selecting a wall material, since the partitioning of the drug between the wall material and the solvent used will affect formation and drug release kinetics of the microcapsule. If a liquid drug is enclosed in the drug soluble coat the drug may leak out of the coat, and the properties of the coat cannot be used effectively to regulate the drug release profile of the resulting microcapsule. The solubility or non-solubility of the solid core material in the polymer solvent also play an important role in microencapsulation success and drug release. If the drug has a high solubility in the polymer solvent, and a pan or air

suspension encapsulation method was employed, the drug will dissolve in the solvent and may salt out to produce undesired drug release properties, or cause an initial drug burst effect. The core polarity and solubility parameter becomes important if the drug is going to be microencapsulated by phase separation method. If the solubility parameter value of the core material approximates the solubility parameter of the polymer solvent, the core may dissolve in the solvent, and monolithic type of microcapsule may result when the aim was to produce a reservoir type of microcapsule. On the other hand if the aim is to produce a matrix type of microcapsule, depending on the manufacturing procedure selected, a core material that is soluble in the polymer solvent can be used. An even dispersal of drug particles throughout the polymer matrix will result and the matrix type of microcapsuleproduced. The core material should maintain its chemical stability in all the solvents used for the microencapsulation work, other wise drug loss and generation of pharmacologically toxic degradation by products may result. Very small sized solid core material tend to give rise to severe aggregation problems during microencapsulation (**46**) due to their surface attractive forces. Large core particles on the other hand tend to sediment, while irregularly shaped core particles are difficult to coat, especially in pan coating. The effect of the core density on the physiological effects such as gastric emptying times

should be considered when using very dense or light core materials. Several researchers (78-79) have demonstrated that core material density has a marked effect on the gastric emptying time.

1.11.2. WALL MATERIALS

A wide variety of high-molecular-weight polymers may be used alone or in combination with other polymers and additives to form the wall material around the core. The polymer choice can range from biodegradable synthetic polymers e.g. cellulose acetate phthalate, to modified natural products such as starch, proteins and waxes. Due to lack of toxicological data on the newly emerging polymers, conventional polymers such as the cellulose derivatives are often chosen in microencapsulation and other controlled release formulation procedures in order to avoid possible problems of toxicity, allergenicity or irritancy. The release kinetics of the final device is another criterion in selecting a film or wall former. Polyelectrolyte polymers containing ionizable carboxyl groups such as cellulose acetate phthalate (CAP) polyvinyl acetate phthalate (PVAP) and hydroxypropylmethylcellulose phthalate (HPMCP) are commonly used as enteric polymers due to their pH dependant erosion properties. These polymers are used to manufacture microcapsules of drugs that are acid labile, or drugs with a

high gastric intolerance. Cationic acrylic polymers on the other hand release in the lower pH environments, and cannot be used to formulate delivery devices of acid labile core, or the core that corrodes the gastric mucosa. Aliphatic polymers such as paraffin, and crystalline polymers such as cellulose are water insoluble, and release the drug slowly throughout the GI tract irrespective of the pH environment. The storage chemical stability of the polymer is another factor for consideration in drug product formulation. Enteric polymers tend to harden with time upon storage, and may result in altered drug release rates in GI fluids (28) due to cross-linking. Factors that affect the adhesiveness of the film former to the core surface have to be considered, because film formers that do not adhere well may detach from the microcapsule wall after some time, or due to handling and storage conditions. The problem of poor film former adhesiveness can be corrected by increasing the core porosity, while increasing the film former thickness may decrease the polymer substrate adhesion (80, 81). Drug release mechanisms have been discussed under the topic Release mechanisms.

1.11.3. SOLVENTS

Solvent selection is made based on the drug and polymer solubility value known as the solubility parameter (δ), and

the polarity of the polymer the solvent and the drug. Knowing these factors will quickly narrow down the solvent choice because materials of approximately equal solubility parameter values will dissolve in each other if they have the same polarity (82). The drug stability in the polymer solution and the process conditions is another point to consider. The presence of hydroxyl ions accelerate the hydrolytic break down of materials such as acetylsalicylic acid in the presence of moisture, or high temperature conditions. For this reason, alcoholic solutions may not be suitable to use as film former solvents in the microencapsulation of acetylsalicylic acid. Other considerations include the process safety, economic aspects and toxicity e.g. methylene chloride has been used as a polymer solvent of choice due to its volatile nature. It has been found to be retained in trace amounts in polymeric coats, and its use is facing ban because of its carcinogenic properties. Even though both aqueous and non-aqueous solvent systems are used in most microencapsulation work, the aqueous based systems are considered first before organic solvent based methods due to environmental and toxicity considerations. Aqueous based methods are safer because of reduced chances of explosion as compared to the solvent based methods during processing. Care should be taken when using core materials that are easily hydrolysed under moisture conditions. In such cases, solvent based systems may provide

a better alternative. Another case where solvent systems are more favourable is when the polymer is insoluble in aqueous based systems, and this happens often. Understanding the behaviour of polymers in solutions and the chain of events that ensue when polymers are solubilized (**Fig. 6**) is crucial in designing microencapsulation methods. This understanding is vital in minimising common problems encountered in most microencapsulation procedures, e.g. the polymer shock problems or failure to form microcapsules in phase separation coacervation techniques.

The process of dissolving a wall forming polymeric material in its solvent is governed by the following Gibb's free energy equation.

$$F = H - T S \quad \text{.....Equation (19)}$$

Where:

F is the change in Gibbs free energy, **H** is the heat of mixing, **T** is the absolute temperature and **S** is the mixing entropy. **S** is always very large in polymer solubilization. According to the solubility parameter theory proposed by Scott, non-crystalline polymers will dissolve in a solvent of similar solubility parameter (**δ**) (see Table 1). The calculation of this value can be done in a number of different ways, which are discussed by Burrel (**84**). Drug

solubility in the membrane substance can determine the drug release rate. High drug solubility in the membrane assists drug exit by diffusion (**27**), while in low membrane drug exit is usually by dissolution. A table of examples of the most commonly used microencapsulation wall forming material solvents/non-solvents and their solubility parameters is given below.

1.11.4. ADDITIVES

These may be used in microencapsulation formulations to give certain properties to microcapsules, or to optimize the microencapsulation process. Depending on their characteristics e.g. lipophilic nature, volatility, solubility, and interaction with the wall formers, materials can be used microencapsulation procedures as porous wall sealants e.g. waxes, enhancement of the pore capillary volume by hydrophillic materials and salts, materials such as polyisobutylene and dibutyl sebacate have been used as plasticisers to improve the chain interaction forces of the wall formers. Polyvalent ions and very soluble salts e.g. sodium sulphate, ammonium fluoride and potassium fluoride have been used as coacervation inducers (**60**). Cetrimide and sodium lauryl sulphate have been used as surface active agents. These additives can have a negative effect on the physico chemical properties stability and drug release from

Table 1. Examples of polymer solvents/non-solvents commonly used in coating and microencapsulation work.

SOLVENT	POLARITY	δ (cal/cm^3)
Acetic acid	Polar	10.1
Acetone	Polar	9.9
Acetonitrile	Non-polar	11.9
Benzene	Non-polar	9.2
Chloroform	Non-polar	9.3
Cyclohexane	Non-polar	8.2
Dibutyl sebacate	Non-polar	9.2
Dichloroacetic acid	Polar	11.0
Diethyl ether	Non-polar	7.4
Ethylene glycol	Non-polar	14.6
Formic acid	Polar	12.1

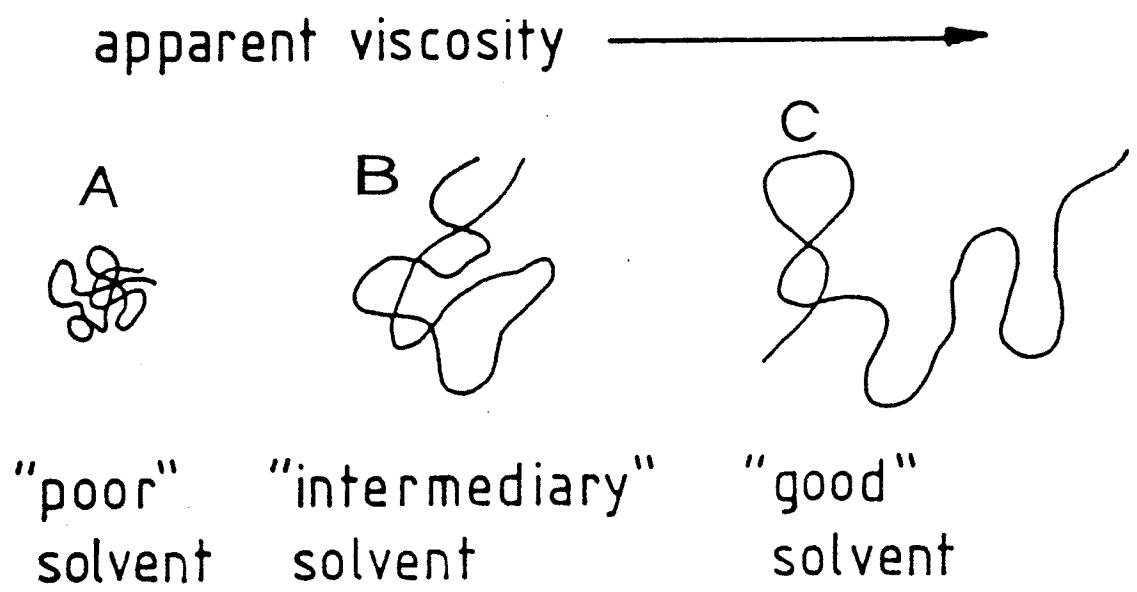


Figure 6. Diagrams depicting polymer (solubility events) swelling and un-coiling in the poor (A), intermediate (B), and good (C) solvent.

microcapsules if used indiscriminately, e.g. high levels of surfactant can affect the dissolution the drug hence the release kinetics of the microcapsule, so they should be used with caution.

1.11.5. FORMULATION AND PROCESS PARAMETERS

Depending on the microencapsulation technique employed, some process parameters, namely temperature, solvent quality, agitation rates and methods, solidification rates, evaporation rates, and solvent-alteration rates can be critical in determining the success or failure to encapsulate the core. All microencapsulation process parameters should preserve the stability of the core material, physico chemical properties of the core should be considered when designing the process, e.g. decomposition activation energy of the core should be considered in processes that require high temperature conditions. All the factors and the catalysts that may lower this energy should be excluded from the process. Microcapsule size, shape, structure, coating efficiency and agglomeration can be affected by a number of factors such as agitation rates and temperatures. There are a multitude of parameters to be considered in microencapsulation, and each process parameter is best discussed within the framework of specific technologies employed in coating specific drug materials. Some process

parameters and their effects on the form, yield and release kinetics of the microcapsules will be discussed in the microencapsulation of ASA by phase separation, using the solvent alteration method.

1.11.6. GENERAL CONSIDERATIONS

Depending on its intended use, the microcapsule size may be an important feature. For microencapsulated drugs used in suspension, or chewable tablet formulations, the mouth feel is a factor for consideration. Particle sizes in the range of 200 to 300 μ are preferred because they will not be broken by mastication, and they will not be held back in the mouth. Enhancement of palatability of bitter drugs, or drugs that leave objectionable after taste can be accomplished by incorporating sweeteners and flavouring agents in the formulation. In order to avoid abuse of such drug products, the sweetening and flavouring agents should be used sparingly. For very small sized (0.9 μ) microcapsules, the effect of body host defense mechanism phenomena where the microcapsules may be engulfed by polymorphonuclear leucocytes should be considered. Several researchers (**84, 85**) have demonstrated a relationship between the phagocyte activity and small sized microcapsule ingestion. Since microcapsules should stay in the body until their function has been carried

out, the effect of phagocytosis on microcapsules should be considered during the formulation stages, and preventive measures effected at this stage if necessary.

1.12. OBJECTIVES OF THE STUDY

The objectives of this study were to develop an ASA formulation that possess the following characteristics:

- To make a low dose (5-10 mg/h for 10 hours) ASA formulation for antiplatelet use.
- Enteric release
- Sustained near zero order drug release kinetics in the intestinal environments
- Reduced drug dumping problems resulting from device failure
- Prepare microcapsules of sufficient physical strength to withstand further coating procedures.

Multiple unit formulations have been reported to provide unit doses that have a shorter and more predictable retention time (65) than single unit formulations such as tablets. Enteric-coated ASA tablets have been studied and have been reported to have reduced incidences of gastric irritation problems when compared with un-coated ASA formulations, only a few reports (20, 65) however have discussed multiple unit dose

formulation of ASA that possess enteric release properties, and this ASA formulation may have a more promising potential to overcome some of the problems associated with ASA therapy. The formulation of a monolithic type of pellet was an attempt to minimise drug dumping problems that could result due to device failure as in the cases of reservoir type of microcapsules. Currently only buffered and enteric coated Acetylsalicylic acid tablets are available in pharmacy outlets. There are no microencapsulated acetylsalicylic acid or any other multiparticulate dosage form of ASA.

CHAPTER II

EXPERIMENTAL

2.0. ASA MICROENCAPSULATION

2.0.1. EQUIPMENT

General laboratory equipment for preparation of microcapsules and for most analytical work.

1. Pump, Masterflex L/S. Barnant Co. IL USA.
2. Masterflex easy load model 7518 Pump Head Barnant Co. IL USA.
3. Fluid bed dryer. Aeromatic Ltd. Muttenz, Basle, Switzerland.
4. Product vessel custom made (**Fig. 7**)
5. Spray nozzle custom made (**Fig. 8**)
6. Dissolution Testing Apparatus, with Gold plated USP dissolution test baskets. Vander kamp 600. Van-kel Ind. N. J. USA.
7. Spectrophotometer, Shimadzu UV-160, with Guartz Spectrophotometer cells. Shimatzu Corp, Kyoto, Japan.
8. Shimadzu HPLC. Fisher Scientific LTD New Jersey.
9. C18 Synchropak (RP-P-100) reverse phase column. Synchro inc.

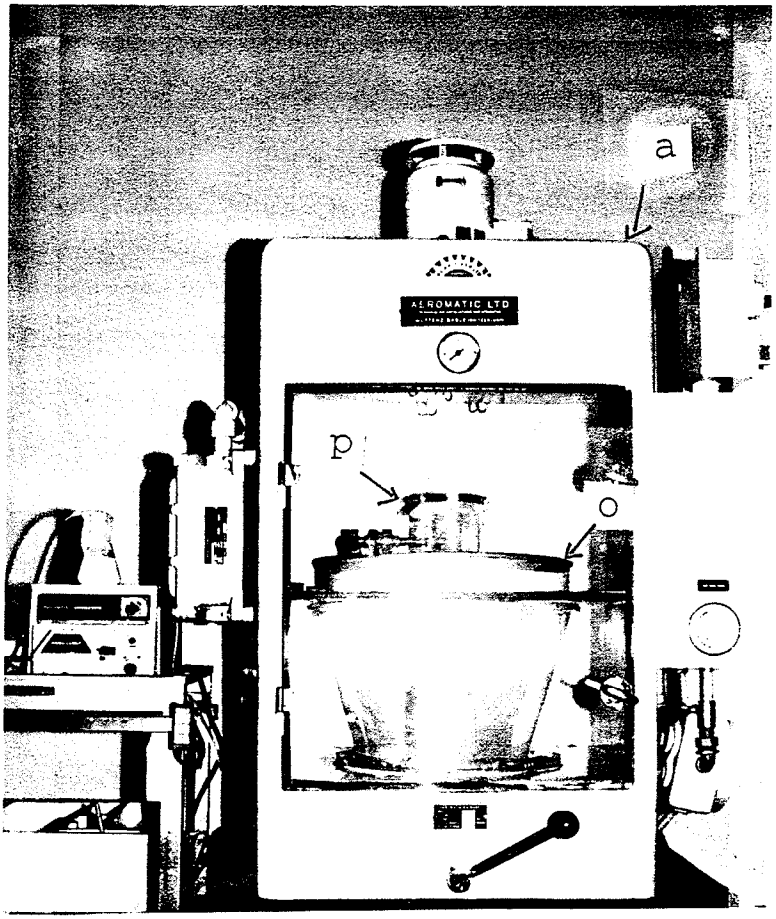


Figure 7. A photograph of the (custom made) product vessel (p) fixed inside the original product vessel (o) of the Aeromatic fluid bed drier (a).

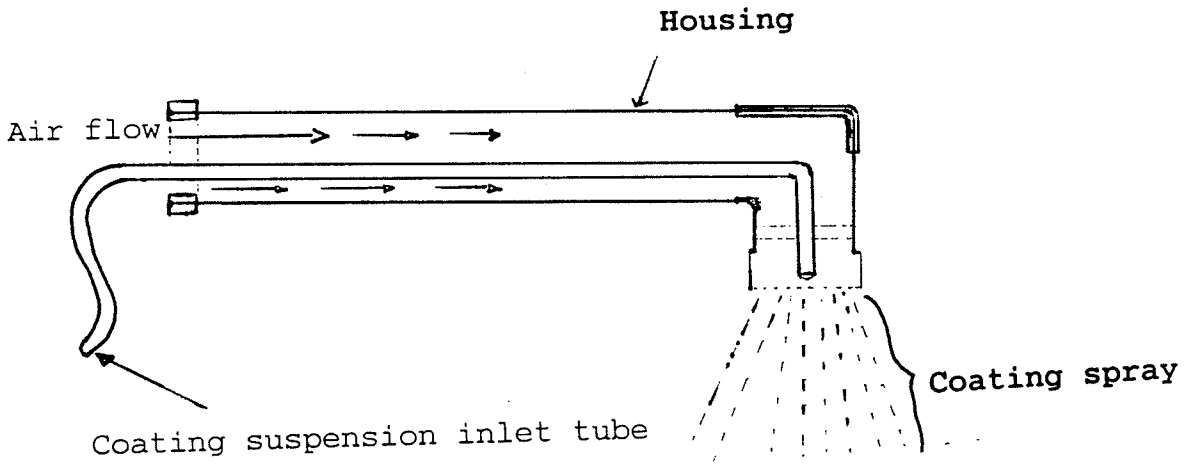


Figure 8. Schematic diagram of the custom made spraying nozzle.

10. Shimadzu CR 601 recorder and integrator. Fisher Scientific LTD New Jersey.
11. Shimadzu SPD-10A uv-vis Detector. Fisher Scientific LTD New Jersey.
12. USP graded sieves mesh sizes 8, 12, 16, 22, 30 and 60.
13. Mettler weighing Balances, PJ 400, AE 160, and PE 360. E. Mettler, Zurich, Switzerland.
14. Camera, Nikon F-601 with a 35-70 mm lens fitted with Kokin A-160, and A-103 + 3 filters. Nikon Corporation, Tokyo Japan.

2.0.2. MATERIALS

1. Aspirin powder USP (ASA). Mallincrodt speciality chemicals. Ontario Canada.
2. Cellulose acetate hydrogen phthalate (CAP). Eastman. Tennessee USA.
3. Diethyl pthalate. Morflex Inc. Greensboro North Carolina USA.
4. Ethylcellulose. Fisher Scientific, New Jersey USA.
5. Entrophen. Merck Frost Canada Inc. Quebec, Canada.
6. Potassium Phosphate (monobasic). Mallincrodt, Kentucky USA.
7. Potassium Chloride. Mallincrodt, Kentucky USA.
8. Sodium Chloride. Merck & Co, Inc. Rahway, New

Jersey USA.

9. Sodium Phosphate (dibasic). Mallincrodt, Kentucky USA.
10. Eudragit RSPM. Rohm Pharmaceutical Darmstadt Germany.
11. Polyethylene glycol (PEG 8000, mw 7 000 - 9 000). Fisher Scientific, New Jersey USA.
12. Polyisobutylene (low molecular weight). Aldrich Chemical Company, Milwaukee USA.
13. Tween 80. Mallincrodt Speciality Chemicals, Kentucky USA.
14. Aquateric®. FMC Corporation, Newark, DE, USA.

2.0.3. SOLVENTS

1. Acetone. Mallincrodt Speciality Chemicals Co. Kentucky.
2. Cyclohexane. Mallincrodt Speciality Chemicals Co. Kentucky.
3. Hydrochloric acid. Baxter Corporation, Toronto.

2.1. PROCEDURE

Cellulose acetate hydrogen phthalate (CAP) was placed in 400 ml acetone under vigorous stirring contained in a 2-litre three necked reflux flask fitted with a condenser and a

thermometer (see Table 2, and **Fig. 9** for amounts and manufacturing flow diagram). Eudragit RSPM was added into the same flask, followed by ASA crystals. The mixture was warmed to 56°C using a water bath. Then allowed to stay at this temperature for about 45 minutes under constant stirring. 50 ml of 0.1% Tween 80 in cyclohexane was carefully added into the reflux flask via a graduated separatory funnel. The reflux condenser was replaced with a distillation unit and the low boiling point organic solvents distilled off. A further 50 ml of 0.1% Tween 80 in cyclohexane was added drop-wise at the rate of about 2 ml/min, followed by the addition of pure cyclohexane at the same rate until the pellets started to form (**Fig. 9, 10**). When all the acetone was removed, 0.2 g of sodium chloride was added into the flask, and the heat turned off. The temperature was allowed to drop to room temperature while maintaining the stirring. The hardened pellets were collected by decantation and filtration over a buchner funnel, washed with 30 ml aliquots of cold cyclohexane and dried in air overnight. Microscopic observation of the produced pellets (complete and sliced) revealed that the spheroids produced are monolithic with the drug crystals evenly dispersed throughout the matrix. The weight of the dried and hardened pellets was recorded, and the pellets sieve sized using standard USP sieves. The weights of all the collected fractions were recorded. Pellets collected on

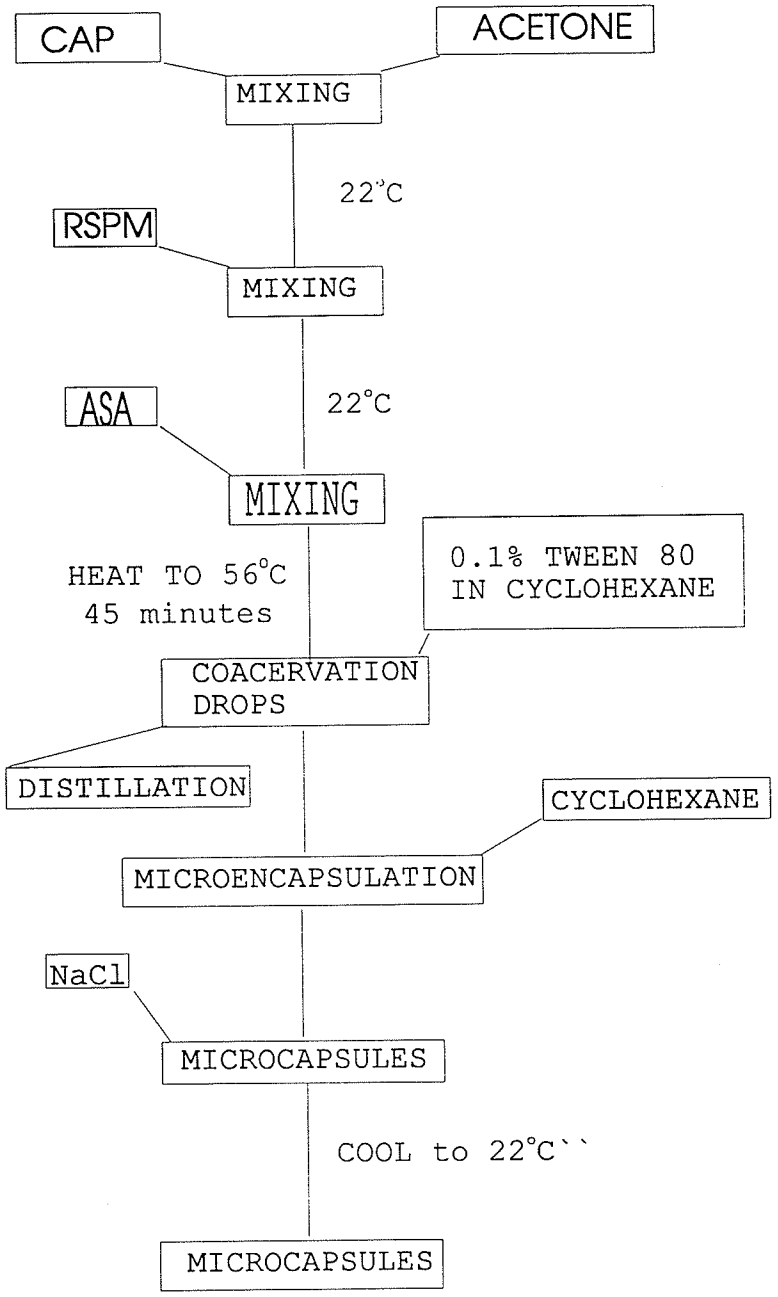


Figure 9. A flow diagram illustrating the preparation of ASA microcapsules by coacervation method, inducing the phase separation of polymers by solvent alteration.

Table 2. Microcapsule formulations.

Materials (g)	FORMULATIONS				
	1	2	3	4	5
ASA	12	4	4	4	4
CAP	6	6	7	8	6
RSPM	6	6	7	6	8

the sieve sizes 12/16 and 16/22 mesh were retained for further chemical and dissolution studies. Only the microcapsules retained on a size 22 mesh screen were photographed (**Fig. 11**) to present a visual record.

2.1.1. PELLET WASHING TREATMENT

50 ml of cyclohexane was warmed to 62°C in a 200 ml two necked round bottom reflux flask. ASA pellets (1.0 g) were added into the cyclohexane while stirring vigorously with a stirrer bar. The mixture was allowed to cool down to about 15°C. The cyclohexane was discarded by decantation, and the pellets washed with two aliquots each of 10 ml of cyclohexane, filtered over a buchner funnel at room temperature, and allowed to dry in air before analysis.

2.2. RECONSTITUTION OF COATING SUSPENSIONS

2.2.1. Aquateric^{R}

	Suspension (g)	Dry Solids (g)
Aquateric^{R} Powder	100	100
Diethyl Phthalate (DEP)	35	35
Tween 80 (emulsifier)	1	

Water, Purified	771	
	907	135

METHOD

1. Tween 80 was added to 20 ml of water placed in a 50 ml beaker, while stirring fast, with a magnetic stirrer. The mixture was stirred for ten minutes, and transferred to about 500 ml of distilled water with stirring placed in a 2 litre flask.
2. Aquateric was carefully added to (1) with stirring.
3. DEP was slowly added to (2) while the mixture was kept under continuous stirring, and the mixing continued for 1.5 Hours, after which the rest of the water (251 ml) added.
4. The resulting dispersion was then screened over a 60 mesh screen and kept for use in a covered stainless steel beaker.

The coating suspension was kept in a closed container for further use. 50 ml of the suspension was diluted to 100 ml with distilled water under stirring and used for coating the ASA microcapsules.

2.2.2. ENTERIC COATING

EXPERIMENTAL PARAMETERS/CONDITIONS:

Inlet air pressure	= 30 - 40 psi
Fluid bed temperature	= 40-45°C
Coating suspension flow rate	= 2 ml/min
Pump setting	= 40.0
Volume of suspension used	= 30.0 ml
Total coating time	= 180 minutes

METHOD. 5.0 g of the ASA pellets were placed in the modified fluid bed drier vessel attached to the bowl. The vessel was then fitted and clamped in place in the fluid bed drier. The pellets were fluidized and preheated at 45°C for 15 minutes. The coating suspension was sprayed onto the pellets for about 12 seconds, and dried for 3-4 minutes. The procedure was repeated until 30 ml of the coating suspension was applied.

2.2.3. ETHYLCELLULOSE COATING

METHOD. 50 ml of cyclohexane was warmed to 50°C in a 200 ml two necked reflux flask fitted with a thermometer and a reflux condenser. 0.2 g of Ethylcellulose was carefully added in the flask while stirring vigorously. The mixture temperature was slowly raised to 55°C over a period of 30

minutes while continuing the stirring. 0.1 g of Polyethylene glycol 8000 (PEG 8000) was added into the flask, and the mixture heated to 62°C over another 20 minutes. 1.0 g of ASA pellets were carefully added into the flask, and the flask allowed to cool down to about 15° C over 25 minutes. The cyclohexane together with any free Ethylcellulose that did not get deposited onto the pellets was decanted, 20 ml of cyclohexane added, and the microcapsules recovered by filtration over a buchner funnel and two washings by 20 ml aliquots of cyclohexane at room temperature. The coated pellets were allowed to dry in air. This method was modified from the method of I. Jalsenjak *et al.* (**54**).

2.2.4. POLY-ISOBUTYLENE COATING

METHOD. A 0.25% w/v of polyisobutylene (PIB) in cyclohexane was prepared by placing finely chopped PIB in enough cyclohexane with stirring in an erlenmeyer flask at room temperature. The flask was covered and allowed to stir over night. 50 ml of the 0.25% PIB was placed in a 200 ml two necked round bottom flask, and heated to 55°C over 15 minutes. 0.2 g of PEG 8000 was added, and the mixture heated to 62°C over 20 minutes. 1.0 g of the ASA pellets were added and the mixture allowed to cool to about 15°C over 25 minutes. 20 ml of the cyclohexane solution was decanted,

and the remaining mixture transferred to the buchner funnel and filtered. The pellets were washed with two aliquots of 10 ml each of cyclohexane and allowed to dry in air.

2.3. PREPARATION OF BUFFER SOLUTIONS

pH 2.0 HCl-KCl BUFFER SOLUTION

(1) 1,416 ml of 0.2 N HCl was carefully added in 4,584 ml of deionized water in an erlenmeyer flask while stirring.

(2) 101.46 g of KCl crystals were quantitatively transfered into (1) and mixed thoroughly.

(3) The above mixture (2) was transferred into a 25 L plastic Container.

(4) Mixture (3) was diluted to 20 L with deionized water and the erlenmeyer flask washed with about 4 litres of deionized water and diluted to a total volume of 24 litres. The mixture was stirred and allowed to stand for at least two hours before checking the pH, or before use.

pH 7.4 BUFFER SOLUTION

(1) 45.35 g of monopotassium phosphate (KH_2PO_4) was dissolved in 5 litres of deionized water contained in an erlenmeyer flask and mixed for about 40 minutes.

(2) 237.4 g of Disodium phosphate ($Na_2PO_4.2H_2O$) was separately dissolved in 20 litres of deionized water contained in a 25 litre plastic container, and stirrerd for a about 2 hours, or until all the crystals have dissolved.

(3) 728 ml was removed from (2) above.

(4) 4,728 ml of (1) was added to (3) and mixed for at least one hour before checking the pH or before use, (total volume is 24 litres).

DISSOLUTION METHOD. The apparatus bath temperature and dissolution test medium were stabilised at 37.0 ± 0.1°C for about two hours, and the rotation speed of the spindles set to 50 rpm.

2.3.1. CALIBRATION CURVES

pH 2.0 KCl BUFFER. The weight of about 5 g of pure ASA was determined, and placed in a desiccator under a partial vacuum over-night. Six different concentrations of ASA solution ranging from 0.0 mg ml^{-1} to 4.0 x 10^{-2} mg ml^{-1} were prepared using pH 2.0 buffer. Absorbances of these solutions were determined at 228 nm wave length, using a pure buffer solution as the reference. A calibration curve of absorbance *vs.* ASA concentration was drawn (**see Fig. 12 A**), and used to read off the concentrations of the sample solutions taken

from the dissolution medium in the acid buffer using their absorbances.

pH 7.4 PHOSPHATE BUFFER. Six different concentrations of ASA solution ranging from 0.0 to 5.0 x 10^{-1} mg ml^{-1} were prepared using the dried ASA, and pH 7.4 phosphate buffer. Absorbances were determined at 262 nm, using pure pH 7.4 buffer solution as reference. A calibration curve of absorbance *vs.* ASA concentration was made (**see Fig. 12 B**).

2.4. DISSOLUTION TESTING PROCEDURE

USP dissolution apparatus No. 1 (basket method) was used for dissolution studies. The weight of the pellets containing 100 mg of ASA (or placebo pellets) were carefully determined, recorded and placed in size 0 gelatin capsules, the capsules were transferred into the basket, covered with a strip of nylon mesh, fixed on to the spindle and placed in the dissolution vessel previously equilibrated at 37.0 ± 0.1°C. The dissolution test was first done in 900 ml of pH 2.0 acid for two hours at 50 rpm taking samples at pre-determined times. After the two hours, the baskets were transferred into the second dissolution vessel containing 900 ml of phosphate buffer pH 7.4 equilibrated to 37°C ± 0.1°C and the test continued for eight more hours, taking samples at pre-

determined times. After the total time of ten hours, the test was continued until all the ASA had dissolved in the phosphate buffer. The U.V. absorbances of samples taken from the dissolution apparatus were determined and their corresponding concentrations calculated from the calibration curves originally constructed. The total amount of ASA eventually dissolved was compared with the assay results obtained by a separate analytical method to confirm the potency of the pellets. The dissolution test was performed in triplicate for each of sample. Graphs of the average percentage amount of ASA dissolved were plotted against time (hours).

2.5. ASA CONTENT DETERMINATION

METHOD

1- ASA calibration curves were constructed by plotting the UV absorbances of ASA standard solutions versus concentrations for concentrations ranging from 0 mg/ml to 4 x 10^{-5} g/ml in PH 7.4 buffer at 262 nm.

2- 2 g of the ASA pellets was finely ground, and an amount of powder equivalent to 23 mg of ASA transferred to a 50 ml erlenmeyer flask. About 20 ml of Phosphate buffer PH 7.4 was added to the flask, sonicated for about 5 minutes and stirred by means of a stirrer bar while warming the flask to

about 35°C for ten minutes. The mixture was allowed to cool to room temperature. The flask contents were quantitatively transferred to a 100 ml volumetric flask, diluted to volume using the phosphate buffer, mixed for five minutes and filtered over a 0.2 µm filter, then the UV absorbance of the resulting clear solution determined at 262 nm.

3- The concentration of ASA in the sample preparation was calculated from the calibration curve, and the ASA content in the pellets determined from the results.

2.6. STABILITY TESTING

METHOD: HPLC

EXPERIMENTAL PARAMETERS:

Flow rate	= 1.00 ml/min.
Working pressure	= 1.80 kgf.
Maximum pressure	= 2.50 kgf.
Range (Auf)	= 1.
Absorbance wave length	= 228 nm.
Column/length/diameter	= C 18 Reverse phase/250 x 4.6mm I.D.
Mobile phase	= H_2O:Methanol:H_2PO_3 at the

ratio 45:55:07 mixing.

Mobile phase PH = 2.35

Injection volume = 20 µl.

METHOD. About 2 g of the pellets were crushed into fine granules. An accurately weighted amount of crushed granules equivalent to 15 mg, were delivered into a 100 ml Volumetric flask. 5 ml of pH 2.0 buffer and about 15 ml of methanol were placed into the volumetric flask and agitated by means of a mechanical shaker for about 20 minutes. 50 ml of distilled water was added to the mixture and sonicated for 5 minutes. The mixture was diluted to volume with distilled water, mixed and filtered through a 0.2 µ filter, 5 ml of the filtrate was reserved for HPLC analysis. Three analysis trials were done for each of the five formulations and their washing/coating treatments.

CHAPTER III

RESULTS AND DISCUSSIONS

3.0. FORMULATION CRITERIA AND CONSIDERATIONS

In the formulation of a solid oral drug delivery product, the formulation method, excipient and the dosage form type will influence the final properties of the drug, which may in turn affect the clinical performance of the formulation as discussed earlier. If the formulation is intended to deliver the active substance in the small intestine, or lower colon, gastric fluid resistant materials such as cellulose acetate hydrogen phthalate and hydroxypropyl methyl cellulose are included in the formulation. In this work, the organic solvent based approach was selected over the aqueous based method (**see Fig. 10**) in order to minimise chances of ASA hydrolytic degradation into salicylic acid and acetic acid during the manufacturing process. The properties of ASA to undergo an accelerated hydrolytic degradation process in moisture and elevated temperature conditions in the presence of air (3), places a limitation in the use of aqueous based methods of microencapsulation. The solvents were selected on the basis of low reactivity, low toxicity, and efficiency to act as solvents or non-solvents of the polymers. A micrograph and photograph of the microcapsules are shown

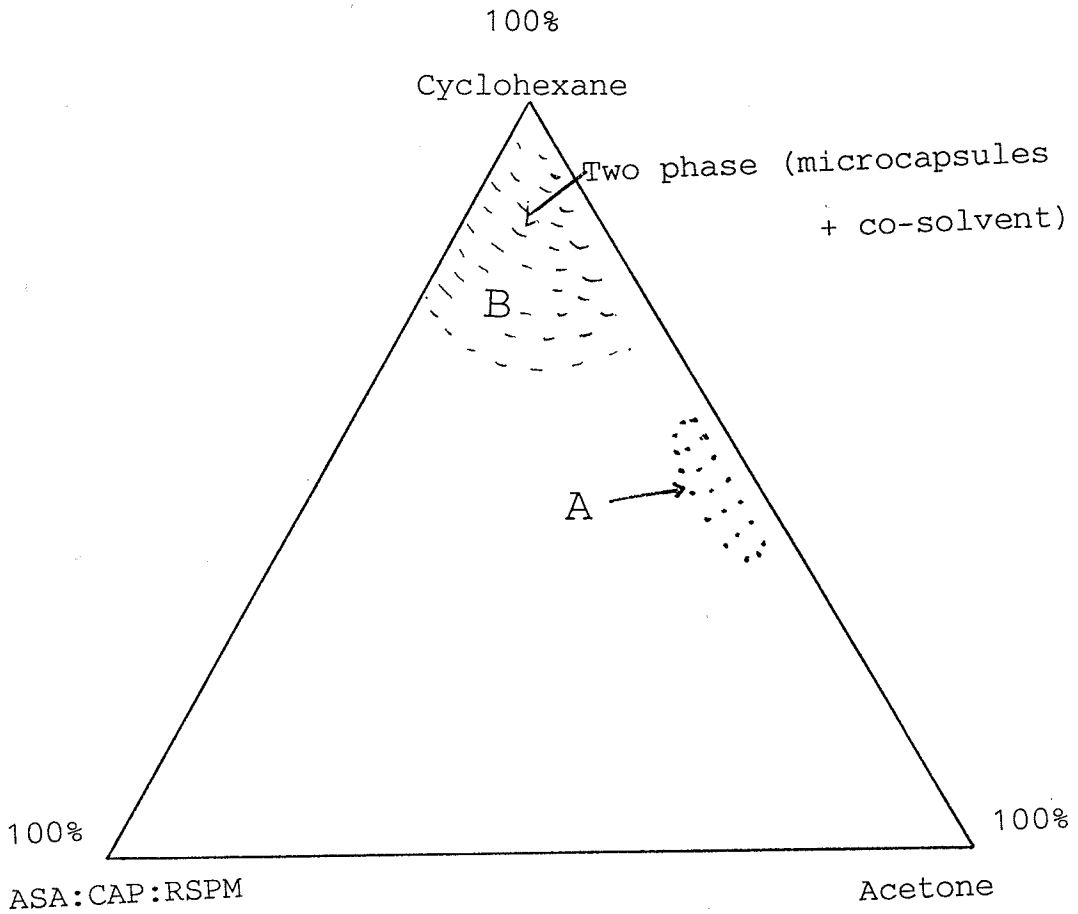


Figure 10. A ternary diagram for the system: (ASA: CAP:RSPM):Acetone:Cyclohexane in the ASA microencapsulation, showing the first appearance of coacervate droplets (A), and the point of the actual formation of the microcapsules (B).

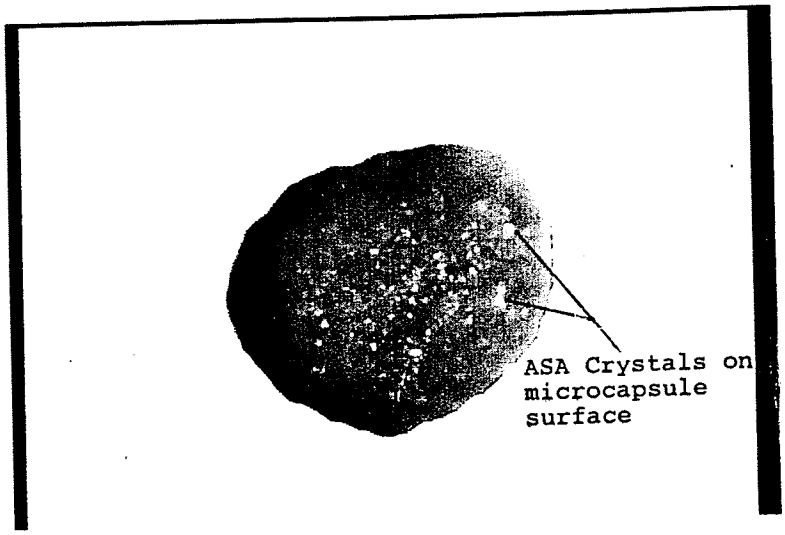


Figure 11 (A). A photo-micrograph of ASA microcapsule, showing the presence of un-coated ASA crystals on the surface (**Magnification x 50**).

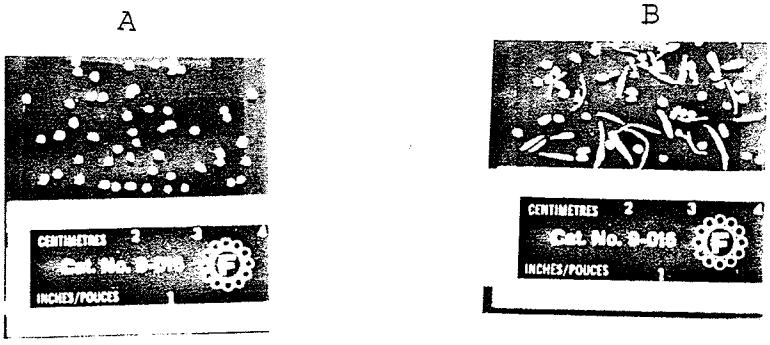


Figure 11 (B). Photograph of Microcapsules collected from size 22 mesh USP screen, showing (A) well formed microcapsules, and (B) elongated microcapsules showing the effect of a high CAP content and the effect of low stirring speeds.

in Figure 11 A and 11 B.

3.1. ANALYSIS OF ASA MICROCAPSULES

GENERAL. In many aspects of pharmaceutical formulation, various analytical procedures are used to assess the product and obtain information about its overall quality. An instrumental, or chemical analysis of degradation product test is a helpful tool used to validate the formulation method to confirm that the integrity of the drug (and sometimes the excipient) has not been compromised by the processing method. The results of a degradation product test of the microcapsules obtained with the help of HPLC will be discussed later in this chapter. The drug content analysis was done in order to assess the encapsulation efficiency and for determining the payload or time for complete payload release of the microcapsules. The drug content results are summarized in Table 3. In conventional oral delivery systems, drug release from the matrix occurs in such a way that the *in-vitro* dissolution profile has a direct correlation to the *in-vivo* bioavailability. The drug delivery design in such systems is aimed at achieving fast drug release rates, and the *in-vitro* testing is based on assessing how fast the drug is released from the device. In controlled and sustained release formulations on the other hand, the emphasis is placed more on the uniformity and

Table 3. Results of total microcapsule yield, micromeritic properties (for the 22 mesh size only), and drug content from the five formulations.

PARAMETER	FORMULATION				
	1	2	3	4	5
% Yield	100	98.5	90.7	90.9	98.1
Angle of repose	29.7	28.6	27.6	28.1	28.6
Bulk density (g/ml)	0.51	0.49	0.47	0.54	0.56
% Drug loaded	50.0	25.0	22.0	22.0	22.0
% Drug calculated	49.5	27.5	31.0	32.1	25.0

predictability of drug release as opposed to rapid drug release rates. Statistical tests were done to assess both the uniformity and predictability of the drug release profiles from the microcapsules.

STATISTICAL ANALYSIS. Statistical analysis can be used to describe and to characterize the relationship between variables and treatments. The researcher may also need to test whether the observed results are due to the differences in experimental treatments, or they are due to chance, hence a null hypothesis is stated and tested against the alternative hypothesis to decide whether the observed means are the same under the specified limits of error. An analysis of variance (ANOVA) is often used to test the hypotheses. The most important assumption in this work was that the experimental conditions and parameters were identical between formulations, and that the differences in the mean percentage amounts of drug released at any given time point are due to the differences in the formulations only and not due to experimental variability. Since in practice it is not possible to keep the microencapsulation procedure parameters identical when using methods that are not automated, when using the statistical method (e.g. ANOVA), the tests should be done in such a way that the method is able to correct for the experimental procedure variations. The ANOVA test was done with the aid of Minitab

release 10.2 statistical software on a personal computer, and regression analysis of the plots of the percentage amount of drug released *vs.* time was done using Quattro software on the personal computer. Due to problems of drug burst effect, the regression analysis of the percentage drug released *vs.* time plot in the acid pH buffer was not done, regression analysis was done only for the graph portion representing the amount of drug released in the phosphate buffer pH 7.4 up to the point where 95% of the drug ($T_{95\%}$) had been released.

ANOVA GROUPING. Because of the way in which the experiment was designed and conducted, the microcapsules were grouped as follows; the washed and untreated microcapsules were grouped together, the ethylcellulose coated, and PIB coated microcapsules were in another group, and the enteric coated microcapsules tested on their own.

3.2. MICROCAPSULE SIZE DISTRIBUTION

The mesh fraction analysis of the microcapsules revealed that in general the 16/22 mesh size formed the bulk in all formulations as illustrated in **Fig. 12.** Formulations (1 and 2) containing a higher drug load (2:1:1 and 2:3:3 ASA:CAP:RSPM respectively) registered very low yields of 12/16 mesh size fractions. The dissolution studies were not conducted using the 12/16 mesh particle size with these two

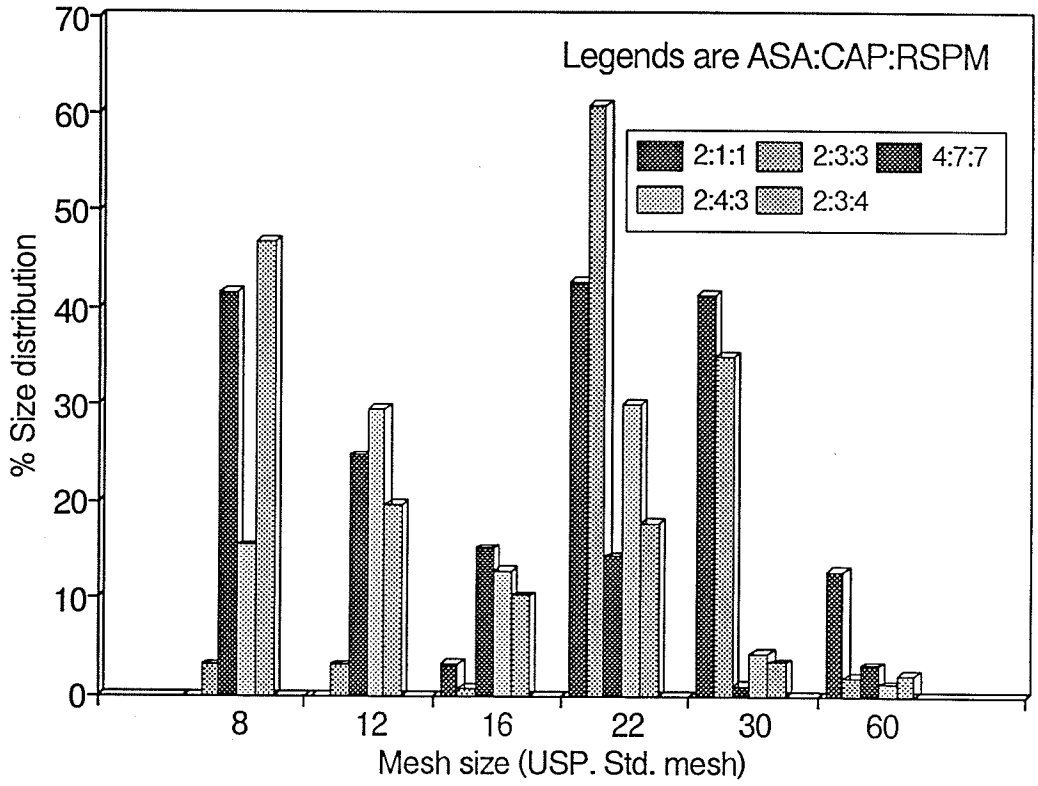


Figure 12. Size distribution of various ASA microcapsules formulations (1-5).

formulations because of the poor yield of microcapsules of that size.

3.3. CONTENT UNIFORMITY % YIELD AND MICROMERITIC PROPERTIES

The ASA content calculated from the microcapsules was higher than the amount loaded (Table 3), in all but formulation 1 which had 50.0% drug load. There appears to be a relationship between the microcapsule % yield and the % ASA calculated from the microcapsule. In formulation 1 where the % product yield was 100.0%, the % ASA calculated was 99.0% of the loaded drug. In all the other formulations, where the % product yield was below 100.0%, the % ASA calculated is inversely proportional to % drug loaded. The interpretation of this observation suggests that the processing loss observed results from the polymer loss, which occurs through the process wall adhesion. This polymer adhesion was actually observed during microencapsulation, and increasing the amount of ASA load seemed to reduce this polymer loss. A more effective solution to this problem lies in the optimization of the manufacturing process parameters, through further study of the effect of amounts of materials and solvents, effect of stirring rate, effect of non-solvent addition rate, types and optimum amounts of anti-adherent and microencapsulation inducers on microencapsulation of ASA by

this method. Due to the small coating batch sizes dealt with in this work, it was not possible to do a quantitative measurement of the flow properties of the coated microcapsules.

3.4. ANALYSIS OF DETERIORATION COMPOUNDS (HPLC METHOD)

The HPLC run time was set at 20 minutes, and the following peak retention times corresponding to ASA and deterioration compounds were observed.

ASA = 4.5
Salicylic acid = 6.3
Phthalic Acid = 3.5
Others = 10.7 (for Entrophen only)
Absorbance wave length at 228 nm.

A sample chromatogram is shown on **Fig. 13** and the results illustrated in Table 4.

The salicylic acid content was calculated on the basis of percentage w/w salicylic/acetylsalicylic acid in each case. The observed phthalic acid should result from the break-down of the cellulose acetate hydrogen phthalate, while the salicylic acid the by product of the ASA break down.

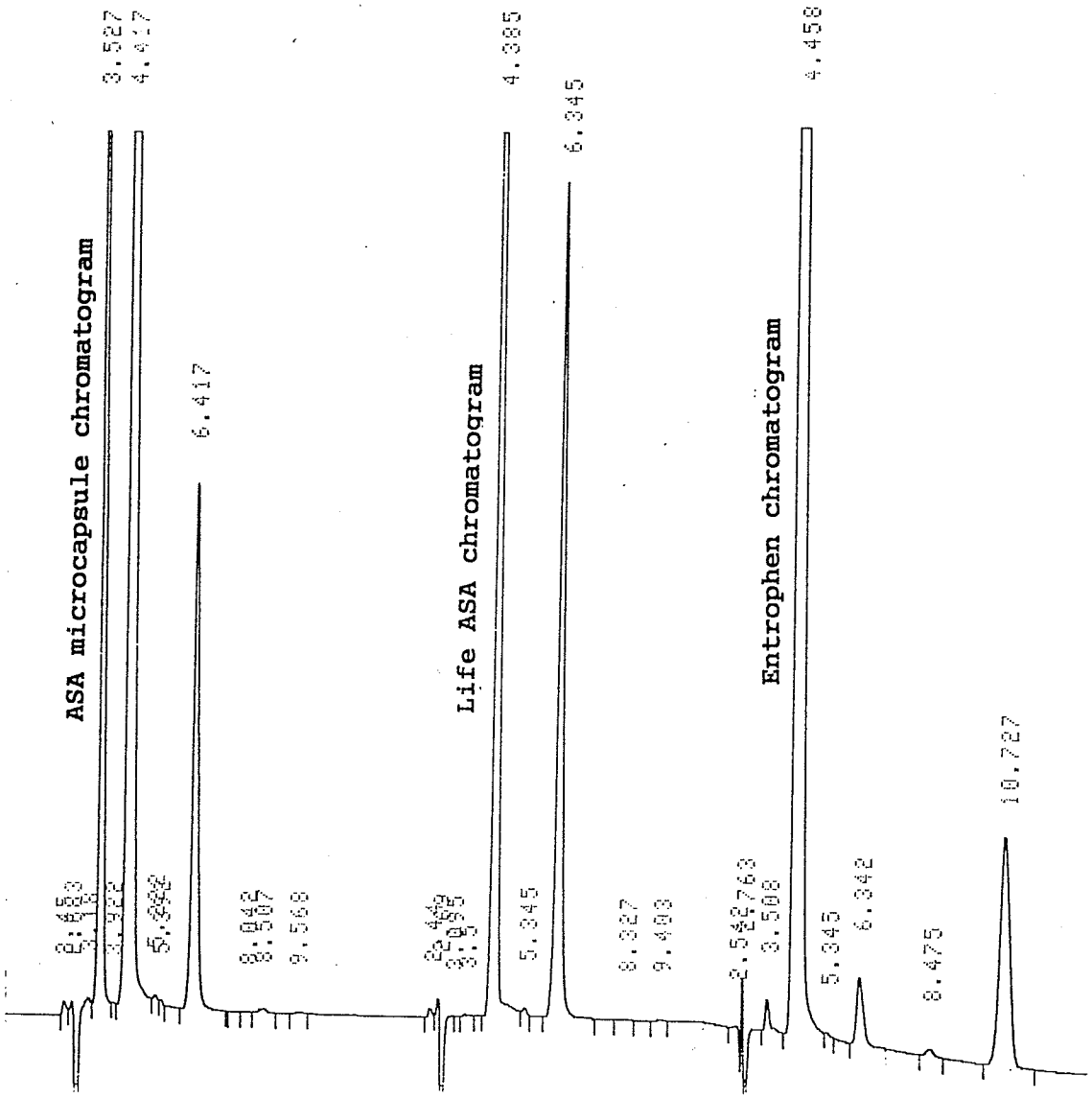


Figure 13. A sample chromatogram showing the peak positions of acetyl salicylic acid and deterioration products in the form of salicylic acid and phthalic acid, for the HPLC analysis of acetylsalicylic acid microcapsules. Peak positions are 3.5 min. for phthalic acid, 4.4 min. for ASA, 6.4 for salicylic acid and 10.7 min. for the unknown peak.

Entrophen, a commercial ASA product that is enteric coated produced an un-identifiable peak at 10.7 minutes position. The levels of salicylic acid detected in the microcapsules was found to be slightly below the levels found in the pure ASA, and were comparable to the levels detected in the Life ASA 325 mg tablets and Entrophen 325 mg enteric coated tablets. Only the microcapsules coated with Aquateric dispersion appeared to have higher levels of salicylic acid content (reaching up to 10.28 % w/w) when compared to those which did not receive Aquateric coating. This could have been caused by the exposure of ASA to high moisture levels and elevated temperature conditions under which the coating procedure was performed. These operating conditions could have resulted in accelerating the hydrolytic degradation of ASA (**88, 89**). It would be expected that the appearance of high levels of phthalic acid be accompanied by reduction or loss of enteric properties due to the break-down of cellulose acetate phthalate. The appearance of phthalic acid determined from the microcapsules did not seem to have any clearly distinct pattern, and therefore the results of this test on phthalic acid are inconclusive.

Table 4. Deterioration products levels % w/w (% Salicylic acid/ASA) determined by the HPLC method in all the microcapsule formulations (A) and two commercial products (B).

A

TREATMENTS	FORMULATIONS									
	1		2		3		4		5	
	Pthal.	Sal.	Phtha	Sal.	Phtha	Sal.	Phtha	Sal.	Phtha	Sal.
Un-coated	0.16	1.10	1.80	7.11	0.00	4.23	1.56	3.98	0.00	5.87
Washed	0.12	1.61	1.23	3.92	4.52	1.91	0.15	5.40	0.00	5.89
PIB.	1.31	1.80	2.21	5.73	4.51	6.52	4.09	4.52	3.28	4.58
Enteric	4.29	4.42	11.57	8.17	5.21	10.28	0.00	5.96	10.90	8.94
Ethylcell.	0.40	1.73	2.12	4.12	4.90	9.78	0.00	2.35	0.00	4.45

Key.

Pthal. refers to phthalic acid, and Sal. to salicylic acid.

B

COMMERCIAL FORMULATIONS	% Phthalic acid	% Salicylic acid
Life ASA 325 mg tablet	0.00	5.89
Entrophen 325 mg tablet	0.00	3.29

3.5. *IN VITRO* RELEASE PROPERTIES

CALIBRATION CURVES

Linear calibration of the absorbance of ASA vs concentration were produced in an acid pH 2.0 buffer, and in phosphate pH 7.4 buffer (**Figure 14 A and B**). The concentration range of the curves were 2.490×10^{-6} g ml^{-1} to 4.064×10^{-5} g ml^{-1} in pH 2.0 buffer, and 2.912×10^{-5} g ml^{-1} to 4.992×10^{-4} g ml^{-1} in phosphate pH 7.4 buffer. Beer's law was obeyed in both curves in these concentration ranges and the slopes of the curves were 39 508 and 3 034, with the y intercepts of 0.0027 and 0.006 for the ASA standard curve in acid and base respectively. The correlation (R^2) was 1.00 in both cases.

DISSOLUTION PROFILES

Dissolution profiles of the microcapsules of the five formulations, were produced in two different media, being the acid buffer pH 2.0 and phosphate buffer 7.4. Dissolution profiles were found to differ depending on the ratio of the polymer : polymer, and polymer : core. Washing or coating

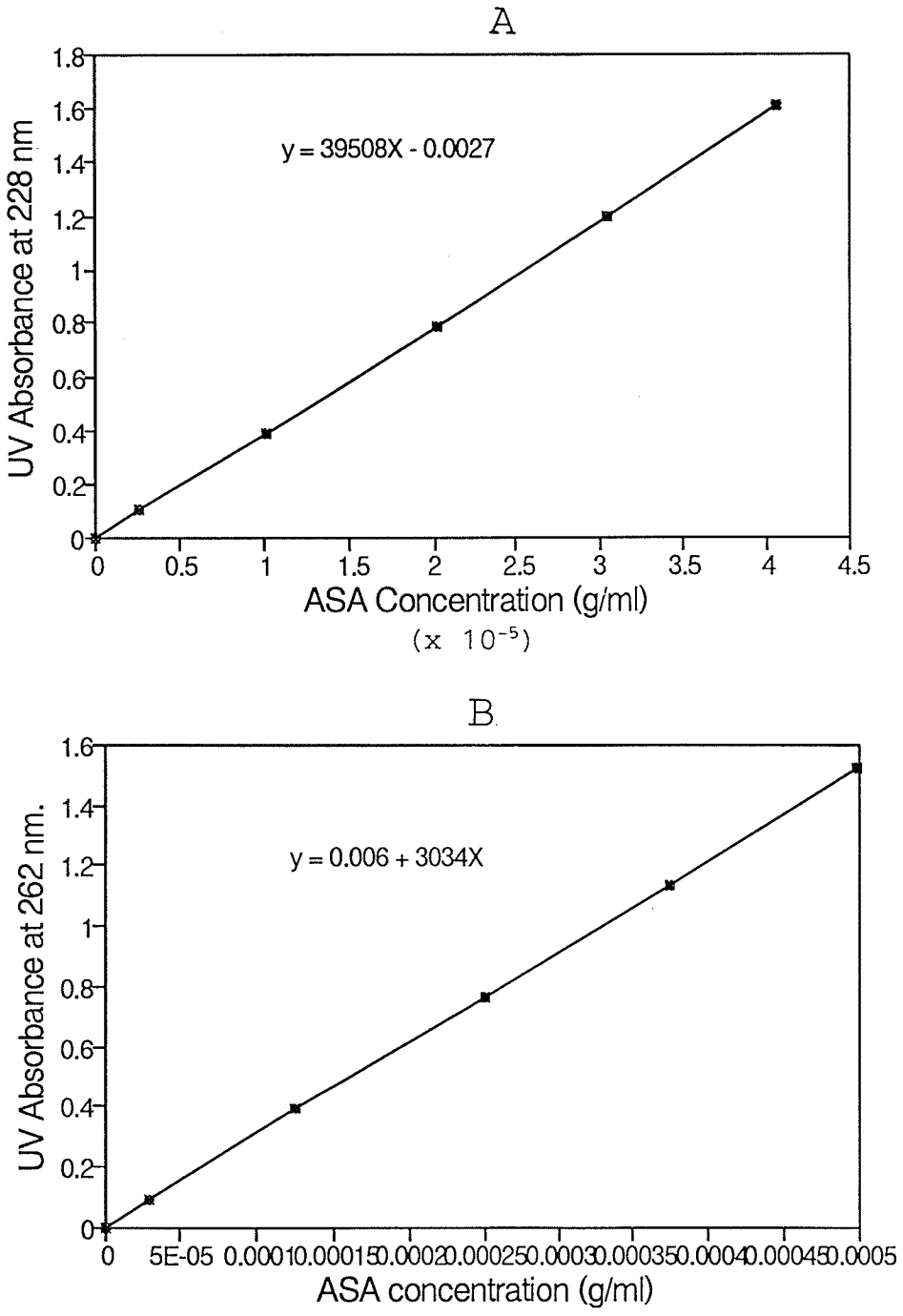


Figure 14. Calibration curves of pure ASA in (A) pH 2.0 (KCl/HCl) buffer and (B) pH 7.4 Phosphate buffer.

treatments were found to modulate the drug release profile, and coating had a slight effect in the drug release kinetics.

3.5.1. REGRESSION ANALYSIS

Regression analysis of the plots was done using Quattro pro 4.0 software for measuring the formulations release characteristics which are summarized in Table 5-16 below. Profiles that did not fit the near zero order models were fitted with the square root of time release model.

UNTREATED MICROCAPSULES. Burst effect (and lag time) are a common phenomenon in most microcapsules of a monolithic type (90). Burst effect was observed in all the five formulations, from plots of % drug released vs time, being more pronounced in untreated 22 mesh than in other microcapsules. Formulations 1 and 5, containing 50% and 29% drug load respectively, both indicated a higher burst effect (**Fig. 15 - 24**) incidence when compared to formulations 2 to 4 both of which contained 22% drug load. From this observation, it seems like, an increase in drug loading, and an increase in Eudragit RSPM content (or a decrease in CAP) lead to an increase in the burst effect. Note how the burst effect observed in formulation 2 is lower than formulation 5, despite the fact that formulation 2 had a higher drug loading (**Figures 15 and 16**), indicating that a higher RSPM content

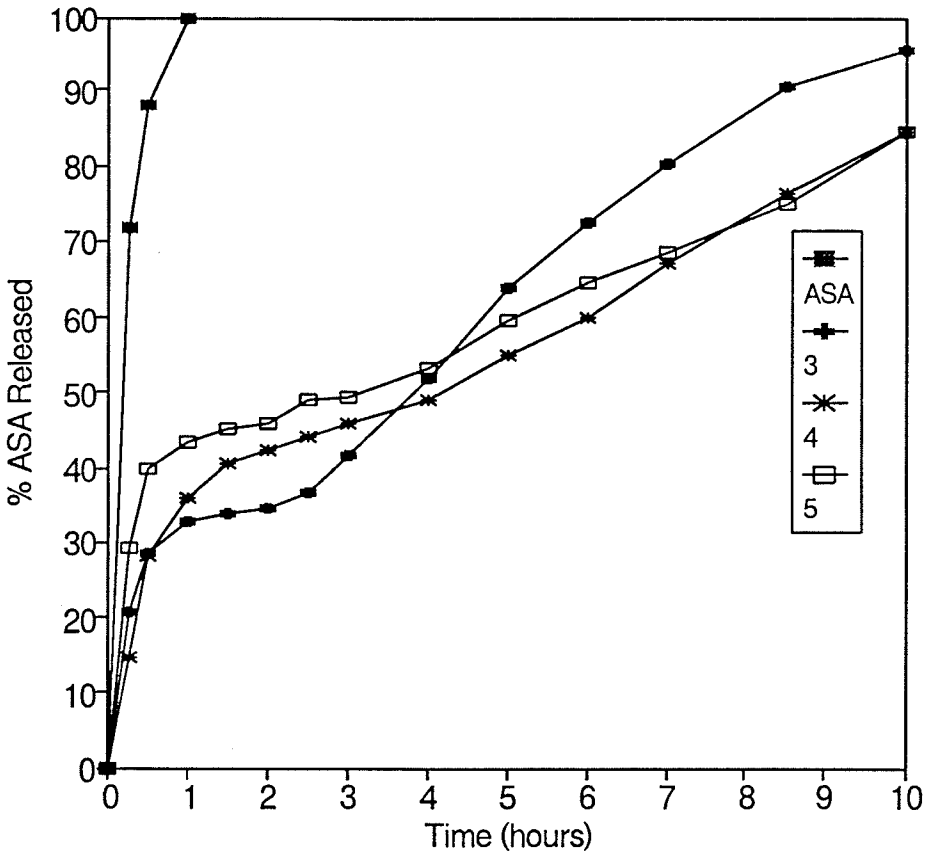


Figure 15. Dissolution profile of un-treated 12/16 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

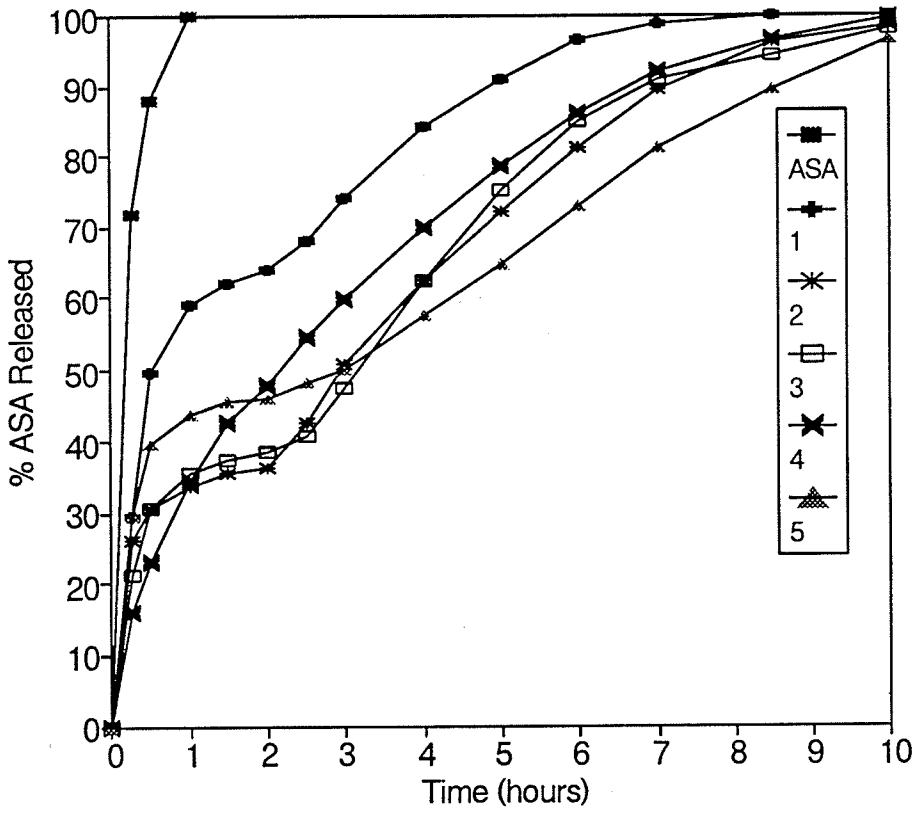


Figure 16. Dissolution profile of un-treated 16/22 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

Table 5. Analysis of variance results for un-treated and washed fractions, comparing the mean amount of drug released after 0.25 hours in pH 2.0 buffer at 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	1718.03	114.54	103.80	0.000
Error	32	35.31	1.10		
Total	47	1753.34			

Level	N	Mean	StDev
16WASH3	3	9.310	0.462
16WASH4	3	12.330	0.293
16WASH5	3	13.700	0.346
16UNTR4	3	14.670	0.568
22UNTR4	3	15.990	0.904
22WASH3	3	17.590	0.360
22WASH4	3	19.010	0.122
22WASH5	3	19.730	1.204
22WASH2	3	20.310	0.642
16UNTR3	3	20.640	1.091
22UNTR3	3	21.020	0.617
22WASH1	3	24.000	0.141
22UNTR2	3	26.250	0.328
22UNTR1	3	29.003	2.866
16UNTR5	3	29.250	0.776
22UNTR5	3	29.250	1.891

Pooled StDev = 1.050

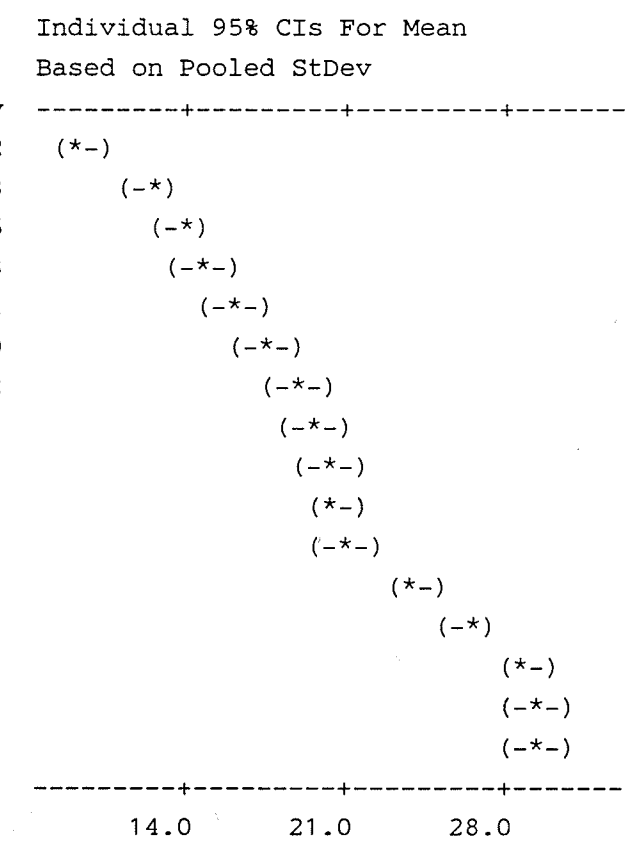


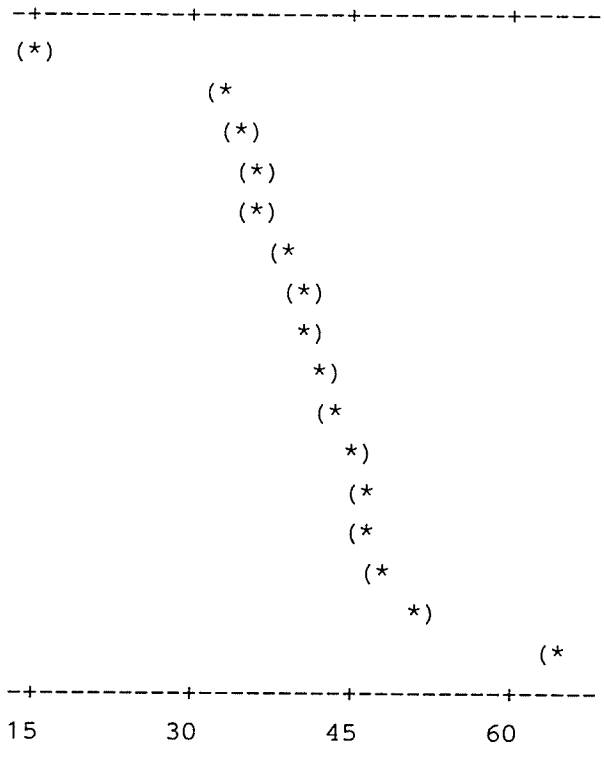
Table 6. One way analysis of variance results for un-untreated and washed fractions, comparing the mean amount of drug released after 2.0 hours in pH 2.0 buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	4818.272	321.218	482.60	0.000
Error	32	21.299	0.666		
Total	47	4839.571			

Level	N	Mean	StDev
16WASH3	3	14.810	0.036
22WASH2	3	32.640	0.615
16UNTR3	3	34.520	0.026
22WASH3	3	36.010	0.644
22UNTR2	3	36.020	1.895
22UNTR3	3	38.540	0.788
16WASH5	3	40.600	0.046
16WASH8	3	41.033	0.201
16UNTR4	3	42.240	0.040
22WASH5	3	43.010	0.115
22WASH4	3	45.280	1.443
16UNTR5	3	45.800	0.046
22UNTR5	3	46.150	1.264
22UNTR4	3	47.690	1.165
22WASH1	3	51.250	0.236
22UNTR1	3	64.020	0.700

Pooled StDev = 0.816

Individual 95% CIs For Mean
Based on Pooled StDev



Key for tables 5 - 16 and 22.

- uncoat = untreated (unwashed and uncoated) microcapsules.
- 16 or 22 preceding untr, cell or teric etc. refers to microcapsule mesh size 12/16 and 16/22 respectively.
- the number (1-5) appearing at the end of the treatment method refers to the formulation number.
- Level refers to the formulation number.
- DF = the degree of freedom.
- DF Factor is the total number of formulations (t) minus 1.
- DF Error is the total number of observations (n) minus t (n - t).
- SS Factor = Sum of squares between samples.
- SS Error = Sum of squares within samples.
- MS Factor = (SS Factor/t-1).
- MS Error = (SS error/n-t).
- F = The test statistic (at the specified α level. e.g. 0.05 in this case.
- α level refers to the probability of rejecting the null hypothesis when it is true. That is committing a type I error. NB, type II error is committed when the false null hypothesis is accepted (ß).
- P = the probability that the observed differences

in mean are due to chance at the specified confidence interval (CIs 95%).

- StDev = Standard deviation.

Table 7. Analysis of variance results for the un-treated and washed fractions, comparing the mean amount of drug released after 5.0 hours in pH 7.4 phosphate buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	7261.246	484.083	2420.42	0.000
Error	32	6.400	0.200		
Total	47	7267.646			

Level	N	Mean	StDev
16WASH3	3	40.950	0.050
16WASH4	3	54.980	0.036
16WASH5	3	56.830	0.128
16UNTR4	3	57.690	0.557
16UNTR5	3	59.530	0.719
22WASH5	3	62.580	0.529
16UNTR3	3	63.820	0.044
22UNTR5	3	64.830	1.140
22WASH3	3	65.930	0.304
22WASH4	3	68.790	0.373
22WASH2	3	72.420	0.347
22UNTR3	3	75.270	0.480
22UNTR4	3	78.550	0.375
22UNTR2	3	82.890	0.111
22WASH1	3	82.960	0.089
22UNTR1	3	91.030	0.168

Pooled StDev = 0.447

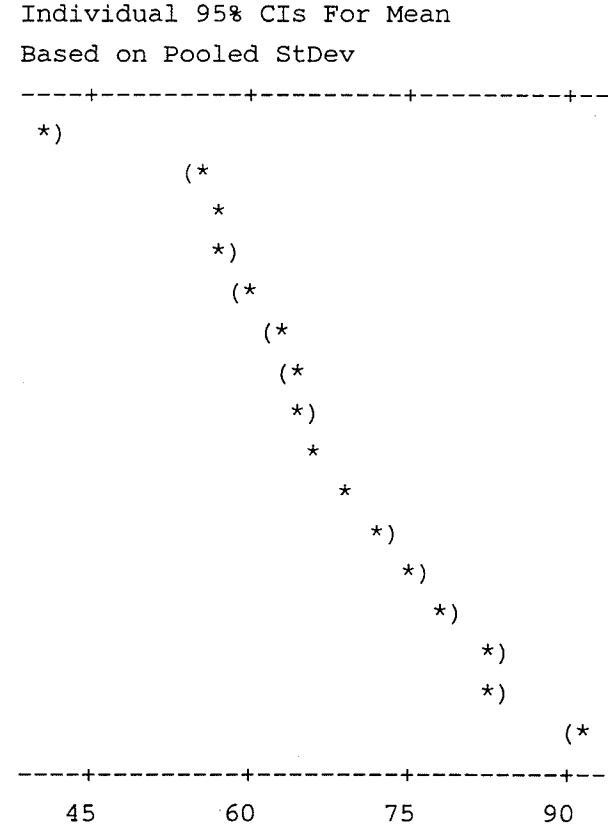


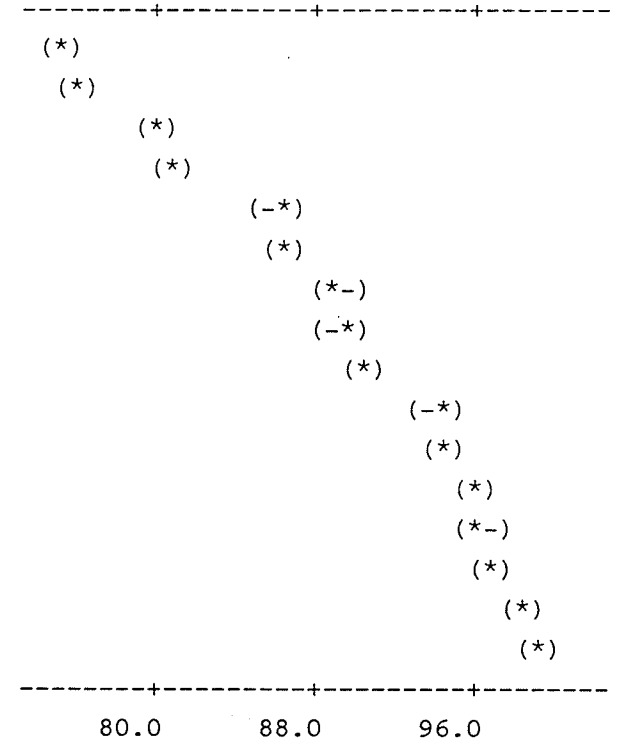
Table 8. Analysis of variance results for the un-treated and washed fractions, comparing the mean amount of drug released after 8.5 hours in pH 7.4 phosphate buffer buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	2831.120	188.741	339.20	0.000
Error	32	17.806	0.556		
Total	47	2848.926			

Level	N	Mean	StDev
16WASH5	3	74.910	0.377
16WASH4	3	76.270	0.913
16UNTR5	3	80.200	0.456
16UNTR4	3	80.750	0.567
16WASH3	3	86.030	0.446
22WASH5	3	86.360	0.344
22WASH4	3	89.140	0.306
22UNTR5	3	89.220	0.684
16UNTR3	3	90.710	0.046
22WASH3	3	94.050	1.110
22UNTR3	3	94.380	1.152
22UNTR2	3	96.220	1.095
22UNTR4	3	96.380	1.017
22WASH2	3	97.110	0.246
22WASH1	3	98.320	0.624
22UNTR1	3	99.343	1.129

Pooled StDev = 0.746

Individual 95% CIs For Mean
Based on Pooled StDev



contributes more to an increased initial burst effect than a higher drug load up to a point. Drug burst effect ranged from a low of 24% to a high of 47% within the first 30 minutes for the microcapsules that were not washed or coated. The burst effect was reduced to 11% and 17% respectively by washing the pellets with cyclohexane or coating them. Considering the solubility of ASA in the solvent system used, and the events that lead to phase separation and subsequent formation of microcapsules, an inference was made that the observed burst effect was caused by the embedding of the un-coated ASA into the microcapsule after it had formed, and before it could harden, and loosely adhered to the microcapsule.

3.5.2. EFFECT OF PELLET WASHING AND COATING

CYCLOHEXANE WASHING. Washing of the dried pellets with cyclohexane after microencapsulation resulted in a considerable decrease in the initial drug burst (from 60% to 39% with formulation 1). Washing also appear to modulate the drug release consistency as witnessed in Figure 17 and 18.

POLYISOBUTYLENE COATING . Coating in the presence of polyisobutylene did not reveal any positive improvement on the release characteristics of the microcapsules. The

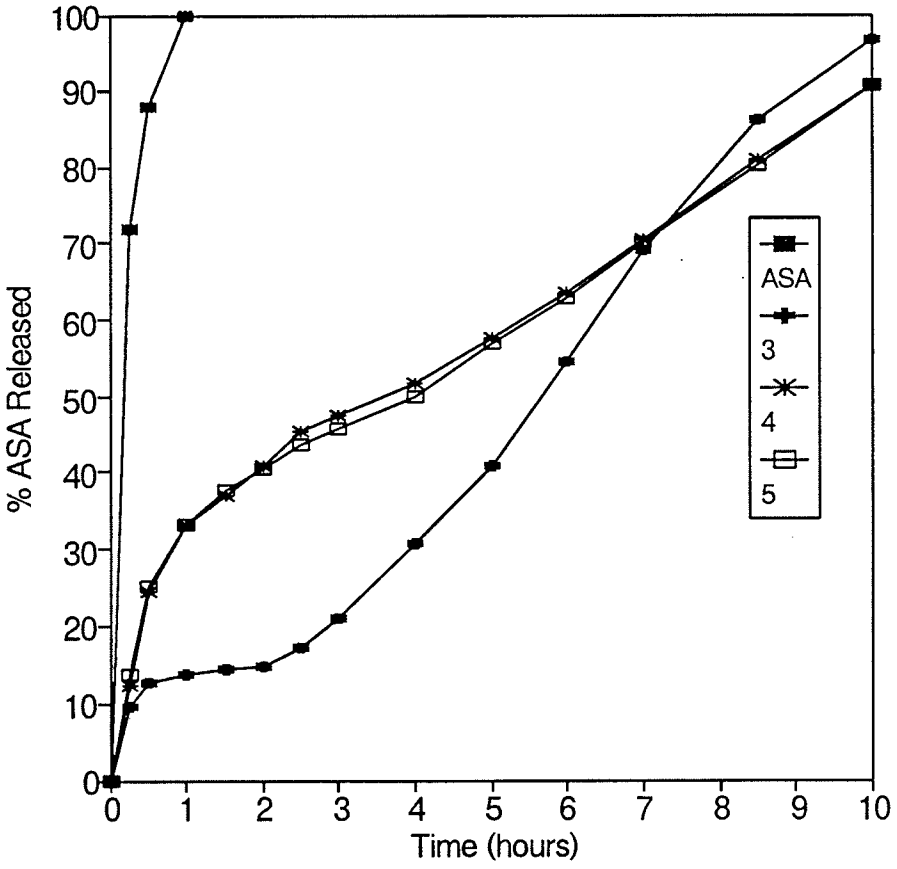


Figure 17. Dissolution profile of cyclohexane washed 12/16 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

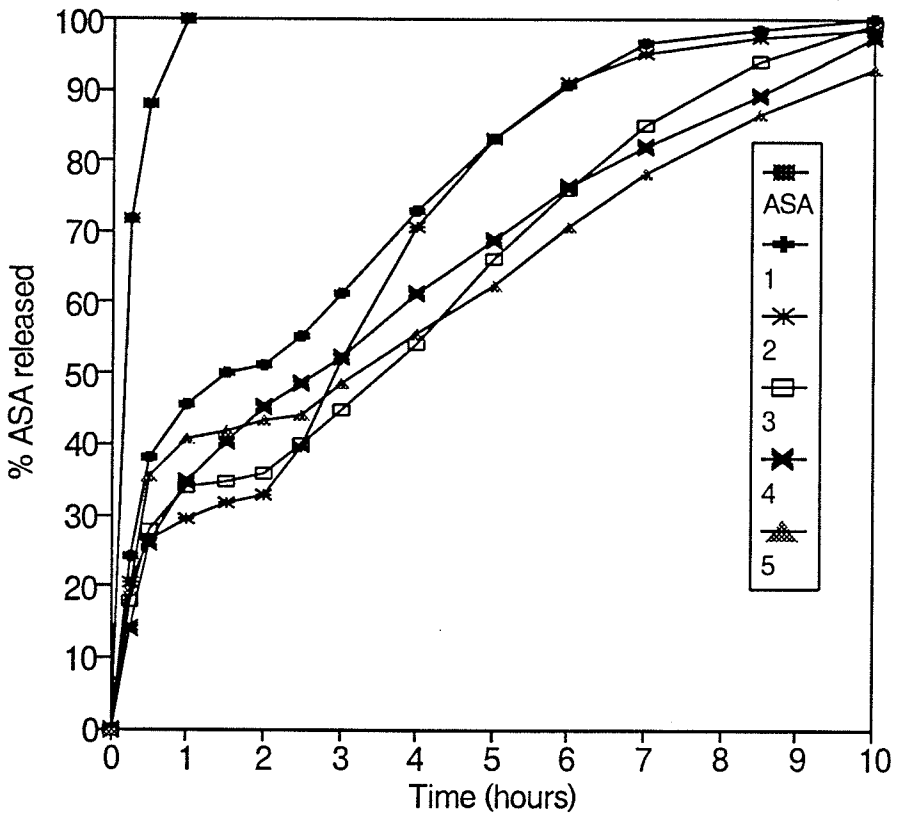


Figure 18. Dissolution profile of cyclohexane washed 16/22 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

release profiles do not follow any specific pattern (**see Figure 19 and 20**).

ETHYLCELLULOSE COATING. Ethylcellylose coating produced a marked effect in reducing the initial drug burst effect, and modulation of the drug release profile in all the formulations (**see Figure 21 and 22**). This observation is in keeping with other findings where ethylcellulose has been found to be an efficient wall former in the formulation of reservoir type of devices by different methods (**54, 91**).

ENTERIC COATING. Enteric coating by Aquateric^{R} did not improve the release properties of the microcapsules (**see Figure 23 and 24**) . This could be attributed to the difficulty in controlling the process parameters of this coating method which are not easy to control when dealing in small batch sizes. More tests would be required before any conclusions on the effectiveness of Aquateric as a microencapsulation wall former can be made. The visual observation of the flow of the microcapsules appeared to be excellent after coating with Aquateric.

3.6. PHYSICAL STRENGTH OF THE PELLETS

The pellets further demonstrated good physical strength by withstanding the rigors of air suspension coating procedure

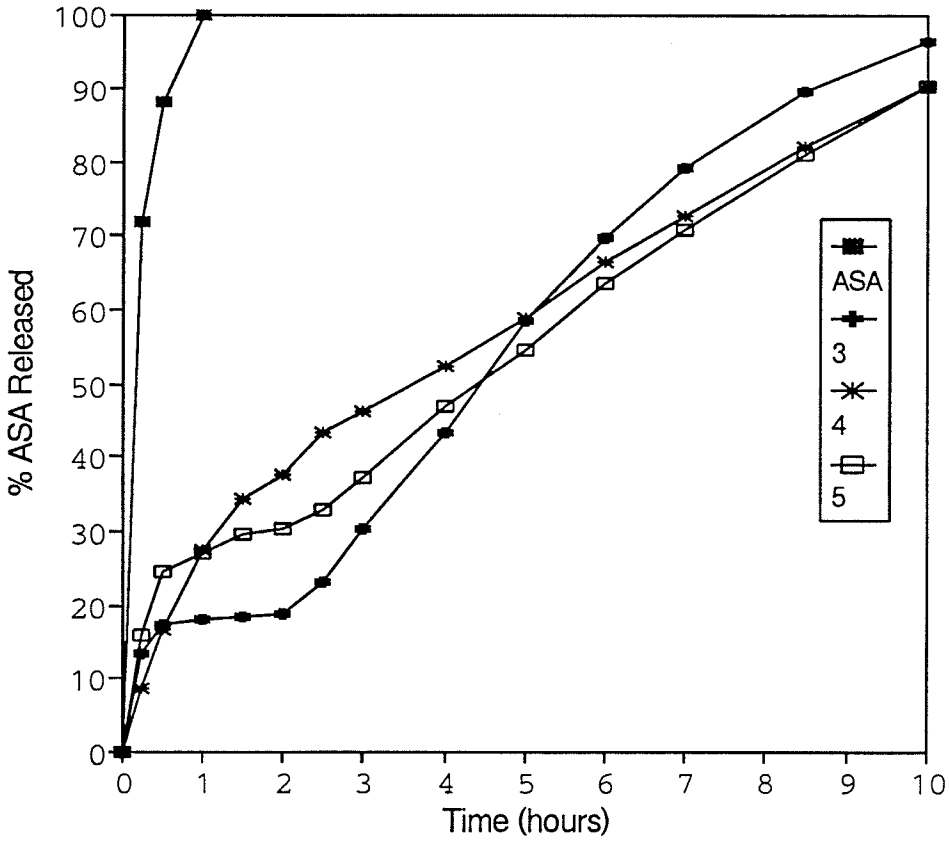


Figure 19. Dissolution profile of Poly-isobutylene coated 12/16 mesh ASA microcapsules showing a plot of percentage ASA released vs. time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

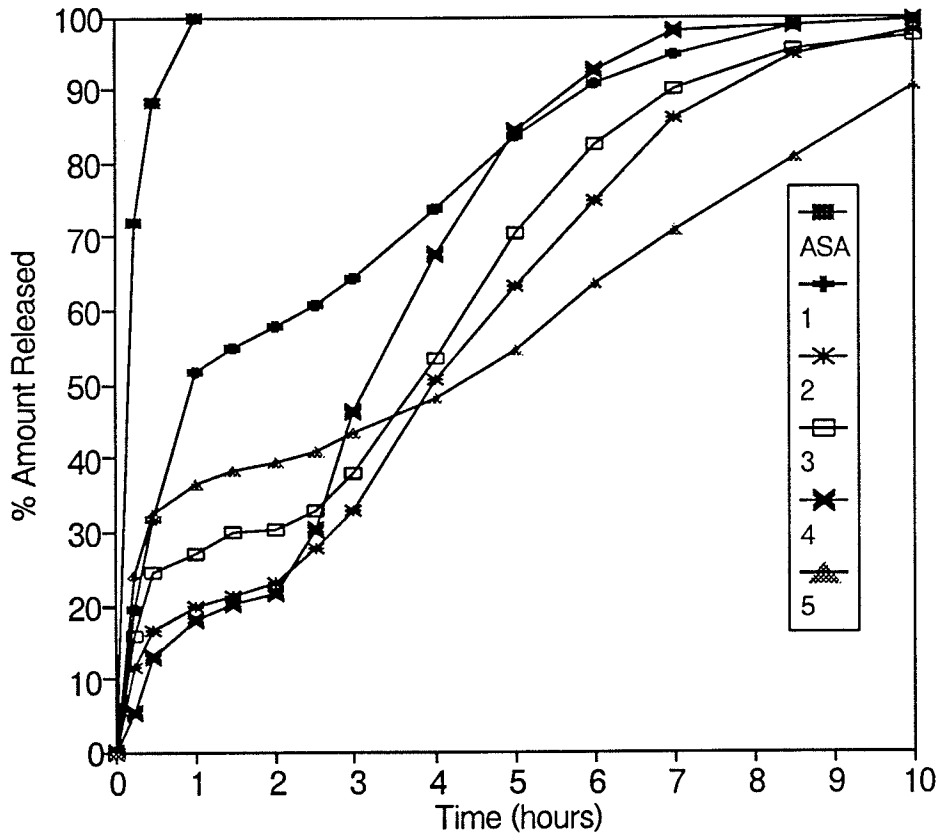


Figure 20. Dissolution profile of poly-isobutylene coated 16/22 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

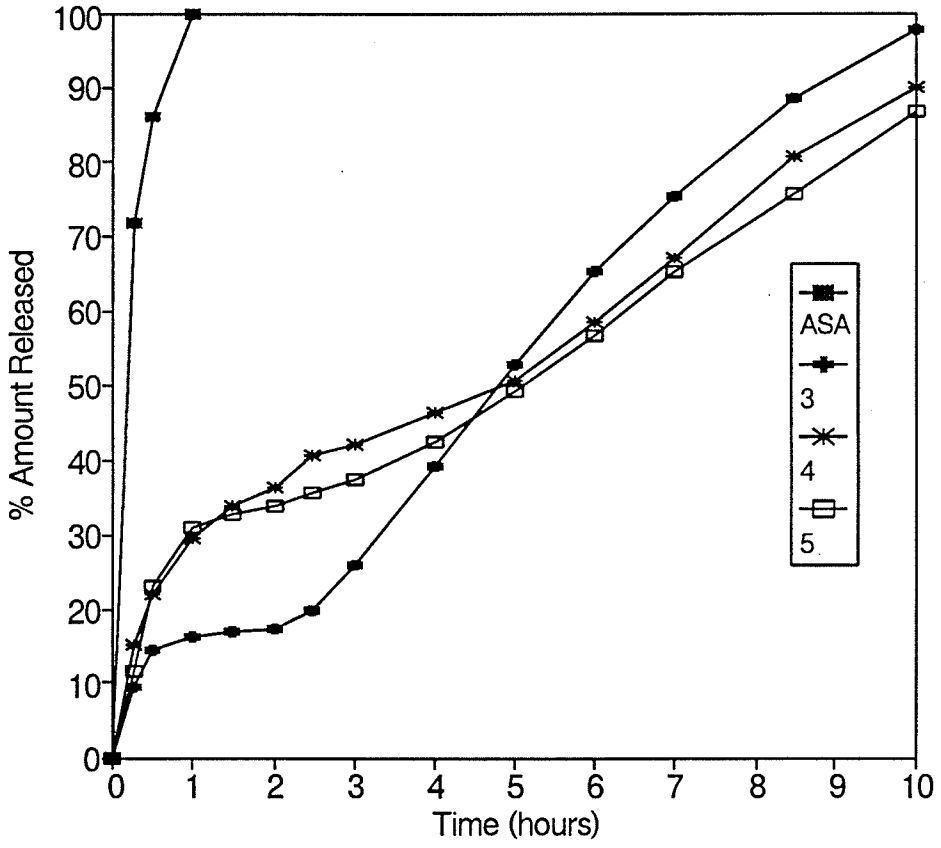


Figure 21. Dissolution profile of Ethylcellulose coated 12/16 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2.0 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

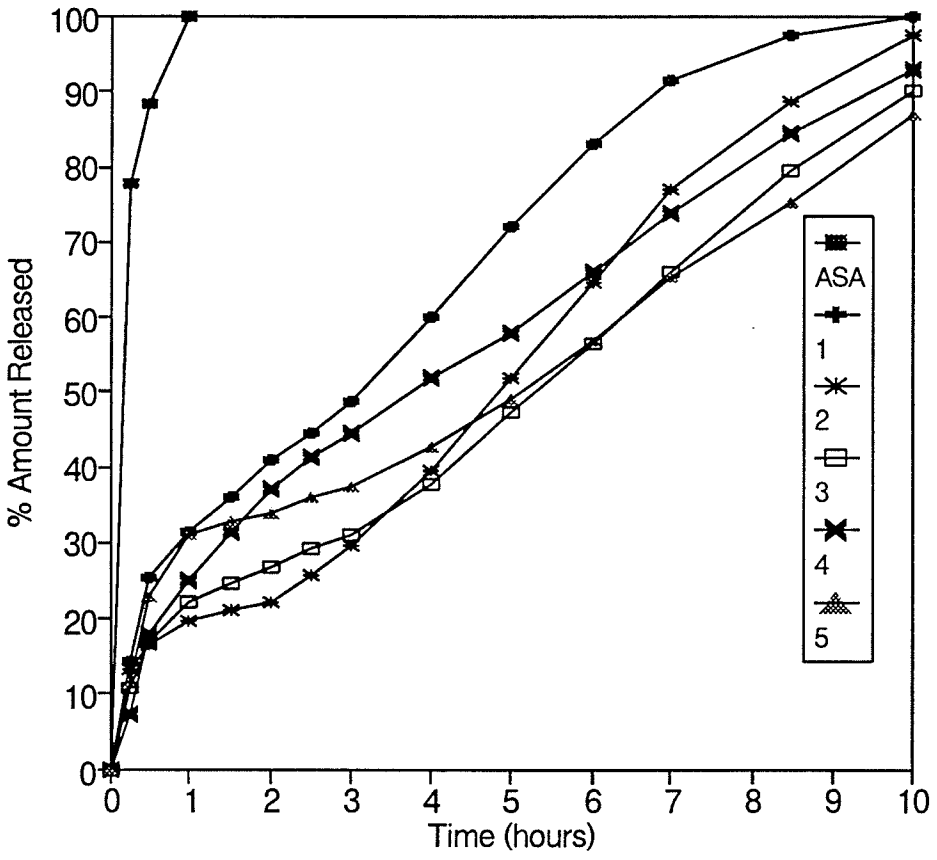


Figure 22. Dissolution profile of ethylcellulose coated 16/22 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

Table 9. One way analysis of variance for ethylcellulose coated and for microcapsules coated in the presence of PIB fractions, comparing the mean amount of drug released after 0.25 hours in pH 2.0 buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	972.968	64.865	201.55	0.000
Error	32	10.299	0.322		
Total	47	983.267			

Level	N	Mean	StDev
22PIB4	3	5.190	0.810
22CELL4	3	7.360	0.195
16PIB4	3	8.620	1.611
16CELL3	3	9.640	0.079
22CELL3	3	10.790	0.026
16CELL5	3	11.390	0.267
22CELL5	3	11.390	0.141
22PIB2	3	11.510	0.458
16PIB3	3	13.370	0.183
22CELL2	3	13.510	0.040
22CELL1	3	14.510	0.066
16CELL4	3	15.200	0.787
16PIB5	3	15.580	0.166
22PIB3	3	15.580	0.473
22PIB1	3	19.520	0.781
22PIB5	3	24.200	0.182

Pooled StDev = 0.567

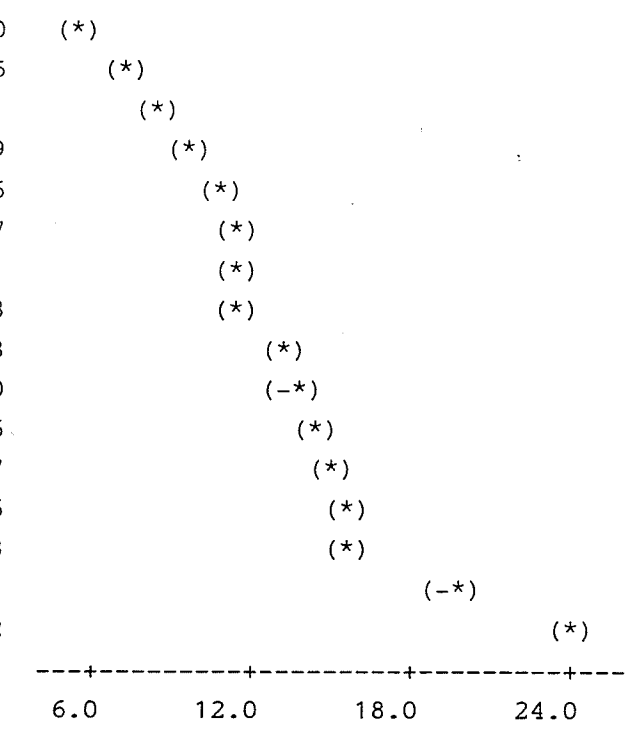


Table 10. One way analysis of variance for ethylcellulose coated, and for microcapsules coated in the presence of PIB fractions, comparing the mean amount of drug released after 2.0 hours in pH 2.0 buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	4784.262	318.951	1119.08	0.000
Error	32	9.120	0.285		
Total	47	4793.382			

Level	N	Mean	StDev
16CELL3	3	17.330	0.096
16PIB3	3	18.640	0.036
22PIB4	3	21.607	0.396
22CELL2	3	22.260	0.473
22PIB2	3	22.903	0.633
22CELL3	3	26.580	0.511
16PIB5	3	30.230	0.036
22PIB3	3	30.230	0.903
16CELL5	3	33.850	0.046
22CELL5	3	33.850	0.269
16CELL4	3	36.200	0.070
22CELL4	3	36.970	0.187
16PIB4	3	37.530	0.115
22PIB5	3	39.090	0.920
22CELL1	3	40.890	0.095
22PIB1	3	57.940	1.306

Pooled StDev = 0.534

Individual 95% CIs For Mean
Based on Pooled StDev

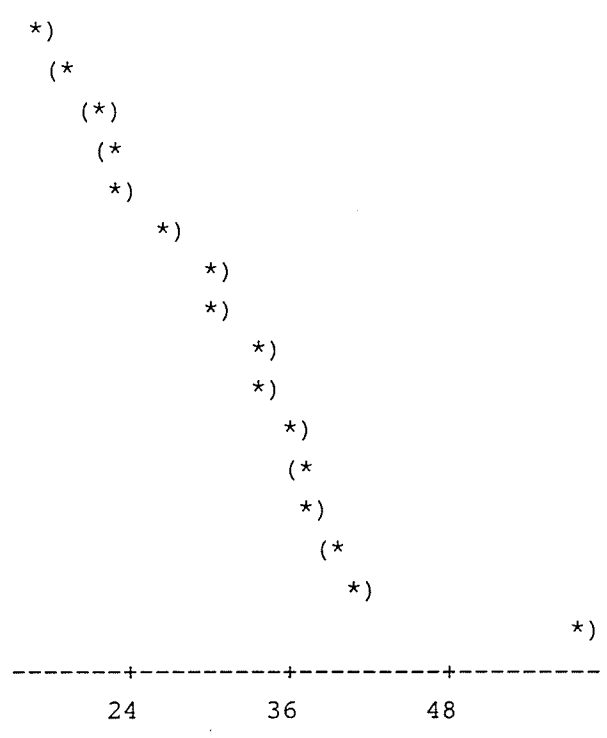


Table 11. One way Analysis of variance results for ethylcellulose coated microcapsules, and for microcapsules coated in the presence of PIB fractions, comparing the mean amount of drug released after 5.0 hours in pH 7.4 phosphate buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	6234.602	415.640	716.31	0.000
Error	32	18.568	0.580		
Total	47	6253.170			

Level	N	Mean	StDev
22CELL3	3	47.410	0.717
22CELL5	3	49.000	0.241
16CELL5	3	49.000	0.089
16CELL4	3	50.610	0.017
22CELL2	3	52.080	0.331
16CELL3	3	52.830	0.026
16PIB5	3	54.620	0.866
22PIB5	3	54.620	1.797
22CELL4	3	57.910	0.252
16PIB3	3	58.240	1.363
16PIB4	3	58.960	1.088
22PIB2	3	63.290	0.295
22PIB3	3	70.470	0.569
22CELL1	3	72.150	0.436
22PIB1	3	83.410	0.330
22PIB4	3	84.350	0.894

Pooled StDev = 0.762

Individual 95% CIs For Mean
Based on Pooled StDev

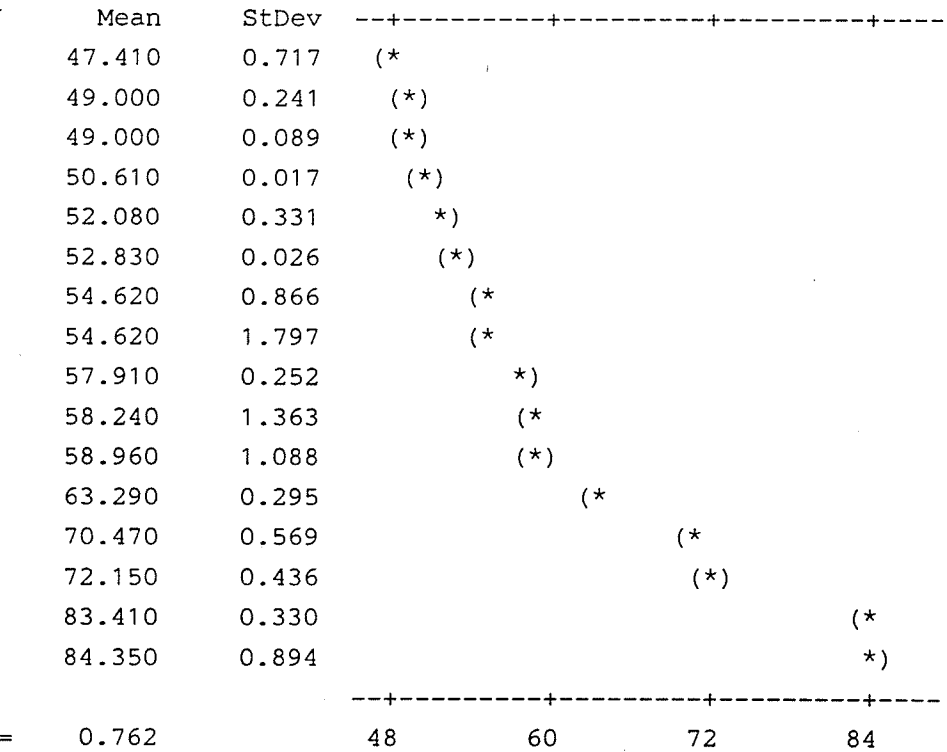
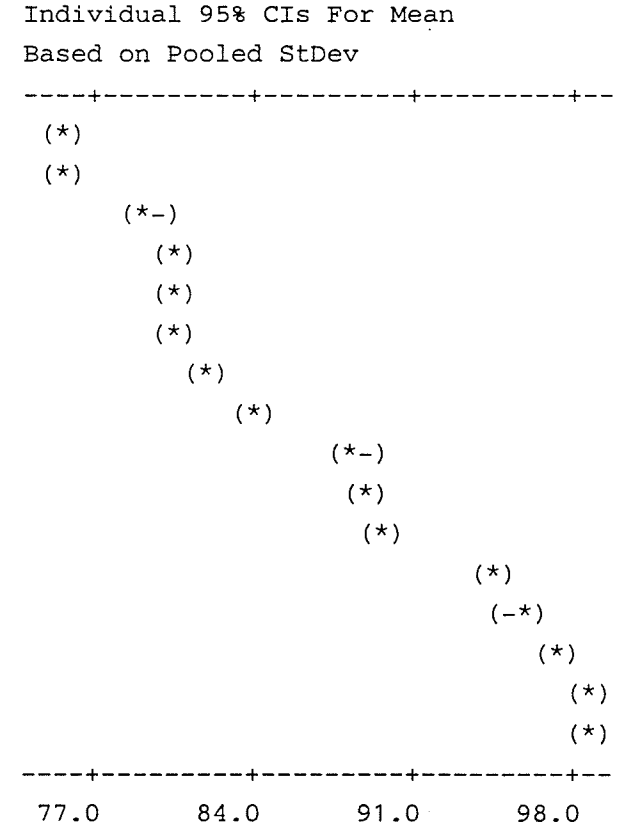


Table 12. Analysis of variance results for ethylcellulose coated microcapsules, and for microcapsules coated in the presence of PIB fractions, comparing the mean amount of drug released after 8.5 hours in pH 7.4 phosphate buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	15	3036.703	202.447	459.26	0.000
Error	32	14.106	0.441		
Total	47	3050.809			

Level	N	Mean	StDev
16CELL5	3	75.440	0.087
22CELL5	3	75.440	0.267
22CELL3	3	79.420	0.410
16CELL4	3	80.480	0.069
16PIB5	3	80.680	0.584
22PIB5	3	80.680	0.330
16PIB4	3	81.810	0.329
22CELL4	3	84.050	0.040
16CELL3	3	88.540	0.135
22CELL2	3	88.750	0.180
16PIB3	3	89.410	0.738
22PIB2	3	94.720	1.819
22PIB3	3	95.583	0.476
22CELL1	3	97.347	0.713
22PIB4	3	98.723	0.431
22PIB1	3	98.863	1.189

Pooled StDev = 0.664



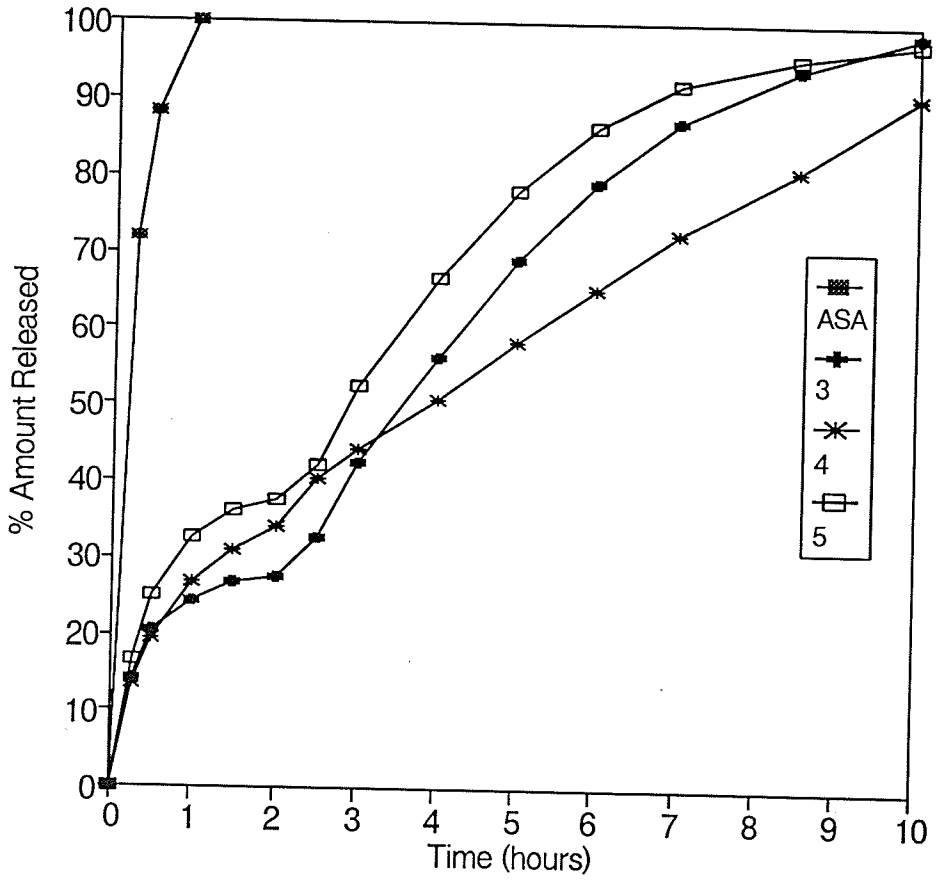


Figure 23. Dissolution profile of enteric coated 12/16 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37° C ± 0.1° C.

Table 13. Analysis of variance results for the enteric coated microcapsules, comparing the mean amount of drug released after 0.25 hours in pH 2.0 buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	7	71.560	10.223	14.69	0.000
Error	16	11.136	0.696		
Total	23	82.697			

Level	N	Mean	StDev
16TERIC4	3	13.480	0.185
16TERIC3	3	14.180	1.287
16TERIC5	3	16.660	0.384
22TERIC5	3	16.690	0.336
22TERIC4	3	17.220	0.453
22TERIC1	3	17.280	0.829
22TERIC3	3	18.380	0.726
22TERIC2	3	18.660	1.483

Pooled StDev = 0.834

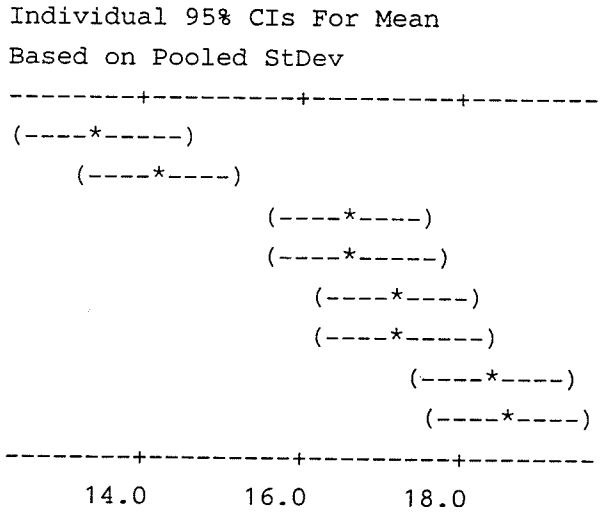


Table 14. Analysis of variance results for the enteric coated microcapsules, comparing the mean amount of drug released after 2.0 hours in pH 2.0 buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	7	453.664	64.809	105.06	0.000
Error	16	9.870	0.617		
Total	23	463.533			

Level	N	Mean	StDev
16TERIC3	3	27.590	1.362
16TERIC4	3	33.910	0.960
22TERIC2	3	34.140	0.185
22TERIC4	3	34.140	1.173
22TERIC3	3	34.570	0.123
22TERIC5	3	37.560	0.507
16TERIC5	3	37.560	0.577
22TERIC1	3	44.050	0.380

Pooled StDev = 0.785

Individual 95% CIs For Mean
Based on Pooled StDev

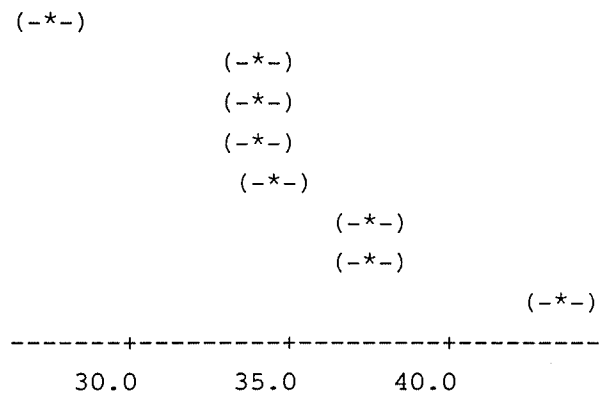


Table 15. Analysis of variance results for the enteric coated microcapsules, comparing the mean amount of drug released after 5.0 hours in pH 7.4 phosphate buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	7	1595.20	227.89	39.21	0.000
Error	16	92.99	5.81		
Total	23	1688.19			

Level	N	Mean	StDev
16TERIC4	3	58.340	0.563
22TERIC4	3	66.180	0.708
16TERIC3	3	69.030	6.710
22TERIC2	3	70.540	0.092
22TERIC3	3	76.270	0.201
22TERIC5	3	77.860	0.208
16TERIC5	3	77.860	0.740
22TERIC1	3	86.810	0.095

Pooled StDev = 2.411

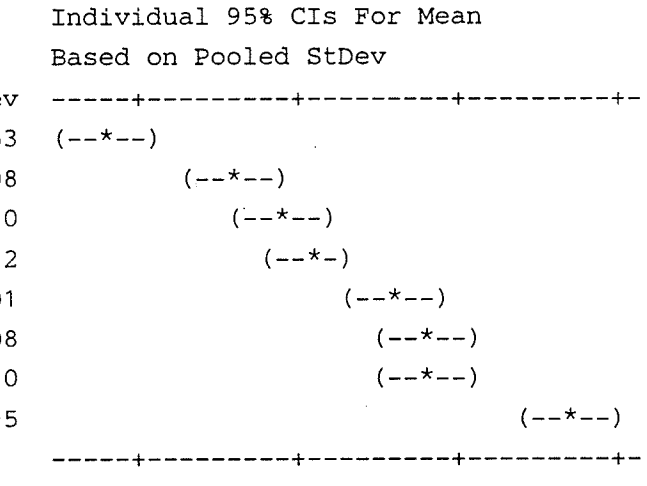
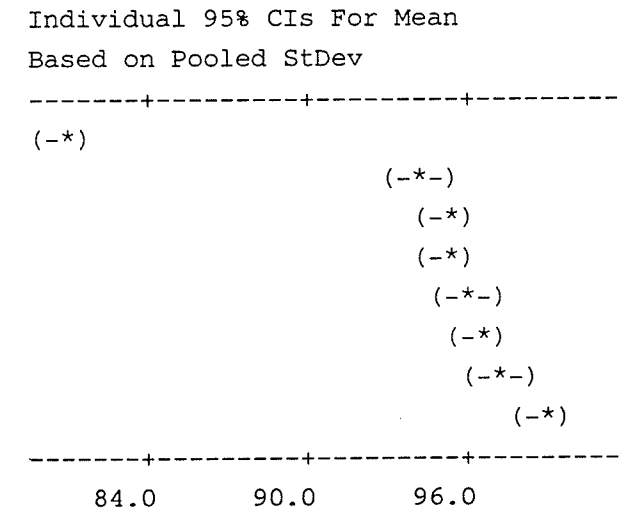


Table 16. Analysis of variance results for the enteric coated microcapsules, comparing the mean amount of drug released after 8.5 hours in pH 7.4 phosphate buffer 37°C ± 0.1°C.

Source	DF	SS	MS	F	p
Factor	7	659.946	94.278	152.50	0.000
Error	16	9.892	0.618		
Total	23	669.838			

Level	N	Mean	StDev
16TERIC4	3	80.850	0.062
16TERIC3	3	94.180	1.025
16TERIC5	3	95.330	0.403
22TERIC5	3	95.330	0.769
22TERIC3	3	96.030	0.075
22TERIC4	3	96.460	0.592
22TERIC2	3	97.230	1.175
22TERIC1	3	98.850	1.183

Pooled StDev = 0.786



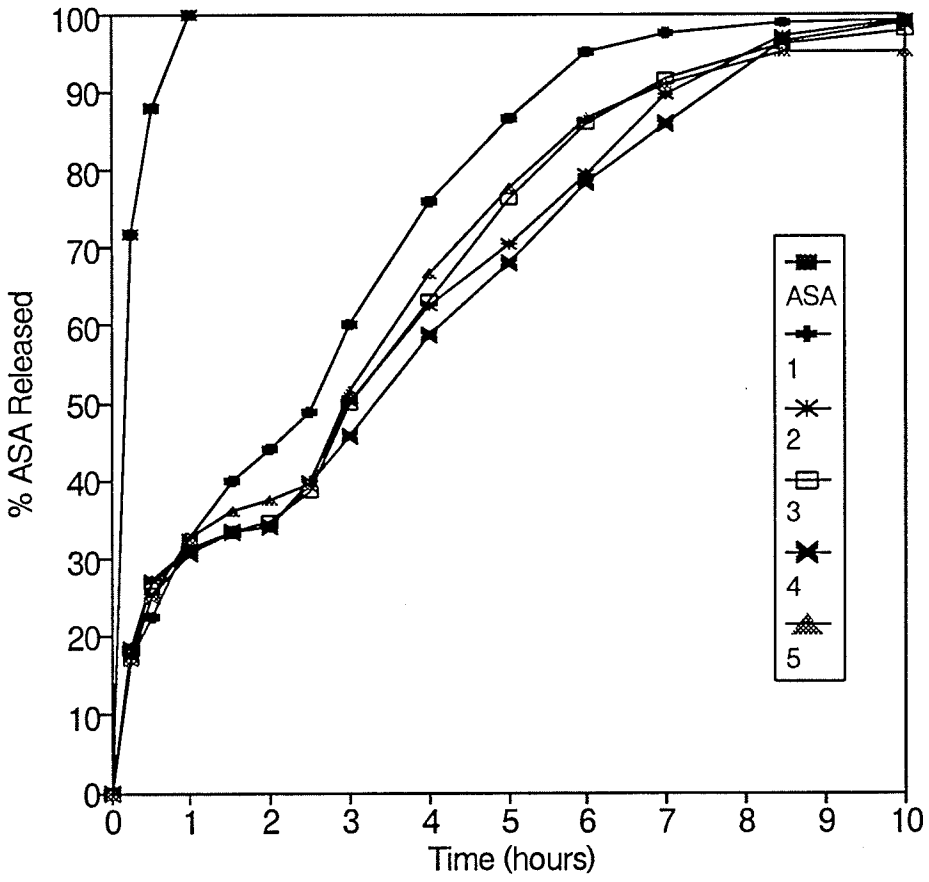


Figure 24. Dissolution profile of enteric coated 16/22 mesh ASA microcapsules showing a plot of percentage ASA released *vs.* time in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

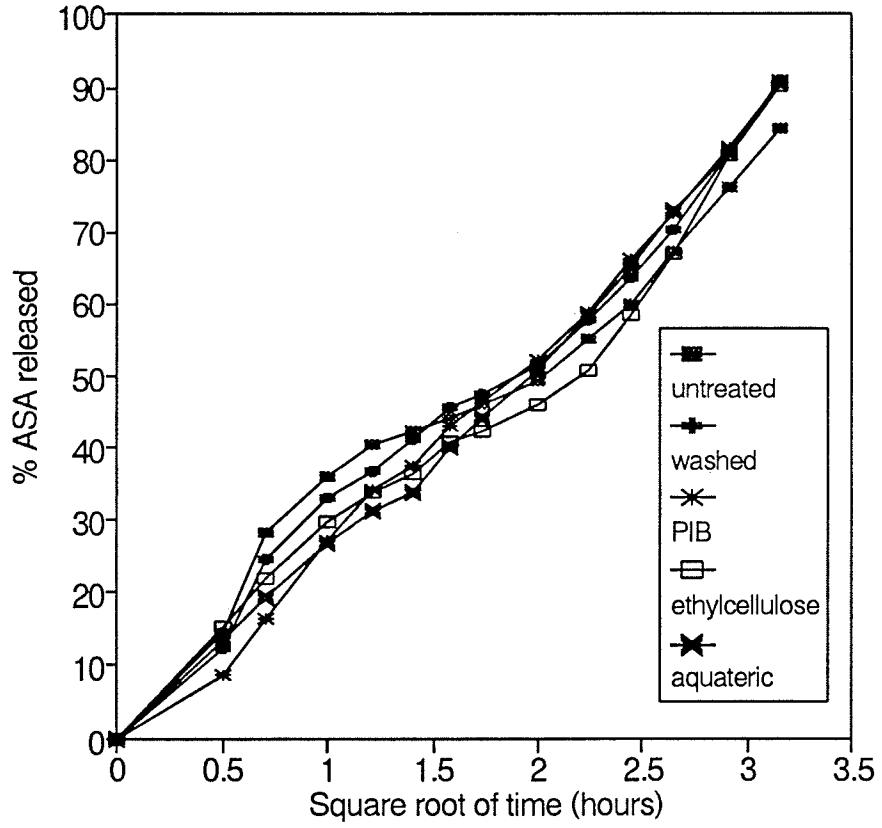


Figure 25. Dissolution profile of formulation 4, the treated and untreated microcapsules, 12/16 mesh showing a plot of percentage ASA released *vs.* square root of time graph in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

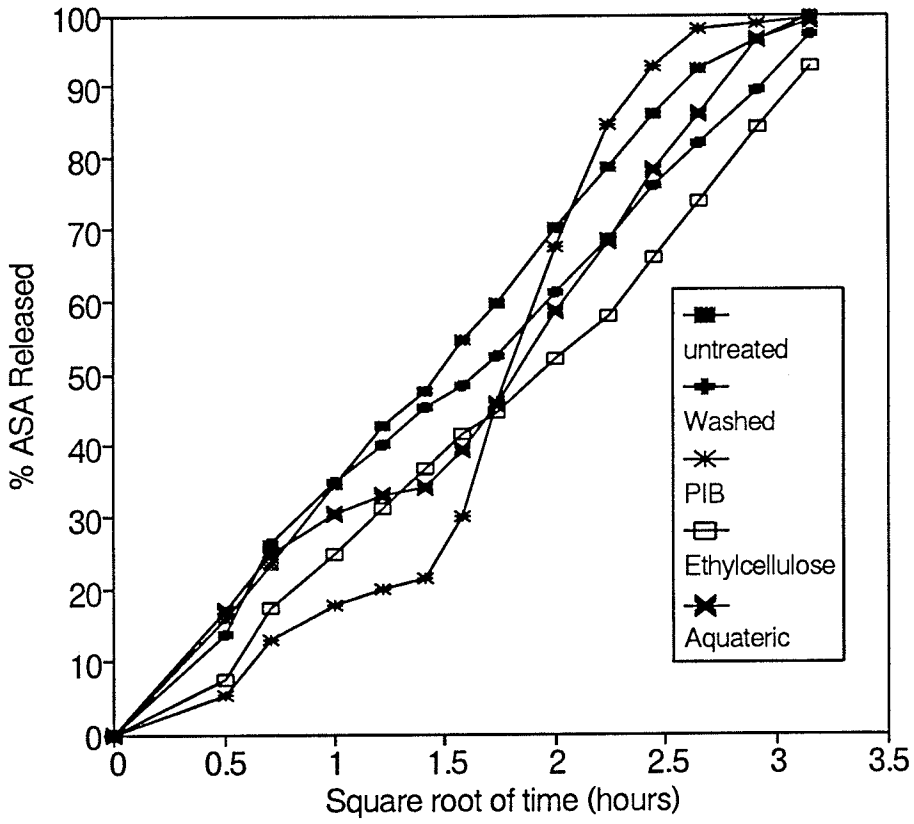


Figure 26. Dissolution profile of formulation 4, the treated and untreated microcapsules, 16/22 mesh showing a plot of percentage ASA released *vs.* square root of time graph in pH 2.0 KCl-HCl buffer for the first 2 hours, and in phosphate 7.4 buffer for the next 8 hours for all the formulations at 37°C ± 0.1°C.

without showing any visible signs of breakage and coat damage. This is a superior property because units fragmenting during coating process is usually a problem, and result in the production of small aggregates that behave as individual sub-unit devices possessing their own release characteristics that differ from the characteristics of the bulk of the microcapsules. The result of which may give different drug release characteristics. More tests would be required to detect the presence of cracks and other flaws that may result from the pellets knocking against each other, and against the vessel walls during the air suspension coating process. These flaws would result in an alteration of the dissolution profile, but could be minimized by an incorporation of a suitable plasticizer material in the microcapsule formulation, or by using a different coating technique. An advantage of microcapsules with a strong physical strength is their ability to withstand further industrial processing, packaging and handling. The drug release characteristics of such pellets would be expected to exhibit a good storage stability.

3.7. ANALYSIS OF VARIANCE

The ANOVA test was done to test the hypothesis (H_o) that the mean % amount of drug of the five formulations and the different mesh sizes released are the same at each time

point. This hypothesis was tested against the alternative hypothesis (H_a) that at least one of the mean % amount of drug released is different. The tests were performed at 95% confidence interval and at the α level of 0.05. The hypotheses are stated as follows;

H_o; $\mu_i - \mu_j = 0$

H_a; $\mu_i - \mu_j \neq 0$

The ANOVA test was done using Minitab release 10.2 statistical programme for time points 0.25 hours showing the early release, 2.0 hours representing the total amount released in the acidic medium, 5.0 hours, which represents the mid-point release in a higher pH medium and 8.5 hours representing late release in the higher pH medium. The ANOVA tables are given below (table 5 to 16), and the results are summarised in Table 17. The null hypothesis is rejected when the F value calculated by the ANOVA exceeds the F value stated in statistical tables [Percentage points of the F distribution (a = 0.10, 0.05, 0.025, 0.01, 0.005 and 0.001)] **(92)**.

3.8. REGRESSION ANALYSIS PARAMETERS

The Y intercept is the point of the graph where the

Table 17. A summary of the ANOVA test results.

Time point (minutes)	GROUP	F value		ACTION
		Observed	Tabled	
0.25 hours	Uncoat	103.80	1.99	Reject H_o
	Coat	201.55	1.99	Reject H_o
	Teric	14.69	2.66	Reject H_o
2.0 hours	Uncoat	482.60	1.99	Reject H_o
	Coat	1119.08	1.99	Reject H_o
	Teric	105.06	2.66	Reject H_o
5.0 hours	Uncoat	2420.42	1.99	Reject H_o
	Coat	716.31	1.99	Reject H_o
	Teric	39.21	2.66	Reject H_o
8.5 hours	Uncoat	339.20	1.99	Reject H_o
	Coat	459.26	1.99	Reject H_o
	Teric	152.50	2.66	Reject H_o

Action = Rejection or acceptance of the null hypothesis.

extrapolated linear part of the graph crosses the y axis for the release in the lower pH (2.0) buffer. The value of the positive Y intercept gives a measure of the magnitude of the burst effect, while the negative Y intercept suggests a lag time. The microcapsule size did not have any significant effect on the burst effect as expected. The relationship between the magnitude of initial burst effect and the microcapsule size was found to be inconsistent, and the results are inconclusive at this point.

R^2 is a unit that measures the validity of the model. In this case it gives a measure of the association of the fraction of drug released *vs.* time or square root of time. When $\mathbf{R^2} = 1$ then good linear relationship exist, and a value of 0 means that there is no linear relationship between the two variables. In experiments, especially where the measured variable is influenced by many factors, and is subject to experimental and instrumental errors, values of $\mathbf{R^2} = 1$ may not be achievable at all times due to experimental difficulties (**87**), and values close to 1.00 may suffice. The correlation of the ethylcellulose coated microcapsules (both size fractions) from the % ASA released *vs.* time plots range from 0.94 to 1.00, indicating good to excellent model representation.

3.9. RELEASE PROFILES

The R^2 values (**see Table 18**) obtained from the regression analysis of the plots are reasonably close to the maximum value of 1, the lowest value being 0.94 observed in the case of formulation 4 which had the highest CAP content (2:4:3 ratio of ASA:CAP:RSPM). When the release profile of formulation 4 was fitted using the Higuchi model, the R^2 values ranged between 0.97 and 1.00 (**Figure 19**). The **n** values calculated from plots of log M vs. log t ranged from 0.81 to 1.3 for the mesh size 12/16 (**Figure 20**), while the range of the **n** values were between 0.82 to 1.3 for microcapsules of 16/22 mesh fraction (**see Figure 21**) which suggests a near zero order release kinetics. The plot of log M vs. log t for formulation 4 were found to be around 0.5 (**Table 22**) suggesting that formulation 4 microcapsules drug release is closer to the matrix release model than it is to zero order release. This observation is in agreement with findings of other CAP based sustained release formulations (**93, 94**).

3.10. ENTERIC PROPERTIES AND RELEASE RATES

All but formulation 4 of the microcapsules demonstrated good enteric properties before and after microcapsule treatment, all formulations demonstrated sustained release properties with release rates ranging from 4 to 11 mg/hour over a period

Table 18. Regression parameters of a plot of % ASA released *vs.* time for the un-treated formulations (3 to 5) for the 12/16 mesh fraction in pH 7.4 buffer.

FORMULATIONS / TREATMENT		Regression parameters and mesh sizes							
		Y intercept		R^2		Slope		$T_{95\%}$	
		12/16	16/22	12/16	16/22	12/16	16/22	12/16	16/22
1	Un-coated	--	47.17	--	0.98	--	8.41	--	5.8
	Washed	--	35.24	--	0.99	--	9.46	--	6.8
	PIB	--	24.28	--	0.96	--	6.88	--	7.5
	Ethylcell	--	20.51	--	0.95	--	8.14	--	8.5
	Enteric	--	16.76	--	0.98	--	13.31	--	6.0
2	Un-coated	--	29.25	--	0.98	--	9.51	--	7.9
	Washed	--	24.93	--	0.95	--	13.09	--	7.0
	PIB	--	15.05	--	0.98	--	11.82	--	8.5
	Ethylcell	--	15.32	--	0.99	--	10.08	--	9.5
	Enteric	--	25.81	--	0.97	--	9.86	--	7.8

3	Un-coated	27.65	28.88	0.98	0.99	8.29	12.41	10.0	8.6
	Washed	12.12	26.99	0.99	0.98	10.95	8.45	9.0	8.6
	PIB	16.97	22.76	0.97	0.96	10.34	11.32	10.0	8.5
	Ethylcell	15.31	14.90	0.99	0.99	10.74	8.29	9.5	10.0+
	Enteric	18.78	25.03	0.98	0.98	9.35	12.03	10.0	7.9
4	Un-coated	24.96	16.63	0.99	0.94	5.38	6.67	10.0+	7.9
	Washed	20.59	21.08	1.00	0.99	6.12	6.64	10.0+	9.5
	PIB	11.52	11.21	1.00	0.95	6.53	15.97	10.0+	6.5
	Ethylcell	23.19	11.72	0.98	1.00	6.72	7.04	10.0+	10.0+
	Enteric	11.80	23.29	1.00	0.99	7.00	10.57	10.0+	7.9
5	Un-coated	38.58	38.38	0.99	0.99	4.74	6.71	10.0+	8.4
	Washed	21.66	34.03	1.00	0.99	6.24	6.62	10.0+	10.0+
	PIB	22.76	31.18	1.00	0.99	7.73	6.63	10.0+	10.0+
	Ethylcell	28.21	21.67	0.99	0.99	6.76	6.76	10.0+	10.0+
	Enteric	22.64	22.64	0.99	0.96	11.11	11.54	10.0	8.4

Table 19. Correlation values (R^2) obtained from the plot of the fraction of drug released *vs.* square root of time for formulation 4 microcapsules.

MESH SIZE	CORRELATIONS				
	Untreated	Washed	PIB	Ethylcell	Aquateric^R
16	0.97	0.98	1.00	0.97	0.99
22	0.99	1.00	0.92	0.99	0.97

Table 20. Values of **n.** calculated from plots of log M *vs.* log t, (in pH 7.4 buffer) for testing the release kinetics of the size 12/16 mesh microcapsules according to **equation 5.**

TREATMENTS	FORMULATIONS		
	3	4	5
Un-treated	1.2	1.2	0.97
Washed	1.3	0.91	1.0
Polyisobutylene	1.0	0.89	1.1
Ethylcellulose	1.2	0.94	1.3
Enteric	0.99	0.81	0.9

Table 21. Values of **n.** calculated from plots of log M *vs.* log t, (in pH 7.4) buffer for testing the release kinetics of the size 16/22 mesh microcapsules according to **equation 5.**

TREATMENTS	FORMULATIONS				
	1	2	3	4	5
Un-treated	1.0	0.87	1.3	0.87	1.2
Washed	1.0	0.95	1.0	1.0	1.3
Polyisobutylene	1.2	0.98	1.2	0.82	1.3
Ethylcellulose	1.2	1.2	1.2	0.93	1.3
Enteric	1.1	0.90	1.0	0.97	1.3

Table 22. Values of **n.** calculated from plots of log M *vs.* log t, for the entire release period in both the pH 2.0 and pH 7.4 buffer solution for testing the release kinetics of the formulation 4 microcapsules according to **equation 5.**

TREATMENT	MESH SIZE	
	12/16	16/22
Untreated	0.41	0.51
Washed	0.47	0.48
PIB	0.56	0.73
Ethylcellulose	0.44	0.62
Enteric	0.51	0.49

extending from 7.5 to over 10 hours. The release rates are represented by the slopes of the graphs shown in Tables 5 through 14. Although there has not been any report on microencapsulation of ASA with a mixture of CAP and methacrylate Eudragit RSPM, the expectation was that given the pH dependence release of CAP (**91**) the formulation that contained a higher CAP content will show the best enteric release properties. On the contrary, formulation 3 containing equal amounts of CAP and Eudragit RSPM at the ratio of 2:3:3 ASA:CAP:RSPM indicated the best enteric release properties, and not formulation 4 which had a higher CAP content. Formulation 5 containing ASA:CAP:RSPM at the ratio of 2:3:4 followed by formulation 4 exhibited the best sustained released properties, with the time taken by the microcapsules to release 95% of the drug ($T_{95\%}$) being over 10 hours for size 16 mesh, and around 8 to 10 hours in the case of size 22 mesh fractions.

CHAPTER IV

CONCLUSIONS

1- The results of this study show that, acetyl salicylic acid can be microecapsulated using the described method and excipient to produce microcapsules that are spheroidal in shape, and have a good flow. These microcapsules are suitable for filling in hard gelatin capsules to produce sustained release unit doses of 100 mg ASA.

2- The microcapsules were found to extend and release the drug in a controlled predictable manner as shown by the coefficient (R^2), that show the strong association between the drug release and the plot of % drug release *vs.* time, and the plots of % release *vs.* time (or SQRT of time) for all the formulations.

3- Varying the polymer:polymer (eudragit RSPM:CAP) or total polymer:drug (eudragit RSPM + CAP:ASA) ratio results in altered drug release rate and kinetics as indicated by formulation 4 containing ASA:CAP:RSPM at the ratios 2:4:3 which followed the Higuchi's drug release model, and formulation 1 containing CAP:RSPM:ASA at the ratio of 1:1:2 which

releases the drug at comparatively higher rates, and does not appear to follow the near zero order, or square root of time release models.

4- Increasing the CAP content beyond 44.4% resulted in elongated microcapsules (**see Fig. 11**).

5- Higher eudragit RSPM values of up to 44.4% results in better drug release rate control, with a release model that best approaches zero order. With eudragit RSPM content higher than 44.4%, microencapsulation could not be achieved due to problems of shock, and a production of large particles that coalesced together resulted.

6- Increasing the drug load resulted in the production of more spherical microcapsules of smaller mesh size, which released drugs at higher rates as indicated by formulations 1 and 2 containing 50% and 25% drug load respectively. Higher microcapsule yields also results.

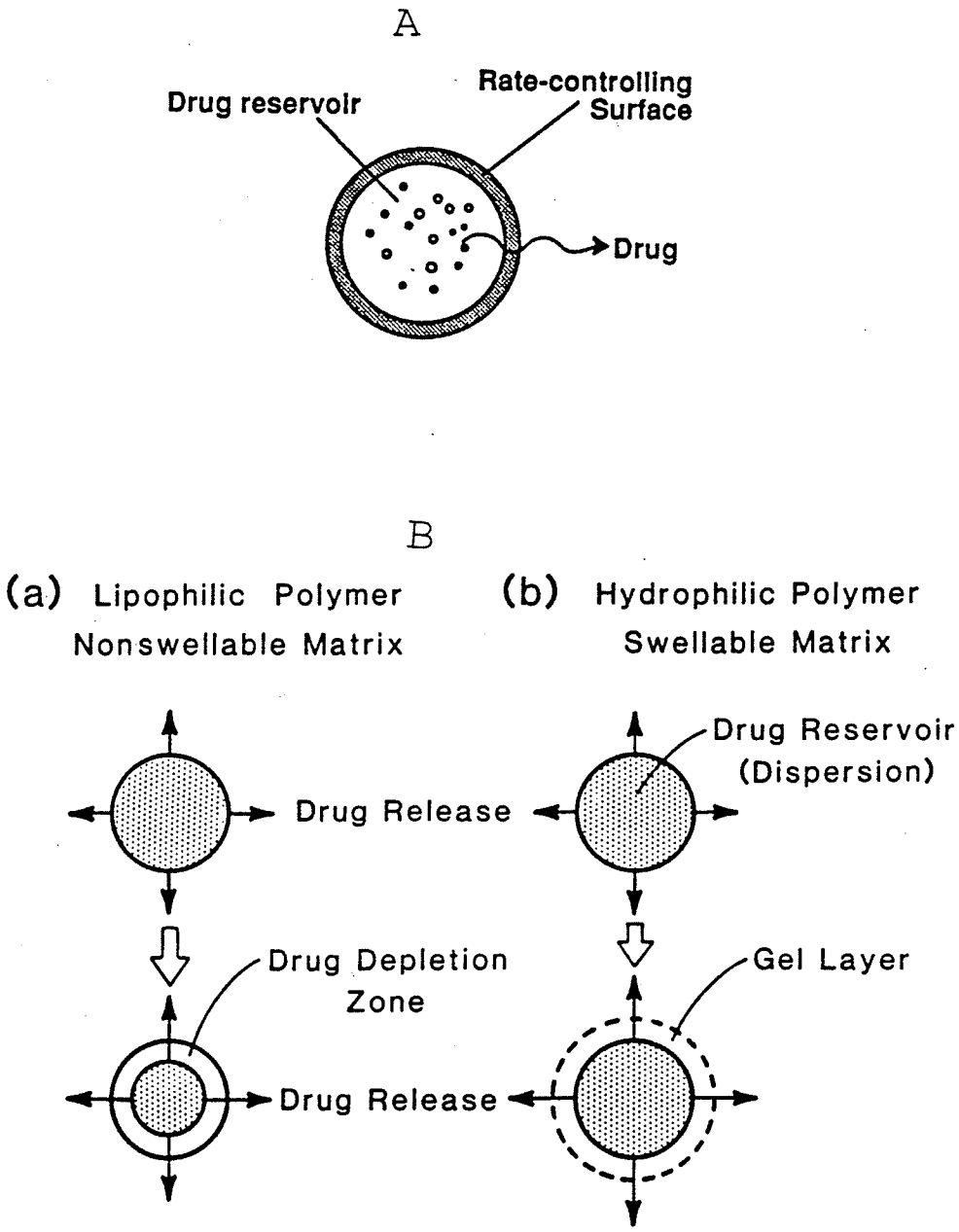
7- The microcapsules did not lose their discreteness when subjected to the air suspension a further phase separation coating procedures.

8- All formulations exhibited an initial drug burst effect that could be reduced by cyclohexane washing, or by further coating of the microcapsules.

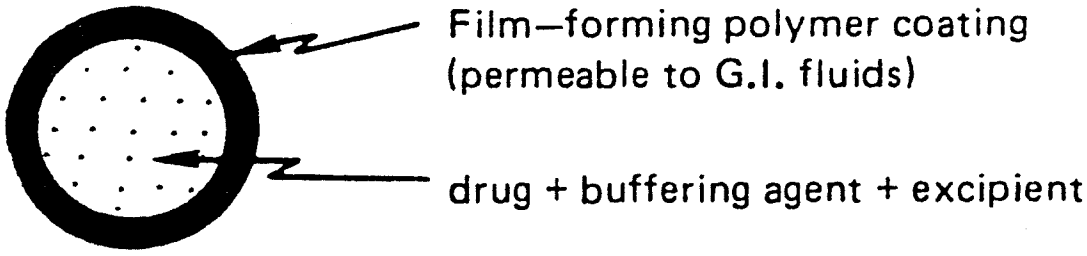
9- Further coating with ethylcellulose resulted in a notable modulation of the drug release rates kinetics, and consistency in all the formulations.

10- The results of the ANOVA test indicate that the null hypothesis (H_o) that the means of the formulations in each group are the same is not valid, even under the lowest probability value (NB. the calculated P = 0.00 in all the cases). Based on these results, the null hypothesis is rejected in favour of the alternative hypothesis (H_a) that at least one of the means is different, and the differences in mean are not due to chance at the confidence interval of 95%.

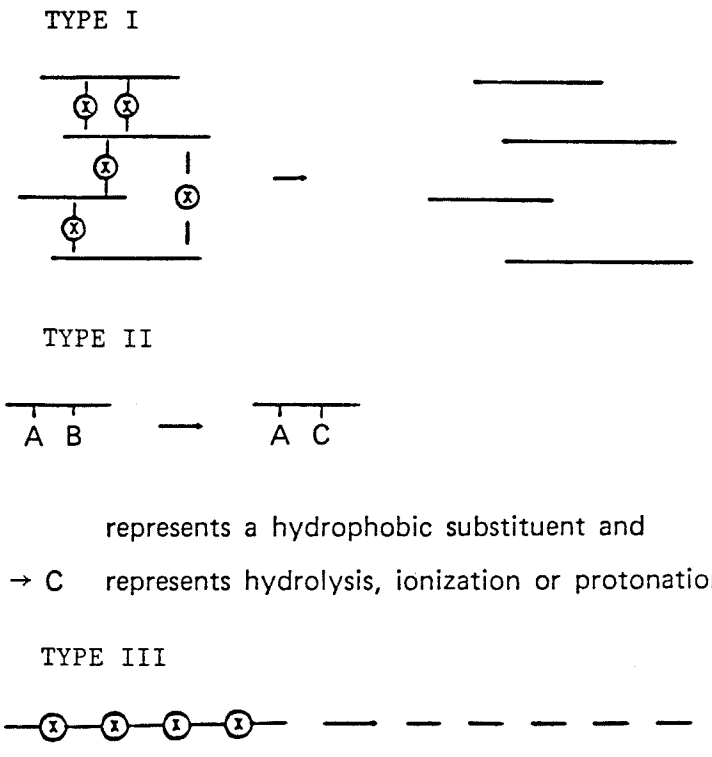
APPENDIX



Appendix 1. A representation of (A) a reservoir device with the rate controlling surface and (B) a matrix device showing the drug release by diffusion through a drug depletion zone, or through a gel layer.

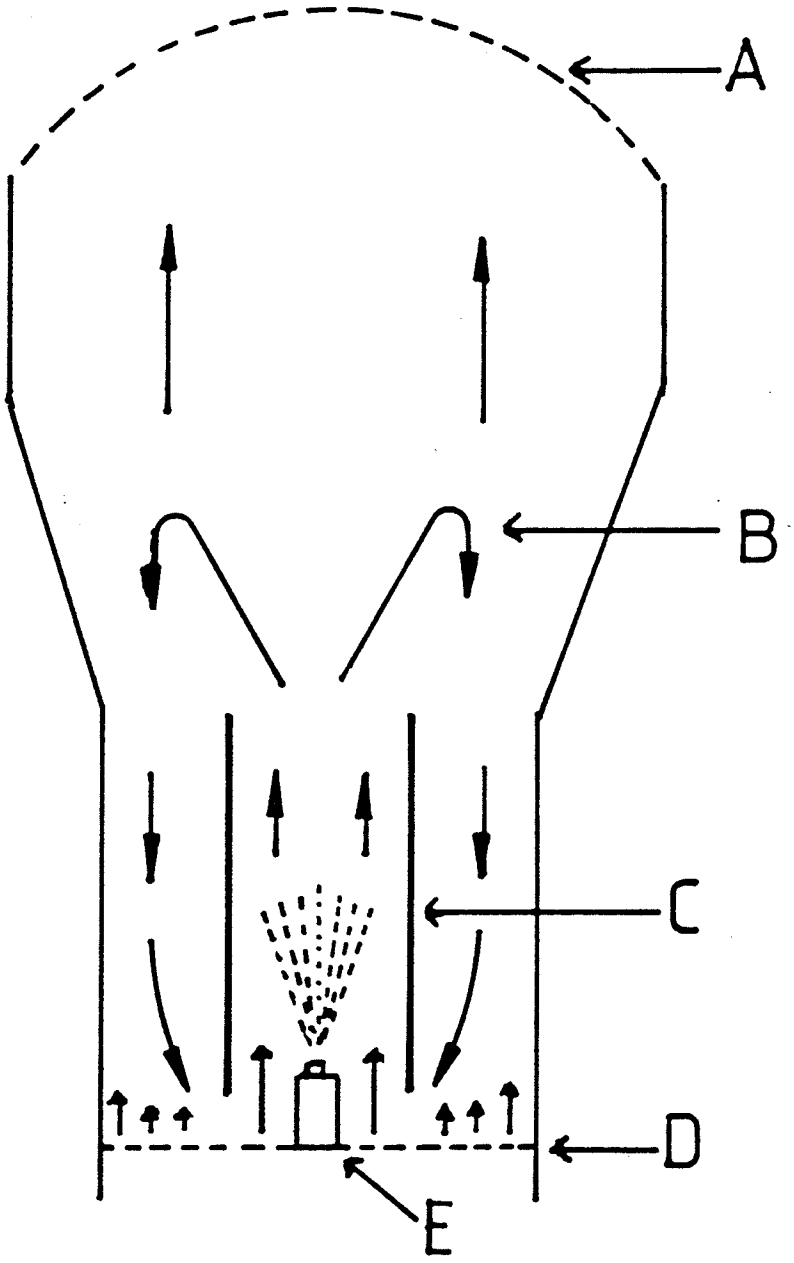


Appendix 2. A sketch of a pH-controlled oral drug delivery device showing the presence of buffering agents used to control drug release.



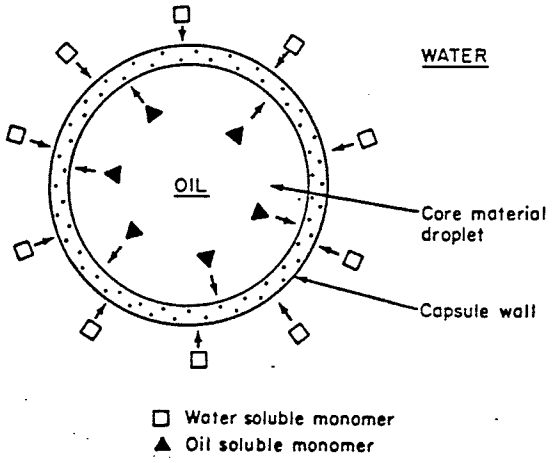
Appendix 3. A schematic representation of polymer erosion showing three different polymer erosion methods. Type I method occurs with cross-linked water soluble polymers. Type II method occurs with initially water insoluble polymers solubilised by hydrolysis, ionization or protonation of pendant group. Type III method occurs by solubilization of small water soluble molecules of hydrophobic polymers. (from Ref. 95).

Appendix 4. A micrograph of a mucoadhessive coated pellet prepared from p-HEMA hydrogel in a swollen state.

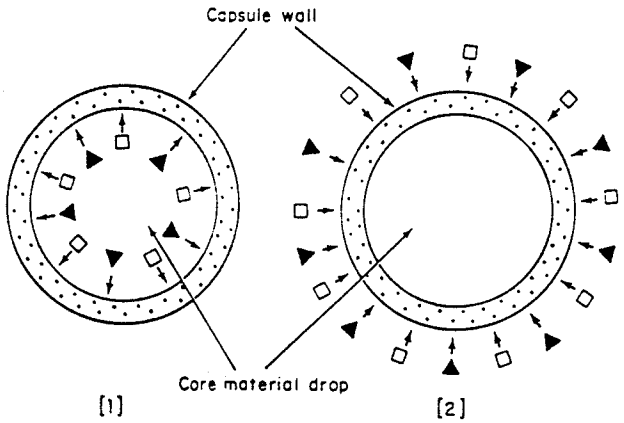


Appendix 5. A schematic diagram of an air suspension coater. A, filter bags; B, particle and air flow; C, cylindrical partition; D, air distribution plates; E, atomizer.

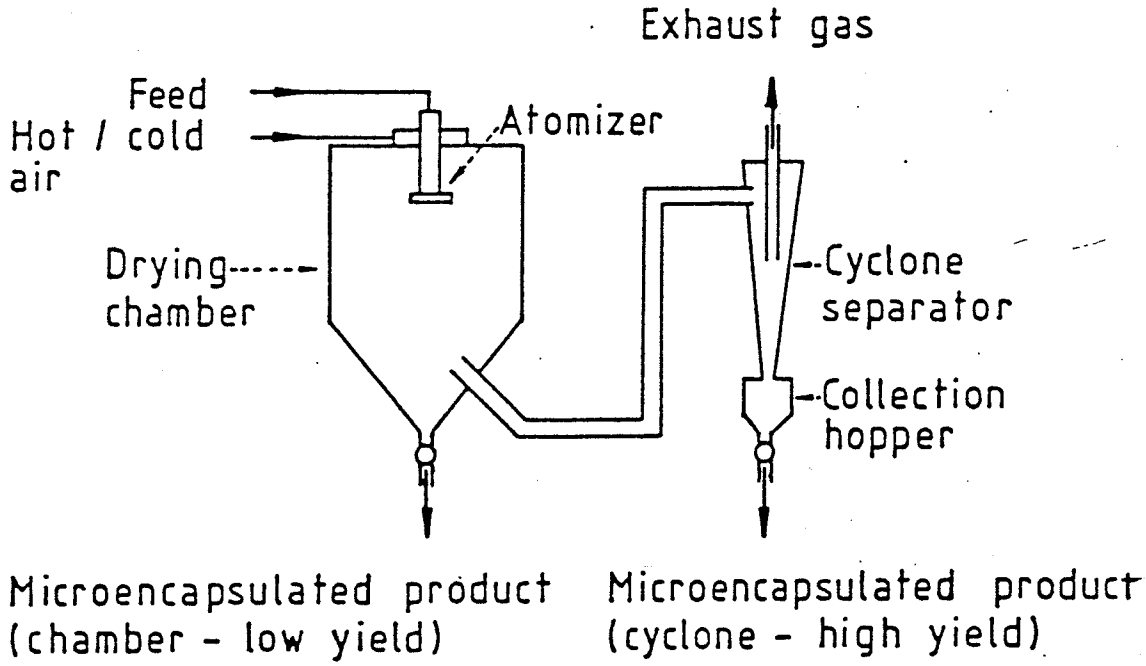
A



B



Appendix 6. A schematic diagram of the interfacial polymerization technique (A). Schematic diagrams of the *in-situ* polymerization (B).



Appendix 7. A diagram of a cocurrent spray dryer.

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