BIODEGRADABLE POLYMERS

AS DRUG DELIVERY SYSTEMS

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SUMMARY

Poly〔1,2-bis(p-carboxyphenoxy) propane:sebacic acid〕(P(CPP:SA)) 20:80 was synthesised by melt polycondensation. *In vitro* the degradation of P(CPP-SA) 20:80 in 0.1M phosphate buffer at 37℃ was followed by ^{1}H-NMR, IR, weight change, pH change, and molecular weight changes. Surface eroding P(CPP-SA) and bulk eroding PLGA 75:25, 50:50 microspheres containing 10% w/w theoretical loading bovine serum albumin (BSA) were prepared by solvent evaporation/ double emulsion, spray drying and solvent extraction/ oil-in-oil techniques. This thesis describes the characterization of the microspheres obtained in terms of morphology, particle size, drug content, and *in vitro* drug release behaviour. A comparison was made between these two kinds of polymers from these aspects. The microspheres containing protein were generally spherical, with diameters around 10-20 μm for double solvent evaporation/ emulsion method; with diameters around 50 μm for the solvent removal method/ oil-in-oil method; and 1-5 μm for spray drying method. *In vitro* release of BSA into 0.1M phosphate buffer at 37℃ from microspheres of three polymers prepared by three different techniques showed that the microspheres prepared by oil-in-oil had an initial 'burst' release; The P(CPP-SA) 20:80 microspheres prepared by double emulsion method had a high rate of BSA release, and the initial BSA release followed zero order kinetics; PLGA microspheres prepared by this method had lower BSA release. BSA released from microspheres prepared by spray drying had linear rates of release and no obvious difference in release was observed between P(CPP-SA) and PLGA microspheres.

Key words: Controlled release; drug delivery; bulk erosion; surface erosion; double emulsion; solvent removal; spray drying.

DEDICATION

I would like to dedicate this thesis to my family and all my friends.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. B. R. Conway and Dr. Y. F. Wang for their expert guidance over one last year.

Thanks for technical assistance go to Mr. Mike Davis for his patient, kind advice and help.

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1. INTRODUCTION:

1.1 Controlled Drug Delivery

An important subset of historical development in pharmaceutics is the area of controlled drug release systems. Drug delivery, which takes into consideration agents such as the carriers as well as the route and the target, has evolved into a strategy using processes or devices designed to enhance the efficacy of therapeutic agents through controlled release. This may involve enhanced bioavailability, improved therapeutic index, and/or improved patient acceptance or compliance.

1.1.1 Controlled Delivery Systems for Peptides and Proteins

Compared to conventional drug compounds, peptides and proteins have unique requirements and restrictions. These properties include molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, sometimes an unusual dose-response curve, immunogenicity, biocompatibility issues, as well as the tendency of a peptide to undergo aggregation, adsorption, and denaturation (Banejee, 1990). Most of these drugs require multiple injections *via* a parenteral route. In general, pharmaceutical proteins and peptides are administered parenterally, because of their poor bioavailability. Two main reasons for this poor bioavailability can be discerned:

1. Protein degradation in gastrointestinal (GI) tract,
2. Poor permeability in the case of a passive transport process.

Regarding point 1: the human body has developed a very efficient system to break

down proteins. Regarding point 2: high molecular weight molecules are poor penetrators of the intact and mature epithelial barrier, if diffusion is the sole driving force for mass transfer. Proteins are no exception to this rule (Crommelin, 1996).

The demand for effective delivery systems for proteins and peptides has brought a tremendous thrust in recent years in both the scope and complexity of drug delivery technology. Some delivery systems have been designed to control the release rate of the proteins and peptides for a long time period, to target to site of action or protect the drug from the harsh environment. Biodegradable polymers are an important approach.

1.1.1.1 Biodegradable polymers

The trend in drug delivery technology has been towards biodegradable polymer excipients requiring no follow-up surgical removal once the drug supply is depleted (Lewis, 1990). Linear polyesters are by far the most widely characterised and utilised group of biodegradable polymers, the most significant among them being copolymers of lactic and glycolic acids (PLGA). Polyanhydrides are one class of these biodegradable polymers. Poly[bis (p-carboxyphenoxy) propane-co-sebacic acid anhydride] P(CPP:SA) has been approved by the United States Food and Drug Administration (FDA) for delivery of drugs to treat brain cancer.

Controlled release can also be achieved with biodegradable hydrogel systems. The hydrogel degradation mechanism may involve the degradation of polymer backbone, cross-linking agent, or pendent chains (Ende, *et al.*, 1997).

1.1.1.2 Microspheres

Biodegradable polymers can be used in many different forms, depending on the application, with microspheres or microcapsules being an important form.

These are free-flowing spherical microparticles, ranging in size from a few micrometers to about 200 μm. The term microcapsule usually refers to a reservoir-type system in which the active molecules are enclosed in the cavity surrounded by the polymeric membrane (Figure 1.1a), whereas the term microsphere usually implies a monolithic system in which the active agent is uniformly distributed through the polymeric matrix. Particles that are smaller than 1 μm are usually termed nanoparticles, but in general, the polymers as well as the processing techniques used for nanoparticles differ considerably from the ones used for microparticles (Bhagat *et al.*, 1996).

Microspheres can be defined as micromatrices in which the drug is uniformally dispersed and / or dissolved in the polymeric network either as fine particles / agglomerates or in a molecular state (Figure 1.1b) (Giunchedi & Conte, 1995). Also in this case, one or more polymers can make up the matrix structure. However, the term "microparticle" should be used because very often it is actually difficult to distinguish between microcapsules and microspheres. The term microcapsule should be reserved for reservoir type devices,

whereas microspheres are monolithic or matrix-type microparticles (Kissel *et al.*, 1996). When the microparticles contain several drug crystals, it is a matter of semantics to decide if they are multinuclear microcapsules (Figure 1.1c) or hetergeneous microspheres (Figure 1.1d) (Aftabroushad & Doelker, 1994).

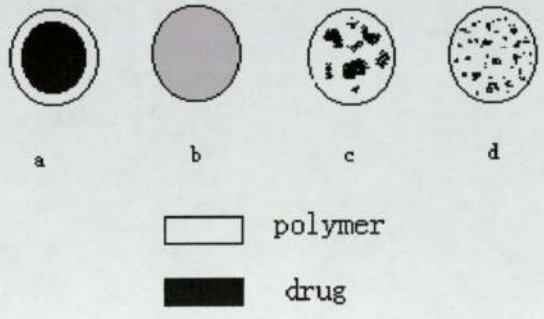


Figure1.1 Microparticles: a. microcapsule; b. microsphere; c. multinuclear microcapsule; d. heterogeneous microsphere (Giunchedi & Conte, 1995).

Microencapsulation is a method of wrapping small entities in individual coatings designed to protect, separate, or aid in storage. The reasons for microencapsulation are:

(a) Sustained release is possible; the coating acts as a barrier to drug release. Various mechanisms of release are possible,

(b) Taste masking (*e.g.* for chloroquine, an anti-malarial drug),

(c) Environmental protection, protection of drug contents from moisture and /or oxygen,

(d) Gastric irritation reduction,

(e) Liquid-solid conversion

(f) To allow the combination of incompatible constituents by the protection of one or

more components by microencapsulation.

(g) Minimising or eliminating side effects (Kas *et al.*, 2000).

Given the wide range of sizes possible, such biodegradable microspheres can be administered intraveneously, intra-arterially, subcutaneously and intra-muscularly (as well as by the oral route where limited uptake is possible for specific applications) (Whateley, 1993).

Microspheres intended for drug delivery can be prepared from a variety of different materials and are of different physical characteristics depending on the application. The choice of material will be directed by the drug, the intended destination, disease condition to be treated and duration of action, *etc* (Davis *et al.*, 1989).

1.1.1.3 Protein Release from Microspheres

The incorporation and release of low molecular weight, water-soluble species from microcapsules and microspheres is reasonably well documented, but unlike low molecular weight drugs, proteins as biopolymers have very large globular structures (typically 2-8 nm or even larger) and possess complex internal architecture that defines their unique biological functions (Schwendeman *et al.*, 1996). When attempts are made to incorporate macromolecules such as bioactive proteins into polymeric systems, there are many difficulties encountered. Injectable PLGA microspheres, which can continuously release small polypeptide analogues of leuteinising hormone releasing hormone (LH-RH) for 1 or 3 months are commercially available for treating prostate cancer and endometriosis. However, the successful development of PLGA

microspheres for the controlled release of small polypeptides cannot be readily extended to protein compounds. Difficulties associated with the encapsulation of proteins are typically related to their high molecular weight and water solubility, and chemical and physical instabilities upon exposure to various microencapsulation process conditions (Mehta *et al.*, 1994).

One approach to achieving oral delivery of complex molecules, including genes, has been the development of polyanhydride microspheres, which display strong adhesive interactions with the intestinal mucosa and cell lining (Langer, 1990). The interaction of P(CPP-SA) (poly(1,3-bis(p-carboxyphenoxy)propane-sebacic acid)) 20:80 microspheres with mucin gels has been characterised by quantification of the mucoadhesive forces between polymer and gel (Tamada & Langer, 1992). Low molecular mass drugs such as dicumarol, as well as larger molecules such as insulin and even genes, can be taken up in animals using this type of approach, presumably because the microspheres maintain contact with the intestinal epithelium for longer times. Microscopic evidence has shown that the microspheres can actually penetrate the epithelium, through and between cells (Mathiowitz *et al.*, 1997).

1.2 Polyanhydrides as Drug Delivery Systems

1.2.1 Historical Development and Presently Used Polyanhydrides

Polyanhydrides were first synthesised in 1909, by Bucher and Slade, and were made of aromatic monomers. In 1930, the first aliphatic polyanhydrides were synthesized as prospective raw materials for the manufacture of textile fibers. As polyanhydrides are not hydrolysis-resistant enough to serve as long-lasting materials, in the early 1980's,

they were 'rediscovered' in the research for fast-degrading polymers that could be used for erosion-controlled drug delivery (Brunner *et al.*, 1996). Langer and co-workers began to explore polyanhydrides as early as 1983, seeking to exploit the inherent instability for drug delivery applications (Roskos *et al.*, 1997). Today, polyanhydrides can be regarded as 'designer polymer' for many reasons:

- Biocompatibility in combination with excellent drug-release control
- Synthesized from a large pool of monomers
- Manufactured with various degrees of crystallinity
- Allow control of degradation rates and water uptake
- Manufactured with a branched structure, or may be cross-linked

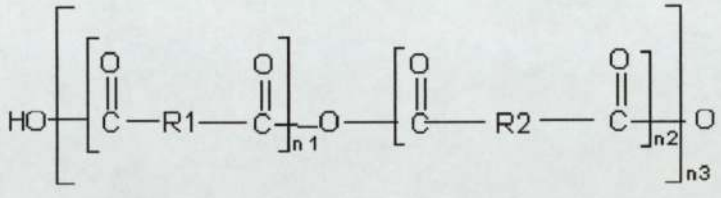
Compared with the relatively short period during which they have been synthesized as drug carriers, polyanhydrides have been very successful. At present, P (CPP-SA) polymers are used as carriers in the treatment of brain cancer in humans after promising clinical trials in the form of Gliadel® wafers. The P (FAD-SA) (poly(erucic acid dimmer-co-sebacic acid)) polymers have been evaluated for the same type of therapy, with microspheres because they allow stereotactic injection (Brunner *et al*, 1996). The biocompatibility and safety of polyanhydrides were established following the 1986 guidelines by the Food and Drug Administration (FDA) for testing and evaluating new biomaterials. Several accepted criteria and tests to evaluate new biomedical materials were used to access the safety of polyanhydride (Leong *et al.*, 1985a; Laurencin *et al.*, 1993).

1.2.2 Polyanhydride composition

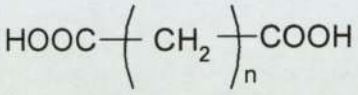
1.2.2.1 Monomers

The general formula of polyanhydrides is shown in Figure 1.2. They are bifunctional carboxylic acids, which differ in the chemical groups R1 and R2, separating the carboxylic acid ends. Polyanhydrides can be synthesised as homopolymers (R1=R2), or as copolymers (R1≠R2). Some of the numerous monomers that have been used for the manufacture of polyanhydrides are shown in Figure1.3.

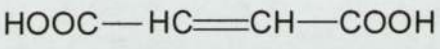
Not all polyanhydrides made from the monomers shown in Figure 1.3 are ideal materials for the manufacture of drug delivery systems. For example, P (SA), is highly crystalline, has poor mechanical properties, and erodes too rapidly, whereas P (CPP) erodes too slowly. Through the synthesis of copolymers, these properties can be tremendously improved (Brunner *et al.,* 1996). By the appropriate choice of monomers, the polyanhydride matrix can degrade over periods ranging from one day to several months or any time in between (Leong *et al.*, 1985b).



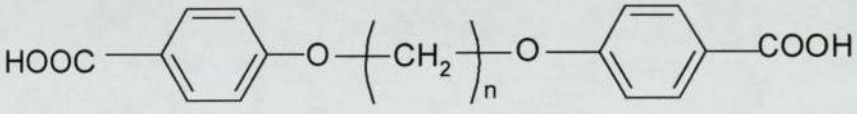
Figture1. 2 General formula of polyanhydrides



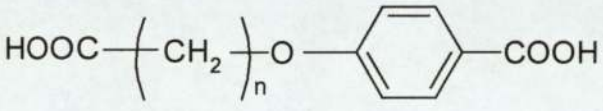
n=4 adipic acid (AA)
n=8 sebacic acid (SA)
n=10 dodecanoic acid (DA)



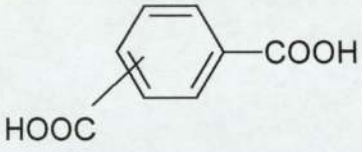
fumaric acid (FA)



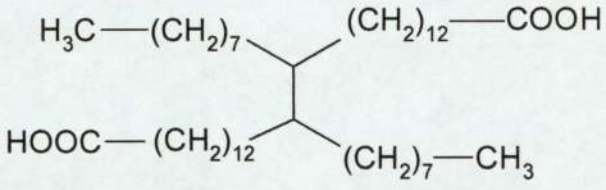
n=1 bis (p-carboxyphenoxy)methane (CPM)
n=3 1,2-bis(p-carboxyphenoxy)propane (CPP)
n=6 1,3-bis (p-carboxyphenoxy)hexane (CPH)



n=1 p-carboxyphenoxy acetic acid (CPA)
n=4 p-carboxyphenoxy valeric acid (CPV)
n=8 p-carboxyphenoxy octanotic acid (CPO)



meta: isophthalic acid (IPA)
para: terephthalic acid (TA)



erucic acid dimer (FAD)

Figure1.3 Monomers used for the synthesis of polyanhydrides

1.2.2.2 Aliphatic Polyanhydrides

Aliphatic polyanhydrides were first prepared in 1932 by Hilland and Carothers. Aliphatic polyanhydrides derived from fatty acids have been used as carriers for

controlling drug delivery (Domb & Maniar, 1993). The common aliphatic diacids in medically used polyanhydrides are sebacic (SA) and the dimer erucic acid (FAD), but P (FAD) is a liquid and not well suited for the manufacture of solid drug delivery systems. However a copolymer that has been proved to be useful for drug delivery purposes is P (FAD: SA). The relative degree of copolymer crystallinity decreased with the increased amount of FAD (Mathiowitz *et al.,* 1990a). The degree of crystallinity may play an important role in preventing water diffusion into the polymer matrix, thus preventing bulk erosion (Tabata *et al.,* 1993). The copolymer degrades and are eliminated from the body within weeks (Domb & Nudelman, 1995).

1.2.2.3 Aromatic polyanhydrides

Aromatic polyanhydrides were first synthesised in 1909 by Bucher and Slade (Domb *et al.,* 1987). Aromatic polyanhydrides generally degrade and erode more slowly than aliphatic compounds (Leong *et al.*, 1985a). Some aromatic polyanhydrides, for example pure P(CPP), at pH 7.4, degrades in about 3 years (Chasin *et al.,* 1990). To give a more constant release, but still offer reasonable erosion rates, a class of polyanhydrides was prepared from aliphatic-aromatic homopolyanhydrides of the structure $-(OOC\text{-}C_6H_4\text{-}O\ (CH_2)_X\text{-}CO\text{-})_n$, where X varies from 1 to 10 (Chasin *et al.,* 1990). With such aliphatic-aromatic homopolymers, there can no longer exist regions of the polymer enriched in aromatic or aliphatic groups. These polymers should give linear release profiles, without rapid initial depletion (Tamada & Langer, 1992).

1.2.2.4 Cross-linked and Branched Polyanhydrides

Branched polyanhydrides possess branches of linked monomer molecules protruding

from various central branch points along the main polymer chain in a random pattern. Crosslinked polyanhydrides are in the form of a three dimensional network and have been developed for high mechanical strength and slow degradation (Kumar *et al.*, 2002).

Unsaturated polyanhydrides of the structure $[-(OOC-CH=CH-CO)_x-(OOC-R-CO)_y-]_n$ have the advantage of being able to undergo secondary polymerisation of the double bonds to create a crosslinked matrix (Chasin *et al.,* 1990). A monomer that has been used for that purpose is FA in combination with SA (Maniar *et al.*, 1990). A series of unsaturated polyanhydrides were prepared by melt or solution polymerisation of fumaric acid (FA), acetylenedicarboxylic acid (ACDA), and 4,4'-stilbendicarboxylic acid (STDA) (Domb *et al.*, 1991). These polymers were prepared from the corresponding diacids polymerised either by melt polycondensation or by polymerisation in solution. Molecular weights of up to 44,000 were achieved for polyanhydrides of p-carboxyphenoxyalkanoic acid and fumaric acid (Domb &Langer, 1988).

The unsaturated homopolymers were crystalline and insoluble in common organic solvents whereas copolymers with aliphatic diacids were less crystalline and were soluble in chlorinated hydrocarbons, and at same time the mechanical stability of polyanhydrides was increased (Domb *et al.*, 1991).

1.2.3 Polyanhydride synthesis

1.2.3.1 High molecular weight polyanhydrides

Several approaches have been examined for synthesising polyanhydrides, including

melt condensation of activated diacids (Domb *et al.*, 1987), ring-opening polymerisation, dehydrochlorination, and dehydrative coupling agents (Leong *et al.*, 1985b; Domb *et al.,* 1988). One major drawback of polyanhydride synthesis is that solution polymerisation generally results in low molecular weight polymers, which made them impractical for many applications. The highest molecular weight polymers are obtained using melt polymerisation techniques, by operating under conditions which optimise the polymerisation process while at the same time minimising the depolymerisation process (Chasin *et al.,* 1990). To manufacture copolymers, all individual monomers are activated separately. The dicarboxylic acid monomers are reacted with excess acetic anhydride to form acetyl terminated anhydride prepolymers (Domb *et al.,* 1997), then reacting individually pure prepared prepolymers under vacuum to produce copolymer by heating. During the polymerisation of the oligomers, acetic anhydride is formed as a side product, and removed by distillation and vacuum during the reaction. Factors, other than the purity of the starting materials, which were found to be critical to achieve high molecular weight polyanhydrides, were the reaction temperature and duration, and the rapid removal of the acetic acid byproduct by maintaining an appropriate vacuum during the polymerisation reaction (Chasin *et al.,* 1990). In previous studies, a molecular weight of 12,030 weight average (Mw) and 5,280 number average (Mn) was reported for a P(CPP:SA) 20:80 coplymer prepared by the melt-polycondensation method at 130°C, as determined by gel permeation chromatography (GPC) analysis (Domb & Langer, 1987). By reacting pure individually prepared prepolymers to produce P(CPP:SA) in a 20:80 molar ratio,

a molecular weight (Mw) of 116,800 was achieved. This is a sharp contrast to the molecular weight of 12,030 obtained when an unisolated and unpurified prepolymer mixture was used. Since the polymerisation and the depolymerisation reactions involve anhydride interchange, which leads to a high molecular weight polymer with the removal of acetic anhydride as the condensation product (polymerisation) and internal ring formation (depolymerisation), catalysts affect both reactions. Optimising the reaction time in the presence of catalysts is therefore critical to achieving high molecular weight polymers (Chasin *et al.,* 1990).

1.2.3.2 Function of Catalysts

Since the polymerisation reaction is an anhydride interchange, which involves nucleophilic attack on a carbonyl carbon, a catalyst, which will increase the electron deficiency of the carbonyl carton, will facilitate the polymerisation. Many effective coordination catalysts have been suggested for the transesterification polymerisation of polyesters, which is a reaction similar to the anhydride interchange. Also, similar catalysts have been found to be effective in ring-opening polymerisation of epoxides due to metal oxygen complexation (Domb & Langer, 1987). Over 20 coordination catalysts were examined in the synthesis of P (CPP:SA 20:80) copolymer. Significantly higher molecular weights in shorter times were achieved by utilising cadmium acetate, earth metal oxides, and $ZnEt_2$-H_2O. The molecular weights ranged from 140,935 to 245,010 with catalysts, in comparison to 116,800 without catalysts (Chasin *et al.,* 1990).

1.2.4 The Composition of Copolymers of Polyanhydrides

In a copolymer with monomers A and B, there are three possible types of bonds, AA, BB, and AB. The relative number of these bonds can be determined experimentally by using NMR (Tamada & Langer, 1992) and compared with predictions based on the random distribution of monomers (Ron *et al.*, 1991). If there is reasonable agreement between predicted and experimental values, one can conclude that copolymer is random. For copolymers made of SA, in combination with CPP or 1,3-bis(p-carboxyphenoxy)hexane (CPH), it was found that the monomers were mainly randomly distributed when the content of both monomers was equal (Ron *et al.*, 1991). The sequence distribution of monomers in the copolymer can help in understanding several effects. Firstly, the segment length affects how crystalline the polymer is likely to be. The long block lengths of a particular monomer that are expected for a high fraction of the monomer in the copolymer suggest a more crystalline structure at the extremes of composition, a hypothesis that was verified experimentally. Secondly, the sequence distribution can help explain erosion behavior. If the different types of bonds have different reactivities, then the appearance of monomers relative to each other would be affected (Tamada & Langer, 1992) and the extent of randomness in the distribution is important with respect to erosion. A block-like arrangement of the monomers inside the polymer chain might lead to the discontinuous erosion of the material when the two blocks exhibit different resistance against degradation and erosion.

1.2.5 Characterisation of Polyanhydrides

To understand the characteristics of polyanhydride microspheres, it is necessary to have a detailed knowledge of the properties of the polymers. Polyanhydrides have been investigated thoroughly during the last 20 years, which makes much physicochemical data available. Homopolyanhydrides of aromatic and aliphatic diacids were found to be crystalline (>50% crystallinity) when examed by X-ray diffraction (Uhrich *et al.*, 1995). The copolymers possessed a high degree of crystallinity at high mole ratios of either aliphatic or aromatic diacids. The heat of fusion values for the polymers demonstrated a sharp decrease as CPP was added to SA or *vice versa*. The trend of decreasing crystallinity, as one monomer is added, was detected using X-ray diffraction or differential scanning calorimetry (DSC). Copolymers with high ratios of SA and CPP, or CPH (1,3-bis(p- carboxyphenoxy) hexane) were crystalline while copolymers of equal ratios of SA and CPP or CPH were amorphous. The poly(FA-SA) series displayed high crystallinity regardless of comonomer ratio (Domb *et al.*, 1997). Important parameters for processing polyanhydrides to dosage forms are the glass transition temperature (Tg) and the melting point (Tm) (Tamada & Langer, 1992). Tg, the glass to rubber transition temperature, indicates a transition from a rigid to a flexible structure causing a change in heat capacity and hence a shift in the baseline of DSC. It influences polymer mechanical properties, polymer forming and processing characteristics, permeability and drug diffusion. Below the Tg, the polymer loses its flexible working behaviour, polymer molecules behave as rigid units. At the Tg, molecular vibrations / oscillations are large enough to overcome intermolecular forces

and the polymer may be deformed comparatively easily. Above the Tg, the thermal energy of molecules is so large that polymer exhibits rubbery behaviour, and eventually the polymer is converted to a liquid if the temperature is raised further.

For all homopolymers made of SA, FA, CPP, and CPH and all copolymers made of SA in combination with FA, CPP, and CPH, Tg values ranged from 2°C to 60°C. P (CPP) is the only exception with a Tg of 90°C. The lowest values of Tg were obtained for the copolymers with equal molar composition (Mathiowitz *et al.*, 1990b).

The Tm depends on the degree of crystallinity and is the crystalline melting temperature. Melting points have been found to be as high as 246°C for P (FA), 240°C for P(CPP), and 143°C for P(CPH) (Mathiowitz *et al.*, 1990b). The melting point drops substantially after copolymerisation.

Anhydrides present characteristic peaks in infrared spectroscopy (IR). In general, aliphatic polymers absorb at 1740 and 1810 cm^{-1} and aromatic polymers at 1720 and 1780 cm^{-1}. A typical IR spectrum of aliphatic and aromatic polymers that contain aliphatic and aromatic hydride bonds may present 3 distinct peaks, where the aliphatic peak is shown at 1810 cm^{-1}, the aromatic peak is shown at 1780 cm^{-1} and the peaks at 1720-1740 cm^{-1} in general overlap (Domb *et al.*, 1997).

1.3 PLGA in Drug Delivery

1.3.1 Historical Use and Development of PLGA

The first synthetic polymers designed specially for use in the body as resorbable materials were the polyglycolides, which were used to make Dexon® sutures in 1970. In parallel, research on aliphatic polyesters derived from lactic acid was initiated and led to the first lactic/glycolic copolymer (PLGA) exploited as the Vicryl® suture. Research on PLGA polymers, copolymers and stereocopolymers has been extensive, resulting in many preparations, formulations, and characterisation techniques for both implantable and injectable controlled delivery systems. By the late 1980s, the number of patents for lactide- and glycolide-based implants and other devices had escalated significantly (Brannon-Peppas *et al.,* 2000). Early efforts were directed towards the homopolymer of lactic acid rather than the copolymers. This was primarily due to the limited availability of the glycolide co-monomer. Recently, the full range of monomers and polymers has become more accessible through major chemical companies. This availability of materials has greatly broadened the scope of possibilities for designing drug delivery systems. Lactide/glycolide copolymers have had such success in drug delivery formulations because their degradation can range from 3 weeks to over a year, depending on the composition of the copolymer as well as the method of preparation and formulation. The fastest degradation is seen for copolymer with a 50:50 ratio of lactide to glycolide and with low molecular weight. The ability of PLGA polymers to dissolve in a variety of organic solvents in addition to being extruded into a number of shapes has been instrumental in exploring their use

from biodegradable sutures into implants, microparticles, and nanoparticles, and fibers for an ever-increasing number of controlled release formulations and devices (Brannon-Peppas *et al.,* 2000).

1.3.2 Significance of PLGA as a biodegradable polymer

Polyglycolide is a crystalline, biodegradable polymer having a melting point (Tm) of ~225°C and a glass transition temperature (Tg) of ~35°C. The heat fusion of 100% crystalline polyglycolide is 45.7cal/gram. Relative to other biodegradable polymers polyglycolide is a highly crystalline polymer, with crystallinity typically reported in the range of 35-75% (Perrin *et al.,* 1997). Polyglycolide biodegrades by hydrolysis of the readily accessible and hydrolytically unstable aliphatic ester linkage. The degradation time is just a few weeks depending on the molecular weight, degree of crystallinity, crystal morphology, physical geometry of the specimen, and the physico-chemical environment (Perrin *et al.,* 1997).

Lactic acid is optically active and can be produced as poly(L-lactide), poly(D-lactide), and the racemic poly(D,L-lactide). Polylactides are soluble in common organic solvents. Although structurally very similar to polyglycolide, the polylactides are quite different in chemical, physical and mechanical properties because of the presence of a pendant methyl group on the alpha carbon (see Figure 1.4). L-polylactide is a crystalline, biodegradable polymer having a melting point of approximately 175°C and a glass transition temperature of approximately 65°C. L-polylactide is generally less crystalline than polyglycolide, with crystallinity

reported in the range of 35% (Perrin *et al.,* 1997). D,L-polylactide is a completely amorphous polymer having a Tg of ~57°C. The methyl group in polylactide causes the carbonyl of the ester linkage to be sterically less accessible to hydrolytic attack; and depending on the type of polylactide, its molecular weight, degree of crystallinity, the physical geometry of the specimen, and the physico-chemical environment the polylactides are typically more hydrolytically stable than polyglycolide. However, the lack of crystallinity in D,L-polylactide causes this polymer to degrade faster (Perrin *et al.,* 1997).

An advantage of the lactide/glycolide copolymers is the well-documented versatility in polymer properties (*via* manipulation of comonomer ratio and polymer molecular weight) and corresponding performance characteristics (predictable *in vivo* degradation rates). The 50:50 D, L-lactide/glycolide copolymer is the vehicle of choice for many drug delivery systems designed for a 30-day duration of action. Another distinct advantage of lactide/glycolide materials for use in drug delivery is their relative flexibility of fabrication. The lactide/glycolide polymers are generally low-melting thermoplastics with good solubility in common solvents, polyglycolic acid and glycolide-rich copolymer being the exceptions. These favourable characteristics have allowed investigators considerable flexibility in the fabrication of drug delivery formulations. At the present, the overall degradation kinetics are fairly well established for the entire family of homopolymers and copolymers (see Table 1.1).

Polymer		Approximate time for biodegradation (months)
Poly (L-lactide)		18-24
Poly (D, L-lactide)		12~16
Poly (glycoside)		2~4
50:50 Poly	(D, L-lactide-co-glycolide)	2
85:15 Poly	(D, L-lactide-co-glycolide)	5
90:10 Poly	(D, L-lactide-co-glycolide)	2

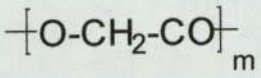
Table 1.1.Degradation times for PLA, PGA and PLGA polymers (Adapted from Lewis, 1990).

PLGA systems degrade *in vivo* to lactic ($C_3H_6O_3$) and glycolic ($C_2H_4O_3$) acids, which are subsequently eliminated as CO_2 and H_2O *via* the Krebs cycles (Reed & Gilding, 1981). A potential problem encountered with the use of high molecular weight lactide/glycolide copolymers as drug delivery matrices is the presence of residual catalyst used in the polymerisation procedure. Further, the processing and fabrication of protein delivery systems often requires the use of solvents and high temperature (Asano *et al.,* 1991). The drug release profile from these systems is frequently reported to be multiphasic due to bulk erosion processes within one polyester.

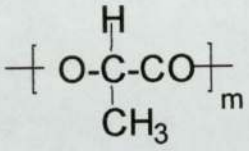
1.3.3 PLGA composition

The PLGA aliphatic polyester family includes an almost infinite number of compounds depending on the gross composition, the distribution of chiral and achiral repeating units, and molecular weight (Figure 1.4).

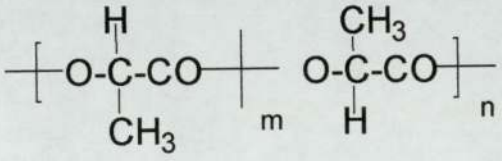
Polyglycolide



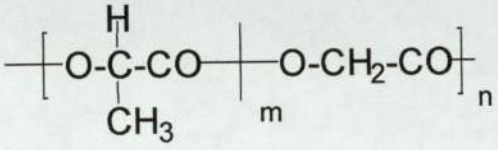
Poly(L-lactide)



Poly(D,L-lactide)



Poly(L-lactide-co-glycolide)



Poly(D,L-lactide-co-glycolide)

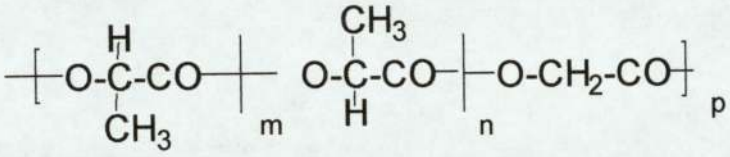


Figure 1.4 Structures of Polylactide, Polyglycolide, and Poly(lactide-co-glycoside).

It is important to note that there is not a linear relationship between the copolymer composition and the mechanical and degradation properties of the materials. For example, a copolymer of 50% glycolide and 50% D, L-lactide degrades faster than either homopolymer (Miller *et al.,* 1977).

Glycolide is much more reactive than D, L-lactide. This leads to copolymers with block sequences along the polymer chain. Resembling the insolubility of the pure polyglycolide in all common organic solvents, block structures of glycolide are only sparingly soluble. However, because these compounds are nearly exclusively used in

the production of drug-loaded microparticles in the pharmaceutical industry, solubility in methylene chloride or acetone is a key factor. The degree of blocking is therefore the most important parameter to be controlled during polymerisation (Bendix, 1998).

1.3.4 PLGA Synthesis

The homo- and copolymers of lactic and glycolic acids are synthesised by the ring-opening melt condensation of the cyclic dimers, lactide and glycolide. Only low-molecular-weight polymers can be derived by the direct condensation of the corresponding alpha-hydroxy acids.

The reaction has been performed as melt or bulk polymerisation, in solution or emulsion (Bendix, 1998). The polymerisations are usually conducted over a period of 2-6 hours at about 175°C. Organotin catalysts are normally utilised with stannous chloride and stannous octoate being the most common. As with most polymerisations, monomer purity is highly critical in the synthesis of PLGA. Differential scanning calorimetry (DSC) purity of 99.9% or greater is usually required with the starting lactide and glycolide materials. Low monomer acidity is also a critical parameter. Free acid of 0.05% or less is normally required for achieving a high molecular weight polymer. Of equal importance, however, are the environmental conditions, particularly humidity levels, in the processing areas. Most failed glycolide polymerisations can be traced to high levels of humidity or higher monomer acidity (Lewis, 1990).

1.3.5 The Characteristics of PLGA

A broad spectrum of performance characteristics of PLGA can be obtained by careful manipulation of four key variables: monomer stereochemistry, comonomer ratios, polymer chain linearity, and polymer molecular weight. Because the mechanism of biodegradation is simple hydrolysis of the ester linkages, it is apparent how each of these factors plays an important role in *in vivo* performance of the lactide/glycolide materials. Crystallinity and water uptake are key factors in determining the rates of *in vivo* degradation. The copolymers of lactide and glycolide are less crystalline than the two homopolymers of the two monomers. In addition, the lactic acid polymer, because of the methyl group, is more hydrophobic than the glycolide polymer (Lewis, 1990). It was demonstrated that water uptake increases as the glycolide ratio in the copolymer increases. The extent of block or random structure in the copolymer can also affect the rate of hydration and the rate of degradation (Dunn *et al.,* 1988).

Solubility of the polymers in common organic solvents is an important factor in regard to fabrication of drug delivery systems. The homopolymers from D,L-, D-, and L-lactide are quite soluble in halogenated hydrocarbons, ethyl acetate, tetra hydrofuran (THF), dioxane, and a few other solvents. At glycolide contents of less than 50%, lactide/glycolide copolymers display characteristics similar to those of the lactide homopolymers. Polyglycolide acid and the glycolide-rich copolymers are quite insoluble materials (Lewis, 1990).

1.4 Polymer Degradation and Erosion

1.4.1 Definition of Degradation and Erosion

Polymer degradation is the collective name given to various processes and is defined as deleterious change in the properties of a polymer due to a change in the chemical structure. Biodegradation will be referred to when it is emphasised that a biological agent (*e.g.*, enzyme or microbe) is a dominant component in the degradation process. Bioabsorption and bioresorption are often used interchangeably and imply that the polymer or its degradation products are removed by cellular activity (*e.g.*, phagocytosis) in a biological environment (Roskos *et al.*, 1997). Generally, polymer degradation is a harmful process, which is to be avoided or prevented, but sometimes, polymer degradation may be useful, for example when polymers are used as drugs carriers. Degradation may happen during every phase of a polymer's life, *i.e.*, during its synthesis, processing and use (Kelen, 1983). Bioerosion is defined as changes in polymer or matrix structure that occur under physiological conditions as a consequence of chemical reaction, dissolution of a water-soluble polymer, dissolution of a water-insoluble lipid, or dissolution of a polymer promoted by ionisation or protonation of functional groups. Bioerosion therefore includes both physical processes (*i.e.*, dissolution) and chemical processes (*i.e.*, polymer backbone cleavage) (Heller, 1987). Biodegradation is the chemical breakdown of materials by the action of living organisms, which leads to changes in physical properties. Within the last decade, a variety of synthetic polymers have been reported to be degradable in mammalian organisms and, for some of them, to be resorbable, *i.e.*, eliminated from

the body either by kidney filtration or by metabolism. Those polymeric materials, which undergo chemical degradation in body fluids, either because of simple reactions or of enzymatic activity, are now designated as biodegradable, the prefix "bio" reflecting that degradation occurs in a living environment which always affects the degradation mechanism in one way or another (Vert *et al.,* 1990).

1.4.2 Surface Erosion and Bulk Erosion

Degradation can be based on enzymatic or hydrolytic breakdown of the polymer. Generally the hydrolytic degradation mechanism is considered to be the more desirable option, because there is less variation in breakdown among different implantation sites and different patients. Erosion due to polymer degradation has been classified into heterogeneous or surface erosion and homogeneous or bulk erosion (Park *et al.,* 1995). To maximise control over the release process, it has generally been considered desirable for a polymer system to undergo 'surface erosion' kinetics, *i.e.,* for the polymer to erode like a bar of soap from the outside to the inside and to exclude water penetration into the bulk of the matrix. However, most currently available biodegradable systems undergo 'bulk erosion'; water penetrates into the polymeric matrix and degrades it internally as well as externally (Tamada & Langer, 1992).

Polyanhydrides are capable of undergoing a hydrolysis process primarily confined to the surface of devices. It has been indicated that the hydrolysis of anhydride linkages is inhibited by the presence of acids; bulk erosion of these materials is therefore

suppressed by the acidity of the carboxylic products produced upon hydrolysis, and the erosion process occurs preferentially towards the outer polymeric surface (Roskos, 1996).

Achieving such a heterogeneous degradation requires that the rate of hydrolytic degradation at the surface of the polymeric system be much faster than the rate of water penetration into the bulk of the matrix. Such a feature may also aid in the delivery of water-labile drugs by making it more difficult for water to interact with these substances until they are released. In designing a biodegradable system that would erode in a controlled heterogeneous manner without requiring any additives, polyanhydrides were proposed as promising candidates, due to the high liability of the anhydride linkage (Chasin *et al.*, 1990).

Degradation of PLGA occurs by a random, nonenzymatic hydrolytic cleavage of ester linkages, usually referred to as a bulk erosion mechanism. When the molecular weight of PLGA reaches the threshold level of water solubility of the oligomeric breakdown products, a rapid mass loss is observed. Rate of hydrolysis depends mainly on co-monomer ratio and molecular weight of PLGA (Kissel and Koneberg, 1996).

1.4.3 Kinetics of Degradation

The erosion of degradable polymers is a complicated process, in which various reaction and transport processes are involved. Erosion starts with the intrusion of water into the polymer bulk and triggers degradation. Degradation is the polymer chain scission process and is the most important part of erosion. Through the degradation, oligomers

and monomers are created that finally diffuse to the polymer surface, where they are released from the polymer bulk (Brunner *et al*, 1996). The degradation velocity depends on the type of hydrolysable functional group from which the polymer is built and determines how a polymer erodes (Göpferich, 1996a). Table 1.2 shows the half-lives of functional groups that are typical for degradable polymers.

POLYMER CLASS	HYDROLYSIS RATE
Polyanhydride	0.1 hours
Polyketal	3 hours
Poly(ortho-ester)	4 hours
Polyacetal	0.8 years
Polyester	3.3 years
Polyurea	33 years
Polycarbonate	42,000 years
Polyurethane	42,000 years
Polyamide	83,000 years

Table 1.2 Degradation times for degradable polymers (Adapted from Brunner *et al.*, 1996.)

The degradation of polyanhydrides, in general, varies with a number of factors. These factors include the nature and hydrophobicity of the monomers used to produce the polymer, the level of drug loading in the polymeric matrix, the pH of the surrounding medium (the higher the pH, the more rapidly the polymers degrade), the shape and geometry of the implant (degradation is a function of the surface area) and the accessibility of the implant to water (porous materials will degrade more rapidly than non-porous) (Domb *et al.*, 1997). For poly(CPP-SA) and poly(FAD-SA), during the initial 10-24 hours of incubation in aqueous medium, the molecular weight dropped rapidly with no mass lost. This period was followed by a fast decrease in mass

accompained by a very small change in polymer molecular weight. The period of extensive mass loss starts when the polymer molecular weight reaches a number average molecular weight (Mn) of about 2,000 regardless of the initial molecular weight of the polymer. During this period which lasted for about one week, sebacic acid, the relatively water soluble co-monomer, was released from the polymer leaving the less soluble co-monomer, CPP or FAD, which is slow to solubilise (Dang *et al.*, 1996). Increasing the content of sebacic acid in the copolymer increased the hydrophilicity of the copolymer, which resulted in a higher erosion rate and hence higher drug release rates. This could be explained by the fact that the anhydride linkages in the polymer are hydrolysed subsequent to penetration of water into the polymer. The water uptake depends on the hydrophobicity of the polymer and therefore, hydrophobic polymers which prevent water uptake, have slower erosion rates and lower drug release rates. This is valuable information since polymer hydrophobicity can be altered by changing the structure and/ or the monomer ratio of the copolymer, thereby being able to alter the drug release rate (Domb *et al.*, 1997). Investigations can reveal the time scale on which degradation occurs, as the chemical degradation of bonds in the polymer chains is important among the variety of parameters affecting the erosion of the polymer bulk, therefore, yielding precious information on the expected time over which drugs may be released. However, the result of investigating large matrix discs does not allow the assessment of the degradation properties unequivocally. With increasing dimensions, the result depends on other processes in addition to degradation, such as the diffusion of water into the

polymer bulk. If water diffusion is slow, the degradation of the polymer matrix disc is affected because the lack of water prevents the degradation inside the polymer matrix. From the resulting molecular weight changes, it can be concluded that polyanhydrides degrade across their entire cross section for geometries of a reasonable size (Göpferich, 1996b). The examples illustrate that polyanhydrides have to be stored under anhydrous conditions.

Polyanhydrides, in general, degrade more rapidly in basic media than in acidic media . At pH 7.4, pure P(CPP) degrades in about 3 years. However, this rate increases markedly as the pH rises, and at pH 10.0, this material degrades in just over 100 days. At very acidic pH values, many of the polyanhydrides virtually do not degrade at all (Santos *et al.*, 1999).

When investigating the degradation behaviour of polyanhydride microspheres, the process is more complex. For P(FA:SA) (poly(fumaric acid-co-sebacic acid)) microspheres made by the hot melt encapsulation process, it appears that although the P(FA:SA) material degrades very quickly (18 h) in an aqueous environment, there is a plateau in the degradation and oligomeric material containing anhydride linkages remains stable for an extended period of time (Santos *et al.*, 1999).

It is possible to construct a composite model of how absorbable devices degrade in living tissues. With the exception of degradation rate, this model can be generally applicable to devices made from PLGA. From a chemical standpoint, absorbable devices are thought to undergo five general stages of degradation (Kronenthal, 1975). These stages are not discrete and may overlap.

Firstly, hydration of the implant begins when the device is placed in the body. During this stage, the device absorbs water from the surrounding environment. Depending upon the mass and surface area of the implant, this diffusion process occurs over the course of days or months.

The second stage of degradation is depolymerisation or chemical cleavage of the polymer backbone which results in a reduction in mechanical properties (strength). In this process, water reacts with the polymer in a hydrolytic fashion resulting in cleavage of covalent chemical bonds with a commensurate reduction in average molecular weight and physical strengh.

The third stage in the degradation process is loss of mass integrity which occurs when the implant has essentially no cohesive strength and begins to fragment into pieces of low molecular weight.

The fourth stage of degradation is absorption which occurs when further hydrolysis causes the fragment size to be suitable for assimilation by phagocytes or when further hydrolysis simply leads to soluble monomeric (lactate or glycolate) anions which dissolve into the intercellular fluid.

The final stage of degradation is elimination (Perrin *et al.*, 1997).

Degradation of PLGA in an aqueous environment occurs through simple hydrolysis of ester bonds autocatalysed by carboxylic groups and hydrolysis rate increases exponentially with degradation time (Hakkarainen *et al.*, 1996). The degradation proceeds in two main stages. Water diffuses first into the amorphous regions which are less organised and allow water to penetrate more easily than highly ordered

densely packed crystalline regions. The second stage starts when most or all of the amorphous regions have been removed and the water slowly penetrates the crystalline regions (Hakkarainen *et al.*, 1996). The PLGA 50:50 copolymer has the fastest degradation rate of the D,L-lactide/ glycolide materials, (about 50-60 days). The 65:35, 75:25, and 85:15 PLGA have progressively longer *in vivo* lifetimes (Lewis, 1990).

To summarise, once a PLGA device is placed in contact with an aqueous medium, water penetrates and the hydrolytic cleavage of ester bonds starts. Each ester bond cleavage generates a new carboxyl end group that, in principle, can catalyse the hydrolytic reaction of other ester bonds as proposed in the case of homogeneous degradation mechanism (Pitt *et al.*, 1981). For a time, the partially degraded macromolecules remain insoluble in the surrounding aqueous medium, regardless of its nature, and the degradation proceeds homogeneously. However, as soon as the molecular weight of some of the partially degraded macromolecules becomes low enough to allow dissolution in the aqueous medium, diffusion starts within the whole bulk, with the soluble compounds moving slowly towards and off the surface while they continue to degrade (Brannon-Peppas *et al.*, 2000).

According to the general degradation mechanism of PLGA polymers, there are four main factors that condition the diffusion-reaction-dissolution phenomena:

(a) the hydrolysis rate constant of the ester bond:

(b) the diffusion coefficient of water within the matix:

(c) the diffusion coefficient of chain fragments within the polymeric matrix:

(d) the solubility of the degradation products, generally oligomers, within the surrounding liquid medium from which penetrating water is issued.

Any additional factors, such as temperature, additives in the polymeric matrix, additives in the surrounding medium, pH, buffering capacity, size and processing history, quenching or annealing, steric hindrance, porosity, and other variables, affect the general balance through their effects on the main factors listed above (Vert, 1998).

1.4.4 The Importance of Erosion for Drug Release

Drug release can be classified into diffusion-, swelling-, and erosion- controlled release (Langer, 1990). A degradable polymer might release drugs by all three mechanisms. The quickest mechanism, however, will dominate the drug release. If the three processes proceed at similar speed, drug release will be controlled by all three simultaneously. In order to have optimal control over drug release from degradable polymers, it is desirable that it should be mainly erosion-controlled (Brunner *et al.*, 1996).

It appears that drug dispersed in the polymer matrix is released when the eroding polymer brings the drug with it into solution. Thus the release rate would depend on the rate of erosion expressed as volume of the matrix dissolved *per* unit time, times the drug load, rather than the rate of polymer degradation. The implication is that drug release should correlate with the weight loss, which is a more appropriate indicator of erosion rate rather than the decrease in molecular weight (Domb *et al.*, 1997).

Polyanhydrides differ from other polymers used for drug delivery by the reactivity of the anhydride bond and polyanhydrides are an ideal material for the manufacture of

erosion-controlled drug-delivery systems. Under the assumption that a saturated solution of SA, which is in equilibrium with suspended SA, exists at the erosion front, a diffusion model simulates the release of SA through the porous and tortuous erosion zone. Assuming further that SA controls the solubility of the CPP, the sigmoidal release profile for CPP was confirmed by this modelling approach. Meanwhile, the poor solubility of the monomers is also reflected by the fast release of drugs from P(CPP:SA) matrix discs. Indomethacin, for example, was found to be released faster than SA (Göpferich & Langer, 1995).

The release of macromolecules from biodegradable microspheres is influenced both by the structure of the microparticles and properties of the biodegradable polymer itself. The biodegradable coating of the microcapsules would be impermeable for proteins, but water could slowly diffuse into the core, creating suffcient osmotic pressure for rupture of the membrane (Kissel & Koneberg, 1996).

Most protein-delivering microparticles exhibit a matrix-type internal, solid dispersion morphological structure. The proteins are insoluble in the polymeric matrix, and the macromolecules are released by a mechanism that combines pore diffusion and polymer erosion (Kissel & Koneberg, 1996). Initially, water diffuses into the matrix, dissolving drug particles adjacent to the surface of the device. The resulting osmotic pressure is relieved by forming a tortuous channel to the surface, releasing a defined amount of protein in the initial drug burst. This burst effect is controlled mainly by three factors: the protein / polymer ratio, the particle size of the dispersed protein, and the size of the microspheres (Kissel & Koneberg, 1996).

PLGA microspheres release macromolecules by this mechanism in the initial phase. Depending on the composition and molecular weight of the PLGA, protein release recommences when the polymer degradation has reached a stage of rapid mass loss. This phase is controlled by the degradation of the polymer, leading to a degradation or erosion of the matrix.

1.4.5 Changes in Polymer Properties During Erosion

1.4.5.1 Changes in Polyanhydride Properties During Erosion

1.4.5.1.1 Morphological Changes During Erosion

Surface and bulk erosion are ideal cases, and most polymers cannot be unequivocally assigned to one of them. It can be concluded, that the crystalline parts of polyanhydrides degrade and erode too slowly to allow perfect surface erosion. Crystalline erosion zones remain on the polymer surface. The amorphous polymer areas erode substantially faster than the crystalline ones, and a consequence, erosion zones formed in which the amorphous polymer disappear first and are replaced by a network of pores that stretch through the crystalline areas of noneroded polymer. The foremost line of eroded polymer, the erosion front, moves from the surface of the polymer matrix into the centre (Brem, 1990). Because of the high porosity in the erosion zone, however, P (CPP: SA) comes close to a perfect surface-eroding polymer. Erosion zones in P (FAD: SA) are different. This is because of the physical state of FAD, which is an oily liquid (Brunner *et al.*, 1996). The porosity of eroding P(CPP:SA) disks has been investigated by mercury intrusion porosimetry (Göpferich,1996). This

method can distinguish between macro- and micropores. Macropores are created immediately after contact with the erosion medium and have a size of approximately 100μm. They result from cracks on the surface of polyanhydride matrix disks. The micropores are gradually created by erosion and result from the faster erosion of amorphous polymer areas compared with crystalline ones; they are approximately 100nm (Göpferich and Langer, 1993).

1.4.5.1.2 Crystallinity Changes During Erosion

The faster erosion of amorphous polymers compared with crystalline ones, changes the overall crystallinity of polymer matrices during erosion. Changes in crystallinity can be followed by wide-angle X-ray diffraction (WAXD) and DSC. When P(CPP:SA) was studied, the spectra obtained by both methods showed very characteristic peak patterns. By determining the DSC and WAXD time series spectra of the monomers SA and CPP, the source of new crystallinity was shown to arise from the crystallisation of monomer (Göpferich and Langer, 1993). The data suggest that these monomers have the tendency to crystallize inside the pores of the erosion zone.

1.4.5.1.3 pH Changes During Erosion

The acid environment produced by the degradation of the polyanhydride would be a substantial hurdle to overcome for delivery of proteins, because acid denaturation of some proteins may be rapid in this acid environment. The pH was measured as a

function of the distance from the surface of the eroding polymer matrix disk (Göpferich & Langer, 1993). The pH drops significantly when approaching the surface of the device, a clear indication that the pH within polymer pores must be even lower to permit compensation of the buffer on the matrix surface. It has been postulated that the pH drop is much less pronounced *in vivo*, than *in vitro,* as the degradation products of the polymer that cause the pH drop will be rapidly cleared from the injection site *in vivo* (Park *et al.*, 1995). One approach that has been taken to counteract the acidity is the incorporation of buffering substances into the matrix during the device fabrication. The incorporation of buffering substances within the polymer does result in an increase of the pH inside the delivery system (Domb *et al.*, 1997).

1.4.5.2 Changes in PLGA During Erosion:

The degradation of bulk-eroding polymers usually follows first-order kinetics and their erosion, which is gauged as mass loss, is substantially more complicated. After an initial period of no significant mass loss, erosion usually sets in spontaneously, leading to a rapid mass loss within short periods of time. All bulk-eroding polymers show this erosion behaviour, however on different time scales (Göpferich, 1997).

Both erosion and molecular weight profiles for PLGA show an induction period where no time-dependent changes occur. For molecular weight loss, the induction period is short and presumably reflects the interval required for water to completely permeate the polymer structure. For the erosion process, the induction period is longer and has mechanistic significance: because onset of erosion lags behind molecular

weight loss, PLGA hydrolysis must proceed throughout the bulk of the polymer structure (Kenley *et al.*, 1987). At later stages of the degradation a bimodal profile of molecular weight distribution with lower molecular weight inside the device compared to the surface was observed, termed as 'bulk degradation' (Vert *et al.*, 1998). This phenomenon was correlated with an autocatalytic acceleration of ester hydrolysis caused by acidic degradation products, which cannot leave the device *via* diffusion (Pitt *et al.*, 1998). For polylactide and poly (lactide-co-glycolide), it was observed that the acidic degradation products accelerate degradation within polymer matrix disks compared to the surface when eroded in buffer solutions of pH 7.4 (Göpferich, 1997). Since degradation of the polymer takes time to generate water-soluble products, PLGA shows a lag phase with respect to erosion (Mainil-Varlet *et al.*, 1997). In the case of PLGA, the molecular weight loss follows a sigmoidal profile. After an initial lag period, the degradation rates increases. In general it is seen that the degradation velocity depends on the molecular weight of the polymers - the lower the molecular weight, the faster the degradation. Most important with respect to interpretation of the shape of the erosion profiles is the fact that degradation starts immediately after the start of the erosion experiment. Therefore, the initially constant mass cannot explain with the lack of degradation early during the experiment (Göpferich, 1997).

For PLGA, approximately 90% of the original tensile strength is retained after degradation for 1 week, 55% is retained at 2 weeks, with essentially no strength remaining after 4-5 weeks (Chu, 1983).

1.5 Biocompatibility and Toxicity of Biodegradable Polymers

Whenever a synthetic material is applied *in vivo*, there are biocompatibility concerns in terms of tissue-implant interaction. In the case of a bioerodible matrix the potential toxicity of the degradation products must also be considered (Leong *et al.*, 1985b). Biocompatibility of monomer is considered as the foundation for biocompatibility of degradable polymer systems, not the polymer itself (Domb *et al.,* 2002).

1. Lactide/Glycolide Copolymers

The lactide/glycolide copolymers have been subjected to extensive animal and human trials without any significant harmful side effects. No evidence of inflammatory response, irritation, or other adverse effects has been reported upon implantation of lactide/glycolide polymer device. Good biocompatibility data were reported with lactide/glycolide copolymer matrices containing antineoplastic drugs, antibiotics, and anti-inflammatory compounds (Perrin *et al.*, 1997)

2. Polyanhydrides

A series of biocompatibility studies reported on several polyanhydrides have shown them to be nonmutagenic and nontoxic. Based on the biocompatibility and safety pre-clinical studies carried out in rats, rabbits and monkeys, polyanhydrides were accepted for human use. A Phase I and II clinical protocol was instituted. In keeping with the results of the earlier pre-clinical studies suggesting a lack of toxicity, no central or systemic toxicity of the treatment was observed during the course of treatment (Domb *et al.,* 2002). Early studies assessed the biocompatibility of P (CPP), poly (terephthalic acid anhydride) [P (TA)], P (CPP:SA), and poly (terephthatic

acid-co-sebacic acid) [P(TA:SA)] as well as the toxicity of their monomers (Leong *et al.*,1985b). The monomers tested were non-mutagenic, non-toxic, and were found to have a low teratogenic potential *in vitro*. The polymers did not lead to inflammatory responses after 6 weeks of implantation into the rabbit cornea and there were no signs of inflammation after subcutaneous implantation into rats (Bakker *et al.*, 1988). The biocompatibility of P (CPP: SA) 20:80 in the brain was first assessed in rodent models (Tamargo *et al.*, 1989; Brem *et al.*, 1989) then verified in a monkey model (Brem, 1990).

1.6 Microsphere Systems

1.6.1 Microsphere preparation

Several techniques are available for microencapsulation, and the choice of a method depends on the physical and chemical properties of the polymer and protein to be encapsulated, and the function and desired size of the microspheres (Bhagat *et al.*, 1996). Several methods have been used in the preparation of microspheres of different biodegradable polymers, including both natural and synthetic polymers. Pharmaceutically acceptable microencapsulation techniques for hydrophilic macromolecules, such as peptides and proteins, using biodegradable polymers as matrix materials can be classified into:

1. Solvent evaporation,
2. Solvent removal,
3. Hot-melt encapsulation,

4. Spray-drying.

In addition, two methods for the manufacture of double-walled microspheres have been reported (see section 1.6.1.5).

1.6.1.1 Solvent Evaporation

In order to improve the loading of water soluble compounds within PLGA microparticles, Ogawa *et al.* developed a water-in oil-in water (W/O/W) solvent evaporation technique to entrap a water soluble peptide into PLGA microparticles (Ogawa *et al.,* 1988). The double-emulsion solvent evaporation technique is commonly used to prepare biodegradable, hydrophobic microspheres containing hydrophilic proteins or polypeptides for sustained-release applications. In this double emulsion-solvent evaporation approach, an aqueous solution of protein is emulsified with the polymer solution to form a primary water-in-oil emulsion (W/ O). This is subsequently emulsified with an aqueous surfactant solution (W/O/W) to induce polymer precipitation and microparticle hardening and to allow solvent removal by evaporation (Yeh *et al.,* 1994). Polyanhydride and PLGA microspheres that are manufactured by solvent evaporation tend to be porous. Matrices of variable porosity facilitate modulation of drug release. Porous microspheres are also essential to deliver high molecular weight substances which can not diffuse out of a nonporous matrix and to deliver substances which have high affinity for polymer and are not released unless the matrix erodes. Matrix porosity is also of significance in polymeric carriers, which deliver drugs, at least in part, by erosion. Altering the porosity of the matrix can control polymer degradation and hence control the rate and extent of drug release (Li

et al., 1995). The disadvantages of solvent evaporation include:

1. Solvent residues in the polymer
2. Polymer degradation during microsphere preparation
3. Risk of changes in biological activity of proteins (Tabata *et al.,* 1993).

1.6.1.2 Solvent Removal:

The solvent-removal technique uses only organic solvents for the manufacture of microspheres. The method is a modification of organic phase precipitation, but it offers a significant advantage: the preparation occurs at room temperature and totally in organic solvents. This latter advantage is particularly important for hydrolytically labile polymers such as polyanhydrides. In this method, the polymer is dissolved in an organic solvent, such as methylene chloride, and is dispersed in a mixture of silicone oil, methylene chloride, and a surfactant, such as Span 85. Adding a nonsolvent, such as petroleum ether, to the suspension, hardens the microspheres. The microspheres obtained by solvent removal are porous (Brunner *et al.,* 1996).

The process is reproducible to within 5% with respect to yield and size distribution, if polymers of the same molecular weight are used (Chasin *et al.,* 1990). A potential problem is the use of organic solvents and the danger of silicone oil residues in the microspheres (Brunner *et al.,* 1996). Compared with the holt-melt technique, this method permits the preparation of microspheres from polymers with a range of melting points, and the encapsulation of drugs, which may lose biological activity at high temperature (Mathiowitz *et al.*, 1988).

1.6.1.3 Hot-Melt Encapsulation:

An interesting approach to reduce organic solvent residues in polyanhydride microspheres is the formation of microspheres from melted polymer (Brunner & Göpferich, 1996). The hot-melt microencapsulation process is analogous to the melt molding process to form flat devices. In the hot-melt microencapsulation process, the drug and polymer are suspended in a polymer immiscible-solvent, such a silicone or olive oil. The matrix is heated to 5°C above the melting point of the polymer and stirred continuously to form a suspension of the polymer in the oil. The liquid is cooled until the polymer solidifies into microspheres. The microspheres are then washed with petroleum ether, dried, sieved and stored under nitrogen at -20°C as a dry powder. The method produced dense spheres, with smooth, even surfaces. Reproducible size distribution of the microspheres was achieved by adjustment of the stirring speed (Tamada and Langer, 1992). The process was quite reproducible with respect to yield, size and loading distribution, if the same molecular weight polymer was used. However, the temperatures to which polymer and drug are exposed limit the broad application of the method (Brunner & Göpferich, 1996).

1.6.1.4 Spray Drying

Among the methods investigated, spray drying has proved to be very convenient. Spray drying is a widely used technology in the pharmaceutical and biochemical fields and in the food industry. The main applications of spray drying in the pharmaceutical field are: drying processes, of plant extracts for example, and of heat sensitive materials; improvement of the flow properties of pharmaceutical powders and their excipient

production, such as spray dried lactose; granulation; preparation of solid dispersions with water-soluble polymers and complexation with cyclodextrins, to improve the dissolution rate of drugs that are poorly soluble in water; alteration of the polymorphism of a drug; preparation dry powder for aerosols; encapsulation volatile products; encapsulation for taste masking and protection from oxidation (Giunchedi & Conte,1995).

Spray-drying converts a liquid into a powder in a single step. The spray drying process involves the following four sequential stages (Broadhead *et al.*, 1992):

1. atomisation of the feed materials into a spray nozzle,
2. spray-air contact,
3. drying of the sprayed droplets,
4. collection of the solid product (spray dried microparticles) obtained.

In this method, the polymer is dissolved in a solvent such as chloroform or methylene chloride along with the drug, either in a dissolved or dispersed form. The solution is sprayed through an atomizer. As the particles fall toward the bottom of the spray dryer, they are simultaneous dried by an upward flow of nitrogen (Tamada and Langer, 1992). For example, during the manufacture of microspheres by spray drying, polyanhydride polymers were dissolved in methylene chloride and were spray-dried with the drug suspended therein. Microspheres made by spray drying tend to have an irregular shape and high porosities that may cause the fast release of drugs (Mathiowitz *et al.*, 1992). Pavanetto *et al* (1993) prepared PLGA microspheres containing a lipophilic model drug (vitamin D_3) by spray-drying and found that

particle size distribution seems to be affected by polymer molecular weight, as it increases with polymer molecular weight, when polymer concentration in the starting solution is kept constant; particle size within the same polymer always increases with polymer concentration.

The main advantages compared with the other microencapsulation methods in the production of microsparticle systems are:

-general applicability, both concerning the drugs (heat-sensitive materials can be used) and concerning the polymers (hydrophilic and hydrophobic can be used);

-continuous in operation: it is one stage process;

-adaptable on the industrial scale.

A possible disadvantages of spray-drying can be the loss of product, the low yields, the use of organic solvent and cost of the spraying equipment (Giunchedi & Conte,1995).

1.6.2 Microsphere Characterisation

Microsphere microstructure, in turn, affects the stability of drugs and drug release so detailed microstructural characterisation of microspheres, therefore, may resolve some of the problems in which drug-release and drug-stability issues are involved (Brunner, 1996). There are numerous physicochemical methods by which they might be characterised, including wide-angle X-ray diffraction (O'Donnell *et al.,* 1997), differential scanning calorimetry (DSC) (Tabata & Langer, 1993), scanning electron microscopy (SEM) (Mathiowitz *et al.*, 1988), transmission electron spectroscopy (Pekarek *et al.*, 1994), gel-permeation chromatography (GPC) (Mathiowitz, 1987).

SEM is a standard technique for microsphere characterisation, as it offers, compared with light microscopy, a much higher resolution. SEM allows investigation of microsphere surfaces (Brunner *et al.*, 1996).

Polymer composition, device fabrication method, size and geometry of the device, particle size of incorporated drug, method of incorporation of drug, drug loading, and drug hydrophilicity all affect the rate of drug release. Generally hydrophilic drugs, which are dispersed as particles within the devices, will display a burst effect. That is, drug on the surface of the device will dissolve very rapidly. The extent of the burst depends on the particle size of the drug compared to that of the device; the finer the drug particles, the smaller the burst effect (Tamada & Langer, 1992). Drug release from degradable microspheres might yield information on the microstructure of particles and the mechanism of erosion.

PLGA microspheres have a typical three phase release pattern:

1. an initial burst due to material at or close to the surface
2. a phase of very little release, followed by
3. a rapid release on collapse of the matrix following hydrolytic degradation.

Gamma-irradiation can alter drug release from PLGA matrices. It does not affect the rate of release in the initial few days, then it causes massive release on collapse of the matrix, which is dramatically affected. The matrix collapse can occur at 10-15 days rather than at 60-70 days in the non-irradiated materials (Whateley, 1993).

For an explanation of release of hydrophilic macromolecules from parenteral depot systems based on PLGA, two mechanisms are discussed, namely pore-diffusion and

polymer-erosion. After an initial burst of drug molecules located at the surface of the polymeric device, the following release is controlled by diffusion through aqueous pores. The low diffusivity of hydrophilic macromolecules, like proteins, leads to low release rates in this phase. The molecular weight degradation decreases the mechanical stability of the device, leading to a collapse of the pore structure (Bodmer *et al.,* 1992). On the other hand, the degradation of PLGA induces formation of acidic mono- and oligomers leading to a decrease in pH within the matrix in which proteins tend to aggregate and denature (Mader *et al.,* 1998). Furthermore, diffusion-controlled release is influenced by the drug/polymer ratio (loading), the dimension of the device, the polymer molecular weight and the particle size of the drug substance (Bodmer *et al.,* 1992). The final release phase is governed by the polymer erosion, which is in general affected by properties of the polymeric device such as polymer molecular weight, comonomer composition and molecular weight distribution (Hora *et al.,* 1990). Even when polymer mass loss occurs, Bodmer *et al.* (1992) found that the loaded bovine serum albumin, BSA, was not completely released.

The release of low molecular weight compounds from polyanhydride microspheres has been studied extensively and has revealed some of the polymer properties. The release of acid orange from P(SA), for example, has been observed to be very rapid. This is because aliphatic polyanhydrides erode faster than aromatic ones (Mathiowitz *et al.*, 1990a). In addition, surface cracking after contact with water is much more pronounced for P(SA) than it is for P(CPP:SA) copolymers. The slower release of drugs from copolymers containing aromatic monomers has been proven by releasing

acid orange from P(CPH:SA) 50:50, which is substantially slower than from p (SA). The decreased release rate is due to the slower degradation of the polymer and the increased hydrophobicity of the matrix owing to the content of aromatic CPH monomer.

An interesting observation is the release of protein from P(FAD:SA). No initial burst was observed in the release profiles of BSA from P(FAD:SA), irrespective of the protein loading. The protein was released for up to 3 weeks at a near-constant rate. The release rate of protein depends on the monomer composition of polyanhydrides used. The deposition of FAD monomer on the surface of particles during erosion might be responsible for the changing release profile (Tabata *et al.*, 1993). Although release profiles can yield valuable information on microspheres, often they can be interpreted only if information on the polymer properties and the erosion mechanism is available.

In the assessment of drug release characteristics, it is important to have *in vitro* models that are good predictors of the *in vivo* situation. Thus it is important to know the probable release mechanism *in vivo*, be it one of diffusion, desorption, erosion or biodegradation or as is more likely, a combination of these. For instance, dramatic differences in release can occur *in vitro* when changes from a simple release medium, such as a buffer, to one containing plasma proteins are carried out (Douglas *et al.*, 1986). The *in vitro* release of protein encapsulated in biodegradable microspheres, however, does not necessarily correlate well with the *in vivo* response (Alonso *et al.*, 1993; Jeffrey *et al.*, 1993).

1.7 Applications of PLGA and Polyanhydrides

The PLGA polymers are presently the most investigated biodegradable excipients for controlled drug delivery. Several major pharmaceutical companies have extensive development programs based on these polymers. The majority of these programs are aimed at injectable microsphere formulations, although implantable rods and pellets are also being investigated. Applications cover both human and veterinary medicine.

Classical or conventional pharmaceutical agents in combination with PLGA polymers have been widely studied since about 1973. In general, these compounds are bioactive agents usually produced by synthetic chemistry, with molecular weights of less than a few hundred and relatively stable structures. Examples include:

1. Steroid systems: A testosterone microsphere system has been developed and clinically evaluated. This formulation is based on a PLGA copolymer and natural testosterone (Asch *et al.,* 1986).
2. Anticancer systems: Controlled release formulations offer the potential of reducing the drug toxicity, which is almost always a serious problem in cancer chemotherapy. Cisplatin, mitomycin, and adriamycin have been studied in biodegradable delivery systems (Lewis, 1990).

Polypeptides, viral and bacterial antigens and many other macromolecules such as those derived from recombinant DNA technology represent a challenge of greater magnitude for designers of drug delivery system. Because of the tremendous clinical and commercial potential, combinations of these agents with PLGA polymers have received considerable attention. For example, in the process of developing a

microsphere formulation for LHRH agonists, Ogawa and colleagues used copolymers of PLGA and found a continuous zero-order peptide release rate for 21 days both *in vitro* and *in vivo* (Ogawa *et al.,* 1988). Now the LHRH system is on the market. This research resulted in clinical testing and eventual approval of Lupron Depot® for use in prostate cancer and endometriosis (Cleland, 1997). In 2002, United Kingdom approved Jassen-Cilag's Risperdal Consta ™ (risperidone). The formulation encapsulates risperidone in microspheres made of PLGA, which is suspended water-based solution and injected into the muscle. Risperdal Consta is administered once every two weeks for the management of schizophrenia—a brain disorder.

The application of polyanhydrides which has advanced the furthest clinically is for the treatment of human brain tumours. Polyanhydride matrices have been used to locally deliver chemotherapeutic drugs such as carmustine (BCNU) to treat brain cancer (Brem *et al.*, 1995). In the therapy, BCNU is incorporated into the polyanhydride, which is then implanted into the site of the excised tumor. The surgeon removes as much of the tumour as possible at the time of operation, but also places up to eight small polymer-drug wafers at the tumour site. The drug is slowly released from the polymer for 1 month to kill remaining tumour cells. Because the drug is delivered locally, harmful side effects that normally occur from systemic chemotherapy are minimized (Valonen, 1997). Phase III human clinical trials demonstrated that site specific delivery of BCNU from a poly (CPP:SA) 20:80 wafer (Gliadel®) in patients with recurring brain cancer (glioblastoma multiforme) significantly prolonged patients' survival (Brem *et al.*, 1995). Gliadel® has won approval from the FDA for

the treatment of brain tumours since 1996.

Insulin has been reported to be encapsulated in polyanhydride, but there are not yet any products on the market. Insulin was loaded at 15% into P(CPP:SA) 50:50 microspheres, which were between 850 and 1000 μm in diameter. Much of insulin was released over the first 1-2 days, but significant amounts continued to be released for 4-5 days (Mathiowitz *et al.*, 1987b; Mathiowitz, *et al.*, 1985). It is therefore possible to incorporate labile biological products into polyanhydrides and to release them in a biologically active form.

Investigations have expanded to newer polymers and other drugs such as 4-hydroperoxy cyclophosphamide (4HC), cisplatin, carboplatin, taxol and several alkaloid drugs in an effort to develop a better system for treating brain tumors (Olivi *et al.*, 1996; Judy *et al.*, 1995). Carboplatin was incorporated into P(FAD-SA), by mixing the drug in the melted polymer and evaluated for the treatment of brain tumours in laboratory animals with promising results (Olivi *et al.*, 1996). P(FAD-SA) has also been used to develop a delivery system for gentamicin sulfate for the treatment of osteomyelitis (Domb and Amselem, 1994b; Laurencin *et al,* 1993). A sustained release of gentamicin sulfate over a period of few weeks was obtained both *in vivo* and *in vitro* using this system. The effect of long term glutamic acid stimulation of trigeminal motoneurons, using P(FAD-SA) microspheres has been explored. This study was undertaken to determine the role of glutamate in possible growth disorders of the craniofacial skeleton. Pronounced skeletal changes in the snout region were observed in rats that received glutamate showing that sustained

release of glutamic acid in vivo can effect the development of skeletal tissue in growing rats (Hamilton-Byrd *et al.,* 1992).

Some results suggest that bioadhesion of polymers lacking chain flexibility could largely be a result of secondary bond formation, such as hydrogen bonding between mucin and free carboxyl groups after polyanhydride hydrolysis. Bioadhesion is a complex phenomenon related to the ability of some natural and synthetic macromolecules to adhere to biological tissues (Tamburic *et al,* 1996). Rapid degradation of P(FA:SA) polymers may enhance their bioadhesive nature through production of carboxylic acid and an increase in surface roughness (Peppas & Sahlin, 1996; Mathiowitz *et al.*, 1997).

1.8 Objectives of This Study

P(CPP:SA), poly [1,3-bis (p-carboxyphenoxy) propane-co-sebacic acid], has been generally been considered to undergo surface erosion kinetics for the polymer to erode from the outside to the inside and poly(lactide-co-glycolide),which is a widely available biodegradable system has been considered to undergo bulk erosion kinetics: water penetrates into the polymeric matrix and degrades it internally as well as externally. This study focuses on comparing these two kinds of biodegradable polymer in terms of degradation, microsphere preparation and drug release under the same experimental conditions. During these studies, P(CPP:SA), PLGA(50:50) and PLGA(75:25) microspheres were prepared by three methods: double emulsion (w/o/w), spraying drying (SD), and oil-in-oil (o/o) microencapsulation (solvent removal).

All the polymers and the resulting microspheres were characterised as follows: polymer or microspheres were examined by infrared (IR) spectroscopy, differential scanning calorimetry (DSC), and nuclear magnetic resonance spectra (NMR). The morphology was examined by scanning electron microscopy (SEM).The moleculer weight was examined by gel permeation chromatography (GPC).

In order to monitor microsphere size distribution, surface morphology, encapsulation efficiency, the character and the result of release studies and differences between different microspheres preparation methods, bovine serum albumin (BSA) was encapsulated, as a model drug.

Experimental

2. EXPERIMENTAL

2.1 Materials

Reagents of analytical grade and double distilled water were used throughout this study. Poly(DL-Lactide-co-Glycolide) 75:25 molecular weight (Mw) 90,000~126,000 and 50:50 Mw 40,000 were supplied by Sigma (UK). Poly (vinyl alcohol) (PVA) with 13,000-23,000 average molecular weight and 87-89% hydrolysed, dichloromethane (DCM) (HPLC grade used without further purification), silicone oil, sodium azide (NaN_3), 1 μm polystyrene latex, Span 85, potassium bromide (KBr, 99% IR grade) and phosphate buffered saline (PBS) tablets were all purchased from Aldrich Chemical Co., Gillingham, Dorset, UK. All other solvents used were of analytical grade. Sebacic acid was recrystallised twice from dry methanol. Bicinchoninic acid (BCA, 4,4`-dicarboxy-2, 2`-biquinoline, sodium salt) protein assay reagent and bovine serum albumin (BSA) were obtained from Sigma Chemical Co., Poole, UK.

2.2 Polymer Synthesis

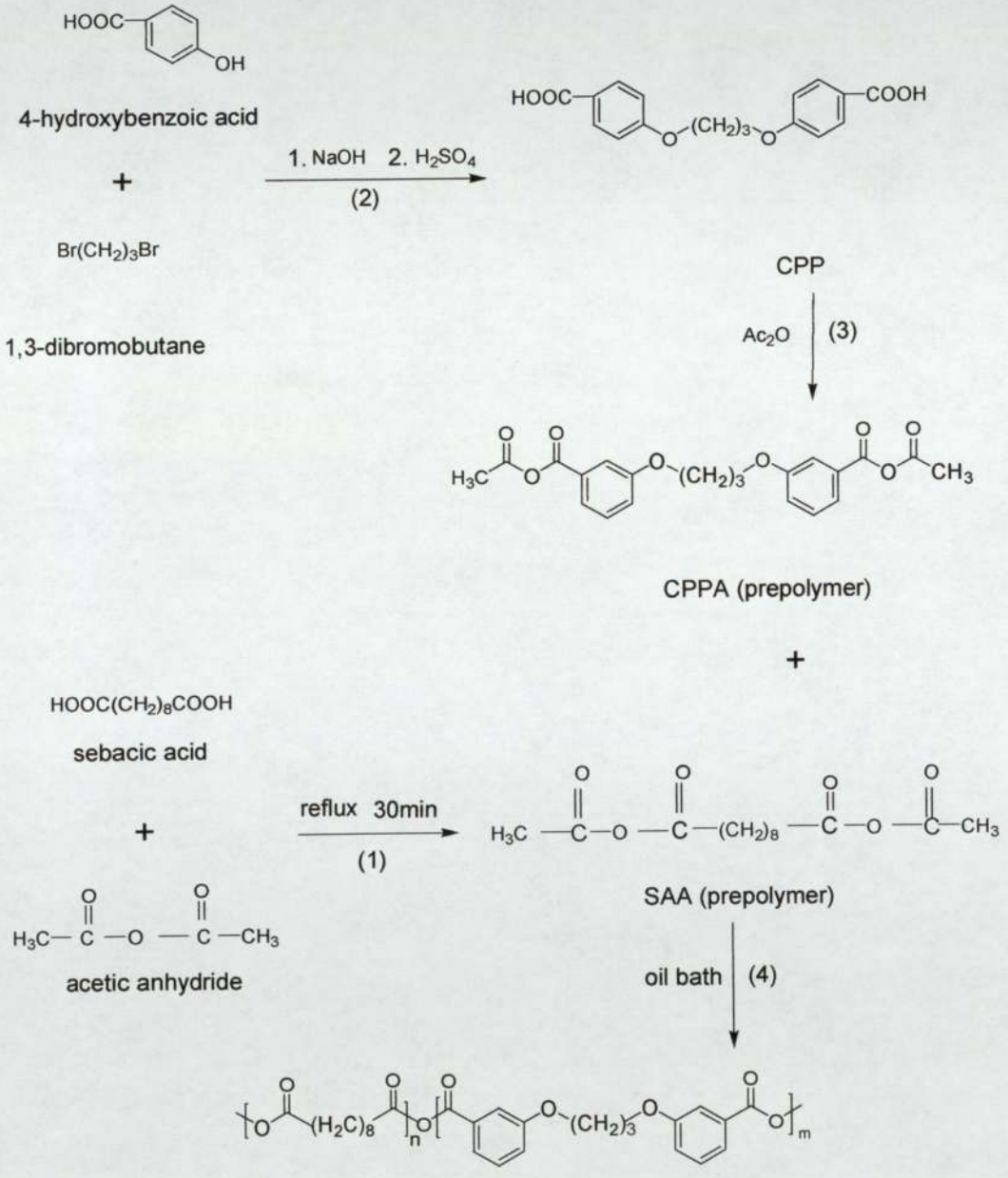
Polyanhydrides were synthesised by melt polycondensation (Figure 2.1) and all the experiment equipments had been dried in oven over night. Briefly, 50 g SA monomer was recrystallisd twice from 150 ml dry methanol, and the excess water in purified SA was removed by heating under vacuum. After purification, purified SA was stored in a sealed flask filled with N_2. The acetic anhydride (AA) was distilled at 138 °C and was also stored in sealed flasks before use. About 10 g purified SA was converted to the anhydride by refluxing in 100 ml purified acetic anhydride for about 30 minutes in an

oil bath (see Figure 2.1 eq 1). After cooling to room temperature, excess acetic anhydride was removed under vaccum at 20 ~ 30°C. Following cooling of the resulting mixture in a freezer, the white crude prepolymer sebacic acid anhydride (SAA) was precipitated, and it was immersed in a 1:1 mixture of dry petroleum ether (dried by 4A molecular sieves) and dry ethyl ether over-night at 4 °C to extract the acetic anhydride. The pure aliphatic prepolymer was washed with petroleum and diethyl ether (1:1), filtrated under a N_2 environment, and dried under vaccum.

Aromatic prepolymer was synthesised according to the method applied by Conix (Conix, 1977). In a three-necked flask equipped with a stirrer, a condenser, and a dropping funnel, a solution of 13.8 g (0.1 mole) of p-hydroxybenzoic acid and 8.0 g (0.2 moles) sodium hydroxide in 400 ml water, was placed. Through the funnel, 10.2 g (0.05 moles) of 1,3-dibromopropane was added over a period of one hour, while the contents of the flask were stirred and kept at the reflux temperature (see Figure 2.1 eq 2). After the addition, the reaction mixture was refluxed for 3.5 hours. Then 2.0 g (0.05 mole) of solid sodium hydroxide was added to the mixture, and the reflux continued for another 2 hours. The reaction mixture was cooled at room temperature overnight. The disodium salt precipitate was isolated by filtration and washed with methanol. The wet precipitate was dissolved in 100 ml distilled water and while the solution was heated to 60 ~70 °C, 6 N sulphuric acid was added until the pH of the solution was about 1 (tested with pH paper). The dibasic acid precipitated in acid was filtered and dried in a vaccum oven at 80 °C.

In a 200 ml three-necked flask, equipped with a stirrer, a condenser, and a gas-inlet tube, 10 g (0.03 moles) of aromatic monomer 1,3-bis(p-carboxyphenoxy) propane and 100 ml of purified acetic anhydride was placed. A slow stream of dry argon, passed through $CaCl_2$, was bubbled through the mixture while it was refluxed for approximately 2 hours (see Figure 2.1 eq 3). Then the unreacted diacid was removed by filtration. The slightly yellow-coloured filtrate was concentrated to a volume of about 25 ml by distilling acetic anhydride under vaccum at a temperature not higher than 65°C. Aromatic prepolymers were isolated by crystallisation from the concentrated acetic anhydride solution, purified with dry diethyl ether, and dried in a vaccum oven (with phosphorus pentoide) at 70°C to dry the water out (normally 2 or 3 days). Both of the prepolymers were characterised by ^{1}H-NMR and stored in a vaccum desiccator before further application. The prepolymers were then subjected to melt-polycondensation as follows: in a typical reaction, CPPA [1,3-bis(p-carboxyphenoxy) propane prepolymer] 1.036g for mol ratio 20:80 was mixed with SAA (sebacic acid prepolymer) 2.964g and 2 molar percent catalyst (0.069g), cadmium acetate, in a mortar, and placed in a glass tube (2 x 20 cm) with a top arm equipped with a capillary nitrogen inlet, passed through the $CaCl_2$. The tube was immersed in an oil bath at 180°C. After the prepolymer was melted, a high vacuum (10^{-4} mm Hg) was applied through the side arm (see eq 4). The condensation product was collected in an acetone/dry ice trap. During the polymerisation, a strong nitrogen sweep, with vigorous agitation of the melt, was performed for 30 seconds every 15 mins. After 30 mins, the reaction was stopped. After cooling to room

temperature, the mixture of polymer and catalyst was dissolved in dry DCM. Catalysts were removed from the polymer DCM solution by filtration. The crude polymer was purified by precipitation in dry petroleum ether from the DCM solution. The precipate was then extracted with anhydrous ether for several hours at room temperature. The purified polymer was characterised by ^{1}H-NMR, IR, GPC, and stored in a vacuum desiccator for further use.



poly [1,3-bis (p-carboxyphenoxy) propane : sebacic acid] CPP:SA

2.3 Determination of Polymer Composition

The composition of P(CPP:SA) copolymer was determined by ^{1}H-NMR (Bruker AC 250 NMR Spectrometer using QNP probe head) from the ratio of the peak integration at 1.3 ppm (8H, SA) and 6.8 ~ 8.2 ppm (8H, CPP) using win –NMR version 3 software. The following copolymer characteristics can also be studied by ^{1}H NMR (Ron *et al.*, 1991): the degree of randomness that suggests whether the polyanhydride is a random or block copolymer; the average length of sequence (Ln) and the frequency of occurrence of specific comonomer sequences. The protons close to electronegative groups, as the aromatic copolymers, experience a lower frequency (deshielding). On the other hand, the protons distant from such groups, as next to aliphatic copolymers, absorb at a higher frequency (all expressed in relation to tetramethylsilane). These long-range effects affect the chemical shifts of the α-protons of the other comonomer. The downfield doublets at 8.1 and 8.0 ppm were the diads CPP-CPP and CPP-SA, respectively. Similarly, the upfield triplets at 2.6 and 2.4 ppm were diads SA-CPP and SA-SA, respectively.

2.4 Polymer Degradation

The polymer degradation experiments were carried out using ~ 3 mg polymer in an ependorff tubes at 37°C (n=3). The polymer suspensions were kept shaking thoroughout the experiment duration. The degradation medium used was 1 ml phosphate buffer saline (PBS). The PBS was prepared by dissolving 1 phosphate buffered saline tablet in 200 ml of double distilled water to obtain 0.1 M phosphate buffer, pH7.4 at 25°C. Every day in the first week and every three days in the next

twenty-one days three samples were collected, centrifuged at 21000 rpm for 10 minutes, removed the supernatent, freeze-dried (Edwards Modylo freeze drier) and stored in a desiccator at room temperature for SEM, GPC, NMR, DSC and IR analyses. The removed solution was collected into one bottle and stored in the freezer for pH analysis. Each experiment was performed in triplicate and results were the mean of three samples.

2.4.1. FTIR

Infrared spectroscopy was performed on a FTIR spectrophotometer (Mattson Galaxy 3020 FTIR Spectrphotometer, Unicam). Polymer and microsphere samples were impressed into potassium bromide (KBr) discs. The spectrum was analysed using Mattson First fourier infrared software tools.

2.4.2 Thermal Analysis

To characterize the thermal properties of polymer and microspheres, differential scanning calorimetry (DSC) thermograms were obtained using a Pekin Elmer DSC-4 differential scanning calorimeter. Samples of about 3mg were sealed into aluminium sample pans. The instrument was manipulated with empty aluminium pans under the same conditions, and the measurements were carried out from -40°C to 200°C under nitrogen at a scan rate of 10°C/min for heating and at 320°C/min for cooling (Perkin Elmer system 4, Thermal analysis microprocessor controller) in a nitrogen atmosphere from ambient temperature. The rate of heating and cooling was controlled by the computer, and the thermograms were analysed by thermal analysis computer

software. The melting point was taken as the maxpoint of the endotherm peak. Tg was taken as the midpoint of the transition curve.

2.4.3 Surface Morphology of Microspheres

The surface morphology of microspheres was studied *via* scanning electron microscopy (SEM) (Cambridge Instruments, ISI Model DS-130). Samples for SEM were thoroughly dried, mounted on aluminium stubs using adhesive tabs, and sputter-coated in an argon atmosphere with gold-palladium. The surface morphology of at least twenty-five randomly selected microspheres for each polymer was monitored using a Cambridge Instruments Stereoscan 5150 scanning electron microscope equipped with a photographic facility. The diameter of microspheres was determined using the scale bar on electron micrographs.

2.4.4 GPC

The molecular weight of the polymers before and after microsphere preparation and during degradation was followed by GPC. An adjustable flow rate pump (Altex model 110 A) preceded by a sintered metal frit was used to pump HPLC grade chloroform at 1 ml/minute around the system. Two 300 x 7.5 mm, 500Å pore size, 5μm mixed pore highly cross-linked spherical macroporous polystyrene-divinylbenzene matrix (PLGel) columns (Polymer Laboratories Ltd, Shropshire, U.K.) were used in series and were protected by a 50 x 7.5 mm 10μm mixed pore guard column (PLGel) (Polymer Laboratories Ltd, Shropshire, U.K.). A Pye Unicam LC3 UV detector at a wavelength of 254nm was used for sample detection. Samples were dissolved in

chloroform, filtered and injected using a 100µl sample size through a Rheodyne injector valve (Waters, CA, U.S.A).

Standardisation of the GPC system was obtained by narrow-MW polystyrene standards (Easical, Polymer Laboratories Ltd, Shropshire UK). Inert PTFE strips coated with polystyrene (~5 mg) were immersed in 5 ml of chloroform. There were two types of strips each representing Mw values of 580, 9200, 66000, 330000, 3040000 and 3250, 28500, 156000, 1030000, 8500000 respectively. The calibration curve of retention time was obtained under the same conditions used for the polymer and microspheres

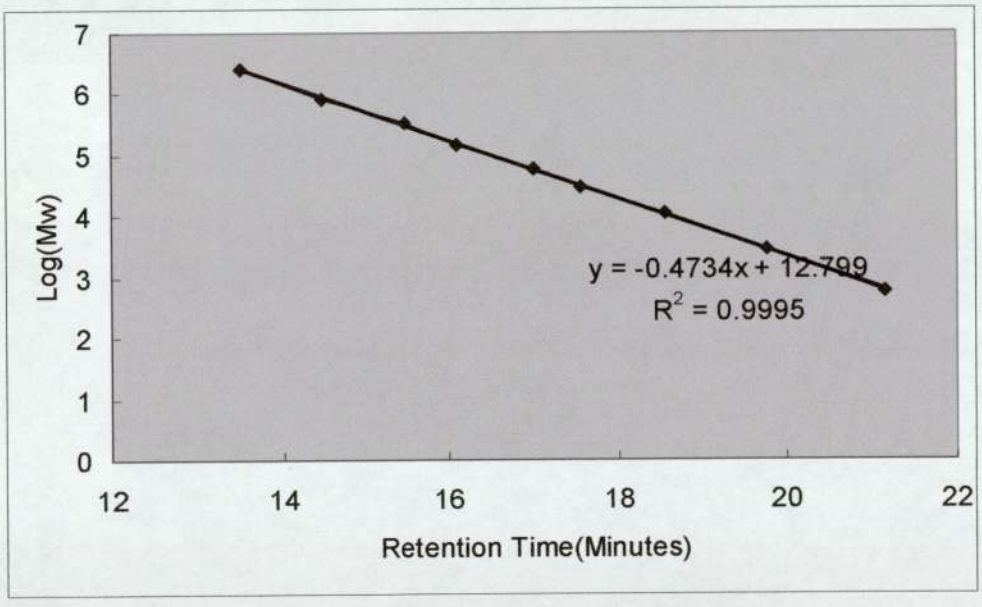


Figure 2.1 A calibration curve for estimation of molecular weight by GPC.
Molecular weight refers to weight average molecular weight.

2.4.5 Weight loss study

Weight change during the degradation studies was using an analysed by the analytical balance (KERN 770, German). Each experiment was performed in triplicate and results were the mean of three samples.

2.4.6 NMR study

The composition of P(CPP:SA) copolymer after degradation was determined by ^{1}H-NMR (Bruker AC 250 NMR Spectrometer using QNP probe head), using win –NMR version 3 software.

2.5 Microsphere Preparation

2.5.1 Preparation of Microspheres by a Double Emulsion Method (w/o/w)

2% w/v and 0.1% w/v polyvinyl alcohol (PVA) solutions were prepared by dissolving 0.2g and 0.1g PVA in 10ml and 100ml double distilled water. The solutions were stored at 4℃ until use. To form primary emulsion, 500 µl of aqueous 2% (w/w) BSA was added to 5ml DCM containing 0.1g polymer(CPP:SA 20:80). These solutions were emulsified by probe sonication (Soniprep 150), output 80w for 3 minutes on ice. (This organic solution of polymer in DCM, 2% and 0.1% PVA solution were cooled in an ice bath for one hour before use.) Next, 10 ml of 2% (w/v) PVA double distilled water solution was poured into the primary emulsion immediately and mixed vigorously on a vortex mixer for 1 minute to form the double emulsion. The resultant double emulsion was added into 100ml 0.1% (w/w) PVA solution and stirred at room temperature for 4 hours on a magnetic stirring plate, to facilitate complete evaporation of the DCM and to harden the microspheres. The suspension obtained was centrifuged at 10000rpm for 35 min (JA-14 rotor, Beckman Centrifuge U.K.). After withdrawing the supernatant the solid microspheres collected were rinsed twice, with 100ml double distilled water

and lyophilized with a freeze dryer. The free-flowing powder was stored in a desiccator in a refrigerator at 4°C.

Under the same experimental conditions, microspheres of PLGA(50:50) ,PLGA(75:25) and PLGA +CPP:SA(50:50) were prepared.

2.5.2 Preparation of Microspheres by Oil-in-Oil Method (o/o)

P(CPP:SA) 20:80 microspheres were prepared as follows: 0.1 g polymer was dissolved in 2 ml DCM, BSA powder (diameter less then 53 μm after sieving) was suspended in the solution by a vortex mixer and dropped into the external oil phase made up of 40 ml silicone oil, 10 ml DCM and 4 ml of Span 85 under stirring using an overhead stirrer (Heidolph) with a three-blade impeller, at 600rpm. The oil-in-oil emulsion was immediately poured into 180 ml petroleum ether [melting point (M.P.) 40~60] and the agitation was continued with a stirring bar for two hours to harden the microspheres. The microspheres were isolated by filtration through a 0.4 μm cellulose acetate filter, washed with petroleum ether, freeze-dried (Edwards Modulyo Freeze-drier), and stored in a desiccator in a refrigerator at 4°C.

When trying to apply the same method to PLGA(50:50) and PLGA(75:25), the above process resulted in rod formation rather than microspheres. In this case a different ratio (1:1) between silicone oil and DCM was applied. 3ml Span 85 and 110ml petroleum ether were used. The other additional procedures were as above.

2.5.3 Preparation of Microspheres by Spray Drying Method (SD)

Microspheres were prepared using a Büchi 191 mini spray dryer (see Figure 2.2,

supplied by SmithKline Beecham Pharmaceuticals). 0.4 ml 10% (w/v) BSA aqueous solution was emulsified into 20 ml 2% (w/v) polyanhydride P(CPB:SA) in DCM solution, using probe sonication at output 70 to 80 W for 4 minutes on ice, until an emulsion was formed. Microspheres were then obtained by spray drying the polymer-drug emulsion through a 0.7 mm nozzle. The emulsion was stirred on ice before feeding to prevent droplet coalescence. The collected microspheres were kept under vacuum for 24 hours. The yield was calculated from the ratio of the weight of microspheres obtained to the total amount of drug and polymer used in the preparation.

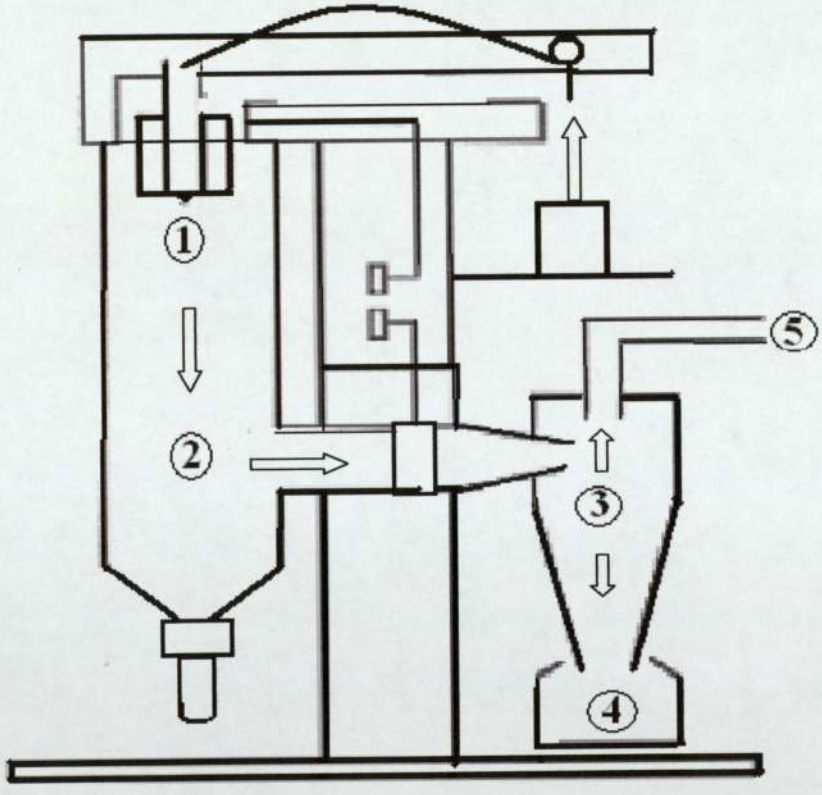


Figure 2.2 Schematic representation of Mini Büchi Spray dryer apparatus: (1) 0.7mm nozzle; (2) spray chamber; (3) cyclone; (4) collector; (5) aspirator (adapted from Fu *et al.*, 2002).

2.6 Bovine Serum Albumin Encapsulation in P (CPP: SA) Microspheres

The method used to determine protein encapsulation was adapted from literature (Hora *et al.* 1990). Briefly, about 3 milligrams of the freeze-dried microparticles, accurately weighed, were incubated in 1ml of 1M NaOH. Sodium hydroxide catalyses the hydrolysis of the polymer. Extraction of the protein occurred after degradation of the polymer for 2-4 hours shaking in a 37°C water bath (until the solution is clear). 1ml of 1M HCL was added to the solution and the BSA concentration determined using the bicinchoninic acid (BCA) assay. From this result, the percentage (w/w) of BSA encapsulated *per* dry weight of microspheres was determined. Each sample was assayed in triplicate. The percentage encapsulation efficiency was expressed by relating the actual BSA encapsulation to the theoretical BSA entrapment.

$$\text{Encapsulation efficiency\%} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100\%$$

2.7 Release study

Release experiments from P (CPP:SA) (20:80), PLGA 75:25 and 50:50 microspheres prepared by different methods were carried out in the same environments as degradation studies. NaN_3 was added at a concentration of 0.02 mg / ml as an antibacterial agent for all release media. 200 µl of sample was removed at predetermined time following centrifugation at 21000rpm for 10 min (Micro Centaur Bench top centrifuge), 200 µl of the appropritate fresh buffer was added to the samples. Each experiment was performed in triplicate and results were the mean of three samples.

The concentration of BSA in the release samples was monitored using the BCA method of protein determination (Smith *et al.*, 1985). The water-soluble sodium salt, BCA is sensitive, stable and highly specific for the Cu (I) ion forming an intense purple complex at 60°C in an alkaline environment (see Figure. 2.3). This colour generation forms the basis of the analytical method, capable of monitoring the amount of Cu (I) ion produced when the peptide bonds of a protein, complex with the alkaline Cu (II) ion (Biuret reaction). The absorbance of the purple complex at room temperature at 572 nm increases proportionally over a broad range of protein concentrations (0.5-1200 μg / ml). 200μl of the working reagent, consisting of 50 parts of BCA and 1 part 4% $CuSO_4$, was added to 10μl of the protein sample on a 96 well microtitre plate (Fisher, U.K.). The solution was incubated at 60°C for one hour, cooled to room temperature and the absorbance was read using an MRX microplate reader (Dynex, Technologies) at 570nm. Each absorbance is the average of at least 4 readings. A standard calibration was carried out each time from 10 μg/ml to 250 μg/ml. The calibration curve was constructed by plotting the absorbance of a series of protein standards subjected to the same conditions as the samples.

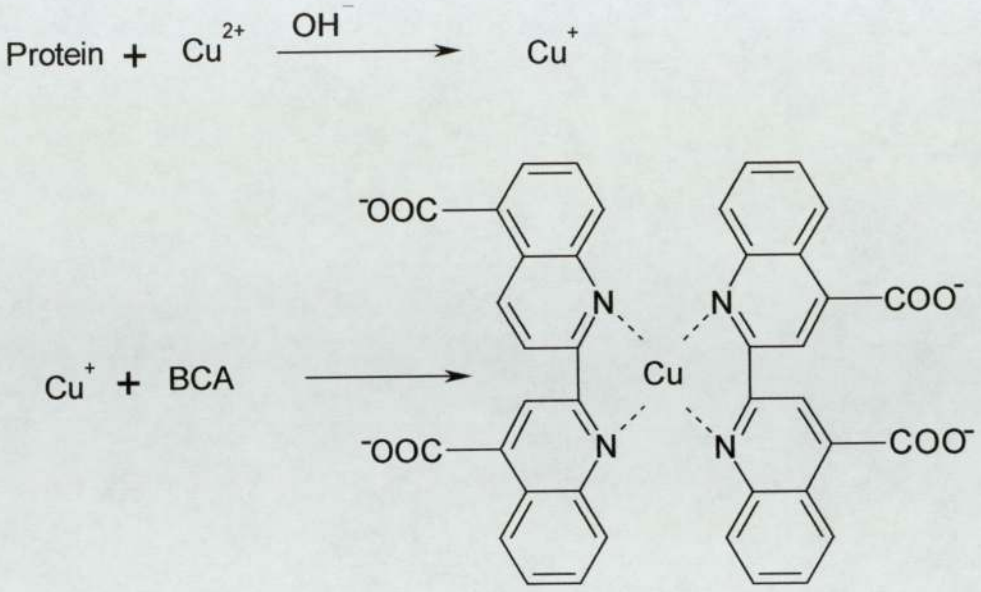


Figure 2.3 Formation of Cu (I) ion purple complex.

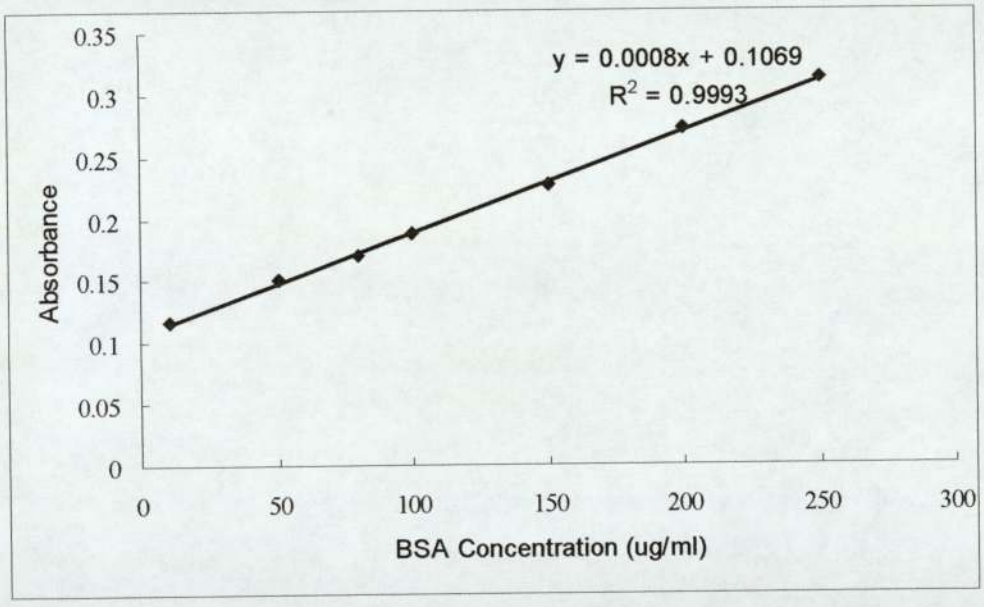


Figure 2.4 A calibration curve constructed by plotting the absorbance of a series of protein standards subjected to the same conditions as the samples.

Results and Discussion

3. Results and Discussion:

3.1Polymer Characterisation

The ^{1}H NMR spectrum of polymer P(CPP:SA) 20:50 after synthesis is shown in Figure 3.3. The composition of P(CPP:SA) copolymer was determined by ^{1}H-NMR from the ratio of the peak integration at 1.3 ppm (8H, SA) and 6.8 ~ 8.2 ppm (8H, CPP). P(CPP:SA) present characteristic peaks in infrared spectroscopy (IR) at 1740 and 1780 cm^{-1} (Figure 3.6a). The melting point of P(CPP:SA) was 68.71 °C and the heat fusion was 66.74 J/gram determined by DSC. The ^{1}H NMR spectrum is shown in the figure 3.1.

3.2 Polymer degradation

Polyanhydrides are composed of a hydrophobic polymer backbone joined by anhydride linkages that readily split in the presence of water to form two carboxylic acid end groups.

Molecular weight change during degradation of P(CPP:SA) was followed by GPC and presented in Figure 3.1. The original molecular weight was measured before the beginning of degradation experiments was 62.9kDa. In the first 24 hours, molecular weight was measured every 6 hours and the respective molecular weights were 43.4kDa, 13.7kDa, and 4.6kDa. An obviously sharp decrease in molecular weight was observed during the first 24 hours. After 24 hours degradation, the molecular weight fell to 3.0kDa. After 3 days degradation, the molecular weight had fallen to 1,000, and

then remained constant. After 2 days degradation, the molecular weight was remained at ~1000 for another 1 day for P(CPP:SA) 20:80.

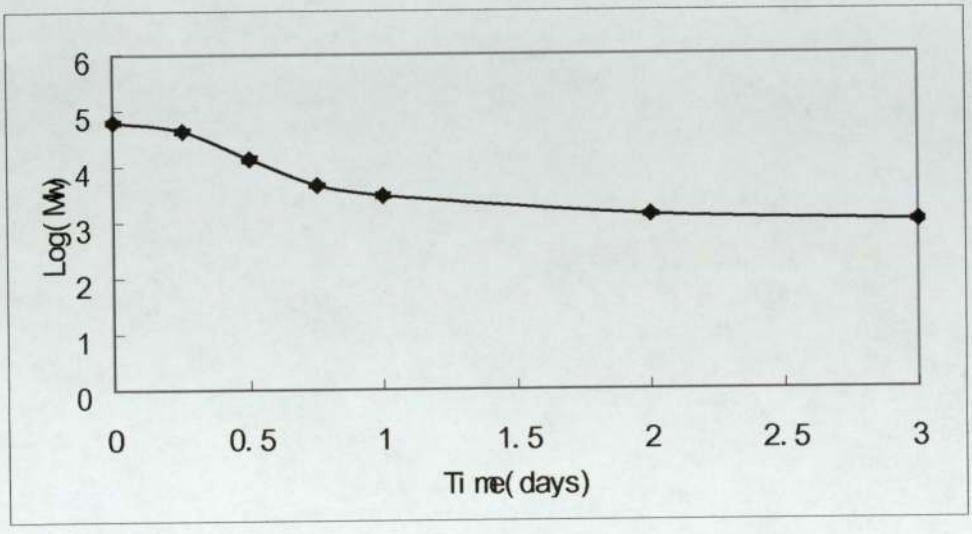


Figure 3.1 Molecular weight changes in P(CPP:SA) during degradation. (n=3).

Degradation was also studied by NMR technology.

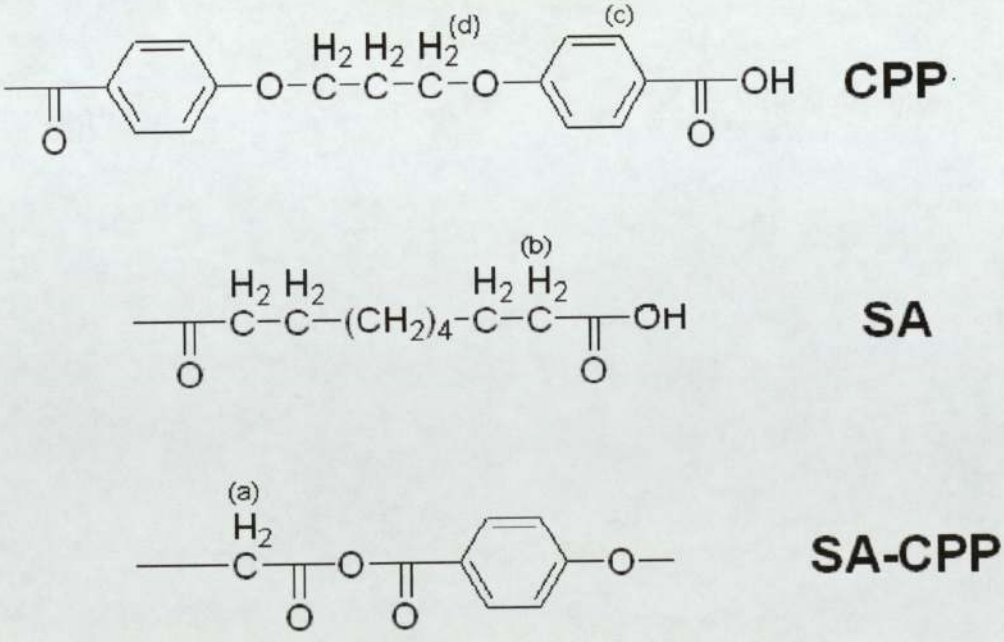


Figure 3.2 The respective H of ^{1}H-NMR spectrum in P(CPP-SA).

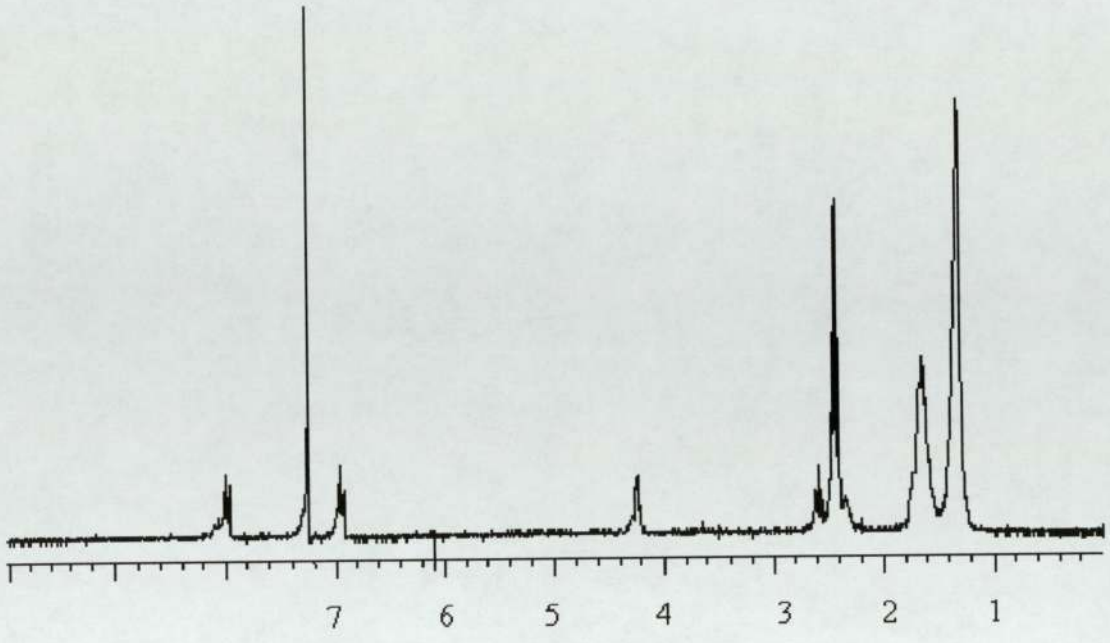


Figure 3.3 ^{1}H NMR spectrum of P(CPP:SA) 20:80

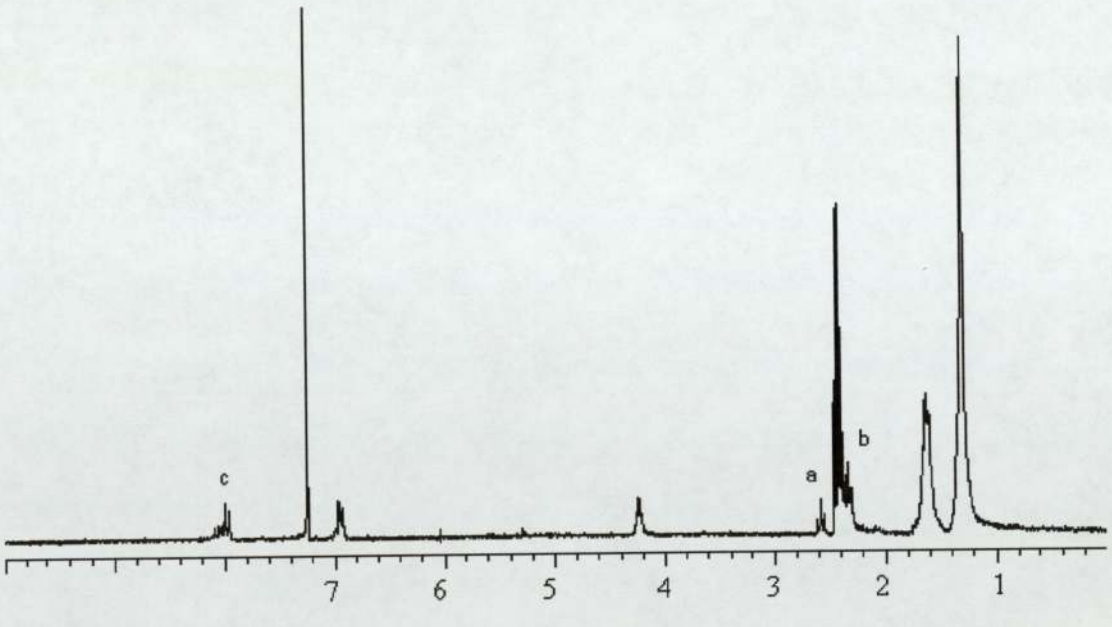


Figure 3.4 ^{1}H-NMR spectra of P(CPP:SA) after 24 hours

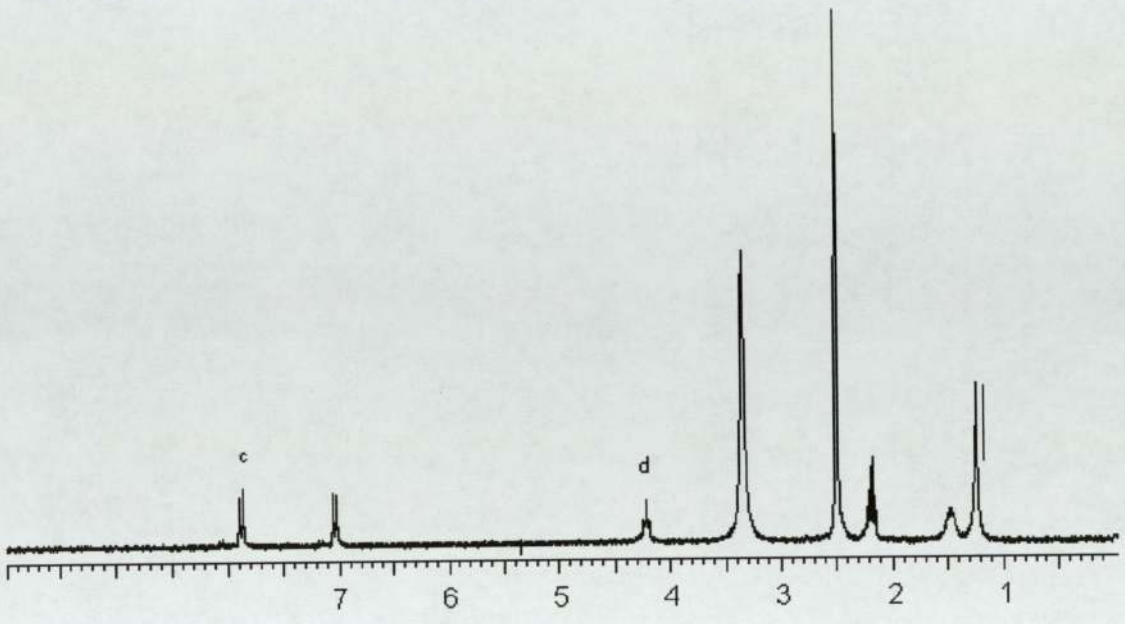


Figure 3.5 ^{1}H-NMR spectra of P(CPP:SA) after 7 days

Figures3.4 and 3.5 show the ^{1}H-NMR spectra of P (CPP:SA) after 24 hours and 7 days degradation. The polymer P(CPP-SA) is a random copolymer. Therefore, the chain contains a distribution of CPP-CPP, CPP-SA, and SA-SA linkages. From the spectra, after 24 hours degradation the peaks of CPP-CPP, SA-SA, and CPP-SA were all visible, but compared to the original spectrum of P(CPP-SA) 20:80, the SA-SA peak had decreased. From the 7 days spectrum of P(CPP-SA), the peaks of SA-SA and SA-CPP have disappeared, but the peak of CPP-CPP (d in Figure 3.5) is still clear and obvious. Degradation is relatively rapid initially as the SA-SA and CPP-SA bonds break. After the SA is depleted, a partially eroded device containing only CPP-CPP bonds is left.

Anhydrides present characteristic peaks in infrared spectroscopy (IR). In general,

aliphatic polymers absorb at 1740 and 1810 cm^{-1} and aromatic polymers at 1720 and 1780 cm^{-1}. The IR spectrum of P(CPP:SA) is shown in figure 3.6. From the IR spectrum of P(CPP:SA) 20:80, after 6 hours degradation, the peak of aliphatic anhydride bond at 1740 cm^{-1} became very small, after 3 days degradation, this peak had disappeared; even after 6 days degradation, the peak for the aromatic anhydride bond at 1780 cm^{-1} still can be seen, though it is smaller; after 30 days degradation both these peaks are eliminated. The anhydride of SA is much more easily attacked by water than CPP, and the aliphatic SA monomers erode significantly more quickly than aromatic CPP entities.

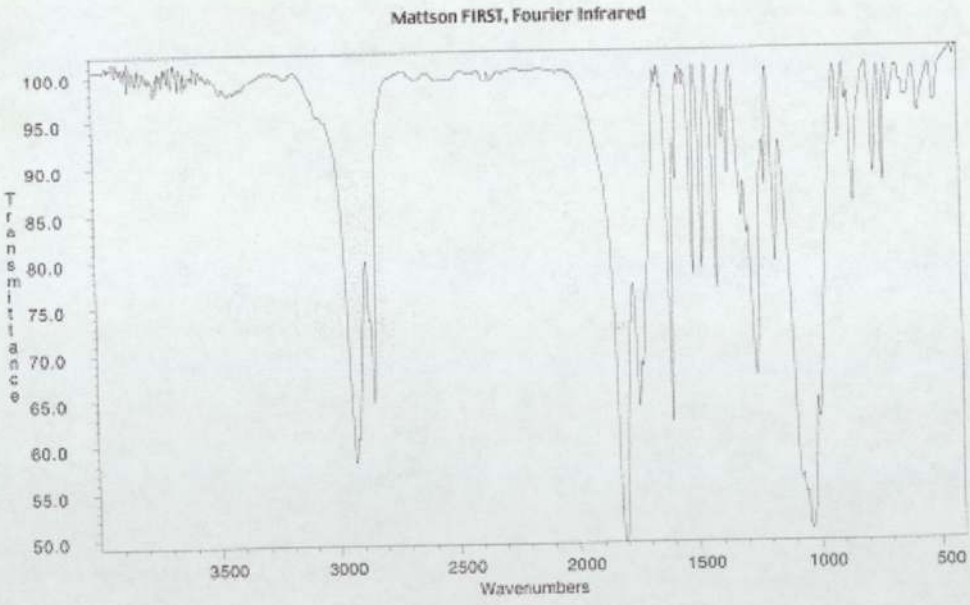


Figure 3.6a IR spectra of P(CPP:SA) 20:80

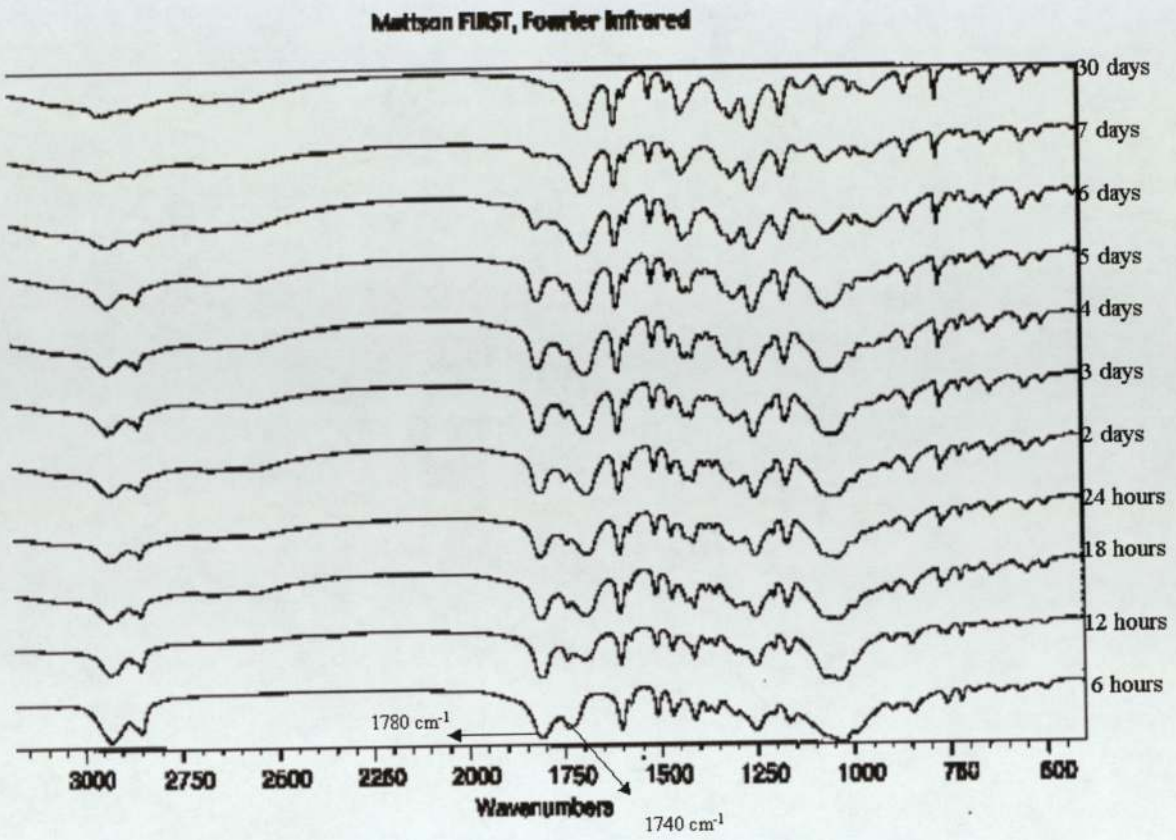


Figure 3.6b IR spectra of P(CPP:SA) 20:80 during degradation

During the copolymer degradation period, weight loss of polymer was observed. For P(CPP:SA), when degradation occurs, sebacic acid, the relatively water soluble comonomer, is released from the copolymer leaving the less water soluble comonomer CPP, which is slow to dissolve. The weight loss of P(CPP:SA) 20:80 is shown in figure 3.7.

During the initial 18 hours, there is no obvious mass loss, however, the following 6 hours, up to 24 hours, there is nearly 7% mass lost; after 5 days degradation more than 40% of the mass was lost and during the following one-month period, no further weight loss was observed. The sharp weight loss is caused by co-monomer, sebacic

acid, dividing from the copolymer. When most of sebacic co-monomer is lost, and only the less water soluble co-monomer CPP and part of sebacic co-monomer are left, the rate of weight loss became very slow. In theory there is 26% CPP in the original polymer by weight. At the end of 30 days degradation, most mass left was CPP.

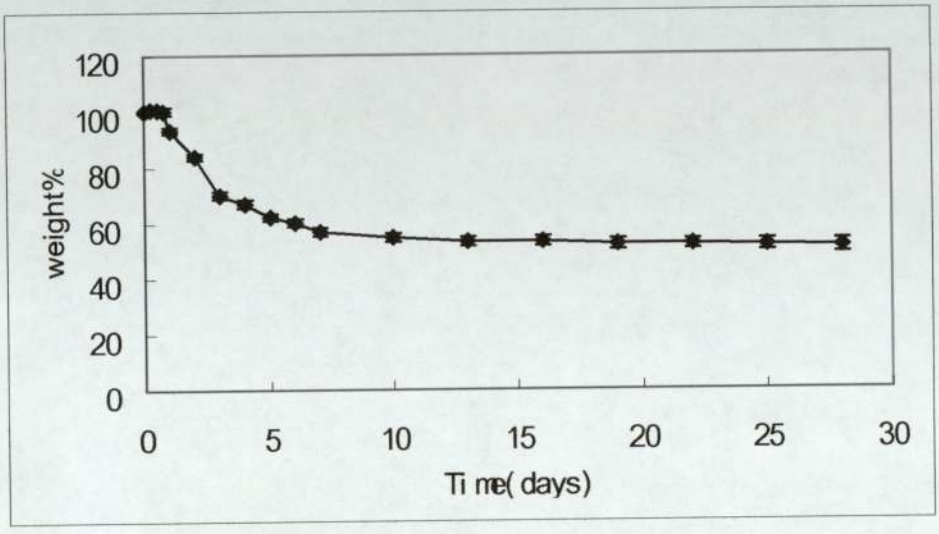


Figure 3.7 Weight loss of P(CPP:SA) 20:80 during degradation. (n=3; mean ± s.d.).

Changes in pH can also be used to monitor same sebacic acid release. Figure 3.8 shows the pH change during the degradation period.

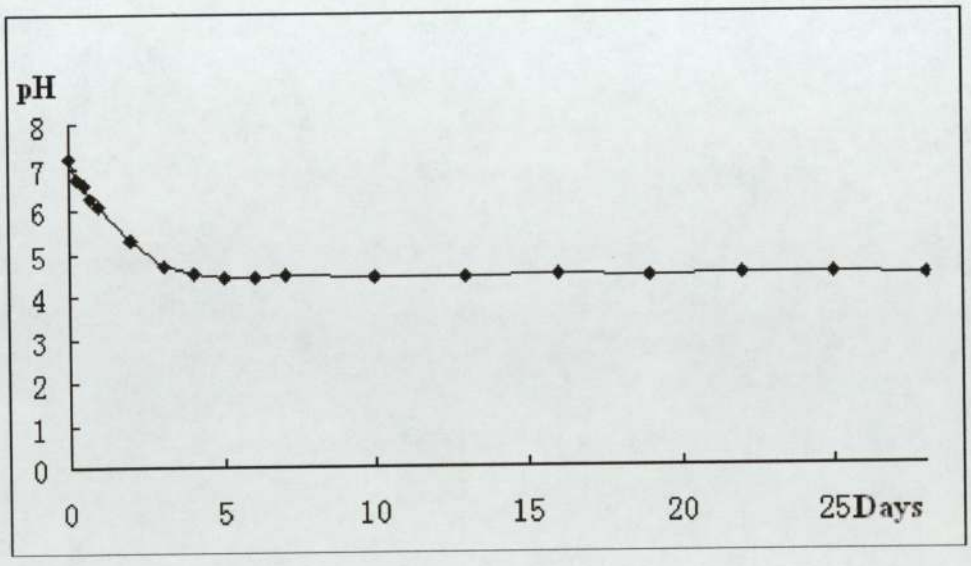


Figure 3.8 shows the pH change during the degradation period.

The starting pH of the solution is about 7. The pH changed sharply during the first 3 days of degradation, but little change was observed after this period. The pH change is caused by the acid co-monomer released from the copolymer. At the beginning, following the degradation, sebacic acid was released rapidly into the solution and the pH of the solution decreased. After a period of degradation, most of sebacic acid had been released into the solution, and then the pH of the solution was maintained at a stable level. The resultant pH of the solution was 4.42.

3.3 Microsphere preparation

3.3.1Double emulsion

Polyanhydride P(CPP:SA) 20:80 microspheres, were prepared by the solvent evaporation method using a double emulsion. All of the encapsulation efficiencies, particle sizes and the morphology of the microsphere used in this thesis are summarized in Table 3.1.

Materials	Method	Encapsulation efficiency	Size (μm)	Morphology of microspheres
P(CPP-SA) 20:80	Double Emulsion	67.6%	10-20	Smooth, porous and spherical.
PLGA 50:50		73.9%	10-20	
PLGA 75:25		70.1%	10-20	

P(CPP-SA) 20:80	Oil-in-oil	75.1%	50-100	Smooth without holes.
PLGA 50:50		76.7%	20-50	
PLGA 75:25		71.6%	10-30	
P(CPP-SA) 20:80	Spray Drying	55.6%	1-2	Aggregated, partly spherical and partly similar spheres.
PLGA 50:50		58.7%	1-2	
PLGA 75:25		60.1%	1-5	

Table 3.1 Characteristics of the microspheres prepared by double emulsion, spray drying and oil-in-oil methods.

The key factor in the size as well as size distribution of particles is the mixing rate used during the inner emulsion preparation. It was very important to control the time and power used to form the initial emulsion, therefore the same sonication conditions were maintained during the prepration process.

The molecular weight of polymer used for microspheres preparation was 60.1 kDa. The diameter and the morphology of microspheres were confirmed by the scale bar on electron micrographs. The total preparation process for the double emulsion microspheres took about 6 hours and the molecular weight following microsphere preparation by this method was 41.2 kDa.

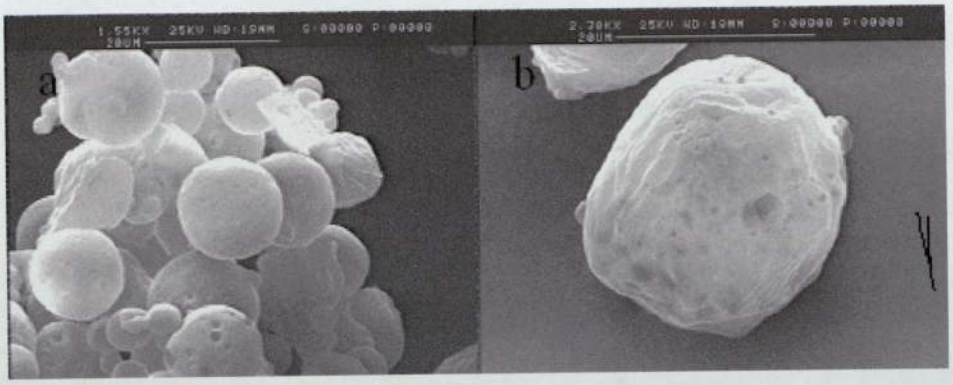


Figure 3.9a and 3.9b SEM of P(CPP:SA) 20:80 BSA-loaded microspheres, prepared by a double emulsion method.

PLGA microspheres (50:50 and 75:25), prepared by the same technique, are shown in Figure 3.10. There was no obvious difference in appearance between PLGA 75: 25 microspheres and PLGA50: 50 under SEM and the particles were porous and spherical.

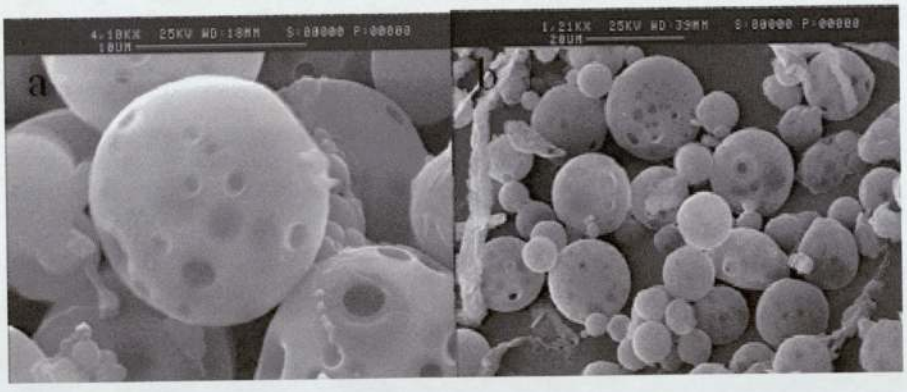


Figure 3.10 PLGA microspheres, prepared by a double emulsion method. PLGA 75:25 (a); PLGA 50:50 (b).

3.3.2 Oil-in-oil (solvent removal)

Compared to double emulsion microsphere preparation, the solvent removal method is more difficult. Successful microsphere preparation by solvent removal depends on two factors: the rate of precipitation of the polymer and the rate of DCM diffusion into the silicone oil. The polymer was dissolved in methylene chloride, and then BSA powder (diameter less then 53 μm after sieving) was dispersed in the polymer solution. The mixture was suspended in silicone oil containing span 85 and also a known amount of methylene chloride. The surfactant Span 85, which is immiscible with silicone oil, was introduced to prevent coagulation of the polymer phase. The ratio of silicone oil to methylene chloride is an important factor for successful microsphere preparation. The amount of methylene chloride depended on the type and the molecular weight of the polymer used. For P(CPP:SA) with higher molecular weight or higher percentages of CPP, the ratio of methylene chloride to silicone oil should be higher. During all preparation processes, almost no precipitation on the stirrer was observed. The diameter of microspheres prepared by this method was much larger than those prepared by double emulsion. The results could be seen by microscopy (x 400).

Polyanhydride P(CPP:SA) microspheres prepared by this method are shown in Figure 3.10. A, and PLGA 50:50 and 75:25 are shown in Figure 3.10 B and C. In all cases, the microsphere external surface was smooth, with no pores visible to SEM examination. The removal of the methylene chloride was accelerated by addition of petroleum ether, but it was always necessary to evaporate the traces of methylene

chloride entrapped in the microspheres.

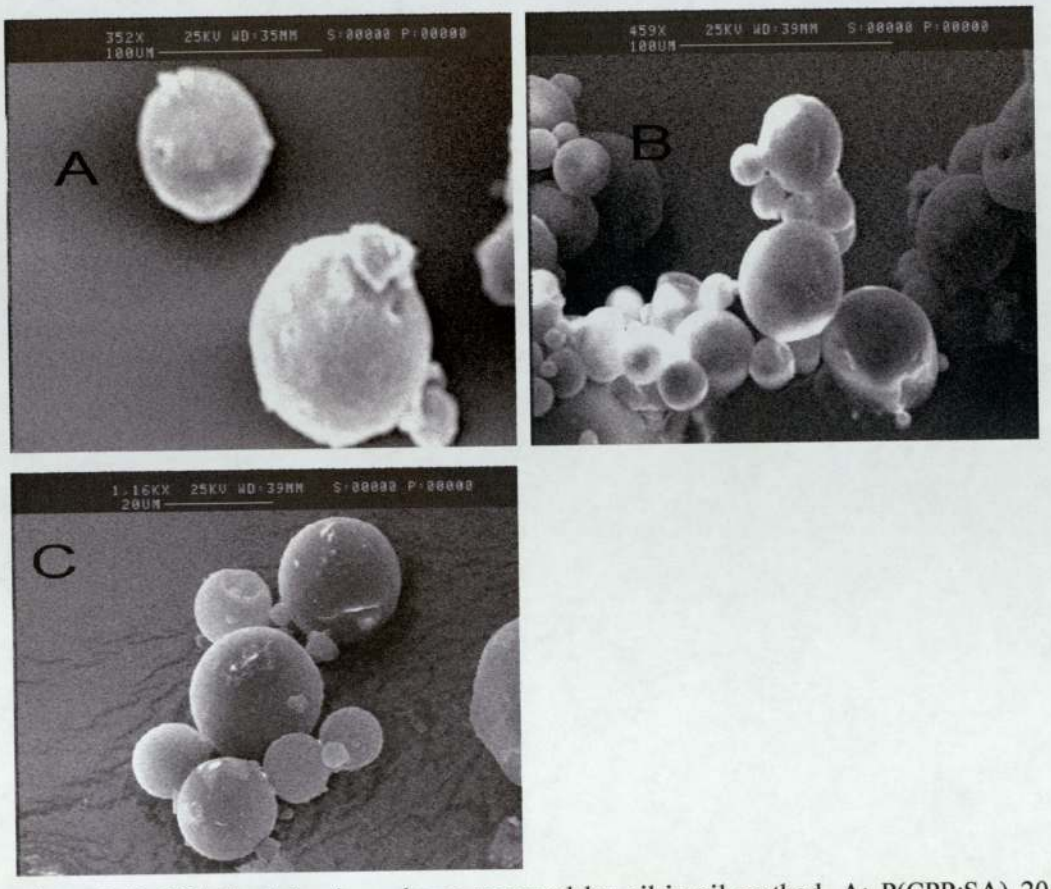


Figure 3.11 BSA-loaded microspheres prepared by oil-in-oil method. A: P(CPP:SA) 20:80; B: PLGA 50:50; C: PLGA 75:25.

A schematic representation of the process is described in Figure 3.12. The polymer solution is introduced into the oil phase; then a fast diffusion of the methylene chloride into the oil phase takes place. The concentration of the polymer near the wall is high, which explains why precipitation of the outer shell occurs first leaving high concentrations of polymer dissolved in methylene chloride inside the core. This organic solvent can be later removed by addition of a nonsolvent or applying high vaccum. The process of microencapsulation is diffusion-controlled, at least in the first stages where the difference in concentration of DCM between the two phases is significant (Mathiowitz & Langer, 1991).

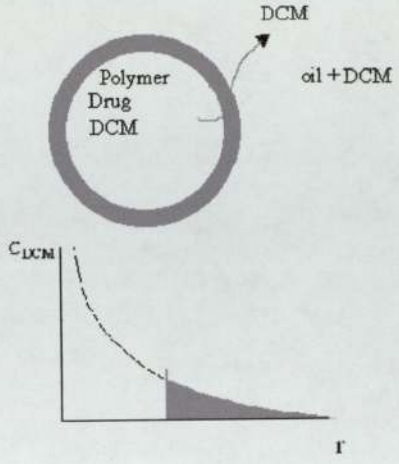


Figure 3.12 Schematic representation of solvent removal process (r) Distance from center of the microspheres; (C_{DCM}) Concentration of DCM inside microspheres during solidification process. (Adapted from Mathiowitz *et al.*, 1990a).

3.3.3 Spray drying

Spray drying techniques have shown considerable potential for formulating biodegradable microparticles. The spray drying process consists of converting liquid into powder by spraying a solution or a liquid dispersion through an atomisation nozzle into hot air. The solvent evaporates very quickly and the solid particle can be collected. It is a reproducible, rapid, and easy to scale up method. In this method, the polymer is dissolved in a solvent such as chloroform or methylene chloride along with the drug in a dispersed form. The solution is sprayed through an atomizer. As the particles fall toward the bottom of the spray drier, they are simultaneously dried by an

upward flow of nitrogen.

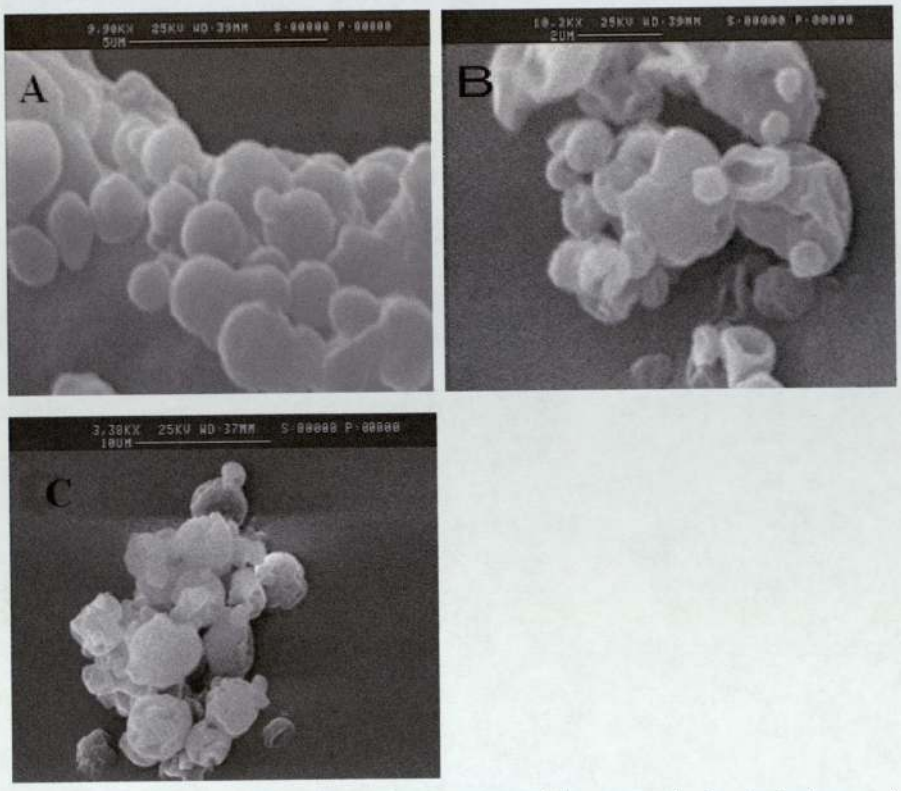


Figure 3.13 BSA-loaded microspheres prepared by spray drying techniques. A: P(CPP:SA) 20:80; B: PLGA 50:50; C, D: PLGA 75:25.

The microspheres prepared by this method are shown in Figure 3.13. Aggregation happened in all cases, and microspheres prepared by this method are only partly spherical.

The polymer solution is atomized into liquid droplets. These droplets then undergo evaporation. During the process, when a critical polymer concentration is reached, there will be a phase transition taking place on the surface of droplets, and sequent form a thin layer (crust) (Giunchedi and Conte, 1995). The solvent, especially the solvent within the crust, will continue to evaporate from the particle. If the crust is sufficiently dried, then dry and spherical particles will be obtained. Microspheres will be deformed or adhere to each other in the spraying chamber and resulting

aggregation will occur if they are not sufficiently dry or hard. This aggregation can also lead to the accumulation of microspheres in the spray drier trap and on the chamber wall and then parts of the microspheres are prevented from reaching the final collecting tube. This could be the cause of the aggregation seen in all these preparations.

3.3.4 Drug release from microspheres

In vitro BSA release from microspheres, prepared by different techniques, in PBS at 37℃ was measured. The drug release behaviour depends on many factors including the choice of polymers and the formulation procedure.

The BSA cumulative release in PBS for P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres prepared by the double emulsion method is shown in figure 3.14.

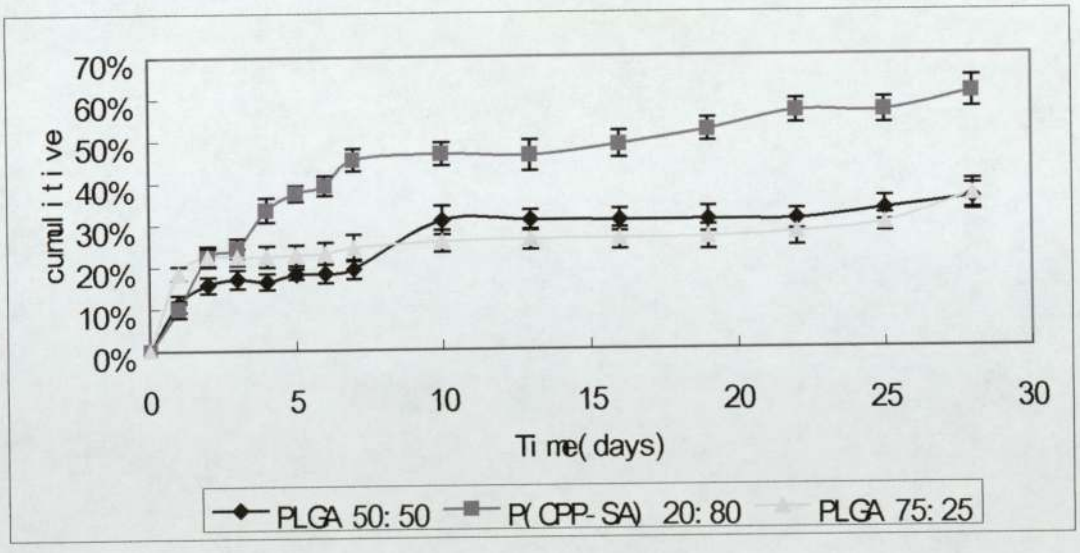


Figure 3.14 P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres, prepared by a double emulsion method, cumulative release, (n=3; mean ± s.d.)

The cumulative release from P(CPP-SA) 20-80 microspheres is the highest of three microsphere preparations and during the first 7 days, the BSA release followed a linear trend (R^2=0.9497). This could be explained by surface erosion of P(CPP-SA). From the graph we can see, for P(CPP-SA) 20-80 microspheres most of BSA release happened in the first 7 days (45%), after that the release was slow, and after one month the cumulative BSA released was about 60%. Degradation of P(CPP:SA) 20:80 is very fast due to the rapid hydrolysis of the polyanhydride bond and this way may lead to this fast BSA release. For PLGA 50:50 and 75:25 microspheres, there was about 15-20% released in the first day, after that the speed of BSA release was very slow. At the end of the experiment, there was only about 25-30% BSA released. Alonso suggested that the percentage of protein released was higher and less consistent for microspheres prepared when the protein powder was dispersed (Alonso *et al.*, 1993). Then the protein could leach out from the porous structure of microspheres. This may explain the fast protein release in the first 24 hours.

The BSA cumulative release in PBS for P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres prepared by the oil-in-oil method are shown in Figure 3.15.

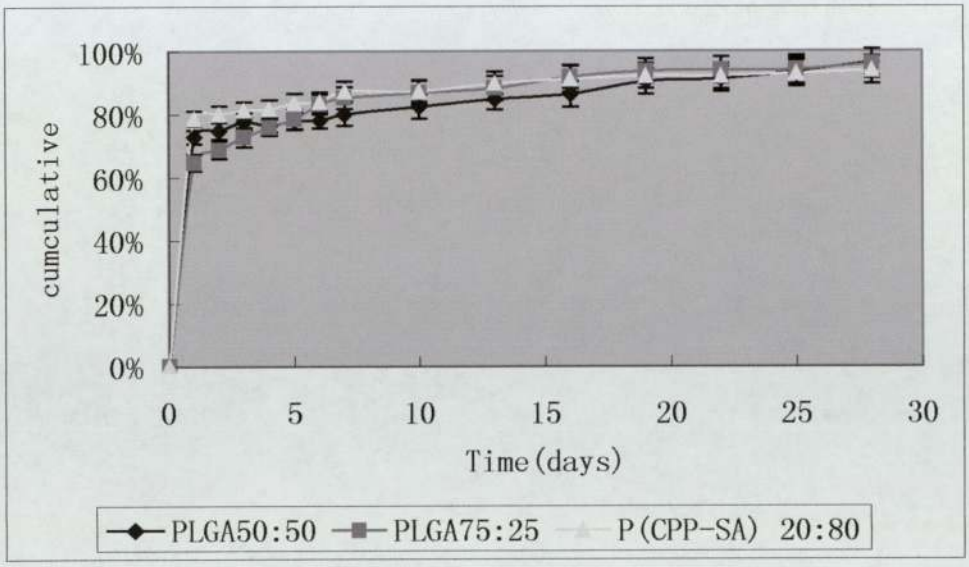


Figure 3.15 P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres, prepared by an oil-in-oil method, cumulative release, (n=3; mean ± s.d.).

In the first 24 hours, the BSA release from three microsphere preparations were high. P(CPP-SA) 20:80 microspheres released more than 60%, PLGA 50:50 microspheres released 70%, and PLGA 75:25 microspheres released about 80% of the BSA. The release of the incorporated material can occur *via* two independent processes. The first is diffusion of the drug through fluid-filled pores, formed by the dissolution of the incorporated drug particles; the second is *via* erosion of the polymer matrix. The total release of drug will be the sum of these two release rates. These processes are based on the dispersion of the material as a powder or as an aqueous solution into the organic solution containing the polymer. The particle size of microspheres prepared by oil-in-oil method is much greater than microspheres prepared by any other methods. The fractured big microspheres also may lead to the 'burst' in the initial release. This may explain the 'burst' release in the first 24 hours from the microspheres prepared by an oil-in-oil method.

In the cases of double emulsion and oil-in-oil prepared microspheres, the release of the BSA is characterised by an initial 'burst effect', the intensity of which ranges from 10% to 20% for double emulsion method to 60% to 80% for oil-in-oil method. This burst effect is followed by a 'plateau' that lasts a few weeks. The presence of part of the drug in the external surface of the microspheres may lead to this 'burst effect'. And the part of the drug entrapped in the polymeric network may lead to this 'plateau' for requiring significant polymer erosion prior to drug release.

The BSA cumulative release in PBS for P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres prepared by spray drying are shown in figure 3.16.

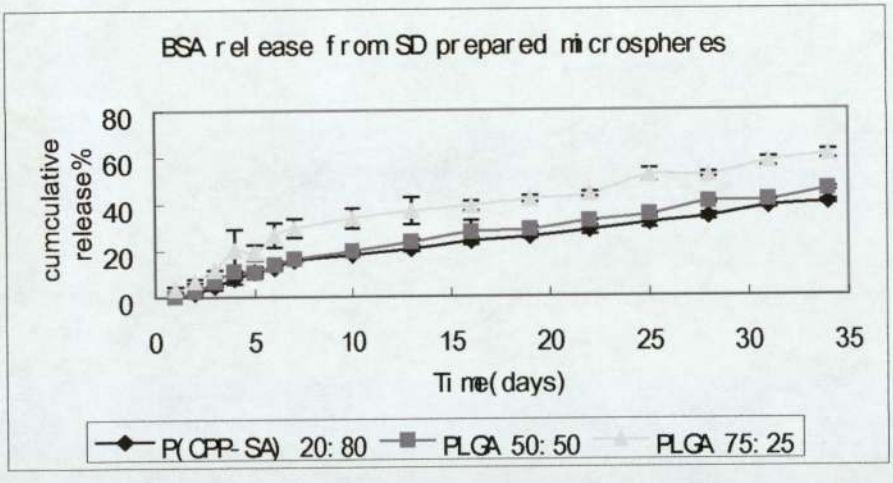


Figure 3.16 P(CPP:SA) 20:80, PLGA 50:50 and PLGA 75:25 microspheres, prepared by spray drying, cumulative release, (n=3; mean ± s.d.).

In the first 24 hours, no BSA was detected. Over the next 24 hours, the BSA release was less than 5% for all microspheres. In the first 7 days, the release of BSA of all microspheres followed a good linear trend [P(CPP-SA)20:80 R^2=0.9939; PLGA50:50 R^2=0.9609; PLGA 75:25 R^2=0.9671]. PLGA 75: 25 microspheres showed the highest cumulative BSA release at the end of 30 days, almost with 60% BSA released. The

cumulative release trends of PLGA 50:50 and P(CPP-SA) 20:80 microspheres were similar to each other, at the end of 30 days around 40% BSA has been released. The cumulative release of microspheres prepared by spray drying technique were all followed zero order kinetics [P(CPP-SA)20:80 R^2=0.963; PLGA50:50 R^2=0.9669; PLGA 75:25 R^2=0.9224]. There is no obvious 'burst effect' observed in microspheres prepared by spray drying and aggregation of microspheres may be the reason for this result. The aggregation could inhibit the contact between microspheres and PBS solution. The smooth surface may inhibit the leach-out of the protein and then led to a slow release rate.

Conclusions

4. CONCLUSIONS

Throughout this study, P(CPP-SA) 20:80 was successfully synthesised and the degradation of P(CPP-SA) 20:80 shows its surface erosion properties. Microspheres were produced using double emulsion, oil-in-oil, and spray drying techniques.

The method of solvent evaporation has been used extensively in preparation of microspheres, but the presence of an aqueous phase used in this method would be expected to initiate polymer hydrolysis. Solvent removal and spray drying methods provide new methods for microsphere preparation. The solvent removal method does not involve any aqueous phase, but microspheres prepared by this method had a relatively large particle size and an uneven distribution of BSA, leading to a initial 'burst' release of BSA.

Spray drying is a reproducible, rapid, and easy to scale up method, but due to aggregation of the microspheres, the yield was low. However with increasing the microspheres batch size to an industrial scale, this problem could be overcome. The BSA release using this method of microsphere prepared followed zero order release and after one-month release, a high percent of BSA has been released.

Theoretically, P(CPP-SA) will undergo surface erosion and PLGA will undergo bulk erosion, and the release properties of microspheres prepared from these two methods should be different. However during the comparison between P(CPP-SA) and PLGA microsphere release properties, difference were only detected in double emulsion prepared microspheres. The release of BSA from microspheres prepared by solvent removal and spray drying method do not show obvious differences.

. Future study

Polyanhydrides, P(CPP-SA), has the potential for use in the preparation of controlled delivery microspheres. The surface erosion properties could be seen during our experiment data. In this study, the degradation properties of P(CPP-SA) 20:80 have been followed by NMR, IR, Mw change and weight change. The monomer release from the polymer investigation could illuminate the degradation properties more precisely. Future work could focus on this aspect of polymer degradation.

For the microspheres preparing method, double emulsion has a limitation for the involving aqueous solution, but it has good encapsulation efficiencies and yields. Solvent removal is a potential method for microspheres preparation, optimising the preparing process is necessary to control the particle size of microspheres. The BSA releases from different microspheres prepared by spray drying show good liner release. But it's still necessary to optimise the process parameter to improve the yield.

The future research on microspheres will focus on a more precise degradation characterisation. And optimise all the preparing methods to control the particle size, and release properties of micrspehres. More comparison should be carried on between polyanhydrides and PLGA systems to illuminate the mechanism of surface erosion and bulk erosion.

References

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