BIODEGRADABLE POLYMERS AS DRUG DELIVERY SYSTEMS

Liang Zhao

Master of Philosophy

ASTON UNIVERSITY

ASTON UNIVERSITY IN BIRMINGHAM

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SUMMARY

Polyanhydrides have been investigated as a biodegradable material used for controlling the release of drugs. Poly1,3-bis(p-carboxyphenoxy) propane:sebacic acid [P(CPP:SA) 20:80] is one of the popular biodegradable polymers for controlling release of drugs as it has a character of surface degradation mechanism. In this thesis P(CPP:SA) 20:80 was synthesised by the melt condensation. The degradation of P(CPP:SA) 20:80 was investigated *in vitro* by using ^{1}H-NMR, IR to determine the manners of degrading process in line with molecular weight changes and effects on the microenvironment such as pH. Paclitaxel was selected to be encapsulated into P(CPP:SA) microspheres using PVA and Vitamin E TPGS as emulsifying agents in order to overcome its poor water solubility and maximise its anti-tumor therapeutic potential. The microspheres were characterized in terms of morphology, particle size drug content, as well as *in vitro* drug release behavior. Two implantable disks were prepared using P(CPP:SA) 20:80 and paclitaxel; one by pressing the mixture of P(CPP:SA) 20:80 and paclitaxel, the other was prepared by pressing P(CPP:SA) microspheres loaded with paclitaxel. Studies of the drug release from the disks were carried out *in vitro*.

Key words: biodegradable polymers; melt condensation; surgical removal; microencapsulate; polyanhydride; paclitaxel.

DEDICATION

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

ACKNOWLEDGEMENTS

I would like to offer thanks to my supervisor, Dr Yongfeng Wang and Dr.B.R. Conway who have provided me with good ideas and valuable advice for the completion of this report.

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Abbreviations

CPPA : 1,3-bis(p-carboxyphenoxy) propane anhydride

SA : sebacic acid

AZ: azelaic acid

FA: fumaric acid

CPA: p-Carboxyphenoxy acetic acid

CPM: Bis (p-carboxyphenoxy) methane

EAD: erucic acid dimmer

Cremphor EL: polyethoxylated castor oil

PLA: poly(lactic acid)

PGA: poly(glycolic acid)

PLGA: poly(lactic-co-glycolic acid)

PVA: polyvinyl alcohol

DPPC: L-α-dipalmitoyl-phosphatidylcholine

Vitamin E TPGS : d-α-tocopheryl polyethylene glycol 1000 succinate

HPLC : high performance liquid chromatography

NMR : nuclear magnetic resonance spectroscopy

IR: infrared spectroscopy

GPC: gel permeation chromatography

Chapter 1

Introduction

1.1 Polyanhydrides as a drug delivery system

1.1.1 The development of polyanhydrides

Polyanhydrides are useful bioabsorbable materials for controlled drug delivery. They hydrolyze to dicarboxylic acid monomers when placed in aqueous medium. Since their introduction to the field of controlled drug delivery, about 15 years ago, extensive research has been conducted to study their chemistry as well as their toxicity and medical applications. Several review articles have been published on polyanhydrides for controlled drug delivery applications (Leong *et al.*,1989; Domb *et al.*,1992; Laurencin *et al.,* 1995).

The earliest report on the synthesis of polyanhydrides was by Bucher and Slade in 1909. Years later, Hill and Carothers in1930s synthesized polymers based on aliphatic diacid monomers for textile applications. During the 1950s and 1960s, Conix and Yoda synthesized over a 100 new polyanhydrides based on aromatic and heterocyclic diacid monomers. In 1980, Langer proposed the use of polyanhydrides as biodegradable carriers for controlled drug delivery systems (Rosen, 1983), resulting so far into implantable devices for human use. For example, in 1996, the carmustine implant (GLIADEL®), a biodegradable, chemotherapy agent-loaded polyanhydride matrix, was approved by the US Food and Drug Administration after it had been demonstrated to be a well tolerated and effective treatment for recurrent malignant glioma.

1.1.2 The synthesis of polyanhydrides

Polyanhydrides have been synthesized by various techniques; melt condensation, dehydrochlorination, and dehydrative coupling agents (Leong *et al.*, 1989; Domb *et al.*, 1993). Methods used for the synthesis of polyanhydrides are listed in Figure1.1.

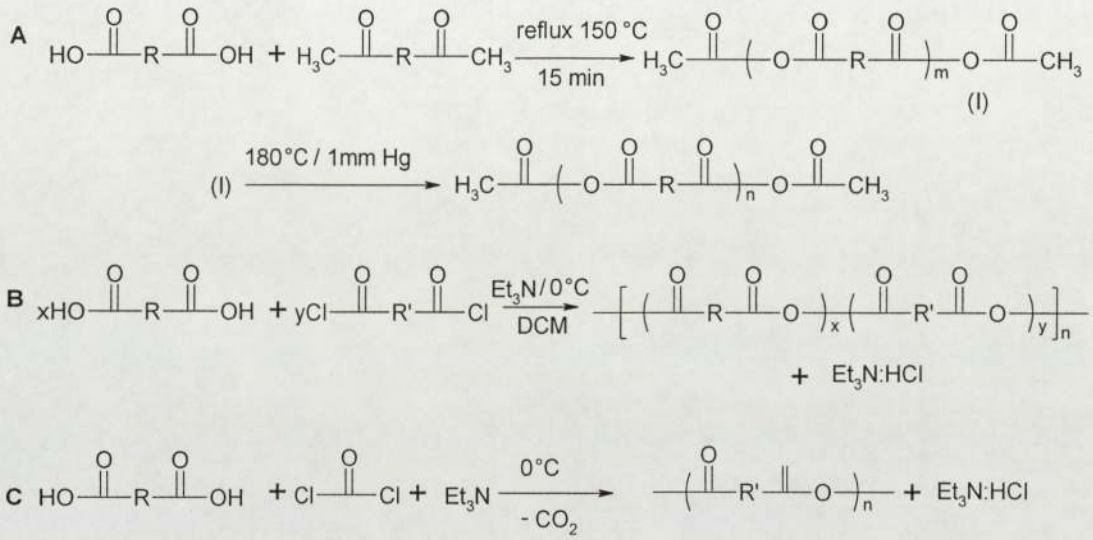


Figure 1.1 Methods for the synthesis of polyanhydrides (Kumar *et al.*, 2002) (A: melt condensation B: dehydrochlorination C: dehydrative coupling agents)

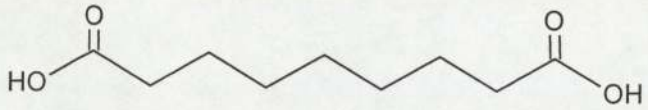
One major drawback of previous work on polyanhydrides was their low molecular weight, which held back the development of polyanhydrides in the medical area. Melt condensation was found to be a good technique to get high molecular weight polymers. Melt polycondensation involves a number of steps. Prepolymers are synthesized by reflux of diacid in excess acetic anhydride for several hours. This forms the mixed anhydride prepolymer. The crude prepolymer is purified by recrystallisation from dry toluene, and then immersed in a 1:1 mixture of dry petroleum ether and diethyl ether to extract traces of acetic anhydride and toluene. The purified prepolymers are placed

into a temperature-controlled vessel under high vacuum, where polymerization takes places. The acetic anhydride, which is produced by the polymerization reaction, is removed by vacuum. The optimum temperature for polymerization was determined to be 180°C to synthesise polyanhydrides with high molecular weight. After polymerisation is completed, the polymer is purified by precipitation in dry petroleum ether from a dichloromethane solution. The precipitate is then extracted with dry diethyl ether for several hours at room temperature. The polymers are stored under dry nitrogen at -20°C (Domb and Langer, 1987). A variety of catalysts have been used in the synthesis of a range of polyanhydrides by melt condensation. Particularly, coordination catalysts facilitate anhydride interchange during the polymerization and enhance the nucleophilicity of the carbonyl carbon. For example, cadmium acetate and earth metal oxides can contribute to polyanhydrides with high molecular weights in a shorter reaction time. However, the potential toxicity of the catalysts is a drawback in the synthesis of polyanhydrides by this method (Domb and Langer, 1987).

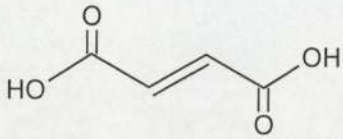
1.1.3 Polyanhydride structures

1.1.3.1 Monomers of polyanhydrides

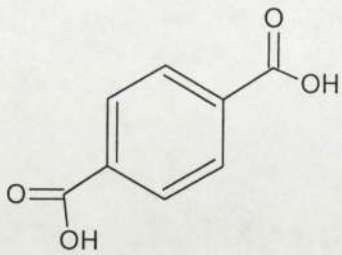
Since polyanhydrides are made of different monomers, the properties of the monomer determines the characteristics of polyanhydrides in some extent. Polyanhydrides can be synthesised as homopolymers (R1=R2), or as copolymers (R1≠R2). Some of the numerous monomers that have been used for the manufacture of polyanhydrides are shown in Figure 1.2.



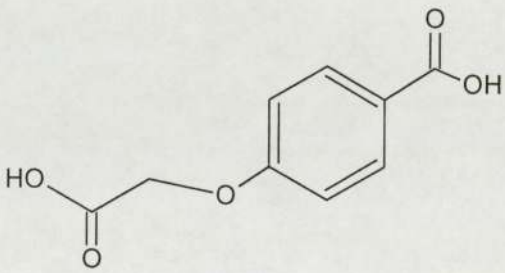
Azelaic acid (AZ)



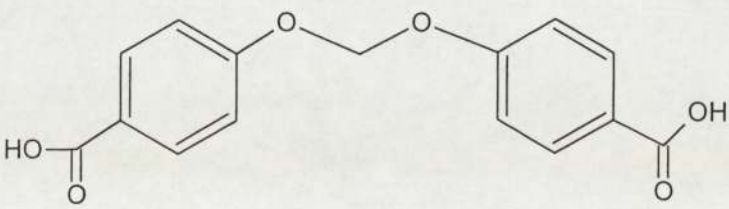
Fumaric acid (FA)



Para: terephtalic acid



p-Carboxyphenoxy acetic acid (CPA)



Bis (p-carboxyphenoxy) methane (CPM)

Figure 1.2 Monomers for the synthesis of polyanhydrides

1.1.3.2 Aliphatic polyanhydrides

Aliphatic polyanhydrides were among the first polyandrides to be investigated for the purpose of drug delivery (Göpferich *et al.*, 2002). General structure of aliphatic polyanhydride is shown in Figure 1.3. Homopolymers are often problematic materials as they are usually highly crystalline with unfavourable mechanical properties. p(SA) (sebacic acid), for example, has a crystallinity of 66% (Mathiowitz *et al.*, 1990). Its microstructure is composed of crystalline and amorphous domains which are of utmost importance for the erosion mechanism. In some cases, aliphatic polyanhydrides have been used for blending with other polymers.

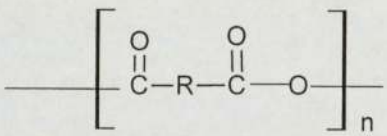


Figure 1.3 General structure of aliphatic polyanhydrides (R=Aliphatic chain and n are the number of units)

1.1.3.3 Aromatic polyanhydrides

The development of aromatic polyanhydrides has been relatively slow over the last decades. Aromatic polyanhydrides have low solubility in common organic solvents and have high melting points (Leong, 1985); therefore, they can not be easily fabricated into films or microspheres using solvent or melt techniques (Domb *et al.*, 1989).

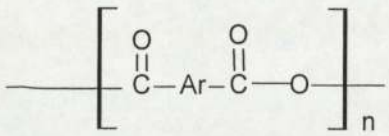
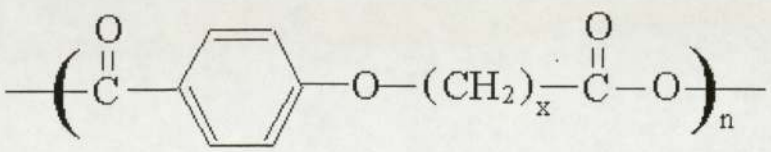


Figure 1.4 Homopolymers of aromatic polyanhydrides (Ar =  n are the number of units)

1.1.3.4 Aliphatic-aromatic homopolyanhydrides

From a historical perspective, the properties of polyanhydrides need to be improved in order to obtain materials with superior properties, i.e., better mechanical characteristics and materials with adjustable erosion times. There have been numerous approaches to do so; however, one of the most successful polymer types was a copolymer made of sebacic acid and 1,3-bis(*p*-carboxyphenoxy)propane (p(CPP-SA)). First reports of using these polymers as a biomaterial goes back to the early 1980s (Rosen *et al.*, 1983). Since then, there have been numerous reports on their synthesis (Domb *et al.*, 1989) and characterization (Maeder *et al.*, 1997). p(CPP-SA) is probably the best characterized material in the family of polyanhydrides.



X=1-10

Figure 1.5 General structure of aliphatic-aromatic polyanhydrides

1.1.3.5 Polyanhydrides derived from fatty acids

The basic idea behind developing polyanhydrides from fatty acids was to obtain materials with a pronounced hydrophobic character and improved mechanical properties. The hope was to increase the hydrolytic resistance of polyanhydrides and concomitantly slow down the intrusion of water into polymer matrices. However it was not successful because a high concentration of acetyl stearate in the reaction mixture resulted in the formation of much amount of stearic anhydride by-product (Kumar *et al.*, 2002).

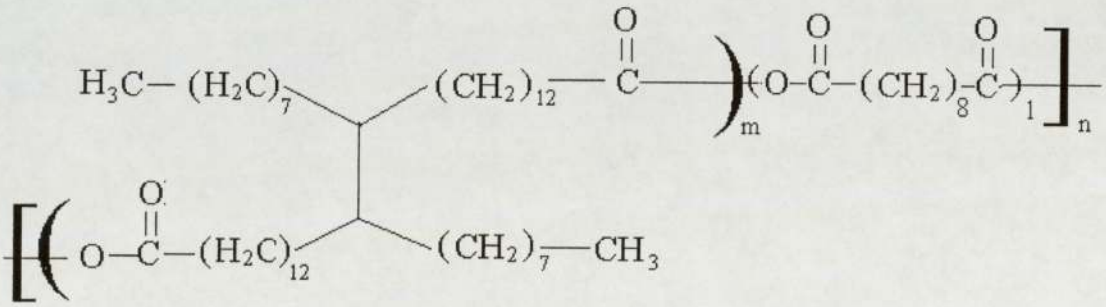


Figure 1.6 Copolyanhydrides of erucic acid dimer (EAD) and sebacic acid (SA)

1.1.3.6 Cross-linked polyanhydrides

The often limited mechanical stability of polyanhydrides has always been a handicap to their use as biomaterials for orthopaedic applications such as temporary replacement in bone defects. Therefore crosslinked polyanhydrides have been

developed for high mechanical strength and slow degradation. The crosslinked polyanhydrides are prepared from the monomers having anhydride bonds and unsaturated endcaps, e.g. vinyl or 2-propenyl groups.

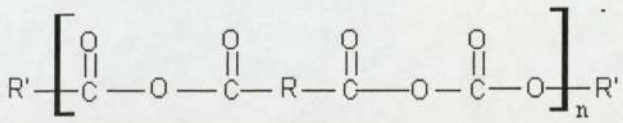


Figure 1.7 General structure of cross-linked polyanhydrides. R is an alkyl or aromatic chain and R' is a vinyl or 2-propenyl group.

1.1.4 Characterization of polyanhydrides

1.1.4.1 Polymer composition by ^{1}H-NMR

By using ^{1}H-NMR, the average length of sequences and the frequency of occurrence of specific comonomer sequences can be determined. The protons on the aromatic ring close to the anhydride groups experience a lower density of shielding electrons and absorb at lower frequency. On the other hand, the protons next to aliphatic comonomers, absorb at higher frequency accordingly.

1.1.4.2 Molecular weight

The molecular weight of polyanhydrides can be detemined by gel permeation chromatography (GPC) (Ron *et al.*, 1991). When sample molecules with different molecular size run through columns which are tightly packed with gel and filled with solvent (mobile phase), they were retarded for different times with column because of

the limitation pore size of gel particles. The retention time is proportional to their molecular size. Traditionally, GPC has been used for the analysis of molecular weight distributions of synthetic polymers. Molecular weight averages (Mn, Mw) indicate the number and length of the polymeric chains formed during manufacture. Mn is the molecular weight of the average chain length in a polymer sample. Mw is the modal molecular weight of polymer chains. Therefore the value of Mw is always larger than Mn if the polymer is not monodisperse. The change of molecular weight during the degradation process of polymer is a significant factor in the investigation of polymer degradation mechanism (see section 3.2.1).

1.1.4.3 Infrared spectroscopy

Anhydrides present characteristic peaks in IR and Raman spectra. 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 containing aliphatic and aromatic anhydride bonds may present 3 distinct peaks, where the aliphatic peak is evident at 1810 cm^{-1}; the aromatic peak at 1780 $cm^{-1}$and the peaks at 1720-1740 cm^{-1} in general overlap. The presence of carboxylic acid groups in the polymer can be determined from the presence of a peak at 1700 cm^{-1} The degradation of polyanhydrides can be followed by IR using the ratio between the anhydride peak at 1810 and 1700 cm^{-1}. The significance of this analysis is that it measures the degradation of the anhydride bonds and not the dissolution of the degradation products which are dependent on the solubility of the degradation products.

1.1.5 Degradable Polymeric Drug Delivery Systems

Biodegradable polymeric drug delivery systems have several advantages compared to conventional drug therapeutics, including improved patient compliance, avoidance of the peaks and valleys of drug plasma levels associated with conventional injections, localized delivery of the drug to a particular body compartment or cell type, thereby lowering the systemic drug levels, protection of drugs that are rapidly degraded in the body, and improved drug efficacy. In addition, compared with non-degradable systems, biodegradable systems have some potential advantages. Firstly, the polymer matrix system plays an important role in controlling the drug release in biodegradable polymeric drug delivery systems. For example, varying a copolymer composition can change its degradation rate which can determine the release rate of contained drug. After the polymer matrix system is degraded fully a nearly complete release can always be achieved in a biodegradable system. Secondly the release rate may be more stable with time compared to conventional drug delivery systems. A biodegradable system may yield constant release even with a simple monolithic device.

1.1.5.1 The definition of polymer degradation and erosion

Degradation is the chain scission process that breaks polymer chains down to oligomers and finally into monomers, and is the most important part of erosion. Through degradation, oligomers and monomers are created that finally diffuse to the polymer surface, where they release from the polymer bulk. Erosion designates the

sum of all processes that can lead to the loss of mass from a polyanhydride matrix irrespective of its geometry, such as slab, cylinder or microspheres. It is obvious that degradation takes place earlier than erosion during the break down of polymer. In addition to degradation, other processes can contribute to erosion as well. For example mechanical instabilities can result in the loss of pieces of non-degraded material and some polyanhydride matrices became fragile and brittle so that parts of the matrix may wear off under weak mechanical forces applied during a *in vitro* erosion experiment (Göpferich and Langer.,1993).

1.1.5.2 The definition of surface erosion and bulk erosion

Surface erosion, also termed heterogeneous erosion, designates degradation and erosion that are limited to the surface of a polymer. In an ideal scenario, the mass loss kinetics are, therefore, linear. Bulk erosion, also termed homogeneous erosion, in contrast, reflects a different mechanism. Bulk eroding polymers degrade all over their cross-section, and erosion kinetics are not linear. The schematic illustration of surface erosion and bulk erosion is seen in Figure 1.8. Surface erosion is much more desirable because

1) it can lead to pseudo zero-order drug release provided diffusional release of the drug is minimal and the overall surface area of the device remains constant.
2) release rate is independent of the chemical and physical properties of the drug.
3) release rate can be varied simply by changing loading, making the device easy to design.

4) mechanical integrity is maintained because erosion is confined to the surface.

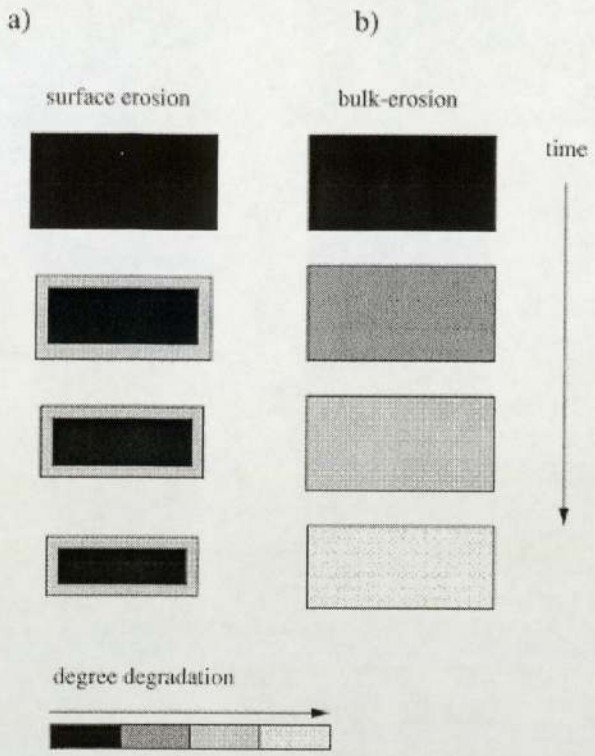


Figure 1.8 Schematic illustration of surface erosion and bulk erosion (Kumar *et al.*, 2002)

1.1.6 Advantages of polyanhydrides for drug delivery systems

Compared with other kinds of polymers polyanhydrides have unique advantages for drug delivery. Firstly, they can be made from cheap material. (Kumar *et al.*, 2002). Secondly they can be prepared easily; no purification steps are needed. Thirdly, it is possible they may have well-defined polymeric structures and degrade hydrolytically at a predictable rate. Finally polyanhydrides are considered as a surface-erosion biodegradable polymer and can lead to zero-order drug release. So it was proposed

that polyanhydrides are an ideal material for producing erosion-controlled drug delivery systems.

1.2 Paclitaxel and its formulation

1.2.1 Introduction

Paclitaxel is considered as an effective anti-tumour agent in the therapy of various cancers, especially for the therapy of ovarian and breast cancers.

Paclitaxel was obtained in early 1960s from the bark of Pacific Yew (*Taxus brebifolia*; family Taxaceae), one of the geographical varieties of yew (Wani *et al.*,1971). Paclitaxel was obtained in a pure form in 1969 and its structure (see Figure 1.9) was published in 1971.The importance of paclitaxel was not recognized until the late 1970s as it can not be synthesized easily, and it was only when its mechanism of action was uncovered, that it generated great interest. Paclitaxel is a white to off-white crystalline powder and melts at around 216-217°C. Paclitaxel is a diterpenoid pseudoalkaloid having molecular formula $C_{47}H_{51}NO_{14}$ with molecular weight of 853 Da. The whole taxane molecule is important for the anti-tumor activity. The ester and the tatraol formed by a low temperature cleavage of paclitaxel did not show the function of curing cancers(Wall and Wani,1996). The generally accepted dose is 200-250 mg per square meter and is given as 3 and 24h infusion. Pharmacokinetics of paclitaxel shows wide variability (Rowinsky and Donehower, 1993).

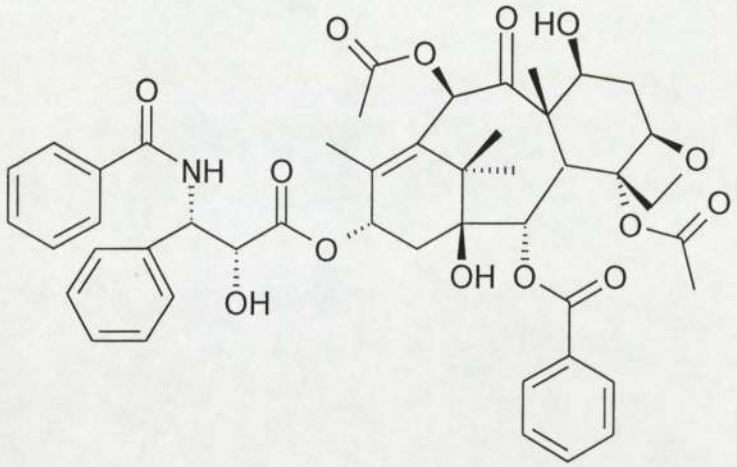


Figure1.9 Structure of Paclitaxel

Although paclitaxel has been shown to exhibit a significant activity against a variety of solid tumours, the success of its clinical application is limited by its low solubility in water. Therefore, other organic solvents such as Cremphor EL (polyethoxylated castor oil) and ethanol have been used to aid in improving the solubility of paclitaxel in water, which resulted in severe hypersensitivity reaction (Lorenz *et al.*,1977; Dye and Watkin, 1980; Weiss *et al.*, 1990). In addition, as there have been difficulties in synthesizing paclitaxel, the majority of paclitaxel was isolated from trees. It is reported that only 2g of the paclitaxel was extracted from 4 trees enough for the chemotherapy of one patient (Oliver 1993). This is not a cost-effective method of producing a drug and an environmentally acceptable practice for pharmaceutical industry.

1.2.2 Problems of formulations available in market

Currently, a 1:1 blend of Cremphor EL (polyethoxylated castor oil) and ethanol are

often used. The pharmaceutical formulation of paclitaxel (Taxol®; Bristol-Myers Squibb) contains 30 mg paclitaxel dissolved in 5 ml of this (1:1, v/v) mixture. One of the substantial problems associated with this formulation is that the use of Cremophor EL (Cr EL). When Cremophor EL as a vehicle was used in some special formulations such as cyclosporine (Howrie *et al.*, 1985) and teniposide (O'Dwyer *et al.*, 1986), its harm to human body is limited because the amount of Cremophor EL in formulations was very small. But, the amount of Cremophor EL necessary to deliver the required doses of paclitaxel is significantly higher than that of administered with any other marketed drug (Rowinsky *et al.*, 1992). The most well known side-effect of paclitaxel formulated with CrEL is a clinical acute hypersensitivity reaction, characterised by dyspnoea, flushing, rash, chest pain, tachycardia, hypotension, angio-oedema, and generalised urticaria (Weiss *et al.*, 1990). Moreover, axonal degeneration and demyelination, one of the principal side-effects of paclitaxel resulting in peripheral neuropathy, is also reputedly a biological effect caused by CrEL. In addition to the toxicity of paclitaxel, the poor physical stability the formulation limited the widely use of the drug (Adams *et al.*, 1993).

1.2.3 Importance of a paclitaxel local drug delivery system

Local drug delivery system is defined as a method of delivery of drug (for its local action) from a dosage form to a particular site in the biological system where its entire pharmacological effect is desired. As most anticancer drugs that are in clinical use are

antiproliferative but have no special target on killing cancer cells, all dividing cells in the body are affected and toxic effects exist. In order to decrease the toxicity of anti-cancer drugs for normal tissues, the drugs were formulated and delivered to body compartment harboring tumour (Dhanikula and Panchagnula 1999). Particularly for patients who received surgical treatment, cancer cells often can not be taken away from human bodies and local recurrence of primary tumors and metastatic spread often occurs. Therefore a delivery system loaded with paclitaxel at a tumour resection site will provide a high local concentration of the drug detrimental to malignant cells which may have survived surgery.

1.2.4 Formulations available in localized paclitaxel delivery

1.2.4.1 Implants

Implants may be rod- or disk-shaped devices for subcutaneous application. These devices can be manufactured by compression molding, injection molding and screw extrusion. Size of 1-1.5mm in diameter and 1-2 cm in length can be administered subcutaneously using a trocar. Disk- or tablet- shaped implants require a small surgical incision for application.

Implants have the advantage that they can be localized to the site of implantation, which lowers the drug dosage, thereby reducing potential systemic side effects. Moreover, implants can be designed and manufactured easily and uniformly (Göpferich, 1996). On the other hand, a major disadvantage is the inconvenience of

application. The injection is often painful and their sizes may not be tolerated when they are applied sucutaneously.

1.2.4.2 Nanospheres

Among the new drug delivery systems, polymeric nanospheres have been considered as promising carriers for anticancer agents. It is reported that nanospheres help improve a drug's specificity of action and change the distribution of drugs in tissue. (Couvreur *et al.*, 1980; Rolland 1989). These modifications may consequently result in a reduction in the side-effects and toxicity of the drug and in an increase in its therapeutic efficacy. Especially, it has been demonstrated that nanospheres can escape from the vasculature through the leaky endothelial tissue and accumulate in certain solid tumours (Leroux *et al.*, 1996; Monsky *et al.*, 1999). A major shortcoming of nanospheres is their rapid plasma clearance.

1.2.5 Microspheres containing paclitaxel

1.2.5.1 Advantages of microspheres containing paclitaxel

During the last 20 years, biodegradable polymer microspheres have been considered as a promising controlled release dosage form (Brunner and Göpferich, 1996). According to definition, the size range of micropheres is between 1 and 1000 μm. The drug is dispersed or dissolved in a polymeric matrix (Figure 1.10 A and Figure 1.10 B). Compared with other formulations, microspheres show some advantages as a

good controlled release system. Firstly, unlike other implants, microspheres can be injected in suspension. Secondly, through encapsulating drugs in a polymer matrix microspheres can improve the stability of sensitive drugs and protect them from oxidative and hydrolytic degradation, thus increasing their therapeutic efficacies (Tabata *et al.*, 1993). Finally, a further advantage is the potential to administer multiple drugs in a single injection.

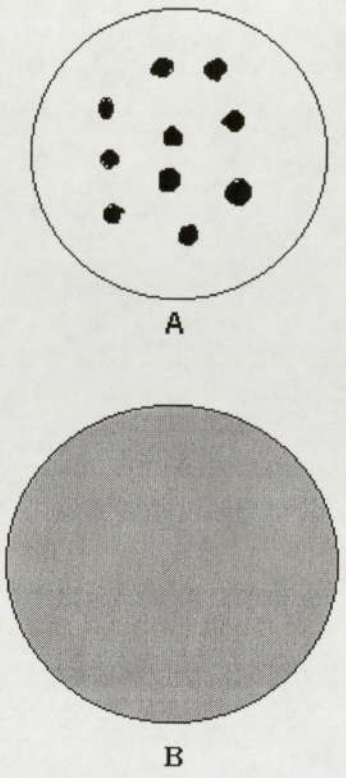
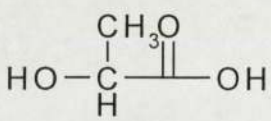


Figure 1.10 Typical structure of microspheres

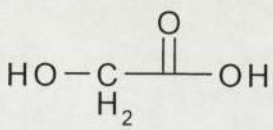
1.2.5.2 The use of PLA and PLGA

A variety of synthetic and natural polymers have been studied over the past 30 years (Chasin *et al.*, 1990) and polyesters have been used widely. That is because

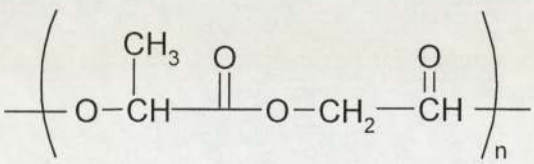
thermoplastic polyesters of poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers poly(lactic-co-glycolic acid) (PLGA) have many advantages, including an excellent record of biocompatibility and lack of toxicity. Through manipulation of comonomer ratio and polymer molecular weight the lactide/glycolide copolymers display different properties and performance characteristics (predictable *in vivo* degradation rates). 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. These favourable characteristics have allowed investigators considerable flexibility in the fabrication of drug delivery formulations. However, PLGA also has a inherent shortcomings. As a polyester PLGA degrades by bulk erosion, which means that drug release from polyester system can be unpredictable.



lactic acid



glycolic acid



poly (lactic acid-co-glycolic acid)

Figure 1.11 Poly (lactic acid-co-glycolic acid) (PLGA)

1.2.5.3 Previous study on microspheres containing paclitaxel

There are a lot of reports on the use of PLA and PLGA for paclitaxel-loaded microspheres. On the one hand investigators studied the release and degradation profiles of paclitaxel-loaded poly(lactic-co-glycolic acid) microspheres *in vitro and in vivo*. It was found the copolymer ratio of lactic acid(LA) and glycolic acid (GA) displayed obvious influences on the characteristics of the microspheres and their *in vitro* release kinetics (Wang *et al.*, 1997). On the other hand, in order to achieve high encapsulation efficiencies of paclitaxel to microspheres and desired properties for a sustained release, various emulsifiers such as cholesterol, polyvinyl alcohol (PVA), gelatin and L-α-dipalmitoyl-phosphatidylcholine (DPPC) were incorporated in microspheres (Mu and Feng., 2001). Moreover, recently it was reported that through co-encapsulating another drug (5-fluorouracil) into PLGA microspheres the cytotoxic activity of paclitaxel microspheres was enhanced (Gupte and Ciftci, 2004).

1.3 VitaminE TPGS as a novel emusifier in the preparation of microspheres

1.3.1 The development of Vitamin E TPGS

d-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS or TPGS) was invented by Eastman Kodak Co and suggested as a solubilizing agent for oil-soluble vitamins in 1960s. During the 1980s Vitamin E TPGS was first used for treating Vitamin E deficient patients and chronic cholestasis. Later Vitamin E TPGS was used as a water-soluble antioxidant and as a P-glycoprotein inhibitor (Dintaman and

Silvenman, 1999).

1.3.2 The structure of Vitamin E TPGS

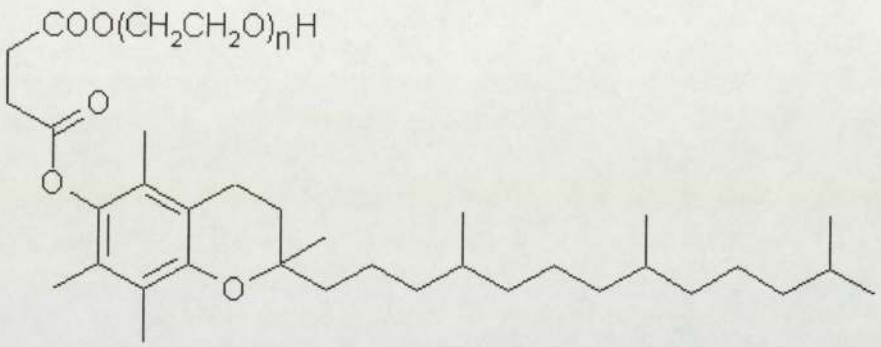


Figure 1.12 the structure of Vitamin E TPGS

Structurally, vitamin E TPGS has a dual nature, similar to an amphiphile, with part of the molecule exhibiting lipophilicity and another part exhibiting hydrophilicity, which is necessary for use as a surface-active agent. It is accepted that the polyethylene glycol portion behaves as the polar head while the tocopherol succinate portion behaves as the lipophilic tail. Moreover, not only does the TPGS molecule possess a bulky shape and large surface area, but it is also miscible with water as well as being soluble in oil. The special structure–property relationship of Vitamin E TPGS suggests its potential use as an emulsifier for various oil–water immiscible systems.

1.3.3 Typical properties of Vitamin E TPGS

The melting temperature of Vitamin E TPGS is 37-40°C and the thermal degradation temperature is about 200°C. Moreover, Vitamin E TPGS is stable under heat sterilization condition at 125°C for at least one hour or as packaged at ambient storage conditions for more than 2 years (Mu and Feng., 2002).

1.3.4 Function of Vitamin E TPGS

Vitamin E TPGS is a water-soluble derivative of natural vitamin E, which is formed by esterification of vitamin E succinate with polyethylene glycol 1000 (Eastman Chemical Company, USA). TPGS is a safe and effective form of vitamin E for reversing or preventing vitamin E deficiency. It can also improve the oral bioavailability of vitamin E. Vitamin E TPGS can be absorbed tract readily in the gastrointestinal tract, and can inhibit P-glycoprotein in the intestine to enhance the cytotoxicity of anticancer drugs such as doxorubicin, vinblastine (a commonly used medication for HIV and AIDS patients), and paclitaxel (Dintaman and Silverman., 1999). Vitamin E TPGS has also been shown to increase the absorption of amprenavir by enhancing its solubility and permeability, which were essential in the development of the soft gelatine capsule formulation for use in the clinic (Yu *et al.*, 1999). It may also have other potential applications such as being used as a solubiliser, absorption enhancer and as a vehicle for lipid-based drug delivery formulations (Khoo *et al.*, 2000).

1.3.5 Adavantages of Vitamin E TPGS as a emulsifier

It is well known that emulsifiers play an important role on the preparation of microspheres and the release profiles of drug. Normally, the emulsifier should be an amphiphilic compound possessing two distinct groups, i.e., a hydrophobic and a hydrophilic group in the same molecule. Although poly(vinyl alcohol) (PVA) has been widely used as a traditional and most popular emulsifier it has some shortcomings in drug formulations. In the fabrication process, it is difficult to completely remove it from the microspheres, causing the difficulty in purification of the product (Lu et al., 1999). Compared with PVA, Vitamin E TPGS as a semi-natural surfactants may have reduced side effects and may have better performance in the preparation of polymeric microspheres for clinical administration of anticancer drugs (Mu and Feng., 2002).

1.3.6 The common use of Vitamin E TPGS

In the past, research was concentrated on applying Vitamin E TPGS as emulsifer. Investigation of the preparation and characterization was carried out. It was reported that Vitamin E TPGS was blended with PLGA to make paclitaxel-loaded nanospheres (Mu and Feng, 2002). The result showed that Vitamin E TPGS was very effective at improving the emulsification process. Under the same fabrication conditions, the required amount of Vitamin E TPGS is an amount 67 times less than the PVA but with

the same emulsifying effects. The drug encapsulation efficiency could be as high as 100% by using Vitamin E TPGS (Mu *et al.*, 2003).

1.4 Objective of the project

The objectives of this project are:

1) 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. This investigation focuses on the study of the degradation of P(CPP:SA) and relationship between polymer degradation and controlled release process of paclitaxel from microspheres.

2) to investigate the effects of PVA and Vitamin E TPGS as emulsifiers on formation of microspheres with P(CPP:SA)

3) to study release of paclitaxel from the P(CPP:SA) microspheres.

4) to explore release characters of paclitaxel from the implantable disks made from (1) directly pressing the mixture of P(CPP:SA) powder and paclitaxel, and (2) pressing paclitaxel-loaded P(CPP:SA) microspheres containing PVA or Vitamin E TPGS as an emulsifier.

Chapter 2

Materials and Instrumentation

2.1 Materials

Sebacic acid (SA) (Sigma-Aldrich)

p-Hydroxybenzoic acid (Sigma-Aldrich)

Dry acetic anhydride (Acros Organics)

Cadmium acetate (BDH Chemicals Ltd. Poole England)

Potassium bromide (KBr, 99% FI-IR grade) (Sigma-Aldrich)

Inert PTFE strips (Easical, Polymer Laboratories Ltd, Shropshire UK)

1,3-Dibromopropane (Sigma-Aldrich)

NaOH (Sigma-Aldrich)

Sulfuric acid (Sigma-Aldrich)

Poly (vinyl alcohol) (PVA) with 13,000-23,000 average molecular weight and 87-89% hydrolysed (Sigma-Aldrich)

Phosphate buffered saline tablets (Sigma-Aldrich)

Vitamin E TPGS (Estman, USA)

Paclitaxel (XI'AN HIGH-TECH INDUSTRIES CO., LTD)

Dry methanol (Fisher scientific)

Dry ethyl ether (Fisher scientific)

Dry dichloromethane (Fisher scientific)

All other solvents of analytical grade (Fisher scientific)

2.2 Instrumentation

Bruker NMR AC250 Spectrometer

2020 Galaxy FT-IR Spectrometer

PERKIN-ELMER Gel Permeation Chromatography (GPC) System

Cambridge Instruments Stereoscan S90B

Malvern Mastersizer

Zentrifugen Universal 32

Agilent 1100 Series HPLC

2.3 Polymer synthesis

2.3.1 Recrystallization of sebacic acid(SA)

Firstly, sebacic acid was recrystallized twice from dry methanol and dried under vaccum. After that, the sebacic acid was heated at 140°C to melt under vacuum. The heating was continued for 20 min in order to remove water. Finally, the heating was stopped, and the purified sebacic acid was cooled and stored under vacuum.

2.3.2 Distillation of Acetic Anhydride(AA)

Acetic anhydride was distilled in a round flask with anti-bumping granules. The distilling liquid was collected at the temperature 138-140°C.

2.3.3 The synthesis of 1,3-bis(p-carboxyphenoxy) propane prepolymer

A solution of 138 g (1.0 mole) of p-hydroxybenzoic acid and 80 g (2.0 moles) of sodium hydroxide in 400 ml of water was added into a flask. Through the funnel, 102 g (0.5 mole) of 1,3-dibromopropane was dropped into the flask over a period of 1 hour and the reaction mixture was refluxed for 3.5 hours. Then 20 g of solid sodium hydroxide was added to the mixture and refluxed for 2.0 hours again. Heating was stopped and the mixture was left standing overnight. The fine, white precipitate was isolated by filtration and washed with 200 ml of methanol. The filtrate was placed in 1L of distilled water and the solution heated to 60-70°C, then 6N sulfuric acid was

added to acidify the solution. CPP was isolated by filtration and dried in a vacuum oven at 80°C.

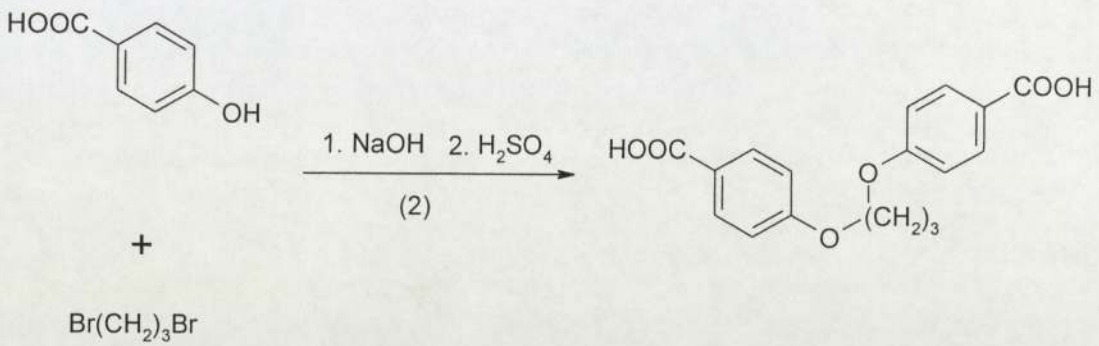


Figure 2.1 Synthesis of of 1,3-bis(p-carboxyphenoxy) propane prepolymer

2.3.4 Synthesis of sebacic acid anhydride (SAA)

8 g of the purified sebacic acid and 100 ml of acetic anhydride was placed in a flask and heated to 180°C and refluxed for 27 min at 180°C and the excess acetic anhydride removed under vacuum. The reaction mixture was stored in a refrigerator overnight. Dry petroleum and dry diethyl ether were added for purification. Finally the pure crystals were obtained by filtration and dried under vacuum.

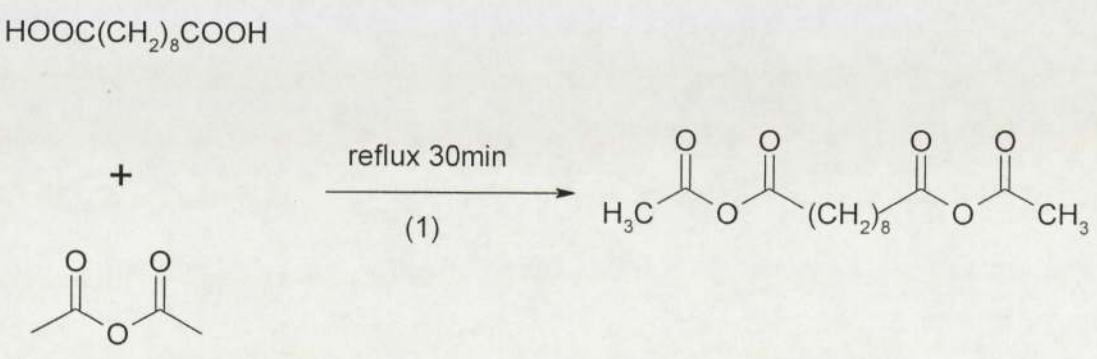


Figure 2.2 Synthesis of sebacic acid anhydride (SAA)

2.3.5 The synthesis of 1,3-bis(p-carboxyphenoxy) propane anhydride(CPPA)

1g of CPP and 10 ml purified acetic anhydride were placed in a flask and heated to 150°C. A slow stream of dry nitrogen was bubbled through the reaction mixture and after 30 min almost all CPP was dissolved. The mixture was filtered when it was still hot and yellow-coloured filtrate was concentrated by distilling excess acetic anhydride under vacuum at room temperature. The reaction mixture was kept in a refrigerator overnight. The white crystals were filtered, washed with dry ethyl ether, and dried in a vaccum oven at 70°C.

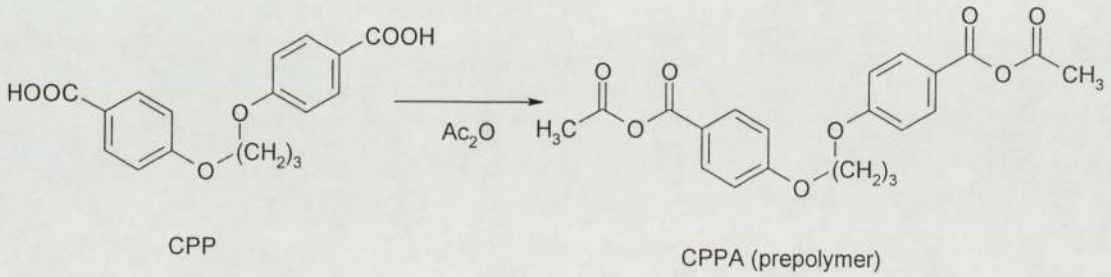


Figure 2.3 Synthesis of 1,3-bis(p-carboxyphenoxy) propane anhydride (CPPA)

2.3.6 The synthesis of poly1,3-bis(p-carboxyphenoxy) propane:sebacic acid(P(CPP:SA)) 20:80

Polyanhydride P(CPP-SA) with a molar ratio of 20:80 has been extensively investigated in vivo as a drug delivery matrix. Compared with other polymers having different ratios, it shows a higher molecular weight and a longer degradation time, which are helpful to control drug release from the matrix.

CPPA and SAA prepolymers were mixed in a mortar and pestle according to the mole ratio of 20:80. The mixture was put into a glass tube with a side arm equipped a nitrogen inlet. 2 molar percent cadmium acetate, a known catalyst in polyanhydride synthesis, was mixed with the prepolymers prior to polymerisation and the tube was immersed in an oil bath at 180°C. After the mixture was melted, a high vacuum was applied through the side arm. The condensation product (acetic anhydride) was collected in an acetone/dry ice trap. During polymerization, a strong nitrogen sweep with vigorous agitation of the melt was performed for 30 s every 15 min. Nitrogen was dried over calcium chloride before passing through the tube and the reaction was continued for 30 min. After cooling to room temperature, the crude copolymer was dissolved in dry dichloromethane, the catalyst removed from the solution by filtration, and the crude copolymer was purified by precipitation in dry petroleum ether from the dichloromethane solution. The precipitate was filtered and extracted with dry ethyl ether for several hours at room temperature. After filtration the pure copolymer was dried under vacuum.

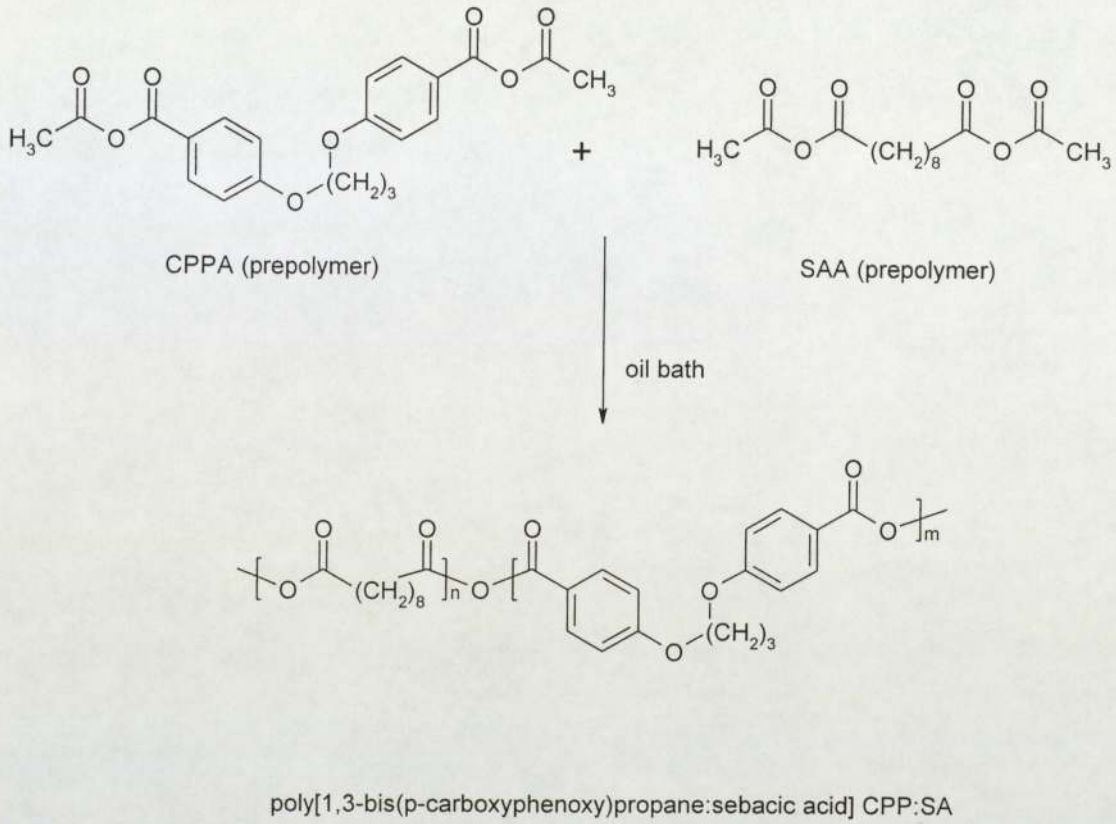


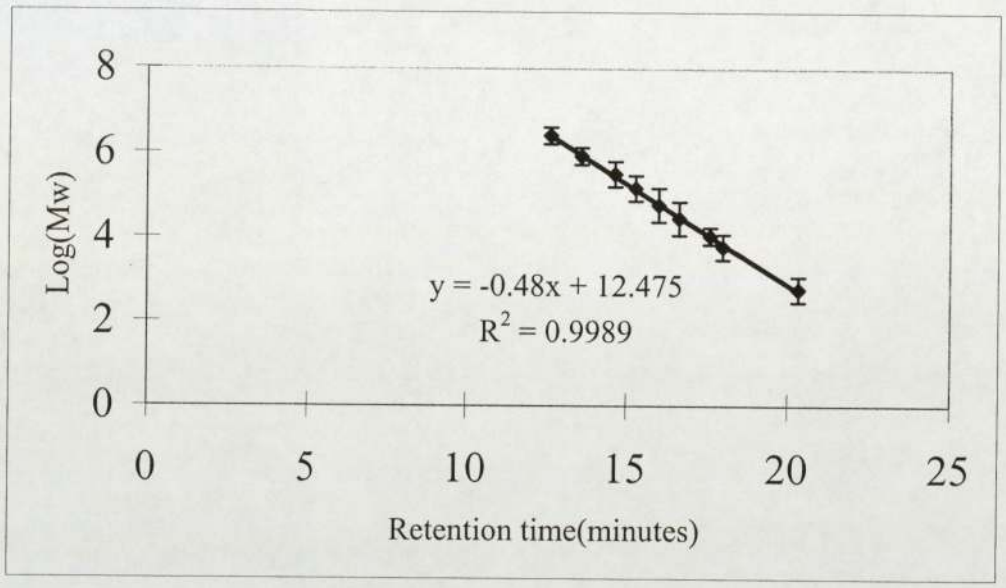
Figure 2.4 Synthesis of Poly1,3-bis(p-carboxyphenoxy)propane:sebacic acid (P(CPP:SA)) 20:80

2.4 Gel Permeation Chromatography

Polymer was dissolved in chloroform and injected through a Rheodyne injector valve (Waters, CA, U.S.A). The sample was detected with a Pye Unicam LC3 UV detector at a wavelength of 254 nm. The objective of using GPC was to find out the changes in molecular weight of polymer before and after degradation.

Narrow-MW polystyrene standards (Easical, Polymer Laboratories Ltd, Shropshire UK) were used to obtain standardisation of the GPC system. Inert PTFE strips with polystyrene (~5 mg) were immersed in 5 ml of chloroform and the solution injected through the valve. Each strip represented Mw values of 580, 10850, 59500, 320000,

2560000 and 5930, 28500, 148000, 841700, respectively. A calibration curve of retention time versus molecular weight was obtained, and each retention time was the average of three readings.



Figur 2.5 A calibration curve for estimation of molecular weight by GPC(n=3; mean ± s.d.). Molecular weight refers to weight average molecular weight.

2.5 FTIR

Infrared spectroscopy was performed on a FTIR spectrophotometer (Mattson Galaxy 2020 FTIR Spectrophotometer, Unicam). Polymer and microsphere samples were impressed into potassium bromide (KBr) discs. The spectrum was analysed using Mattson First fourier infrared software tools.

2.6 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.

2.7 Polymer degradation

About 3 mg of polymer was suspended in 1 ml of PBS (pH=7.4) in an ependorff tube (n=3) and shaken in an orbital shaker bath which was maintained at 37°C. The tubes containing suspended polymer were centrifuged at 11000 rpm for 15 min and the supernatant was removed. The pellets were washed with double-distilled water three times, freeze-dried (Edwards Modylo freeze drier) and stored in a refrigerator for SEM, GPC, NMR and IR analyses. The supernatant was collected for pH analysis. During the first day, samples of polymers were collected every 6 hours and over the second day samples were taken every 12 hours. Then samples were removed every day. After a week samples were obtained every three days.

2.8 Microsphere preparation

2.8.1 Preparation of microspheres by an oil- water single emulsion solvent evaporation technique.

250 mg polymer and 25 mg paclitaxel were dissolved in 5 ml dichloromethane. 1%w/v 3%w/v 5%w/v and 10%w/v of poly (vinyl alcohol) (PVA) was added into 75 ml double-distilled water and heated until the PVA was dissolved. The oil phase and the water phase were cooled on ice for one hour. The solution of organic phase was slowly poured into the stirred aqueous solution (8000 rpm, 8min). The formed o/w emulsion

was gently stirred at room temperature by magnetic stirrer overnight to evaporate the organic solvent. The sample was centrifuged (4,000 rpm, 20 min) and washed three times with double-distilled water to remove the excess PVA. After that, the solid microspheres were frozen and freeze-dried. The free-flowing powder was stored in a desiccator in a refrigerator at 4°C untill use.

2.8.2 Preparation of microspheres with VitaminE TPGS by an oil- water single emulsion solvent evaporation technique.

Vitamin E TPGS was heated to 60°C. After melting they was slowly added to 75 ml pre-heated water (80°C) to form 0.05%w/v, 0.1%w/v and 0.5%w/v solutions of Vitamin E TPGS. The solutions were kept stirring for 30 min to ensure that all materials were dissolved.

The organic phase and microspheres were prepared as described in section 2.8.1.

2.9 The preparation of disks

2.9.1 The preparation of disks with polymer and paclitaxel

55 mg of polymer and 5 mg of paclitaxel were dissolved in 5 ml DCM. The solution containing polymer and taxol was evaporated under vacuum to remove DCM, and yielded dry powders. 20 mg of the taxol-polymer powder was pressed using Specac 15.011 Vacuum die press. The press force continued for 4 min at 2 tons and the radius of die was 5mm.

2.9.2 The preparation of disks containing microspheres

Microspheres were compressed into discs (20 mg) with a stainless steel mold as described in section 2.9.1.

2.10 Surface morphology of microspheres

The surface morphology of microspheres was studied *via* scanning electron microscopy (SEM) (Cambridge Instruments, ISI Model DS-130). Dry samples were placed on the aluminum stubs and coated with gold under a stream of argon for about 10 min. A Cambridge Instruments Stereoscan 5150 scanning electron microscope was used to monitor the surface morphology of the microspheres

2.11 The determination of paclitaxel content in the microspheres

The paclitaxel entrapped in the microspheres was determined by HPLC. A reverse phase Hypersil ODS – 3 column (250 × 5 mm) was used. First a 3 mg sample of microspheres was dissolved in 2 ml DCM and evaporated to dryness. As the polymer dose not dissolve in methanol, then 2 ml methanol was used to redissolve the sample to extract paclitaxel from polymer. The solution was filtered into a vial for HPLC analysis. The mobile phase of HPLC consisted of a mixture of methanol and water and was delivered at a flow rate of 1.00 ml/min. The proportion of methanol in the mobile phase is increased from 50% to 100% over 20 min. A 50-µl aliquot of the sample was injected with an injector. The column effluent was detected at 227nm with a variable

wavelength detector (VWD). The calculation curve used for the quantification of paclitaxel in the microspheres was linear over the range of 1 μg-150 μg/ml (standard concentration of paclitaxel).

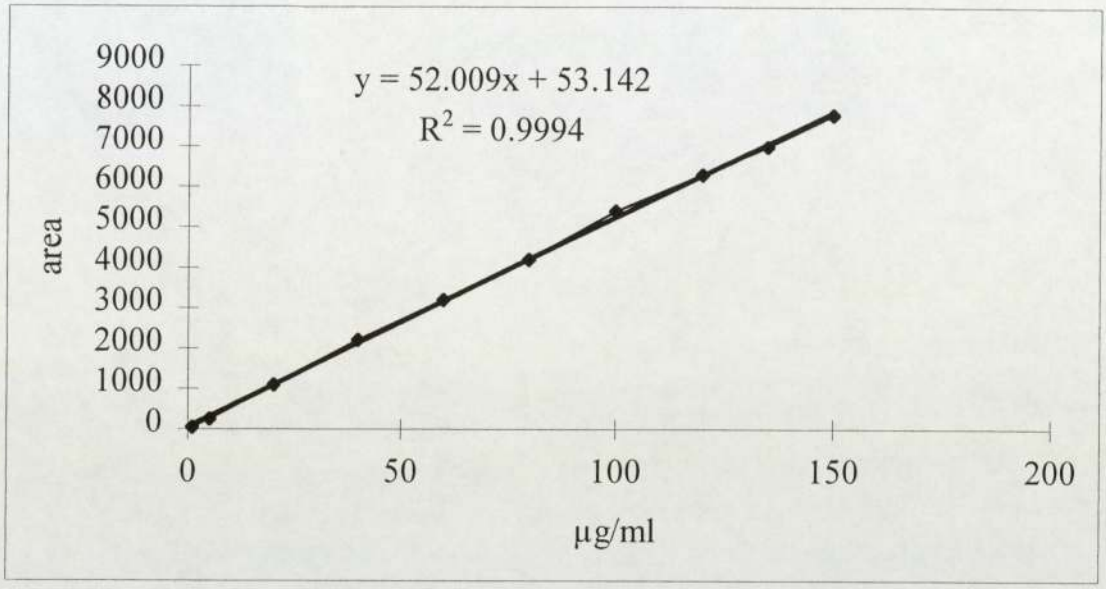


Figure 2.6 A calibration curve for the quantification of paclitaxel in the microspheres (n=3; mean ± s.d.)

The efficiency of the extraction procedure was determined as follows. Known weights of pure paclitaxel from 30 to 300μg and 3mg polymer were dissolved in 2 ml of DCM and then subjected to the same extraction procedure as described above. About 99.4% of the original amount of the paclitaxel was recovered, therefore the extraction procedure was considered efficient.

Original amount of paclitaxel(μg)	31	62	94	128	189	246	301
Paclitaxel detected by HPLC (μg)	30.8	61.9	93.8	127.5	188.5	245.4	300.8
Recovery efficiency(%)	99.4	99.8	99.9	99.6	99.7	99.8	99.9

Table 2.1 The recovery efficiency of the extraction procedure for paclitaxel (n=3)

$$\text{Drug content} = \frac{\text{amount of paclitaxel in the microspheres}}{\text{weight of microspheres}} \times 100$$

Figure 2.7 General formula for drug content

$$\text{Loading efficiency} = \frac{\text{amount of paclitaxel in the microspheres}}{\text{feeding amount of paclitaxel}} \times 100$$

Figure 2.8 General formula for loading efficiency

2.12 Release study

2.12.1 Release study of paclitaxel from microspheres

The release rate of paclitaxel from the microspheres was measured in PBS (pH 7.4) by HPLC. It was reported that the solublility of paclitaxel in aqueous condition is about 0.5 g/L (Tarr and Yalkowsky, 1987; Swindell and Krauss, 1991). A large volume of buffer solution was used to ensure the solute concentration did not exceed 10% of the concentration of solute required to saturate the solvent, namely under 'sink' conditions. Paclitaxel-loaded microspheres (10 mg) were suspended in 1000 ml of buffer solution in a beaker and placed in a shaker bath. In the first week, 200 ml of supernatant was removed every day and the same amount of fresh buffer was added. The removed solution was extracted using 20 ml of DCM, followed by evaporating to dryness. The dry powder was dissolved in 2 ml methanol and a 50-μl aliquot of the sample was injected onto the HPLC. Paclitaxel was detected as described in section 2.11.

2.12.2 Release study of paclitaxel from disks

The release of paclitaxel from disks was measured in PBS by HPLC. Paclitaxel loaded disks (20 mg) were suspended in 2000 ml of buffer solution in a beaker and placed in a shaker bath and 400 ml solution was removed for sampling analysis is described in section 2.12.1.

Chapter 3

Results and discussion

3.1 Polymer Characterisation

The composition of CPP-SA copolymer was determined by ^{1}H NMR from the ratio of the peak integration at 1.3ppm (8H, SA) and 6.9-8.2 ppm (8H, CPP). The CPP-CPP and CPP-SA were represented by peaks at 8.1 and 8.0 respectively, and the triplets at 2.6 and 2.4 represented the SA-CPP and SA-SA diads, respectively.

3.2 Polymer degradation

3.2.1 Molecular weight changes during degradation

Molecular weight changes were determined by GPC. The original molecular weight of polymer was 62,373±400Da, and the data on molecular weight changes were collected every 6 hours in the first day. The respective molecular weights were 15,848 ±100, 10,471±80, 5,128±50 and 2884±30Da. After 4 days the molecular weight decreased to about 1000±10Da (Figure 3.1). The results coincided with what we expected. When degradation occurs, sebacic acid, the relatively water soluble comonomer, is released from the copolymer rapidly, resulting in the fast decrease of molecular weight.

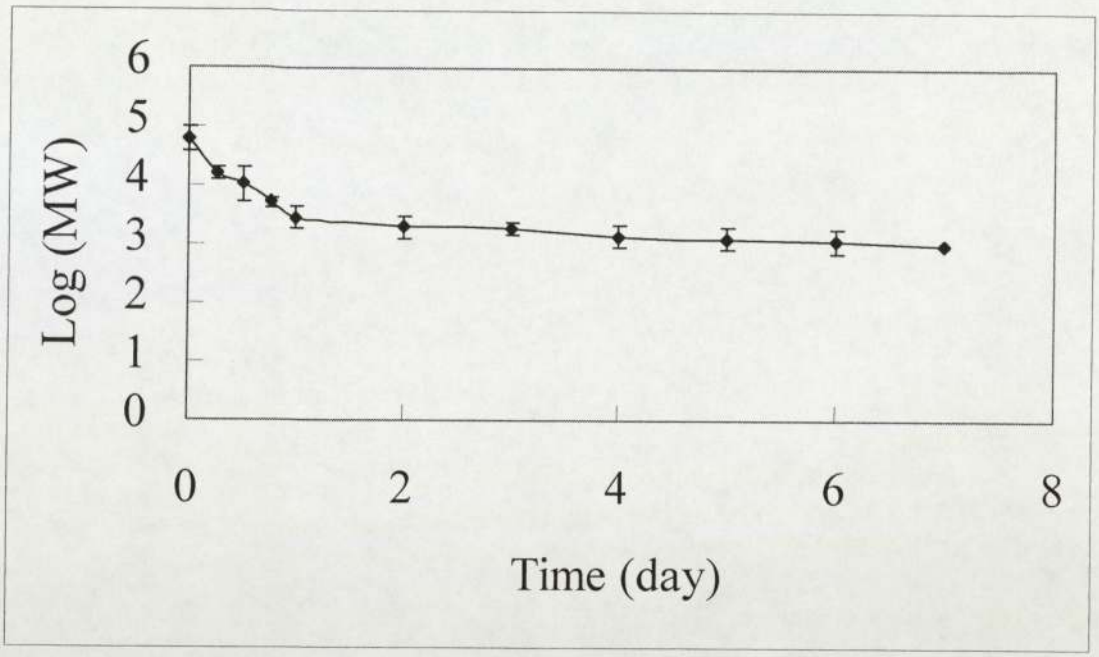


Figure 3.1 Molecular weight changes in P(CPP:SA) during degradation. (n=3; mean ± s.d.).

3.2.2 Degradation studied by ^{1}H-NMR

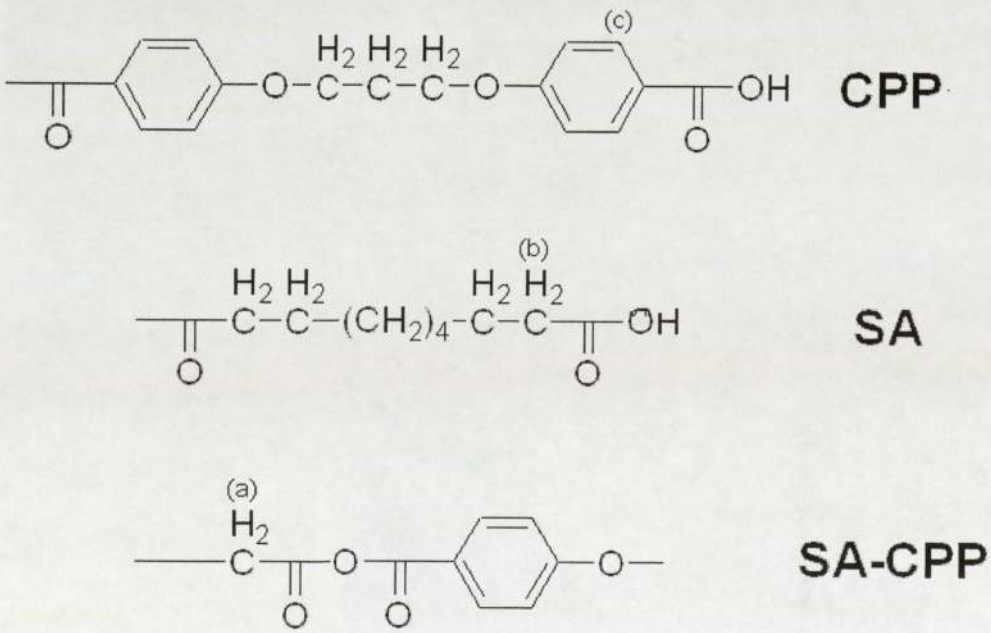


Figure 3.2 The respective H of ^{1}H-NMR spectrum in P(CPP-SA)

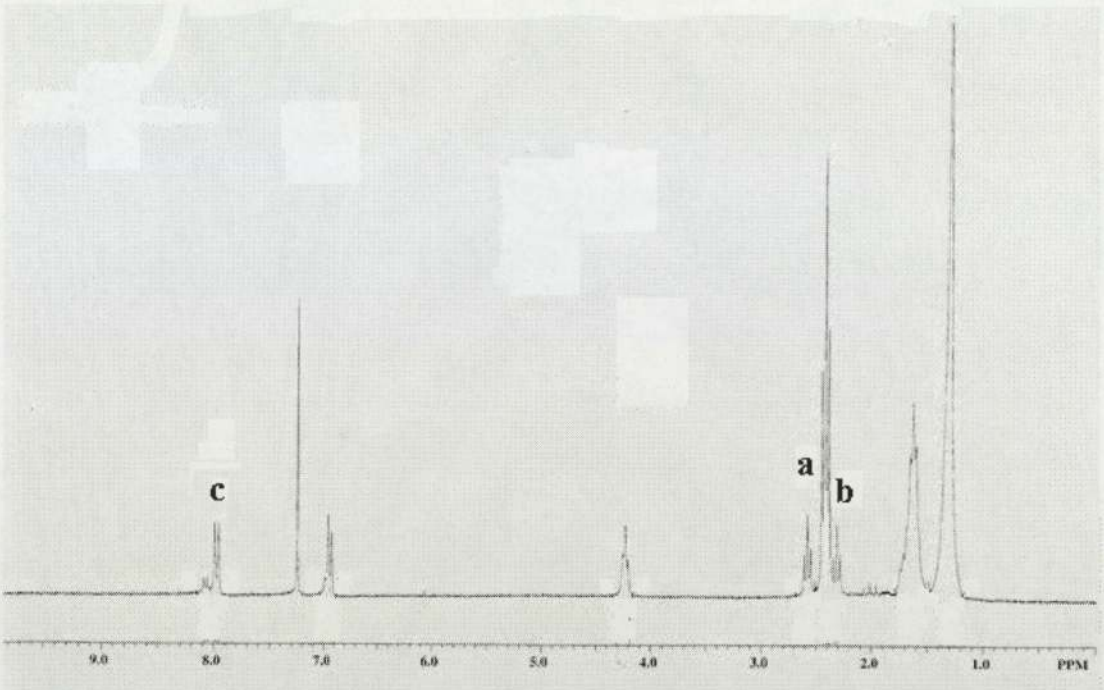


Figure 3.3 ^{1}H-NMR spectrum of P (CPP:SA) 20:80 (a, b, c are shown on Figure 3.2)

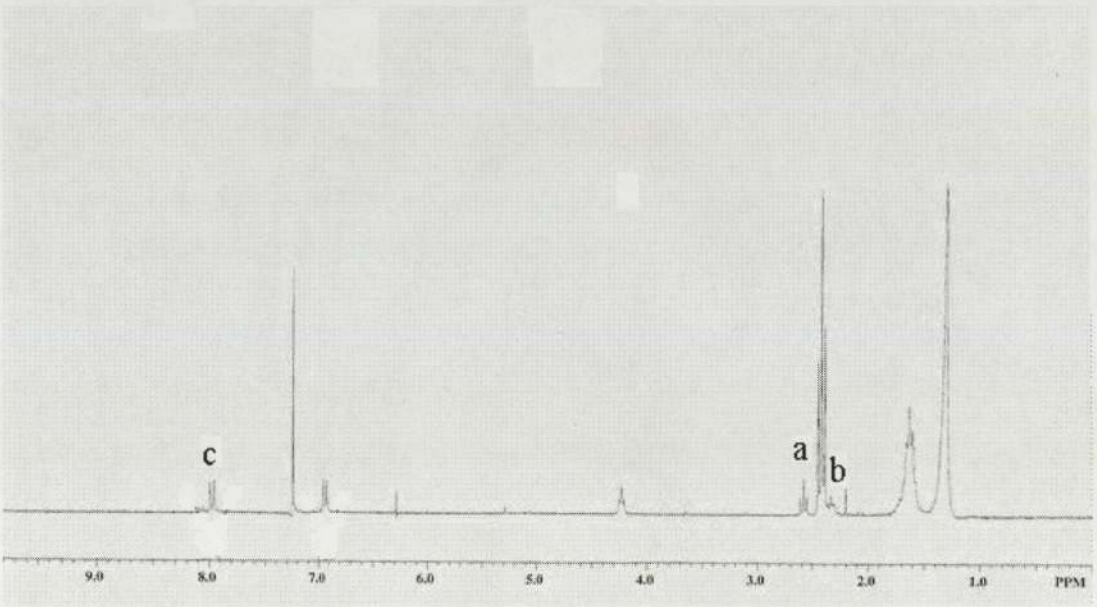


Figure 3.4 ^{1}H-NMR spectrum of P (CPP:SA) after 24 hours degradation

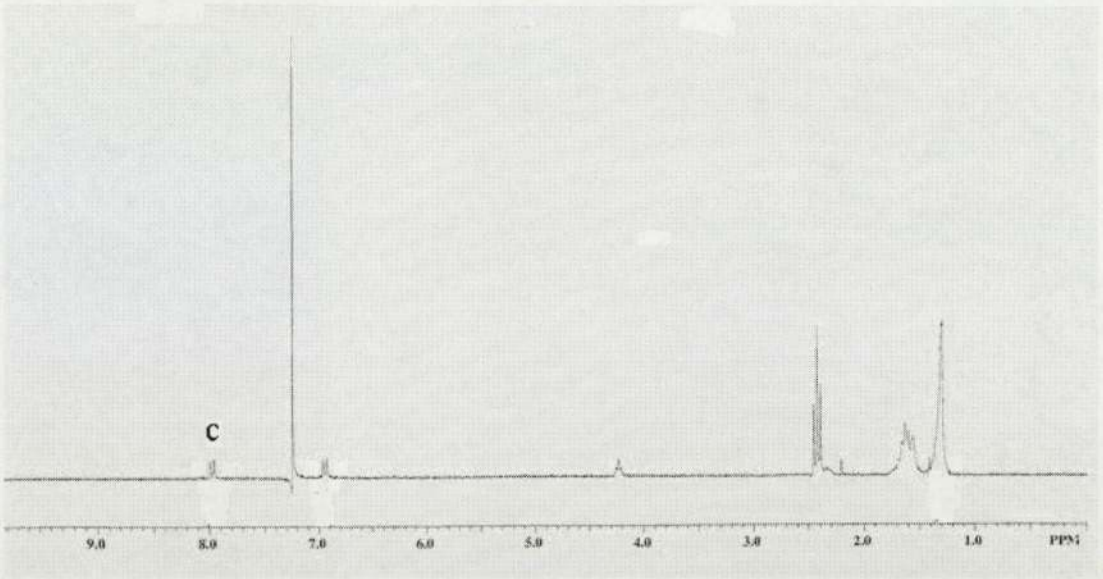


Figure 3.5 ^{1}H-NMR spectrum of P (CPP:SA) after 7 days degradation

From Figure 3.4 after 24 hours degradation, CPP-CPP, SA-SA, and CPP-SA could be seen, but compared with the original spectra the peaks of SA-SA (b) and SAA-CPP (a) became smaller. When the polymer was incubated in buffer for one week, the peaks of SA-SA and SA-CPP had disappeared. However, the peaks of CPP-CPP (c) still were visible. 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.

3.2.3 Degradation studied by IR

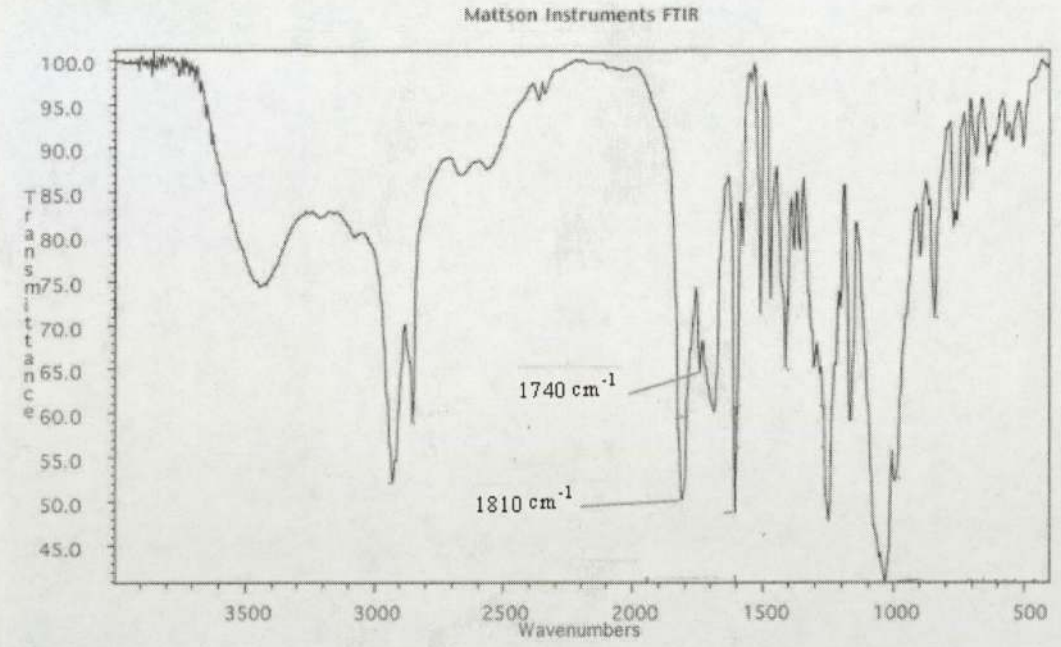


Figure 3.6 Spectrum of P (CPP:SA) after 1 day degradation

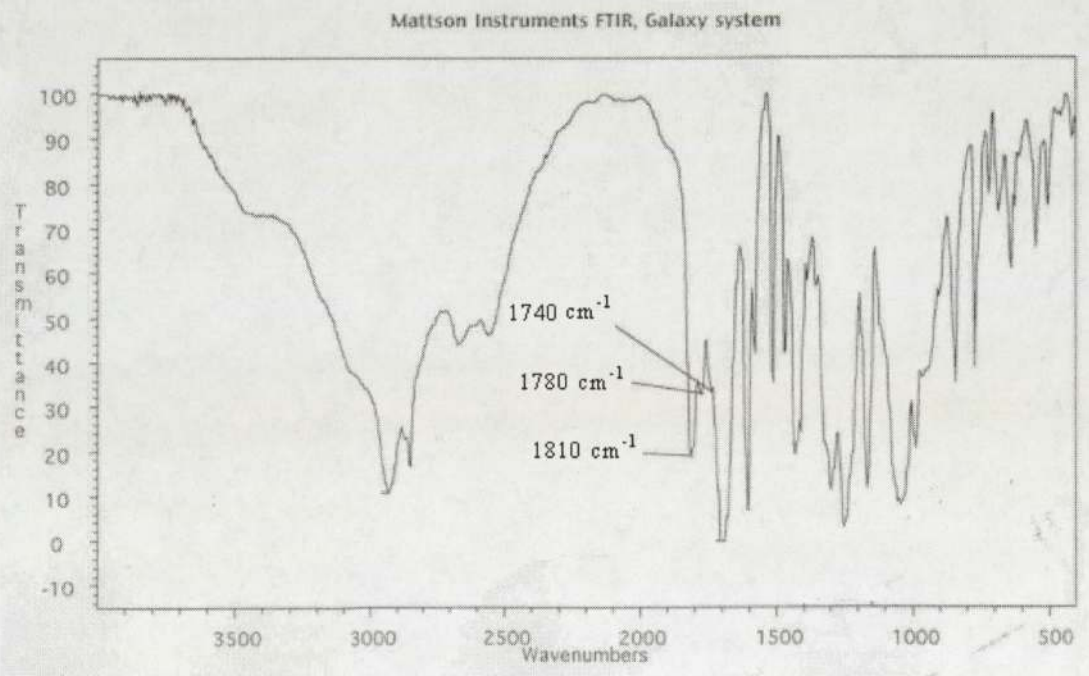


Figure 3.7 Spectrum of P (CPP:SA) after 3 days degradation

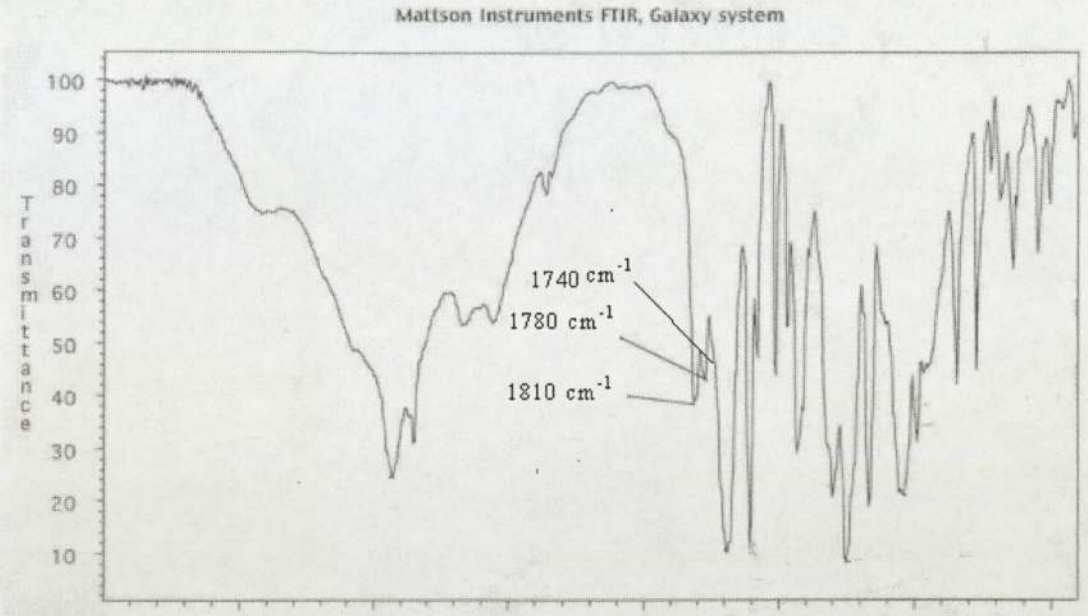


Figure 3.8 Spectrum of P (CPP:SA) after 6 days degradation

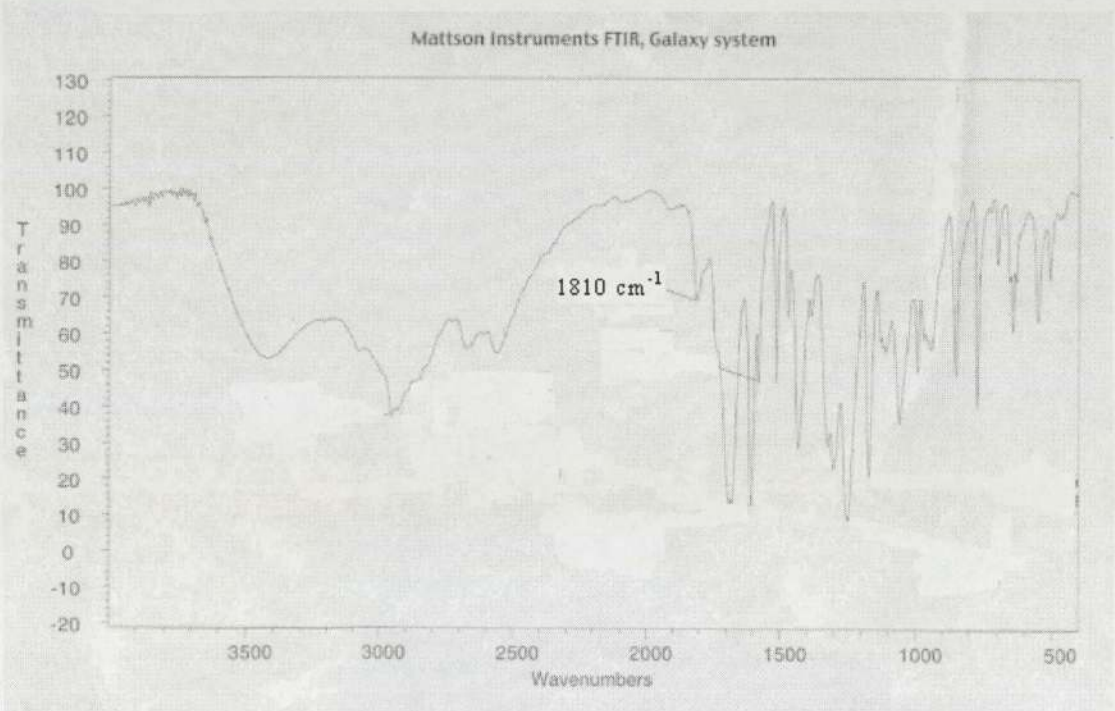


Figure 3.9 Spectrum of P (CPP:SA) after 20 days degradation

SA, as an aliphatic polymer absorbs at 1740 and 1810 cm^{-1}. On the other hand CPP has the characteristic absorbances of aromatic polymers, at 1720 and 1780 cm^{-1}. From the IR spectrum of P(CPP:SA) 20:80, the peak of the aliphatic anhydride bond at 1740 cm^{-1} and 1810 cm^{-1} became weaker following degradation, and after 3 days, this peak at 1740 cm^{-1} had disappeared (Figure 3.7); even after 6 days degradation, the peak for the aromatic anhydride bond at 1780 cm^{-1} still can be seen (Figure 3.8) . After 20 days degradation both these peaks are eliminated (figure 3.9). So it demonstrated that 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.

3.2.4 Degradation studied by weight loss

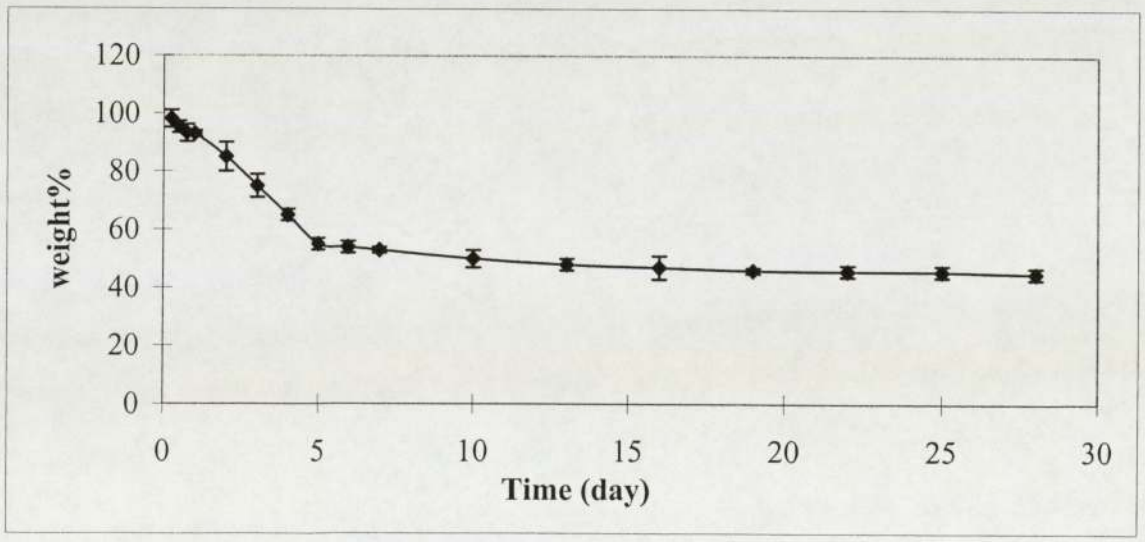


Figure 3.10 Weight loss of P(CPP:SA) 20:80 during degradation (n=3; mean ± s.d.)

Through comparing the differences on the mass of polymer and before degradation

and after degradation, it was found that there was a rapid decrease in weight from 100% to 55% over 5 days (Figure 3.10). The fast weight loss was caused by the degradation of comonomer, SA. As water penetrated into the polymer, the anhydride linkages were broken. Compared with CPP, SA will more readily dissolve in the buffer, which resulted in the rapid mass loss. The less water-soluble comonomer CPP and part of the sebacic comonomer are left, and the rate of weight loss decreased. Compared reports in literature, my results are similar to these in literature (Göpferich and Tessmar, 2002). We can find that SA showed a faster degradation than CPP similar to the study and about 60% of mass weight was lost during 5 days.

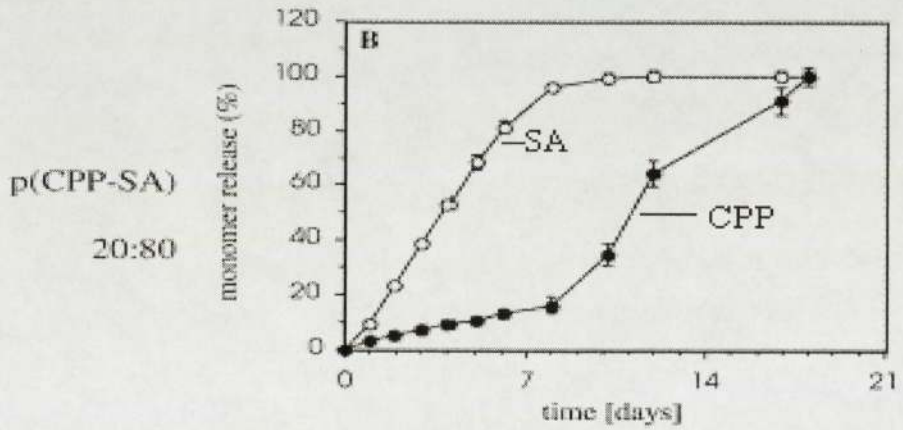


Figure 3.11 Monomer release from p(CPP-SA)20:80 reproduced from (Göpferich and Tessmar., 2002)

3.2.5 pH changes during polymer degradation.

The supernatant fluid during degradation was used to monitor the change in pH over the degradation period. The pH experienced a significant drop from 7.4 to 5.1 over the first 5 days. After that slight decreases were observed (figure 3.12).

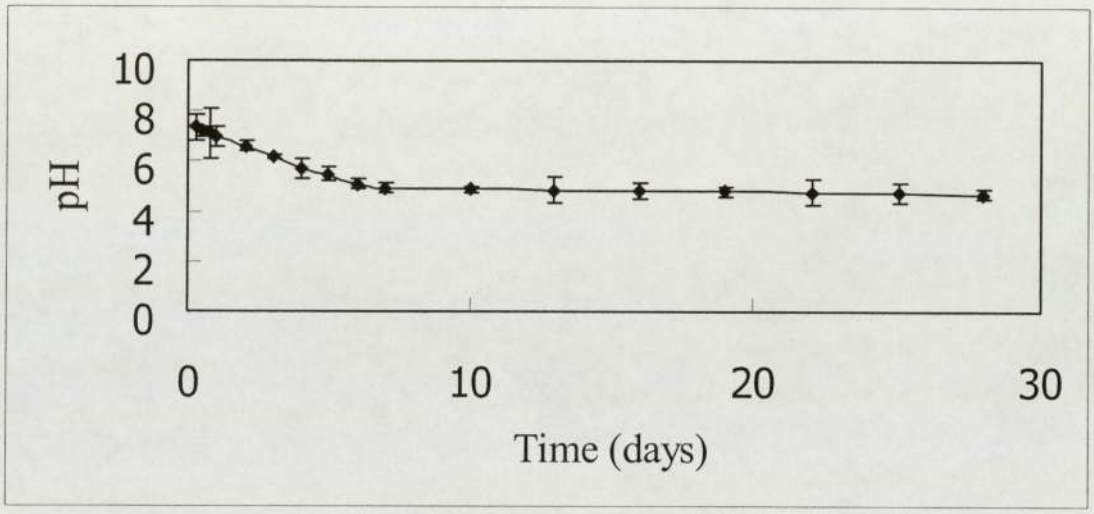


Figure 3.12 pH changes during the degradation period(n=3; mean ± s.d.)

The pH change is caused by the acid comonomer being released from the copolymer. At the beginning, sebacic acid was released rapidly into the solution resulting in a rapid decrease in the pH of the solution. 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. It has been reported that after one-day degradation of CPP-SA, the pH of the buffer dropped from 7.4 to 6.6 (Göpferich, 1997). The shorter the half-life of polymer bonds, the faster monomers are created upon degradation causing a rapid decrease in pH (Göpferich, 1997).

3.3 Microsphere preparation

Microspheres were prepared with P(CPP:SA) 20:80 using solvent evaporation and different percentages of PVA in the emulsion (Table 3.1). Microspheres were suspended in double-distilled water and sonicated. The size was determined using a Malvern Mastersizer/E laser diffraction analyser. The characteristics of microspheres are summarized in Table 3.1.

PVA concentration (% g/ml)	Drug content (%)	Loading Efficiency (%)	Size (μm)	Morphology of microsphere
1	6.34	60.8	52±2	Spherical
3	4.55	44.1	35±1	Spherical
5	2.04	20.0	24±1	Spherical
10	1.02	10.2	18±0.4	Spherical

Table 3.1 The physicochemical properties of microspheres with different percentage of PVA (n=3)

As shown in Table 3.1, particle size, loading efficiency, and morphology of the microspheres showed variation, depending on the PVA in the emulsion. As shown, drug content and loading efficiency were decreased with increasing PVA. This result was due to PVA in water phase increasing the viscosity and stabilizing the emulsion

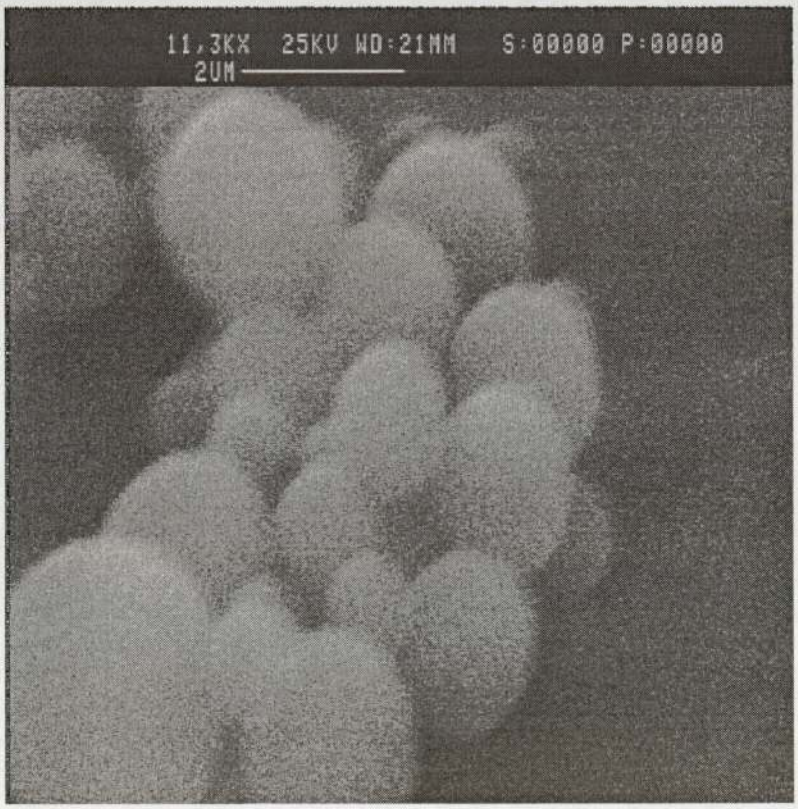
droplets against coelascence, resulting in smaller droplets. It can be concluded that the stabilization effect is dominant at higher PVA concentrations and leads to the decrease in the size of microspheres. In additon, there is a possible interaction between excess PVA and paclitaxel, thus the drug diffusing into micelles which excess PVA has formed. Therefore, with the removal of PVA paclitaxel was lost and resulted in lower loading efficiency. Compared with a traditional emulsifier, PVA, Vitamin E TPGS was used in the preparation of taxol-loaded microspheres.

Vitamin E TPGS concentration (% g/ml)	Drug content (%)	Loading Efficiency (%)	Size (μm)	Morphology Of microsphere
0.05	4.01	41.7	45±2	Not found
0.1	8.51	89.9	21±0.8	Spherical
0.5	4.11	42.4	14±0.3	Spherical

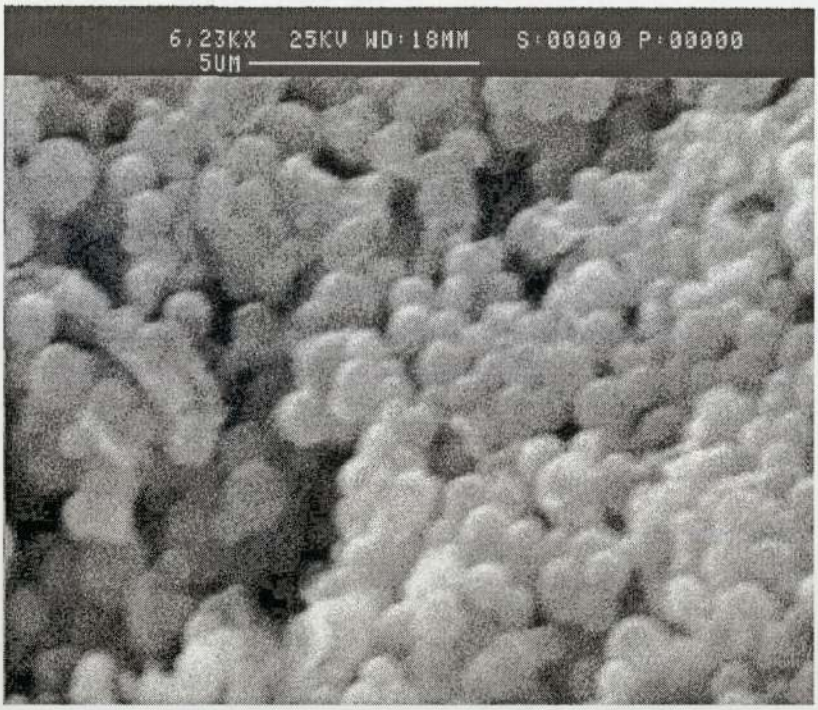
Table 3.2 The physicochemical properties of microspheres with Vitamin E TPGS (n=3)

As the critical micelle concentration (CMC) of Vitamin E TPGS is about 0.02 wt%, a lower concentration of Vitamin E TPGS was used in micropheres formulation. From Table 3.2, it can be seen that the microspheres with 0.1%Vitamin E TPGS have higher drug content and loading efficiency, whereas higher or lower Vitamin E TPGS concentration resulted in lower drug content and loading efficiency. The main reason

is that the role of the surfactant stabiliser is to stabilise the dispersed-phase droplets and inhibit coalescence. The amphipathic surfactants align themselves at the droplet surface so as to promote stability by lowering the free energy at the interface between two phases and resisting coalescence and flocculation of the microspheres. At higher concentrations, the state of Vitamin E TPGS in the aqueous dispersing phase has changed and it can not exert a stabilising effect on the formation of the emulsion system, with droplet separation and unstabilisation. However, when the concentration is too low it does not act as an emulsifier, so microspheres are not found. Compared with literature (Mu *et al.*, 2003) the particles in this study had higher encapsulation efficiency and a large size.



a



b

Figure 3.13 (a.b) SEM of microspheres with 1% PVA

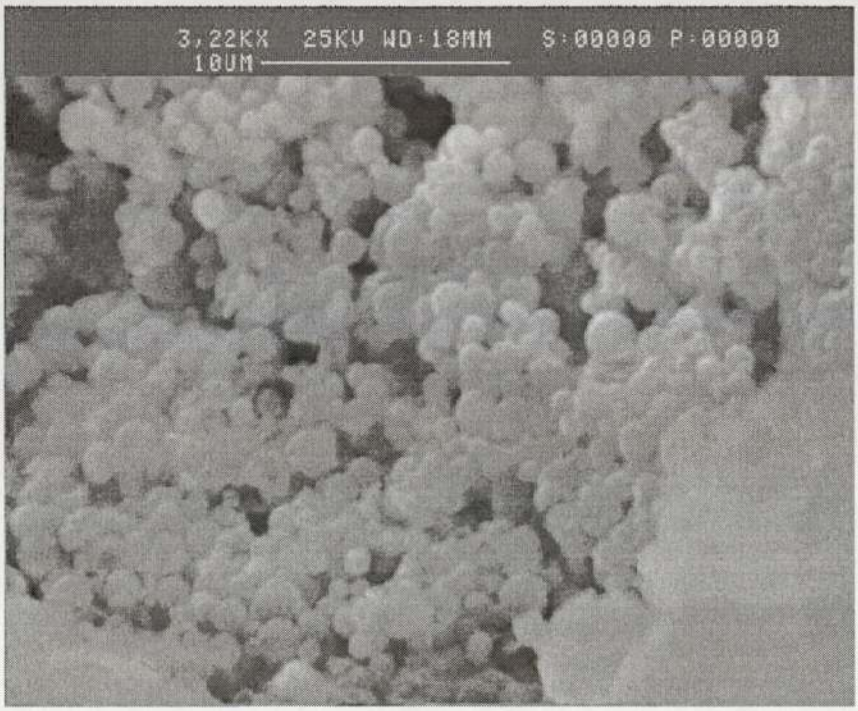


Figure 3.14 SEM of microspheres with 3%PVA

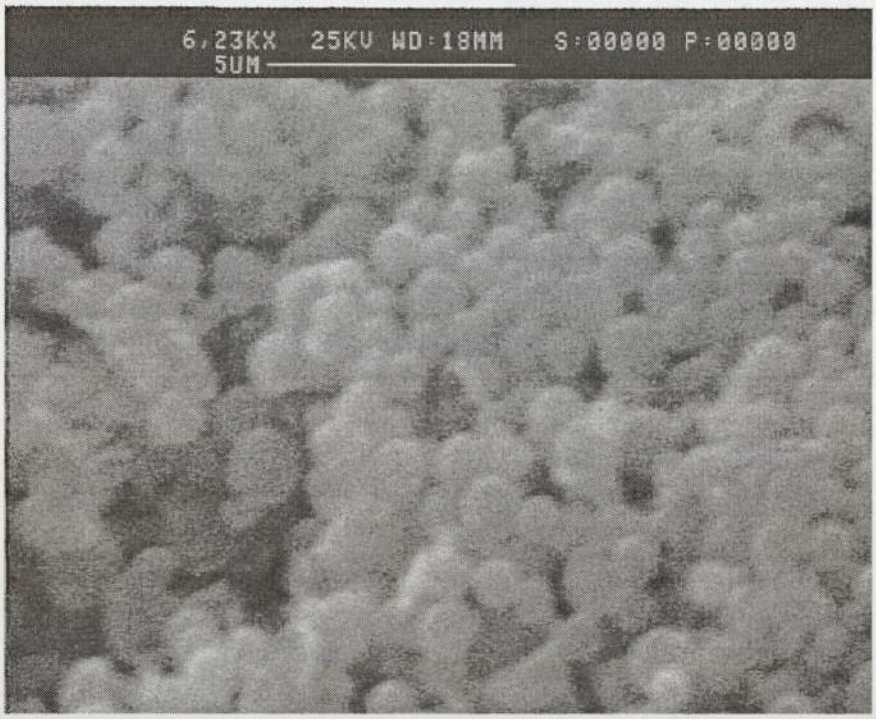


Figure 3.15 SEM of microspheres with 5% PVA

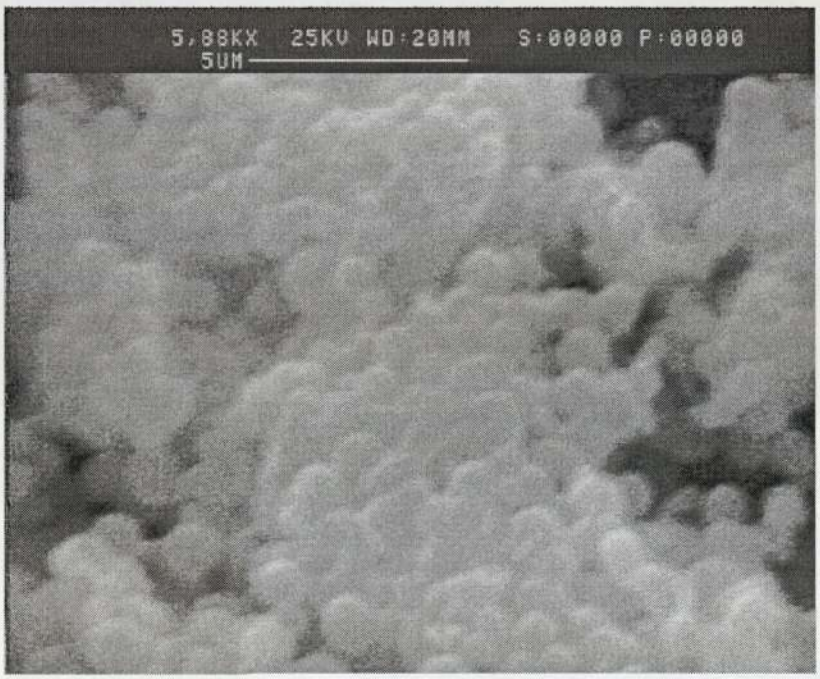


Figure 3.16 SEM of microspheres with 10% PVA

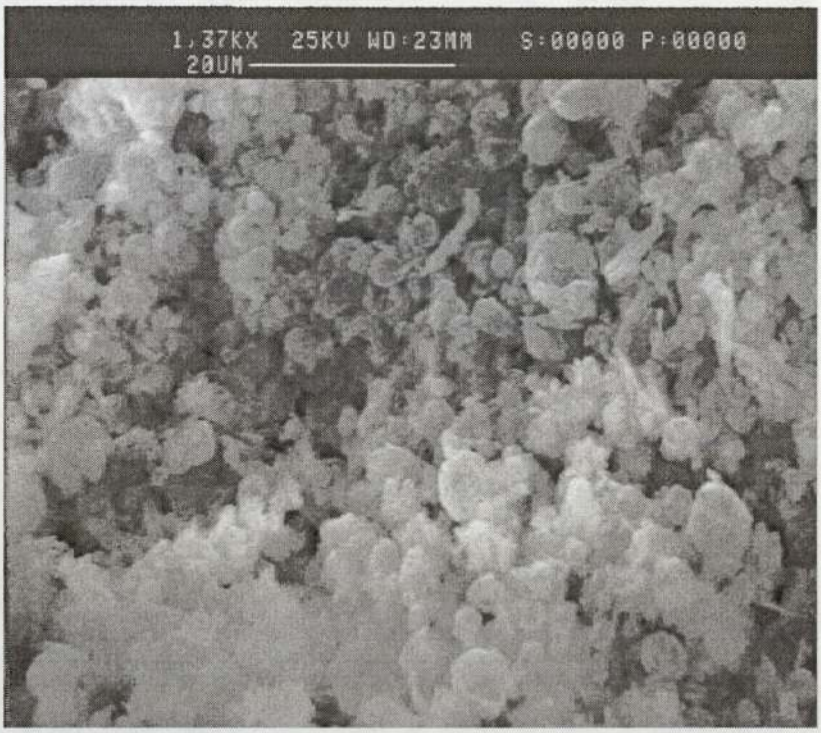


Figure 3.17 SEM of microspheres with 0.05% Vitamin E TPGS

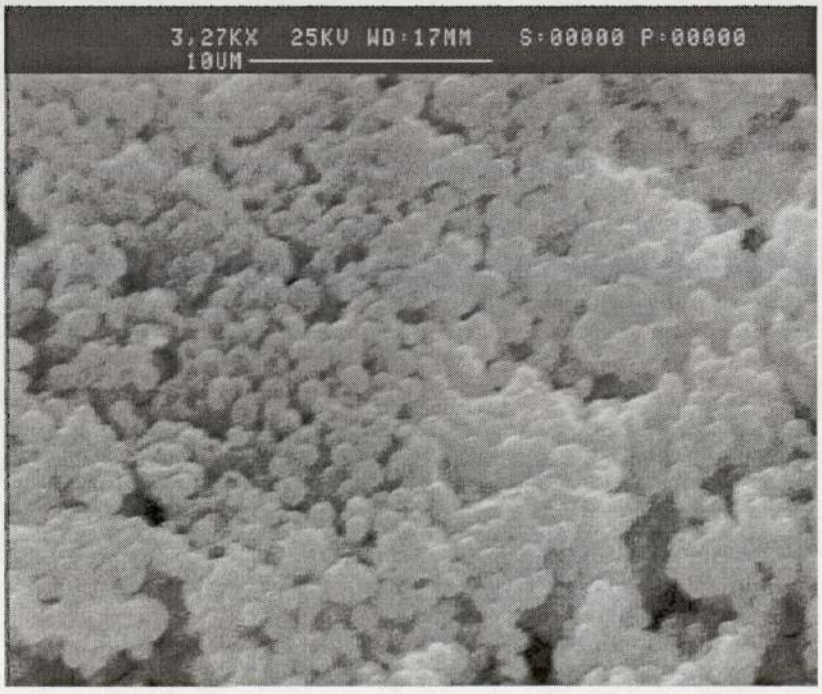


Figure 3.18 SEM of microspheres with 0.1% Vitamin E TPGS

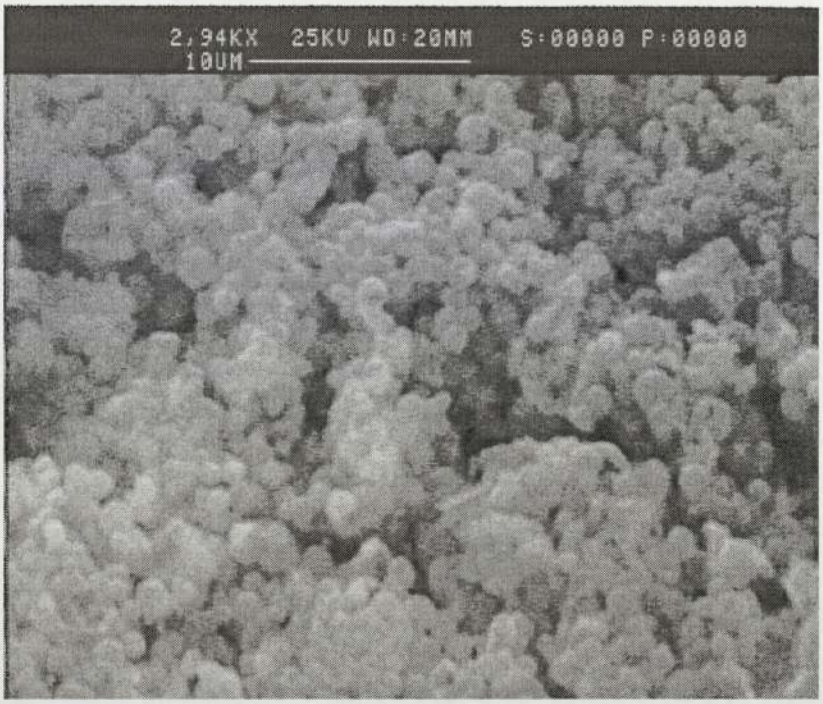


Figure 3.19 SEM of microspheres with 0.5% Vitamin E TPGS

SEM images (Figure 3.13 to Figure 3.19) indicate a smaller paticle size than laser diffractometry. The main reason is posssibly that microspheres must be dispersed in water before using Malvern Mastersizer to measure size. Therefore microspheres tend to aggregate in aqueous medium, resulting in large size. The hydrophobicity of microspheres can cause the poor dispersibility in water.

3.4 Release of paclitaxel from microspheres

The release rate of paclitaxel from microspheres with different percentages of PVA was studied.

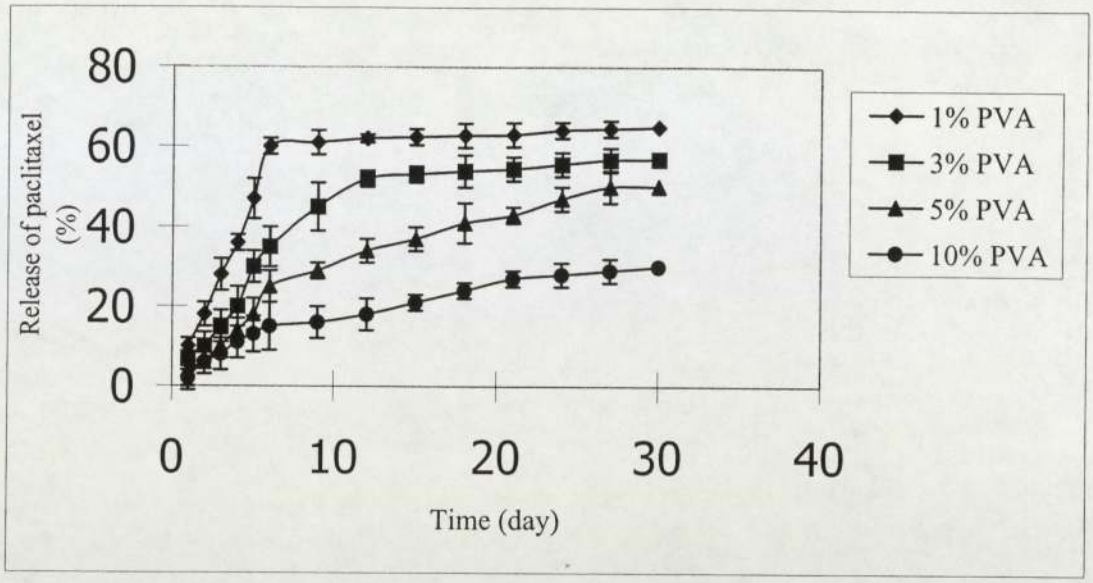
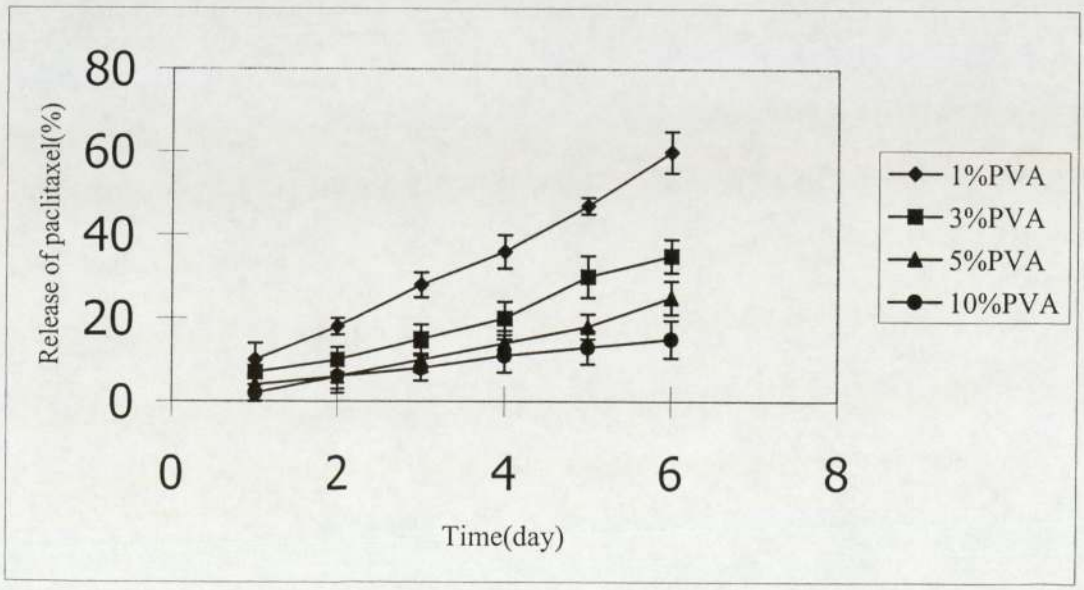


Figure 3.20 The release of paclitaxel from microspheres with PVA(n=3; mean ± s.d.)

As shown in Figure 3.20, there was a rapid release of paclitaxel from the microspheres with 1% of PVA; about 60% of paclitaxel was released over the first five days. However, with increasing PVA, microspheres showed a slower release. Residual PVA may dissolve in buffer therefore increasing the viscosity of the buffer and resulting in decreased drug diffusion (Yang *et al.*, 2001).



As shown in Figure 3.21, microspheres displayed a nearly linear drug release in the first 6 days, which could be due to mechanism of surface erosion of polymer. Most importantly, it demonstrated that the release of paclitaxel can be controlled by the erosion of polymer. Therefore release rate of drug can be adjusted by controlling the rate of erosion.

The release rate of taxol from microspheres with different percentages of Vitamin E TPGS was studied.

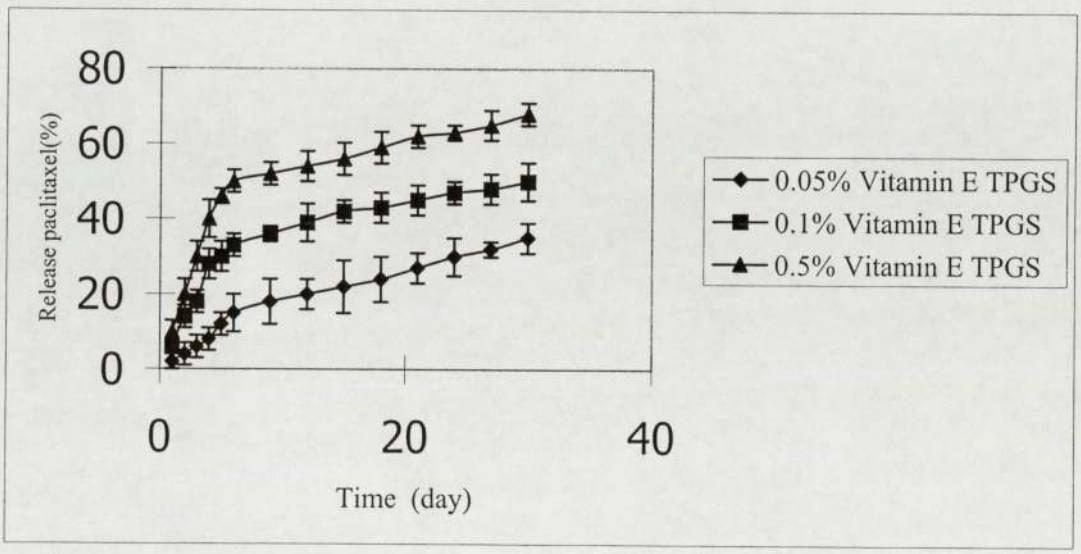


Figure 3.22 The release of paclitaxel from microspheres with Vitamin E TPGS(n=3; mean ± s.d.)

As shown in Figure 3.22, microspheres containing different vitamin E TPGS showed different pattern of drug release. With increasing vitamin E TPGS, the rate of drug release from microspheres became faster. Although about 50% of paclitaxel was released from particles containing 0.05% of vitamin E TPGS, microspheres were

poorly formed. When more TPGS was used, the microspheres became more hydrophilic. When exposed to an aqueous environment, the microspheres degraded more easily with increased water uptake, which resulted in faster release of paclitaxel.

3.5 The release of paclitaxel from disks

Disks were prepared using paclitaxel-loaded microspheres and a mixture of polymer and paclitaxel respectively.

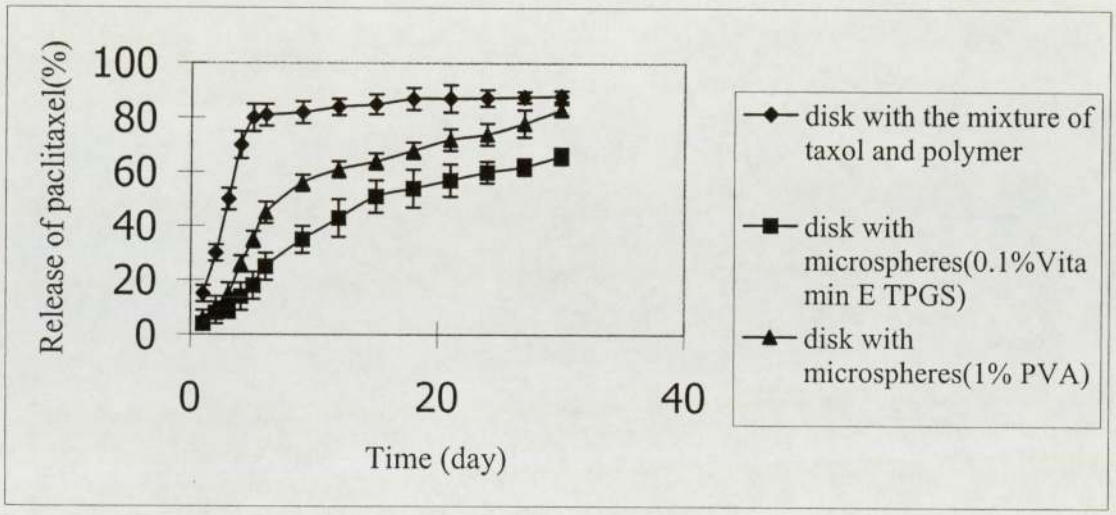


Figure 3.23 The release of paclitaxel from disks(n=3; mean ± s.d.)

As shown in Figure 3.23, disks with a mixture of polymer and paclitaxel had a faster release than disks with paclitaxel-loaded microspheres. About 80% of paclitaxel was released over 5 days and only less than 5 percent over the next days. In contrast, paclitaxel-loaded microspheres showed a slow drug release. Only 35% and 18% of paclitxel after 5 day was released from disks containing PVA and Vitamin E TPGS each respectively. This result just coincides with the release of paclitaxel from microspheres containing PVA and Vitamin E TPGS respectively. In conclusion,

paclitaxel-loaded microspheres containing PVA or Vitamin E TPGS may help extend the release of paclitaxel from disks and slow the initial burst of paclitaxel from disks.

Chapter 4

Conclusions

P(CPP-SA) 20:80 was successfully synthesised. The degradation of polymer coincided with the mechanism of surface erosion. Microspheres were prepared with paclitaxel and different percentages of PVA by a single-emulsion evaporation method. Microspheres with 1% PVA not only showed the highest drug content and loading efficiency but also displayed fast drug release. Compared with the use of PVA which is a traditional emulsifier, Vitamin E TPGS showed promising results as a new emulsifier. Firstly, Vitamin E TPGS was very effective at improving the emulsification process for microencapsulation. In fabrication of microspheres by the solvent evaporation technique, the concentration needed for the traditional emulsifier PVA was normally at 1%(w/v). However, under the same fabrication conditions, the required amount of TPGS is only 0.1%, an amount 10 times less than the PVA but with the same emulsifying effects. In addition microspheres with Vitamin E TPGS showed a longer controlled release of drug than microspheres containing PVA as an emulsifier. Compared with the fast drug release from microspheres with PVA or Vitamin E TPGS disk with drug-loaded microspheres showed a slow and controlled release. Therefore, it is concluded that disks with microspheres may be a promising controlled-release preparation for paclitaxel.

Chapter 5

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