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THE UNIVERSITY OF ASTON IN BIRMINGHAM

MICROENCAPSULATION STUDIES WITH P(HB-HV) POLYMERS

JONATHAN KENNETH EMBLETON

MICROENCAPSULATION STUDIES WITH P(HB-HV) POLYMERS.

SUMMARY

Microencapsulation processes based upon the concept of solvent evaporation, have been considered within three studies to prepare microcapsules from poly-β-hydroxybutyrate-co-valerate (P(HB-HV)) polymers. The first study has facilitated the fabrication of the electrically charged hollow microcapsules. Thus, monolithic microcapsules and reservoir-type microcapsules have been respectively fabricated by single and double emulsion solvent evaporation processes.

JONATHAN KENNETH EMBLETON

Doctor of Philosophy

The objective of this study is to assess how a range of microcapsule morphologies can be achieved and how the morphology of P(HB-HV) microcapsules. The following chapter describes how microcapsule morphology in general, and microcapsule morphology in particular, can be regulated by blending the polymer with poly-ε-caprolactone (PCL). One revelation of this study is that porous microcapsules can be fabricated from P(HB-HV) polymers with a low molecular weight. The reason for this is that the porous microcapsules are formed because they may be formed via an evaporation process.

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October 1991

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JONATHAN KENNETH EMBLETON

Submitted for the degree of Doctor of Philosophy, October 1991

SUMMARY

Microencapsulation processes, based upon the concept of solvent evaporation, have been employed within these studies to prepare microparticles from poly- β -hydroxybutyrate homopolymers and copolymers thereof with 3-hydroxyvalerate [P(HB-HV) polymers]. Variations in the preparative technique have facilitated the manufacture of two structurally distinct forms of microparticle. Thus, monolithic microspheres and reservoir-type microcapsules have been respectively fabricated by single and double emulsion-solvent evaporation processes.

The objective of the studies reported in chapter three is to assess how a range of preparative variables affect the yield, shape and surface morphology of P(HB-HV) microcapsules. The following chapter then describes how microcapsule morphology in general, and microcapsule porosity in particular, can be regulated by blending the fabricating P(HB-HV) polymer with poly- ϵ -caprolactone [PCL]. One revelation of these studies is the ability to generate uniformly microporous microcapsules from blends of various high molecular weight P(HB-HV) polymers with a low molecular weight form of PCL. These microcapsules are of particular interest because they may have the potential to facilitate the release of an encapsulated macromolecule via an aqueous diffusion mechanism which is not reliant on polymer degradation. In order to investigate this possibility, one such formulation is used in chapter five to encapsulate a wide range of different macromolecules, whose *in vitro* release behaviour is subsequently evaluated.

The studies reported in chapter six centre on the preparation and characterization of hydrocortisone-loaded microspheres, prepared from a range of P(HB-HV) polymers, using a single emulsion-solvent evaporation process. In this chapter, the influence of the organic phase viscosity on the efficiency of drug encapsulation is the focus of initial investigations. Thereafter, it is shown how the strategies previously adopted for the regulation of microcapsule morphology can also be applied to single emulsion systems, with profound implications for the rate of drug release.

Keywords : microencapsulation, microcapsule, microsphere, poly- β -hydroxybutyrate / poly-3-hydroxyvalerate polymers, drug delivery devices.

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*to my Mother, my Father,
and
the memory of their Mothers*

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PLA	poly(l-lactide)
PCL	poly(ϵ -caprolactone)
PLGA	poly(D,L-lactide/glycolide)
PCL II	poly(ϵ -caprolactone)
PHB	poly(β -hydroxybutyrate)
PHB-HV	poly(β -hydroxybutyrate homopolymers and / or copolymers thereof with 3-hydroxyvalerate)
PLA	poly(lactide acid)
PCL	poly(ϵ -caprolactone)
PCL II	poly(ϵ -caprolactone)
PVA	poly(vinyl alcohol)
RFS	reticular fibrous system
rpm	revolutions per minute
SEM	scanning electron microscopy

LIST OF ABBREVIATIONS.

BSA	bovine serum albumin
DCM	dichloromethane [methylene chloride]
FITC	fluorescein isothiocyanate
FM	fluorescence microscopy
GPC	gel permeation chromatography
HV	3-hydroxyvalerate
HC	hydrocortisone
M_n	number-average molecular weight
M_w	weight-average molecular weight
o/w	oil-in-water
PBS	phosphate buffered saline
PCL	poly- ϵ -caprolactone
PGA	poly(glycolic acid)
PHB	poly- β -hydroxybutyrate
P(HB-HV) polymers	poly- β -hydroxybutyrate homopolymers and / or copolymers thereof with 3-hydroxyvalerate.
PLA	poly(lactic acid)
poly (α, β esters)	poly(alpha esters) and / or poly(beta esters)
PVA	poly(vinyl alcohol)
RES	reticuloendothelial system
rpm	revolutions per minute
SEM	scanning electron microscopy

T1-T4	the temperature treatment used for the preparation of microparticles by an emulsion-solvent evaporation process [see section 2.2.4].
TCM	trichloromethane [chloroform]
T _g	glass transition temperature
w/o	water-in-oil
w/o/w	water-in-oil-in-water

CHAPTER ONE

INTRODUCTION AND LITERATURE SURVEY

1.1. The products of microencapsulation processes.

Articles of manufacture by the process of microencapsulation can be collectively described as colloidal carriers¹, and include nanoparticles [e.g. 2,3,4], microparticles and liposomes [e.g. 5]. The latter are microscopic lipid vesicles composed of phospholipid bilayers, and can serve as carriers for both hydrophilic and hydrophobic species⁶. Although liposomes can offer the advantages of being biodegradable and non-toxic⁷, and have received considerable commercial interest⁸, they present significant control and stability problems and are not well suited to long term release applications^{9,10}. These problems are not to particulate systems. Thus,

CHAPTER ONE.

INTRODUCTION AND LITERATURE SURVEY.

In contrast microparticles are frequently spherical particulate devices, with diameters in the 1-100µm range, and are usually classified as either microspheres or microcapsules. However, an obvious problem which becomes apparent when reviewing the microencapsulation literature is that there are varying definitions of the terms microcapsule and microsphere. Thus, the terminology adopted by Davis and Illum¹⁰ embodies the word microsphere in relation to a solidly uniform [i.e. monolithic] polymer particle, whereas microcapsule is the label they apply to a device which consists of a non-polymer core surrounded by a polymer membrane. Likewise, in the work of Chou and Pitt¹¹, a microsphere is described as a monolithic polymer particle containing randomly distributed drug, whilst drug crystals that are enclosed within a polymer shell constitute a microcapsule. Similar uses of the term microsphere are adopted by many other researchers including Dubernet *et al.*¹², Bodamer and McGinity¹³, Saito *et al.*⁹, Fong *et al.*¹⁴, Spentlove *et al.*¹⁵, Benita

1.1. The products of microencapsulation processes.

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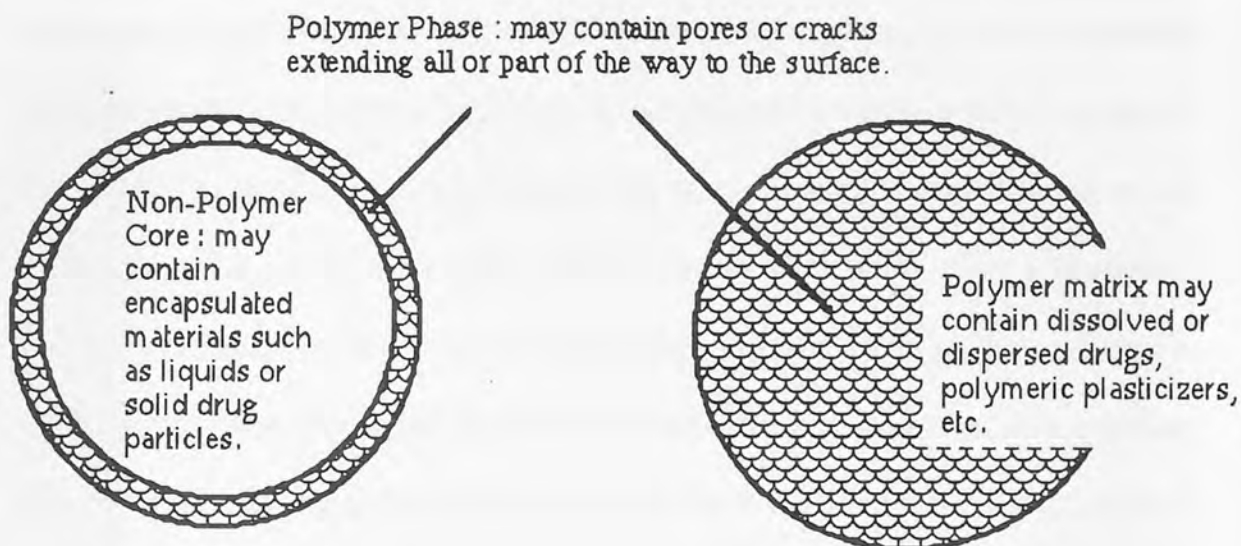
*et al.*¹⁶ and Cavalier *et al.*¹⁷. However, Ogawa *et al.*¹⁸ describe monolithic particles as microcapsules, but closer examination of their work reveals that they are actually referring to reservoir-type devices with an aqueous-based centre surrounded by a polymer membrane. Moreover, Bodmeier *et al.*¹⁹ describe the preparation of both monolithic polymer particles and reservoir-type devices, but apply the term microsphere to each type of particle. In contrast, Tsai *et al.*²⁰ refer to monolithic polymer particles containing randomly distributed hydrocortisone as microcapsules, whereas Maulding²¹ uses the two terms synonymously.

In this thesis, a microcapsule is defined as a reservoir-type particulate device which consists of a non-polymer core surrounded by a polymer membrane. The core may be for example an aqueous solution or a mass of solid drug. Conversely, a microsphere is defined as a monolithic polymer particle, which may contain other substances [such as polymeric plasticizers or drug particles] dispersed throughout the polymer bulk.

Although, as Davis and Illum emphasize, strictly speaking microspheres should also be entirely solid in nature¹⁰, this is not usually the case in practice. Indeed, the microspheres described in the literature, which have been prepared from polyesters by single emulsion-solvent evaporation processes, are sometimes porous with honeycombed interiors¹⁴. Diagrams 1 (a) and (b) illustrate the difference in structure between the two types of microparticle, whilst figures 1 (a) and (b) are respectively scanning electron micrographs of a sectioned microcapsule and a sectioned microsphere [prepared from P(HB-HV) polymers using the procedures described in chapter two].

Diagram 1.1. An illustration showing the difference in structure between :

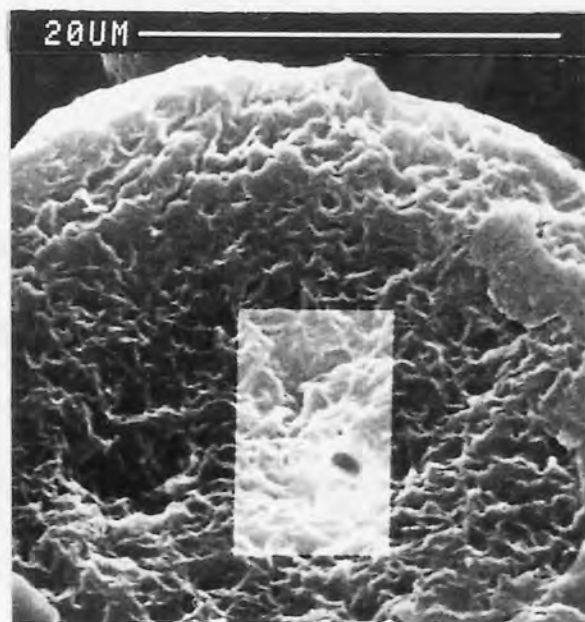
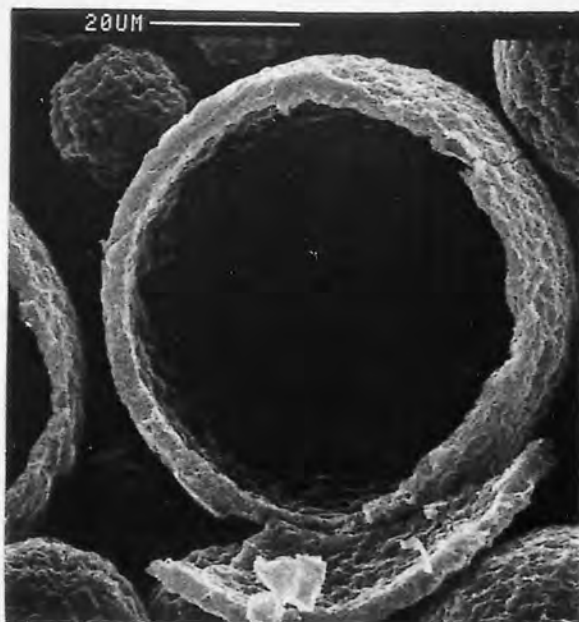
(a) a reservoir-type microcapsule ; and (b), a monolithic microsphere.



A. Reservoir-Type Microcapsule

B. Monolithic Microsphere

Figure 1.1. Scanning electron micrographs of : (a) a full section through a reservoir-type microcapsule, prepared from a 43 000 molecular weight PHB homopolymer [43PHB] by a double emulsion-solvent evaporation process ; and (b) a top section through a monolithic microsphere, prepared from 43PHB by an o/w single emulsion solvent evaporation process.



Of the many different colloidal carrier systems, the forthcoming literature review, and the remaining work in this thesis, are concerned only with microparticles [microcapsules and microspheres]. In addition, particular emphasis is directed towards microencapsulation processes which utilize either poly(alpha esters) or poly(beta esters) [collectively termed poly(α,β esters)] as the fabricating polymers. Included in the former group are poly(glycolic acid) [PGA] and poly(lactic acid) [PLA]. However, due to the presence of a chiral centre, there are three possible forms of PLA, which are those deriving from the optically active D(-) and L(+) forms of the parent acid, together with the racemic D,L form. These are respectively abbreviated to P(D-LA), P(L-LA) and P(D,L-LA) in this thesis, although P(D-LA) is rarely featured, because it is the L form of lactic acid that is a normal mammalian metabolite.

Poly(beta esters) include poly- ϵ -caprolactone [PCL], poly- β -hydroxybutyrate [PHB], and copolymers of the latter with 3-hydroxyvalerate [HV]. The abbreviation P(HB-HV) polymers is widely used in this thesis, and envelops both PHB homopolymers and the copolymers with HV.

1.2. Applications of microencapsulation.

Over the last twenty years, microencapsulation has found applications in a multitude of industries ranging from aerospace ²², computing ²³, horticulture ²⁴ photography ²⁵, and printing ²⁶, to healthcare - its apparent forte. Within the latter field, there are many distinct niches for the application of microencapsulation. For example, microencapsulated cells or microcells have an important role in the development of implantable systems that may be effective in the treatment of diseases requiring organ transplantation, such as diabetes. Thus, a recent patent filed in Canada ²⁷ describes a

process for the microencapsulation of Islet cells within a semipermeable polylysine membrane. The patent claims that the polymer is well tolerated *in vivo* , and immunoglobins are unable to traverse the membrane, with the result that the system can survive for a considerable period of time. Additionally, since the microencapsulated cells bath in a physiologically-acceptable medium, they remain viable and capable of ongoing metabolism. A similar formulation, administered to diabetic rats via intraperitoneal injection, was found to maintain normal blood glucose levels for up to a year ²⁸.

An additional application of microencapsulation in the treatment of organ failure and similar conditions [e.g. drug overdose] lies in the area of hemoperfusion. Hemoperfusion systems are utilized *in vitro* , and typically consist of columns packed with microparticles through which the blood of an overdosed or hepatically compromised patient flows. The microparticles consist of an active substance, such as activated charcoal, which adsorbs the toxic molecules from the blood and is coated with an ultrathin, microporous, biocompatible polymer membrane. This polymer membrane does not cover the surface pores of the charcoal granules, with the result that the rate of adsorption of toxic molecules is not compromised. The coating, however, forms a blood compatible surface which reduces the likelihood of emboli ²⁸.

However, by far the most important role for microencapsulation in the healthcare industry is as a drug delivery technology. Drug delivery is a headline term that embodies a number of separate, though closely related, modalities. Principally these are controlled drug release and drug targeting, although in practice drug delivery systems may be required to meet both objectives. The following two sections [1.2.1 and 1.2.2] briefly review these two aspects of drug delivery, starting with controlled release.

This section opens with a general overview of the scope and purpose of controlled release technology with regard to conventional drugs, and then outlines the advantages which microparticulate systems offer in relation to other forms of device. Included in this section are definitions of the terms biodegradation and bioerosion, together with a list of some of the more popular biodegradable polymers used to fabricate controlled release devices. This section then concludes by looking at the opportunities and problems associated with the development of polymeric controlled release devices for macromolecular drugs.

The following section [1.2.2] examines the role of microparticles as devices for targeted drug delivery. Initial attention in this section is directed towards simple systems where the particles are administered directly to the target areas. Thereafter the concept of 'site-specific drug delivery' is introduced, which is then discussed in relation to both orally administered particles and those injected directly into the bloodstream.

1.2.1. Controlled drug release.

The ongoing efforts of the pharmaceutical industry to develop extended release formulations for conventional drugs is in part a reflection of the long time-scale, the high cost and the enormous risk that are associated with the development of new drug molecules. Indeed, it can take up to 10 years and US\$50m⁷⁵ to bring a new drug to the market, with most of this time and expense being consumed during the advanced stages of clinical trials. The development of more durable formulations, which can deliver existing drugs over extended periods, is widely seen as a more cost-effective, risk averse product strategy because of the possibilities offered for reducing the scale of official testing and extending the patent life^{29, 30}. Extended release is typically referred

to as either sustained or controlled release in the literature, and in this thesis no distinction is made between these terms.

The advantages of having a drug released for an extended period in a controlled manner are easily appreciated by comparing the delivery profiles from controlled release and conventional dosage forms. Thus, instead of the peak-and-valley drug concentrations associated with conventional administration, whereby the initial doses are often inadequate to reach the therapeutic range, but the high concentrations associated with later dosage may lead to dangerous side effects, controlled release products offer far more idealized release profiles. Indeed, the formulation of potentially toxic pharmaceuticals into controlled release dosage forms may facilitate the maintenance of a constant plasma drug level that is within the therapeutic range yet too low to cause toxicity problems. In this way the total amount of absorbed drug can be lowered and the efficiency of the treatment can be increased. Clearly therefore, controlled release formulations are particularly desirable for drugs that exhibit bioavailability problems and have toxic side-effects such as nitrofurantoin ¹², and those, such as L-methadone, that are associated with poor patient compliance as a result of the need for regular administration and frequent clinical visits ¹¹.

Initial investigations into controlled release polymeric devices focused on implantable systems prepared from highly permeable [at least to low molecular weight hydrophobic molecules], but non-biodegradable, materials such as silicone rubber [i.e. cross-linked poly(dimethylsiloxane)] ³¹. The first clinically-tested controlled release system was the 'Ocusert' device designed to release pilocarpine into the eye to combat glaucoma, and this was made from the non-biodegradable ethylene-vinyl acetate

copolymer³². However, implants prepared from non-biodegradable materials must be surgically removed once depleted of drug in order to avoid the problems associated with allowing an alien substance to remain indefinitely within the body³³, and this important drawback focused attention onto biodegradable polymers. There are varying definitions of the terms biodegradable and bioerodible in the literature and a selection of these are contained in the review of Holland *et al.*³⁴. In this thesis, biodegradation is taken to refer to hydrolytic, enzymatic or bacteriological degradation processes which do not necessarily change the physical form of the polymer. In contrast, bioerosion implicitly involves physical [i.e. weight] loss from the polymer matrix, although the mechanism by which this occurs need not be clearly defined. These definitions are consistent with those adopted in previous studies within the Speciality Materials Research Group [e.g. ³⁵].

To date, a wide range of biodegradable polymers have been utilized for the fabrication of controlled release devices, and these can be described as either natural or synthetic materials³⁶. Polymers in the former category that have been used to prepare microparticles for controlled drug release include albumin³⁷, fibrinogen³⁸, collagen³⁹ and gelatin [denatured collagen]⁴⁰. However there are several problems, such as uncertain purity, associated with the use of these polymers as medical devices³⁶.

Consequently the favoured materials for the manufacture of controlled release devices at the present time are synthetic biodegradable polymers. The number of different materials within this category is large and includes cross-linked polyester hydrogels⁴¹, surface eroding polymers [such as polyanhydrides^{42, 43} and poly(ortho esters)^{44, 45}] and poly(α, β esters). The latter have been widely utilized for the manufacture of microparticulate controlled release devices and include : poly(glycolic acid)⁹, poly(lactic acid)^{11, 15, 19, 20, 46, 47}, poly(glycolic-co-lactic acid)^{11, 15, 18,}

poly- ϵ -caprolactone ^{12, 48, 49, 50}, poly- β -hydroxybutyrate ⁵¹, and copolymers of the latter with 3-hydroxyvalerate ⁵². The poly(α,β esters) are popular choices for the fabrication of microparticles, and a wide range of other forms of controlled release device, because they offer the twin advantages of biodegradability and favourable biocompatibility [^{34, 53} for detailed reviews].

In relation to other forms of drug delivery device, such as implants, microparticles have gained in popularity over the last decade for several reasons. For parenterally administered systems, these include ease of administration, where injection can replace surgical implantation. Additionally, the products of microencapsulation offer opportunities for targeting to specific body sites [see section 1.2.1]. With regard to oral dosage forms, microparticulate systems distribute more uniformly in the gastrointestinal tract, thereby resulting in a more uniform drug absorption. They also present fewer parochial adverse reactions, and ensure the avoidance of inadvertent intestinal retention on chronic dosing, when compared to single unit dosage forms ^{19, 54}.

Moreover, it is the advantages which microencapsulation offers in the administration of highly toxic anti-cancer drugs which represents one of the technology's most promising uses : chemoembolization. This modality involves the selective arterial administration of microencapsulated anti-cancer drugs. The microparticles occlude the small arterioles in the malignant region close to the injection site and discharge their payloads in a controlled manner. These systems not only offer the benefits of increasing the local drug concentration whilst reducing the general systemic toxicity, but they can also extend the drug-tumour contact time and increase

the permeation of the drug into the tissues by altering the local environment ⁵⁵.

Chemoembolization systems which have presently been developed include PLA microspheres containing either floxuridine ⁵⁵ or mitomycin C ²⁰, and poly- β -hydroxybutyrate-encapsulated aclarubicin ⁵¹.

Whilst controlled release technology was initially conceived in order to improve the delivery of synthetic or conventional drugs, it is now finding a new area of application in the pharmaceutical industry. Thus, in recent years, major advances in genetic engineering and other protein production technologies have led to the commercial availability of numerous naturally occurring, biologically-active proteins and high molecular weight polypeptides with a diverse array of potential therapeutic applications. These "biopharmaceuticals" include interferons, interleukins and colony stimulating factors ; hormones such as serotonin, insulin and human growth hormone ; and a host of others including tissue plasminogen activator and growth factors such as epidermal growth factor ^{58, 61}.

However, these advances have, as yet, largely served to highlight the immense difficulties associated with the delivery of such macromolecules to the target tissues. Thus, oral administration is usually ineffective, either as a result of proteolytic activity in the gastrointestinal tract, or non-absorption through the intestinal wall. Intranasal, buccal, intravaginal and rectal routes of administration are all reported to be associated with a low and variable bioavailability ⁵⁹. In order to overcome these problems, one line of current drug delivery research is concerned with finding ways to enhance the permeability of mucosal surfaces ⁶⁰. Nevertheless, at the present time, parenteral administration is normally the chosen route, but this involves the same drawbacks that

have been mentioned with regard to conventional drugs. These include the need for frequent injections, together with the constant under- and overdosing caused by the inability to maintain constant, idealized, plasma drug concentrations. In addition, protein drugs have only very short elimination half-lives ⁵⁹.

The need to develop practical and effective delivery systems is a critical precursor for exploitation of the full therapeutic and commercial potentials of these macromolecular drugs ; indeed, the market for protein delivery systems has recently been forecast to reach around US\$2 billion annually by 1999 ⁶¹. The key to competitive advantage in situations where companies are competing with similar macromolecules will lie in the product differentiation conferred by the delivery mechanism ⁵⁸.

A diverse array of potential protein delivery systems are the subject of current investigations ⁶¹. In some cases, the delivery mechanism is required merely to afford better internal targeting to the drug molecule ; for example via its conjugation to a monoclonal antibody in an extension of the " magic bullet " idea developed for cancer chemotherapy. The principle objective, however, for many research programs lies in the development of controlled release formulations whereby the protein or polypeptide is protected from its environment by a rate-controlling matrix or membrane whose properties determine the kinetic profile and mechanism of macromolecular release.

Controlled release from a polymeric matrix is far more difficult to achieve with macromolecules, such as proteins and high molecular weight polypeptides, than it is with conventional drugs. Thus, whereas the latter, when entrapped within amorphous polymers above the glass transition temperature, may escape by partition-dependent [or Fickian] diffusion through the polymer matrix, this is not an option usually open to macromolecular species. Holland *et al.* ³⁴ identified four mechanisms from the literature which could account for the release of macromolecules from polymeric

matrices : macromolecule discharge by aqueous diffusion, with the consequential emergence of matrix porosity ; diffusion controlled release through swollen colloids such as poly(vinyl alcohol) ; release by diffusion following the erosion of cross-linked polyester hydrogels ; and, finally, release as a consequence of the erosion of poly(α,β esters), such as PLA and PGA.

In general, macromolecular release from a poly(α,β ester) matrix can only be effected by the leaching of the entrapped species into the dissolution medium. For macromolecules not at the surface of the polymeric device, release may occur as a result of the dissolution medium imbibing through aqueous pores and channels. These are typically generated *in situ* , as mentioned above, either by drug leaching or polymer erosion. However, considerable porosity may also be an inherent morphological feature of the polymeric device, and one of the objectives of the work described in this thesis was to develop a strategy for introducing controlled porosity into poly(beta ester) microcapsules, so that polymer erosion would not be a factor in dictating the release of encapsulated species.

Polymeric controlled release devices that require the entrapped macromolecule to diffuse through aqueous pores and channels in order to be released present an important drawback. This arises because many proteins and high molecular weight polypeptides are unstable in the concentrated solutions that form in the pores. For example, Edelman *et al.* ⁶² found that 99% of the fibroblast growth factor that was released via aqueous diffusion from a polymeric matrix over 14 days was biologically inactive. Furthermore, in cases where macromolecular release occurs alongside polymer breakdown, there is a danger that the polymer decomposition products may adversely affect the active species. For example, Maulding ²¹ reported that the decomposition of an octapeptide

somatostatic analogue, entrapped within a polyester microparticle, was enhanced as a consequence of its interaction with the products of polymer hydrolysis.

A problem frequently encountered during the development of macromolecular controlled release formulations relates to the difficulties associated with incorporating macromolecules [i.e. those with molecular weights exceeding 1.5 KD] into polymeric devices. This is partially a reflection of polymer-polymer incompatibility, but is also due to solubility differences ³⁴. Thus, macromolecules with biological activity such as proteins and high molecular weight polypeptides are generally hydrophilic, water-soluble, and have little affinity for organic solvents. Indeed, exposure to certain organic solvents can result in protein denaturation and a loss of biological activity. In contrast, polymers such as poly(α,β esters) are hydrophobic materials which require organic solvents in order to dissolve. Therefore the incorporation of macromolecules into polymeric matrices frequently requires the difficult combination of an aqueous solvent system and an organic solvent system ³⁴. One technique for the manufacture of controlled release devices, from polymers such as poly(α,β esters), which effectively utilizes this combination is the double emulsion-solvent evaporation process. This technique typically results in the preparation of reservoir-type microcapsules, and is used in chapter five to encapsulate bovine serum albumin and bovine insulin. The incorporation of proteins and high molecular weight polypeptides into polymeric controlled release devices is also frequently complicated by the poor stability these molecules have at high temperatures and extreme pHs.

The release kinetics of both conventional and macromolecular drugs from polymeric matrices [including microspheres, e.g. ^{12, 21, 48}] are rarely zero-order and often

follow a square root of time dependence³⁴. However, alteration of the matrix geometry has facilitated the attainment of pseudo-zero-order release for both monolithic⁶³ and swellable hydrogel systems⁶⁴. Clearly this strategy cannot be applied to modify the kinetics of drug release from microsphere matrices, although Cha and Pitt¹¹ obtained nearly zero-order kinetics for the release of L-methadone by combining different microsphere formulations. The release of some drugs may appear to be nearly zero-order due to poor sink conditions^{11, 21}. Reservoir-type microcapsules are potentially capable of releasing drugs, which are able to diffuse through the polymer membrane, at a constant non-time-dependent rate, provided that the core is made from an aqueous suspension of dissolved and excess drug molecules⁶⁵. In addition, there is considerable evidence to suggest that zero-order kinetics are not desirable for many macromolecular drugs because of the potential down-regulation of biological receptors^{59, 60}.

1.2.2. Drug targeting.

The simplest method of targeting microparticulate drug delivery devices to their intended sites of action is by direct administration. One illustration of this technique that has already been mentioned [see section 1.2.1] is the process of chemoembolization whereby microparticles are injected into, and occlude, small blood vessels close to the target area. Direct administration has also been used to localize drug-loaded microparticles within the ocular environment⁶⁶ and the periodontal pocket⁶⁷.

However, a considerable quantity of drug delivery research currently centres on the concept of 'site-specific drug delivery' which refers for example to the development of microparticulate systems that are able to direct themselves to specific targets within the body⁶⁸. Poste and Kirsh have classified these targets into three groups, which are

organs or tissues, cells and intracellular structures ⁶⁹.

A critical factor in determining the effectiveness of microparticles that are intended for site-specific drug delivery relates to their interaction with the phagocytic cells of the reticuloendothelial system [RES]. This point is relevant to both oral systems and those which are injected directly into the bloodstream. For example, orally administered microparticles can only be taken up from the gastrointestinal tract as a result of phagocytosis by the microfold cells which line the lumen. It is therefore imperative that ingested microparticles are readily phagocytosed by these cells if they are to reach target tissues in the gut area such as the Peyer's patches ⁷⁰. In contrast, if microparticles that have been injected directly into the bloodstream are recognized as foreign by the phagocytic cells of the RES they are likely to be totally removed from the circulation within a few minutes ⁷¹. Clearly therefore, unless the RES is actually the target, it is essential that these microparticles are not phagocytosed if they are to reach their site of action. In addition microparticles injected into the circulation cannot be more than a few microns in size if they are not to occlude in the small arterioles of the lungs.

Eldridge *et al.* ⁷⁰ administered microspheres prepared from various different polymers to mice, and found that the uptake of these particles by the microfold cells lining the gut was dependent on their size and hydrophobicity. Microspheres in the 1-10 μ m size range, made from hydrophobic polymers such as poly- β -hydroxybutyrate, were the most readily phagocytosed. Similarly, Davis and Illum ¹ emphasized the importance of surface charge and hydrophilicity in relation to the removal of microparticles from the circulation by phagocytic cells of the RES. Moreover these researchers have actually demonstrated that microparticle sequestration by the RES can be prevented via the application of hydrophilic coatings based on the poloxamer and

poloxamine series. This procedure also offers some degree of targeting selectivity, for example microparticles coated with poloxamine 908 remain for extended periods in the circulation whilst those coated with poloxamer 407 are directed to the bone¹. It was suggested that the attachment of a homing ligand to the surfaces of the former group of microparticles provided opportunities for more sophisticated site-specific targeting¹.

However, in the short term these strategies for microparticle targeting may be more suitable for diagnostic imaging purposes than for use in controlled drug delivery, since the rate of drug release is likely to be altered by the application of a polymer coating. Indeed, the development of microparticulate drug delivery devices with special coatings for *in vivo* targeting purposes, which also offer the desired release kinetics, is likely to be a complex exercise.

1.3. Techniques for microencapsulation.

1.3.1. Introduction.

Numerous techniques for the process of microencapsulation have been described in the literature. Each has its own advantages and disadvantages, and most are only suitable for certain types of polymer and / or core material. Some preparative procedures lead exclusively to the formation of monolithic microspheres, whilst others generate only reservoir-type microcapsules. However, there are a few production techniques which can be adapted to produce either type of microparticle. The method of manufacture may also have a profound effect on numerous other characteristics of the final product. These typically include particle size and morphology together with the release profile of the encapsulated material. The microparticles' intended end-use should therefore be taken into account when choosing between alternate preparative

techniques, but so also should factors relating to the production process itself, such as batch-to-batch reproducibility, scale-up and efficiency. In addition, for microparticles that are intended for use as *in vivo* drug carriers, consideration must also be given to sterilization and toxicological issues. To illustrate the latter, it would be advantageous for microparticulate drug delivery devices to be prepared by a technique that did not involve the use of toxic organic solvents.

Li *et al.*⁷² divided the different microencapsulation techniques into four general groups : electrostatic methods ; interfacial polymerization ; mechanical methods ; and phase separation or coacervation. However, the work in this thesis involves microencapsulation exclusively with poly(beta esters), and therefore detailed attention in this section is only directed towards those techniques which are suitable for use with these materials.

Electrostatic methods of microencapsulation are intrinsically rare, and involve mixing the encapsulating polymer and the core material when both are aerosolized. Each phase must be a liquid during the encapsulation stage and the fabricating polymer must be capable of surrounding the oppositely charged core material⁷².

In contrast to the electrostatic methods, interfacial polymerization has been widely applied as a microencapsulation technique. Using interfacial polymerization, microencapsulation is achieved via the polymerization and precipitation of a monomer at the interface of two immiscible liquids. Thus, a monomer-containing dispersed phase is emulsified into a continuous phase until the desired particle size is reached, at which point a cross-linking agent is added⁷². Arshady has listed several different types of interfacial polymerization : suspension, emulsion, soapless emulsion, dispersion, precipitation, and bimodal [referring to combinations of the previous five]⁷³. However, vinyl monomers are usually used to form the encapsulating polymers,

although interfacial polymerization has been extended to form hydrogel [poly(2-hydroxyethyl)-methacrylate] microparticles ⁷⁴. In addition, Jalil and Nixon ³⁶ highlighted an *in situ* polymerization process, which was patented by Speiser and Hijnsbroek ⁷⁶, for microencapsulation using poly(lactic acid). Nevertheless, microencapsulation with poly(α,β esters) is more typically practised using mechanical or phase separation processes.

1.3.2. Mechanical methods.

A considerable number of mechanical methods for the process of microencapsulation have been described in the literature, and most of these utilize special equipment. As the title suggests, the formation of microparticles via these methods occurs primarily as a result of mechanical rather than physico-chemical phenomena ⁷². In addition, mechanical processes are generally highly suitable for the production of microparticles on an industrial scale. One such process for example, called multi-orifice centrifugation, is reported to produce units at rates in excess of 300 000 per second ⁷⁷.

Li *et al.* ⁷² in a comprehensive review of microencapsulation technologies and equipment, list several additional mechanical methods for microencapsulation, which include spray drying, spray congealing, pan coating, spheronization, and fluid-bed coating. Of these, spray drying is most widely featured in the literature, and this technique has been used to form monolithic microspheres from a variety of poly(α,β esters), including poly(D,L-lactic acid) ⁷⁸ and poly- β -hydroxybutyrate ⁷⁹.

Microencapsulation using spray-drying is effected via the solvation or dispersion of a core material in a casting solvent which also contains the dissolved wall-forming

polymer. This solution or dispersion is then forced out of a fine nozzle, and thereby atomized, into a heated air stream which supplies the latent heat of vapourization required to remove the solvent and precipitate the polymer⁷².

Bodmeier and Chen⁷⁸ suggested that the ideal method for microencapsulation should be simple, reproducible, easy to scale-up, and essentially independent of the solubility characteristics of the drug and polymer. Spray-drying was thought by these researchers to approach the desired objectives, and was consequently studied using poly(lactic acid) as the encapsulating polymer, with progesterone, theophylline and caffeine as model drugs. With progesterone, spray-drying led to higher levels of drug entrapment than alternate [solvent evaporation] microencapsulation processes, and Bodmeier and Chen concluded their report with the comment 'spray-drying ... appears to be an attractive alternative to the conventional microencapsulation techniques'. However, a detailed examination of their work highlights a number of disadvantages associated with the use of spray-drying. For example, the microparticles exhibited considerable deviations from sphericity, although with progesterone this could be reduced by increasing the drug loading. However, when caffeine was employed as the model drug, it crystallized to a considerable extent outside the polymeric matrix, supporting the contention of Maulding²¹ that spray-drying is 'probably best with drugs that are not too water soluble'.

The most serious flaw Bodmeier and Chen⁷⁸ encountered with spray-drying, however, was the failure of the casting solution to form microdroplets in subsequence to its elution from the nozzle. This problem led to the formation of fibrous strands instead of microparticles, and was found to be predominantly dependent on the nature of the polymer. Indeed, even 1% solutions of PLA in methylene chloride gave rise to

fibres.

These findings are consistent with the work of Akhtar ⁷⁹ who attempted to form microspheres from poly- β -hydroxybutyrate using a Buchi B190 Minispray dryer [the same model as employed by Bodmeier and Chen]. Akhtar found that high molecular weight poly- β -hydroxybutyrate homopolymers, and copolymers thereof with 3-hydroxyvalerate [P(HB-HV) polymers], gave rise to a poor quality product - even when the concentration of the casting solution was only 0.5%.

In contrast, however, when spray-drying was performed using low molecular weight samples of these polymers and copolymers [i.e. molecular weights of less than 140 000 for the homopolymer], a fine-free flowing powder resulted. Akhtar ⁷⁹ concluded that non-film-forming polymers were the most suitable for spray-drying, since a high degree of chain entanglements made the formation of microdroplets practically impossible. Although Bodmeier and Chen ⁷⁸ make no mention of using different molecular weight samples of PLA, they do emphasize the importance of structural considerations which lead to a high level of intermolecular bonding in their explanation for the fibre production.

It is interesting to note that the fine-free flowing powder, which Akhtar ⁷⁹ obtained as a result of spray-drying low molecular weight P(HB-HV) polymers, was revealed by scanning electron microscopy to actually consist of fused particulate aggregates and not individualized microspheres. In addition, the release of methyl red from spray-dried poly- β -hydroxybutyrate microspheres was subject to 'some variance between batches'. Spray-drying also typically involves temperatures well in excess of 37°C, and this could effectively prohibit the encapsulation of heat-sensitive core materials such as proteins and high molecular weight polypeptides.

As was mentioned earlier in this section, Bodmeier and Chen ⁷⁸ have suggested that

one feature of the ideal microencapsulation technique is simplicity. In comparison, for example with solvent evaporation processes, spray-drying is not complicated. Nevertheless, variables such as the viscosity, uniformity and concentration of the core and coating materials, plus the inlet and outlet temperatures, offer some degree of control over particle characteristics ⁷². However, although Akhtar ⁷⁹ found that microsphere size was related to the polymer concentration, the surface morphology of the particles was constant irrespective of the operational settings chosen. Clearly this is useful in terms of batch-to-batch reproducibility, but it significantly diminishes the possibility of regulating important functional parameters, such as the rate of release of encapsulated material, via intentional changes in particle morphology. Thus, phase separation techniques such as solvent evaporation may be considerably more complicated than mechanical processes such as spray-drying, and as a result present some difficulties in terms of reproducibility, but they compensate by offering greater opportunities for the regulation of particle morphology. In chapter three of this thesis, the effects of variables such as polymer molecular weight, polymer composition, polymer concentration and temperature on the morphology of microcapsules prepared by a solvent evaporation process are demonstrated. Some of these control strategies are then utilized in chapter six to regulate the rate of release of encapsulated hydrocortisone from monolithic microspheres.

One additional mechanical method for microencapsulation which warrants discussion is a technique called 'melt dispersion' or 'hot-melt microencapsulation'. This technique has been employed to produce drug-loaded microspheres from polymers such as polyanhydrides ⁸⁰, carnauba wax ⁸¹ and poly- ϵ -caprolactone ⁵⁰. In the latter instance, indomethacin - poly- ϵ -caprolactone films [which had been prepared by

solvent casting] were cut into small pieces and then dispersed into a hot 0.25% w/v aqueous poly(vinyl alcohol) solution. The temperature of the aqueous phase [80°C] exceeded the melting point of the polymer [approximately 60°C], with the result that the film fragments became a molten mass. Following homogenization and cooling, solid microspheres without drug crystals were obtained.

The advantage of the melt-dispersion technique, for the production of microspheres from polymers such as poly(α,β esters), is that it makes little use of toxic organic solvents. Clearly, the procedure described above for the formation of indomethacin-loaded poly- ϵ -caprolactone microspheres still required an organic solvent to form the films, but this stage could easily be omitted via the utilization of a high shear-mixing device, which could facilitate the direct emulsification of the polymer-drug melt into the aqueous phase ⁵⁰. Nevertheless, melt-dispersion involves exposing the core drug material to temperatures in excess of the melting point of the encapsulating polymer. In view of very high melting points of most poly(α,β esters) [e.g. 223-227°C for PGA ⁵³, 130-135°C for P(D,L-LA) ³⁴, approximately 180°C for P(L-LA) ^{34, 53}, and 175-177°C for PHB ⁵³], melt-dispersion is effectively limited to poly- ϵ -caprolactone, and copolymers thereof with other lactones which have low melting points. However, it is of interest to note in this regard that annealed copolymers of poly- ϵ -caprolactone with 20 to 25 mole percent of δ -valerolactone have melting points below 37°C ⁵³, and are therefore potentially suitable for encapsulating heat-sensitive drugs, such as proteins and high molecular weight polypeptides, using the melt-dispersion process.

1.3.3. Phase separation techniques.

There are several different phase separation techniques which can be used to microencapsulate core materials within poly(α,β esters). In general, these processes

achieve polymer precipitation, and thus microparticle formation, as a consequence of either non-solvent addition or solvent evaporation, although in some instances both mechanisms may have a role [e.g. ⁶⁵].

Various forms of non-solvent addition are described in the literature. For example, Sato *et al.* ⁹ used a variant called 'solvent-extraction-precipitation' to microencapsulate core materials such as methylene blue and prednisolone acetate within poly(glycolic acid) microspheres. This process involved dissolving the core material and the encapsulating polymer in hexafluoroacetone sesquihydrate [HFA] and then dispersing this solution at 37°C into a continuous phase of carbon tetrachloride. The continuous phase was then agitated via the use of either a vibromixer or a dispersator to produce an emulsion of dispersed phase microdroplets. Then, in order to remove the dispersed phase solvent and thereby precipitate the microdroplets, the emulsion was rapidly added to a miscible non-solvent [1,4-dioxane] and stirred with a vibromixer. After approximately 30 minutes, the HFA had been extracted from the dispersed phase microdroplets, which had consequently precipitated to give monolithic microspheres.

Sanders *et al.* ⁸² described the use of a non-solvent addition microencapsulation technique to effect the entrapment of nafarelin acetate within devices made from poly(glycolic acid-co-D,L-lactic acid). An aqueous solution of the drug and a solution of the copolymer in methylene chloride were combined to form a water-in-oil [w/o] emulsion. An unspecified non-solvent was then added to the initial w/o emulsion to initiate polymer precipitation.

The resulting mixture was then added to a larger volume of non-solvent in order to extract the residual methylene chloride from the partially precipitated microdroplets, and thereby form solid microparticles. In theory, these final microparticles should have

been true reservoir-type devices or microcapsules, consisting of an aqueous solution of drug encapsulated within a polymer membrane. However, no information concerning the structure and morphology of the particles was presented by Sanders *et al.*⁸², and the release data did not suggest that the drug was entirely present as an aqueous solution located at the centre of reservoir-type microcapsules. Additionally, Ogawa *et al.*¹⁸ stated that 'particles prepared by this method tend to be large and transformed', a comment based on the work of Sakatoku *et al.*⁸³.

In 1987, Gardener⁶⁵ filed a US patent for a phase-separation microencapsulation process that was claimed to lead to the production of reservoir-type microcapsules from homo- and copolymers of glycolic and lactic acids. This process was partially based upon the concept of non-solvent addition, but it also utilized solvent evaporation as a means of inducing polymer precipitation. This hybrid process involves dissolving the encapsulating polymer in a mixture of two miscible organic liquids, one of which is a solvent for the polymer, whilst the other is a non-solvent; the solvent has the higher vapour pressure. The two liquids are employed in a ratio such that the resulting polymer solution is very close to its phase separation or cloud point. An aqueous solution or suspension of the material for encapsulation is then emulsified into the organic phase. Vigorous agitation is required to create the emulsion and thereafter to maintain it whilst solvent / non-solvent evaporation is allowed to proceed. The lower vapour pressure of the solvent ensures that the proportion of non-solvent in the solvent / non-solvent mixture increases as a result of solvent / non-solvent evaporation. This, coupled with the fact that the polymer solution was initially close to its cloud point, ensures that phase separation, and thus polymer precipitation, occurs rapidly. The phase separated polymer then migrates to the surface of the aqueous microdroplets and

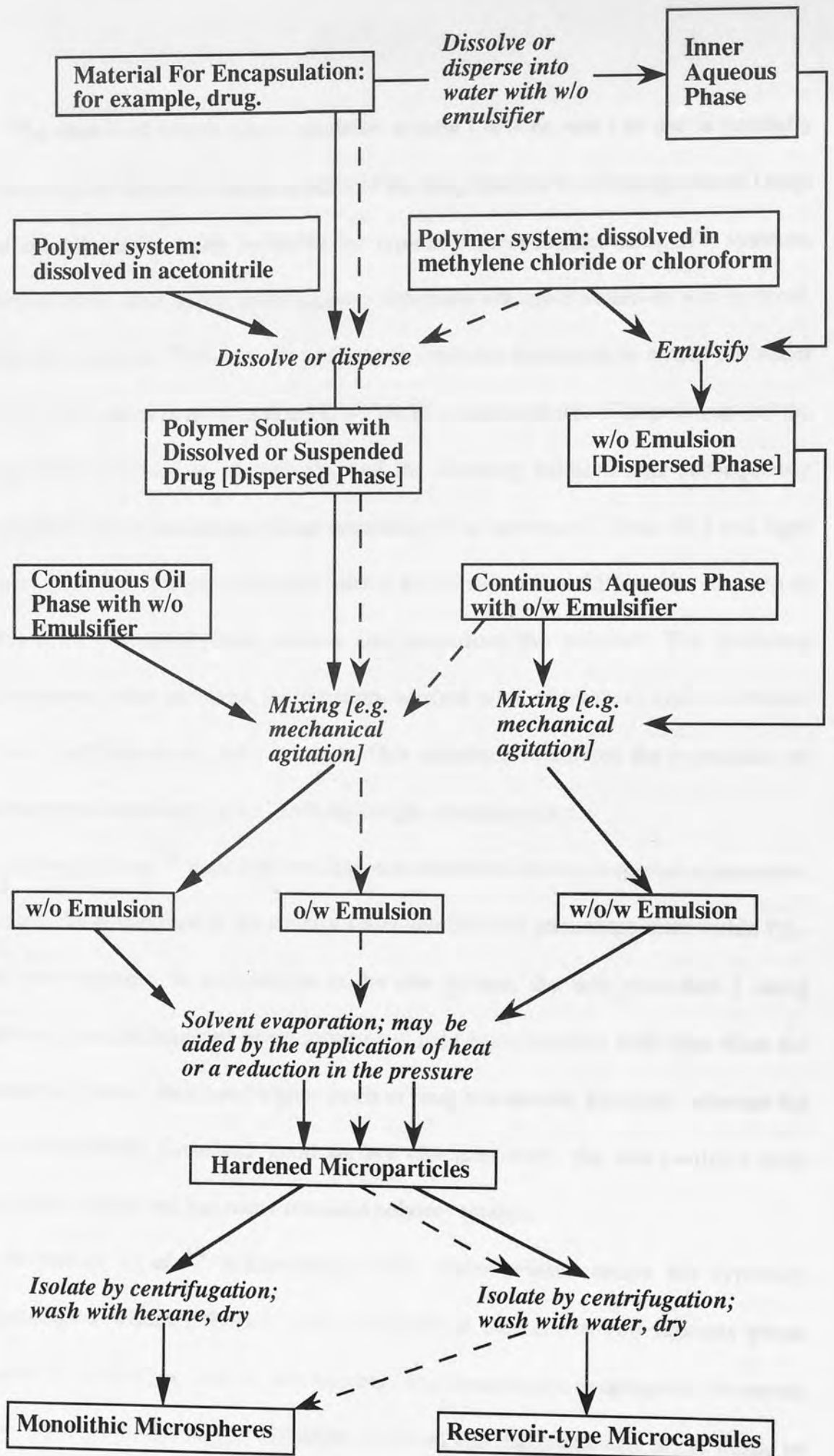
begins to encapsulate them. When encapsulation has been completed [an experimental determination of the appropriate time interval for the system under study is required], the solvent evaporation phase is over, and non-solvent addition is utilized to effect the hardening of the microparticles. Thus, the suspension of nascent microparticles is added to a large volume of non-solvent, which facilitates the extraction of residual solvent from the polymer membrane.

One of the most extensively utilized methods of microencapsulation is the phase separation process known as emulsion-solvent evaporation. This technique is most commonly employed using poly(α,β esters) such as P(D,L-LA) ^{13, 15, 16, 17, 47} P(L-LA) ^{11, 84, 85, 86} and, to a lesser extent, PGA ⁹, PHB ^{51, 70, 87} and PCL ^{12, 48, 49} as the fabricating materials. However, emulsion-solvent evaporation has also been used to form microparticles from numerous other polymers, including cellulose acetate butyrate ^{19, 49}, ethylcellulose ^{50, 70} poly(methyl methacrylate) ^{19, 50, 70} and poly(styrene) ^{50, 70}.

Variations in the preparative procedure can lead to the production of either reservoir-type microcapsules or monolithic microspheres. To generate the former, a double emulsion technique must be utilized, whereas the latter can be produced via a single emulsion process. Single emulsion processes may be based on either oil-in-water [o/w] or water-in-oil [w/o] emulsion systems. Three different forms of the emulsion-solvent evaporation process are outlined schematically in diagram 1.2.

Diagram 1.2. Schematic representation of three different types of emulsion-solvent evaporation process.





The choice of which single emulsion system [o/w or w/o] to use is normally dictated by the solubility characteristics of the drug material to be encapsulated. Drugs that are essentially water insoluble are typically encapsulated using o/w systems, whereas those with appreciable aqueous solubility are more suited to w/o systems. Thus, for example, Tsai *et al.*²⁰ used a w/o emulsion technique to entrap the water soluble anti-cancer drug mitomycin C within PLA microspheres. The polymer and the drug were dissolved in acetonitrile, and the resulting solution was subsequently emulsified into a continuous phase consisting of a surfactant [Span 65] and light mineral oil. The emulsion was then stirred for 25 minutes at 55°C under vacuum to remove the dispersed phase solvent and precipitate the polymer. The hardened microspheres were removed by filtration, washed with mineral oil and *n*-hexane-Arlacel, and then dried under vacuum. This technique facilitated the production of microspheres containing up to 13.8% by weight of mitomycin C.

Jalil and Nixon⁸⁶ used both o/w and w/o emulsion-solvent evaporation processes to effect the entrapment of the slightly water-soluble drug phenobarbitone within P(L-LA) microspheres. In comparison to the o/w system, the w/o procedure [using acetonitrile as the dispersed phase solvent and light liquid paraffin with Span 40 as the continuous phase] facilitated higher levels of drug entrapment. However, whereas the o/w microspheres possessed 'good surface characteristics', the w/o particles were irregularly shaped and had many uncoated polymer crystals.

Bodmeier *et al.*¹⁹ acknowledged that water-soluble drugs are typically encapsulated within polymers such as poly(α,β esters) via non-aqueous phase separation techniques, such as non-aqueous emulsion-solvent evaporation. However, these researchers then suggested that the use of an aqueous continuous phase would be

more economically desirable, and would present additional advantages in terms of product clean-up and continuous phase recovery. The use of a double emulsion [i.e. water-in-oil-in-water, or w/o/w] solvent evaporation technique was investigated by these researchers as a means to effect the entrapment of a highly water soluble drug [pseudoephedrine HCL : aqueous solubility approximately 2g / ml] within microparticles prepared from various polymers, including P(D,L-LA). Whereas the use of an o/w emulsion-solvent evaporation process frequently results in the partitioning of water-soluble drugs from the organic dispersed phase into the continuous phase, this problem is reduced via the use of a double emulsion process, because the organic polymer solution forms a physical barrier between the inner aqueous phase and the continuous aqueous phase ¹⁹. Indeed, with P(D,L-LA) as the encapsulating polymer, Bodmeier *et al.* ¹⁹ found that the w/o/w emulsion system gave rise to particles containing more than twice as much pseudoephedrine HCL as those correspondingly prepared using the o/w system. However, the formation of drug containing microcapsules from poly(α,β esters), via the double emulsion-solvent evaporation process, is reported infrequently in the literature. This technique appears to have originated from the work of Vranken and Claeys ⁸⁸ as detailed in US Patent 3 523 906.

Ogawa *et al.* ¹⁸ described the encapsulation of the hydrophilic peptide leuprolide acetate within poly(D,L-lactic acid) and poly(glycolic-co-D,L-lactic acid) microcapsules using a similar technique, based on US Patent 4 652 441 ⁸⁹.

In contrast, microencapsulation using single emulsion-solvent evaporation processes has been widely reported, particularly with o/w systems to effect the entrapment of drugs with little aqueous solubility. The popularity of this technique is probably a reflection of its technical simplicity. However, a wide range of preparative variables can affect the nature of the final product. These include factors related to the

nature of the polymer [such as the polymer type, its molecular weight and the polydispersity], the organic solvent system [such as the heat of evaporation, the level of water-miscibility] and the drug [such as its solubility in each phase] ; the polymer concentration ; the drug loading ; polymer-organic solvent-drug-aqueous phase interactions ; the dispersed to continuous phase ratio ; the type and concentration of the aqueous phase emulsifier ; the physical emulsification process ; the temperature and pressure ; and the solvent evaporation period. The role of the different preparative variables in controlling the efficiency of drug encapsulation, when microencapsulation is performed using an o/w emulsion-solvent evaporation process with poorly water soluble drugs, is discussed in some detail in chapter six, and will therefore not be addressed further in this section. Instead, the remainder of this discussion will focus on how a selection of the various preparative variables, listed above, affect microparticle formation, and particle characteristics such as size, shape and morphology.

Several different workers have reported a relationship between the viscosity of the organic dispersed phase and the size of the resulting particles. Thus, Spenlehauer *et al.*⁴⁷ noted that increases in either the drug or the polymer concentration enhanced the mean diameter of cisplatin-loaded P(D,L-LA) microspheres. Similarly, in a comprehensive series of investigations, Jalil and Nixon demonstrated that the mean volume diameter of P(D,L-LA) microspheres, prepared using a w/o [acetonitrile / light liquid paraffin] emulsion-solvent evaporation process, increased as a result of the use of either a more concentrated polymer solution⁹⁰ or a higher molecular weight polymer⁹¹. However, in the latter instance, the variations were 'only minor', although to some extent this may have been a consequence of the narrow molecular weight range [from

5200 to 20500] employed. The higher dispersed phase viscosities were believed to lower the stirring efficiency and make it more difficult to produce smaller emulsion droplets.

Wakiyama *et al.*⁴⁶ also reported the effects of P(D,L-LA) molecular weight on microsphere size. The polymer molecular weights used in these investigations ranged from 9 100 to 25 000, which implied intrinsic viscosity values in benzene at 30°C of between 0.32 and 0.70 dL/g. Methylene chloride was utilized as the dispersed phase solvent, and three different drugs were separately encapsulated. Butamben-loaded microspheres prepared from the lowest molecular weight polymer had a mean diameter of 50.6µm, but this increased to 60.2 µm when the molecular weight of the fabricating polymer was raised to 25 000. In contrast, when either dibucaine or tetracaine was used as the encapsulated species, microspheres prepared from the lowest and highest molecular weight polymers had diameters so close that they were within the margins of error.

The presence of a surfactant [such as a hydrophilic colloid] in the continuous phase is required in order to stabilize the initial o/w emulsion, and to subsequently prevent the inchoate microparticles from agglomerating. Gelatin has been employed as the continuous phase surfactant in several reported studies^{46, 51}, but Fong *et al.*⁹⁴ have suggested that this colloid is associated with particle agglomeration, and the production of few microspheres with diameters below 150µm, which is the maximum size suitable for injection with a 20 gauge syringe needle.

The hydrophilic colloid most commonly employed as a continuous phase surfactant in microencapsulation work with poly(α,β esters) is poly (vinyl alcohol) [PVA],

which may be used either alone [e.g. 11, 19, 92] or in combination with other colloids such as methylcellulose [e.g. 15, 17, 47]. The work of Cavalier *et al.* 17 with hydrocortisone-loaded PLA microspheres clearly demonstrates the important influence that the continuous phase surfactant system can have on microsphere formation and particle shape. Thus, when 0.27% PVA [partially hydrolysed, 88%] was exclusively used as the aqueous phase emulsifier, spherical microspheres were produced at drug : polymer ratios below 0.4. However, increasing the drug : polymer ratio to 0.56 resulted in the production of mis-shapen particles, whilst at drug : polymer ratios exceeding 0.6%, microsphere formation was not possible due to the instability of the emulsion and the consequent production of large aggregates. The use of 400-cps grade methylcellulose [methylcellulose-400], at concentrations ranging from 0.05% to 0.3%, instead of PVA, as the aqueous phase emulsifier facilitated the production of what appeared to be largely oval-shaped microspheres at drug : polymer ratios ranging from 0.6 to 1.0. It was concluded that the methylcellulose-400 generated a stable emulsion, but that the high solution viscosity prevented the formation of spherical particles. However, a combination of 0.05% methylcellulose-400 and 0.27% PVA in the continuous phase led to the production of spherical particles at these drug : polymer ratios. The rheological properties of the mixture were therefore judged to be superior to those of the individual colloids. Moreover, a less viscous form of methylcellulose [10-cps grade] resulted in the production of spherical particles at drug : polymer ratios exceeding 0.6, with or without the presence of 0.27% PVA 17.

There is considerable similarity between the work of Cavalier *et al.* 17 and the research reported by Spenlehauer *et al.* 47 into the formation and characterization of cisplatin-loaded P(D,L-LA) microspheres. Thus, the latter group were also able to induce the formation of microspheres at high drug : polymer ratios [1.0] via the

inclusion of methylcellulose-400 in the aqueous phase. Furthermore, the resulting particles were mis-shapen [i.e. non-spherical] when this colloid was used alone at a concentration of 1% [w/v]. The deviations from sphericity appeared to result from a collapse in the polymer matrix, an effect that was attributed to the high viscosity of the methylcellulose-400 solution. As in the hydrocortisone-P(D,L-LA) system reported by Cavalier *et al.*¹⁷ spherical microspheres were produced from equal ratios of drug and polymer when the aqueous phase contained a mixture of 0.05% methylcellulose-400 and PVA. However, Spenlehauer *et al.*⁴⁷ demonstrated that the viscosity of the PVA was of importance in determining the level of aggregation amongst the particles. Thus, microspheres prepared from equal ratios of cisplatin and P(D,L-LA) were spherical, but aggregated, when formed using an aqueous phase which contained 0.05% methylcellulose-400 and 1% 4 mPa-s grade PVA. Substitution of the 4mPa-s grade with an 8 mPa-s grade PVA, however, led to the production of spherical and individualized microspheres. The longer polymer chains of the latter were believed to induce a greater degree of steric repulsion between the microdroplets, thereby reducing the tendency for aggregation. Increasing the concentration of the 8 mPa-s grade PVA from 1% to 4% [whilst leaving the methylcellulose-400 concentration unchanged at 0.05%] did not lead to a change in microsphere morphology, although it did decrease the particle size⁴⁷. This effect may have resulted from the increased viscosity of the continuous phase and the consequent increase in the shear stress. In addition, at higher surfactant concentrations, a greater interfacial area can be stabilized.

Fong *et al.*⁹⁴ added the fatty acid salt emulsifier sodium oleate to the aqueous continuous phase in order to form drug-loaded P(D,L-LA) microspheres. With thioridazine, ketotifen or hydrocortisone acetate as the encapsulated species, this

process led to the production of high yields of microspheres [74-96%], most of which [> 80%] were smaller than 150 μ m, and thus suitable for injection via a 20 gauge needle. The high efficiency of the sodium oleate emulsifier was believed to account for the observation that the size distribution of the particles was not affected by process variables such as the stirring speed, the initial polymer concentration and the emulsifier concentration.

A subsequent paper by Fong *et al.*¹⁴ described the effects of adding sodium hydroxide to the aqueous continuous phase during the microencapsulation of thioridazine. As in their previous work⁹⁴, sodium oleate was used as the continuous phase surfactant, and P(D,L-LA) as the wall-forming polymer. In aqueous solutions, sodium oleate undergoes hydrolysis to form oleic acid, a reaction which reduces the oleate ion concentration, and thus the effectiveness of the compound as an emulsifier. The addition of sodium hydroxide to the continuous phase of the experimental system described above, opposed the hydrolysis of sodium oleate, and as a result facilitated the production of more porous microspheres.

The preceding text has highlighted the role of variables such as viscosity [dispersed and continuous phase] and interfacial tension in controlling the size and / or shape of microparticles, prepared using the emulsion-solvent evaporation technique. These characteristics are also governed to a considerable extent by the physical emulsification process. Thus, Dubernet *et al.*¹² reported that the size of drug-free poly- ϵ -caprolactone microspheres, prepared using an o/w solvent evaporation process, was influenced by both the stirring rate and the nature of the shearing. Agitation at 700 revolutions per minute [rpm], using a one-bladed stirrer, produced a range of different

microsphere diameters, which adopted a bimodal distribution with the main peak situated at between 400 and 630 μm . In contrast, when the agitation was effected using a Microvortex propeller at 3700 rpm, a contraction in the size distribution was noted, and all the particles had a diameter of less than 315 μm . However, replacement of the Microvortex propeller with an Ultraturax turbine at 1500 rpm for 5 minutes led to an even narrower size distribution and a mean microsphere diameter of only 30 μm . These workers were unable to explain why the lower stirring rate of the Ultraturax turbine led to smaller particles than the more rapid agitation provided by the Microvortex propeller.

Benita *et al.*¹⁶ found that the mean diameter of both drug-free and progesterone-loaded P(D,L-LA) microspheres, prepared using an o/w solvent evaporation process, progressively decreased when the stirring rate was elevated. This experimental system was also used by Benoit *et al.*⁹⁵, who found a similar linkage between the rate of agitation and the size of both drug-free and progesterone-loaded microspheres.

Jalil and Nixon⁹³ examined the effects of the stirring rate on the size of phenobarbitone-loaded P(L-LA) microspheres, prepared using a w/o [acetonitrile / light liquid paraffin] emulsion-solvent evaporation process. These researchers reported a narrowing of the size distribution, and a progressive decrease in the mean volume diameter [as measured following particle deaggregation by sonication], from 344.78 to 80.77 μm , when the stirring rate was raised from 500 to 2000 rpm. Moreover, when the mean volume diameter was plotted against the stirring speed on a log-log scale, a straight line resulted. The effect of the stirring speed on the particle size was attributed to the production of a 'finer emulsion' at higher stirring speeds, and a reduced tendency for fusion amongst the 'immature' particles. Unimodal volume size distributions were a ubiquitous feature of this work.

Chang *et al.*⁴⁹ noted that particle size could be 'largely controlled by the rate of

agitation', but they did not include any experimental evidence to substantiate this claim. These authors did, however, make the important point that there are limits on the extent to which the rate of agitation can be used to control particle size. Indeed, the essential requirement for the formation of a successful emulsion, and thus spherical microparticles, is that the shear stress provided by the agitator gives droplet dimensions which are stabilized by the formulation. The influence of the stirring rate on the particle size will therefore be dependent on the other experimental variables [the emulsifier type and concentration, the dispersed phase viscosity, the temperature, etc.] in the particular system under study.

It is of interest to note that a previous study performed within the Speciality Materials Research Group ⁹⁷ found that microparticles prepared at higher stirring speeds were generally larger and less spherical than those similarly prepared at lower stirring speeds. Whilst this work appears to be at variance with the findings of Dubernet *et al.* ¹², Benita *et al.* ¹⁶, Benoit *et al.* ⁹⁵, and Jalil and Nixon ⁹³, the discrepancy was actually an experimental artefact caused by the utilization of an inefficient mixing system, which led to severe vortexing at higher stirring speeds. This problem can be eliminated via the adoption of various process modifications. An example is the inclusion of 'baffles' within the mixing vessel ⁹⁸. Thus, four vertical baffles [each with a width of 9-10% of the mixing vessel diameter] can be employed to neutralize the formation of a strong vortex and thereby enhance the mixing efficiency ⁹⁹. Alternately, baffle use can be avoided completely when mixing with an overhead unit simply by adopting an angular or off-centre position for the impeller ⁹⁹. Bodmeier and McGinity ⁹⁸ reported that the shift to effective top-to-bottom mixing eliminated foaming, air bubbles and the formation of 'sticky' agglomerates, improved the particle

yields, and reduced the mean particle diameter, when microencapsulation was performed using an o/w solvent evaporation process.

In order for emulsion-solvent evaporation processes to successfully produce microparticles, it is essential that the dispersed phase solvent evaporates more rapidly than the continuous phase solvent. Methylene chloride is the most widely featured dispersed phase solvent in the literature, particularly when the fabricating polymers are poly(α,β esters). This solvent has a solubility in water of 1.961 percent by weight¹⁰⁰, and can therefore be readily mixed into an aqueous continuous phase to form an o/w emulsion. Moreover, Bodmeier and McGinity¹³ demonstrated that solvents with lesser degrees of water miscibility, such as chloroform and benzene, were actually unsuitable for the effective encapsulation of quinidine sulphate, because of the long droplet precipitation times. These workers found that, in comparison with chloroform and benzene, the extraction of methylene chloride into the aqueous phase, and its subsequent evaporation, occurred very rapidly. The latter event was a consequence of its lower boiling point [41°C¹⁰¹] and heat of evaporation. The rapid vapourization of methylene chloride from an o/w emulsion typically enables solvent evaporation to be effected at room temperature, and under ambient pressure [e.g. ^{13, 16, 49}].

However, when solvents with higher boiling points than methylene chloride are used as the dispersed phase, a reduction in the pressure, or an increase in the temperature, may be necessary to induce polymer precipitation. This situation is particularly relevant to w/o solvent evaporation experiments, such as the acetonitrile / mineral oil system adopted by Tsai *et al.*²⁰ or the acetonitrile / light liquid paraffin system utilized by Jalil and Nixon [e.g. ⁸⁶]. In the former instance, the emulsion was heated to 55°C and a vacuum was applied in order to remove the acetonitrile, whilst

Jalil and Nixon effected acetonitrile evaporation by heating the emulsion system to 55°C and passing a gentle stream of air of the reaction vessel. The latter workers also investigated the effects of different temperatures [ranging from 50-85°C] on the characteristics of phenobarbitone-loaded P(D,L-LA) microspheres ⁹⁰. With regard to particle size, increasing the temperature from 50°C to 85°C did not change the distribution profile, although it did produce a very slight reduction in the mean volume diameter, an effect that was attributed to the reduced viscosity of the emulsion. The preparative temperature was also found to influence the surface morphology of the resulting microspheres. Indeed, particles prepared at 55°C were spherical with smooth surfaces, whereas those prepared at 85°C had highly irregular surfaces. This morphological effect was attributed to more rapid rates of solvent evaporation, and interfacial drug crystallization, at the higher temperatures ⁹⁰.

1.4. Scope and objectives of the research presented in this thesis.

The work of Jalil and Nixon ⁹⁰, described above, is one of only a small number of research articles to examine the linkage between microparticle morphology and a preparative variable other than the drug / polymer ratio. Moreover, in addition to temperature, these authors have reported the effects of polymer concentration ⁹⁰ and polymer molecular weight ⁹⁶ on the morphology of microspheres, prepared using a w/o emulsion-solvent evaporation process.

The dearth of investigative attention on the subject of microparticle morphology, and its relationship with different preparative variables, is particularly surprising given that this characteristic typically plays a salient role in determining the release kinetics of an encapsulated drug. In addition, elucidation of the factors influencing particle

morphology is also of interest from a theoretical viewpoint. Thus, in comparison for example to the formation of organic polymer films by solvent evaporation, the morphology of polymer surfaces which have been produced as a consequence of solvent / non-solvent diffusion has not been a topic that has received comprehensive research attention.

The central objective of the work described in this thesis was to investigate the effects of selected preparative variables on the morphology of microcapsules, prepared using the double emulsion-solvent evaporation technique. The fabricating materials utilized are poly- β -hydroxybutyrate homopolymers and copolymers thereof with 3-hydroxyvalerate [P(HB-HV) polymers] [for details about these materials, see section 1.5]. The investigations described in chapter three assess how the molecular weight, 3-hydroxyvalerate [HV] content and concentration of the fabricating polymer, and the temperature at which solvent evaporation is conducted, affect the characteristics of the resulting microcapsules. In this regard, assessments have been made of microcapsule yield, shape and surface morphology. Chapter four then investigates how microcapsule morphology in general, and microcapsule porosity in particular, are influenced by blending the fabricating P(HB-HV) polymer with poly- ϵ -caprolactone [PCL].

One revelation of these studies is the ability to generate uniformly microporous microcapsules from blends of various high molecular weight P(HB-HV) polymers with a low molecular weight form of PCL. These microcapsules are of particular interest because they may have the potential to facilitate the release of encapsulated macromolecules via an aqueous diffusion mechanism that does not rely on polymer breakdown. In order to test this possibility, one such formulation was used in chapter five to encapsulate a range of different macromolecules, whose *in vitro* release

behaviour was subsequently evaluated.

The studies detailed in chapter six centre on the preparation and characterization of hydrocortisone [HC]-loaded microspheres, prepared using an o/w single emulsion-solvent evaporation process. In this chapter, the influence of the organic phase viscosity on the efficiency of drug encapsulation is the focus of initial investigations. The viscosity changes are produced by altering the concentration and molecular weight of the fabricating P(HB-HV) polymer.

In the following section, microspheres are prepared at a 20% hydrocortisone loading from the full range of P(HB-HV) polymers used in chapter three, and are then evaluated in terms of surface morphology. This investigation was effected primarily in order to permit a subsequent analysis of the drug release kinetics, but it also facilitated a comparison to be made between the morphology of each drug-loaded microsphere and the corresponding drug-free microcapsule. In addition, the influence of the solvent evaporation temperature and the drug loading on microsphere morphology were investigated for selected formulations.

The final objective of the studies described in chapter six was to determine the *in vitro* hydrocortisone release kinetics for the different microsphere formulations, and interpret these data in terms of particle morphology [i.e. the level of porosity], drug loading and polymer biodegradation / bioerosion.

1.5. Poly- β -hydroxybutyrate homopolymers and copolymers thereof with 3-hydroxyvalerate [i.e. P(HB-HV) polymers].

Poly- β -hydroxybutyrate [PHB] is an aliphatic polyester of D(-)- β -hydroxybutyrate ¹⁰², and was discovered in bacteria [*Bacillus megatarium*] by Lemoigne in 1925 ¹⁰³. This polymer is now known to be produced by numerous microorganisms, such as those found in the soil ¹⁰⁴ and on raw sewage ⁷⁹, where it serves as an energy and carbon reserve. Bacteria store PHB as highly crystalline hydrophobic granules of 0.2 to 0.7 μ m in diameter ⁵³, from which it may be extracted using organic solvents such as chloroform ¹⁰⁶. Typically the amount of PHB in a bacterial cell is between 1% and 30% of the dry weight, but under special fermentation conditions, unregulated polymer production can increase this level to approximately 80% ¹⁰⁴. The ICI subsidiary, Marlborough Biopolymers Ltd, has exploited this behaviour in order to manufacture multi-kilogram batches of PHB from *Alcaligenes eutrophus* cultures ^{79, 102}. In addition, these bacteria have been induced to synthesize a range of random copolymers of poly- β -hydroxybutyrate with hydroxypentanoate.

The latter is more typically referred to as D(-)- β -hydroxyvalerate [HV], and copolymers containing up to 30% of this monomer are manufactured by ICI, and marketed under the trade name Biopol[®].

In the following three sub-sections, P(HB-HV) polymers are respectively reviewed in terms of their physico-chemical properties, biocompatibility and applications in microencapsulation. A discussion concerning the degradation of these materials is included in chapter six.

1.5.1. Physico-chemical properties of P(HB-HV) polymers.

P(HB-HV) polymers are members of the general class of compounds known as polyhydroxyalkanoates, and exhibit the unusual property of isodimorphism. This means that both HB and HV units can co-crystallize within the same crystal lattice, probably because of their similar main-chain conformations and molecular dimensions^{107, 108}. An important consequence of isodimorphism is believed to be that the copolymers are capable of attaining the same high levels of crystallinity as the PHB homopolymer [typically > 60%]¹⁰⁸. This, in turn, is likely to profoundly affect polymer properties relevant to controlled release applications such as permeability and degradation.

As a result of the high levels of crystallinity, the glass transition temperature [T_g] values of P(HB-HV) polymers are difficult to determine^{79, 109}. However, Akhtar⁷⁹ reported that the T_g values [as determined by differential scanning calorimetry] of P(HB-HV) polymers, containing from 0 to 27 mole percent HV, varied non-sequentially between -5 and 20°C. This suggests that at normal physiological temperatures, the amorphous regions of the polymer will be largely in the rubbery state.

Akhtar also used DSC to characterize the melting behaviour of this range of P(HB-HV) polymers. Thus, the peak melting temperature of a 380 000 molecular weight PHB homopolymer was located at 174°C. However, increasing the HV content diminished this value and broadened the range over which melting occurred. It is of interest to note that the copolymer containing 27 mole percent HV [of unreported molecular weight] had a peak melting temperature of approximately 51°C. This value is lower than the melting point of pure PHV which is reported to lie between 105 and 108°C¹¹⁰. Molecular weight differences notwithstanding, it appears that P(HB-HV)

polymers exhibit eutectic-like minima in their melting points, which is a probable consequence of their isodimorphic behaviour^{107, 108}.

P(HB-HV) polymers are highly soluble in common halogenated solvents such as methylene chloride, chloroform and dichloroacetate, but they are insoluble in water, methanol, ethanol, hexane, benzene, ethylacetate and tetrahydrofuran.

Bloembergen *et al.*¹¹¹ have demonstrated that PHB homopolymers crystallize more rapidly than the HV copolymers from chloroform solutions. The explanation forwarded for this behaviour was that the additional methyl group in the HV unit retarded the copolymer crystallization kinetics. PHB is known to crystallize as a helix, and there is some evidence that the helical conformation is retained even in solution¹¹².

1.5.2. Biocompatibility of P(HB-HV) polymers.

Extensive *in vitro* biocompatibility work on P(HB-HV) copolymers using cell culture techniques has been performed within the laboratories of the Speciality Materials Research Group¹¹³. This work, using mammalian fibroblasts, has demonstrated the cytocompatibility [as evidenced by cell attachment and spreading] and non-cytotoxicity [as evidenced by the trypan blue exclusion test] of several different P(HB-HV) polymers.

These materials undergo degradation *in vivo* to produce D(-)- β -hydroxybutyrate and D(-)- β -hydroxyvalerate. Although both monomers are normal physiological metabolites, there is some evidence to suggest that they can inhibit cellular growth at high concentrations. Thus, Pouton *et al.*¹¹⁴ reported that the population doubling time of CHO-K1 [Chinese Hamster Ovary] cells in culture was extended at D(-)- β -hydroxybutyrate concentrations exceeding 10 mg / ml [relative to control samples grown in a medium free of this monomer], and that growth was totally inhibited at

levels above 25 mg / ml. However, this is unlikely to be a serious concern in relation to the use PHB-based internal medical devices, because slow degradation rates and rapid metabolism should ensure that the *in vivo* concentration of D(-)- β -hydroxybutyrate remains at a tolerable level ¹¹⁴.

Satisfactory *in vivo* biocompatibility and toxicological acceptability of the PHB homopolymer was indicated by the early work of Korsatko *et al.* ¹¹⁵, involving the subcutaneous implantation of compressed PHB tablets [molecular weight : 260 000] into the neck folds of mice. Subsequent work by these researchers ¹¹⁶ using the same experimental system showed, via histological examinations, that an acute inflammatory response [with the appearance of neutrophils] was initially observed around the implant site, and that was then followed by a chronic inflammatory response [with increased lymphocytes]. This behaviour is a typical host response to a foreign implant, and no necrotic tissue was formed around the tablet. The satisfactory biocompatibility of the PHB was evident at the end of the 20-week study period, at which point the tablets were encapsulated in a soft and well-vascularized connective tissue.

Pouton *et al.* ¹¹⁴ investigated the *in vivo* [rat] biocompatibility of a PHB homopolymer fabricated as microspheres. Following administration via intramuscular [thigh] injection, the inflammatory response was assessed by monitoring the level of alkaline phosphatase around the injection site. This enzyme is a lysosomal hydrolase and is particularly abundant in the polymorphonuclear leukocytes which are present during both acute and chronic inflammatory responses. The injection of either the PHB microspheres or a placebo [1% methylcellulose in saline] prompted an elevation in the level of alkaline phosphatase near the site of administration, suggesting acute inflammatory reactions. However, the increased enzyme levels were similar for each

formulation, and were normal again after 10 days. It was therefore concluded that the inflammation detected was only related to the injection trauma and not the PHB.

Juni and Nakano⁵³ also evaluated the biocompatibility of PHB microspheres following their injection into the thigh muscles of rats. Although this work differed from that of Pouton *et al.*¹¹⁴ in that Juni and Nakano assessed the effects of the polymer via histological examinations, the findings of the two studies were very similar. Thus, Juni and Nakano detected a mild inflammatory response in the local area around the injection site which ended after 7 days. After 4 weeks post-injection, the microspheres were completely encapsulated within connective tissue⁵³.

In summary, the evidence available from the literature at the present time suggests that the PHB homopolymer and its principle degradation product, D(-)- β -hydroxybutyrate, are well-tolerated *in vivo*. However, little information regarding the *in vivo* acceptance of copolymers of PHB with HV is available, although *in vitro* cell culture work, recently performed within the laboratories of the Speciality Materials Research Group, has indicated that these materials have no deleterious effects on cell growth¹¹³. In addition, PHB is not licensed in either the UK or the US with the regulatory authorities for use as an internal medical device.

1.5.3. Microencapsulation with P(HB-HV) polymers.

Brophy and Deasy¹¹⁷ employed an unusual technique to produce sulphamethizole-loaded microparticles from various P(HB-HV) polymers. The preparative procedure involved the preparation of a drug / polymer matrix via solvent evaporation, and the subsequent grinding of this matrix to produce microparticles. The latter were observed to be very irregular, and had pores, cracks and occasionally exposed crystals present on

their surfaces. The rate of drug release from microparticles prepared from PHB homopolymers was enhanced by reducing the particle size and by increasing the polymer molecular weight. The former observation was attributed by the authors to smaller particles having a greater surface area per unit weight, a higher concentration of surface drug, and less depth in the matrix in relation to larger particles. However, as a consequence of both solubility and permeability effects, the rate of transport of small molecules [e.g. sulphamethizole] through a polymer matrix or membrane should diminish as the polymer molecular weight rises. That the opposite trend was observed by Brophy and Deasy suggested that partition-dependent diffusion through the polymer matrix was not the primary mechanism of release in their system. Instead the sulphamethizole was probably discharged by aqueous diffusion through the pores and cracks present in the microparticle matrices. It was suggested that the higher molecular weight forms of the PHB homopolymer deposited more unevenly in the matrix than the lower molecular weight forms, and therefore gave rise to more porous products.

Bissery *et al.*⁸⁷ reported the preparation of drug-loaded PHB microspheres via the use of an o/w single emulsion-solvent evaporation technique. The encapsulated drugs included progesterone, 5-fluorouracil and lomustine at payloads of up to 17 weight percent. It was noted that microspheres with highly porous surfaces were produced.

Juni *et al.*¹¹⁸ encapsulated the anticancer drug, aclarubicin, within PHB microspheres using an o/w single emulsion-solvent evaporation process. However, the *in vitro* rate of drug release from particles containing 12.7 weight percent aclarubicin was found to proceed slowly. Indeed, only about 10% of the encapsulated material was released during 120 hours of dissolution. In order to enhance the rate of aclarubicin discharge, a series of fatty acids and their alkyl esters were incorporated into the microspheres. This was done by co-dissolving the steroid with the polymer in the

dispersed phase solvent. Ethyl and butyl esters of fatty acids, with more than 12 and 10 carbon atoms respectively in the acyl chain, were found to substantially enhance the rate of drug release, an effect that became more marked as the steroid / polymer ratio increased. A subsequent paper by these researchers attempted to identify the mechanism by which the fatty acids enhanced the aclarubicin release kinetics ⁵¹. This work suggested that the drug probably diffused through channels, formed by the esters in the microsphere matrices.

A German patent, filed by Sandow and Seidel ¹¹⁹, describes three different emulsion-solvent evaporation processes for the preparation of PHB microparticles. Each of these processes utilizes a halogenated hydrocarbon, such as chloroform or methylene chloride, as the dispersed phase [i.e. polymer] solvent. However, in one case, silicone oil is the continuous phase solvent and this is therefore a non-aqueous single emulsion process. A second method for the production of monolithic microspheres is described, via the use of an o/w single emulsion process with an aqueous continuous phase. The third process described is a double emulsion [i.e. w/o/w] technique for the manufacture of reservoir-type microcapsules. The o/w single emulsion, and the w/o/w double emulsion, processes described in this patent are similar to the microencapsulation techniques utilized in this thesis.

2.1. Materials.

In the following subsections, the various reagents and materials used in the experimental work are listed together with details of source and batch number [where applicable].

2.1.1. Polymers.

POLY- ϵ -CAPROLACTONE [PCL] : Obtained in pellet form as two different batches [PCL-I and PCL-II] from the Aldrich Chemical Company Ltd, Gillingham, Dorset, UK. The weight-average and number-average molecular weights [M_w and M_n respectively] were determined by gel permeation chromatography [GPC] at the Polymer Supply and Characterisation Centre [PSCC], RAPRA Technology Ltd, Swadby, Shrewsbury, Shropshire, UK. For PCL-I, $M_w = 132\ 000$, $M_n = 70\ 500$ and PCL-II, $M_w = 64\ 000$, $M_n = 34\ 200$. The utilization of two different batches was necessitated by the supplier changing from PCL-I to PCL-II during the course of the experimental work.

CHAPTER TWO.

MATERIALS AND METHODS.

POLY- β -HYDROXYBUTYRATE HOMOPOLYMERS AND COPOLYMERS THEREOF WITH 3-HYDROXYVALERATE [PHB-HV] POLYMERS] .

For the structural formulae, see diagram 2.1.

Obtained from ICI Biopolymers Ltd, Stockton-on-Tees, Cleveland, UK. The polymer employed, together with their β -hydroxyvalerate content and batch numbers are summarized in table 2.1. Also included are M_w and M_n as determined by GPC at the PSCC, RAPRA Technology Ltd.

The various PHB-HV polymers utilized in these studies are identified in the text in terms of both molecular weight and β -hydroxyvalerate [HV] content. The former is

2.1. Materials.

In the following subsections, the various reagents and materials used in the experimental work are listed together with details of source and batch number [where applicable].

2.1.1. Polymers.

POLY- ϵ -CAPROLACTONE [PCL] : Obtained in pellet form as two different batches [PCL I and PCL II] from the Aldrich Chemical Company Ltd, Gillingham, Dorset, UK. The weight-average and number-average molecular weights [M_w and M_n respectively] were determined by gel permeation chromatography [GPC] at the Polymer Supply and Characterization Centre [PSCC], RAPRA Technology Ltd, Shawbury, Shrewsbury, Shropshire, UK. For PCL I, $M_w = 132\ 000$, $M_n = 70\ 500$ and PCL II, $M_w = 64\ 000$, $M_n = 34\ 200$. The utilization of two different batches was necessitated by the supplier changing from PCL I to PCL II during the course of the experimental work.

POLY- β -HYDROXYBUTYRATE HOMOPOLYMERS AND COPOLYMERS THEREOF WITH 3-HYDROXYVALERATE [P(HB-HV) POLYMERS] :

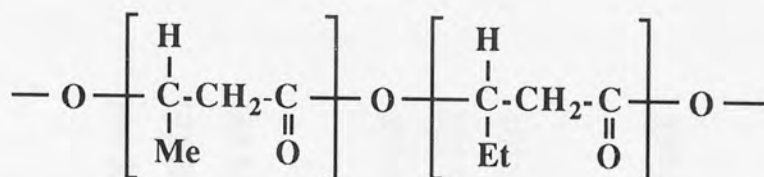
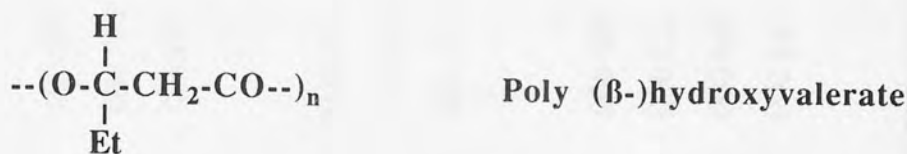
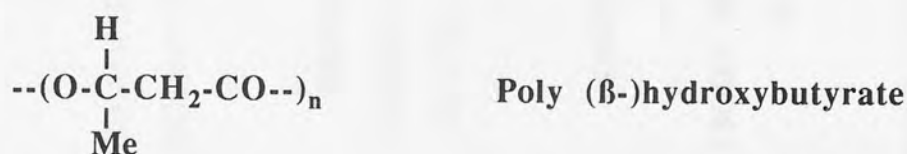
For the structural formulae, see diagram 2.1.

Obtained from ICI Biopolymers Ltd, Stockton-on-Tees, Cleveland, UK. The batches employed, together with their ' as supplied ' molecular weights and batch numbers are summarized in table 2.1. Also included are M_w and M_n as determined by GPC at the PSCC, RAPRA Technology Ltd.

The various P(HB-HV) polymers utilized in these studies are identified in the text in terms of both molecular weight and 3-hydroxyvalerate [HV] content. The former is

expressed as 10^{-3} x the ' as supplied ' molecular weight. For the copolymers, the HV content follows in parentheses as a percentage . Thus, for example, 350 PHB (12% HV) is the 12% 3-hydroxyvalerate copolymer with a molecular weight of 350 000. In all instances, the polymers were used without further purification for the production of microparticles.

Diagram 2.1 : Structural formulae of poly- β -hydroxybutyrate, poly- β -hydroxyvalerate and poly- β -hydroxybutyrate / poly- β -hydroxyvalerate copolymers.



Poly(β -hydroxybutyrate)/Poly(β -hydroxyvalerate) copolymer

2.1.2. Solvents.

All solvents were used without further purification.

METHYLENE CHLORIDE = DICHLOROMETHANE [DCM] : Reagent grade, purchased from the Aldrich Chemical Company Ltd.

CHLOROFORM = TRICHLOROMETHANE [TCM] : Spectral grade, purchased

Table 2.1. Batch and full molecular weight details of the P(HB-HV) polymers used in these studies.

POLYMER	Manufacturer's Batch Number	'As Supplied" Molecular Weight	M_w	GPC determined:		M_w / M_n
				M_n		
<u>PHB HOMOPOLYMERS:</u>						
43PHB	BX GV6 [EE] T-82	43 000				
159PHB	BX GC8/89B MBL 100/985	159 000	199 000	71 700		2.78
273PHB	BX GV6 [EE] T-44	273 000				
540PHB	BX GO8 MBL 100/985	540 000	539 000*	131 000*		4.10*
<u>10.8% HV COPOLYMERS:</u>						
83.1PHB (10.8% HV)	BX PV12B MBL 100/1083	83 100	101 500	47 800		2.12
180PHB (10.8% HV)	BX PV12/1 MBL 100/1083	180 000	227 900	98 250		2.32
330PHB (10.8% HV)	BX PO28 MBL 100/806	330 000	335 700	125 600		2.67
<u>20.1% HV COPOLYMERS:</u>						
32.8PHB (20.1% HV)	BX P025 RUN 256 MBL 100/1083	32 800	52 550	30 990		1.70
140PHB (20.1% HV)	BX P025 RUN 89D MBL 100/1083	140 000	173 200	68 570		2.53
390PHB (20.1% HV)	BX P025 MBL 100/806	390 000	394 500	83 610		4.72

* As determined by the manufacturer [ICI Biopolymers Ltd].

from the Aldrich Chemical Company Ltd.

2.1.3. Colloids.

GELATIN [Type 1, approximately 300 bloom, derived from swine skin] and low gelling temperature AGAROSE [type VII] were supplied by the Sigma Chemical Company, Poole, Dorset, UK.

POLY(VINYL ALCOHOL) [PVA], 88% hydrolysed, average $M_w = 77\ 000-79\ 000$, was purchased from the Aldrich Chemical Company Ltd.

2.1.4. Materials for encapsulation.

FLUORESCEIN ISOTHIOCYANATE-LABELLED-DEXTRANS [FITC-DEXTRANS] : Having molecular weights of 4 KD [FD-4], 20 KD [FD-20] and 150 KD [FD-150] were purchased from the Sigma Chemical Company Ltd.

BOVINE SERUM ALBUMIN [BSA] and BOVINE INSULIN [crystallized] were supplied by the Sigma Chemical Company Ltd.

HYDROCORTISONE [HC] : Manufactured by the Upjohn Company Inc, Kalamazoo, Michigan, USA was generously supplied by Squibbderm, Deeside, UK, as a micronized powder.

2.1.5. Dissolution media.

BORAX BUFFER [pH 7.4] : Prepared by adding 1 volume of 0.05M borax [19.07 g borax / 1L distilled water] to 9 volumes of 0.02M boric acid [12.37 g boric acid / 1L distilled water]. Borax and boric acid were purchased from the Sigma Chemical Company Ltd.

PHOSPHATE BUFFERED SALINE [PBS] : "PBS Dulbecco's", obtained from Gibco Ltd, Uxbridge, Middlesex, UK.

2.1.6. Materials used in the Lowry Method of total protein determination.

LOWRY REAGENT : Prepared by combining 50 mls of 2% [w/v] Na_2CO_3 [in 0.1M NaOH] with 0.5 mls of 1% [w/v] CuSO_4 solution and 0.5 mls of 2% [w/v] sodium / potassium tartrate solution.

FOLINS AND CIOCALTEU'S PHENOL REAGENT [FOLINS REAGENT] : Obtained from BDH Chemicals Ltd, Poole, Dorset, UK.

2.1.7. Materials used for insulin radioimmunoassay.

ANTI-INSULIN ANTI-BODY : Purchased from Wellcome PLC.

BOVINE INSULIN : ^{125}I -labelled, was purchased from Amersham International PLC.

POLYETHYLENE GLYCOL [PEG] : Purchased from BDH Chemicals Ltd.

γ -GLOBULIN [Sheep] : Purchased from Wellcome PLC.

2.2. Experimental technique for the production of microcapsules for the morphological investigations described in chapters three and four.

2.2.1. Polymer solution preparations.

The work described in chapter three concerns microcapsules prepared from nine different P(HB-HV) polymers, and these are divided into three groups on the basis of

HV content : group 1 contains PHB homopolymers, group 2 contains copolymers with 10.8% HV, and group 3 contains copolymers with 20.1% HV. Molecular weight variations are divided into three molecular weight bands : band A contains the lowest molecular weight polymers [molecular weight 32,800 - 83,100], band C the highest [molecular weight 330,000 - 540,000], and band B those polymers with molecular weight values intermediate between groups 1 and 3 [molecular weight 140,000 - 180,000]. Table 2.2 details the polymers within the framework of this classification system.

Table 2.2. Classification of the P(HB-HV) polymers used for the preparation of microcapsules in chapter three.

	BAND A	BAND B	BAND C
GROUP 1	43PHB	159PHB	540PHB
GROUP 2	83.1PHB (10.8% HV)	180PHB (10.8% HV)	330PHB (10.8% HV)
GROUP 3	32.8PHB (20.1% HV)	140PHB (20.1% HV)	390PHB (20.1% HV)

The work in chapter four involves the formation of microcapsules from blends of P(HB-HV) polymers with PCL. For this purpose, the polymers listed in table 2.2 were employed, as were two further P(HB-HV) polymers. These are 273PHB, which is described alongside 540PHB as a band C, group 1 polymer, and 350PHB (12% HV), which is not included in a classification system.

Polymer solutions [typically 6% w/v] were prepared by adding the P(HB-HV) polymer and / or PCL to DCM [50 mls] in a glass reagent bottle, which was then tightly sealed with a PTFE screw cap. Polymer solvation was effected via

ultrasonication at 40°C for 30 minutes. However, optically clear solutions were produced in only two cases, those of PCL [forms I and II] and 43 PHB. 540PHB appeared to be predominantly undissolved, whilst the other polymers appeared to have solvated, giving slightly hazy solutions that were noticeably more viscous than the pure solvent. The 540PHB solution obtained these characteristics only after being placed in a water bath at 90°C for 10 minutes. The general lack of optical clarity of the P(HB-HV) polymer solutions can be attributed to the presence of bacterial contaminants, largely high molecular weight polysaccharides [although 350PHB (12% HV) also contained 1% [w/w] hydroxyapatite, which was insoluble in DCM].

2.2.2. Preparation of the continuous aqueous phase.

The continuous aqueous phase was a 0.5% [w/v] solution of PVA. This was prepared in 2L batches by adding 10 g of colloid to 2L of distilled water, and stirring at approximately 2000 rpm with a high shear blade until the PVA had dissolved.

2.2.3. Preparation of the inner aqueous phase.

This consisted of a 6% [w/v] gelatin solution, typically prepared by ultrasonicing 3 g of gelatin in 50 mls of distilled water at 35-40°C.

2.2.4. Microcapsule preparation.

The procedure described in this section is outlined schematically in diagram 2.2.

A small aliquot [typically 1.5 mls] of inner aqueous phase at 37°C was added [in two steps, via a disposable 1 ml plastic pipette, prewarmed to 37°C] to a 10x volume of 6% polymer solution at the same temperature in a screw-capped glass reagent bottle. The sealed bottle was then vigorously shaken at room temperature for 1 minute to create

a fine water-in-oil [w/o] emulsion. This mixture will subsequently be referred to as the dispersed phase.

After the initial w/o emulsion had been rapidly cooled to approximately 20°C, phase combination was effected immediately by adding 15 mls of dispersed phase [in three stages, via a disposable 5 ml glass pipette] to 150 mls of continuous phase, held at room temperature [20-22°C, unless otherwise stated] in a 250 ml glass beaker, and stirred at approximately 700 rpm with a 3-blade propeller. The propeller was positioned in an off-centre position to ensure efficient mixing.

Solvent evaporation was then allowed to proceed for 5 hours under an extraction hood. However, the temperature conditions prevalent during solvent evaporation were an important experimental variable. Essentially, four different treatments were employed and these are referred to as T1 to T4. For experiments conducted at :

T1 - room temperature was maintained throughout the 5 hour evaporation period ;

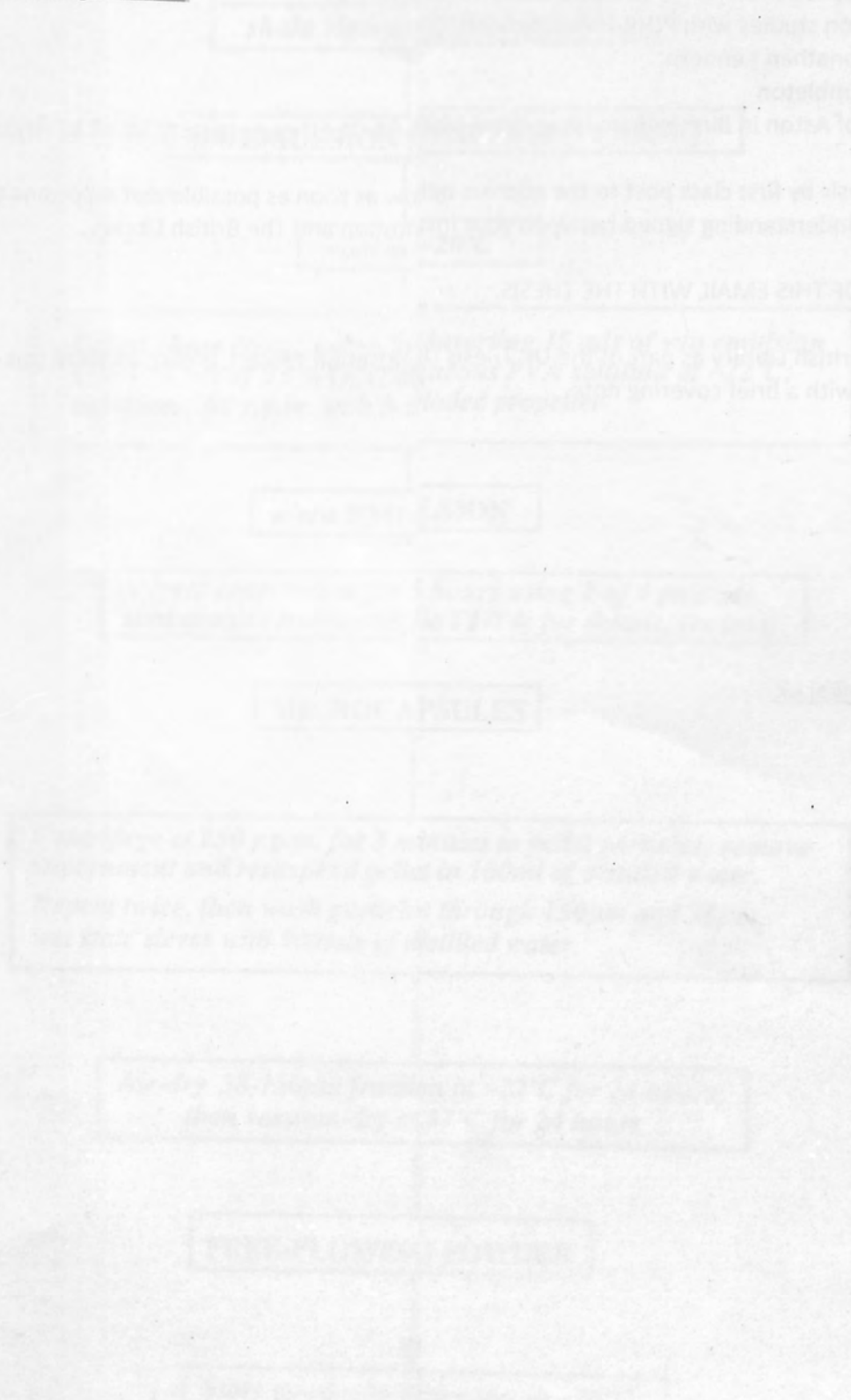
T2 - room temperature was maintained for 70 minutes after phase combination, and then raised to 40°C [by immersing the beaker in a water bath] ;

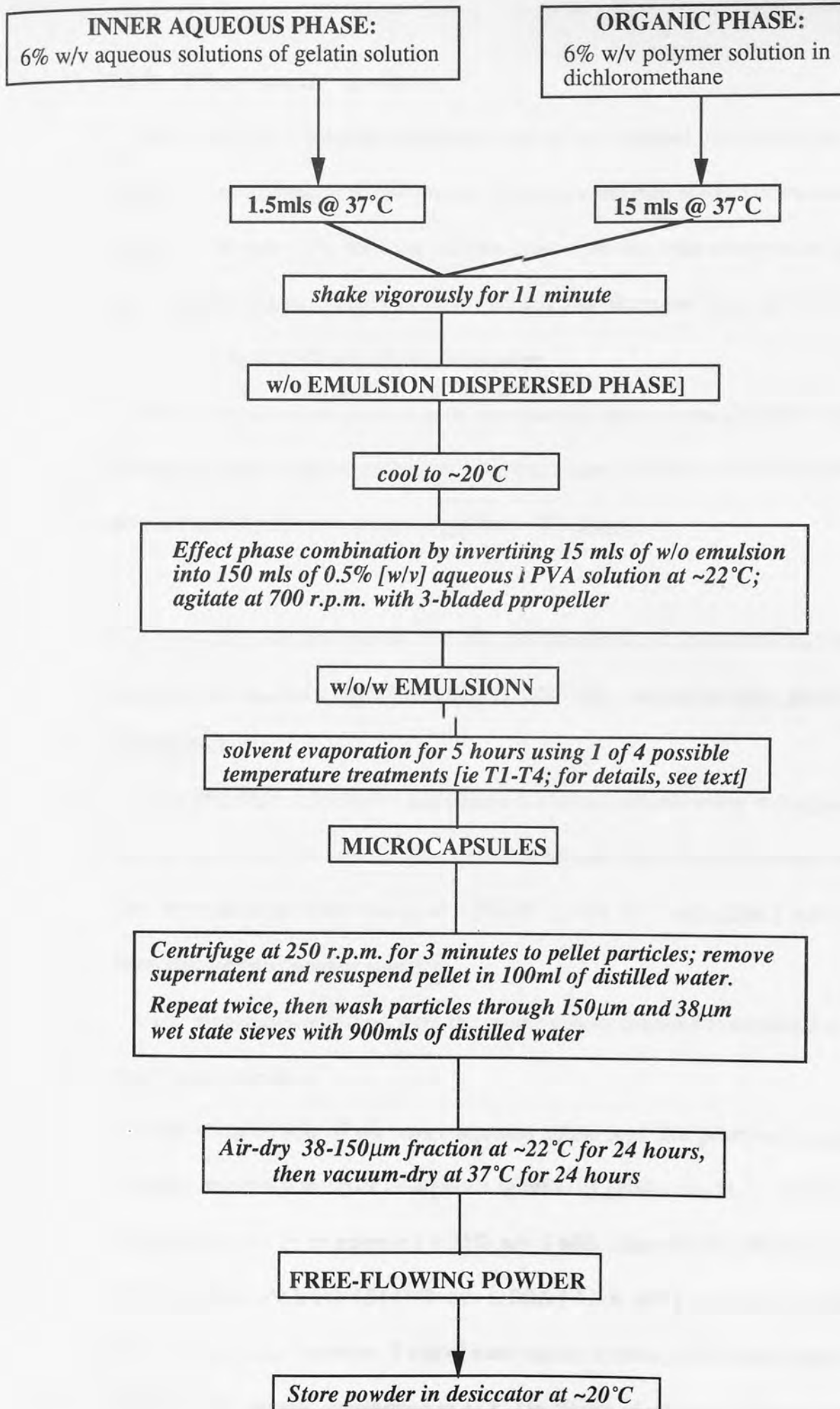
T3 - room temperature was maintained for 35 minutes after phase combination, and then raised to 40°C ;

T4 - room temperature was maintained for 2 minutes after phase combination and then then raised to 40°C.

When temperature treatments T2 - T4 were employed, once the temperature had been raised to 40°C, it was held at this level for the remainder of the 5 hour total solvent evaporation period.

Diagram 2.2. Schematic representation of the procedure described in section 2.2 for the production of microcapsules for morphological investigations.





2.2.5. *Microcapsule isolation.*

After the 5 hour solvent evaporation period had elapsed, the microcapsules were isolated by centrifugation at 250 rpm for 3 minutes and then washed twice with distilled water [2x 100 mls]. The resulting pelleted microcapsules were resuspended in distilled water and thoroughly washed through 150 μ m and 38 μ m wet state sieves [Endecotts Ltd.] with a further 900 mls of distilled water.

The microparticulate product to be retained for analysis was allowed to air-dry for 24 hours at room temperature before being vacuum dried for at least 24 hours at 37°C and subsequently stored at room temperature in a desiccator.

2.3. Experimental technique for the production of macromolecule-loaded microcapsules for the controlled release experiments described in chapter five.

The procedure described in this section is outlined schematically in diagram 2.3. All the microcapsules used for the controlled release experiments described in chapter five were prepared from blends of 330PHB (10.8% HV) with 20% [w/w] PCL II. Each polymer was used as supplied.

The preparative variables were the same as those described in section 2.2, with the following exceptions :

The composition of the inner aqueous phase was the principal experimental variable, and this was either an aqueous solution of gelatin [6, 11, or 16% w/v] with FD-20 [6% w/v] ; or agarose [2.25% w/v] with either FD-4 [9% w/v], FD-20 [4.5, 9 or 18% w/v], FD-150 [9% w/v], BSA [1.6% w/v] or bovine insulin [1.6% w/v]. In each case, however, 2 mls of inner aqueous phase at 37°C was added [via a 2 ml disposable pipette, prewarmed to 37°C] to 30 mls of polymer solution at the same

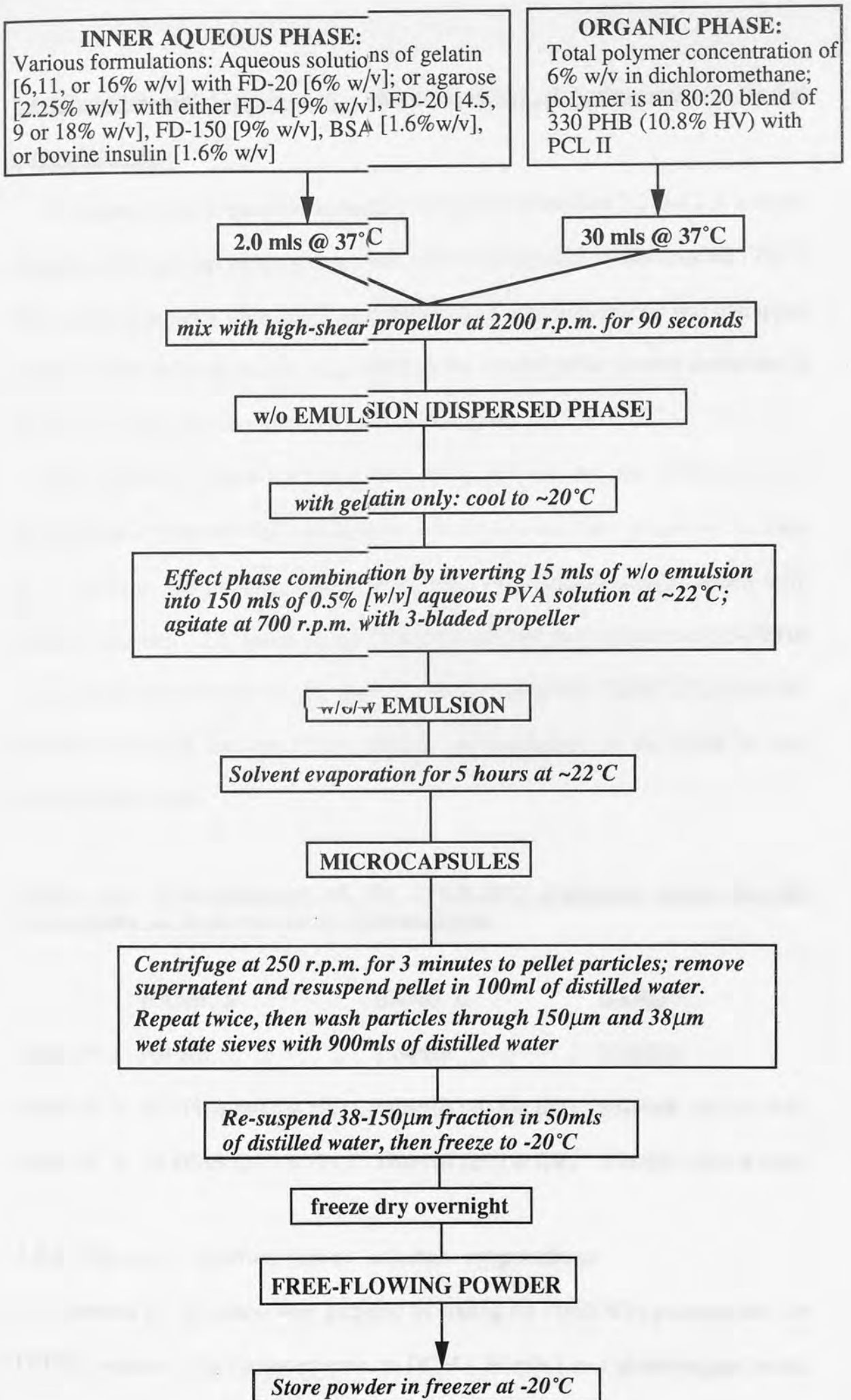
temperature in a specially constructed sealed mixing unit. Mixing was then effected with a high shear propeller at 2200 rpm for 90 seconds. 15 mls of the resulting w/o emulsion was then used for microencapsulation, as described in section 2.2.5, at T1.

However, when the inner aqueous phase contained agarose, the initial w/o emulsion was not cooled to 20°C prior to phase combination. Additionally, during all the FITC-dextran encapsulation experiments, the beaker containing the w/o/w emulsion was protected from the light using aluminium foil.

After the 5 hour solvent evaporation period had elapsed, the microcapsules were isolated by centrifugation at 250 rpm for 3 minutes and then washed twice with distilled water [2x 100 mls]. The resulting pelleted microcapsules were resuspended in distilled water and thoroughly washed through 150µm and 38µm wet state sieves [Endecotts Ltd.] with a further 900 mls of distilled water. The fraction retained in the 38µm sieve was then washed into a beaker with approximately 50 mls of distilled water and frozen to -20°C. The microcapsules were then isolated by freeze-drying overnight in an Edwards 'Modulyo' 4L freeze-dryer to produce a free-flowing powder, which was stored in a freezer at -20°C until the onset of the dissolution experiments.

For each different inner aqueous phase formulation, two separate batches of microcapsules were simultaneously produced, and these were then pooled during the sieving procedure. All samples for dissolution analysis were obtained from the 38-150µm pooled fraction.

Diagram 2.3. Schematic representation of the procedure described in section 2.3 for the production of macromolecule-loaded microcapsules.



2.4. Experimental technique for the production of hydrocortisone-loaded microspheres.

In contrast to the preparative techniques described in sections 2.2 and 2.3, a single emulsion-solvent evaporation process was used to encapsulate hydrocortisone [HC]. The resulting particles were monolithic devices, as the hydrocortisone was entrapped within a solid polymer matrix. According to the classification system described in chapter one, these particles will be termed microspheres.

Nine different P(HB-HV) polymers were utilized for the preparation of hydrocortisone microspheres, and these are divided into the three groups on the basis of HV content, and the three bands on the basis of molecular weight, which were detailed in section 2.2.1. However, for the hydrocortisone microsphere work, 540PHB was replaced by 273PHB as the band C, group 1 polymer. Table 2.3 details the polymers used to prepare hydrocortisone microspheres on the basis of this classification system.

Table 2.3. Classification of the P(HB-HV) polymers used for the preparation of hydrocortisone microspheres.

	BAND A	BAND B	BAND C
GROUP 1	43PHB	159PHB	273PHB
GROUP 2	83.1PHB (10.8% HV)	180PHB (10.8% HV)	330PHB (10.8% HV)
GROUP 3	32.8PHB (20.1% HV)	140PHB (20.1% HV)	390PHB (20.1% HV)

2.4.1 Polymer / hydrocortisone solution preparations.

Polymer / HC solutions were prepared by adding the P(HB-HV) polymer and / or PCL II, together with hydrocortisone, to DCM [50 mls] in a glass reagent bottle,

which was then tightly sealed with a PTFE screw cap. Polymer solvation was effected by ultrasonication at 40°C for 30 minutes, but the resulting solutions were all milky in appearance because most of the hydrocortisone remained undissolved.

The concentration of polymer in the polymer / HC solutions was dependent on the molecular weight of the P(HB-HV) polymer. Band A, B, and C polymers were respectively employed at concentrations of 15%, 12% and 8% [w/v], and the reason for this is discussed in chapter six. There were three experiments in which the microencapsulation of hydrocortisone was performed using a P(HB-HV) polymer blended with 20% [w/w] PCL II. In these instances, the *total* polymer concentrations were either 15%, 12% or 8% [w/v], depending on the molecular weight band of the P(HB-HV) polymer as previously described.

With each polymer listed in table 2.3, microencapsulation was performed using hydrocortisone at a 20% loading. The **hydrocortisone loading** [expressed as a percentage] is defined as the ratio, by dry weight, of hydrocortisone to total solids [i.e. drug + total polymer]. The concentration of hydrocortisone in the polymer solutions was therefore dependent on the concentration of the polymer. Additionally, with selected polymer formulations, microencapsulation was also performed using hydrocortisone at 10% and 30% loadings.

To identify a particular hydrocortisone microsphere formulation within the text, an abbreviation system is used which incorporates the molecular weight and HV content details of the P(HB-HV) polymer, the proportion of PCL II [if present], and the hydrocortisone loading.

For example, 43PHB / 20% PCL II / 20% HC characterizes microspheres prepared from a 43,000 molecular weight PHB homopolymer, blended with 20% PCL II , which have a 20% hydrocortisone loading. The exact formulation details and

abbreviations for the various hydrocortisone microsphere batches are summarized in table 2.4.

2.4.2. Preparation of the continuous aqueous phase.

Within the text, unless it is otherwise stated, the hydrocortisone microspheres have been prepared using the 0.5% PVA solution, described in section 2.2.2, as the continuous phase. However, some microspheres were produced using an aqueous phase that was presaturated with hydrocortisone. This solution was prepared by adding approximately 1 g of hydrocortisone to 200 mls of distilled water in a glass reagent bottle, which was then sealed and shaken for 48 hours at room temperature.

Approximately 150 mls of the resulting solution was then removed with a syringe and filtered through a 0.8 μ m 'Millex' filter unit [Millipore Inc.] into a glass reagent bottle. Approximately 0.75 g of PVA was added, the bottle was sealed, and the mixture was then ultrasonicated until the PVA dissolved. Although the PVA may have had some effect on the solubility of the hydrocortisone, this solution was regarded as a 0.5% PVA solution saturated with hydrocortisone for the purposes of the microencapsulation experiments.

2.4.3. Preparation of hydrocortisone microspheres.

The procedure described in this section is outlined schematically in diagram 2.4.

The polymer / HC solution was violently shaken within the sealed glass reagent bottle for approximately 1 minute at room temperature [20-22°C], to ensure that the undissolved hydrocortisone was uniformly suspended throughout the solution.

Phase combination was then effected immediately by adding 15 mls of polymer / HC

Table 2.4. Formulation details for the hydrocortisone microspheres described in section 2.4.

FORMULATION CODE	POLYMER	HC LOADING [% w/w]		CONCENTRATION [% w/v] OF :		
		HC	POLYMER	HC	TOTAL SOLIDS	
<u>BAND A POLYMERS :</u>						
43PHB / 10% HC	43PHB	10.0	15.0	1.7	16.7	
43PHB / 20% HC	43PHB	20.0	15.0	3.8	18.8	
43PHB / 30% HC	43PHB	30.0	15.0	6.4	21.4	
83.1PHB (10.8% HV) / 20% HC	83.1PHB (10.8% HV)	20.0	15.0	3.8	18.8	
32.8PHB (20.1% HV) / 20% HC	32.8PHB (20.1% HV)	20.0	15.0	3.8	18.8	
<u>BAND B POLYMERS :</u>						
159PHB / 10% HC	159PHB	10.0	12.0	1.3	13.3	
159PHB / 20% HC	159PHB	20.0	12.0	3.0	15.0	
159PHB / 30% HC	159PHB	30.0	12.0	5.1	17.1	
180PHB (10.8% HV) / 20% HC	180PHB (10.8% HV)	20.0	12.0	3.0	15.0	
140PHB (20.1% HV) / 20% HC	140PHB (20.1% HV)	20.0	12.0	3.0	15.0	
<u>BAND C POLYMERS :</u>						
273PHB / 10% HC	273PHB	10.0	8.0	0.9	8.9	
273PHB / 20% HC	273PHB	20.0	8.0	2.0	10.0	
273PHB / 30% HC	273PHB	30.0	8.0	3.4	11.4	
330PHB (10.8% HV) / 20% HC	330PHB (10.8% HV)	20.0	8.0	2.0	10.0	
390PHB (20.1% HV) / 20% HC	390PHB (20.1% HV)	20.0	8.0	2.0	10.0	
<u>PCL II BLENDS AND UNBLENDED PCL II :</u>						
43PHB / 20% PCL II / 20% HC	43PHB / 20% PCL II	20.0	15.0	3.8	18.8	
159PHB / 20% PCL II / 20% HC	159PHB / 20% PCL II	20.0	12.0	3.0	15.0	
273PHB / 20% PCL II / 20% HC	273PHB / 20% PCL II	20.0	8.0	2.0	10.0	
100% PCL II / 20% HC	100% PCL II	20.0	15.0	3.8	18.8	

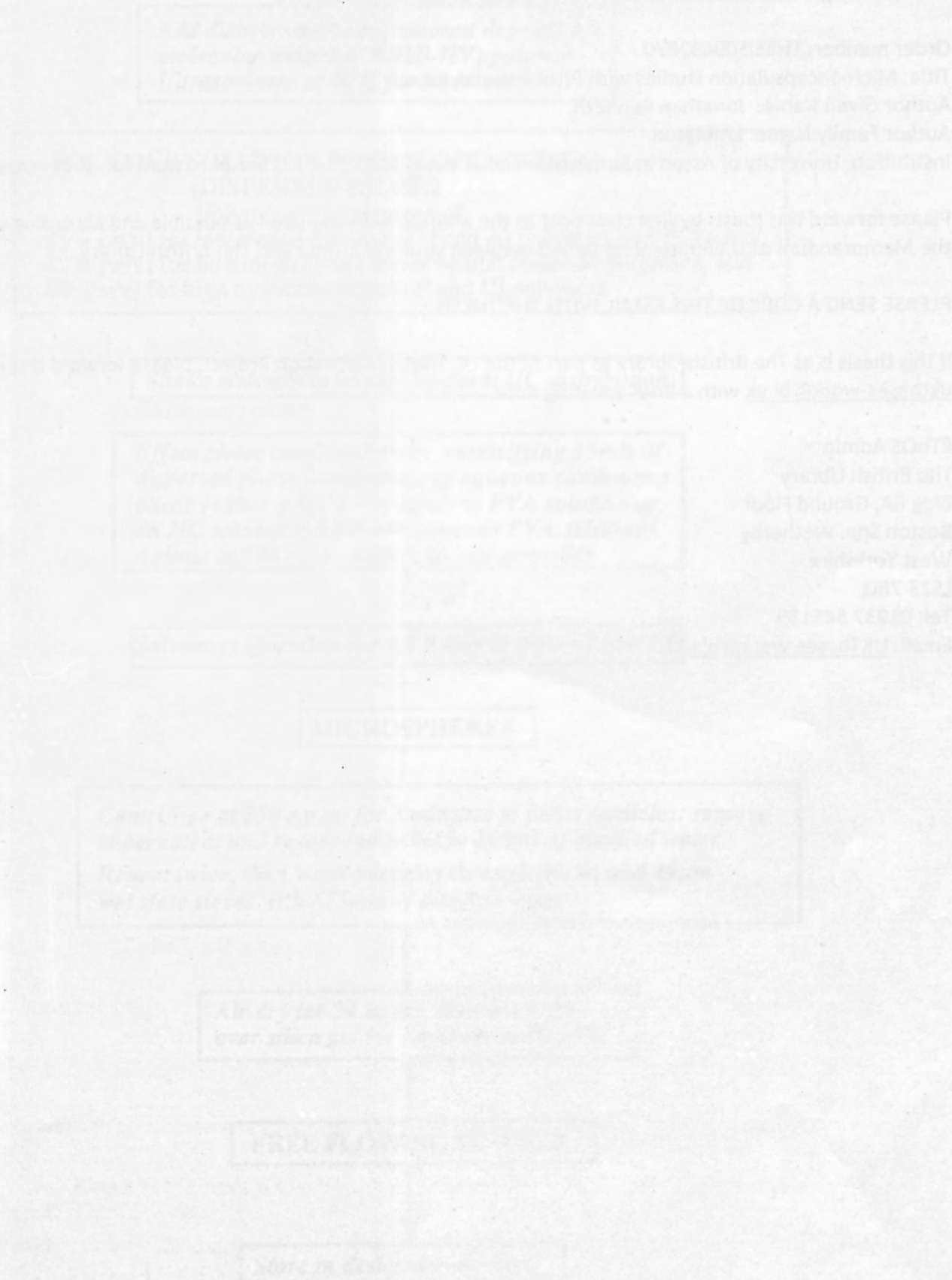
solution [in three stages, via a disposable 5 ml glass pipette] to 150 mls of continuous phase, held at room temperature [20-22°C] in a 250 ml glass beaker and stirred at approximately 700 rpm with a 3-blade propeller. The propeller was positioned in an off-centre position to ensure efficient mixing.

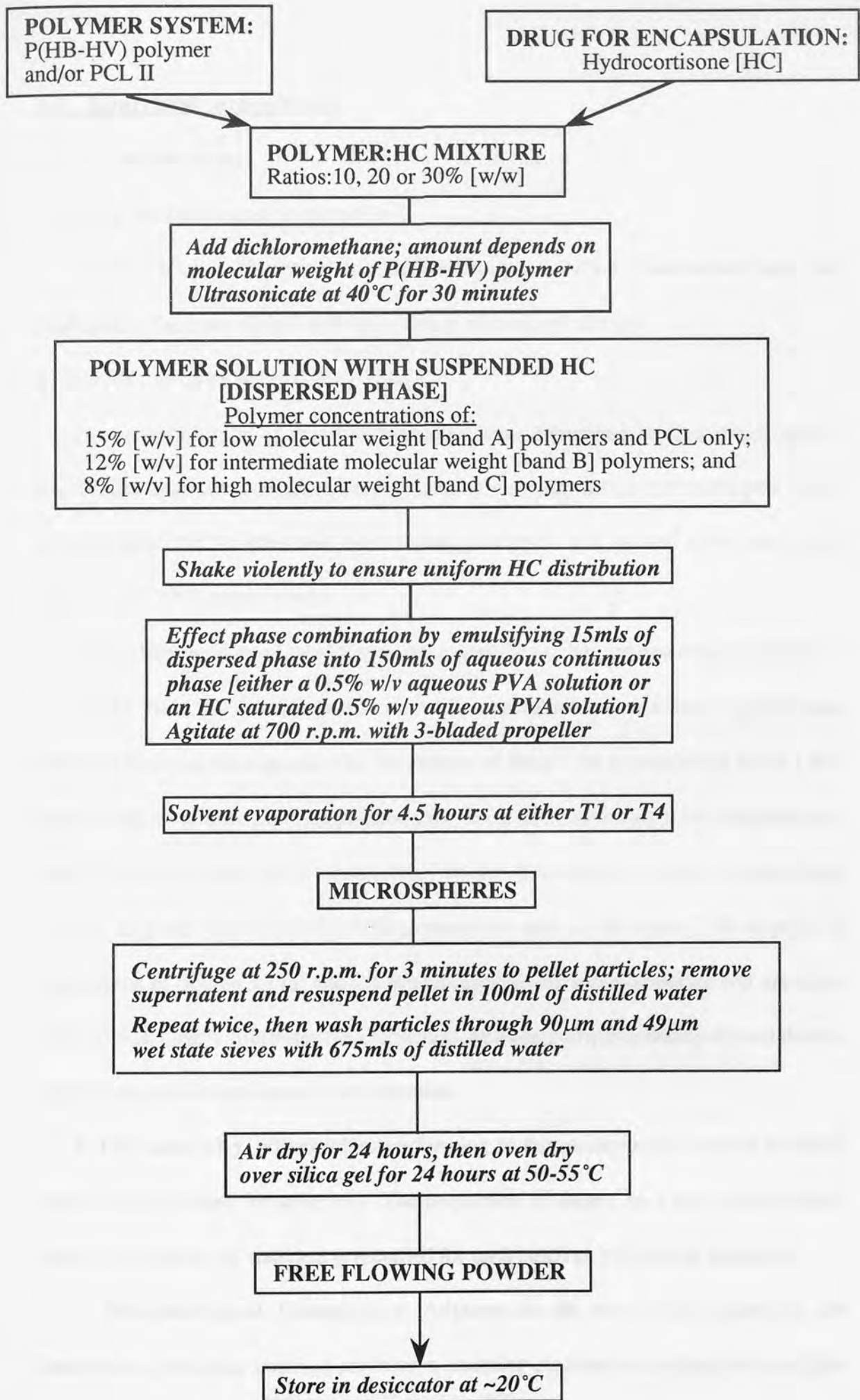
Solvent evaporation was then allowed to proceed for 4.5 hours [at T1, unless otherwise stated] in a fume cupboard.

2.4.4. Microsphere isolation procedure.

After the 4.5 hour solvent evaporation period had elapsed, the microspheres were isolated by centrifugation at 250 rpm for 3 minutes and then washed twice with distilled water [2x 100 mls]. The resulting pelleted microspheres were resuspended in distilled water and thoroughly washed through 90 μ m and 49 μ m wet state sieves [Endecotts Ltd.] with a further 675 mls of distilled water, and the 49-90 μ m fraction was retained for analysis. However, some formulations did not give rise to particles >49 μ m in diameter, and in these cases, 100 mls of the washings were re-centrifuged at 250 rpm for 3 minutes, and the resulting pelleted microspheres were carefully placed on a filter paper to dry. Microspheres retained for analysis were allowed to air-dry for 24 hours at room temperature before being oven dried over silica gel for 24 hours at 50-55°C, and subsequently stored at room temperature in a desiccator.

Diagram 2.4. Schematic representation of the procedure described in section 2.4 for the production of hydrocortisone microspheres.





2.5. Analytical procedures.

2.5.1. Microscopy.

A. Optical and fluorescence microscopy.

A Leitz 'Dialux 20' optical microscope equipped with a fluorescence unit was employed to facilitate optical and fluorescence microscopy studies.

B. Scanning electron microscopy [SEM].

The morphologies of the microparticles were examined in fine detail with a Cambridge Instruments Stereoscan 90 scanning electron microscope equipped with a camera, after the samples had been thoroughly dried and coated with gold in an Emscope SC2200 sputter coater.

Three characteristics of the different microparticle batches are described in the text :

1. The yield. 50 discrete polymer entities were observed on a low magnification scanning electron micrograph. The proportion of these [on a percentage basis] that were judged to be intact, non-aggregated particles is taken as the yield for that particular batch. In the text, batch yields exceeding 75% are described as excellent, yields from 50-75% as good, yields from 25-50% as moderate, and yields below 25% as poor. A comparison of figures 3.3 (a) and (b), which respectively show moderate and excellent batch yields, clearly illustrates the distinction between non-particulate polymer debris, aggregated particles and intact microcapsules.

2. The sphericity. 50 particles, conforming to the quality requirements outlined above, were assessed for sphericity. The proportion of these [on a percentage basis] that were judged to be spherical is recorded for each batch as the level of sphericity.

3. Morphological Consistency. Adjacent to the text which describes the features of a particular batch of particles, a scanning electron micrograph of a particle

that is considered to be representative will normally be shown. In order to provide an indication of the degree to which the morphological characteristics of this particle typify those of the batch as a whole, the micrograph has been compared to at least 20 randomly selected, additional microcapsules. The proportion of these [on a percentage basis] that were judged to clearly have the same morphological features is referred to as the morphological consistency. However, in those instances where a scanning electron micrograph of a typical particle is shown alongside the text, but the morphological consistency is not detailed, it can be assumed that this measure was not less than 75%.

For sectioning, the microparticles were embedded in glue on an aluminium specimen mount and then fractured with a razor blade.

2.5.2. Macromolecular release studies.

The release of either FITC-dextran, BSA or bovine insulin from uniformly microporous microcapsules was measured *in vitro* at 4°C and / or 37°C. For this purpose, accurately weighed microcapsule samples [approximately 250 mg, unless otherwise stated] in LP3 tubes were allowed to equilibrate for 30 minutes at the intended dissolution temperature in constant temperature rooms. Thereafter, 2 mls of sterile buffer [at the intended dissolution temperature] was added to each tube, which was then capped and placed on a Denley Spiramix 5 rotator inside the constant temperature room. For periodic sampling, the tubes were gently centrifuged at 250 rpm for 3 minutes to pellet the particles. The supernatant was subsequently removed for analysis [using a syringe equipped with a hypodermic needle] and replaced by 2 mls of fresh buffer [at the intended dissolution temperature]. The microcapsules were then resuspended and dissolution was continued as before.

For each microcapsule formulation, a single preliminary dissolution experiment was

performed in order to establish the general profile and time scale of release. From this experiment, the sampling times in the subsequent accurate dissolution experiments were then selected, and, for each formulation, 10 days was taken as a suitable experimental end-point. The accurate dissolution readings are reported as the mean of duplicate runs and do not take into account the data obtained from the preliminary experiments.

Placebo microcapsules, made from the same formulation, but without the macromolecular marker, were used as controls. The dissolution data is presented graphically in chapter five.

With the FITC-dextran and BSA microcapsules, absorbances were measured using a Pye Unicam SP800 UV spectrophotometer against buffer blanks, and the mean of the duplicate runs was corrected according to the mean of the duplicate controls [in the latter case, the experimental and control samples were first subjected to the Lowry treatment described in section 2.5.4.]. The concentration of FITC-dextran or BSA in each sample was then calculated by correlating the corrected absorbance reading with a predetermined standard curve. The release of ^{125}I -labelled bovine insulin was measured using radioimmunoassay with the same control procedure.

In every experiment, the readings from the duplicate tubes were extremely similar. Indeed, there were no instances where an individual data point deviated from the mean of the duplicates by more than 4.5%. As a result of this high degree of reproducibility, the graphical representation of the data in chapter five includes only the mean of the duplicate values for each data point.

After the final readings [at 10 days] had been taken from the duplicate tubes used in the accurate dissolution runs and the controls, the microcapsules were resuspended

in 2 mls of the appropriate buffer solution and ultrasonicated at 40-50°C for 4 hours.

The quantity of macromolecule was then determined according to the appropriate analytical method. The total quantity of macromolecule released during the dissolution experiment, plus the quantity released during this ultrasonication was taken as the total quantity of macromolecule present in the initial weight of microcapsules. This facilitated an estimate of the **encapsulation efficiency** for each formulation, which is defined as the ratio of the macromolecule content to the macromolecule loading. The **macromolecule content** is itself defined as the total weight of macromolecular marker [FITC-dextran, BSA or bovine insulin] released per unit weight of dried microcapsules [from the 38-150 μ m size range], and the **macromolecule loading** as the weight of macromolecular marker [FITC-dextran, BSA or bovine insulin] per unit weight of total dissolved solids in the initial w/o emulsion. In the text, the macromolecule loading, the macromolecule content and the encapsulation efficiency are all expressed as percentages.

2.5.3. FITC-dextran assay.

Dissolution was effected in a borate buffer medium which stabilized the absorbtion maxima of the FITC-dextrans. The absorbtion maxima of each FITC-dextran was found to be 494nm.

Gel filtration was used to check that the fluorescein isothiocyanate had not dissociated from the dextran during the fabrication process or dissolution study. Thus, a concentrated sample of fresh FITC-dextran in borate buffer was eluted down a Sephadex G50 [medium] column at a flow rate of 1.25 mls per minute. The absorbance was monitored until fluorescein could no longer be detected. The column was subsequently washed [with borate buffer] and the dissolution sample was eluted

down the column. The FITC-dextran eluted as a single band from both the control and experimental samples, implying that no adjustment for dissociation was necessary.

2.5.4. BSA assay.

Dissolution was effected in PBS and quantitative determination of the BSA released was performed using the Lowry method of total protein determination at an absorbance of 750nm. Thus, 2 mls of Lowry Reagent was mixed thoroughly with 0.4 mls of the dissolution sample and left for 10 minutes. This solution was then mixed with 0.2 mls of Folin's Reagent and left for 30 minutes before having its absorbance measured. A standard curve was constructed by applying this procedure to protein solutions of known concentration.

2.5.5. Bovine insulin assay.

Dissolution was effected in PBS containing 0.04% sodium azide as a preservative.

Radioimmunoassay, using PEG to precipitate the antibody-bound insulin from free insulin and antibody, was employed to quantify the amount of insulin released. Thus, 50 μ l of the dissolution sample and 50 μ l of anti-insulin antibody were combined and incubated at 4°C for 4 hours. Thereafter 50 μ l of 125 I-insulin was added and the resulting solution was incubated at 4°C for a further 20 hours. 80 μ l of PEG / γ -globulin solution was subsequently added and the solution was then centrifuged at 2900 rpm for 20 minutes. The supernatant was decanted and its radioactivity measured in a gamma counter [LKB Computa-Gamma].

A standard curve was constructed by applying this procedure to insulin solutions of known concentration.

2.5.6. Microsphere hydrocortisone content determination.

Spectral grade TCM was accurately transferred, from a glass burette, into a screw-capped glass reagent bottle containing an accurately weighed quantity of microspheres [approximately 10 mg]. The volume of solvent added was exactly that required [approximately 20 mls] to produce a microsphere concentration of 0.5 mg / ml. The sealed bottle was then ultrasonicated at 40-45°C for 1 hour, with occasional shaking.

After cooling, a small aliquot of the microsphere solution was removed from the bottle with a glass syringe and filtered through a 0.5µm Millex filter unit into a quartz cuvette. The UV absorbance was then measured at 254nm with a Pye Unicam SP800 UV spectrophotometer against a similarly filtered TCM blank. After correcting for polymer absorbances, which had been predetermined using filtered [with a 0.5µm Millex filter unit] 0.5 mg / ml TCM-polymer solutions, the hydrocortisone concentration was calculated from a standard curve. The results were taken as the mean of duplicate measurements [using two batches of microspheres from the same preparation experiment], and no reading deviated from the mean by more than 2.5%.

The ratio of the hydrocortisone concentration to the original microsphere concentration corresponded to the proportion by weight of hydrocortisone in the undissolved microspheres, and when expressed as a percentage, represents the microsphere **hydrocortisone content**. The **hydrocortisone encapsulation efficiency** is defined as the ratio of the hydrocortisone content to the hydrocortisone loading [as defined in section 2.4.1], and is also expressed as a percentage.

A comprehensive attempt to evaluate the batch-to-batch reproducibility of the hydrocortisone encapsulation efficiency for each different formulation and set of processing conditions was not undertaken due to the large number of samples involved.

However, an indication of the reproducibility of the preparative process was obtained by preparing three separate batches of microspheres from the 159PHB / 20% HC formulation at T1. The three batches were found to have hydrocortisone contents of 16.3%, 16.4% and 17.7%, with a mean of 16.8% and a standard deviation of 0.8%.

2.5.7. Hydrocortisone solubility determination.

The solubility of hydrocortisone in PBS at 37°C was determined by the following procedure. Approximately 100 mg of drug was added to approximately 20 mls of PBS in a screw-capped dissolution tube, which was sealed and placed on a Denley Spiramix 5 rotator inside a 37°C constant temperature room. 5 ml samples were removed after 24 and 48 hours, passed through 5µm and 0.22µm Millex filter units, diluted 1:20 with PBS, and then assayed at 248nm using a Pye Unicam SP800 UV spectrophotometer. The concentration of hydrocortisone in each sample was then calculated by correlating the absorbance reading with a predetermined standard curve. The two readings differed by less than 1%, and the original 48 hour sample was found to contain 0.287 mg / ml of dissolved hydrocortisone. This figure is comparable to the 0.28 mg / ml value for the solubility of hydrocortisone in water at 25°C reported by Cavalier *et al.*¹⁷, and will subsequently be taken as the saturation solubility of hydrocortisone in PBS at 37°C.

2.5.8. Hydrocortisone release studies.

An accurately weighed quantity of microspheres [approximately 10 mg] in a screw-capped dissolution bottle was allowed to equilibrate for 30 minutes at 37°C in a constant temperature room. Thereafter, precisely 10 mls of buffer [also at 37°C] was added with a pipette. The sealed bottle was then placed on a Denley Spiramix 5 rotator inside the 37°C constant temperature room. For periodic sampling, the tubes were

gently centrifuged at 250 rpm for 3 minutes to pellet the particles. Exactly 4 mls of the supernatant was removed for analysis and the remaining dissolution medium was made up to its original volume with 4 mls of fresh buffer [prewarmed to 37°C]. The samples were collected using a fin-pipette equipped with a disposable plastic tip. A specially constructed filter unit, made from the wire-mesh of a 38 μ m wet-state sieve, was placed over the front of the disposable pipette tip as the dissolution sample was withdrawn so as to prevent the removal of any large particulate matter. [Calibration checks using distilled water showed that the pipette was accurate to within 0.01% when this filter unit was in place]. The samples were subsequently passed through 5 μ m Millex filter units to ensure the removal of effectively all particulate matter.

For each microcapsule formulation, a single preliminary dissolution experiment was performed in order to establish the general profile of hydrocortisone release over the first week. On the basis of these results, the sampling times in the subsequent accurate dissolution experiments were selected so as to ensure that [as far as was practically possible] the hydrocortisone concentration in the dissolution medium did not exceed 10% of its solubility, thereby maintaining good a sink condition ¹²⁴. The accurate dissolution readings are presented as the mean of duplicate runs and do not take into account the data obtained from the preliminary experiments. Placebo microcapsules, were made from each polymer formulation, but without hydrocortisone, for use as controls.

Sample absorbances were measured using a Pye Unicam SP800 UV spectrophotometer against buffer blanks at 248nm, and the mean of the duplicate runs was corrected according to the mean of duplicate controls. The concentration of hydrocortisone in each sample was then calculated by correlating the corrected

absorbance reading with a predetermined standard curve. The dissolution data is presented graphically in chapter six, and is tabulated in the appendix. Generally the data points are within 5% of the mean, but there are exceptions. In addition, because of the long periods over which many of the dissolution experiments were continued, it was not possible to replace tubes as a result of sampling errors, and therefore in some instances late data points are from single experiments. The appendix shows where this has been the case, and also includes the standard deviation of the mean for data points derived from duplicate experiments.

2.5.9. Isolation of microspheres from the dissolution medium for SEM analysis.

Bodmeier and McGinity¹²⁵ found that P(D,L-LA) microspheres, which had been hydrated for a few days, underwent structural changes as a consequence of the coating procedure and vacuum treatment associated with SEM analysis, unless they were extensively pre-dried. Since some of the microsphere samples required for SEM in chapter six had been in the dissolution medium for several months, a very careful drying procedure was adopted. Thus, the microspheres were gently removed from the dissolution medium, without the use of a syringe, and placed on a filter paper. They were subsequently left at room temperature and pressure in a desiccator for at least two weeks, and then transferred to an oven where the samples were dried over silica gel at 50-55°C, for at least 72 hours, before being subjected to the SEM procedure described in section 2.5.1. Additionally, samples of dissolution media still containing hydrated microspheres were observed using an optical microscope in order to confirm that the particle shapes detected by SEM were genuine.

3.1. Introduction.

For the studies reported in this chapter, a double emulsion-solvent evaporation process was employed to prepare microcapsules from the nine different poly- β -hydroxybutyrate homopolymers, and copolymers thereof with 3-hydroxyvalerate (P(HB-HV) polymers), listed in table 3.1. The objective was to assess how the preparative variables highlighted in diagram 3.1 affected the yield, shape and surface morphology of the resulting microcapsules. The methods utilized for the quantification of these features are detailed in section 3.3.1.

CHAPTER THREE.

3.2. Effects of polymer composition and molecular weight.

THE EFFECTS OF POLYMER COMPOSITION, MOLECULAR WEIGHT, TEMPERATURE AND POLYMER CONCENTRATION ON P(HB-HV) MICROCAPSULE MORPHOLOGY.

3.2.1. P(HB) Homopolymers.

Scanning electron micrographs of several neat capsules, prepared from the three different molecular weight P(HB) homopolymers which comprise group 1, are shown in figures 3.1 (a-c). Table 3.1 details the yield, sphericity and morphological consistency values determined for these three batches of particles.

The microcapsule prepared from the low molecular weight P(HB) homopolymer (40P(HB)) has a uniform, non-porous, but highly wrinkled surface morphology. The latter feature correlates with the striated appearance noted by Biscery *et al.* (12) when observing P(HB) microspheres prepared using a similar, though single emulsion, process. The degree of morphological consistency within this batch was extremely high, indeed all 20 of the particles closely examined had the same surface features as

3.1. Introduction.

For the studies reported in this chapter, a double emulsion-solvent evaporation process was employed to prepare microcapsules from the nine different poly- β -hydroxybutyrate homopolymers, and copolymers thereof with 3-hydroxyvalerate [P(HB-HV) polymers], listed in table 2.2. The objective was to assess how the preparative variables highlighted in diagram 3.1 affected the yield, shape and surface morphology of the resulting microcapsules. The methods utilized for the quantification of these features are detailed in section 2.5.1.

3.2. Effects of polymer composition and molecular weight.

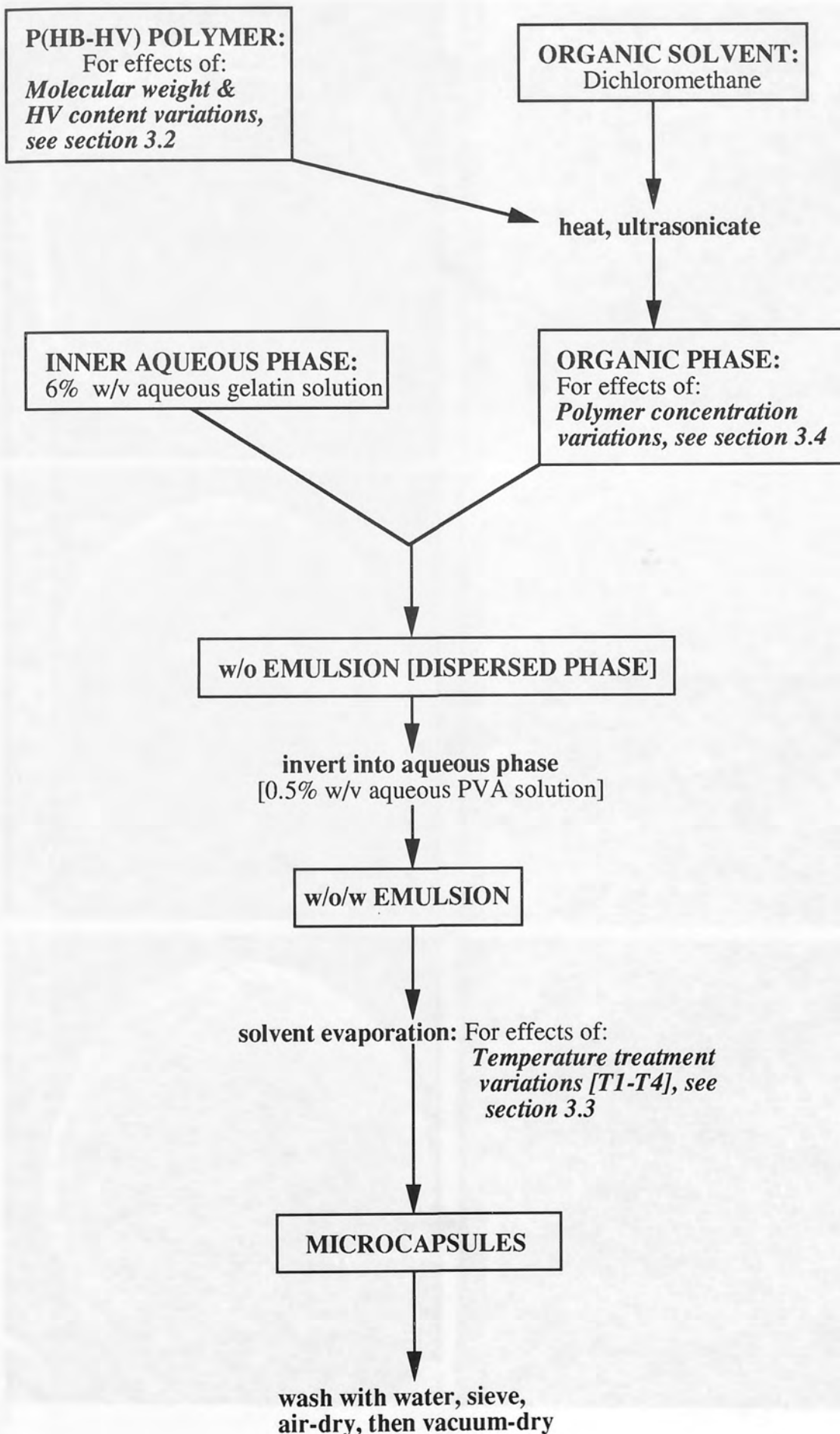
The microcapsules described in sections 3.2.1 - 3.2.3 were prepared using the procedure described in section 2.2, at temperature treatment 1 [T1].

3.2.1. PHB homopolymers.

Scanning electron micrographs of typical microcapsules, prepared from the three different molecular weight PHB homopolymers which comprise group 1, are shown in figures 3.1 (a-c). Table 3.1 details the yield, sphericity and morphological consistency values determined for these three batches of particles.

The microcapsule prepared from the low molecular weight PHB homopolymer [43PHB] has a uniform, non-porous, but highly wrinkled surface morphology. The latter feature correlates with the shriveled appearance noted by Bissery *et al.*¹²³ when observing PHB microspheres prepared using a similar, though single emulsion, process. The degree of morphological consistency within this batch was extremely high, indeed all 20 of the particles closely examined had the same surface features as

Diagram 3.1. Schematic representation of the experimental variables which are investigated for their effects on microcapsule morphology in chapter three.



Figures 3.1 (a)-(c). Scanning electron micrographs of microcapsules prepared at temperature variation T1 from PHB homopolymers with different molecular weights :

(a) 43 000

Low magnification [x1260]

High magnification [x2780]

(b) 159 000

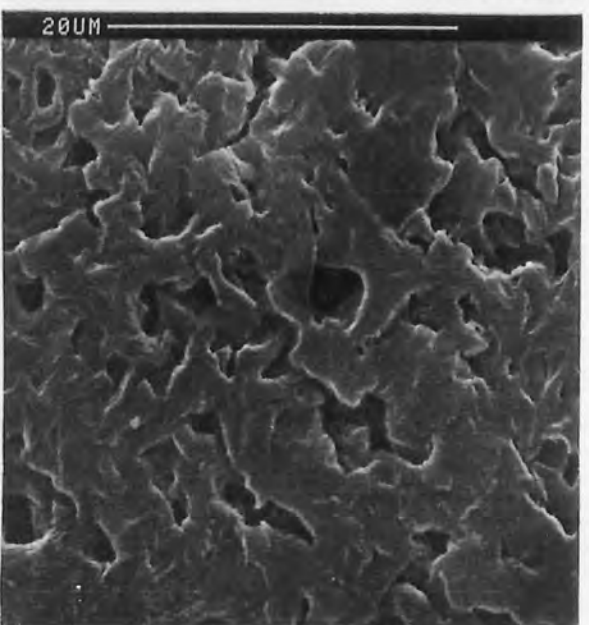
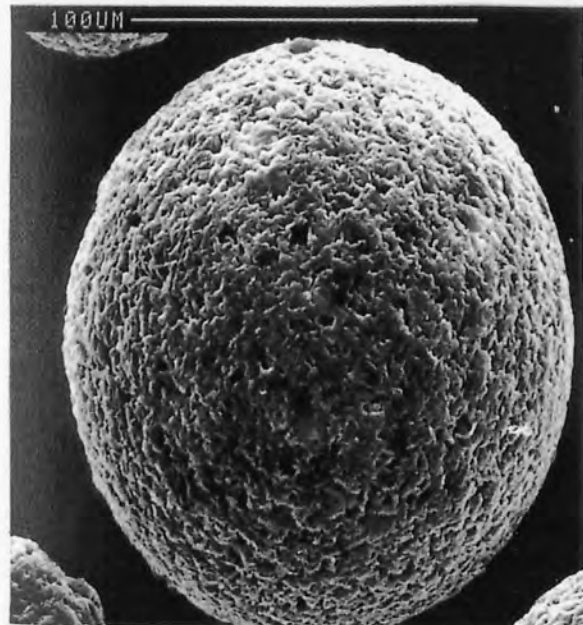
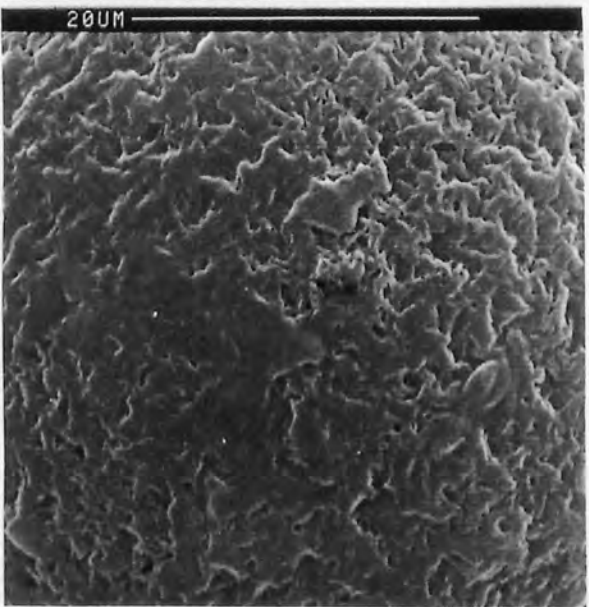
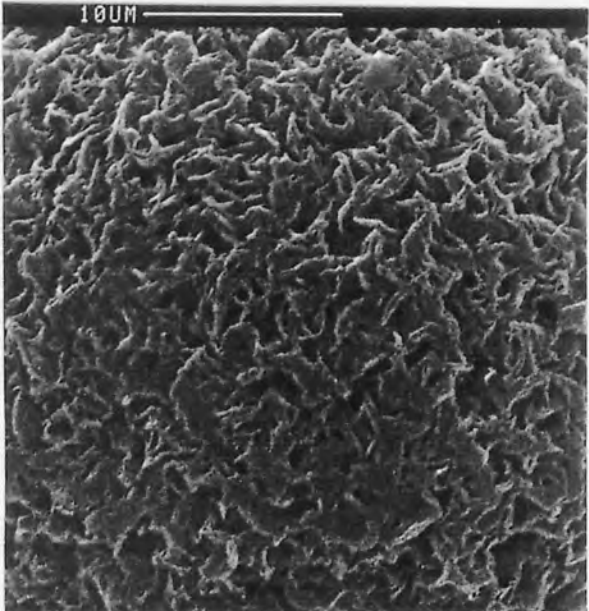
Low magnification [x1060]

High magnification [x2410]

(c) 540 000.

Low magnification [x458]

High magnification [x2410]



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the one shown in figure 3.1 (a). All the particles observed were intact, most were spherical, and there was no polymer debris.

In contrast, many of the microcapsules prepared from 159PHB were mis-formed or aggregated. A considerable quantity of non-particulate polymer debris was also encountered which led to the yield being assessed as only 44%. Figure 3.1 (b) shows a typical particle from this batch as having a well-formed wall that is very slightly wrinkled at the surface, but essentially non-porous. The sphericity and morphological consistency were quite high amongst the intact particles.

The highest molecular weight homopolymer [540PHB] gave rise to an excellent yield of mostly spherical microcapsules. The morphological consistency was 85% in favour of the features exhibited by the particle in figure 3.1 (c). Thus, most particles had compact walls, but surfaces covered by discrete micropores.

Table 3.1. Yield, sphericity and morphological consistency of microcapsules prepared from different molecular weight PHB homopolymers.

POLYMER	YIELD [%]	SPHERICITY [%]	CONSISTENCY [%]
43PHB	100	94	100
159PHB	44	78	80
540PHB	96	82	85

3.2.2. PHB (10.8% HV) copolymers.

Scanning electron micrographs of typical microcapsules, prepared from the three different molecular weight PHB (10.8% HV) copolymers which comprise group 2, are shown in figures 3.1 (d-f). Table 3.2 details the yield, sphericity and morphological consistency values determined for these three batches of particles.

Figures 3.1 (d)-(f). Scanning electron micrographs of microcapsules prepared at temperature variation T1 from PHB (10.8% HV) copolymers with different molecular weights :

(a) 83 100

Low magnification [x842]

High magnification [x2410]

(b) 180 000

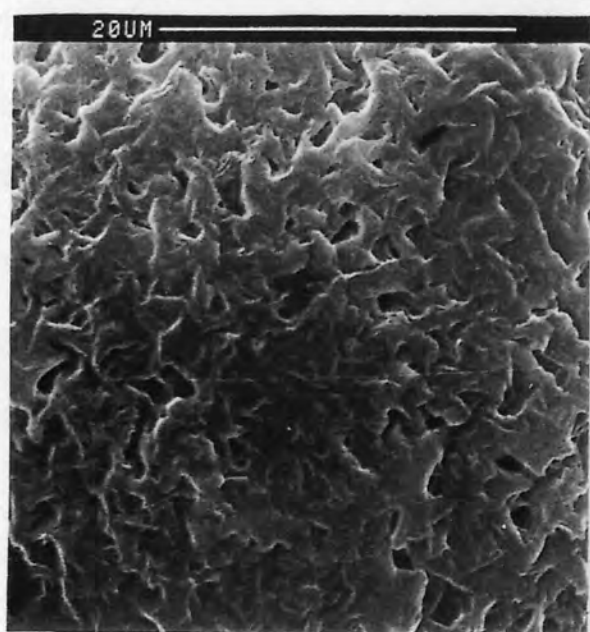
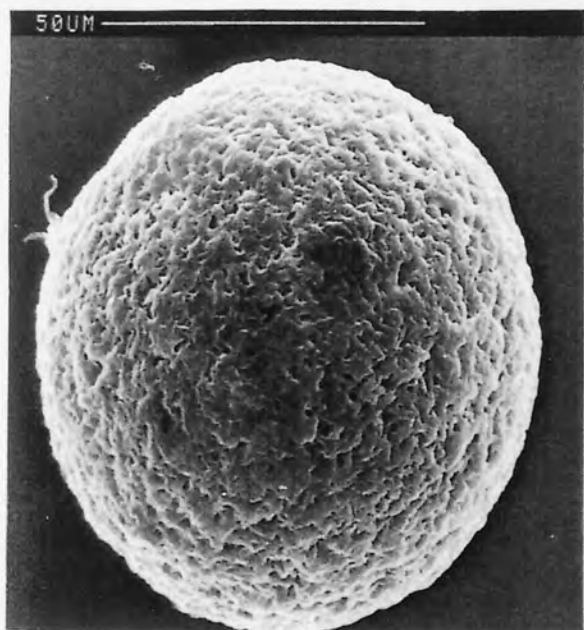
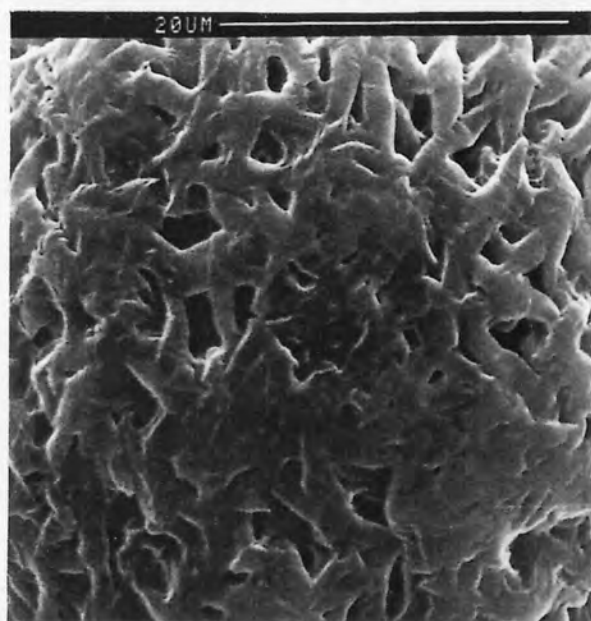
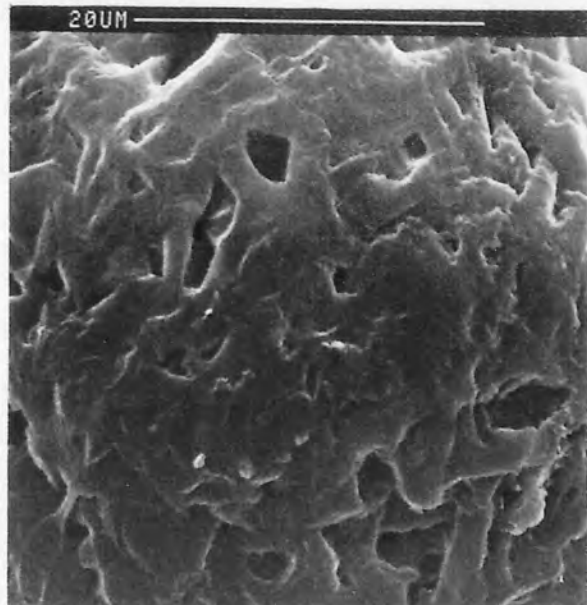
Low magnification [x1060]

High magnification [x2410]

(c) 330 000.

Low magnification [x872]

High magnification [x2370]



Microencapsulation with 83.1PHB (10.8% HV) resulted in an excellent yield of particles, but most were distorted in shape and had irregular surface morphologies. Thus, only 22% of the microcapsules observed were judged to be spherical, and a representative particle is shown in figure 3.1 (d). The polymer chains in the wall of this particle appear to be loosely bound giving rise to a heavily structured and macroporous surface. The irregular nature of particles in this batch complicated the assessment of morphological consistency, which was taken as only 75%.

Microcapsules prepared from 180PHB (10.8% HV) also had heavily structured and macroporous surfaces, with the result that only 16 of the 20 closely examined particles from this batch had the same morphological features as the one shown in figure 3.1 (e). However, in relation to the microcapsules prepared from 83.1PHB (10.8% HV), the 180PHB (10.8% HV) particles displayed a higher level of sphericity, but were produced in much lower yields due to the presence of a substantial quantity of polymer debris.

The high molecular weight polymer within this group [i.e. 330PHB (10.8% HV)] gave rise to a good yield [72%] of mostly spherical particles. The defects in this batch were essentially well-formed particles whose walls had exploded outwards. The representative microcapsule shown in figure 3.1 (f) has a surface morphology reminiscent of particles prepared from 540PHB. Thus, whilst there is a high level of porosity, it does not appear to be a consequence of poor chain deposition, as has been observed with the other polymers in this group. Instead, the pores are similarly-sized and discretely impressed upon a well-formed polymer wall.

Table 3.2. Yield, sphericity and morphological consistency of microcapsules prepared from different molecular weight PHB (10.8% HV) copolymers.

POLYMER	YIELD [%]	SPHERICITY [%]	CONSISTENCY [%]
83.1PHB (10.8% HV)	96	22	75
180PHB (10.8% HV)	54	44	80
330PHB (10.8% HV).	72	72	85

3.2.3. PHB (20.1% HV) copolymers.

Scanning electron micrographs of typical microcapsules, prepared from the three different molecular weight PHB (20.1% HV) copolymers which comprise group 3, are shown in figures 3.1 (g-i). Table 3.3 details the yield, sphericity and morphological consistency values determined for these three batches of particles.

The surface morphologies of particles prepared from these copolymers adopted a similar pattern to the one observed for the 10.8% HV copolymers. Thus, as figure 3.1 (g) shows, microcapsules prepared from 32.8PHB (20.1% HV) typically had heavily structured and macroporous surfaces, although these features were not apparent on a quarter of the 20 particles closely examined. In addition, many of the microcapsules from this batch were distorted in shape, and a large quantity of polymer debris resulted in a yield of only 54%.

The yield of intact microcapsules produced from 140PHB (20.1% HV) was not materially different from the 54% derived from 32.8PHB (20.1% HV), although with the higher molecular weight copolymer, more particles were distorted in shape. In addition, as a comparison of figures 3.1 (h) and (g) reveals, the microcapsules prepared from 140PHB (20.1% HV) had more compact and less porous surfaces than were

Figures 3.1 (g)-(i). Scanning electron micrographs of microcapsules prepared at temperature variation T1 from PHB (20.1% HV) copolymers with different molecular weights :

(a) 32 800

Low magnification [x1100]

High magnification [x2370]

(b) 140 000

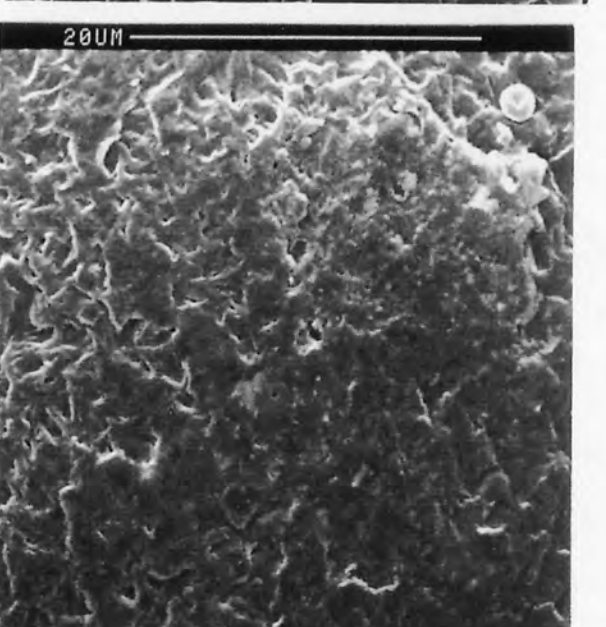
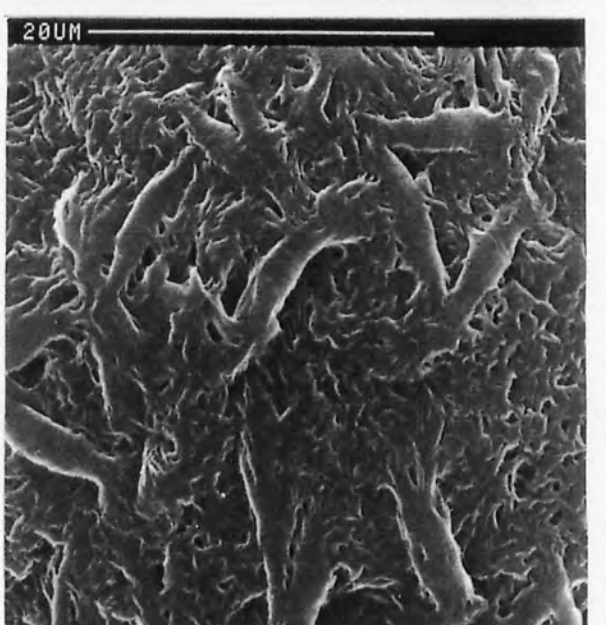
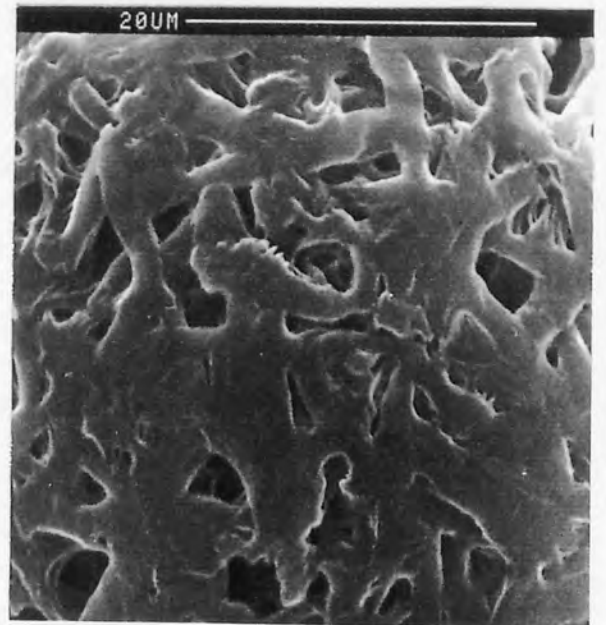
Low magnification [x1130]

High magnification [x2370]

(c) 390 000.

Low magnification [x647]

High magnification [x2370]



typically observed on 32.8PHB (20.1% HV) particles.

A high level of sphericity was found amongst the microcapsules prepared from 390PHB (20.1% HV), but the yield was taken as only 20%. This poor yield was a consequence of a high level of agglomeration amongst otherwise good quality particles. These particles had compact, well-formed walls with slightly wrinkled, but essentially non-porous, surfaces of the type shown by the representative microcapsule in figure 3.1 (i).

Table 3.3. Yield, sphericity and morphological consistency of microcapsules prepared from different molecular weight PHB (20.1% HV) copolymers.

POLYMER	YIELD [%]	SPHERICITY [%]	CONSISTENCY [%]
32.8PHB (20.1% HV)	54	46	75
140PHB (20.1% HV)	52	36	85
390PHB (20.1% HV)	20	78	80

3.3. Effects of temperature.

The microcapsules described in sections 3.3.1-3.3.3 were prepared using the procedure described in section 2.2, but at the temperature treatment specified.

3.3.1. Temperature treatment 2 [T2].

Microcapsules were prepared at T2 from 43PHB, 540PHB, 330PHB (10.8% HV) and 390PHB (20.1% HV).

SEM analysis revealed that there were no significant changes in either the batch or morphological characteristics in comparison to particles prepared from the same

polymer at T1.

3.3.2. Temperature treatment 3 [T3].

Microcapsules were prepared at T3 from 43PHB, 540PHB, 330PHB (10.8% HV) and 390PHB (20.1% HV).

SEM analysis revealed that, for the three high molecular weight band polymers, there were no significant changes in either the batch or morphological characteristics in comparison to particles prepared from the same polymer at T1.

In the case of 43PHB however, there was some evidence that the temperature elevation to 40°C, 35 minutes after phase combination, had an effect on surface morphology. The particles prepared at T3 from this polymer were judged to be slightly smoother and less wrinkled than the sphere shown in figure 3.1 (a).

3.3.3. Temperature treatment 4 [T4].

Microcapsules were prepared at T4 from each of the nine P(HB-HV) polymers listed in table 2.2, and representative particles from these batches are shown in figures 3.2 (a) - (i).

In the case of every polymer investigated, particles prepared at T4 were different in some respect from those prepared at T1. With the exception of particles prepared from 159PHB, T4 particles had smoother and / or less porous or wrinkled surfaces. This trend is clearly illustrated by comparing the features of a microcapsule from figure 3.1 with the corresponding particle in figure 3.2.

Figures 3.2 (a)-(c). Scanning electron micrographs of microcapsules prepared at temperature variation T4 from PHB homopolymers with different molecular weights :

(a) 43 000

Low magnification [x10³0]

High magnification [x2080]

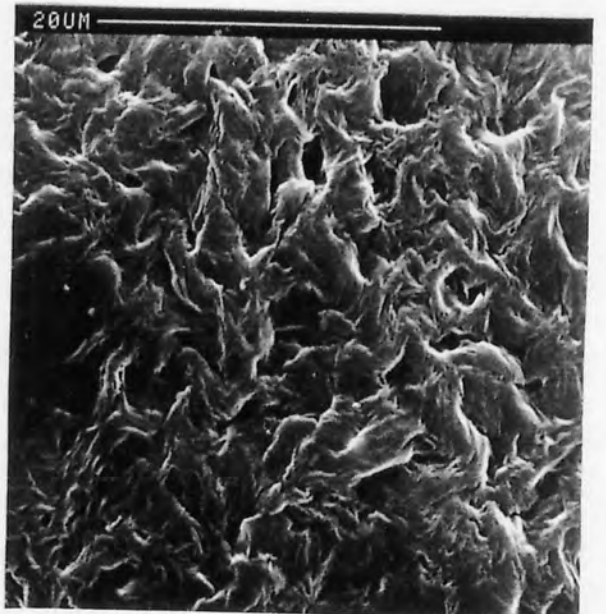
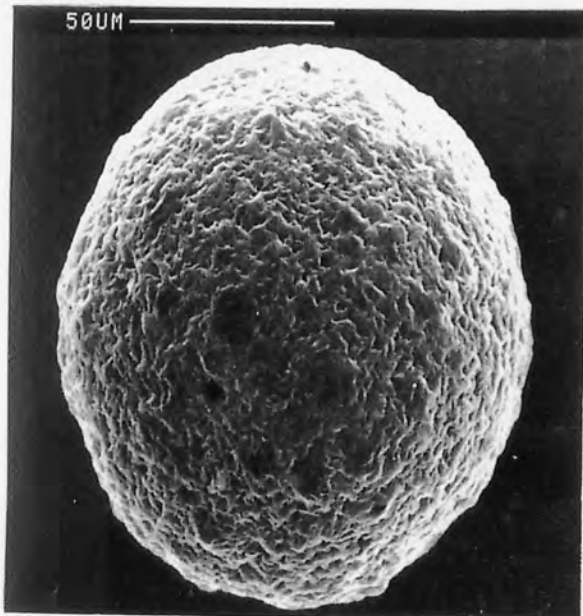
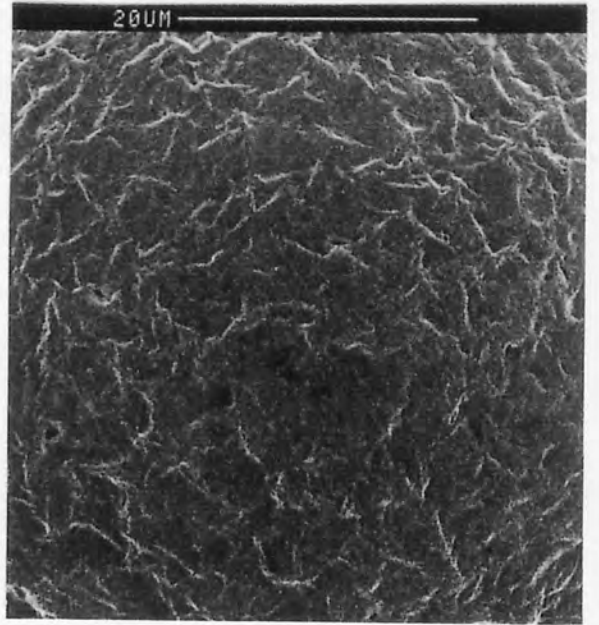
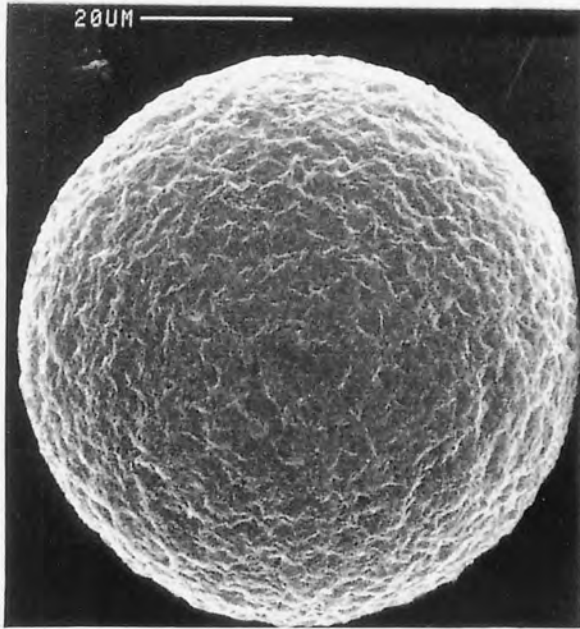
(b) 159 000

Unchanged from T1 - see figure 3.1 (b)

(c) 540 000.

Low magnification [x575]

High magnification [x2410]



Figures 3.2 (d)-(f). Scanning electron micrographs of microcapsules prepared at temperature variation T4 from PHB (10.8% HV) copolymers with different molecular weights.:

(d) 83 100

Low magnification [x1640]

High magnification [x2700]

(e) 180 000

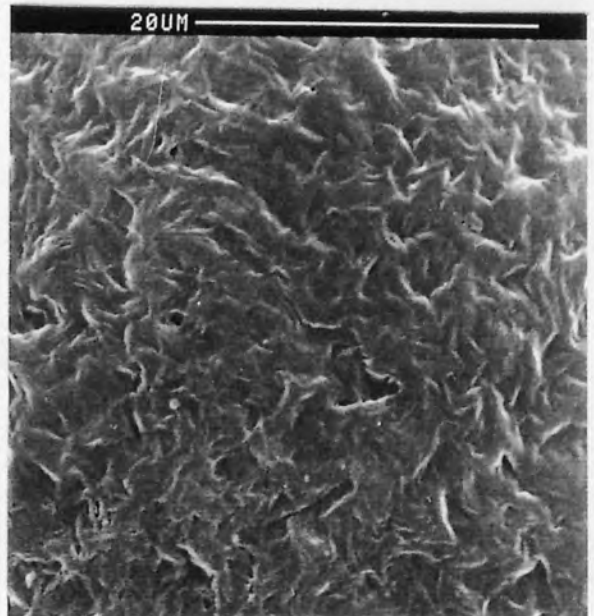
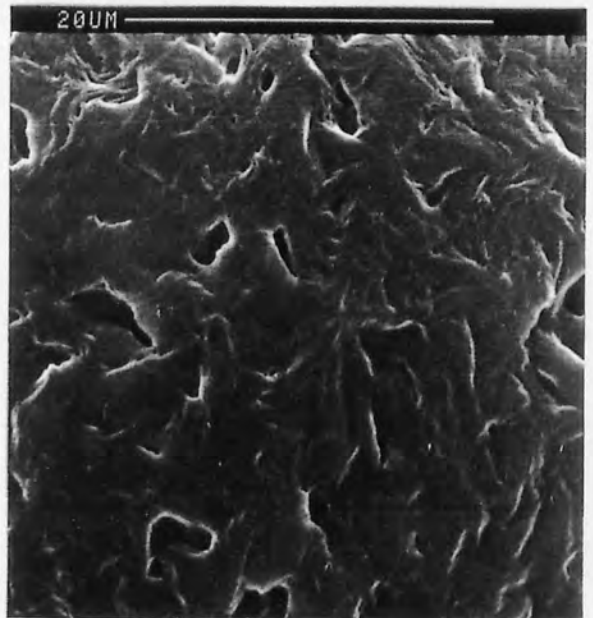
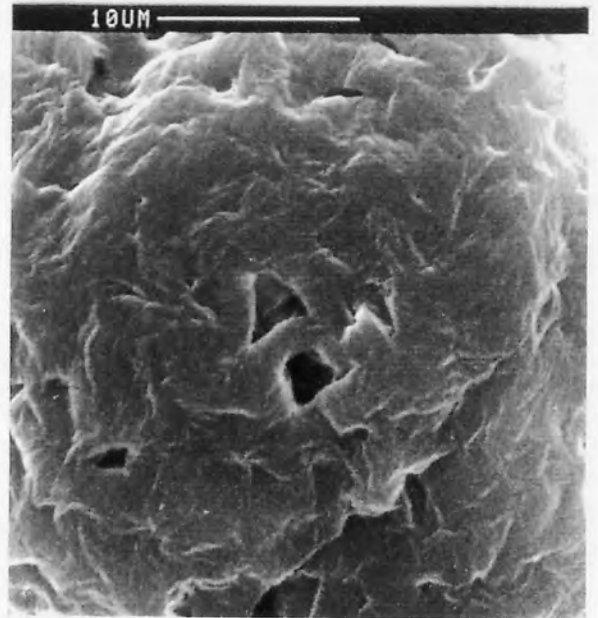
Low magnification [x746]

High magnification [x2440]

(f) 330 000.

Low magnification [x1100]

High magnification [x2370]



Figures 3.2 (g)-(i). Scanning electron micrographs of microcapsules prepared at temperature variation T4 from PHB (20.1% HV) copolymers with different molecular weights :

(g) 32 800

Low magnification [x1200]

High magnification [x2370]

(h) 140 000

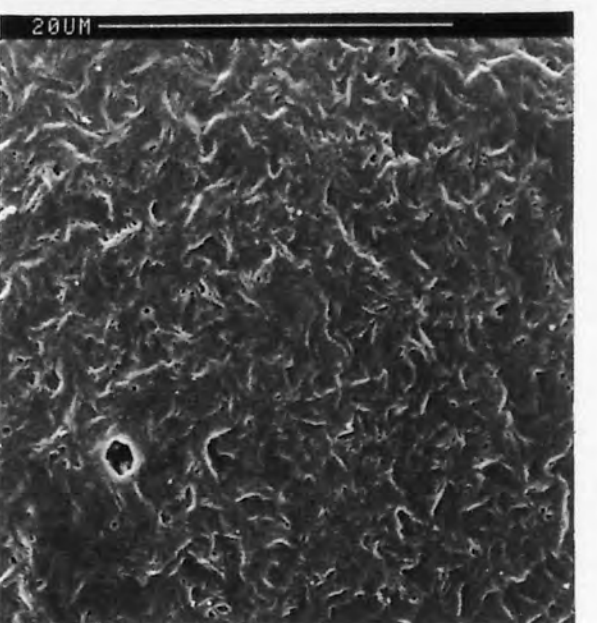
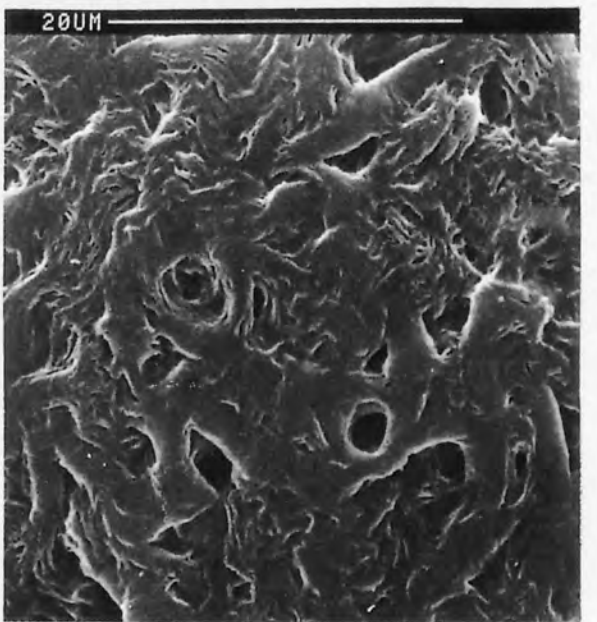
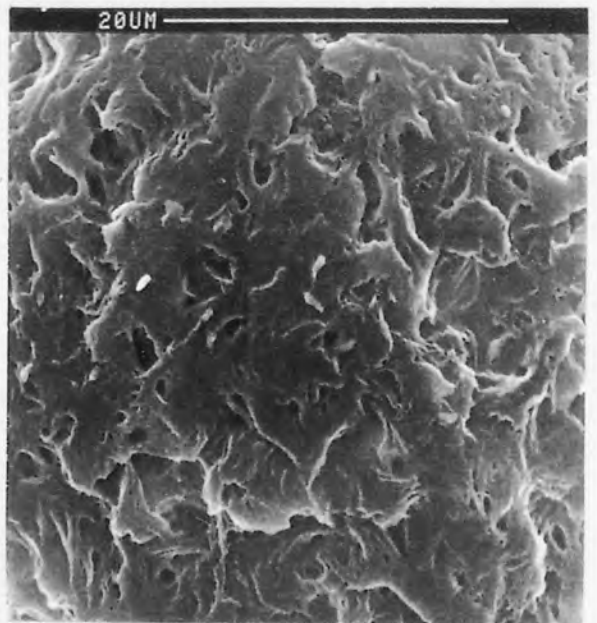
Low magnification [x746]

High magnification [x2410]

(i) 390 000.

Low magnification [x709]

High magnification [x2410]



The temperature treatment was also found to have a significant effect on the batch yield. In contrast to T1, where only 4 of the 10 polymers investigated gave rise to yields exceeding 72%, the lowest yield at T4 was 86%. The dramatic reduction in the level of polymer debris, when microencapsulation was performed at T4 instead of T1 with 159PHB, is illustrated in figures 3.3 (a) and (b). Furthermore, it is of interest to note that, whilst virtually no intact microcapsules were produced from a 3% solution of 330PHB (10.8% HV) at T1, a yield of 78% was obtained at T4, although few of the particles were spherical. Conversely, when forming microcapsules from a 9% solution of the same polymer, a near 100% yield of highly spherical particles was obtained at each temperature treatment. [The effects of polymer solution concentration on microparticle characteristics are discussed in more detail in section 3.4.]

Microcapsules prepared from 6% solutions of the three highest molecular weight materials [540PHB, 330PHB (10.8% HV) and 390PHB (20.1% HV)] were observed to become more distorted in shape when solvent evaporation was conducted at T4 instead of T1. Apart from a modest increase noted for 159PHB, the level of sphericity was not found to be affected by temperature with the other polymers.

Table 3.4 highlights the changes in microparticle yield and sphericity produced by changing the preparative temperature treatment from T1 to T4.

Figure 3.3. Scanning electron micrographs of microcapsules prepared from a 159 000 molecular weight PHB homopolymer at temperature variation :

(a) T1

Magnification x 73.3

(b) T4

Magnification x 83.6

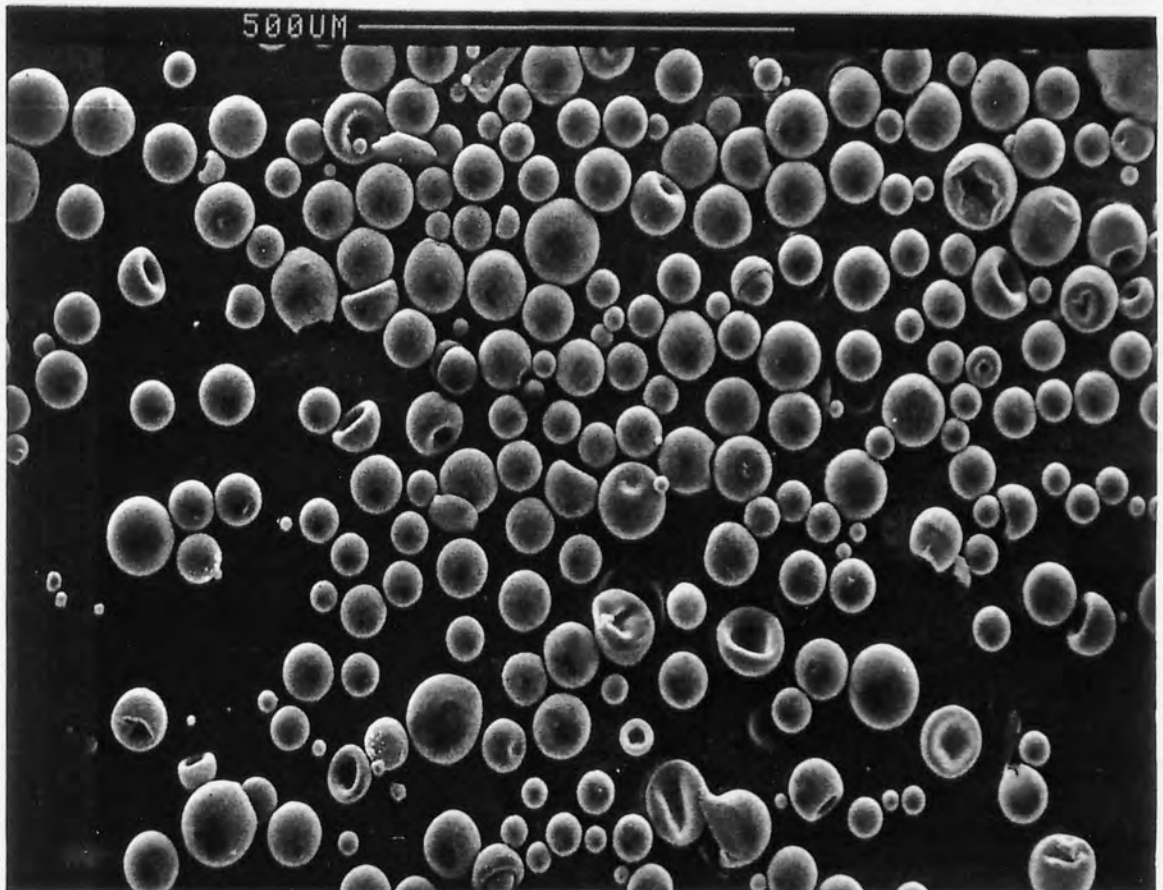
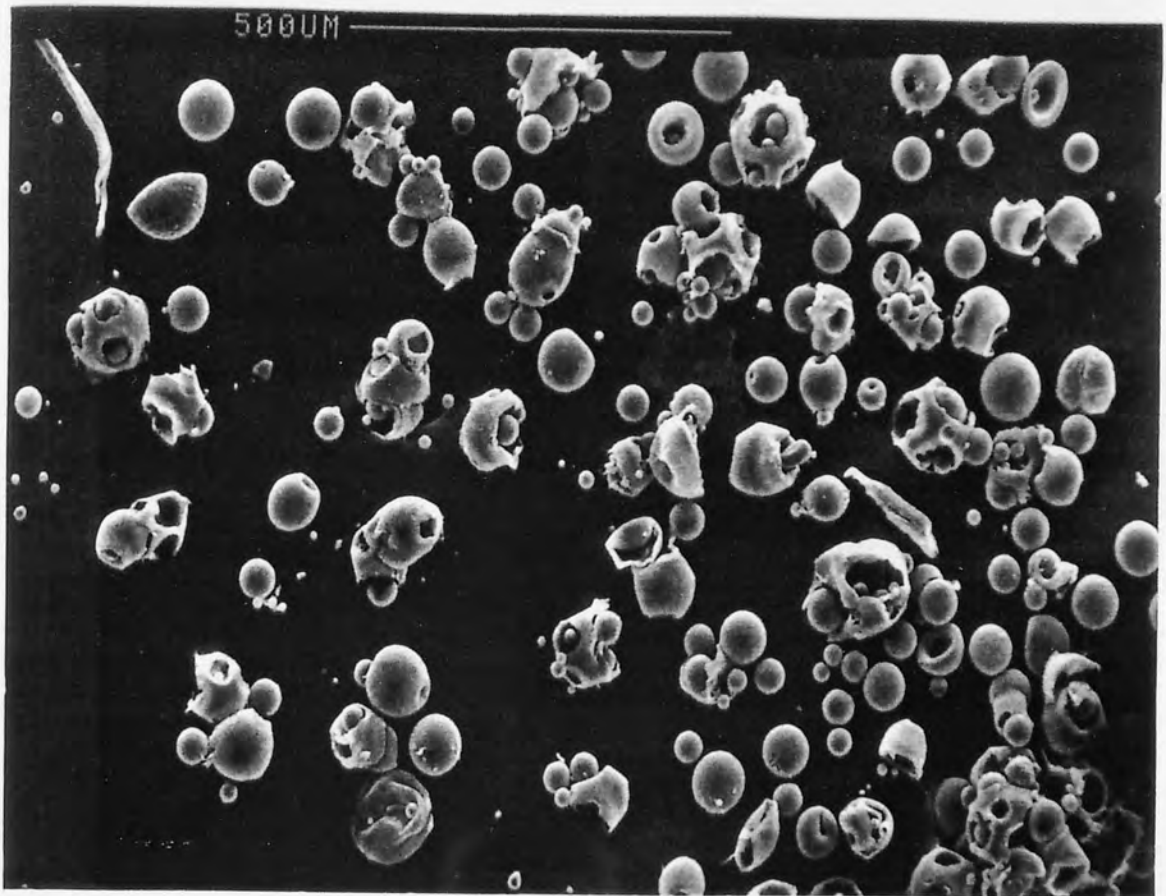


Table 3.4. Changes in microcapsule yield and sphericity produced by changing from preparative temperature treatment T1 to T4. [Note : Polymers which generated batches with yield and sphericity differences estimated at less than 10% between T1 & T4 are not included].

Polymer	Yield Change*	Sphericity Change*
43PHB	negligible	negligible
159PHB	+48	negligible
540PHB	negligible	-34
83.1PHB (10.8% HV)	negligible	negligible
180PHB (10.8% HV)	+40	negligible
330PHB (10.8% HV)	+20	-30
32.8PHB (20.1% HV)	+34	negligible
140PHB (20.1% HV)	+34	negligible
390PHB (20.1% HV)	+66	-50

*Calculated as % yield [or % sphericity] at T4 - % yield [or % sphericity] at T1.

3.4. Effects of polymer concentration.

The characteristics of microcapsules prepared from a 6% solution of 330PHB (10.8% HV) have been previously described : in section 3.2.2 [figure 3.1 (f)] for particles prepared at T1, and in section 3.3.3. [figure 3.2 (f)] for particles prepared at T4. In order to determine whether the initial polymer solution concentration had an influence on particle characteristics, microencapsulation was additionally performed using 3% and 9% solutions of this polymer, at both T1 and T4.

The 3% solution at T1 largely gave rise to a collection of polymer debris, but one of the few intact particles is shown in figure 3.4 (a) as having a highly structured wall with a macroporous surface.

Figure 3.4 (b) is a repeat picture of the particle prepared from a 6% solution of 330PHB (10.8% HV) at T1, previously shown as figure 3.1 (f). The polymer has been more uniformly deposited in the wall of this particle than in the one prepared at T1 from the 3% solution [c.f. figure 3.4 (b) with figure 3.4 (a)], and the surface has smaller,

more discrete, pores. Additionally, the 72% yield derived from 330PHB (10.8% HV) at a concentration of 6%, was a vast improvement on that obtained using the more dilute solution.

Increasing the polymer concentration to 9% had the effect of improving the yield still further. In this instance, virtually no mis-formed particles or polymer debris were encountered. A typical particle from this batch is shown in figure 3.4 (c), and has a well-formed wall whose surface is occasionally interrupted by some very small, irregular, pores.

Table 3.5. Yield, sphericity and morphological consistency of microcapsules prepared from different concentrations of 330PHB (10.8 % HV) solution at T1 and T4.

POLYMER CONCENTRATION	[%]		[%]		[%]	
	YIELD		SPHERICITY		CONSISTENCY	
	T1	T4	T1	T4	T1	T4
3	<10	78	n/a*	<10	n/a*	70
6	72	92	72	42	85	90
9	98	96	84	80	80	95

* In this batch the yield was too low to facilitate an assessment of these characteristics.

Microcapsules were also prepared from each of these differently concentrated polymer solutions at T4. With the 3% solution, T4 resulted in an increased yield of 78% relative to T1, although most of the intact particles were highly distorted in shape. One of the more spherical microcapsules from the T4 batch is shown in figure 3.4 (d), and has a contoured but fairly smooth surface with only a few small pores.

Figure 3.4 (e) is a repeat of figure 3.2 (f), and shows a microcapsule prepared from

Figures 3.4 (a)-(c). Scanning electron micrographs of microcapsules prepared from differently concentrated 330PHB (10.8% HV) solutions at temperature variation T1 :

(a) 3% [w/v]

Magnification [x1720]

(b) 6% [w/v]

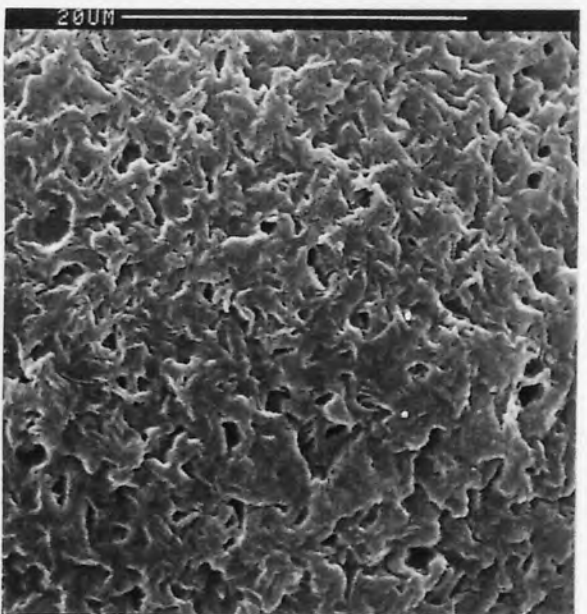
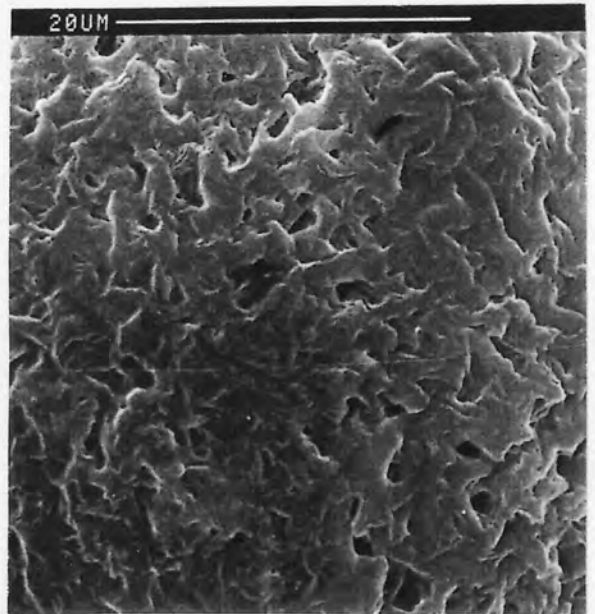
Low magnification [x924]

High magnification [x2370]

(c) 9% [w/v]

Low magnification [x626]

High magnification [x2410]



Figures 3.4 (d)-(f). Scanning electron micrographs of microcapsules prepared from differently concentrated 330PHB (10.8% HV) solutions at temperature variation T4 :

(d) 3% [w/v]

Low magnification [x1680]

High magnification [x2470]

(e) 6% [w/v]

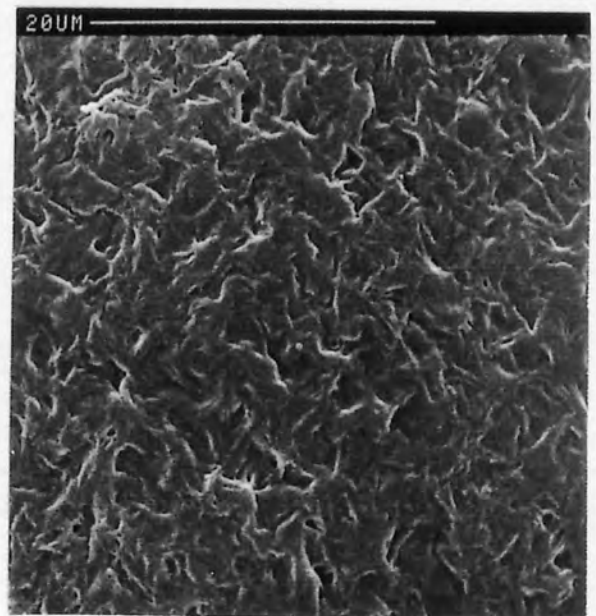
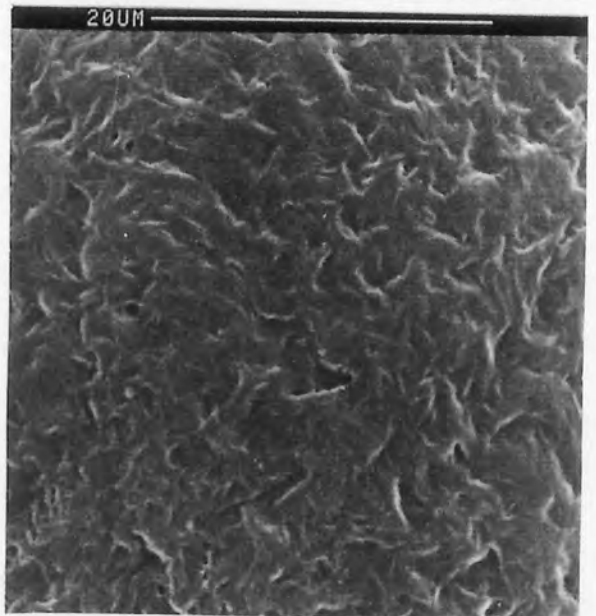
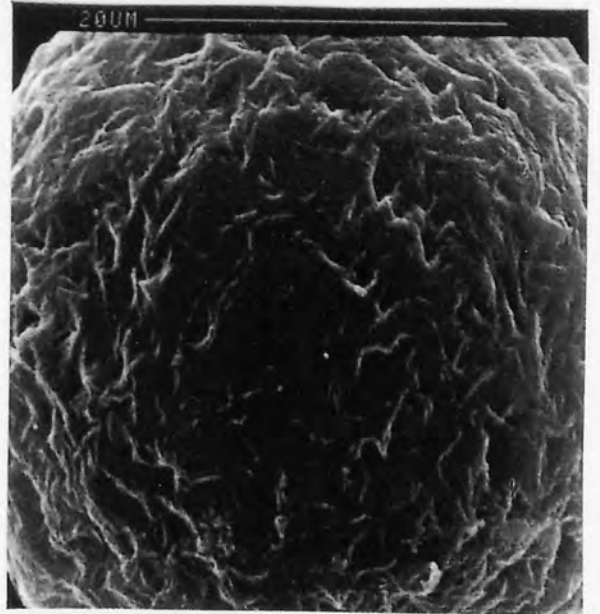
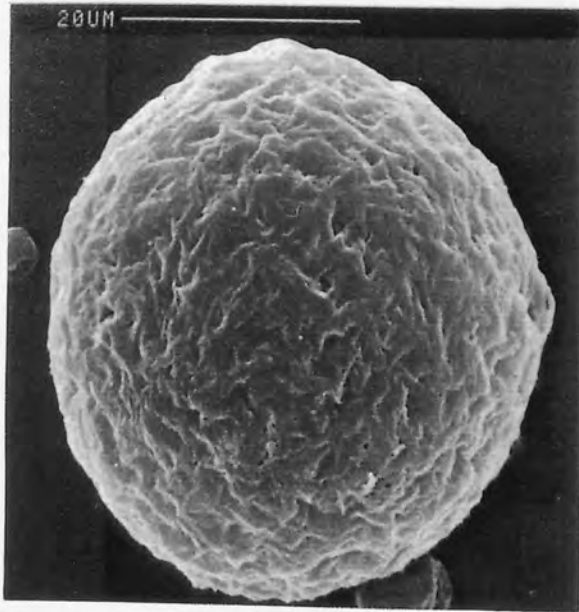
Low magnification [x1100]

High magnification [x2370]

(f) 9% [w/v]

Low magnification [x599]

High magnification [x2410]



6% solution of 330PHB (10.8% HV) as having a very slightly contoured, but essentially smooth and non-porous surface. As was detailed in section 3.2.3, changing from T1 to T4 in this instance resulted in a 20% rise in the yield to 92%.

The high yield of microcapsules obtained from the 9% solution at T1 was repeated at T4. However, as is evident from a comparison of figures 3.4 (f) and (c), the T4 particles had smoother and less porous surfaces than those prepared at T1.

3.5. Discussion.

The double emulsion-solvent evaporation process can be successfully employed to produce spherical microcapsules, provided that a stable microemulsion can be generated whilst the polymer remains appreciably solvated. The morphological characteristics which the nascent particles then develop will be governed by factors that determine polymer precipitation behaviour, such as solubility, crystallinity and mechanical properties, the rate of solvent extraction and the viscosity at a given level of solvent loss. Experimental variables which have the capacity to influence the precipitation process by affecting these factors can therefore be considered as having a possible role in the development of particulate features. Intrinsic properties of the material, such as molecular weight, molecular weight distribution and the co-monomer ratio, may be important in this regard. Similar importance may be attributed to external variables related to the preparative process, such as temperature and polymer concentration.

Moreover, alteration of even one such variable may influence the precipitation process in several ways, which may not be synergistic. For example, an increase in temperature is typically associated with enhanced polymer solubility, but in the solvent evaporation process, this could be expected to compete with the opposite effect caused

by a faster rate of solvent removal. Thus, for a particular set of investigative parameters, the relative importance of the different events which influence the precipitation process can only be judged on the basis of the experimental observations.

The nine experiments reported in section 3.1, which employed polymers differing in molecular weight and / or HV content, provide practical support for the supposition that particle morphology is determined by the complex interaction of several factors. Thus, whilst many different morphologies are exhibited by these particles, there appear to be no clear trends attributable to molecular weight or HV content changes.

Precipitation from solution will be accompanied by a change in conformation and nett chain contraction of the polymer, which act as a deforming force on the developing wall. Precipitating polymers whose mechanical properties are weak may be unable to withstand these pressures resulting in mis-shapen particles with heavily structured and porous walls. The microcapsules produced from the two copolymer series in the lower molecular weight bands are believed to illustrate this behaviour.

The lowest molecular weight homopolymer, which has been observed to give rise to wrinkled, non-porous and spherical particles, is believed to precipitate as a more mechanically stable gel. The forces caused by contraction, as solvent extraction progresses, are therefore unable to rupture the developing wall. Instead, the wall shrinks as a continuous film during these final stages of polymer precipitation and emerges intact, but with a shrivelled appearance. The greater viscoelasticity of precipitating 159PHB in comparison to 43PHB is believed to be responsible for the less shrivelled appearance of particles prepared from the former polymer.

The behaviour described above may be partially a consequence of crystallinity differences between the homopolymer and the copolymers. Whilst the copolymers are

believed to be capable of attaining the same high levels of crystallinity exhibited by PHB, as a result of isodimorphic behaviour, their rates of crystallization are retarded by the presence of HV¹¹¹. Furthermore, equilibrium aged, solid P(HB-HV) polymers have been shown to become less rigid and more ductile as the HV content increases⁷⁹, although the applicability of these properties to the case of a heavily plasticized, viscous polymer gel precipitating from solution is questionable.

The materials in the highest molecular weight band have the good mechanical properties found with PHB-based materials having molecular weights above 250 000¹⁰⁹, and typically associated with a high degree of chain entanglement. This is consistent with the observation that the polymer chains are densely packed into the walls of particles prepared from the band C polymers.

The microporosity seen on the surfaces of microparticles prepared from 540PHB and 330PHB (10.8% HV), is a probable consequence of differential precipitation caused by a wide molecular weight distribution. The solubility of a polymer in a particular solvent generally decreases with increasing molecular weight¹²⁰. Consequently, polymer solutions containing chains of widely differing size may well exhibit heterogeneous precipitation whereby the high molecular weight chains precipitate before the more soluble low molecular weight fraction. Whilst all nine polymers used for microencapsulation in this chapter all had quite wide molecular weight distribution values, this was particularly so for the polymers in the highest molecular weight band which had polydispersity values between 4.1 [540PHB] and 4.72 [390PHB (20.1% HV)]. Additionally, these materials have the greatest absolute differences between M_w and M_n , and are therefore the most likely to undergo

heterogeneous precipitation.

The surface morphologies of the particles prepared from 540PHB and 330PHB (10.8% HV) may reflect this type of non-uniform precipitation. In these instances, the range of polymer chain lengths are believed to be miscible in the initial microdroplets, formed shortly after phase combination. However, as the organic solvent diffuses into the continuous phase, the predominant, high molecular weight fractions begin to precipitate whilst the smaller chains remain fully solvated, but randomly dispersed, throughout the viscous wall. This situation is assumed to be maintained until solvent loss had reached the point where the high molecular weight regions, which form the framework of the particle wall, have become too viscous to flow. Any redistribution in the position of the more mobile low molecular weight segments at this stage will therefore directly influence the final particle morphology. The observed surface microporosity is explained by the actual elution of the small, solvated chains from, and consequent contraction of, the highly viscous polymer wall. Chapter four demonstrates how the enhanced solubility of poly- ϵ -caprolactone [PCL] can produce a similar, though more pronounced, micropore-inducing effect, when microencapsulation is practiced with miscible PCL / high molecular weight P(HB-HV) polymer blends.

It is of interest to note that the pores are more marked on particles prepared from 540PHB than they are on particles prepared from 330PHB (10.8% HV) [c.f. figures 3.1 (c) and (f)]. In the case of 540PHB, the differential between low and high molecular weight chains is greater than in the case of 330PHB (10.8% HV) and their elution will more effectively form pores.

The lack of surface microporosity on particles prepared from 390PHB (20.1% HV) is a probable consequence of the extra HV enhancing the solubility of this material and

reducing the solubility difference between the extreme fractions.

The experiments reported in section 3.4, which involved the production of microcapsules from 3%, 6% and 9% solutions of 330PHB (10.8% HV), did not provide clear evidence that polymer concentration significantly affected particle morphology. Thus, the yield obtained from the 3% solution at T1 was so low [< 10%] that intact particles, such as that shown in figure 3.4 (a), cannot be considered as representative. Furthermore, microcapsules prepared from the 9% solution at T1 had fairly similar morphological features and levels of sphericity to those obtained from the 6% solution.

Microparticle morphologies are reported to be dependent upon the temperature of solvent evaporation. For example, when the boiling point of the polymer solvent is exceeded, it may boil out of the emulsion droplets resulting in enhanced porosity³⁶. The boiling point of DCM is 41°C¹⁰¹, whereas the microcapsules prepared at T1 experienced a maximum temperature of 22°C during fabrication. In contrast, temperature treatments 2-4 involved a maximum temperature of 40°C at some stage during the 5 hour solvent evaporation period. Although this temperature was marginally below the boiling point of DCM, it was not possible to generate stable emulsions if the organic phase was actually added to a PVA solution at 40°C. Therefore the PVA solution was maintained at room temperature during phase combination and then raised to 40°C by immersing the reaction vessel in a water bath at a specified time after this event [see section 2.2].

A comparison of figures 3.1 and 3.2 reveals that temperature can affect particle morphology, even below the boiling point of the organic solvent. With the exception of

159PHB, the surfaces of all microcapsules which received exposure to the higher temperature, 2 minutes after phase combination [i.e. T4], are smoother and / or less porous than those of similar particles prepared at T1. However, the time interval after which temperature elevation occurs is of great importance. This is illustrated by the fact that a temperature rise to 40°C, 70 minutes after phase combination, produced particles with unchanged characteristics from those prepared at T1 for each of the four polymer systems investigated. Furthermore, only the most soluble polymer [43PHB] was influenced by a temperature rise to 40°C, 35 minutes after phase combination.

During microparticle preparation, higher temperatures can be expected to enhance the rate of solvent / non-solvent diffusion by lowering the viscosity of the microdroplets and increasing the rate of solvent evaporation from the air / water interface. Conversely, polymer solubility will be maintained to a greater extent and the tendency for precipitation and crystallization reduced. Therefore at a particular level of solvent loss the polymer will have a greater ability to flow. With 540PHB and 330PHB (10.8% HV), the heterogeneous precipitation behaviour observed at T1 will be less pronounced, and porosity avoided by chain re-organization in the bulk. Thus, the increased fluidity of the polymer at higher temperatures becomes particularly important at high degrees of solvent loss when the nascent wall is precipitating and contracting. In the case of the lower molecular weight materials, the deforming forces that emerge at this stage will be applied to a more mobile polymer film which will be able to respond by flowing in the direction of the applied stress instead of undergoing the degree of rupture or shrinkage observed at T1.

Clearly, if most of the solvent has already been extracted from the microdroplet when the temperature is raised, the polymer may be too viscous to respond. Of the four

polymers investigated at T2 and T3, this situation appeared to have been reached by 35 minutes after phase combination for all except 43PHB.

Unfortunately, the use of SEM only produced qualitative measures of the microparticle characteristics discussed in this chapter. Techniques such as porosimetry and gas adsorption, which have been used to quantify the specific surface area, pore volume and pore size distribution of porous solid microparticles⁹, cannot be effectively applied to hollow devices with possible transmembranous porosity. Furthermore, whilst the batch yield is frequently expressed in the literature as a percentage, obtained by comparing the weight of recovered particulate matter to the initial weight of total solids [e.g. ^{9, 121}], this method takes no account of particle structural integrity. This point may be of only limited significance in the case of single emulsion processes, which produce drug-polymer composites, but when manufacturing reservoir-type devices, any loss of structural integrity will be catastrophic in terms of drug encapsulation. Consequently, it was decided that the most appropriate measure of the yield in these studies was the proportion of discrete polymer entities in the 38-150 μ m size range that were judged to be individual, structurally intact, microcapsules.

The intermediate molecular weight polymers, which comprise band B, were observed to give rise to low yields of particles at T1, principally as the result of a large quantity of polymer debris. With the exception of 32.8PHB (20.1% HV) [whose anomalous behaviour will be discussed later], significant debris was not generated from 6% solutions of either the low or high molecular weight band polymers, when solvent evaporation was conducted at T1.

The presence of mis-formed particles and polymer debris from band B polymers, as described above, is believed to be due to precipitation of the polymer before a stable microemulsion has been formed. However, the fact that the increase in molecular weight associated with a change from band A to band B polymers appears to encourage such premature precipitation, whilst the additional molecular weight rise that results from a move from band B to band C polymers seemingly has the opposite effect, suggests that at least two molecular weight-dependent factors are influencing the rate of precipitation. Clearly, to have the observed effects, these factors must move in opposite directions as the molecular weight changes. Furthermore, at a particular molecular weight level between band A and band C, a crossover point must be reached where the position of dominance is reversed.

As has been previously mentioned, an increase in molecular weight is typically associated with reduced polymer solubility. On this basis, a tendency for more rapid precipitation can be expected from a particular polymer at higher molecular weight. In the solvent evaporation process, however, the rate of precipitation may be affected by factors other than polymer solubility. An important example is the rate of solvent / non-solvent diffusion¹³ which is directly influenced by the viscosity of the polymer microdroplets.

The experiments reported in section 3.1 were all performed with 6% polymer solutions which varied in viscosity, principally according to molecular weight. The low molecular weight materials in band A gave rise to relatively low viscosity solutions and, as a result, quite rapid rates of DCM diffusion into the aqueous continuum after phase combination. However, the enhanced solubility of the low molecular weight polymers meant that a very high degree of DCM partitioning had to occur before the onset of precipitation. The high yields of microcapsules obtained from 43PHB and

83.1PHB (10.8% HV) suggest that, despite a favourable rate of DCM diffusion, the inherent solubility of these polymers ensured that they remained solvated long enough for a stable microemulsion to be established.

The molecular weight increase associated with a move to band B resulted in a reduction in polymer solubility. The high level of debris encountered in these instances suggests that this outweighed any reduction in the rate of solvent diffusion and led to the premature precipitation of these materials from solution. In contrast, the effect of the molecular weight changes involved with a move from band B to band C on the solubility of the polymers appeared to be vastly outweighed by the effects of a viscosity increase on the rate of solvent diffusion. The dramatically retarded rate at which DCM was removed from microdroplets of these polymers meant they were able to form stable microdroplets whilst still in the gel state.

The poor yields obtained from 390PHB (20.1% HV), and to a lesser extent 330PHB (10.8% HV), appeared to be a consequence of this very low rate of solvent / non-solvent diffusion. In the former case, 80% of the particles were well formed but aggregated. This aggregation must have occurred after the 5 hour solvent evaporation period and the washing and isolation procedure, because the aggregated particles would not have passed through the sieves. It therefore appears that the surfaces of these particles were still tacky due to the presence of residual solvent at the drying stage. The 28% defect level encountered with the batch prepared from 330PHB (10.8% HV) appeared to be a result of the particle walls exploding outwards after the surface layers had fully precipitated. This may be explained by the presence of a considerable quantity of residual DCM in the middle regions of the wall which was violently removed during the vacuum procedure.

As the viscosity of the organic phase decreases, it becomes increasingly difficult to form a stable o/w microemulsion since the droplets coalesce more readily. This behaviour is believed to explain why 32.8PHB (20.1% HV) and the 3% solution of 330PHB (10.8% HV) respectively produced moderate and poor yields of microcapsules when solvent evaporation was conducted at T1.

The use of temperature treatment T4 resulted in excellent yields of microcapsules from all the polymer systems investigated. As has been previously mentioned, temperature affects the rate of polymer precipitation by enhancing the polymer's solubility, lowering the viscosity at a given level of solvent loss and increasing the rate of DCM diffusion. Of these events, the solubility effect is believed to be responsible for the improved particle yields from the band B polymers, and the faster rate and greater magnitude of DCM diffusion, the result for 330PHB (10.8% HV) and 390PHB (20.1% HV). The poor sphericity of particles prepared from all the high molecular weight polymers at T4 can be similarly explained. Thus, when the microdroplets are initially formed, they will be elongated in shape as a result of the mixing action. At T1, the rate of solvent extraction is slow enough to enable the droplets to undergo an elastic recovery and assume an approximately spherical shape. In contrast, the increased rate of solvent removal at T4 reduces the opportunity for such a recovery by facilitating the more rapid attainment of a prohibitively high viscosity. Interestingly, however, 80% of microcapsules prepared from the 9% solution of 330PHB (10.8% HV) at T4, were spherical. In this case, the higher viscosity at a given level of solvent loss which resulted from the extra polymer appears to override the temperature effects.

Temperature elevation, especially in systems which contain dissolved organic

material in an aqueous phase, frequently produces an increase in micellization and emulsion formation. This effect could account for the increased microcapsule yields from the two least viscous polymer solutions [32.8PHB (20.1% HV) and the 3% solution of 330PHB (10.8% HV)] when microencapsulation was practised at T4 instead of T1. Phenomena such as the Krafft Temperature have a similar cause and, although there may be no exact parallel with the situation in this work, at least demonstrate that the above explanation is consistent with normal physico-chemical parameters.

The range of effects described in this chapter are well recognized in the formation of organic polymer films by solvent evaporation. Morphological phenomena described as 'orange peel', 'alligator skin', and 'cissing effects' are similarly related to solution viscosity and show the importance of volatile content, polymer molecular weight and molecular weight distribution, solvent volatility and evaporation temperature. These effects, described for example in texts on print film defects ¹²², have many parallels with the phenomena described in the formation of microcapsules by solvent evaporation in this chapter.

The investigations contained within this thesis, have not addressed microparticle size distribution profiles and their determining factors. Whilst this is an area of considerable practical importance, it was considered that previous researchers have comprehensively addressed this topic [see section 1.3.2.]. Therefore, the work in this chapter has focused on the factors which influence microparticle morphology, a relatively under-reported subject. The microcapsules whose features have been described are all in the 38-150 μ m size range. An upper limit of 150 μ m was selected

because this is reported to be the maximum particle size suitable for injection with a 20 gauge syringe needle ^{14, 92}. The lower limit of 38 μ m corresponded to the smallest diameter Endecotts wet-state sieve available ; in addition SEM became increasingly difficult as the size of the particles diminished. Moreover, the SEM observations revealed that, within the 38-150 μ m size range, there was no relationship between particle morphology and particle size in any given batch. The possibility that such a relationship existed for particles that did not fall within this range was not investigated. Confirmation that the microcapsules produced in these experiments were indeed hollow, reservoir-type, devices was obtained by direct visualization of sectioned particles using SEM. Figure 1.1 (a) shows a scanning electron micrograph of a sectioned microcapsule prepared from 43PHB at T1.

4.1. Introduction.

Previous studies within the Speciality Materials Research Group, on solvent cast films¹⁰³, have demonstrated [using phase contrast microscopy] that miscible blends can be formed between high molecular weight P(HB-HV) polymers and poly- ϵ -caprolactone [PCL], when the latter is present in low proportions [typically < 20% by weight]. In addition, preliminary microencapsulation work has suggested that particles prepared from these blends have very different morphologies from those correspondingly prepared from unblended P(HB-HV) polymers⁹⁷.

CHAPTER FOUR.

In this chapter, microcapsules prepared from blends of P(HB-HV) polymers with varying proportions of poly- ϵ -caprolactone, and a comprehensive **MORPHOLOGICAL STUDIES OF MICROCAPSULES PREPARED FROM BLENDS OF P(HB-HV) POLYMERS WITH** **POLY- ϵ -CAPROLACTONE.**

employed, PCL I and PCL II, and the mole- en in section 2.1.1. All the blends are identified by the ratio of poly- ϵ -caprolactone to total polymer on a weight percentage basis.

The first section is concerned with PCL I, and describes the features of microcapsules prepared with up to 20% of this polymer, blended with either 350PHB (12% HV) or 43PHB.

Microencapsulation in the second section of this chapter has been performed with blends of 20% PCL II and, individually, ten different P(HB-HV) polymers. Additionally, microcapsules have been prepared from blends of 330PHB (10.8% HV) with up to 90% PCL II.

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Previous studies within the Speciality Materials Research Group, on solvent cast films¹⁰⁵, have demonstrated [using phase contrast microscopy] that miscible blends can be formed between high molecular weight P(HB-HV) polymers and poly- ϵ -caprolactone [PCL], when the latter is present in low proportions [typically < 20% by weight]. In addition, preliminary microencapsulation work has suggested that particles prepared from these blends have very different morphologies from those correspondingly prepared from unblended P(HB-HV) polymers⁹⁷.

In this chapter, microcapsules have been prepared from blends of P(HB-HV) polymers with varying proportions of poly- ϵ -caprolactone, and a comprehensive assessment has been made concerning the effects of polymer blending on microcapsule morphology. Two different batches of poly- ϵ -caprolactone have been employed, PCL I and PCL II, and the molecular weight details of each are given in section 2.1.1. All the blends are identified by the ratio of poly- ϵ -caprolactone to total polymer on a weight percentage basis.

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4.2. Microencapsulation with PCL I blends.

4.2.1. 350PHB (12% HV) / PCL I blends.

The microcapsules described in this section were prepared from blends of 350PHB (12% HV) with PCL I, under the experimental conditions described in section 2.2, and at temperature treatments T1, T3 and T4. Three blends of differing composition were utilized [Blends 5, 10 and 20], and these respectively contained 5, 10 and 20% PCL I.

The morphologies of typical microcapsules prepared from each of these blends, together with unblended 350 PHB (12% HV), at the three different temperature treatments, are shown in figure 4.1.

Microcapsules prepared from unblended 350PHB (12% HV) at T1 and T3 clearly have highly structured polymer walls and, consequently, surface macroporosity [figures 4.1 (a) and (e)]. The same features are apparent on microcapsules prepared from Blends 5 and 10 at these temperature treatments [figures 4.1 (b), (c), (f) and (g)]. In contrast, when microencapsulation was performed using these formulations at temperature treatment T4, the polymer was more evenly deposited in the walls of the resulting particles, and their surfaces were smoother and less macroporous [figures 4.1 (i), (j), and (k)]. In addition, all the microcapsules prepared from Blends 5 and 10 have small pin-holes dispersed throughout the surface, but these indentations are particularly apparent on particles prepared at temperature treatments T1 and T3.

The microcapsules prepared from Blend 20 at T1 [figure 4.1 (d)] also possess surfaces scarred by both macropores and smaller indentations. With these particles, however, the latter are far more pronounced than on any of the Blend 5 and Blend 10 microcapsules. In contrast, the morphological characteristics of particles prepared from

Figures 4.1 (a), (b), (e), (f), (i) and (j). Scanning electron micrographs of microcapsules prepared from 350PHB (12% HV) and blends thereof with 5% PCL I, at temperature treatment :

T1:

(a) unblended
350PHB (12% HV)

(b) 350PHB (12% HV)
+ 5% PCL I

T3:

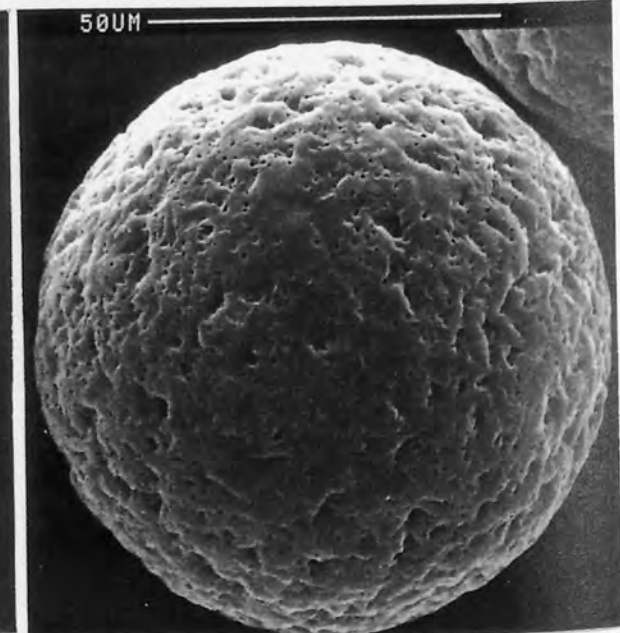
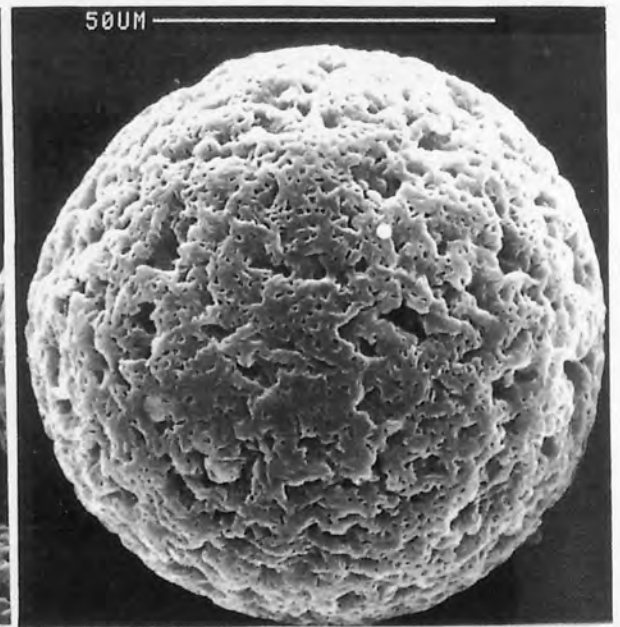
(e) unblended
350PHB (12% HV)

(f) 350PHB (12% HV)
+ 5% PCL I

T4:

(i) unblended
350PHB (12% HV)

(j) 350PHB (12% HV)
+ 5% PCL I



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0001 0001
0001 0001

Figures 4.1 (c), (d), (g), (h), (k) and (l). Scanning electron micrographs of microcapsules prepared from blends of 350PHB (12% HV) with 5% and 10% PCL I, at temperature treatment :

T1 :

(c) 350PHB (12% HV)
+ 10% PCL I

(d) 350PHB (12% HV)
+ 20% PCL I

T3 :

(g) 350PHB (12% HV)
+ 10% PCL I

(h) 350PHB (12% HV)
+ 20% PCL I

T4 :

(k) 350PHB (12% HV)
+ 10% PCL I

(l) 350PHB (12% HV)
+ 20% PCL I



this blend at T3 have a very different appearance. Thus, as figure 4.1 (h) demonstrates, the polymer in the walls of these microcapsules appears to be uniformly deposited, with the result that there is no irregular surface macroporosity. However, the surfaces are regularly perforated by numerous, discrete micropores of approximately 2-4 μ m in size.

Irregular macropores are again observed on the surfaces of microcapsules prepared from Blend 20 at T4. However, as figure 4.1 (l) demonstrates, the remainder of the surface is reasonably smooth, although saturated by the tiny indentations typical of particles prepared from the lower PCL content blends.

The small indentations seen on the surfaces of Blend 5 and Blend 10 particles are attributed to the presence of PCL, which is believed to be highly miscible with the 350PHB (12% HV) in the unprecipitated polymer microdroplets. With these formulations at T1 and T3, a high level of miscibility appears to be maintained until solvent removal reaches the point at which the 350PHB (12% HV) loses its ability to flow. The PCL, which remains solvated until after the 350PHB (12% HV) has precipitated, because of its relatively greater solubility in the casting solvent, appears to be subsequently eluted from the hardened particle wall.

The increased fluidity of the P(HB-HV) polymer at high levels of solvent loss, which was forwarded as the explanation in chapter three for the smoother morphologies of particles prepared at T4, is also applicable to the work described in this section, and has the additional effect on Blend 5 and 10 formulations of partially covering the PCL-induced microporosity. This suggests that PCL is eluted from the walls of microcapsules made from Blends 5 and 10 at T4 in a solubilised form.

The miscibility and solubility effects, which are responsible for the development of

a particular morphology, become considerably more complex when the proportion of PCL in the blend reaches 20% [Blend 20]. At this ratio, temperature appears to affect these processes to a far greater extent than in the simpler situations represented by Blends 5 and 10. The importance of temperature is reflected in the widely differing morphological features of Blend 20 particles prepared at T1, T3 and T4.

The microcapsule prepared from Blend 20 at T3 is of particular interest. The temperature rise is believed to occur at such a point on the phase diagram that considerable polymer-solvent-polymer interactions develop, which have the effect of significantly enhancing the solubility of the least soluble component [i.e. 350PHB (12% HV)]. Indeed, particles prepared from unblended 350PHB (12% HV) at T1 and T3 are not materially different, suggesting that the polymer has essentially fully precipitated after 35 minutes in the absence of PCL. Therefore, the fact that a temperature rise after this time with Blend 20 formulations produces a more uniform deposition of 350PHB (12% HV) in the particle wall, suggests that the solubility of this component is enhanced by the presence of PCL.

As with the preparation of Blend 5 and Blend 10 microcapsules, a high level of miscibility between the two polymers appears to be maintained until solvent removal approaches the point at which the 350PHB (12% HV) becomes too viscous to flow. However, in this instance, the PCL-induced microporosity is considerably more pronounced [c.f. Blends 5 and 10], which implies a greater degree of association between precipitating PCL chains. Thus, with 350PHB (12% HV) / 20% PCL I particles, prepared at T3, the PCL may have partially separated from the PHB-rich phase prior to the full precipitation of the latter. Thereafter, poor adhesion of the two polymers in the absence of solvent could be responsible for the loss of PCL fragments from the solidified particle wall. In support of this suggestion, there was a high level of

polymer debris generally associated with the Blend 20 microcapsules prepared at T3, and this appeared to be compatible in size with the observed microporosity.

4.2.2. 43PHB / PCL I blends.

The microcapsules described in this section were prepared from blends of PCL I and 43PHB. The preparative variables and blend compositions are as described for the 350PHB (12% HV) / PCL I blends in section 4.2.1. The blend compositions are listed in table 4.1, which also briefly describes the surface morphologies of all the particles prepared in this section and identifies the corresponding scanning electron micrographs.

Table 4.1 : Composition details of microcapsules prepared from 43PHB / PCL I blends and a summary of their surface morphologies.

	Unblended	Blend 5	Blend 10	Blend 20
T1	figure 3.1 (a) <i>highly wrinkled but non-porous</i>		figure 4.2 (a) <i>highly wrinkled but non-porous with some very smooth patches</i>	figure 4.2 (c)
T3	<i>highly wrinkled but non-porous</i>		<i>highly wrinkled but non-porous with some very smooth patches</i>	
T4	figure 3.2 (a) <i>fairly smooth and non-porous</i>		figure 4.2 (b) <i>highly wrinkled with small pores</i>	figure 4.2 (d)

At each of the three temperature treatments, particles derived from Blend 5 showed no discernible differences from those correspondingly prepared from the unblended homopolymer.

Blend 10 microcapsules prepared at T1 and T3, however, tended to have a feature

Figures 4.2 (a) and (b). Scanning electron micrographs of microcapsules prepared from 43PHB blended with 10% PCL II at temperature treatment :

(a) T1

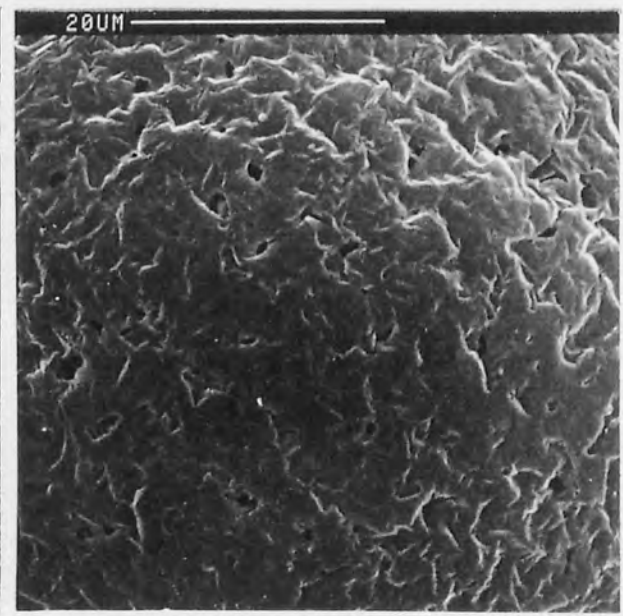
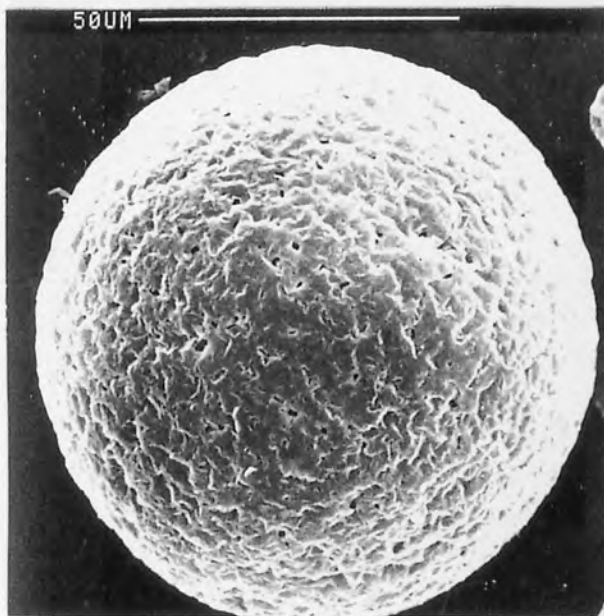
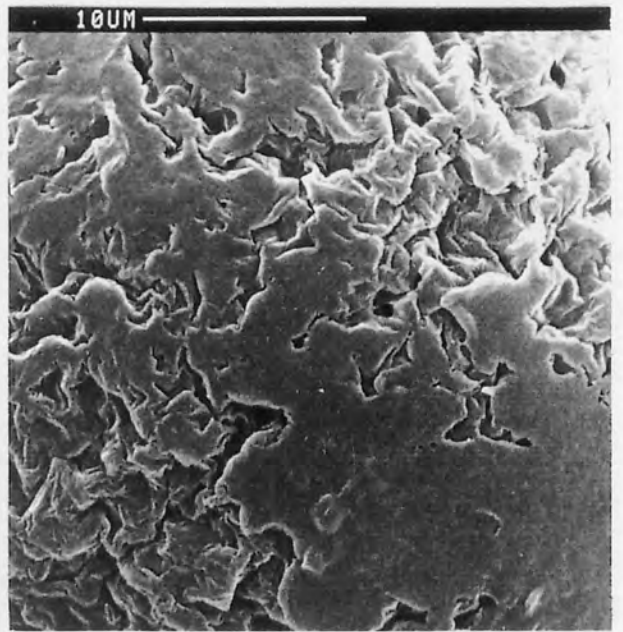
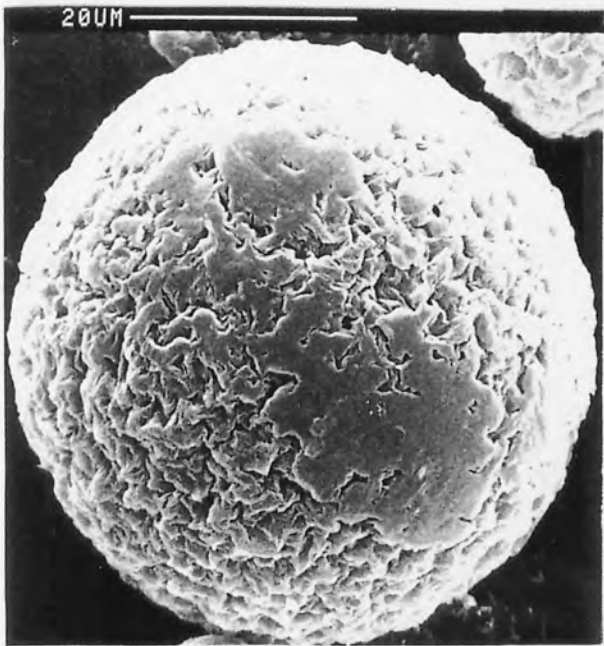
Low magnification [x1500]

High magnification [x2950]

(b) T4

Low magnification [x872]

High magnification [x1750]



Figures 4.2 (c) and (d). Scanning electron micrographs of microcapsules prepared from 43PHB blended with 20% PCL II at temperature treatment :

(c) T1

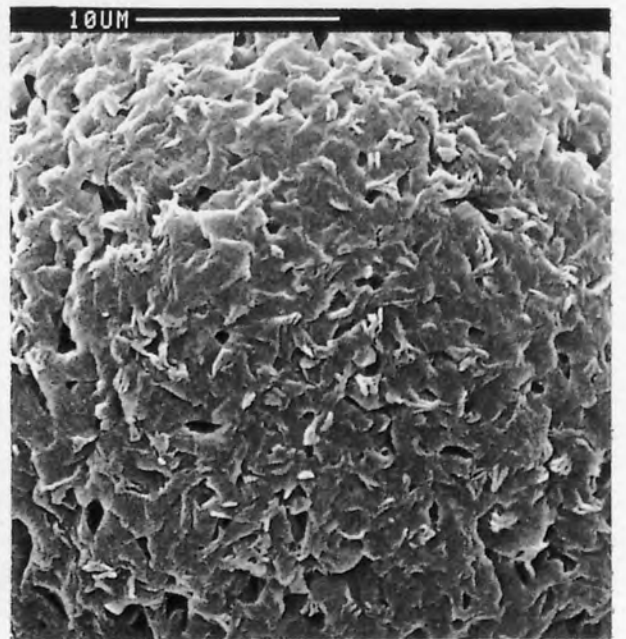
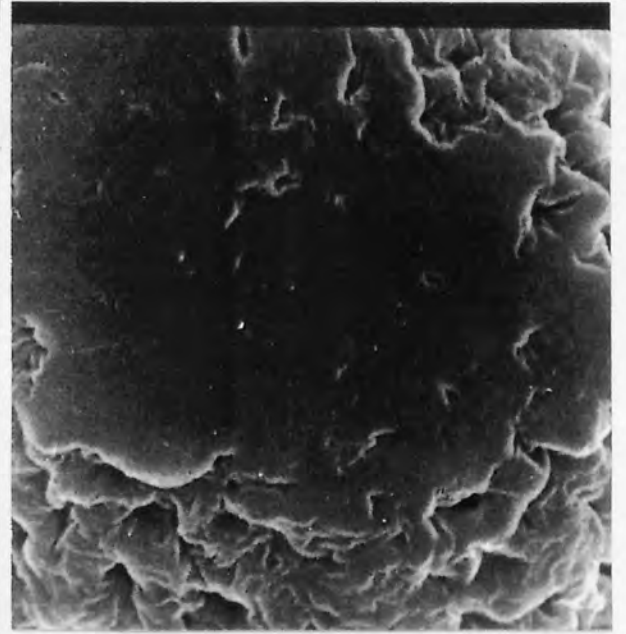
Low magnification [x1770]

High magnification [x2620]

(d) T4

Low magnification [x1500]

High magnification [x2700]



that was not encountered in the absence of PCL. Thus, whilst the surfaces of these particles were highly wrinkled, they had regions of unusual smoothness. Figure 4.2 (a) shows a typical particle prepared from Blend 10 at T1. In contrast, these smooth surface patches were not characteristics displayed by microcapsules prepared from this formulation at T4. These particles, which are represented by figure 4.2 (b), had smoother and less wrinkled surfaces than those prepared from this blend at T1 and T3, and were also slightly porous.

As was noted for the Blend 10 microcapsules, particles prepared from Blend 20 at T1 and T3 were indistinguishable, having wrinkled surfaces partially covered by a highly smooth "plate". However, as can be seen by comparing figures 4.2 (a) and (c), the increased PCL content typically enhanced the area covered by this smooth plate. This plating effect was not exhibited by microcapsules prepared from Blend 20 at T4, however. Instead, as figure 4.2 (d) demonstrates, the surfaces of these particles were regularly perforated by small micropores, and also littered with numerous, similarly sized, pieces of polymer debris.

In the previous section [4.2.1], it was demonstrated that the morphologies of microcapsules, prepared from blends of 350PHB (12% HV) with PCL I, were influenced by the degree of polymer-polymer miscibility and the relative solubility of the two polymers. In those systems, it was shown that a high level of miscibility, at least in the presence of DCM, and a considerable solubility differential between the two polymers led to the development of surface microporosity.

Microcapsules prepared at T1 or T3 from the 43PHB / PCL I blends discussed in this section did not appear to be porous but had plated surface regions. Whilst these

characteristics are the result of polymer-polymer immiscibility, it does not necessarily follow that these two polymers are any less miscible in the solvent-rich microdroplets than PCL I and 350PHB (12% HV). Instead, the appearance of a PCL plate rather than PCL-induced microporosity may be a consequence of the different solubilities of the two P(HB-HV) polymers in the dispersed phase solvent.

Thus, in both systems [350PHB (12% HV) / PCL I and 43PHB / PCL I], it is likely that there was a linkage between the level of residual organic solvent in the microdroplets and the degree of polymer-polymer miscibility. The expected result of this was that the migration of DCM into the continuous phase, and its subsequent evaporation, encouraged the formation of two distinct polymer phases. However, the loss of the dispersed phase solvent has the additional effect of increasing microdroplet viscosity. The experimental observations reported in section 4.2.1, suggested that 350PHB (12% HV) precipitated whilst the level of polymer-polymer miscibility was still high. As the latter diminished, the microdroplet was too viscous to allow pronounced phase separation to occur, and the PCL consequently remained quite uniformly distributed throughout the 350PHB (12% HV) wall, as the overall precipitation process concluded.

As a consequence of its lower molecular weight, 43PHB is far more soluble in DCM than 350PHB (12% HV), and only slightly less soluble than PCL I. Therefore, when the level of solvent in the polymer microdroplet diminished to the point that there was an appreciable reduction in the level of polymer-polymer miscibility, the 43PHB was probably insufficiently viscous to prevent phase separation from occurring. To a considerable extent, the two polymers then precipitated as distinct phases, with the PCL being deposited on the surface of the largely PHB microcapsule. Microparticles with similar surface features are reported to have been prepared from blends of PCL with

either cellulose propionate⁴⁸ or cellulose acetate butyrate⁴⁹.

Particles prepared at T4 from Blends 10 & 20, however, had discrete surface pores and were not plated by PCL. This suggests that, whilst the temperature increase to 40°C after 2 minutes reduced the viscosity of the microdroplets at a given level of solvent loss, it had the additional effect with these formulations of significantly maintaining polymer-polymer miscibility. As a result, the PCL I remained quite uniformly distributed throughout the PHB wall, until after the polymer had effectively lost its fluidity.

The debris on the surface of the Blend 20 microcapsule appears to correlate with the porosity, suggesting that poor adhesion between the two polymers in the absence of solvent resulted in the fragmentation of PCL I from the surface of the solidified particle during the isolation procedure. The lack of debris on the surfaces of Blend 10 microcapsules may result from a subtle and unintentional variation in the isolation procedure.

4.3. Microencapsulation with PCL II blends.

4.3.1. 330PHB (10.8% HV) / PCL II blends.

The studies described in this section are based on microparticles prepared at T1 from blends of 330PHB (10.8% HV) and PCL II, in which the proportion of the latter was varied from 10% to 90%, at 10% intervals. The blend nomenclature that was used in section 4.2 is repeated in this section so, for example, Blend 60 refers to a blend in which the proportion of PCL II is 60%. Representative microparticles prepared from each formulation are shown in figures 4.3 (b-j). Additionally, figures 4.3 (a) and (k) represent particles prepared from unblended 330PHB (10.8% HV) and unblended PCL

Figures 4.3 (a)-(c). Scanning electron micrographs of microcapsules prepared from different blend ratios of 330PHB (10.8% HV) with PCL II [330PHB (10.8% HV) / PCL II respectively] :

(a) 100-0

Low magnification [x872]

High magnification [x2370]

(b) 90-10

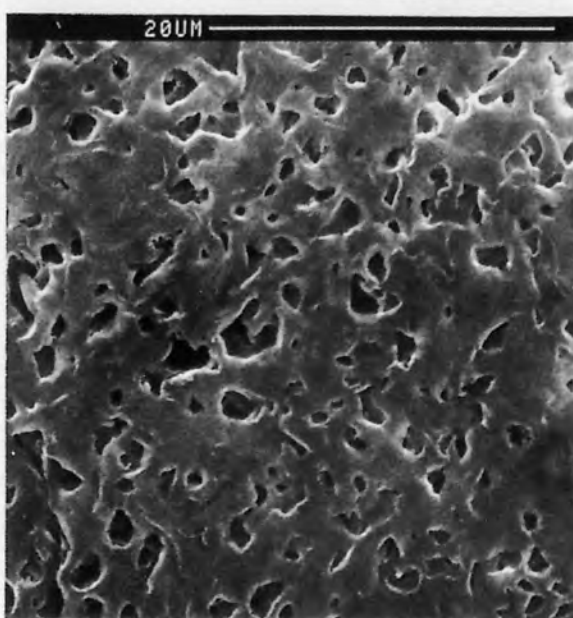
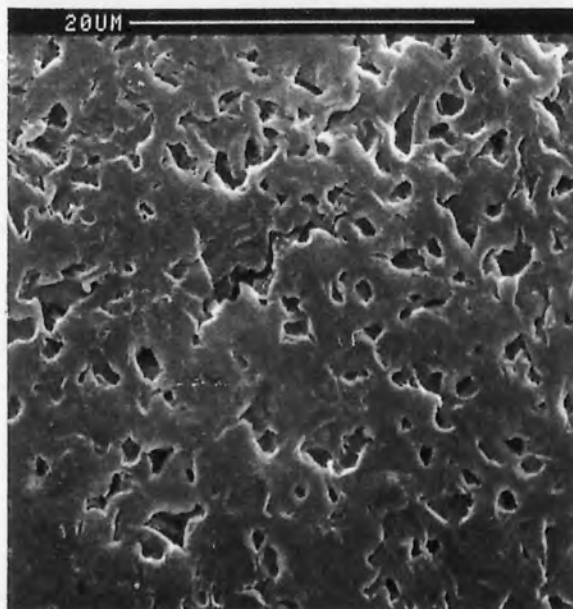
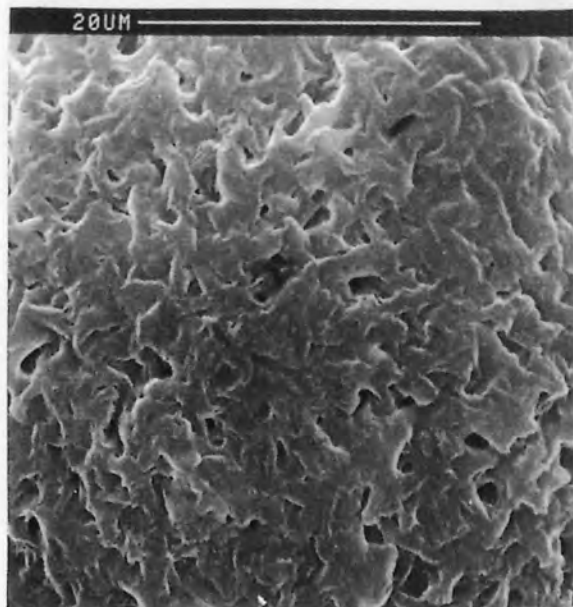
Low magnification [x599]

High magnification [x2410]

(c) 80-20

Low magnification [x638]

High magnification [x2370]



Figures 4.3 (d)-(f). Scanning electron micrographs of microcapsules prepared from different blend ratios of 330PHB (10.8% HV) with PCL II [330PHB (10.8% HV) / PCL II respectively] :

(d) 70-30

Low magnification [x538]

High magnification [2340]

(e) 60-40

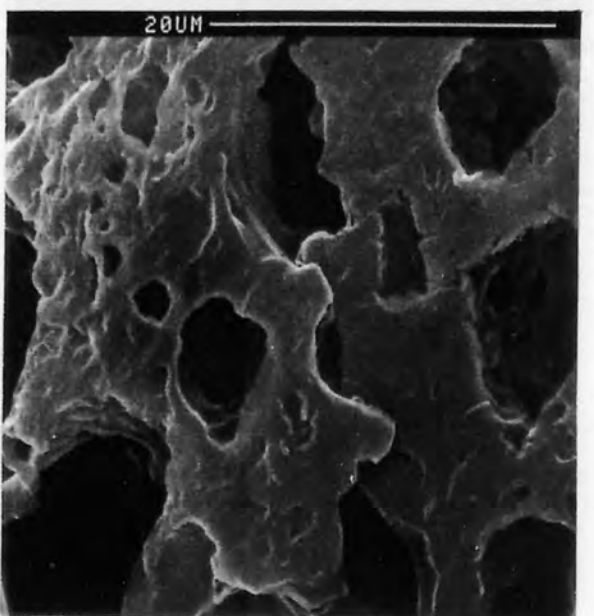
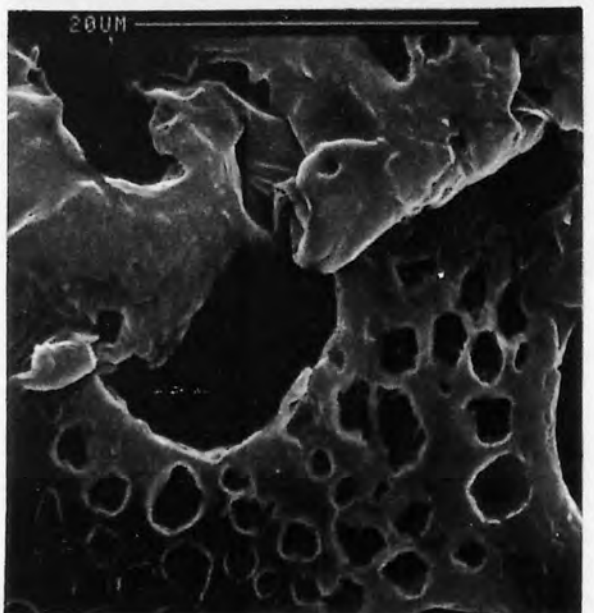
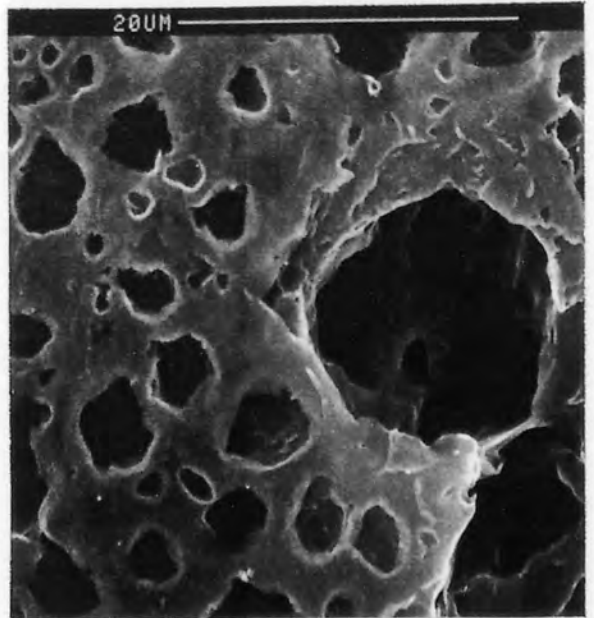
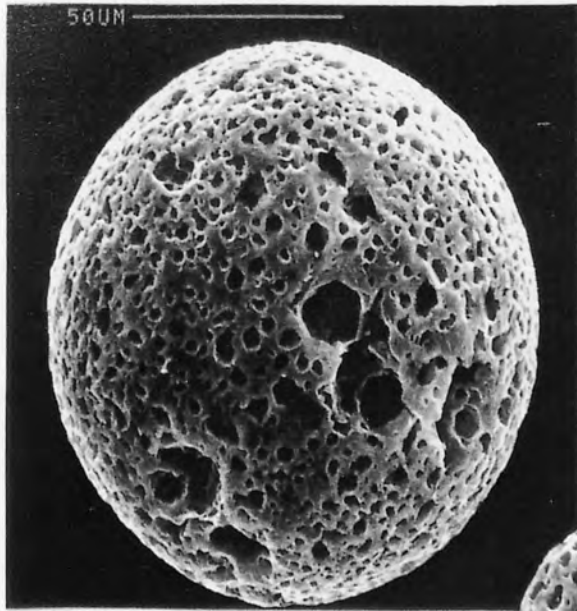
Low magnification [x677]

High magnification [x2370]

(f) 50-50

Low magnification [x617]

High magnification [x2370]



Figures 4.3 (g)-(i). Scanning electron micrographs of microcapsules prepared from different blend ratios of 330PHB (10.8% HV) with PCL II [330PHB (10.8% HV) / PCL II respectively] :

(g) 40-60

Low magnification [x773]

High magnification [x2410]

(h) 30-70

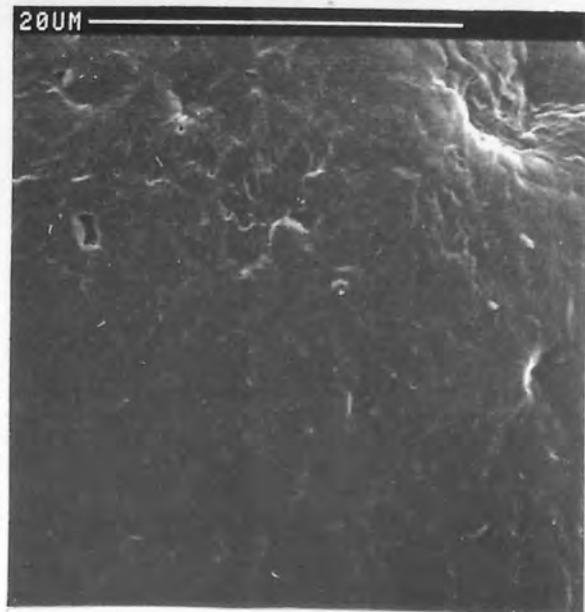
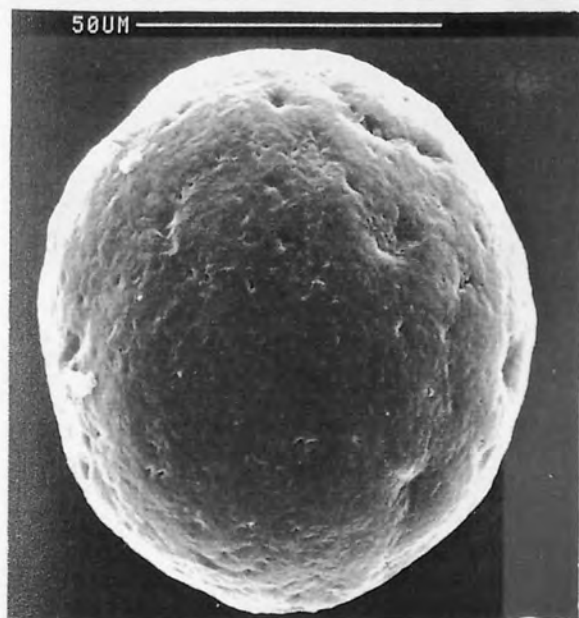
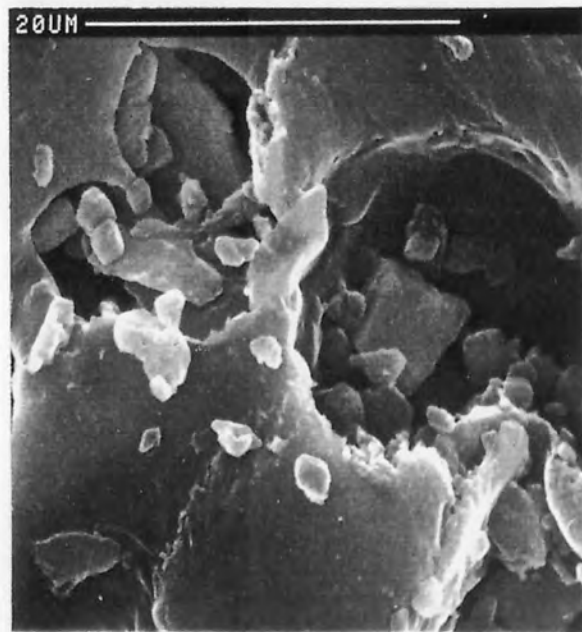
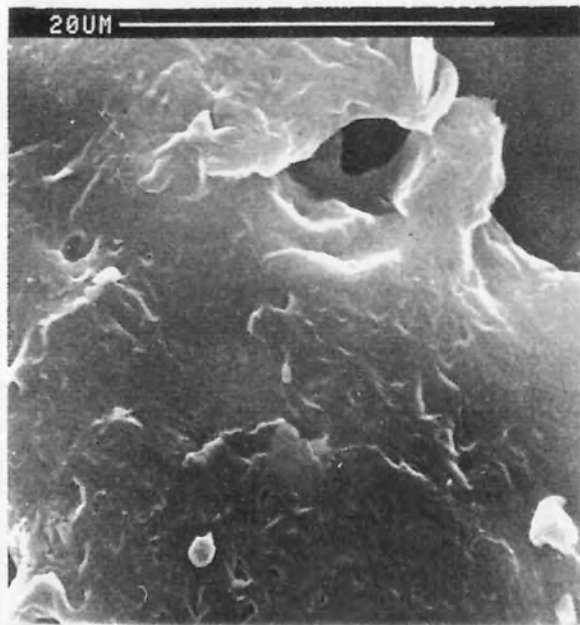
Low magnification [x626]

High magnification [x2410]

(i) 20-80

Low magnification [x853]

High magnification [x2410]



Figures 4.3 (j) and (k). Scanning electron micrographs of microcapsules prepared from different blend ratios of 330PHB (10.8% HV) with PCL II [330PHB (10.8% HV) / PCL II respectively] :

(j) 10-90

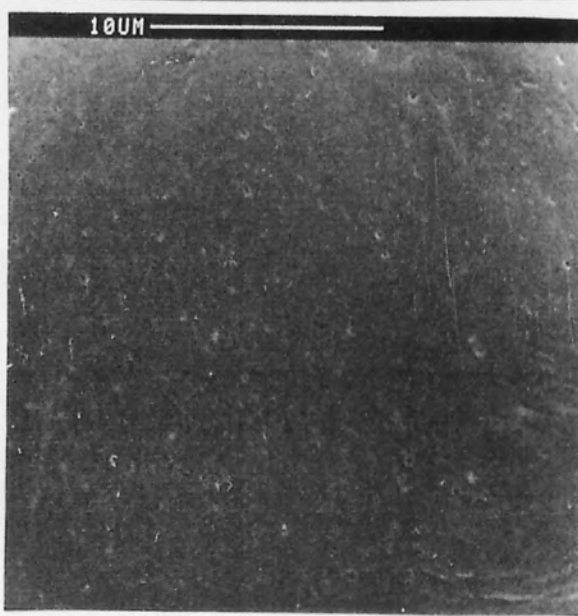
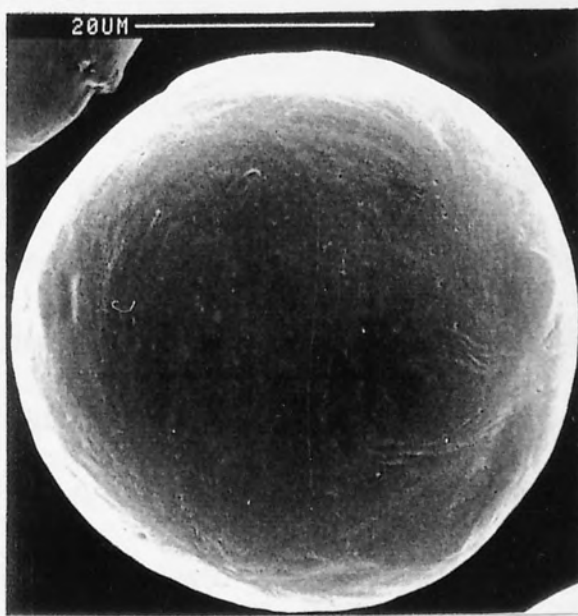
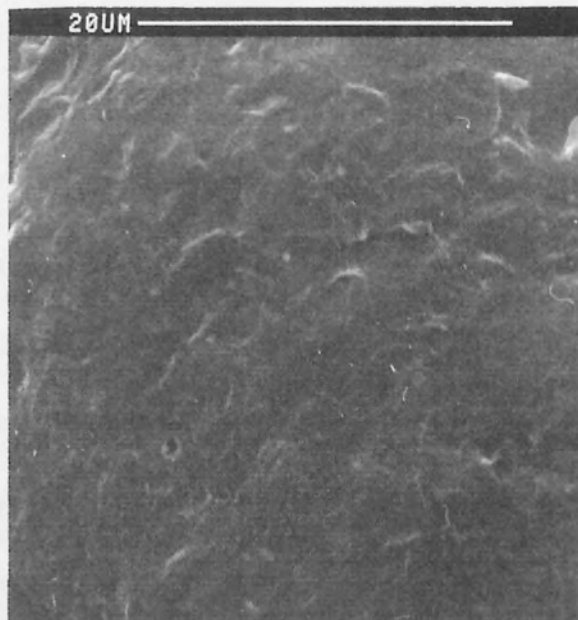
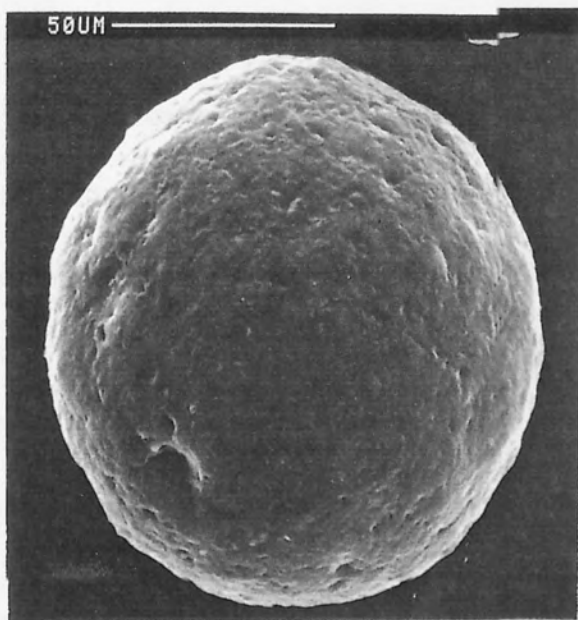
Low magnification [x626]

High magnification [x2410]

(k) 0-100

Low magnification [x1860]

High magnification [x3390]



II respectively.

Microcapsules prepared from Blends 10 and 20 [figures 4.3. (b) and (c) respectively] have well formed walls and surfaces covered by numerous discrete micropores of approximately 2-4 μm in size.

Blend 30 microparticles exhibit two distinct types of porosity [figure 4.3 (d)]. Thus, the majority of the surface is covered by individual pores, fairly consistent in size, but larger [at approximately 4-6 μm] than those observed on Blend 10 and 20 particles. There are, however, also several much larger holes, which are over 10 μm in size, extending deep into the wall. Similar features are found on particles prepared from Blend 40 [figure 4.3 (e)], although in this instance a greater number of large holes are encountered. This trend continues as the proportion of PCL II in the fabricating blend reaches 50%, at which stage little more than a particle framework remains [figure 4.3 (f)].

When PCL II becomes the predominant component, the microparticles produced have very different morphologies. Blend 60 particles have grossly deformed walls with large quantities of small polymer debris hidden within the holes [figure 4.3 (g)]. This latter feature is even more pronounced when the PCL II level reaches 70%, although, with these particles, the wall itself is structurally more intact [figure 4.3 (h)].

Microcapsules produced from Blends 80 and 90 are indistinguishable, and have intact walls with a fairly smooth surface. Some small dents are obvious, but the particles are essentially non-porous [figures 4.3 (i) and (j)]. These characteristics are similar to those of microcapsules prepared from unblended PCL II, although in the latter case the particle surfaces are even smoother [figure 4.3 (k)].

The surface morphologies of the Blend 10 and 20 microcapsules described in this section are similar to those of particles prepared from 350PHB (12% HV) with 20% PCL I at T3.

The explanation that was forwarded in section 4.2.1 to account for the latter situation is also considered to be applicable to these blends. However, the lower molecular weight of PCL II in comparison with PCL I, is believed to encourage the polymer-solvent-polymer interactions which lead to the enhanced solubility and more uniform precipitation of the P(HB-HV) copolymer, and as a result, the importance of temperature in determining particle morphology is diminished.

Indeed, additional experiments showed that particles prepared from 330PHB (10.8% HV) with 20% PCL II at T1, T3 and T4 were not dissimilar.

The level of miscibility between 330PHB (10.8% HV) and PCL II in the presence of DCM appears to diminish when the proportion of PCL II exceeds 20%. This is clearly reflected in the morphologies of the microparticles prepared from Blends 30, 40, and 50. In these instances, the two polymers are believed to be only partially miscible in the solvent-rich microdroplets, with the result that two distinct polymer phases [a PHB-rich phase containing PCL and a PCL-rich phase containing PHB] are present. The precipitation events which are associated with the PHB-rich phase appear similar to those described for the Blend 10 and 20 formulations, although the micropores are larger because the actual quantity of PCL II in this phase is likely to be slightly greater than 20%.

PCL-rich regions non-uniformly dispersed in the more voluminous PHB-rich phase are believed to give rise to the large pores observed on microparticles prepared from blends containing 30%-50% PCL II. After crystallizing from solution, the PCL II in these regions probably flakes out of the particle wall in the same way as the smaller

fragments in the PHB-rich phase.

When the PCL II content in the fabricating blend reaches 60%, this polymer forms the superstructure of the particle. At this level, and also at 70% PCL II, the two polymers appear to have little mutual miscibility, even in the presence of solvent. Thus, PHB-rich regions non-uniformly dispersed in the more voluminous PCL-rich phase precipitate and then fragment leaving only the PCL particle framework. In these instances, as figures 4.3 (g) and (h) clearly demonstrate, large quantities of PHB debris are retained within the particle crevices. As was mentioned in section 4.2.2, the visualization of polymer debris in some instances where it has been postulated to occur, but not in others, may be the result of a subtle and unintentional variation in the isolation procedure.

Microcapsules prepared from Blends 80 and 90 have similar appearances which closely resemble those of particles prepared from unblended PCL II. PCL is a smooth film-forming polymer and this is manifested in the surface morphologies of particles prepared from these formulations. There is little indication that the PHB polymer has any appreciable solubility in the bulk PCL phase.

4.3.2. Blends of PCL II with different P(HB-HV) polymers.

The microcapsules described in this section were prepared at T1, from blends of 20% PCL II, and, individually, ten different P(HB-HV) polymers.

The yields from the different formulations were assessed according to the procedure described in section 2.5.1, and the results are summarized in table 4.2. This table also compares the yields from the 20% PCL II blends with those from the corresponding unblended P(HB-HV) polymers.

Table 4.2. The microcapsule yields obtained from blends of different P(HB-HV) polymers with 20% PCL II. Also shown are the differences between the yield from each blend and the corresponding unblended P(HB-HV) polymer formulation [yield differences estimated at less than 10% are described as "negligible"]. All experiments were performed at temperature treatment T1.

Polymer	Yield from 20% PCL II blend	Yield Change*
43PHB	92	negligible
159PHB	73	+29
273PHB	88	negligible
540PHB	96	negligible
83.1PHB (10.8% HV)	96	negligible
180PHB (10.8% HV)	76	+22
330PHB (10.8% HV)	72	negligible
32.8PHB (20.1% HV)	60	negligible
140PHB (20.1% HV)	82	+30
390PHB (20.1% HV)	90	+70

*Calculated as % yield from [20% PCL II / P(HB-HV) polymer blend] - [% yield from corresponding unblended P(HB-HV) polymer].

Table 4.2 shows that, with most of the P(HB-HV) polymers, the yields resulting from the unblended formulations were very similar to those produced from the corresponding 20% PCL II blends. However, a dramatic 70% yield increase over the unblended formulation was detected when 390PHB (20.1% HV) was blended with 20% PCL II. In addition, blending also produced higher yields of microcapsules from the three band B polymers [for classification details, see section 2.2.1].

The poor yield [20%] of microcapsules produced from unblended 390PHB (20.1% HV) at T1 was attributed in chapter three to the retention of a significant level of solvent in the microdroplets after the solvent evaporation period had elapsed. This was primarily due to the high dispersed phase viscosity which resulted from the

polymer's high molecular weight. Blending with 20% PCL II has the effect of lowering the dispersed phase viscosity in comparison to the unblended formulation, and the high microcapsule yields in the former instance are therefore probably due to the resulting higher flow of solvent from the microdroplets during the evaporation period and the consequent increased ease of polymer precipitation.

In contrast, the low yields of microcapsules produced from the unblended band B polymers were a consequence of a high ratio of prematurely precipitated debris to perfect and near perfect particles. Polymer precipitation prior to the formation of a stable microemulsion was forwarded in chapter three as the explanation for this behaviour. The yield improvements, which resulted from the blending of these polymers with 20% PCL II, are therefore probably due to an enhancement in their solubilities and a consequent reduction in the level of prematurely precipitated debris. This could have been produced as a consequence of a co-solubility effect by the PCL II.

Figures 4.4 (a)-(i) represent scanning electron micrographs of typical microcapsules obtained from a selection of the P(HB-HV) polymer / 20% PCL II blends detailed in table 4.2.

Figure 4.4 (a) shows that, as with the 43PHB / 20% PCL I blend detailed in section 4.2.2, microencapsulation at T1 with 43PHB blended with 20% PCL II resulted in the production of wrinkled particles whose surfaces were partially covered by a smooth PCL plate.

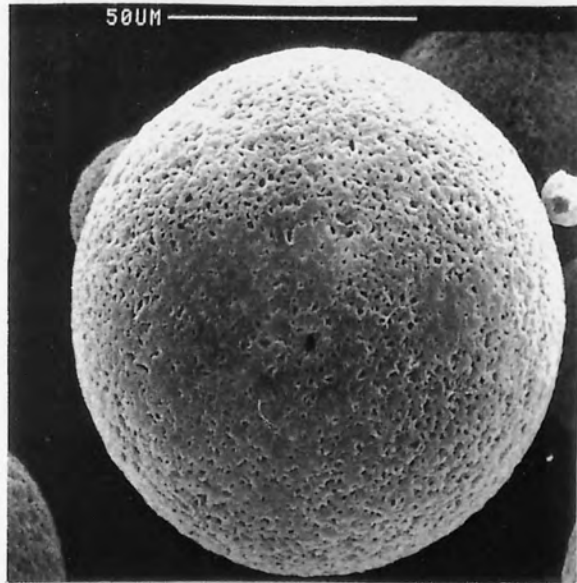
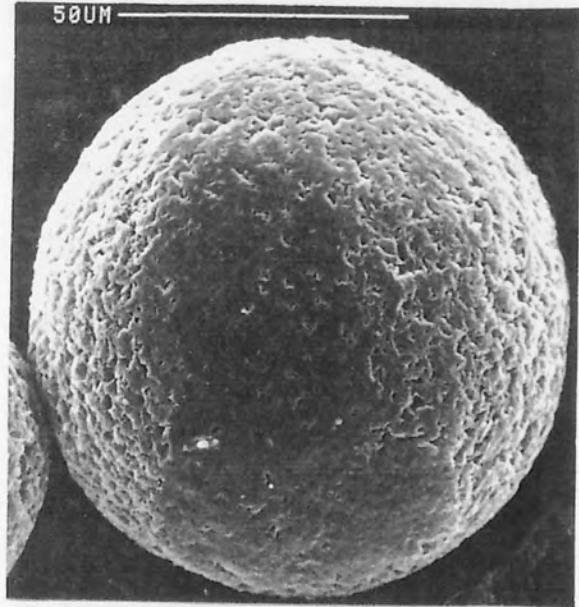
The microcapsules prepared from the 159PHB / 20% PCL II blend were essentially well-formed and spherical, but exhibited an unusually high degree of variation in terms of surface morphology. As a result, it is not possible to offer meaningful comment in

Figures 4.4 (a)-(c). Scanning electron micrographs of microcapsules prepared at temperature treatment T1 from blends of different PHB homopolymers with 20% PCL II :

(a) 43 000

(b) 273 000

(c) 540 000.



relation to the latter for these particles.

Microencapsulation with 273PHB blended with 20% PCL II resulted in the production of particles with well-formed walls, but surfaces uniformly perforated by discrete micropores [figure 4.4 (b)]. In contrast, as figure 4.4 (c) demonstrates, microcapsules prepared from a blend of the highest molecular weight PHB homopolymer [i.e. 540PHB] with 20% PCL II, exhibited two distinct types of porosity. Thus, the majority of the surface was covered by individual micropores, but there were also several larger holes [of approximately 10 μ m in size], which extended deep into the wall. These features closely resemble those described in section 4.3.1 for microcapsules prepared from blends of 330PHB (10.8%HV) with 30% PCL II.

Indeed, increasing the molecular weight of the fabricating PHB homopolymer, whilst maintaining a constant [20%] proportion of PCL II, appears to have a similar effect on particle morphology to that caused by increasing the PCL II / 330PHB (10.8% HV) blend ratio [see section 4.3.1]. Thus, taking microcapsules prepared from the 43PHB, 273PHB and 540PHB blends with 20% PCL II, it would appear that as the molecular weight of the PHB rises, there is an increased solubility differential between the two polymers, coupled with a lower degree of polymer-polymer miscibility in the solvent-rich microdroplets.

Figure 4.4 (d) shows a typical microcapsule obtained from 83.1PHB (10.8%HV) blended with 20% PCL II. In this instance, the presence of PCL appears to have had little effect on surface morphology [c.f. figure 3.1 (d)]

Microcapsules prepared from unblended 180PHB (10.8%HV) at T1 were shown in chapter three to have heavily structured and macroporous surfaces as a consequence of

Figures 4.4 (d)-(i). Scanning electron micrographs of microcapsules prepared at temperature treatment T1 from blends of different P(HB-HV) polymers with 20% PCL II :

(d) 83.1PHB (10.8% HV)

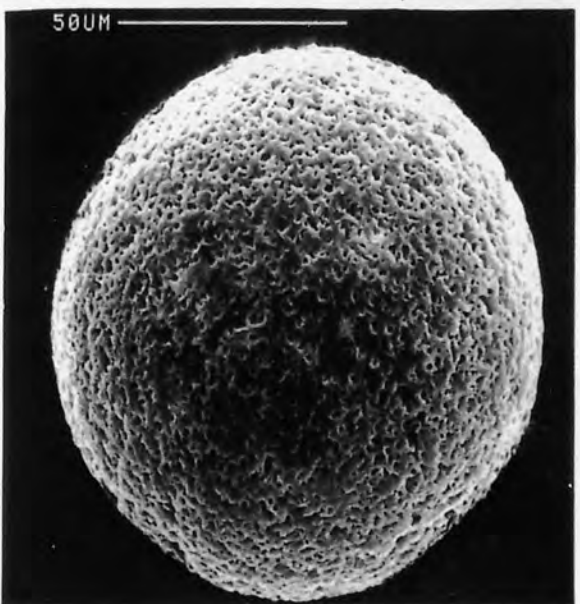
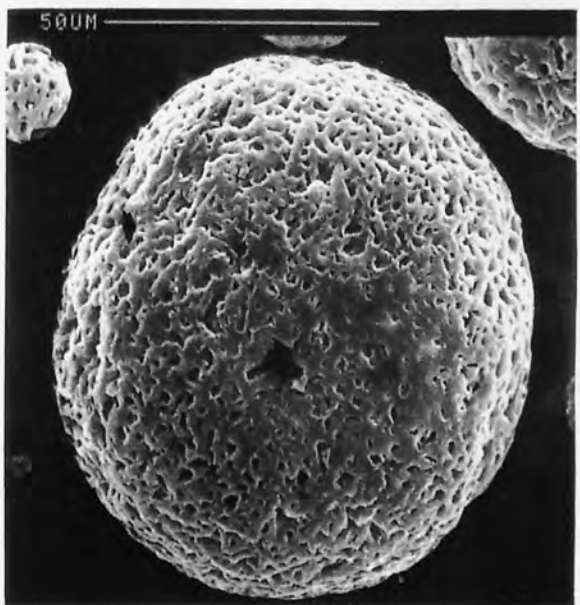
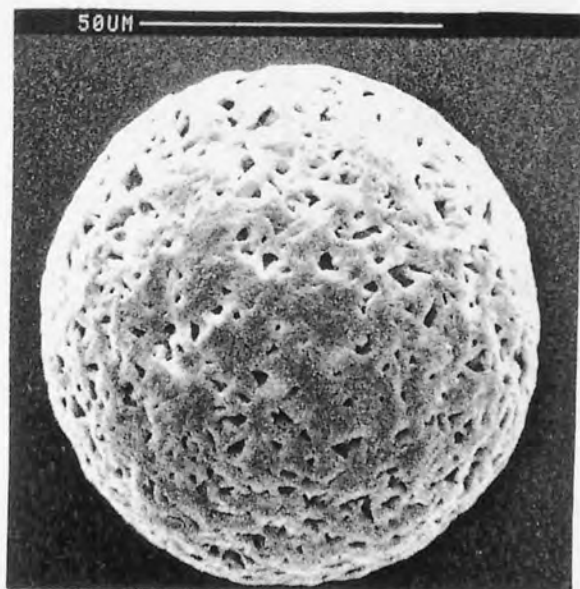
(g) 32.8PHB (20.1% HV)

(e) 180PHB (10.8% HV)

(h) 140PHB (20.1% HV)

(f) 330PHB (10.8% HV)

(i) 390PHB (20.1% HV)



irregular chain deposition in the particle wall [figure 3.1 (e)], and this also appears to be a feature of microcapsules prepared from a blend of this polymer with 20% PCL II, albeit to a reduced extent. However, as figure 4.4 (e) demonstrates, the presence of PCL II does appear to induce the formation of considerable microporosity on the surfaces of 180PHB (10.8%HV) / 20% PCL II microcapsules.

Microencapsulation with 32.8PHB (20.1%HV) blended with 20% PCL II resulted in the formation of particles with badly formed walls, and a high level of surface macroporosity [figure 4.4. (g)]. However, these characteristics are also typical of microcapsules prepared from unblended 32.8PHB (20.1%HV) at T1 [figure 3.1 (g)], and therefore the presence of PCL was not judged to have a significant effect on the morphology of particles prepared from this particular P(HB-HV) polymer. Similarly, microcapsules prepared from 140PHB (20.1%HV) blended with 20% PCL II are also very similar to those correspondingly prepared from the unblended P(HB-HV) polymer. However, as a comparison of figure 4.4 (h) and figure 3.1 (h) reveals, the presence of PCL II appears to slightly enhance particle porosity.

As was noted with 273 PHB, the blending of either 330PHB (10.8%HV) or 390PHB (20.1%HV) with 20% PCL II, led to the formation of microcapsules with well-formed walls, but surfaces uniformly perforated by discrete micropores [figures 4.4 (g) and (j)].

It is virtually impossible, given the number of variables involved in these systems, to make incontrovertible statements about cause and effect. It must be continuously borne in mind that the formation of microcapsules by the double emulsion solvent evaporation process is not the simplest of experimental procedures. Choice of polymer,

polymer concentration and processing conditions can all have dramatic effects, not only on the properties of the microcapsules, but also on whether they are actually formed or not! There is therefore a conjunction or window of conditions in which microcapsule formation is feasible. An alteration to the system, for example by accelerating the precipitation of the polymer, will have an effect that is related to the position of the experimental system within this window. Thus, at one extreme, enhancing precipitation favours microcapsule formation, whereas at the other, enhancing precipitation leads to a high level of prematurely precipitated debris with few intact microcapsules. As a result of the fact that changing even single variables, such as copolymer composition and molecular weight, can have several knock-on effects, it is always a matter of judgement, rather than certainty, in interpreting morphological features in terms of normal physico-chemical parameters.

5.1. Introduction.

The previous two chapters have demonstrated a number of ways in which the morphologies of reservoir-type microcapsules, prepared from P(HB-HV) polymers using a double emulsion-solvent evaporation process, can be modified. The investigations described in this chapter are directed towards the inclusion of macromolecules (FTTC-dextran, BSA and bovine haemoglobin) in the colloidal gel at the centre of such microcapsules and the subsequent evaluation of their *in vitro* release profiles.

CHAPTER FIVE.

MACROMOLECULAR RELEASE STUDIES.

The release of entrapped macromolecules from (HB-HV) microcapsules cannot be expected to occur by partition-dependent diffusion through the polymer wall, because the free volume available for diffusion is small. Additionally, transportation by this mechanism requires appreciable drug solubility within the polymer phase and so it cannot be expected to facilitate the release of hydrophilic macromolecules such as sugars and polypeptides. These molecules are unlikely to have any appreciable solubility in a polyester because of structural dissimilarities²⁹ and mutual incompatibility³⁰.

However, since an alternate route for drug release is by diffusion through aqueous pores and channels, the microcapsules for evaluation as macromolecular carriers in this chapter have been made from a blend of 330PHB (10.8% HV) with 20% PCL. It was shown in chapter four to generate uniformly macroporous particles.

5.2. Microcapsules with a gelatin-based inner aqueous phase.

Studies by Ogawa *et al.*¹⁸, aimed at encapsulating the peptide drug leuprolide acetate within poly(lactic) acid and poly(glycolic acid-co-lactic acid) microcapsules

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The release of entrapped macromolecules from P(HB-HV) microcapsules cannot be expected to occur by partition-dependent diffusion through the polymer wall, because the free volume arising from segmental motion will be too small. Additionally, transportation by this mechanism requires appreciable drug solubility within the polymer phase and so it cannot be expected to facilitate the release of hydrophilic macromolecules such as sugars and polypeptides. These molecules are unlikely to have any appreciable solubility in a polyester because of structural dissimilarities⁵⁹ and mutual incompatibility³⁴.

However, since an alternate route for drug release is by diffusion through aqueous pores and channels, the microcapsules for evaluation as macromolecular carriers in this chapter have been made from a blend of 330PHB (10.8% HV) with 20% PCL II. This formulation was shown in chapter four to generate uniformly microporous particles.

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Studies by Ogawa *et al.*¹⁸, aimed at encapsulating the peptide drug leuprolide acetate within poly(lactic) acid and poly(glycolic acid-co-lactic acid) microcapsules

using a double emulsion-solvent evaporation technique, found that the encapsulation efficiency was critically dependent on the viscosity of the inner aqueous phase. Thus, when microencapsulation was attempted with a leuprolide acetate / water solution as the inner aqueous phase, only 6.7% of the starting drug was encapsulated. In contrast, up to 70% of the initial leuprolide acetate could be entrapped when 16% [w/v] gelatin was incorporated into the inner aqueous phase, provided that the viscosity was increased by temperature reduction prior to phase combination. A further increase in the gelatin concentration to 20% [w/v], however, reduced the proportion of encapsulated drug and resulted in the production of deformed microcapsules.

The above findings suggest that it may be possible to experimentally determine an inner aqueous phase viscosity that will optimize the encapsulation efficiency, within non-deformed microcapsules. However, since the polymer formulation for encapsulating macromolecules in this chapter [330PHB (10.8% HV) / 20% PCL II] was selected on the basis of the morphological studies reported in chapter four, which employed 6% gelatin solutions as the inner aqueous phase, microcapsules prepared at other gelatin levels may not have the desired morphologies.

Consequently, three experiments were performed in order to assess how the proportion of encapsulated macromolecule, and microcapsule morphology, were affected by changes to the inner aqueous phase viscosity [i.e. gelatin concentration], when microencapsulation was practised using the procedure described in section 2.3.

In these investigations, FD-20 was used as the macromolecular marker at a concentration of 6% [w/v] in the inner aqueous phase, regardless of the gelatin concentration. Experiment 1 had an inner aqueous phase gelatin concentration of 6% ; experiment 2, 11% ; and experiment 3, 16% [all w/v]. The microencapsulation procedure was the same for each experiment, as described in section 2.3.

The results obtained from experiments 1-3 [detailed above] are summarized in table 5.1.

Table 5.1. Inner aqueous phase formulation details for experiments 1-3, and some characteristics of the resulting microcapsules.

	Inner aqueous phase CONCENTRATION [w/v] of :		ENCAPSULATION EFFICIENCY [%]	SURFACE MORPHOLOGY
	<u>GELATIN :</u>	<u>FD-20 :</u>		
EXP ^T . 1	6	6	negligible	uniformly microporous
EXP ^T . 2	11	6	negligible	as above, but many particles mis-formed
EXP ^T . 3	16	6	34	gelatin-polymer conjugate

In all three experiments, the continuous phase [PVA solution] was orange-coloured at the end of the 5 hour solvent evaporation period, indicating the presence of FITC-dextran.

A significant fraction of the microcapsules produced in experiments 1 and 2 were in the 38-150 μ m size range, and had the appearance of a white powder when dried.

However, these microcapsules were not visible under the fluorescence microscope and content analysis did not reveal the presence of any FITC-dextran.

SEM demonstrated that the microcapsules prepared in experiment 1 were largely spherical, and had similar morphologies to the corresponding particles prepared for the morphological investigations. This confirmed that freeze-drying did not alter particle

morphology.

In contrast, experiment 2 was revealed by SEM to have generated many mis-formed particles and a high level of polymer debris. The morphologies of the observed intact particles were, however, the same as those obtained in experiment 1.

The microparticles produced in experiment 3 tended to be aggregated, and as a result were generally too large to pass through the 150 μ m sieve. These particles had a bright orange colour, and as figure 5.1 shows, they were clearly visible under the fluorescence microscope, although they did not appear to be particularly spherical.

Moreover, SEM revealed that the high inner aqueous phase viscosity had resulted in the production of a highly irregular particle wall [figure 5.2]. Thus, the dye and gelatin appeared to be loosely held in an entangled polymer cage, and were quickly flushed out [total release within 5 hours] when immersed in borate buffer at 37°C.

The results obtained from these preliminary experiments suggest that it is not possible to successfully entrap a 20KD macromolecule at the centre of a structurally intact, reservoir-type microcapsule, using the procedure described in section 2.3. Thus, although particles prepared with a 16% inner aqueous phase gelatin concentration contained 34% of the initial macromolecule, they were totally inadequate as a controlled release formulation. Furthermore, their considerable structural irregularity suggested that batch to batch reproducibility would not be easily achieved.

Whilst Ogawa *et al.*¹⁸ claimed successful encapsulation using the double emulsion technique, a number of their preparative variables were different from those used in these studies. Of prominent importance in this regard are probably the nature of the encapsulating polymer and of the gelatin binder. Indeed, Ogawa *et al.*¹⁸ highlighted

Figure 5.1. Fluorescence micrograph of the particles prepared in experiment 3 [16% w/v inner aqueous phase gelatin concentration].
Magnification : x125.

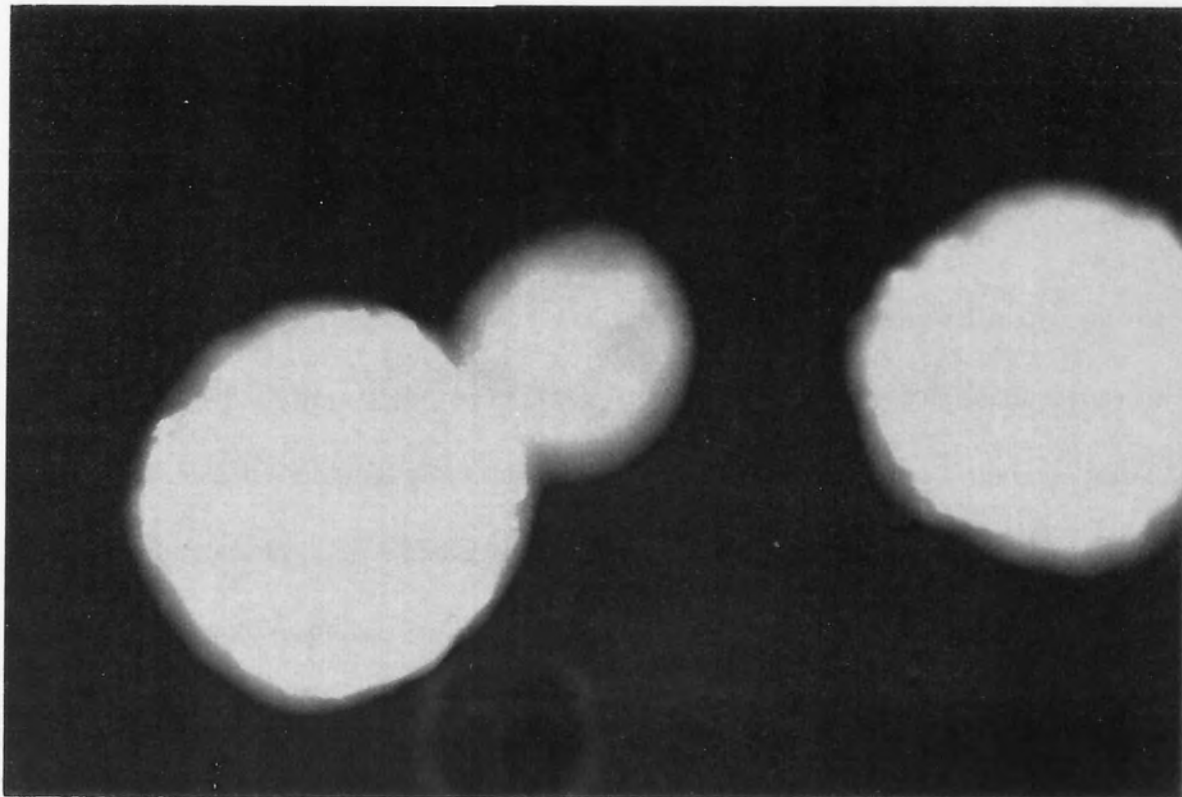
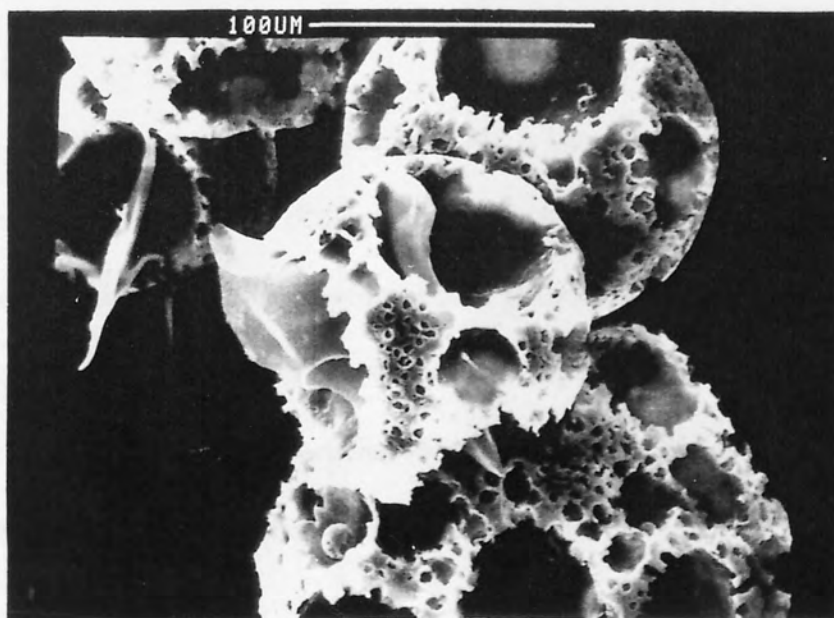


Figure 5.2. Scanning electron micrograph of the particles prepared in experiment 3 [16% w/v inner aqueous phase gelatin concentration].



the importance of the former by showing that the encapsulation efficiency fell from 70% to 44% by only slightly changing the composition of the encapsulating polymer.

The type of gelatin employed in their work was not specified, but the 300 bloom employed in these studies was the highest viscosity grade available from the Sigma Chemical Company. It is also interesting to note that Ogawa's paper did not provide a detailed account of microcapsule morphology.

An explanation for the effects of inner aqueous phase viscosity on microcapsule morphology, which were observed in the experiments described in this section, may be as follows. When the initial w/o emulsion is added to the continuous aqueous phase, the organic microdroplets, which usually form, will contain numerous smaller droplets of the inner aqueous phase. In instances where microencapsulation is successful, and hollow devices with intact polymers walls are formed, the collection of inner aqueous phase droplets are believed to coalesce into one large droplet whilst the organic phase still contains a high level of solvent. This appears to happen during the production of microcapsules from 330PHB (10.8% HV) with 20% PCL II, when the inner aqueous phase contains 6% gelatin.

However, as the inner aqueous phase viscosity is increased by the addition of more gelatin, it becomes increasingly difficult for the droplets within the organic phase to fuse. Moreover, when the inner aqueous phase gelatin concentration reaches 16%, gelation occurs so readily that the polymer effectively precipitates around a colloidal suspension. The resulting microparticles therefore have the appearance of a gelatin-polymer composite of the type shown figure 5.2.

5.3. Microcapsules with an agarose-based inner aqueous phase.

The unsuccessful encapsulation attempts reported in section 5.2 led to a second series of investigations, which employed a low gelling temperature agarose, instead of gelatin, to increase the viscosity of the inner aqueous phase. Preliminary investigations revealed that inner aqueous phase agarose concentrations between 1.5 and 3.0% [w/v] were suitable for microencapsulation, provided that the initial w/o emulsion was not cooled from 37°C before phase combination. In all the following experiments, the inner aqueous phase agarose concentration was 2.25% [w/v], and microencapsulation was performed as described in section 2.3. The macromolecules used, and their loadings, are detailed in table 5.2, which also summarizes the extent to which encapsulation was successful in each case.

Table 5.2. The different macromolecules encapsulated during the experiments described in sections 5.3.1 - 5.3.4 [for details, see text], together with their loading, content and encapsulation efficiency details.

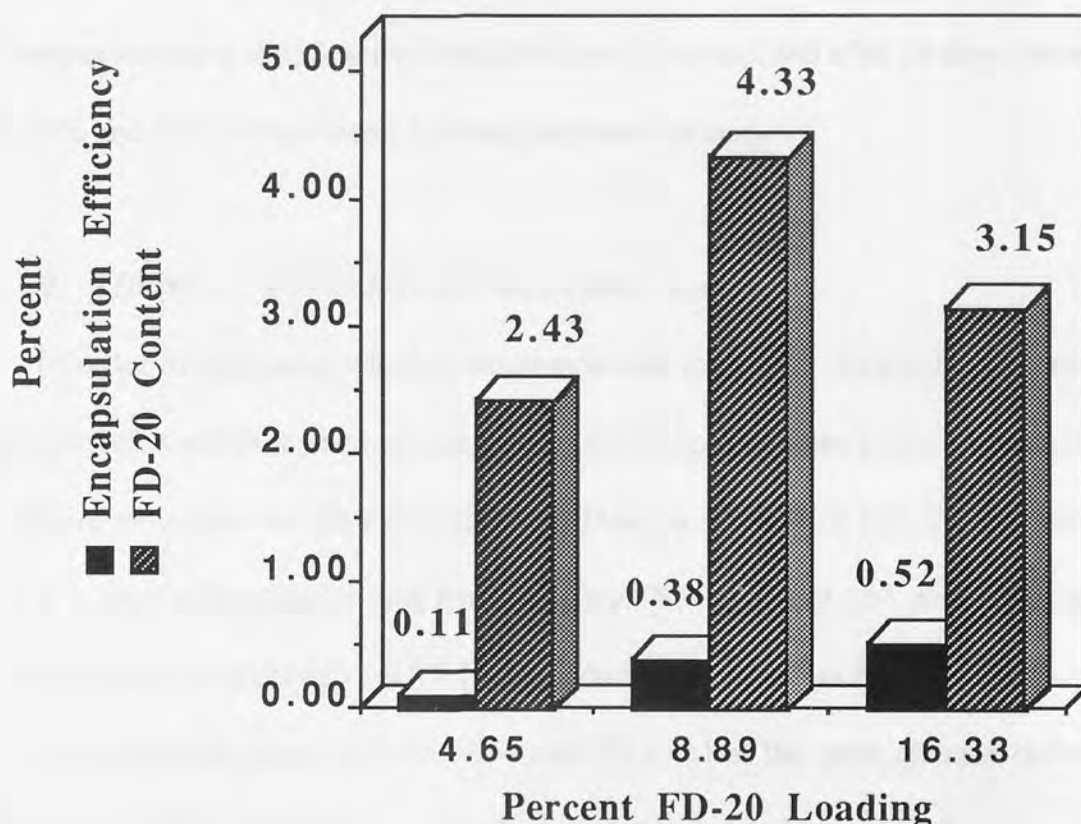
TYPE	<u>MACROMOLECULE :</u>		
	LOADING [%]	CONTENT [%]	ENCAPSULATION EFFICIENCY [%]
FD-4	8.89	0.42	4.82
FD-20	4.65	0.11	2.43
	8.89	0.38	4.33
	16.33	0.52	3.15
FD-150	8.89	0.61	6.90
BSA	1.70	0.19	11.39
INSULIN	1.70	0.05	2.85

5.3.1. Effects of FITC-dextran loading.

The importance of the FITC-dextran loading in governing the encapsulation efficiency and dissolution kinetics, was investigated by preparing microcapsules with FD-20 loadings of 4.65%, 8.89% and 16.33%. These loadings were respectively achieved by employing FD-20 inner aqueous phase concentrations of 4.5%, 9.0% and 18.0% [w/v]. Each of these formulations gave rise to an excellent yield of microcapsules in the 38-150 μ m size range, having the desired [uniformly microporous] morphologies. However, the continuous phase [PVA solution] was orange-coloured at the end of the solvent evaporation period in all the experiments, and the particles were barely visible under the fluorescence microscope.

The three microcapsule formulations were found to have FD-20 contents ranging from approximately 0.11% to 0.52%. This implied encapsulation efficiencies of between 2.43% and 4.33%. However, as is evident from graph 5.1 ,although the quantity of encapsulated macromolecule appeared to be directly related to the initial loading, there was no such correlation with regard to the encapsulation efficiency, which was highest in particles prepared with an 8.89% FD-20 loading.

Graph 5.1. The effects of macromolecule loading on microcapsule FD-20 content and the encapsulation efficiency.



Graphs 5.2 and 5.3 compare the dissolution profiles, obtained over 10 days at 37°C, from microcapsules prepared at the three different FD-20 loadings. Graph 5.2 shows that, throughout the dissolution procedure, the cumulative quantity of FD-20 released increased as the amount of entrapped macromolecule increased. However, when the release rates were plotted in terms of the cumulative proportion of encapsulated FD-20 released over time, as in graph 5.3, this relationship no longer held. Instead, microcapsules from each formulation actually had very similar release profiles, which consisted of a rapid initial burst, whereby between 26.50% and 42.83% of the encapsulated FD-20 was released in the first 90 minutes, followed by more sustained release over an approximate 4 day period.

At this stage, the different microcapsule formulations had discharged between 71.73% and 92.93% of their FD-20 payloads. The final six days of all three dissolution experiments saw a significantly diminished rate of release, and after 10 days, between 93.99% and 100% of the detectable drug had been released.

5.3.2. Effects of FITC-dextran molecular weight.

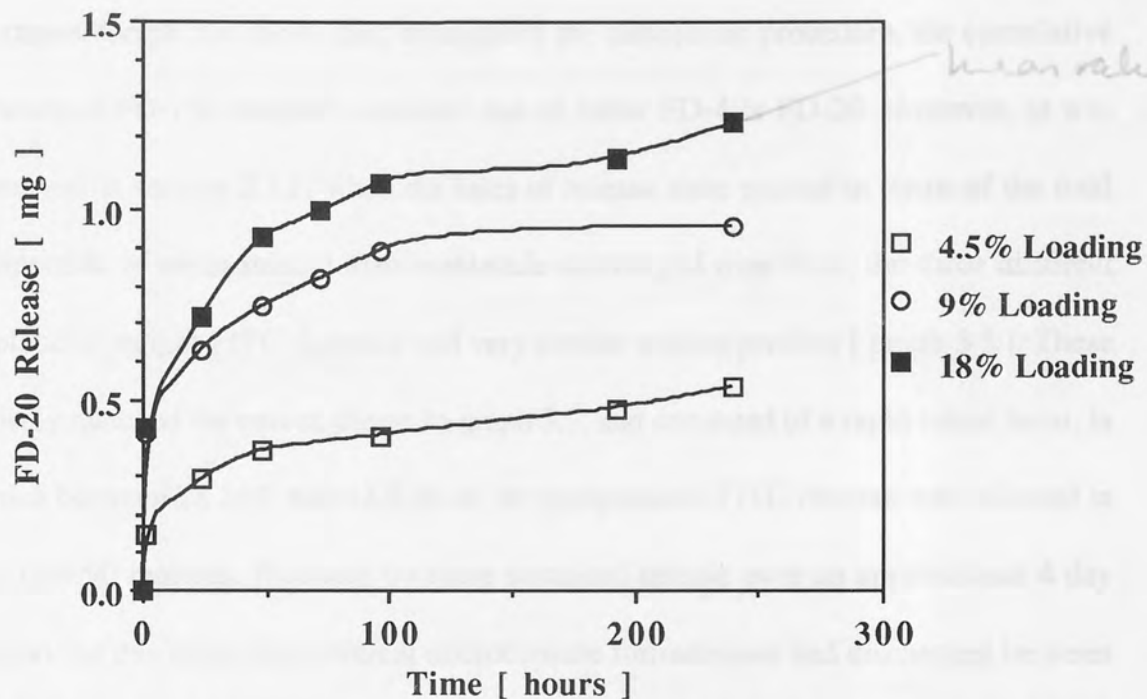
In order to determine whether macromolecule molecular weight influenced the encapsulation efficiency and release profile, microcapsules were prepared using three different molecular weight FITC-dextran. Thus, in addition to FD-20 [see section 5.3.1.], microencapsulation was performed with FD-4 and FD-150, each at an inner aqueous phase concentration of 9% [w/v], which represented an 8.89% loading.

Microcapsules prepared from FD-4 and FD-150 had the same characteristics as those prepared from FD-20, and described in section 5.3.1. Thus, in each case, a good yield of 38-150 μ m particles was obtained, and these had the desired [uniformly microporous] morphologies, but were barely visible using fluorescence microscopy.

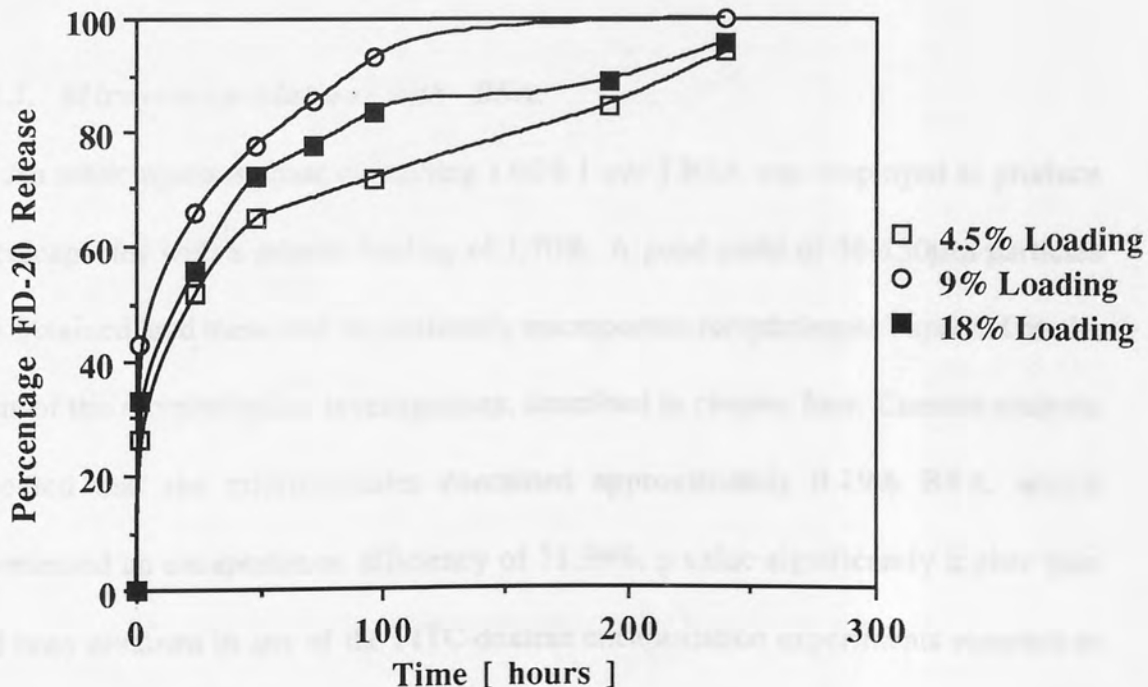
The continuous phase was again orange-coloured at the end of the solvent evaporation period in each experiment.

Microcapsules, produced using FD-4 and FD-150, respectively had macromolecule contents of approximately 0.42% and 0.61%. Interestingly, both these values exceeded the 0.38% content of microcapsules prepared using an FD-20 solution of the same concentration. Since all these microcapsule formulations had the same FITC-dextran loading, the encapsulation efficiencies adopted the same order as the macromolecule contents. Thus, FD-150 had the highest encapsulation efficiency at 6.91%, which was followed by 4.82% for FD-4, and 4.33% for FD-20.

Graph 5.2. Dissolution profiles showing the cumulative release of FD-20 from microcapsules prepared with different macromolecule loadings :



Graph 5.3. Dissolution profiles showing the proportion of total encapsulated FD-20 released with time from microcapsules prepared with different macromolecule loadings :

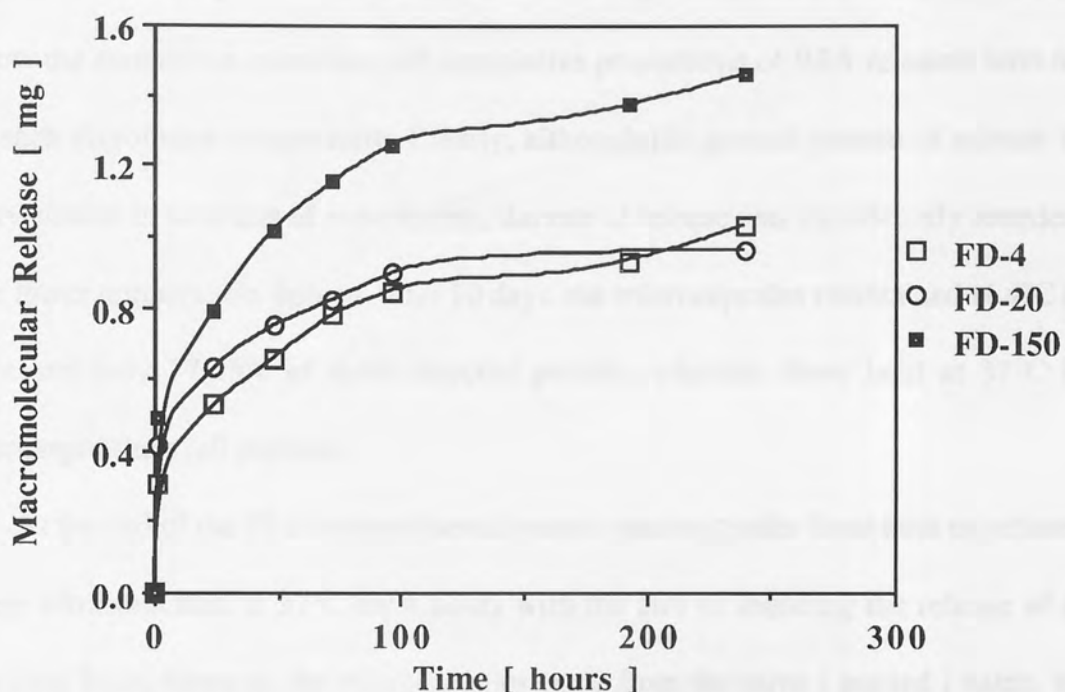


Graphs 5.4 and 5.5 compare the dissolution profiles, obtained over 10 days at 37°C, from microcapsules prepared using the three different molecular weight FITC-dextran. Graph 5.4 shows that, throughout the dissolution procedure, the cumulative amount of FD-150 released exceeded that of either FD-4 or FD-20. However, as was observed in section 5.3.1, when the rates of release were plotted in terms of the total proportion of encapsulated macromolecule discharged over time, the three different molecular weight FITC-dextran had very similar release profiles [graph 5.5]. These closely matched the curves shown in graph 5.3, and consisted of a rapid initial burst, in which between 28.54% and 42.83% of the encapsulated FITC-dextran was released in the first 90 minutes, followed by more sustained release over an approximate 4 day period. At this stage, the different microcapsule formulations had discharged between 78.73% and 92.93% of their FITC-dextran payloads. The final six days of all three dissolution experiments saw a significantly diminished rate of release, and after 10 days, between 95.05% and 100% of the detectable macromolecule had been released.

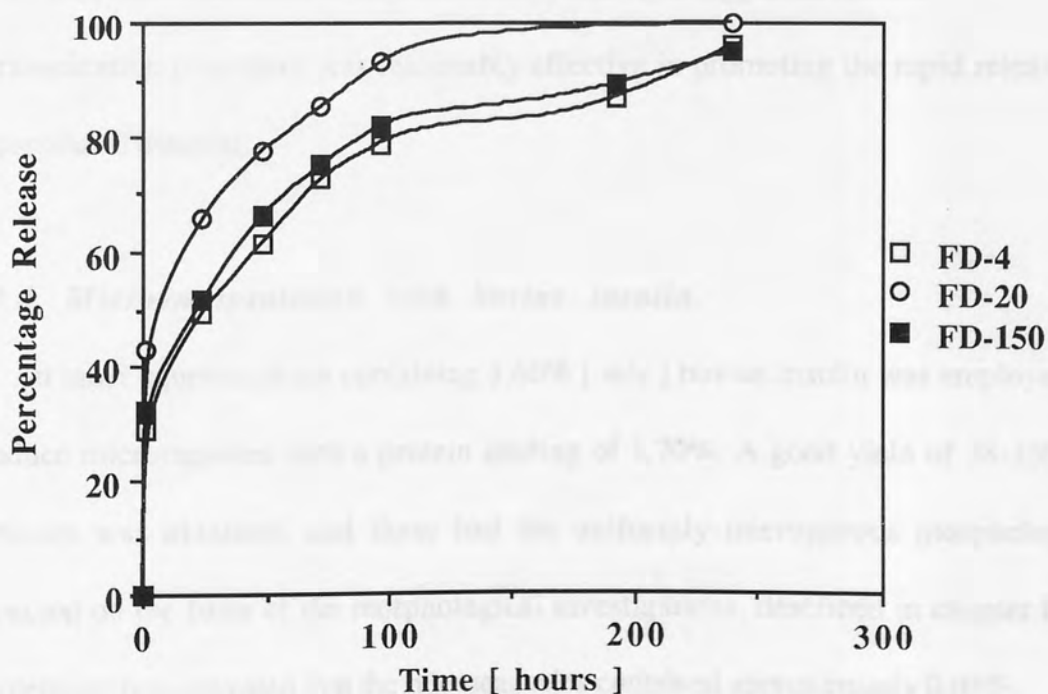
5.3.3. Microencapsulation with BSA.

An inner aqueous phase containing 1.60% [w/v] BSA was employed to produce microcapsules with a protein loading of 1.70%. A good yield of 38-150µm particles was obtained, and these had the uniformly microporous morphologies expected on the basis of the morphological investigations, described in chapter four. Content analysis revealed that the microcapsules contained approximately 0.19% BSA, which represented an encapsulation efficiency of 11.39%, a value significantly higher than had been obtained in any of the FITC-dextran encapsulation experiments reported in sections 5.3.1 and 5.3.2.

Graph 5.4. Dissolution profiles showing the cumulative release of different molecular weight FITC-dextrans from microcapsules prepared with a 9% macromolecule loading :



Graph 5.5. Dissolution profiles showing the proportion of total encapsulated FITC-dextran released with time from microcapsules containing different molecular weight macromolecules at a 9% loading :



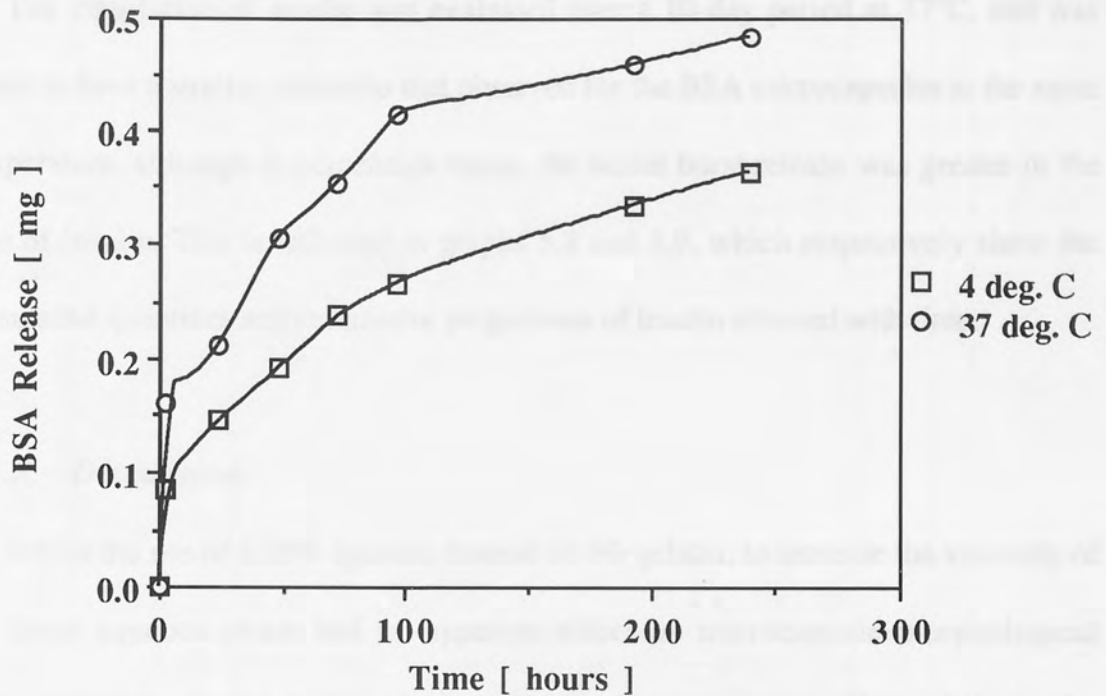
The dissolution profile of BSA from microcapsules was evaluated over a 10 day period, but in order to assess the effects of temperature on the release kinetics, experiments were performed at both 37°C and 4°C. Graphs 5.6 and 5.7 respectively show the cumulative quantities and cumulative proportions of BSA released with time at each dissolution temperature. Clearly, although the general pattern of release was very similar in both sets of experiments, the rate of release was significantly retarded at the lower temperature. Indeed, after 10 days, the microcapsules maintained at 4°C had released only 77.45% of their detected protein, whereas those held at 37°C had discharged their full payload.

At the end of the 10 day experimental period, microcapsules from both experiments were ultrasonicated at 37°C for 4 hours with the aim of inducing the release of any residual BSA. Since all the microcapsules came from the same [pooled] batch, they should have had very similar BSA contents. In practice, the microcapsules dissolved at 4°C were found to have 97.11% of the BSA found within the particles dissolved at 37°C. This was considered to be a satisfactory ratio and suggested that the ultrasonication procedure was reasonably effective in promoting the rapid release of encapsulated material.

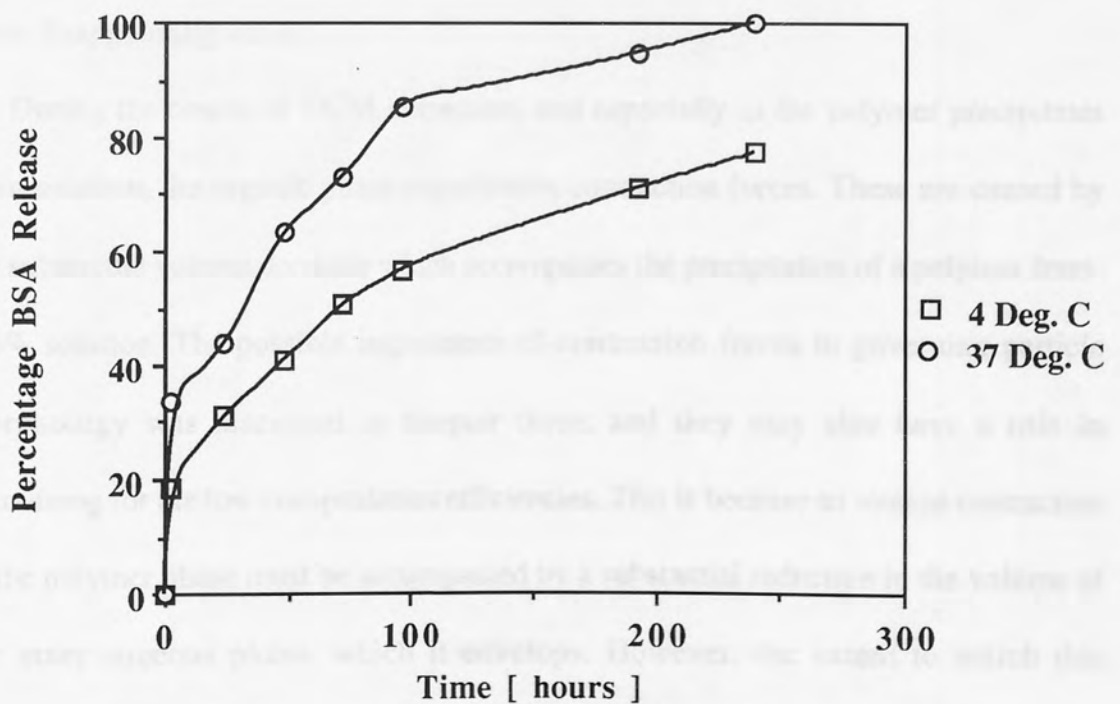
5.3.4. Microencapsulation with bovine insulin.

An inner aqueous phase containing 1.60% [w/v] bovine insulin was employed to produce microcapsules with a protein loading of 1.70%. A good yield of 38-150µm particles was obtained, and these had the uniformly microporous morphologies expected on the basis of the morphological investigations, described in chapter four. Content analysis revealed that the microcapsules contained approximately 0.05%

Graph 5.6. Dissolution profiles showing the cumulative release of BSA from microcapsules at different temperatures :



Graph 5.7. Dissolution profiles showing the proportion of total encapsulated BSA released with time from microcapsules at different temperatures :



insulin, which represented an encapsulation efficiency of 2.85%, a value significantly lower than was obtained with BSA at the same loading.

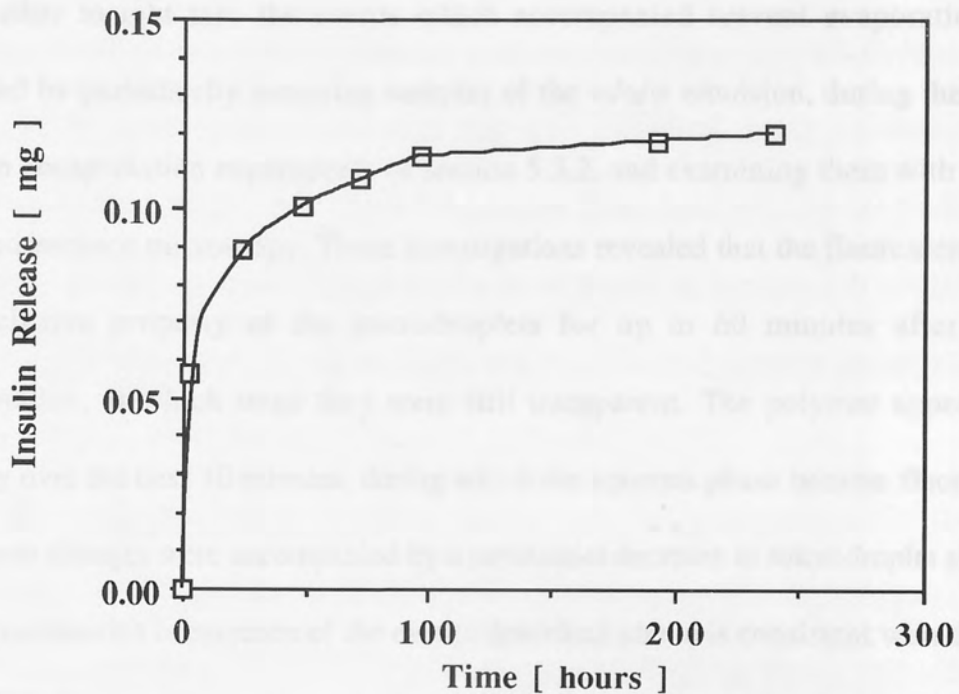
The dissolution of insulin was evaluated over a 10 day period at 37°C, and was found to have a similar pattern to that observed for the BSA microcapsules at the same temperature, although in percentage terms, the initial burst release was greater in the case of insulin. This is reflected in graphs 5.8 and 5.9, which respectively show the cumulative quantities and cumulative proportions of insulin released with time.

5.3.5. Discussion.

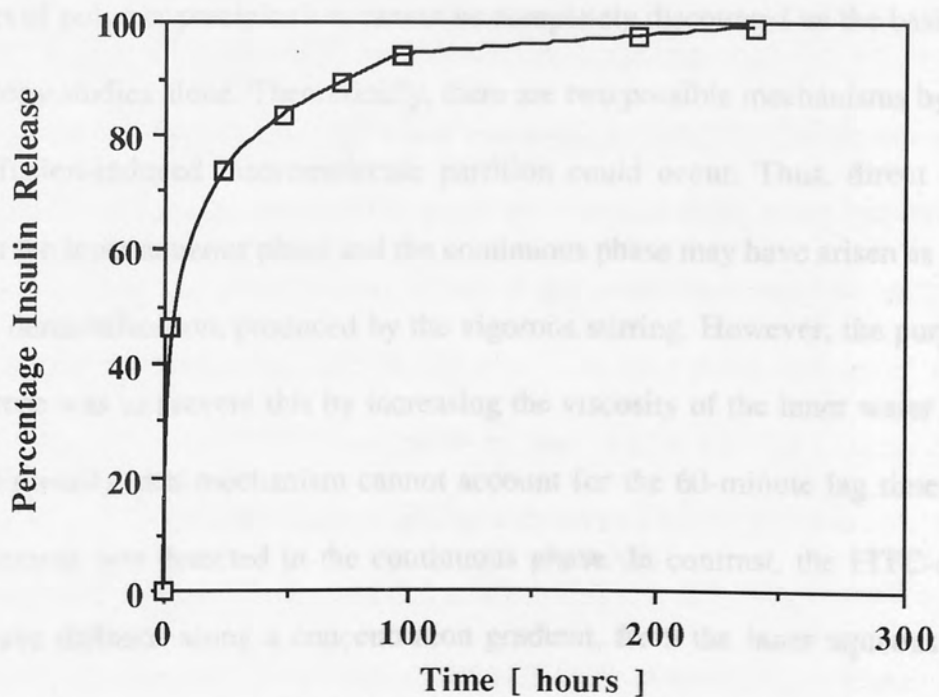
Whilst the use of 2.25% agarose, instead of 6% gelatin, to increase the viscosity of the inner aqueous phase had no apparent effect on microcapsule morphological characteristics, it did facilitate macromolecule entrapment. Nevertheless, the experiments reported in sections 5.3.1 - 5.3.4 resulted in low encapsulation efficiencies, ranging from 2.43% to 11.39%, and a maximum macromolecule content of just 0.61%. The preparative procedure gives rise to various possible explanations for these disappointing values.

During the course of DCM extraction, and especially as the polymer precipitates from solution, the organic phase experiences contraction forces. These are caused by the substantial volume decrease which accompanies the precipitation of a polymer from a 6% solution. The possible importance of contraction forces in governing particle morphology was discussed in chapter three, and they may also have a role in accounting for the low encapsulation efficiencies. This is because an intense contraction in the polymer phase must be accompanied by a substantial reduction in the volume of the inner aqueous phase, which it envelops. However, the extent to which this behaviour is responsible for the poor encapsulation efficiencies cannot be assessed

Graph 5.8. Dissolution profile showing the cumulative release of Insulin from microcapsules at 37°C :



Graph 5.9. Dissolution profile showing the proportion of total encapsulated insulin released with time from microcapsules at 37°C :



without further evidence. This is because the macromolecule may diffuse out of the inner aqueous phase along a concentration gradient before the onset of polymer precipitation.

Further insight into the events which accompanied solvent evaporation was obtained by periodically removing samples of the w/o/w emulsion, during the FITC-dextran encapsulation experiments of section 5.3.2, and examining them with optical and fluorescence microscopy. These investigations revealed that the fluorescence was an exclusive property of the microdroplets for up to 60 minutes after phase combination, at which stage they were still transparent. The polymer appeared to solidify over the next 10 minutes, during which the aqueous phase became fluorescent.

These changes were accompanied by a substantial decrease in microdroplet size. The simultaneous occurrence of the events described above is consistent with the idea that FITC-dextran migration into the continuous phase is largely induced by contraction pressures. However, the possibility that the macromolecule partitions into the continuous phase by simple diffusional release along a concentration gradient, before the onset of polymer precipitation, cannot be completely discounted on the basis of the microscopy studies alone. Theoretically, there are two possible mechanisms by which this diffusion-induced macromolecule partition could occur. Thus, direct contact between the inner aqueous phase and the continuous phase may have arisen as a result of local demulsification, produced by the vigorous stirring. However, the purpose of the agarose was to prevent this by increasing the viscosity of the inner water phase.

Additionally, this mechanism cannot account for the 60-minute lag time before FITC-dextran was detected in the continuous phase. In contrast, the FITC-dextran could have diffused along a concentration gradient, from the inner aqueous phase, through the organic phase, and into the continuous phase. This situation is analogous to

a diffusion cell experiment, used for measuring the transport properties of polymer membranes. The release kinetics observed in such an experiment typically consist of a lag time, followed by sustained release until equilibrium is established, and are therefore consistent with the microscopy observations. However, if FITC-dextran was lost from the inner aqueous phase in this way, the close proximity between the appearance of macromolecule in the continuous phase and polymer precipitation is surprising. Indeed, the maximum rate of release would be expected to occur shortly after phase combination, whilst the organic phase was still heavily plasticized with solvent.

Confirmation that simple diffusion was not responsible for the low FITC-dextran encapsulation efficiencies, was provided by repeating the microencapsulation experiment with FD-4, at a 4.65% loading, using a continuous phase preloaded with 4.65% dextran. This particular formulation was chosen because it enabled the macromolecule concentration gradient between the inner aqueous and continuous phases to be eliminated, whilst having a minimal impact on the viscosity of the latter. An FD-4 concentration gradient still remained between the inner aqueous phase and the organic phase, but the partition coefficient was small, and any FITC-dextran which did diffuse into the organic phase would be expected to remain there, eventually becoming embedded within the particle wall. Thus, if the poor encapsulation efficiencies described in sections 5.3.1 and 5.3.2 were due to simple diffusion along a concentration gradient, then this experiment, with 4.65% dextran in both aqueous phases, should have produced microcapsules with a much higher FD-4 content than the 0.42% obtained when the continuous phase was initially dextran-free.

However, the aqueous phase preloaded with dextran was orange-coloured at the

end of the solvent evaporation period, and the resulting microcapsules were found to have an FD-4 content of approximately 0.47%, which implied an encapsulation efficiency of only 5.29%. Clearly, the FD-4 concentration gradient between the two aqueous phases had no significant impact on the level of encapsulation, suggesting that simple diffusion was not the mechanism by which the macromolecule partitioned into the continuous phase.

The three experiments with FD-20 reported in section 5.3.1 demonstrated that, although an increase in the FITC-dextran loading marginally increased the quantity of macromolecule encapsulated, it had no consistent effects on the encapsulation efficiency. Similarly, in section 5.3.2, it was shown that the FITC-dextran molecular weight had no consistent effects on either the macromolecule content or the encapsulation efficiency. The diffusion coefficients of molecules with similar physico-chemical properties, through viscous media, normally decrease with increasing molecular weight. Consequently, the increase in molecular weight associated with a change from FD-4 or FD-20 to FD-150 might have improved the encapsulation efficiency, if diffusional processes had a prominent role in the partitioning of macromolecule from the microdroplet. In practice, the encapsulation efficiency with FD-150 was higher than with the lower molecular weight FITC-dextran at the same loadings, but the increase was negligible in absolute terms.

In order to evaluate the reproducibility of the preparative process, a second batch of microcapsules [also pooled from two duplicate preparations], was produced from FD-20 at an 8.89% loading. These were found to have a macromolecule loading of 0.32%, in comparison with 0.38% for the original batch. Whilst this indicates satisfactory

reproducibility, it clearly shows that the FITC-dextran contents of all the microcapsules produced from these experiments were really too similar in view of their low magnitudes for meaningful comparisons to be made.

BSA and bovine insulin microcapsules, prepared with loadings of 1.70%, were found to have encapsulation efficiencies of 11.39% and 2.85% respectively. However, although the former figure compares favourably with the results obtained from the FITC-dextran experiments, the actual BSA content was only 0.19%. This reflected the lower loading levels employed in the protein experiments, which were in response to these macromolecules' lower aqueous solubilities. *of these macromolecules*

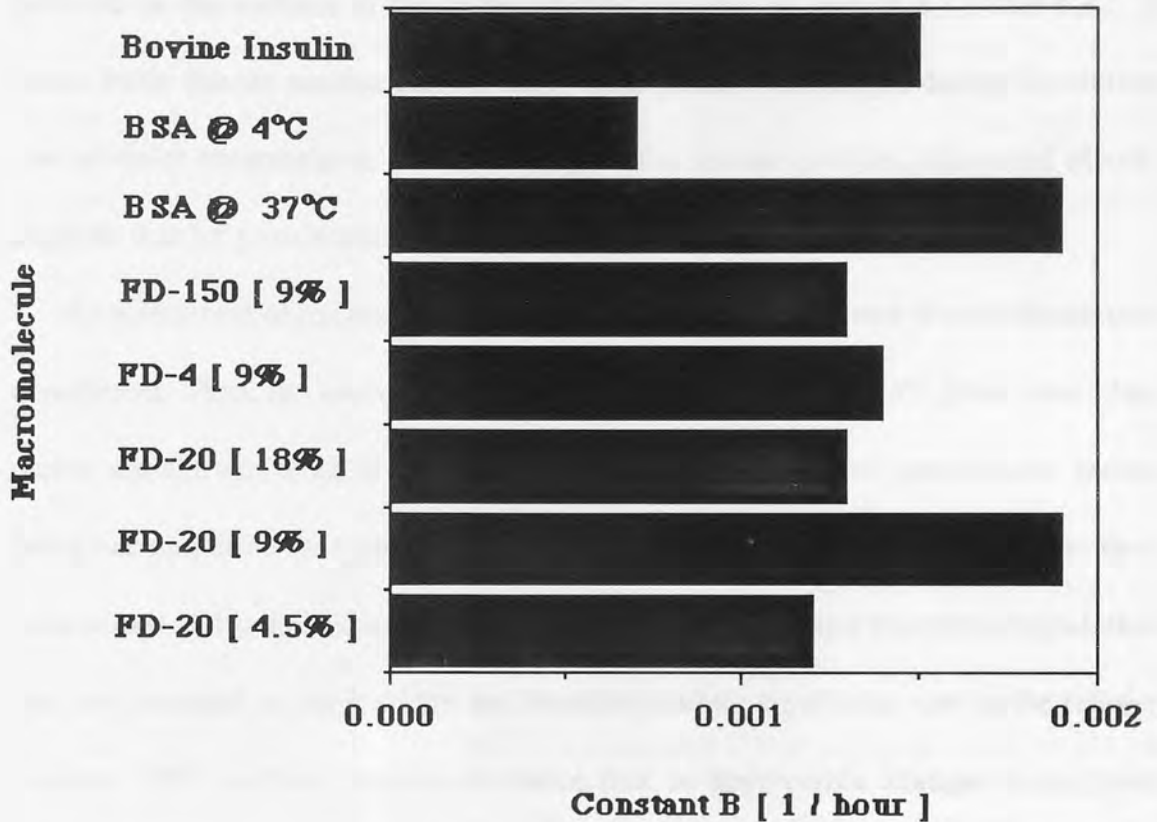
A number of mathematical models are available to analyse the release kinetics of a drug from a spherical particle. Baker and Lonsdale's adaptation of Higuchi's spherical matrix model has been widely applied in the literature [e.g. ^{121, 49}] and may be summarized by the following equation :

$$1.5 [1 - (1 - M_t / M_i)^{2/3}] - [M_t / M_i] = Bt$$

Where M_t and M_i respectively represent the proportions of macromolecule released at time t and infinite time, and B is a constant which describes a combination of parameters.

The release kinetics from the various microcapsule formulations described in this section were treated according to the above equation. In each case, a plot of $1.5 [1 - (1 - M_t / M_i)^{2/3}] - [M_t / M_i]$ versus time produced an approximately linear relationship, from which the constant B was calculated to give a quantitative representation of the rate of macromolecule release. Correlation coefficients ranged from 0.97 to 1.00, except from the insulin release data, in which case it was 0.94. Graph 5.10 compares the B values derived from the macromolecule release data described in this section.

Graph 5.10. Comparison of the Baker and Lonsdale constants, B, from microcapsules with FD-20 loadings of 4.65%, 8.89% and 16.33% ; FD-4 and FD-150 loadings of 8.89% ; and BSA and bovine insulin loadings of 1.70%, dissolved at 37°C, and with BSA, also at 4°C.



The good correlation coefficients show that the kinetics of macromolecule release, from the various microcapsule formulations described in sections 5.3.1 - 5.3.4, can be adequately [though not necessarily optimally] described by the Baker and Lonsdale model. The dissolution experiments carried out at 37°C produced B values ranging from 0.0012 to 0.0019 hour⁻¹. This very narrow variation clearly shows that that the nature of the macromolecule, and its level of incorporation in the microcapsule, had a negligible impact on the release process. The latter is probably of little significance because the macromolecule contents were not materially different in absolute terms. However, the similar release profiles shown by molecules with different physico-chemical properties and molecular weights does strongly suggest that the overall rate-determining process was unrelated to the properties of the encapsulated macromolecule.

In section 5.2, it was demonstrated that fluorescence microscopy could reveal the presence of non-encapsulated FITC-dextran. Therefore, since FITC-dextran was not detected on the surfaces of the microcapsules prepared in section 5.3.1 and 5.3.2, it seems likely that the macromolecule which these particles discharged during dissolution was initially encapsulated. The similarity of the release profiles, discussed above, suggests that the protein macromolecules were distributed in a similar manner.

An assessment of microcapsule degradation was made at the end of each dissolution experiment. Thus, the microcapsules were transferred from the LP3 tubes onto filter paper, and allowed to air dry for 72 hours in a desiccator at room temperature, before being vacuum dried for a further 24 hours at 37°C. The dried microcapsules were then gold-coated and examined using SEM, which revealed that major structural degradation had not occurred in any instance and therefore had no significant role in the release process. GPC analysis provided evidence that no appreciable changes in polymer molecular weight occurred during the dissolution studies.

The findings discussed so far suggest that the macromolecule discharged during the dissolution experiments was not initially fully entrapped within the polymer dense regions of the particle wall. This is because the release mechanisms potentially relevant to such a situation are inconsistent with the evidence. As was mentioned in section 5.1, the hydrophilic macromolecules used for encapsulation had no appreciable solubility in the polymer phase, and so could not diffuse through the polymer dense regions, with their small free volumes. Additionally, degradation was not detected by either SEM or GPC. With the possibility of surface macromolecule already discounted on the basis of fluorescence microscopy studies with the FITC-dextran microcapsules, it must be

concluded that the macromolecule discharged during the dissolution experiments was initially located within the microcapsules' hollow centres, very probably in association with the agarose.

It is, of course, possible that additional macromolecule, relative to that discharged during the dissolution experiments, was located within the polymer dense regions of the particle wall, and this would not have been included in the macromolecule content and encapsulation efficiency measurements presented in table 5.2.

SEM examinations of sectioned microcapsules revealed that the regular microporosity was present on both sides of the polymer membrane. Whilst it was not possible from this evidence to estimate the extent of intrinsic transmembranous porosity, any polymeric barrier between individual pores would have to be very thin, and as a result, susceptible to hydrolytic erosion that would probably evade detection by GPC or SEM. The inherent presence, or erosion-induced generation, of open channels from the interior of the capsule to the external phase does provide a possible mechanism for the release of macromolecule entrapped within the core of the device.

The retarded rate of BSA release seen at 4°C in comparison to 37°C, shows that temperature is an important factor in determining the rate of release. This suggests that polymer degradation is not the rate-limiting step. However, diffusional processes are generally accelerated at higher temperatures and so changes in either the rate at which the macromolecule dissociated from the agarose, or the rate at which the macromolecule diffused through the aqueous membrane channels, or a combination thereof, could be responsible for this effect.

6.1 Introduction

Hydrocortisone (HC) is a low molecular weight steroid drug that has applications as an anti-inflammatory agent. It is typically administered topically in the form of a cream in order to alleviate dermatological conditions. However, the incorporation of this agent into a sustained release formulation, which can be readily and conveniently applied to the skin, is presently desirable, both in therapeutic and commercial terms.

One possible mechanism by which this objective may be achieved involves microencapsulation of the drug. Indeed, with liposome-encapsulated hydrocortisone,

CHAPTER SIX.

an improved concentration-time profile (layers of human skin) following topical application) has been noted, in comparison with a conventional hydrocortisone ointment [3].

HYDROCORTISONE MICROSPHERES.

Such as those prepared from P(HB-HV) polymers. Moreover, because hydrocortisone is a hydrophobic molecule, with little aqueous solubility (i.e. 0.23 mg/ml; see section 2.5.7), its microencapsulation can be conveniently effected via an o/w single emulsion-solvent evaporation process.

In these studies, microspheres have been thus prepared from an organic phase which contained dissolved P(HB-HV) polymer and partially dissolved hydrocortisone (for preparative details, see section 2.4). These devices were therefore structurally different from the reservoir-type microcapsules described in chapters three to five, as they were comprised of a solid polymer matrix in which the drug was dispersed (see diagram 1 (b)).

The first experimental section of this chapter (section 6.2) examines the influence of polymer concentration and polymer molecular weight (i.e. dispersed phase viscosity) on the efficiency of drug encapsulation. This work is then extensively discussed in

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One possible mechanism by which this objective may be achieved involves microencapsulation of the drug. Indeed, with liposome-encapsulated hydrocortisone, an improved concentration-time profile in the different layers of human skin [following topical application] has been noted, in comparison with a conventional hydrocortisone ointment ¹³¹. This drug can also be encapsulated within biodegradable microspheres, such as those prepared from P(HB-HV) polymers. Moreover, because hydrocortisone is a hydrophobic molecule, with little aqueous solubility [*c.* ~ 0.28 mg / ml : see section 2.5.7], its microencapsulation can be conveniently effected via an o/w single emulsion-solvent evaporation process.

In these studies, microparticles have been thus prepared from an organic phase which contained dissolved P(HB-HV) polymer and partially dissolved hydrocortisone [for preparative details, see section 2.4]. These devices were therefore structurally different from the reservoir-type microcapsules described in chapters three to five, as they were comprised of a solid polymer matrix in which the drug was dispersed [see diagram 1 (b)].

The first experimental section of this chapter [section 6.2] examines the influence of polymer concentration and polymer molecular weight [i.e. dispersed phase viscosity] on the efficiency of drug encapsulation. This work is then extensively discussed in

relation to the factors which have been reported in the literature to influence both the efficiency of drug encapsulation, and its physical state and distribution in the final microspheres.

In the subsequent section [6.3], microspheres, prepared at a 20% hydrocortisone loading from the full range of P(HB-HV) polymers used in chapter three, are evaluated in terms of surface morphology. Although this investigation was effected in order to permit a subsequent analysis of the drug release profiles, it also facilitated a comparison to be made between the morphology of each drug-loaded microsphere and the corresponding drug-free microcapsule. In addition, the influence of the solvent evaporation temperature, the drug loading, and PCL II blending, on microsphere morphology were investigated for selected formulations.

The final part of the work described in this chapter [section 6.4] involves an evaluation of the *in vitro* drug release profiles for the different microsphere formulations. The importance of polymer hydrolysis, particle morphology [i.e. the level of porosity], and drug loading in determining the rate of hydrocortisone discharge from P(HB-HV) microsphere matrices are then discussed.

6.2. Hydrocortisone content determinations.

An assessment of the effects of polymer molecular weight on the encapsulation efficiency of hydrocortisone was made by preparing microspheres from 8% and 4% solutions of 32.8PHB (20.1%HV) and 390PHB (20.1%HV), at a 20% drug loading, using the procedure described in section 2.4.

Table 6.1 shows that polymer molecular weight had only a small impact on the encapsulation efficiency when microspheres were prepared from 8% polymer

Table 6.1 : The effects of polymer concentration on the encapsulation efficiency of hydrocortisone in microspheres made from either 32.8P(HB(20.1%HV) or 390P(HB(20.1%HV) with 20% HC. Shown also are the proportions of drug that were present in a solubilised form within the initial casting solutions.

POLYMER CONCENTRATION [% w/v]	32.8P(HB (20.1%HV) MICROSPHERES :		390P(HB (20.1%HV) MICROSPHERES :		HC INITIALLY SOLUBILISED ^b [%]
	HC CONTENT [% w/v]	E.E. ^a [%]	HC CONTENT [% w/w]	E.E. ^a [%]	
15.0	13.5	67.5	-	-	7.6
12.0	16.0	80.0	-	-	9.6
8.0	13.2	65.8	14.4	72.0	14.4
4.0	7.0 ^c	35.0 ^c	11.1 ^c	55.5 ^c	28.8
1.3	0.7 ^c / 1.2 ^{c, d}	3.5 ^c / 5.8 ^{c, d}	-	-	100.0

^a EE represents the encapsulation efficiency, defined in section 2.5.6 as the ratio of the hydrocortisone content to the hydrocortisone loading, and expressed as a percentage.

^b The proportion of hydrocortisone that was present in the original 15mls of DCM in a solubilised form. The figures are based on a hydrocortisone saturation solubility of 2.88 mg/ml at each of the different polymer concentrations and for both polymers.

^c No particles above 49µm in size were produced during these experiments.

^d Prepared using an aqueous continuous phase which had been presaturated with hydrocortisone.

solutions. In contrast, the hydrocortisone content of microspheres prepared from 4% polymer solutions was found to be markedly dependent on the molecular weight of the fabricating material. This is reflected in an increase of approximately 58% in the encapsulation efficiency when microencapsulation was performed using 390PHB (20.1%HV) instead of 32.8PHB (20.1%HV).

Table 6.1 also shows the effect of varying the 32.8PHB (20.1%HV) concentration on the hydrocortisone content and encapsulation efficiency, whilst maintaining a constant 20% drug loading. The optimum concentration for maximizing the encapsulation efficiency seems to be something over 10%, since reducing the polymer concentration from 15% to 12% produced an increase in the microsphere drug content and encapsulation efficiency, whereas further dilutions had the opposite effect. Indeed, lowering the polymer concentration from 8% to 4% reduced the encapsulation efficiency by approximately 47%, to just 35%, and microspheres prepared from a 1.3% polymer solution retained only 3.5% of the original drug.

An explanation for the influence of polymer molecular weight and concentration on the proportion of drug that is retained, during the production of microspheres by a single emulsion-solvent evaporation process, clearly depends on an understanding of the mechanism by which drug loss occurs. However, an examination of the literature reveals that there is no straightforward or universal process that can comprehensively account for drug loss in all the systems which have been studied.

Bodmeier and McGinity¹³, investigating quinidine and quinidine sulphate-loaded P(D,L-LA) microspheres prepared by an o/w solvent evaporation process, attributed drug loss to the diffusion of solubilised drug across the interface of the unprecipitated

microdroplets. The partitioning of drug from the dispersed to the continuous phase by simple diffusion along a concentration gradient is indeed plausible, provided that it is thermodynamically favourable and kinetically achievable. The thermodynamic feasibility is dictated by the relative solubility of the drug in each phase and the relative volume of each phase. However, the presence of the polymer may affect the solubility of the drug within the dispersed phase⁹⁵, whilst the presence of emulsifier may affect its solubility in the continuous phase⁹⁴.

The flow of solubilised drug into the continuous phase, by simple diffusion, is likely to remain thermodynamically favourable until equilibrium partitioning has been achieved, and there is no longer a gradient in drug concentration between the two phases. However, because the experimental system is itself dynamic, as a consequence of differential solvent evaporation, the continuous / dispersed phase ratio will be continuously increasing. This progressive decrease in the relative volume of the dispersed phase should constantly alter the position of the partitioning equilibrium, so as to favour the sustained migration of drug out of the microdroplets, at least until the continuous phase becomes saturated.

An additional effect of the changing phase ratio will be on the kinetics of drug partitioning. As the dispersed phase solvent is lost, microdroplet viscosity will be enhanced, as the polymer starts to precipitate. This will progressively reduce the rate of drug, and, to a lesser extent, solvent diffusion into the continuous phase, until the viscosity becomes so high that drug partitioning is effectively inhibited. The kinetics of drug flux may therefore make the attainment of thermodynamic equilibrium unachievable within the time frame of the experiment. At this stage, there may still be a significant quantity of residual dispersed phase solvent, containing solubilised drug, in

the nascent microparticles. When this is removed, the drug will solidify, although its actual physical form will be dependent on the precise nature of the system under study.

Consequently, in an emulsion-solvent evaporation experiment, the rate of polymer precipitation in relation to the rate of drug partitioning may be influential in determining the proportion of drug retained within the microspheres. Indeed, Bodmeier and McGinity¹³ provided experimental evidence to show that an increase in the rate of polymer precipitation, and thus a reduction in the rate of drug partitioning, could improve the quinidine sulphate encapsulation efficiency. Thus, when the rate of polymer precipitation was enhanced, by employing a dispersed phase solvent with a lower heat of evaporation and a higher solubility in the continuous phase, the proportion of encapsulated drug was dramatically increased. Moreover, these researchers also found that the quinidine sulphate encapsulation efficiency could be enhanced by increasing the molecular weight of the P(D,L-LA), although the magnitude of this effect was generally small and highly dependent on the underlying polymer concentration. However, viscosity increases produced by the use of more concentrated polymer solutions, significantly improved the proportion of encapsulated drug, regardless of the fabricating polymer's molecular weight. This relationship between the polymer concentration or molecular weight and the drug encapsulation efficiency was attributed to both an increase in the rate of polymer precipitation and a decrease in the rate of drug diffusion.

The diffusion of solubilised drug out of the microdroplets can also be reduced by lowering the solubility of the drug in the continuous phase. Thus, Bodmeier and Chen⁵⁰ demonstrated that the encapsulation efficiency of three different anti-inflammatory agents in ethylcellulose microspheres, was inversely related to each drug's solubility in

the continuous phase. Additionally, Bodmeier and McGinity¹³ improved the encapsulation efficiency of quinidine sulphate by using pH changes to lower the solubility of this ionizable drug in the continuous phase. Moreover, a ubiquitously applied method for improving the proportion of encapsulated drug involves presaturating the continuous phase with drug, thereby eliminating the concentration gradient [e.g. 12, 86].

Although the preceding text has shown that kinetic considerations may result in less drug partitioning into the continuous phase than would be expected on a purely thermodynamic basis, there are reports that, in some systems, more drug partitions into the aqueous phase than is actually required to saturate it. Indeed, whilst Jalil and Nixon⁸⁶ found that presaturation of the aqueous phase with phenobarbitone enhanced the encapsulation efficiency of this drug, in microspheres prepared by an o/w solvent evaporation process, 15.5% of the original dispersed phase drug still ended up in the continuous phase. Similarly, as can be seen from table 6.1, when microspheres were prepared from a 1.3% 32.8PHB (20.1%HV) / 20% HC solution, using an aqueous phase presaturated with hydrocortisone, only a very minor improvement in the encapsulation efficiency, from 3.5% to 5.8%, was observed. These observations suggest that simple diffusion is not the only mechanism by which drug can be lost from the microdroplets.

An alternate mechanism could involve the simultaneous loss of both drug and dispersed phase solvent from the microdroplets by syneresis. However, the phenobarbitone partitioning was attributed to spontaneous crystal growth, which occurred via a stage of supersaturation and precipitation of drug crystals, whilst the polymer was still in solution, followed by the splitting of the crystals from the droplet

surface⁸⁶. Moreover, the crystallization of initially solubilised drug within the microdroplets is not necessarily restricted to systems where the continuous phase has been presaturated with drug, although a number of conditions would have to be met for this to occur. Firstly, thermodynamic considerations should favour the retention of drug in the microdroplets, but the drug must also have little miscibility with the polymer⁹⁵, otherwise it is likely to remain molecularly dispersed throughout the precipitation of the polymer, and be present as a solid solution within the final microspheres. Secondly, the drug should have a lower degree of solubility in the dispersed phase solvent than the polymer, and ideally, the initial drug concentration in the dispersed phase solvent should be close to its saturation level. These features should ensure that the drug starts to precipitate within the microdroplets whilst the polymer is still fully solvated. This is a critical point in view of the nature of the solvent evaporation process. Thus, if drug crystallization within the microdroplets is to occur, it is essential that, as the drug precipitates, the viscosity is sufficiently low to enable the drug molecules to diffuse together to nucleate and develop crystal domains. If the microdroplet is too viscous when the saturation solubility of the drug is reached, drug crystallization may be effectively prevented. In this manner, drug that has little mutual miscibility with the polymer could be in a molecularly dispersed state within the final microspheres. The physical state of drug in this situation has been described as a "metastable molecular dispersion"⁹⁵, although this does not necessarily mean that all the drug molecules are individually dispersed within the polymer. Clusters of drug molecules could be associated within the polymer phase, but at concentrations too low to lead to crystallization.

Whilst the studies of Jalil and Nixon⁸⁶ demonstrated that some interfacial drug

crystals were able to split off into the continuous phase, others remained attached to the droplet surface during the precipitation period. Thereafter, these unprotected drug crystals were either removed during the washing stages, and so contributed to a lower encapsulation efficiency, or became an inherent morphological feature of the final product. The dissolution of surface drug crystals during washing was dependent upon the solubility of the drug in the washing solvent. Moreover, the presence of free drug crystals on the surface of the final microspheres can have a major impact on the drug release kinetics ¹².

In some systems, drug crystallization has been observed only at the droplet interface and in the continuous phase ¹⁶. In such instances, a process modification called "interrupted solvent evaporation" has been successfully employed to prevent drug crystallization, although its effectiveness can diminish as the drug loading increases ⁹⁵. Thus, at an experimentally determined time after phase combination, the continuous phase is removed and replaced with either the same or a different solvent, with or without emulsifier, after which solvent evaporation is taken to completion ¹²⁷.

In systems where the continuous phase is not saturated with drug, interfacial drug crystals could split off and / or dissolve into the continuous phase. In either case, the fundamental prerequisite for the loss of crystalline drug into the continuous phase is the actual presence of the crystals at the phase boundary. Therefore, in systems either where drug crystallization is not an exclusively interfacial event, or which inherently contain suspended drug, an understanding of the factors which influence the distribution of the drug crystals in the unprecipitated microdroplets is of considerable importance. The work of Spenlehauer *et al.* ¹⁵, involving cisplatin-loaded microspheres, prepared using an o/w solvent evaporation process, from a dispersed

phase that initially contained dissolved polymer and predominantly non-solubilised drug, is particularly informative in this regard. These researchers suggested that the stirring employed to create and maintain the initial o/w emulsion produced centrifugation forces which encouraged the migration of the drug particles towards the periphery of the microdroplets. When the continuous phase was not saturated with cisplatin, the interfacial drug crystals were then able to dissolve out from the microdroplets. In contrast, the migration of cisplatin particles was opposed by an increase in the viscosity of the dispersed phase. Consequently, the use of a more concentrated polymer solution or a higher molecular weight polymer, reduced the concentration of drug particles at the phase boundary and improved the encapsulation efficiency.

Although the centrifugation effects in the system studied by Spenlehauer *et al.*¹⁵ may have been exaggerated by the high density of the metallic drug particles, the physical state of the drug in the microdroplets appears to be an influential factor in determining the mechanism of partition into the continuous phase. Moreover, as solubilised drug may crystallize within the microdroplets during the experiment, the mechanism of partitioning is not necessarily governed by the physical state of the drug in the initial casting solution. Indeed, it is also possible for drug that was initially present as a crystalline suspension to dissolve in the dispersed phase during the course of the experiment. Clearly, in order for this to occur, the relative rates of solvent and drug migration into the continuous phase must be such that the concentration of solubilised drug in the dispersed phase solvent is brought below the saturation level.

Although the likelihood of this occurring will be dictated by the precise nature of the polymer / drug / dispersed phase solvent / continuous phase solvent interactions in the

particular system under study ¹³, the following features of the solvent evaporation process may be of importance in this regard.

Immediately after phase combination, the dispersed phase solvent can migrate from the microdroplets into the continuous phase. This initial migration should be effected largely by diffusion, and could possibly continue until the continuous phase became saturated with the dispersed phase solvent. At this point, the rate of solvent diffusion out of the microdroplets should slow considerably, since it could then only proceed [by diffusion] in subsequence to the restoration of the concentration gradient by differential solvent evaporation. This reduced rate of solvent diffusion may provide an opportunity for the concentration of solubilised drug in the microdroplets to fall, provided that the initial, rapid, flux of dispersed phase solvent did not produce a concomitant increase in microdroplet viscosity sufficient to inhibit further drug migration. Moreover, if the concentration of solubilised drug in the microdroplets slipped below the saturation level, it would then be thermodynamically possible - though, not necessarily, kinetically feasible - for suspended drug to dissolve in the dispersed phase solvent. The subsequent diffusion of this drug into the continuous phase, and a sustained repetition of these events until the level of solvent loss had reached the point where the microdroplet viscosity became prohibitively high, could provide an alternative mechanism for the loss of initially non-solubilised drug.

Although most of the mechanisms for drug loss that have been illustrated in this section are conditional upon the drug being in a specific physical form, it is the combined influence of all the variables in the particular experimental system under study that will ultimately determine which, if any, of the potentially-applicable mechanisms are actually operational. Moreover, since the physical form of the drug

may also change during the experimental period, it is, in practice, very difficult to gain a comprehensive understanding of the way(s) in which drug loss occurs.

The improved hydrocortisone encapsulation efficiencies, which typically accompanied increases in the molecular weight or concentration of the fabricating polymer [as detailed in table 6.1], may be attributed to the reduced loss of either solubilised or suspended drug, or a combination thereof. Thus, the models proposed by Bodmeier and McGinity ¹³ and Spenlehauer *et al.* ¹⁵ to respectively explain the reduced loss of solubilised and particulate drug that occurred when the molecular weight or concentration of the fabricating polymer was increased, could each have a role in accounting for the results obtained in these studies. Indeed, most of the initial casting solutions actually contained both forms of hydrocortisone, and in every case, changes in the physical state of the drug could have occurred after phase combination.

The work of Cavalier *et al.* ¹⁷, involving hydrocortisone-loaded P(D,L-LA) microspheres, prepared by an o/w solvent evaporation process, from a DCM solution containing partially solubilised drug, is probably the closest parallel to these studies available from the literature. When microspheres were prepared at an initial drug loading of 21.9%, these researchers experienced an encapsulation efficiency of approximately 55%, but the influence of polymer molecular weight and concentration on the efficiency of hydrocortisone encapsulation were not investigated. Interestingly, the drug which partitioned into the continuous phase during microsphere preparation was claimed to be the fraction that was initially present in a non-solubilised form.

Moreover, thermal analysis revealed the presence of crystalline drug in the final microspheres, and this was believed to have resulted from the crystallization of the initially solubilised fraction. However, the heat of fusion values suggested that very

little drug was actually present in a crystalline form within the final microspheres, and the fusion temperature itself was significantly lower than the 228°C that is normally associated with well-defined hydrocortisone crystals. Although, these observations were attributed to the difficulty of crystallization in the viscous microdroplets, this interpretation ignores an important drawback of thermal analysis when used as a tool for determining the physical state of drug molecules within polymers. The difficulty can arise when the polymer melts at a lower temperature than the drug, so that inherent drug crystals could dissolve in the molten polymer during the heating cycle and thereby falsely indicate the absence or reduced presence or perfection of crystalline drug in the initial samples. This behaviour has been quite widely encountered^{12, 50}, and probably occurred in Cavalier's system. Indeed, P(D,L-LA) normally melts at around 130°C³⁴, and X-ray analysis was reported to have indicated the presence of hydrocortisone crystals in the original microspheres.

An alternate way of using thermal analysis to determine the physical state of a drug in a polymer, involves looking at how the drug affects the glass transition temperature [T_g] of that polymer. When the drug loading is low, a molecularly dispersed drug should reduce and broaden the T_g of the polymer, whereas a crystalline drug should have little effect⁹⁵. Since Cavalier *et al.*¹⁷ reported that the T_g of PLA, in microspheres containing 12.6% hydrocortisone, was not affected by the presence of drug, it therefore seems likely that the steroid was largely present in a crystalline form within the original microspheres, but that it partially dissolved in the molten polymer during the heating cycle.

Thermal analysis was not considered to be a suitable technique for assessing the physical state of the drug in the microspheres prepared in these studies for two reasons.

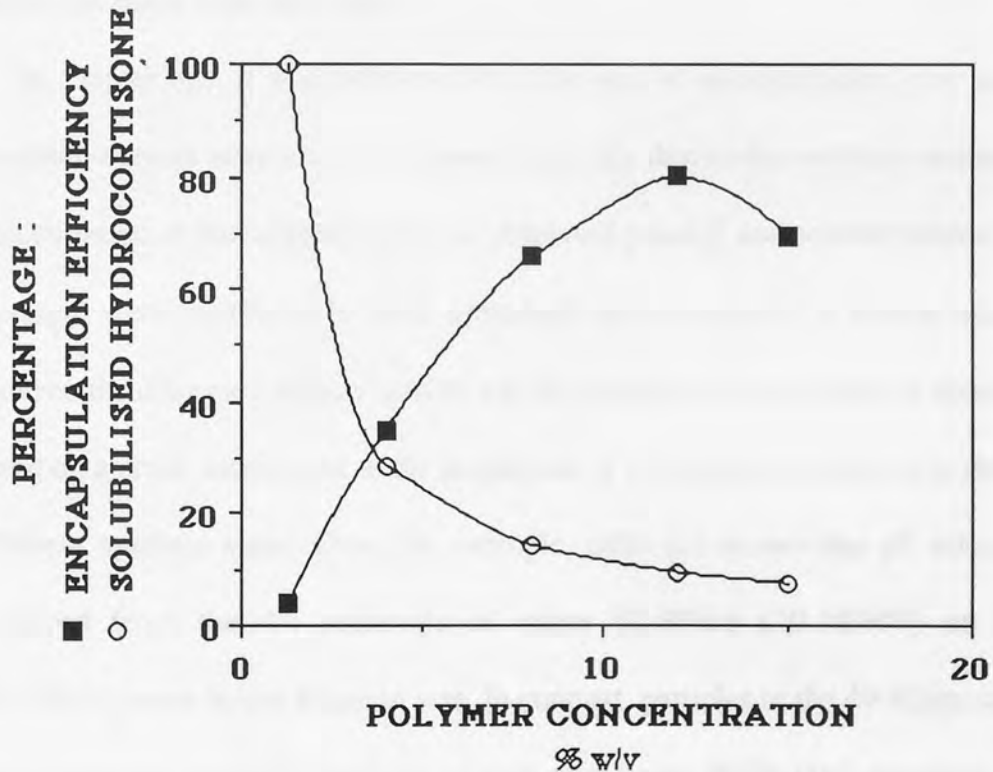
Firstly, the T_g of P(HB-HV) polymers is intrinsically difficult to determine as a consequence of these materials high crystallinities^{79, 109}, and secondly, because all these polymers melt at a lower temperature than the drug.

The probability that Cavalier *et al.*¹⁷ misinterpreted their thermal data clearly does not diminish their claim that the crystalline hydrocortisone in the final microspheres was produced during the experimental period from the initially solubilised drug, or that the initially suspended fraction migrated into the continuous phase. Unfortunately, no information regarding the relative proportions of suspended and solubilised drug in the initial casting solutions was presented, and the only reported technique - a visual examination - used to establish the physical form in which the drug partitioned could only detect solid drug. In contrast, the drug loss which occurred during the production of the microspheres listed in table 6.1 could not have resulted entirely from the initially suspended fraction in every instance. Indeed, with the 32.8PHB (20.1% HV) / 20% HC formulation, graph 6.1 shows that the hydrocortisone encapsulation efficiency actually rose as the proportion of initially suspended drug increased, and 96.5% of the initial drug was not encapsulated in microspheres prepared from a 1.3% polymer solution, even though all the drug was initially dissolved.

However, as has been mentioned previously, the physical state of the drug in the initial casting solution does not necessarily define either the form(s) in which it is lost or the mechanism(s) of that process, and, in the case of the microspheres prepared using an initially drug-free continuous phase, there is too little information to support more conclusive comment. In contrast, when microspheres were prepared from the 1.3% polymer solution, using a continuous phase that had been presaturated with hydrocortisone, the diffusion of solubilised drug was unlikely to have been a major

source of drug loss, as there was not a hydrocortisone concentration gradient between the two phases. Nevertheless, although the drug content of microspheres prepared from the 1.3% polymer solution was not materially affected by presaturating the continuous phase with drug, it does not necessarily follow that the mechanism of drug loss was the same in each experiment.

Graph 6.1 : The effects of polymer concentration on the encapsulation efficiency of hydrocortisone in microspheres made from 32.8PHB (10.8%HV) with a 20% drug loading. Also shown are the proportions of hydrocortisone that were present in a solubilised form within the initial casting solutions.



Note : The 'HC initially solubilised' represents the actual quantity of hydrocortisone present in the original 15 mls of DCM that was in a solubilised form. The figures are based on a hydrocortisone saturation solubility of 2.88 mg / ml at each of the different polymer concentrations.

The hydrocortisone content and encapsulation efficiency details for the different microsphere formulations described in table 2.4 are summarized in table 6.2. As was mentioned in section 2.4.1, the concentration of polymer in the initial casting solution was dependent upon its molecular weight. Thus, band A, B and C polymers were respectively employed at concentrations of 15%, 12% and 8% [w/v]. Table 6.1 demonstrated that [with the 32.8PHB (20.1% HV) / 20% HC formulation] reducing the polymer concentration from 15% to 8% had only a minor effect on the encapsulation efficiency, and so these variations were not employed to regulate the microsphere drug contents. Instead, the polymer concentrations were related to the molecular weight in order to maximize the proportion of microspheres within the 49-90 μ m size range from each batch.

In chapter one, it was mentioned that the size of microparticles, prepared using emulsion-solvent evaporation processes, typically diminishes with an increase in the concentration of the polymer solution [dispersed phase], and several reports from the literature were referenced in order to support this contention. A similar relationship between the dispersed phase viscosity and the particle size was found in these studies, based on a crude assessment of the proportion of microspheres retained in sieves with different aperture sizes. Thus, for example, table 6.1 shows that all microspheres prepared from the 4% solutions of either 32.8PHB (20.1%HV) or 390PHB (20.1%HV) were below 49 μ m in size. In contrast, particles in the 49-90 μ m size range were produced from 8% solutions of each of the nine P(HB-HV) polymers listed in table 6.2, although the proportion in this size range from the band A and B materials was small. This was compensated for by increasing the concentrations of these polymers to 15% and 12% respectively.

Table 6.2. Encapsulation efficiency details for different hydrocortisone microsphere formulations.

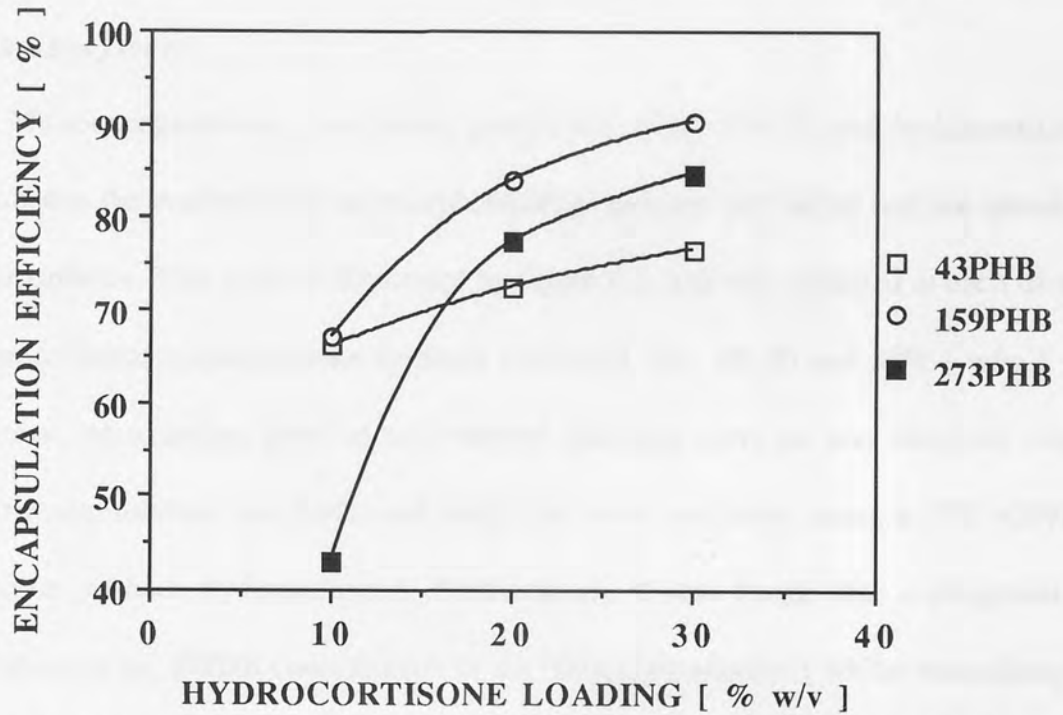
FORMULATION CODE	HC LOADING [% w/w]	HC CONTENT [% w/w]	ENCAPSULATION EFFICIENCY [%]
<u>BAND A POLYMERS:</u>			
43PHB / 10% HC	10.0	6.6	66.0
43PHB / 20% HC	20.0	14.5	72.5
43PHB / 30% HC	30.0	23.0	76.6
83.1PHB (10.8% HV) / 20% HC	20.0	14.2 / 15.6 ^a	71.0 / 78.0 ^a
32.8PHB (20.1% HV) / 20% HC	20.0	13.5	67.5
<u>BAND B POLYMERS:</u>			
159PHB / 10% HC	10.0	6.7	67.0
159PHB / 20% HC	20.0	16.8 ^b	84.0 ^b
159PHB / 30% HC	30.0	27.1	90.3
180PHB (10.8% HV) / 20% HC	20.0	13.9 / 14.3 ^a	69.5 / 71.5 ^a
140PHB (20.1% HV) / 20% HC	20.0	15.5	77.50
<u>BAND C POLYMERS:</u>			
273PHB / 10% HC	10.0	4.3	43.0
273PHB / 20% HC	20.0	15.5	77.5
273PHB / 30% HC	30.0	25.4	84.7
330PHB (10.8% HV) / 20% HC	20.0	15.2 / 13.4 ^a	76.0 / 67.0 ^a
390PHB (20.1% HV) / 20% HC	20.0	14.4	72.0
<u>PCL II BLENDS:</u>			
43PHB / 20% PCL II / 20% HC	20.0	15.9	79.5
159PHB / 20% PCL II / 20% HC	20.0	16.1	80.5
273PHB / 20% PCL II / 20% HC	20.0	12.8	64.0
100% PCL II / 20% HC	20.0	15.1	75.5

^a Microspheres prepared at temperature variation T4. ^b The values for 159PHB are the mean of three batches [section 2.5.6].

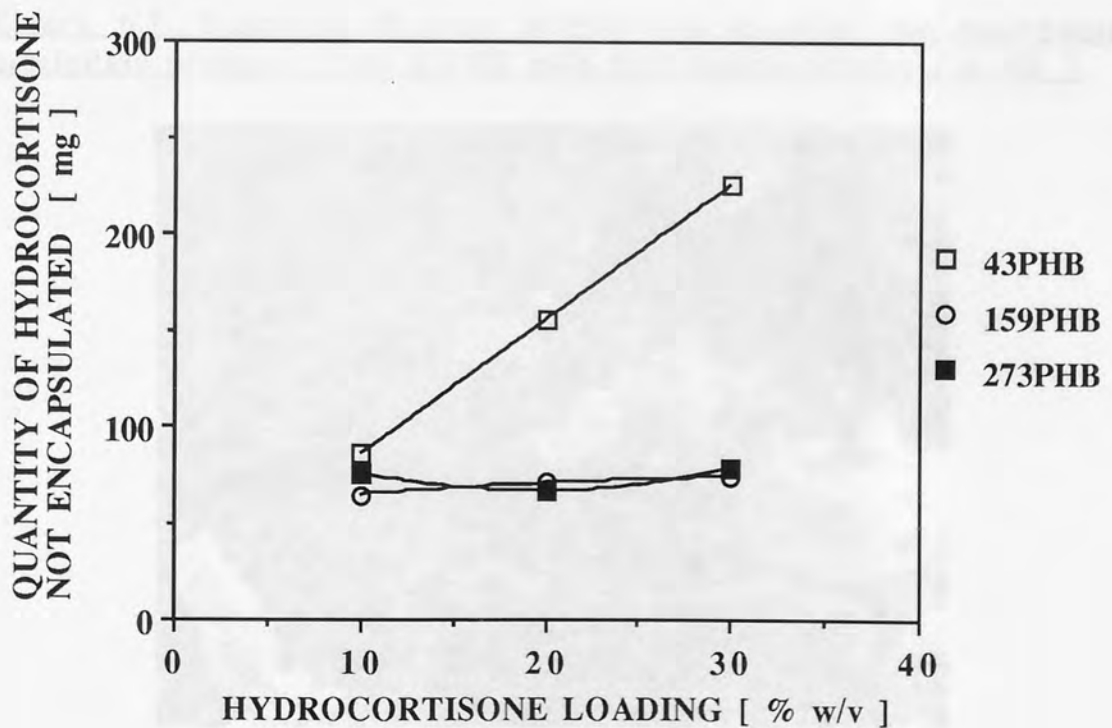
As graph 6.2 illustrates, the hydrocortisone encapsulation efficiency in microspheres prepared from each of the three different molecular weight homopolymers increased with the drug loading. This effect was most pronounced in the case of 273PHB, where an increase in the the drug loading from 10% to 30% improved the encapsulation efficiency from 43.0% to 84.7% .

In several different reported studies ^{17, 50, 91, 128} increased drug loadings have resulted in enhanced encapsulation efficiencies . These include the work of Cavalier *et al.* ¹⁷ with HC-loaded P(D,L-LA) microspheres, where an increase in the HC-loading from approximately 13.0% to 21.9% improved the encapsulation efficiency by nearly 72% [as calculated from the data in table 1 of reference ¹⁷]. However, these researchers noted that the actual amount of steroid which partitioned into the continuous phase during the microsphere preparation experiments remained "essentially constant" as the drug loading was increased. A similar observation was made by Jalil and Nixon ¹²⁸ when phenobarbitone was encapsulated in P(L-LA) microspheres. With the 159PHB and 273PHB formulations shown in graph 6.2, the actual quantity of non-encapsulated hydrocortisone also remained essentially constant as the drug loading was increased. This is demonstrated in figure 6.3, which also shows that the actual quantity of non-encapsulated hydrocortisone increased linearly with the drug loading when 43PHB was used as the fabricating polymer. The anomalous behaviour of 43PHB may have been due to the fact that amorphous drug-polymer composites, and not intact microspheres, were produced from solutions of this polymer and hydrocortisone. Clearly, if the quantity of drug which is not encapsulated during the experimental procedure is independent of the drug loading, then the proportion of drug that is encapsulated, i.e., the encapsulation efficiency, will increase with the drug loading.

Graph 6.2. The relationship between the encapsulation efficiency of hydrocortisone [HC] and the initial drug loading, for microspheres prepared from three different molecular weight PHB homopolymers.



Graph 6.3. The actual quantities of HC not encapsulated during the preparation of microspheres with initial drug loadings of 10%, 20% and 30% [w/w], from three different molecular weight PHB homopolymers

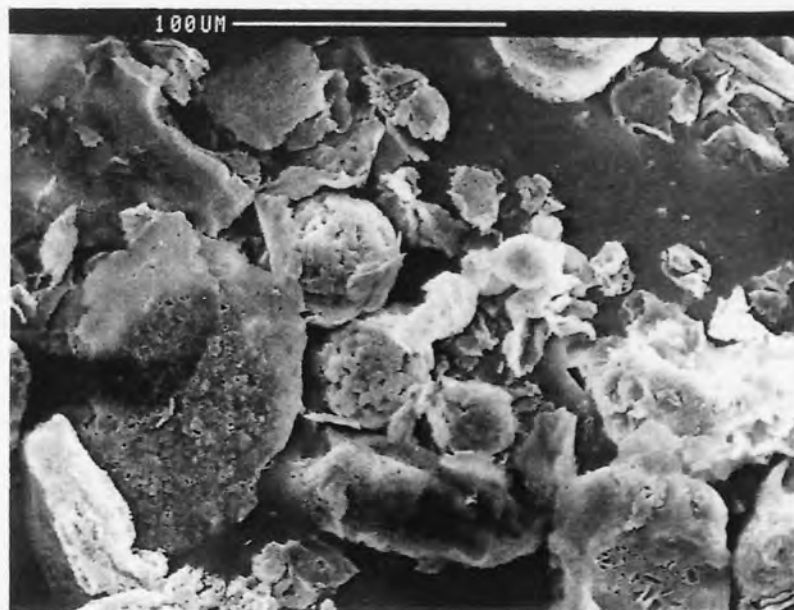


6.3. Morphological characteristics.

6.3.1. Hydrocortisone - loaded microspheres prepared from PHB homopolymers.

Microencapsulation experiments performed using 43PHB and hydrocortisone resulted in the production of an amorphous drug / polymer precipitate and not spherical microspheres. This result is illustrated by figure 6.1, and was obtained at each of the three different hydrocortisone loadings employed, i.e., 10, 20 and 30% [w/w]. In contrast, an excellent yield of well-formed spherical particles was obtained when microencapsulation was performed under the same conditions using a 15% 43PHB solution without hydrocortisone. Furthermore, it was found that a progressive reduction in the 43PHB concentration of the fabricating solution [whilst maintaining a constant 20% HC loading] from 15% to approximately 1.3% led to a substantial improvement in the yield of intact microspheres.

Figure 6.1. Scanning electron micrograph showing the amorphous precipitate produced from 43PHB with 20% hydrocortisone [x 381].



Each of the three different 159PHB / HC formulations listed in table 2.4 gave rise to an excellent yield of spherical particles. Figure 6.2 (a) shows a typical microsphere from the 10% hydrocortisone batch as having a slightly wrinkled, but essentially non-porous, surface. In contrast, surface holes were observed, although in small numbers, on the surfaces of microspheres prepared from the 159PHB / 20% formulation [figure 6.2 (b)]. Moreover, as figure 6.2 (c) demonstrates, in relation to the latter, a comparatively greater level of porosity was observed on particles prepared from 159PHB with 30% hydrocortisone.

The morphological characteristics of the microspheres prepared from the three different 273PHB / HC formulations listed in table 2.3 are shown in figures 6.3 (a)-(c), and were similar to those of the corresponding 159PHB formulations. Thus, in each case an excellent yield of spherical particles was obtained. Additionally, microspheres from the 10% HC batch were essentially non-porous, whereas the 20% and 30% HC formulations generated particles with surface holes, which became more frequent as the drug loading increased.

The small pores which appeared on the surfaces of particles prepared from 159PHB and 273PHB at the higher drug loadings can be attributed to the presence of hydrocortisone crystals which became embedded in the unprecipitated polymer wall and were subsequently removed after the microdroplet had hardened.

Figure 6.2. Scanning electron micrographs showing hydrocortisone microspheres prepared from 159PHB with different drug loadings :

(a) 10% [w/w]

Low magnification [x911]

High magnification [x2370]

(b) 20% [w/w]

Low magnification [x746]

High magnification [x2410]

(c) 30% [w/w].

Low magnification [x700]

High magnification [x2370]

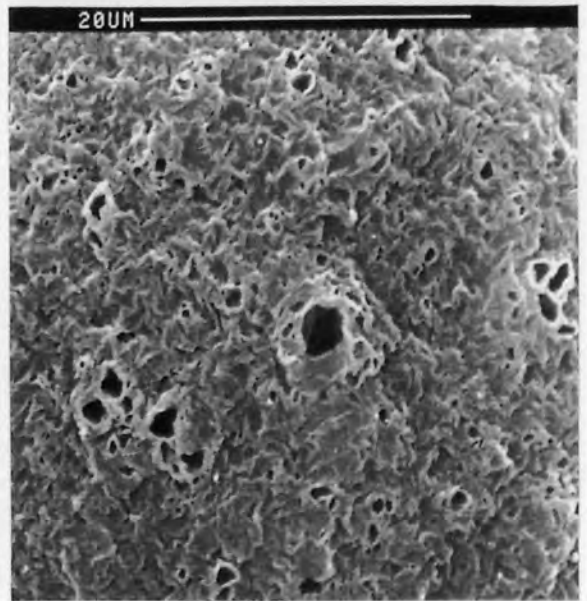
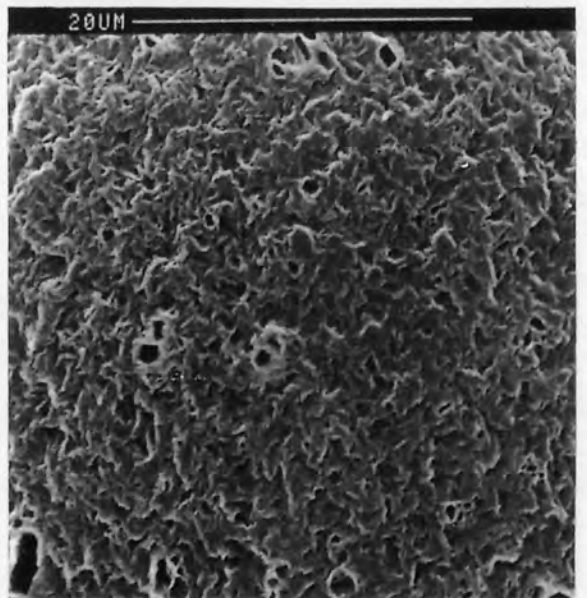
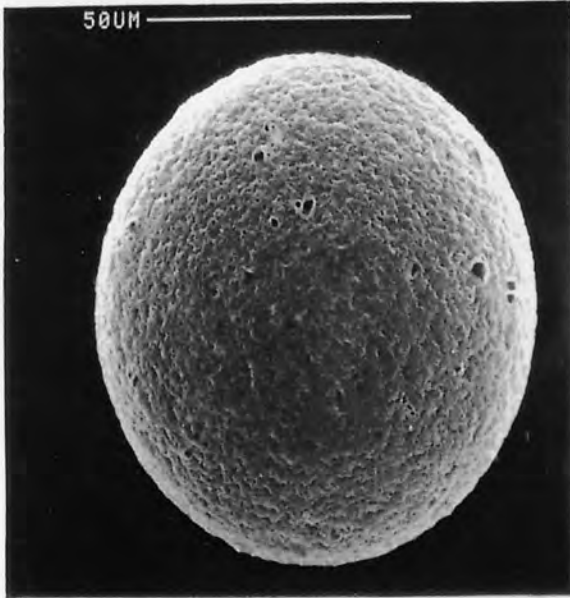
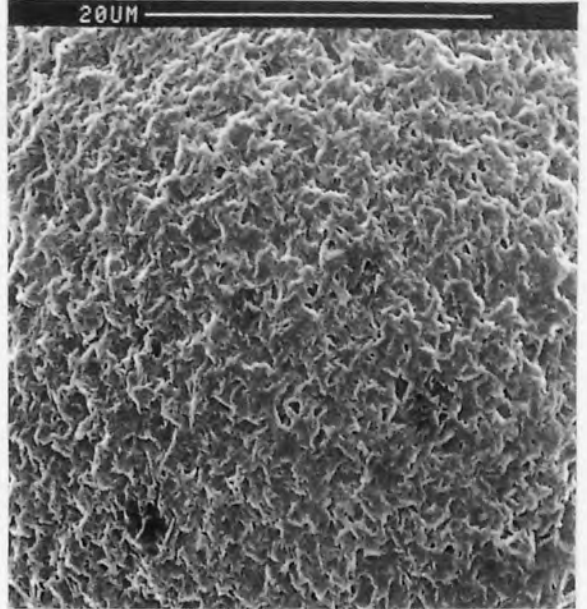


Figure 6.3. Scanning electron micrographs showing hydrocortisone microspheres prepared from 273PHB with different drug loadings :

(a) 10% [w/w]

Low magnification [x883]

High magnification [x2410]

(b) 20% [w/w]

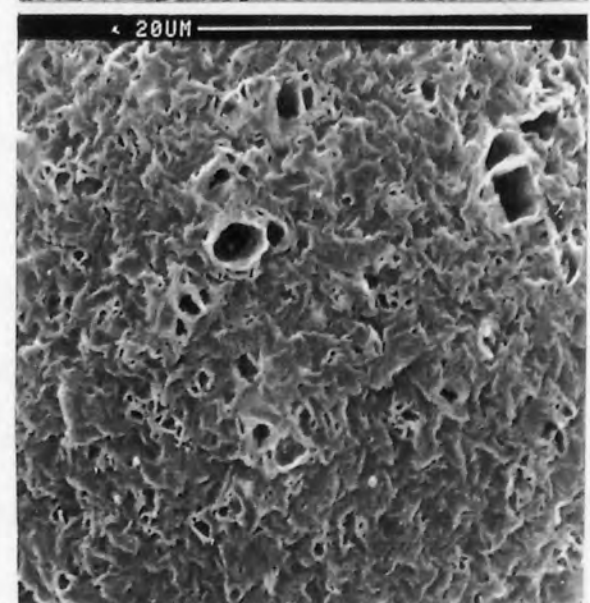
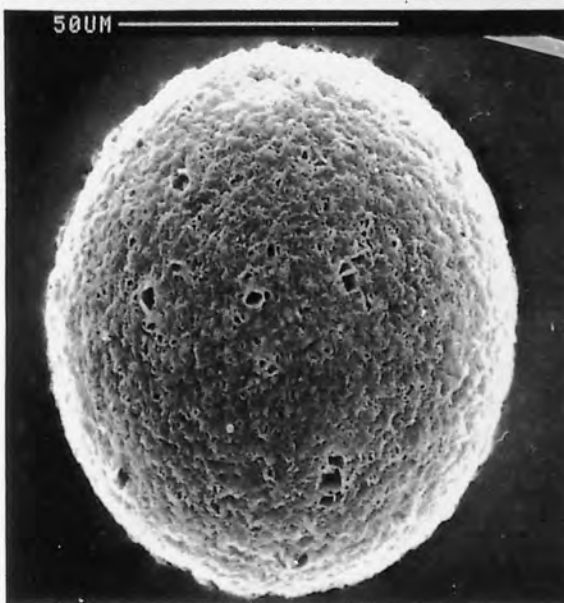
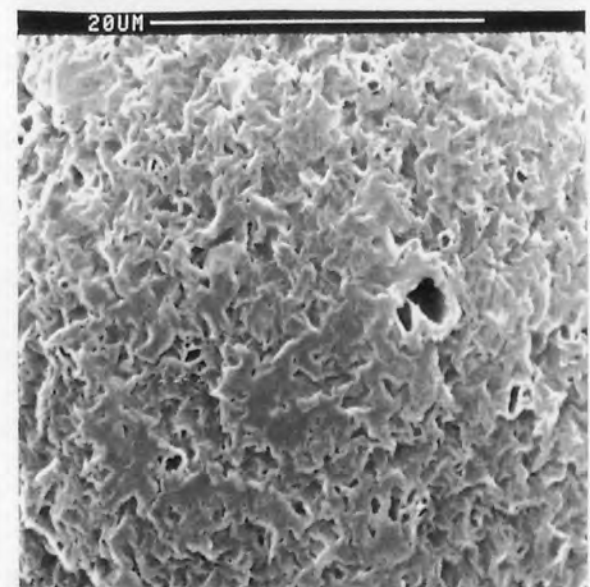
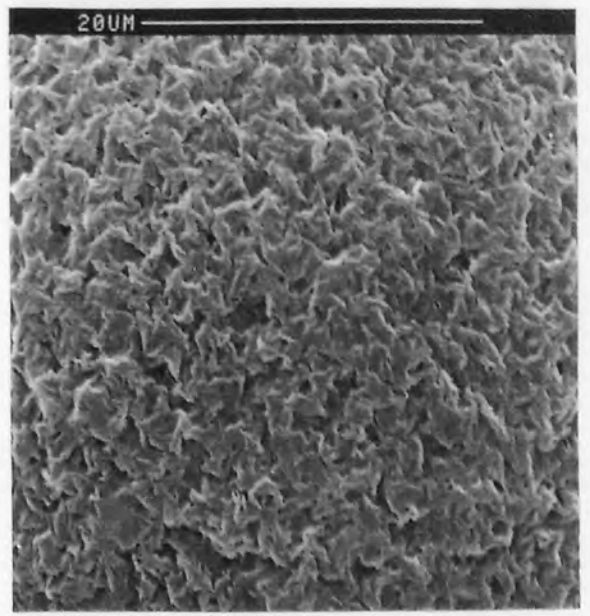
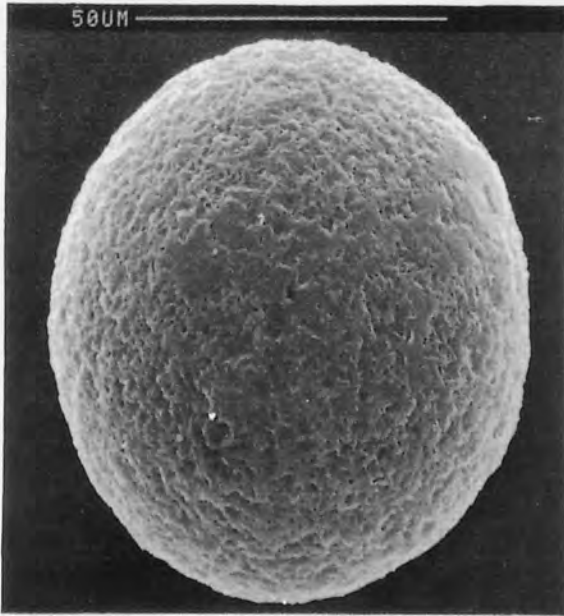
Low magnification [x820]

High magnification [x2410]

(c) 30% [w/w].

Low magnification [x773]

High magnification [x2410]



6.3.2. Hydrocortisone - loaded microspheres prepared from PHB (10.8% HV) copolymers.

Microencapsulation experiments with each of the three different 10.8% HV copolymer / 20% HC formulations listed in table 2.4, resulted in the production of an excellent yield of spherical particles. Typical microspheres obtained from 83.1PHB (10.8% HV) and 180PHB (10.8% HV) with 20% hydrocortisone are respectively shown in figures 6.4 (a) and (b). In each case, the polymer chains in the particle wall appear to be loosely bound, producing heavily structured and macroporous surfaces. However, these features are consistent with those of the corresponding microcapsules described in chapter three, and there was no porosity that could be attributed to the presence of drug in either batch. The latter feature was also true of microspheres prepared from 330PHB (10.8% HV) with 20% hydrocortisone. A representative particle from this batch is shown in figure 6.4 (c), and this has a less structured surface than the microspheres prepared from the lower molecular weight 10.8% HV copolymers, which is also consistent with the microcapsule work described in chapter three.

Microspheres were also produced from the three different 10.8% HV copolymer / 20% HC formulations at T4, in order to determine whether the solvent evaporation temperature influenced the particle characteristics. In each case, the excellent yields obtained at T1 were repeated at T4, and the T4 microspheres all had smoother and / or less structured surfaces than those correspondingly prepared at T1 [figures 6.5 (a), (b) and (c)]. Whilst the latter feature was encountered and discussed in chapter three, the microspheres prepared at T4 appeared to have the small holes which typified some of the homopolymer formulations [see section 6.3.1], and were attributed to the removal of surface drug crystals from the solidified particle walls.

Figure 6.4. Scanning electron micrographs of 20% hydrocortisone-loaded microspheres prepared at temperature variation T1 from PHB (10.8% HV) copolymers with different molecular weights :

(a) 83 100

Low magnification [x820]

High magnification [x2410]

(b) 180 000

Low magnification [x809]

High magnification [x2370]

(c) 330 000.

Low magnification [x853]

High magnification [x2410]

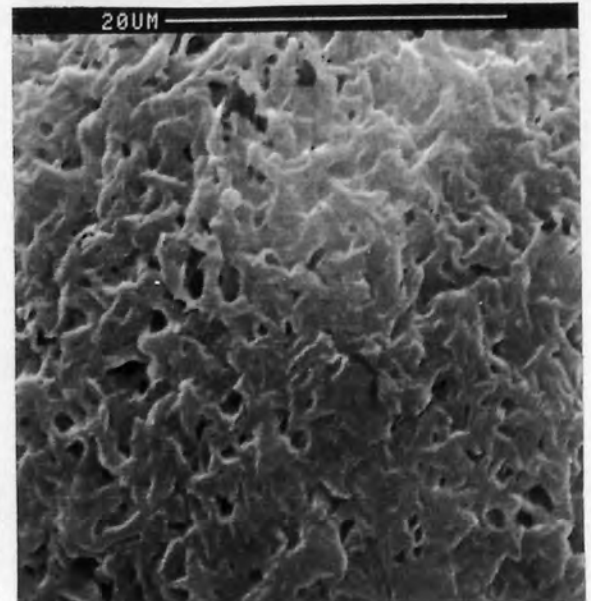
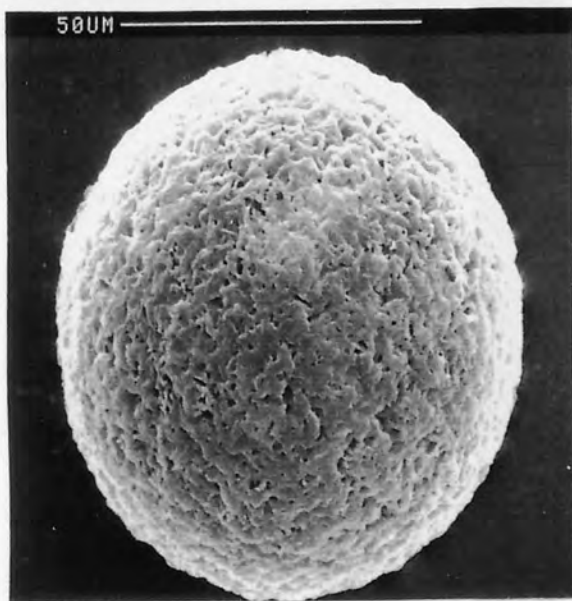
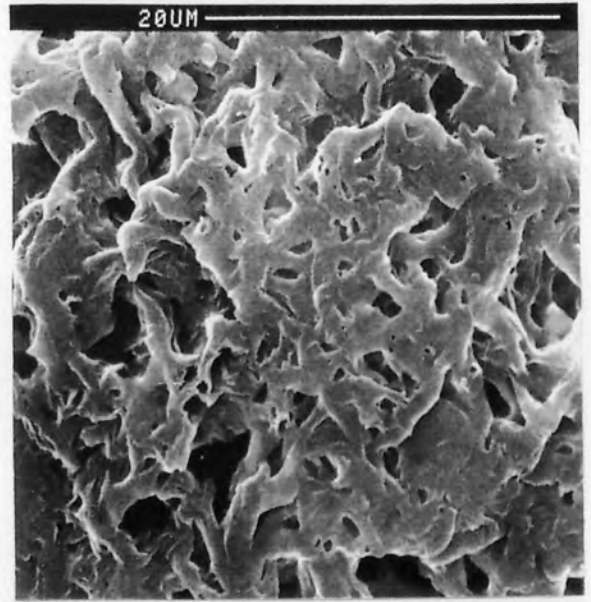
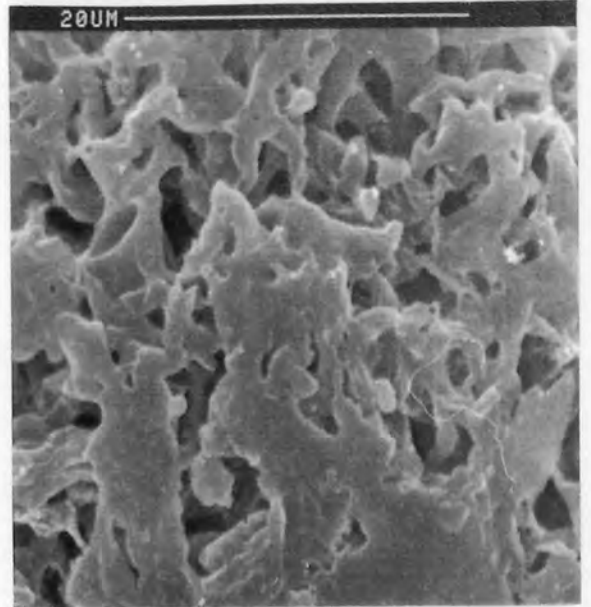
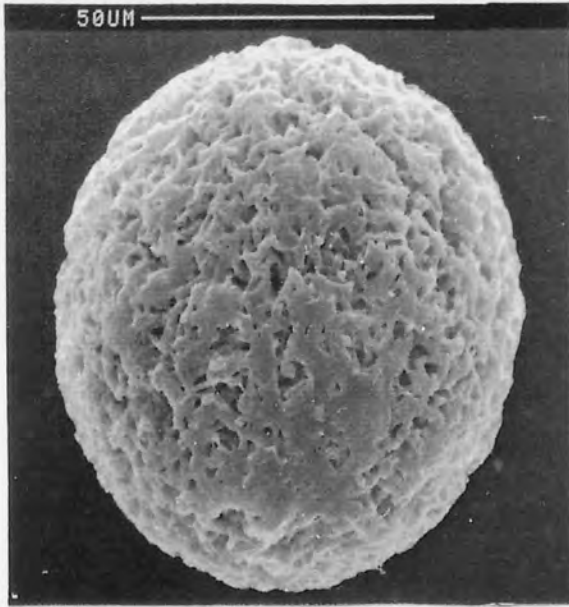


Figure 6.5. Scanning electron micrographs of 20% hydrocortisone-loaded microspheres prepared at temperature variation T4 from PHB (10.8% HV) copolymers with different molecular weights :

(a) 83 100

Low magnification [x700]

High magnification [x2410]

(b) 180 000

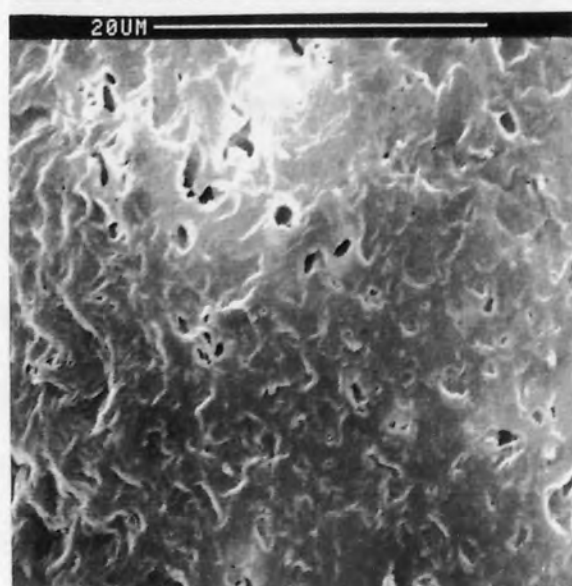
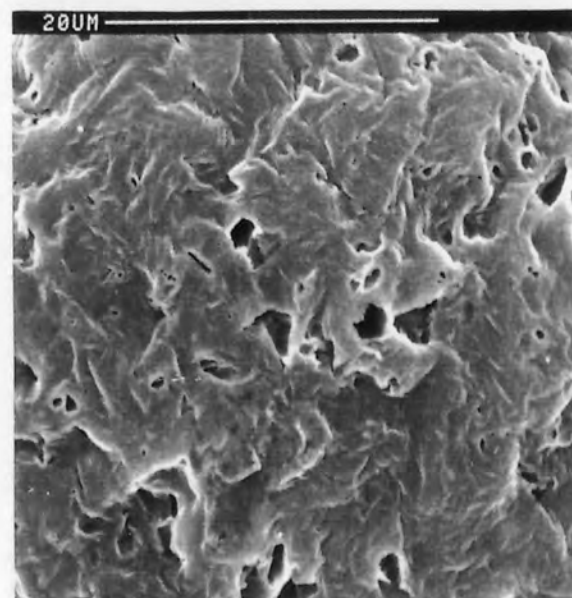
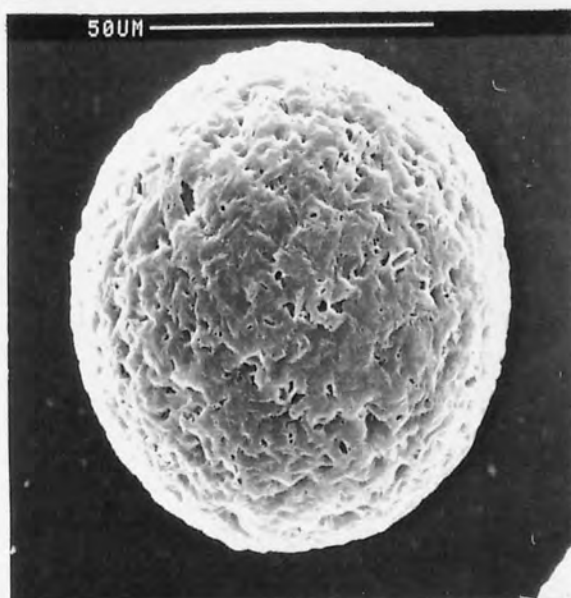
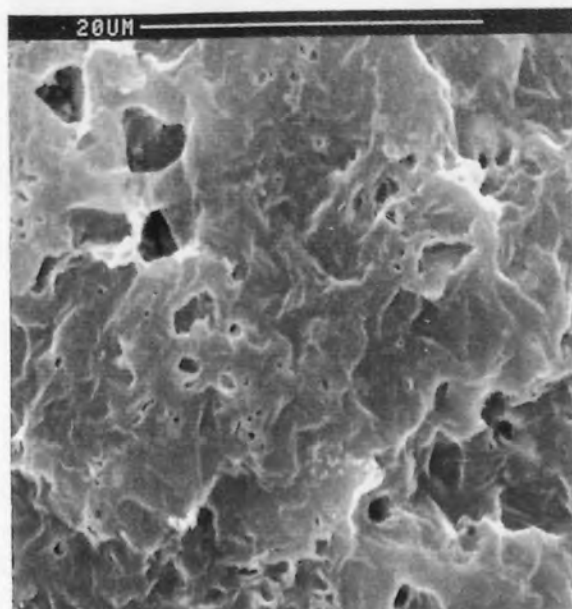
Low magnification [x820]

High magnification [x2410]

(c) 330 000.

Low magnification [x686]

High magnification [x2410]



6.3.3. Hydrocortisone - loaded microspheres prepared from PHB (20.1% HV) copolymers.

A moderate yield of spherical microspheres was obtained from the 32.8PHB (20.1% HV) / 20% HC formulation. In contrast, excellent particle yields were produced from 140PHB (20.1% HV) or 390PHB (20.1% HV) with 20% hydrocortisone.

The surface morphology of a typical particle from the 32.8PHB (20.1% HV) / 20% HC batch is shown in figure 6.6 (a). A high level of macroporosity can be seen extending from the surface deep into the matrix of this microsphere.

The microspheres prepared from 140PHB (20.1% HV) with 20% hydrocortisone, had a considerably higher level of structural integrity than those represented by figure 6.6 (a), although, as figure 6.6 (b) demonstrates, appreciable surface macroporosity was still a typical feature.

In contrast to the particles prepared from the two lower molecular weight 20.1%HV copolymer / 20% HC formulations, microspheres prepared from 390PHB (20.1%HV) had essentially well-formed and non-macroporous walls. However, as figure 6.6 (c) shows, these particles did have a few of the small surface holes that have been attributed to the removal of drug crystals from the solidified polymer wall.

6.3.4. Hydrocortisone - loaded microspheres prepared from PHB homopolymers blended with 20% PCL II.

Microencapsulation with 43PHB / 20% PCL II / 20% HC was slightly more successful than with the 43PHB formulations discussed in section 6.3.1, but the yield of spherical microspheres was still only poor [i.e. < 25%]. However, one of the more perfect particles is shown in figure 6.7 (a).

Figure 6.6. Scanning electron micrographs of 20% hydrocortisone-loaded microspheres prepared from PHB (20.1% HV) copolymers with different molecular weights :

(a) 32 800

Low magnification [x820]

High magnification [x2410]

(b) 140 000

Low magnification [x709]

High magnification [x2370]

(c) 390 000.

Low magnification [x853]

High magnification [x2410]

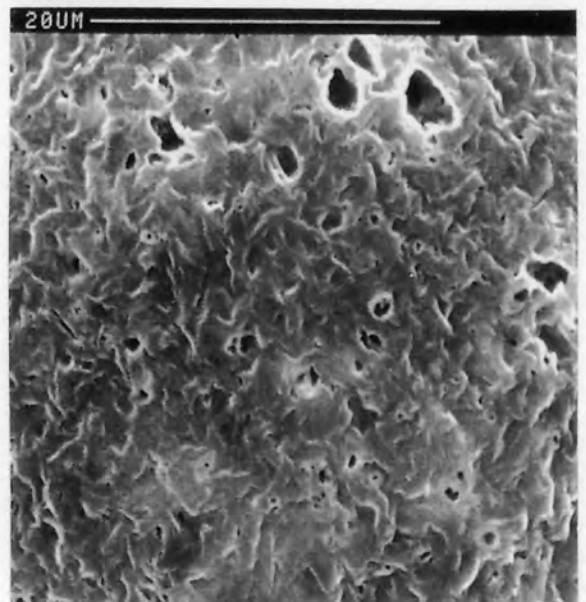
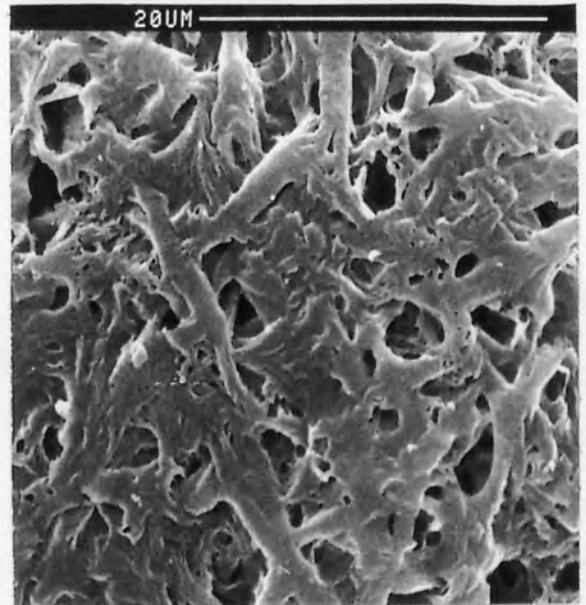
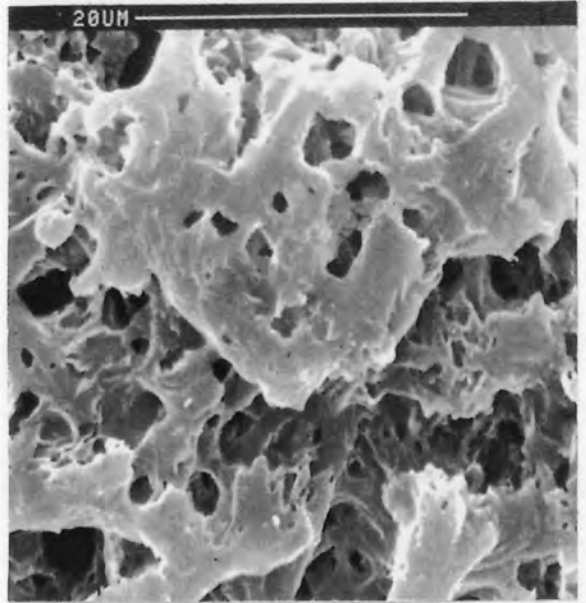


Figure 6.7. Scanning electron micrographs of 20% hydrocortisone-loaded microspheres prepared from blends of different molecular weight PHB homopolymers with 20% PCL II :

(a) 43 000

Low magnification [x842]

High magnification [x2370]

(b) 159 000

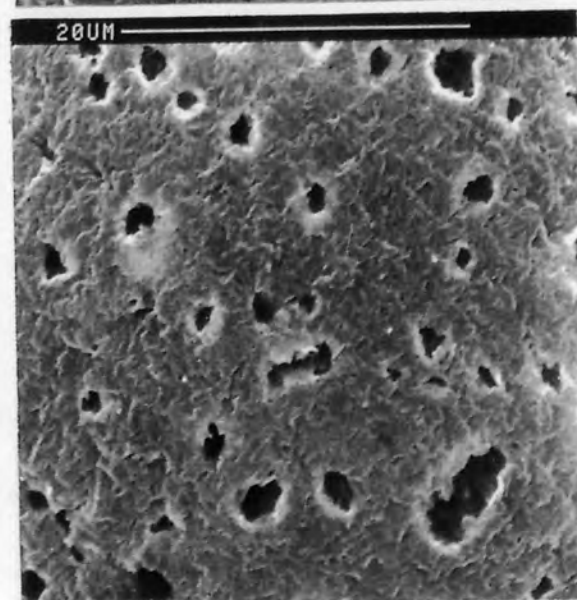
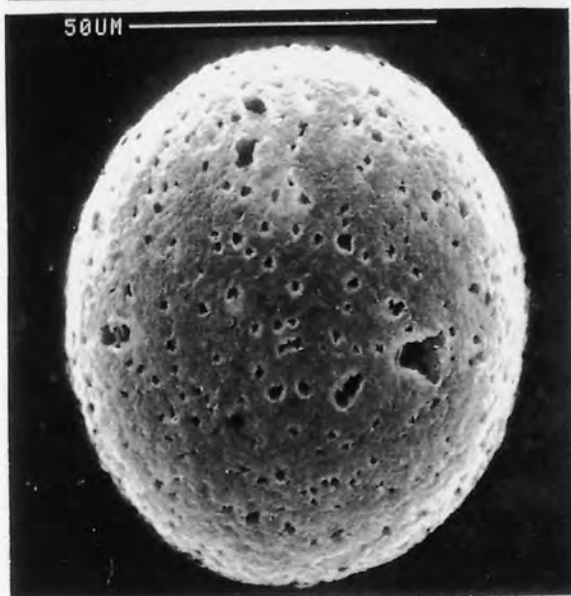
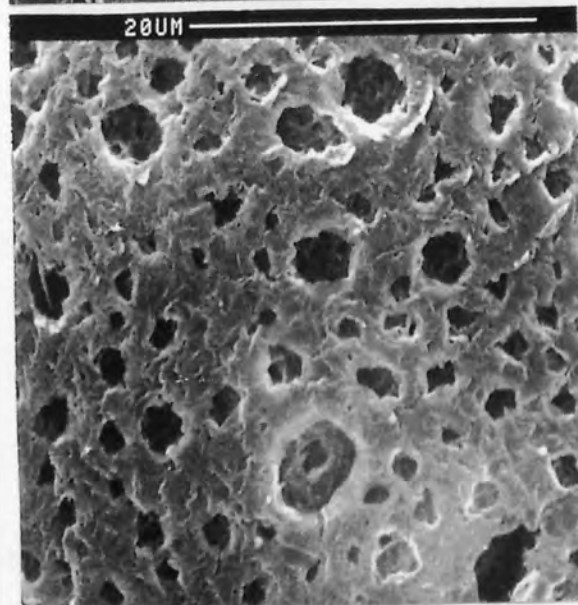
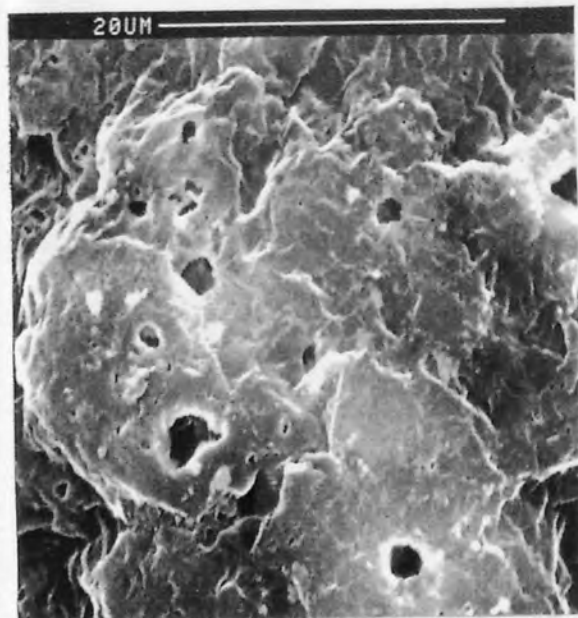
Low magnification [x809]

High magnification [x2370]

(c) 273 000.

Low magnification [x853]

High magnification [x2410]



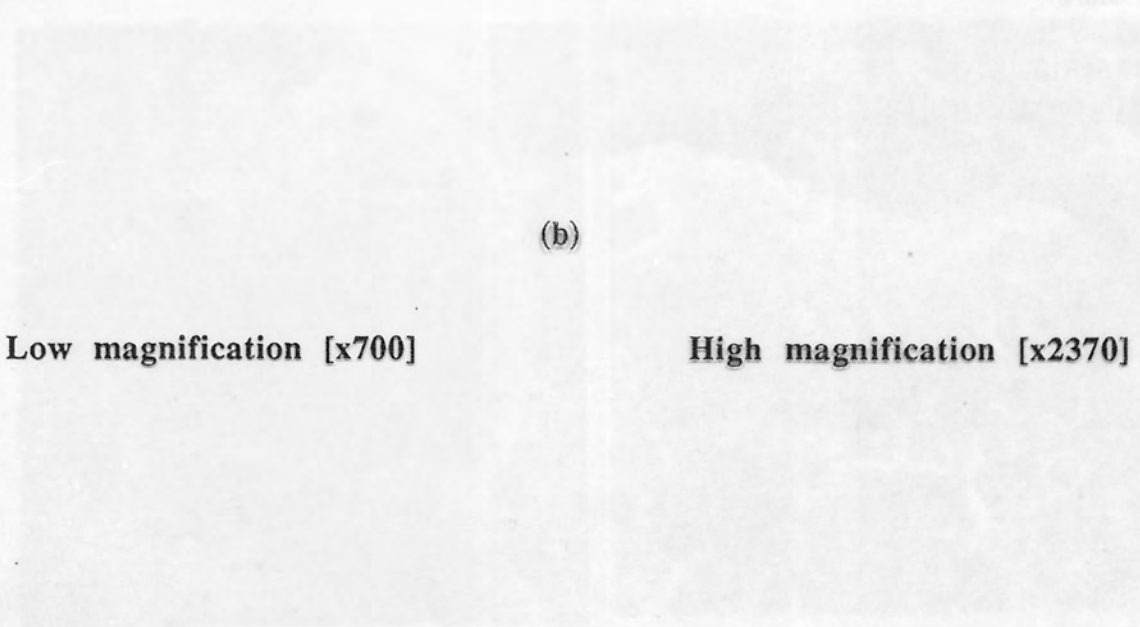
In contrast to the latter, excellent yields of spherical microspheres were prepared from the 159PHB / 20% PCL II / 20% HC and 273PHB / 20% PCL II / 20% HC formulations. A typical microsphere from the former batch is shown in figure 6.7 (b), and this has considerable porosity. Microspheres prepared from the 273PHB / 20% PCL II / 20% HC formulation also had PCL-induced surface porosity, although in this instance, as figure 6.7 (c) demonstrates, there were fewer pores than on the particles typified by figure 6.7 (b).

Figure 6.8 (a) shows the excellent yield of spherical particles prepared from unblended PCL II with 20% HC. A typical microsphere from this batch is shown in figure 6.8 (b). The polymer appears to be unevenly deposited in the wall of this particle thereby creating some irregular pores. In the smoother surface regions, some very fine microporosity can also be seen, although these pores do not appear to extend far into the matrix.

The production of high yields of spherical microspheres from every formulation listed in table 2.4, except for those which contained 15% 43PHB, is consistent with principles of polymer precipitation. Thus, higher molecular weight polymers precipitate more readily than low molecular weight polymers, and, at a given dispersed to continuous phase ratio, *complete* precipitation is more easily achieved from dilute, rather than from concentrated, solutions. If we now consider a competing precipitation process, where hydrocortisone forms a dispersed particulate phase within the precipitation medium, and in which polymer precipitation is occurring less readily and less effectively, then competition between these two characteristics will control the nature, and especially the physical form, of the observed precipitate. In the particular set of microsphere preparation experiments described in sections 6.3.1 - 6.3.3, it appears that this balance favours polymer precipitation as the morphology-controlling

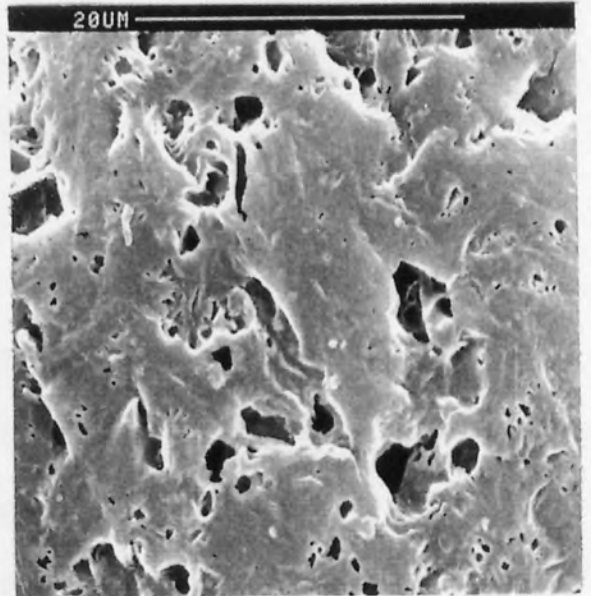
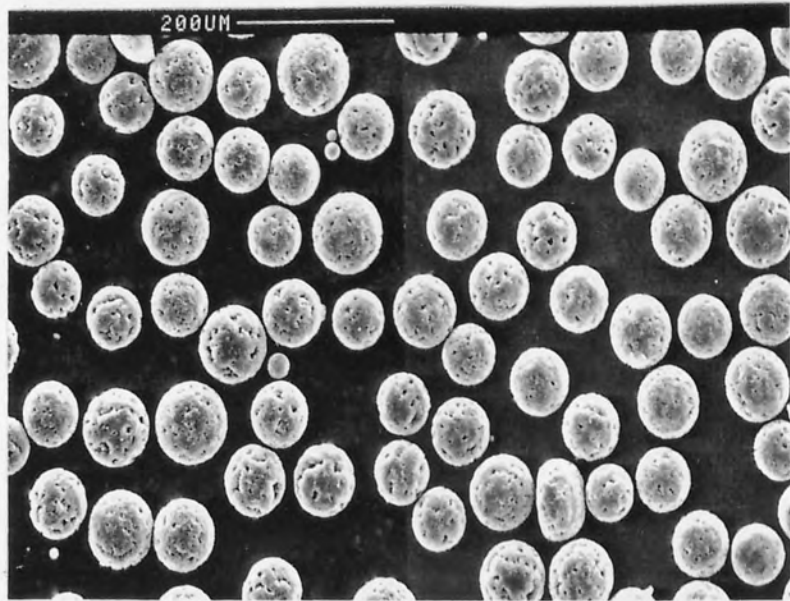
Figure 6.8. Scanning electron micrographs of 20% hydrocortisone-loaded microspheres prepared from PCL II :

(a) Very low magnification [x113]



Low magnification [x700]

High magnification [x2370]



event until the increased concentration and reduced molecular weight converge on the 43,000 molecular weight 15% PHB homopolymer solution. In this case, polymer precipitation is not the dominant morphology-controlling event, and consequently intact microspheres are not produced. Rather, the product is a finely divided amorphous precipitate.

Additionally, it should be noted that, although the less perfectly crystalline P(HB-HV) copolymers are easier to dissolve in chlorinated solvents than is PHB itself, once in solution, the more hydrophobic copolymer chains should be more readily precipitated in the presence of non-solvent than those of the homopolymer. This behaviour may not have significantly influenced the precipitation of the polymer at the droplet interface, and is therefore not relevant to the precipitation events that determined microcapsule morphology, as discussed in chapter three. However, it does account for the more rapid precipitation of 83.1PHB (10.8%HV) and 32.8PHB (20.1%HV) compared with 43PHB from 15% polymer solutions, and therefore the production of intact microspheres from the low molecular weight band copolymer / 20% HC formulations.

In addition, with regard to the improved yield of intact particles from the 43PHB / 20% HC formulation at lower polymer / drug concentrations, it should be noted that a decrease in the concentration of the polymer solution, whilst maintaining a constant drug loading, diminishes the proportion of particulate drug.

Whilst the individual experiments needed to verify the component parts of this explanation were not performed [as this would hardly have been a profitable exercise given the scope of this work], all the experimental observations are consistent with the explanation advanced.

6.4. Hydrocortisone dissolution experiments.

The dissolution profiles of hydrocortisone from microspheres prepared using the three different molecular weight PHB homopolymers, with a 20% drug loading, are compared over 8 day and 300 day experimental periods in graph 6.4.

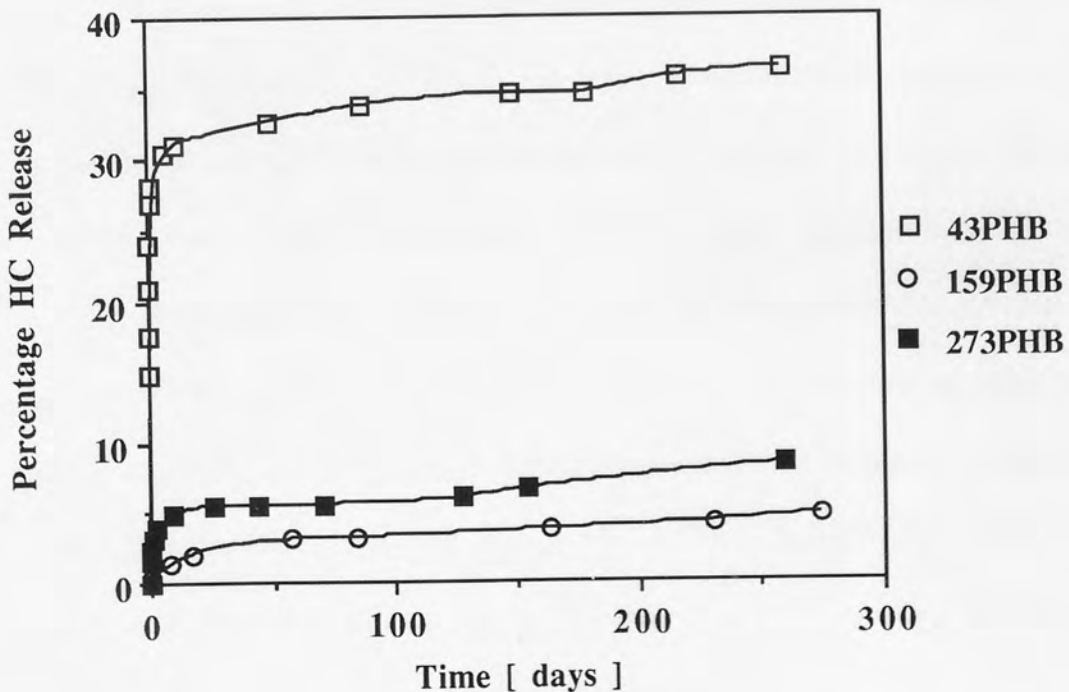
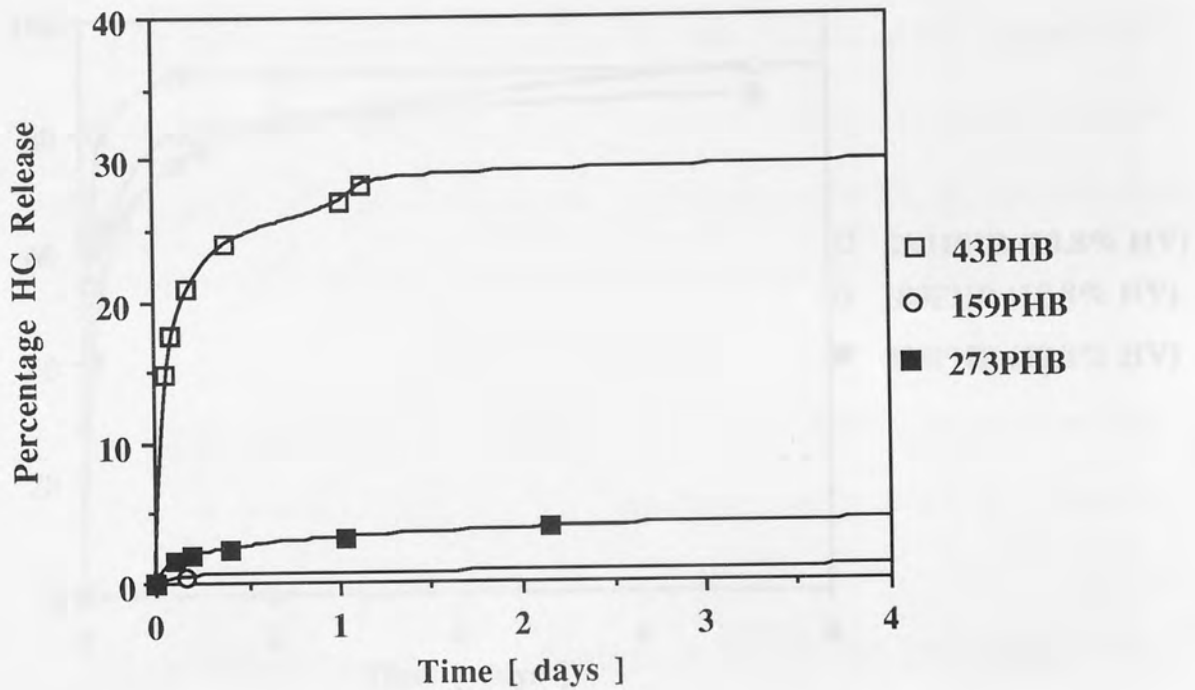
The release of drug from the amorphous precipitate that was produced from the 43PHB / 20% HC formulation occurred rapidly at first, as approximately 30% of the entrapped steroid was discharged within the first two days. However, this initial burst was not sustained, and after 9 months of dissolution less than 40% of the available hydrocortisone had been released.

Protracted phases of impoverished drug discharge also characterized the hydrocortisone dissolution profiles of microspheres prepared from the 159PHB / 20% HC and 273PHB / 20% HC formulations. Moreover, in these instances, near-negligible release phases were not preceded by significant a burst effect, and consequently the particles still retained more than 90% of their original steroid payloads after 9 months of dissolution.

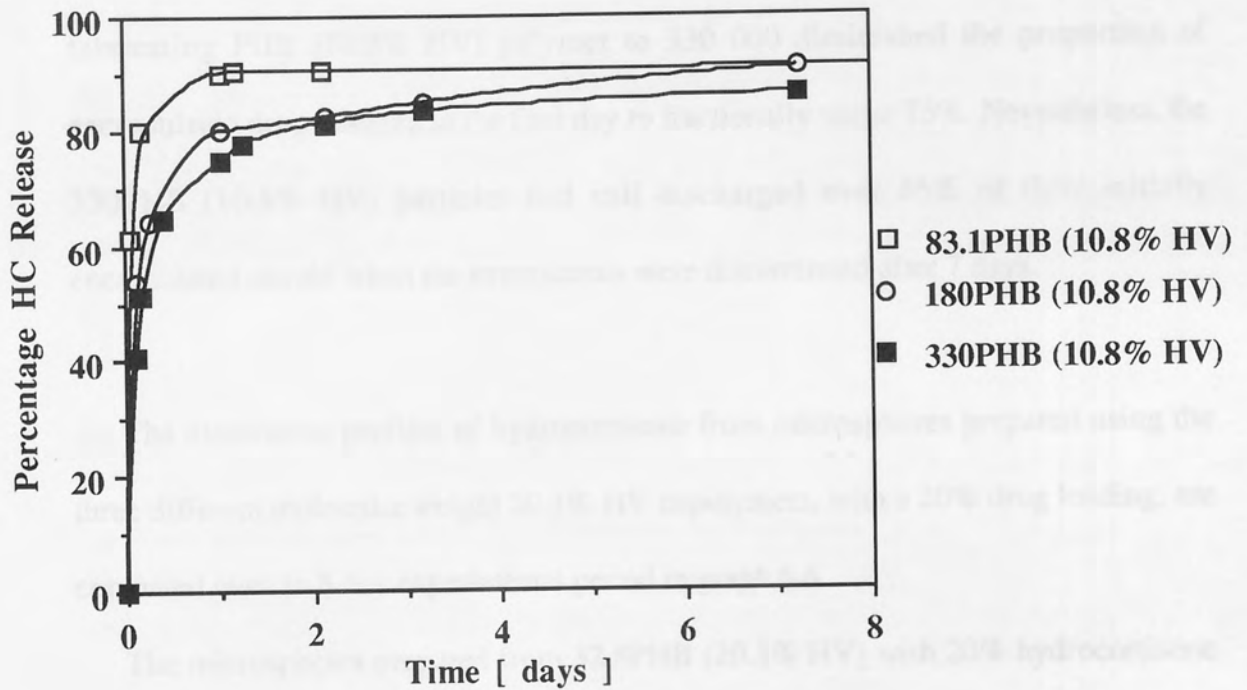
The dissolution profiles of hydrocortisone from microspheres prepared using the three different molecular weight 10.8% HV copolymers, with a 20% drug loading, are compared over an 8 day experimental period in graph 6.5.

Microspheres, prepared from the three different molecular weight 10.8% HV copolymers with 20% hydrocortisone, released most of their encapsulated steroid in a rapid initial burst. Thus, the 83.1PHB (10.8% HV) / 20% HC particles discharged approximately 90% of their hydrocortisone payload within the first day of dissolution [although only a further 3% followed in the next 4 months]. In comparison with the

Graph 6.4. Dissolution profiles showing the effects of PHB homopolymer molecular weight on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20%. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.



Graph 6.5. Dissolution profiles showing the effects of PHB(10.8% HV) copolymer molecular weight on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20%. The dissolution profiles are shown over an 8 day period.

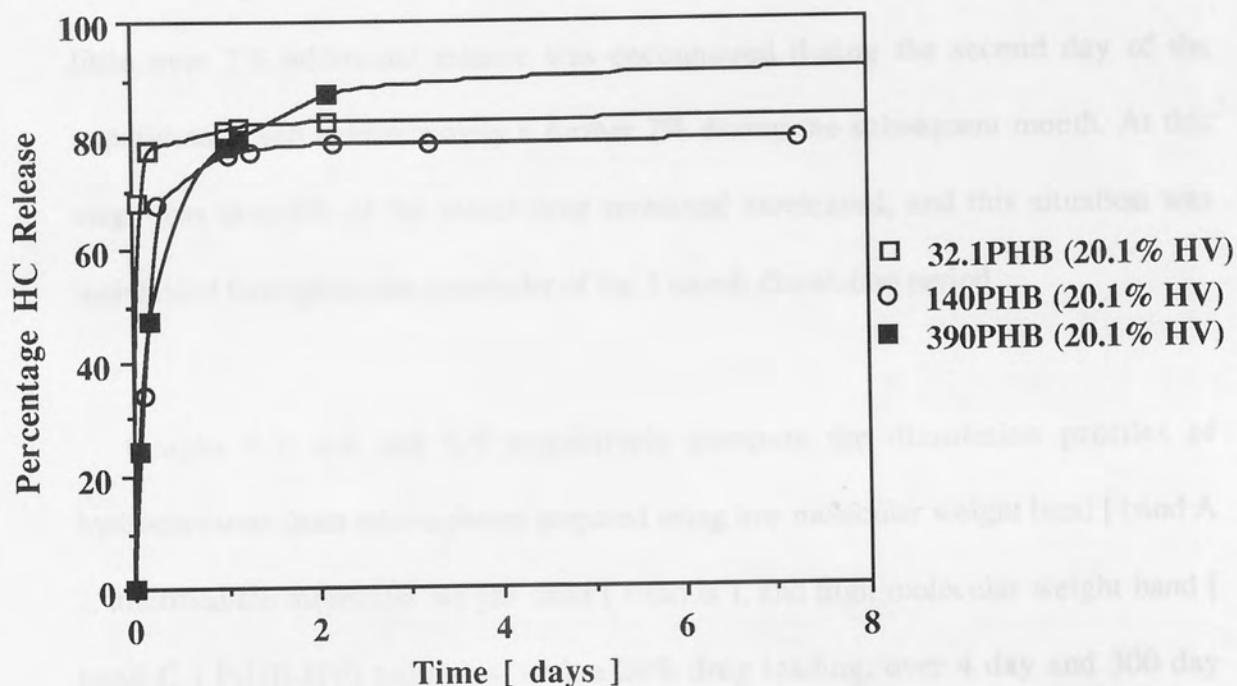


latter, a slightly slower initial drug release rate was observed from the 180PHB (10.8% HV) / 20% HC microspheres, as day 1 discharge was fractionally under 80%. However, by the end of the first week, when the experiments were abandoned, over 90% of the encapsulated steroid had escaped. Increasing the molecular weight of the fabricating PHB (10.8% HV) polymer to 330 000 diminished the proportion of encapsulated drug released in the first day to fractionally under 75%. Nevertheless, the 330PHB (10.8% HV) particles had still discharged over 86% of their initially encapsulated steroid when the experiments were discontinued after 7 days.

The dissolution profiles of hydrocortisone from microspheres prepared using the three different molecular weight 20.1% HV copolymers, with a 20% drug loading, are compared over an 8 day experimental period in graph 6.6.

The microspheres prepared from 32.8PHB (20.1% HV) with 20% hydrocortisone displayed a profound burst effect when initially immersed in the dissolution medium, as over 68% of the encapsulated drug was released within the first 80 minutes. The rate of release then slowed sharply, with the result that by the end of the first day barely 80% of the available steroid had been discharged. Little subsequent release was detected, and when the experiments were abandoned after 3 months, approximately 17% of the initially encapsulated drug remained unreleased. In comparison with the latter, a less pronounced burst effect characterized the release of drug from microspheres prepared using 140PHB (20.1% HV) with 20% hydrocortisone. In this case, approximately 34% of the available steroid was released in the first 3 hours of dissolution, and this had increased to 76% by the end of the first day. Thereafter, there was little additional discharge in the remainder of the 7 day experimental period.

Graph 6.6. Dissolution profiles showing the effects of PHB(20.1% HV) copolymer molecular weight on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20%. The dissolution profiles are shown over an 8 day period.



The microspheres prepared from 390PHB (20.1% HV) with 20% hydrocortisone also released a significant proportion of the encapsulated steroid in a rapid initial burst.

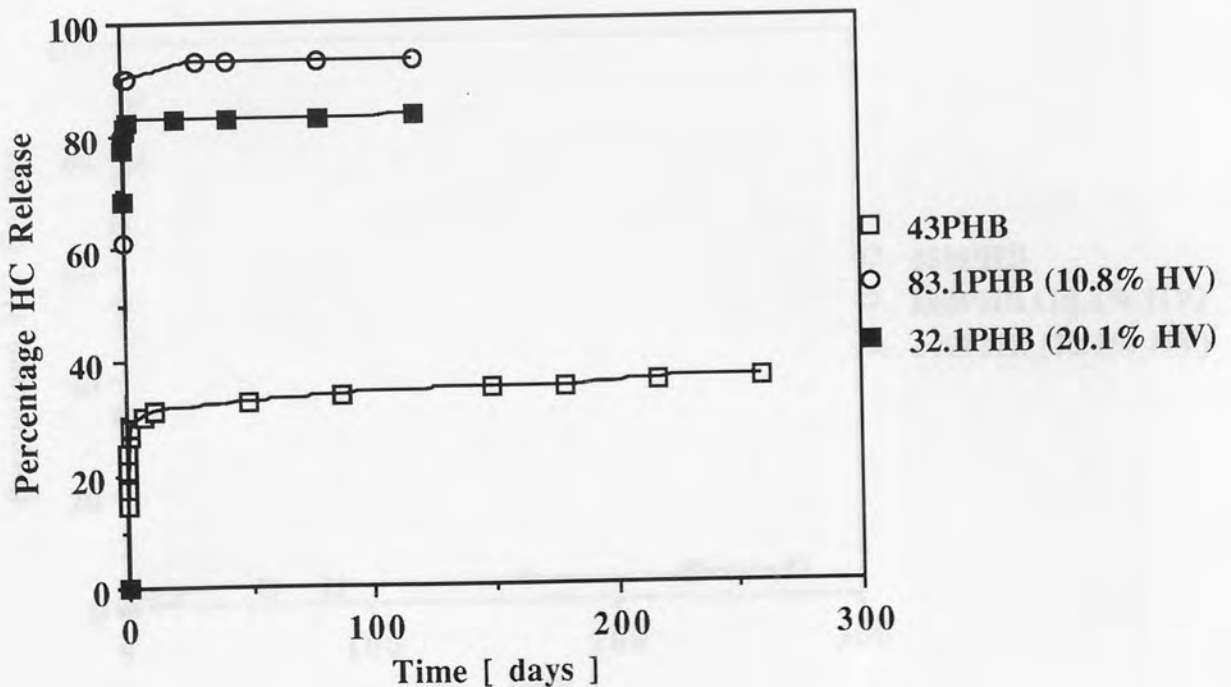
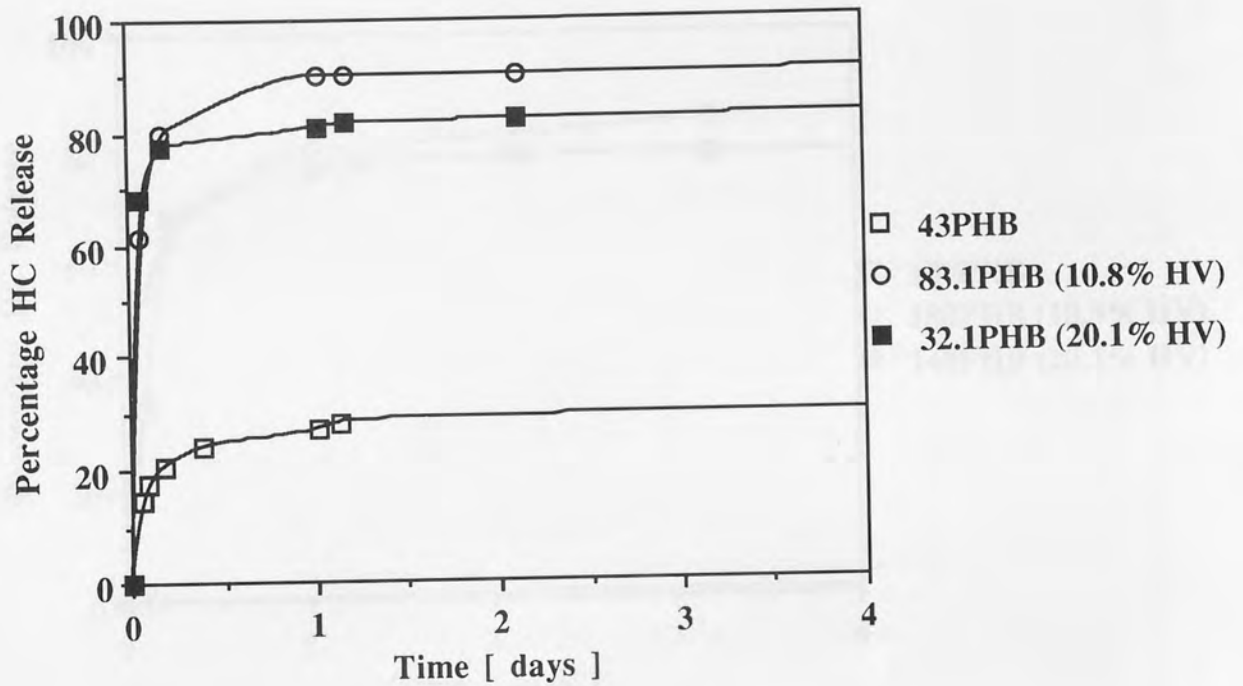
Thus, after 80 minutes of dissolution, approximately 24% of the available drug had been discharged, and this had increased to nearly 80% by the end of the first day. A little over 7% additional release was encountered during the second day of the experiments, and approximately a further 7% during the subsequent month. At this stage less than 6% of the initial drug remained unreleased, and this situation was maintained throughout the remainder of the 3 month dissolution period.

Graphs 6.7, 6.8 and 6.9 respectively compare the dissolution profiles of hydrocortisone from microspheres prepared using low molecular weight band [band A], intermediate molecular weight band [band B], and high molecular weight band [band C] P(HB-HV) polymers, with a 20% drug loading, over 4 day and 300 day experimental periods. These graphs therefore highlight the effects of the fabricating polymer's HV content on the release kinetics.

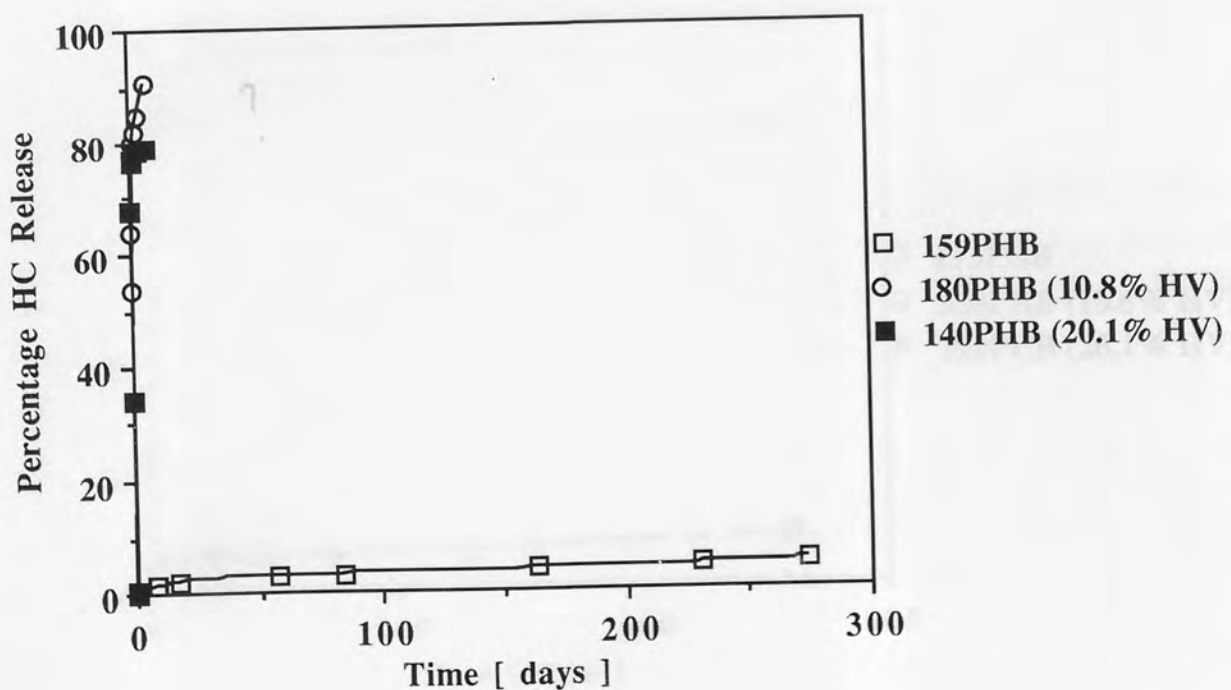
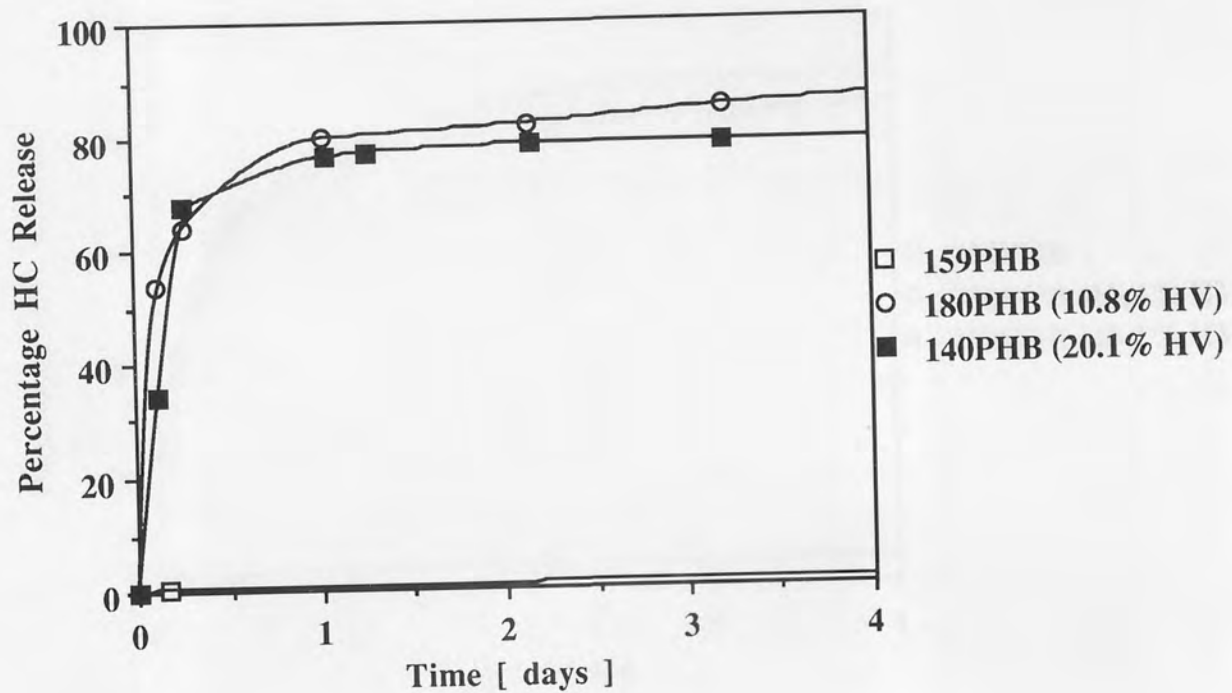
In order to test whether there was a linkage between the microsphere hydrocortisone loading and the drug release profile, particles were prepared from the three different molecular weight PHB homopolymers [43PHB, 159PHB and 273PHB] with three different steroid loadings [10%, 20% and 30%]. The actual hydrocortisone contents of microspheres produced from these formulations are detailed in table 6.2.

The effects of the drug loading on the dissolution profile of hydrocortisone are shown in graphs 6.10, 6.11 and 6.12, which respectively relate to microspheres prepared from 43PHB, 159PHB and 273PHB.

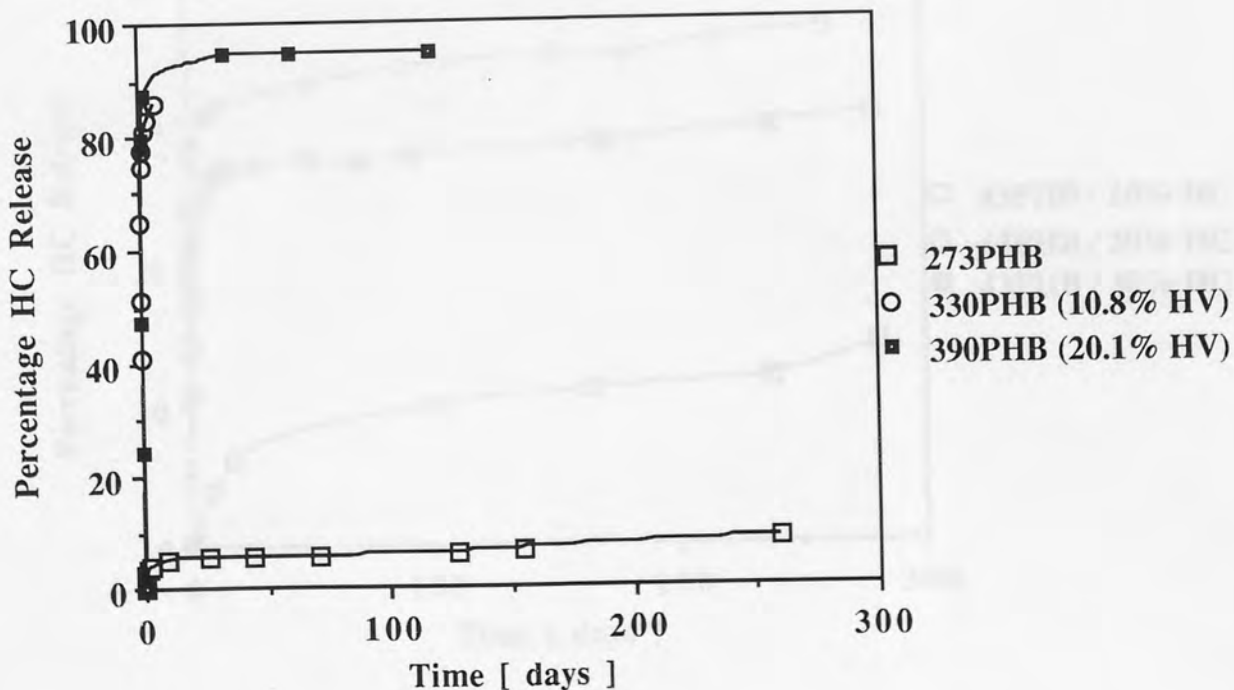
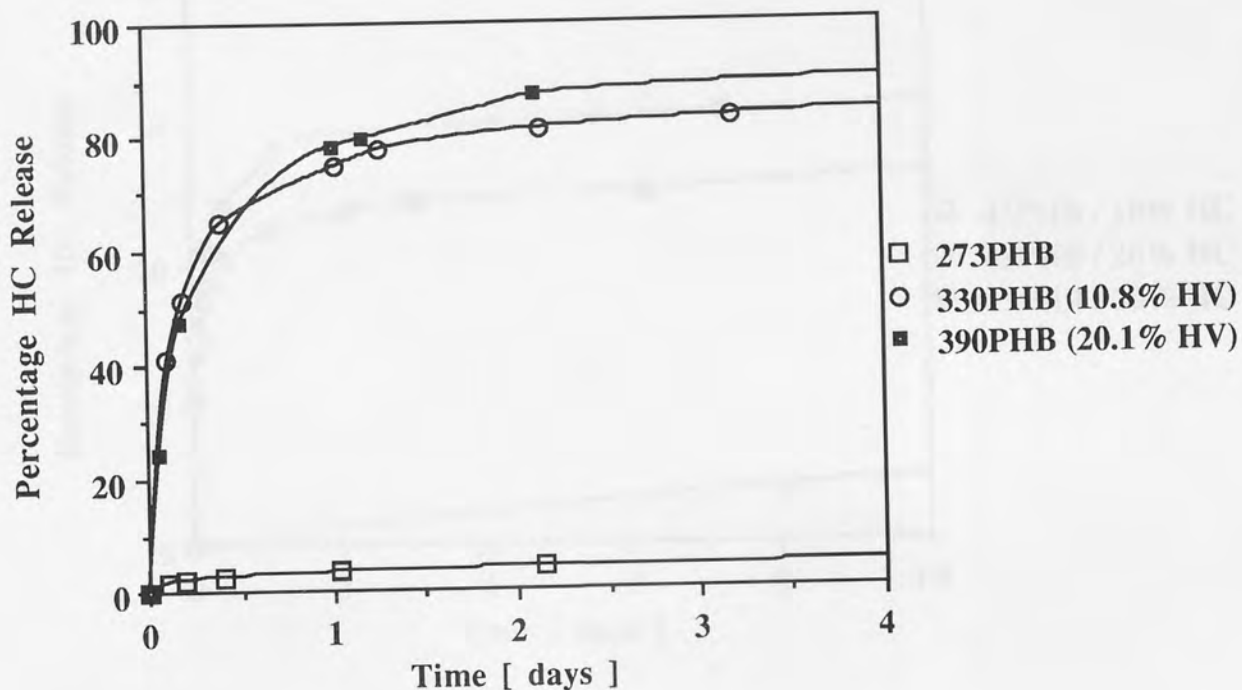
Graph 6.7. Dissolution profiles showing the effects of HV content on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20% from three low molecular weight [band A] polymers. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.



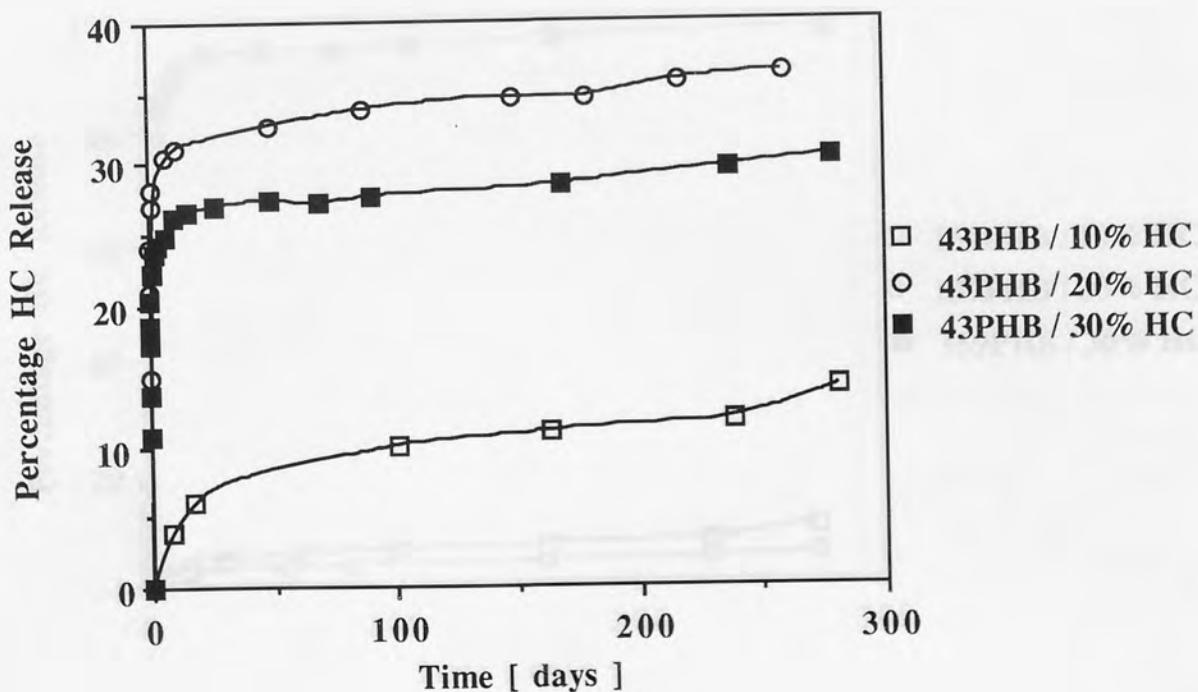
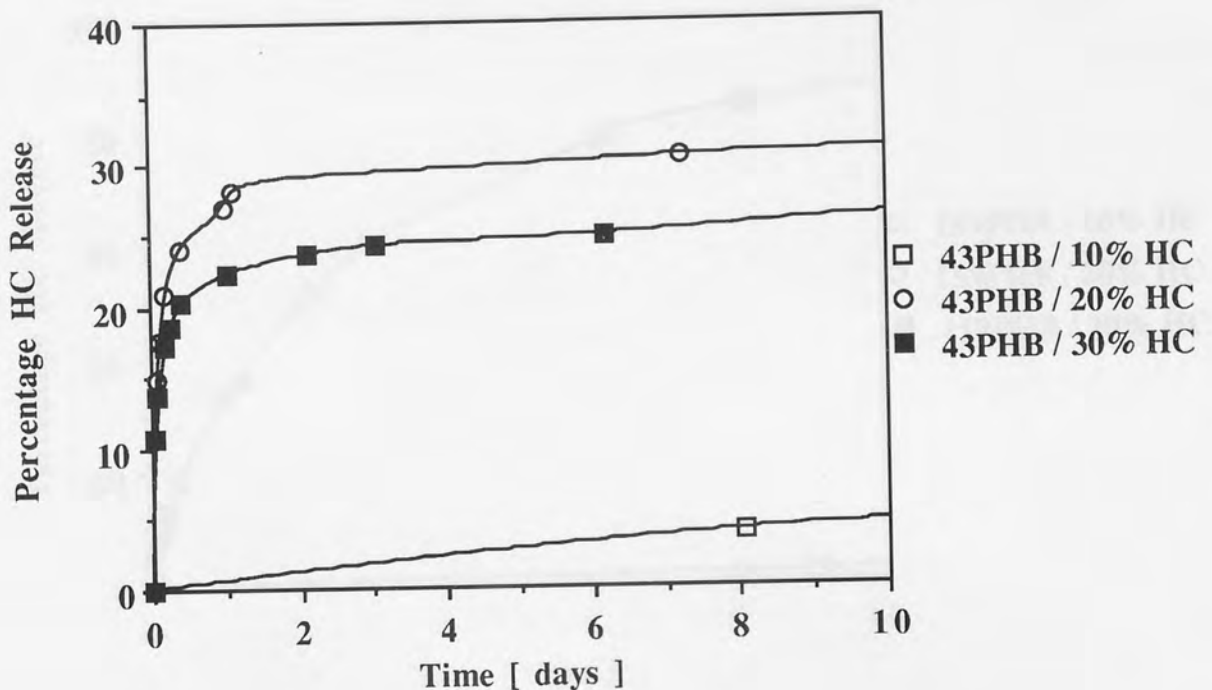
Graph 6.8. Dissolution profiles showing the effects of HV content on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20% from three intermediate molecular weight [band B] polymers. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.



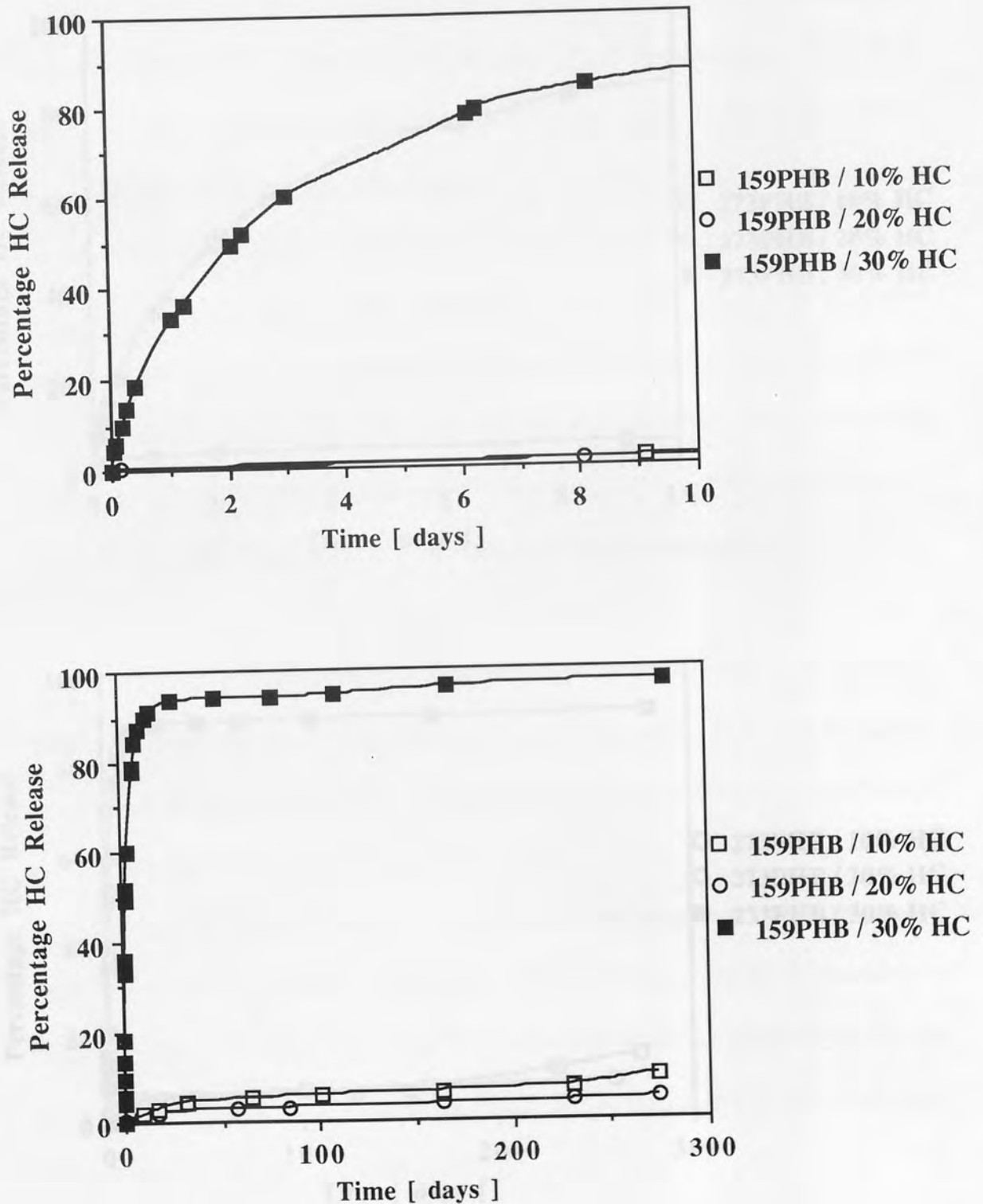
Graph 6.9. Dissolution profiles showing the effects of HV content on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20% from three high molecular weight [band C] polymers. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.



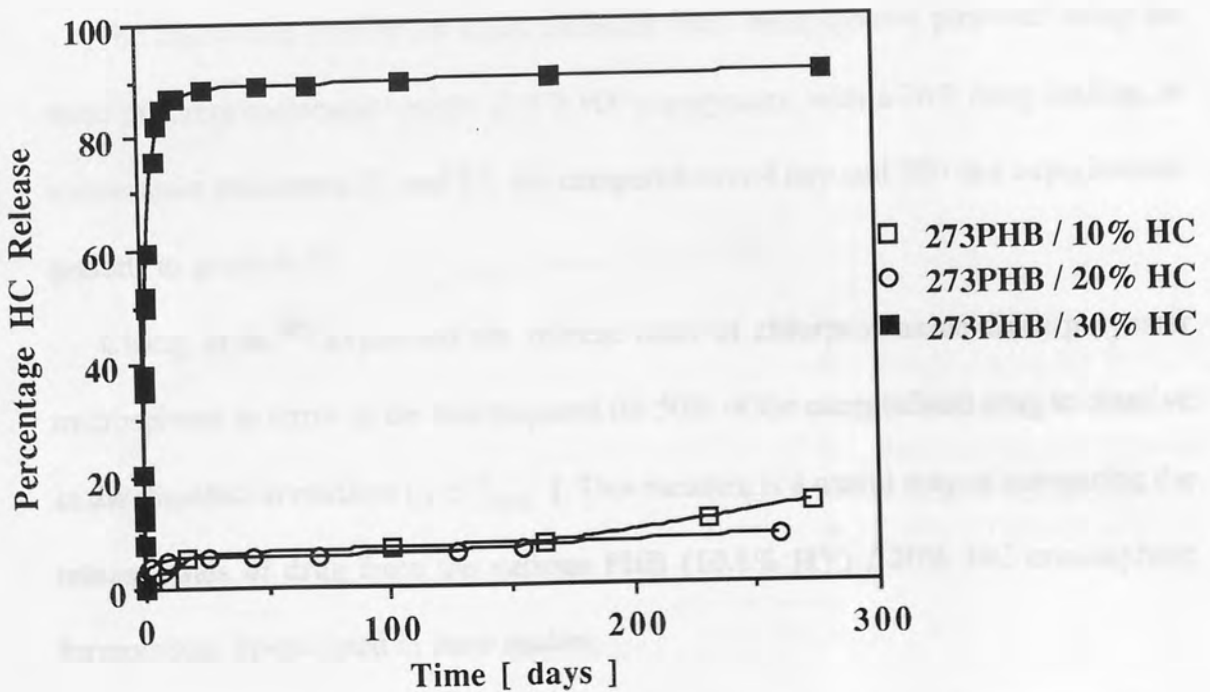
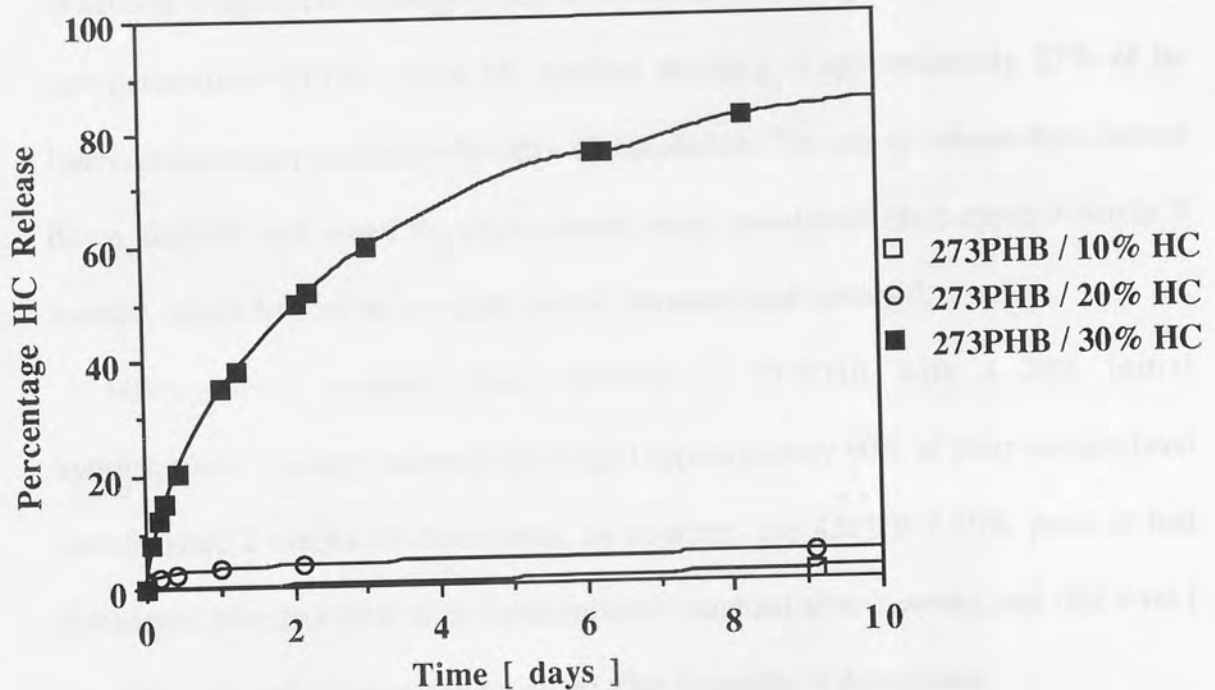
Graph 6.10. Dissolution profiles showing the effects of drug loading on the cumulative release of hydrocortisone from microspheres prepared from 43PHB. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 10 days, whereas the bottom graph shows the total release over approximately 300 days.



Graph 6.11. Dissolution profiles showing the effects of drug loading on the cumulative release of hydrocortisone from microspheres prepared from 159PHB. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 10 days, whereas the bottom graph shows the total release over approximately 300 days.



Graph 6.12. Dissolution profiles showing the effects of drug loading on the cumulative release of hydrocortisone from microspheres prepared from 273PHB. The dissolution profiles are expressed in two time scales ; the top graph shows the initial release over 10 days, whereas the bottom graph shows the total release over approximately 300 days.



Microspheres prepared with an initial hydrocortisone loading of 10%, released very little drug during 9 months of dissolution, regardless of the molecular weight of the fabricating polymer. A similar situation was also encountered with the two higher molecular weight PHB homopolymers when the drug loading was 20%. However, the non-particulate 43PHB / 20% HC product discharged approximately 27% of its hydrocortisone payload in the first day of dissolution. The rate of release then slowed down sharply, and when the experiments were abandoned after approximately 9 months, nearly 64% of the available steroid remained undischarged.

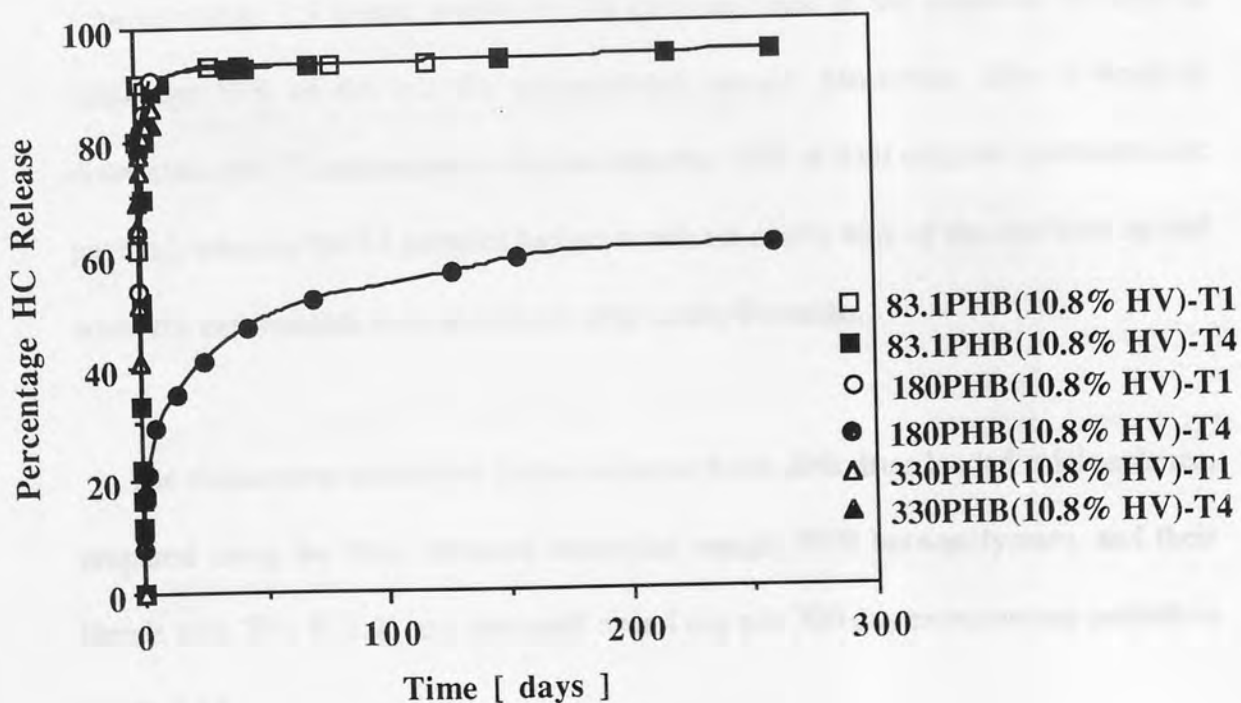
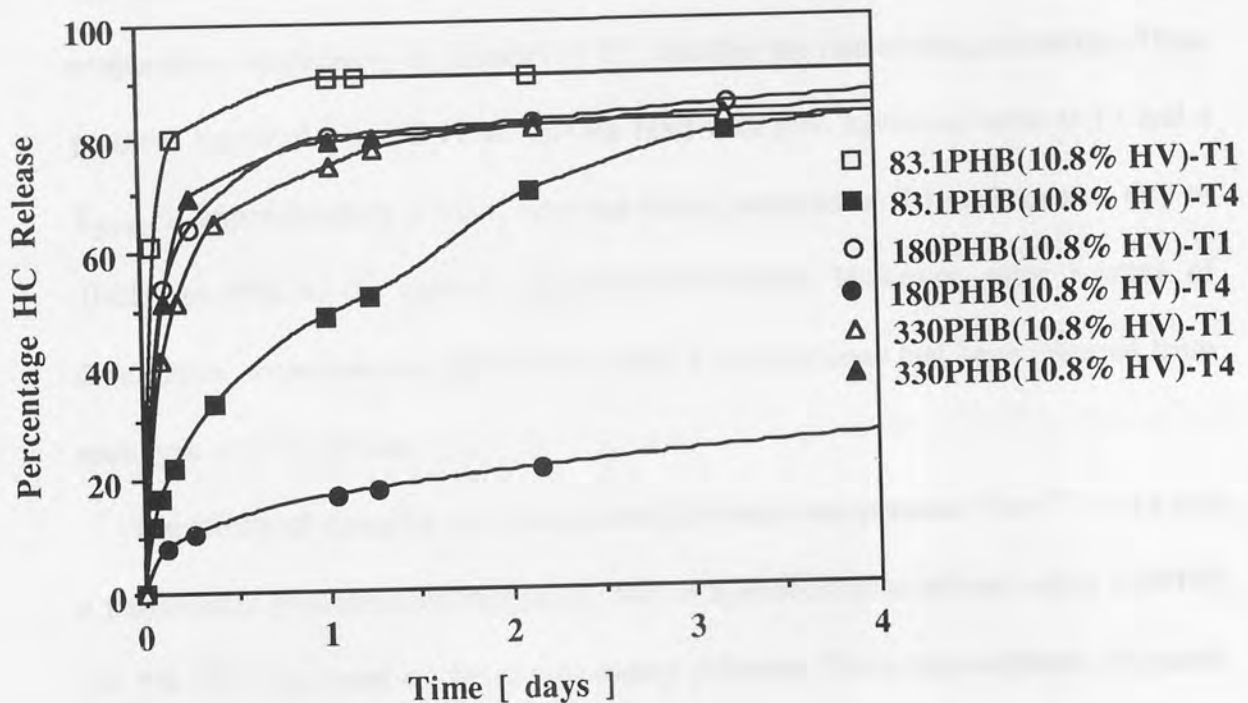
Microspheres prepared from 159PHB or 273PHB with a 30% initial hydrocortisone loading had each discharged approximately 90% of their encapsulated steroid after 2 weeks of dissolution. In contrast, the 43PHB / 30% product had discharged less than 30% of its hydrocortisone payload after 2 weeks, and this level [i.e. 30%] was only fractionally exceeded after 9 months of dissolution.

The dissolution profiles of hydrocortisone from microspheres prepared using the three different molecular weight 10.8% HV copolymers, with a 20% drug loading, at temperature treatments T1 and T4, are compared over 4 day and 300 day experimental periods in graph 6.13.

Chang *et al.*⁴⁸ expressed the release rates of chlorpromazine from polyester microspheres in terms of the time required for 50% of the encapsulated drug to dissolve in the dissolution medium [i.e. $T_{50\%}$]. This measure is a useful way of comparing the release rates of drug from the various PHB (10.8% HV) / 20% HC microsphere formulations investigated in these studies.

The three batches of microspheres prepared at T1 released most of their steroid

Graph 6.13. Dissolution profiles showing the effects of solvent evaporation temperature on the cumulative release of HC from microspheres prepared at an initial drug loading of 20% from three different molecular weight PHB (10.8% HV) copolymers. The dissolution profiles are expressed in two time scales ; the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.

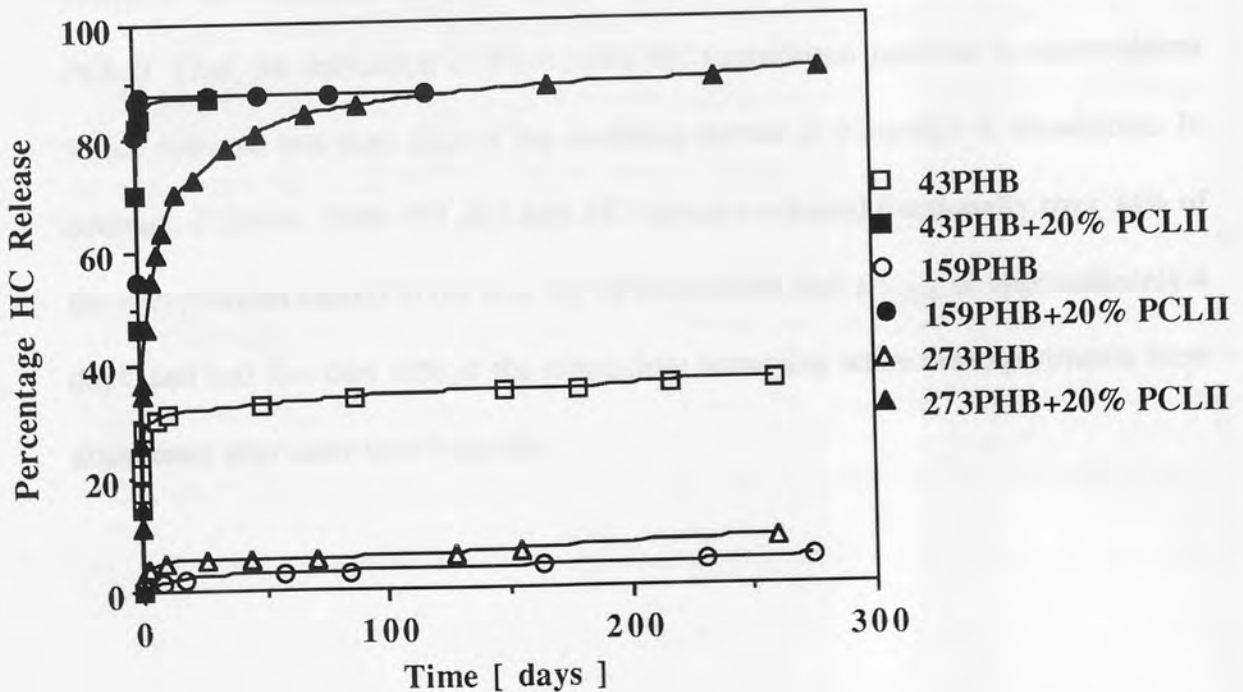
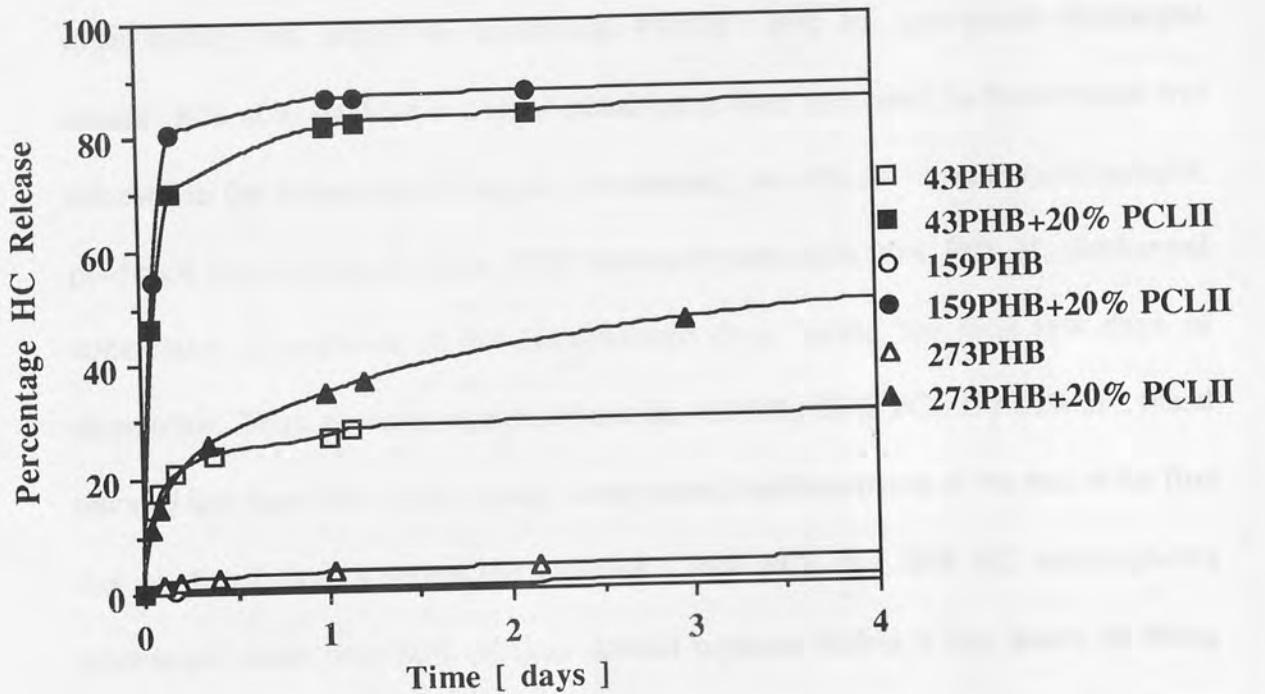


payload in the first two days of dissolution. This was also the case for the 330PHB (10.8% HV) / 20% HC particles prepared at T4, which retained less than 19% of the original hydrocortisone by the third day of the dissolution experiments. However, with the two lower molecular weight (10.8% HV) copolymers, the use of solvent evaporation temperature T4, instead of T1, retarded the rate of drug discharge. Thus, particles prepared from 83.1PHB (10.8% HV) with 20% hydrocortisone at T1 had a $T_{50\%}$ of approximately 1 hour, whereas those prepared at T4 took over a day to discharge 50% of the initially encapsulated steroid. However, after 1 week of dissolution, approximately 90% of the initial hydrocortisone had been released from each type of microsphere.

The effects of changing the microsphere fabrication temperature from T1 to T4 had a particularly pronounced effect on the rate of hydrocortisone release when 180PHB (10.8% HV) was used as the encapsulating polymer. Thus, microspheres prepared from 180PHB (10.8% HV) with 20% hydrocortisone at T1 exhibited a $T_{50\%}$ of approximately 2.5 hours, whilst the T4 particles took in the order of 55 days to discharge 50% of the initially encapsulated steroid. Moreover, after 1 week of dissolution, the T1 microspheres retained less than 10% of their original hydrocortisone payload, whereas the T4 particles had yet to release nearly 40% of the available steroid when the experiments were abandoned after nearly 9 months.

The dissolution profiles of hydrocortisone from 20% drug-loaded microspheres, prepared using the three different molecular weight PHB homopolymers, and their blends with 20% PCL II, are compared over 4 day and 300 day experimental periods in graph 6.14.

Graph 6.14. Dissolution profiles showing the effects of 20% PCL II blending on the cumulative release of hydrocortisone from microspheres prepared at an initial drug loading of 20% from three different molecular weight PHB homopolymers. The dissolution profiles are expressed in two time scales : the top graph shows the initial release over 4 days, whereas the bottom graph shows the total release over approximately 300 days.



Microspheres prepared from the three different unblended PHB homopolymer / 20% HC formulations released only minor proportions of their encapsulated steroid during 9 months of dissolution. Thus, the 159PHB / 20% HC and the 273PHB / 20% HC particles still retained over 90% of the initial drug at the end of the dissolution experiments, and, whilst the amorphous 43PHB / 20% HC precipitate discharged around 30% of its payload in a rapid initial burst, little additional hydrocortisone was released in the subsequent 9 months. In contrast, the 20% HC-loaded microspheres, produced from blends of these PHB homopolymers with 20% PCL II, discharged substantial proportions of the encapsulated drug during the first few days of dissolution. Thus, particles prepared from the 43PHB / 20% PCL II / 20% HC blend retained less than 20% of the initially encapsulated hydrocortisone at the end of the first day of dissolution, whereas the 159PHB / 20% PCL II / 20% HC microspheres discharged more than 80% of their steroid payload within a few hours of being introduced to the dissolution medium. A less pronounced, but nonetheless significant, change in the drug discharge rate was observed when 273PHB was blended with 20% PCL II. Thus, the unblended 273PHB / 20% HC formulation gave rise to microspheres which released less than 10% of the available steroid in 9 months of dissolution. In contrast, 273PHB / 20% PCL II / 20% HC particles released fractionally over 34% of the encapsulated steroid in the first day of dissolution, had a $T_{50\%}$ of approximately 4 days, and had less than 10% of the initial drug remaining when the experiments were abandoned after more than 9 months.

The release of small hydrophobic drug molecules from polyester microspheres may occur by one, or a combination of two, distinct diffusional processes. One of these, typically termed "partition-dependent" diffusion, involves the migration of individual drug molecules through the polymer dense regions of the matrix. This mechanism utilizes the free-volume which arises as a result of the rotation and translation of polymer chains, and is therefore a characteristic of amorphous regions at temperatures above the glass transition. For drug release by partition-dependent diffusion to make a significant contribution to the overall process of drug loss from a polymeric device, it is essential that the drug has appreciable solubility in the polymer phase and that there is a high degree of polymer chain mobility. Cha and Pitt¹¹ explained the release of methadone from PCL-P(L-LA) microspheres in terms of this type of diffusion. These researchers also demonstrated that a wide range of low molecular weight steroids were able to diffuse through intact PCL-based membranes in diffusion cell-experiments³³.

Poly- ϵ -caprolactone is typically a highly crystalline polymer, but a very low glass transition temperature [T_g] ensures that it is appreciably rubbery at physiological temperatures. The result of this is a large free-volume which, in combination with the high solubility most lipophilic molecules have in this extremely hydrophobic polyester, ensures a rapid rate of transit for most small steroids.

In comparison with PCL, P(HB-HV) polymers are less hydrophobic, generally more crystalline, and have lower free volumes. As a result, a steroid such as hydrocortisone, when entrapped within a P(HB-HV) polymer matrix, will not to enjoy the same freedom of movement via partition-dependent diffusion that would typically arise in a comparable PCL system. One result of this, in terms of drug discharge from P(HB-HV) matrices, is that drug molecules which are, in the felicitous words of Siegel

*et al.*¹²⁶, "entirely surrounded by a sea of polymer", can only be released as a consequence of polymer hydrolysis. Moreover, it is apparent from graphs 6.4 - 6.14 that a ubiquitous feature of the microsphere systems studied in this work is the presence of unreleased hydrocortisone after 9 months of dissolution. This non-discharged drug probably corresponds to that fraction of the encapsulated material which was totally surrounded by the polymer in the microsphere matrix. Discovering that this fraction of encapsulated drug [which could probably only be released as a result of polymer breakdown] did in fact remain in the microspheres throughout the 9-month dissolution study is totally consistent with previous studies performed within the Speciality Materials Research Group on the hydrolytic degradation of P(HB-HV) polymers [e.g. Holland *et al.*¹²⁹]. Thus, polymer hydrolysis may facilitate the release of an entrapped drug from a poly(α,β ester) matrix by a mechanism related to biodegradation [i.e. a molecular weight reduction and a diminution in the level of crystallinity] and / or bioerosion [i.e. physical breakdown of the polymer matrix and the generation of porosity]. In the former case, partition-dependent diffusion leads to drug release, since diffusion coefficients are expected to increase as the molecular weight and chain entanglements of a polymer decrease¹¹. Alternately, erosion-related drug release typically involves either the diffusion of the active species through water-filled pores which arise from the elution of solubilised low molecular weight polymer fractions into the dissolution media, or a total collapse of the polymer matrix.

Holland *et al.*¹²⁹ reported that the weight-average molecular weight [M_w] of an injection moulded plaque, made from a P(HB-HV) copolymer containing 12% HV, declined only very slowly when immersed in aqueous buffer at 37°C. Indeed, from an initial M_w of approximately 300 000, M_w was still over 200 000 by day 250 of the

study. This time period [i.e. 250 days] is broadly in line with the 9 months of dissolution given to the hydrocortisone-loaded microspheres in this work.

Holland *et al.*¹²⁹ also found that the most marked period of molecular weight loss took place between days 250 and 318, at which point M_w was less than 50 000. However, in terms of crystallinity, the degradative profile was very different. Indeed, from an initial value of 69%, the matrix crystallinity [as measured by X-ray diffraction] had increased to 77% by day 318. An increase in crystallinity with degradation time is not uncommon with poly(α,β) esters, and is accounted for by the chain fragments of the hydrolysed amorphous regions re-aligning themselves into more ordered [crystalline] states³⁴.

In view of the biodegradation studies performed by Holland *et al.*¹²⁹, it is not surprising to find that hydrocortisone release from P(HB-HV) microsphere matrices was not apparent after 9 months of dissolution. Indeed it is not unreasonable to speculate that, in order for partition-dependent diffusional discharge of hydrocortisone to become significant, a substantial decline in both polymer molecular weight and the level of crystallinity would need to occur. With regard to the latter, the data presented by Holland *et al.*¹²⁹ showed that P(HB-HV) polymers hydrolysed for 531 days were still more crystalline than they were at the outset. It would therefore appear that more than two years is required for P(HB-HV) polymers to suffer the appreciable reductions in the level of crystallinity probably needed to facilitate the partition-dependent diffusional release of encapsulated materials such as hydrocortisone [although, as is mentioned below, there are several reasons for believing that the microspheres should degrade more rapidly than injection moulded plaques].

Holland *et al.*¹²⁹ also measured the weight loss after 531 days from the polymer

sample, and this was approximately 5% [of the initial dry weight]. This small decrease suggests drug release from P(HB-HV) polymers as a consequence matrix bioerosion is also unlikely to occur within a time frame of approximately two years.

However, injection moulding typically produces more crystalline P(HB-HV) polymers than solvent casting ¹³⁰, and so the microsphere matrices prepared in these studies probably degraded more rapidly than the injection moulded plaques employed in the work of Holland *et al.* ¹²⁹. This may also have been helped by the fact that many of the microspheres had very porous morphologies, and were prepared from polymers with initial molecular weights as low as 32 800 [c.f. ~ 300 000 for the samples of Holland *et al.*].

For the microsphere formulations which retained the largest proportions of hydrocortisone during the dissolution studies, the extent of polymer hydrolysis was monitored after approximately 9 months, by GPC and SEM. The GPC data was difficult to interpret, as a result of the small sample sizes, but generally suggested that no extensive molecular weight reductions had occurred. Similarly, the SEM observations revealed that no detectable changes in either surface morphology or particle shape had been produced as a consequence of the dissolution.

For drug particles that are not completely protected by the polymer, there is an alternate diffusional process [c.f. to partition-dependent diffusion] by which release from the undegraded polymeric matrix may occur. This involves the leaching of exposed drug particles directly into the dissolution medium, and is generally responsible for the 'burst effect' or rapid initial discharge of entrapped [but not fully encapsulated] material that is a ubiquitous feature of microsphere systems [e.g. ^{15, 121}

J. The exposed drug particles may be at the surface of the polymeric device or actually within the matrix. In the latter instance, rapid release can only occur provided that the dissolution medium can readily reach the drug particles by imbibing through pores and channels. Whilst these may be present as a result of intrinsic matrix porosity, they are frequently generated during dissolution, for example by the progressive leaching of drug from the surface inwards.

The formation of connected pores spaces in a polymer matrix as a consequence of drug leaching has been discussed by, amongst others, Siegel, Kost and Langer ¹²⁶. These researchers observed polypeptide-containing polymeric matrices before and after release, and noted that, as the drug content increased, the drug particles became more closely associated, and a greater number came into direct contact with the microsphere surface or an open channel. An important consequence of this behaviour is that the drug content can have a profound effect on the rate of drug discharge from a polymeric matrix. Thus, at higher contents, when the initially-exposed drug particles leach into the penetrating front of the dissolution medium, more interconnected pores spaces are formed [than at lower contents] and a greater proportion of the entrapped drug is able to escape by aqueous diffusion. Since this is often a more rapid mechanism of drug discharge than would otherwise be available, an increase in the rate of drug release [or more precisely an increase in the proportion of drug that is released rapidly] with an increase in the drug content is a common phenomenon in polymeric controlled release systems. Indeed, a study of graphs 6.10 - 6.12 reveals that this was generally the case with the hydrocortisone microspheres investigated in this work. Thus, particles prepared with a 10% drug loading from the three different molecular weight PHB homopolymers had hydrocortisone contents of between 4.3% and 6.7% [from table 6.2], and discharged less than 15% of the available steroid during the 9 months of

dissolution. In contrast, microspheres prepared from 159PHB or 273PHB with 30% hydrocortisone had drug contents of 27.1% and 25.4% respectively, and retained less than 10% of the initially encapsulated steroid after 9 months of dissolution. These findings are consistent with the model proposed by Siegel *et al.*¹²⁶. The observation that the 43PHB / 20% HC product had released a greater proportion of the initially-encapsulated material after 9 months than the 43PHB / 30% HC product was a probable consequence of the non-uniform nature of the polymeric matrices produced from these formulations.

The production of intrinsically porous particles [in order to enable encapsulated drug to be released independently of polymer erosion] was the objective of the studies reported by Sato *et al.*⁹. These researchers prepared highly porous PGA microspheres by using solvent-extraction-precipitation and freeze-drying, instead of solvent evaporation, to effect the removal of the dispersed phase solvent. In these devices, the drug [e.g. prednisolone acetate] was assumed to reside along the lining of fine pores which extended deep into the microsphere matrix, and the solubility of the drug in the dissolution medium was deemed to be the rate limiting step for its release. The importance of intrinsic microsphere porosity in governing the release of an encapsulated steroid was also an important objective of the work described here. Indeed, the studies reported in chapters three and four of this thesis demonstrated a number of different ways in which the morphologies of microcapsules could be regulated, and section 6.3 showed the applicability of this work to microsphere systems.

When the hydrocortisone dissolution profiles shown in graphs 6.4 - 6.14 are correlated with the various microsphere morphologies shown in figures 6.4 - 6.7, the

importance of intrinsic matrix porosity in governing the release of the encapsulated material is plainly apparent. Thus, the 20% hydrocortisone-loaded microspheres, prepared at T1 from the three different molecular weight 10.8% HV and 20.1% HV copolymers, all discharged a major proportion of their steroid payloads very rapidly [i.e. $T_{50\%} \ll 1$ day]. Surface drug crystals were not observed by SEM, but these particles all had highly porous morphologies. As a result, it is proposed that the majority of the encapsulated drug particles in these microspheres were "networked" , i.e. had access to water-filled open channels, either directly or following the dissolution of other drug particles. After dissolving in the penetrating front of the dissolution medium, the drug could then escape from the microspheres by diffusion through the water-filled macropores.

Microspheres were prepared from the three different molecular weight 10.8% HV copolymer / 20% HC formulations at both T1 and T4. The morphological studies reported in section 6.3.2 suggested that, by altering the solvent evaporation temperature in this way, particle porosity could be manipulated. With the two lower molecular weight 10.8% HV copolymers, the use of T4 instead of T1 during particle preparation led to a reduction in the rate of hydrocortisone discharge as measured by $T_{50\%}$. Thus, microspheres prepared from 83.1PHB (10.8% HV) with 20% hydrocortisone at T1 exhibited a $T_{50\%}$ of approximately 90 minutes, whilst a $T_{50\%}$ of fractionally over 1 day was observed for particles prepared from this formulation at T4. The use of solvent evaporation T4 instead of T1 induced a far more pronounced extension in the time required for 50% of the initially encapsulated drug to be released when microencapsulation was performed using 180PHB (10.8% HV) with 20% hydrocortisone. In this instance, a $T_{50\%}$ of approximately 3 hours for the T1 particles

contrasted sharply with a $T_{50\%}$ of in the order of 10 weeks for the T4 microspheres.

Indeed, in the latter instance, drug release was sustained for the first 5 months of dissolution, at which point approximately 60% of the initial hydrocortisone payload had been discharged.

These effects of the solvent evaporation temperature on the rate of drug discharge are probably a consequence of the morphological changes described in section 6.3.2. Thus, T4 microspheres [prepared from either 83.1PHB (10.8% HV) or 180PHB (10.8% HV)] appear less porous than the corresponding T1 particles. Therefore, a diminution in the size, or an increase in the tortuosity, of the pores through which the entrapped drug particles have access to the dissolution medium would be expected to extend the release period. Moreover, discovering that the rate of discharge of drug from deep within the microsphere matrix, by dissolution followed by diffusion through water-filled pores, can actually be a lengthy process [i.e. approximately 5 months for the 180PHB (10.8% HV) / 20% HC / T4 particles] is not unreasonable from a theoretical viewpoint ¹²⁶. In addition to the size and tortuosity of the pores, factors such as the hydrophobicity of the polymer, the solubility of the drug in the dissolution medium, and the system hydrodynamics can all assume important roles in determining the time scale for release.

It is interesting to note that microspheres prepared from 83.1PHB (10.8% HV) with 20% hydrocortisone had discharged approximately 90% of their steroid payloads after 7 days, regardless of the temperature at which they were fabricated. It would therefore appear that the change from T1 to T4, with the 83.1PHB (10.8% HV) / 20% HC formulation, did not materially increase the proportion [< 10%] of entrapped drug that was fully protected by the polymer [and was therefore not released at all during the

9-month dissolution study].

In contrast, whereas microcapsules prepared from 180PHB (10.8% HV) with 20% hydrocortisone at T1 had discharged over 90% of the available steroid after 7 days, the particles prepared from this formulation at T4 had still to release nearly 40% of the initially encapsulated drug after approximately 9 months of dissolution. It therefore appears that, with the 180PHB (10.8% HV) / 20% HC formulation, the use of solvent evaporation temperature T4 instead of T1 results in a substantial increase in the proportion of drug that is entirely surrounded by polymer in the microsphere matrix.

Microspheres prepared from the three different molecular weight PHB homopolymers with 20% hydrocortisone released only small fractions of the encapsulated steroid in approximately 9 months of dissolution [graph 6.14]. It therefore appears that most of the hydrocortisone in these microspheres was totally surrounded by polymer and [in the absence of polymer breakdown] had no access to the dissolution medium. In contrast, when microencapsulation was performed using either 43PHB or 159PHB blended with 20% PCL II, at a 20% HC loading, the resulting particles discharged approximately 80% of their drug payloads within 1 day.

In relation to either of the latter, the release of hydrocortisone from the 273PHB / 20% PCL II / 20% HC microspheres was more protracted, although after 100 days, approximately 80% of the available drug had still been released . The release profiles of hydrocortisone from the three different PHB homopolymer / 20% PCL II / 20% HC microsphere formulations are shown in graph 6.14.

These effects of PCL blending on the profile of hydrocortisone discharge can be attributed to the morphological changes described in section 6.3.4. Thus, the microparticles prepared from the two higher molecular weight PHB homopolymer /

20% PCL II blends are highly porous in comparison with the particles prepared from the corresponding unblended formulations. This was probably also the case with the 43PHB product, although its irregular nature made this suggestion impossible to verify via SEM. It is proposed that the increased porosity caused by PCL blending dramatically increased the proportion of drug particles which had access to an open channel, and thus could escape from the microsphere matrix by aqueous diffusion.

6.5. Conclusions.

In order to modify the release rate of aclarubicin from PHB microspheres, Juni *et al.*¹¹⁸ incorporated a series of fatty acids and their alkyl esters into the microsphere matrices. However, these agents did not enhance the rate of drug discharge by acting as conventional polymeric plasticizers, and thus making the polymer dense regions intrinsically more permeable. Instead the steroids were believed to form discrete channels within the microsphere matrices, which enabled the aclarubicin to escape by simple diffusion⁵¹.

Another strategy which has been adopted to enhance drug release from P(HB-HV) matrices involves the incorporation of hydrophilic additives, such as polysaccharides, into the polymer phase. In these instances, when the device is immersed in water, the "filler" rapidly dissolves out of the polymer matrix, leaving behind a porous network through which the entrapped drug can escape by aqueous diffusion³⁵.

The studies which have been described in this chapter demonstrate a novel strategy for effecting the regulation of drug release from P(HB-HV) microsphere matrices. In this work, microsphere morphology [i.e. porosity] been extensively manipulated by

varying preparative variables such as polymer molecular weight, polymer HV content, and the solvent evaporation temperature. These changes in microsphere morphology have led to significant changes in the release profile of an encapsulated species [hydrocortisone].

Moreover, even more profound changes in the drug discharge rate were induced by blending the fabricating P(HB-HV) polymer with 20% poly- ϵ -caprolactone. Thus, for example, microspheres prepared from unblended 159PHB [drug content : 16.8%] released a mere 4.7% of their payload in 9 months of dissolution. In sharp contrast, microspheres prepared from a 159PHB / 20% PCL II blend [drug content : 16.1%] discharged nearly 90% of the initial drug in the first two days of dissolution.

The use of PCL blending in order to control the rate of drug release from a microsphere matrix has been previously reported^{48, 49}. However, in those studies, the PCL had its effect by facilitating the partition-dependent diffusional release of the encapsulated species. In contrast, the increased hydrocortisone discharge rates induced by PCL blending in these studies were believed to be primarily a consequence of the morphological changes caused by polymer-polymer incompatibility phenomena [as described in chapter four].

The studies which have been reported in this thesis are directed towards the attainment of two strategic goals. One of these is to assess the potential of microparticles as devices for effecting the encapsulation and controlled release of drugs : both novel biopharmaceuticals and low molecular weight steroids. The second objective is to broaden an established base of research within these laboratories, namely to investigate the suitability of P(HB-IV) polymers as materials for the fabrication of biodegradable medical devices. Consequently, the work in this thesis is directed towards the development of microparticle drug delivery devices, using P(HB-IV) polymers.

CHAPTER SEVEN.

The process evaporation process was selected as the technique for microparticle fabrication for its versatility and product characteristics. In addition, this process can be modified to generate two distinct forms of microparticles. Thus, in this work, reservoir-type microcapsules and monolithic microspheres (diagram 1.1 / figure 1.1) have been respectively fabricated by o/w single emulsion, and w/o/w double emulsion-solvent evaporation processes. The latter is a particularly suitable technique for the encapsulation of water-soluble macromolecules, because it does not require the direct exposure of such species to organic solvents. The encapsulation of a range of different molecular weight FITC-dextran, BSA and insulin is effected via use of this process in chapter five.

In contrast, the o/w single emulsion technique is highly suitable for the encapsulation of hydrophobic molecules ; for example hydrocortisone, in the work reported in chapter six.

The studies which have been reported in this thesis are directed towards the attainment of two strategic goals. One of these is to assess the potential of microparticles, as devices for effecting the entrapment and controlled release of drugs : both novel biopharmaceuticals and low molecular weight steroids. The second objective is to broaden an established line of research within these laboratories, namely to investigate the suitability of P(HB-HV) polymers as materials for the fabrication of biodegradable medical devices. Consequently, the work in this thesis is directed towards the development of microparticulate drug delivery devices, using P(HB-HV) polymers.

The solvent evaporation process was selected as the technique for microparticle preparation in these studies, because of its technical simplicity, and the opportunities it offers for controlling product characteristics. In addition, this process can be modified to generate two distinct forms of microparticle. Thus, in this work, reservoir-type microcapsules and monolithic microspheres [diagram 1.1 / figure 1.1] have been respectively fabricated by o/w single emulsion, and w/o/w double emulsion-solvent evaporation processes. The latter is a particularly suitable technique for the encapsulation of water-soluble macromolecules, because it does not require the direct exposure of such species to organic solvents. The encapsulation of a range of different molecular weight FITC-dextran, BSA and insulin is effected via use of this process in chapter five.

In contrast, the o/w single emulsion technique is highly suitable for the encapsulation of hydrophobic molecules ; for example hydrocortisone, in the work reported in chapter six.

In comparison with other poly(α,β esters), particularly PLA, only a small volume of work has been published in relation to the potential uses of P(HB-HV) polymers as drug carriers. This is primarily a consequence of the fact that the latter materials have only recently become available on a commercial basis. Indeed, P(HB-HV) polymers appear to offer advantages over poly(lactides) and poly(glycolides), for *in vivo* applications, as a result of their synthetic origin. Thus, whilst the two previously mentioned poly(alpha esters) are typically associated with the retention of catalytic residues, P(HB-HV) polymers are biologically produced ¹¹⁹.

Nevertheless, in terms of their physico-mechanical and degradation properties, P(HB-HV) polymers are not particularly well suited for use as controlled release matrices or membranes. Thus, the high crystallinity of P(HB-HV) polymers ¹³⁰ is likely to render the polymer-dense regions essentially impermeable to even the smallest, most hydrophobic, drug molecules. In addition, the greater hydrolytic stability of P(HB-HV) polymers, under physiological conditions, in relation for example to poly(lactides), suggests that degradation or erosion induced drug release is unlikely to occur within a time frame of < 2 years ¹²⁹.

In the light of these salient disadvantages, which are verified to a considerable extent in chapter six of this thesis, P(HB-HV) polymer matrices or membranes must be modified, if pharmaceutically-acceptable rates of drug discharge are to be achieved. One such method of modification involves the creation of a highly porous matrix structure ⁹, and consequently, the initial objective of this work was to develop strategies for the regulation of P(HB-HV) microparticle morphology. Chapters three and four detail the morphological investigations, which were performed in relation to this objective, using a double emulsion system.

A range of preparative variables are shown in these chapters to affect particle characteristics, such as yield, shape and - most particularly - surface morphology. With regard to the latter, a general theme which emerges from this work, is that particle porosity is diminished by temperature elevations [which do not exceed the boiling point of the dispersed phase solvent], and enhanced by polymer [poly- ϵ -caprolactone] blending. This broad theme is shown in chapter six to be equally applicable in relation to the surface morphologies of monolithic microspheres, prepared via an o/w single emulsion process.

The microporosity, induced into P(HB-HV) matrices or membranes by poly- ϵ -caprolactone blending, is shown, in chapters five and six, to be an effective mechanism for the regulation of drug release.

However, although hydrocortisone could be effectively entrapped within monolithic P(HB-HV) microspheres [see tables 6.1 and 6.2], the investigations reported in chapter five revealed that an inherent feature of the double-emulsion technique is the expulsion of most of the [active species-containing] inner aqueous phase, into the continuous phase, during microcapsule formation. Moreover, this expulsion was found, using FITC-dextran as the encapsulated species, to commence concurrently with polymer precipitation. In addition, considerable evidence was obtained to suggest that the mechanism of this expulsion was not simple diffusion, suggesting that manipulation of microcapsule drug contents would not be easily achieved. Contraction forces, induced during solvent extraction and polymer precipitation, appeared the most obvious cause of this phenomenon. However, it is possible that the use of more concentrated polymer solutions, different w/o phase ratios, and substantial elevations in the macromolecule loading might improve the drug contents, and this is an area for

further work.

Functional efficiency is, of course, only one aspect in the development of a microparticulate drug delivery system. Equal consideration must be given, for example, to toxicity. The latter can be assessed at several different levels, which include the fabricating material, the preparative process, the sterilization of the device, and the risks of dose-dumping.

With regard to the former, the polymer must be available in a highly pure form, it must be intrinsically non-toxic, and its degradation products must have no deleterious effects on the host. As was mentioned in chapter one, poly(α,β esters) are generally non-toxic, and their degradation products are typically normal mammalian metabolites. However, it was pointed out earlier in this discussion that poly(α,β esters), which are produced via organic synthesis [e.g. ring-opening polymerization reactions], may contain catalytic residues.

The solvent evaporation process is certainly not the ideal microencapsulation technique for large scale-production. In addition, this process typically utilizes toxic organic solvents [e.g. methylene chloride or chloroform], traces of which will remain in the polymer matrix or membrane. Indeed, Benoit *et al.*⁹⁵ found that PLA microspheres, prepared using an o/w [methylene chloride / aqueous PVA solution] emulsion-solvent evaporation process, typically retained 2.8 - 3.5 weight percent of the organic solvent, when dried. The tenacity with which PLA retains methylene chloride was also highlighted in the work of Cavalier *et al.*¹⁷, who found that microspheres, prepared via an o/w emulsion-solvent evaporation process, which had been dried at 22°C under vacuum for 72 hours, still contained 2.56 weight percent of the organic

solvent.

However, it is of interest to note, that Benoit *et al.*⁹⁵ were typically able to drive off all traces of methylene chloride from the microspheres by heating them under vacuum at 110°C for 22 hours. Unfortunately, because they used the racemic form of PLA, the microspheres fused together during this treatment. Nevertheless, the use of higher melting point polymers [e.g. PHB homopolymers, with a melting point of 175-177°C⁵³] for microsphere fabrication could enable solvent removal at these temperatures to be effected without causing the gross structure of the device to be lost. Clearly this procedure could only be applied in cases where the drug also had a high degree thermal stability [e.g. hydrocortisone, with a melting point of 228°C¹⁷]. In addition, for microsphere systems, where a drug is dispersed within the polymer phase, the application of heat could affect the physical distribution of the drug, and thus its release profile⁹⁵.

Microcapsules, containing heat-sensitive, macromolecular, biopharmaceuticals within their hollow centres, clearly could not be subjected to strong heat treatment. However, one strategy by which the twin problems of obtaining satisfactory encapsulation, and the removal of toxic solvent residues, could be concurrently overcome, would be to prefabricate a placebo microcapsule, subject it to strong heat under vacuum, and to subsequently fill it with drug. Unfortunately it is not obvious how this idea could be effected !

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Experiment Number	Dissolving Time (min)	Total HC Release (µg)	SD
1	15	14.5	0.5
1	30	17.8	0.6
1	45	20.8	0.8
1	60	24.1	0.7
1	75	27.0	1.0
1	90	28.2	2.5
1	105	28.4	2.6
1	120	31.0	3.4
1	135	30.0	3.4
1	150	33.2	4.8
1	165	36.4	7.0
1	180	38.4	7.9
1	195	37.8	7.8
1	210	40.7	8.4

APPENDIX

TABULATED HYDROCORTISONE DISSOLUTION DATA.

This appendix tabulates the hydrocortisone dissolution data which formed the basis of graphs 6.4 - 6.14. However, the data presented in the latter graphs represented only the mean value of duplicate dissolution experiments, and did not give any indication as to the extent to which the two readings may have differed. In addition, some of the late readings in a number of the experiments were based on readings from one experiment only.

In this appendix, readings which were based on duplicate experiments are presented as the mean value [in the column labelled "Total HC Release"], together with the standard deviation [in the column labelled "SD"]. In those instances where the reading was drawn from a single experiment only, the letters " n.a." appear in the standard deviation column.

2	15	15.0	2.4
2	30	17.5	3.0
2	45	19.0	3.4
2	60	21.4	3.3
2	75	23.0	3.5
2	90	24.0	3.5
2	105	25.0	3.5
2	120	26.0	3.5
2	135	27.0	3.5
2	150	28.0	3.5
2	165	29.0	3.5
2	180	30.0	3.5
2	195	31.0	3.5
2	210	32.0	3.5

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
43PHB / 20% HC		81	14.9	0.5
		135	17.6	0.6
		259	20.8	0.8
		569	24.1	0.7
	1	21	27.0	1.0
	1	193	28.2	2.6
	7	367	30.4	2.6
	11	374	31.0	3.4
	49	205	32.6	3.4
	87	169	33.8	2.8
	149	451	34.6	2.9
	179	451	34.6	2.9
	217	232	35.8	2.8
	259	247	36.3	n.a.
159PHB / 20% HC		228	0.4	0.0
	8	97	1.3	0.3
	17	85	2.0	0.2
	57	54	3.0	0.5
	84	81	3.2	0.1
	163	283	3.7	0.4
	231	65	4.2	n.a.
	274	75	4.7	n.a.
273PHB / 20% HC		150	1.6	0.4
		273	1.9	0.3
		574	2.4	0.5
	1	34	3.2	0.8
	2	208	3.8	1.2
	9	132	4.8	2.0
	25	317	5.5	2.4
	43	227	5.5	2.4
	70	254	5.5	2.4
	127	399	6.0	3.0
	154	202	6.6	3.6
260	247	8.4	3.1	
43PHB / 10% HC	8	111	3.9	0.1
	17	21	6.0	1.6
	101	2	10.0	4.0
	163	286	10.9	4.5
	238	68	11.7	3.6
	281	75	14.0	1.7
159PHB / 20% HC	9	146	1.7	0.6
	17	46	3.1	0.7

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
159PHB / 10% HC, [continued]	31	2	4.5	0.9
	65	42	5.3	n.a.
	101	3	5.9	n.a
	163	287	6.6	n.a
	231	70	7.5	n.a
	274	75	10.0	n.a
273PHB / 10% HC	9	166	1.7	0.1
	17	66	5.2	1.4
	101	5	6.8	2.2
	163	289	7.4	2.2
	231	72	11.1	3.1
	273	75	14.2	4.0
330PHB (10.8% HV) / 20% HC / T1		151	40.9	2.6
		274	51.1	1.5
		575	64.7	1.4
	1	35	74.6	0.8
	1	387	77.6	1.3
	2	208	81.1	1.6
	3	287	83.1	1.2
	7	341	86.0	1.6
330PHB (10.8% HV) / 20% HC / T4		163	51.1	1.1
		381	69.4	0.6
	1	48	79.0	1.9
	1	387	79.5	2.6
	2	220	81.5	4.3
	3	288	82.0	4.5
	7	342	83.1	3.1
390PHB (20.1% HV) / 20% HC		79	24.3	0.7
		252	47.4	1.4
	1	30	78.2	0.1
	1	256	79.5	0.2
	2	175	87.1	1.3
	35	112	94.5	n.a.
	62	122	94.5	n.a
	119	269	94.5	n.a
180PHB (10.8% HV) / 20% HC / T1		165	53.5	2.5
		382	64.1	1.6
	1	49	79.8	2.8
	2	222	82.1	1.7
	3	304	84.7	1.9

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
180PHB (10.8% HV) / 20% HC / T1, [continued]	7	345	90.7	2.2
180PHB (10.8% HV) / 20% HC / T4		174	7.9	0.7
		392	10.4	0.3
	1	64	16.8	0.7
	1	388	17.8	0.5
	2	236	21.1	0.6
	5	1155	29.3	0.9
	14	267	35.2	1.2
	25	310	41.0	1.2
	43	230	47.0	1.6
	70	260	51.7	1.2
	127	407	56.0	1.1
	154	203	58.3	n.a.
260	247	60.4	n.a.	
140PHB (20.1% HV) / 20% HC		175	34.1	0.5
		393	67.6	0.1
	1	65	76.5	0.7
	1	389	77.2	1.3
	2	237	78.4	0.0
	3	305	78.5	0.1
7	349	79.0	0.4	
32.8PHB (20.1% HV) / 20% HC		80	68.2	2.1
		258	77.6	2.1
	1	35	80.9	4.0
	1	252	81.6	3.2
	2	171	82.3	2.4
	21	212	83.0	n.a.
	43	102	83.0	n.a.
	79	64	83.0	n.a.
119	294	83.3	n.a.	
83.1PHB (10.8% HV) / 20% HC		93	61.1	1.0
		260	80.0	0.3
	1	34	90.2	1.0
	1	254	90.2	1.2
	2	174	90.3	1.3
	30	229	93.2	n.a.
	43	103	93.2	n.a.
	79	68	93.2	n.a.
119	296	93.2	n.a.	

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
83.1PHB (10.8% HV) / 20% HC		67	11.9	0.2
		121	16.4	0.1
		246	22.1	0.8
		559	33.0	1.4
	1	4	48.5	2.1
	1	337	51.7	0.2
	2	179	69.7	1.5
	3	274	79.8	0.6
	7	373	88.8	1.7
	10	282	90.5	1.9
	38		92.7	5.0
	45	193	92.7	5.0
	70	263	93.2	4.2
	149	464	93.6	3.8
	217	246	94.2	4.0
	260	247	94.7	4.0
43PHB / 30% HC		70	10.7	0.0
		126	13.7	0.0
		253	17.2	0.1
		366	18.5	0.4
		613	20.3	0.3
	1	81	22.3	0.5
	2	172	23.6	0.5
	3	105	24.2	0.5
	6	293	24.8	0.8
	10	207	26.1	0.6
	16	299	26.5	0.6
	27	304	26.9	0.5
	49	390	27.3	0.5
	90	333	27.5	n.a.
	169	536	28.3	n.a.
	237	318	29.5	n.a.
279	316	30.3	n.a.	
159PHB / 30% HC		70	4.3	0.2
		127	6.1	0.3
		264	9.6	0.5
		376	13.7	1.6
		612	18.5	1.3
	1	80	33.0	1.9
	1	380	36.3	1.8
	2	115	49.2	2.1
	2	405	51.8	2.1
	3	51	60.0	2.3

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
159PHB / 30% HC, [continued]	6	237	78.2	2.1
	6	440	79.1	1.2
	8	293	84.2	1.4
	10	207	87.2	1.3
	13	382	89.9	n.a.
	16	273	91.3	n.a.
	27	366	93.8	n.a.
	49	388	94.0	n.a.
	78	357	94.0	n.a.
	111	170	94.7	n.a.
	169	524	96.0	n.a.
	280	301	97.2	n.a.
273PHB / 30% HC		131	7.7	0.0
		264	12.0	0.1
		377	15.1	0.0
		623	20.7	0.0
	1	81	34.9	0.2
	1	379	38.2	0.1
	2	113	49.6	0.2
	2	367	51.7	0.4
	3	62	59.3	0.7
	6	236	75.4	2.0
	6	440	75.4	2.0
	8	290	81.7	n.a.
	10	218	84.7	n.a.
	13	286	86.7	n.a.
	16	176	86.8	n.a.
	27	262	88.3	n.a.
	49	374	88.8	n.a.
	69	264	88.9	n.a.
107	255	89.5	n.a.	
169	540	89.6	n.a.	
280	201	90.3	n.a.	
43PHB / 20% PCL II / 20% HC		95	46.2	1.0
		254	70.4	2.7
	1	32	81.4	1.1
	1	250	82	1.7
	2	177	83.5	2.9
	30	235	87.0	0.5
159PHB / 20% PCL II / 20% HC		97	54.5	1.0
		256	80.7	2.2
	1	47	86.3	2.6
	1	267	86.5	2.3

<u>Microsphere Formulation</u>	<u>Dissolution Time :</u>		<u>Total HC Release [%]</u>	<u>SD</u>
	<u>days</u>	<u>minutes</u>		
159PHB / PCL II / 20% HC	2	172	87.3	1.3
	30	236	87.5	n.a.
	50	304	87.5	n.a.
	79	70	87.5	n.a.
	119	297	87.5	n.a.
273PHB / PCL II / 20% HC		61	11.4	0.4
		119	14.6	1.1
		525	26.0	0.1
	1	9	34.6	1.3
	1	306	36.7	0.5
	2	1404	46.5	0.9
	6	164	54.7	0.7
	8	223	59.4	0.7
	10	118	63.3	0.6
	16	208	70.1	0.7
	23	131	72.7	0.0
	37	160	77.8	0.0
	49	291	80.6	0.3
	69	180	83.7	0.0
	90	220	85.2	0.3
	169	425	88.2	n.a.
	237	203	89.4	n.a.
280	201	90.9	n.a.	