MECHANISTIC ASPECTS OF THE DEGRADATION AND DRUG RELEASE PROPERTIES OF POLY-ALPHA-HYDROXYL ALIPHATIC ESTER NANO- AND MICROPARTICLES

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DECLARATION

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SUMMARY

Particulate systems based on the polymers of lactic and glycolic acids have been extensively investigated as drug delivery technologies, particularly as vehicles to achieve controlled release. The release kinetics from these systems is a consequence of the physicochemical properties of the system. It is generally recognised that the polymer degradation plays a crucial role in determining the release profile from PLA/PLGA systems. The release of drug from such carrier systems is actually a combination of a range of processes that are attributed to either diffusion or degradation. A physicochemical understanding of these processes is the key to a better understanding of these systems.

The purpose of this work was to examine the degradation phenomena of a range of polyesters based on lactide and glycolide copolymers. Drug free micro and nanoparticles were manufactured using a range of process variables. A systematic investigation into the effects of microparticle size, polymer molecular weight and polymer composition was carried out. The physicochemical and morphological changes associated with the degradation and erosion process were monitored. Kinetic equations to describe the degradation process were developed and applied.

The mechanism of degradation of polylactide and polylactide-co-glycolide is primarily dependent on the physicochemical characteristics of the polymer. In this study it was shown that the particle size influenced the degradation of PLA and PLGA particles. Polymer hydrolysis produces a degradation profile that contains distinct components of polymer degradation and polymer erosion. The concept of multiphasic degradation patterns, which were influenced by the physicochemical characteristics of the polymer and dissolution conditions, was introduced. The three possible phases are (a) the induction phase where no property of the polymer apparently changes during incubation under a defined set of experimental conditions (lag phase), (b) a rapid degradation of the polymer to lower molecular weights by scission of high molecular weight polymer chains without an accompanying weight loss (phase 1) and (c) a slow loss in molecular weight as the polymer erodes with an accomanying slow decrease in polymer molecular weight (phase 2). The application of mathematical models to the polymer degradation and erosion profiles allowed the estimation of parameters that could be used to study the factors

influencing polymer degradation and erosion. Comparison of two mathematical models to the polymer profiles established that the second phase of degradation is associated with the onset of mass loss.

The effects of polymer microenvironment and incubation conditions were also examined for the degradable polymer matrix. The degradation of PLGA was shown to undergo different polymer degradation profiles under the influence of acid/base conditions. The end products of the degradation process lactic/glycolic acid were also shown to increase the rate of degradation of a PLGA polymer. In this work the activation energy for PLGA hydrolysis was calculated. Rate constants calculated based on Phase 1, Phase 2 or based on polymer erosion (mass loss) were used to calculate the activation energy for PLGA. Three comparable values (Ea: 26.7, 28.5 and 23.62 kcal/mol) for the activation energy of PLGA were obtained which indicates that the underlying process of polymer degradation and erosion is hydrolysis. A lower activation energy (Ea: 8.8 kcal/mol) was calculated for the induction phase in the degradation phase which suggests that this is a dissolution controlled phase.

The release mechanism of an encapsulated drug fluphenazine HCl from a sustained release system with concurrent polymer degradation was investigated using a selection of PLA/PLGA systems. The presence of fluphenazine was shown to influence the physicochemical characteristics of the microspheres and the rate of polymer degradation. The release of fluphenazine showed a burst effect followed by a sigmoidal release profile. A model containing diffusion controlled and degradation components of release was used to describe the release of fluphenazine from nano- and microspheres. The use of the various models to describe fluphenazine release, polymer degradation and polymer erosion demonstrated their utility for the elucidation of the factors controlling the release mechanism from PLA/PLGA particulate systems.

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Release mechanisms of Fluphenazine HCI from Polylactide-co-glycolide microspheres

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ABREVIATIONS AND SYMBOLS

a	*constant related to polymer composition*
a_{MH}	*Mark-Houwink constant*
A	*unit area*
b	*constant related to polymer composition*
B_e	*erosion rate*
C	*concentration*
c	*amount of sample*
ca.	*circa*
CD	*measure of total variance*
C_o	*drug loading*
C_s	*saturated solubility of drug*
C_t	*amount of drug per unit volume of matrix*
C_m	*solubility of the drug in the polymer matrix*
CTAB	*cetyltrimethyl ammonium bromide*
Cyc A	*Cyclosporin A*
D	*diffusion coefficient*
D_o	*observed diffusion coefficient*
D_r	*relative diffusion coefficient*
DCM	*dichloromethane*
dC/dx	*concentration gradient*
dM/dt	*fraction of drug released by a diffusion mechanism*
DMSO	*dimethylsulphoxide*
dn/dt	*rate of production of oligomeric active sites*
DP	*degree of polymerisation at time t*
DP_0	*degree of polymerisation at time zero*
DSC	*differential scanning calorimetry*
F	*fraction of drug released at time t*
$F_{BIN(R)}$	*fraction of drug released in the burst phase at time infinity*
F_{TOT}	*Fraction of total drug released at time t*
F-HCl	*Fluphenazine Hydrochloride*
FITC-BSA	*FITC labelled bovine serum albumin*
G	*Gibbs free energy*

G_1, G_2, G_3	*shape factors*
GPC	*gel permeation chromatography*
H	*enthalpy*
hi	*height of the peak at Mi*
HCl	*hydrochloride*
Hr	*hour*
im	*intramuscular*
ip	*intraperitoneal*
iv	*intravenous*
I.V.	*inherent viscosity*
J	*flux*
k	*rate constant*
k_a	*rate constant of component a of the profile*
k_b	*rate constant of component b of the profile*
K	*temperature in degrees kelvin*
k_1	*degradation rate constant of the first phase of degradation*
k_2	*degradation rate constant of the second phase of degradation*
k	*erosion rate constant*
k_3	*surface erosion rate constant*
$k_{1(R)}$	*release rate constant for the drug released in the burst phase*
$k_{2(R)}$	*release rate constant for the drug released in the degradation phase*
k_{FF}	*constant in the Flory-Fox equation*
KCl	*potassium chloride*
kg	*kilograms*
L	*half thickness of the device*
l	*length*
LH-RH	*Luteinizing hormone releasing hormone*
M	*mass*
m_t	*amount of drug released at time t*
m_∞	*amount of drug released at time* ∞
m_0	*amount of drug released at time zero*
mg	*milligrams*
min	*minute*
Mi	*molecular weight of the* i^{th} *component*

Mn	*number average molecular weight*
mm	*millimeter*
Mp	*peak average molecular weight*
Mp_0	*peak average molecular weight at time zero*
Mp_1	*peak average molecular weight at time Tau*
MSC	*model selection criteria*
Mw	*weight average molecular weight*
Mz	*z-average molecular weight*
n	*number of data points*
Ni	*number of molecules of the molecular weight Mi*
n_o	*initial number of active sites*
nm	*nanometers*
o/w	*oil in water*
o/o	*oil in oil*
P	*polydispersity*
P	*drug permeability in the matrix*
p_p	*number of parameters estimated*
PEG	*polyethylene glycol*
pH	*minus the log of the hydrogen ion concentration*
PLA	*poly(lactide)*
PLGA	*poly(lactide-co-glycolide)*
PM	*physical mix*
PVP	*polyvinylpyrrolidone*
psi	*pounds per square inch*
PVA	*polyvinylalcohol*
Q	*amount of drug release per time t, per unit area*
®	*registered*
r	*radius*
rpm	*revolutions per minute*
S	*entropy*
SD	*standard deviation*
Sec	*second*
So	*solubility of the substrate*
St	*total concentration of the substrate*

sc	*subcutaneous*
SDS	*sodium dodecylsulphate*
SEM	*scanning electron miscroscopy*
SLS	*sodium laurylsulphate*
spm	*shakes per minute*
t	*time*
T	*absolute temperature*
T_g	*glass transition temperature*
$T_{g(p)}$	*peak temperature of the* T_g *transition*
T_m	*temperature of melting*
Tau	*time where the second phase in the degradation phase begins*
*T*max	*time to 50% polymer degradation*
$T\max_{(R)}$	*time to 50% drug release via polymer degradation*
Tlag	*time during which no change in the molecular weight is observed*
V	*volume*
V_d	*volume of disc*
w	*weight*
W_d	*dry weight of sample after incubation*
W_i	*initial weight of sample*
w/o	*water in oil*
w/w	*weight per weight*
w/v	*weight per volume*
w/o/w	*water in oil in water*
w/o/w/o	*water in oil in water in oil*
Y_i	*observed data point*
$\hat{Y}_i$	*mode predicted data point*
Xc	*the degree of crystallinity*
°C	*temperature in degrees Celsius*
%	*percentage*
% w/w F	*percentage weight per weight fluphenazine*
ε	*porosity*
γ	*gamma*
η	*intrinsic viscosity*

η_{inh}	*inherent viscosity*
η_r	*relative viscosity*
η_{red}	*reduced viscosity*
μm	*micrometers*
ρ	*density*
τ	*tortuosity*
ΔHm	*enthalpy of melting*
ΔHm_0	*enthalpy of melting for 100% crystalline polymer*
[COOH]	*concentration of free carboxylic acid end groups*
[ester]	*concentration of free ester groups*
$[H_2O]$	*concentration of water*

CHAPTER 1

INTRODUCTION TO POLYESTERS AS DRUG DELIVERY SYSTEMS

1.1 INTRODUCTION TO POLYMERS AS DRUG DELIVERY DEVICES

Polymers exist in a natural form as macromolecules in structures such as DNA, RNA, proteins and carbohydrates that are found in both plant and animal life. Recognition of the chemical structure of polymers did not occur until the 1920s, when Hermann Staudinger introduced the concept of a macromolecule to describe the structure of polymeric material.

The use of polymers in drug formulation is a very large and diverse area of pharmaceutical research and development. Traditionally, polymers have been used as coating agents, capsule material, binders and thickening agents in various types of therapeutic dosage forms. In addition to polymers being used as excipients, some drugs are themselves polymers e.g. polysaccarides such as heparin and proteins such as insulin. More recently, various types of polymers have been extensively investigated as drug delivery systems.

Drug delivery technology can bring both therapeutic and commercial value to health care products as it has the potential for reducing both the amount of total drug dosed and the associated side effects as well as the elimination of the discomfort associated with multiple dosing/injection. Drug delivery technology also has the potential to protect drugs that have short in vivo half-lives and to improve the bioavailability of drugs with poor permeabilities or low aqueous solubilities (Langer and Peppas 1981; Deasy 1984).

Historically, polymer based drug delivery systems have borrowed materials originally developed for other applications (Lewis, 1990). Early studies in the field of controlled delivery focused on the use of biostable commercial polymers used as subdermal implants. These devices had to be surgically removed after drug depletion since leaving nondegradable foreign materials in the body for an indefinite time period constituted an undesirable toxicological hazard. Diffusion-controlled release from biostable polymeric matrices or reservoir devices was limited by the polymer permeability and the characteristics of the drug. The focus of drug delivery devices thus changed to the use of biodegradable polymers as drug carriers (Rande and Hollinger, 1990).

1.2 BIODEGRADABLE POLYMERS FOR DRUG DELIVERY

There are four major strategies for the use of polymers as controlled release systems. These strategies are based on the mechanism controlling the release of the incorporated drug. These are diffusion controlled systems, solvent controlled systems, externally activated or modulated and chemically controlled systems (Langer and Peppas 1981, Smith *et al.* 1990, Heller 1995). Biodegradable or bioerodible polymers constitute the vast majority of polymers based on the chemically controlled drug delivery systems.

Many authors have defined the terms biodegradable and bioerodible; Heller (1983) has defined bioerosion as the conversion of an initially water-insoluble material to one that is water-soluble. Williams (1982) defined biodegradation as the biological breakdown of the polymer, as opposed to simple hydrolysis. Graham and Wood (1982) define biodegradable systems as those which degrade after a period of time to soluble products that can be easily removed from the implant site and excreted by the body.

Many of the definitions for the terms biodegradable and bioerodible presented in the literature overlap which makes their exact definition difficult. Biodegradation has therefore been defined as a broad term that refers to hydrolytic, enzymatic or bacteriological degradation processes that occur on or within a polymer matrix which do not necessarily proceed to a stage where the matrix morphology is affected. Bioerosion however refers to an actual physical loss from the polymer matrix (Holland and Tinge 1992). The terms bioabsorbable and bioresorbable are used to infer ultimate assimilation of the material in vivo by the host tissues after enzymatic degradation, hydrolytic degradation or simple dissolution of the polymer matrix (Holland and Tinge, 1992).

There exists a large pool of biodegradable polymers that have potential to act as controlled release materials. These can be derived from either natural sources or synthesised chemically by either addition reactions or condensation reactions. The majority of biodegradable biomaterials are synthetic polymeric materials that are available as custom-synthesised polymers. Table 1.1 gives an outline of the range of biodegradable polymers available for drug delivery technology complied from a number of key reviews and articles on natural and synthetic drug vehicles (Holland *et al.* 1986, Schacht 1990, Arshady 1991, Okada and Toguchi 1995). Certain authors have further subdivided these classes into hydrophobic and hydrophilic polymers (Kopecek and Ulbrich 1983, Heller 1993).

Table 1.1 Some examples of biodegradable polymers investigated for drug delivery

Natural polymers
Proteins and polypeptides (albumin, antibodies, fibrinogen, gelatin, casein and collagen)
Polysaccharides (alginic acid, hyaluronic acid, starch, dextran, dextrin, chitin and chitosan)
Polyesters (poly(hydroxybuterate), poly(hydroxyvalerate))
Synthetic Polymers
Polyesters (poly(lactide), poly(glycolide), poly(ε-caprolactone))
Polycyanoacrylate (poly(alkylcyanoacrylate))
Polyanhydrides (poly(carboxyphenoxy)propane:sebacic acid))
Poloxamer (pluronic polyols)
Poly orthoesters (1,2,6-hexanetriol and alkyl orthoacetate)
Polycarbonates (poly(ethylene carbonate), poly (propylene carbonate))
Polyurethanes (poly(oxytetramethylene, hexamethyl diisocynate and dioxaoctane-diol))
Polydioxanones and Polyoxalates (poly(para-dioxanone), poly(1,4-dioxane-2,5-diones))
Polyamides (poly (α-amino acids), (polyhydroxypropyl-L-glutamate)
Polyvinyl (polyvinyl alcohol, polyvinyl pyrrolidone)
Polyphosphazenes (poly[bis(glycine ethyl ester)phosphazene])

The significant advantages of utilising a biodegradable/bioerodible polymer is that removal of the device is not a problem. The most ideal properties of a biodegradable polymer are as follows: it must be biocompatible, produce non-toxic degradation products and be degradable either hydrolytically, enzymatically or microbially with a rate that can be modulated according to polymer properties and environmental conditions, have suitable mechanical and physicochemical properties, be economically viable, sterilizable, suitable for processing and free from elutable impurities that may be toxic (Mills and Davis 1987, Schacht 1990).

1.3 ALIPHATIC POLYESTERS OF HYDROXY ACIDS AS DRUG CARRIERS

A vast proportion of the current interest in biodegradable systems focuses on the use of the polyester series based on lactic and glycolic acid. These polymers have an additional advantage over other biodegradable polymers in that they are widely investigated in terms of toxicological and clinical data. Biocompatibility and regulatory approval have attracted investigators to these systems (Lewis 1990). In 1966 Kulkarni *et al.* reported that PLA (polylactic acid) was a suitable material for surgical implantation because it was found to biodegrade to normal physiological components. The biocompatibility of polylactide and polylactide-co-glycolide was demonstrated using sutures by Cutrigh *et al.* (1971) and Craig *et al.* (1975) in rat tissue. These materials are well tolerated in living tissue and do not cause irritation (Bos 1991), and are progressively degraded then resorbed followed by complete tissue recovery (Robert *et al.* 1993). Visscher and co-workers (1987) confirmed the biocompatibility of polylactide and poly (lactide-co-glycolide) injectable microspheres by examining tissue from the injected site over time. The biocompatibility of these polymers is attributed to the facts that the biodegradation of these polymers follows normal physiological pathways. Poly (lactide) and poly (glycolide) undergo biodegradation to form lactic and glycolic acid monomers, which are eliminated from the body via Krebs cycle. The l- (+) form of lactic acid is naturally produced and metabolised in the body while lactate is an intermediate or end product found in carbohydrate metabolism in all life forms. Lactate is oxidised to pyruvate, which is either converted to glucose or decomposed to carbon dioxide and water. Glycolic acid is a physiological intermediate product in the degradation of the amino acid gylcine and is converted to carbon dioxide and active formate by oxidative carboxylation (Boehringer Ingelheim KG, 1985). The only condition required for degradation to occur seems to be the presence of an aqueous environment.

Polyesters had been used for decades as synthetic resorbable sutures and evaluated for other medical applications, in fact the development of new polyester materials came about as a result of a search for improvements in the properties of these materials for medical applications. In 1970, Yolles became the first researcher to achieve systemic delivery of a therapeutic agent using a biodegradable polymer composite in a system that released cyclozacine from a poly (lactide) implant. Beck *et al.* (1979) developed a long acting injectable progesterone microcapsule system using these polymers. These polymers are attractive because they are now commercially available for use as a matrix for drug delivery. A broad spectrum of performance characteristics with the polyesters can be obtained by careful manipulation of four key variables: monomer stereochemistry, comonomer ratio, polymer chain linearity and polymer molecular weight.

1.4 SYNTHESIS AND STRUCTURE OF BIODEGRADABLE POLYESTERS

A polymer is a substance composed of molecules which have long sequences of one or more species of atoms or groups of atoms linked to each other by primary, usually covalent, bonds. The structure of the polymer depends on the basic unit or monomer(s) used in it's preparation. The lactide contains an asymmetric carbon atom, hence two steroisomers d-lactide, l-lactide (or S, R as referred to absolute configurations) and the racemic d,l-lactide exist. Glycolic acid has no optical isomers (Figure 1.1).

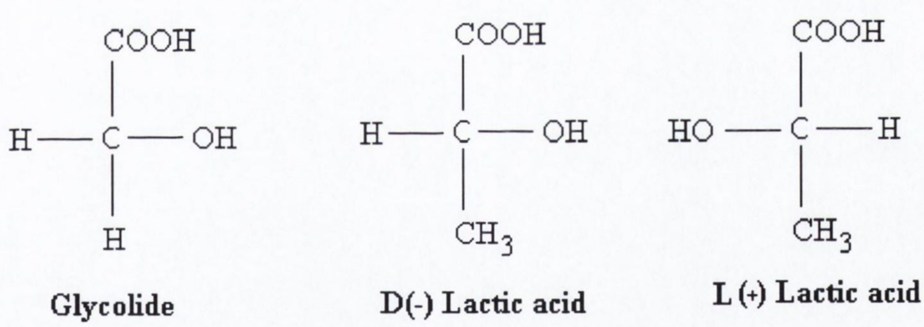


Figure 1.1 Structure of glycolic and lactic acid monomers

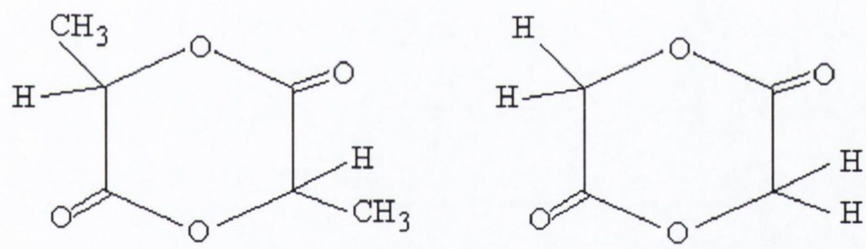
Low molecular weight (molecular weight polymers <10,000) homo- and co-polymers of lactic and glycolic acid (ca. molecular weight 2000) can be synthesised by direct condensation of lactic acid and gylcolic acid (Suzuki and Price 1985, Asano *et al.* 1989) or by azeotropic distillation with an aromatic hydrocarbon. High molecular weight polymers are produced by a ring-opening melt condensation of the cyclic dimers, lactide and glycolide (Kulkarni *et al.* 1966, Gilding and Reed 1979, Hutchinson and Furr 1985).

The low molecular weight polymers are produced according to the following reaction.

$$nHOCH_2COOH \xrightarrow[180^{\circ}C/5mmHg]{Sb_2O_3} H\{OCH_2COOCH_2CO\}OH + nH_2O$$

Figure 1.2 Preparation of polyglycolide.

If the temperature of this reaction mixture given in Figure 1.2 is increased to 255-270°C, and if an air condenser is substituted for the water condenser under a vacuum of 0.1-0.2mmHg, the cyclic dimer Glycolide sublimed. Lactide is prepared in a similar manner (Figure 1.3) (Gilding and Reed 1979).

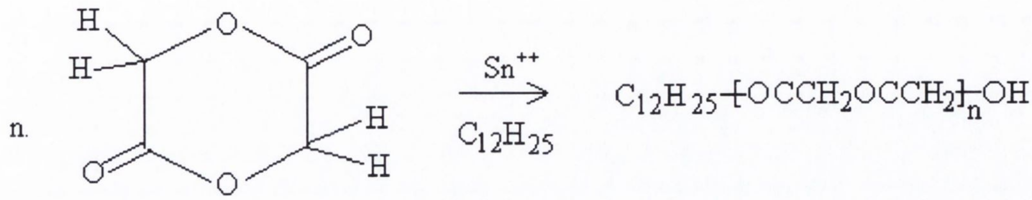


Lactide **Glycolide**

Figure 1.3 The structure of cyclic glycolide and lactide dimers

The production of high molecular weight polymers requires the addition of a catalyst and a molecular weight controller (Figure 1.4). A range of acid catalyst material is suitable including compounds of antimony, cadmium, lead, tin, titanium or Zinc and a variety of amines. Organic tin catalysts are mainly utilised, stannous chloride ($SnCl_2.2H_2O$) and stannous octate (Tin [II]-2-ethylhexanoate) (FDA acceptance as a food stabiliser) being the most common. Other catalysts have also been utilised on a limited basis. Laural alcohol (1-Dodecanol $C_{12}H_{26}O$, MW 186.3) 0.01% is added as a catalyst initiator and its concentration can be used to control molecular weight during synthesis (Gilding and Reed, 1979, Deasy

1989, Fukuzaki *et al.* 1991). The cyclic dimers are polymerised over a period of 2-6 hours at approximately 175-220°C.

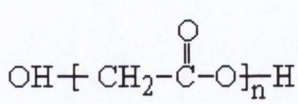


Glycolide

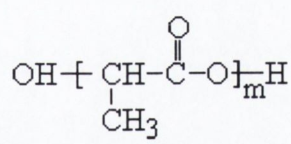
Polyglycolic acid

Figure 1.4 Polymerisation of glycolide to produce high molecular weight polymers

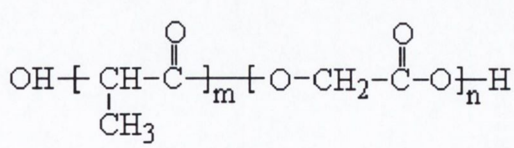
Custom synthesised polymers can be produced by the selection of adequate polymerisation conditions. The polymerisation reaction is terminated by rapidly cooling the molten polymer. Residual monomer is removed by purification of the polymer by extraction with an organic solvent. Lactic and glycolic acid form linear homo and co-polymers with the basic structure as shown in Figure 1.5. The steriochemistry of the asymmetric carbon atom is unaffected during the polymerisation process and as a result polylactides of the d-, l- and d, l- lactide are available.



Polygylcolide



Polylactide



Polylactide-co-glycolide

Figure 1.5 The structure of poly(d,l-lactide), poly(glycolide) and poly(d,l-lactide-co-glycolide).

From these basic polymer units a variety of polymers can be derived. The homopolymers of lactic acid have the following nomenclature: poly(d-lactide) PDLA and poly(l-lactide)

PLLA (or poly-l-lactic acid) while polyglycolide is denoted as PGA. The co- polymers available are poly(d,l-lactide) PDLLA and poly(d,l-lactide-co-glycolide) PDLLGA usually denoted PLGA. It is possible to synthesise co-polymers with variable ratios of either monomer and the ratio of one monomer is indicated before the polymer name, so a 50:50 PLGA polymer indicates 25% l-LA, 25% d-LA and 50% GA.

1.5 CHARACTERISTICS OF LACTIDE GLYCOLIDE BASED POLYMERS

Polymer characteristics contribute to the mechanical, physical, or chemical properties observed in polymer materials. In the lactic acid polymer, the additional methyl group results in a more hydrophobic polymer than the glycolide polymer. The rate of hydration of the polymer material is an important consideration in respect to drug release. Gilding and Reed (1979) demonstrated that water uptake increases as the glycolide ratio in the polymer increases.

Polymers tend to have two types of morphology within the material crystalline and amorphous. A crystalline region is a physical state where the chains exist in an ordered fashion analogous to crystal lattice packing. An amorphous region is a physical state where the chains are in an unordered fashion. The crystalline phases of polymers are characterised by their melting temperature (T_m) where as amorphous phases are characterised by their glass transition temperature (T_g). This transition occurs at a specific temperature and its value may be influenced by both instrumental variations and the polymer history. The T_g corresponds to the onset of chain motion in the polymer. Below the glass transition temperature the polymer chains can only undergo low amplitude vibratory motion and the polymer is usually in a brittle inelastic state as the temperature increases the chains begin to under go rotational and diffusional movement, for this to occur the space between the atoms (the free volume) must increase and the polymer material transforms abruptly from the glassy state to the rubbery state. The molecular weight of the polymer, position of the pendant side chains, chain stiffness and the polarity of the chain can affect the transition. The formation of amorphous polymers is favoured in polymers that contain bulky side chains, irregular substitutions, or that have an irregular repeating sequence of dissimilar monomer units, which frequently occurs in co-polymers (Deasy 1984). In polyesters the sterioregular polyglycolic acid and the d- and l-lactic acid polymers are highly crystalline, while the racemic polymer is amorphous. Poly-l-lactic acid has a crystallinity of over 80% and a melting point of 185°C and degrades into the cyclic monomer lactide after prolonged heating above 200°C. The degree of crystallinity (Xc) of a sample can be calculated using the following equation:

$$Xc = \frac{\Delta H_m}{\Delta H_m^0} \quad 1.1$$

Where ΔH_m is the measured enthalpy of melting and $\Delta H^0{}_m$ the enthalpy of melting for 100% crystalline polymer (for PLLA $\Delta H^0{}_m$=203.4 Jg^{-1}) (Jamshidi *et al.* 1988, von Recum *et al.* 1995). The crystallinity of a PLLA was calculated from the melting enthalpy assuming 93.7 cal/g for a theoretically 100% crystalline PLLA polymer (Pistner *et al.* 1993). Crystallinity can also be induced in a number of other ways, cooling of a molten polymer, evaporation of polymer solutions or by annealing. Introducing small quantities of the d-lactide into the l-lactide structure causes a much lower initial crystallinity. Gilding and Reed (1979) reported that most of the copolymer composition range consists of amorphous polymers. For the (l-)LA/GA system 25-75% Glycolide compositions are amorphous, while for the (d,l) LA/GA series, compositions of 0-70% copolymers are amorphous. Thus copolymer compositions rich in poly (l-)LA or PGA are much more stable to hydrolytic attack than the intermediate compositions since amorphous polymers of polyesters degrade faster than crystalline ones.

Solubility of the polymer in common organic solvents is an important factor in regard to fabrication of drug delivery systems. The homopolymers of lactide are soluble in halogenated hydrocarbons, ethylacetate, dioxane, tetrahydrofuran and a few other solvents (Lewis, 1990). Glycolide polymers are a much more insoluble material but at concentrations less than 50% in the copolymers they exhibit solubilities comparable to that of the lactide. Poly d,l-lactide has a relatively good solubility in most organic solvents with a solubility of >60% (g polymer per 100ml solvent) quoted for acetone, dichloromethane and ethylacetate (Boehringer Ingelheim, 1988). These polymers are insoluble in highly polar solvents such as water, ethanol and methanol (Conti *et al.* 1992).

The molecular weight characteristics of these polymers are frequently determined by size exclusion chromatography (SEC), a detailed account of this technique is given in chapter 5. SEC separates molecules according to their molecular size in solution using porous packing. Large molecules move fastest through the column and appear first in the chromatogram. Because of the nature of the polymerisation process the molecular weight of polyester polymers is polydisperse and thus forms a distribution of molecular weights. The broadness of the molecular weight distribution depends on the preparative method used to synthesise the polymer. The determination of the molecular weight and distribution is important because of the relationship that exists between molecular weight and polymer properties. Many of the properties exhibited by polymers such as viscosity, mechanical strength and thermal properties can be related to the polymer molecular weight.

1.6 APPLICATIONS OF LACTIDE/GLYCOLIDE BASED POLYMERS

Due to the excellent biocompatibility and bioresorbability of these polymers, some of these bioresorbable materials have successfully been used in products for the medical industry. Examples include Dexon® Maxon® and Vicryl® as sutures as well as Biofix® and Phusiline® bone fracture internal fixation devices. These materials have also been investigated for many medical applications including dental applications (Robert *et al.* 1993), internal fracture fixation devices (Leenslag *et al.* 1987) and prosthetic material (Hoffman 1977).

These materials have been investigated for the controlled release of a broad range of actives for a wide range of applications. These include veterinary (Gupta *et al.,* 1992), pesticide (Langer, 1980) and pharmaceutical (Deasy, 1984) applications in various forms such as microparticles, implants and fibres (Lewis, 1990). Microparticles offer a flexible choice of administration route; they can be injected by i.v., s.c., i.m. or i.p. routes (Linhardt, 1990) for either sustained release depots or targeted drug carriers. Sustained release products have been formulated for injectable, topical, implantation and pulmonary applications. There is now plenty of evidence to support gastrointestinal (GI) uptake of particulates making them feasible as oral carriers (Florence and Jani, 1993) however the full potential for the use of particulates for drug deliver has not been achieved because of their low uptake in vivo.

A diversity of drug categories with varying physical and chemical properties have been evaluated for controlled release purposes in polyester matrices. These include narcotic antagonists (Yolles *et al.,* 1973; Mason *et al.*, 1976), contraceptive steroids (Beck *et al.,* 1979; Benita *et al.,* 1984), anti-inflammatory steroids (Heller *et al.*, 1980; Leelarasamee *et al.*,1986; Lalla and Sapna, 1993), anticancer agents (Wakiyama *et al.*, 1981; Spenlehauer *et al.*, 1988), local anaesthetics (Wakiyama *et al.*, 1981; 1982a), antimalarial agents (Wise *et al.*, 1984) as well as antibiotics (Vidmar *et al.*,1984;Vert *et al.,*1994b; Zhang *et al.*, 1994, Schmidt *et al.,* 1995) and non steroid anti-inflammatory drugs (Armstrong *et al.*, 1995). Recent interest in these carriers has focused on their potential to deliver biologically active agents. Compounds with short biological half-life, instability, degradability in the GI tract and high toxicity are good candidates for incorporation into these systems (Conti *et al.,* 1992). The search for appropriate delivery systems for these biological agents is due to the fact that many of these molecules are not orally active and their molecular weights are often too high for absorption through the intestinal wall to occur. Nano- and microparticles have been reviewed for the delivery of peptides and proteins (Pitt *et al.,* 1990; Couvreur and Puisieux, 1993; Gombotz and Pettit, 1995) and have been successfully encapsulated within PLGA with retention of structural integrity and activity (Cohen *et al.,* 1991; Yeh *et al.,* 1995). In the 1980's several potent LH-RH agonists were successfully encapsulated in polyester microspheres and produced commercially successful 1-month injectable depots (Table 1.1).

Table 1.1 Commercially produced PLGA based products

Trade Name	Manufacturer	Polymer	Form of delivery system	Method of manufacture	Active
Zoladex®	ICI	50:50 PDLLGA	monolithic cylindrical implant	-	Goserelin acetate
Decapeptyl LP®	lpsen Biotech	50:50 PDLLGA	Microparticulate injection	Coacervation	Triptoreline
Prostap SR®	Lederle	50:50 PDLLGA	Microparticulate injection	Coacervation	Leuprolide acetate
Lupron Depot	TAP	PLGA (75:25)	Microparticulate injection	Double emulsion technique	Leuprolide acetate

CHAPTER 2

DRUG DELIVERY TECHNOLOGIES BASED ON POLYESTER HOMO AND COPOLYMERS

2.1 INTRODUCTION TO PARTICULATE DRUG DELIVERY TECHNOLOGY

One of the reasons for the popularity of the polylactide and lactide co-glycolide polymers is their relative ease of fabrication. A range of pharmaceutically active agents has been investigated in controlled release dosage forms using a variety of production techniques, which were subsequently released in an active form. The physicochemical and pharmacological properties of drugs and their intended use will dictate technology used to manufacture these devices. Microencapsulation embodies a series of techniques for the entrapment of solids or liquids within polymer coats or matrices. Table 2.1 shows the range of techniques available for microencapsulation within microparticulate drug delivery systems (Watts *et al.* 1990, Conti *et al* 1992).

Table 2.1 Preparative methods for polymeric particulate systems

Coacervation-phase separation	Spray coating
Emulsion polymerisation	Spray drying
Extrusion-spheronization	Melting
Interfacial polymerisation	Milling
Emulsification/solvent evaporation	Supercritical fluids
Emulsification/solvent extraction	

The microencapsulation method most widely used for the production of biodegradable PLA/PLGA microparticles has been solvent evaporation/extraction from an emulsion system. Intensive research efforts have been made to develop this technique for the production of sustained release formulations for therapeutic applications. A patent issued to Vraken and Claeys in 1970 described the original microencapsulation process using an emulsion solvent evaporation and solvent extraction technique; both of these procedures have been adopted for the microencapsulation of drugs using biodegradable polyesters. Numerous reviews have been published on this procedure (Arshady 1991, Watts *et al.* 1990, Holland and Tighe 1986, Conti *et al.* 1992, Okada and Toguchi 1995).

2.2 MICROPARTICLE AND NANOPARTICLE TECHNOLOGY USING THE SOLVENT EVAPORATION PROCESS

The solvent evaporation technique involves the following basic process (Figure 2.1), the polymer is dissolved in an organic solvent and the drug is dispersed in or dissolved in an organic solvent. The polymer solution is added to the drug solution/suspension and mixed together (disperse phase). This organic solution is then emulsified into an aqueous solution of a macromolecular stabiliser (continuous phase). This type of emulsion has been termed an oil-in-water (o/w) emulsion system. Emulsification can be achieved by rapid stirring, vortexing, homogenisation, microfluidisation or by sonication. The organic solvent is then allowed to evaporate by stirring the suspension at room temperature leading to microsphere solidification.

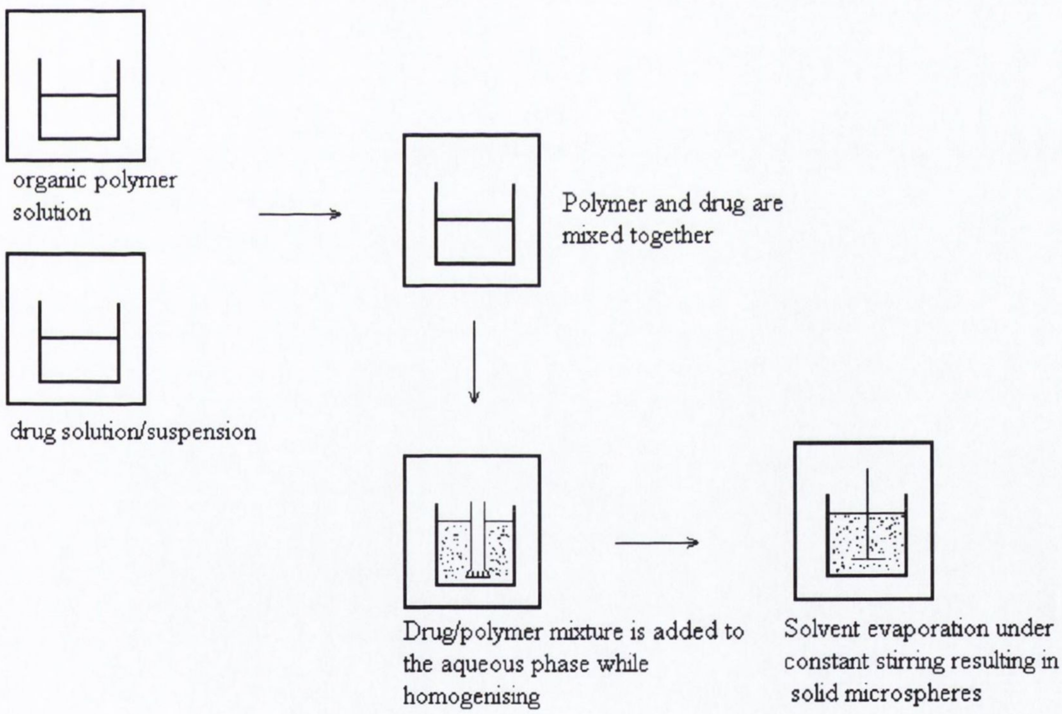


Figure 2.1 Schematic diagram of a basic solvent evaporation procedure

The evaporation process can be accelerated by the application of heat or a vacuum (*Aso et al.* 1993, Mehta *et al.* 1994), or by rotary evaporation (Fong *et al.* 1986). The emulsion must be stirred continuously during the evaporation process to prevent particle aggregation. The drug containing particles are then recovered from the suspension by filtration or by centrifugation, then washed and dried under vacuum or recovered by lyophilization. This process has been used to produce microparticles with high yields and reproducible drug release profiles (Fong *et al.* 1986, Parikh *et al.* 1993). Essentially the same process is used to produce nanoparticles except the droplet size is reduced by either increasing the shear rate or adjusting the formulation parameters to produce a smaller droplet size.

Many modifications of this procedure have been developed to suit the particular combination of materials used in the formulation. This basic procedure works well for molecules that are low-to-moderate water solubility. Highly water-soluble compounds are difficult to entrap in these systems because of the high degree of drug partitioning into the aqueous phase.

The inability of the o/w method to entrap highly water-soluble drugs in these systems resulted in this procedure being modified to an o/o type emulsion where the polymer and drug solution in an organic solvent such as acetonitrile are emulsified into an immiscible lipophilic phase such as mineral oil. A range of hydrophilic drugs have been encapsulated using this technique (Tsai *et al.* 1986, Jalil and Nixon 1990a). This technique requires that the microspheres must be recovered from the external phase and cleaned by use of an organic solvent such as hexane. The water-in oil-in water (w/o/w) technique first proposed by Ogawa and co-workers (1988) was developed to entrap water-soluble compounds. In this procedure an aqueous drug solution (usually containing a surfactant) is emulsified into the polymer solution to form water in oil (w/o) emulsion. This emulsion is then emulsified into an aqueous phase containing a surfactant to produce a w/o/w dispersion. The solvent is subsequently allowed to evaporate leaving an aqueous suspension of microspheres than can be recovered and dried as before. A multiphasic w/o/w/o technique for the entrapment of water-soluble compounds has also been described (Iwata and McGinity 1992/3, Yeh *et al.* 1995). The sustained release of proteins by adsorption onto the surface of pre-formed particles has also been described by Mehta *et al.* (1994).

The solvent evaporation procedure is a conceptually simple process, however many process variables can affect the properties of the particles produced. Some process parameters produce a '*microcapsule*' structure where the active ingredient is located in a central core in the form of a suspension, solid or a liquid (Linhardt 1990), surrounded by a wall of polymer material in the mononuclear or polynuclear state (Okada and Toguchi 1995), other formulations produce a matrix type system or '*microsphere*'. Microspheres are solid polymeric homogeneous or monolithic structures in which the active is dispersed through out the polymer matrix or dissolved in the polymer matrix. Nanocapsules or nanospheres are submicron (<1μm) equivalent of the corresponding microstructure. In practice, especially in older publications these terms have been used interchangeably and structures assigned without any actual knowledge of the particle internal morphology.

The particular formulation variables used in the microparticle manufacturing method influence the characteristics of the product. Many of these variables are designed to improve the drug loading of the microspheres. Drug loading is expressed as a % w/w of the overall material in a particular formulation while the % encapsulation efficiency is the amount of drug loaded relative to the amount of drug used in the particular formulation. Both drug loading and encapsulation efficiency are important terms in the characteristics and viability of the final formulation.

2.3 SELECTION CRITERIA FOR THE SOLVENT EVAPORATION PROCESS

2.3.1 Selection of a suitable solvent

The microencapsulation process involves the selection of two liquid phases, one that will contain the drug and the polymer (disperse phase) and one to contain the emulsifier (continuous phase) and the selection of these phases is critical to the overall process (Watts *et al.* 1990).

Important criteria for the disperse phase solvent:

- Ability to dissolve a chosen polymer
- Ideally, the solvent should be able to dissolve the drug
- Immiscibility with the continuous phase solvent
- Lower boiling point than the continuous phase solvent
- Low toxicity

Important criteria for the continuous phase solvent:

- Immiscibility with the disperse phase solvent
- Inability to dissolve the polymer
- Low solubility toward drug

- Higher boiling point than dispersed phase solvent
- Low toxicity
- Allows easy recovery and clean up of microspheres

The most common disperse phase solvent employed for the o/w procedure is dichloromethane (Suzuki and Price 1985, Spenlehauer *et al.* 1986, Benita *et al.* 1984, Bodmeier and McGinity 1987a,b) however the draw back in the use of dichloromethane is its toxicity (Martindale) as quantities of solvent can remain in the microspheres after drying. Benoit and Theis (1986) reported between 1.8 and 4.7% dichloromethane content in PLA microspheres by thermogravemitric analysis. Attempts have been made to substitute chlorinated solvents such as dichloromethane with alternative solvents such as ethyl acetate (McGee *et al.* 1995) and halothane (Armstrong *et al.* 1994).

Bodmeir and McGinity (1988a) carried out a detailed investigation on the importance of choice of solvent system. The solvents evaluated were acetone, acetonitrile, benzene, dichloromethane, chloroform, ethyl acetate, dimethyl sulphoxide (DMSO), methanol, ethanol and methylacetate. The rate of polymer precipitation is related to the rate of diffusion of the organic solvent into the aqueous phase. The rate of solvent diffusion is furthermore related to the miscibility of the solvent with the continuous phase. In the o/w system solvents with high aqueous solubility such as acetone or DMSO do not produce well-formed particles. High miscibility with the aqueous phase which results in rapid diffusion of the solvent causes the formation of hollow microspheres. Dichloromethane was determined by Bodmeir and McGinity (1988a) to have the most suitable properties (high water solubility and low boiling point) for the solvent evaporation process.

In a solvent extraction system the solvent is chosen to be mutually miscible with oil and the continuous phase so that solvent evaporation is accelerated. Acetone/water or dichloromethane/mineral oil is the most commonly employed systems. For the o/w system the mixture (dichloromethane/water) can also be poured into a mutually miscible solvent such as methanol. Adding a cosolvent to the organic phase or employing large volumes of continuous phase are also considered solvent extraction procedures (Arshady 1991, Conti 1992,) A mixture of dichloromethane and acetone is commonly used (Bodmeir and McGinity 1988a).

For the disperse phase water is the most common suspension medium. Partitioning of the drug between the organic and aqueous phase can cause the loss of some of the drug to the aqueous phase. In order to optimise the encapsulation of drug in these microspheres a number of strategies have been developed to reduce drug partitioning. The encapsulation of drug within the polymer matrix is highly dependent on the solubility of the drug in the aqueous phase. At pH values where the drug is highly soluble in the aqueous phase, the drug can essentially fully partition into the aqueous phase resulting in drug free microspheres. Loss of drug can be minimised by adjusting the pH of the aqueous phase to pH values of low drug solubility. Bodmeier and McGinity (1987a) showed the relationship between % drug content and pH of the aqueous phase for quinidine sulphate and quinidine base. In this case the drug was highly soluble below pH 7.5 therefore a range of external phases with different pH values were evaluated. At extreme pH values >pH 10.0 the surface of the microspheres became porous probably due to polymer hydrolysis. pH values around pH 8 were the optimum for this process.

As an alternative to this adjustment of the aqueous phase pH, presaturation of the continuous phase with the active to be encapsulated was found to improve the loading of microspheres (Bodmeier and McGinity 1987a and b, Wakiyama *et al.* 1981, Jalil and Nixon 1989). Even at aqueous phase pH values of high drug solubility when phase saturation was applied, high drug loadings were achieved. A linear relationship was observed between the amount of drug dissolved in the aqueous phase and the final drug loading, indicating that drug also can diffuse from the external medium into the polymer droplet (Bodmeier and McGinity, 1987b). In certain cases this can lead to the formation of drug crystals on the surface of the microsphere. Investigators found that the formation of drug crystals can be greatly reduced if the particles are recovered from the continuous phase before solvent evaporation is complete and allowed to complete solvent evaporation in stirred emulsifier-free water (Benita *et al.* 1984).

Solvent loss from the droplet causes an increase in polymer concentration at the droplet surface and when the limiting concentration is reached the polymer precipitates. While the droplet remains in the liquid state partitioning of the drug into the external aqueous phase and diffusion of the components of the external phase into the droplet can also occur. Bodmeier and McGinity (1987b) illustrated this concept in a study by investigating the effect of a time dependent pH change on the drug content. The pH of the external medium was changed from a low aqueous solubility to a high solubility over time. The drug content in the microspheres stabilised almost immediately and once polymer precipitation occurred no diffusion of drug from the microspheres occurred even with a change in external medium pH.

For compounds with higher water solubilities the emulsion used can be based on acetonitrile dispersed in a mineral oil o/o, with the aid of a surfactant (Span) for entrapment for a range of drugs such as Mitomycin C (Tsai *et al.* 1986). Phenobarbitone was encapsulated by dissolving the polymer and drug in acetonitrile and emulsifying this into light liquid paraffin (Span 40). Petroleum ether was used to wash the microspheres (Jalil and Nixon 1990a). Leelarasamee *et al.* (1986) employed a dichloromethane/mineral oil emulsion and washed the microparticles with hexane.

2.3.2 Selection of a suitable emulsifier

The role of the emulsifier in microsphere production by solvent evaporation is the short-term stabilisation of the suspended polymer droplets until microsphere hardening occurs. The presence of an emulsification agent was shown to be critical for the formation of individual well formed spherical microparticles and the concentration of the emulgent was found to effect the loading and yield of microspheres (Parikh *et al.* 1993).

O/w stabilisers include polyvinyl alcohol (PVA) (Benoit *et al.* 1986), polyvinylpyrrolidone (PVP) (Cha and Pitt 1988, Yan 1995), alginates, tween 20 (Denkbas *et al.* 1994) methylcellulose (MC) (Benita *et al.* 1984) and gelatin (Wakiyama *et al.* 1982a). Cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS) (Jeffery *et al.* 1991), 5% PVA/0.025%-0.05% sodium lauryl sulphate (SLS) (Suzuki and Price 1985), sodium oleate (Fong *et al.* 1986) and tween 80 0.05% (Bodmeier and McGinity 1987a) have also been utilised.

The preparation of surfactant free microspheres has recently been demonstrated to be possible. PLA oligomers which have an amphiphilic surfactant-like structure consisting of a polar head and a hydrophobic tail have the ability to stabilise an emulsion. PLA oligomers self stabilised the emulsion to produce microspheres (Carrio *et al.* 1995).

For the o/o emulsion solvent evaporation system span 40 (Jalil and Nixon 1990a-d), span 65 (Tsai *et al.* 1986) or span 80 (Iwata and McGinity 1992) are suitable emulsifying agents. A range of Spans (20-85) was utilised by McGee *et al.* (1995) to prepare microparticles and it was shown that Span 40 in low concentrations was the optimum. Insulin was encapsulated by an o/o technique using 10% aqueous acetonitrile/cotton seed oil with 0.05% soybean lecithin as an emulsifier (Wada *et al.* 1990).

2.3.3 Selection of suitable processing parameters

Processing parameters can be used to control particle morphology, particle size is determined by the mixing speed, equipment, and technique used (Watts *et al.* 1990). Particle size tends to decrease exponentially with increased mixing speed and is usually accompanied by a reduction in the particle distribution. Jalil and Nixon (1990a) showed a log-log linear relationship between the mean volume diameter of the microspheres with both the stirring rate and the concentration of the emulsifier in the external phase when all other parameters were kept constant. Increasing the rate and time length of the emulsification process decreased the mean diameter of the microspheres and also reduced the particle size distribution (Benita *et al.* 1984, Jeffery *et al.* 1991 and Sansdrap *et al.* 1993). The particle size can be adjusted through process parameters to produce particles in nano and microcapsule (100nm-2mm) range (Arshady *et al.* 1991). Yields of microspheres were also related to the microsphere size, since smaller particles are lost more easily during the recovery phase (Benita *et al.* 1984)

Jalil and Nixon (1990d) showed that the temperature of the evaporation process affected the microsphere properties. An increase in temperature of the process resulted in lower encapsulation efficiency and a smaller microcapsule size. This was attributed to the increased solubility of the phenobarbitone in the continuous phase and the lowering of the solution viscosity at higher temperatures resulting in the formation of smaller emulsion droplets. An increase in temperature of both phases resulted in a decrease in drug loading due to a higher solubilisation of the drug at higher temperatures (Bodmeier and McGinity, 1987b). Mehta *et al.* (1994) showed that the particle morphology could be affected by temperature of the evaporation and the gradient employed to raise the temperature. A rapid rise in temperature caused the formation of a large hollow core surrounded by a thin wall.

Utilising low temperatures of ~2°C could also produced misshapen particles (McGee *et al.* 1995).

The design of the equipment and the type of process used also affect the particle size. Bodmeier and McGinity (1987b) demonstrated the effect of apparatus design on the microsphere particle size where cylindrical vessels with side baffles were shown to produce relatively smaller particle sizes. A variety of mixing techniques have been utilised to produce spherical particles, including vortexing (Yan *et al.* 1994), sonication (Scholes *et al.* 1993, Yan *et al.* 1994, Park 1995), spray drying (Giunchedi and Conte 1995), stirring (Wakiyama *et al* 1982a, Parikh *et al* 1993, Lalla and Sapna 1993) homogenisation (Jeffery *et al.* 1991, O'Hagan *et al.* 1994) and microfluidisation (Verrecchia *et al.* 1993). Vortex mixing was unable to produce a fine and uniform emulsion, which resulted in large microspheres where the active was clustered within the polymer core and a porous outer structure. Homogenisation or sonication produced a more homogeneous matrix consisting of smaller microspheres that were more uniform in size and shape (Yan *et al.* 1994). High stirring speed increased the rate of solvent evaporation and decreased the drug loss to the external phase (Ramtoola *et al.* 1991). Spraydrying generally results in larger microparticles than can be formed by homogenisation since the size of the microsphere is limited by the diameter of the atomising system nozzle. Microfluidisation generally results in the formation of sub-micron particles.

2.4 FORMULATION VARIABLES AFFECTING MICROSPHERE CHARACTERISTICS AND MORPHOLOGY

In the previous section (Section 2.3) it was shown that there are many formulation variables that can be adjusted to optimise the microencapsulation process. Microspheres prepared by an emulsion method can exhibit different characteristics and morphologies depending on the particular formulation variables used. Many of the processes that are used to prepare drug-loaded particles involve the intrinsic mixing of the polymeric carrier with the drug molecules. This creates a location where a variety of physiochemical interactions are possible. These physiochemical interactions can influence the morphology and performance of drug loaded particles (Benoit *et al.* 1986, Yeh *et al.* 1995).

The state a drug molecule assumes in a polymeric environment is defined by the drug-polymer interactions that occur during the solvent evaporation process, the presence of residual solvent and the level of drug loading in the final device. These interactions between the drug and polymer can influence the release and performance of the drug from the final formulation. Crystalline drugs which are freely soluble in the casting solvent may exist in the final microsphere as either a molecular dispersion, as well defined crystalline domains or as a combination of both. When a crystalline drug is soluble in the casting solvent its ability to crystallise in the polymer is inhibited at low drug loadings because of the nature of the evaporation process. When the polymer solution containing the drug is emulsified into the aqueous phase the solvent begins to partition into the aqueous phase where it evaporates. This causes an increase in the viscosity of the remaining polymer solution. If the polymer T_g is above the required evaporation temperature then the polymer solution viscosity tends to infinity as the solvent concentration tends to zero. If the drug-polymer solvent phase is sufficiently viscous when the saturated solubility of the drug in the solvent is reached and if the rate of removal of the remaining solvent is fast enough then crystallisation of the drug is prevented. The dispersion is a meta-stable state formed because the drug does not have time to crystallise before the polymer phase forms an organic glass. In this state a drug that has no natural miscibility with the polymer is molecularly dispersed in the polymer. Benoit *et al.* (1986) carried out a physiochemical study on the morphology of progesterone-loaded poly (d,l-lactide) microspheres. Microspheres with no detectable crystalline material present on the surface were prepared. As the loading of drug was increased some crystallisation of material inside the particle occurred. It was shown that as the loading of the microspheres increased from 23% to 35% and then to 68% the presence of drug crystals on the surface of the spheres also increased. The formation of free drug crystals at the microsphere surface can be minimised by removing the emulsifier before the solvent evaporation process is complete and completing the evaporation in non-emulsified aqueous phase. Differential thermal analysis (DTA) of these microspheres revealed that the progesterone was molecularly dispersed in the polymer as no endothermic peaks due to progesterone were observed in the thermogram. Annealing experiments where the polymer is heated above the T_g of the polymer but below the melting temperature of the progesterone revealed that drug and the polymer are mutually immiscible as the drug crystallised out of the polymer upon the reheat followed by rapid cooling. It is assumed that what occurred in this case is that the progesterone molecules did not have time to crystallise during the evaporation process. The T_g of the polymer was shown to remain unchanged and therefore these states are not true molecular solutions since a true molecular solution would lower the T_g of the polymer on addition of drug molecules (Benoit *et al.* 1986).

Rosilio *et al.* (1991) also prepared progesterone loaded PLGA microspheres in a range of different loadings. As in the case with the PLA loaded particles (Benoit *et al.* 1986) the external appearance of the microspheres varied with loading; however the variation was more pronounced. As the progesterone loadings of the microspheres was increased the presence of drug crystals at the surface of the microsphere was observed. At >50% loading the microspheres became porous with drug crystals at the surface. The thermal analysis of the microspheres with greater than 22.8% loadings exhibited thermograms consistent with the presence of crystalline domains within the microspheres. At lower loading the amorphous microspheres showed crystalline domains after the samples were annealed; the progesterone was deemed to be present in a metastable molecular dispersion. Many of these microspheres exhibit an endotherm at 60-80°C associated with volatilisation of residual dichloromethane. The residual dichloromethane was found to diffuse from the samples when aged. The boiling point of dichloromethane is 40-41°C while the dichloromethane endotherm occurs at a much higher temperature. This suggests that the solvent is interacting with the polymer and is difficult to remove. This residual solvent observed may have an effect on the overall morphology and drug stability of these microspheres.

A diverse range of variables can influence microsphere morphology, these factors include the amount of drug in the particle, the nature of the drug and the nature of the polymer. Morphology and surface properties of the l-PLA microcapsules were found to be influenced by the loading of the microcapsules, as the loading increased an increase in the number of phenobarbitone crystals at the surface of the particles were observed. Microcapsules with low loadings showed a uniform smooth surface (Jalil and Nixon 1990a, d). Low drug loadings promote the dispersion of the drug in the polymer matrix and as the loading increased drug crystals began to appear at the microsphere surface and within the microsphere (Spenlehauer *et al.* 1988, Kishida *et al.* 1990). At high theoretical loadings the encapsulation efficiency was >90% but this gradually fell to 72.55% when the initial theoretical loading was 20% w/w phenobarbitone (Jalil and Nixon 1990e). A lowering of encapsulation efficiency with lowering of initial core loading was also observed with L-PLA (Jalil and Nixon 1990a). Microparticles produced by an o/w technique at high drug loadings produced distorted particles or unstable emulsions (Benita *et al.* 1984, Ramtoola *et al.* 1991). Microcapsule size was also found to reduce with reduced loading. High loadings resulted in an increase in the total solid content in the system which increased the collision rate between droplets resulting in a larger particle size after solidification of the microspheres (Jalil and Nixon 1990a and d).

The physicochemical characteristics of the active agent to be encapsulated can determine microsphere characteristics. The solubility of the drug in the solvent of choice and in the continuous phase determines the emulsion system required (o/w, o/o or w/o/w) and the internal morphology of the microsphere. Igwata and McGinity (1993) demonstrated the internal structure of conventional w/o and multiphasic microspheres. Cross sectional analysis of the o/w microspheres revealed a homogeneous matrix while multiphasic microspheres contained pockets of drug within the microsphere and at the polymer wall.

Some investigations have also reported the effects of drug characteristics on the polymer matrix. Bodmeier and McGinity (1987) reported the degradation of the polymer material when quinidine free base or its sulfate was encapsulated. The enhancement of the degradation in the presence of basic loads was also studied by Cha and Pitt 1989 where they showed that this catalytic effect followed the order meperidine>methadone> promethazine>>naltrexon. Schwope *et al.* (1975) showed that naltrexon free base in PLA had the ability to act as a plasticizer thus making processing of the polymer difficult. Vert *et al.* (1994) showed that gentamycin base and gentamycin sulphate interact with carboxylic end groups in an acid base reaction. The basic form of the drug that was soluble in the solvent reacted during the manufacture process while gentamycin sulphate required the presence of water to react with the polymer. Protein and peptide groups also have the ability to interact or form complexes with polymer carboxyl groups. Interactions between proteins tend to be protein and polymer specific (Gombotz and Pettit 1995). Adsorption of BSA onto PLA nanoparticles was demonstrated by Verrecchia *et al.* (1993).

The nature of the polymer, even the particular properties of equivalent polymers produced by different manufacturers (Wang *et al.* 1991, O'Hagan *et al.* 1994) also affects microsphere properties. Bodmeier *et al.* (1989) investigated the behaviour of different blends of low (2,000) and high (120,000) molecular weight PLA polymer. It was found that the formation of microspheres did not occur at ratios smaller than 25/75. The molecular weight of the polymer can affect the properties of the microspheres, higher molecular weight d,l-PLA polymers produced larger microcapsules (Jalil and Nixon 1990e). Microsphere size increased with increased molecular weight of the polymer for l-PLA of molecular weight 61300, 43200 and 2400 due to the increase in solution viscosity of the higher molecular weight polymer (Jalil and Nixon 1990b).

A number of formulation variables were effective in controlling the particle size, these include variations in surfactant type and concentration, the viscosity of the organic and aqueous phase and varying the ratios of organic to aqueous phase. For a constant surfactant concentration (1%) microspheres are produced in the following rank order of decreasing particle size: gelatin>MC>SDS>PVA>CTAB (Jeffery *et al.* 1991).

Increasing the surfactant concentration in the external phase reduced the particle size of the resultant microspheres (Spenlehaeur *et al.* 1988, Jeffery *et al.* 1991, and Sansdrap *et al.* 1993). Increases in PVA concentration from 8-15% decreased particle size, after which efficient homogenisation is not possible and particle size increases (Scholes *et al.* 1993). Above this concentration the effect of the emulsifier levelled off because optimum packing of the surfactant around the emulsion droplet was achieved.

The effect of changing emulsifier was demonstrated using insulin-loaded particles. PVA was shown to cause a higher burst effect and subsequent release than did gelatin when used as the emulsifier (Kwong 1986) and this difference was attributed to differences in the solubility of the drug by the emulsifier. In the production of progesterone loaded microparticles the presence of drug crystals on the microsphere surface could be eliminated by the removal of the emulsifier solution halfway through the process and then re-suspending the particles in water. In this system the presence of the drug crystals was attributed to the enhanced solubilisation of the drug by the emulsifiers at the microsphere surface (Benita *et al.* 1984).

The viscosity of the organic phase is a significant factor in the formulation, increases in the disperse phase viscosity due to an increase in polymer or drug concentration was found to increase the particle size (Maulding *et al.* 1986 Denkbas *et al* 1994, Yan *et al.* 1994). Morphology rather than drug loading was affected by altering this process parameters. Smaller microcapsules produced had a slightly lower loading due an increased surface area, which contribute to a higher loss of drug from the microcapsule surface during manufacture (Jalil and Nixon 1990a and d). The mean particle size decrease of nifedipine PLGA microspheres was accompanied by a corresponding reduction in particle size distribution when the volume of the internal oil phase was increased (Sansdrap *et al.* 1993). Lowering the molecular weight of the polymer dissolved in the solvent also caused a lowering of particle size due to a reduction in organic phase viscosity (Scholes *et al.* 1993). Yan *et al.* (1994) examined the effects of polymer concentration on average particle size, encapsulation efficiency and release properties of FITC-BSA PLGA microparticles. As the PLGA concentration increased from 5% w/v to 35% w/v the particle size increased from 5.4μm to 64.4μm. The core loading and the encapsulation efficiency also increased.

The effect of viscosity of the organic phase on the distribution of the drug within the microsphere matrix was examined by optical imaging of cisplatin microspheres. Microspheres prepared with a polymer:dichloromethane ratio of 1:12.6 (g:ml) showed drug loading near to the polymer wall, however when this was compared to microspheres prepared with a polymer:dichloromethane ratio of 1:8.4 (g:ml) the drug dispersed more evenly through the microsphere matrix (Spenlehaeur *et al.* 1988). The appearance of drug crystals is a function of how well the drug can migrate through the polymer solution. Decreases in the polymer viscosity increases the number of crystals at the surface of the microsphere, increases in the molecular weight of the polymer indirectly decreases the number of drug crystals by increasing the polymer solution viscosity (Spenlehaeur *et al.* 1988).

The art of removal of the solvent is also a determinant of microsphere morphology. Li *et al.* (1995) carried out a detailed study on the effects of solvent removal rate from microspheres. In this study it was observed that a low solvent:continuous ratios (1/100) provides a driving force for solvent removal due to better sink conditions for the solvent compared to higher solvent:continuous ratios (1/10). Low solvent:disperse ratios causes earlier hardening of the microspheres that in turn affects other properties such as residual solvent drug loading and internal morphology. Low solvent:disperse ratios gave higher residual dichloromethane and slower release rates due to a higher distribution of drug away from the surface of the sphere. Spenlehauer *et al.* (1986) showed that the presence of small amounts of cyclohexane in the solvent mixture (cyclohexane/dichloromethane) leads to the formation of highly porous microcapsules. Cyclohexane is a poor solvent for PLA and is less volatile than dichloromethane and when the dichloromethane evaporates it entraps cyclohexane, eventual evaporation of cyclohexane creates pores in the microparticle surface.

The volume, pH and ionic strength of the external medium can influence particle properties. Jeffery *et al.* (1991) noted a linear reduction in particle size when the volume of the external phase was reduced. The structure of the microspheres is also a function of the electrolyte concentration added to the buffer by altering the osmotic pressure gradient. The effect of this concentration was demonstrated by adding varying amounts of KCl to the aqueous phase buffer used to prepare quinidine sulphate and quinidine base microspheres at pH 13 (Bodmeier and McGinity, 1987a). The addition of electrolytes to the buffer solution resulted in a flow of water and ions into the microspheres resulting in an increasingly irregular microsphere structure with increasing amounts of KCl. Hermann *et al.* (1995) used this same principal to control the microstructure and loading of somatostatin microspheres using sodium and calcium chloride and a range of various buffers from pH 2.2 to 5.5. Varying the osmotic gradient through altering the salt concentration or pH of the buffer produced porous microparticles that gave an increased release rate of somatostatin acetate from poly(lactide) microspheres.

CHAPTER 3

MECHANISMS OF DRUG RELEASE FROM AND POLYMER DEGRADATION OF POLYESTER MATRICES

3.1 INTRODUCTION TO DRUG RELEASE FROM BIODEGRADABLE POLYMERS

The poly-α-hydroxy aliphatic esters display many characteristics that make them attractive as sustained drug delivery systems. The performance of these devices is usually assessed on their ability to release the active form of an encapsulated drug at a rate dictated by its therapeutic function. Drug release is a combination of a range of processes that are attributed to three basic phenomena, diffusion through the polymer, release as a result of matrix degradation and solubilisation followed by diffusion through pores and channels (Vert 1991). This mechanism is related to the physicochemical properties of the drug and the polymer matrix. Drug release data have a number of potential applications. At the simplest level it can be used for quality control purposes to ensure batch to batch reproducibility and can also be used to try to understand the physiochemical structure of the delivery system and its release mechanism. Furthermore this data can be used in an attempt to predict the likely performance of the device *in vivo* (Washington 1996).

Drug release from microsphere systems is usually assessed by dispersion in an agitated buffered release medium that is withdrawn at specific time intervals and assayed for drug content. Drug release has been evaluated using the following procedures; Wakiyama *et al.* (1982a) used the 'shaken flask' method to determine drug release while the 'rotating bottle' procedure has also been used to evaluate drug release (Bodmeir and McGinity 1887a, Iwata and McGinity 1993). Drug dissolution may also be evaluated using the Vankel U.S.P dissolution apparatus (Bodmeier *et al.* 1989, Jalil and Nixon, 1990f). As an alternative to these closed rotational systems, an open-flow arrangement where drug release is measured in the elution medium which is passed over the device, may also be utilised (Gould 1983, Koosha *et al.* 1988). An equilibrium dialysis technique involves placing a small volume of the concentrated drug-particle suspension in a dialysis bag, which is immersed in a larger volume of continuous phase buffered acceptor fluid (Miyazaki *et al.* 1986, Cha and Pitt 1988, Park *et al.* 1995a). While a vast array of dissolution methodologies exist it is the data interpretation and correlation with the *in vivo* performance of the device that still remains a challenge.

It is difficult to attribute drug release to specific parameters when a great number of variables that can affect the release properties exist. The release profile of a drug from a polymer system involves a complex mechanism of many factors. Several attempts at describing the release processing from a polymer system have been evaluated using mathematical models; these are discussed in the following sections.

3.2 MATHEMATICAL DESCRIPTION OF DRUG RELEASE FROM A POLYMER MATRIX.

The majority of PLA/PLGA drug delivery systems are based on monolithic systems where the drug is physically incorporated into the polymer matrix. Monolithic systems can be divided into two different types of system: those that contain drug which is dissolved in the polymer matrix or those where the drug is dispersed in the polymer matrix and which is released by diffusion through the polymer and/or through pores and channels created in the polymer matrix.

3.2.1 Diffusion of a dissolved drug through the polymer matrix

The release process of a drug dissolved or molecularly dispersed in a spherical polymer matrix with a radius *r* was described using the model proposed by Baker and Lonsdale (1974):

$$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{r^2\pi}\right)^{1/2} - \frac{3Dt}{r^2}$$ For release $0<M_t/M_\infty<0.4$ Equation 3.1

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\exp\left[\frac{-\pi^2 Dt}{r^2}\right]$$ and For release $0.6<M_t/M_\infty<1$ Equation 3.2

where M_t/M_∞ is the fraction of drug with diffusion coefficient *D*, released over a time *t*. This model states that the release of the first 40% of the drug is linearly related to the square root of reciprocal time $t^{-1/2}$ and that the final 40% of drug is released in an exponential manner. Vandelli *et al.* (1993) studied the release of progesterone from microspheres and found at low drug loading, drug release fitted the model proposed by Baker and Lonsdale (1974) for a molecularly dispersed drug. However as the drug loading increased, the goodness of fit to the model reduced due to crystallisation of the drug in the polymer matrix. The physical state of the drug in the matrix was confirmed by DSC analysis.

3.2.2 Diffusion of a dispersed drug through the polymer matrix

Early theories which have been put forward to describe the dissolution process from matrix systems were very often based on Fick's laws of diffusion. Fick's first law of diffusion states that the flux (*J*) or drug mass *dM* in time *dt*, of a compound across a unit area (*A*) of a predetermined reference plane is given by:

$$J = -D\frac{dC}{dx} \qquad \text{Equation 3.3}$$

Where C is the concentration in g/cm^3 and x is the distance in cm of movement perpendicular to the surface of the barrier. Based on Fick's first law of diffusion Higuchi (1961) developed an equation for release of solid drug from an ointment base and later applied it to the diffusion of solid drugs in a granular matrix dosage system (Higuchi 1961).

$$Q=(DC_m(2Ct-Cs)\,t\,)^{1/2} \qquad \text{Equation 3.4}$$

where Q is the amount of drug released per unit area of matrix, D is the diffusivity of the drug in the matrix, C_t is the total amount of drug per unit volume of matrix, C_m the solubility of drug in the polymer matrix, C_s is the solubility of the drug in the sink phase and t is time. This model assumes: the presence of excess drug compared to the solubility of the drug in the polymer matrix ($C_t \gg C_m$) where C_t should preferably be at least ten times that of C_s, the diffusion coefficient is constant, the drug particle size is small relative to that of the polymer matrix, sink conditions prevail and that there is no interaction between the drug and the polymer (Deasy 1984). Assuming that diffusivity and the other parameters are constant this equation may be expressed as (Higuchi 1963):

$$Q = kt^{1/2} \qquad \text{Equation 3.5}$$

when k is a constant. This equation has been used to describe the release of phenobarbitone from PLA microspheres (Jalil and Nixon 1990h) and the release of progesterone from ethylene-vinyl acetate microspheres (Vandelli *et al.* 1993) and PLA microspheres (Yoshioka *et al.* 1995). While Pradhan and Vasavada (1995) fitted the release of a range of peptides from PLA to this equation, when no degradation of the polymer matrix occurred.

Higuchi subsequently described the release from an irregular matrix such as a granular matrix or a cluster of microspheres when Q is the amount of drug released at time t:

$$Q = \left[\frac{D\varepsilon}{\tau} \left(2C_t - C_s \right) C_s t \right]^{1/2} \qquad \text{Equation 3.6}$$

where D is the diffusion coefficient of the drug in the permeating fluid, C is the drug solubility, ε is the porosity and τ is the tortuosity of the matrix.

A common microsphere structure is where the drug and the polymer exist in a phase-separated state. This occurs when the drug is in excess of its solubility in the matrix and crystallises into pure domains which are released by diffusion through the polymer. The release rate for this type of system was derived by Higuchi (1963):

$$\frac{3}{2}\left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{2/3} \right] - \frac{M_t}{M\infty} = Bt \qquad \text{Equation 3.7}$$

$$B = \frac{3C_s D}{r^2 A} \qquad \text{Equation 3.8}$$

where M_t and M_∞ are the amounts of drug released at time t and infinite time respectively, and B is a constant which describes the combined effect of drug solubility in the dissolution medium (C_s), drug diffusivity (D), and radius of the matrix (r). The initial drug content per unit volume M_t/M_∞ is the fractional drug release. This model is based on a flux mechanism where drug, which dissolves in the penetrating solvent, diffuses from the polymer matrix.

The Higuchi spherical matrix model has been used to describe the release of oxytetracycline HCl (Vidmar *et al.* 1984), hydrocortisone (Leelarasamee *et al.* 1986), phenobarbitone (Jalil and Nixon 1990g) and oestrogen (Parikh *et al.* 1993) from PLA particles. The release curves from hydrocortisone loaded d,l-PLA microspheres developed by Leelarasamee *et al.* (1986) showed a fast first stage and a slow second phase drug release. The diffusion-controlled release could be fitted to Higuchi (1963). Studies by Leelarasamee *et al.* (1988), Suzuki and Price (1985), Parikh *et al.* (1993) and Tsai *et al.* (1986) have shown that both the solubility of the drug and the particle size of the polymeric system affect the rate of drug release as described by Equation 3.6. An exponential increase in drug release occurs with decrease in particle size consistent with that predicted in the above equation since the rate constant B is proportional to $1/r_0^2$. Aso *et al.* (1994) demonstrated that drug release at different incubation temperatures could be fitted to the Higuchi equation, therefore the mechanism of drug release was independent of temperature.

Leelarasamee *et al.* (1986) demonstrated that when log B was plotted against log C_0 a linear relationship was observed indicating an empirical relationship existed between D and the drug loading C_0 where,

$$D = kC_0^n \quad \text{and} \qquad \text{Equation 3.9}$$

$$B = \frac{3C_{ms}kC_0^{(n-1)}}{r_0^2} \qquad \text{Equation 3.10}$$

The value of n for the polylactic acid-hydrocortisone system was determined to be 4.1±0.1, this implies that a two fold increase in drug loading will result in an eight fold increase in release rate for this particular system studied.

3.2.3 Diffusion of a drug through an eroding polymer matrix

These equations represent release from a matrix by a diffusion process, however because these polymers undergo hydrolysis in an aqueous medium, the properties of the matrix may also change with time due to diffusion through aqueous pores in the matrix. Modifications of the Higuchi model that represent changes in the drug permeability and porosity of the matrix have been developed to fit drug release from a polymer matrix that is degrading and hence is its properties are continuously changing (Chiu *et al.* 1995).

$$\frac{Q_t}{Q_\infty} = \frac{1}{L}\sqrt{\frac{2C_w^*}{A}\int_0^t P(t)dt} \qquad \text{Equation 3.11}$$

Where L is the half-thickness of the matrix, and $Q_\infty = AL$ is the drug loading per unit release area. By determining P experimentally of polymer films, which incorporates the effects of changes in molecular weight and matrix porosity, at different times the release profile can be calculated to account for these effects.

Empirical models of drug release have also been proposed in the literature. The diffusion exponent approach described by Peppas *et al.* (1984) was used to describe diffusion behaviour by hydrating or eroding systems. The diffusional exponent method proposes a power law relationship for drug release according to:

$$\frac{M_t}{M_0} = kt^n \qquad \text{Equation 3.12}$$

The constant n the diffusional exponent should equal 0.5 for diffusional (Fickian) release from a planar slab and 0.43 from a spherical geometry. Values greater than these are indicative of anomalous diffusion such as that which occurs in a system that swells prior to release. The value of n decreases when diffusion through the polymeric network or through pores occurs. Parikh *et al.* (1993) evaluated n as 0.46 for 3.5% oestrogen loaded PLA microspheres suggesting diffusion through the matrix, n values decreased when the drug loading increased.

Another popular empirical relation is to assign release to a bi-exponential process:

$$\frac{M_t}{M_0} = 1 - \left[A\exp(-k_A t) + B\exp(-k_B t)\right] \qquad \text{Equation 3.13}$$

where k_A and k_B are the rate constants of the two components usually assigned to the burst and sustained release components of the release profile (Washington 1996).

Using the same diffusion principle as Higuchi, Cobby *et al.* (1974), presented equations describing the release of drug from matrix tablets having either a spherical, cylindrical or a biconvex shape. In each case the three dimensional equation has the cubic form:

$$F = G_1(kt^{1/2}) - G_2(kt^{1/2})^2 + G_3(kt^{1/2})^3 \qquad \text{Equation 3.14}$$

where F = fraction of drug released in time t, k is the release rate constant, G_1 - G_3 are shape factors. For a spherical shape, G_1 and $G_2 = 3$ and $G_3 = 1$.

The kinetics of drug release by polymer erosion have been investigated by Baker and Lonsdale (1976) for a system where the drug is dispersed in a polymer that erodes at a constant rate per unit surface area:

$$\frac{d\left(M_t / M_\infty\right)}{dt} = n(1 - kt / C_0 r_0)^{n-1}(k / C_0 r_0) \qquad \text{Equation 3.15}$$

where C_0 is the initial drug concentration, r_0 is the radius or thickness of the device, k is the erosion rate constant. For a cylinder n=3, n=2 for a sphere and n=1 for a slab or film. Singh *et al.* (1991) used this model to describe the release of a protein from PLA microspheres, which was released by a polymer degradation mechanism. This model assumes that the drug does not migrate within the polymer matrix. In the case where drug migration is accounted for, the drug release rate depends on the relative rates of drug diffusion, polymer erosion and on the geometry of the device. In this case drug release tends towards zero order as the film thickness, drug concentration and erosion rate are increased relative to the drug solubility and the diffusion coefficient. For example, the expression for a slab is given as:

$$\frac{\Delta l}{l_0} = \frac{M}{M_0} - \left[1 - \exp\left(\frac{-B_e l_0 M}{M_0}\right)\right] / B_e P l_0 \qquad \text{when } C_0 >> C_S \qquad \text{Equation 3.16}$$

where B_e is the erosion rate, P is the ratio C_0/DC_s, and Δl and l_0 are the thickness and the initial thickness of the device respectively.

Wada *et al.* (1995) modified the equations originally derived by Baker and Lonsdale to allow for changes in the diffusivity D as a result of matrix degradation. An empirical relationship between D and the molecular weight Mn of the matrix as:

$$D = D_0 - k \ln Mn \qquad \text{Equation 3.17}$$

where D_0 and K are constants. Since,

$$\ln M_n = \ln M_{n,initial} - k_d t \qquad \text{Equation 3.18}$$

where k_d is the degradation rate constant. Combining these two equations gives the following relationship between D and t:

$$D(t) = D_{init} + kt \quad (\text{k=constant}=k_d \times k) \qquad \text{Equation 3.19}$$

where $D_{init}=(D_0\text{-}k\ln Mn,\ init)$ is the diffusion coefficient at t=0. The Equations 3.1 and 3.2 can be rewritten as:

$$\frac{Mt}{M_\infty} = 6\sqrt{\frac{D(t).t}{\pi r^2}} - 3\frac{D(t).t}{r^2} \qquad 0<Mt/M\infty<0.4 \qquad \text{Equation 3.20}$$

$$\frac{Mt}{M\infty} = 1 - \frac{6}{\pi^2}\exp\left(-\frac{\pi^2 D(t).t}{r^2}\right) \qquad 0.6<Mt/M\infty<1.0 \qquad \text{Equation 3.21}$$

This model was used to fit the release of aclarubicic hydrochloride from poly (lactide) microspheres (Wada *et al.* 1995).

Hixson and Crowell (1931) derived the 'cube root law' from the recognition that the surface area of a regular particle is proportional to two-thirds power of its volume according to:

$$\left(\frac{W_d}{W_i}\right)^{1/3} = 1 - k_3 t \qquad \text{Equation 3.22}$$

where W_i is the initial weight of the sample, W_d is the dry weight of the sample after incubation in the dissolution medium for a time t, k_3 is a rate constant having the dimensions of the cube root of mass per unit time. This relationship assumes that the dimensions of the device decrease proportional to one another.

A model described by Fitzgerald and Corrigan (1993) has been used to fit polymer degradation and drug release from PLGA matrices. This model is based on a solid decomposition model first described by Prout and Tompkins (1944). This model has been used to describe polymer decomposition dependent drug release from PLA/PLGA systems for the release of fluphenazine (Ramtoola *et al.* 1992), procaine (Fitzgerald *et al.* 1991) and diltiazem base (Fitzgerald and Corrigan 1993).

$$ln\frac{c}{1-c} = kt + m, \text{ where } m = -kt_{max} \qquad 3.23$$

Polymer decomposition is initiated by polymer hydrolysis at or near the device surface. The size and shape of the device determine the number of active sites where hydrolysis is initiated. Hydrolysis of the polymer chains results in the formation of low molecular weight chains that eventually become water soluble and diffuse from the matrix, this creates a more porous matrix where further active sites are formed.

3.3 DEGRADATION OF POLYESTERS DERIVED FROM LACTIDE AND GLYCOLIDE

The degradation properties of poly (lactide) and poly (lactide-co-glycolide) matrices are central to their performance as controlled release systems. The degradation of the polymer with concomitant release of the entrapped drug plays an important role in the release mechanism from biodegradable polymeric matrices. In this section the degradation properties of PLA/PLGA type systems is examined.

3.3.1 Mechanism of hydrolytic degradation of aliphatic polyesters

The hydrolytic degradation of aliphatic polyesters of the PLA/PLGA type is known to primarily depend on the kinetics of the cleavage of main chain ester bonds according to a well-known reaction:

$$[COOH]_t = k'[COOH]_0[H_2O][Ester] \qquad \text{Equation 3.24}$$

The homologous and heterologous polyesters of α-hydroxy carboxylic acid are hydrolytically degraded into their corresponding monomeric hydroxyacids under environmental as well as under physiological conditions.

Degradation via chain cleavage can occur either by random chain scission of ester bonds (Wang *et al.* 1990), by chain end scission or 'unzipping' of the polymer chains or by a combination of both (Shih 1995). Random chain scission is defined as the statistical random process of chain cleavage where all links between the monomer units in the chain molecules are of equal strength and accessibility while chain end scission or 'unzipping' is a depolymerisation process where small fragments or monomer units are broken off the chain ends (Jellinek 1955).

The polymer degradation mechanism can also be described in physical terms which are based on the location of polymer breakdown (Heller 1983, Holland and Tighe 1992) either homogeneous, occurring a the same rate throughout the polymer matrix or heterogeneous, where surface-centre differential rates exist. It was originally assumed that PLA and PLGA polymers degraded via a homogeneous degradation mechanism (Kenley *et al.* 1987), however, recent studies showed a heterogeneous degradation mechanism in large polymer devices (Li *et al.* 1990, Therin *et al.* 1992). SEM analysis of cross-sections of degraded polyester devices, which after advanced degradation times showed a hollow interior, support this mechanism (Ali *et al.* 1993).

When a polymeric device is placed in an aqueous environment, water uptake by diffusion into the device occurs. Initially, hydrolysis of the ester bonds begins homogeneously in the device where water uptake has begun resulting in the cleavage of polymer chains to lower molecular weight chains. Several workers have shown that the degradation of polylactic acid and polylactide-co-glycolide occurs in two stages. In the first stage, random hydrolytic chain scission to produce a linear decline in molecular weight occurs, while the second stage involves gravimetric weight loss from the polymer (Pitt *et al.* 1981, Kenley *et al.* 1987, Fitzgerald 1994). Other investigations showed that that measurable weight loss did not occur until a critical molecular weight of Mn<5000 was achieved (Pitt *et al.* 1992, Hausberger *et al.* 1995). Sah *et al.* (1995) showed that PLA and PLGA polymers with a molecular weight of 5,000 demonstrated immediate mass loss without an induction period.

Degradation of the ester bonds causes an increase in the number of carboxylic end groups that are known to autocatalyse the ester hydrolysis. Oligomers that are soluble in the surrounding medium and are close to the surface of the device can leach out and escape from the device, but those that are located in the centre of the device are trapped by the remaining polymer where they contribute to the autocatalytic effect. The catalytic effect of carboxyl groups has been attributed to hydrogen bonding of carboxyl hydrogens produced according to equation 3.26, to the ester linkages of the internal polymer chain (Pitt *et al.* 1987). A non-invasive magnetic resonance (EPR) technique was used to demonstrate the development of an acidic microenvironment in PLGA tablets (Mader *et al.* 1995).

The kinetic laws governing chain cleavage are derived from the assumption that cleavage is catalysed by the carboxyl end groups generated during degradation and have been described by the rate equation in 3.24.

The two relationships between [COOH] and [Ester] are expressed as:

$$[COOH] = \frac{w}{Mn}.V = \rho Mn$$ Equation 3.25

$$[COOH] = \frac{[Ester]}{DP - 1}$$ Equation 3.26

where w is the weight of the polymer sample, V is its volume and ρ is its density the following equation is obtained:

$$\frac{d(1/DP)}{dt} = k(\rho / Mn)[H2O](DP - 1)DP^{-2}$$ Equation 3.27

Integration leads to:

$$\ln\frac{(1 - DP)}{1 - DP_0} = -k^{'}t$$ Equation 3.28

where $k' = k\,[H_2O]\,\rho$/Mn and DP and DP_0 are the degrees of polymerisation at time t and zero respectively. This kinetic expression holds when there is no mass loss from the polymer (Zhu *et al.* 1991, Pitt *et al.* 1981,1987, 1992). When DP>>1, equation 3.30 can be simplified to:

$$\ln\frac{DP}{DP_0} = \ln\frac{Mn}{Mn_0} = -k^{'}t$$ Equation 3.29

Thus, a semi-log plot of Mn versus time is linear during this first phase of degradation. Kenley *et al.* (1987), Pitt (1990) and Shah *et al.* (1992) demonstrated a first order reduction in molecular weight for PLA/PLGA. In these reports, random scission of the backbone ester bonds was assumed and the degradation kinetics was described by a singe rate constant.

3.3.2 The degradation of PLA and PLGA based devices

The pioneering study on degradation of PLA/PLGA type polymers was carried out using a series of polyglycolic/polylactic acid homo and copolymers (Gilding and Reed 1981). Degradation studies were carried out on lengths of suture material and on discs prepared from cast films. The test material was degraded in buffer at 37°C. Degradation was measured in terms of loss of tensile strength, mass loss and molecular weight changes. The tensile strength, mass and molecular weight of the polymer were shown to decrease with incubation time.

The biodegradation of polyglycolic acid proceeds more quickly than that of polylactic acid. The reduced reactivity of polylactic acid relative to polyglycolic acid is a reflection of the steric effect of the methyl side chain. The order is: glycolide>d-lactide>l-lactide. Polylactide and polyglycolide are highly crystalline while copolymers of both acids are amorphous. Amorphous polymers degraded more rapidly than the corresponding crystalline homopolymers (Gilding and Reed 1979, 1981). Differential degradation rates between amorphous and crystalline regions in polyester devices have been shown (Asano *et al.* 1989, Pistner *et al.* 1993). Amorphous regions are more accessible to water than crystalline regions and hence degradation proceeds faster in these regions (Park 1995b). Increasing the glycolide content in the copolymer increases the rate of degradation (Spenlehauer *et al.* 1989, Asano *et al.* 1989, Wang 1990 and Park 1995b).

The profile of water uptake by polyester devices has also been examined by Hutchinson and Furr (1990) where water uptake was governed by two events. The first event is diffusion of water into the polymer matrix, then further uptake of water occurs due to erosion of the polymer. For a high molecular weight polymer a lag phase during which no further water is taken up occurs between the diffusion mediated and the erosion mediated phases. Water uptake into a thin film of molecular weight Mn and polydispersity (*P*) in the absence of hydrolytic degradation is governed by the following relationship:

$$[H_2O] = a + \frac{b}{PMn} \qquad \text{Equation 3.30}$$

where a and b are constants related to the polymer composition.

If the initial stage is instantaneous, the approximate expression for the degradation induced change in molecular weight and water uptake can be obtained. Polymer degradation was defined by the rate of production of $-CO_2H$ according to:

$$\frac{d[COOH]}{dt} = k[H_2O][Ester][COOH] \qquad \text{Equation 3.31}$$

Where [COOH] $\propto 1/Mn$ and Mn is the number average molecular weight at time *t*. Assuming ester concentration is constant and using the relationship 3.32 then equation 3.33 was be rewritten as:

$$\frac{d\left[1/Mn^t\right]}{dt} = k'\left(a + \frac{b}{PMn^t}\right)\frac{1}{Mn^t} \qquad \text{Equation 3.32}$$

where k'=k [Ester], and at time zero $Mn^t = Mn^0$ then,

$$Mn^t = Mn^0 e^{-akt} + \frac{b(e^{-akt} - 1)}{aP} \qquad \text{Equation 3.33}$$

$$\left[H_2O\right] = a\left[1 + \frac{b}{aPMn^0 e^{-akt} + b\left(e^{-akt} - 1\right)}\right] \qquad \text{Equation 3.34}$$

Hutchinson and Furr have used this model to successfully fit the water uptake during the degradation of poly (lactide-co-glycolide) films of varying molecular weights (Hutchinson and Furr 1990).

Poly (d,l-lactic acid) microcapsules were found to swell in an aqueous environment and the mean size was found to increase linearly with time over the study period. This rate of swelling was higher with the low molecular polymer and also showed a dependence on the drug loading of the microcapsule (Jalil and Nixon 1990e).

Many of the studies that have been carried out on these polymers have focused on implant systems. Most of the initial studies carried out have been investigated under different incubation conditions and therefore only generalised observations can be compared between different investigators, however, a number of authors have taken a standardisation approach to the investigation of the degradation properties of polyester implants (Li *et al.* 1990, Vert *et al.* 1991).

A standard protocol was developed by Li *et al.* (1990) to investigate the degradation of parallelepipedic samples (15x10x2mm) cut from a compressed solid polymer. Poly d,l-lactic acid (PLA50) was allowed to degrade in isotonic saline and in phosphate buffered saline (PBS) and the changes were monitored in the medium and in the polymer. The pH of the incubation medium was found to dramatically reduce in the saline after an 8-week period however the drop was not as substantial in PBS due to the buffering capacity. The degradation of massive PLA50 specimens proceeds more rapidly at the centre of the device than at the surface. When degradation was well advanced the interior of the device became hollow. This phenomenon was related to the formation of an outer layer of slowly degrading polymer that entraps the inner polymer macromolecules. As the degradation proceeds the number of carboxylic acid groups in the interior of the device increases resulting in an autocatalytic effect on degradation. The difference between the molecular weight of the surface and the core was demonstrated by bimodal size exclusion chromatogram (SEC). When the surface of the device becomes permeable to oligomers a weight loss is observed. (Li *et al.* 1990 Part 1). In a second study by these authors the degradation of PLA37.5GA25 (75% d,l-lactide, 25% glycolide) and PLA75GA25 (75% l-lactide and 25% glycolide) was examined in isotonic saline, phosphate buffered saline and in water. The surface centre differential rate was also demonstrated for these polymers. The rates of degradation were compared and it was observed that intrinsically amorphous PLA75GA25 crystallised as degradation proceeded, in contrast to PLA37.5GA25. The rate of degradation was faster in iso-molar solutions than in water, since the carboxyl-terminated inner oligomers dissolved more easily in the buffer than in water, the carboxylic form RCOO-Na+ of organic acids being more hydrophilic than the carboxylic form RCOOH. (Li *et al.* 1990 Part 2). In the third study by this group, the effects of morphology on the degradation characteristics of poly (l-lactic acid) PLA100 were examined. Amorphous (quenching) or semicrystalline (annealing) devices were examined.

Degradation of the Pl-LA was considerably slower than the other polymers. Degradation proceeded more rapidly in the centre than at the outside for both devices. Initially, amorphous PLA100 was found to crystallise as degradation proceeded and this material was found to be very resistant to degradation. The morphology was a critical factor in the degradation rate, and device degradation depends on the thermal history and the initial crystallinity of the polymer (*Li et al. 1990* Part 3).

It has been reported that in PLGA, glycolide linkages (G-G and G-L) will be preferentially cleaved compared to that of lactide linkages (L-L) because it is more hydrophilic (Reed and Gilding 1981, Wang *et al.* 1990). It is therefore possible that as degradation advances the percentage of lactide increases and the situation may occur where lactic acid sequences may become crystalline (Li *et al.* PartII 1990, Vert 1994b). Degradation of d,l-LA chains can produce isotactic sequences therefore stereoregular fragments can be formed during degradation which have the ability to crystallise as a low molecular weight residue (Vert 1994). A further study attributed this residue to an oligomeric PLA sterocomplex formed from the degradation products (Li and Vert 1994). Both DSC and x-ray scattering showed evolution of crystallinity within the material. Aso *et al.* (1993) reported the crystallisation of previously amorphous PLA when it was stored under humid conditions above its glass transition temperature.

The hydrolytic degradation of polymeric devices of different dimensions was compared in a study carried out by Grizzi *et al.* (1995). The degradation of compression moulded plates (15x10x2mm), millimetric beads (0.5-1.0mm), microspheres (0.125-0.250mm) and cast films of d,l-PLA were compared in isotonic phosphate buffer pH 7.4 at 37°C. A visual examination of a transverse cut of the plates revealed a heterogeneous degradation mechanism, with the plate exhibiting a whitish skin and a yellowish transparent core. As degradation advanced this was replaced by a thin shell of about 200μm thickness which surrounded an oligomeric viscous liquid. The films underwent a different degradation mechanism. Initially the films were transparent and flexible with a distinct presence of pores due to evaporation of residual solvents, but after one week they were whitish and rigid with brittleness increasing with ageing time. The degradation of the beads and microspheres was observed as large cracks occurring on the particle surface. The size exclusion chromatograms of these devices showed an initially small decrease in molecular weight of the plates attributed to a slower water uptake mechanism, followed by a faster decrease that superseded that of the other devices. The degradation of the polymeric devices of different dimensions had the rank order plates > beads >microspheres > films (in order of increasing degradation). Plates exhibited bimodal size exclusion chromatograms typical of heterogeneous degradation while the size exclusion chromatograms of the other devices exhibited monomodal distributions typical of homogeneous degradation.

There are few studies in the literature on how the molecular weight influences the degradation behaviour of PLA/PLGA. Fukuzaki *et al.* (1991) showed polymers of the same composition but of different molecular weight appeared to have the same percentage molecular weight loss during degradation. The molecular weight Mw decreased during the mass loss induction phase and higher molecular weight polymers degraded at a similar or faster rate than lower molecular weight polymers. The weight loss from the polymer followed an 'S-type pattern' for a range of molecular weight poly (-l-LA/GA) characterised by an induction phase, then rapid mass loss and final slow phase until completion (Fukyzaki *et al.* 1991). Different molecular weight samples were also compared by Pistner *et al.* (1993) and it was shown that the two samples degraded rapidly to reach a common molecular weight after 20 weeks after which they degraded simultaneously. The relative exponential decay for the two different molecular weight samples reveal that the higher the starting molecular weight the faster it has to degrade to reach the final common level of molecular weight. Asano *et al.* (1989) plotted % degradation for four poly (lactide) polymers and found a linear decrease in molecular weight for them all, however the time required to reach complete degradation increased with increase in molecular weight. Wada *et al.* (1995) showed a linear decrease in molecular weight for three different molecular weights of PLA, however the onset of mass loss was faster for the lower molecular weight polymer. The corresponding water penetration and water uptake was faster with the lowering of polymer molecular weight.

In a study by Park (1994), it was demonstrated that poly (d,l-lactic acid) microspheres of different molecular weight exhibited different degradation behaviour. The lower molecular weight PLA 17.0k degraded during the study period while no degradation of the higher molecular weight polymer 41.0K was observed. However these polymers were in different states in the incubation medium at 37°C. The lower molecular weight polymers became rubbery when placed in PBS at 37°C and hence degraded faster than the higher molecular weight microspheres which were still in a glassy state. A detailed DSC study revealed the presence of two different polymer domains supporting the heterogeneous degradation mechanism. At the later stages of degradation the initially amorphous PLA showed the presence of crystalline material residues that were more resistant to degradation.

The blending of low molecular weight polymers with higher molecular weight polymers in order to tailor a profile is a common strategy in controlled release from these systems. The enhancement of degradation rates of high molecular weight polymers by the incorporation of low molecular weight polymers in the blend is thought to be caused by an increase in hydrophilicity of the polymer due to an increasing concentration of polar carboxyl end groups and an increase in porosity due to extensive microsphere degradation (Sah and Chein 1995). Blending polymers of different molecular weights also increases the polydispersity of the polymer material. The effect of polymer polydispersity on the degradation rate of these polymers has been indirectly investigated by blending different molecular weights of polymer and studying their degradation pattern. Blends of high molecular weight (HMW) and low molecular weight (LMW) PLA were used as model polydisperse polymers. In these blends the degradation rate as measured by the amount of lactic acid produced increased as the amount of LMW polymer increased. In LMW compositions of >50%, pores were created in the degrading blends due to the degradation of the LMW PLA (von Rectum *et al.* 1995).

3.3.3 External factors that influence the degradation properties of PLA, PLGA and their co-polymers

The kinetics of hydrolysis can be strongly influenced by a number of factors. The release of the soluble oligomeric materials depends on their solubility in the bulk medium and therefore factors such as pH, ionic strength, buffering capacity and temperature can effect the degradation rate of the polymeric devices (Vert 1990).

The effect of temperature on the degradation profile of these polymers was shown to increase the degradation rate with increasing incubation temperature (Makino *et al.* 1985, Wang *et al.* 1990). The Arrhenius equation was used to calculate an activation energy of 19.9 and 20.0kcal/mol for the d,l -PLA and l-PLA microspheres respectively (Makino *et al.*

1985). The effect of temperature on the mass loss and loss of tensile strength of PGA sutures was demonstrated by Gilding and Reed (1981). Both parameters were found to increase with increase in the incubation medium temperature.

Gamma (γ) irradiation has been investigated as a means of sterilisation of polyesters for implant and injectable microspheres formulations. Certain reports have shown that the polymer molecular weight is significantly decreased by this process (Chu and Campbell 1982, Spenlehauer 1989, Beck and Tice 1983). The effects of γ-irradiation dose on the molecular weight and on the in-vitro degradation of PLGA microparticles was determined by Hausberger *et al.* (1995). The microparticles were subjected to 0, 1.5, 2.5, 3.5, 4.5, and 5.5 Mrad doses of γ-irradiation, examined by size exclusion chromatography and subsequently allowed to degrade under physiological conditions. The γ-irradiation dose had a substantial effect on the molecular weight of the polymer and this effect increased with increasing dose. In the degradation profile the radiation was shown to influence the onset of mass loss (through the effects of γ-irradiation dose on the molecular weight of the polymer) but had no effect on its subsequent rate. In another study, Yoshioka *et al.* demonstrated degradation of d,l-PLA microspheres by irradiation in terms of carboxylic acid content, changes in molecular weight and T_g. Carboxylic content of the microspheres increased, while weight average molecular weight and the T_g of the polymer were found to decrease during irradiation indicating that ester bonds had been cleaved by the irradiation process. These effects were also shown to be dose dependant (Yoshioka *et al.* 1995).

The nature of the incubation medium can also influence the degradation rate of PLA/PLGA. In buffer solution the hydrolytic degradation was enhanced when the ionic strength of the medium was increased (Makino *et al.* 1985). This was attributed to a change in the thickness of the electric double layer which makes the microcapsules more accessible to attack. The degradation of PLA microcapsules was examined under different conditions of pH. Degradation was accelerated in extreme acidic and extreme basic solutions, with the basic conditions giving the greater effect. Slightly acidic conditions did not affect the degradation rate of the polymer (Makino *et al.* 1985). In a basic medium, the degradation was assumed to proceed via an unzipping process and not by a random chain scission process (Makino *et al.* 1985). Brizzolara *et al.* (1990) reported identical degradation rates for PLGA degraded in pH 4.2 to 8.9, while the rate of degradation and erosion of PLGA are reported to be identical in various aqueous buffers (pH 4.5-7.4) by Kenley *et al.* (1987).

3.4 THE RELEASE PROPERTIES OF POLYESTER SUSTAINED RELEASE SYSTEMS

Drug release from the lactide glycolide polymers can be influenced by a number of different factors. Some of these will already have become apparent based on the preceding discussions of drug release models and polymer degradation. Release rate depends on many factors some of which have been outlined by Maulding *et al.* (1987) including physicochemical properties of the drug, drug loading, the homogeneity of the matrix, particle size and the stability and rate of degradation of the polymer matrix. Depending on the properties of the system, drug release may occur over a period of hours/days to over one year.

Beck *et al.* (1983) showed sustained release from microspheres of poly (d,l) LAGA based on a two-stage process involving diffusion and degradation. Tri-phasic release patterns are often observed involving initial diffusion/dissolution controlled burst release of surface-associated drug, followed by a lag phase and then degradation controlled release (Bodmer *et al.* 1992, O'Hagan *et al.* 1994). The initial process involving surface controlled diffusion or dissolution is usually termed as the 'burst effect'. The magnitude of the percentage drug released during the burst effect is an important consideration with respect to the overall profile of drug release. The remainer of drug in the device is usually released in a controlled manner as the device degrades. The burst effect and the degradation controlled phase are influenced by the particular set of formulation variables.

3.4.1 Factors controlling drug release from PLA/PLGA systems

Release of low molecular weight drugs from microcapsules has been demonstrated to mainly occur predominately by a diffusion mechanism over short periods of time (hours), which can be described by classical Higuchi diffusion controlled kinetics (Yoshioka *et al.* 1995). For diffusion of a drug to occur through a polymer matrix the drug would presumably need to have some solubility in the polymer (Hutchinson and Furr 1987). Pitt *et al.* (1979) found a very poor solubility (0.65mg/g) for hydrophobic drugs such as Progesterone in d,l-PLA matrices, furthermore as the molecular weight of the drug increases its ability to diffuse through the polymer diminished according to:

$$\log D = a - b \log M \qquad \text{Equation 3.36}$$

There is an approximate log-log correlation between molecular weight M and the diffusion coefficient D, where a and b are arbitrary constants. As the molecular weight increases, D becomes smaller because the drug cannot be contained in the polymer free volume. Therefore diffusion-controlled release is an unlikely mechanism from these polymers (Furr and Hutchinson 1992).

The dependence of drug release rate on the drug loading of the matrix has now been well established by many studies (Benoit *et al.* 1984, Jalil and Nixon 1990d, Bodmer *et al.* 1992, Ramtoola *et al.* 1992, Fitzgerald and Corrigan 1993, O'Hagan *et al.* 1994). Polymer devices of higher drug loadings exhibit faster rates of drug release than those of lower drug loading (Leelarasaaamee *et al.* 1986, Parikh *et al.* 1993). The length of the post burst lag phase was linearly dependent on the % progesterone loading (Jalil and Nixon 1990d).

Benoit *et al.* (1984) demonstrated how the drug loading determined the rate of drug release from microspheres; high drug loadings resulted in a higher incidence of drug crystals at the surface of the microsphere that could immediately be released into the dissolution medium by surface diffusion. For a drug that is phase-separated from the polymer matrix, a high drug loading will favour immediate release by diffusion through drug filled pores in the polymer matrix (Shah *et al.* 1992). Where no surface crystals are present at the microsphere surface, the increased loading increases the drug release rate due to the fact that at higher drug loadings, channels formed by loss of drug are more closely connected and offer less resistance to further drug loss. Higher drug loadings are also associated with a higher burst effect from the microspheres (Ramtoola *et al.* 1992, O'Hagan *et al.* 1994).

Internal morphology of the device can influence the release of drug from the polymer. Confocal laser scanning microscopy analysis of BSA loaded microspheres revealed that two types of structure existed dependent on the particular formulation used. Microspheres contained either a homogeneous dispersion of the drug through out the matrix or a heterogeneous matrix where the drug existed in pockets randomly distributed throughout the matrix. In vitro dissolution indicated that heterogeneous microspheres in which BSA was not evenly distributed provided a faster release profile and larger burst effect (62%) while homogeneous microspheres spheres had a lower burst effect (7%) and a slower release profile (Yan *et al.* 1994). Iwata and McGinity (1992, 1993) prepared conventional and multiphase microspheres. SEM of the cross-sections revealed that microspheres prepared by the conventional o/w technique had a homogeneous internal structure that developed with incubation time into concentric lamella-like structures to eventually give a porous sponge like matrix. Multiphase structures produced pockets in the microsphere where the internal emulsion was distributed. A porous structure was also formed during incubation, and pores became enlarged and connected with each other over time. The release of a dye was faster and had a larger burst effect from multiphase microspheres than from the conventional structures even thought the degradation rate of the polymer was equivalent. Spenlehauer *et al.* (1988) observed that the morphology of the microsphere was sensitive to viscosity changes induced by changing molecular weight or polymer:solvent ratios. Optical micrograms of cisplatin microspheres showed that decreases in viscosity induced a higher rate of drug migration towards the microsphere wall.

The release of drug from PLA and PLGA microspheres can be altered by the inclusion of additional excipients in the formulation. The presence of hydrophilic excipients increases the porosity of the matrix by acting as 'channelling agents'. A study by Leelarasamee *et al.* (1986) demonstrated that when microcapsules were manufactured using the same drug loading but with increasing dextrose content in the microcapsules the rate of drug release increased with increased concentration of the dextrose. PVP and PEG 600 were incorporated into PLA microspheres where they affected the drug loading and subsequent release from piroxicam loaded microspheres (Lalla and Spana 1993). A similar approach was adopted by Yeh *et al.* (1995) when they blended PEG 8000 with PLGA in the manufacture of ovalbumin loaded microspheres. Incorporation of the hydrophilic PEG increased the release rate by altering the matrix hydration rate and the development of microsphere porosity. The addition of hydrophilic compounds was also shown to increase the initial dissolution rate of leuprolide acetate from PLA microcapsules (Ogawa *et al.* 1988a). Other rate modifiers such as hydrophilic monoglycerides (Yamakawa *et al.* 1992) and sodium chloride (Bodmeier and Chen 1989) perform the same functional alteration in the matrix.

Varying the blend composition could be used to control the release rate (Bodmeier *et al.* 1989) from the polymer matrix. The faster release rate was attributed to the reduction in T_g of the polymer as a result of blending the two polymers and to leaching of the low molecular weight polymers from the polymer. The T_g of the polymer was intermediate between the T_g of its component polymers; therefore introducing a low molecular weight polymer also increases the permeability of the polymer.

Pitt and co-workers demonstrated that the T_g of amorphous copolymers containing d,l-lactide could be estimated by the empirical equation:

$$(1/T_g)_{AB}=(W/T_g)_A+W/T_g)_B \qquad \text{Equation 3.37}$$

where W represents the weight fraction of each monomer A and B in the copolymer (Ford and Timmins 1989). Blending polymers can also vary the porosity and degree of water uptake and degradation rate. Sah *et al.* (1995) demonstrated how the release of BSA could be modified by the blending of different molecular weight polymers prior to preparation of the delivery system. Zero or first order release kinetics were achievable depending on the blend ratios. Low molecular weight poly (d,l-Lactic acid) (PLA) when blended with high molecular weight PLA was found to increase the release rate of caffeine and salicylic acid from PLA films and microspheres. The blending of oligomers with HMW polymers increased the rate of drug release and matrix degradation in Gentamycin/PLA blends (Vert 1994). Shah *et al.* (1995b) demonstrated that water uptake into microspheres increased with the inclusion of low molecular weight PLA or PLGA by forming pores in the matrix with degradation.

The choice of polymer molecular weight can also influence the release rate of the drug from PLA/PLGA polymers. Yamakawa *et al.* (1992) demonstrated that sustained release of a neurotensin analogue over a 20-60 day period was controlled by the molecular weight of the PLA. Using PLA 2000, release was immediate, while release was slower for PLA 4000 and PLA 6000, both of which had a lag phase. The length of the lag phase was reduced with a lowering of the molecular weight of the polymer. Wakiyama *et al.* (1982a) and Suzuki and Price (1985) also demonstrated that microspheres prepared from high molecular weight polymers showed a slower release rate than low molecular weight polymers. The release rate of leuprolide acetate from PLA microspheres into pH 7.4 tris buffer decreases in the order molecular weight PLA 6000 >12,200 > 22,500 (Ogawa *et al.* 1988, Okada *et al.* 1994). In some cases a lag period is observed in drug release, it this case it was thought that further drug release does not occur until the polymer has degraded to a critical molecular weight.

In a study reported by Bodmer *et al.* (1992) they reported that the release rate was fastest from higher molecular weight PLGA in contrast to that reported by other investigators. Jalil and Nixon (1990c) also showed that the release rate of phenobarbitone was faster from high molecular weight polymers than from the corresponding low molecular weight polymers. This phenomenon was attributed to the ability of the lower molecular weight polymer to form a more uniform film. Fong *et al.* (1986) found the differences in molecular weight between polymers did not influence the release rate when the concentrations of the polymer solution were constant. Controlled release of leuprorelin from PLA was dependent on the molecular weight of the polymer with low molecular weight polymers exhibiting a faster release rate that was also dependent on polymer composition.

In a study by Yoshioka *et al.* (1995) drug release was altered by subjecting the microspheres to different levels of γ-irradiation. This was achieved by irradiating the microspheres to induce changes in the molecular weight and T_g of the polymer that in turn effected the release of progesterone from these systems. The initial release rate constants estimated from the slopes of the Higuchi plots were plotted against T_g and showed a linear relationship suggesting that the release rate of d,l -PLA microspheres can be controlled by adjusting the T_g of the polymer. Aso *et al.* (1993) reported that Progesterone release was also controlled by the T_g of the polymer. In this work, alterations in the T_g occurred by storing the microspheres under different conditions of temperature and humidity for different lengths of time.

Microsphere particle sizes usually exist as a distribution with a corresponding range of release rates. Smaller particles release drug at a faster rate than the larger particles. The time required for 50% release of chlorpromazine was linearly related to the mean particle size (Suzuki and Price, 1985). Leelarasamee *et al.* (1988) showed an exponential increase in the rate of drug release with decreasing particle size for the initial diffusion component of the release profile. Visscher *et al.* (1988) showed that the in vitro drug release was faster for the larger microspheres over a 32-day period although they were unable to show the same trend in degradation.

The incorporation of a basic compound into these polymers can accelerate the rate of polymer hydrolysis. When thioridazine was encapsulated in PLA microspheres the polymer molecular weight was reduced by half during the processing technique. Accelerated release of drug from these systems was due to an increase in polymer hydrolysis by the amine functional group (Maulding 1986). A similar effect was observed on polyesters in the presence of methadone (Cha and Pitt 1988). This effect was observed only for some amine-containing compounds that were encapsulated in PLA/PLGA. In a subsequent study by Cha and Pitt (1989) where a range of basic compounds were incorporated, it was demonstrated that the acceleration of degradation by these compounds was related to the nucleophilicity of the amine group rather than its pKa or concentration. Studies have also demonstrated acceleration of degradation of PLA/PLGA polymers by incorporation of a basic compound, diltiazem (Fitzgerald and Corrigan 1993).

Controlled release studies for polypeptide release in vitro and in vivo from (d,l) PLGA copolymers containing from 25-100% poly (d,l-lactide) have been reported (Hutchinson and Furr 1990). The release of polypeptide was triphasic with an initial surface release followed by a lag period during which little or no release occurred, followed by a period of continuous release until exhaustion. The initial matrix diffusion was at a minimum for high molecular weight polymers and high molecular weight polypeptides. During the erosion period drug release was through aqueous channels formed by matrix erosion. Shah *et al.* (1992) also demonstrated that the release of testosterone was related to initial diffusion followed release that was attributed to the erosion of the polymer matrix. Cohen *et al.* (1991) demonstrated matrix erosion release kinetics for the release of fluorescent labelled-bovine serum albumin from PLGA microspheres. The release of ovalbumin from PLGA microparticles also showed a significant degradation controlled component (Yeh *et al.*

1995). A progressive formation of pores resulted in a continuous release of the active agent from these systems.

Zhang *et al.* (1994) in a mechanistic study of antibiotic release from PLA cylinders demonstrated that the mechanism was controlled by diffusion, osmotic pressure and polymer degradation. Osmotic pressure turned isolated drug clusters into connected channels over time through fracturing of the polymer matrix. O'Hagan *et al.* (1994) demonstrated that release and concurrent degradation was fastest from polymers with the higher content of glycolide and lower molecular weight. GPC analysis of the polymers showed a steady decrease in weight average molecular weight with degradation time for the PLGA polymers studied. The release profile of ovalbumin not attributed to the burst was in good agreement with the degradation profile of the corresponding blank microspheres. Constant release of ovalbumin was achieved during the degradation phase of the polymer. The length of the lag phase increased proportionally with the molecular weight of the polymer.

McGee *et al.* (1995) reported that release from PLGA (RG503) was faster than PLA (R208). Microparticles initially released 8-15% of the encapsulated ovalbumin before the remaining ovalbumin was released at a constant rate. Zero order release was achievable in both systems but at different loading levels, 20 days for PLGA and 40 days for PLA. Ramtoola *et al.* (1992) studied the effects of polymer composition on the release mechanism and found that the copolymers released at a faster rate than the homopolymers. Drug release profiles were sigmoidal and were polymer decomposition controlled.

A lipophilic peptide, cyclosporin A (Cyc A), was entrapped in PLGA (50/50) with a high efficiency by an o/w emulsion method (Sanchez *et al.* 1993). An 8°C reduction in the T_g occurred suggesting that the drug was dissolved in the polymer. Cyc A was released in a biphasic manner over 28 days. The initial release could be enhanced by reducing the particle size of the microspheres but not by reduction in the molecular weight of the polymer. Later release was dependent on the molecular weight of the polymer and was mediated by polymer erosion.

3.4.2 Incubation conditions that affect drug Release

The temperature of the dissolution medium influences the dissolution of drug from microcapsules. Jalil and Nixon (1990g) and Aso *et al.* (1994) investigated the release of Phenobarbitone and Progesterone from PLA microspheres respectively and it was reported that the rate of dissolution increased with increasing incubation temperature. The release rate followed Higuchi's square root of time relationship showing that variations in temperature did not influence the release mechanism but did affect the release rate. The influence of increasing dissolution temperature on the rate of release was attributed to an increase in the solubility of the drug in the bulk solution and an increase in the intrinsic diffusivity of these polymers because of their thermoplastic nature. Incubation temperature was found to increase the rate of drug release and concurrent polymer degradation in rifampicin loaded microspheres (Denkbas *et al.* 1994).

The properties of the dissolution medium have also been shown to influence the release rate from PLA/PLGA based systems. The pH of the incubation medium can influence the release process; Bodmeier and Chen (1989) demonstrated a faster release of quinidine sulphate in 0.1M HCl due to higher drug solubility at this pH and possibly a higher rate of polymer degradation. Leelarasamee *et al.* (1986) demonstrated that when a cationic or anionic surfactant was added to the dissolution medium, regardless of their ionic characteristics the release rate of hydrocortisone was increased. The reduced surface tension created by the presence of the surfactant caused an increase in the wetting of the microcapsules which therefore increased the rate of drug release. The ionic strength of the dissolution medium also has the ability to alter the release process from microspheres. The ionic strength was adjusted by the addition of NaCl to the incubation medium. The release of drug was found to decrease with an increase in the ionic strength, while the lag time before the onset of drug release increased with increasing ionic strength. As the ionic strength increases, the water associates with the inorganic ions and the rate of penetration of the water molecules into the microsphere is reduced (Bodmeier and McGinity 1987c,) Bodmer *et al.* (1992) also demonstrated that increases in ionic strength decreased the release rate from PLGA microspheres which they attributed to reduction in the swelling of the polymeric backbone due to ion-shielding effect.

3.5 IN VITRO/IN VIVO CORRELATION OF DRUG RELEASE FROM POLYESTER MATRICES

Excellent tissue biocompatibility and no significant foreign body reaction to injected microspheres have been demonstrated by some workers (Csernus *et al.* 1990, Visscher *et al.* 1988 and Asano 1989b). Other studies have reported on tissue reaction to PLA/PLGA implants. When a polymer matrix is placed in a space it becomes enclosed in an envelope of scar tissue composed of collagen fibres and forgein body giant cells. Certain factors dictate the level of this response such as implant shape and rigidity, surface roughness and porosity, dictated by the mechanical traumatization induced. When implants are in contact with blood the surface chemistry can also determine the response by interaction with serum proteins (Bagnall 1980, Anderson *et al.* 1993).

Visscher *et al.* (1987) also reported the tissue response after injection of PLA and PLGA lysine-8-vasopressin loaded microspheres. A minimal inflammatory reaction was observed post injection characterised by infiltration of lymphocytes, plasma cells and histocytes, and acute myositis was observed. Four days post injection a subacute response had occurred and the microspheres were surrounded by a thin connective tissue capsule and an accumulation of macrophages, giant cells, neutrophils, fibroblasts, lymphocytes and plasma cells. From days 11 to 42 the microspheres were surrounded by these chronic inflammatory cells. From day 30 to 42 some infiltration of hollowed microspheres had occurred by the cells, however no evidence of substantial phagocytosis was observed. At 63 days complete erosion of the polymer had occurred and tissue response retracted.

The role of enzymes in the degradation process is not very clear, however there are indications that certain enzymes influence the degradation of these polymers. Their role in the *in vivo* degradation process has been controversial. Williams and Mort tested a range of enzymes and found four that showed evidence of affecting the rate of degradation of PGA including ficin, carboxypeptidase A, alpha-chymotrypsin and clostridiopeptidase A (Williams and Mort 1977). Carboxylic esterase showed a concentration dependant increase in the degradation of PLA microcapsules (Makino *et al.* 1985). Based on a literature review Holland *et al.* (1986) concluded that polymers in the glassy state showed little evidence of enzymatic involvement whereas polymers in the rubbery state were susceptible to enzymatic involvement.

Several reports have assumed an absence of enzymatic degradation on the basis that they can show good *in vivo-in vitro* correlation (Ray *et al.* 1981; Beck and Tice, 1983). Poor correlation has often been attributed to the presence of enzymatic effects (Korsatko *et al.* 1983). Polymer breakdown product removal would be expected to occur via an outer boundary layer, and in vivo tissue encapsulation may retard this process. Therefore a poor correlation of *in vitro-in vivo* results does not imply enzymatic involvement. Much of the speculation is based on the differences that have been observed between *in vivo* and in vitro degradation rates and release (Holland and Tighe 1992). The lack of correlation of *in vitro* and *in vivo* data has been observed in degradation studies and in drug released into plasma. Williams (1982) observed a faster degradation *in vivo* than was the case *in vitro* by measuring the loss in tensile strength of a polymer suture. A comparison of the *in vitro* and *in vivo* degradation behaviour of PLGA 75:25 with a molecular weight of 100,000 (GPC) showed the rate of *in vivo* degradation to be almost twice that of the *in vivo* rate. Therin *et al.* (1992) reported a faster degradation of a PLA implant *in vivo*, which they suggested might be due either to mechanical stress due to muscular movement or a different solubility of PLA oligomers in-vivo compared to phosphate buffered saline. Schmidt *et al.* (1995) observed non-correlation for a PLGA/PLA implant releasing gentamycin where release in vitro was faster than that observed *in vivo*. They attributed this effect to faster water uptake in the *in vitro* experiment due to agitation of the suspended implant. Visscher *et al.* (1988) reported a faster release *in vitro* than *in vivo*, which they attributed to stirring effects and a total aqueous environment. Fong *et al.* (1986) reported a faster release *in vivo* in dogs compared to that in vitro for thioridazine base release from d,l PLA. Maulding *et al.* (1986) reported that the in vivo release of thioridazine occurred over a 10-day period compared to the *in vitro* release, which lasted for 15 days. This was attributed to catastrophic failure of the microspheres after 5 days implantation leading to dose dumping. Wakiyama *et al.* (1982) determined that the release rate and the microsphere disintegration rate of PLA containing dibucane were faster *in vivo* than that observed *in vitro*.

Many of the observations regarding polymer molecular weight, loading and composition demonstrated *in vitro* were also shown to apply *in vivo*. Zoladex® polyester implant, by optimisation of polymer molecular weight, composition and loading was shown to produce a continuous release system *in vitro* that showed good *in vivo* agreement (Hutchinson and Furr 1990). PLGA (85:15) microcapsules containing norethisterone were prepared by Cowsar *et al.* (1985). These microcapsules provided a constant release in vivo over a three-month period. The *in vivo* degradation period of a radiolabelled polymer material implanted in rats correlated with the *in vitro* drug release profile.

A one-month depot of microspheres of leuprorelin release correlated well with the *in vitro* release profile (Ogawa 1988). However they were unable to achieve *in vivo* agreement for a three-month depot. The *in vivo* release from this system was constant, however, in vitro a lag phase was observed and after 8 weeks only 10% of leuprorelin was released *in vitro* compared to ~45% *in vivo* (Okada *et al.* 1994). They attributed this difference to complex cellular responses that exist *in vivo*. Asano *et al.* (1989) studied the degradation of low molecular weight PLGA placebo and LHRH loaded cylindrical implants and observed that the *in vivo* release was faster than the *in vivo* degradation rate of the polymer. They attributed the increase to an increase in matrix hydrophilicity due to the presence of LHRH which was shown to increase water uptake. Csernus *et al.* (1990) concluded that the release of peptide was controlled by the degradation of the polymer and the physicochemical characteristics of the matrix. The formation of biological barriers around the particles and the tissue reaction dictates the speed of degradation.

CHAPTER 4

ORIGIN AND SCOPE

4.1 ORIGIN AND SCOPE OF THESIS

The development and application of biodegradable polymers to drug delivery technology has increased dramatically over the last two decades. A substantial volume of this interest has focused on polymers of lactic and glycolic acid and their copolymers, mostly in the form of microsphere systems. The behaviour of these polymers is dictated by their physical and chemical properties such as size and shape of the device, molecular weight and molecular weight distribution, polymer composition, hydrophilicity, glass transition temperature and/or melting temperatures. The physicochemical properties of the active to be delivered are also an important consideration.

Degradation of the polymer is a source of dramatic chemical and morphological change in the polymer matrix. The chemical environment of an eroding polymer is constantly changing due to the formation of carboxylic end groups within the polymer matrix, indeed the microenvironment within the solid matrix can be quite different to the bulk solution. A number of investigations have been carried out on the degradation of polyesters based on lactide and glycolide copolymers. Most of the initial studies carried out have been investigated under different incubation conditions for a particular polymer and therefore the only generalised observations can be compared between different investigators. Many of the recent studies that have been carried out on these polymers have been focused on implants or are done concurrent with drug release where the contribution factors cannot be easily ascertained.

The purpose of this work was to examine the degradation phenomena of a range of polyesters based on lactide and glycolide copolymers. Drug free micro and nanoparticles were manufactured using a range of PLA and PLGA polymers. A systematic investigation on the effects of microparticle size and the influence of the chemical properties of molecular weight, polymer composition, hydrophilicity and glass transition temperature was carried out. The physicochemical and morphological changes associated with the degradation and erosion process were monitored. The effects of polymer microenvironment and incubation conditions were also examined for the degradable polymer matrix. Kinetic equations to describe the degradation process were developed and applied. The contribution factors from polymer degradation to erosion in relation to drug release are explored.

The release of an active component from a polymer matrix is governed by a combination of three basic phenomena, diffusion controlled release through the polymer, solubilisation and then diffusion through pores and channels or liberation as a result of matrix degradation. All of these release mechanisms depend on the behaviour of the degradable matrix. The release kinetics from these systems is often harder to control and predict because of the nature of this release process. Many aspects of the performance of such systems remain undefined. A physical and chemical understanding of the complex process of degradation and erosion is a prerequisite to controlling the performance of these devices. The continuously changing morphology and polymer properties signify the complex nature of release processes from these systems.

Drug loaded microspheres were formulated using a selection of the polymers used to investigate the polymer degradation process. Fluphenazine HCl microspheres were chosen for study because, as they had previously demonstrated sustained release behaviour (Ramtoola *et al.* 1992) and they could be used to investigate the process of degradation controlled release. The release and concurrent degradation of fluphenazine loaded microspheres was monitored. Previous investigations on the sustained release from PLGA implant and microsphere systems proposed the Prout-Tompkin model to describe the degradation controlled release component (Ramtoola *et al.* 1992, Fitzgerald and Corrigan 1993). More recently this model was also used to describe both the diffusion controlled and degradation components of release from PLA and PLGA polymer implants (Gallagher *et al.* 1998). This model is also applied in this work to describe the release process from a range of fluphenazine HCl microsphere systems.

CHAPTER 5

MATERIALS AND EXPERIMENTAL METHODS

5.1 MATERIALS

5.1.1 Reagents

5.1.1 Reagents	**Manufacturer/Supplier**
Acetic acid ($C_2H_4O_2$) 99.8%	Riedel de Haën
di-Sodium hydrogen phosphate anhydrous (Na_2HPO_4)	Riedel de Haën
Hydrochloric acid (HCl) 0.985N	Riedel de Haën
Sodium acetate-3-hydrate ($C_2H_3NaO_2.3H_2O$)	Riedel de Haën
Sodium carbonate anhydrous (Na_2CO_3)	Riedel de Haën
Sodium chloride (NaCl)	Riedel de Haën
Sodium di-hydrogen phosphate anhydrous (NaH_2PO_4)	Riedel de Haën
Sodium hydrogen carbonate ($NaHCO_3$)	Riedel de Haën
Sodium hydroxide pellets (NaOH)	Riedel de Haën
Polyvinyl alcohol $(C_2H_4O)_n$ MW 71400-75600	Honeywill & Stein
Tween 20	Aldrich
d,l lactic acid ($C_3H_6O_3$; FW 90.08)	Sigma
Glycolic acid ($C_2H_4O_3$; FW 70.05)	Sigma

5.1.2 Solvents

5.1.2 Solvents	**Manufacturer/Supplier**
Acetone (C_3H_6O)	Lab-Scan
Dichloromethane (CH_2Cl_2) HPLC	Lab-Scan
Ethyl Acetate ($C_4H_8O_2$) HPLC	Lab-Scan
Tetrahydrofuran (C_4H_8O) Analar	BDH
Stabilised with approx. 250ppm 2,6-Di-tert-butyl-4-methylphenol	
Water (H_2O)	In- house
Purified by Millipore Reverse Osmosis '6' and Millipore Q Plus systems to reagent grade.	

5.1.3 Drug

5.1.3 Drug	**Manufacturer/Supplier**
Fluphenazine HCl	Fine Chemicals Limited

5.1.4 Molecular weight standards

5.1.4 Molecular weight standards	**Manufacturer/Supplier**
Polystyrene molecular weight standards	TOSOH
Molecular weight : 456-190,000	

5.1.5 Biodegradable polymers	**Manufacturer/Supplier**
Poly-(d,l- lactide) $((C_3H_4O_2)_n)$	
Resomer® R104 Lot. 13030	Boehringer Ingelheim
Poly (d,l-lactide) inherent viscosity ~0.14dl/g	
Resomer® R203 Lot. 15004	Boehringer Ingelheim
Poly (d,l-lactide) inherent viscosity 0.33dl/g	
Resomer® R206 Lot. 241888	Boehringer Ingelheim
Poly (d,l-lactide) inherent viscosity 0.90dl/g	
Poly-(lactide-co-glycolide $((C_3H_4O_2)n(C_2H_2O_2)))$	
Resomer® RG502 Lot. 15068	Boehringer Ingelheim
Poly (d,l-lactide-co-glycolide) inherent viscosity 0.21dl/g	
Resomer® RG503 Lot. 211687	Boehringer Ingelheim
Poly (d,l-lactide-co-glycolide) inherent viscosity 0.33dl/g	
Resomer® RG504 Lot. 34015	Boehringer Ingelheim
Poly (d,l-lactide-co-glycolide) inherent viscosity 0.48dl/g	
Resomer® RG502H Lot. 24035	Boehringer Ingelheim
Poly (d,l-lactide-co-glycolide) inherent viscosity 0.17dl/g	
Resomer® RG504H Lot. 34018	Boehringer Ingelheim
Poly (d,l-lactide-co-glycolide) inherent viscosity 0.52dl/g	

5.2 EQUIPMENT

5.2.1 Instrumentation	Manufacturer/Supplier
Analytical Balance Model No. 1601	Sartorius
Analytical Balance Model No. MC210P	Sartorius
Biofuge Centrifuge Model No. 28R8	Heraeus
Centrifuge Model No. GS-6R	Beckman
Differential Scanning Calorimeter Series 7	Perkin Elmer
Filtration unit	Millipore
Glass Syringe	Fortuna
Gel Permeation Chromatographic System	Waters
Hot Plate stirrer	Stuart Scientific Top
Mastersizer sizer S version 2.14	Malvern Instruments
Microfluidiser Model No. M120E	Microfluidics Corp.
Pan Balance Model No. LC2200P	Sartorius
pH meter Model No. 420A	Orion
pH probe Model No. 81-63	Orion
Probe sonicator	Misonix
Scanning electron microscope SEM S360	Leica Cambridge
Scanning electron microscope S4300	Hitachi
Shaking Water bath Model No. 25	Precision
Stirrers Model No. RW25	IKA-labortechnik
Stop watch	Quartz
Spray dryer Büchi Model No. 190	Büchi Labortechnik
Thermogravemetric Analyser Model Number 50	Mettler
Ultra Turrax Homogeniser Model No. T25	IKA-labortechnik
Ultra-Violet Spectrophotometer Model No. 160A	Shimadzu
Ultra sonic bath	Kerry Ultrasonics
Vacuum Oven	Gallenkamp
Vacuum pump	KNF Neuberger
Zeta Sizer version 3000	Malvern Instruments
X-ray diffractometer	Siemens

5.2.2 Miscellaneous materials	**Manufacturer/Supplier**
Visking dialysis tubing 18/32	Lennox
Elastic Bands/String for bag ties	Reads
Syringes	Omnifix
Filters	Millipore
Filter Paper (0.45 and 0.22µm)	Millipore
Needles	Braun
Cuvettes	Starstedt
10 ml Glass Tubes	LIP
Aluminium pans	Perkin Elmer
Centrifuge Tubes	Costar

5.3 METHODS

5.3.1 Conversion of fluphenazine HCl to fluphenazine base

Fluphenazine HCl was converted to the base form by preparing an aqueous solution of the hydrochloride and then adding an excess of 1M NaOH until precipitation occurred. The base was recovered washed with NaOH and dried in a vacuum oven.

5.3.2 Solubility measurements

A saturated solution of the drug was prepared using an excess of drug in the dissolution medium that had been maintained at 37°C. The dissolution flasks were incubated at 37°C in a shaking water bath at 60 shakes per minute (s.p.m). At selected intervals of time a 2ml sample was taken and filtered using a pre-equilibrated needle, syringe and filter. The sample was immediately diluted as appropriate. The drug content of the sample was determined spectrophotometrically. Samples were taken until equilibrium had been established. All determinations were taken in triplicate and averaged.

5.3.3 Preparation of drug free microparticles

Drug free microspheres were prepared by a modified aqueous emulsification and solvent evaporation technique described by Beck *et al.* (1979), this process is shown schematically in Figure 2.1. The polymer was dissolved in the designated amount of dichloromethane

(Table 5.1). This was then added while emulsifying to 100mls of the aqueous solution of polyvinyl alcohol 0.27% w/v.

Emulsifying process

IKA Ultra Turrax dispenser model No.T25 with an S25 head homogeniser shaft for two minutes at the required speed (rpm), 24,000 rpm (**Process B**) or 8,000 rpm (**Process C**).

The resulting mixture was then stirred at 1,200 rpmwith an IKA RW25 motor with a four-blade stirrer for 2 hours to allow droplet hardening. Particles were recovered by centrifugation (Heraeus Biofuge 28RS) using a HFA 13.5 rotor at 12,500 rpm for 15 minutes after which the supernatant was decanted. The particles were dried under vacuum at 1,000mbar over silica gel for at least 72 hours. The particles were stored in opaque airtight containers at 5°C.

Table 5.1 Specific polymer:dichloromethane ratios in the formulation for 1.8g batch for each PLA/PLGA polymer evaluated

Polymer	CH_2Cl_2 (mls)	Polymer	CH_2Cl_2 (mls)	Polymer	CH_2Cl_2 (mls)
R206	5.0	RG504	6.8	RG504H	7.3
R203	1.8	RG503	4.8	RG502H	2.4
R104	1.0	RG502	3.0	-	-

5.3.4 Preparation of drug free nanoparticles

Microfluidisation is a procedure that can be used to produce very fine emulsion droplets therefore reducing the resulting particle size. A microfluidised™ is high pressure homogenisation capable of making very small emulsion droplets by pumping the emulsion through microchannels into an interaction chamber wher shear and cavitation produce a size reduction (Microfluidics Corporation).

After homogenisation using an Ultra Turrax at 24,000 rpm for 30 seconds, the suspension was microfluidised for 4 cycles at 10,000 psi. The suspension was recovered from the microfluidiser and then stirred at 1,200rpm for 2 hours (**Process A**). Particles were recovered and stored as described in section 5.3.3.

5.3.5 Preparation of drug loaded microparticles (Process D)

Drug loaded microspheres were prepared by the aqueous emulsification and solvent evaporation method as described above. The polymer was dissolved in the designated amount of dichloromethane. The drug (as a percentage of the polymer weight) was suspended in the designated amount of acetone (3mls for a 10% theoretical loaded batch). Table 5.2 shows preliminary attempts (2 batches ± acetone) at encapsulation of fluphenazine HCl in PLGA microspheres resulted in better drug loadings when acetone was used in the formulation.

Table 5.2 Comparison of the % fluphenazine HCl loading (2 batches ± acetone) in PLGA microspheres

Polymer	CH_2Cl_2 (mls)	Acetone (mls)	% Fluphenazine HCl (encapsulated)
RG504	6.8	1.5	8.42
RG504	6.8	1.5	8.12
RG504	6.8	0.0	6.31
RG504	6.8	0.0	6.97

The polymer solution was added to the drug suspension and then mixed. This was then added while emulsifying to 100mls of polyvinyl alcohol solution at pH 10.0 to prevent partition of the drug into the aqueous phase. (0.27% w/v PVA in carbonate buffer pH 10.0). The resulting mixture was stirred at 1,200 rpm for 2 hours. Particles were recovered and stored as described in section 5.3.3.

5.3.6 Preparation of drug loaded nanoparticles

The drug/polymer suspension (as per 5.3.5) was homogenised at 24,000 rpm for 30 seconds and then the suspension was microfluidised for 4 cycles at 10,000 psi. The suspension was recovered from the microfluidiser and then stirred at 1,200 rpm for 2 hours. Particles were recovered and stored as described in section 5.3.3.

5.3.7 Preparation of microspheres by stirring

The polymer was dissolved in the designated amount of dichloromethane. This was then added while stirring at 1,200 rpm with an IKA RW25 motor with a four-blade stirrer to the aqueous solution of polyvinyl alcohol 0.27% w/v. The suspension was allowed to stir at 1,200 rpm for 2 hours. Particles were recovered and stored as described in section 5.3.3.

5.3.8 Preparation of microspheres by Probe Sonication

Microspheres were prepared by dissolving 1g of polymer in 1ml of dichloromethane and this was then sonicated (output 4 for 2 minutes) into 0.27% PVA. The particles were stirred at 1,200 rpm for 2 hours to remove the solvent then recovered by centrifugation and dried under vacuum as described previously in section 5.3.3.

5.3.9 Preparation of microspheres by spray drying.

Recently, spray drying has also been investigated for the formulation of drug-loaded PLA and PLGA microparticles. This method is independent of drug solubility and usually results in high drug entrapment efficiency as this process consists of the transformation of liquid feed into a dried particulate form by spraying the feed into a hot drying gaseous medium. The spray drying process involves the following four sequential stages: atomisation of the feed materials into a spray nozzle, spray-air contact, drying of the sprayed droplets, collection of the dried particles obtained (Broadhead *et al.* 1992).

Drug free microspheres were prepared by spray drying by dissolving 1g of polymer in 5mls of ethyl acetate. The polymer solution was spray-dried under the following operating parameters:

inlet temperature	59°C
Outlet temperature	46°C
Pump control	10ml/min.
Aspirator	10
Heating control	1
Flow control	600-700

The particles were then recovered from the spray drier apparatus.

5.4 DRUG RELEASE AND POLYMER DEGRADATION STUDIES

5.4.1 Degradation studies of polymer systems

Particle in Visking bags

The degradation characteristics of the polymer systems were evaluated by placing an accurately weighted sample of particles of approximately 100 mg into pre-weighed and pre-equilibrated (washed and equilibrated in PBS) visking bags (fixed size of 2.5 inches). The bags were then placed in a flask containing 100ml of phosphate buffer, at 37°C. The flasks were placed in a shaker water-bath maintained at 37°C and were horizontally shaken at a rate of 60cpm. At various intervals samples were removed from the flask dried under vacuum and over silica gel for at least 72hours.

Dispersed Particles

The degradation characteristics of the polymer systems were evaluated by placing an accurately weighed sample of particles of approximately 100 mg into 100 ml of phosphate buffer pH 7.4 at 37°C in a conical flask. The flasks were placed in a shaker water-bath maintained at 37°C and were horizontally shaken at a rate of 60 cpm. At various intervals samples were removed from the flask dried under vacuum and over silica gel for at least 72 hours.

Modifications: For certain experiments variations in the dissolution conditions were investigated such as pH, temperature and volume. Details of these modifications are outlined where the experiment results are discussed.

5.4.2 In-vitro dissolution studies

Dissolution studies were carried out in isotonic phosphate buffer pH 7.4 (NaCl 4.4g/L, NaH_2PO_4 1.615g/L and Na_2HPO_4) in stoppered 100ml dissolution flasks. The dissolution medium was measured out into a flask, placed in a shaking waterbath and pre-equilibrated at 37°C. The sample was weighed out and added to the flasks. The dissolution flasks were shaken at 60 spm. At selected intervals of time a 3ml sample was taken and filtered using a needle, syringe and 0.45μm filter. After each sample collection, the sample was replaced with 3mls of fresh phosphate buffer maintained at 37°C. The sample was diluted as appropriate and the drug content of the sample was determined spectrophotometrically. All determinations were taken in triplicate and averaged.

5.4.3 Preparation of pH titration profiles

A pH titration profile of an equimolar d,l-lactic acid: glycolic acid in isotonic phosphate buffer and dH_2O was carried out by adding aliquots of the equimolar d,l-lactic acid:glycolic acid solution into the buffer or dH_2O, mixing the solution and measuring the resulting pH. The concentration of lactic/glycolic acid was then plotted against pH of the solution.

5.5 CHARACTERISATION METHODS

5.5.1 Calculation of % yield from a formulation process

Dried microspheres were weighed to determine the % yield of the process, which was calculated as follows:

$$\frac{\textit{Weight of recovered microspheres}}{\textit{Weight of polymer and drug used}} \; X\,100 \qquad \text{Equation 5.1}$$

5.5.2 Mass loss analysis by gravimetry

Gravimetric analysis for polymer mass loss was accomplished on an electrobalance. One hundred-milligram portions of the dry polymer samples were carefully weighed prior to introduction (W_i) into the medium. The particle samples were first transferred to pre weighed and wetted visking bags that were then secured and placed in the dissolution medium. The samples were removed from the dissolution medium after a designated time and dried in a vacuum oven. The dried samples were weighed (W_d) and the % mass loss was determined using the equation.

$$\%\,Mass\ loss = \frac{Wi - Wd}{Wi} \times 100 \qquad \text{Equation 5.2}$$

5.5.3 Particle size analysis

Particle size analysis was carried out using a Malvern Mastersizer S Ver. 2.14 (Malvern Instruments Ltd., U.K.). Approximately 10mg of microparticles was suspended in 3.0ml of filtered 0.1% Tween 20, sonicated for 5-10 minutes to deaggregate particles, and analysed under continuous stirring. This was carried out in triplicate for each product and the average of the three readings obtained.

The result from the analysis is expressed as the relative distribution of volume in the range of size classes. The interpolated results allow the cumulative undersize result to be determined for any size. The percentile is the size determined for any percent of the total volume of particles under that size, e.g. the 90% volume percentile (D90%) is the size under which 90% of the total volume of particles exist. The percentile sizes for 10%, 50% and 90% were obtained. The $D_{50\%}$ value is also known as the median of the volume distribution.

5.5.4 Scanning Electron Microscopy (SEM)

The morphological characteristics of the particles were studied by Scanning Electron Microscopy (Cambridge Lieca S360). Particles were fixed to metal stubs and sputter coated with gold prior to examination

5.5.5 Differential Scanning Calorimetry

The DSC technique was accomplished using a calorimeter connected to a refrigeration system (Perkin Elmer DSC 7). System calibration was performed using an indium standard. Samples 5-10mg were weighted into aluminium pans and crimped. An empty crimped pan was used as a reference. Sample analysis was carried out under nitrogen purge. Samples were heated at a rate of 10°C per min. The glass transition temperature was taken as the midpoint of the transition curve.

5.5.6 Thermogravimetric analysis

Samples were weighed into an open aluminium pan and placed in the sample chamber of a Mettler Thermogravimetric analyser. Weight loss due to water loss by the sample was analysed between 25°C to 100°C. The % water content in the original sample was determined from the loss in mass.

5.5.7 Assay of the drug loading of microspheres

A sample of 20-25 mg of microspheres was weighted out in triplicate and to each sample 1ml of dichloromethane was added to dissolve the polymer. Then 5ml of 0.1N HCl was added to each vial to dissolve the drug. The samples were sonicated for 1 hour and then centrifuged at 3,500rpm for 15minutes. A 2ml sample was withdrawn from the aqueous layer filtered and an appropriate dilution was made. The samples were assayed spectrophotometrically at 306nm using 0.1N HCl as a reference. The quantity of drug in the microspheres was calculated using this absorbance reading from a linear calibration curve of Fluphenazine in 0.1N HCl using standards in the range 0.01-0.05 mg/ml (Appendix I). Assays and calibrations were performed in triplicate.

5.5.8 X-ray diffraction

X-ray diffraction analysis of samples (polymer, drug or particles) was carried out using nickel filtered copper radiation. Powder samples were dispersed and compacted on the surface of a glass slide. The samples were evaluated in the 2θ range of 5-35°.

5.5.9 Determination of the zeta potential

Zeta potential measurements were made on a Malvern Zetasizer 3000. Approximately 20mgs of sample was dispersed in 10^{-3} M NaCl and phosphate buffer pH 7.4 using 2 minutes sonication. Zeta potential readings were carried out five times and the average determined. A zeta standard DTS5050 was analysed before and after the samples to determine if reliable zeta potential readings were obtained. The standard readings were carried out by preparing a standard sample in a 1 in 10 dilution of the stock buffer solution provided in manufacturers kit. Samples were deemed acceptable if the zeta potential readings were within the 50mV±5mV range.

5.5.10 Principles of Gel Permeation Chromatography

Gel permeation chromatography or size exclusion chromatography is a technique designed to separate high molecular weight compounds. This analytical technique separates molecules on the basis of their size in solution dependent on the solvent and temperature used. It offers an equivalent variety of operating and optimisation techniques as other liquid chromatographic methods. Polymers are by definition polydisperse and thus have a distribution of various molecular weights. The eluting chromatogram of such a polymer will have a shape that represents how the chain lengths are distributed throughout the polymer.

Gel Permeation Chromatography (GPC) system

The Gel permeation Chromatography system consisted of a solvent delivery device (Waters 510 HPLC Pump), a column (Waters HR4E), RI detector (Waters 410 Differential Refractometer), column oven (Jones chromatography) a computer installed with software capable of directly analysing molecular weight data (Millenium version) and an autosampler (Waters 717 plus autosampler). THF was used as both the solvent and the mobile phase.

The following operating conditions were used:

Column temperature	25°C
Mobile phase	THF
Flow rate	1.0ml/min.
Run time	15 mins

Calibration curve

Molecular weights relative to polystyrene are quoted. GPC calibration can be carried out using a variety of methods and operating conditions (Gilding *et al.* 1981). This technique has been described by a number of authors to characterise polymers for medical and pharmaceutical applications (Van Dijk and Smit, 1983). System calibration was carried out using polystyrene standards with molecular weights ranging from 456 to 190,000 daltons (Appendix II). Polystyrene standards in degassed prefiltered stabilised THF at a concentrations of 1mg/ml. Waters recommend a concentration of <2.5mg/1ml for molecular weights 0-25,000 and for molecular weights 25-200,000 up to 1mg/1ml. Samples (PLA/PLGA) were prepared by dissolving the 5 mg of the polymer in 10ml of tetrahydrofuran. The solutions were prepared and analysed in duplicate or triplicate for each sample.

The molecular weight moments calculated describe the polymer's molecular weight distribution, these moments are different statistical representations of the molecular weight. Because polymers within a given polymer mass are polydisperse, molecular weights of the polymers are described in terms of average molecular weights. Mn, Mp, Mw, Mv and Mz are number average, peak average, weight average, viscosity average and Z-average molecular weights respectively. The polydispersity (Mw/Mn) indicates the broadness/narrowness of polymer distributions. Values <1.1 indicate monodispersity. Values around 2 indicate a broad distribution. The average molecular weights are defined as follows (Gilding *et al.* 1981, Young and Lovell 1990):

$$Mn = \frac{w}{\sum Ni} = \frac{\sum NiMi}{\sum Ni} = \frac{\sum hi}{\sum hi / Mi}$$ Equation 5.3

where $w = \sum NiMi$ Equation 5.4

$$Mw = \frac{\sum wiMi}{w} = \frac{\sum NiMi^2}{\sum NiMi} = \frac{\sum hiMi}{\sum hi}$$ Equation 5.5

$$Mv = \left[\frac{\sum NiMi^{1+a}}{\sum NiMi}\right]^{1/a} = \left[\frac{\sum hiMi^{a}}{\sum hi}\right]^{1/a}$$ Equation 5.6

$$Mz = \frac{\sum NiMi^3}{\sum NiMi^2} = \frac{\sum hiMi^2}{\sum hiMi}$$ Equation 5.7

Ni is the number of polymer molecules of molecular weight Mi, hi is the height of the chromatogram at Mi where $hi \propto NiMi$, ie the weight fraction of the species Mi. Mn, Mv, Mw and Mz are the average molecular weights obtained from osmometry, solution viscosity, light scattering, and sedimentation measurement respectively. Initial analysis of day to day variation in the calibration standards indicated that Mp was the most consistent parameter to track polymer molecular weight.

5.6 MATHEMATICAL MODELING OF POLYMER DEGRADATION AND DRUG RELEASE PROFILES

Micromath® SCIENTIST® for Windows™, version 2.1 was used to fit polymer degradation and drug release profiles. The coefficient of determination(CD) and the model selection criteria (MSC) were used as the measures of goodness of fit when evaluating the suitability of a model. Where the coefficient of determination is defined by the following equation:

$$CD = \frac{\sum_{i=1}^{n} w_i \left(Y_i - \overline{Y}\right)^2 - \sum_{i=1}^{n} w_i \left(Y_i - \hat{Y}_i\right)^2}{\sum_{i=1}^{n} w_i \left(Y_i - \overline{Y}\right)^2}$$ Equation 5.8

where n is the number of data points, w_i are the weights applied to each point, and Y_i, $\hat{Y}_i$ and $\overline{Y}$ are the observed data points, the model-predicted data points and the weighted mean of the observed data respectively. The CD is a measure of the fraction of the total variance accounted for by the model (Scientist User Handbook, 1995). The MSC is a normalised modification of the Akaike Information criteria and gives the same rankings between models but is independent of the scaling of the data points. The MSC is defined by:

$$MSC = \ln\left(\frac{\sum_{i=1}^{n} w_i \left(Y_i - \overline{Y}\right)^2}{\sum_{i=1}^{n} w_i \left(Y_i - \hat{Y}_i\right)^2}\right) - \frac{2p_p}{n}$$ Equation 5.9

where p_p is the number of parameters calculated. The appropriateness of fit of a particular model to a set of data is judged based on the MSC. A model that gives the largest MSC is the best model to fit that particular data set. Equal weighting was used in the fitting of all polymer degradation and drug release profiles. Parameters are compared ±1 standard deviation. Micromath scientist models used in this thesis are given in Appendix IV.

CHAPTER 6

CHARACTERISATION OF POLYMERS AND PARTICLE PREPARATION METHOD

6.1 INTRODUCTION

Biodegradable polymers of polylactide, glycolide and their copolymers have specific physical and chemical properties such as molecular weight and distribution, polymer composition, hydrophilicity, glass transition and/or melting temperatures which have the potential to influence the physical behaviour of the polymer. Their versatility is primarily due to the poly (2-hydroxy-acid) backbone that allows property adjustments during polymerisation or through copolymerisation of lactic acid with its optical isomer and/or with glycolic acid. Several authors have described the manufacture of their own range of PLA, PGA and PLGA polymers and their subsequent characterisation (Deasy *et al.* 1989, Gilding and Reed 1979), however interest in these polymers for medical applications means that these polymers are now widely available commercially.

This chapter will discuss the initial characterisation of the range of polymers used in this study. These same techniques will be later used to characterise drug free and drug loaded particles for the purpose of investigating particle properties and performance in aqueous medium. Different methodologies and process parameters for the preparation of drug free microparticles were evaluated in terms of microparticle characteristics.

6.2 CHARACTERISATION OF PLA AND PLGA POLYMERS

A series of poly d,l-lactide (PLA) and polylactide-co-glycolide (PLGA) polymers manufactured by Boehringer Ingelheim with differing inherent viscosities were chosen for study. Information from the manufacturer's certificate of analysis is shown in Table 6.1. The polymers used in this study had low residual monomer levels and trace metal content including tin of <200ppm. These polymers were characterised before further use. In all cases the polymer was supplied as an off white powder and was characterised by GPC and DSC.

Table 6.1 Characteristics of PLA and PLGA polymers as supplied, taken from the Boehringer Ingelheim certificates of analysis.

Polymer	I.V. (dl\g)	molar ratio d,l-LA:GA	Residual monomer (%)
PLA(R104)	~0.14	100:0	3.0
PLA(R203)	0.33	100:0	0.24
PLA(R206)	0.90	100:0	<0.5
PLGA(RG502)	0.21	48:52	<1.0
PLGA(RG503)	0.33	50:50	<0.3
PLGA(RG504)	0.48	48:52	<1.0
PLGA(RG502H	0.17	50:50	<0.5
PLGA(RG504H)	0.52	48:52	<1.0

6.2.1 Characterisation of PLA and PLGA by Gel Permeation Chromatography

The statistical molecular weights of the polymers to be investigated were determined by GPC analysis. The polymers as supplied were dissolved in tetrahydrofuran (THF) for analysis by GPC. The GPC elution chromatogram of the PLA and PLGA polymers showed a polydisperse unimodal distribution of molecular weight moments (Figure 6.1). The statistical molecular weights of the polymers were calculated based on a relative calibration curve (Appendix II) of molecular weight versus retention time for a series of molecular weight standards. Three different molecular weights of PLA (R206, R203 and R104) and of PLGA (RG504, RG503 and RG502) were used for the purpose of this work. In the PLGA series of polymers two uncapped polymers denoted as the H-group of polymers were also investigated (RG504H and RG502H). These polymers are two of the new hydrophilic copolymers based on d,l-lactide and glycolide. The H series product line contains hydrophilic adjusted homo- and copolymers with free carboxylic end groups (Boehringer Ingelheim Product Literature).

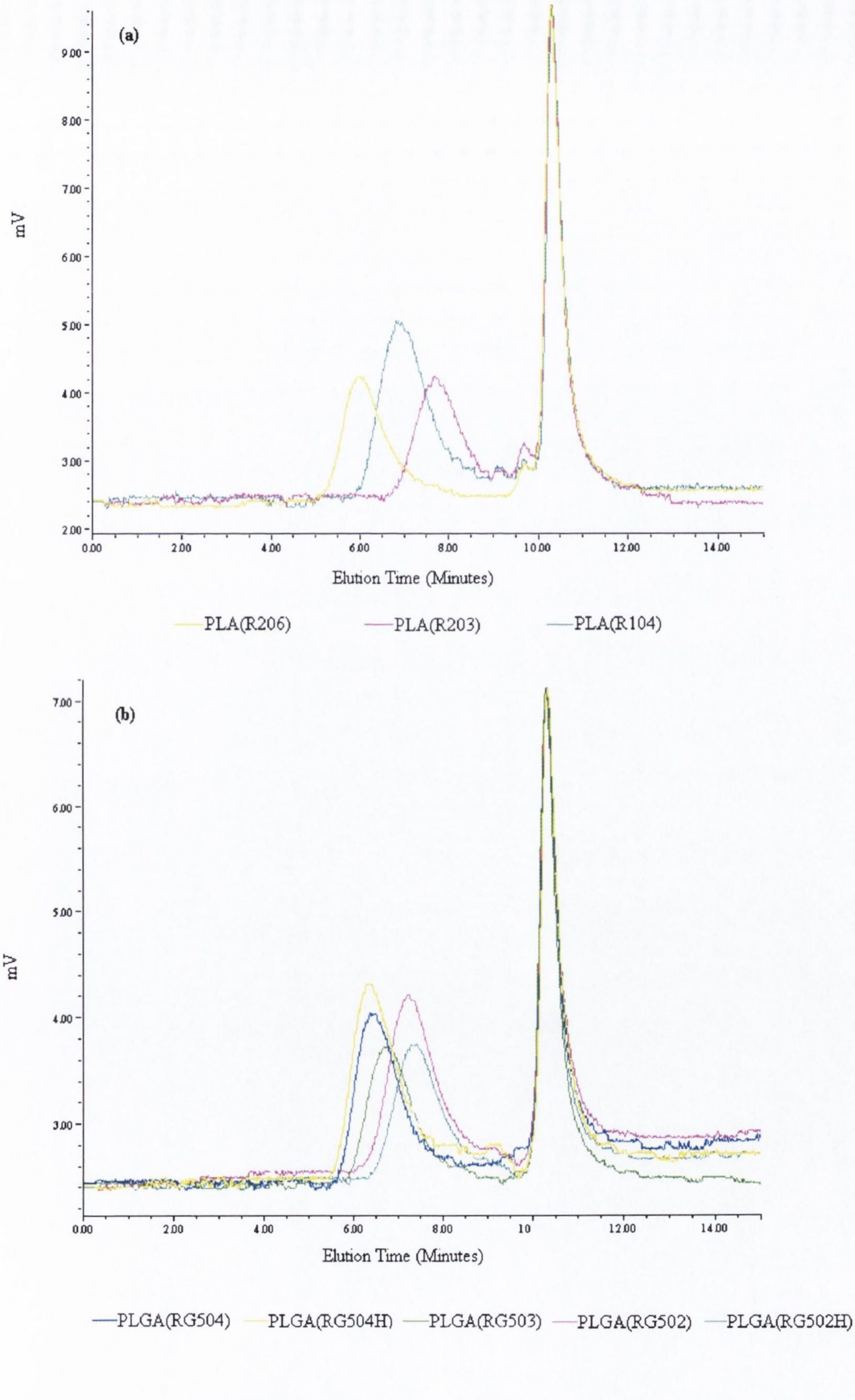


Figure 6.1 Gel permeation chromatograms of a range of poly (alpha-hydroxyl aliphatic esters), for (a) PLA series and (b) PLGA series of polymers.

The molecular weight moments determined for PLA and PLGA polymers are shown in Table 6.2 and 6.3 respectively. For each polymer the molecular weight is expressed as the mean ± the standard deviation, the deviation of each data point from the mean was not greater than 5%.

Table 6.2.2 Determination of molecular weight of PLA as supplied *(mean of three determinations ± standard deviation).*

Resomer	Mn	Mp	Mw	Mz	P
R104	2130	4654	4768	7702	2.242
	±109	±263	±134	±289	±0.0672
R203	12146	20015	19759	33634	1.6268
	±520	±568	±467	±221	±0.0878
R206	45900	92526	84385	127463	1.8406
	±2055	±4401	±625	±2155	±0.0737

Table 6.2.3 Determination of molecular weight of PLGA as supplied using gel permeation chromatography *(mean of three determinations ± standard deviation).*

Resomer	Mn	Mp	Mw	Mz	P
RG504	27161	45963	44059	65030	1.622
	± 612	± 479	± 1187	± 2889	± 0.0165
RG504H	28250	51015	49575	72939	1.755
	±327	±155	±1150	±1680	±0.0204
RG503	14731	25976	25112	37928	1.705
	± 56	± 264	± 282	± 920	± 0.0129
RG502	6591	12220	11846	17911	1.978
	± 180	± 311	± 418	± 668	± 0.032
RG502H	5232	8974	9394	13681	1.796
	±231	±411	±402	±572	±0.068

All polymers were polydisperse and exhibited P values greater than 1.6, smaller molecular weight polymers exhibited higher P values than the larger molecular weight polymers. A broad range of statistical molecular weight moment values ranging from Mn to Mz were calculated using the GPC software for each polymer sample. The statistical molecular weight value of Mn reflects the smaller molecular weight chains in the polymer distribution, while the statistical molecular weight Mz reflects the higher molecular weight chains.

Product Literature received from Boehringer Ingleheim indicated the following guideline molecular weights for the polymers studied (Table 6.4).

Table 6.4 Molecular weight of PLA and PLGA from Boehringer Ingelheim product literature.

Product	Mn	Mw
R104	Not given	Not given
R203	12000	30000
R206	60000	126000
RG502	9000	17000
RG502H	4000	10000
RG503	13000	40000
RG504	22500	57000
RG504H	11500	50500

The values determined in this work (Table 6.2.2 and Table 6.2.3) are quite different to those given in Table 6.2.4 but are similar to those reported by Gallagher (1998). GPC molecular weights are highly influenced by the mobile phase therefore differences between polymer molecular weights may be expected. Characterisation of the raw polymer is therefore necessary for any further polymer molecular weight investigations.

6.2.2 Relationship between inherent viscosity and polymer molecular weight

The PLA and PLGA series of polymers are supplied with a certificate of analysis detailing the polymer inherent viscosity value however no polymer molecular weight value is given.

The correlation of molecular weight determined by GPC with the inherent viscosity values given in the certificates of analysis was therefore explored.

For an unbranched linear polyester, average molecular weight Mw can be directly correlated to the viscosity by the Mark-Houwink Equation:

$[\eta]=K_{MH} \times (Mw)^{a}{}_{MH}$ Equation 6.1

Where $[\eta]$ stands for the intrinsic viscosity and K_{MH} and a_{MH} are the Mark-Houwink parameters for a given polymer-solvent combination at a particular temperature (Schindler and Harper, 1979; Van Dijk et al., 1983). Figure 6.2 shows that when the molecular weight Mw values determined by GPC were plotted against the inherent viscosity a linear correlation was observed.

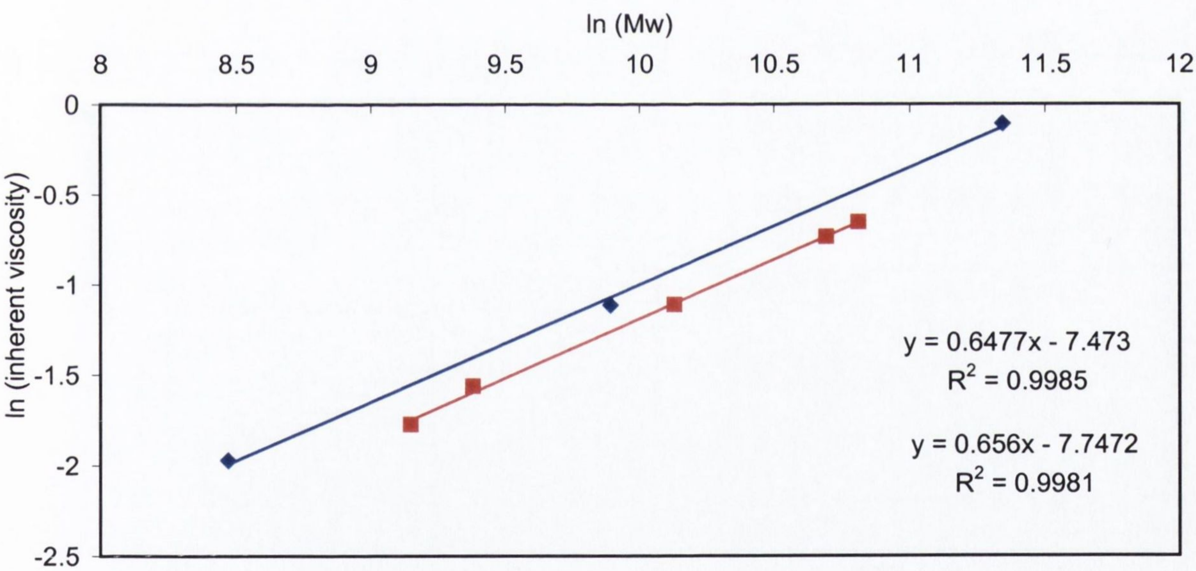


Figure 6.2 Plots of polymer Mw with inherent viscosity, for ■ PLGA series and ▲ PLA series of polymer (data points and fitted using linear regression).

The relationship shown in Figure 6.2 is useful for the selection of polymers for investigation where the inherent viscosity is the only information made available to the user as the plot can be used to empirically determine the molecular weight of unknown samples.

The Mark-Houwink constants for poly(d,l-lactide) were calculated as a_{MH}=0.65 and K_{MH}=5.68x10^{-4} and for poly(d,l-lactide-co-glycolide) as a_{MH}=0.66 and K_{MH}=4.31x10^{-4}. Values calculated for poly(d,l-lactide) by Omelczuk and McGinity (1992) were a_{MH}=0.66 and K_{MH}=3.3x10^{-4}.

6.2.3 Characterisation of PLA and PLGA by thermal analysis

The thermal characteristics of a polymer are an important factor in determining the properties the polymer will exhibit. The DSC thermograms, in which heat flow dH/dt is plotted against temperature (°C), of a PLA polymer (R203) and a PLGA polymer (RG504) are shown in Figure 6.3.

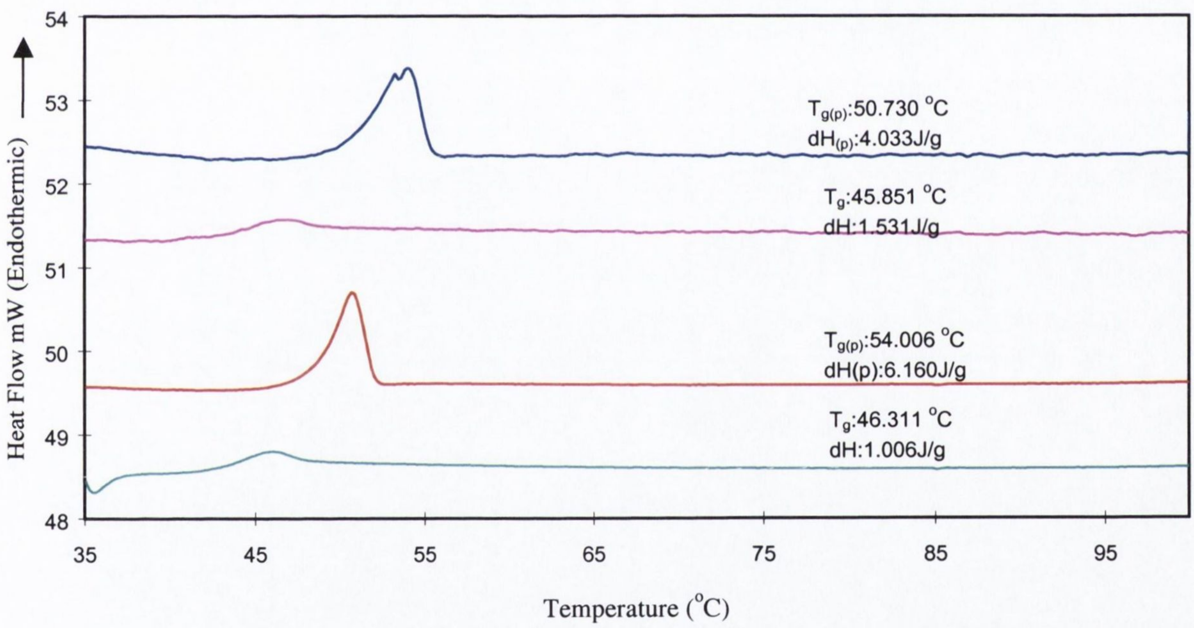


Figure 6.3 The DSC thermograms of PLA (R203) and PLGA (RG504) first heat (Run 1) followed by sample cooling then a second heat (Run 2), for ___ PLGA (Run 1), ___ PLGA (Run 2), ___ PLA (Run 1) and ___ PLA (Run 2).

Two heating scans were performed on each polymer sample to demonstrate how the thermal history of the polymer can effect the T_g of the polymer. The first heating cycle for each polymer shows an endothermic peak rather than a typical glass transition baseline shift, this peak at the glass transition temperature is known as kinetic overshoot and occurs due to the structure introduced into the polymer as a result of its thermal history (Richardson 1988, Hausberger and DeLuca 1995). By heating the polymer once past the T_g

then cooling the polymer below the T_g at a faster rate than that used for heating any 'history' of the polymer can be eliminated and a 'true T_g' of the polymer is then observed. Heat-cool-heating the polymer in this manner was observed to cause a reduction in temperature and in dH where the thermal event associated with the T_g occurs. As T_g values determined using the heat-cool-heat method are different to the starting polymers these values may not represent the thermal properties of the polymers as used. Values obtained from the first heating cycle (Run 1) are used for the purpose of this work and these values are denoted $T_{g(p)}$ to differentiate them from T_g values.

Thermal analysis of PLA and PLGA polymers used for this work was carried out and is shown in Table 6.5 and Table 6.6 respectively. The thermal analysis of the polymers show a glass transition temperature $T_{g(p)}$ which was associated with a low heat of transition ΔH. There were no further transitions in the DSC thermogram until an endothermic drift due to decomposition of the polymer sample. Thermal analysis of PLGA revealed that compositions in the range 25-70 mole % glycolic acid were amorphous (Gilding and Reed 1979) and exhibited a glass transition temperature followed by an endotherm due to decomposition.

Table 6.5 Thermal characteristics of PLA polymers determined by DSC (mean of three determinations ± standard deviation).

Polymer	$T_{g(p)}$ (°C)	ΔH (J/g)	Onset decomposition temperature (°C)
R104	35.6	4.6	283.7
	±0.78	±0.16	±3.03
R203	55.7	5.36	302.5
	±.15	±0.25	±3.65
R206	59.8	7.1	305.4
	±1.06	±0.25	±0.48

Table 6.6 Thermal characteristics of PLGA polymers determined by DSC *(mean of three determinations ± standard deviation).*

Polymer	$T_{g(p)}$ (°C)	ΔH (J/g)	Onset decomposition temperature (°C)
RG502	45.7 ±0.52	7.0 ±0.18	304.6 ±0.55
RG503	48.9 ±0.25	4.3 ±0.03	307.6 ±0.75
RG504	53.2 ±0.19	4.7 ±0.12	311.0 ±0.44
RG502H	42.6 ±0.23	8.2 ±0.11	302.6 ±1.48
RG504H	53.7 ±0.28	5.6 ±0.14	312.8 ±0.95

T_g represents a measure of the flexibility of the polymer chains (*Ford and Timmons 1989*) and therefore can be related to the polymer molecular weight for a specific polymer. The $T_{g(p)}$ and decomposition temperature of the range of PLA and PLGA polymers showed a dependence on the molecular weight of the polymer. As the molecular weight Mw increased the $T_{g(p)}$ and the temperature at which the onset of decomposition occurred increased. The T_g and melting temperature of poly-l- LA and T_g of poly-d,l-LA has also been shown to be related to the molecular weight (Mn) (Jamshidi *et al.* 1988).

Plots of reciprocal Mn (and 1/Mw) versus $T_{g(p)}$ are linear (Figure 6.4) according to the Flory-Fox equation:

$$T_g = T_g^{\infty} - K_{FF} / Mn$$ Equation 6.2

Where T_g^{∞} is the T_g at infinite molecular weight, and K_{FF} is a constant representing the excess free volume of the polymer chain-end groups. The relationship between molecular weight and $T_{g(p)}$ for the PLA and PLGA polymers was investigated by plotting $T_{g(p)}$ against reciprocal molecular weight (Mn). For both polymer series a linear relationship was observed (Figure 6.4).

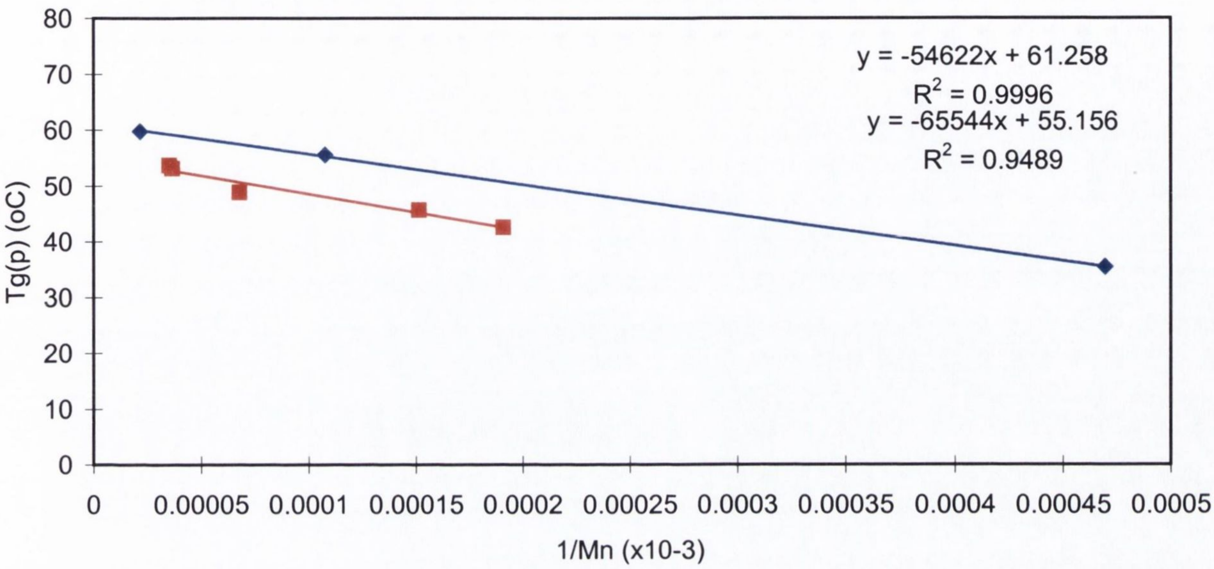


Figure 6.4 Flory-Fox plots for PLA and PLGA polymers, for ■ PLGA Series and ▲ PLA series data points (data fitted using linear regression).

T_g^{∞} occurs because as the molecular weight of the polymer increases the free volume of the polymer decreases. The decrease in free volume eventually reaches a limit as the molecular weight increases due to chain entanglements. As a consequence the energy is required to provide extra motion to chain segments at the T_g also reaches a limit

The T_g^{∞} determined for PLA from the Flory-Fox relationship was determined as 61.25°C which was similar to that reported by Omelczuk and McGinity 1992(60.1°C) while K_{FF} was calculated as 5.5×10^4. For PLA as the T_g approaches 61.25°C the Mw becomes infinite. The corresponding values for PLGA were T_g^{∞} 55.16 and $K=6.55\times10^4$, the higher K_{FF} value for PLGA represents a higher free volume due to the absence of the methyl side chain for this series of polymers.

6.3 PREPARATION AND PHYSICOCHEMICAL CHARACTERISATION OF PLA (R203) PARTICLES PRODUCED USING DIFFERENT METHODS AND PROCESS VARIABLES

The particles used for investigation in this work were prepared using a solvent evaporation technique. Two different solvent evaporation methods were first evaluated, emulsification solvent evaporation (Method I) or spray drying (Method II) using particles preparing using drug free PLA (R203). For the emulsification solvent evaporation method (Method I), the characteristics of particles using different techniques to form the emulsion were investigated. The emulsion was formed by dispersing the polymer solution for two minutes in the aqueous phase by either stirring using an Ultra Turrax four blade stirrer at 1,200rpm (Method II.1), or by sonication using a sonic probe at maximum output (Method II.2), homogenisation using an Ultra Turrax dispersing tool at the maximum speed of 24,000rpm (Method II.3) or by microfluidisation (Method II.4). The solvent was then evaporated by stirring the particle suspension at 1,200 rpm for 2 hours. The characteristics of the drug free PLA (R203) microspheres are shown in Tables 6.7 and 6.8.

Table 6.7 Effect of processing method on PLA (R203) microsphere characteristics *(mean of three determinations ± standard deviation).*

Method	Procedure	Yield (%)	$T_{g(p)}$ (°C)	Mp	Mw	P
I		22.8	51.4	20127	20051	1.622
		±0.57	±0.68	±935	±689	±0.0105
II	II.1	85.2	55.3	20560	21232	1.682
		±2.68	±0.66	±569	±1123	±0.0863
	II.2	68.7	55.2	14812	15259	1.874
		±1.94	±0.56	±626	±350.54	±0.0926
	II.3	80.7	55.7	19914	20801	1.638
		±0.19	±0.13	±703	±964	±0.0656
	II.4	83.9	55.5	19130	20215	1.744
		±1.27	±0.17	±1828	±313	±0.061

Table 6.8 Effect of processing method on PLA (R203) microsphere particle size *(mean of three determinations ± standard deviation).*

Method	Procedure	D10% (μm)	D50% (μm)	D90% (μm)	Span
I		1.9 ±0.05	9.7 ±0.57	27.7 ±0.30	2.7 ±0.13
II	II.1	96.7 ±14.16	298.9 ±6.95	544.6 ±4.16	1.7 ±0.31
	II.2	27.2 ±9.45	327.7 ±39.27	633.1 ±23.84	1.9 ±0.19
	II.3	1.5 ±0.03	5.9 ±0.23	18.3 ±0.13	2.8 ±0.13
	II.4	0.3 ±0.01	0.6 ±0.02	1.0 ±0.05	1.2 ±0.05

6.3.1 Spray drying(Method I)

Spray drying was a relatively fast procedure to produce microparticles and because of the high temperatures used in this procedure it allowed the used of a non-chlorinated solvent ethyl acetate to produce microparticles. The use of this solvent in the solvent evaporation procedure would considerably lengthen the evaporation step due to the higher boiling point of ethyl acetate (77.1°C) compared to dichloromethane (39.5°C). The molecular weight characteristics of the microspheres were similar to that of the starting polymer however the $T_{g(p)}$ of the particles was lower that that of the starting polymer attributed to residual solvent in the microparticles (Table 6.7). The yield produced by this process was low for the 1.8g batch size, this disadvantage has been also reported by other authors (Wang *et al.* 1990, Volland *et al.* 1994). Microparticles produced were smooth and spherical in nature (Figure 6.6) and from the particle size distribution the $D_{90\%}$ was determined as 27.7μm.

6.3.2 Emulsification/solvent evaporation(Method II)

Stirring/solvent evaporation(Method II.1)

Particles produced by stirring the polymer solution into the aqueous phase produced a high yield of microparticles. Thermal and molecular weight characteristics of the microspheres were comparable to those of the starting polymer (Table 6.7). The particles produced were spherical but displayed some polymer strings in the electron micrographs (Figure 6.6) attributed to inefficient mixing of the polymer solution into the aqueous phase. Particles produced at stirring speed 1,200 rpm had a $D_{90\%}$ of 544.56μm (Table 6.8).

Probe sonication/solvent evaporation(Method II.2)

When Probe sonication/solvent evaporation was utilised to produce microparticles a reduction in the statistical molecular weights of the PLA particles was observed due to the disruption of the polymer chains by the sonication process (Table 6.7). The yield of particles was also lower (Table 6.7) for this process as the polymer solution was sonicated first in a small volume of 0.27% PVA and then transferred into the remaining 0.27% PVA solution causing polymer material to be lost on transfer. Particles produced were large, D90% was 633.1μm (Table 6.8) and while spherical particles were produced, a large amount of irregular particles were observed in the SEM (Figure 6.6). Park (1994) also reported a reduction in the molecular weight compared to the raw polymer for PLA microspheres produced using an ultrasonic process. Studies by Reich (1998) showed that probe sonication of PLA and PLGA solutions in dichloromethane produced a decrease in polymer molecular weight relative to the duration and intensity of the sonication process. This effect was shown to be more pronounced with polymers of high molecular weight and increased glycolide content.

Homogenisation/solvent evaporation((Method II.3)

Particles prepared using the homogenisation/solvent evaporation procedure produced particles that had thermal and molecular weight characteristics similar to that of the starting polymer, a high yield of microspheres was obtained (>80%) for a 1.8g batch (Table 6.7). Microparticles produced were smooth spherical discrete particles (Figure 6.6). The particle size distribution gave a $D_{90\%}$ of <20μm at maximum speed of 24,000 rpm (Table 6.8).

Selection of alternative homogenisation speeds (8,000, 13,500 and 24,000 rpm) allowed for the production of different particle size distributions. Using the homogenisation/solvent evaporation technique PLGA and PLA particles were prepared using variations in homogenisation speeds. Figure 6.5 shows the relationship between particle size and homogenisation speed for PLA (R203) particles.

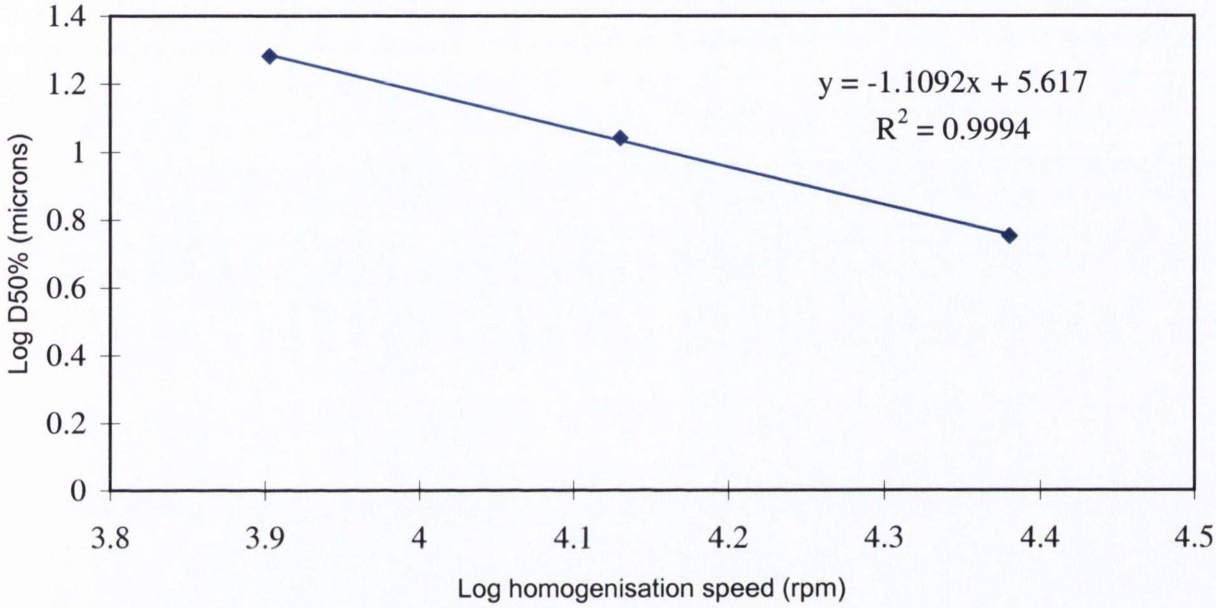


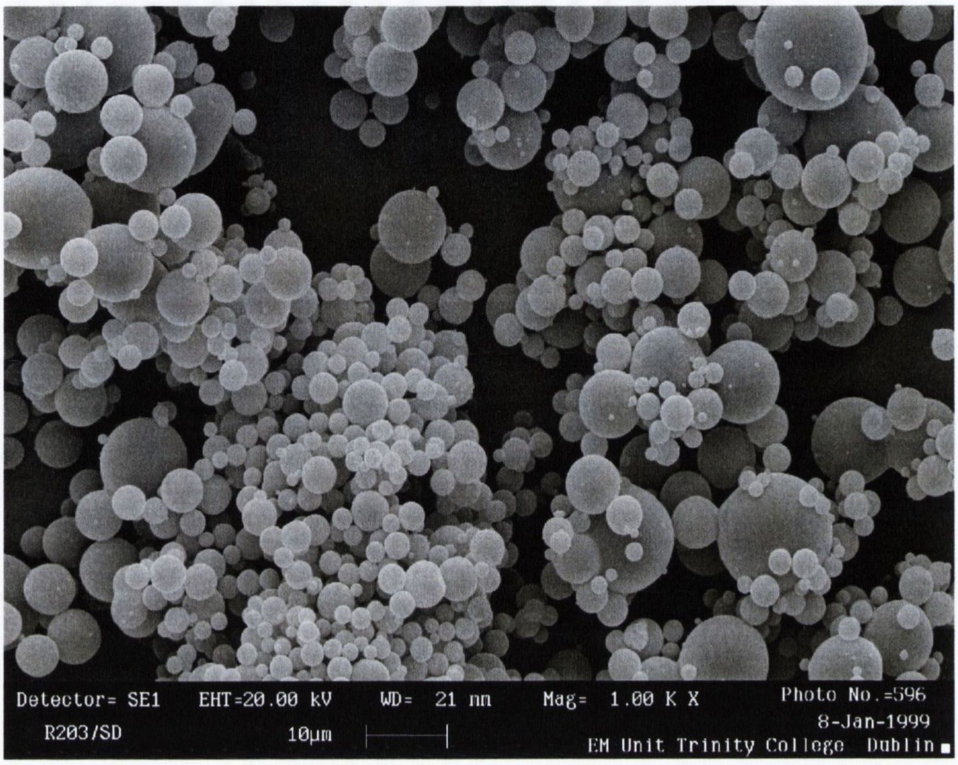
Figure 6.3.1 Log-log relationship between particle size $D_{50\%}$ and homogenisation speed for the production of PLA(R203) microspheres.

When particles were prepared by variations in the mixing speed the particle size decreased with a corresponding increase in homogenisation speed when all other parameters were kept constant. This relationship was observed to be log-log linear (Figure 6.5) consistent with what has been observed previously in the literature (Jalil and Nixon 1990a).

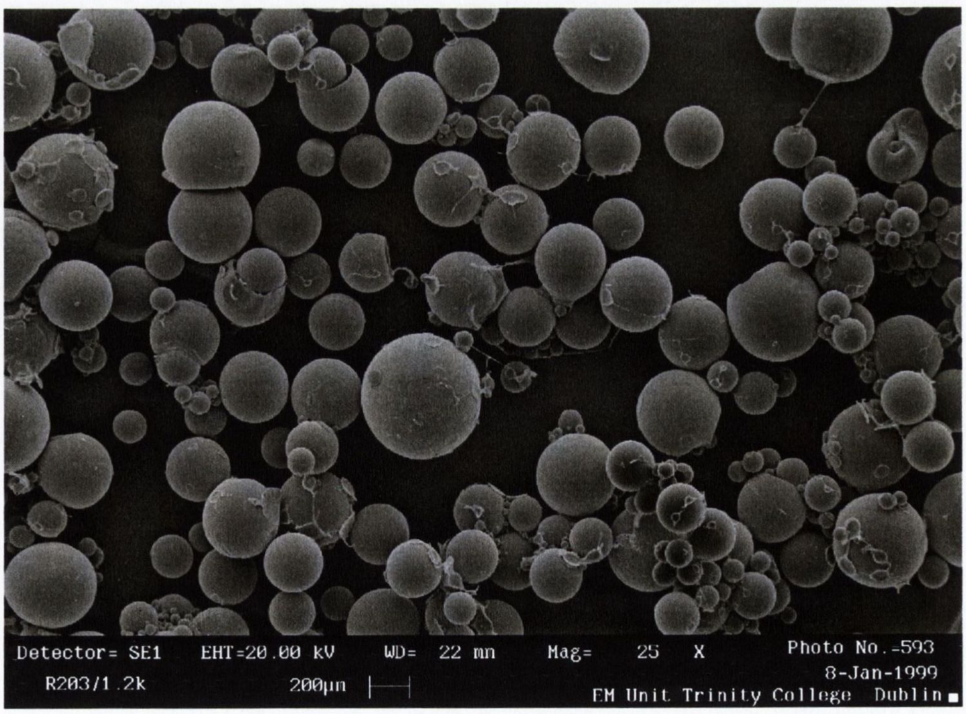
Microfluidisation/solvent evaporation (Method II.4)

Particles produced using microfluidisation also produced particles with thermal and molecular weight characteristics similar to the starting polymer (Table 6.7). Particles produced were in the nanometer range and were more homogeneous in size than particles produced using the other techniques (Table 6.8). Electron micrographs showed discrete spherical nanospheres were produced using this technique (Figure 6.6).

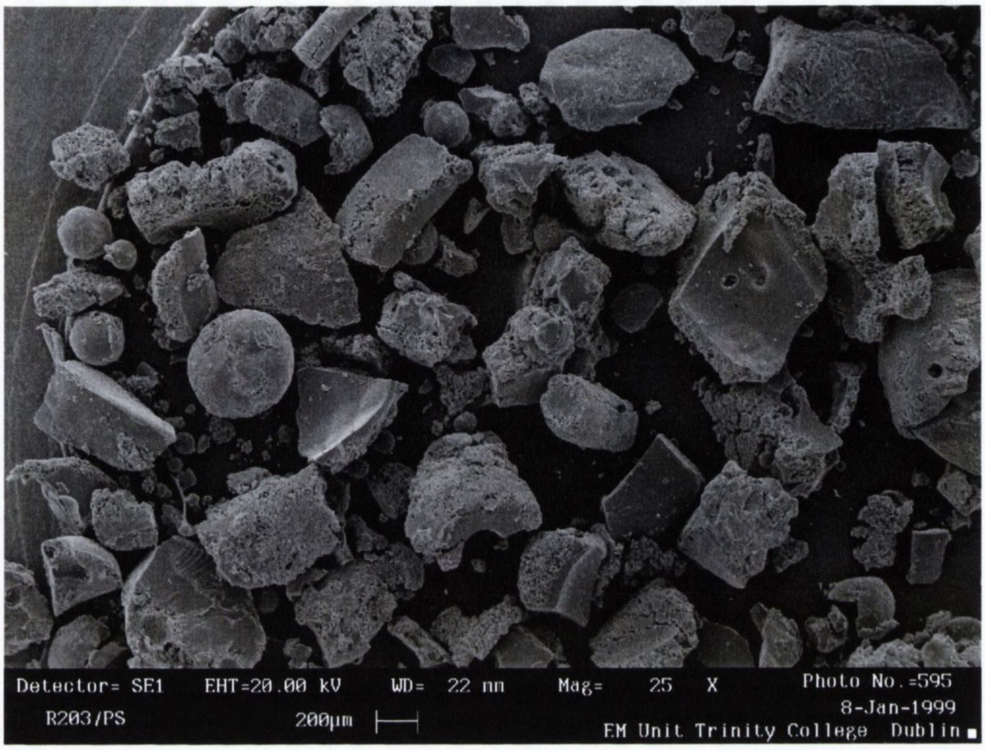
(a) Spray Dried (×1.0K)



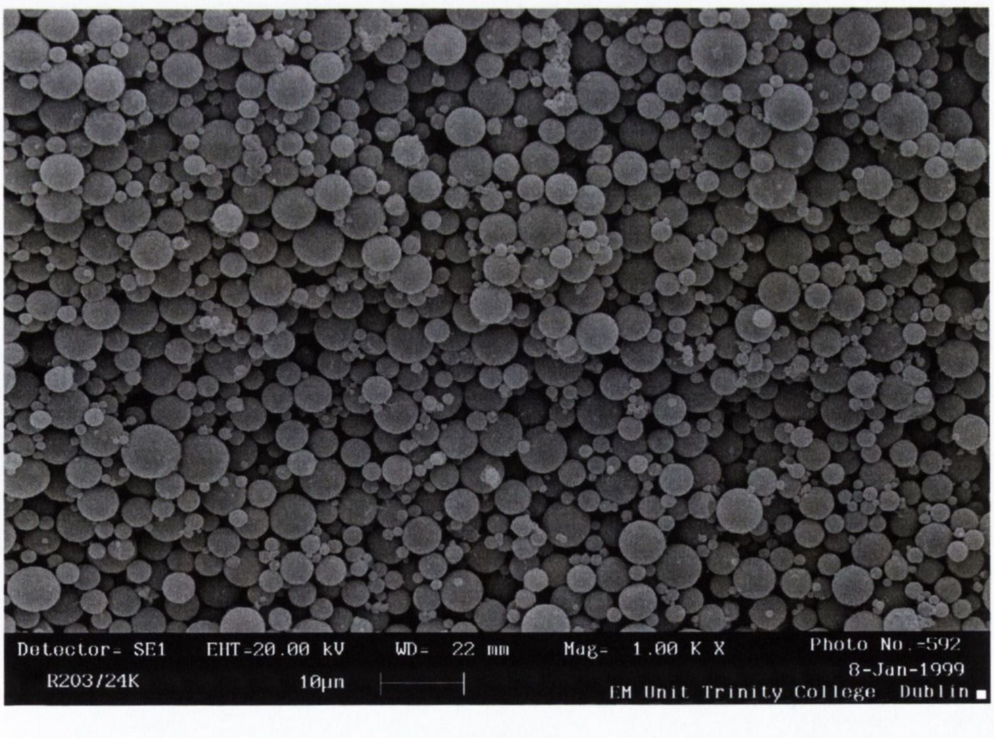
(b) Ultra Turrax Stirrer (×25)



(c) Probe Sonication (×25)



(d) Ultra Turrax Homogenisation at 24,000rpm (×1.0K)



(e) Microfluidised (× 15.0K)

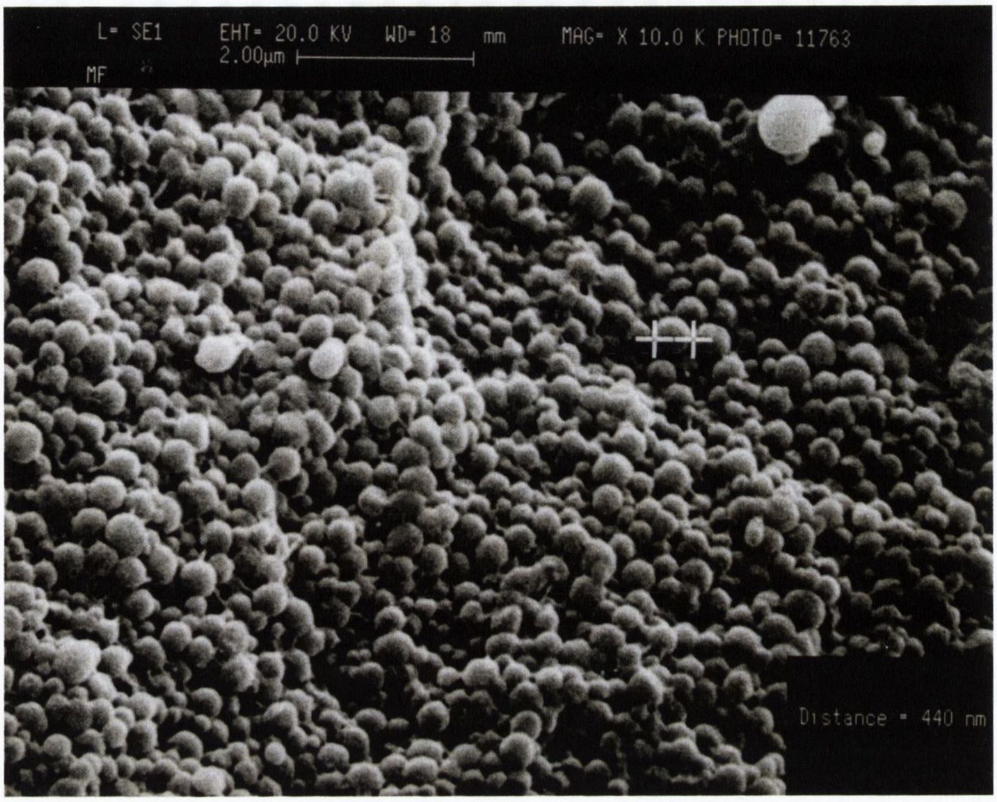


Figure 6.3.2 Electron micrographs of PLA(R203) particles prepared using different modifications of the solvent evaporation procedure (a) Spray dried (b) Stirred at 1,200 rpm (c) Probe sonicated (d) Homogenised at 24,000 rpm and (e) Microfluidised.

6.4 SUMMARY

In this chapter the molecular weight and thermal properties of a series of PLA and PLGA polymers were investigated. This relationship between polymer inherent viscosity and molecular weight along with the relationship between polymer molecular weight and $T_{g(p)}$ were established using the Mark-Houwink and Flory-Fox equation. Parameters calculated using these equations allow the determination of molecular weight and T_g of unknown PLA and PLGA polymer samples.

The physicochemical characteristics of PLA(R203) particles produced using different methods and processing variables were investigated to determine the optimum method to produce microparticles. Homogenisation/solvent evaporation was deemed to be the best method to produce microparticles based on the results shown in this work since high yields, minimal alteration in the polymer molecular weight and thermal properties, and controllable particle size was achievable. Three particle size fractions (<1, <20 and <50 microns) were chosen for further studies. These size fractions were chosen to reflect the size distribution produced by particles made using the single emulsion solvent evaporation method and the double emulsion solvent evaporation procedure to entrap water soluble molecules (Okada and Toguchi 1995). Nanoparticles were also prepared because of recent interest in these systems as drug delivery systems.

CHAPTER 7

THE DEGRADATION PROPERTIES OF PLGA PARTICLES

7.1 INTRODUCTION

In sustained release systems based on a polyester matrix, degradation that occurs via the cleavage of polymer chains to smaller molecular weights combined with polymer erosion usually plays a crucial role in drug release, particularly at low drug loadings. Transport of degradation products or drug molecules through the polymer matrix depends on both the morphology and the properties of the polymer matrix. In order to elucidate the mechanism governing the release process from these systems it appears fundamental to determine the degradation profile of the polymer matrix.

In this chapter polylactide-co-glycolide (PLGA) particles were prepared using the emulsification-solvent evaporation method with size ranges: <1μm, <20μm and <50μm in diameter. Nanoparticles were prepared by microfluidisation (Process A), while microparticles were prepared to give microspheres <20μm by homogenisation at 24,000rpm (Process B) and <50μm in diameter by homogenisation at 8,000 rpm (Process C) based on the relationship between homogenisation speed and particle size established in Chapter 6 (see Figure 6.5). Nano and microparticles were first characterised, and then the degradation profile of the particles was examined with respect to physicochemical and morphological changes that occur during this process. The influence of particle characteristics and environmental conditions on the degradation behaviour of poly-d,l-lactide-co-glycolide nano- and microparticles are examined. The degradation of PLGA was monitored by time dependent changes in molecular weight, polymer mass and glass transition temperature.

7.2 PHYSICOCHEMICAL CHARACTERISATION OF PLGA (RG504) PARTICLES WITH DIFFERENT PARTICLE SIZE DISTRIBUTIONS

PLGA particles prepared in the three size distributions using polylactide-co-glycolide (RG504) were smooth and spherical in nature with no surface defects as observed in the scanning electron micrographs (Figure 7.1). The particles were first characterised in terms of particle size and thermal properties (Table 7.1). The thermal properties were compared to that of the starting polymer (% differences from the starting polymer are indicated in parenthesis).

Table 7.1 Characteristics of PLGA particles ranked in order of increasing particle size *(mean of three determinations ± standard deviation).*

Process	$D_{10\%}$ (μm)	$D_{50\%}$ (μm)	$D_{90\%}$ (μm)	$T_{g(p)}$ (°C)	Yield (%)
Process A	0.3 ±0.01	0.5 ±0.03	0.9 ±0.05	52.4 (-1.5%) ±0.15	82.2 ±3.89
Process B	1.4 ±0.07	6.9 ±0.25	15.1 ±0.46	52.1 (-2.0%) ±1.46	86.6 ±1.89
Process C	4.6 ±0.16	22.5 ±1.07	45.8 ±1.83	52.4 (-1.5%) ±0.15	87.6 ±2.47

(% difference from the starting polymer are indicated in parenthesis)

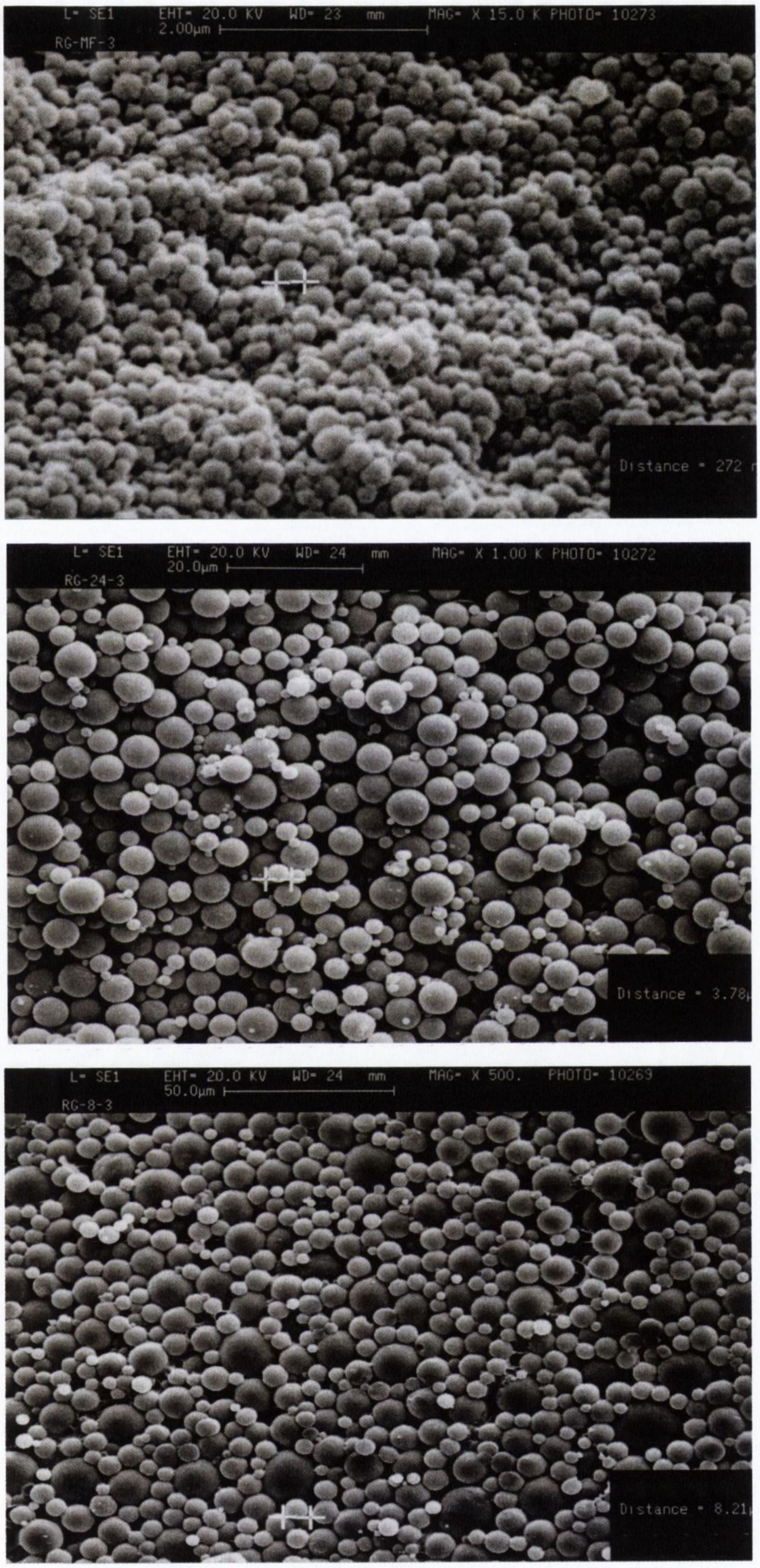
The $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ of the microspheres decreased with an increase in the homogenisation speed from 8,000 rpm to 24,000 rpm using the IKA Ultra Turrax. Nanoparticles were produced by microfluidisation. The yield of particles was high relative to the 1.8g batch size used to prepare particles for this work, for all three processes. The thermal properties $T_{g(p)}$ of the particles were similar to that of the starting polymer.

GPC analysis of the particles (Table 7.2) showed the Mn values remained relatively unchanged (<5%) compared to that of the unprocessed polymer. However decreases in molecular weight of greater than 5% were observed for the other molecular weight moments indicating that some degradation of the polymer chains does occur during the manufacturing process.

Table 7.2 GPC characteristics of PLGA (RG504) polymer and RG504 particles ranked in order of increasing particle size *(mean of three determinations ± standard deviation).*

Process	Mn	Mp	Mw	Mz	P
PLGA	27161	45963	44059	65030	1.622
	±612	±479	±1187	±2889	±0.0165
Process A	26203	41152	40957	58511	1.563
	±709	±926	±650	±1265	±0.0342
Process B	26109	42124	41312	59255	1.586
	±598	±930	±2104	±1370	±0.0335
Process C	26567	42916	41851	61113	1.575
	±1043	±1455	±1756	±1183	±0.0760

Microfluidisation, which involves forcing the polymer droplets at high pressures of 10,000 psi through small-bore valves, produced the largest drop in molecular weight. Homogenisation speed also affected the mean amount of degradation produced with the higher homogenisation speed of 24,000 rpm used in Process B producing a larger drop in molecular weight, however Table 7.2 shows that mean values ± the standard deviation shows no significant differences between the three processes.



(a)

(b)

(c)

Figure 7.1 Electron micrographs of PLGA (RG504) particles (a) Process A (b) Process B and (c) Process C.

7.3 THE INFLUENCE OF PARTICLE SIZE ON THE DEGRADATION BEHAVIOUR OF PLGA (RG504) PARTICLES.

The degradation profile of PLGA particles for particles produced in the three size ranges: <1μm, <20μm and <50μm in diameter was examined. The degradation profile of PLGA particles were examined by placing 100mg of particles into pre-equilibrated visking bags which were secured and then placed into 100mls of PBS phosphate buffer at 37°C. This method was utilised because it facilitates complete sample recovery from the dissolution medium. Polymer molecular weight, mass loss, polymer hydration, thermal properties and matrix morphology were used to monitor the degradation.

7.3.1 Polymer Molecular weight degradation profiles of PLGA (RG504) particles as a function of incubation time in PBS at 37°C

When the degradation of RG504 particles in phosphate buffer at 37°C was examined for the three size ranges the molecular weight profile measured using the molecular weight parameter Mp, was found to decrease with time (Figure 7.2).

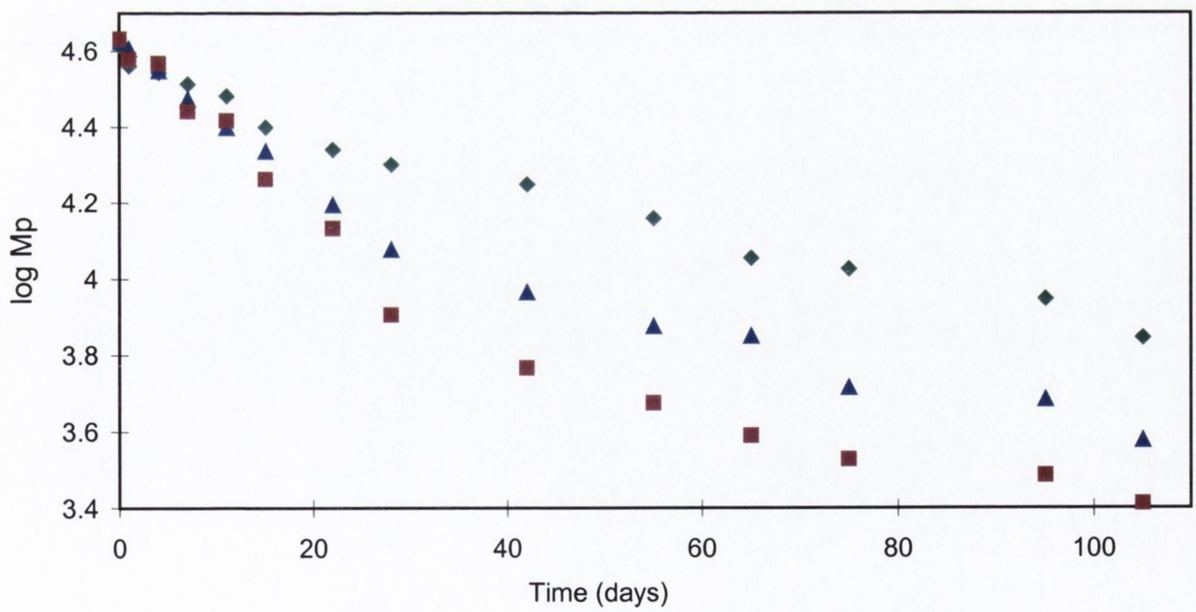


Figure 7.2 Plots of log molecular weight (Mp) with incubation time for PLGA (RG504) particles incubated in PBS pH 7.4 at 37°C, for ◆ < 1 micron, ▲ <20 micron and ■ <50 micron particles (*data points represent mean of two samples*).

The relationship between polymer molecular weight and incubation time was found to be log–linear with incubation time (Figure 7.2) similar to that reported by Kenley *et al.* (1987), Tracy *et al.* (1995) and Reich (1998).

Figure 7.3 shows that the molecular weight decrease occurs in an exponential mode similar to that first described by Gilding and Reed (1979). Plots of molecular weight against incubation time and log molecular weight against incubation time showed that the molecular weight profile of the microspheres was dependent on the particle size. A larger decrease in molecular weight was observed with increased particle size, particularly at the later time points.

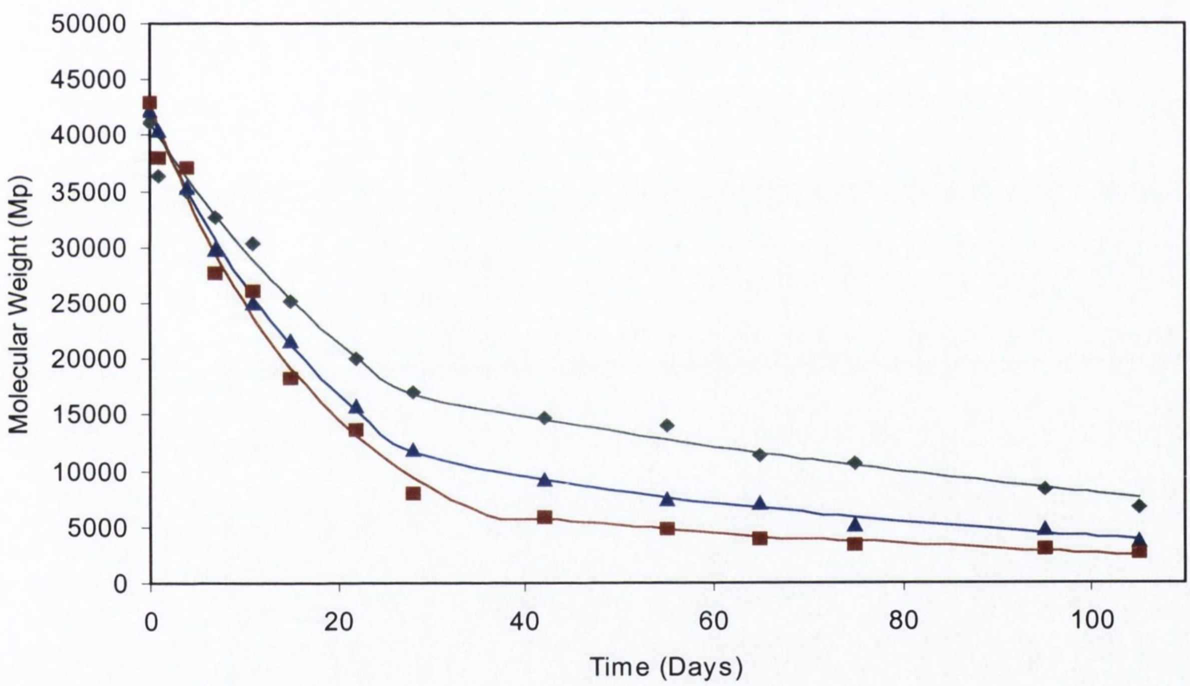


Figure 7.3 Influence of particle size on the molecular weight profiles of PLGA (RG504) particles incubated at pH 7.4 and 37°C, for ◆ < 1 micron, ▲ <20 microns and ■ <50 microns particles (*data points represent mean of two samples*). Data points fitted to equation 7.3.

The molecular weight degradation demonstrated a biphasic profile consisting of an initial fast rate of reduction k_1 over time t_1 followed by a slower rate of reduction k_2 of the molecular weight of the remaining polymer Mp_1 over time t_2 which occurred after a time, *Tau*. It was not possible to determine if a third phase existed at the later degradation points due limited sample and low resolution of the polymer molecular weight peak from the solvent peak in the GPC chromatogram.

The molecular weight profile could be described by the following equations:

$Mp = (\mathrm{Mp}_0 \exp(-k_1 t_1)) + (Mp_1 \exp(-k_2(t_2 - Tau)))$ However, Equation 7.1

$Mp_1 = Mp_0 \exp(-k_1 Tau)$ therefore Equation 7.2

$Mp = (Mp_0 \exp(-k_1 t_1)) + ((Mp_0 \exp(-k_1 Tau))\exp(-k_2(t_2 - Tau)))$ Equation 7.3

When Mp_o is the molecular weight of the starting microspheres and Mp is the molecular weight of the degradation samples measured over time t, the parameters k_1, k_2 and Tau can be determined using scientist software (Appendix IV), from which Mp_1 can also be calculated (Table 7.3).

Table 7.3 Parameters for the degradation of PLGA particles at 37°C in phosphate buffer saline pH 7.4 fitted to Equation 7.3 *(standard deviation represents error determined by the model).*

Particle Size (μm)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1 Calculated	CD	MSC
< 1	0.032 ±0.0022	0.010 ±0.0021	27.554 ±3.5754	16873	0.989	4.112
< 20	0.046 ±0.0011	0.014 ±0.0012	29.789 ±1.7491	10833	0.999	6.655
< 50	0.054 ±0.0013	0.013 ±0.001	36.199 ±1.5240	6094	0.995	5.860

The degradation kinetics was found to be dependent on the particle size. The rate constant k_1 calculated for the first phase of degradation was found to increase with increasing particle size while degradation rate k_2 calculated for the second phase was less dependent on the particle size. The time Tau at which the second phase begins was also related to the particle size, with the phase change occurring at longer times and lower molecular weights Mp_1 as the particle size increased.

The GPC chromatograms over the study period exhibited a broad uni-modal molecular weight distribution. A typical evolution of the molecular weight distribution over the study period is represented in Figure 7.4. Figure 7.4 shows the GPC chromatograms for the <20 micron microspheres (Process B) over a 105 day study period. The chromatograms are expressed as detector response (mV) versus elution time (Minutes), where longer elution times indicate a lower molecular weight. Figure 7.3.3 shows the movement of the polymer molecular weight peak towards the solvent peak that occurs after 10 minutes. Degradation resulted in the gradual movement of the peak towards longer retention times in the first phase of the degradation profile, indicative of a lowering of the molecular weight. In the chromatograms of samples recovered between days 28 to 75 which represent the second phase of the degradation profile further decreases in the molecular weight accompanied by peak broadening were also observed while at the later time points of day 95 and day 105 the peak then narrows again.

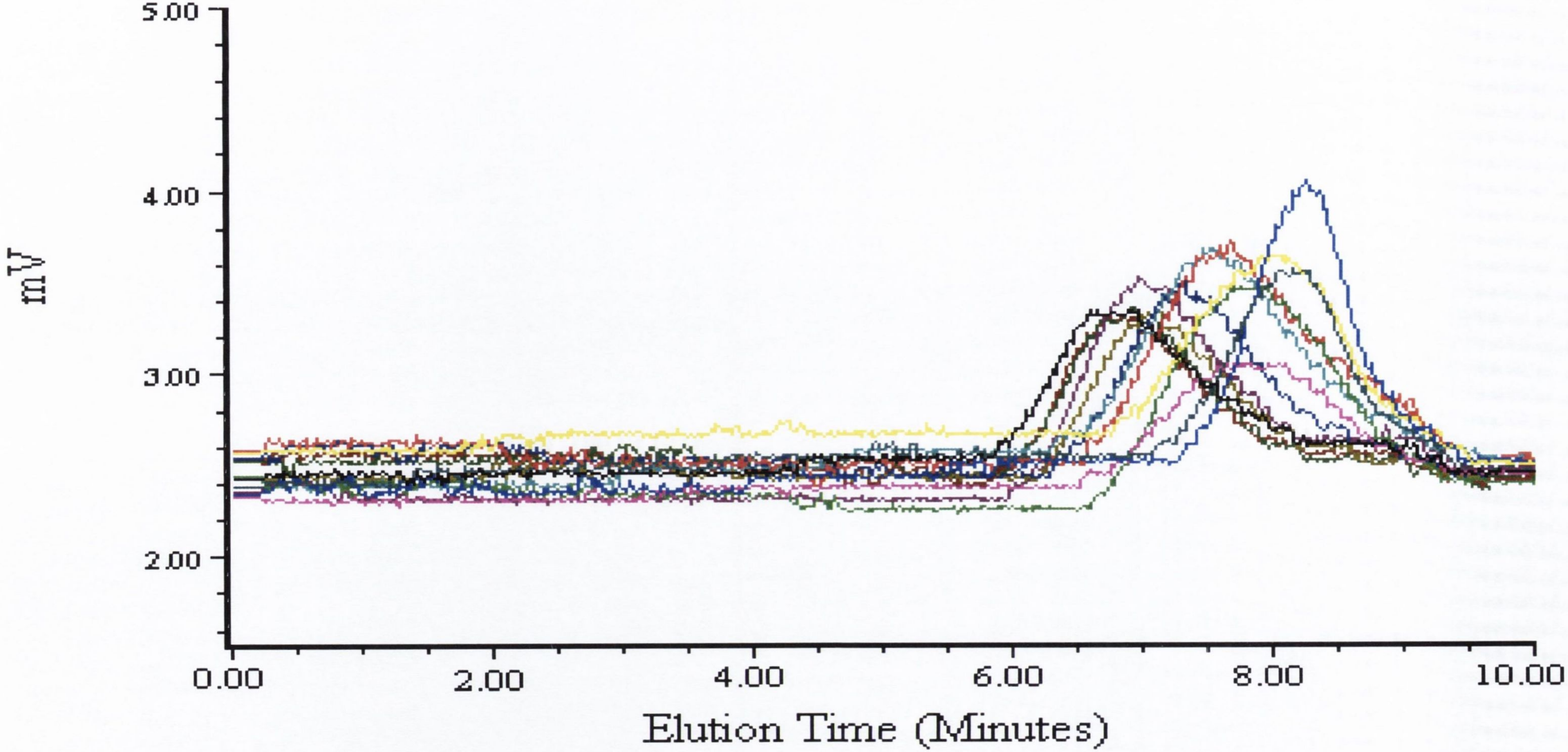


Figure 7.4 GPC Chromatograms of PLGA microspheres (<20 microns) after incubation at different time points after incubation in PBS at 37°C

— Day 1 — Day 4 — Day 7 — Day 11 — Day 15 — Day 22 — Day 28 — Day 42 — Day 55 — Day 65 — Day 75 — Day 95 — Day105

A plot of the polydispersity index with incubation time for the three particle sizes reveals that the molecular weight distribution broadens as the degradation proceeds and then at the later part of the degradation profile narrows again (Figure 7.5).

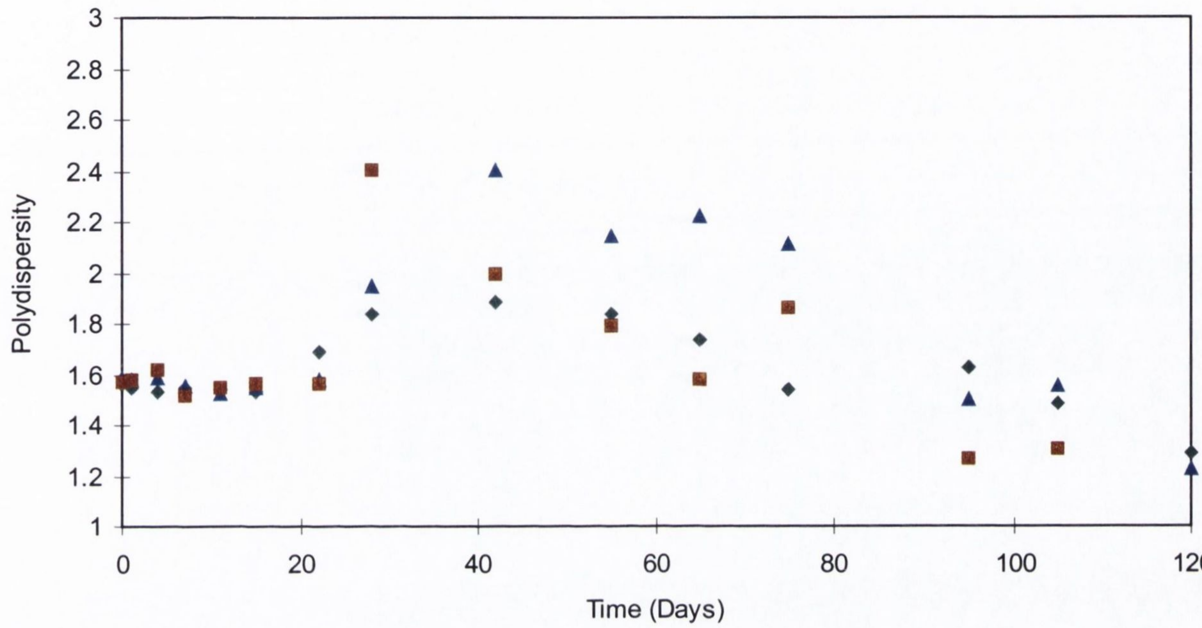


Figure 7.5 Plots of polydispersity as a function incubation time for PLGA (RG504) particles incubated in PBS pH 7.4 at 37°C, for ◆ < 1 micron, ▲ <20 micron and ■ <50 micron particles (*data points represent mean of two samples*).

During the first phase of the degradation profile the polydispersity index remains relatively unchanged compared to that of the starting particles, this indicates that the ratio of Mn to Mw remains relatively unchanged during this period. Between days 22 to 42 the polydispersity index increases and reaches a maximum, with the increase in polydispersity being larger with the larger particle sizes attributed to a build up of low molecular weight chains within the microspheres. During the second phase of the degradation the polydispersity decreases as degradation progresses until near degradation completion the polydispersity value is lower than that of the starting particles.

Park (1995b) also observed the evolution of molecular weight peak broadening and then narrowing demonstrated in this work. GPC elution profiles showed gradual peak broadening with degradation over time that then narrowed. Plots of GPC peak area as a function of time for a range of PLGA of different lactide:glycolide ratio demonstrated this effect. PLGA50:50 reached maximum peak area at day 20 and then reached a plateau, however the study was only carried out up to day 50 (Park 1995b).

7.3.2 Polymer mass loss profile from PLGA (RG504) particles as a function of incubation time in PBS at 37°C

Weight loss from PLGA (RG504) particles was also monitored over the study period. The Prout-Tompkins model was previously used to describe polymer erosion (Fitzgerald and Corrigan 1996) from PLGA systems and this model was therefore utilised to fit the erosion profile (Figure 7.6). The model was applied to the polymer erosion by multiplying the rate constant *k* by -1 to account for a decreasing mass profile.

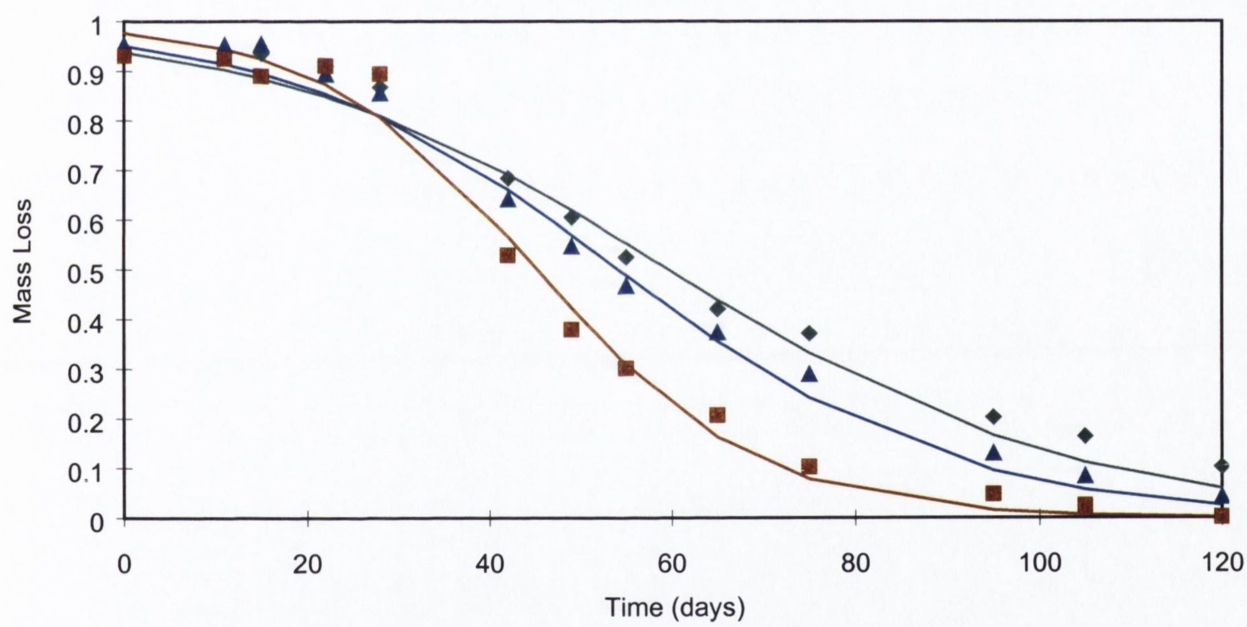


Figure 7.6 Mass loss from PLGA (RG504) particles incubated in PBS at 37°C, for ◆ < 1 micron, ▲ <20 micron and ■ <50 micron particles (*data points represent mean of two samples*). Data points fitted to Equation 3.23.

Some loss in weight (≤ 7%) was observed for all particles during the first 24 hours, this was attributed to the dissolution of low molecular weight oligomers produced during the manufacturing process, from the particle surface. This was followed by a period of negligible weight loss after which weight loss increased dramatically. The weight loss profile shows an induction period because the polymer initially undergoes chain scission that does not create low molecular weight polymer chains capable of being solubilised. The polymer must undergo extensive degradation until the critical molecular weight distribution is reached after which water soluble monomers and oligomers are produced thus no reduction in mass is observed until degradation of the polymer is well advanced. It was observed that the phase change at time *Tau* demonstrated in the molecular weight profile (Table 7.3.1) occurred just after the onset of mass loss. Mass loss from the particles has two consequences on the rate of degradation, it decreases the amount of low molecular weight components in the particles and therefore reduces the autocatalytic effect and it changes the molecular weight distribution, demonstrated by the polydispersity profile during this phase. Continuous loss of the low molecular weight chains influences the rate of decrease in the peak average molecular weight Mp.

Model parameters (*k* and *Tmax*) representing the induction and acceleration of decomposition were determined for the three particle sizes using Equation 3.23 and are shown in Table 7.4.

Table 7.4 Parameters for the erosion of PLGA particles determined using Equation 3.23 *(standard deviation represents that determined by the model).*

Particle Size	*k* (day^{-1})	*Tmax* (days)	CD	MSC
<1 micron	0.045 ±0.0028	59.690 ±1.8756	0.995	3.469
<20 micron	0.054 ±0.0048	54.150 ±1.4377	0.995	3.926
<50 micron	0.083 ±0.0056	45.290 ±1.0664	0.996	4.193

It was observed that as with the plots of molecular weight against time the rate constants *k* and *Tmax* determined using the Prout-Tompkins equation also showed a size dependent increase in rate with increase in particle size. The relationship between particle size and degradation rate is in good agreement with the heterogeneous degradation mechanism recently proposed by Li *et al.* (1990) and Grizzi *et al.* (1995). This heterogeneous degradation mechanism is a combination of autocatalysis due to carboxylic chain ends and the inability of oligomers to immediately diffuse from the polymer matrix. As the device size increases this effect becomes more pronounced. The rate constants for the mass loss profile were dependent on the particle size (Table 7.4) in contrast to k values determined for the change in molecular weight (Table 7.3) which showed no particle size dependence during this phase.

7.3.3 Particle morphology of degrading PLGA (RG504) particles as a function of incubation time in PBS at 37°C

The morphology of degrading PLGA particles was monitored by scanning electron microscopy of samples recovered from the incubation medium at intervals of time and dried under vacuum. Scanning electron microscopy of particles was carried out on samples taken from both phases of the degradation profile and are shown at different stages and compared for all three particle sizes (Figure 7.7). SEM of particles before incubation in phosphate buffer pH 7.4 at 37ºC were shown in Figure 7.1 for comparison.

The first morphological changes that are observed occur at the particle surface. SEM of particles taken after 1 day of incubation showed a fusion of the nanoparticles but not of the larger particles. The larger microparticles retain their shape and a smooth particle surface with no evidence of coalescence (Figure 7.7a). After 7 days incubation the SEM features of the degradation samples had not changed (Figure 7.7b). At 11 days incubation the surface of the microspheres was observed to develop some roughness (Figure 7.7c).

The development of a porous structure that is visible under scanning electron microscopy is detected at 15 days with some pitting of the surface of the larger particles observed (Figure 7.7d). After 28 days large pores were observed by electron microscopy in all particle sizes. SEM of particles after 28 days incubation show particle coalescence in the larger particle sizes. As degradation proceeds all particle sizes show fusion and gradual loss of shape of the particles. The spherical nature of the starting particles is no longer evident at later degradation time and the remaining polymer sample exists as a polymer mass (Figure 7.7g and h).

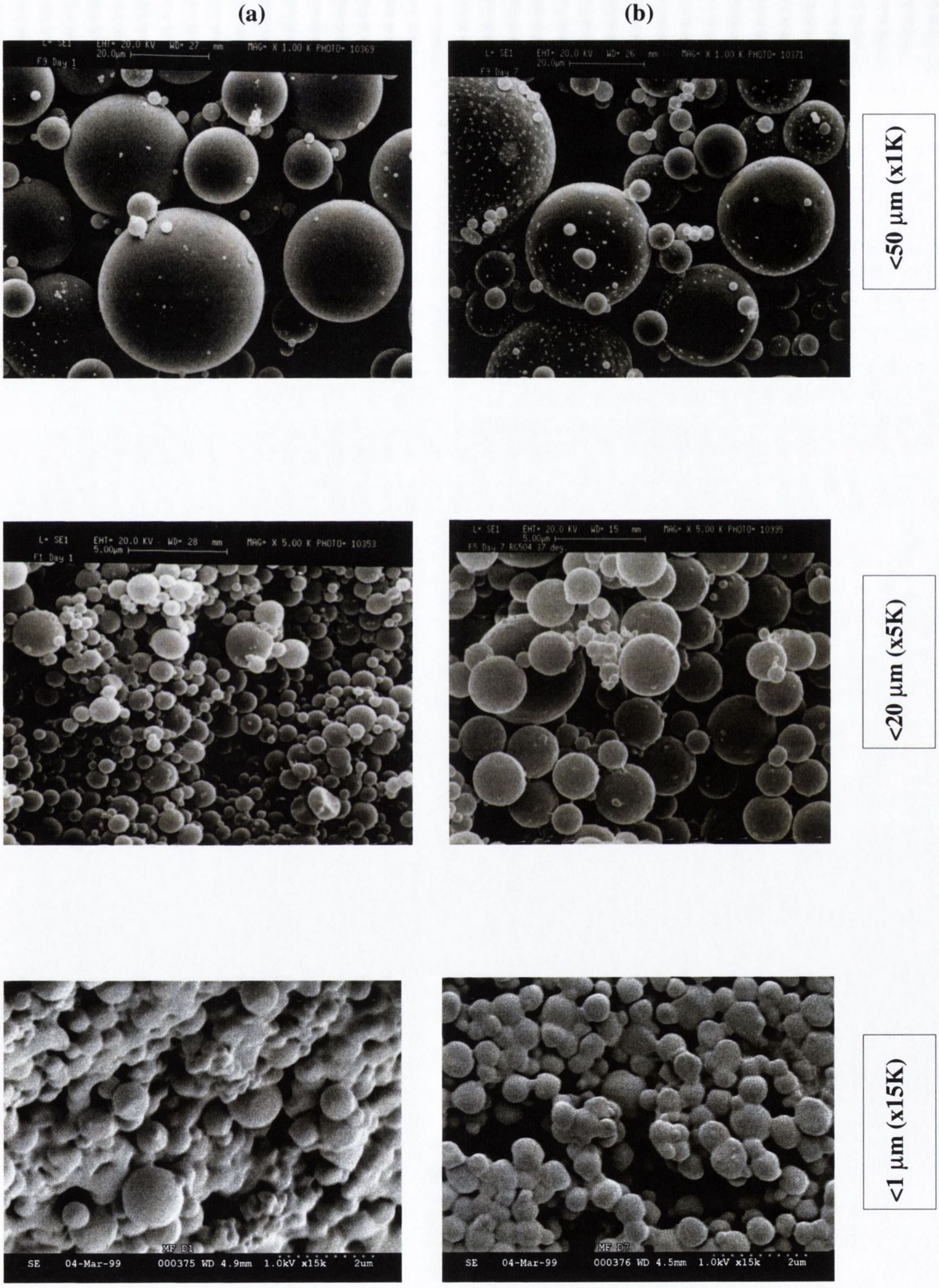


Figure 7.7 Electron micrographs of PLGA particles after incubation in PBS pH7.4 buffer saline at 37°C, for (a) 1 day (b) 7 days.

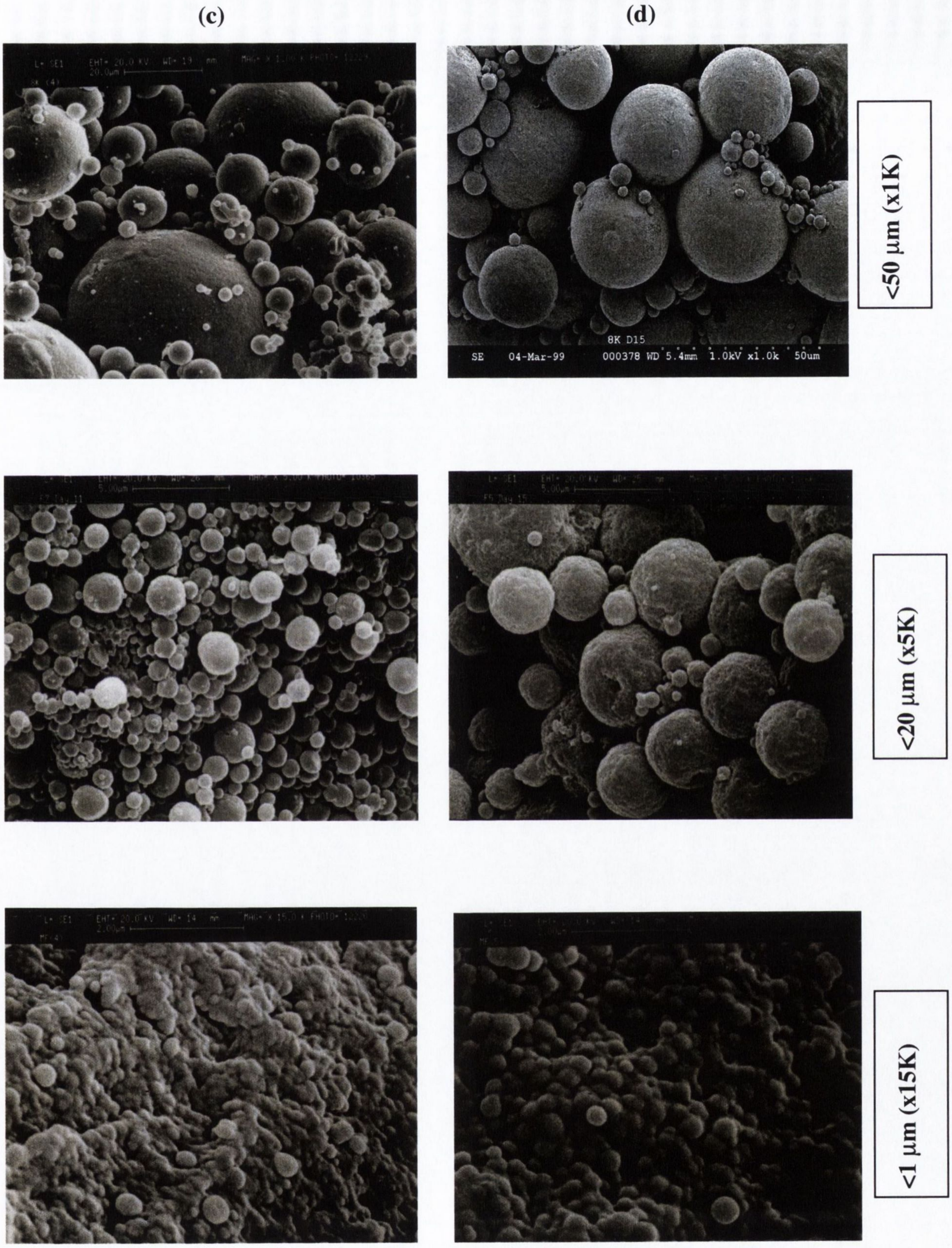


Figure 7.7 Electron micrographs of PLGA particles after incubation in PBS pH7.4 at 37°C, for (c) 11 days (d) 15 days.

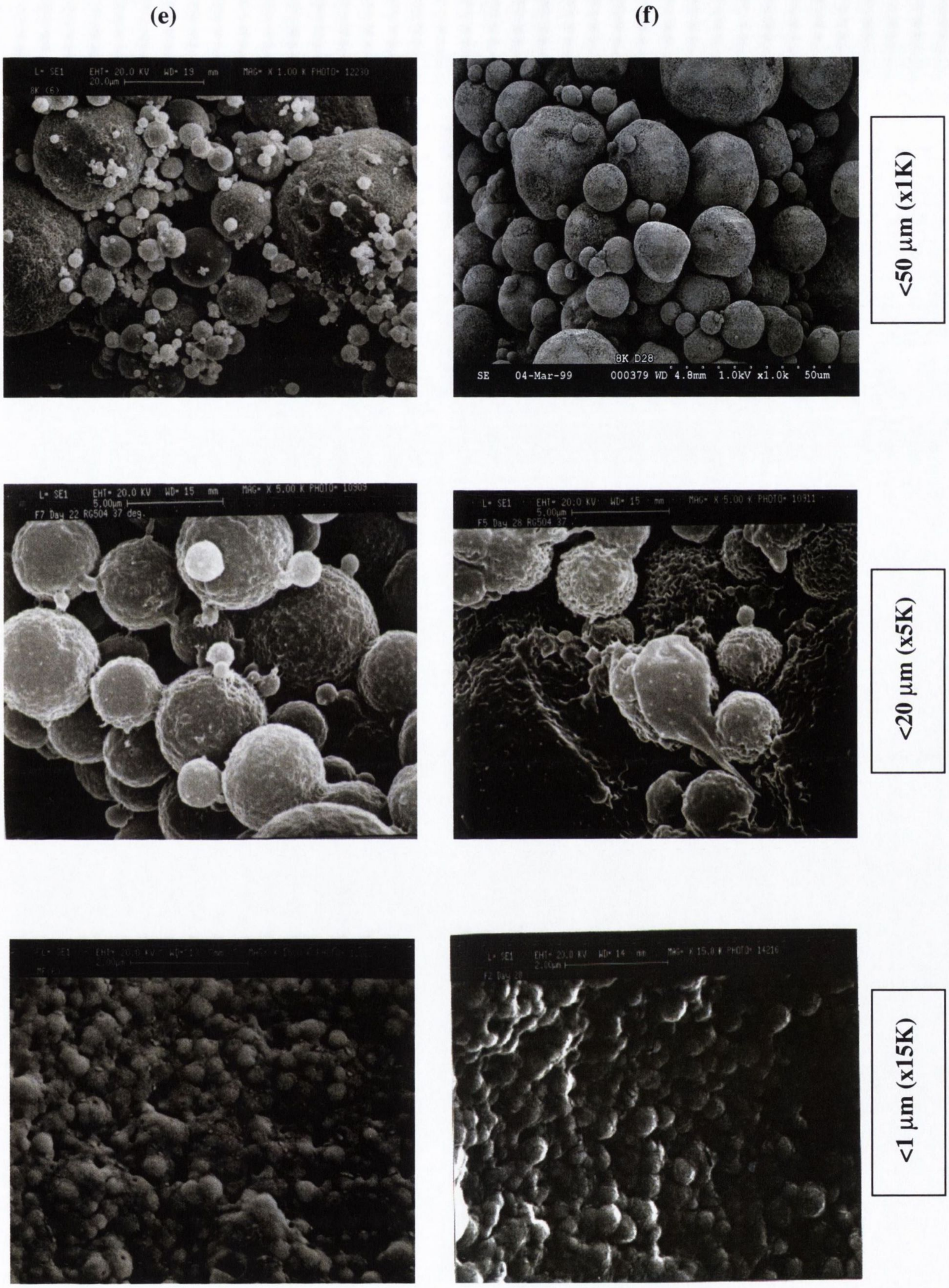


Figure 7.7 Electron micrographs of PLGA particles after incubation in PBS pH7.4 at 37°C, for (e) 22 days (f) 28 days.

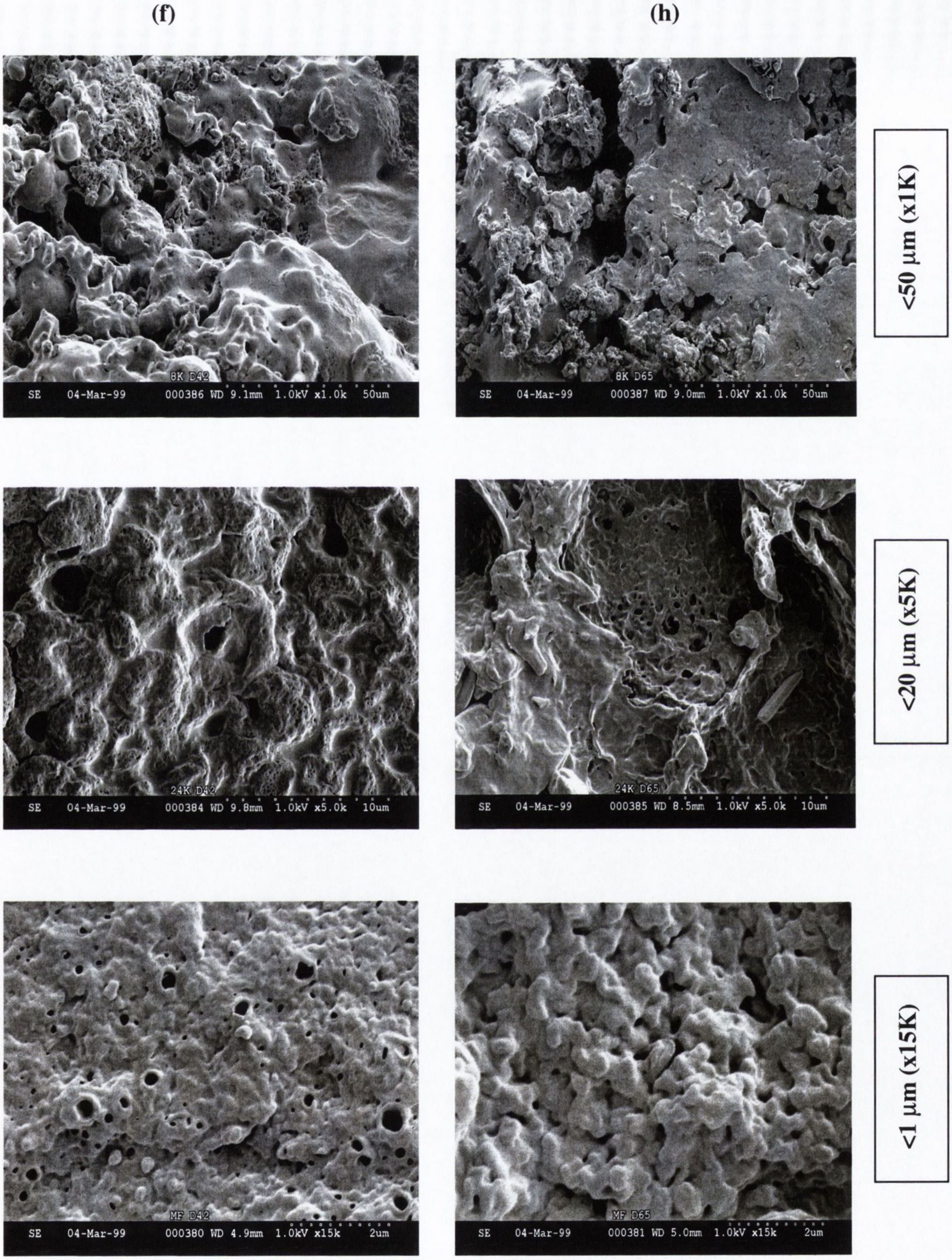


Figure 7.7 Electron micrographs of PLGA particles after incubation in PBS pH7.4 at 37°C, for (g) 42 days (h) 65 days.

7.3.4 Investigation of water uptake and its influence on the thermal properties of PLGA microspheres

The water uptake by degrading polymer microspheres was investigated using the median particle size (<20μm) microspheres. The water uptake by microspheres >20μm was determined by measuring the water loss from the samples by TGA after they were removed from the incubation medium. (Figure 7.8).

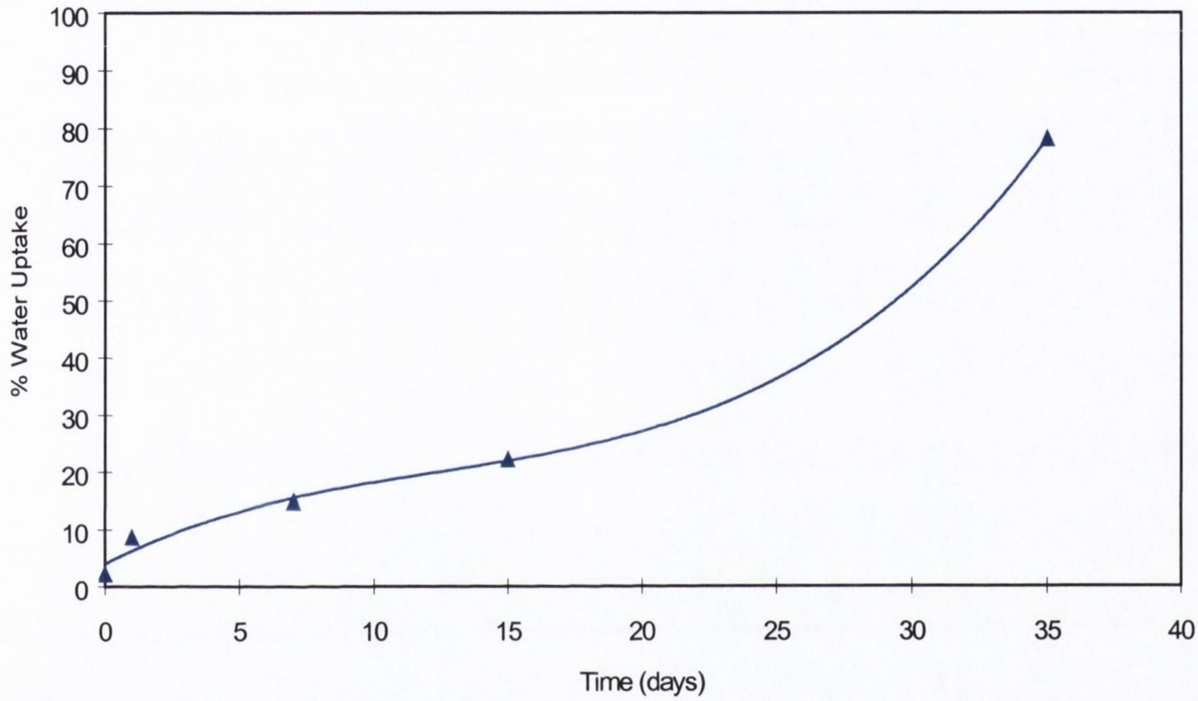


Figure 7.8 Water uptake by microspheres (<20 micron) in PBS pH 7.4 at 37°C as a function of incubation time *(samples represent the mean of two determinations).*

Polymers of lactic/glycolic acid and their copolymers are hydrophobic polymers. When microspheres are placed in the incubation medium very little water uptake by the microspheres occurs initially (Figure 7.8). The permeability of the polymer becomes enhanced as the polymer degrades and the water uptake by the microspheres increases with incubation time.

The rate of water uptake observed in the microspheres occurred in conjunction with the development of visible pores in the microspheres as observed in the electron micrographs (Figure 7.7). When the surface of the particles develop pores, water can diffuse rapidly into the device and the percentage water uptake for the microspheres increases rapidly, the time span during which this event corresponds with the second phase of the polymer degradation profile.

DSC analysis was also carried out on wet samples of the microspheres (<20μm) after they were taken from the dissolution medium. The purpose of this experiment was to investigate the effect of water uptake by the microspheres on the $T_{g(p)}$ of the polymer and the results are shown in Table 7.5.

Table 7.5 Aqueous DSC of microparticles <20μm after removal from dissolution medium *(samples represent the mean of two determinations).*

Incubation Time (Days)	$T_{g(p)}$ (°C)	Nature of $T_{g(p)}$
0	52.1	unimodal peak
1	31.5	unimodal peak
4	31.3	unimodal peak
7	32.9	unimodal peak
22	19.6	Diffuse
28	-	No peak discernible

The addition of water to the polymer causes a decrease in the $T_{g(p)}$ of the polymer due to a plasticizing effect on the polymer, the $T_{g(p)}$ remains constant up to day 22 after which further water uptake reduces the $T_{g(p)}$ to 19.62°C and peak broadening occurs. By day 28 the amount of water uptake further reduces the $T_{g(p)}$ so that it is not detected by the DSC or is not discernible from thermal events associated with water at low temperatures.

Shah *et al.* (1992) also demonstrated a similar effect in PLGA films. A sigmoidal buffer uptake and a corresponding reduction in polymer $T_{g(p)}$ with % w/w water content was shown. This was comparable to the effects of water uptake demonstrated in this work. When PLGA films were immersed in water for periods of time the $T_{g(p)}$ of the polymer initially reduced and reached a plateau after 5 days and then remained constant up to 20 days.

7.3.5 Thermal analysis of PLGA (RG504) degrading particles after incubation in PBS at 37°C

Thermal analysis of the wet polymer samples was limited because of the lowering of the $T_{g(p)}$ values to values close to 0°C, furthermore from the values it was not possible to determine at what point the polymer changes from a glassy to a rubbery state. DSC analysis of the dried degradation samples was then carried out for the three particle sizes (Table 7.6).

Table 7.6. Measured values of $T_{g(p)}$ (°C) for the degradation of PLGA particles.

Time (Days)	< 1 μm $T_{g(p)}$ (°C)	< 20 μm $T_{g(p)}$ (°C)	< 50 μm $T_{g(p)}$ (°C)
0	52.3	52.1	51.7
1	51.6	50.7	49.9
7	51.7	49.7	48.6
11	50.3	50.2	46.9
15	45.7	46.4	45.4
22	46.3	43.8	40.6
28	45.2	43.6	40.5
42	44.2	41.8	36.0

DSC analysis of the dried samples showed a continuous decrease in $T_{g(p)}$ for all particle sizes with incubation time (Table 7.6). The decrease in $T_{g(p)}$ was greater for the larger sized microspheres consistent with the faster degradation rate for the larger microparticles.

Figure 7.9 shows the DSC thermograms of PLGA (RG504) microparticles (<20μm) taken at different time intervals. As degradation proceeds thermal peak broadening was observed in the thermograms. After 42 days incubation DSC analysis of polymer samples showed a very diffuse peak from which it was not possible to determine peak values.

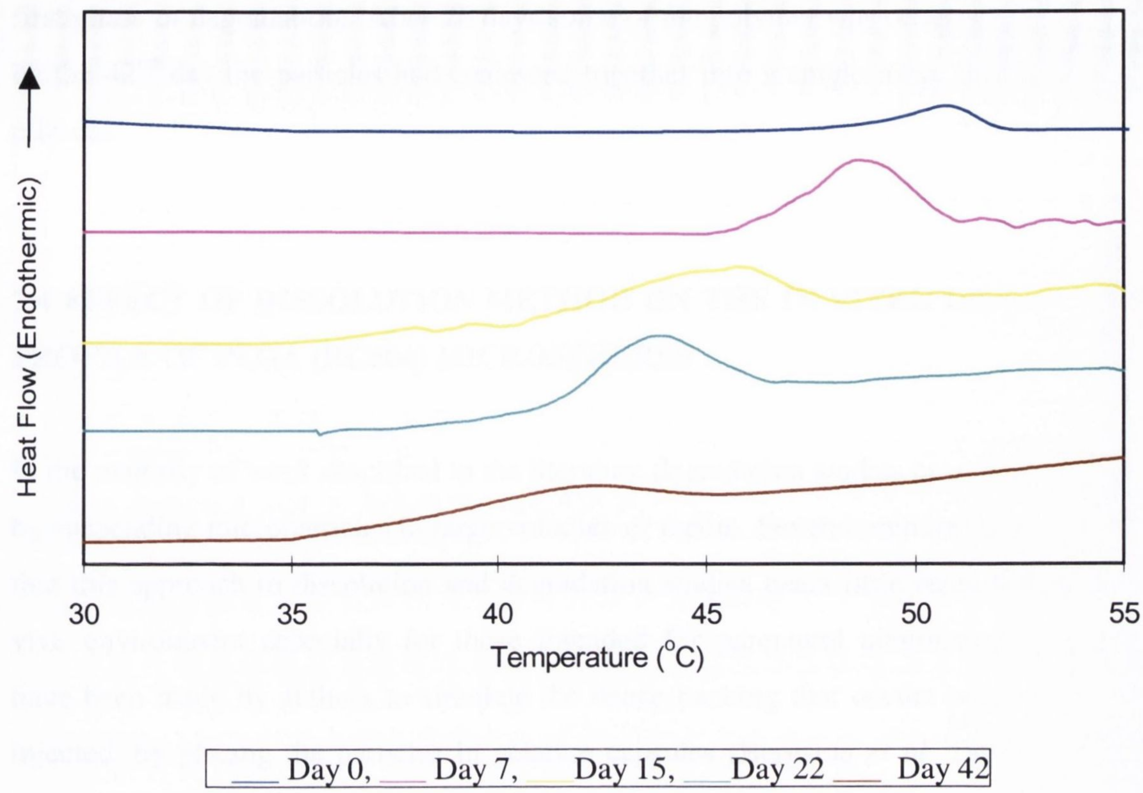


Figure 7.9 DSC thermograms of dried PLGA microparticles (< 20μm) taken after various incubation time-points in PBS at 37°C.

It is observed that for the time points that occur during the first phase of the degradation profile (up to day 22) no polymer chains have a $T_{g(p)}$ below 37°C. However during the second phase of the degradation profile, at the time point where weight loss of polymer had occurred, polymer chains with $T_{g(p)}$ below 37°C had been produced even though the main peak temperature was still above 37°C. At day 42 the thermal peak onset was 35.10°C and the peak temperature is 41.80°C, therefore during this phase of the degradation the polymer can exist in the rubbery state. In this state the polymer chains are free to move within the polymer matrix.

This observation was consistent with visual examination of the polymer samples within the visking bags at the sampling time points. In the visking bag system the physical appearance of the sample could be observed easily by lifting the total sample from the dissolution medium and noting the appearance of the sample at each sample time point. At the onset of degradation the particles were observed to become hydrated as water was absorbed into the polymer. Particles remained independent of each other and had a coarse texture during the first phase of degradation. After 28 days some of the polymer was observed to go soft and by the 42nd day the particles had coalesced together into a single mass and were soft and pliable.

7.4 EFFECT OF DISSOLUTION METHOD ON THE IN VITRO DEGRADATION PROFILE OF PLGA (RG504) MICROSPHERES

In the majority of work described in the literature degradation studies have been carried out by suspending microparticles in large volumes of media. Several authors have commented that this approach to dissolution and degradation studies bears little resemblance to an in vivo environment especially for those intended for parenteral administration. Attempts have been made by authors to simulate the dense packing that occurs when particles are injected, by placing the particles in gelatine capsules (Bergsma *et al.* 1995) or injecting them into gels (Sansdrap *et al.* 1996). In the visking bag method, the particles were transferred into pre-equilibrated visking bags and then placed in the incubation medium (100mls phosphate buffer saline). In the visking bag the particles are placed in close proximity in a smaller volume of liquid which has access to a larger bulk volume (100mls phosphate buffer saline). It was observed that particles can fuse together when placed in close proximity and this alteration of the device morphology may be an influential factor in release and degradation performance. In this section the influence of the use of two different experimental incubation conditions on the polymer degradation profile, using PLGA microspheres (<20 microns) was compared. In the first method the particles were dispersed in a fixed volume of incubation medium (100mls phosphate buffer saline) similar to the shaken flask method described by Wakiyama *et al.* (1982a). These were compared to the degradation profile exhibited by microspheres contained in visking bags that was discussed in the previous section of this chapter.

7.4.1 Comparison of the degradation profile of dispersed microspheres compared to microspheres confined to a visking bag.

The degradation profiles of PLGA (RG504) microspheres incubated using two different experimental incubation conditions is compared. The degradation profile of PLGA(RG504) microspheres dispersed in the incubation medium are compared to the degradation profile exhibited by microspheres contained in visking bags of incubation medium (100mls phosphate buffer saline) in a flask (Figure 7.10).

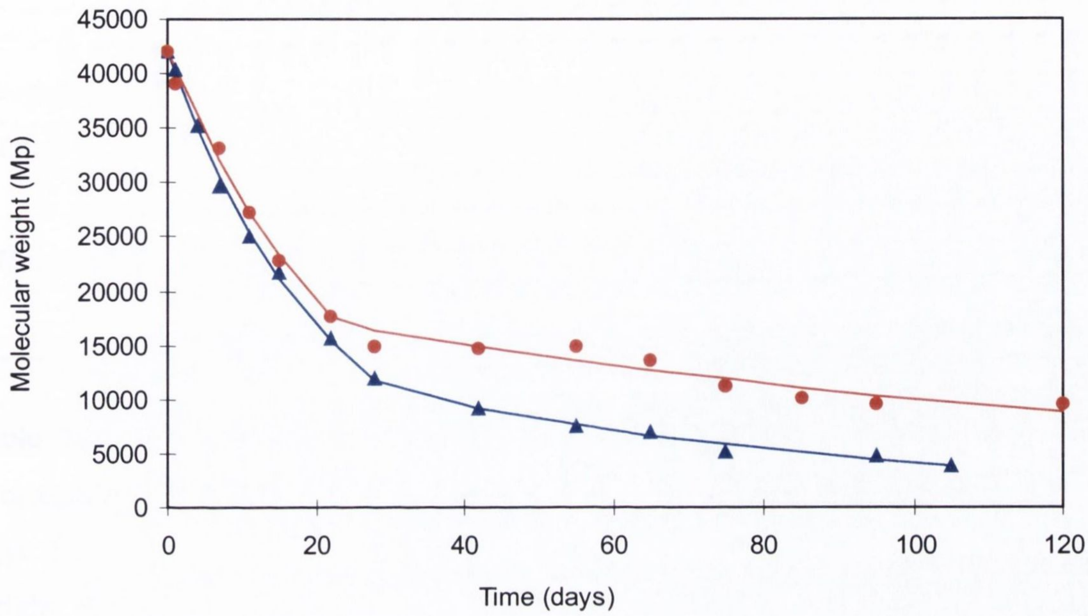


Figure 7.10 A comparison of the degradation profile of PLGA(RG504) microparticles using different dissolution techniques, for ▲ microspheres in visking bags in PBS and • microspheres dispersed in PBS *(data points are based on the mean of two samples).* Data points fitted to Equation 7.3.

Figure 7.10 shows that the first phase of degradation profile of the microspheres was similar when the dissolution techniques were compared. During the second phase of the degradation profile of the microspheres differences in degradation profile were observed. Microspheres contained in the visking system degraded to a lower molecular weight than the dispersed microspheres. The degradation profile of the dispersed microspheres was fitted to equation 7.3 and the degradation parameters were compared for both systems (Table 7.7).

Table 7.7 Parameters for the degradation of PLGA particles at 37°C in phosphate buffer saline fitted to Equation 7.3 *(standard deviation represents error determined by the model).*

Incubation Conditions	k_1 (day^{-1})	k_2 (day^{-1})	Tau (days)	Mp_l	CD	MSC
Dispersed	0.039 ±0.0012	0.007 ±0.0014	23.130 ±1.6585	16873	0.995	4.780
Restricted	0.046 ±0.0011	0.014 ±0.0012	29.789 ±1.7491	10833	0.999	6.655

Table 7.7 shows that the rate constants calculated for the degradation of the dispersed microspheres is slower compared to that observed for microspheres restricted in the visking bags. The time required for the phase change also occurs earlier and at a higher molecular weight for the dispersed microspheres. This is attributed to the increased surface area available for degradation products to escape, which also minimises the autocatalytic effect, and therefore the molecular weight at which the phase change occurs is higher.

Electron micrographs of dispersed microspheres recovered after incubation for 65 days (Figure 7.11) show that these particles do not aggregate in the same manner as particles recovered from the visking bags (Figure 7.7h). In the visking system the microparticles are in contact because of the restricted volume of the visking bag and as the degradation proceeds the polymer becomes soft and the particles coalesce into a single mass when the polymer T_g is lowered below the incubation medium temperature (37°C). During the degradation phase acidic oligomers are generated as a result of hydrolysis and if these are not released from the vicinity of the polymer they have the ability to autocatalyse the degradation of the remaining polymer chains to a lower molecular weight.

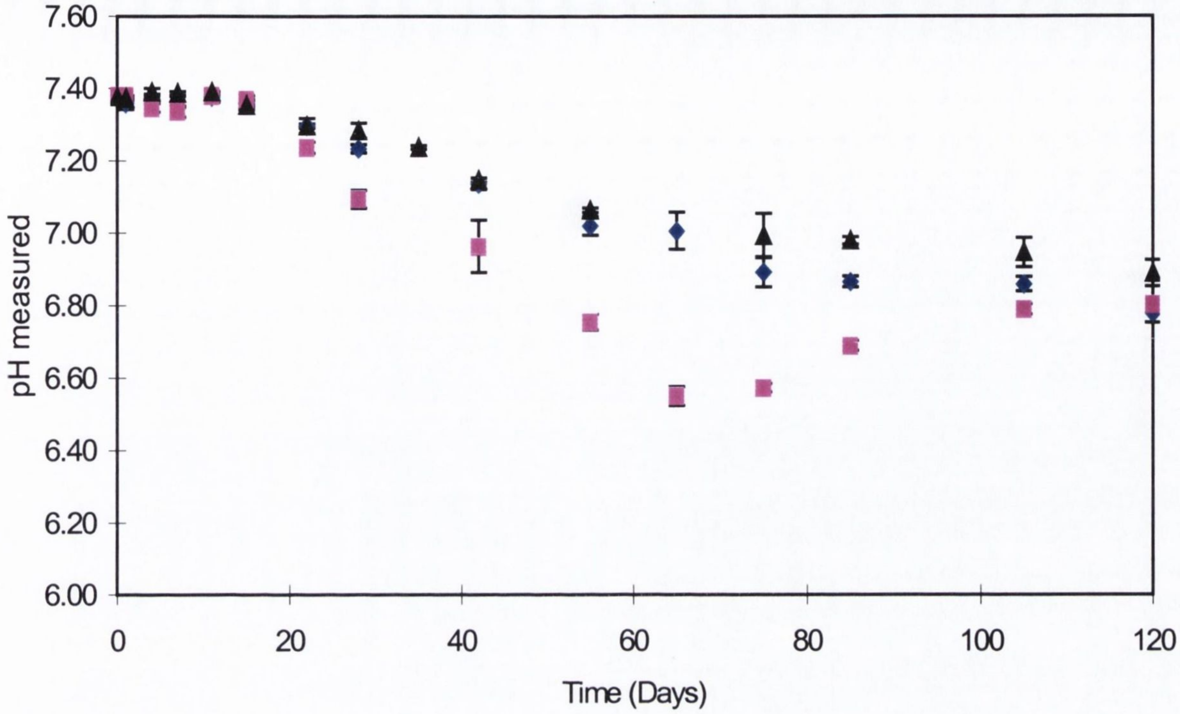


Figure 7.12 The pH profile of PLGA microsphere (Process B) degradation in phosphate buffer saline at 37°C, for ◆ pH of incubation medium containing dispersed microspheres ■ pH measured inside the visking bag ▲ pH of the bulk medium outside visking bag *(data points represent mean of three determinations ± standard deviation).*

Figure 7.12 shows that when the microspheres are placed in the incubation medium, no initial change in pH occurs. The commencement of the pH reduction corresponds to the determined T_{onset} calculated for the mass loss for the <20 μm microspheres at 28 days. Mass loss from the microspheres occurs as monomers and oligomers are solubilised in the surrounding medium, when this occurs the acidic end-groups also generate a reduction in the medium pH.

In the visking bag system the bulk medium (medium in which the visking bag is immersed) also showed a slow reduction in the pH. While inside the visking bags the pH recorded was lower than measured in the bulk medium due to the build up of acidic end products at the site and the inability of trapped degradation products to diffuse into the bulk medium from the glassy polymer. Degradation products have the ability to create a microenvironment that is significantly different from the bulk solution.

7.4.2 The effect of lactic acid and glycolic acid on the pH of phosphate buffer saline

The degradation of PLGA can produce d-lactic acid, l-lactic acid and glycolic acid as monomers or oligomers consisting of mixtures of these monomers. The ability of end products of degradation to alter the pH of the dissolution medium was demonstrated by adding known amounts of a d,l-lactic/glycolic acid solution to phosphate buffer. The ability of lactic and glycolic acid to alter the pH of the phosphate buffer solution was tested by the addition of lactic and glycolic acid. Kricheldorf and co-workers (Kricheldorf *et al.* 1985) have shown that PLGA prepared at high temperature with approximately equal monomer ratios exhibit essentially random co-monomer distributions. Therefore a 50:50 mixture of glycolic acid: d,l lactic acid was prepared for titration into phosphate buffer (Figure 7.13).

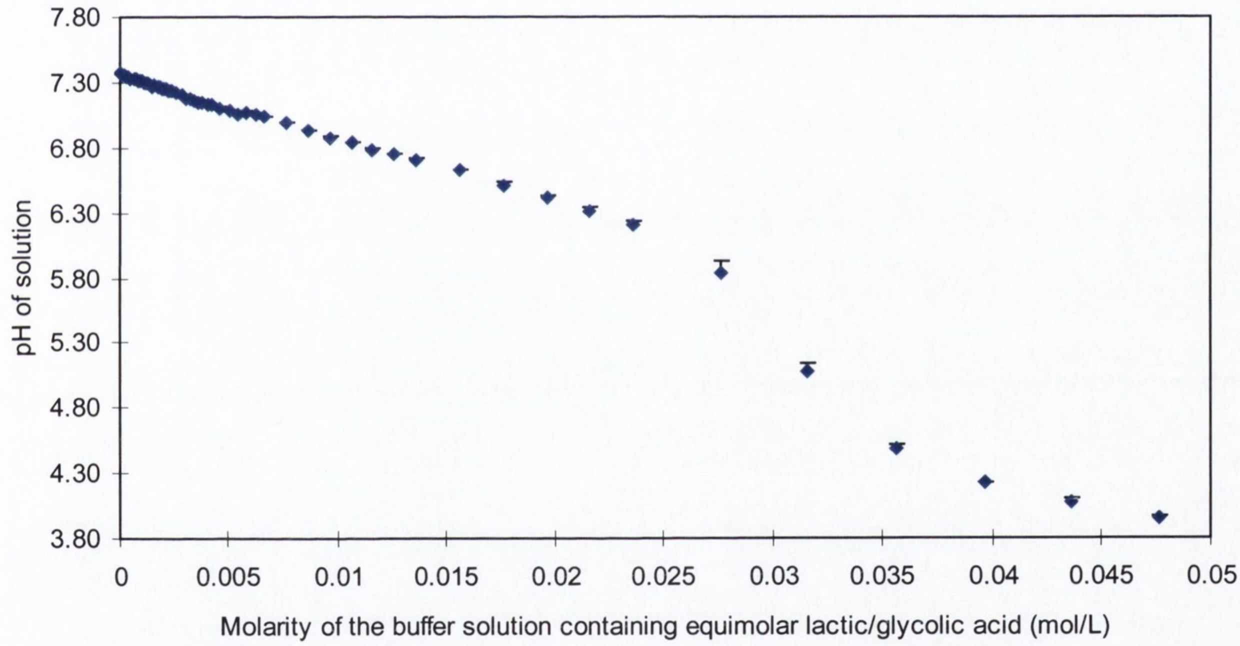


Figure 7.13 The effect of addition of a d,l-lactic/Glycolic acid solution on the pH of a phosphate buffer saline *(mean of three determinations ± standard deviation).*

Since l-lactic acid (pKa′=3.79), d-Lactic acid (pK=3.83), pK=3.86 for d,l -lactic acid and pK=3.83 for glycolic acid all at 25°C (Merck index), in dilute solution the conjugate acids are ionised and it is possible to correlate buffer pH to [H^+] present in the buffer, as the pH of the solution approaches the pKa this relationship becomes non-linear.

7.5 THE EFFECT OF LACTIC: GLYCOLIC ACID CONCENTRATION ON THE DEGRADATION PROFILE OF PLGA MICROSPHERES (< 20 MICRONS)

The effect of lactic and glycolic acid on the degradation process was further investigated by incubation of dispersed microspheres in the presence of lactic:glycolic acid. Microspheres were incubated with two different concentrations of lactic:glycolic acid (Figure 7.14).

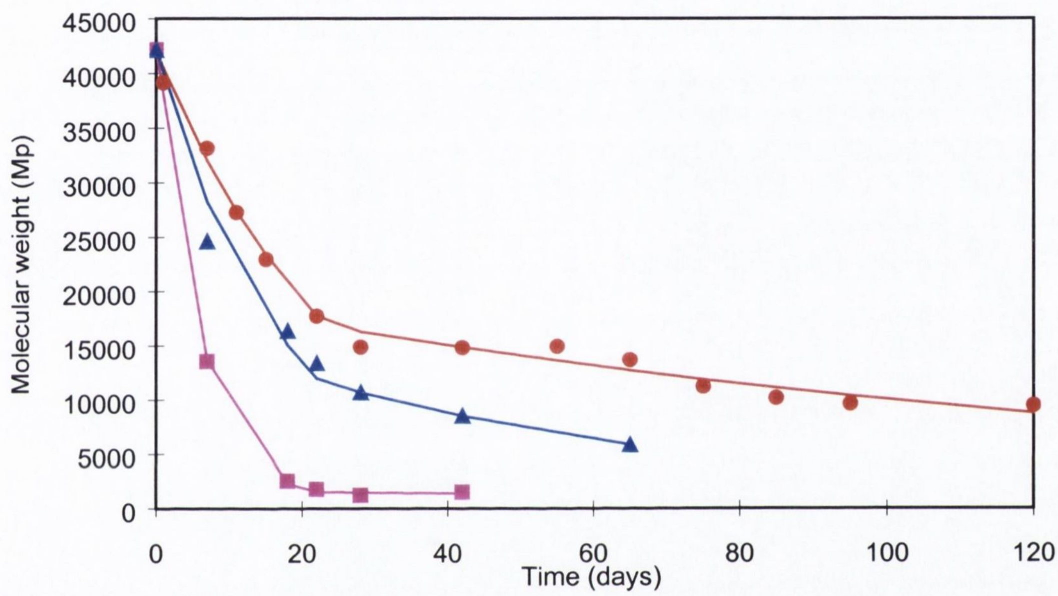


Figure 7.14 The effect of d,l-lactic/glycolic acid concentration on the degradation of 100mg of PLGA microspheres, for ● 0 M ▲ 0.012M ■ 12 M d,l-lactic/glycolic acid *(data points represent mean of two determinations).* Data points fitted to Equation 7.3.

The concentration of equimolar lactic/glycolic acid required to reduce the pH of phosphate buffer to pH 6.8 (equivalent to the pH change observed by the degradation of 100mg of PLGA microspheres in 100mls of PBS) was evaluated using the relationship shown in Figure 7.13. The amount of lactic/glycolic acid calculated to produce a pH drop to 6.8 in 100ml phosphate buffer was 1.15ml of a 1M d,l-lactic/glycolic acid solution. The actual pH of the solution was recorded as 6.78. The amount of acid d,l-lactic acid and glycolic acid in 1.15ml 1M d,l-lactic/glycolic acid solution was calculated as 0.10155 ml of 85% w/w d,l lactic acid and 0.0875g glycolic acid. These quantities of acid were then added to 100mg of microspheres dispersed in 1ml phosphate buffer to investigate the concentration dependent effect of the acids on the degradation process and the pH of this solution was recorded as

1.90. The samples were incubated as before at 37°C in a shaking waterbath at 60 rpm and sampled at intervals of time

The purpose of incubating microspheres with equivalent moles of lactic/glycolic acid was to confirm the local effect of these ions, and its concentration dependence on the degradation process. Parameters for the degradation of dispersed PLGA microspheres and the effect of d,l-lactic and glycolic acid monomers are shown in Table 7.8.

Table 7.8 Parameters for the degradation of PLGA particles at 37°C in phosphate buffer saline pH 7.4 in the presence of lactic:glycolic acid and fitted to Equation 7.3 *(standard deviation represents error determined by the model).*

pH of PBS	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1 Calculated	CD	MSC
pH 7.4	0.039 ±0.0012	0.007 ±0.0014	23.130 ±1.6585	16873	0.995	4.780
pH 6.8	0.057 ±0.0048	0.016 ±0.0107	22.374 ±6.024	11114	0.981	3.142
pH 1.90	0.161 ±0.0045	0.005 ±0.0105	20.457 ±1.203	1585	0.999	7.458

The addition of these acids to the incubation medium caused an increase in the degradation rate of the polymer. The addition of lactic/glycolic to the dissolution medium to pH 6.8 caused an increase in the rate of degradation of the PLGA microspheres for the rate constants k_1 and k_2 of both the first and second phase of the degradation (Table 7.8). The time *Tau* required for the phase change to occur was the same for both incubation conditions however the molecular weight was considerably lower for the microspheres incubated at pH 6.8. When the equivalent amount of lactic/glycolic acid was added to 1ml of PBS the degradation rate of the microspheres was dramatically accelerated (Figure 7.13). The degradation of the microspheres at pH 1.90 could not be evaluated using this equation. Studies by Fu *et al.* (1998) demonstrated that the pH within PLGA (RG503) microspheres can reach a pH range of 1.5 to 3.0 after incubation in vitro in PBS for 10 days.

7.6 SUMMARY

In this chapter the degradation profile of PLGA particles was determined, and the morphological and microenvironmental factors that influence the degradation profile were examined. The reduction in the polymer molecular weight was observed to proceed via a biphasic degradation mechanism. In the first phase chain scission of the polymer chains produces a continuous lowering of the molecular weight. The loss of soluble low molecular weight compounds produces a gravimetric weight loss from the particles, which produces the second phase in the degradation mechanism. During the second degradation phase the molecular weight profile changes and the rate of degradation becomes slower. During this phase the molecular weight measured is that of the solid material remaining from which mass loss is continuously occurring.

The kinetic patterns observed by these polymers are consistent with an autocatalytic process. The process of autocatalysis occurs whereby the liberated carboxylic end groups catalyse the hydrolysis of the remaining polymer (Vert 1998). The presence of the autocatalytic effect within particles in the nanosphere to microsphere range was demonstrated by the differing degradation rates in vitro.

The incubation conditions used to monitor the degradation rate were also shown to influence the polymer degradation rate. In the visking bag the particles are placed in close proximity in a smaller volume of liquid which has access to a larger bulk volume. This experimental design can be used to investigate the behaviour of microparticles in close proximity in a small volume of liquid where the effects of stirring are also minimised in order to simulate in vivo conditions since injected particles are reported to be encapsulated within the tissue and the flow rate of body fluids is slow in the soft and hard tissues (Maulding *et al.*1987, Sandstrap and Moes 1997). Particles placed in close proximity coalesce together into a mass where degradation products can remain trapped and a further contribution to the catalytic effects of the degradation products is attained. The ability of the end products of the degradation process, namely lactic and glycolic acid, to influence the degradation process was confirmed.

CHAPTER 8

FACTORS INFLUENCING THE DEGRADATION OF PLGA

8.1 INTRODUCTION

In this chapter factors that can influence the degradation rate of (polylactide-co-glycolide) PLGA microparticles will be investigated. These factors include polymer molecular weight and PLGA endgroup series. The process of ester bond cleavage is susceptible to rate changes dependent on the properties of the incubation medium. The influence of temperature and pH of the medium were also determined for PLGA.

8.2 PREPARATION OF PLGA PARTICLES OF DIFFERENT MOLECULAR WEIGHTS

Poly (lactide-co-glycolide) particles in the same size distribution, with different polymer molecular weights were prepared. The characteristics of polylactide-co-glycolide microparticles prepared using polymers of different molecular weight are shown in Table 8.1 and Table 8.2. Thermal and molecular weight characteristics were compared to that of the starting polymer and the % differences are indicated in parenthesis.

Table 8.1 Characteristics (n=3) of PLGA microspheres (<20μm) of different polymer molecular weight *(samples represent mean of three determinations ± standard deviation).*

Polymer	D10% (μm)	D50% (μm)	D90% (μm)	$T_{g(p)}$ (°C)	Yield (%)
RG504	1.4	6.9	15.1	52.1 (-2.1%)	86.5
	±0.07	±0.25	±0.46	±1.46	±1.89
RG503	1.4	6.1	14.6	47. (-3.6%)	87.7
	±0.01	±0.21	±0.07	±0.72	±2.80
RG502	1.4	6.3	15.3	42.5 (-7.0%)	86.0
	±0.02	±0.41	±0.99	±0.24	±1.75

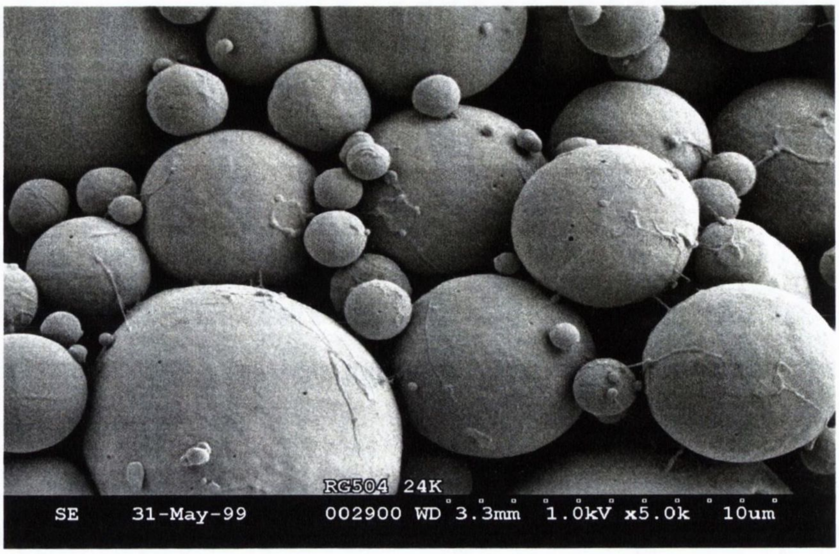
The change in $T_{g(p)}$ values was found to be related to the molecular weight of the polymer. Lower molecular weight polymers produced a larger decrease in $T_{g(p)}$ value on processing. The particle size distribution and yield of microspheres was comparable for microspheres manufactured using the three different polymer molecular weights. Molecular weight characteristics of the microspheres are shown in Table 8.2 and the % differences are indicated in parenthesis.

Table 8.2 GPC characteristics of PLGA microspheres (<20μm) of different polymer molecular weight (*samples represent mean of three determinations ± standard deviation).*

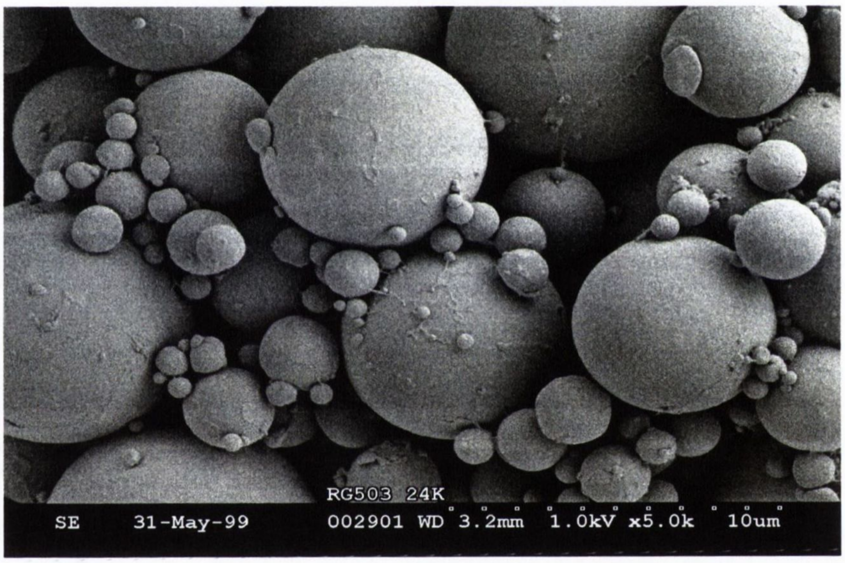
Polymer	Mn	Mp	Mw	Mz	P
RG504	26109(3.9%)	42124(-8.4%)	41312	59255	1.586
	±598	±930	±2104	±1370	±.0335
RG503	14932(1.0%)	25206(-2.9%)	25134	37824	1.683
	±69	±446	±346	±1127	±0.0158
RG502	5801(12.0%)	12194(-0.2%)	11477	17643	1.978
	±226	±381	±512	±818	±0.0386

Table 8.2 shows that the value of Mp demonstrated a larger difference for the higher molecular weight polymer. It has been postulated that a certain non-random type of chain scission is possible during mechanical degradation in which the polymer repeat units near the centre of the polymer chain have a higher probability of breaking than those near the chain ends (Scott 1974). Thus polymers with a higher molecular weight will undergo a larger drop in polymer molecular weight. In contrast, Mn values were most significantly changed for the lower molecular weight polymer. This is presumably because of the low molecular weight of these chains, some of which may have been solubilised during the manufacturing process, giving rise to the larger drop in polymer molecular weight Mn.

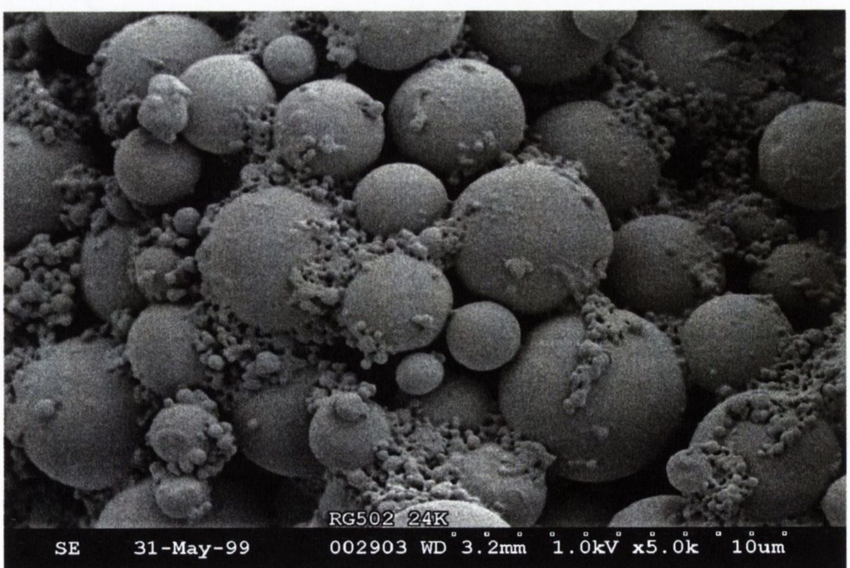
Scanning electron micrographs of microspheres manufactured using the three different polymer molecular weights are shown in Figure 8.1. In all cases smooth and discrete microspheres were produced. Microspheres manufactured from PLGA (RG502) were observed to have many particles with a very small particle size compared to the other two polymers.This was not reflected in the particle size distribution given in Table 8.1.



(a)



(b)



(c)

Figure 8.1 Electron micrographs of PLGA particles made from different PLGA molecular weight polymers, for (a) RG504 (b) RG503 and (c) RG502.

8.3 EFFECT OF POLYMER MOLECULAR WEIGHT ON DEGRADATION OF PLGA MICROSPHERES USING THREE DIFFERENT MOLECULAR WEIGHTS OF PLGA

The degradation profile of three different molecular weight PLGA polymers was investigated by incubation of microspheres (<20μm) manufactured from the three polymers under equivalent conditions, in visking bags, and determining the degradation and erosion rate of the microspheres.

8.3.1 The influence of polymer molecular weight on the degradation profile of PLGA microspheres as a function of incubation time at 37°C and pH 7.4.

The degradation profile was measured as changes in the peak molecular weight Mp and by examining the evolution of the molecular weight distribution or polydispersity. The degradation profile of the three molecular weight polymers is shown in Figure 8.2.

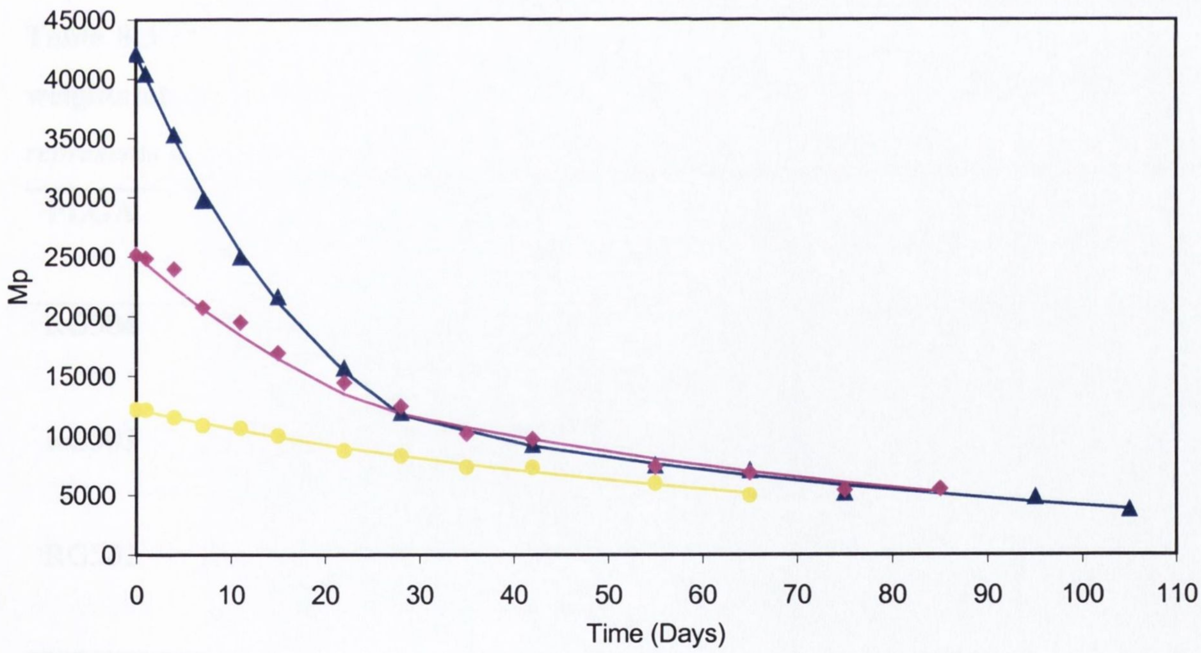


Figure 8.2 Influence of polymer molecular weight on the degradation of PLGA microspheres, for ▲ RG504, ◆ RG503 and ● RG502 particles *(data points represent mean of two determinations)*. Data points fitted to Equation 7.3.

The molecular weight degradation of the PLGA polymers demonstrated a biphasic profile for the three different molecular weights. Plots of log molecular weight as a function of incubation time were also bi-phasic and log-linear during both degradation phases, similar to that shown for RG504 in Figure 7.2.

The exponential decay of the molecular weight showed a dependence on the starting molecular weight of the polymer. By day 42 the three polymers had degraded to approximately equivalent molecular weights. The bi-phasic molecular weight degradation, consisting of an initial fast rate of reduction k_1 over time t_1 followed by a slower rate of reduction k_2 of the molecular weight of the remaining polymer Mp_1 over time t_2, which occurred after a time *Tau,* was observed for the three different molecular weight polymers. The molecular weight profiles were fitted to equation 7.3 from which the degradation parameters could be evaluated. The degradation rate constants were evaluated for microspheres manufactured from the three polymers and are shown in Table 8.3.

Table 8.3 Parameters for the degradation of PLGA particles of different molecular weights at 37°C in phosphate buffer saline fitted to Equation 7.3 *(standard deviation represents error determined by the model)*

PLGA	Mp	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
RG504	42124 ±930	0.046 ±0.0011	0.014 ±0.0012	29.789 ±1.7491	10833	0.999	6.655
RG503	25206 ±446	0.028 ±0.0024	0.014 ±0.0020	25.350 ±2.1801	12342	0.991	4.231
RG502	12194 ±381	0.014 ±0.0012	0.013 ±0.0015	21.990 ±2.8520	8983	0.993	4.513

The rate chain cleavage for the polymers was found to be dependent on the polymer molecular weight. The rate constant k_1 calculated for the first phase of degradation was found to increase with increasing molecular weight of the polymer. The degradation rate k_2 calculated for the second phase was less dependent on the initial microsphere polymer molecular weight.

The time *Tau* where the transition between the first and second phase of the degradation profile occurs was also related to the molecular weight of the polymer. The lower molecular weight of the polymer the faster the phase transition occurred. There was no observable relationship between the initial polymer molecular weight and the critical molecular weight Mp_l where mass loss occurs. In these studies it was observed that the higher the molecular weight of the starting polymer the larger the rate of change of polymer molecular weight (Figure 8.2).

In a study by Pistner *et al.* (1993) it was shown that the molecular weight degradation of three different poly l-lactides with time revealed that the higher the molecular weight of the starting polymer the larger the rate of change of polymer molecular weight. Studies by Fukuzaki *et al.* (1991) and O'Hagan *et al.* (1994) reported a similar result for PLGA polymers. It is thought that random chain cleavage of higher molecular weight chains produces a larger drop in high molecular weight chains by the production of low molecular weight chains. However for a lower molecular weight polymer the scission of low molecular weight chains into smaller molecular weight chains did not result in such a dramatic fall in molecular weight.

8.3.2 The influence of polymer molecular weight on the polydispersity of PLGA as a function of incubation time at 37°C and pH 7.4.

GPC chromatograms over the study period demonstrated broad unimodal molecular weight distributions. It was observed that the lower molecular weight polymers were associated with a higher polydispersity than the higher molecular weight polymers (Table 8.2). The relative change in polydispersity over time was studied for the three PLGA polymers of different molecular weights. The evolution of polydispersity for the three different polymer molecular weights as a function of incubation time was examined (Figure 8.3).

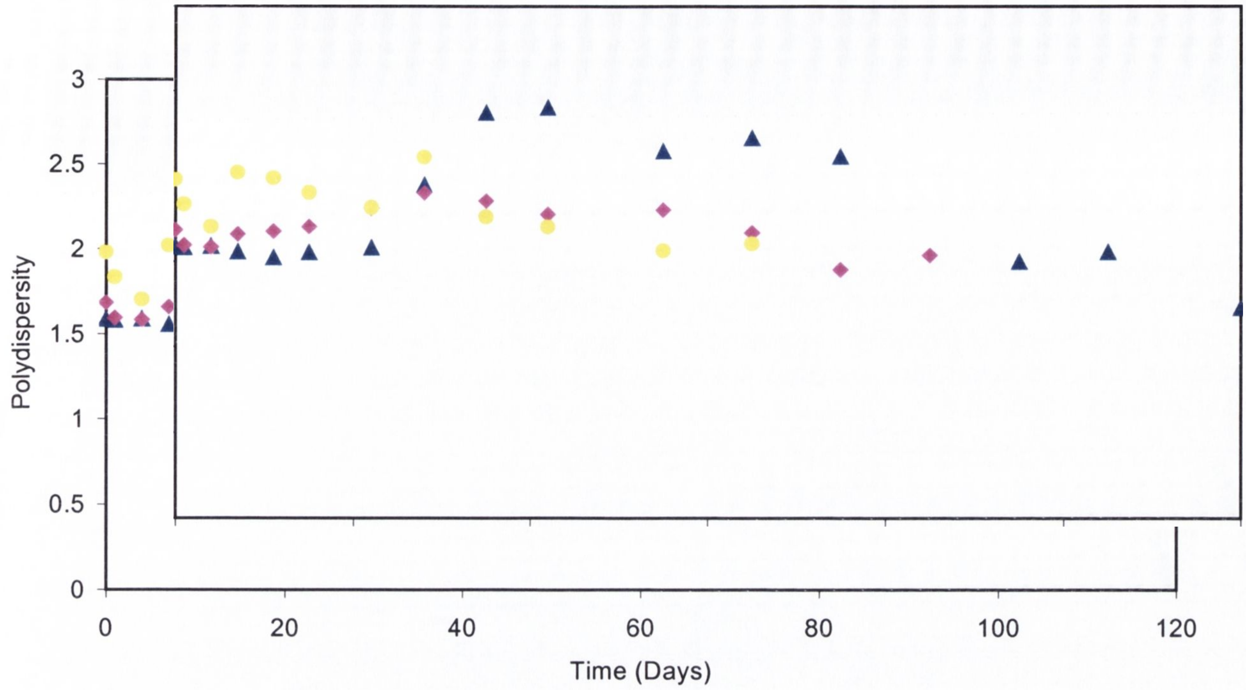


Figure 8.3 Evolution of the molecular weight distribution for the degradation of PLGA of different starting molecular weights, for ▲ RG504, ◆ RG503 and ● RG502 particles *(data represent the mean of two determinations).*

Figure 8.3 shows that for PLGA (RG502) the polydispersity starts high relative to the other two polymers and remains constant up to day 28 after which a slow decrease is observed. For PLGA (RG503) and PLGA (RG504) the polydispersity value is lower during the initial stages of the degradation profile and then begins to increase. The increase in polydispersity appeared to occur at an earlier stage for the lower molecular weight polymers PLGA (RG503). It was observed that increased polydispersity was associated with the time period before *Tau* and the onset of mass loss in the three polymer systems. In the lower molecular weight polymers, the magnitude of change in the polydispersity is not as great as that observed for the RG504. This is attributed to a greater build-up of degradation products within the RG504 microspheres, due to the faster rate of polymer molecular weight degradation.

8.3.3 Polymer Mass Loss from PLGA polymer of different polymer molecular weights as a function of incubation time at 37°C and pH 7.4.

The rate of polymer erosion from the microspheres was also shown to be dependent on the molecular weight of the polymer. Mass loss from microspheres of different polymer molecular weight fitted to the Prout-Tompkins equation (Equation 3.23) is shown in Figure 8.4.

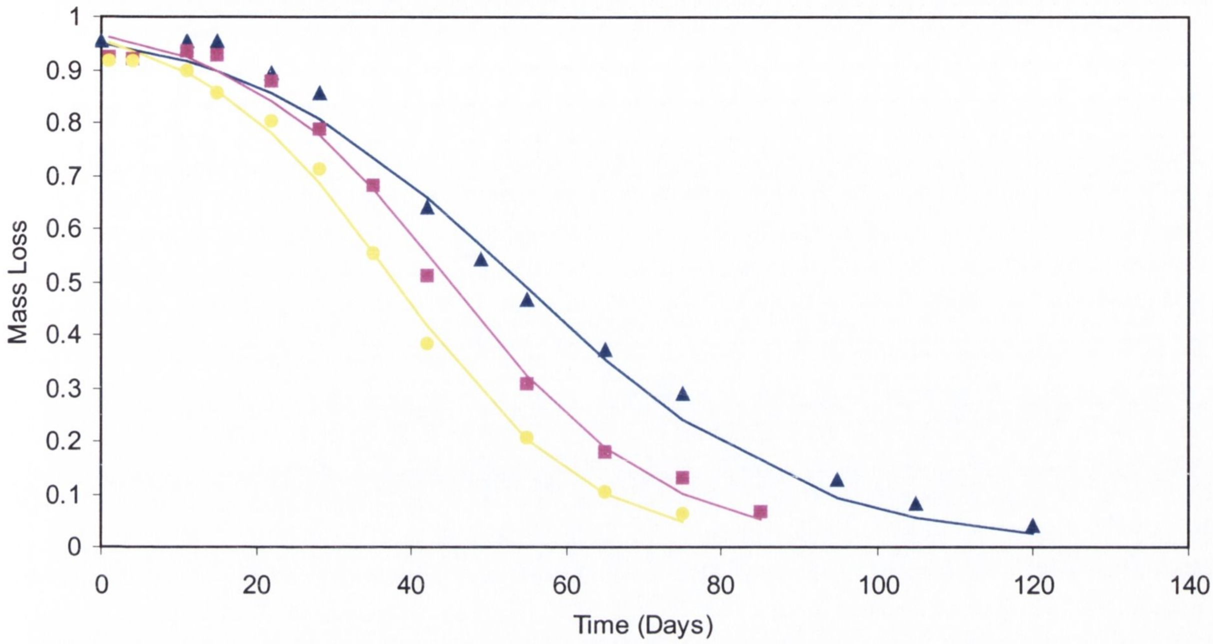


Figure 8.4 Mass loss profiles for the degradation of PLGA microspheres of different polymer molecular weight, for ▲ RG504, ◆ RG503 and ● RG502 particles *(data represent the mean of two determinations).* Data points fitted to Equation 3.23.

The parameters k and $Tmax$ determined for each polymer system using Equation 3.23 are given in Table 8.4. The rate of erosion increased with a corresponding decrease in molecular weight, $Tmax$ decreased with decrease in polymer molecular weight (Table 8.4) The relative rates of mass loss compared to that of the molecular weight decrease are consistent with a bulk hydrolysis and erosion mechanism rather than a surface controlled mechanism.

Table 8.4 Parameters evaluated for polymer erosion of PLGA microspheres of different molecular weights fitted to Equation 3.23 *(standard deviation represents error determined by the model).*

PLGA Polymer	Molecular weight (Mp)	k (day^{-1})	*Tmax* (days)	CD	MSC
RG504	42124	0.054	54.150	0.995	3.926
	±930	±0.0038	±1.4377		
RG503	25206	0.073	45.037	0.992	4.516
	±446	±0.0039	±0.8780		
RG502	12194	0.080	37.830	0.996	5.270
	±381	±0.0031	±0.5360		

8.3.4 SEM study of the effect of polymer molecular weight on the degrading microsphere morphology as a function of incubation time at 37°C and pH 7.4.

Scanning electron microscopy was used to determine morphological changes of the microspheres induced with incubation compared to the morphology of the microspheres before incubation (Figure 8.1). Samples were recovered after incubation in visking bags in phosphate buffer saline (PBS) at 37°C at different time points and are shown in Figure 8.5. The evolution of the matrix morphology with in vitro degradation, followed by SEM, showed a dependence on the molecular weight of the polymer. PLGA RG504 at day 11 initially retained their original morphology, PLGA RG503 microspheres showed fusion of the microspheres and for PLGA RG502 the sample was highly fused together with only some evidence of the initial microsphere structure (Figure 8.5a). At day 15 (Figure 8.5b) microspheres manufactured from the three polymer molecular weights showed the formation of pores in the matrix. For the later time points of day 22 and 28 (Figure 8.5c and d), the polymer samples showed gross deformation of the matrix and the formation of large pores. This was more pronounced in the microspheres formed from the lower molecular weight polymers.

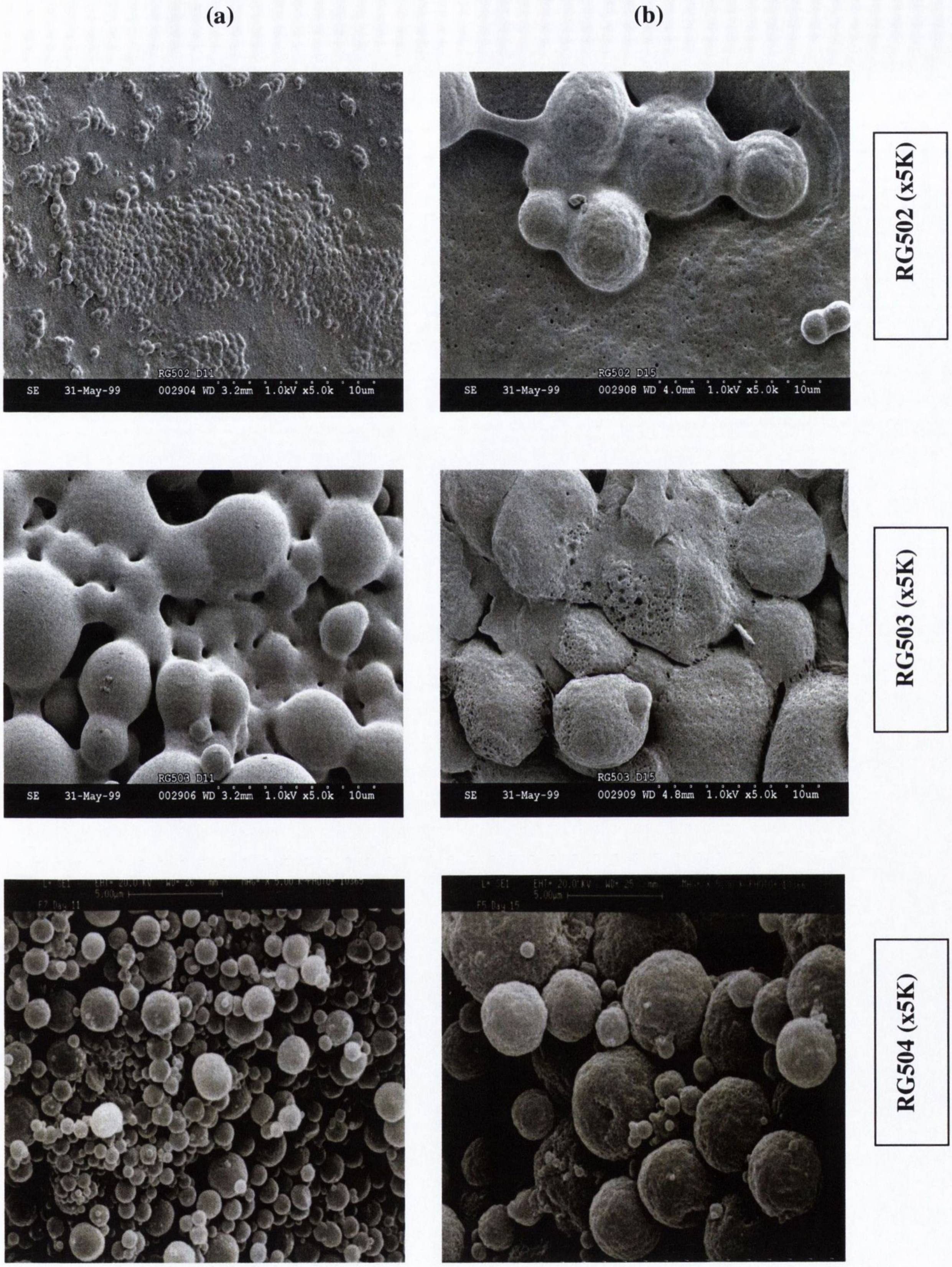


Figure 8.5 Electron micrographs of PLGA microspheres of different molecular weights after (a) 11 days and (b) 15 days incubation in PBS pH 7.4 at 37°C.

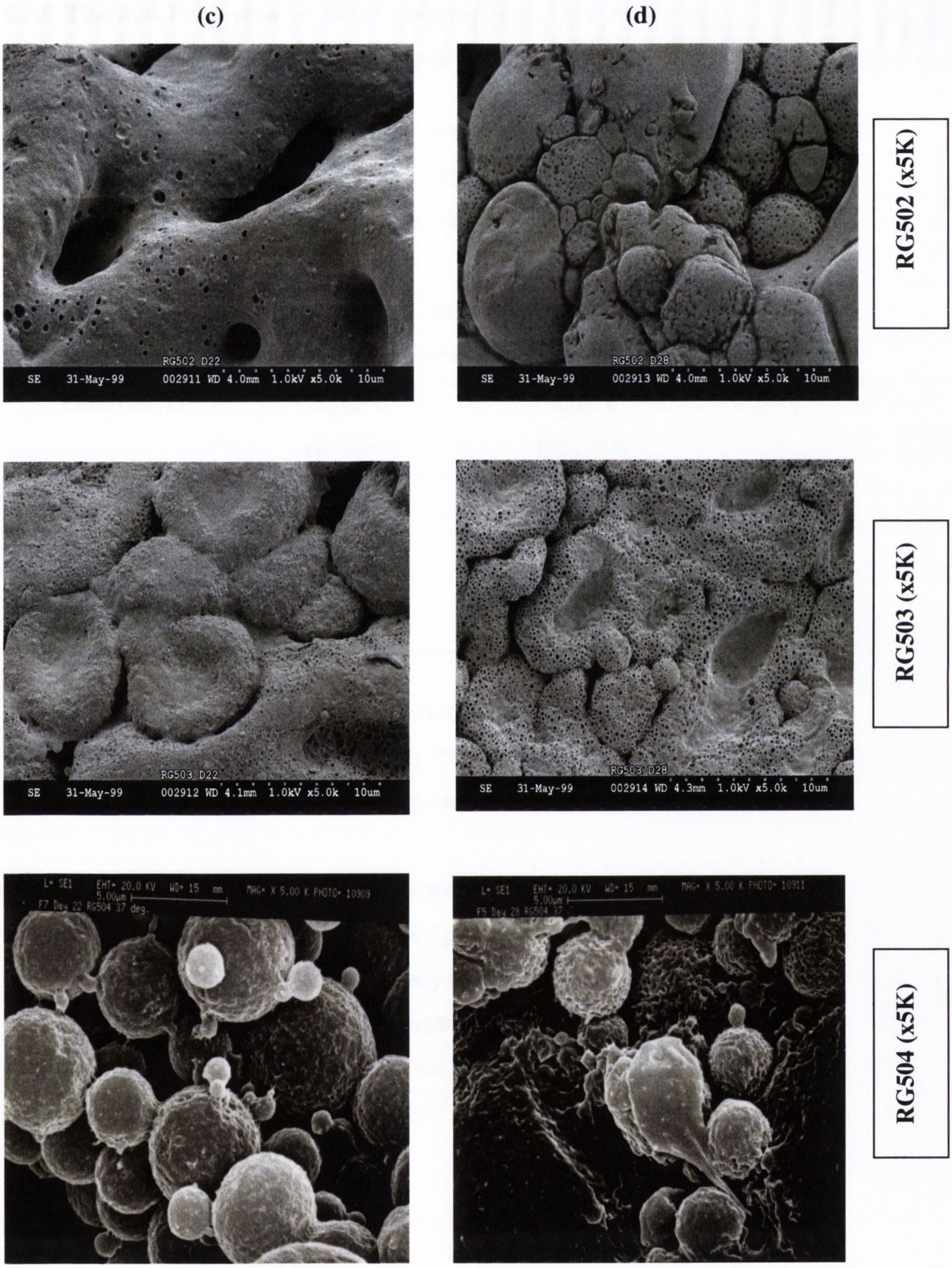


Figure 8.5 Electron micrographs of PLGA microspheres of different molecular weights after (c) 22 days and (d) 28 days incubation in PBS pH 7.4 at 37°C.

8.3.5 Thermal Properties of PLGA ~~microspheres~~ particles with different polymer molecular weights as a function of incubation time at 37°C and pH 7.4.

Changes in thermal properties were monitored for the three polymers over the degradation period. The change in $T_{g(p)}$ for polymer samples recovered after incubation in visking bags in PBS at 37°C and dried is shown in Table 8.5.

Table 8.5 Change in polymer $T_{g(p)}$ °C with incubation time for PLGA microspheres *(data points represent the mean of two determinations).*

Time (days)	$T_{g(p)}$ °C (RG504)	$T_{g(p)}$ °C (RG503)	$T_{g(p)}$ °C (RG502)
0	52.1	47.1	42.5
15	46.4	46.3	42.3
22	43.8	45.2	40.9
28	43.6	43.7	37.2

The decrease in $T_{g(p)}$ corresponded to the lowering of the molecular weight as observed in the molecular weight profile (Figure 8.2). The change in $T_{g(p)}$ of the polymer increased as the molecular weight of the polymer increased.

DSC thermograms of the three molecular weight polymers (RG504, RG503 and RG502) at day 15 show that the three molecular weight polymers have a $T_{g(p)}$ that is above 37°C. Therefore the three polymers are in a glassy state at this time point (Figure 8. 6). Change in $T_{g(p)}$ over 15 days was greater for the higher molecular weight polymers. The change in average $T_{g(p)}$ is 5.7°C, 0.8°C and 0.2°C for the RG504, RG503 and RG502 microspheres respectively.

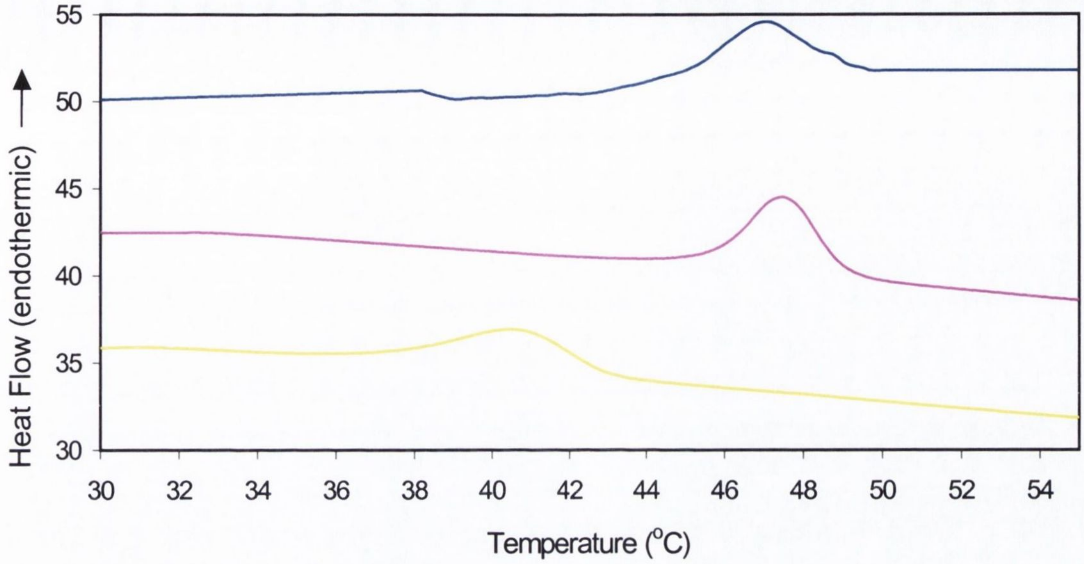


Figure 8.6 DSC thermograms of PLGA microspheres after incubation for 15 days in PBS pH 7.4 at 37°C, for ___ RG504 ___ RG503 and ___ RG502 particles.

At day 22, the thermogram for PLGA RG502 showed that some polymer chains have a $T_{g(p)}$ that occurs below 37°C (Figure 8.7). This time point corresponds with the onset of mass loss and the change from the first phase of the degradation profile to the second phase of the degradation profile. The $T_{g(p)}$ peaks for the two other polymers were still above 37°C.

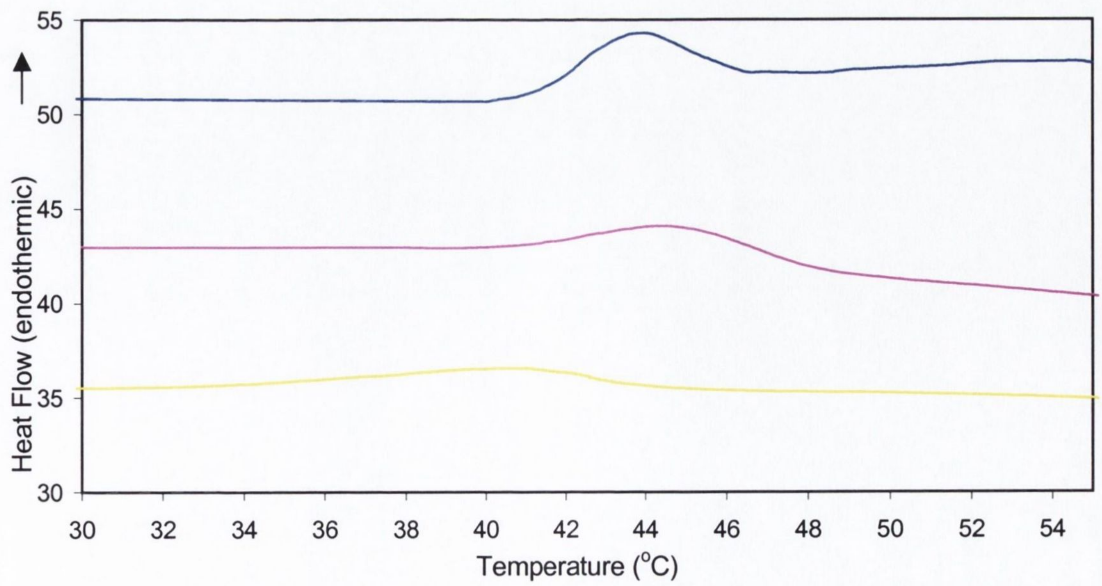


Figure 8.7 DSC thermograms of PLGA microspheres after incubation for 22 days in PBS pH 7.4 at 37°C, for ___ RG504 ___ RG503 and ___ RG502 particles.

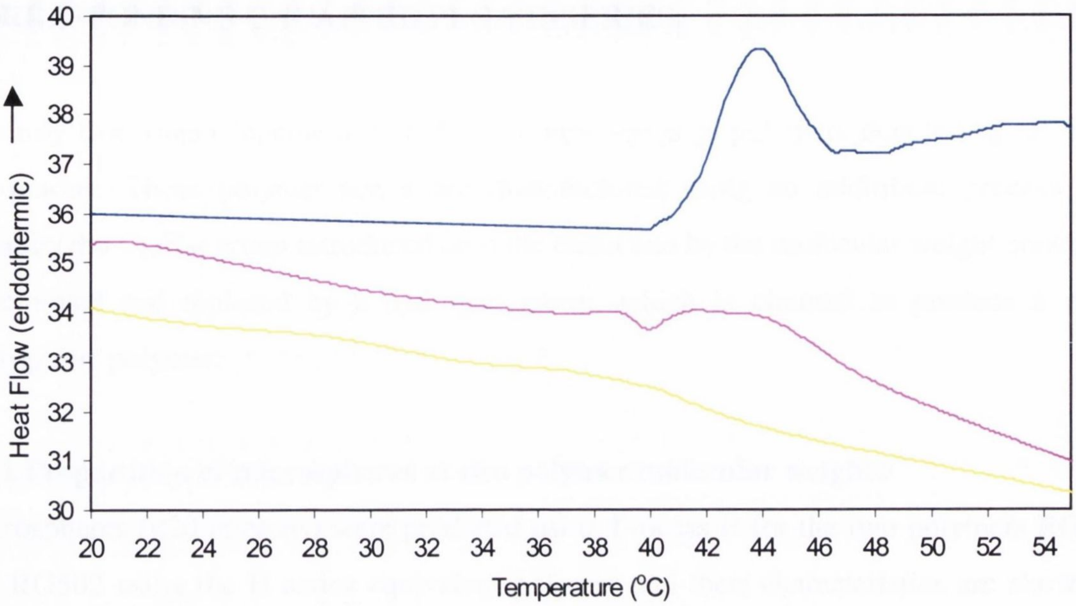


Figure 8.8 DSC thermograms of PLGA microspheres after incubation for 28 days in PBS pH 7.4 at 37°C, for ___ RG504 ___ RG503 and ___ RG502 particles.

After 28 days the RG503 polymer also exhibits polymer chains with a $T_{g(p)}$ below 37°C which also occurs after the onset of erosion for this polymer. In the two lower molecular weight polymers, thermal analysis of the degraded microspheres exhibited bimodal transition peaks, both of which occurred at too low a temperature to be assigned to melting transitions. This suggests the presence of two different degrading polymer domains within the microspheres consistent with the heterogeneous mechanism exhibited by these polymers (Figure 8.8). A DSC study by Park (1995b) on degrading PLGA microspheres of different copolymer ratios also demonstrated the evolution of double glass transition temperatures and the evolution of crystalline melting peaks due to preferential degradation of glycolide linkages.

For all three polymers the time point where the glass transition temperature was below 37°C and the polymer was in the rubbery state correlated to the time point where mass loss proceeded for all three polymers.

8.4 COMPARISON OF THE DEGRADATION PROFILE OF PLGA WITH THE CORRESPONDING UNCAPPED PLGA H-SERIES.

Recently Boehringer Ingelheim introduced a new series of polymers denoted by 'H' onto the market. These polymer series are manufactured using an additional process step whereby the $C_{12}H_{25}$ group introduced onto the chain end by the molecular weight controller is removed and replaced by a hydrogen group, which is claimed to produce a more hydrophilic polymer.

8.4.1 Preparation of microspheres at two polymer molecular weights

Microspheres (<20 microns) were produced using Process B for the two polymers RG504 and RG502 using the H series equivalent polymer and their characteristics are shown in Table 8.6 and Table 8.7 (%) differences from the starting polymer are indicated in parenthesis).

Table 8.6 Characteristics of PLGA-H microspheres (<20microns) of different molecular weight *(data represent mean of three determinations ± standard deviation).*

Polymer	D10% (μm)	D50% (μm)	D90% (μm)	$T_{g(p)}$ (°C)	Yield (%)
RG504H	0.5	3.8	15.8	52.4(-2.6%)	81.5
	±0.01	±0.18	±0.62	±0.53	±3.54
RG502H	1.3	5.4	15.3	38.7(-9.2%)	79.8
	±0.01	±0.27	±0.76	±0.54	±3.47

Table 8.7. GPC characteristics of PLGA-H microspheres (<20microns) of different molecular weight *(data represent mean of three determinations ± standard deviation).*

Polymer	Mn	Mp	Mw	Mz	PD
RG504H	26925	47025(-7.8%)	46125	70129	1.734
	±312	±1896	±1673	±1082	±0.0350
RG502H	5923	8474(-2.2%)	9254	13222	1.598
	±221.3	±411	±199	±103	±0.0510

For the more hydrophilic polymer the change in thermal and polymer molecular characteristics after processing compared to the supplied polymer was more marked than for the uncapped series shown previously in Table 8.1 and 8.2. The H-series of polymers being more hydrophilic are more susceptible to hydrolytic degradation during the manufacturing process compared to the corresponding capped polymer series

8.4.2 Influence of polymer end group on the degradation of PLGA microspheres as a function of incubation time at 37 °C and pH 7.4.

The polymer molecular weight profiles for the H series of polymers (RG504H and RG502H) and their equivalent end capped polymers (RG504 and RG502) is shown in Figure 8.9.

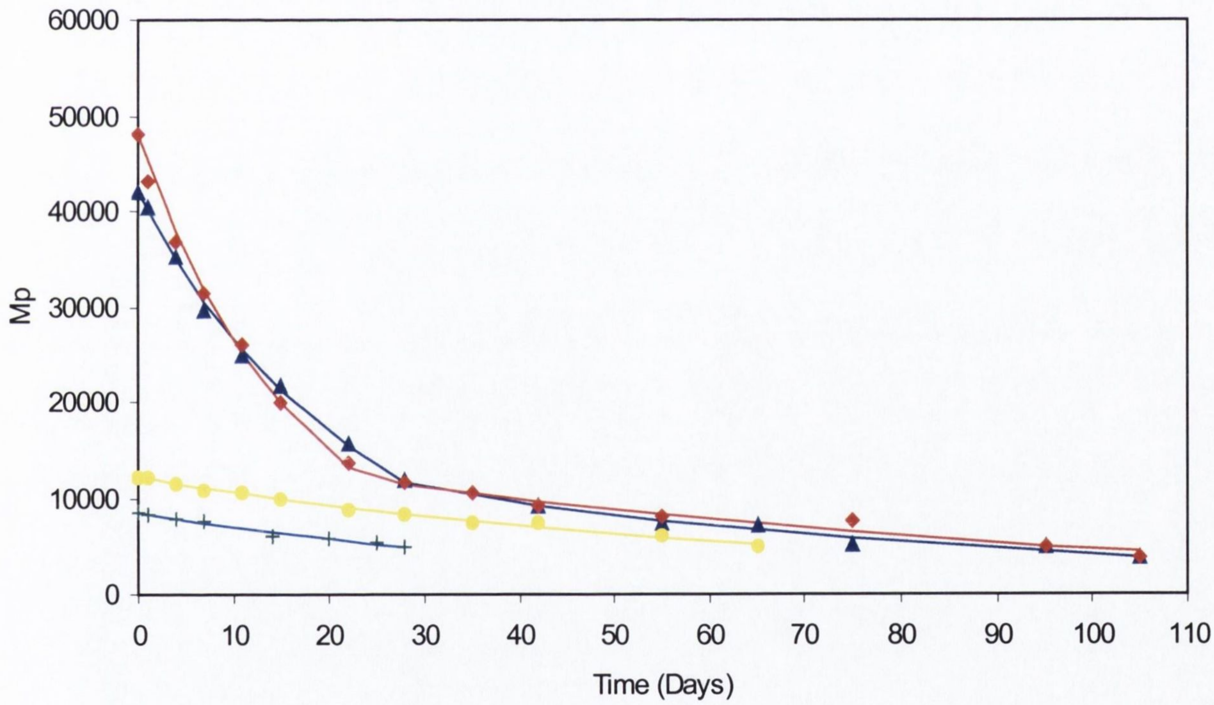


Figure 8.9 Influence of polymer end group on the degradation of PLGA microspheres incubated at 37°C and pH 7.4, for ▲ RG504, ◆ RG504H, ● RG502 and × RG502H particles *(data points represent the mean of two determinations)*. Data points fitted to Equation 7.3.

The initial decrease in molecular weight exhibited by the H-series polymers appears greater than that observed by the corresponding RG504 or RG502 (Figure 8.9). The molecular weight profiles for RG504, RG504H, RG502and RG502H were fitted to equation 7.3 from which the degradation parameters could be evaluated (Table 8.8).

Table 8.8 Parameters for the degradation of PLGA particles of different molecular weights *(standard deviation represents that determined by the model).*

Polymer	Mp	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
RG504	42124 ±930	0.046 ±0.0012	0.014 ±0.0014	29.789 ±1.7492	10833	0.999	6.655
RG504H	48025 ±1896	0.059 ±0.0021	0.012 ±0.0024	23.452 ±1.5233	12179	0.998	5.340
RG502	12194 ±381	0.014 ±0.0014	0.013 ±0.0014	21.990 ±1.5241	8983	0.998	4.517
RG502H	8474 ±411	-	0.019 ±0.0011	-	-	0.998	3.916

For the low molecular weight RG502H, the degradation profile was monophasic and therefore Equation 7.3 could not be used to describe the release kinetics. A single rate constant which was equivalent to k_2 was used to describe the degradation kinetics. This profile was fitted to the following equation:

$$Mp = Mp_0 \exp(-k_2 t_1) \qquad \text{Equation 8.1}$$

where Mp_0 is the molecular weight of the starting microspheres and *Mp* is the molecular weight of the degradation samples measured over time *t*, the parameter k_1 was determined using Scientist® software (Appendix IV).

Table 8.8 showed that the rate of degradation was considerably faster for the H-series of polymers compared to the corresponding uncapped polymer series. For the low molecular weight polymer (RG502H) the degradation profile did not display a biphasic profile but was described by a single molecular weight phase. When this molecular weight phase was fitted to equation 8.1, the rate constant value was approximately equivalent to the parameter k_2. The most important property differentiating the uncapped polymer is their carboxylic acid content. A study by Mehta *et al.* (1996) showed that the uncapped polymers showed the lowest A (acid number).

8.4.3 Influence of polymer end group on the Zeta Potential of PLGA microspheres

The increased rate of degradation was thought to be due to a more hydrophilic surface on the particle due to the presence of hydrogen groups that have orientated towards the surface of the microsphere during the manufacturing process, since water uptake into bulk microsphere would not have been sufficient to alter the rate at this stage of the degradation profile. Zeta potential measurements of the microparticles were carried out in two media. 10^{-3}M NaCl is a common dispersant recommended for zeta potential measurement and phosphate buffer was the dispersant used in this work for dissolution and degradation studies. The differences in surface charge between the two types of polymers was measured by determining the zeta potential difference of a particle suspension (Table 8.9).

Table 8.9 Zeta Potential measurements for microspheres dispersed in phosphate buffer pH 7.4 and 10^{-3} M NaCl pH 7.8 (*samples represent mean of three determinations*).

Polymer	Zeta Potential(mV) phosphate buffer pH 7.4	Zeta Potential(mV) 10^{-3}M NaCl pH 7.8
None	-6.2±2.91	-0.7±0.22
RG504	-3.5±1.10	0.2±0.63
RG504H	-4.5±2.22	1.8±1.80
RG502	-3.4±0.41	1.2±0.72
RG502H	-2.2±1.10	4.1±0.47

Note: the ± indicates the width of the peak not the statistical standard deviation.

The values shown in Table 8.9 for PLGA in PBS are comparable to those (ca. –1.3) determined by Conway and Alpar (1996). Surface groups OH^-, H^+ and COOH can ionise to give a surface charge. Zeta potential gives a measure of the sum of the charges measured in the dispersant media. Particles were dispersed in either 10^{-3}M NaCl or phosphate buffer and measured immediately. Particles in either media demonstrated an overall positive zeta potential whose magnitude depended on the polymer used and its molecular weight. The dispersant medium will affect the surface chemistry of the particles, thus the ionic environment and the pH can influence the zeta potential measured. Chain end-groups in PLA and PLGA at the particle surface are ionized at the pH of the solutions in which they were measured. The difference in values can therefore be attributed to differences in surface groups, which for lower molecular weight polymers are present in greater concentration due to less steric resistance to the alignment of polar end groups in the polar aqueous phase during emulsion formation.

8.4.4 Influence of polymer end group on the erosion of PLGA microspheres as a function of incubation time at 37 °C and pH7.4.

The effect of PLGA endgroup on the erosion of the polymer was also compared for RG504, RG504H, RG502 and RG502H polymers (Figure 8.10). The loss in mass for the polymers shown in Figure 8.10 was also fitted to the Prout-Tompkins equation (Equation 3.23) and the parameters evaluated are shown in Table 8.10

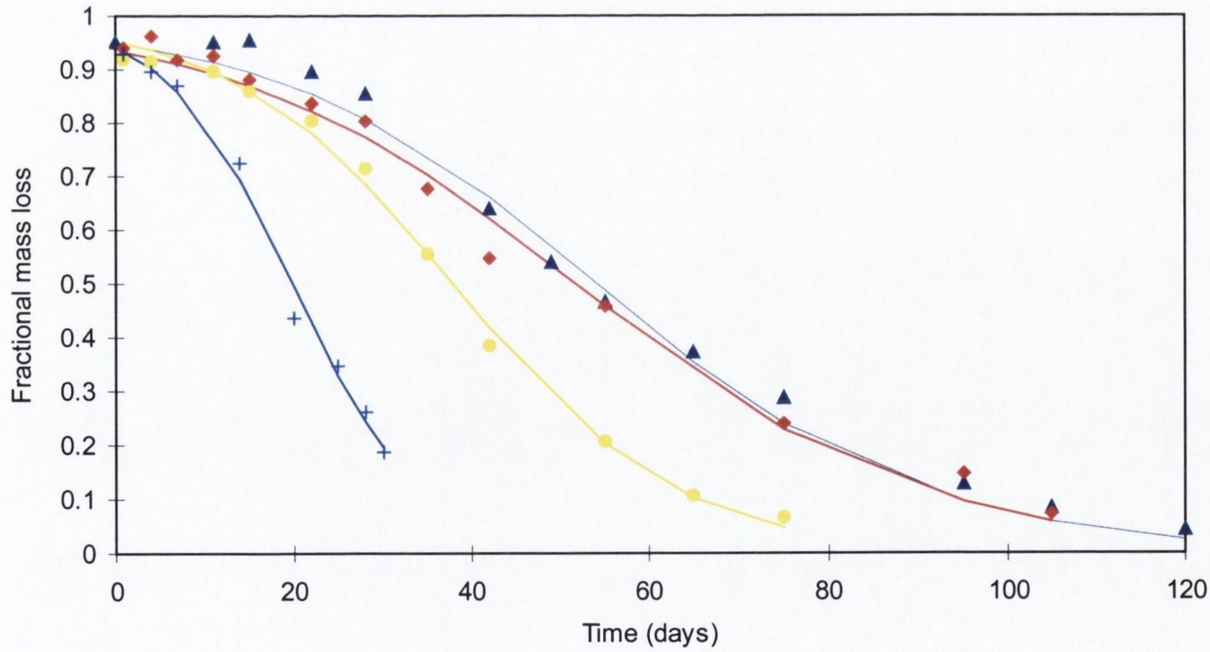


Figure 8.10 Effect of polymer end group on the mass loss from PLGA microspheres at 37°C and pH 7.4, for ▲ RG504, ◆ RG504H, ● RG502, and × RG502H *(data represent the mean of two determinations)*. Data points fitted to Equation 3.23.

Table 8.10 Effect of end group on the parameters for the erosion of PLGA microspheres fitted to Equation 3.23 *(standard deviation represents error determined by the model).*

Polymer	k (day^{-1})	*Tmax* (days)	CD	MSC
RG504	0.054 ±0.0038	54.150 ±1.4377	0.995	3.926
RG504H	0.052 ±0.0002	51.725 ±1.2587	0.998	4.012
RG502	0.080 ±0.0031	39.36 ±0.5360	0.996	5.270
RG502H	0.140 ±0.0083	19.907 ±0.4411	0.998	4.779

The loss in mass from the RG504H microspheres was only marginally faster than from the corresponding RG504 microspheres, however the erosion rate was considerably faster for RG502H microspheres compared to the RG502 microspheres. Based on this observation it was demonstrated that low molecular weight polymers tend to be more hydrophilic and degrade faster than high molecular weight polymers, especially when the end groups are free acid rather than end capped with other groups.

8.5 CHARACTERISATION OF PLGA RG504H PARTICLES OF DIFFERENT SIZE DISTRIBUTIONS

The influence of particle size on the degradation rate of PLGA was also investigated for the H series of polymers using RG504H. Microspheres produced using PLGA RG504H were manufactured and their thermal and molecular weight characteristics are shown in Tables 8.11 and 8.12 and compared to that of the starting polymer (% differences are indicated in parenthesis).

Table 8.11 Characteristics of PLGA-H microspheres <20μm *(samples represent mean of three determinations ± standard deviation).*

Processing conditions	D10% (μm)	D50% (μm)	D90% (μm)	Tg (°C)	Yield (%)
Process A	0.3	0.6	1.2	51.9(3.5%)	93.5
	±0.01	±0.01	±0.02	±0.39	±1.63
Process B	0.5	3.8	15.8	52.4(2.6%)	81.5
	±0.01	±0.18	±0.62	±0.53	±3.54
Process C	3.3	23.1	48.8	53.1(1.1%)	92.1
	±0.17	±1.12	±0.79	±1.05	±2.63

Table 8.12 GPC characteristics of PLGA-H microspheres <20μm *(samples represent mean of three determinations ± standard deviation).*

Processing conditions	Mn	Mp	Mw	Mz	P
Process A	24889	47940(6.0%)	42926	65169	1.725
	±658	±952	±581	±3301	±0.0689
Process B	26925	47025(7.8%)	46125	70129	1.734
	±312	±1896	±1673	±1082	±0.035
Process C	26995	47366(7.2%)	46393	72118	1.719
	±858	±894	±856	±2293	±0.0259

Size fractions for these microspheres are comparable to that obtained for PLGA RG504. The thermal characteristics were dependent on the process used to manufacture the particles with higher shear rates producing a larger drop in the $T_{g(p)}$ of the polymer. The polymer molecular weight characteristics were shown to be independent of the shear rate used.

8.6 STUDY OF THE INFLUENCE OF MICROSPHERE PARTICLE SIZE ON THE DEGRADATION PROFILE FOR PLGA (RG504H) AT pH 7.4 AND 37°C.

8.6.1 Polymer molecular weight profile

The molecular weight profiles of PLGA (RG504H) particles produced in the three size ranges is shown in Figure 8.11. The mechanism of degradation of PLGA RG504H was equivalent to that observed for PLGA RG504. PLGA-H slowed the same dependance between particle size and molecular weight as that observed for PLGA RG504.

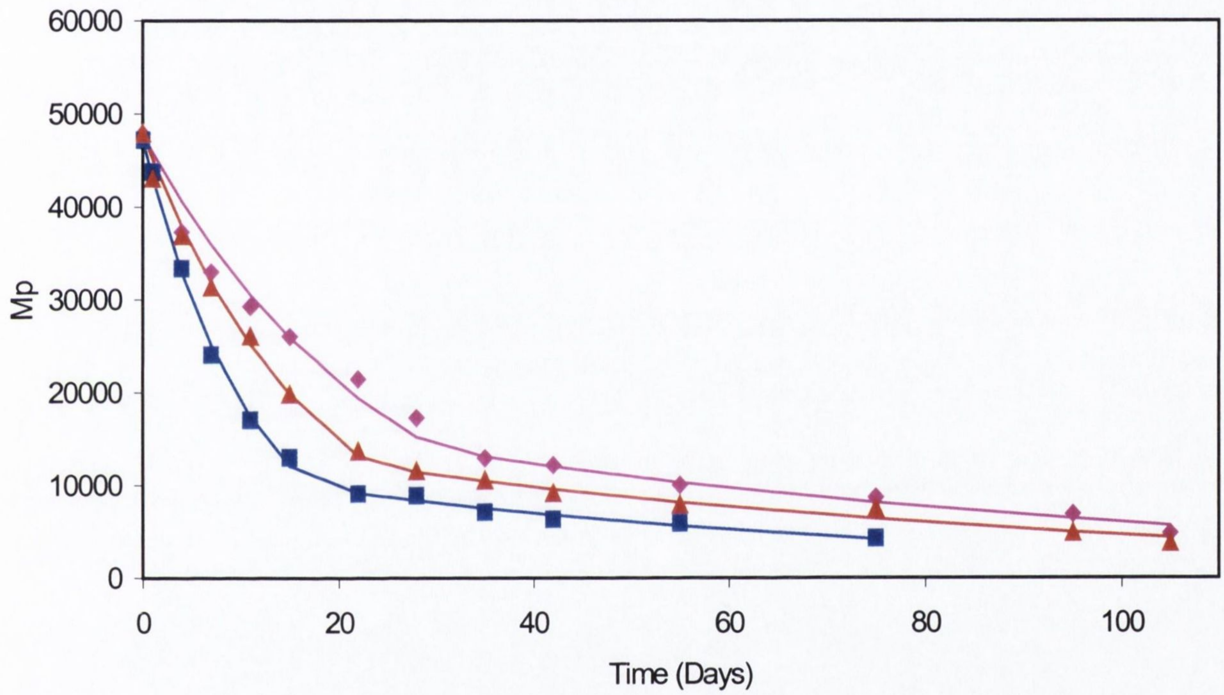


Figure 8.11 Plots of polymer Mp with incubation time for different particle size RG504H microspheres, for ◆ <1 micron ▲ <20micron ■ <50micron particles *(data points represent mean of two determinations).* Data points fitted to Equation 7.3.

The parameters evaluated for the degradation of RG504H particles of different particle sizes are shown in Table 8.13.

Table 8.13 Parameters for the degradation of PLGA-H particles at 37°C in phosphate buffer saline fitted to Equation 7.3 *(standard deviation represents that determined by the model).*

Particle Size (μm)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1 Calculated	CD	MSC
<1	0.041 ±0.0021	0.012 ±0.0035	30.260 ±4.4281	13990	0.996	3.868
<20	0.059 ±0.0016	0.012 ±0.0019	23.452 ±1.5233	11926	0.998	5.340
<50	0.090 ±0.0018	0.015 ±0.0024	17.308 ±0.8411	9959	0.997	6.130

The degradation kinetics which were found to be dependent on the particle size using RG504 were also evident for the corresponding RG504H and confirmed the influence of particle size on the degradation rate. The rate constant k_1 calculated for the first phase of degradation was found to increase with increasing particle size while degradation rate k_2 calculated for the second phase, was less dependent on the particle size. The time *Tau* at which the second phase begins was also related to the particle size, with the phase change occurring at shorter times and lower molecular weights Mp_1 as the particle size increased. At all particle sizes, the degradation rate k_1 was considerably faster for the RG504H particles than for the corresponding RG504 particles.

8.6.2 Polydispersity of PLGA (RG504H) particles of different particle sizes as a function of incubation time at 37°C and pH 7.4

Studies of the polydispersity of the samples taken over the degradation time of the polymer revealed the same trend towards increased polydispersity during the second phase of the degradation profile (Figure 8.12).

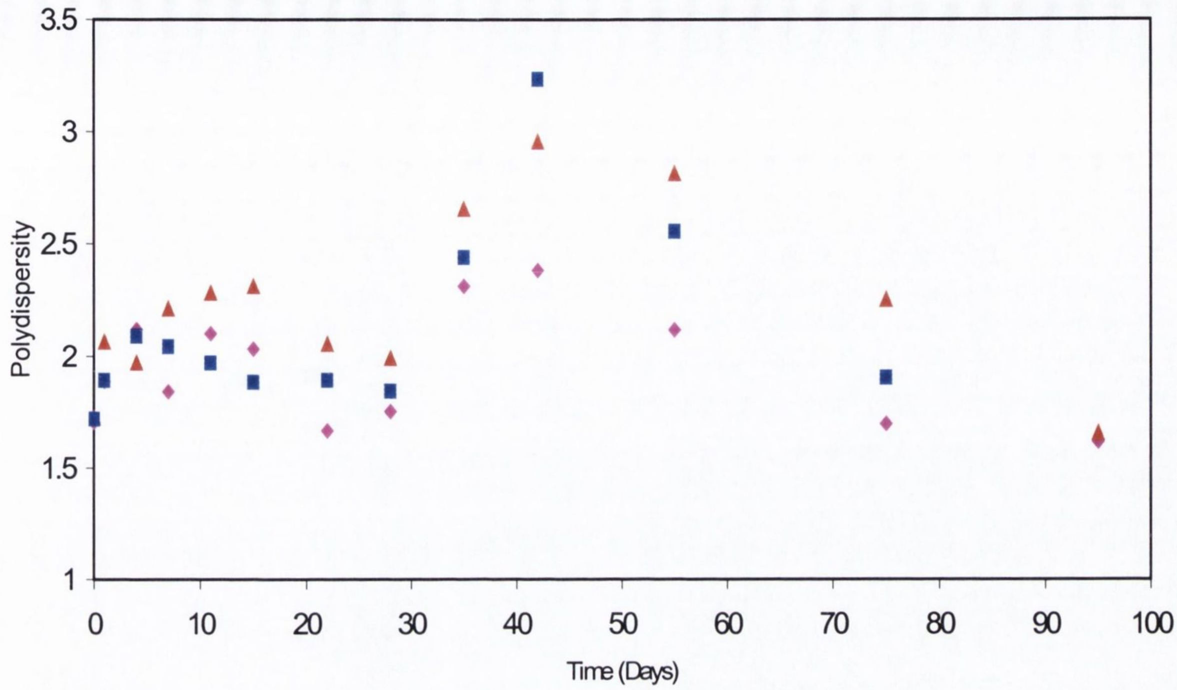


Figure 8.12 Evolution of the polydispersity with degradation time for PLGA RG504H particles incubated at 37°C in Phosphate buffer saline, for ◆ <1 micron, ▲ <20micron and ■ <50micron particles *(data points represent mean of two determinations).*

The increase in polydispersity was shown to be greater for the larger particles than for the corresponding nanoparticles. The increase in polydispersity was shown to occur during the erosion phase when low molecular weight polymer chains capable of dispersing from the matrix are produced.

8.6.3 Polymer Mass Loss of PLGA (RG504H*)* particles of different particle sizes as a function of incubation time at 37°C and pH 7.4

The mass loss from particles of different particle size distributions as a function of incubation time was determined and is shown in Figure 8.13. The mass loss profile was fitted to the Prout-Tompkins equation (Equation 3.23) and the parameters *k* and *Tmax* were evaluated for the polymer erosion (Table 8.14).

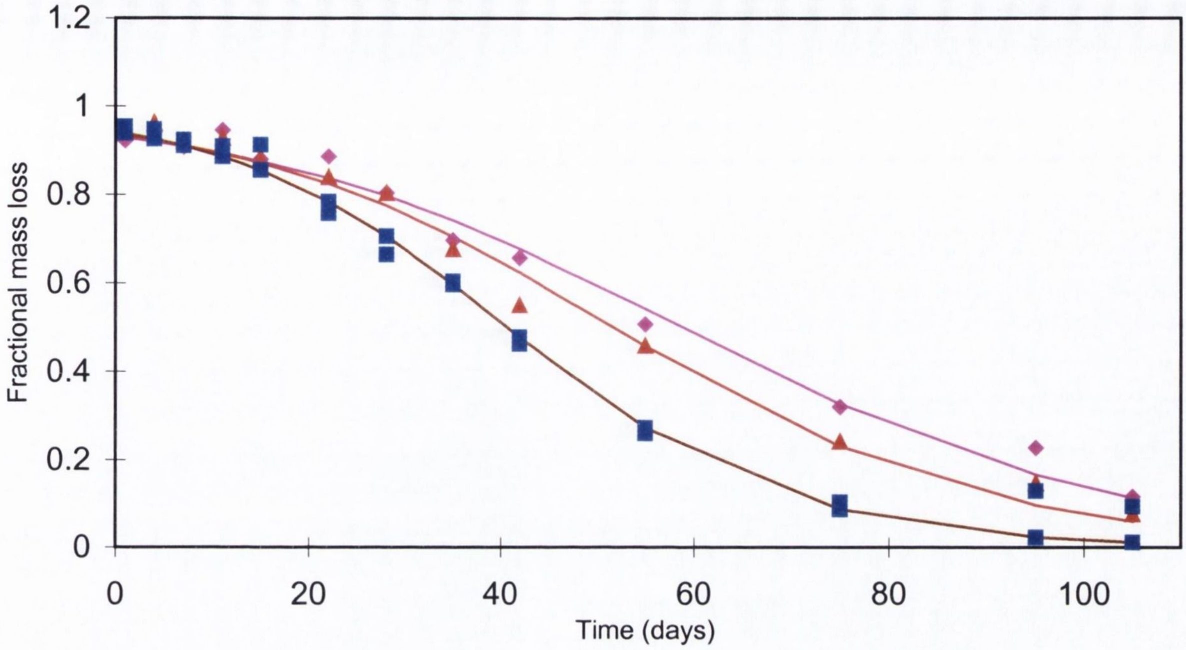


Figure 8.13 Weight loss of PLGA (RG504H) particles at 37°C and pH 7.4 , for ◆ <1 micron, ▲ <20micron and ■ <50micron particles *(data points represent mean of two determinations).* Data points fitted to Equation 3.23.

Table 8.14 Parameters for polymer erosion of PLGA (RG504H) particles determined using Equation 3.23 *(standard deviation represents that determined by the model).*

Particle Size	*k* (day^{-1})	*Tmax* (days)	CD	MSC
(<1 micron)	0.044 ±0.0006	58.752 ±1.3254	0.998	3.857
(<20 microns)	0.052 ±0.0002	51.725 ±1.2587	0.998	4.016
(<50 microns)	0.069 ±0.0006	40.690 ±2.6942	0.995	4.360

Table 8.14 showed values that were comparable to that of the capped polymer. For a polymer that has as high a molecular weight as RG504, the concentration of ester groups is relatively low compared to a corresponding low molecular weight polymer, therefore the increased concentration of COOH groups does not have a significant effect on the polymer erosion rate.

8.7 INFLUENCE OF THE INCUBATION MEDIUM pH ON THE DEGRADATION RATE OF PLGA MICROSPHERES (<20 MICRONS) AT 37°C.

The degradation of PLGA (RG504) in extreme acidic and extreme basic conditions was examined by dispersing microspheres (100mg) in 100 ml 0.1M HCl (pH 1.0) or 100 ml Carbonate buffer (pH 10.0). HCl (pH 1.0) was investigated because it represents the main component of simulated gastric fluid while carbonate buffer was used as it is utilised in the manufacture of Fluphenazine HCl loaded microspheres in Chapter 10. The visking bag experiment was not suitable for use in this experiment because the extreme pH had a detrimental effect on the bag and could not be recovered properly from the incubation medium.

8.7.1 Effect of pH on the polymer molecular weight profile of PLGA (RG504) microspheres

The effect of incubation medium on the degradation was studied by comparing PLGA RG504 microsphere degradation at pH 1.0, 7.4 and 10.0 (Figure 8.14).

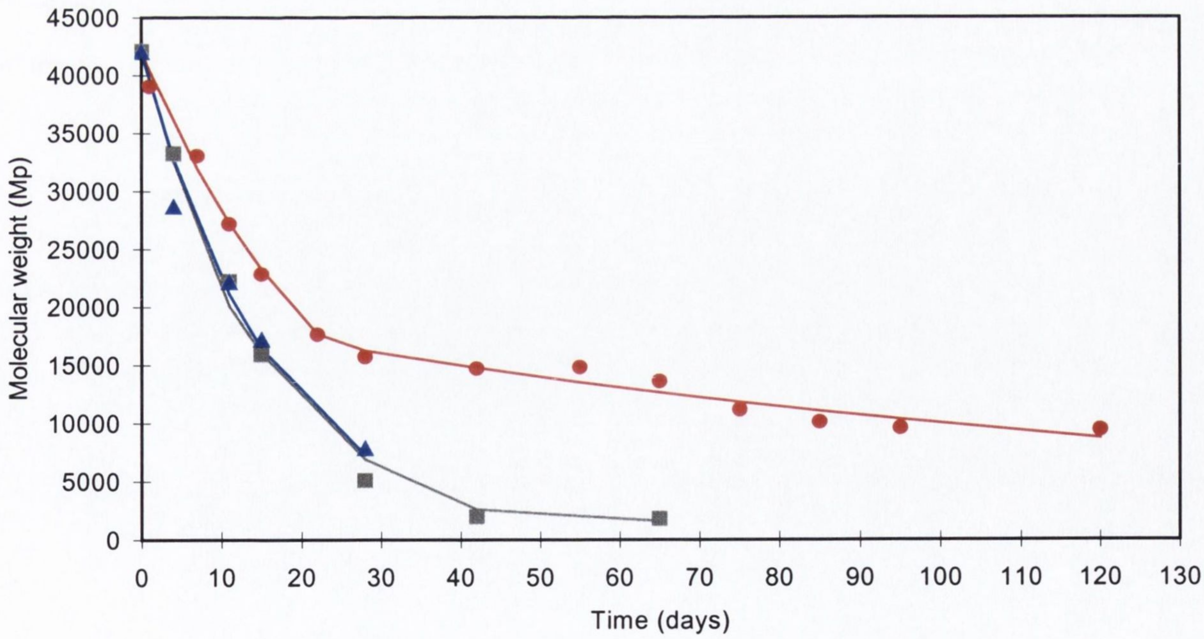


Figure 8.14 Influence of incubation medium pH on the degradation rate of PLGA microspheres (<20 microns) at 37°C, for ● pH 7.4, ▲ pH 10.0 and ■ pH 1.0 media *(data points represent mean of two samples).*

The degradation profiles of the microspheres show that at the initial time points (less than day 15) there is no substantial acceleration of the degradation profile considering the extent of the pH difference (Figure 8.14). It is thought that the extreme pH does not affect the polymer chains within the microsphere because of the impermability of the matrix during this stage of the degradation. Pitt *et al.* (1992) also stated that they thought it was improbable that inorganic ions could affect the rate of biodegradation since it was unlikely that ionic salts would be able to diffuse into the matrix. The molecular weight of the polymer (Mp) decreases rapidly after this and by day 42 there was not enough sample remaining for GPC analysis at pH 10.0 (Figure 8.14). Belbella *et al.* (1996) also demonstrated that the effect of pH (2-10) was more pronounced for PLA after 100 days incubation compared to that observed at 24 days. The degradation parameters for the decrease in polymer molecular weight are shown in Table 8.15.

Table 8.15 Parameters for the degradation of PLGA particles at 37°C and at different pHs *(standard deviation represents error determined by the model).*

Incubation Conditions	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_I	CD	MSC
pH 1.0	0.064 ±0.0031	-	-	-	0.994	4.946
pH 7.4	0.0393 ±0.0012	0.00673 ±0.0014	23.13 ±1.6585	16873	0.995	4.780
pH 10.0	0.062 ±0.0054	-	-	-	0.971	3.165

The degradation of PLGA (RG504) microspheres at pH 1.0 and 10.0 could not be fitted to the bi-exponential equation (Equation 7.3) used to describe the degradation of PLGA microspheres at pH 7.4. Therefore a rate constant was derived using the single exponential equation (Equation 8.1). Table 8.15 shows that the rate constants derived for the polymer degradation at pH 1.0 and 10.0 were comparable. The rate constant k_1 for the degradation of PLGA at pH 1.0 and 10.0 was greater than the rate constants k_1 and k_2 derived for the degradation of PLGA at pH 7.4.

8.7.2 Effect of pH on the polymer mass loss of PLGA (RG504) microspheres

Mass loss from the microspheres at different incubation pH was also examined. The loss in mass showed a different profile in extreme pH values compared to that at pH 7.4 in phosphate buffer (Figure 8.15).

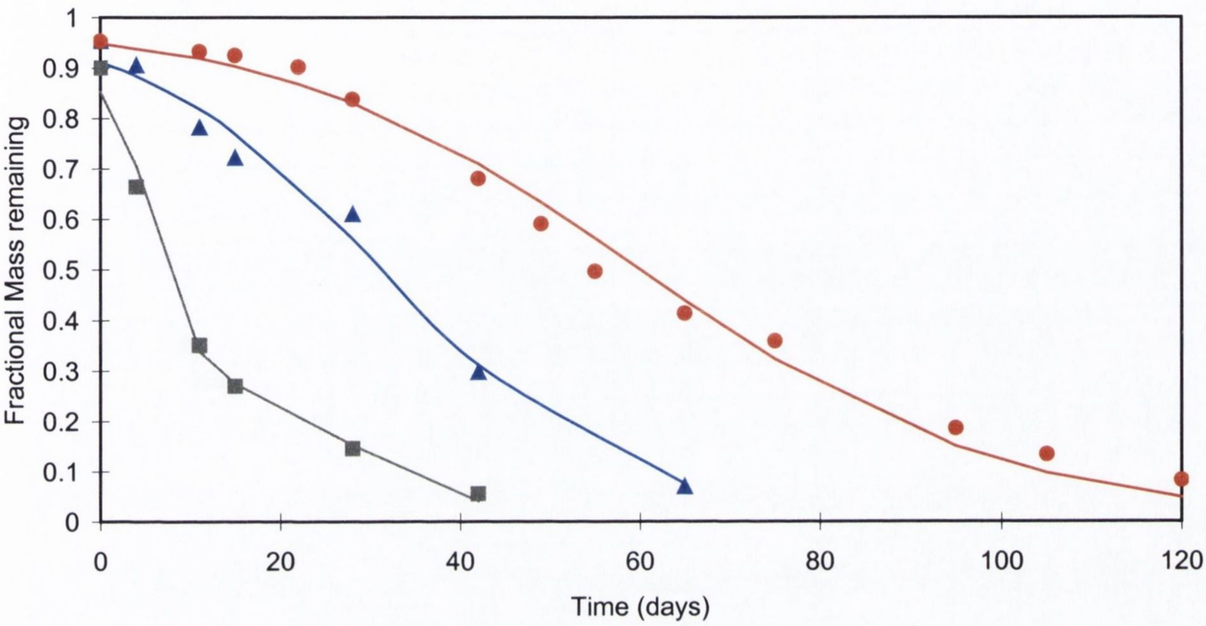


Figure 8.15 Influence of incubation medium pH on the mass loss from PLGA(RG504) microspheres (<20 microns) at 37°C, for ● pH 7.4, ▲ pH 10.0, and ■ pH 1.0 media *(data points represent mean of two samples).* Data points are fitted to Equation 3.23.

The loss in mass from the microspheres was immediate when the microspheres were incubated in extreme pHs compared to the lag observed at pH 7.4. The mass loss from the microspheres was faster in the basic media compared to the acidic media which was faster that that observed in phosphate buffered saline pH 7.4. The parameters for the erosion of PLGA (RG504) microspheres degraded in different incubation media were determined using the Prout-Tompkins equation (Equation 3.25) and are shown in Table 8.16.

Table 8.16 Parameters evaluated for the erosion of PLGA microspheres at different incubation pH fitted to Equation 3.23 *(standard deviation represents error determined by the model).*

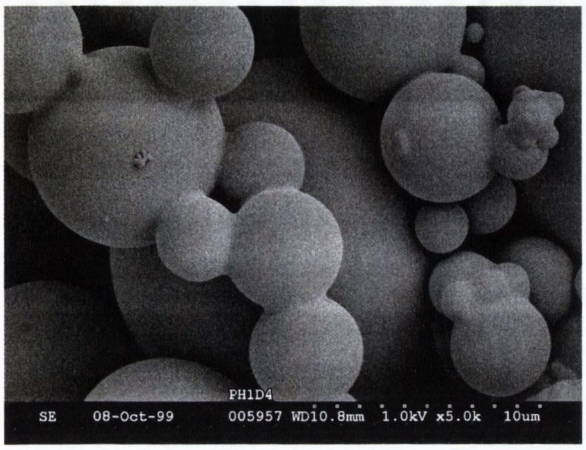
PLGA Polymer	k (day^{-1})	*Tmax*	CD	MSC
pH1.0	0.074 ±0.0068	31.383 ±1.4671	0.986	3.723
pH 7.4	0.050 ±0.0038	60.148 ±1.6262	0.983	3.727
pH 10.0	0.221 ±0.0250	7.964 ±0.0560	0.988	3.601

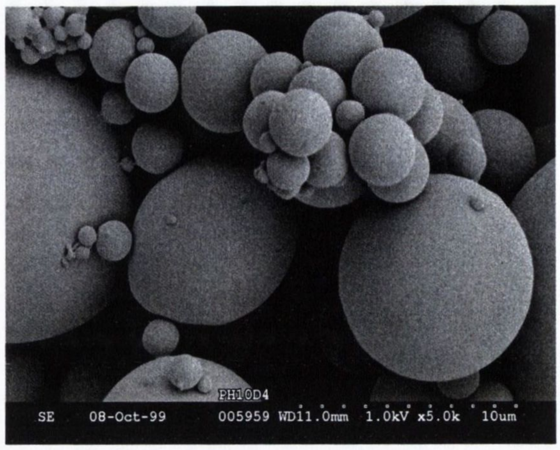
Parameters for the degradation of PLGA microspheres in incubation media of different pH, showed that for polymer erosion, the basic media had the most profound effect. The lack of correlation between the molecular weight loss and the polymer mass loss are attributed in this study to initial surface accelerated degradation of the polymer that is in contact with the extreme acidic and basic conditions. When the undegraded polymer is removed from the incubation medium the polymer inside the matrix retains molecular weight characteristics. The influence of the pH on the polymer molecular weight can only occur when the matrix is sufficiently degraded to allow the ions to penetrate into the matrix. Surface controlled erosion produces an immediate loss in polymer material that is accelerated as the ions have access to increasing amounts of ester bonds.

8.7.3 Effect of pH on the microsphere morphology of PLGA (RG504) microspheres

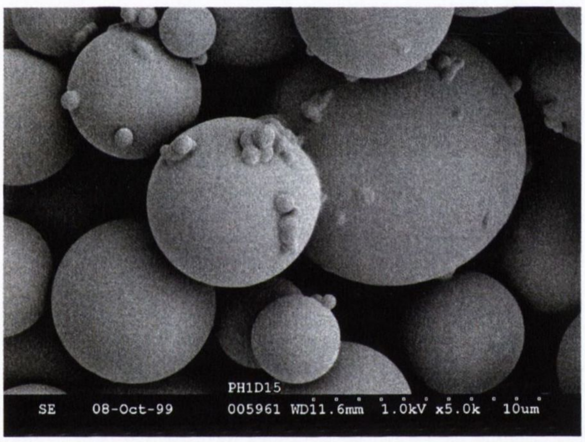
Electron micrographs of PLGA (RG504) microspheres after incubation in pH 1.0 and 10.0 media are shown in Figure 8.16. These micrographs were compared to un-incubated PLGA (RG504) microspheres shown in Chapter 7, Figure 7.1.

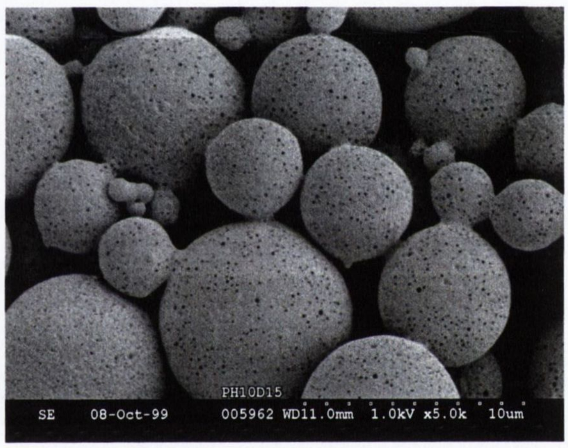
(a) Day 4 pH 1.0 **pH 10.0**



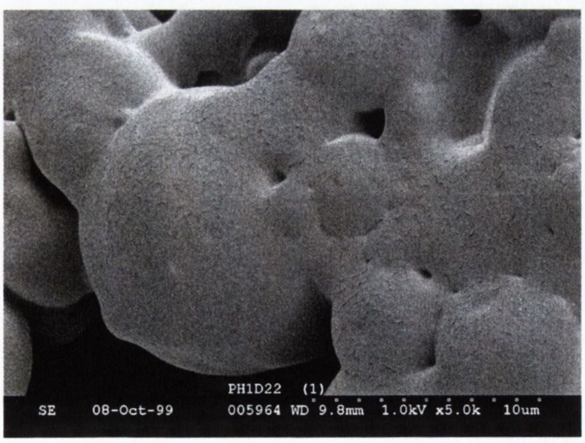


(b) Day 15 pH1.0 **pH 10.0**





(c) Day 22 pH 1.0 **pH 10.0**



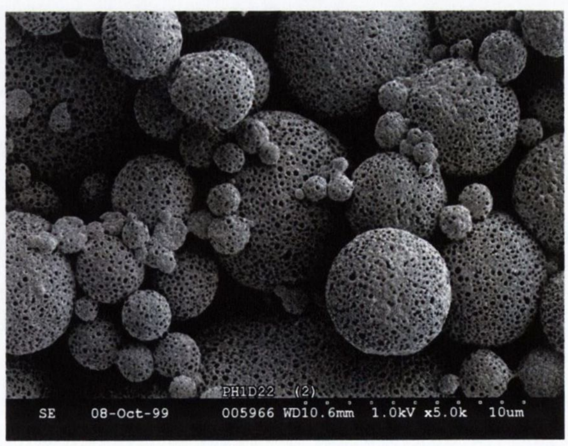


Figure 8.16 Electron micrographs of PLGA RG504 microspheres (<20 microns) incubated in pH 1.0 and 10.0 media for 4, 15 and 22 days at 37°C.

Figure 8.16 revealed that the microsphere morphology was not affected after 4 days incubation at pH 1.0 or pH 10.0. After 15 days incubation the microspheres incubated at pH 1.0 show no gross morphological changes compared to microspheres incubated at pH 10 where severe pitting of the microsphere has occurred. Day 22 electron micrographs show fusion and surface roughness of the remaining microspheres that were incubated at pH 1.0 while those incubated at pH 10.0 are completely porous.

8.7.4 Effect of pH on the polydispersity of PLGA (RG504) microspheres

Polydispersity for the degrading PLGA (RG504) microspheres incubated at pH 1.0, 7.4 and 10.0 were plotted as a function of incubation time and are shown in Figure 8.17.

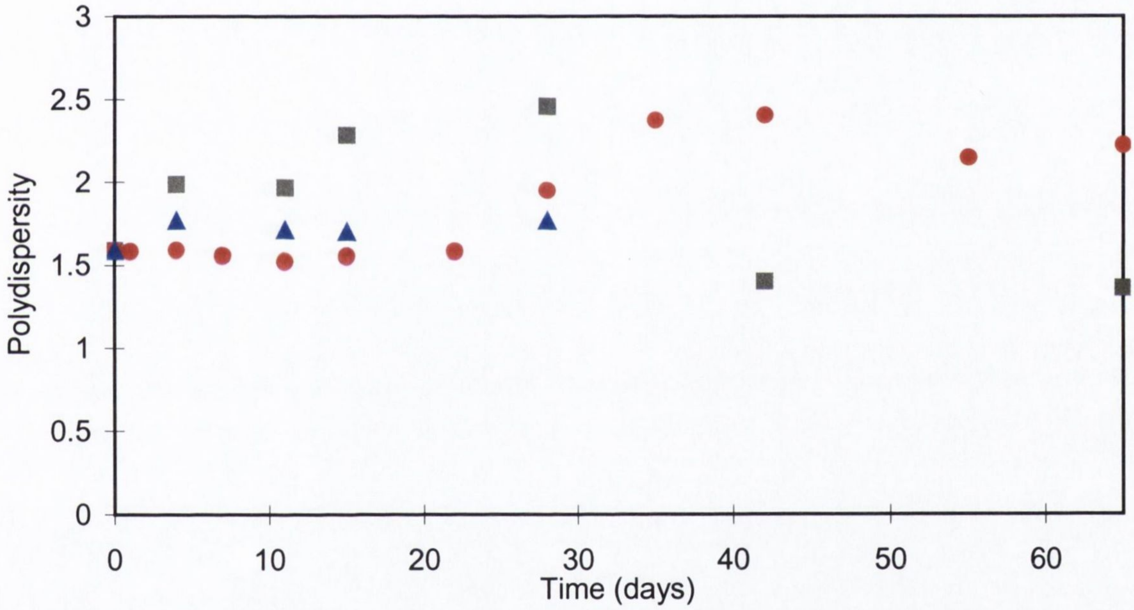


Figure 8.17 Change in polydispersity of PLGA (RG504) microspheres with incubation time, for ● pH 7.4 ▲ pH 10.0 ■ pH 1.0 media *(data points represent mean of two samples).*

Figure 8.17 shows that at pH 1.0 and pH 7.4, the molecular weight distribution pattern of peak broadening with incubation time is demonstrated, while at later incubation times the molecular weight distribution narrows. This pattern occurs fastest in microspheres incubated at pH 1.0. Contrary to that observed at acidic and neutral pH, the polydispersity of the polymer does not change with degradation in basic media (Figure 8.17).

Belbella *et al.* (1996) attributed the acid accelerated hydrolysis to a random chain scission of the polymer backbone due to the observation of intense broadening of the distribution of the polymer chain lengths in the GPC chromatograms or an increase in polydispersity. This is in good agreement with the acid catalysed hydrolysis demonstrated in this work. Makino *et al.* (1985) assumed the degradation at pH 10.0 to proceed via an unzipping process and not by a random chain scission process. The polydispersity observed in the base catalysed degradation of PLGA is in agreement with this mechanism.

The acceleration of polymer hydrolysis is in accordance with general acid-base catalysis. The acceleration of the degradation rate in the presence of acidic (Makino *et al.* 1985, Belbella *et al.* 1996) or basic (Maulding 1986, Cha and Pitt 1989, Belbella *et al.* 1996, Li *et al.* 1996) components has been reported. The mechanism of this catalysis is different depending on the ionic species H^+ or OH^-. Several investigators have studied the mechanism of bond cleavage for the polyester backbone. A ^{1}H-NMR study by Shih (1995) suggested that the scission of the main chain ester bond was not truly a random scission process, but contained a substantial contribution from chain end scission also when studied in acidic medium.

8.8 INFLUENCE OF INCUBATION MEDIUM TEMPERATURE ON THE RATE OF POLYMER DEGRADATION OF PLGA RG504 MICROSPHERES (<20 MICRONS) IN PHOSPHATE BUFFER SALINE pH 7.4.

Investigations on the degradation of polyesters have revealed that the degradation is a result of hydrolysis of ester bonds (Makino *et al.* 1985). Like most reactions, the rate of hydrolysis is dependent on the incubation temperature at which the reaction takes place. The rate of degradation of these polymers will be sensitive to variation in incubation temperature. The effect of temperature on the degradation rate of microspheres was studied by incubating PLGA (RG504) microspheres in visking bags under different incubation temperatures.

8.8.1 Influence of incubation medium temperature on the polymer molecular weight degradation profile of PLGA (RG504) microspheres (<20 microns) in PBS pH 7.4

The molecular weight profiles for microparticles incubated under different conditions of temperature show the relationship between degradation rate of the polymer as a function of the incubation medium temperature (Figure 8.18).

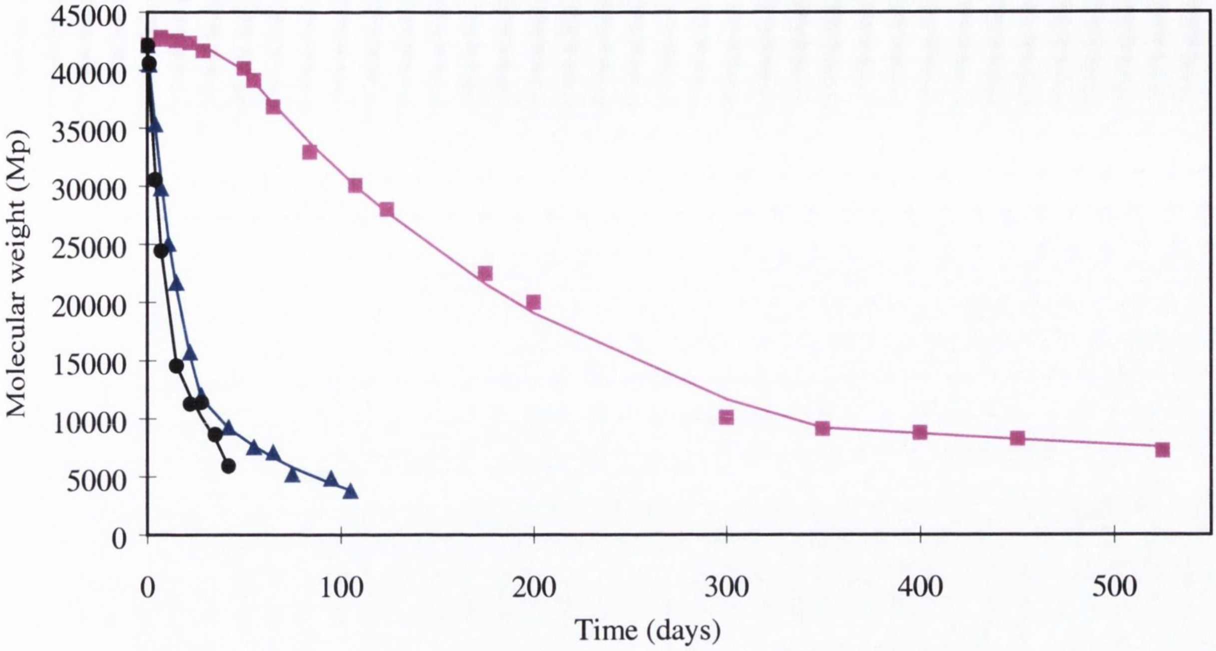


Figure 8.18 Effect of incubation medium temperature on the degradation of PLGA(RG504) microspheres, for ● 45°C, ▲ 37°C and ■ 25°C temperatures *(data points represent mean of two samples).* Data points fitted to Equation 7.3 or 8.1.

It was observed that as incubation temperature of the medium increased the rate of polymer degradation increased correspondingly (Figure 8.18). The relationship between polymer increased degradation rate with incubation temperature is in agreement with that observed by Gilding and Reed (1981) for PLGA polymer rods and by Makino *et al.* (1985) and Wang *et al.* (1990) for PLA polymer microspheres. A more recent study by Li and McCarty (1999) also showed acceleration in PLA degradation at 60°C when compared to PLA degradation at 37°C.

At the lower incubation temperature of 25°C, a tri-phasic profile was observed which consisted of an initial induction phase during which no reduction in the polymer molecular weight was observed. This was followed by a fast reduction in the molecular weight of the polymer and in the final molecular weight degradation phase, the remaining polymer degrades at a slower rate. As the temperature of the incubation medium increases and the rate of polymer degradation increases, the profile becomes biphasic. Parameters for the triphasic degradation profile observed at 25°C were determined using a modification of equation 7.3 according to:

$$Mp = Tlag + (Mp_0 \exp(-k_1 t_1)) + ((Mp_0 \exp(-k_1 Tau))\exp(-k_2(t_2 - Tau))) \qquad \text{Equation 8.2}$$

Where *Tlag* represents the length of the induction phase where no polymer molecular weight decrease is observed. The parameters for the induction, first and second phase were determined from the molecular weight profile for the three temperatures (25, 37 and 45°C) and are shown in Table 8.17.

Table 8.17 The influence of incubation temperature on the degradation parameters of RG504 microspheres (<20microns) fitted to Equation 8.2 and 7.3 *(standard deviation represents that determined by the model).*

Temperature (°C)	*Tlag*	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	CD	MSC
25	39.171 ±2.0900	0.005 ±0.0001	0.001 ±0.0006	307.669 ±19.6220	0.998	5.860
37	0	0.046 ±0.0011	0.014 ±0.0012	29.789 ±1.7491	0.999	6.655
45	0	0.077 ±0.0045	0.020 ±0.0047	12.956 ±1.7861	0.996	4.920

The rate constants increased with an increase in incubation temperature during both phases of the degradation profile. At 25°C the lag phase was determined to be 39 days using equation 8.2.

The effect of temperature on the reaction rate is given by the equation suggested by Arrhenius:

$$k = A\, e^{-Ea/RT} \qquad \text{Equation 8.3}$$

where k is the specific reaction rate, A is a constant known as the Arrhenius factor, Ea is the energy of activation, R is the gas constant (1.987 calories/deg mole), and T is the absolute temperature. The energy of activation was evaluated by determining k at several temperatures and plotting 1/T against log k (Figure 8.19).

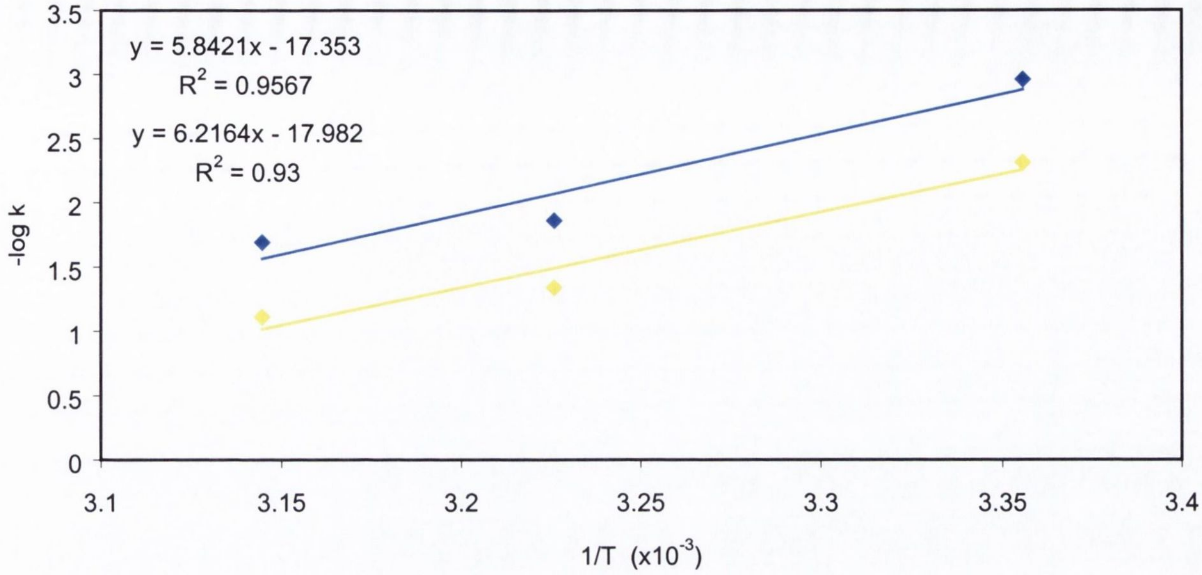


Figure 8.19. Arrhenius plots for the degradation of PLGA (RG504) microspheres, where ___ k_1 and ___ k_2 degradation rate constants for PLGA(RG504).

When 1/T is plotted against log *k* a linear relationship between degradation rate and temperature is observed for both phases of the degradation profile (Figure 8.19). The slope of the line so obtained is -Ea/2.303R and the intercept on the vertical axis is log A (Martin, 1993). The energy of activation was calculated for both phases as (1) 26.734 kcal/mol and (2) 28.447 kcal/mol. In a study of the influence of incubation temperature on the degradation rate of poly (d,l-Lactide) and poly(l-Lactide) microcapsules Makino *et al.* (1985) determined the activation energy of both of these polymers to be 19.9 kcal/mol and 20.0 kcal/mol respectively. The rate constant k was determined for both phases at each temperature and these values were used to calculate the activation energy similar to that described by Makino *et al.* (1985) for PLLA and PDLLA. These values could not be compared directly with the results given here because of differences between particle morphology and incubation conditions. However it was observed that they found these values to be comparable to the activation energy determined for the hydrolysis of alkyl acetates, and consequently the degradation mechanism can be ascribed to hydrolysis.

8.8.2 Effect of incubation temperature on the polymer mass loss from PLGA (RG504) microspheres

The mass loss profiles of the microspheres show a temperature dependence on the degradation rate consistent with that observed in the molecular weight profile (Figure 8.20).

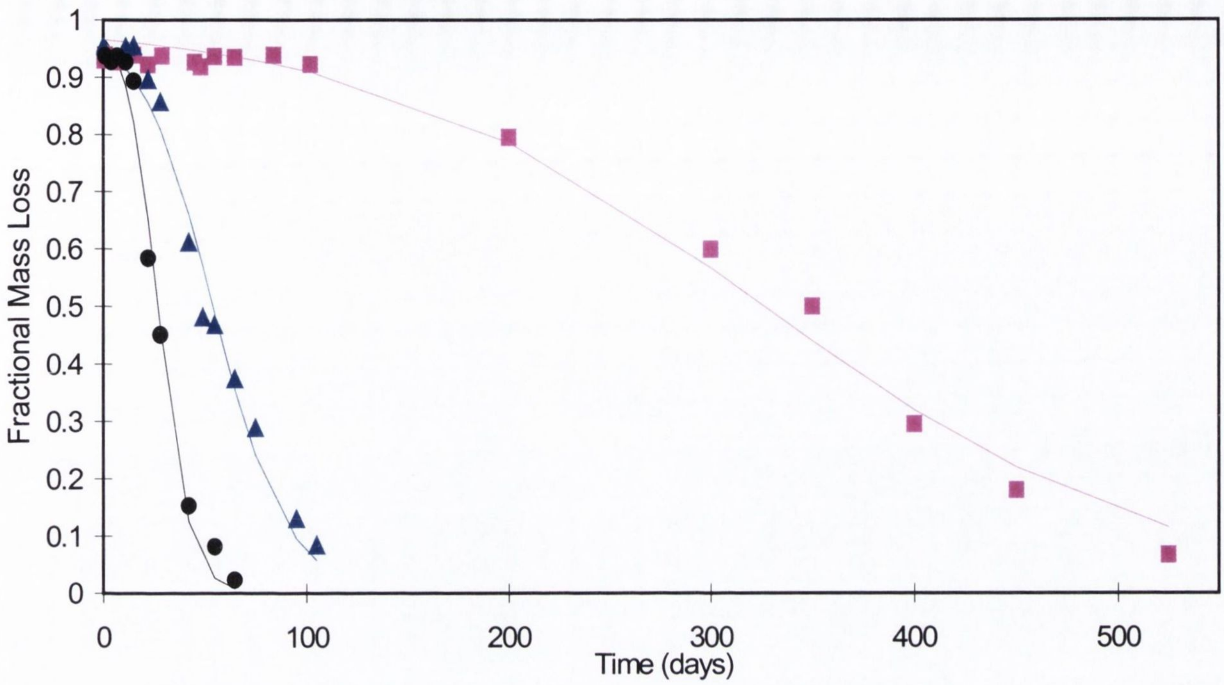


Figure 8.20 Effect of incubation temperature on the mass loss from PLGA(RG504) microspheres, for ● 45°C, ▲ 37°C, and ■ 25°C temperatures *(data points represent mean of two samples).* Data points fitted to Equation 3.23.

Figure 8.20 shows that mass loss from the microspheres occurred faster with an increase in incubation temperature. The profiles of mass loss at 25, 37 and 45°C were fitted to the Prout-Tompkins equation (Equation 3.23) and the rate constants k and $Tmax$ were evaluated for each temperature (Table 8.17).

Table 8.17 The relationship between incubation temperature and the parameters *k* and *Tmax* determined using Equation 3.23 *(standard deviation represents that determined by the model).*

Incubation Temperature (°C)	*k* (day^{-1})	*Tmax* (days)	CD	MSC
25	0.011 ±0.0007	328.000 ±7.5541	0.998	3.856
37	-0.054 ±0.0048	54.150 ±1.4377	0.996	3.926
45	-0.129 ±0.0142	26.880 ±1.0046	0.996	3.84

The parameters *k* and *Tmax* were found to decrease with a corresponding increase in incubation temperature (Table 8.17). When the log of the rate constant *k*, calculated using the Prout-Tompkins equation, was plotted against 1/T a linear relationship was observed (Figure 8.21). Using this relationship the activation energy was calculated for the erosion of PLGA microspheres (<20 microns) as 23.62 kcal/mol. Log *Tmax* was also linear with reciprocal temperature (K) and the slope of this relationship could also be used to determine Ea as 23.929 kcal/mol, indicating that the time required to reach 50% mass loss could also be used to determine the influence of temperature on the polyester hydrolysis rate (Figure 8.22).

This value is in agreement with the value calculated using the plot of molecular weight with temperature. This agreement demonstrates that during both phases of the degradation profile, the underlying process is a hydrolysis controlled degradation mechanism and that the change in the rate constant is due to a transfer from a degradation controlled process to an erosion controlled process.

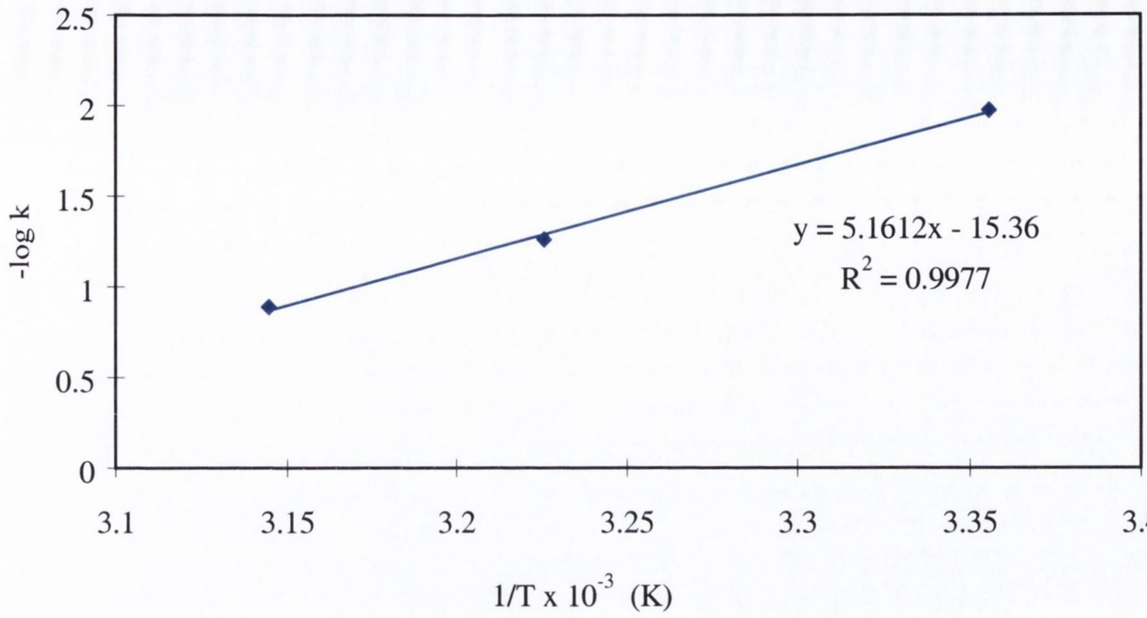


Figure 8.21. Arrhenius plots for the degradation of RG504 microspheres using rate constants determined using the Prout-Tompkins equation (Equation 3.23).

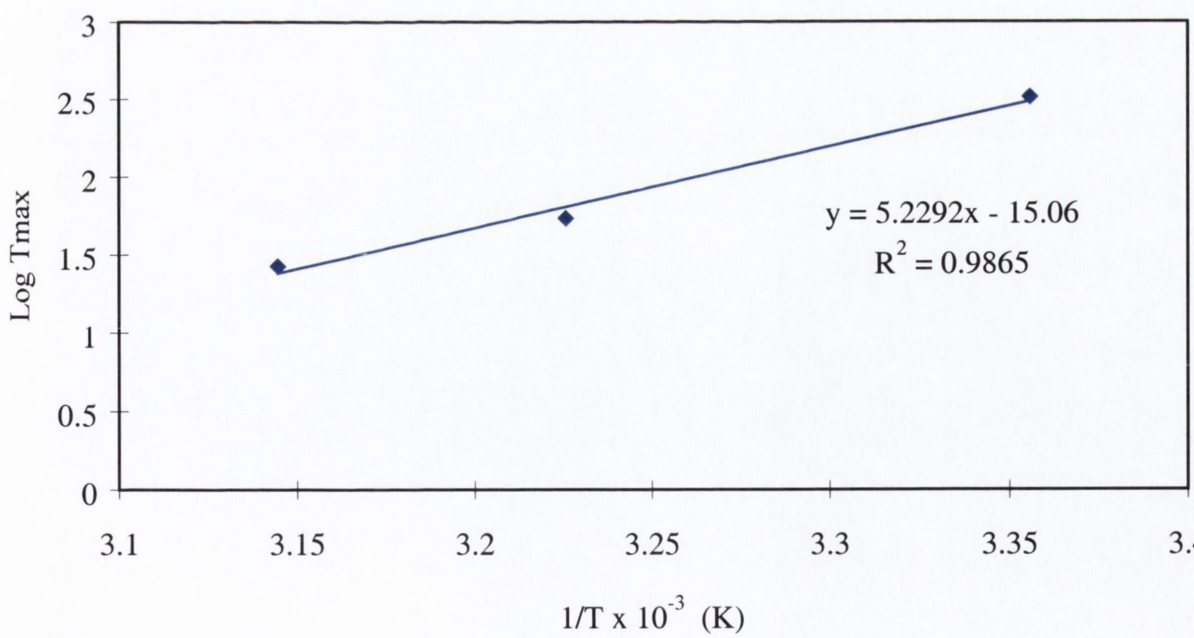


Figure 8.22 Arrhenius plots for the degradation of RG504 microspheres using *Tmax* determined using the Prout-Tompkins equation (Equation 3.23).

8.9 A COMPARISON OF THE THERMAL PROPERTIES OF PLGA (RG504) MICROSPHERES AT DIFFERENT INCUBATION TEMPERATURES AS A FUNCTION OF TIME

Thermal analysis of polymer samples taken at different incubation time points was carried out for the microspheres incubated at 25ºC and 45ºC. The thermal properties at 37ºC were presented in Chapter 7. Samples that were picked for analysis represented the induction, polymer degradation and the erosion phase.

Figure 8.23 shows DSC thermograms of PLGA (RG504) microspheres incubated in phosphate buffered saline pH 7.4 at 25ºC over 400 days.

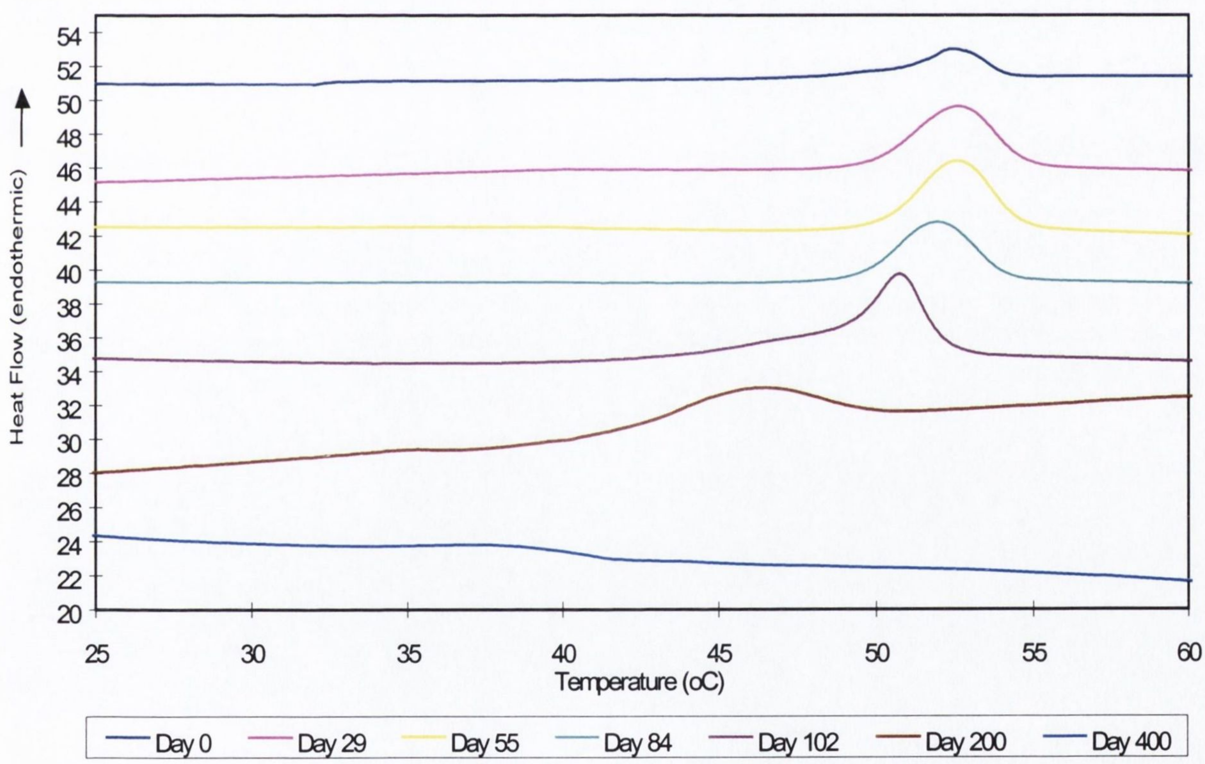


Figure 8.23 DSC thermograms of the degradation of PLGA (RG504) microspheres (<20 microns) incubated at 25°C in phosphate buffer saline pH 7.4.

DSC analysis revealed that the glass transition peak remains constant over the induction period (Figure 8.23), after which a gradual decrease in peak temperature occurs consistent with the lowering of the polymer molecular weight phase. At day 400, when mass loss of the polymer has proceeded the peak temperature is at 37 °C, indicating that the majority of the polymer chains are in the glassy state and loss of degradation products can occur. Figure 8.24 shows DSC thermograms of PLGA (RG504) microspheres incubated in phosphate buffered saline pH 7.4 at 45°C over 28 days.

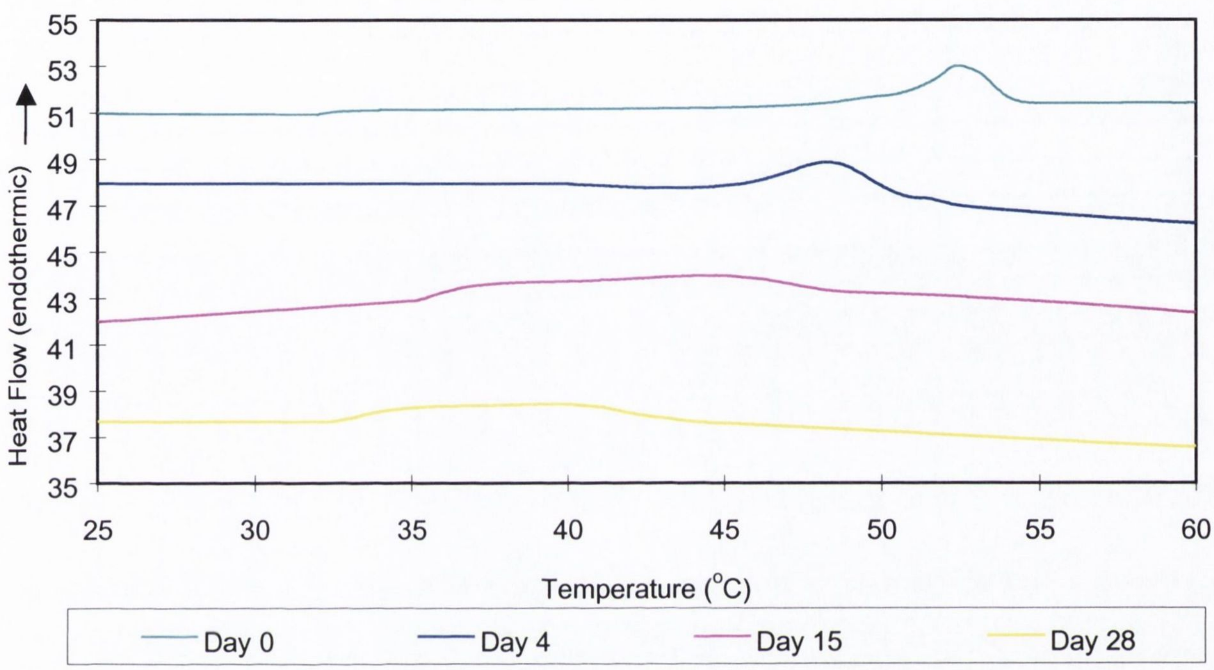


Figure 8.24 DSC thermograms of the degradation of PLGA (RG504) microspheres at 45°C in phosphate buffer saline pH 7.4.

Figure 8.24 shows the $T_{g(p)}$ profile for microspheres incubated at 45°C. A rapid reduction in $T_{g(p)}$ accompanied by peak broadening was demonstrated. At day 15 in the mass loss profile of PLGA microspheres incubated at 45°C, mass loss is occurring. The DSC thermogram shows the development of a broad flat peak in this phase of the degradation profile.

For PLGA (RG504) microspheres incubated at different incubation temperatures where the complete degradation profile was studied, it was observed that in each case the onset of mass loss, the molecular weight degradation, phase change and the reduction in $T_{g(p)}$ to the temperature of incubation were simultaneous.

8.10 INFLUENCE OF INCUBATION TEMPERATURE ON THE MORPHOLOGY OF PLGA MICROSPHERES

Electron micrographs of PLGA microspheres degraded at 25°C are shown in Figure 8.25. Electron micrographs taken of PLGA samples at 25°C during the induction phase show no loss in microsphere shape or fusion. Microspheres recovered at day 29 and day 45 show the microspheres as discrete particles with no evidence of surface deformation despite their exposure to an aqueous environment.

Micrographs taken after the induction phase day 84 and day 102 (Figure 8.25 c and d) show a gradual microsphere fusion. Particle fusion is obviously a consequence of polymer chain scission, not the presence of an aqueous environment. The development of a porous matrix eventually produces mass loss and gross morphological changes in the matrix (Figure 8.25 e and f).

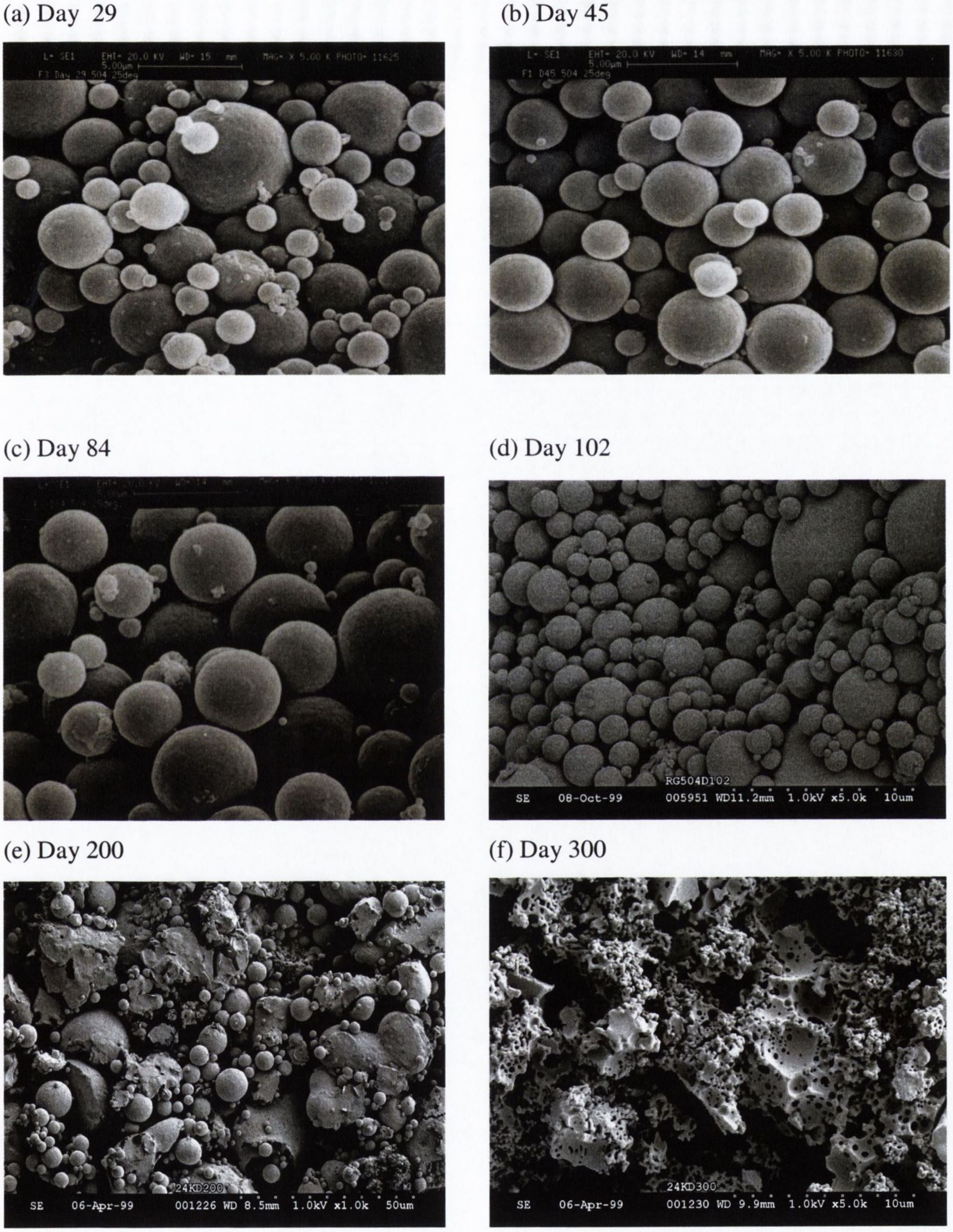


Figure 8.25 The evolution of microsphere morphology with the degradation for PLGA (RG504) microspheres incubated at 25°C.

8.11 STUDY OF THE INFLUENCE OF MICROSPHERE PARTICLE SIZE AT pH 7.4 AND 5°C ON THE DEGRADATION OF PLGA (RG504) MICROSPHERES

The degradation properties of PLGA particles of different particle size were also investigated in phosphate buffer saline. It was postulated at the beginning of this study that a slowed degradation rate might give additional information about the degradation profile.

8.11.1 Polymer molecular weight profile for PLGA (RG504) particles incubated at pH 7.4 and 5°C

The degradation profile for PLGA (RG504) particles < 1, <20 and <50 microns incubated at 5°C is shown in Figure 8.26.

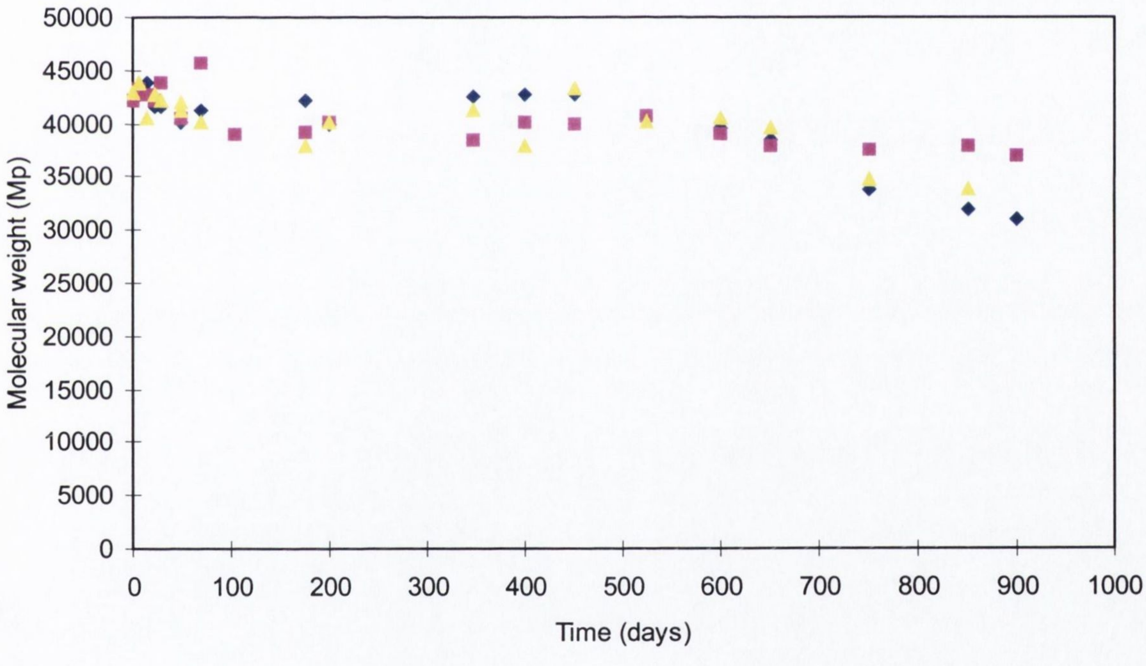


Figure 8.26 Molecular weight profile for PLGA particles incubated in phosphate buffer saline pH 7.4 at 5°C, where ◆ < 1 micron ■ < 20 micron and ▲ <50 microns *(data points represent the mean of two samples).*

Figure 8.26 shows that the degradation profile for all particle sizes was considerably slower than that observed at 37°C. In fact almost no reduction in polymer weight is observed over the study period. Molecular weight (Mp) was constant over 600 days after which a gradual decrease in the molecular weight is observed in the profile. Unfortunately, with the number of samples taken over these time points, no remaining samples were left to determine the full degradation profile. However the length of the induction phase was determined from these results as being 650 days.

8.11.2 Polymer mass loss profile for PLGA (RG504) particles incubated at pH 7.4 and 5°C.

Mass loss from the particles incubated in phosphate buffer saline at 5°C was also monitored as part of this study. The mass loss profile for PLGA (RG504) microspheres under these incubation conditions is shown in Figure 8.27.

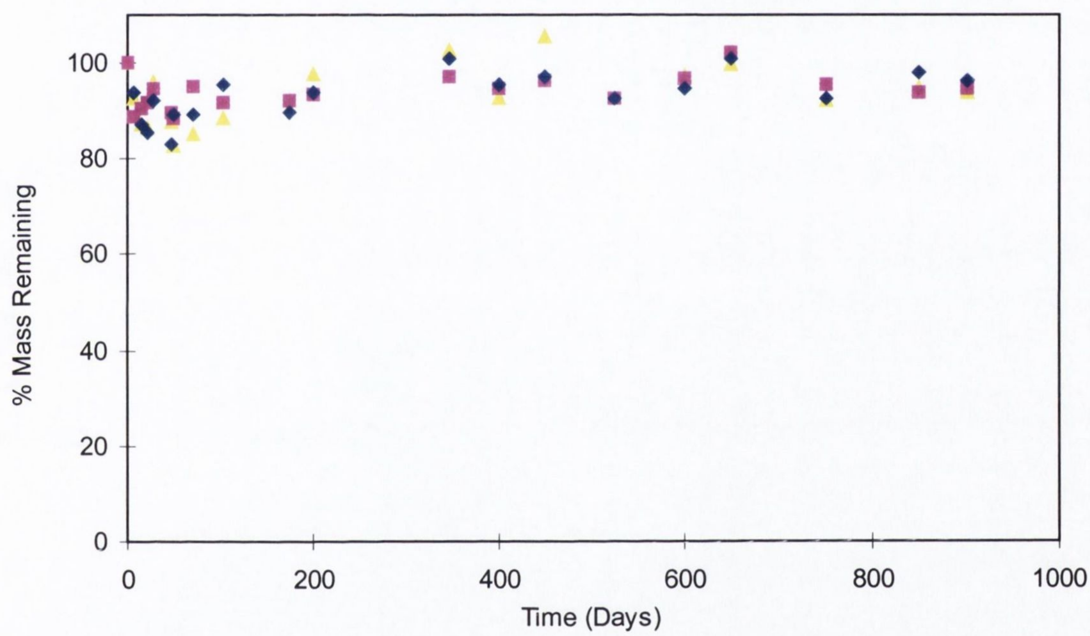


Figure 8.27 Mass loss profile for PLGA (RG504) particles incubated in phosphate buffer saline pH 7.4 at 5°C, where ◆ < 1 micron ■ < 20 micron and ▲ <50 microns *(data points represent the mean of two samples).*

Figure 8.27 shows that after an initial mass loss recorded at the first time point which was 3.60-7.29% at day 7, there was no further loss in weight over the study period (900 days). Constant polymer molecular weight and mass loss over the study period for all particle sizes indicated minimal polymer degradation over this time. When particles were incubated at 5°C, weight loss and polymer degradation were significantly slower than that observed at 37°C.

The previous studies on PLGA incubated at different incubation temperatures have shown that only the rate of polymer degradation is affected by the temperature and since these studies are identical except for the variation in temperature, the activation energy evaluated in the preceding section can be used to determine the time for $T_{50\%}$ erosion. *Tmax* at 5 ºC was determined as 5623 days. This profile demonstrates the stability of these polymers when stored at low temperatures even in the presence of an aqueous environment.

8.11.3 The thermal properties of particles incubated in phosphate buffer saline pH 7.4 at 5°C as a function of incubation time

The thermal properties of the polymer matrix after incubation in phosphate buffer saline pH 7.4 at 5°C as a function of incubation time are shown in Table 8.18.

Table 8.18 Thermal characteristics of particles incubated over time at 5°C in phosphate buffer saline *(samples represent the mean of two determinations)*

Incubation time (days)	Process A $T_{g(p)}$ (°C)	Process B $T_{g(p)}$ (°C)	Process C $T_{g(p)}$ (°C)
0	52.4	52.1	52.4
15	51.3	53.4	50.6
70	51.0	52.9	53.1
104	51.4	50.1	51.2
175	48.4	50.4	50.9
400	49.3	50.2	49.7
600	47.9	48.9	48.9
850	47.2	49.2	49.5

DSC analysis of the particles in the three size ranges after incubation at 5°C did show a gradual drop in $T_{g(p)}$ ranging from 5.2°C for the nanoparticles to 2.9°C for the <50 micron particles (Table 8.18). The DSC thermograms for PLGA (RG504) (<20micron) microspheres are shown in Figure 8.28.

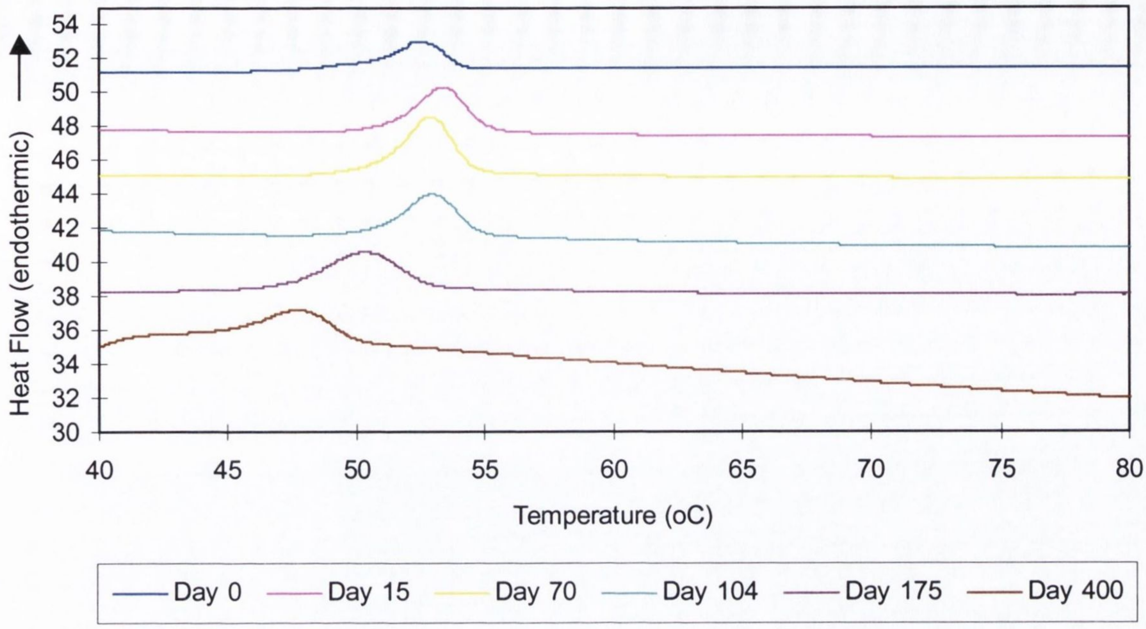
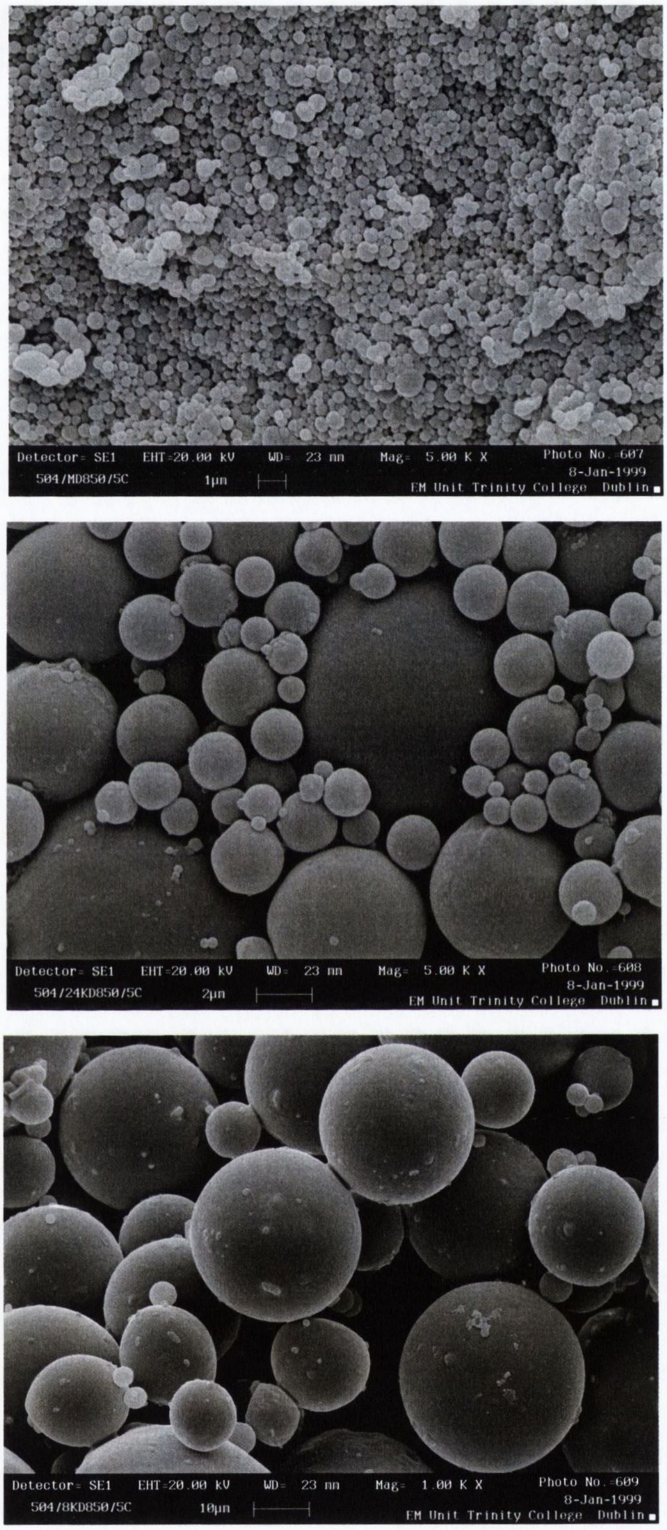


Figure 8.28 DSC thermograms of PLGA (RG504) microspheres incubated at 5°C in phosphate buffer saline pH 7.4

Figure 8.27 shows a shift in the peak temperature was observed after 175 days incubation while no corresponding reduction of the molecular weight Mp was observed. This decrease in peak temperature was also observed for the < 1 micron and <50 micron particles. This peak reduction is thought to occur due to trapped buffer components in the particles that are retained in the particles after recovery and drying.

8.11.4 SEM of particles incubated in phosphate buffer saline pH 7.4 at 5°C as a function of incubation time

Scanning electron micrographs of PLGA (RG504) particles after 850 days incubation in phosphate buffer saline pH 7.4 at 5°C are shown in Figure 8.29. No coalescence or evidence of degradation was observed at or before this time point. Particles in the three size distributions were recovered as discrete particles that showed no morphological differences compared to the particles before incubation.



(a)

(b)

(c)

Figure 8.29 Scanning electron micrographs of particles incubated for 850 days at 5°C in phosphate buffer saline, for (a) <1 micron (b) <20 microns (c) <50 microns particles.

8.12 SUMMARY

In this chapter, the physicochemical factors, which can influence the degradation of PLGA particles, was examined. In general, the influence of the morphology of the particles as shown in Chapter 7 for PLGA (RG504) was also demonstrated using PLGA (RG504H). Properties of the polymer such as polymer molecular weight and the presence/absence of an end-cap on the polymer were shown to influence the degradation and erosion of PLGA microspheres. These properties were then subsequently shown to also influence thermal and morphological changes in the polymer matrix. The influence of incubation conditions (pH and temperature) on the hydrolytic degradation of PLGA was demonstrated. PLGA degradation was accelerated at both extreme acidic and basic conditions however the mechanism of degradation appeared significantly different. The influence of incubation temperature on the degradation rate showed that PLA/PLGA degradation has three phases in its degradation pattern: an incubation phase, a polymer degradation phase and a polymer erosion phase and depending on the incubation temperature all or some of these phases will be followed. In general the rate of polymer degradation increased with increasing incubation temperature.

It is known that the degradation of PLA devices proceeds at a considerably slower rate than for PLGA. In the next chapter, the degradation mechanism of PLA polymer particles will be examined.

CHAPTER 9

THE CHARACTERISATION OF POLY D,L-LACTIC ACID PARTICLES AND THEIR DEGRADATION PROPERTIES

9.1 INTRODUCTION

In this chapter particles prepared using the poly-d,l-lactic acid series of polymers are characterised and their degradation characteristics examined. These studies were carried out to determine if the lactide series of polymers exhibited different degradation characteristics to that of the PLGA polymers. The effects of particle size and polymer molecular weight on PLA degradation were examined. The degradation of PLA was monitored in terms of physicochemical and morphological changes that occur during the degradation process by time dependent changes in molecular weight, polymer mass, glass transition temperature and microscopy.

9.2 PHYSICOCHEMICAL CHARACTERISATION OF POLY D,L-LACTIC ACID (R203) PARTICLES OF DIFFERENT PARTICLE SIZE DISTRIBUTIONS

Scanning Electron micrographs of PLA particles in the three size distributions were smooth and spherical in nature with no surface defects (Figure 9.2.1). Table 9.1 and 9.2 show the properties of particles prepared from poly d,l-lactide (R203). Thermal properties of the particles are compared to that of the starting polymer (% differences are indicated in parenthesis).

Table 9.1 Characteristics of PLA (R203) particles ranked in order of increasing particle size (*data points represent mean of three determinations ± standard deviation)*

Processing conditions	$D_{10\%}$ (μm)	$D_{50\%}$ (μm)	$D_{90\%}$ (μm)	$T_{g(p)}$ (°C)	Yield (%)
Process A	0.3	0.6	1.0	55.5 (-0.3%)	83.9
	±0.01	±0.01	±0.006	±0.17	±1.27
Process B	1.2	4.2	18.2	55.7 (+0.0%)	85.7
	±0.03	±0.23	±0.13	±0.13	±0.19
Process C	3.9	22.48	49.8	55.1 (-1.0%)	81.0
	±0.12	±1.08	±2.38	±0.05	±3.81

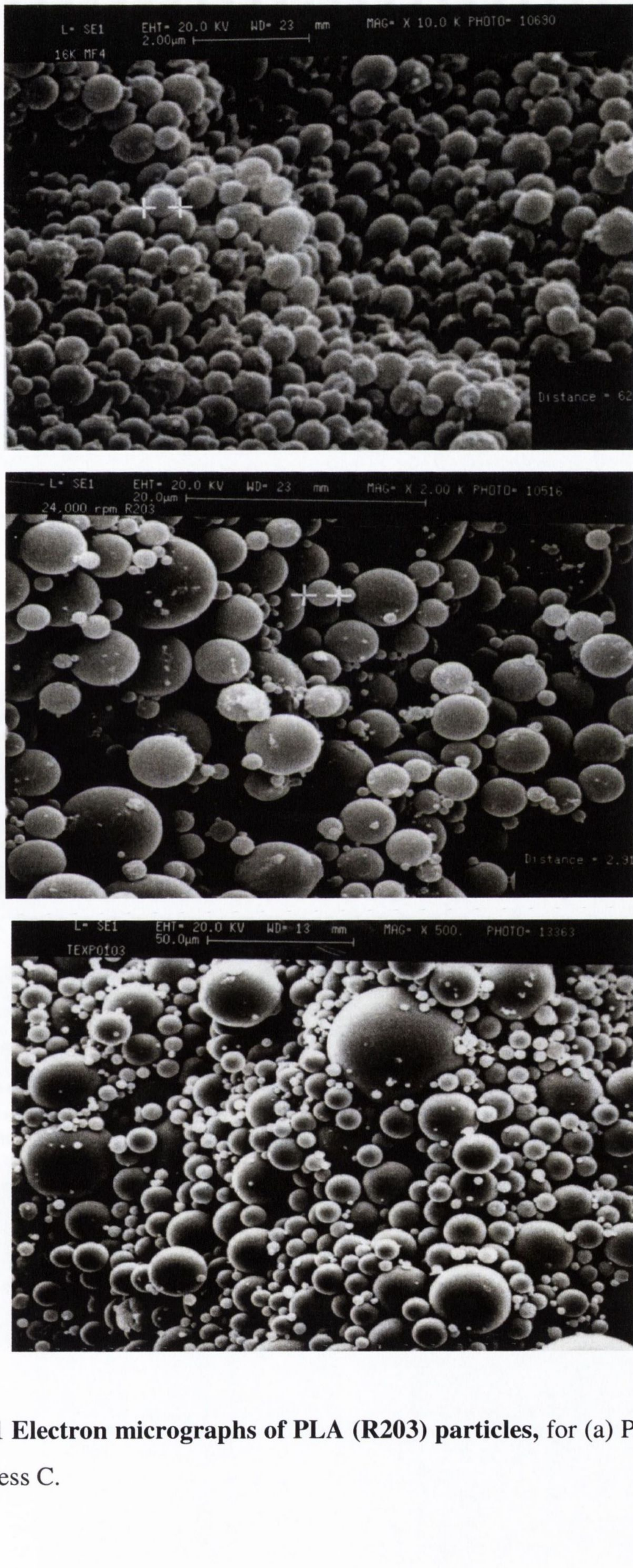
The particle size $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ of the microspheres reduce with increasing homogenisation speed, nanoparticles were produced by microfluidisation. The yield of particles was high relative to the 1.8g batch size used to prepare particles for this work, for all three processes. Thermal properties $T_{g(p)}$ of the particles were similar to that of the starting polylactide polymer.

The GPC characteristics of the blank particles prepared were similar to those of the starting polymer (Table 9.2). Table 9.2 shows that the mean values ± the standard deviation shows no significant differences between the three processes.

Table 9.2 GPC characteristics of PLA (R203) and PLA (R203) particles ranked in order of increasing particle size *(data points represent mean of three determinations ± standard deviation)*

Process	Mn	Mp	Mw	Mz	P
PLA	12146	20015	19759	33634	1.627
	±109	±568	±467	±221	±0.0875
Process A	11840	21130	20215	30898	1.744
	±575	±1828	±313	±1334	±0.0606
Process B	12302	20914	20801	30218	1.638
	±347	±703	±964	±1294	±0.0656
Process C	12612	20694	21097	33282	1.673
	±248	±608	±1030	±1201	±0.0748

The molecular weight parameters of the particles were similar to that of the unprocessed polymer, apart from some shearing of the higher molecular weight chains due to the reduction in Mz and the increase in Mn (Table 9.2). These results indicate that no appreciable polymer degradation occurred during particle preparation, using either homogenisation or microfluidisation as an emulsification technique, similar to that observed for PLGA (RG504) particles in chapter 7.



(a)

(b)

(c)

Figure 9.1 Electron micrographs of PLA (R203) particles, for (a) Process A (b) Process B (c) Process C.

9.3 THE INFLUENCE OF PARTICLE SIZE ON THE DEGRADATION BEHAVIOUR FOR PLA (R203) IN VISKING BAGS IN PHOSPHATE BUFFER SALINE pH 7.4 AND 37°C

The degradation profile of PLA particles produced using three process modifications of the solvent evaporation procedure were <1μm, <20μm and <50μm in diameter as outlined in Table 9.1. The degradation profile of these particles was by monitored by time dependent changes in the polymer molecular weight, mass loss, thermal properties and matrix morphology.

9.3.1 Polymer Molecular Weight

The molecular weight profile of PLA (R203) particles of <1μm, <20μm and <50μm in diameter with incubation time in phosphate buffer saline pH 7.4 at 37°C is shown in Figure 9.2.

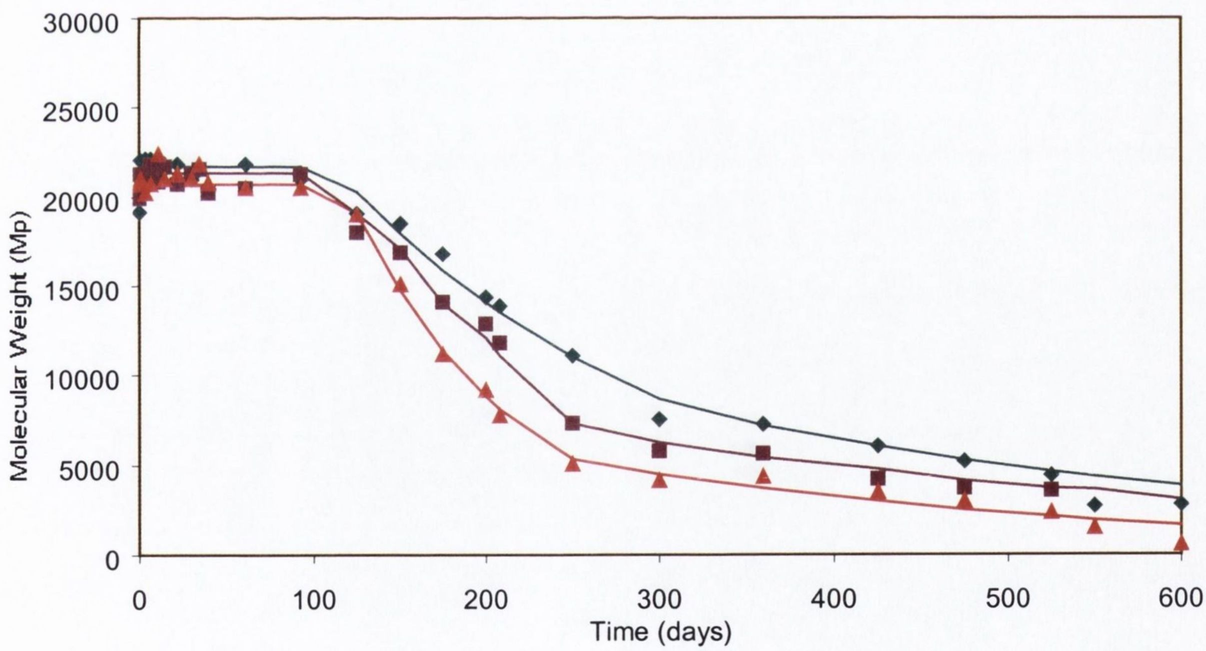


Figure 9.2 Influence of particle size on the molecular weight profiles of PLA (R203) particles incubated at pH 7.4 and 37°C, where ◆ <1 micron, ■ <20 micron and ▲<50micron particles *(data points represent mean of two determinations)*. Data fitted to Equation 8.2.

The degradation of PLA (R203) shows a tri-phasic degradation pattern, consisting of an initial lag phase *Tlag* during which time no change in the molecular weight was observed. In the second phase of the degradation profile the molecular weight underwent a fast rate k_1 of reduction over a time t_1 followed by a the third phase of degradation during which the molecular weight of the remaining polymer Mp_1 degraded at a slower rate k_2 of over a time t_2 which occurred after a time *Tau*. The tri-phasic molecular weight profile observed for the degradation of PLA (R203) could be described by the Equation 8.2,

$$Mp = Tlag + (Mp_0 \exp(-k_1 t_1)) + ((Mp_0 \exp(-k_1 Tau)) \exp(-k_2 (t_2 - Tau)))$$

where Mp_o is the molecular weight of the starting microspheres and *Mp* is the molecular weight of the degradation samples measured over time *t*, the T_{lag}, k_1, k_2 and *Tau* can be determined from which Mp_1 can also be calculated (Table 9.3).

Table 9.3 Parameters for the degradation of PLA particles at 37°C in phosphate buffer saline fitted to Equation 8.2 *(standard deviation represent that determined by the model)*

Particle Size (μm)	*Tlag* (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
< 1	99.114 ±6.2935	0.005 ±0.0005	0.003 ±0.0005	317.499 ±12.6450	7296	0.994	4.765
< 20	100.987 ±5.3978	0.006 ±0.0003	0.003 ±0.0006	282.532 ±19.2740	6891	0.994	4.732
< 50	117.085 ±3.2164	0.010 ±0.0007	0.003 ±0.0006	261.412 ±18.3046	4695	0.995	5.007

The degradation kinetics of PLA particles were described by Equation 8.2 and the data showed good fit to this model Table 9.3. The kinetics of PLA R203 degradation was also found to be dependent on the size of the PLA (R203) particles. The rate constant k_1 calculated for the first phase of degradation was found to increase with increasing particle size. The degradation rate k_2 calculated for the second phase was less dependent on the particle size. For PLA the time *Tau* at which the second phase begins or the molecular weight Mp_1 occurred at lower values as the particle size decreased.

A plot of the polydispersity index for the PLA particles with incubation time is shown in Figure 9.3.

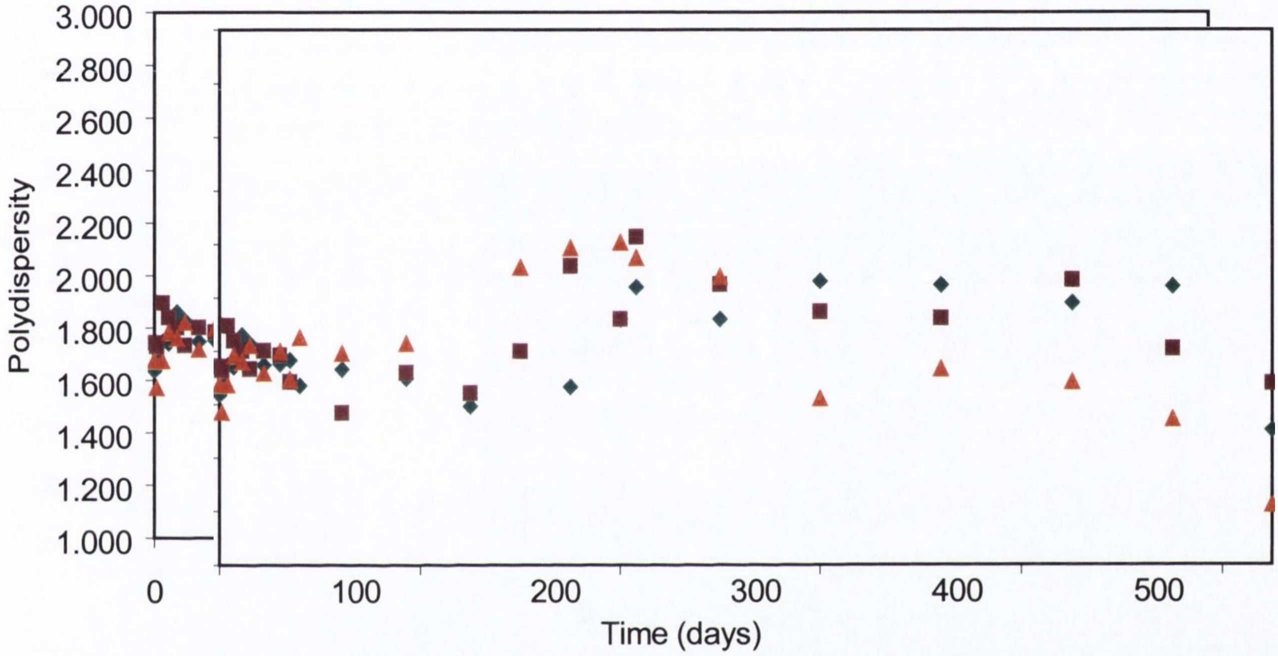


Figure 9.3 Evolution of polydispersity with incubation time for PLA R203 particles, for ◆ <1 micron, ■ <20 microns and ▲<50microns (*data points represent mean of two determinations).*

Figure 9.3 shows that during the lag phase the polydispersity remains constant consistent with the constant molecular weight profile demonstrated in Figure 9.2. A gradual increase in polydispersity was observed after the initial induction period. The increase in polydispersity reaches a maximum during the second molecular weight phase and then decreases after time *Tau*, to a value lower than the starting particle polydispersity. The increase in the polydispersity was fastest with a larger particle consistent with the faster rate of chain cleavage k_1 observed with increased particle size (Table 9.3). When time *Tau* is reached the reduction in polydispersity was also related to the particle size particularly for the <50 micron microspheres.

9.3.2 Polymer Mass Loss from PLA particles incubated in PBS pH 7.4 at 37°C

Weight loss from PLA particles was monitored as a function of incubation time and the profile is shown in Figure 9.4. An initial weight loss (5.9-7.2%) was observed for all particle sizes during the first 24 hours. This was followed by a period of negligible weight loss over the next approximately 200 days after which weight loss proceeded.

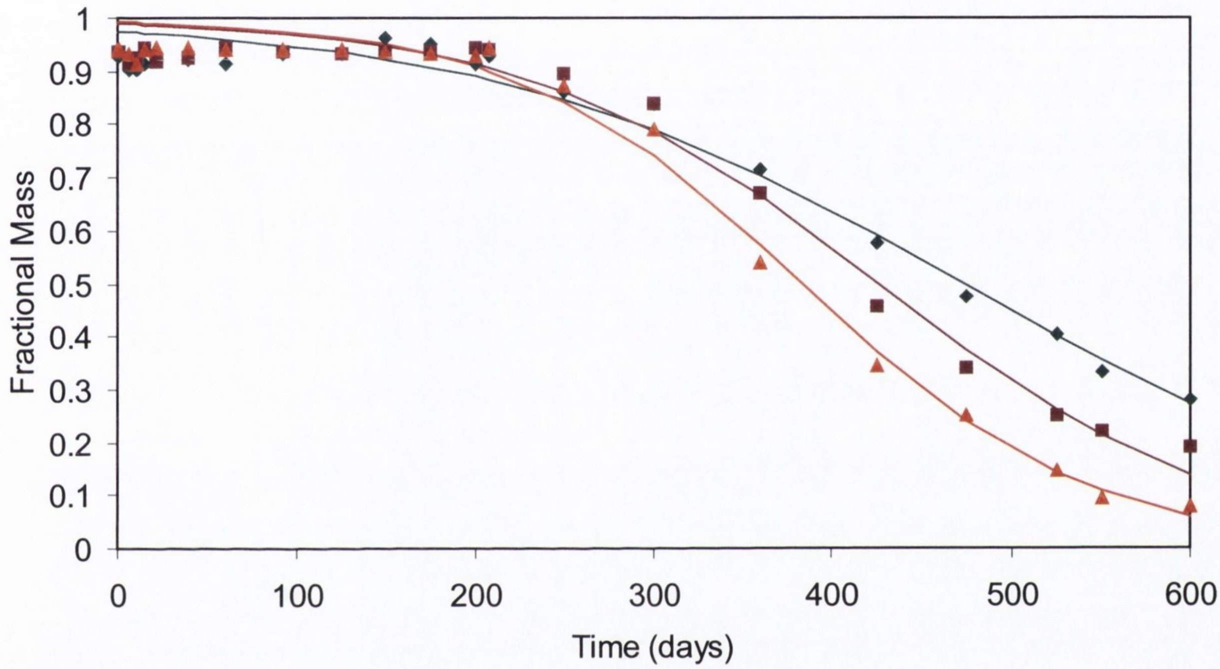


Figure 9.4 Weight loss of PLA particles at 37°C and pH 7.4, for ◆ <1 micron ■ <20 micron ▲ <50micron particles *(data points represent mean of two determinations)*. Data fitted to Equation 3.23.

It was observed that the phase change at time *Tau* demonstrated in the molecular weight profile (Table 9.3) occurred just after the onset of mass loss observed in Figure 9.4 similar to that observed for the PLGA systems studied in Chapter 7 and 8. Mass loss from the particles has two consequences for the rate of degradation, it decreases the amount of low molecular weight components in the particles and therefore reduces the autocatalytic effect and it changes the molecular weight distribution, demonstrated by the reduction in polydispersity during this phase. The mass loss from the PLA (R203) was fitted to the Prout-Tompkins equation (Equation 3.23) and the parameters for the erosion of PLA (*k* and *Tmax*) were evaluated (Table 9.4) for the three particle sizes.

Table 9.4 Parameters evaluated for the erosion of PLA particles determined using the Prout Tompkins Equation 3.25 *(standard deviation as determined by the model)*

Process	k (day^{-1})	*Tmax*	MSC	CD
< 1 micron	0.008	473.380	3.200	0.998
	±.0005	±9.0712		
< 20 microns	0.009	428.970	3.420	0.997
	±0.0006	±8.2501		
< 50 microns	0.013	383.122	3.995	0.998
	±0.0008	±6.5011		

Table 9.4 shows that the model parameter k and *Tmax* for the erosion of PLA particles were shown to be significantly different with differences in particle size. A comparison of the erosion parameters for PLA showed that the larger particles eroded at a faster rate than the corresponding smaller particle sizes.

9.3.3 Particle Morphology of PLA particles as a function of incubation time in phosphate buffer saline pH 7.4 at 37°C

SEM of the PLA (R203) particles taken after incubation for intervals of time in visking bags in phosphate buffer saline at 37°C is shown in Figure 9.5. Figure 9.5(a) shows that particles retain their initial morphology during the induction phase of the degradation profile. A gradual loss in shape and fusion of the microparticles was observed over the course of the degradation experiment however microsphere fusion was faster for the smaller particles. After 125 days some fusion of the particles was observed in the electron micrographs (Figure 9.5(b)). After 200 days electron micrographs (Figure 9.5(c)) show the presence of pores and pitting on the particle surface. Figure 9.5(d) shows that after 350 days the particles have become porous and have lost their original shape.

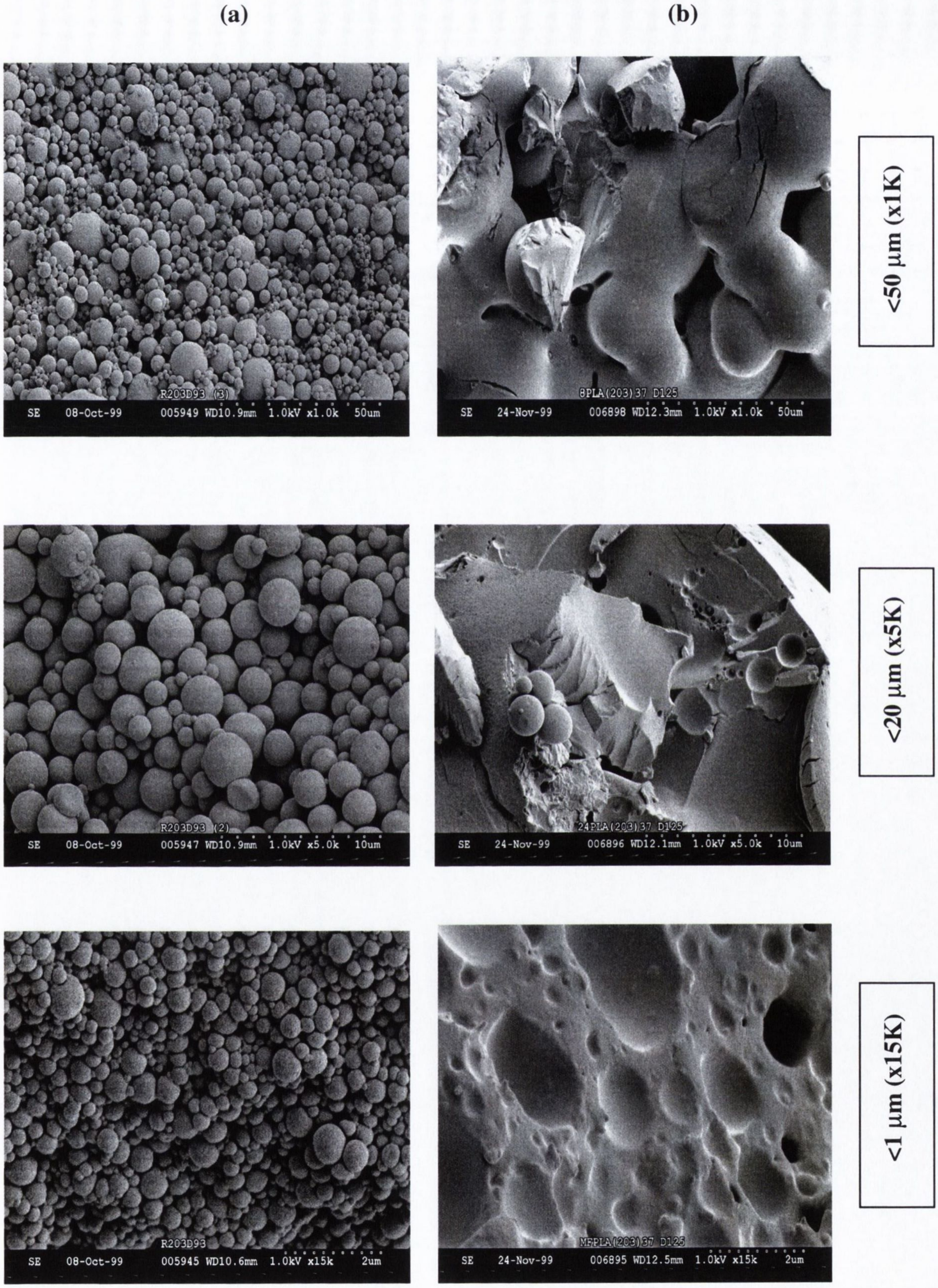


Figure 9.5 Electron micrographs of PLA (R203) particles after incubation in phosphate buffer saline at 37°C, for (a) 93 days and (b) 125 days.

(c) **(d)**

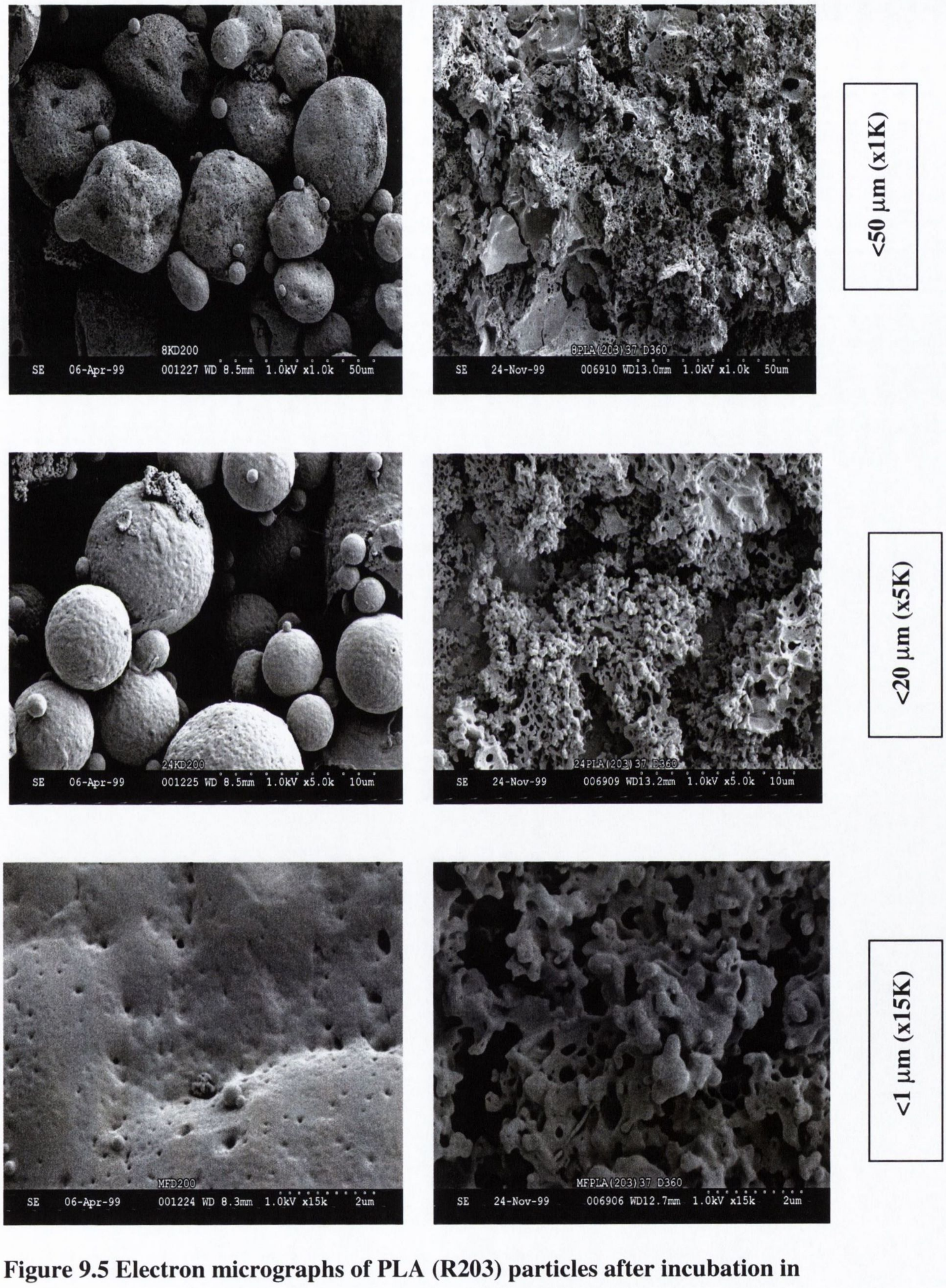


Figure 9.5 Electron micrographs of PLA (R203) particles after incubation in phosphate buffer saline at 37°C, for (c) 200 days and (d) 350 days.

9.3.4 Thermal Properties of PLA microspheres as a function of incubation time

DSC analysis was carried out on samples taken during each phase of the degradation profile of PLA R203 particles recovered from the incubation medium. Thermal analysis of the particles is shown in Table 9.5.

Table 9.5 Measured values of $T_{g(p)}$ (°C) for the degradation of PLA (R203) particles in phosphate buffer saline at 37°C *(data points represent the mean of two determinations).*

Time (Days)	<1μm $T_{g(p)}$ °C	<20μm $T_{g(p)}$ °C	<50μm $T_{g(p)}$ °C
0	55.6	55.7	55.1
35	55.4	55.6	55.9
93	54.6	54.1	54.8
150	51.8	51.4	51.4
208	51.9	50.2	49.0
250	48.1	47.0	44.9
300	47.6	48.0	44.8
360	48.3	47.9	43.9
525	41.4	42.42	40.1

Sample days 0 to 93 represent time-points that occur during the induction phase, sample days 150 to 250 are from the molecular weight decrease portion of the degradation profile while sample time points 300 to 525 are indicative of the erosion phase of the particles.

During the lag phase where no decrease in molecular weight or no mass loss is observed, samples were taken after 35 and 93 days incubation. DSC showed no change in $T_{g(p)}$ of the particles compared to the starting particles (day 0). Three samples were taken during the first phase of the degradation period during which the molecular weight of the polymer is decreasing according to a rate constant k_1, however no mass loss from the polymer has yet occurred. DSC analysis of these samples shows a progressively decreasing $T_{g(p)}$ consistent with a lowering of the molecular weight of the polymer. The decrease in the $T_{g(p)}$ occurred fastest from the larger particles during this phase. An overall decrease of ~4-6.5°C was observed over 100 days for the three particle sizes. In the erosion phase of the profile further reduction in the $T_{g(p)}$ of the polymer is observed with incubation time. The decrease in $T_{g(p)}$ from the larger microspheres has decreased to ~4.8°C over 225 days this is attributed to loss by erosion, of the low molecular weight polymer chains from the bulk polymer sample.

DCS thermograms of the degraded polymer particles are shown in Figure 9.6, these show the progressive decrease in the thermal peak associated with the $T_{g(p)}$ of the PLA polymer. Peak broadening is also observed for the degradation of the PLA polymer. At the later time points e.g. day 525 close inspection of the thermal peak also demonstrated evidence of bi-modality.

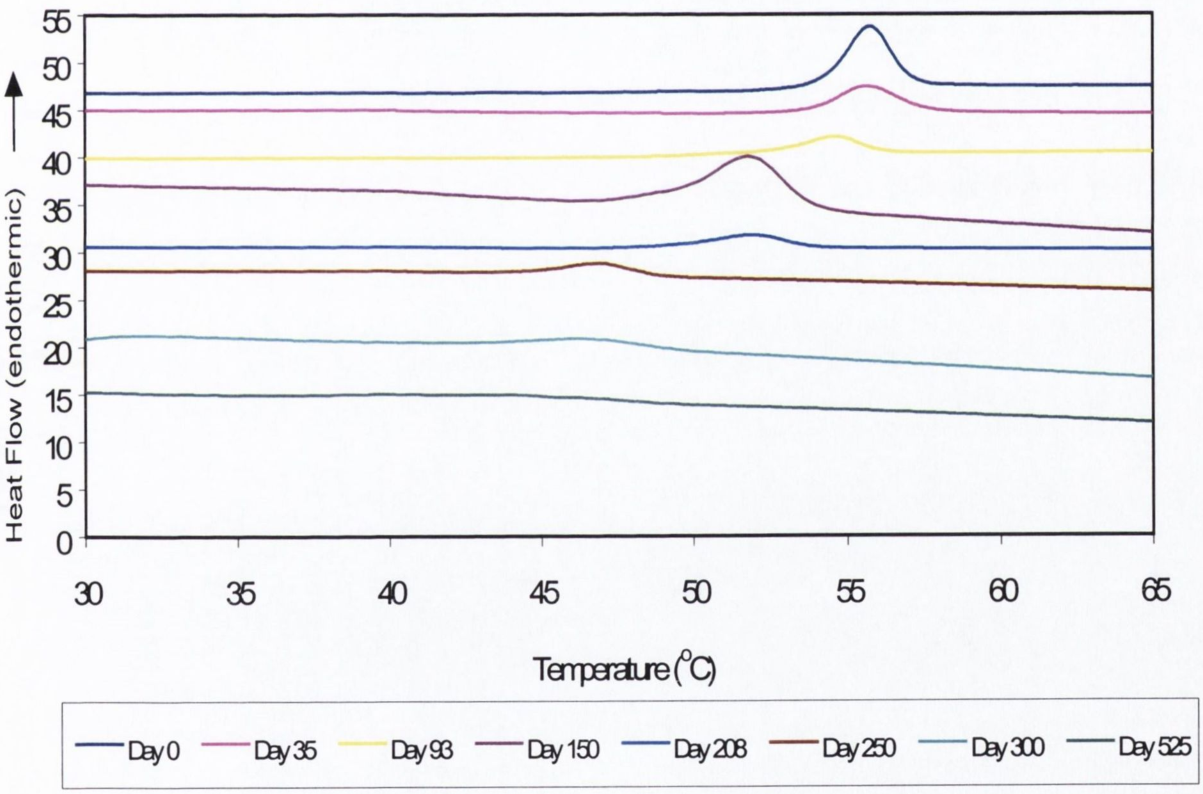


Figure 9.6 DSC thermograms for the degradation of PLA particles (<20 microns) as a function of incubation time in PBS at 37°C.

9.4 STUDY OF THE INFLUENCE OF MICROSPHERE PARTICLE SIZE ON THE DEGRADATION PROFILE FOR PLA (R203) PARTICLES INCUBATED IN VISKING BAGS AT pH 7.4 AND 5°C

The degradation properties of PLA at reduced incubation temperature of 5°C in phosphate buffered saline was also examined. PLA particles were incubated in visking bags and at intervals of time samples were taken and analysed.

9.4.1 Polymer molecular weight profile of PLA particles

The degradation profile of PLA particles at a reduced incubation temperature of 5°C was also evaluated for the three particle sizes. The molecular weight profile for PLA particles incubated at 5°C is shown in Figure 9.7.

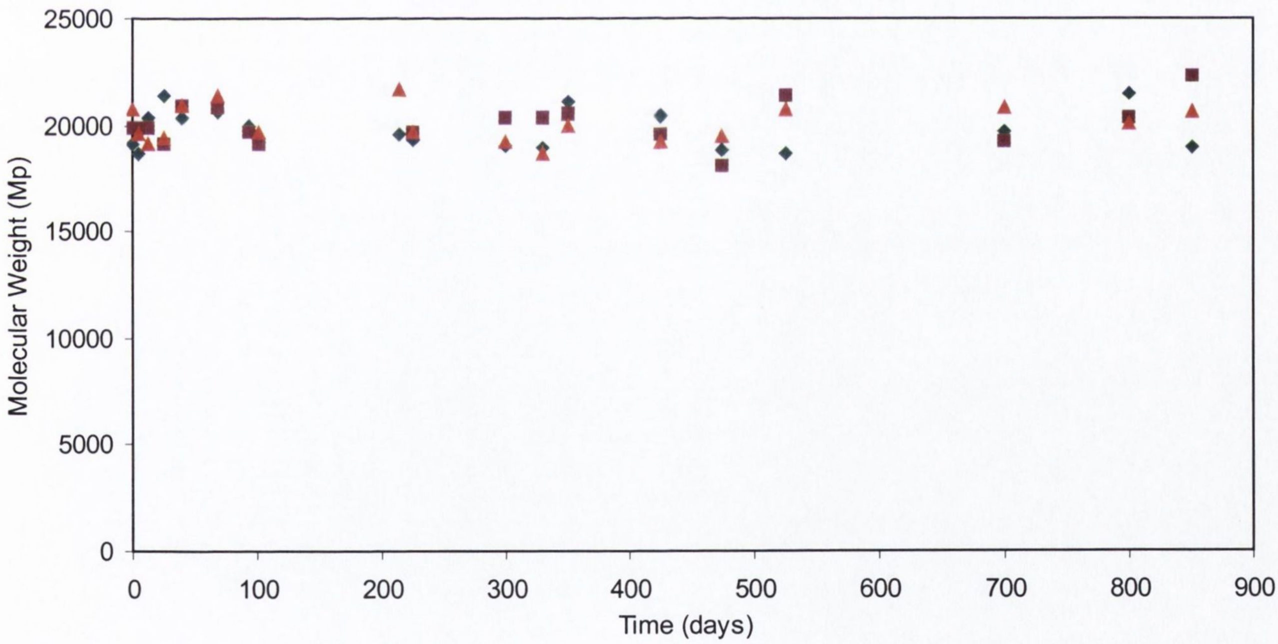


Figure 9.7 Influence of particle size on the molecular weight profiles of PLA particles incubated at pH 7.4 and 5°C, for ◆ <1 micron, ■ <20 micron and ▲<50micron particles *(data points represent the mean of two determinations)***.**

Figure 9.7 shows that over the 850-day study period no reduction in the polymer molecular weight was observed. The molecular weight was similar for all three particle sizes over the 850 day period studied. For PLA the polymer degradation of the PLA microspheres and nanospheres was also substantially slowed at lower incubation temperatures in agreement with that observed for PLGA microspheres in Chapter 8.

8.4.1 Polymer mass loss profile of PLA particles

Polymer mass loss from PLA (R203) microspheres of different particle sizes was also monitored over the study period of 850 days and is shown in Figure 9.8.

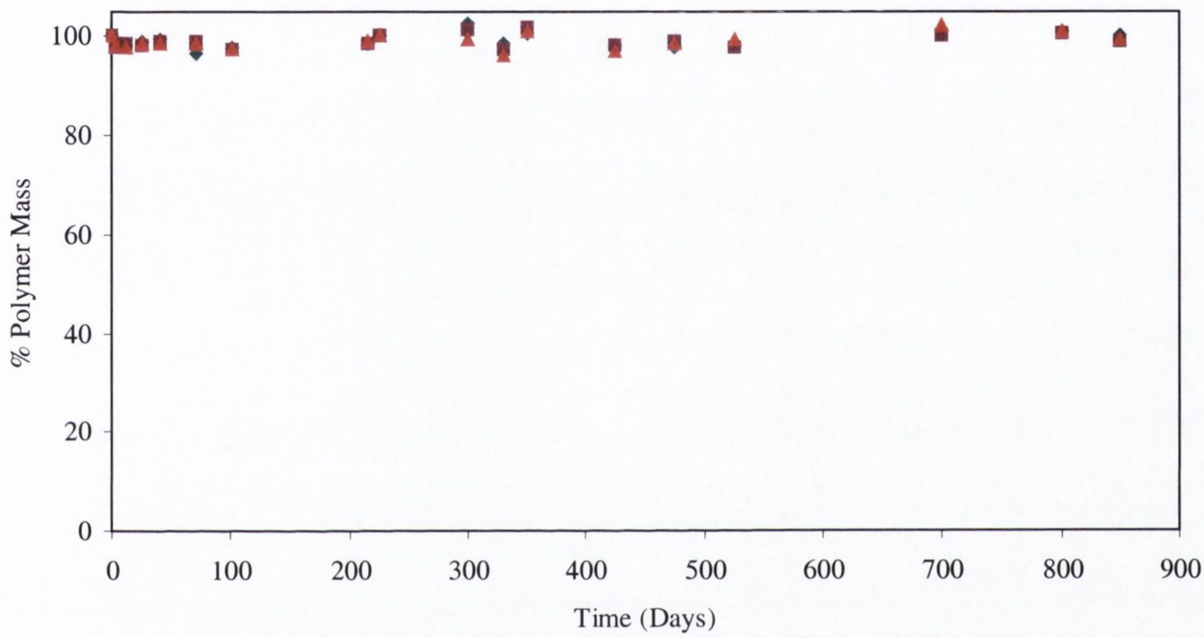
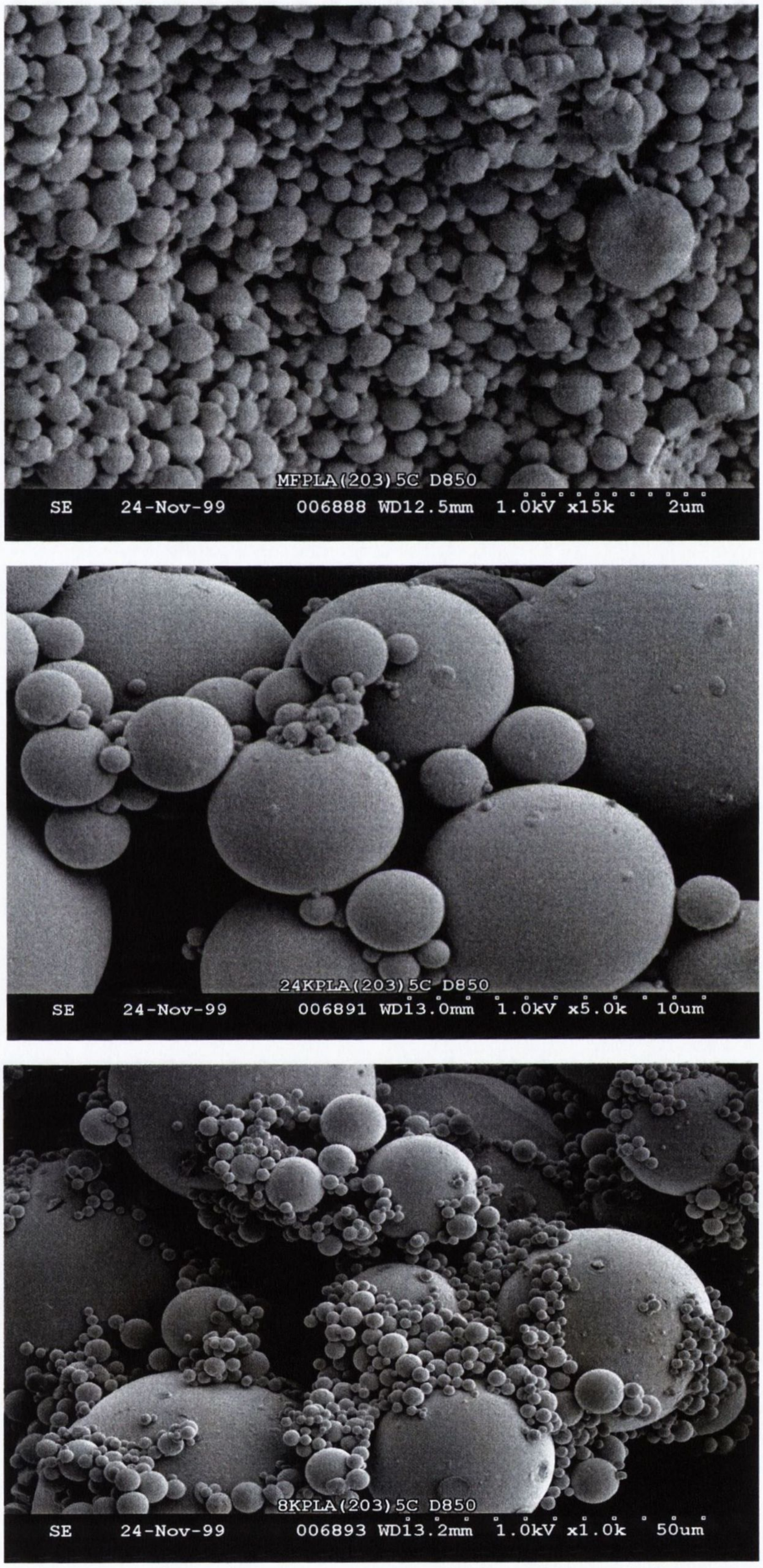


Figure 9.8 Influence of particle size on the weight loss of PLA particles incubated at pH 7.4 and 5°C, for ◆ <1 micron, ■ <20 micron and ▲<50micron particles *(data points represent the mean of two determinations).*

Figure 9.8 shows that no loss in mass was observed from the particles consistent with minimal degradation that was observed in the molecular weight profile. At 5°C polymer degradation was substantially slowed so that no polymer molecular weight or mass loss occurred during this study.

8.4.3 Scanning Electron Micrographs of PLA particles

Scanning electron micrographs of the particles were taken after day 850 incubation in visking bags at pH 7.4 and 5°C are shown in Figure 9.8.



(a)

(b)

(c)

Figure 9.8 Electron micrographs of PLA (R203) particles after incubation for 850 days at pH 7.4 and 5°C, for (a) <1 micron (b) <20 micron and (c) <50 micron particles.

SEM showed no visible loss in integrity of the microspheres over the study period compared to unincubated particles (Figure 9.1). Microspheres after 330 and 850 days are shown in Figure 9.8(a) and (b) and show no evidence of microsphere fusion or porosity that was demonstrated in the previous section to be indicative of polymer degradation.

9.4.4 Thermal Properties of PLA particles

Thermal analyses of samples taken at various time points over the study period were carried out by DSC. The values for $T_{g(p)}$ of the of PLA (R203) polymer, for the three particle sizes are shown in Table 9.6 as a function of incubation time.

Table 9.6 Measured values of $T_{g(p)}$ (°C) for the degradation of PLA (R203) particles in phosphate buffer saline at 5°C

Time (Days)	<1μm $T_{g(p)}$ °C	<20μm $T_{g(p)}$ °C	<50μm $T_{g(p)}$ °C
0	55.6	55.7	55.1
4	53.8	56.6	56.9
40	56.7	56.4	56.2
70	56.8	56.8	56.6
101	56.0	56.2	56.1
215	54.9	55.6	55.0
330	54.5	55.6	54.2
425	51.1	51.0	51.9
700	52.3	52.0	52.0
850	51.5	52.2	51.3

The $T_{g(p)}$ of the polymer also remained constant for the three particle sizes over the experimental time period up to day 330. After day 330 a small decrease in $T_{g(p)}$ was observed at the later time points. This decrease is thought to occur due to the retention of buffer salts in the microsphere samples after drying the incubated particles, this process occurs as a gradual accumulation in the polymer samples.

9.5 PREPARATION AND CHARACTERISATION OF MICROSPHERES OF DIFFERENT PLA POLYMER MOLECULAR WEIGHT

Polylactide particles were prepared in the <20 micron size range using Process B. Three different molecular weights of PLA were used to prepare microparticles and the characteristics of these microspheres were then determined. The effect of polymer molecular weight on the characteristics of PLA microparticles is shown in Table 9.7 and in Table 9.8. Thermal characteristics were compared to that of the starting polymer and the % differences are indicated in parenthesis.

Table 9.7 Characteristics of microparticles from different PLA polymers *(data points represent mean of three determinations ± standard deviation)*

Polymer	D10% (μm)	D50% (μm)	D90% (μm)	$T_{g(p)}$ (°C)	Yield (%)
R104	0.5	3.7	14.3	35.9(+0.9%)	81.5
	±0.02	±0.19	±0.46	±0.52	±1.58
R203	1.2	4.9	18.3	55.7(+0.0%)	85.7
	±0.03	±0.23	±0.13	±0.13	±0.91
R206	1.5	4.7	17.7	57.5(-3.9%)	91.5
	±0.01	±0.01	±0.50	±0.08	±4.01

Table 9.7 shows that the yield of microspheres was high for all manufacturing processes ≥80%. Particle size was comparable for all particles produced using varying molecular weight polymers. The thermal characteristics of the microparticles were similar to that of the corresponding starting polymers for the two low molecular weight polymers, while a 3.9% decrease in the $T_{g(p)}$ was observed for PLA R206.

Table 9.8 shows the polymer molecular weight characteristics of PLA microspheres made using three different polymer molecular weights. Molecular characteristics were compared to that of the starting polymer and the % differences are indicated in parenthesis.

Table 9.8 GPC characteristics of PLA microspheres *(data points represent mean of three determinations ± standard deviation).*

Polymer	Mn	Mp	Mw	Mz	PD
R104	2061 (3.24%)	4184(10.10%)	4736	7245	2.285
	±64.53	±140	±187	±344	±0.05153
R203	12302 (0.27%)	20914(1.56%)	20801	30218	1.6379
	±347	±703	±964	±1294	±0.0656
R206	44032(4.09%)	90062(2.66%)	81978	121082	1.86306
	±2322	±3566	±1440	±5302	±0.07959

Molecular weight determined by GPC of the PLA R104 particles showed a decrease in the molecular weight Mn and Mp (Table 9.8). The molecular weight of PLA R104 is very low therefore it is possible that some low molecular weight chains are solubilised during the manufacturing process. A negligible change in the molecular weight of PLA R203 is observed while for the high molecular weight polymer PLA R206 Mp and Mw values remained relatively unchanged indicating minimal polymer degradation during the production step. Molecular weight moments Mn and Mz have decreased relative to the starting polymer indicative of some long chain breakage due to mechanical degradation induced by the high shear rates involved in this process.

In chapter 8, the effect of polymer molecular weight on the degradation of PLGA was demonstrated. In this chapter the effect of polymer molecular weight will also be examined using the PLA series of polymers. The degradation profile of PLA R203 (Mp 19914) has already been discussed in section 9.3. The degradation of the low molecular weight PLA R104 (Mp 4184) is compared to the degradation profile of PLA R203 in this section. It was decided not to determine the degradation profile of PLA R206 (Mp 90062) at pH 7.4 and 37°C because this polymer would have an extremely long degradation profile.

9.6 THE EFFECT OF POLYMER MOLECULAR WEIGHT ON THE DEGRADATION PROFILE OF PLA MICROSPHERES IN VISKING BAGS IN PHOSPHATE BUFFER SALINE pH 7.4 AND 37°C

The degradation properties of the low molecular weight PLA (R104) were determined in visking bags in phosphate buffer saline pH7.4 and 37°C. PLA (R104) particles were incubated in visking bags and at intervals of time samples were taken and analysed. The degradation of the low molecular weight PLA R104 (Mp 4184) is compared to the degradation profile of PLA R203 in this section.

9.6.1 Polymer Molecular Weight profile for the degradation of PLA R104 microspheres incubated in PBS pH 7.4 at 37°C

The polymer molecular weight degradation profile of the low molecular weight PLA R104 particles (<20 microns) was first examined. The molecular weight degradation profile of the PLA R104 microspheres (<20 micron) is shown in Figure 9.9.

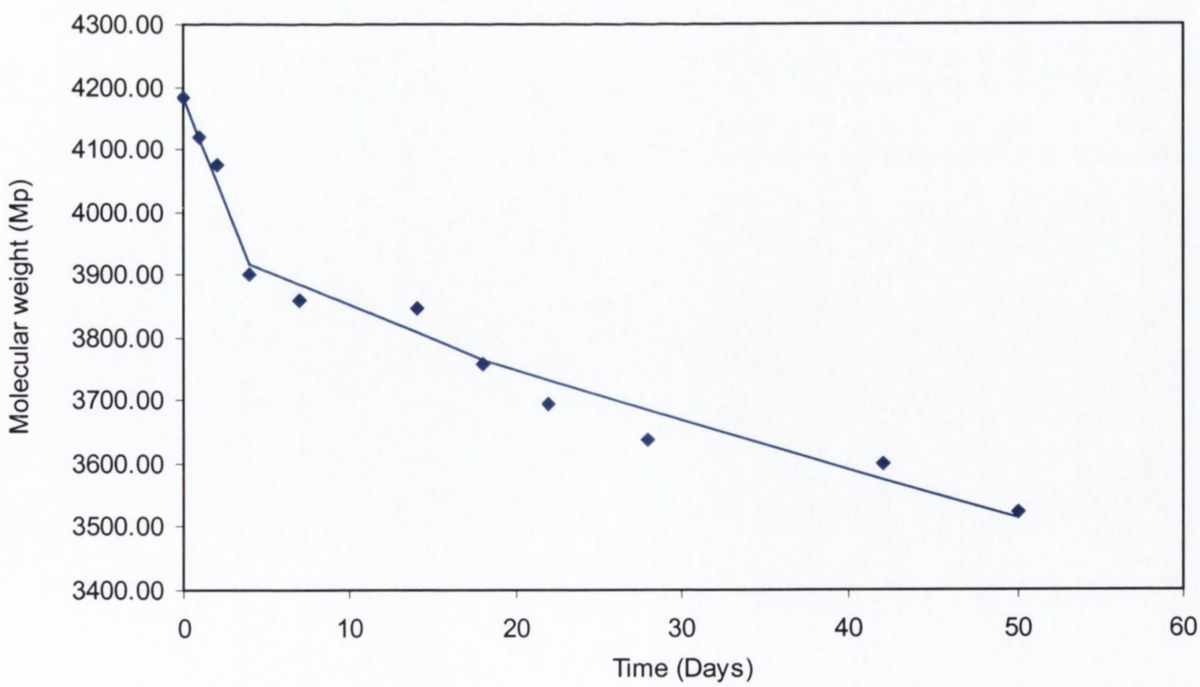


Figure 9.9 The degradation profile of PLA R104 microspheres (<20microns) in phosphate buffer saline pH 7.4 at 37°C fitted to Equation 7.3 *(data points represent mean of two determinations).*

The degradation profile of the PLA R203 polymer showed a molecular weight lag phase followed by a period of rapid reduction in molecular weight and in the final phase a slow loss of molecular weight (Figure 9.2). The lower molecular weight polymer PLA R104 also demonstrated a biphasic degradation profile (Figure 9.9). For PLA R104 microspheres an initial fast decrease in molecular weight was followed by a slow decrease in the molecular weight with incubation time. The molecular weight profile for PLA (R104) was fitted to equation 8.3 and the degradation parameters evaluated are shown in Table 9.9 where they are compared to the corresponding parameters for PLA (R203).

Table 9.9 Parameters for the degradation of PLA particles of different polymer molecular weight at 37°C in PBS pH 7.4 *(standard deviation represents error determined by the model).*

Polymer	*Tlag* (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
R104	0	0.017 ±0.0017	0.002 ±0.0002	4.688 ±0.6985	3874	0.983	3.544
R203	100.987 ±5.3978	0.006 ±0.0003	0.003 ±0.0006	282.532 ±19.2740	6891	0.994	4.732

Table 9.9 shows that for the degradation of PLA particles the lower molecular weight PLA (R104) polymer degraded at a faster rate of k_1 compared to the higher molecular weight PLA (R203) polymer. *Tau*, the time determined for the change in degradation phase also occurred at a much earlier stage for PLA (R104) compared to that for PLA (R203). The parameter k_2 was comparable for both polymers while the critical molecular weight was estimated to be 3874 for PLA (R104) and 7401) for PLA (R203) microspheres.

9.6.2 Polydispersity profile for the degradation of PLA R104 microspheres incubated in PBS pH 7.4 at 37°C

The polydispersity profile of the PLA R104 over the study period was also monitored and is shown in Figure 9.10. For PLA R104 the polymer and starting microspheres have a high polydispersity value of ~2. When the microspheres are placed in the incubation medium initially a drop in the polydispersity is observed (Figure 9.10).

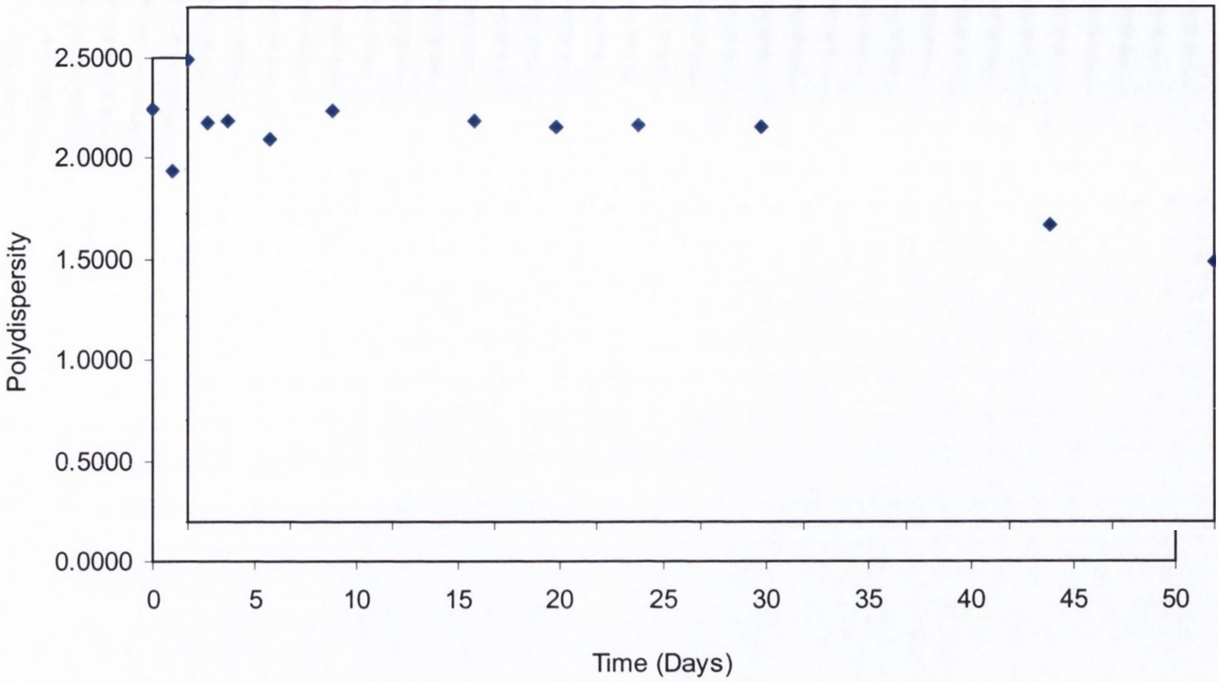


Figure 9.10 Polydispersity profile of PLA R104 microspheres in phosphate buffer saline pH 7.4 at 37°C *(data points represent the mean of two determinations).*

This drop in initial polydispersity is attributed to the loss of low molecular weight chains from the polymer distribution. After the initial reduction in the polydispersity value the polydispersity remains high and constant until day 42 when its value begins to decrease further (Figure 9.10).

9.6.3 Polymer Mass Loss profile for the degradation of PLA R104 microspheres incubated in PBS pH 7.4 at 37°C

The mass loss profile for the PLA R104 microspheres < 20 microns is shown in Figure 9.11. No mass loss lag phase was observed for this polymer in contrast to that observed to PLA (R203). An initial loss in weight loss (3.45%) was observed from the microspheres after the first 24 hours. This was followed by a gradual loss in weight from the microspheres over the rest of the profile. The parameters for the erosion phase of PLA (R104) are shown in Table 9.10 and are compared to the corresponding parameters for PLA (R203).

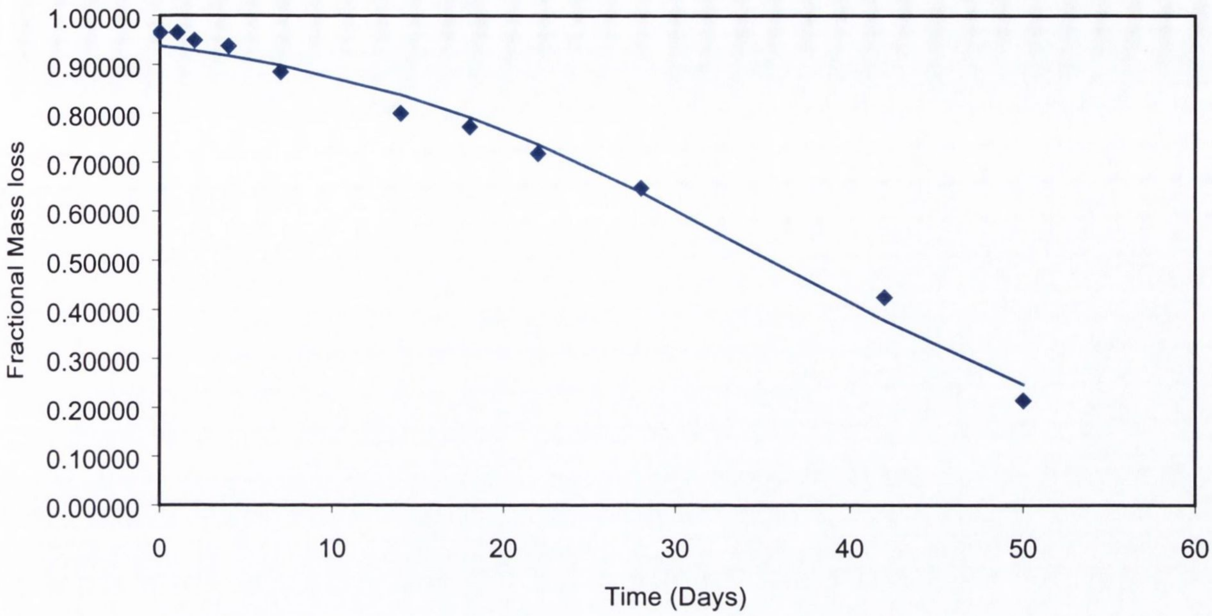


Figure 9.11 The degradation profile of PLA R104 microspheres (<20microns) in phosphate buffer saline pH 7.4 at 37°C *(data points represent the mean of two determinations).* Data fitted to Equation 3.23.

Table 9.10 Parameters evaluated for the erosion of PLA particles determined using Equation 3.23 *(standard deviation as determined by the model).*

Polymer	k (day^{-1})	*Tmax*	MSC	CD
R104	0.076 ±0.0031	35.496 ±4.2512	3.904	0.986
R203	0.009 ±0.0006	428.970 ±8.2501	3.420	0.997

For the R104 polymer the starting molecular weight of the polymer is low enough that release of low molecular weight oligomers can occur at a faster rate. Parameters for the erosion of PLA of two different molecular weights show that both the erosion rate constant and Tmax were dependent on the polymer molecular weight. Table 9.10 shows that both parameters were reduced for the lower molecular weight polymer.

9.8 SUMMARY

PLA was shown to be resistant to degradation during the processing used to produce either microparticles or nanoparticles. No appreciable polymer degradation was observed when either homogenisation or microfluidisation was used to produce PLA particles. Microspheres were produced in a range of particle sizes using PLA R203 and using Process B to produce microspheres with a range of PLA polymer molecular weights.

When particles from the three size ranges were incubated at 37°C polymer degradation was observed to be faster for the larger sized microspheres than for the nanoparticles this is consistent with that observed for the corresponding microspheres of the more hydrophilic polymer poly(d,l lactide-co-glycolide) particles in Chapter 7. The degradation rates observed for the molecular weight and erosion of PLA microspheres and nanospheres was much slower than that for PLGA. Because PLA 203 had a molecular weight of ~20,000 and was more hydrophobic than the PLGA that had been previously studied a triphasic degradation profile consisting of a lag phase, a loss in polymer molecular weight phase and an erosion phase, was demonstrated for this polymer. Polymer molecular weight and polydispersity, mass loss, thermal properties and particle morphology could be examined during the three phases of the degradation profile exhibited by these polymers.

CHAPTER 10

INVESTIGATING THE RELEASE MECHANISM OF FLUPHENAZINE FROM PLA/PLGA PARTICLES

10.1 INTRODUCTION

The poly-α-hydroxy aliphatic esters display many characteristics that make them attractive as sustained drug delivery systems. The performance of these systems is usually assessed by their ability to release the active form of an encapsulated drug. The release of drug from such carrier systems is actually a combination of a range of processes that are attributed to four basic phenomena, diffusion of drug from the particle surface 'burst effect', diffusion through the polymer, liberation as a result of matrix degradation and solubilisation followed by diffusion through pores and channels (Vert *et al.* 1991). Drug release from microparticles was examined to investigate the relationship between degradation of the polymer and the release process. Drug loaded PLA/PLGA particles were prepared by the solvent evaporation method. Fluphenazine HCl (F-HCl) was chosen for encapsulation into the particles because it had previously demonstrated sustained release behaviour (Ramtoola *et al.* 1992) and was therefore considered an appropriate model compound to study degradation controlled release from polyester microspheres.

10.2 PREPARATION AND CHARACTERISATION OF FLUPHENAZINE LOADED PLA AND PLGA MICROPARTICLES OF DIFFERENT DRUG LOADINGS

Particles were prepared using a modified single emulsion solvent evaporation procedure similar to that used for the production of drug free particles. In the case of drug loaded particles a suspension of the drug in acetone was added to the polymer solution before emulsification. The oil phase was emulsified into an external aqueous phase (Chapter 5, 5.3.5) that was buffered to pH 10.0 in order to minimise drug loss to the external phase.

10.2.1 Fluphenazine loading and particle size of PLA and PLGA microspheres

Microspheres were prepared and characterised using three different theoretical loadings namely 5, 10 and 20% w/w in a PLGA (RG504) and a PLA (R203) using Process B. The solvent evaporation method proved effective for the encapsulation of fluphenazine HCl in the microsphere system. An encapsulation efficiency greater than 80% was achieved at all three loadings for both polymer systems. Table 10.1 and Table 10.2 show the characteristics of the drug loaded PLA and PLGA microspheres respectively.

Table 10.1 Characteristics of fluphenazine HCl loaded PLA (R203) microspheres *(data represents mean of three determinations± standard deviation)*

Theoretical Fluphenazine HCl loading (%)	Actual Loading(%)	Encapsulation efficiency (%)	Particle size $D_{50\%}$ (μm)	Particle size $D_{90\%}$ (μm)
5	4.5	89.9	6.0	18.5
	±0.06	±1.11	±0.64	±2.12
10	9.9	98.6	6.0	17.2
	±0.47	±4.70	±1.20	±1.34
20	16.8	84.1	7.0	19.1
	±0.78	±3.91	±0.26	±0.80

Table 10.2 Characteristics of fluphenazine HCl loaded PLGA (RG504) microspheres *(data represents mean of three determinations±standard deviation)*

Theoretical Fluphenazine HCl loading (%)	Actual Loading(%)	Encapsulation efficiency (%)	Particle size $D_{50\%}$ (μm)	Particle size $D_{90\%}$ (μm)
5	4.2	84.0	4.1	19.5
	±0.12	±2.30	±0.15	±0.01
10	8.2	81.7	7.7	20.5
	±0.22	±2.21	±0.41	±0.68
20	16.6	83.1	7.9	20.5
	±0.33	±1.67	±0.16	±0.10

Particle size distributions were monomodal for the microspheres at the three drug loadings. For PLA and PLGA the particle size of the fluphenazine loaded microparticles was similar to that of the corresponding drug free microspheres. The $D_{90\%}$ for all particle sizes was ~20microns.

10.2.2 Polymer molecular weight characteristics of fluphenazine HCl loaded PLA and PLGA microspheres

GPC analysis of the fluphenazine HCl loaded microsphere systems was carried out and compared to that of the starting polymer and that of drug free microspheres manufactured using the same formulation as that of the drug loaded microspheres. The molecular weight characteristics of the fluphenazine HCl loaded PLA and PLGA microsphere are shown in Tables 10.3 and 10.4 respectively.

Table 10.3 Effect of formulation parameters on molecular weight of PLA (R203) microparticles *(data points represent mean of three determinations ± standard deviation)*

Matrix	Mn	Mp	Mw	Mz	P
PLA Polymer	12146	18015	19759	33643	1.627
	±520	±568	±467	±221	±0.0878
Drug Free microspheres	12302	19914	20801	30218	1.638
(Process B)	±347	±703	±964	±1294	±0.0661
Drug Free microspheres	12076	20071	19786	30605	1.640
(Process D)	±564	±889	±820	±1327	±0.0798
4.5% w/w F-HCl	13011	19353	20691	30784	1.592
loaded microspheres	±445	±673	±91	±963	±0.0565
9.9% w/w F-HCl	12030	19580	20167	31293	1.659
loaded microspheres	±322	±504	±561	±616.6	±0.0014
16.8% w/w F-HCl	12041	19520	20291	29539	1.616
loaded microspheres	±408	±829	±948	±1154	±0.0507

The presence of the additional solvent, added to accelerate solvent removal or the buffering of the emulsification medium to pH 10.0 using a carbonate buffer (Chapter 5, section 5.3.5, Process D) caused only a slight decrease in the molecular weight of the polymer which was comparable to that of drug free microparticles prepared without acetone or carbonate buffer (Process B). Microspheres produced using PLA (R203) showed no polymer degradation due to the presence of the drug in the formulation. Using the PLA (R203) polymer it was possible to produce microspheres with the same molecular weight characteristics as that of the starting polymer (Table 10.3).

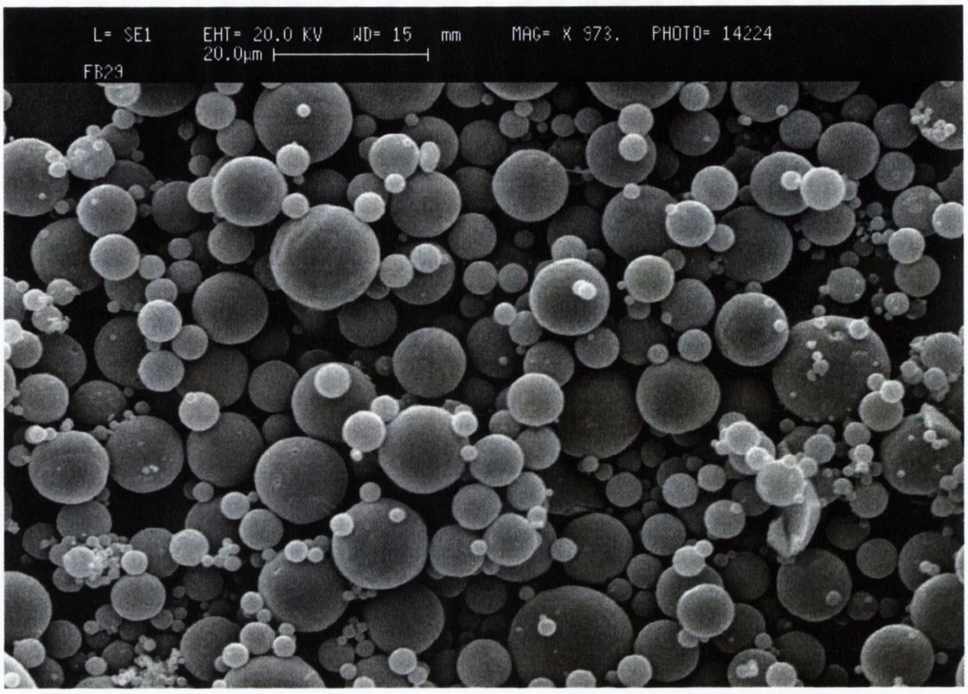
Table 10.4 Effect of formulation on molecular weight of PLGA (RG504) microparticles *(data points represent mean of three determinations ± standard deviation)*

Matrix	Mn	Mp	Mw	Mz	P
PLGA Polymer	27161	45963	44059	65030	1.622
	±612	±479	±1187	±2889	±0.0165
Drug Free PLGA microspheres	26109	42124	41312	59255	1.586
(Process B)	±598	±930	±2104	±1370	±0.0335
Drug Free PLGA microspheres	24714	40918	42032	63020	1.700
(Process D)	±368	±2037	±1973	±2801	±0.0554
4.2% w/w F-HCl loaded PLGA	19690	32919	31649	43956	1.608
microspheres	±421	±1223	±1292	±1884	±0.0324
8.2% w/w F-HCl loaded PLGA	15590	27294	27173	42384	1.745
microspheres	±458	±1159	±616	±2095	±0.0861
16.6% w/w F-HCl loaded PLGA	15411	22721	25280	37297	1.641
microspheres	±550	±356	±470	±695	±0.0287

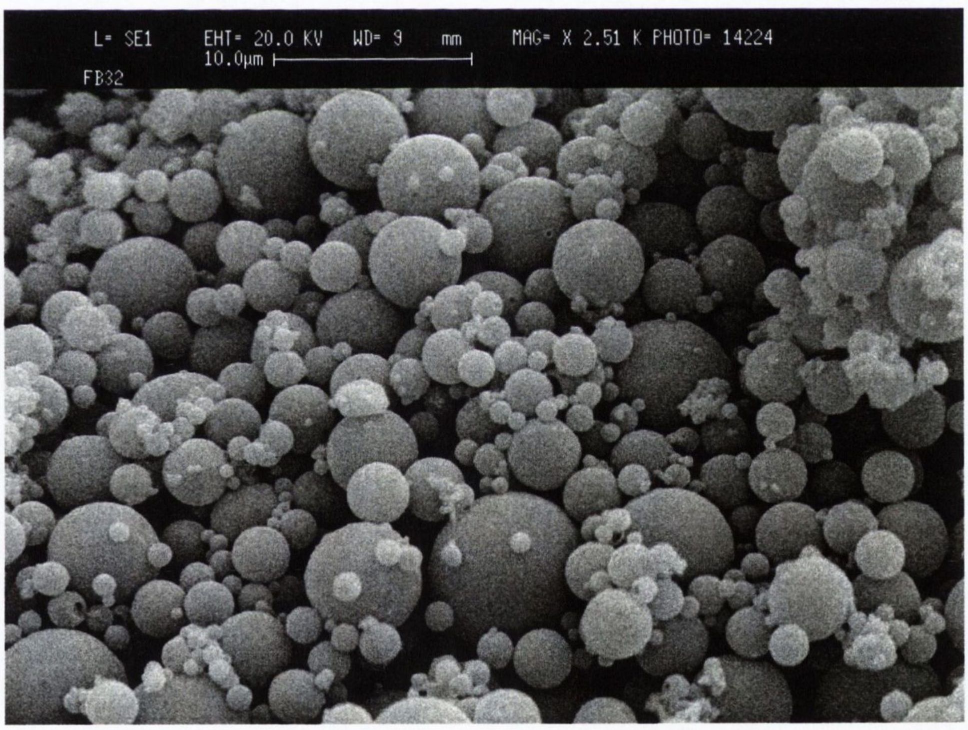
The presence of the drug considerably decreases the molecular weight of the PLGA polymer during the manufacturing process. The effect of drug loading on the molecular weight of the polymer was examined at the three drug loadings and it was observed that the presence of the fluphenazine HCl in the microspheres during processing considerably decreased the molecular weight of the starting polymer. Furthermore it was observed that the decrease in molecular weight was proportional to the amount of drug in the formulation with higher loadings causing a larger decrease in the molecular weight. Several authors have documented the ability of acidic/basic components, either in the form of encapsulated drug or by the use of different buffer systems, to catalyse the degradation of these polymer systems.

10.2.3 Scanning Electron Microscopy

Scanning electron micrographs of all fluphenazine loaded microspheres were smooth and spherical in nature with no evidence of drug crystals. Electron micrographs of the 10% theoretical loaded PLA and PLGA microspheres are shown in Figure 10.1 for illustration.



(a)



(b)

Figure 10.1 Electron micrographs of fluphenazine HCl loaded microspheres, for (a) 9.9% w/w fluphenazine HCl loaded PLA (R203) and (b) 8.2% w/w fluphenazine HCl loaded PLGA (RG504) microspheres.

10.2.4 Differential scanning Calorimetry

The 10% theoretical loaded fluphenazine HCl polymer system was used to characterise the PLA (R203) and PLGA (RG504) microspheres. DSC showed the glass transition temperature of the amorphous copolymer at 53.2°C for PLGA and at 55.7°C for PLA. fluphenazine HCl has a crystalline melting temperature of 238°C. Endotherms corresponding to both the copolymer and the crystalline drug were evident in thermograms of the physical mixtures (Appendix V).

There was no evidence of crystalline drug material in the microspheres at any drug loadings investigated. The presence of fluphenazine HCl caused a loading dependant reduction in the $T_{g(p)}$ of the polymer compared to the starting polymer (Figure 10.3).

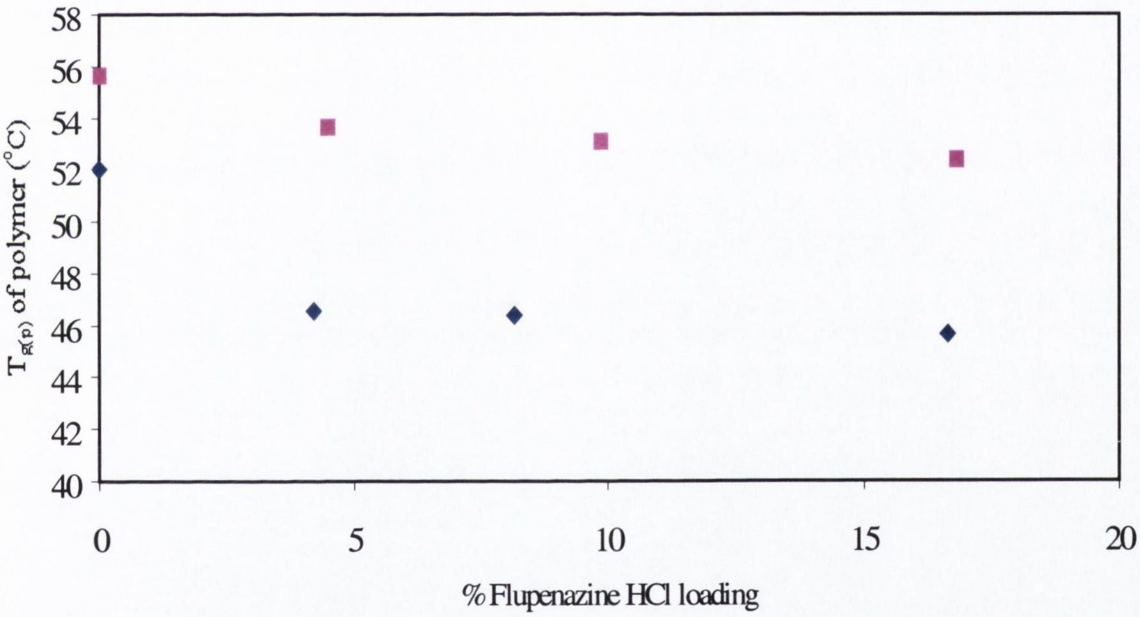


Figure 10.2 The influence of fluphenazine HCl on the $T_{g(p)}$ of PLA and PLGA polymer microspheres, for ■ PLA (R203) ◆ PLGA (RG504) microspheres.

Figure 10.3 shows the effect of incorporation of fluphenazine HCl in the formulation of PLA and PLGA microparticles. The presence of even low loadings of fluphenazine HCl in the microspheres caused a significant reduction in the $T_{g(p)}$ of the polymer. As the fluphenazine HCl loading in the microspheres increased a gradual reduction in the $T_{g(p)}$ of the microspheres was observed for fluphenazine HCl loaded PLA and PLGA microspheres.

10.2.5 X-ray diffraction

Powder x-ray diffraction analysis carried out on PLA (R203) and PLGA (RG504) showed that both polymers were amorphous, while fluphenazine HCl was a crystalline material (Appendix V). For the PLA and PLGA fluphenazine HCl loaded systems, 10% theoretical loaded microspheres were used to characterise the properties of the matrix. Analysis of a 10% physical mix of the drug and polymer was also carried out. The polymer drug physical mixture showed a diffraction pattern consistent with a mixture of amorphous and crystalline material. The diffraction pattern of the PLA and PLGA microspheres showed no evidence of crystalline material indicating that the drug molecules are most probably molecularly dispersed within the polymer matrix (Appendix V).

10.2.6 X-ray elemental analysis

The 10% theoretical loaded system was also evaluated by x-ray elemental analysis to detect the presence of the hydrochloride salt in the matrix (Table 10.5).

Table 10.5 Elemental analysis of PLA (R203) and PLGA (RG504) blank microparticles and drug loaded microparticles *(data points represent mean of three determinations ± standard deviation).*

Element	R203	RG504	9.9% F-HCl R203	8.2% F-HCl RG504	10% F-HCl R203 PM	10% F-HCl RG504 PM
C	90.01	87.40	87.89	85.66	78.61	70.14
	±0.795	±1.937	±1.605	±0.528	3.74	1.578
O	9.93	12.58	8.33	10.42	17.74	27.03
	±0.808	±1.939	±2.304	±1.218	4.455	1.051
S	0.00	0.01	3.04	2.64	0.81	0.40
	±N/A	±0.023	±2.304	±1.479	0.422	0.413
Cl	0.05	0.00	0.37	0.31	1.82	0.82
	±0.021	±N/A	±0.117	±0.147	0.563	0.089

Blank microspheres did not show the presence of chlorine in the matrix; therefore any residual chlorine that could remain in the matrix was not detected by x-ray elemental analysis. In contrast the PLA and PLGA microspheres contain 9.9 and 8.2% w/w Fluphenazine HCl did show the presence of chlorine in the x-ray pattern. The detected chlorine content was considerably reduced to the corresponding physical mix. It is therefore assumed that the majority of encapsulated Fluphenazine is present as the free base.

To account for the difference between the state of Fluphenazine through this work where the actual loaded drug is discussed Fluphenazine is denoted as 'Fluphenazine HCl' to account for the fact that the drug is utilised as the HCl form, in discussions of the quantitated % loading, release and release mechanism it will be denoted 'Fluphenazine'

10.3 THE EFFECT OF FLUPHENAZINE HCl LOADING ON THE RELEASE MECHANISM FROM PLA AND PLGA MICROSPHERES

Prior to carrying out dissolution studies the saturated solubility of Fluphenazine HCl was determined in phosphate buffer pH 7.4 (Method 5.3.2, Appendix III), all dissolution studies were then carried out under sink conditions. Release studies were carried out by dispersing the microspheres in the dissolution medium to first ascertain the physiochemical properties that influence the release process in this dissolution system. Later in this chapter release from microspheres contained within visking bags will also be evaluated. The effect of drug loading was examined by comparing the release profile of the 5, 10 and 20% w/w theoretical loaded fluphenazine HCl microspheres of PLA and PLGA.

10.3.1 The effect of Fluphenazine loading on the Release from PLA (R203) and PLGA (RG504) microspheres

Microspheres demonstrated a triphasic release pattern that consisted of a burst effect, a lag phase and a polymer decomposition controlled release phase. The % drug released in the burst effect, the length of the lag phase and the polymer decomposition controlled release phase were all dependent on microsphere loading. Previously a drug release model used to describe polymer decomposition dependent drug release from PLA/PLGA systems for the release of fluphenazine (Ramtoola *et al.* 1992), procaine (Fitzgerald *et al.* 1991) and diltiazem base (Fitzgerald and Corrigan 1993) was evaluated (Equation 3.23). Modifications of this model allowed its evaluation to predict diffusion controlled release associated with the surface or the microsphere 'burst effect' and decomposition controlled release from levamisole discs (Corrigan *et al.* 1998) using the following equation:

$$F_{tot} = (1 - F_{BIN})x + F_{BIN}\left(1 - e^{-k1(R).t)}\right) \qquad 10.1$$

Where F_{tot} is the total fraction of drug released at time t, F_{BIN} is the total burst fraction at time t, $k_{1(R)}$ is the rate constant and x is given by Equation 3.23. The parameters k, $Tmax$ and k_1 are denoted by the subscripts (R) to denote they are the rate constants of the release profile. This model was evaluated to describe the release of fluphenazine at different loadings from PLA and PLGA microspheres systems. fluphenazine release was used as a marker to measure surface controlled and decomposition controlled release from PLA and PLGA microspheres fitted using Equation 10.2 (Figure10.3 and 10.4).

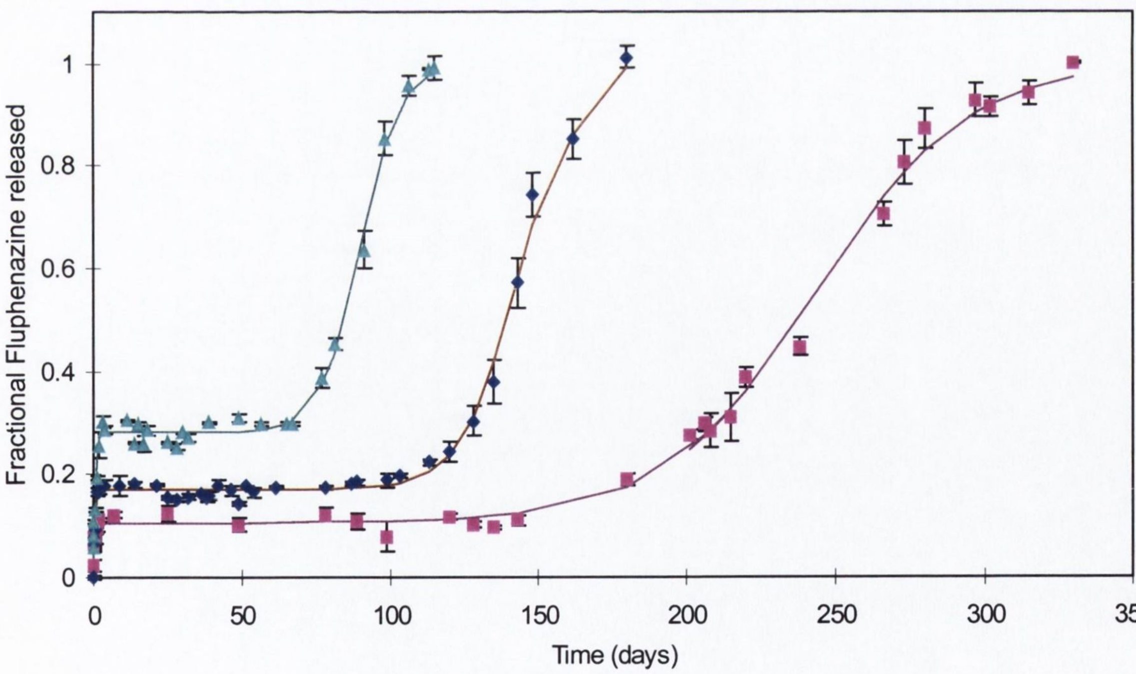


Figure 10.3 Influence of fluphenazine loading (w/w) on the release from PLA (R203) microspheres at 37°C in phosphate buffer saline pH 7.4, for ■ 4.5%, ◆ 9.9 % and ▲ 16.8% fluphenazine loaded microspheres *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

The release profiles of fluphenazine from PLA microspheres in phosphate buffer was shown to be dependent on the drug loading of the microspheres (Figure 10.3). Release was slower as the loading of the microspheres decreased. Previous reports have also shown a relationship between loading and release rate (Tsai *et al.* 1986, Spenlehauer *et al.* 1988, and Ramtoola *et al.* 1992). Microspheres demonstrated a triphasic release pattern that consisted of a burst effect, a lag phase and a polymer decomposition controlled release phase. A higher percentage burst effect was observed for the higher loaded microspheres this can be attributed to a higher concentration of Fluphenazine at the surface of the microparticles. In the second phase of the release profile, the lag no drug release occurs from the polymer matrix as the polymer has not yet sufficiently degraded to allow release of drug trapped within the polymer. During the third phase of the release profile, decomposition of the polymer controls the release of the remaining drug material until complete dissolution occurs.

Figure 10.3.2 shows the effect of drug loading on the release from RG504 microspheres. The effect of drug loading on the release rate was again apparent for the PLGA microspheres and was considerably faster compared to the PLA microspheres (Figure 10.4).

The modified Prout-Tompkins model (Equation 10.1) was found to describe the release kinetics of Fluphenazine from the PLA and PLGA microspheres. Parameters obtained for the dissolution controlled and degradation components of the model were determined for PLA and PLGA and are shown in Table 10.6 and Table 10.7 respectively. The modified Prout-Tompkins equation assumes two additive release processes: an exponential fast release phase with parameter F_{BIN} and $k_{1(R)}$ and the degradation controlled release phase with parameters $Tmax_{(R)}$ and $k_{(R)}$.

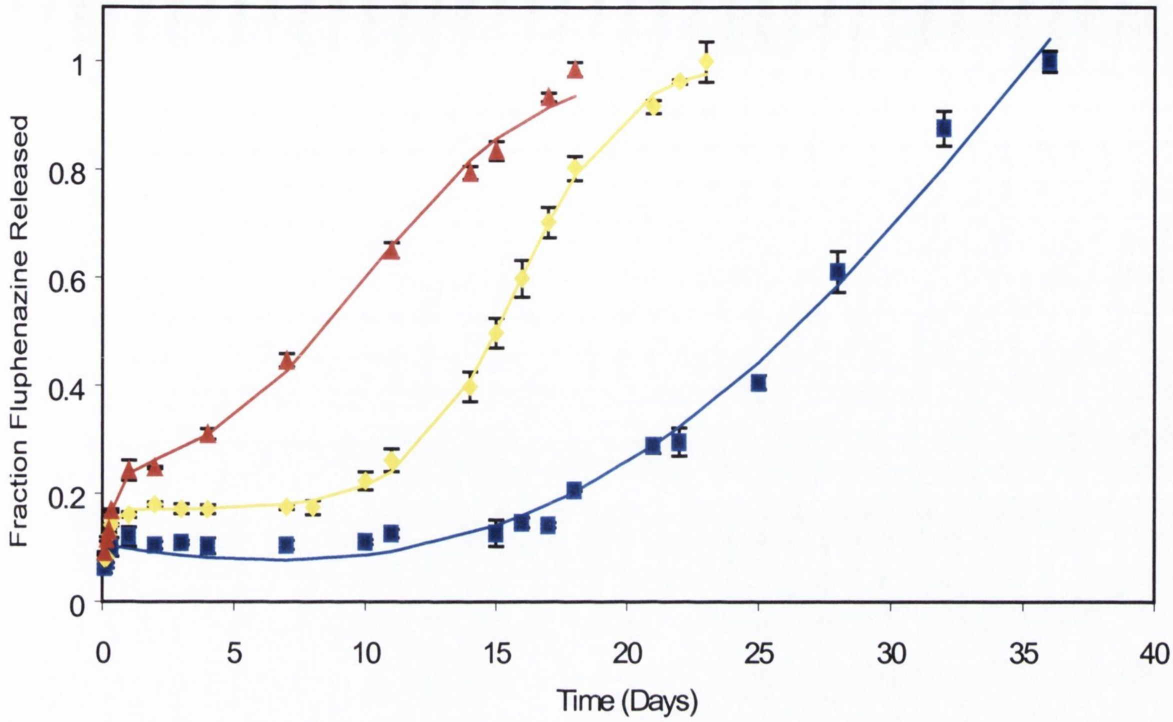


Figure 10.4 Influence of fluphenazine loading (w/w) on the release from PLGA (RG504) microspheres at 37°C in phosphate buffer saline pH 7.4, for ■ 4.50%, ◆ 9.86% and ▲16.81% fluphenazine loaded microspheres *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

Table 10.6 Parameters for the release of fluphenazine from PLA (R203) microspheres fitted to Equation 10.1 *(standard deviation represents that determined by the model).*

% w/w Fluphenazine	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
4.50	0.0396 ±0.0015	243.273 ±1.2635	0.107 ±0.0067	4.490 ±1.5212	5.192	0.996
9.86	0.105 ±0.0057	143.230 ±0.511	0.170 ±0.0405	2.656 ±0.4831	4.707	0.993
16.81	0.149 ±0.0091	89.788 ±0.512	0.282 ±0.0053	1.675 ±0.2064	5.000	0.995

Table 10.7 Parameters for the release of fluphenazine from PLGA (RG504) microspheres fitted to Equation 10.1 *(standard deviation represents that determined by the model).*

% w/w Fluphenazine	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
4.20	0.303 ±0.0181	26.751 ±0.2307	0.113 ±0.0082	7.724 ±3.0262	4.603	0.993
8.17	0.491 ±0.0192	15.836 ±0.0761	0.170 ±0.0061	5.112 ±0.7170	6.056	0.998
16.62	0.304 ±0.0255	10.029 ±0.4461	0.285 ±0.0341	2.967 ±0.6540	5.035	0.996

During the dissolution controlled release process F_{BIN} was found to increase with increase in loading and $k_{1(r)}$ was found to reduce. This was true for both PLA and PLGA microspheres with different loadings of fluphenazine. For the degradation component of the release *Tmax* was also found to be dependent on the % drug loading. $Tmax_{(R)}$ decreased as the % fluphenazine loading of the microspheres increased while in general $k_{(R)}$ increased with drug loading although for PLGA RG504 microspheres at the 16.6% loading the relationship is not continued. For this case the discontinuation of the linear relationship is thought to be due to the lack of sufficient data points in the 16.6% loaded microspheres during the lag phase which influences the values obtained by the model.

A comparison of the parameters for release process from the PLA loaded microspheres and PLGA loaded microspheres shows that during the diffusion process in general the % surface diffusion controlled release as determined by F_{BIN} was comparable for the two polymers. At loadings of ~ 5% the percentage of fluphenazine released from the surface was ~11%, at 8-10% the percentage of fluphenazine released from the surface was ~17% while at 16-17% the range was 20-28% (Table 10.6 and Table 10.7). However the rate of release of fluphenazine by surface diffusion as denoted by $k_{1(r)}$ was observed to be faster from PLGA polymer microspheres than from the corresponding PLA microspheres, at all drug loadings studied. This is attributed to the considerably more hydrophilic nature of PLGA and therefore the surface of the PLGA microspheres probably exhibits a higher wettability than PLA microspheres.

10.3.2 The effect of fluphenazine loading on the polymer molecular weight profile of PLA (R203) microspheres

In order to elucidate the factors that govern the release of fluphenazine from PLA and PLGA based systems it was deemed necessary to follow parallel changes in polymer matrix properties with release of the drug. In section 10.2.3 it was shown that the presence of fluphenazine HCl during formulation had the ability to influence the molecular weight of the resultant microspheres it was therefore decided to monitor the polymer degradation process of the drug loaded microspheres as they released fluphenazine. A comparison with the corresponding drug free microspheres whose degradation profile were determined in Chapters 7 and 9 was carried out.

At intervals of time concurrent with drug release polymer samples were recovered from the dissolution medium, dried and analysed by gel permeation chromatography to determine if the dependence of rate of release on the drug loading was mirrored in the evolution of the molecular weight. Polymer samples for PLA taken and analysed by GPC for the three loadings of fluphenazine are shown in Figure 10.5.

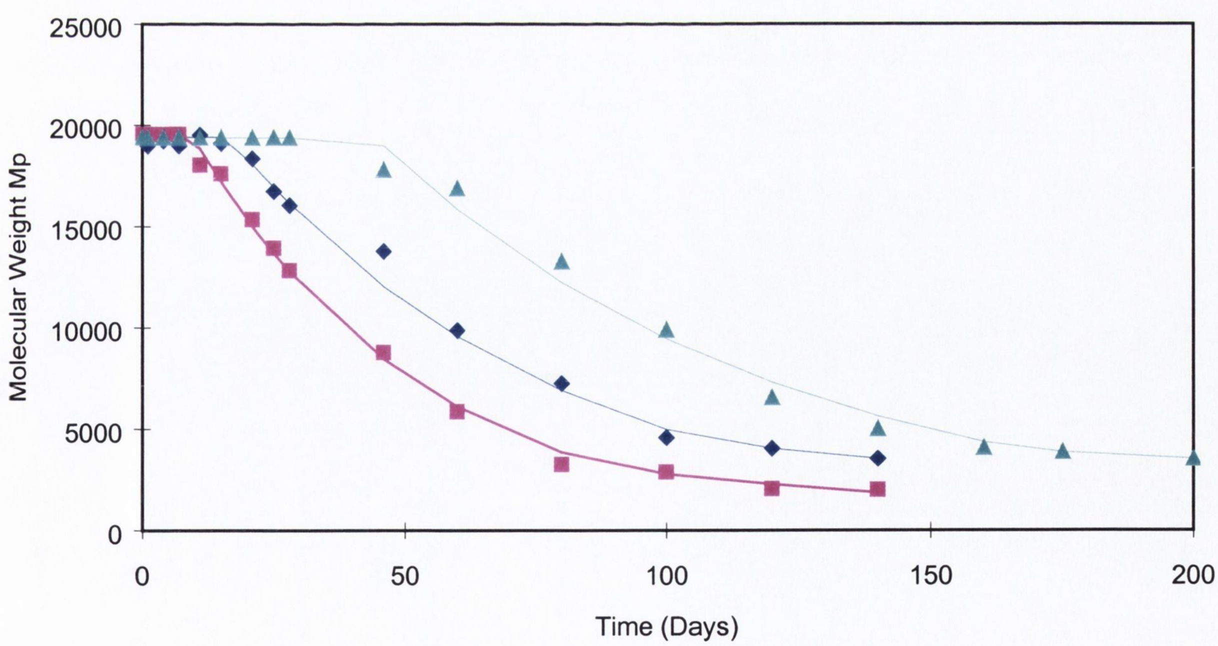


Figure 10.5 The influence of %w/w fluphenazine loading on the degradation of the PLA (R203) microspheres, for ▲ 4.50 %, ◆ 9.86 % and ■ 16.81% fluphenazine loaded microspheres *(data points represent mean of two determinations).* Data points fitted to Equation 8.2.

Figure 10.5 shows that the fluphenazine loaded PLA (R203) microspheres demonstrated a tri-phasic degradation pattern similar to that shown for PLA (R203) blank microspheres. A loading dependent decrease in the molecular weight of the polymer was observed for the PLA (R203) microspheres. As the %w/w fluphenazine loading in the microspheres increased the degradation rate of the polymer increased. A comparison of the degradation of the 9.9% fluphenazine HCl loaded PLA (R203) microspheres with the corresponding degradation rate of PLA (R203) blank microspheres showed that the presence of fluphenazine considerably shortened the degradation profile of the polymer (Figure 10.6).

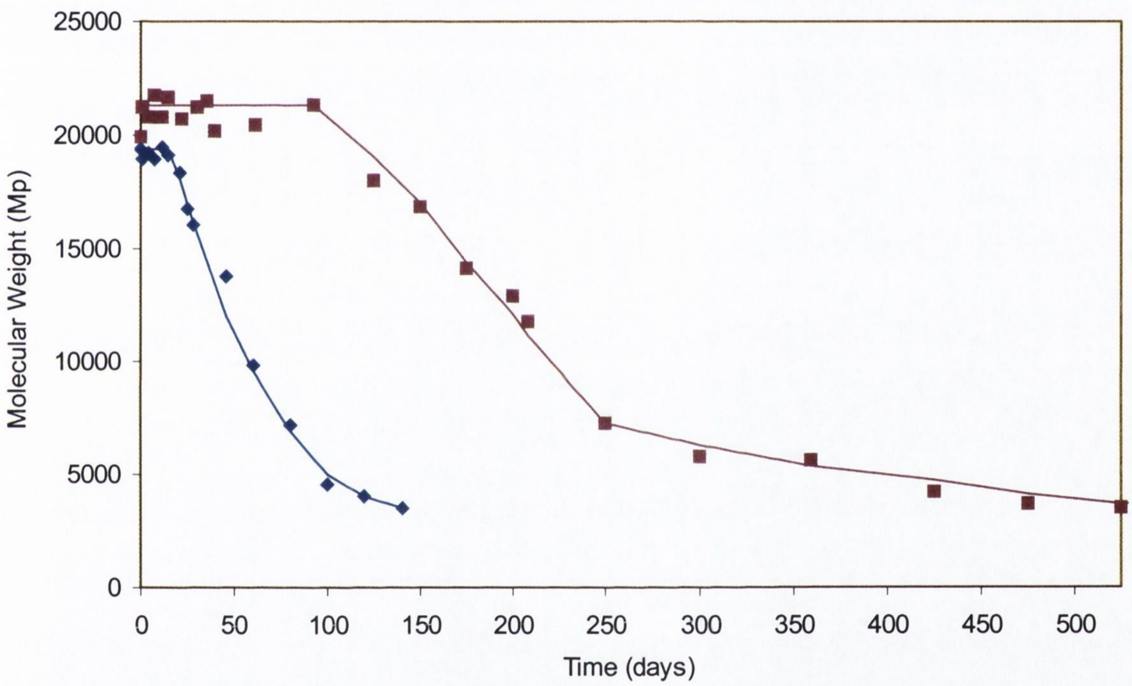


Figure 10.6 The influence of fluphenazine on the degradation of PLA (R203) microspheres, for ◆ 9.9% w/w fluphenazine HCl loaded PLA microspheres and ■ drug free PLA microspheres *(data points represent mean of two determinations).* Data points fitted to Equation 8.2.

Figure 10.6 shows the influence of the presence of 9.9% w/w fluphenazine on the degradation of PLA (R203) microspheres. The length required for the polymer to degrade to a specified molecular weight was considerable shortened for the 9.86% w/w fluphenazine HCl loaded microspheres e.g. to reach a molecular weight Mp of 10,000 the time required for PLA (R203) blank microspheres was approximately 240 days compared to ~60 days for 9.86% w/w fluphenazine loaded microspheres.

The degradation profile of PLA (R203) fluphenazine loaded microspheres was fitted to Equation 8.2. The parameters T_{lag}, k_1, k_2 and *Tau* were determined from which Mp_1 can also be calculated and are shown in (Table 10.8). The parameters for the degradation of fluphenazine HCl loaded PLA (R203) microspheres during the release of fluphenazine are compared to those of PLA (R203) blank microspheres (Table 10.8).

Table 10.8 Parameters for the degradation of PLA particles in PBS pH 7.4 at 37°C *(standard deviation represents that determined by the model).*

% w/w Fluphenazine	T_{lag} (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
0.00	100.987 ±5.3978	0.006 ±0.0003	0.003 ±0.0006	282.532 ±19.2740	6891	0.994	4.732
4.5	44.398 ±1.9611	0.013 ±0.0007	0.004 ±0.0090	122.413 ±23.8101	3991	0.994	4.601
9.9	16.659 ±0.7307	0.016 ±0.0005	0.007 ±0.0056	91.2707 ±13.9987	4363	0.998	5.820
16.8	9.454 ±0.0643	0.02298 ±0.0009	0.010 ±0.0062	80.6028 ±14.9861	3063	0.998	5.537

Table 10.8 shows that the length of the induction phase T_{lag}, the rate of polymer degradation k_1 were dependent on the %w/w Fluphenazine HCl loading. The time required to reach time *Tau* or the rate of degradation k_2 was similar for the three %w/w fluphenazine loadings examined. Table 10.3.3 shows that the critical molecular weight parameter for polymer degradation in drug loaded microspheres is lower that observed for drug free microspheres.

10.3.3 The effect of fluphenazine loading on the polymer molecular weight profile of PLGA (RG504) microspheres

The corresponding polymer degradation profile was also followed for the release of 4.20%, 8.2% and 16.6% w/w fluphenazine loaded PLGA (RG504) microspheres (Figure 10.7). Figure 10.7 shows that the polymer degradation profile for each % w/w fluphenazine has different starting molecular weights depending on the w/w Fluphenazine loaded. This is due to the effect of fluphenazine loading on the polymer during the manufacturing process.

The degradation profile of the PLGA fluphenazine loaded microspheres followed a biphasic pattern similar to that demonstrated for drug-free microparticles PLGA (RG504).

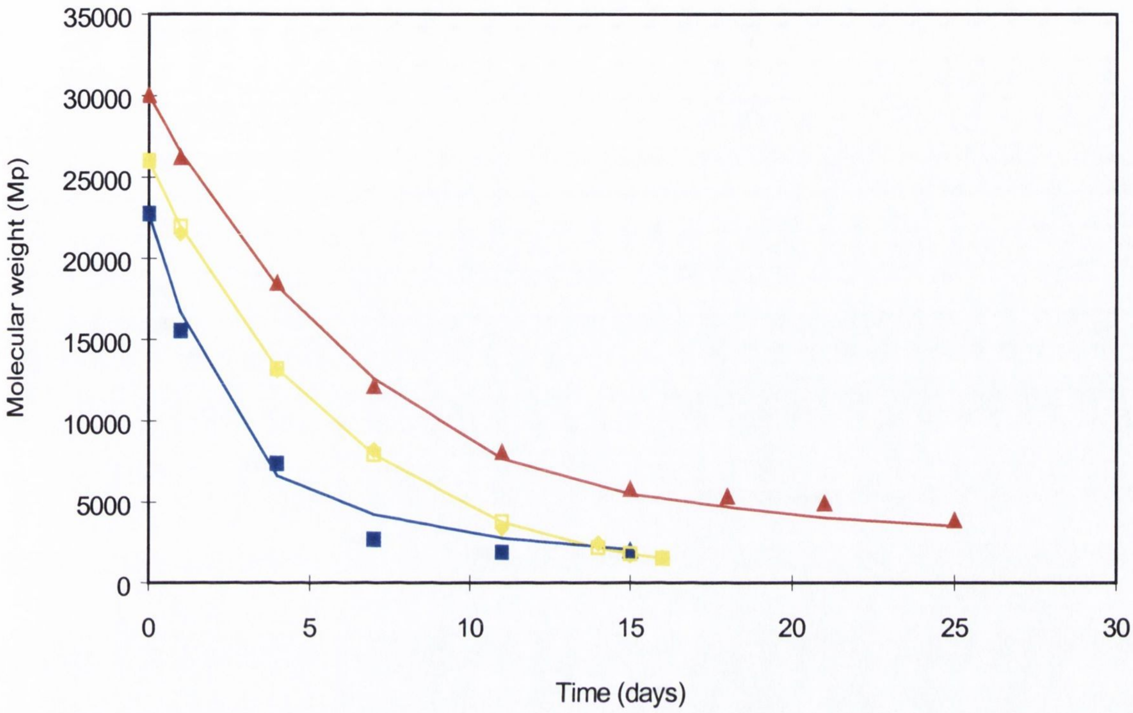


Figure 10.7 The influence of Fluphenazine loading (w/w) on the degradation of the PLGA (RG504) microspheres at 37°C in PBS pH 7.4, for ▲ 4.5 %, ◆ 9.9 % and ■ 16.8% Fluphanazine loaded microspheres*(data points represent mean of two determinations).* Data points fitted to Equation 7.3.

Figure 10.7 shows that the polymer rapidly degrades to a certain level and then levels off. The polymer degradation rate was also found to be faster for the PLGA (RG504) microspheres that contain higher fluphenazine loadings. The polymer degradation profile of the 8.2% w/w fluphenazine loaded PLGA RG504 microspheres is compared to the degradation of drug free microspheres made from PLGA RG504 in (Figure 10.8).

PLGA RG504 microsphere (<20 microns) have a molecular weight Mp of 42124, however when they are formulated into 8.2% w/w fluphenazine loaded PLGA RG504 microspheres the molecular weight of the polymer was reduced to an Mp of 27294. In order to distinguish between changes in degradation rate caused by the presence of the Fluphenazine HCl in the degrading matrix from changes in the degradation rate caused by the reduction in the polymer molecular weight during formulation, PLGA (RG503) drug free microspheres with a molecular weight Mp of 25206 were also compared.

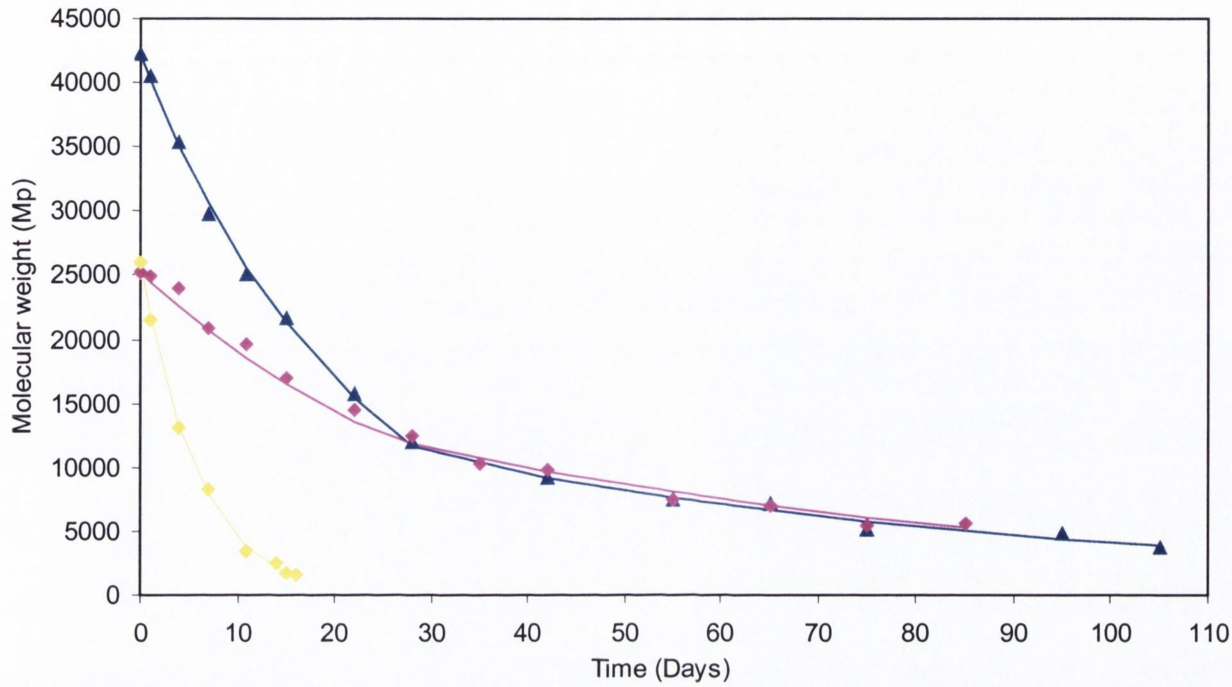


Figure 10.8 The influence of fluphenazine HCl on the degradation of the PLGA microspheres compared to drug free PLGA microspheres, for ▲ drug free RG504, ◆ drug free RG503 and ◆ 8.2 % w/w fluphenazine loaded RG504 *(data points represent mean of two determinations).* Data points fitted to Equation 7.3.

Figure 10.8 shows that the degradation of the 8.2% w/w fluphenazine HCL loaded microspheres was significantly faster than drug free microspheres manufactured from PLGA RG504. The degradation profile of the 8.2% w/w fluphenazine HCl loaded microspheres was also shown to be considerable different compared to drug free PLGA (RG503) microspheres with a polymer molecular weight of Mp=25206 which is comparable to the polymer molecular weight of microspheres containing 8.17% w/w fluphenazine Mp=27294. The presence of fluphenazine encapsulated in the microspheres had the ability to influence the degradation rate of the polymer during the release process

The parameters for the degradation of 4.2%, 8.2% and 16.6% w/w fluphenazine loaded PLGA microspheres evaluated using Equation 7.2 are shown in Table 10.9. These values are compared to the degradation parameters evaluated for PLGA (RG504) and PLGA (RG503).

Table 10.9 Parameters for the degradation of PLGA particles of different molecular weights at 37°C in PBS *(standard deviation represents that determined by the model).*

% w/w Fluphenazine (Polymer)	Mp	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_t	MSC
0.00	42124	0.046	0.014	29.789	10833	6.655
(RG504)	±930	±0.0011	±0.0012	±1.7491		
0.00	25206	0.028	0.014	25.350	12342	4.231
(RG503)	±446	±0.0024	±0.0020	±2.1801		
4.20	32919	0.124	0.038	12.269	4302	6.487
(RG504)	±1223	±0.0025	±0.0094	±0.8330		
8.17	27294	0.169	0.086	7.015	4776	6.420
(RG504)	±1159	±0.0039	±0.0335	±1.1635		
16.62	22721	0.308	0.073	4.518	3838	4.186
(RG504)	±356	±0.0266	±0.0051	±1.2758		

The polymer degradation model described in Chapter 7 was also shown to describe the degradation profile of PLGA during the release process. The fit of data to this model was good for the fluphenazine loaded PLGA degradation process (Table 10.9).

The degradation of polymers of equivalent starting molecular weights was shown to be influenced by the presence of fluphenazine. The presence of fluphenazine within the microspheres therefore accelerated the degradation of the polymer during the release process. The acceleration in the release was dependent on the %w/w fluphenazine in the microspheres. The parameter *Tau* occurred at much shorter intervals than for the drug free microspheres and was associated with a molecular weight of approximately 4000. The time to reach *Tau* was also dependent on the % w/w fluphenazine.

10.3.4 The effect of fluphenazine loading on weight loss profile of PLA (R203) and PLGA (RG504) microspheres

Weight loss experiments were carried out for the 10% theoretical loaded microspheres for both PLA (R203) and PLGA (RG504). Figure 10.9 shows a comparison of the weight loss of PLA (R203) microspheres and 9.9% w/w fluphenazine loaded PLA (R203) microspheres.

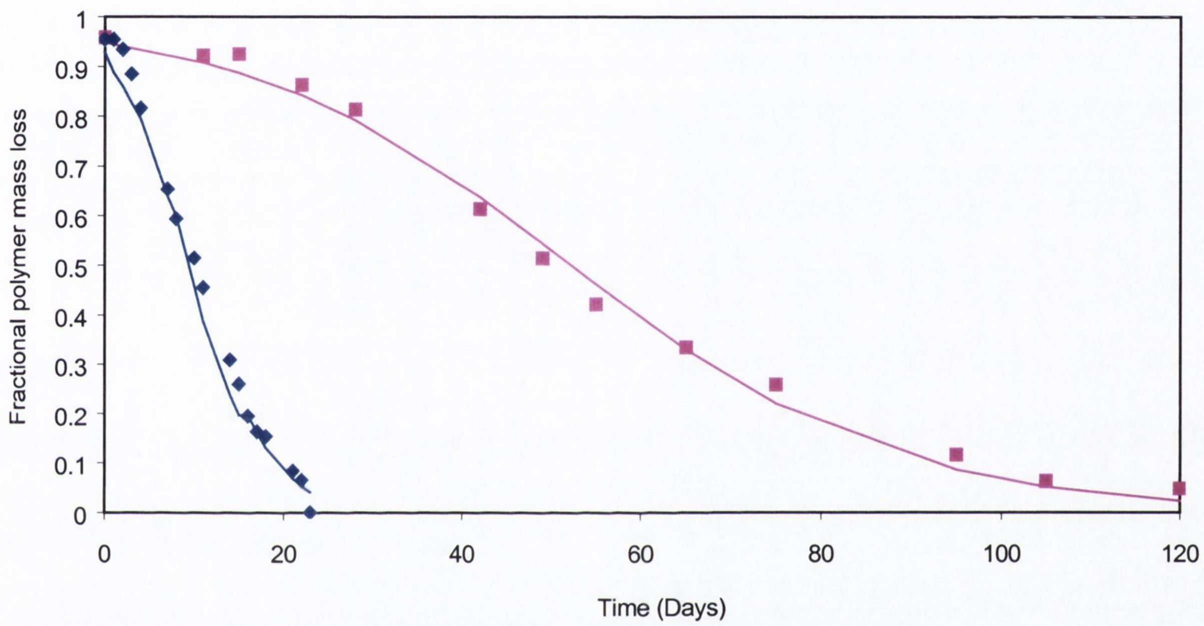


Figure 10.9 The influence of fluphenazine on the weight loss from PLA microspheres, for ◆ Drug free R203 ◆ 9.9% w/w Fluphenazine loaded R203 microspheres *(data points represent mean of two determinations).* Data points fitted to Equation 3.23.

For 9.9% w/w fluphenazine loaded PLA (R203) microspheres the mass loss was observed to be faster than the corresponding drug free PLA (R203) microspheres. A short lag phase was observed at the start of the profile followed by continual mass loss from the polymer with drug release occurring after 100 days. Figure 10.10 shows a comparison of the weight loss of the polymer from PLGA (RG504) microspheres and from 8.17% w/w fluphenazine loaded PLGA (RG504) microspheres.

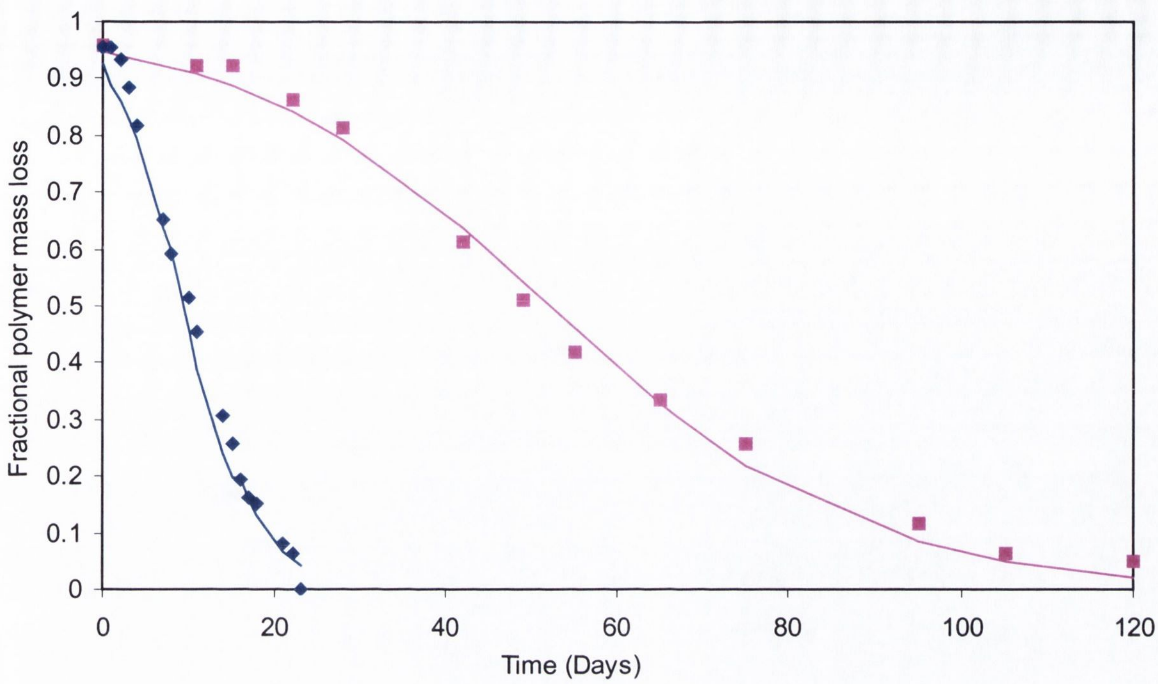


Figure 10.10 The influence of fluphenazine on the polymer weight loss from PLGA microspheres, for ■ drug free RG504 ◆ 8.17% w/w fluphenazine loaded RG504 microspheres *(data points represent mean of two determinations).* Data points fitted to Equation 3.23.

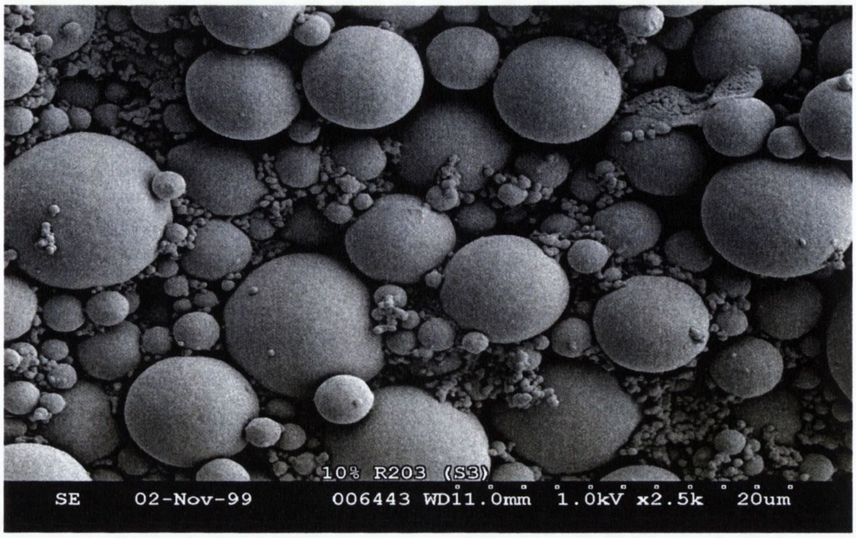
Figure 10.10 shows that the weight loss from the 8.2% w/w fluphenazine loaded PLGA (RG504) microspheres is considerably faster than for the drug free PLGA (RG504) microspheres. The onset of mass loss from the 8.2% w/w fluphenazine loaded microspheres was immediate from the drug-loaded microspheres. Initial polymer weight loss from the PLGA (RG504) microspheres was not accompanied by concurrent drug release. The mass loss from the microspheres appeared to follow a profile similar to the acid-catalysis of PLGA degradation that was observed in Chapter 8. The parameters for the mass loss for PLGA and PLA microspheres are compared to the corresponding fluphenazine HCl loaded microspheres in Table 10.10. The parameters determined for the weight loss from PLA and PLGA microsphere and fluphenazine loaded PLA and PLGA shown in Table 10.10 demonstrate the influence of the fluphenazine on the weight loss profile. In each case the weight loss from the polymer is increased compared to the drug-free particles. The acceleration in the degradation rate *k* and the reduction in the *Tmax* were observed to be greater for the PLGA system compared to PLA.

Table 10.10 Parameters evaluated for the erosion of PLA and PLGA particles determined using Equation 3.23 *(standard deviation represents that determined by the model)*

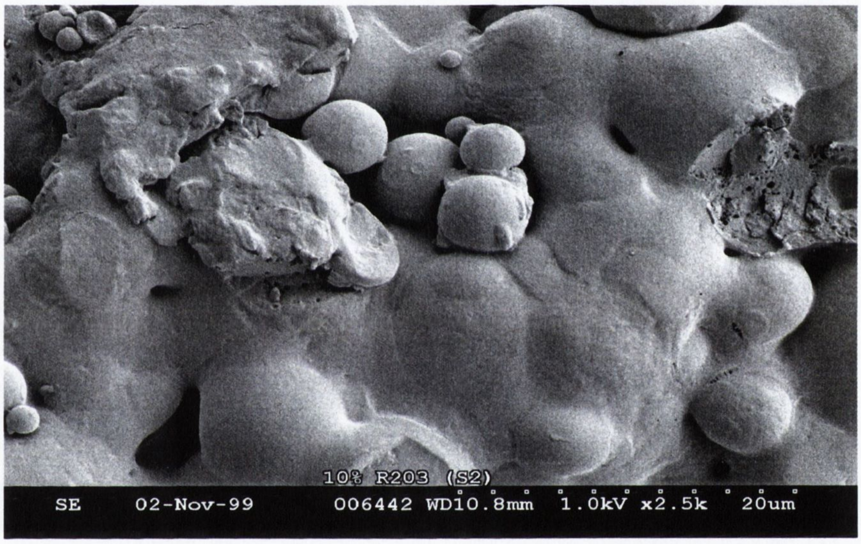
Polymer (%w/w Fluphenazine)	k (day^{-1})	*Tmax*	CD	MSC
R203 (0.00)	0.009 ±0.0006	428.970 ±8.2500	3.420	0.997
R203 (9.86)	0.028 ±0.0019	106.248 ±2.795	0.996	3.74
RG504 (0.00)	0.054 ±0.0038	54.150 ±1.4377	0.995	3.926
RG504 (8.17)	0.248 ±0.0099	10.319 ±0.1850	0.998	4.744

10.3.5 Electron Micrographs of PLA (R203) and PLGA (RG504) microspheres after removal from dissolution in phosphate buffer saline at 37°C

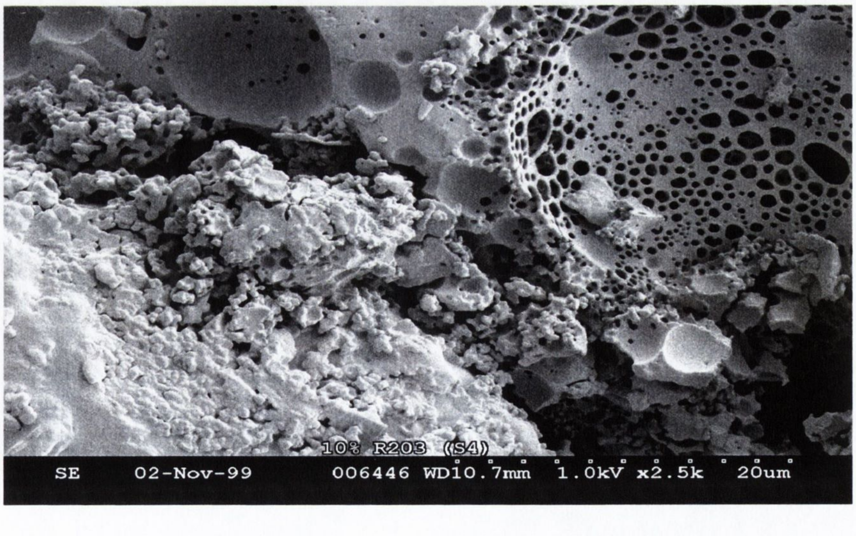
SEM's of the fluphenazine HCl loaded PLA and PLGA microspheres before incubation showed smooth discrete microparticles (Figure 10.1). Figure 10.11 and 10.12 show electron micrographs of 9.9% w/w and 8.2% w/w fluphenazine loaded PLA and PLGA microspheres taken at time intervals corresponding to time points that describe the surface controlled release phase, the lag phase and the degradation controlled release phase of the release profile. Figure 10.11 shows that after 1-day incubation in PBS at 37°C, corresponding to the surface controlled phase, microspheres retain their individual shape. The 46 days sample representing the lag phase shows fusion of the polymer matrix while a 120-day sample taken during the release phase shows a highly degraded matrix. Figure 10.12 shows the corresponding profile for PLGA microspheres. After 1 day incubation in PBS at 37°C the microspheres had lost some shape and fusion was evident, after 7 days the particles still retained some of their individual shape though some degradation was evident. A sample taken at day 14, during the degradation controlled release phase of the release profile shows complete loss in shape of the particulate matrix and the development of a highly porous structure.



(a)

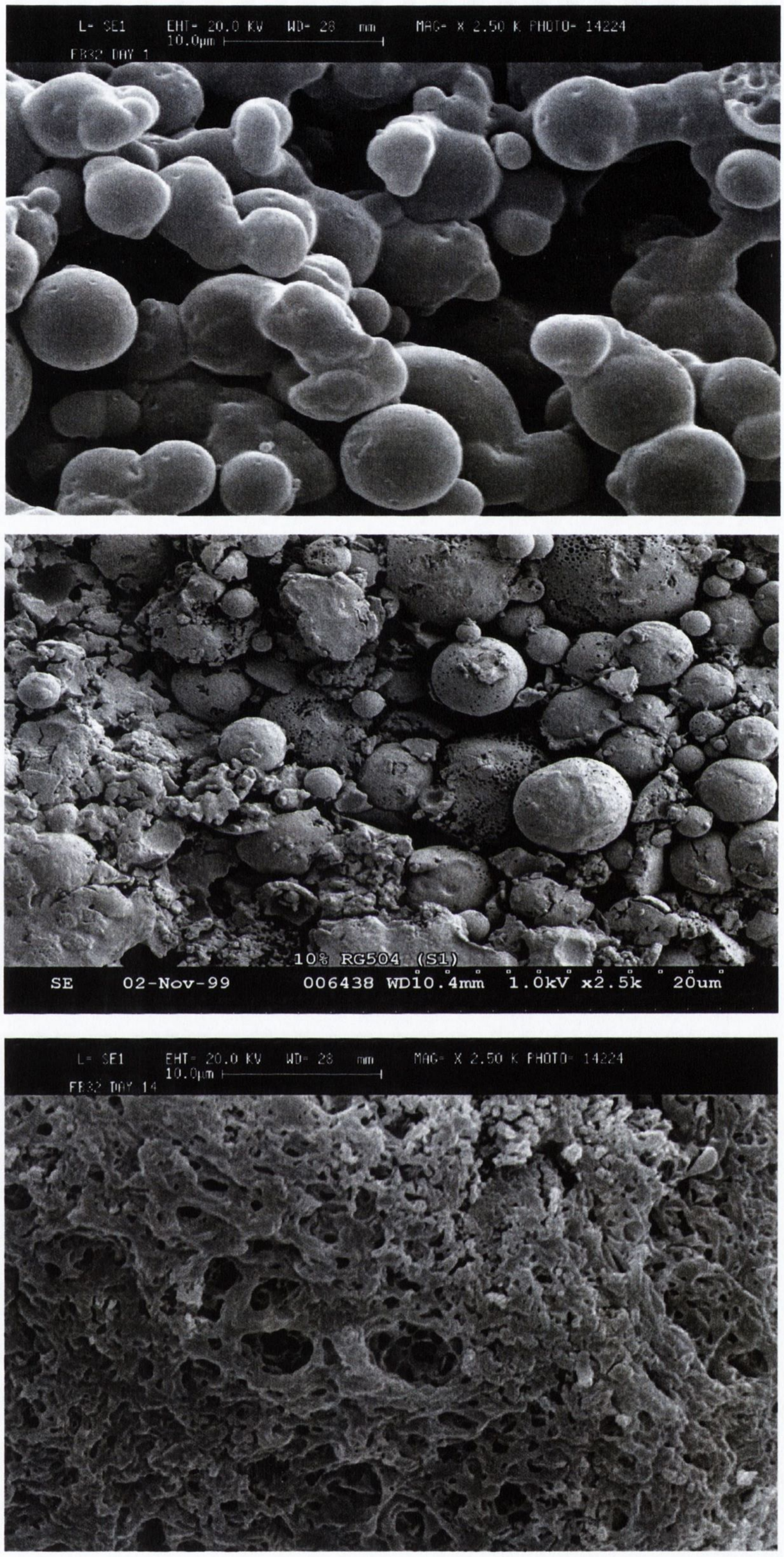


(b)



(c)

Figure 10.11 SEM of 9.9% w/w fluphenazine loaded PLA (R203) microparticles, after (a) 1 day (b) 46 days and (c) 120 days incubation in phosphate buffer saline at 37°C.



(a)

(b)

(c)

Figure 10.12 SEM of 8.2% w/w fluphenazine loaded PLGA (RG504) microparticles, after (a) 1 day (b) 7 days and (c) 14 days incubation in phosphate buffer saline at 37°C.

10.4 THE EFFECT OF PARTICLE SIZE ON THE PHYSIOCHEMICAL CHARACTERISTICS AND RELEASE PROFILE OF FLUPHENAZINE FROM PLGA (RG504) PARTICLES

The influence of particle size (<1 micron, <20 microns and <50 microns) on the release of Fluphenazine from PLGA was examined. These particles were produced using modifications of the emulsification solvent evaporation procedure (Method 5.3.5-6). The characteristics of the particles produced are shown in Table 10.11 and Table 10.12.

Table 10.11 Characteristics of fluphenazine loaded PLGA (RG504) particles made using three different processing conditions *(samples represent mean of three determinations±standard deviation).*

Process	Actual Loading (%)	Encapsulation efficiency (%)	Particle size $D_{50\%}$ (μm)	Particle size $D_{90\%}$ (μm)
A	7.6	82.5	2.8	6.9
	±0.332	±3.32	±0.14	±0.27
B	8.2	81.7	7.7	20.5
	±0.221	±2.21	±0.41	±.0.68
C	8.0	80.4	20.9	48.8
	±0.083	±0.833	±0.93	±2.35

Table 10.12 Effect of Processing technique on the molecular weight of fluphenazine loaded PLGA (RG504) particles *(samples represent mean of three determinations±standard deviation).*

Process	Mn	Mp	Mw	Mz	P
A	16023	24855	25819	38182	1.612
	±429	±686	±474	±941	±0.0192
B	15590	27294	27173	42384	1.745
	±458	±1159	±616	±2095	±0.0866
C	17942	27075	29997	46281	1.672
	±563	±786	±969	±1023	±0.0634

Fluphenazine loaded PLGA particles prepared using three different processing methods all encapsulated >80% of the theoretical drug loading. As with the production of drug free microspheres the size distribution of the microspheres decreases with increasing shear forces. For the particles prepared using Process A it was observed that the particle size $D_{50\%}$ and $D_{90\%}$ were greater for the drug loaded particles compared to the drug free nanoparticles. This is attributed to the presence of suspended drug particles in the polymer solution during the process. There were no obvious effects of particle size on either drug loading or encapsulation efficiency for the particles examined. GPC analysis of the microspheres also showed a shear force related decrease in the molecular weight of the microspheres. The molecular weight of the particles was observed to decrease with increasing shear force used to form the particles.

The release of fluphenazine HCl was measured from particles produced using Process A, Process B and Process C in phosphate buffer saline at 37°C. The effects of particle size on the release profile of fluphenazine HCl loaded PLGA particles is shown in Figure 10.13.

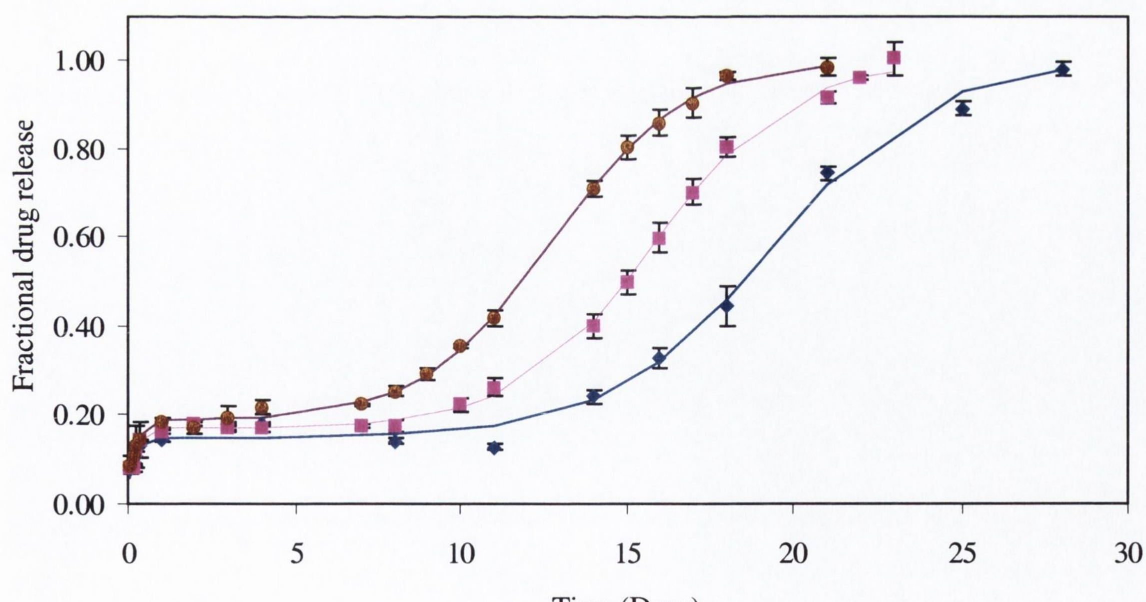


Figure 10.13 The effects of particle size on the release profile of fluphenazine HCl loaded PLGA particles, for ● <50 microns, ■ <20 microns and ◆ < 1 microns *(data points represent mean of three determinations ±standard deviation).* Data points fitted to Equation 10.1.

Figure 10.13 shows that as the particle size of the PLGA particles decreases the rate of fluphenazine release also decreases. The parameters for the release of fluphenazine HCl from PLGA particles are given in Table 10.13.

Table 10.13 Parameters for the release of fluphenazine HCl from PLGA (RG504) particles fitted to equation 10.1 *(standard deviation represents that determined by the model).*

Process	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ *(days)*	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
A	0.419 ±0.0332	19.311 ±0.2165	0.149 ±0.0121	6.221 ±1.9601	4.688	0.995
B	0.491 ±0.0190	15.836 ±0.0766	0.170 ±0.0062	5.112 ±0.7168	6.056	0.998
C	0.509 ±0.0515	12.783 ±0.1681	0.186 ±0.0254	5.133 ±1.2304	5.163	0.996

The parameters for the release of fluphenazine HCl from different sized PLGA microspheres shown in Table 10.13 demonstrate the effect of particle size on the release profile of fluphenazine HCl. F_{BIN} and $k_{1(R)}$ was comparable for all three particle sizes. The parameters *Tmax* and $k_{(R)}$ were shown to depend on the particle size of the microspheres. *Tmax* decreases with an increase in particle size while the corresponding $k_{(R)}$ increased.

Smaller particles usually release drug at a faster rate than the larger particles. A study by Suzuki and Price (1985) showed that the time required for 50% release of chlorpromazine release was linearly related to the mean particle size, this release was over periods of hours. Leelarasamee *et al.* (1988) showed an exponential increase in the rate of drug release with decreasing particle size for the initial diffusion component of the release profile. A study by Visscher *et al.* (1998) showed that the in vitro release from ergot containing microspheres was faster for the larger microspheres over a 32-day period. It is postulated in this study that the degradation of the polymer controls the release rate from the microspheres. Any increase in dissolution rate expected due to the larger surface area of the smaller particles is not observed due to the miscibility observed between the polymer and the drug.

10.5 PHYSICOCHEMICAL CHARACTERISATION OF FLUPHENAZINE LOADED PLA AND PLGA PARTICLES OF DIFFERENT POLYMER MOLECULAR WEIGHTS

The standard 10% formulation made using process B was used to manufacture microparticles using different PLA and PLGA molecular weight polymers. The different polymer molecular weight formulations are characterised in terms of loading and particle size in Table 10.14 and 10.15 for PLA and PLGA respectively.

Table 10.14 Characteristics of fluphenazine loaded PLA microspheres of different polymer molecular weights *(data represent mean of three determinations ±standard deviation).*

PLA	Actual Loading (%)	Encapsulation efficiency (%)	Particle size $D_{50\%}$ (μm)	Particle size $D_{90\%}$ (μm)
R104	9.1	91.31	6.2	16.0
	±0.35	±3.54	±1.16	±1.29
R203	9.9	98.58	6.0	17.2
	±0.47	±4.704	±1.20	±1.336

Table 10.15 Characteristics of fluphenazine loaded PLGA (<20microns) prepared using different molecular weights PLGA *(data represent mean of three determinations ±standard deviation).*

PLGA	Actual Loading (%)	Encapsulation efficiency (%)	Particle size $D_{50\%}$(μm)	Particle size $D_{90\%}$(μm)
RG504	8.2	81.7	7.7	20.5
	±0.22	±2.21	±0.41	±0.68
RG503	9.3	92.5	5.4	18.3
	±0.05	±0.49	±0.26	±0.68
RG502	8.7	87.3	5.9	19.5
	±0.09	±0.87	±0.24	±0.45
RG502H	8.1	81.24	6.2	17.2
	±0.37	±3.71	±0.37	±0.82

The viscosity of the polymer solution was controlled by varying the amount of solvent used in the formulation in order to maintain a constant particle size between batches. Particle size analysis revealed a distribution that was ≤20 microns in all cases (Table 10.14 and 10.15) for the PLA and PLGA polymers. The encapsulation efficiency was >80% in all cases for both the PLA and PLGA microspheres of different polymer molecular weights at the 10% theoretical loading of fluphenazine HCl. GPC analysis of the PLA and PLGA microspheres produced are shown in Tables 10.16 and 10.17 for PLA and PLGA respectively.

Table 10.16 GPC characteristics of PLA and Fluphenazine loaded PLA microparticles of different polymer molecular weight *(data represent mean of three determinations ±standard deviation).*

Polymer	Matrix	Mn	Mp	Mw	Mz	P
R104	Drug free	2130	4654	4768	7702	2.242
	Microparticles	±109	±263	±134	±289	±0.0671
	9.1% fluphenazine	2046	4486	4556	7686	2.226
	Microparticles	±203	±250	±462	±575	±0.0542
R203	Drug free	12302	19914	20801	30218	1.638
	Microparticles	±347	±703	±964	±1294	±0.0661
	9.9% fluphenazine	12030	19580	20167	31293	1.659
	Microparticles	±322	±504	±561	±616.6	±0.0012

Table 10.16 shows for PLA microspheres that the molecular weight moments for the Fluphenazine loaded microspheres are comparable to the molecular weight moments for the drug free microspheres at both molecular weights of PLA studied.

For the PLGA series of polymers, three different molecular weight PLGA microspheres were compared. 10% theoretical loaded fluphenazine HCl loaded microspheres were formulated using PLGA (RG504), PLGA (RG503) and PLGA (RG502). Microspheres prepared using PLGA (RG502) were also compared to 10% theoretical loaded fluphenazine formulated using the more hydrophilic polymer PLGA (RG502H). The GPC characteristics of the PLGA microspheres are shown in Table 10.17.

Table 10.17 GPC characteristics of PLGA and fluphenazine loaded PLGA microparticles of different polymer molecular weight *(data represent mean of three determinations ±standard deviation).*

Polymer	Matrix	Mn	Mp	Mw	Mz	P
RG504	Drug Free	26109	42124	41312	59255	1.586
	Microparticles	±598	±930	±2104	±1370	±0.0335
	8.2% fluphenazine	15590	27294	27173	42384	1.745
	Microparticles	±458	±1159	±616	±2095	±0.0860
RG503	Drug Free	14932	25206	25134	37824	1.683
	Microparticles	±69	±446	±346	±1127	±0.0158
	9.3% fluphenazine	12314	18969	20247	29914	1.644
	Microparticles	±107	±512	±165	±1241	±0.0216
RG502	Drug Free	5801	12194	11477	17643	1.978
	Microparticles	±226	±381	±512	±818	±0.0386
	8.7% fluphenazine	5978	10800	11057	16126	1.785
	Microparticles	±175	±290	±776	±620	±0.0378
RG502H	Drug Free	5923	8474	9254	13222	1.598
	Microparticles	±221.3	±411	±199	±103	±0.0513
	8.0% fluphenazine	5795	8369	9104	12695	1.571
	Microparticles	±268	±469	±623	±875	±0.0262

Table 10.17 shows the GPC analysis of Fluphenazine loaded microparticles produced using three different polymer molecular weighs. The molecular weight moments of the drug-loaded particles are compared to drug free microspheres. It was observed that as the molecular weight of the polymer increased the effect of the presence of fluphenazine HCl in the formulation on the molecular weight of the polymer was increased. The molecular weight moments of the RG502H microspheres were reduced more by the inclusion of fluphenazine compared to the corresponding RG502 fluphenazine loaded microspheres.

10.6 EFFECT OF POLYMER MOLECULAR WEIGHT ON THE RELEASE OF FLUPHENAZINE FROM PLA AND PLGA MICROSPHERES IN PHOSPHATE BUFFERED SALINE pH 7.4 AT 37°C

The effect of polymer molecular weight on the release profile of fluphenazine HCl from PLA and PLGA microspheres was investigated using microspheres characterised in the previous section. Release was monitored as a function of incubation time with parallel polymer degradation sampling.

10.6.1 The influence of polymer molecular weight on the release of Fluphenazine from PLA microspheres

The effect of molecular weight on the release properties of PLA was examined using 10% theoretical fluphenazine loaded PLA microspheres (Table 10.16). The release of fluphenazine from PLA (R104) and PLA (R203) microspheres is shown in Figure 10.14.

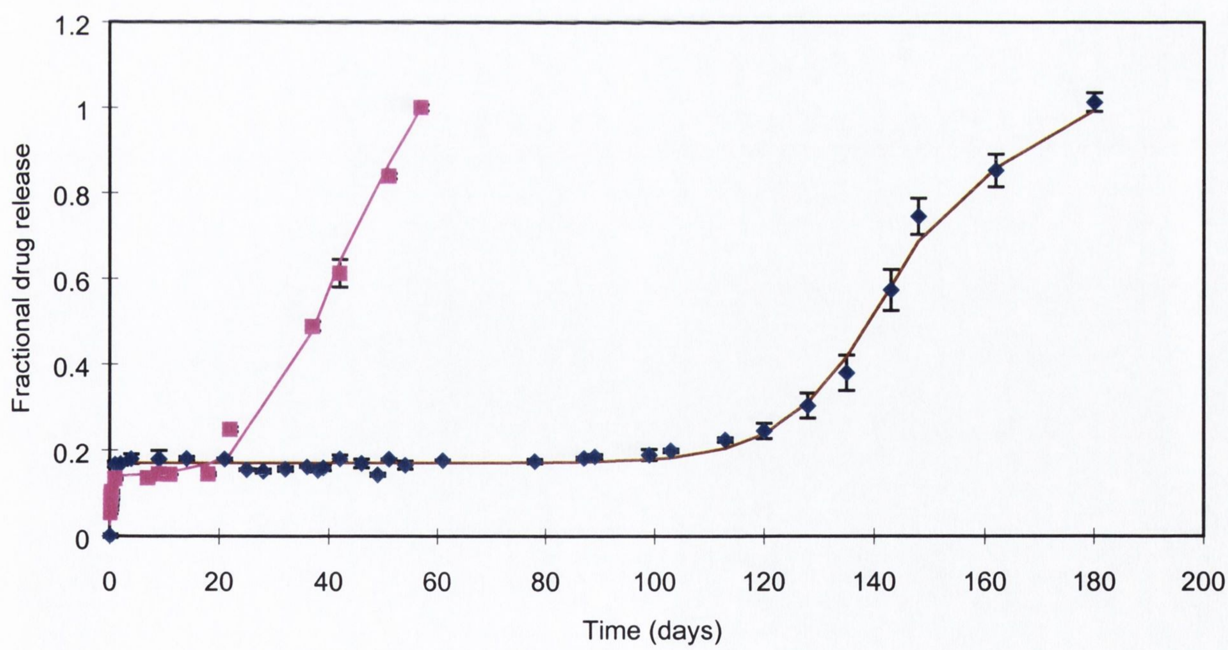


Figure 10.14 The influence of PLA molecular weight on the release of fluphenazine HCl from PLA microspheres in PBS at 37°C, for ■ 9.1% w/w Fluphenazine HCl loaded R104 microspheres and ◆ 9.9% w/w fluphenazine HCl loaded R203 microspheres *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

Figure 10.14 shows that the release of fluphenazine HCl from PLA microspheres was highly dependent on the molecular weight of the polymer. Fluphenazine HCl was released at a faster rate for PLA (R104) Mp 4486 compared to PLA (R203) Mp 19580. The parameters for the release of fluphenazine HCl from PLA (R104) and PLA (R203) microspheres were evaluated using equation 10.1 and are given in Table 10.18.

Table 10.18 Parameters for the release of 9% fluphenazine from PLA particles fitted to Equation 10.1 *(standard deviation represent that determined by the model)*

Polymer (Mp)	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
R104 (4486)	0.154 ±0.0221	39.82 ±0.8900	0.139 ±0.0192	4.6 ±2.1190	3.936	0.989
R203 (19580)	0.105 ±0.0057	143.230 ±0.5112	0.170 ±0.040	2.656 ±0.4832	4.707	0.993

Parameters for the release of fluphenazine show that the dissolution parameters $k_{1(R)}$ and F_{BIN} for the release of fluphenazine were comparable for both polymer molecular weights. Parameters for the polymer degradation controlled component of the release profile k and $Tmax_{(R)}$ show that the release of fluphenazine was faster for the lower molecular weight polymer. The corresponding molecular weight profile of PLA (R104) and PLA (R203) during the release process is shown in Figure 10.15. Parameters for the degradation of PLA with the concurrent release profile are shown in Table 10.19.

Table 10.19 Parameters for the degradation of 9% Fluphenazine loaded PLA particles in PBS pH 7.4 at 37°C fitted to Equation 8.2 (R203) and Equation 8.1 (R104) *(standard deviation represent that determined by the model).*

Polymer (Mp)	T_{lag} (days)	k_1 (day^{-1})	k_2 (day^{-1})	Tau (days)	Mp_1 Estimated	CD	MSC
R104 (4486)	-	-	0.0133 ±0.0005	0.00	-	0.982	3.795
R203 (19580)	16.659 ±0.7307	0.016346 ±0.0005	0.00666 ±0.0056	91.2707 ±13.9990	4363	0.998	5.820

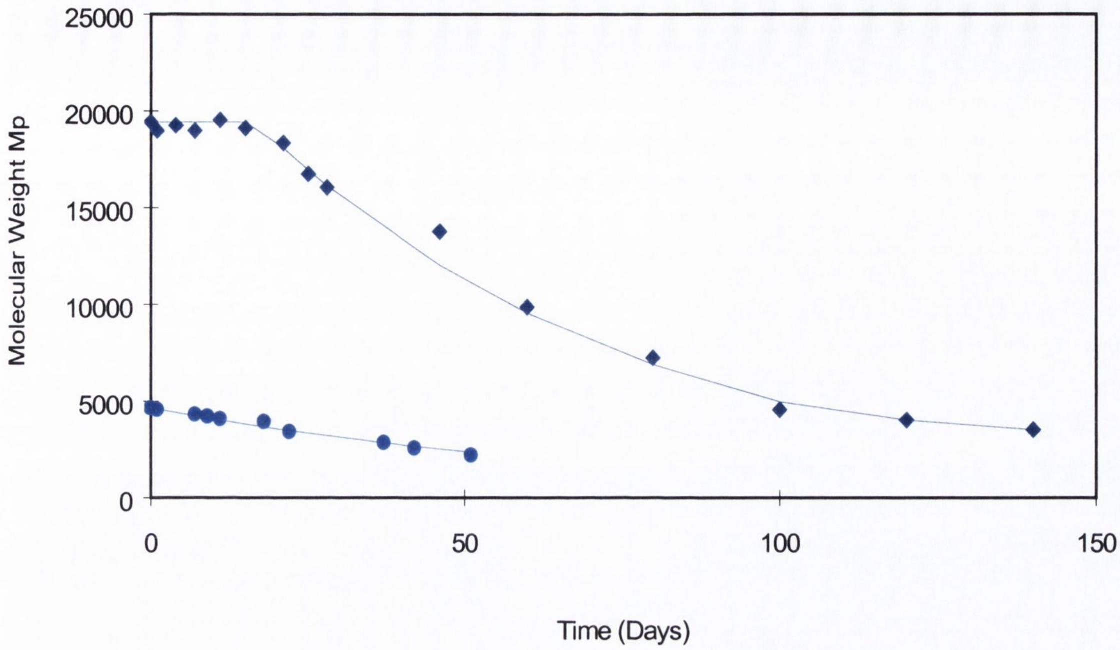


Figure 10.15 The degradation profile of fluphenazine loaded PLA microspheres fitted to Equation 8.2 (R203) and Equation 8.1 (R104), where ◆ 9.9% w/w F-HCl loaded R203 microspheres and ● 9.139% w/w F-HCl loaded R104 microspheres *(data points represent mean of two determinations)*

The degradation kinetics for the PLA (R104) microspheres showed only one phase that was described by a single rate constant compared to that observed for PLA (R203), which demonstrated a triphasic degradation pattern.

10.6.2 The influence of polymer molecular weight on the release of Fluphenazine from PLGA microspheres

The effect of molecular weight on the release properties of PLGA was examined using 10% theoretical loaded PLGA microspheres (Table 10.20). The release of fluphenazine HCl from PLGA (RG504), PLGA (RG503) and PLGA (RG502) microspheres is shown in Figure 10.16. The parameters for the release of fluphenazine HCl from PLGA (RG504), PLGA (RG503) and PLGA (RG502) microspheres were evaluated using Equation 10.2 and are given in Table 10.20.

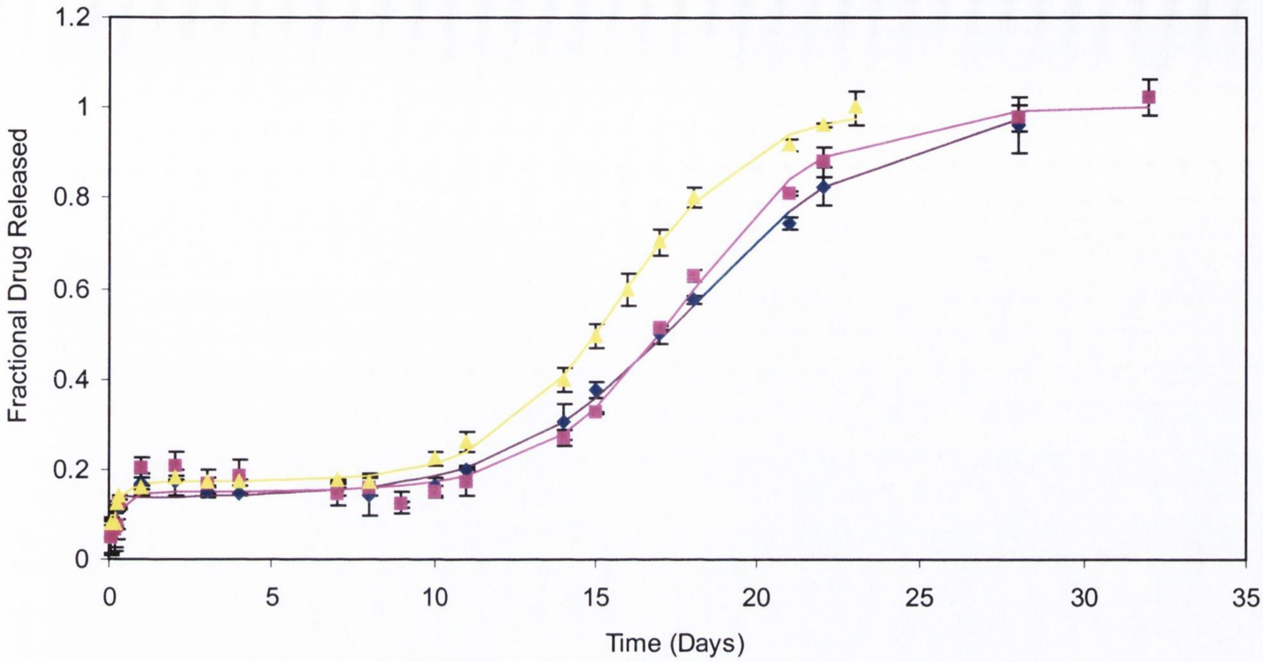


Figure 10.16 The effect of polymer molecular weight on the release of Fluphenazine from PLGA microspheres, for ▲ RG504, ■ RG503 and ◆ RG502 *(samples represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

Table 10.20 Parameters for the release of fluphenazine from PLGA microspheres of different molecular weights fitted to Equation 10.1 *(standard deviation represent that determined by the model).*

Polymer (% w/w fluphenazine)	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
RG504	0.491	15.836	0.170	5.112	6.056	0.998
(8.17)	±0.019	±0.076	±0.006	±0.717		
RG503	0.454	17.767	0.149	3.843	5.226	0.996
(9.25)	±0.026	±0.134	±0.008	±0.897		
RG502	0.446	18.065	0.135	4.229	4.895	0.995
(8.73)	±0.019	±0.165	±0.009	1.116		

Table 10.20 shows the parameters that describe the release of fluphenazine from PLGA microspheres of different molecular weights. For the surface controlled component of the release process as measured by F_{BIN} and $k_{1(R)}$ the burst effect appeared to decrease with decreasing molecular weight of the polymer while no relationship was evident for the

release rate $k_{1(R)}$. For the degradation component of the release process the release rate was observed to be similar for the three polymer molecular weights Figure 10.16. The parameters for the degradation component of the release were also similar for the release from the three PLGA molecular weight polymers (Table 10.20).

The degradation profile concurrent with drug release process was examined for the RG504, RG503 and RG502 fluphenazine loaded microspheres. Figure 10.17 shows the degradation profile for the three polymers during the release process. The molecular weights of the polymers were observed to decrease exponentially similar to that observed for drug free microspheres in Chapter 8. The rate of polymer degradation for the different molecular weights relative to each other also followed the same pattern as that observed for drug free microspheres in Chapter 8.

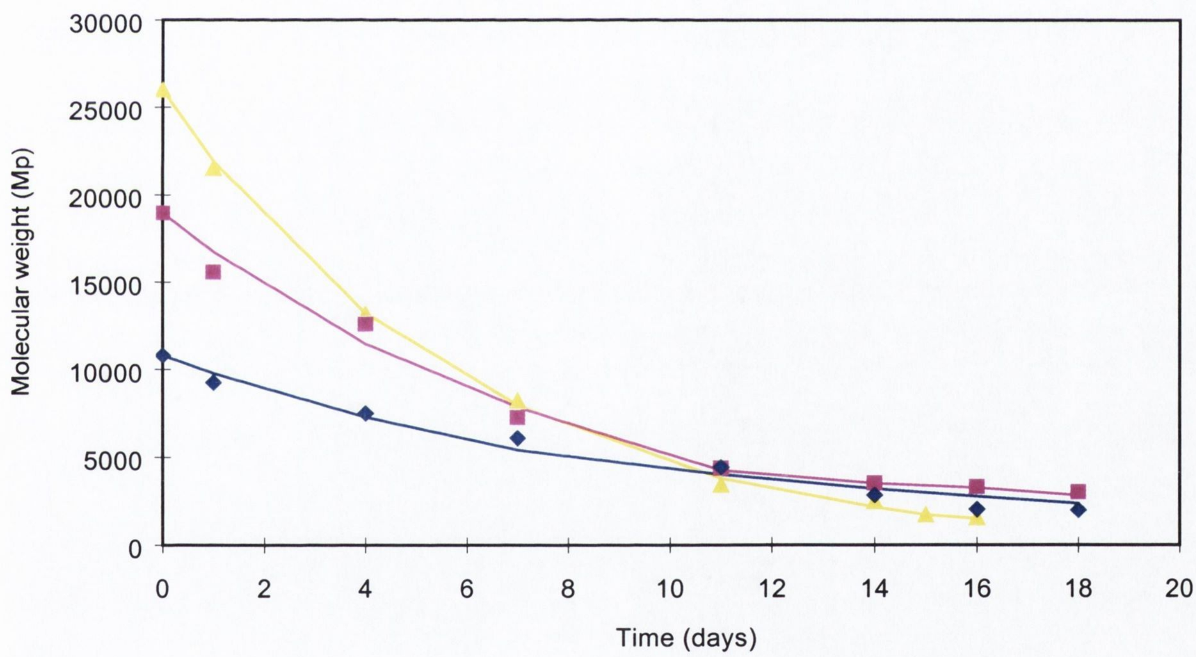


Figure 10.17 The degradation profile of fluphenazine loaded PLGA microspheres of different polymer molecular weights, for ▲ RG504, ■ RG503 and ◆ RG502 *(samples represent mean of two determinations).* Data fitted to Equation 7.3.

During the release process the onset of the degradation controlled phase of the release process was observed to occur at approximately day 10 for the release of fluphenazine from the three different molecular weight polymers (Figure 10.16). Figure 10.17 shows that after day 10 on the degradation profile the polymer molecular weight is considerably reduced (Mp<5000). At day 11 the molecular weight of the three polymers has become equivalent. This implies that while the starting molecular weight of the polymers were different degradation induced changes in the molecular weights produced three polymer systems with equivalent polymer molecular weights at the onset of degradation controlled drug release. This equivalence of polymer molecular weight at the onset of degradation controlled drug release may explain why drug release from the three different molecular weight polymers are similar. Parameters for the degradation of PLGA microspheres of different polymer molecular weights are given in Table 10.21.

Table 10.21 Parameters for the degradation of PLGA particles of different polymer molecular weights at 37°C in PBS fitted to Equation 7.3 (*standard deviation represents that determined by the model).*

% w/w Fluphenazine (Polymer)	Mp	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (*days*)	Mp_t	CD	MSC
8.2 (RG504)	27294 ±1159	0.169 ±0.0039	0.086 ±0.0330	7.000 ±1.1635	4776	0.991	6.422
9.3 (RG503)	18969 ±512	0.126 ±0.0120	0.088 ±0.0488	9.371 ±2.3442	5835	0.987	3.460
8.7 (RG502)	10800 ±290	0.098 ±0.0127	0.075 ±0.0322	6.999 ±1.4682	5426	0.980	3.166

Parameters for the degradation of PLGA of different molecular weights show that during the first phase of the degradation profile during which no release of fluphenazine occurs the polymer degradation rate is faster for the higher molecular weight polymer. The time for the three polymers is comparable and after *Tau* the rate constant for the second phase of the degradation profile is comparable for the three different polymer molecular weights. At the time *Tau* where the phase change occurs the molecular weight Mp_t is comparable for the three different polymers.

10.6.3 The effect of polymer end-group on the release of Fluphenazine HCl from PLGA microspheres

The release of fluphenazine from PLGA (RG502) was compared to the release of fluphenazine from PLGA (RG502H) to determine the effect of the end-group on the release process. The release of fluphenazine HCl from PLGA microspheres with different end-groups is shown in Figure 10.18.

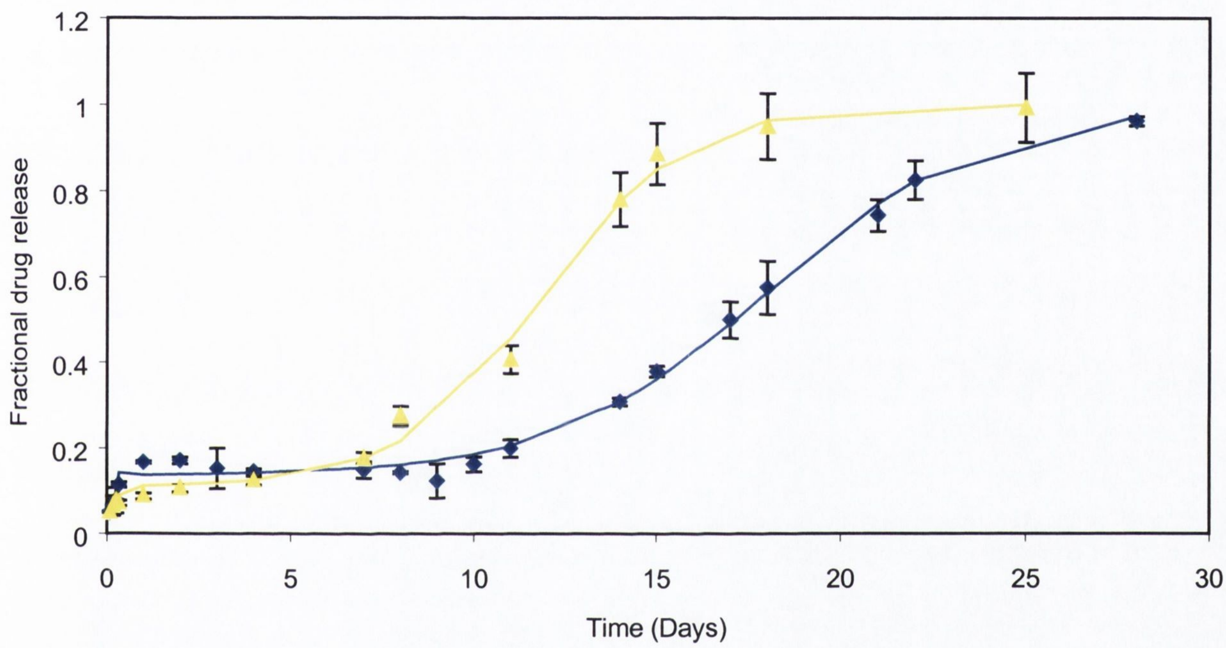


Figure 10.18 Effect of polymer end group on the release of Fluphenazine from PLGA microspheres, for ◆ RG502 and ▲ RG502H *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

The parameters for the release of fluphenazine from the RG502 and RG502H systems are shown in Table 10.22. For the surface controlled release the % burst effect and the rate of drug release during this phase are comparable. The degradation controlled release component of the release profile was highly influenced by the polymer type. The substitution of RG502 with the more hydrophilic RG502H in the microspheres manufacturing method caused a marked increase in the release rate of fluphenazine. Parameters for the release of fluphenazine from PLGA (RG502H) were considerably faster when compared to the corresponding parameters for PLGA (RG502) determined using Equation 10.1.

Table 10.22 Effect of polymer end group on the parameters for the release of Fluphenazine from PLGA microspheres fitted to Equation 10.1 *(standard deviation represents that determined by the model).*

Polymer (% w/w fluphenazine)	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
RG502	0.346	18.065	0.135	4.229	4.895	0.995
(8.7)	±0.0192	±0.1651	±0.0087	1.1161		
RG502H	0.512	11.875	0.106	4.608	4.789	0.998
(8.0)	±0.0451	±0.2215	±0.0185	±2.3391		

Figure 10.19 shows the degradation of RG502H compared to RG502 microspheres as they release Fluphenazine. The degradation of RG502H could not be fitted using Equation 7.3 because it was an approximately mono-phasic profile and was therefore fitted to Equation 8.1. Parameters for the degradation are shown in Table10.23.

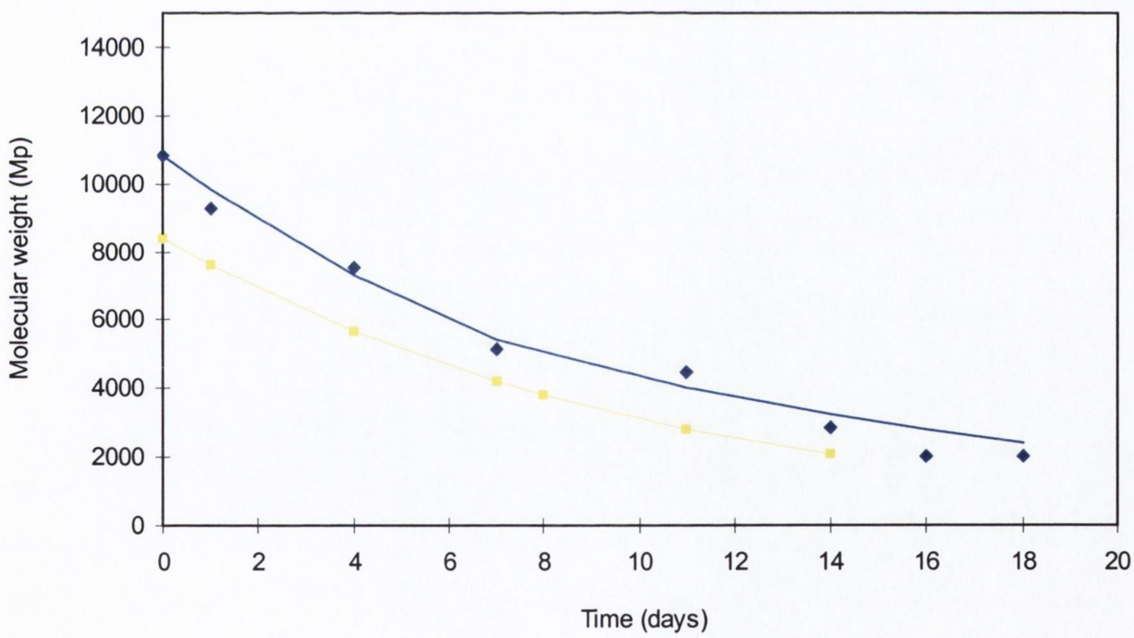


Figure 10.19 Effect of polymer end group on the degradation of Fluphenazine loaded PLGA microspheres, for ◆ RG502 and ▲ RG502H *(data points represent mean of two determinations).* Data points fitted to Equation 7.3 (RG502) and 8.1 (RG502H).

Table 10.23 Effect of polymer end group on the parameters for the degradation of fluphenazine loaded PLGA microspheres fitted to Equation 8.1 (RG502H) and 7.3 (RG502) *(standard deviation represents that determined by the model).*

% w/w fluphenazine (Polymer)	Mp	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (*days*)	Mp_i	CD	MSC
8.2 (RG502H)	8369 ±469	-	0.099 ±0.0038	-	-	0.989	4.267
8.7 (RG502)	10800 ±290	0.098 ±0.0127	0.075 ±0.0322	6.999 ±1.4682	5426	0.980	3.166

Parameters for the degradation of RG502 fitted the bi-phasic degradation profile that exhibited a *Tau* of 7 days. Therefore the RG502 microspheres are required to reach the critical molecular weight after which release occurs. The lower polymer molecular weight and the faster degradation profile of the H series polymer it thought account for the difference in release profile observed between RG502H and RG502.

10.7 COMPARISON OF FLUPHENAZINE RELEASE FROM DISPERSED MICROSPHERES AND MICROSPHERES CONTAINED WITHIN VISKING BAGS IN PHOSPHATE BUFFER SALINE pH 7.4 AT 37°C

The release of fluphenazine from microspheres was monitored using the two different dissolution methods that have been utilised throughout this work. In Method A the particles were dispersed in the dissolution medium while in Method B particles were placed in visking bags, then put into the dissolution medium. The system selected for examination under different dissolution conditions was the 10% theoretical loaded PLGA (RG504) microspheres. In Method B release was monitored by sampling from the bulk medium.

A comparison of the release profile of 8.2% fluphenazine release from PLGA (RG504) when monitored using the two different dissolution methods, is shown in Figure 10.20

Parameters for the release of fluphenazine from PLGA (RG504) microspheres incubated in visking bags and free to disperse are compared in Table 10.24.

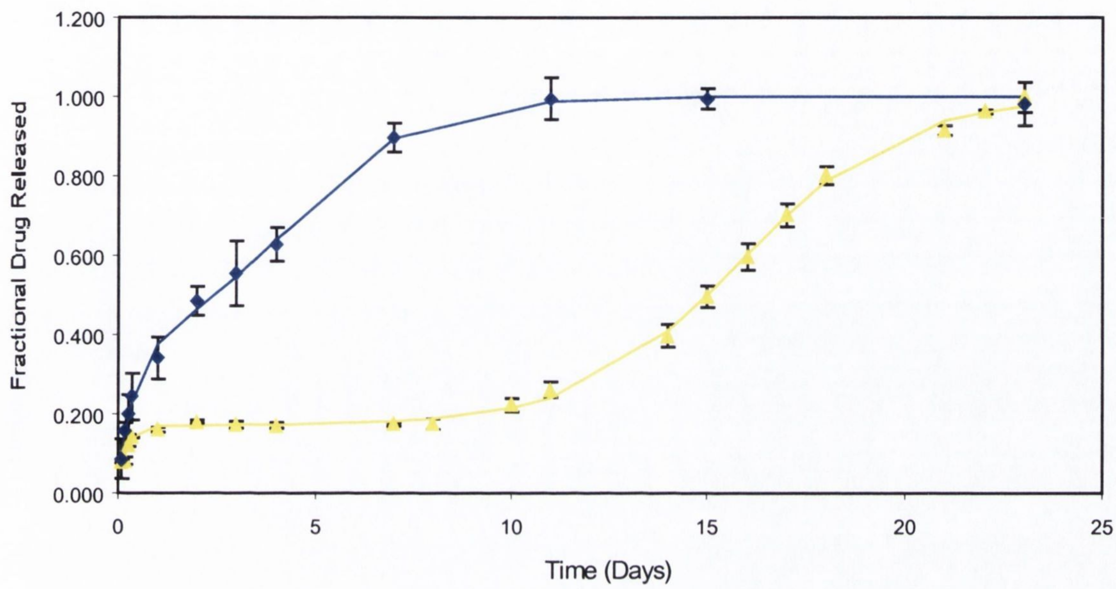


Figure 10.20 Comparison of the release of 8.2% fluphenazine loaded PLGA (RG504) microspheres, for ▲Method A and ◆ Method B *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 10.1.

Table 10.24 Parameters for the release of fluphenazine from RG504 microspheres fitted to Equation 10.1 *(standard deviation represents that determined by the model).*

Method	$k_{(R)}$ (day^{-1})	$Tmax_{(R)}$ (days)	F_{BIN}	$k_{1(R)}$ (day^{-1})	MSC	CD
A	0.491 ±0.019	15.836 ±0.076	0.170 ±0.006	5.112 ±0.717	6.056	0.998
B	0.602 ±0.062	4.242 ±0.281	0.330 ±0.041	2.138 ±0.315	5.505	0.998

Table 10.24 shows that the release parameters determined using equation 10.1 demonstrate that the release profile obtained using Method B was considerably faster. This effect is attributed to the effect of microsphere microenvironment created by the degrading polymer and the released drug. The degradation of the corresponding PLGA microparticles for both systems is shown in Figure 10.21.

The degradation of 8.2% fluphenazine loaded PLGA was also faster when the microspheres were incubated within the visking bags when compared to the degradation profile of the dispersed microspheres. The corresponding degradation parameters for the polymer during the release process were also significantly different.

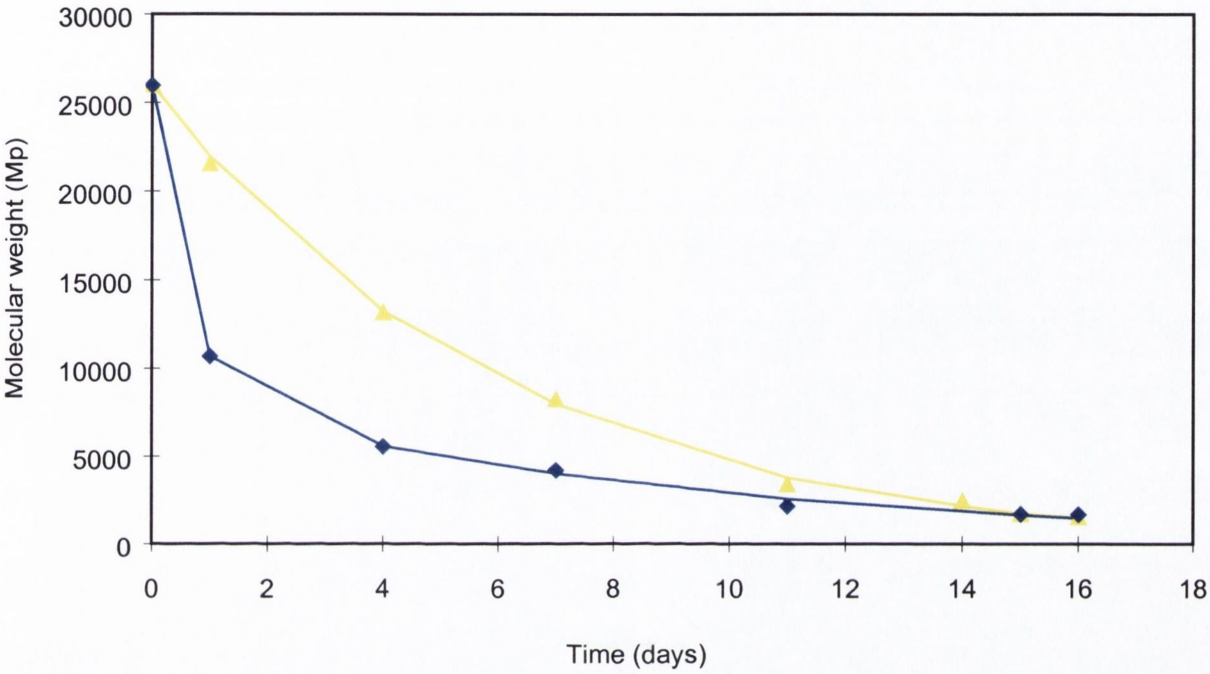


Figure 10.21 A comparison of the degradation of 8.17% fluphenazine HCl loaded PLGA (RG504) microspheres, for ▲Method A and ◆ Method B *(data points represent mean of three determinations ± standard deviation).* Data points fitted to Equation 7.3.

Table 10.25 Parameters for the degradation of 8.2% w/w fluphenazine loaded PLGA (RG504) using two different incubation methods at 37°C in PBS fitted to Equation 7.3 *(standard deviation represents that determined by the model)*

Method	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1	CD	MSC
A	0.169 ±0.0039	0.086 ±0.033	7.00 ±1.16	4776	0.998	6.420
B	0.8901 ±0.0241	0.11085 ±0.00958	1.4064 ±0.08822	12068	0.999	6.607

It was observed in chapter 7 that even the degradation of drug free PLGA microspheres was able to create a transient acidic microenvironment with PLGA microspheres. In the same way the pH of the incubation medium was observed to decrease with time for the fluphenazine loaded PLGA systems incubated as dispersed microspheres and contained within visking bags (Figure 10.22).

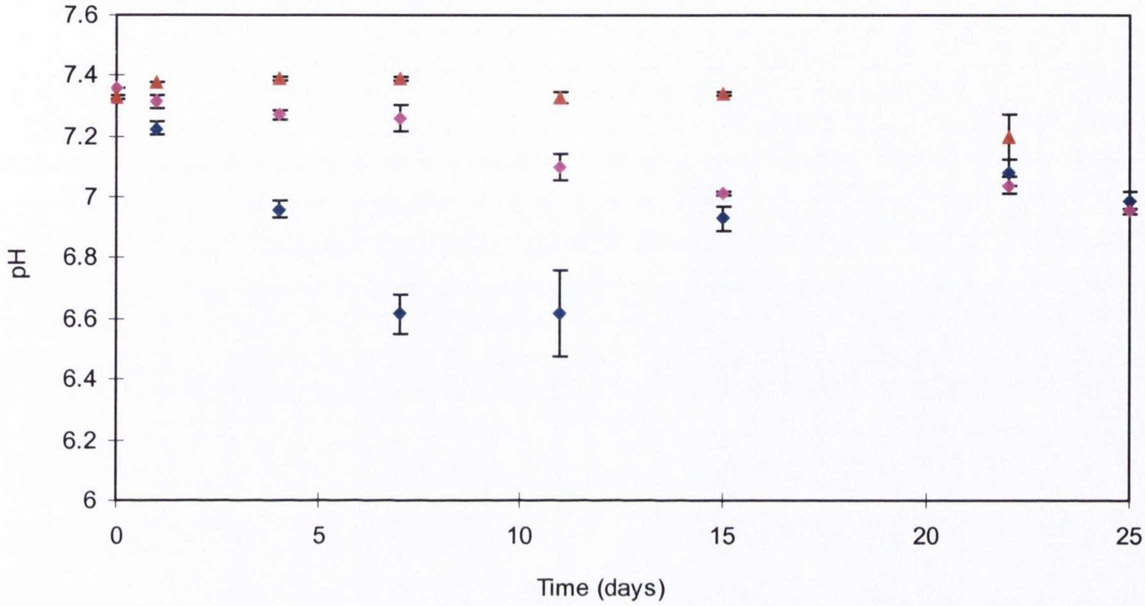
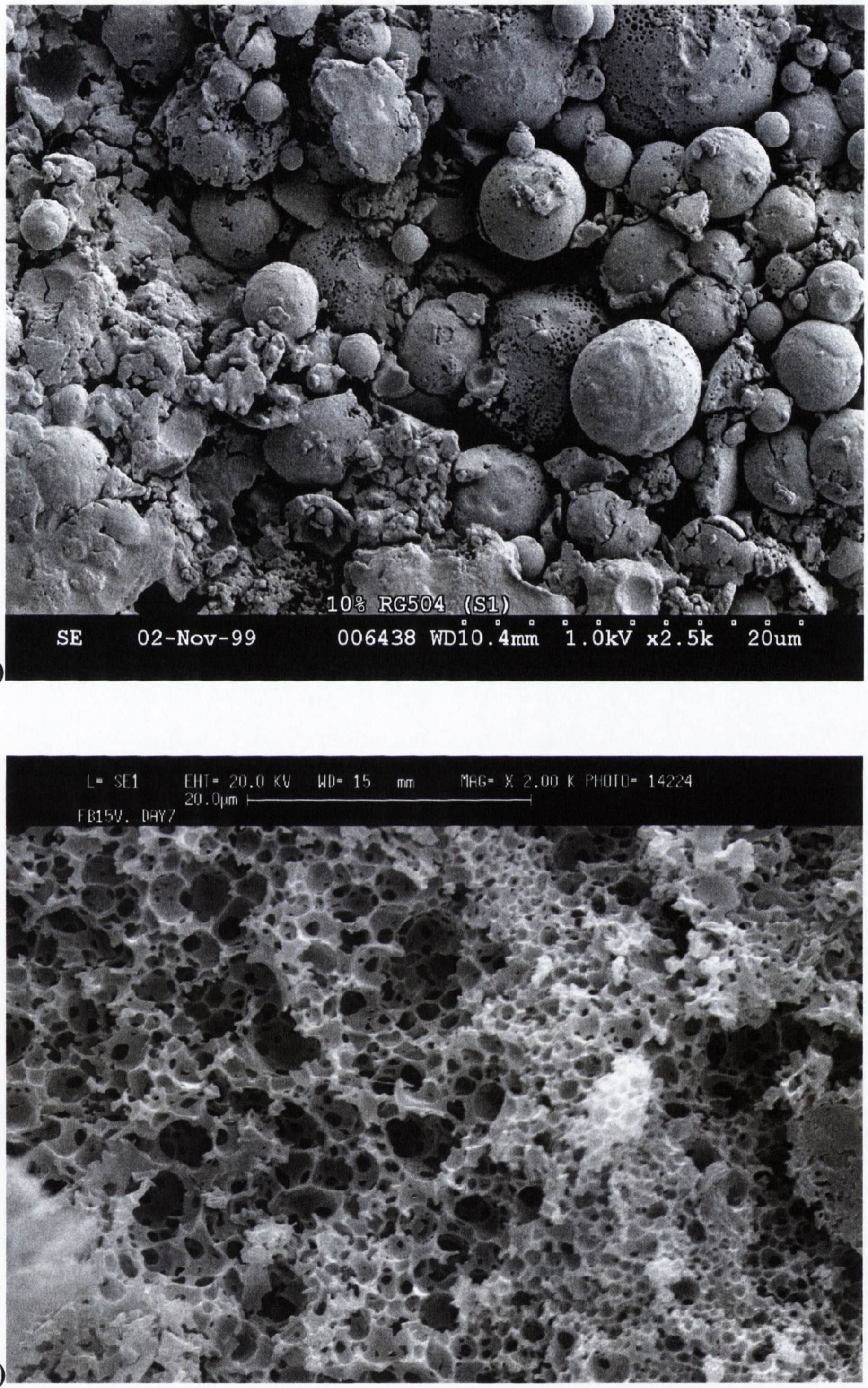


Figure 10.22 pH profile during Fluphenazine HCl release from PLGA microspheres incubated as dispersed microspheres within the dissolution medium (A) and in visking bags (B), for ▲ Method A ◆ Method B bulk media ◆ Method B within visking bag *(data points represent mean of three determinations ± standard deviation).*

Figure 10.22 shows the decrease in dissolution medium pH over time during the release process. For the dispersed microspheres the pH profile shows an initial lag after which a gradual decrease in the incubation medium pH is observed to the end of the profile. When microspheres are incubated in the visking bag the pH in the visking bag is decreased compared to the bulk medium. The acidic microenvironment created by the systems within the visking bag catalyses drug release and polymer degradation of the matrix.

Figure 10.23 shows SEM of 8.17% fluphenazine HCl loaded microparticles after 7days incubation using the two different methods. The electron micrographs also show that the polymer sample in the visking bag exhibits more degradation compared to the polymer sample recovered from the dissolution medium.



(a)

(b)

Figure 10.23. SEM of 8.2% fluphenazine loaded PLGA microparticles after 7 days incubation bags in phosphate buffer saline pH 7.4 at 37°C (a) dispersed microspheres and (b) in visking.

10.8 SUMMARY

Fluphenazine HCl loaded microspheres were prepared by an o/w solvent evaporation technique based on a method adapted from Ramtoola *et al.* (1992). Polymer molecular weight was significantly reduced by the formulation of fluphenazine using the solvent evaporation procedure. Formulation of Fluphenazine with PLGA microspheres caused significant degradation of the polymer during processing. A comparison of the $T_{g(p)}$ of drug free PLGA RG504 microspheres with the $T_{g(p)}$ of the drug-loaded microspheres indicated that a molecular dispersion of the polymer and drug may be present

Drug release showed a burst effect followed by a sigmoidal release profile at the three theoretical loadings investigated (5%, 10.0% and 20.0%). The model developed to describe drug release from polymer discs (Corrigan *et al.* 1998) was also found to describe the release kinetics of fluphenazine from microspheres. Parameters for the release process were obtained for the dissolution and degradation components of the release profile. These parameters were evaluated for different loadings, polymer type, polymer molecular weights and particle size. These effects are drawn together in Chapter 11.

The presence of fluphenazine in the polymer also accelerated the degradation rate of the polymer during the release process. The acceleration of the polymer degradation by the fluphenazine was found to be loading dependent. The effect of accelerated degradation on the release profile was also shown for PLGA and PLA systems.

The incubation of fluphenazine microspheres in visking bags considerably increased the rate of release of fluphenazine from PLGA and the concurrent polymer degradation. This enhancement was attributed to the effect of an acidic microenvironment created by polymer degradation and also to the effect of released fluphenazine in the microenvironment on the matrix. The evaluation of drug release in both models gives a different perspective on drug release profile.

CHAPTER 11

GENERAL DISCUSSION

11.1 INTRODUCTION

Particulate systems based on the polymers of lactic and glycolic acids have been extensively investigated as drug delivery technologies, particularly as vehicles to achieve controlled release. The release kinetics from these systems is a consequence of the physicochemical properties of the system. It is generally recognised that the polymer degradation plays a crucial role in determining the release profile from PLA/PLGA systems. There are very few studies in the literature that examine the degradation properties of the polymer through the full degradation profile particularly for particulate systems. Furthermore degradation effects have in the past been attributed to the composition of the starting polymer, however polymer properties and particle morphology continuously change during the course of degradation. In this work the degradation profile across a range of polymeric particulate variables is examined. The degradation profile was monitored. The influence of hydrolytic degradation induced effects on the degradation profile of PLA and PLGA microspheres is examined. A physicochemical understanding of these processes is the key to a better understanding of these systems. The release of drug from such carrier systems is actually a combination of a range of processes that are attributed to either diffusion or degradation. To investigate the mechanism of release of an encapsulated drug from a sustained release formulation, fluphenazine HCl was formulated into a selection of PLA/PLGA microspheres with varying physicochemical properties. The release mechanism and concurrent polymer degradation was followed from a selection of PLA/PLGA systems.

11.2 CHARACTERISATION OF POLYMERS AND THE SELECTION OF A PARTICLE MANUFACTURING PROCESS

A series of poly d,l-lactide (PLA) and polylactide-co-glycolide (PLGA) polymers manufactured by Boehringer Ingleheim were chosen. These polymers are usually classified according to an inherent viscosity value. Properties of the polymers were determined and related to the inherent viscosity of the polymer. A correlation between the inherent viscosity and polymer molecular weight was observed for both PLA and PLGA. The reciprocal of polymer molecular weight showed a correlation with polymer thermal properties. Since many of the properties of the polymer matrix depend on these parameters, this information is useful for the selection of alternative polymers of the PLA and PLGA series.

For the selection of a particle manufacturing process two methods were used to evaluate the formation of placebo PLA microparticles. These methods were particle production by spray drying or the emulsification solvent evaporation procedure. Smooth discrete particles were obtained by spray drying. For research purposes a small batch size is usually utilised and for the work planned a good yield from the process was a prerequisite. However using the spray drying method a low yield was obtained from this process and it was therefore decided to choose the solvent evaporation method over spray drying to produce placebo microparticles. Three different emulsification procedures were compared for a 1g PLA /1ml dichloromethane solution to produce microparticles in a 0.27% PVA solution. (1) Sonication/solvent evaporation was not sufficient to produce small discrete microparticles for the formulation conditions used. Further optimisation of this process was not carried out because it was observed that the sonication process was decreasing the molecular weight of the polymer. Reich (1998) also demonstrated sonication-induced degradation of polyesters. (2) Stirring/solvent evaporation was deemed not sufficient to produce small discrete microparticles. Homogenisation using the IKA ultra turrax was shown to produce smooth discrete microparticles whose size distribution could be altered by variation in homogenisation speed. A log-log relationship between particle size and homogenisation speed was observed similar to that observed by Jalil and Nixon (1990a). (3) Microfluidisation of the polymer solution in the aqueous phase produced nanoparticles. Nanoparticles and microparticles produced by the emulsification process were shown to retain the molecular weight and thermal properties of the original PLA polymer.

11.3 EFFECT OF PROCESSING ON THE PRODUCTION OF NANO AND MICROPARTICLES

PLGA and PLA particles were prepared using the emulsification-solvent evaporation method with size ranges: <1μm, <20μm and <50μm in diameter. Nanoparticles were prepared by microfluidisation (**Process A**), while microparticles were prepared to give microspheres <20μm by homogenisation at 24,000rpm (**Process B**) and <50μm in diameter by homogenisation at 8,000 rpm (**Process C**). The nano and microparticles were first characterised in terms of their particle size, process yield, thermal properties and molecular weight properties. Consistent particle size could be obtained between polymers of lower molecular weights of different end-group moiety by variations in the formulation.

For PLA and the high molecular weight PLGA microspheres the change in polymer molecular weight was not significantly different to that of the starting polymer indicating that minimal change in polymer properties occurred during processing. For the lower molecular weight PLGA and PLGA-H series a decrease in polymer molecular weight was observed. All property changes were <10% of the starting polymer material. From this work it was concluded the polymer molecular weight is a good indication of the resultant microsphere polymer molecular weight and that these polymers are robust to the processing techniques commonly employed to produce particulate systems. When homogenisation or microfluidisation was used to produce PLGA microspheres and nanospheres respectively no appreciable polymer degradation was observed as a result of processing.

11.4 THE EFFECT OF PARTICLE SIZE ON THE DEGRADATION PROPERTIES OF PLA AND PLGA MICROSPHERES

Polylactide-co-glycolide (PLGA RG504) polylactide-co-glycolide (PLGA RG504H) polylactide (PLA R203) particles were prepared using the emulsification-solvent evaporation method with size ranges: <1μm, <20μm and <50μm in diameter. Estimates for the degradation and erosion of the polymer were calculated and related to the particle size as follows. Plots of the polymer degradation rate constants k_1 and k_2 of PLGA (RG504), PLGA (RG504H) and PLA (R203) particles with size ranges: <1μm, <20μm and <50μm in diameter are shown (Figure 11.1). Figure 11.1 shows that for the three polymer systems studied the rate constant k_1 of the polymer degradation process was linearly dependent on the particle size where larger particle sizes degraded at a faster rate compared to the nanoparticles. In contrast the rate constant k_2 of the polymer degradation process appeared to be independent of the particle size. Two degradation phases were identified in the study period and the rate constants were calculated for each of these phases at 37°C, the rate constants for the degradation of the particles are shown in Figure 11.1.

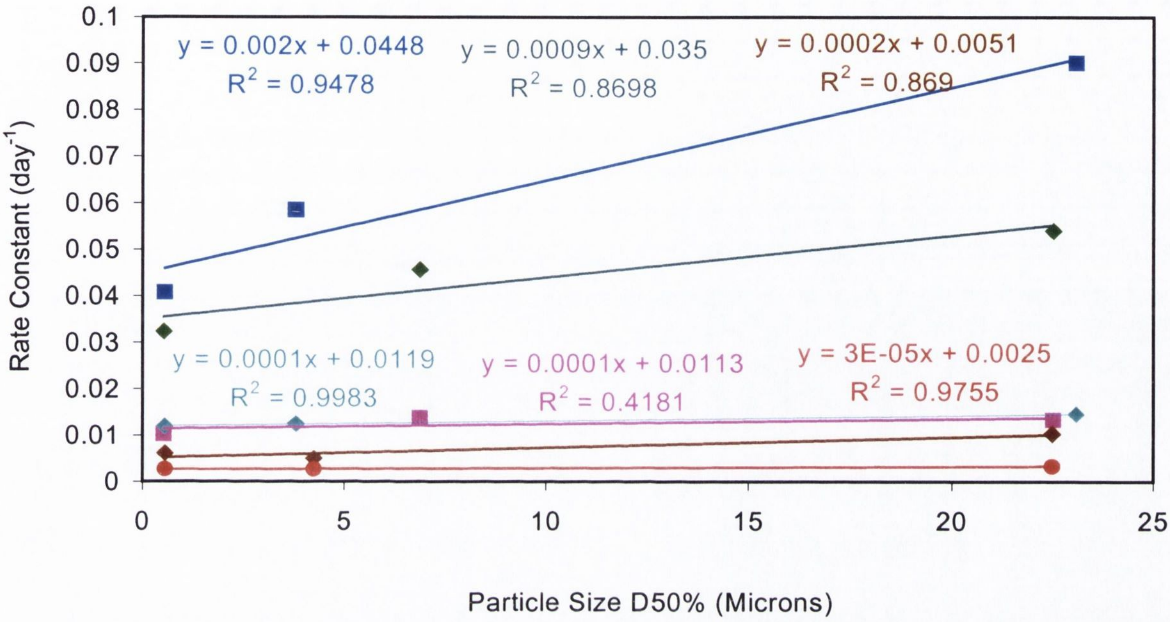


Figure 11.1 Effect of particle size on the polymer degradation rate constants of PLA and PLGA particles, for ■ PLGA (RG504H) k_1, ◆ PLGA (RG504) k_1, ◆ PLA (R203) k_1 and ◆ PLGA (RG504H) k_2, ■ PLGA (RG504) k_2 , ● PLA (R203) k_2.

Morphology studies carried out as part of the investigation into the degradation phase indicate that during the degradation phase k_2 the initial discrete particles were reduced to a porous polymer mass and therefore the effect of initial particle size is no longer relevant. No relationship between particle size and value *Tau* was evident. The critical molecular weight (Mp_1) for PLGA and PLA was also found to decrease with increase in particle size.

The relationship between the particle size and the erosion (mass loss) rate for the three polymer systems was also explored (Figure 11.2 and 11.3). When estimates of the parameters determined using the Prout-Tompkins equation (Equation 3.23) were plotted against the particle size $D_{50\%}$ a linear relationship was observed between the particle size and the erosion rate of the polymer *k* and *Tmax* (Figure 11.2 and 11.3). Similar trends were also true for plots of particle $D_{90\%}$ with the erosion parameters.

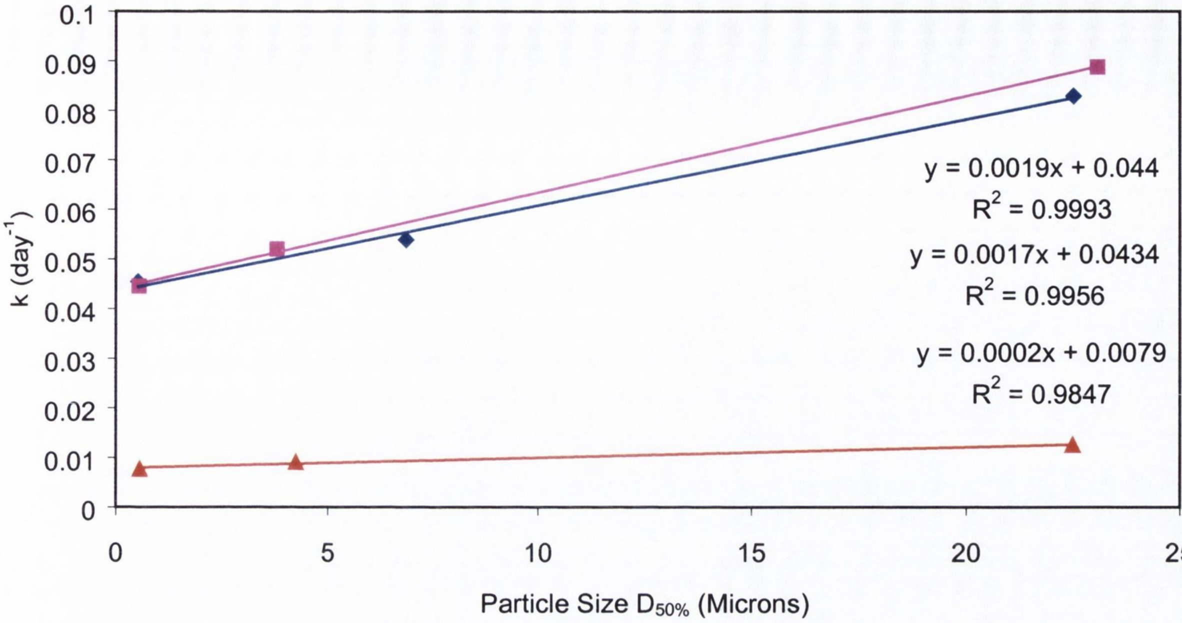


Figure 11.2 The relationship between *k* and particle size $D_{50\%}$ for the erosion of PLA and PLGA microspheres, for ▲ PLA (R203), ◆ PLGA (RG504), and ■ PLGA (RG504H).

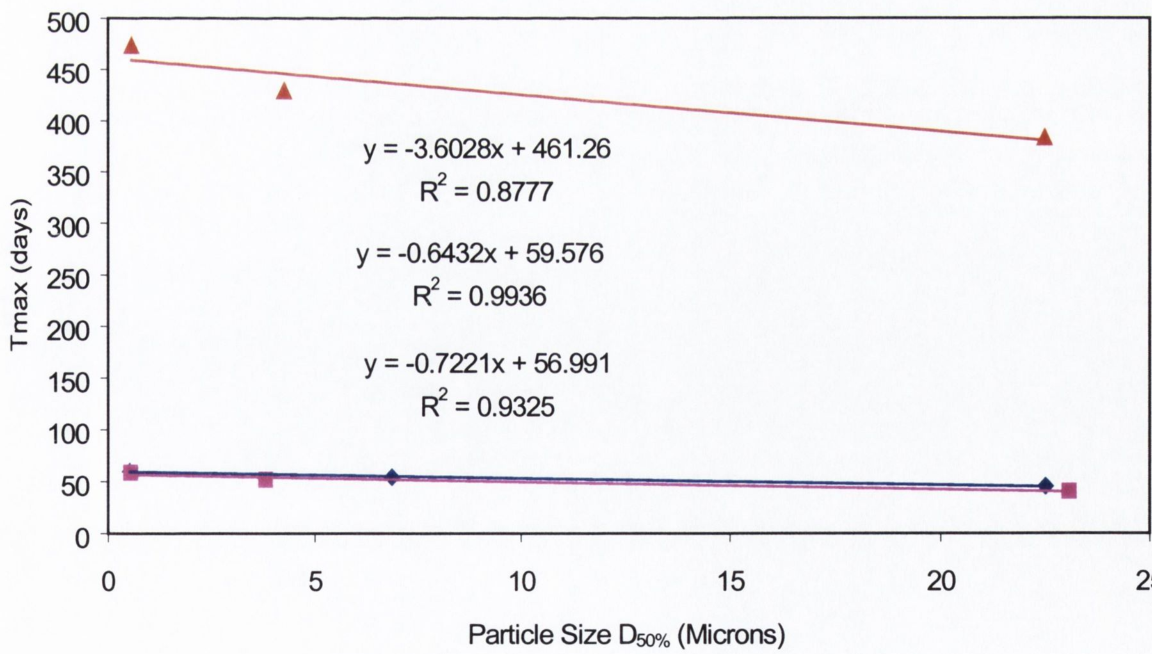


Figure 11.3 The relationship between *Tmax* and particle size $D_{50\%}$ for the erosion of PLA and PLGA microspheres, for ▲ PLA (R203), ◆PLGA (RG504) and ■ PLGA (RG504H).

For the Prout-Tompkins equation (Equation 3.23) the parameter m which is given by equation 11.2:

$$m = -kT\max \qquad \text{Equation 11.2}$$

When –m was calculated from the parameters estimated using the Prout-Tompkins equation and plotted against particle size a linear relationship was also observed (Figure 11.4).

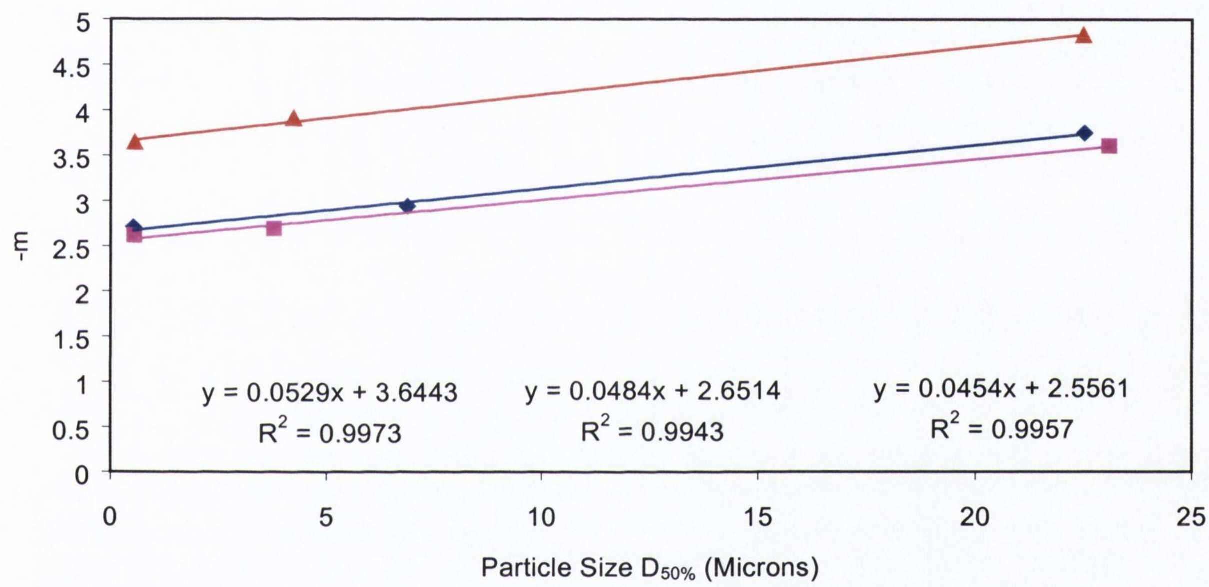


Figure 11.4 The relationship between -m determined using equation 3.25 and particle size $D_{50\%}$ for the erosion of PLA and PLGA microspheres, for ▲ PLA (R203), ◆PLGA (RG504) and ■ PLGA (RG504H).

The linear relationship established between particle size and the parameters estimated from the Prout-Tompkins equation was demonstrated in both PLA and PLGA polymers. The relationship between the erosion parameters determined using Equation 3.23 and particle size $D_{50\%}$(microns) were also true when these parameters were plotted against particle $D_{90\%}$ (microns).

The influence of particle size on the degradation is attributed to the fact that in smaller particles degradation products formed within the particle have a shorter path to the surface while in the larger particles degradation products have a longer path to the surface of the particle. Degradation products trapped within the particle have the potential to catalyse the degradation of the remaining polymer material. In a publication by Grizzi *et al.* (1995) the degradation of compression moulded plates (15*10*2mm), millimetric beads (0.5-1.0mm), microspheres (0.125-0.250) and cast films of d,l-PLA were compared in isotonic phosphate buffer pH 7.4 at 37ºC. The degradation of the plates was found to be faster than beads, followed by microspheres. The polymer films showed the slowest degradation rate.

11.5 THE EFFECT OF POLYMER PROPERTIES ON THE DEGRADATION PROPERTIES OF PLA AND PLGA MICROSPHERES

The relationship between the polymer molecular weight and the degradation rate of PLA and PLGA microspheres was explored. A systematic study of the degradation properties of a range of biodegradable polymers with a comparable particle size (≤20μm) was carried out. Parameters for the polymer degradation of the range of these systems are shown in Table 11.1.

Table 11.1 Parameters for the degradation of PLA and PLGA particles at 37°C in PBS using different polymer molecular weight PLA and PLGA .

Polymer	Mp	$T_{g(p)}$ (ºC)	T_{lag} (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1
RG502H	8474	38.730	-	-	0.019	-	N/A
RG502	12194	42.506	-	0.014	0.013	21.990	8983
RG503	25206	47.122	-	0.028	0.014	25.350	12342
RG504	42124	52.083	-	0.046	0.014	29.788	10833
RG504H	48025	52.379	-	0.059	0.012	23.542	12179
R104	4184	35.865	-	0.017	0.002	4.688	3874
R203	19914	55.671	100.987	0.006	0.003	282.532	6891

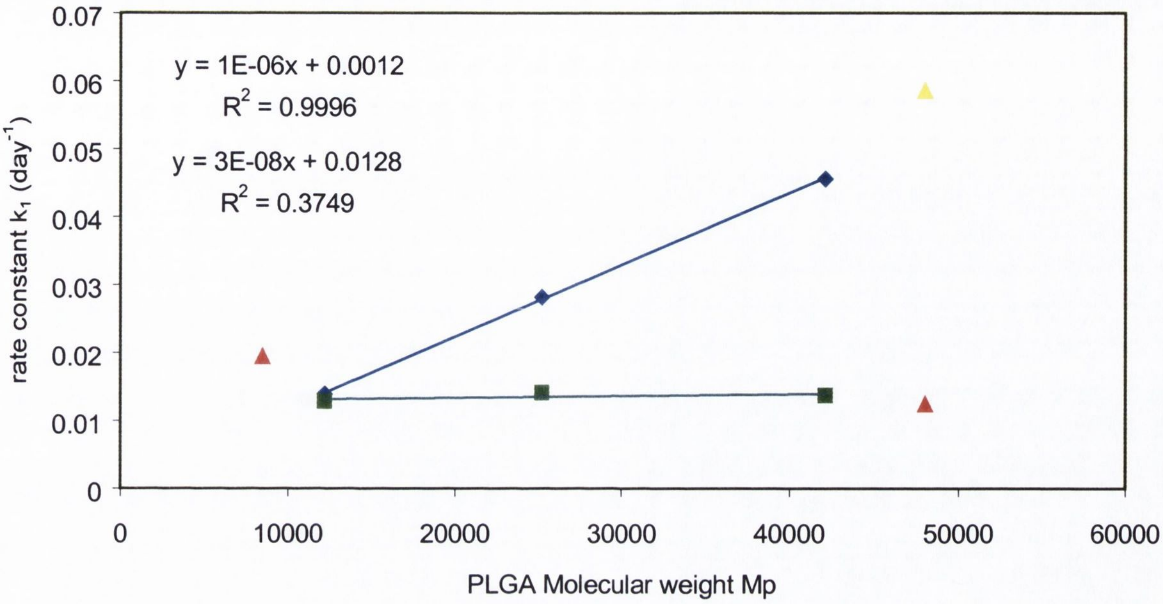
Table 11.1 shows that the degradation rate of the polymer was dependent on the initial molecular weight of the polymer. GPC analysis of all the PLGA series of polymers studied showed a steady decrease in the average molecular mass of the polymer from the onset of

incubation in an aqueous environment at 37°C. Polymers of higher initial polymer molecular weight underwent a change in molecular weight at a faster rate (k_1) than the corresponding lower molecular weight polymer (Figure 11.5a). For molecular weights of 10,000-60,000 this relationship was linear. This observation is in agreement with that observed by Pistner *et al.* (1993), O'Hagan *et al.* (1994). Cleavage of the low molecular weight chains does not produce such a dramatic decrease in molecular weight as the cleavage of a higher molecular weight chain. In contrast the rate constant k_2 of the polymer degradation process appeared to be independent of the initial polymer weight.

The polymer degradation profile for PLA microspheres showed that the polymer molecular weight degraded at a faster rate (k_1) for PLA (R104) compared to PLA (R203) (Figure 11.5b). The degradation pattern for the PLA (R203) showed a triphasic profile compared to the low molecular weight PLA (R104) and to the series of PLGA polymers. A study by Park on a high molecular weight PLA (Mw 18,000) and a low molecular weight PLA (7,4000) also showed an increased polymer degradation rate for the low molecular weight compared to the corresponding high molecular weight polymer.

Two factors are thought to influence the relationship between polymer molecular weight and polymer degradation. First is the selection of individual polymer molecular weights relative to each other and the second is shape of the overall degradation profile. Polymers which exhibit the same degradation mechanism (bi-phasic) e.g. RG504-RG502 will exhibit a lowering of polymer degradation rate (k_1) with a decrease in polymer molecular weight.

(a)



(b)

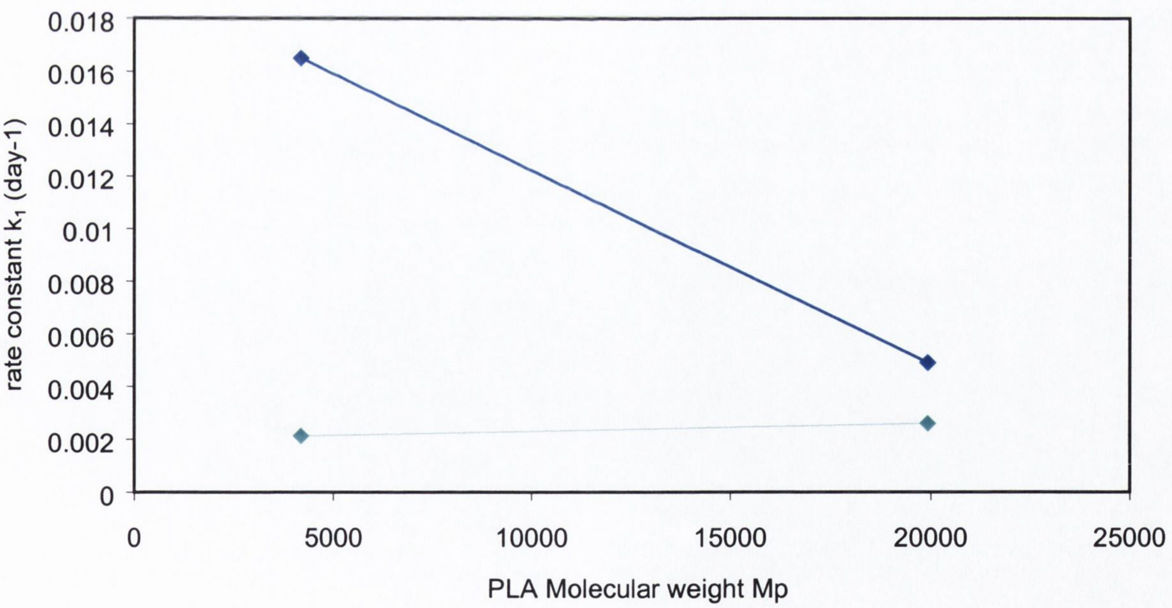
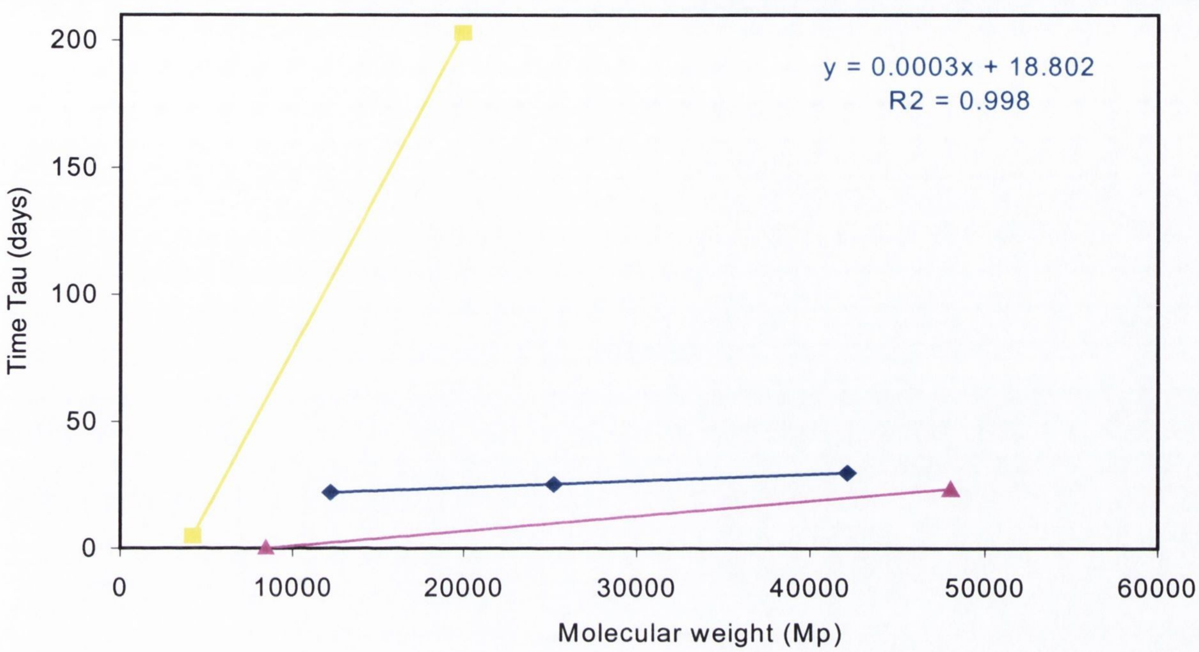


Figure 11.5 The effect of molecular weight on the rate of polymer degradation of (a) PLGA and (b) PLA microspheres, for ◆ PLGA k_1, ■ PLGA k_2, ▲ PLGAH k_1, ▲ PLGAH k_2, ◆ PLA k_1 and ◆ PLA k_2.

A plot of the parameter *Tau* as a function of polymer molecular weight is shown in Figure 11.6a. This plot shows a linear relationship between time *Tau* and polymer molecular weight for PLGA. Values for the more hydrophilic PLGAH series and the low molecular weight PLA (R104) of polymers exhibited considerably shorter *Tau*. PLA (R203) had a time *Tau* of approximately 200 days; this is almost eight times slower that that of the polymer of nearest molecular weight for the PLGA polymer (RG503). The time *Tau* represents the time point where the start of the second molecular weight phase in the degradation profile occurs. This occurred faster for lower molecular weight and more hydrophilic polymers.

Figure 11.6(b) shows the critical molecular weight estimated for the PLGA and PLA series. The critical molecular weight represents the value of the polymer molecular weight when the onset of mass loss occurred from the polymer. It is observed that lower molecular weight polymers were associated with a lower critical molecular weight. This relationship is thought to be a complex combination of the rate of hydration, microsphere morphology and thermal properties of the matrix (discussed in the next section). Figure 11.6(b) shows that PLGA polymers with a molecular weight of ~8000 will immediately undergo monophasic erosion controlled degradation kinetics while for the PLA series of polymers the value is ~3000. The value for PLA is postulated to occur at a lower value because the glycolide units, which are more hydrophilic than the lactide units, are thought to promote water uptake into the polymer, which initiates hydrolytic degradation (Dunn *et al.* 1995) faster. These values may also represent a lower permeability in PLA compared to PLGA.

(a)



(b)

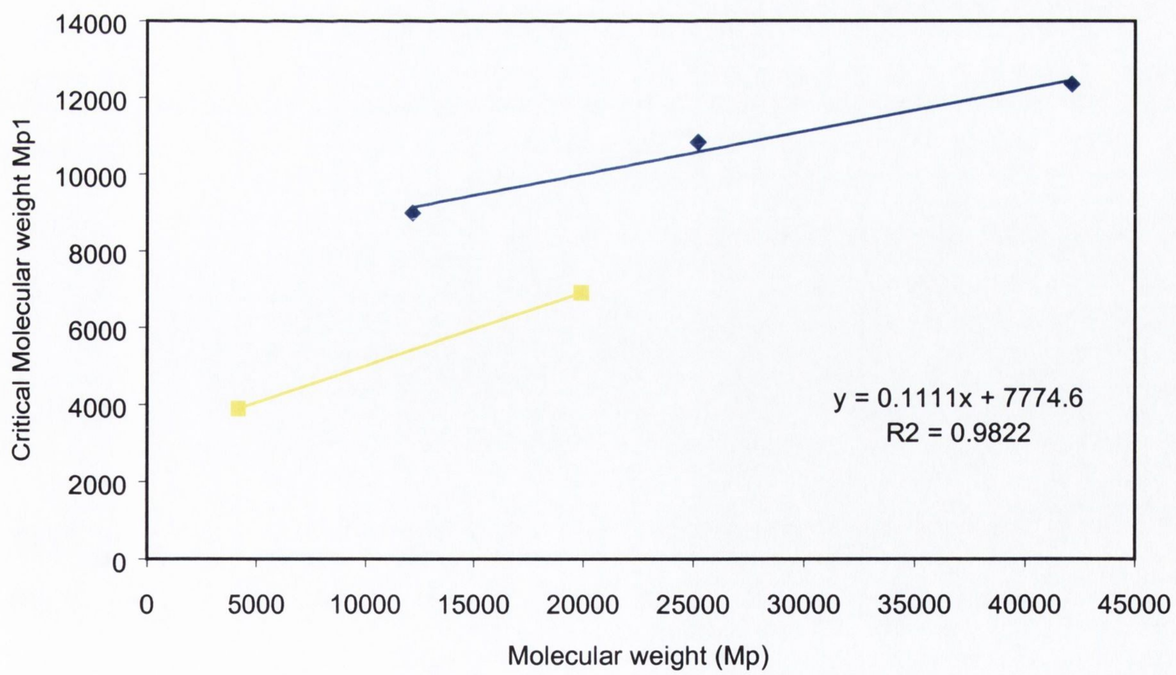
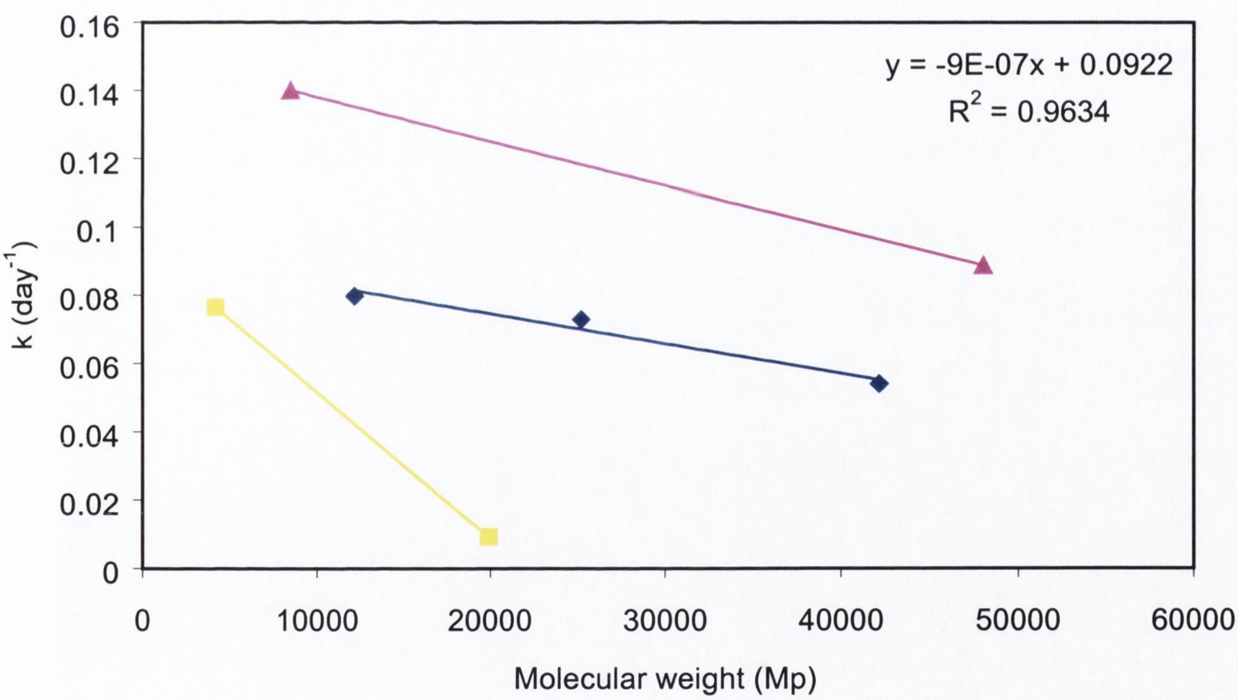


Figure 11.6 The effect of polymer molecular weight on (a) Time *Tau* and (b) critical molecular weight Mp_1, for ◆PLGA, ▲ PLGAH and ■ PLA series.

11.6 THE EFFECT OF POLYMER PROPERTIES ON THE EROSION OF PLA AND PLGA MICROSPHERES

The process of mass loss or erosion occurs from PLA and PLGA matrices when the polymer has sufficiently degraded to produce low molecular weight soluble polymer fragments in the polymer distribution profile of the polymeric deliver system. Fitzgerald and Corrigan (1996) showed that mass loss from drug loaded microspheres followed Prout-Tompkin decomposition kinetics (Equation 3.23). The erosion profile of drug free PLA and PLGA microspheres were fitted to that equation in this work. Estimates for the erosion of PLA and PLGA and PLGAH polymers of different polymer molecular weights are compared in Figure 11.7.

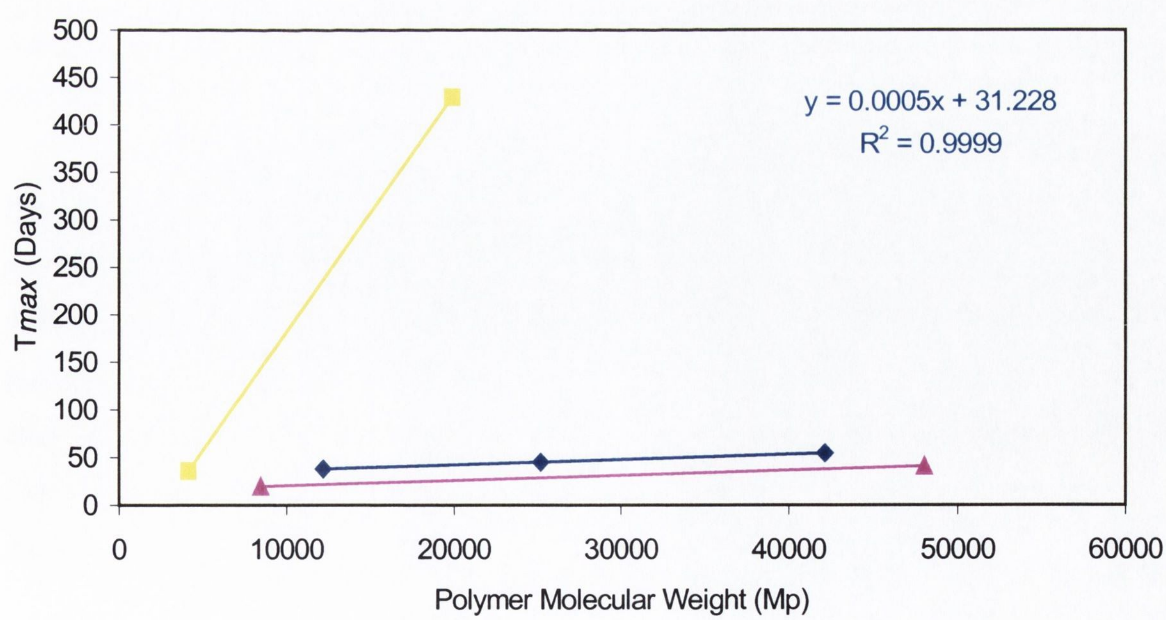


11.7 Effect of polymer molecular weight on the rate of erosion of PLA/PLGA microspheres, for ◆PLGA ▲ PLGAH ■ PLA series.

Figure 11.7 shows that the lower molecular weight polymer erodes at a faster rate than the higher molecular weight polymer for the three polymer systems studied. For PLGA RG504 a comparable onset of mass loss (25-28 days) and erosion profile has been reported (Kenley *et al.* 1987 and Ramchandani *et al.* 1997).

Despite similar physicochemical properties the two PLGA types RG504, RG502 and the corresponding RG504H and RG502H demonstrate significantly different degradation behaviour particularly for the lower molecular weight polymer. Low molecular weight PLA shows fast erosion kinetics compared to the high molecular weight PLA. Park (1994) showed that the critical molecular weight of oligomers which can be solubilised in water was found to be around 1100. The number of units in the water soluble oligomers was estimated as 14-15 lactic acid units (Park 1994). The low molecular weight PLA would therefore have polymer molecular weight chains that could be immediately solubilised upon hydration. In fact low molecular weight PLA (R104) has been utilised for blending with high molecular weight polymers to induce immediate loss from the polymer thereby accelerating the release profile (Grandfils *et al.* 1996).

The *Tmax* of the polymer refers to the time when the weight of residual polymer becomes half the weight of the starting polymer. *Tmax* was shown to decrease as the molecular weight of the PLA or PLGA polymer decreased and was further lowered for the more hydrophilic polymer. *Tmax* for the erosion of PLA and PLGA also demonstrated a relationship with the initial molecular weight of the polymer (Figure 11.8).



11.8 Effect of polymer molecular weight on the rate of erosion of PLA/PLGA microspheres, for ◆PLGA, ▲ PLGAH and ■ PLA series.

11.7 THE DEGRADATION MECHANISM OF PLA AND PLGA PARTICLES

In the preceding sections the effect of the investigators' choice of processing technique, particle size and polymer on the particle properties and degradation profile were discussed. Models applied to the polymer degradation and erosion profile were utilised to determine the relationship between microsphere properties and the degradation rate. In this section the physicochemical and morphological changes that occur during the degradation process are examined.

11.7.1 Polymer Molecular Weight degradation profile

The physicochemical properties of the polymer and the incubation conditions determine the number of degradation phases exhibited by a polymer. The three possible phases are (a) the induction phase where no property of the polymer apparently changes during incubation under a defined set of experimental conditions (lag phase). (b) A rapid degradation of the polymer to lower molecular weights by scission of high molecular weight polymer chains without an accompanying weight loss (phase 1). (c) A slow loss in molecular weight as the polymer erodes (phase 2). In the past most degradation modelling approaches involved assuming first order degradation kinetics and the calculation of the rate constant from the semi-log plot (Kenley *et al.* 1987). In this work models that described the three phases of the degradation profile were developed.

Hydrophobic and/or high molecular polymer or polymers incubated at low temperatures exhibit lag phase kinetics. PLA R203 (Mw 20K) showed tri-phasic degradation kinetics similar to that observed by Reich (1998) for PLA (Mw 16K and 10K). Most of the polymers studied followed a combination of Phase 1 and 2 kinetics. Fukuzaki *et al.* (1991) also showed a biphasic degradation process for three molecular weight PLGA's (Mw 16900-41300) studied. Polymers with the starting molecular weight below the critical molecular weight (RG502H) exhibited only phase 2 kinetics.

11.7.2 Polydispersity

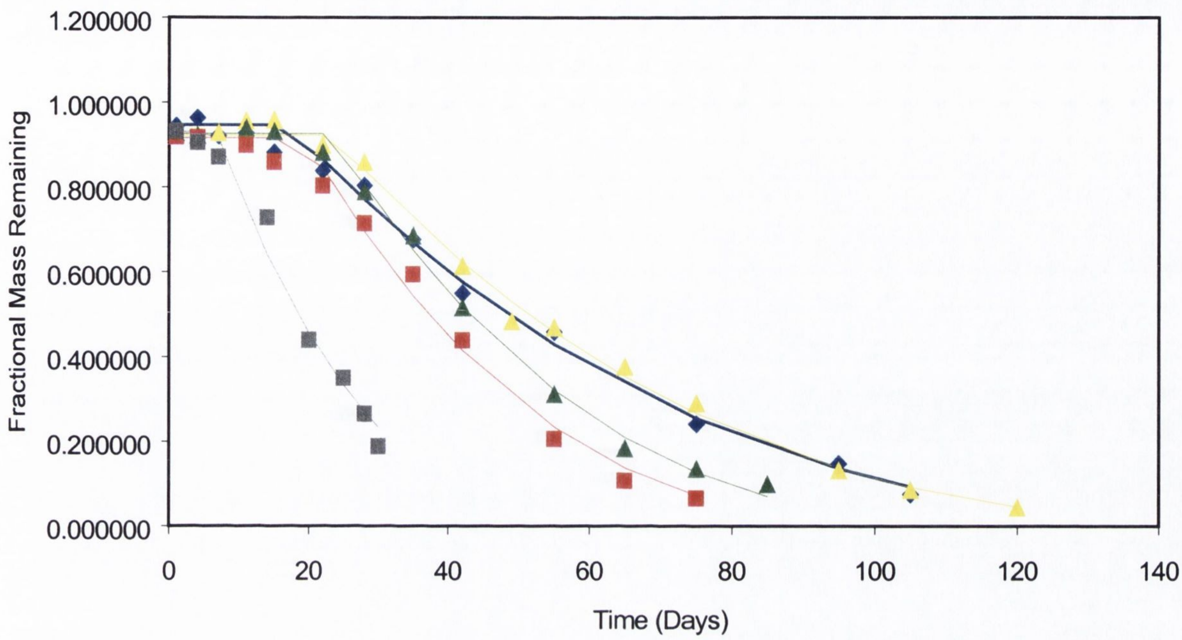
In this work it was observed that polymers initially had a low polydispersity ca. 1.6 and as the study progressed the longer chains of low molecular weight polymer broke into different sizes thus rapidly increasing its polydispersity index to approximately 2. This

Recently Mollo (1999) used the Hixson and Crowell (1931) equation to describe polymer erosion from Amoxycillin loaded PLGA discs. The Hixson and Crowell equation was modified to describe mass loss in terms of the uneroded polymer and to take account of the lag phase observed in the profile according to Equation 11.1.

$$W_d = W_i[1-k_3(t_{elapsed}-t_{lag})]^3 \quad \text{Equation 11.1}$$

where W_i is the initial weight of the microspheres used, W_d is the weight of microspheres after the elapsed incubation time $t_{elapsed}$. t_{lag} is the induction period observed in the weight loss profile during which time no loss in mass is observed after which mass loss occurs at a rate k_2. This equation proves quite versatile for the modeling of PLA/PLGA erosion profile as it enables one to determine the T_{onset} of polymer erosion using a mathematical model. For these polymer systems T_{onset} was shown to be an important parameter in terms of the degradation of the polymer. Mass loss profiles for the erosion of PLA and PLGA were fitted to Equation 11.1 are shown in Figure 11.9 a and b.

(a)



(b)

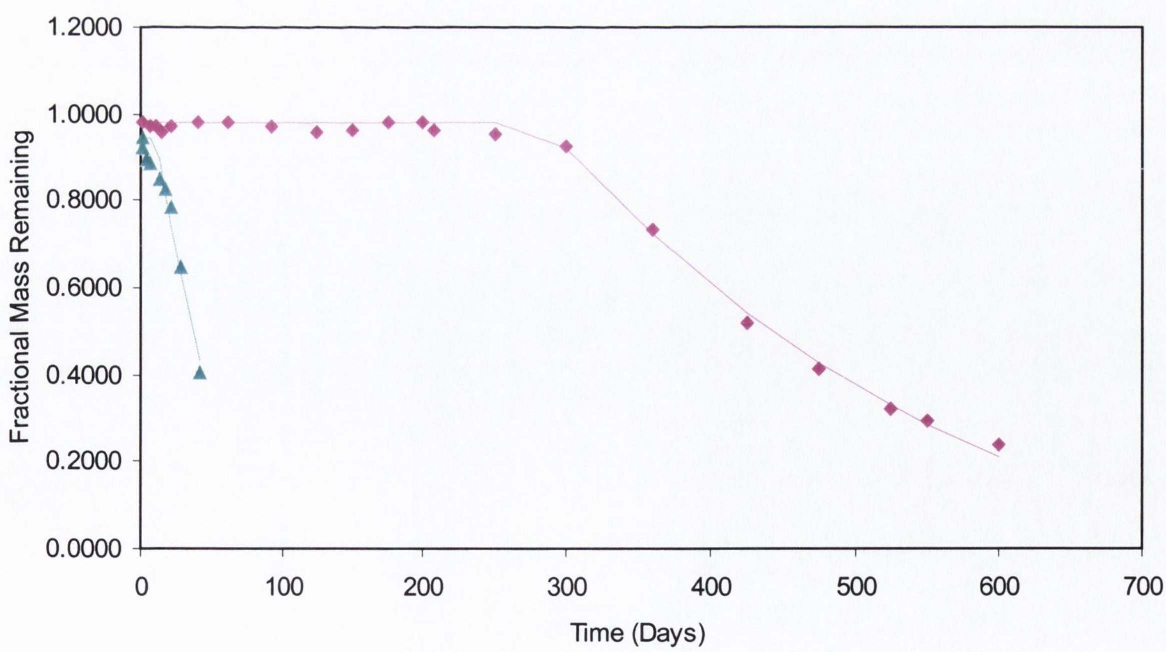


Figure 11.9 Mass loss from (a) PLGA and (b) PLA microspheres fitted to Equation 11.1, for ◆RG504H, ▲ RG504, ▲ RG503, ■ RG502, ■ RG502H, ◆ R203 and ▲ R104.

When the polymeric microspheres were incubated in phosphate buffered saline it was found that for all polymers mass loss was associated with the second phase of the degradation profile. Using the Equation 11.1 it was possible to provide a relationship between the time *Tau* which indicates the change from phase 1 to phase 2 kinetics determined from the molecular weight profile, with T_{onset} the onset of mass loss (Table 11.2).

Table 11.2 A comparison between the parameter *Tau* and T_{onset} for a range of PLA and PLGA microspheres of different molecular weights.

Polymer	Mp	*Tau* (days)	T_{onset}(days)
RG502H	8474	0	6.630
RG502	12194	21.991	19.516
RG503	25206	25.351	21.111
RG504	42124	29.788	23.384
RG504H	48025	23.542	17.230
R104	4184	4.688	11.417
R203	19914	282.532	277.832

Table 11.2 shows that the time *Tau* (the parameter determined from the polymer degradation) occurred just after the onset of mass loss from the polymer determined using equation 11.1 for all the polymers with the exception of the very low molecular weight PLA and PLGA (this is discussed later). These observations indicate that degradation of the polymer progresses until the properties of the polymer are sufficiently altered to allow mass loss from the polymeric device occur. The onset of mass loss correlated with the change in degradation phase, this observation was also demonstrated by Reich (1998).

The two polymer erosion models (Equation 3.23 and 11.1) could be utilised to fit the erosion profile of PLA and PLGA microspheres however the two models represent alternative mechanisms of mass loss. A comparison of the fit of the erosion data to both models may give further insight to the erosion mechanism (Table 11.3 and 11.4).

Table 11.3 Estimates of the parameters for the erosion of PLA and PLGA particles of different polymer molecular weight at 37°C pH7.4 fitted to Equation 3.23.

Polymer	Mp	k (day^{-1})	$Tmax$(days)	CD	MSC
RG502H	8474	0.140	19.907	0.998	4.779
RG502	12194	0.080	37.830	0.996	5.270
RG503	25206	0.073	45.037	0.992	4.516
RG504	42124	0.054	54.150	0.995	3.926
RG504H	48025	0.052	51.725	0.998	4.012
R104	4184	0.076	35.500	0.986	3.904
R203	19914	0.009	428.970	0.997	3.420

Table 11.4 Estimates of the parameters for the erosion of PLA and PLGA particles of different polymer molecular weight at 37°C pH7.4, fitted to Equation 11.1.

Polymer	Mp	T_{onset} (days)	k_3 (day^{-3})	CD	MSC
RG502H	8474	6.630	0.016	0.979	3.406
RG502	12194	19.516	0.010	0.990	4.286
RG503	25206	21.111	0.009	0.995	5.157
RG504	42124	23.384	0.007	0.994	4.923
RG504H	48025	17.230	0.006	0.992	4.574
R104	4184	11.417	0.008	0.948	2.512
R203	19914	277.832	0.001	0.997	5.556

Equation 11.1 incorporates the Hixson and Crowell cube root equation (1931) which is consistent with a surface eroding system however homogeneous bulk erosion is the process commonly reported to occur in eroding PLA/PLGA systems (Gopferich, 1996). The Prout –Tompkins equation expresses polymer mass loss in terms of a homogeneous erosion mechanism. An initial comparison between the models for the polymers PLA (R203) and PLGA (RG504) showed that the fit of the polymer erosion data for these polymers was better using Equation 11.1 which indicates an unexpected surface erosion controlled mechanism. This would be inconsistent with other mechanistic findings for polymer

degradation in this work. The full set of polymers fitted to both equations and compared in Table 11.3 and 11.4 show some interesting observations regarding the erosion mechanism of PLA and PLGA microsphere systems.

Comparison of the MSC values for the PLGA polymers indicated that at high polymer molecular weights the erosion of the polymer was found to give a better fit than the surface erosion controlled model (Equation 11.1). Hence polymers RG504, RG503 and RG502 fitted Equation 11.1 better than the Prout–Tompkins homogeneous erosion mechanism. However as the polymer molecular weight lowered this relationship between erosion models switched. The measure of goodness of fit (the MSC values) became better for the homogeneous erosion mechanism. This relationship was also observed for the two PLA polymers. In fact for the low molecular weight PLA R104 and PLGA RG502H the fit to the model was poor and the T_{onset} values were significantly over estimated (Figure 11.9).

It was observed that the Prout–Tompkins homogeneous erosion mechanism was associated with the low molecular polymers. It is postulated that in the low molecular weight polymers water can immediately diffuse into the polymer and the polymer then undergoes a homogeneous erosion mechanism. However in the higher molecular weight polymer water diffusion and degradation is considerably slower. When the microsphere structure breaks down due to the polymer being sufficiently degraded the loss of erosion products is akin to a surface erosion or surface dissolution mechanism.

Brunner *et al.* (1999) also demonstrated that polymer degradation products are not readily released from degrading PLA/PLGA microspheres as measured by an increase in osmotic pressure inside the degrading microspheres. These authors demonstrated a drop in internal pH to ≤4.7 in PLGA microspheres after 14 days using a pH sensitive electron paramagnetic resonance (EPR) probe. Visscher *et al.* (1986) and Vert *et al.* (1991) have observed significant differences in the characteristics of the surface and the core of microparticles under degradation. Degradation and erosion are the decisive performance parameters of a polymeric system. In the presence of polymer degradation and erosion the matrix progressively alters its thermal and morphological properties.

11.7.4 Thermal properties

The relationship between $T_{g(p)}$ of the polymer and the molecular weight for a given polymer series was demonstrated in Chapter 6. The thermal properties of PLA and PLGA microspheres were followed using dried microspheres recovered from the dissolution medium. Recent studies by Park (1994,1995b) also used dried microspheres to study the degradation properties of PLA/PLGA microspheres. Figure 11.10 shows that the $T_{g(p)}$ of the polymer decreased with a linear relationship as a function of reciprocal Mp. DSC thermograms of PLA/PLGA microspheres showed peak broadening with incubation time that was concurrent with increases in polydispersity observed. In contrast to work with PLA/PLGA implants by Li *et al.* (1990), no evidence of crystallisation of the degradation products was observed in this study. It is considered that in smaller devices such as microspheres this phenomenon may not occur due to the lower concentration of degradation products compared to an implant.

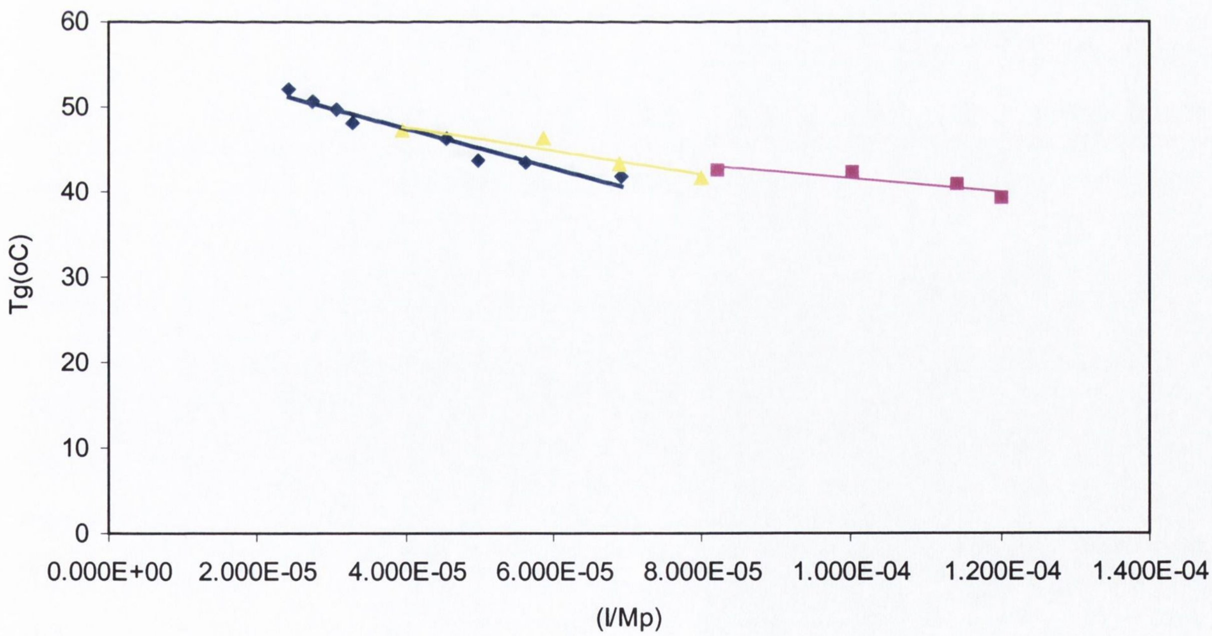


Figure 11.11 The decrease in glass transition temperature as a function of decreasing polymer weight for PLGA microspheres, for ◆ RG504, ▲ RG503 and ■ RG502.

The relationship between the $T_{g\,(p)}$ of the polymer and the onset of erosion determined using Equation 11.1 was examined from the data presented in Table 11.5

Table 11.5 A comparison between the T_{onset} and $T_{g(p)}$ for a range of PLA and PLGA microspheres of different molecular weights.

Polymer	$T_{g(p)}$ (°C)	Mp	T_{onset}(days)
RG502H	38.730	8474	6.630
RG502	42.506	12194	19.516
RG503	47.122	25206	21.111
RG504	52.083	42124	23.384
RG504H	52.379	48025	17.230
R104	35.865	4184	11.417
R203	55.671	19914	277.832

Table 11.5 shows that polymers with a low $T_{g(p)}$ were associated with lower T_{onset} as determined using Equation 11.1. It was observed that for the three PLGA polymer series (RG504, RG503, RG502) a linear relationship between the T_{onset} and the $T_{g(p)}$ of the polymer existed ($y = 0.4046x + 2.2268$, $R^2 = 0.9936$). It is therefore concluded that the thermal properties of the polymer matrix play an important role in determining the degradation profile of PLA/PLGA polymers.

11.7.5 Morphological evaluation of microsphere degradation

The first morphological changes observed occurred at the particle surface. SEM studies of nanoparticles after incubation showed that the smaller particles were more prone to fusion. Throughout this work it was demonstrated that loss of microsphere shape and microsphere fusion were indicators of polymer degradation. SEM time points for different polymer systems when compared, showed that pores occurred just before the onset of detectable mass loss. The morphological changes in the particle structure were associated with the degradation process. The development of a porous structure that is visible under scanning electron microscopy was linked to the particle size, polymer composition and the polymer molecular weight.

An interesting finding was observed in the morphological investigations of the degradation at low incubation temperatures. At 5°C no polymer degradation occurred for PLA and PLGA nano and microspheres. No loss in microsphere integrity over 850 days for PLGA and PLA particles was observed. This finding implies that the morphological changes observed in these systems are related to the degradation of the polymer and are not just induced by the presence of an aqueous environment.

11.8 DISSOLUTION FACTORS THAT INFLUENCE THE DEGRADATION MECHANISM OF PLA/PLGA MICROPARTICLES

11.8.1 The pH effect on the degradation mechanism

The pH of the incubation medium was observed to have a dramatic effect on both the rate and mechanism of polymer degradation. The morphology studies of the microspheres incubated at pH 1 and pH 10 given in Chapter 8 showed a dramatic difference in microsphere morphology at different incubation medium pHs. This observation lead to the deduction that the base catalysed hydrolysis of polyesters may proceed by a different mechanism than observed for acid catalysed hydrolysis. In Table 11.5 the rate of polymer degradation is given with the corresponding rates of erosion at different pH media and their interrelationship is examined.

Table 11.3 The effect of incubation medium pH on the degradation *(k_1)* and erosion (*k* and *Tmax*) parameters of PLGA (RG504) microspheres

Incubation Conditions	k_1 (day^{-1})	k (day^{-1})	*Tmax*
pH 1.0	0.064	0.0743	31.383
pH 7.4	0.0393	0.0496	60.148
pH10.0	0.062	0.221	7.964

Table 11.3 demonstrated that changes observed in the acceleration of molecular weight decrease (k_1) did not correlate with changes in the polymer mass loss (k and *Tmax*). This difference in degradation mechanism is attributed to initial surface accelerated degradation of the polymer that is in contact with the extreme acidic and basic conditions. When the

undegraded polymer is removed from the incubation medium the polymer inside the matrix retains molecular weight characteristics comparable to the molecular weight expected at pH 7.4. The influence of the pH on the polymer molecular weight can only occur when the matrix is sufficiently degraded to allow the ions to penetrate into the polymer matrix. Surface controlled erosion produces an immediate loss in polymer material, which is accelerated as the ions have access to increasing amounts of ester bonds.

Belbella *et al.* (1996) also demonstrated that effect of pH (2 and 10) was more pronounced for PLA after 100 days incubation compared to that observed at 24 days. The acceleration of polymer hydrolysis is in accordance with general acid-base catalysis, the acceleration of the degradation rate in the presence of acidic (Makino *et al.* 1985, Belbella *et al.* 1996) or basic (Maulding 1986, Cha and Pitt 1989, Belbella *et al.* 1996, Li *et al.* 1996) components have been reported. The mechanism of this catalysis is different depending on the ionic species H^+ or OH^-.

Several investigators have studied the mechanism of bond cleavage for the polyester backbone. Makino *et al.* (1985) assumed the degradation at pH 10.0 to proceed via an endchain scission process and not by a random chain scission process. The polydispersity changes observed in the base catalysed degradation of PLGA is in agreement with a faster chain end scission mechanism. Contrary to that observed at acid and neutral pH the polydispersity of the polymer does not change with degradation in basic media (Figure 8.5.3). Belbella *et al.* (1996) attributed the acid accelerated hydrolysis to a random chain scission of the polymer backbone due to the intense broadening of the distribution of the polymer chain lengths in the GPC chromatograms or an increase in polydispersity. These observations are in good agreement with the acid catalysed degradation demonstrated in this work. The results of this study confirm the hypothesis of St. Pierre *et al.* (1986) proposed that if the contribution of the buffer components to catalysis can be disregarded, the following order :kOH>kH>>kH2O usually applies to the decomposition of simple esters with the pH rate profile passing through a minimum between pH 5 and 6. Belbella *et al.* (1996) also suggested that two main hydrolysis mechanisms can be proposed base on pH of the medium, random scission at acidic pH and a sequential cleavage from the chain end in alkaline media.

11.8.2 Effect of temperature on the degradation profile of polyester systems

In Chapter 8 the rate of polymer degradation was found to increase with increasing incubation temperature. The activation energies for the degradation of PLGA was calculated for both phases of the degradation profile as (1) 26.734 kcal/mol and (2) 28.447 kcal/mol. The activation energy was calculated from the erosion of PLGA microspheres <20 microns as 23.62 kcal/mol. These values are comparable to literature values determined shown in Table 11.6 (Connors *et al.* 1985, Makino *et al.* 1985). For comparison the activation energies of dissolution of an active from a polymer system are shown in Table 11.7 (Chien 1974, Jalil and Nixon 1990).

Table 11.6 Literature values for the Activation energies for some hydrolysis reactions

Compound	Reaction	Ea (Kcal/mol)
Poly (D,L-lactide)	Hydrolysis	19.9
Poly (L-lactide)	Hydrolysis	20.0
Aspirin	Hydrolysis	14
Atropine	Hydrolysis	14
Benzocaine	Hydrolysis	19
Chloramphenicol	Hydrolysis	20
Procaine	Hydrolysis	14
Thiamine	Hydrolysis	20

Table 11.7 Literature values for the Activation energies for diffusion process

Polymer	Reaction	Ea (Kcal/mol)
Poly (D,L-lactide) 20.5K	Drug dissolution	2.4
Poly (D,L-lactide) 13.3K	Drug dissolution	4.16
Poly (D,L-lactide) 5.2K	Drug dissolution	5.54
Methacrylate	Drug dissolution	7.71

The values presented here for PLGA degradation and erosion are attributed to a hydrolysis mechanism. The activation energies for the degradation and erosion phases were comparable for PLGA. This agreement demonstrates that during both phases of the degradation profile the underlying process is a hydrolysis of ester bonds. The control of the rate polymer degradation by the incubation temperature was demonstrated by reducing the incubation temperature to give a triphasic degradation profile at 25°C by the inducement of an induction period after which polymer degradation proceeded by a biphasic mechanism.

The activation energy for the lag phase was calculated from the rate constants of the induction phase observed in the 5°C and 25°C PLGA (RG504) degradation profile. The induction phase was taken as 650 days for PLGA incubated at 5°C and 40 days for PLGA incubated at 25°C. The activation energy was calculated as 8.85 kcal/mol, this indicates that the lag phase is diffusion related.

11.9 EFFECT OF MICROENVIRONMENT ON THE DEGRADATION AND RELEASE PROPERTIES OF PLA AND PLGA SYSTEMS

The majority of polymer degradation studies described in the literature are carried out by suspending microparticles in large volumes of media. Several authors have commented that this approach to dissolution and degradation studies bears little resemblance to an in vivo environment especially those intended for parenteral administration. Attempts have been made by authors to simulate the dense packing that occurs when particles are injected, by placing the particles in gelatine capsules (Bergsma *et al.* 1995) or injecting them into gels (Sansdrap *et al.* 1996). A more recent report (Dash *et al.* 1999) describes the development of a microdialysis technique similar to the one used in these studies to serve as an in vitro dissolution method for implantable drug delivery systems.

When the microspheres are placed in the incubation medium, no initial change in pH occurs. The commencement of the pH reduction corresponds to the determined T_{onset} calculated for the mass loss for the microspheres (<20 μm) as 28 days. Mass loss from the microspheres occurs as monomers and oligomers dissolve in the surrounding medium. When this occurs the acidic end-groups also generate a reduction in the medium pH. Inside the visking bags the pH recorded was lower than measured in the bulk medium due to the build up of acidic degradation products at the site and the retarded diffusion of trapped degradation products in polymer in the visking bag. Degradation products have the ability to create a microenvironment that is significantly different from the bulk solution. The effect of lactic:glycolic acid (1:1) concentration on the degradation of PLGA RG504 microspheres is shown in Figure 11.10.

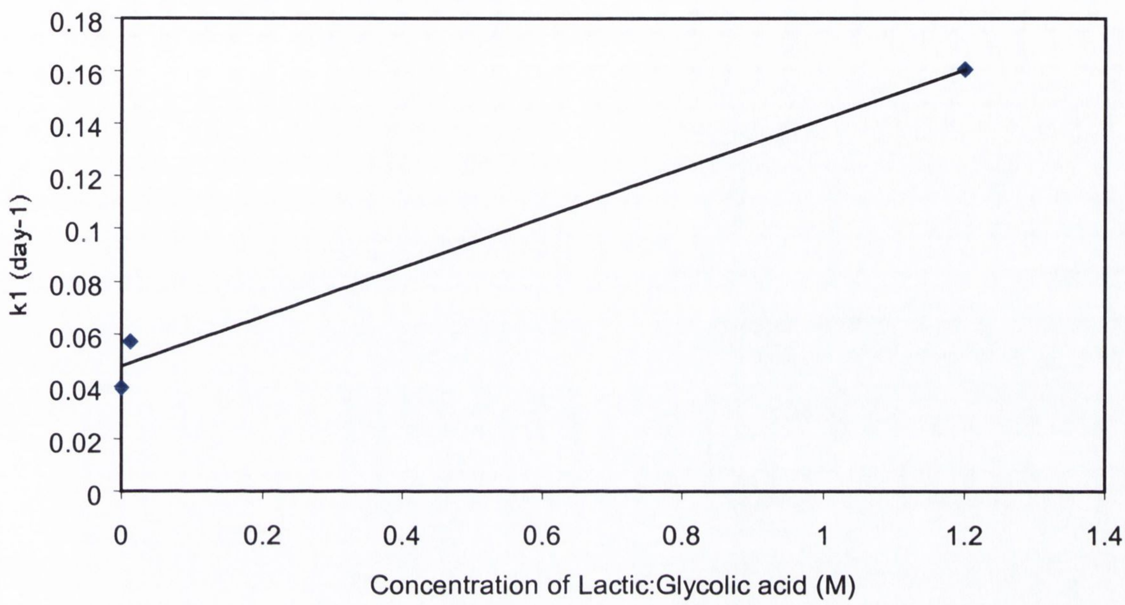


Figure 11.10 The effect of Lactic:Glycolic acid concentration on the degradation of PLGA RG504 microspheres.

The lowering of polymer microenvironmental pH and the ability of degradation products to influence the degradation rate has important implications for the use of PLA/PLGA polymers for drug delivery systems. In Chapter 10 it was also shown that the use of the visking bag method significantly altered the release profile of Fluphenazine from PLGA microspheres. In this case it is thought that the release of Fluphenazine into the small volume containing the polymer microspheres creates a local high concentration of the degrading polymer and released Fluphenazine. This microenvironment can further increase the release rate of the remaining drug and degradation of the polymer.

In chapter 7 it was shown that the rate constants calculated for the degradation of the dispersed microspheres are reduced compared to that observed for microspheres restricted to the visking bags. This is in agreement with a recent publication by Sandstrap and Moes (1997) where the degradation of confined microspheres (in a gel) was faster than dispersed microspheres. This was attributed to the increased surface area available for degradation products to escape that also minimises the autocatalytic effect. The stability of sensitive compounds such as peptides and proteins in the constantly changing chemical environment of an eroding polymer is an important issue. The encapsulation of an acid-base indicator, bromophenol blue, in 50:50 PLGA microspheres (as a probe to estimate pH within the microspheres during accelerated stability studies) indicated that the pH decreased to approximately 3.8 after 3 weeks (Shao *et al.* 2000). Certain substances such as proteins or pH sensitive drugs may be sensitive to this microenvironment or their release kinetics may be altered. Degradation, aggregation, solubilisation, adsorption and desorption events may occur. The effect of degradation products on the rate of polymer degradation may also influence the performance of these devices in vivo. A report by Tracy *et al.* (1998) showed that first order rate constants for the degradation of PLGA microspheres in vivo was about 2-3 times faster than in vitro.

11.10 INCORPORATION OF FLUPHENAZINE HCl INTO PLA AND PLGA MICROSPHERES AND MECHANISM OF RELEASE

11.10.1 Effect of fluphenazine HCl on the microsphere characteristics

Initial characterisation of the fluphenazine loaded microspheres indicated that the fluphenazine HCl was incorporated as an amorphous chemical mix within the polymer and existed as the free base after formulation. Figure 11.11 shows the relationship between the polymer molecular weight of the fluphenazine loaded microspheres with increasing fluphenazine loading for PLA(R203) and PLGA(RG504). Figure 11.11 shows that the presence of fluphenazine in the formulation had a significant effect on the polymer molecular weight of the hydrophilic PLGA RG504 microspheres but did not influence the molecular weight of the PLA microspheres. It was shown that the presence of fluphenazine in the formulation also influenced the microsphere polymer thermal characteristics (Figure 11.12). Figure 11.12 shows that the $T_{g(p)}$ of the polymer was reduced by the presence of fluphenazine in the formulation. This decrease was more evident for the PLGA microspheres compared to the PLA microspheres however both polymer systems showed a decrease in $T_{g(p)}$ of the polymer on inclusion of a low % w/w loading of fluphenazine followed by a more gradual decrease as the %w/w loading of fluphenazine increased. This interaction is thought to be between the basic groups on the fluphenazine and the carboxylic groups of the polymer.

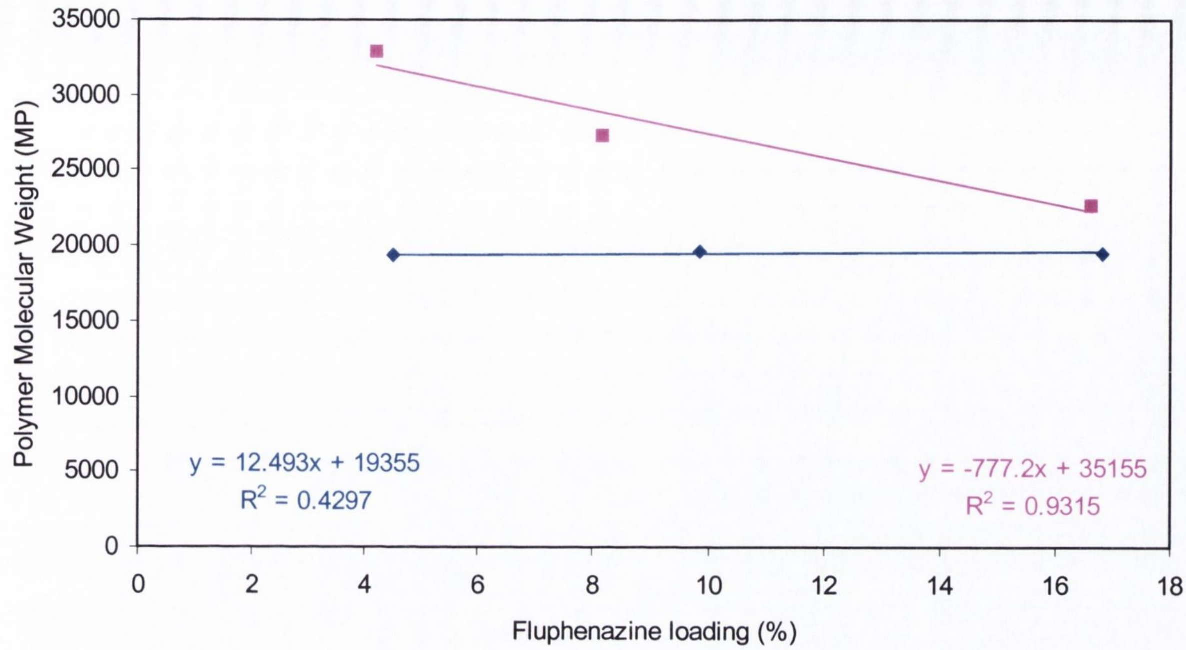


Figure 11.11 The influence of fluphenazine loading on the polymer molecular weight of Fluphenazine loaded microspheres, for ◆ PLA (R203) and ■ PLGA (RG504).

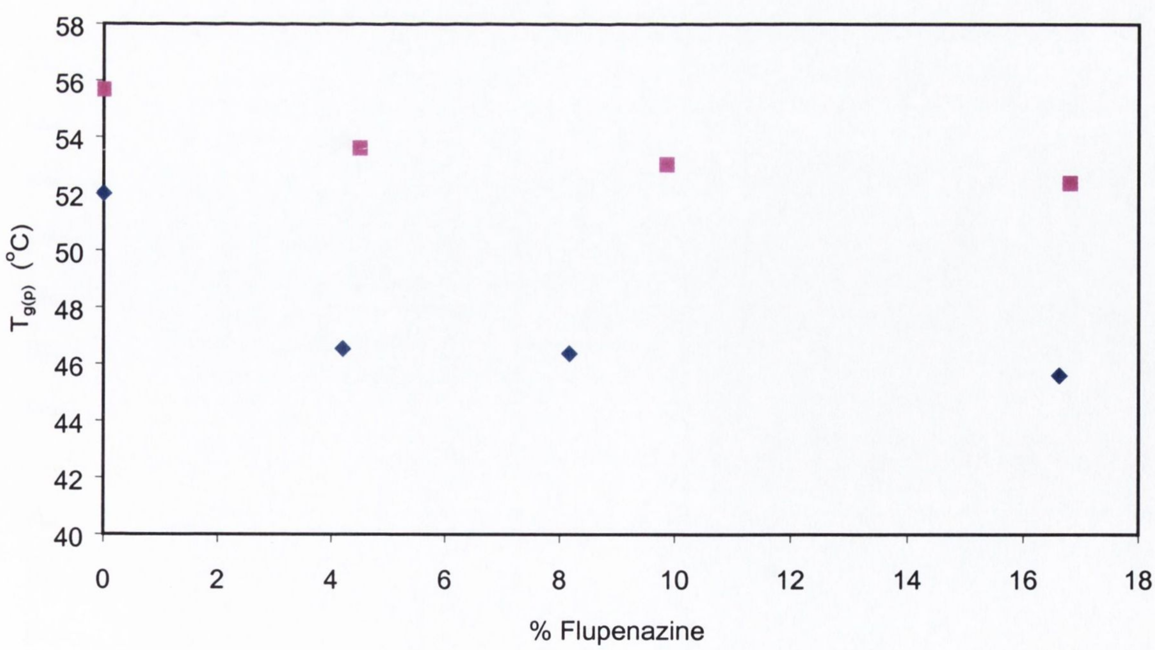


Figure 11.12 The influence of fluphenazine HCl on the $T_{g(p)}$ of PLA and PLGA polymer microspheres, for ■ PLA (R203) and ◆ PLGA (RG504).

11.10.2 The release mechanism of fluphenazine from PLA and PLGA microspheres

Tables 11.8 to 11.10 compare the fluphenazine dissolution, polymer degradation and polymer erosion parameters respectively for PLA (R203) and PLGA (RG504) for the drug free and fluphenazine loaded microspheres. The release and concurrent degradation is examined for the two polymer systems. A comparison of the erosion profile of PLA (Table 11.8) demonstrated that the release, polymer degradation and erosion components of PLA were considerably slower compared to the PLGA polymer microspheres. The fold difference between PLA and PLGA $T_{max(r)}$ of the release profile was directly comparable to the fold difference between PLA and PLGA *Tmax* erosion (10 fold).

The incorporation of fluphenazine in the microspheres was shown to considerably accelerate the rate of polymer degradation of the microspheres. For PLA an 8.5 fold reduction in the T_{lag} time was observed. The rate of PLA erosion for the Fluphenazine loaded PLA was 3 times (x 3.33, 2.56 for k_1 and k_2 respectively) faster than for the drug free microspheres. For PLGA Fluphenazine loaded microspheres the rate of polymer degradation was 6 times faster that for the drug PLGA polymer microspheres of similar starting molecular weight. (x 5.99, 6.10 for k_1 and k_2 respectively).

From the polymer degradation parameters the calculated value of Mp_1 was demonstrated to occur at a lower value for the drug loaded microspheres compared to the drug free microspheres (Table 11.7). A recent publication by Miyajima *et al.* (1998) reported that the basic drug papaverine remained dissolved in the PLA/PLGA matrices due to strong polymer drug interactions and hence the polymer was required to degrade to a lower molecular weight before release occurs. This effect is also observed in this work for the release of Fluphenazine from both PLA and PLGA.

A comparison of the concurrent drug release profile from both PLA and PLGA microspheres indicated that drug release was occurring at a faster rate than the concurrent polymer erosion however the time to reach T*max* for erosion was shorter than the corresponding $Tmax_{(R)}$ for the release profile. This is attributed to a faster loss of polymer material to create the porous matrix shown in Chapter 10 after which drug release proceeds.

11.8 Parameters for the release of fluphenazine from PLA/PLGA particles in PBS pH 7.4 at 37°C determined using Equation 10.1.

Polymer (% w/w F)	$k_{2(R)}$ (day^{-1})	*Tmax(R)*	$F_{BIN(R)}$	$k_{1(R)}$ (day^{-1})	MSC
PLA (9.86)	0.105	143.230	0.170	2.656	4.707
PLGA (8.17)	0.491	15.836	0.170	5.112	6.056

Table 11.9 Parameters for the degradation of PLA/PLGA particles in PBS pH 7.4 at 37°C determined using Equation 8.2(PLA) and 7.3 (PLGA).

Polymer (% w/w F)	Mp	T_{lag} (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1 Estimated
PLA 0.000	19914	100.987	0.006	0.003	282.532	6891
(PLA) 9.86	19580	16.659	0.016	0.007	91.271	4363
PLGA (0.000)	25206	0.000	0.028	0.014	25.350	12342
PLGA (0.000)	42124	0.000	0.046	0.014	29.789	10833
PLGA (8.174)	27294	0.000	0.169	0.086	7.000	4776

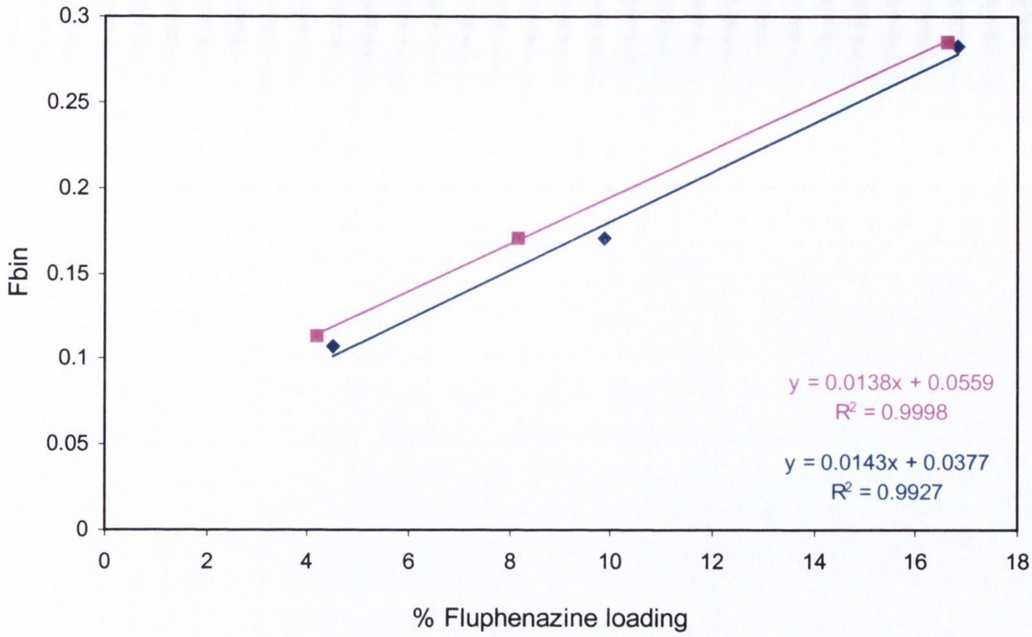
Table 11.10 Parameters evaluated for the erosion of PLA and PLGA particles in PBS pH 7.4 at 37°C determined using Equation 3.23.

Polymer (% w/w F)	*k* (day^{-1})	*Tmax*	CD	MSC
R203 (0.00)	0.009	428.970	0.997	3.42
R203 (9.86)	0.028	106.248	0.996	3.74
RG504 (0.00)	0.055	54.150	0.998	4.76
RG504 (8.17)	0.248	10.319	0.998	4.74

11.10.3 Effect of fluphenazine loading on the dissolution and degradation components of the release profile from PLA and PLGA microspheres.

The effect of % fluphenazine loading on the parameters for the dissolution and degradation components of the release was examined. The dissolution-controlled parameters of the release profile represent drug that is present at the surface of the microspheres and is released immediately upon immersion in the incubation medium. Figure 11.13 shows that for this process the % of fluphenazine released by surface diffusion controlled release increases (F_{BIN}) as the % loading of fluphenazine increases. The rate of surface diffusion controlled release was however found to decrease as the % loading of fluphenazine increased. It is thought that at the low loading most of the surface controlled release is due to drug at the surface of the microspheres which can be liberated quickly. Aas the loading increases the % drug at the surface and close to the microsphere surface increases. The drug located near the microsphere surface is liberated at a slower rate than drug at the surface of the microspheres.

Parameters for the effect of fluphenazine loading on the degradation component are plotted in Figure 11.14. It was shown that the parameter *Tmax(r)* decreased with the % Fluphenazine loading. This is as expected due to the effect of inclusion of fluphenazine on the polymer degradation and erosion mechanism shown in this work. It was also shown that the rate of the erosion controlled component of the release profile increased with increased fluphenazine loading, though this is discontinued after the 16.8% loading. However this data point is thought to be an anomaly due to the data point at day 7 in the release profile (Chapter 10) rather than the true value. In a study by Vert *et al.* (1998) PLA blended with caffeine (a tertiary amine) at concentrations 0-20% w/w. At low levels ca. 2% the presence of the basic material accelerated the degradation of PLA, however at higher levels the PLA degraded at a shower rate. At the low levels caffeine formed a molecular dispersion with the polymer chains however increasing the concentration of caffeine allowed the caffeine to crystallise out where the effect of the presence of the caffeine was observed as a neutralisation of the acid end groups of the polymer.



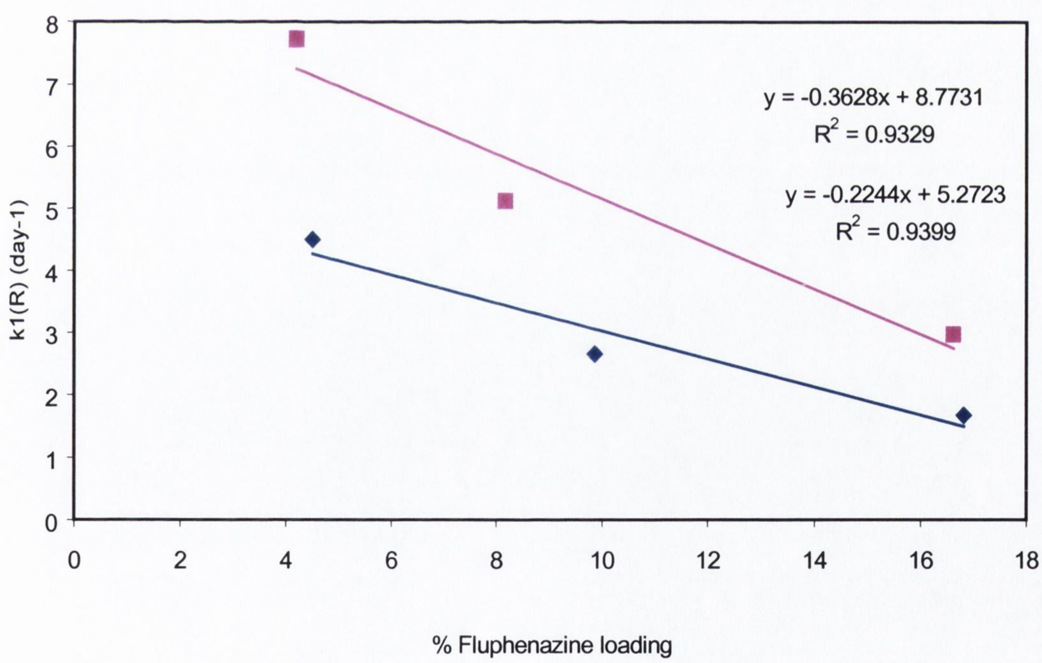
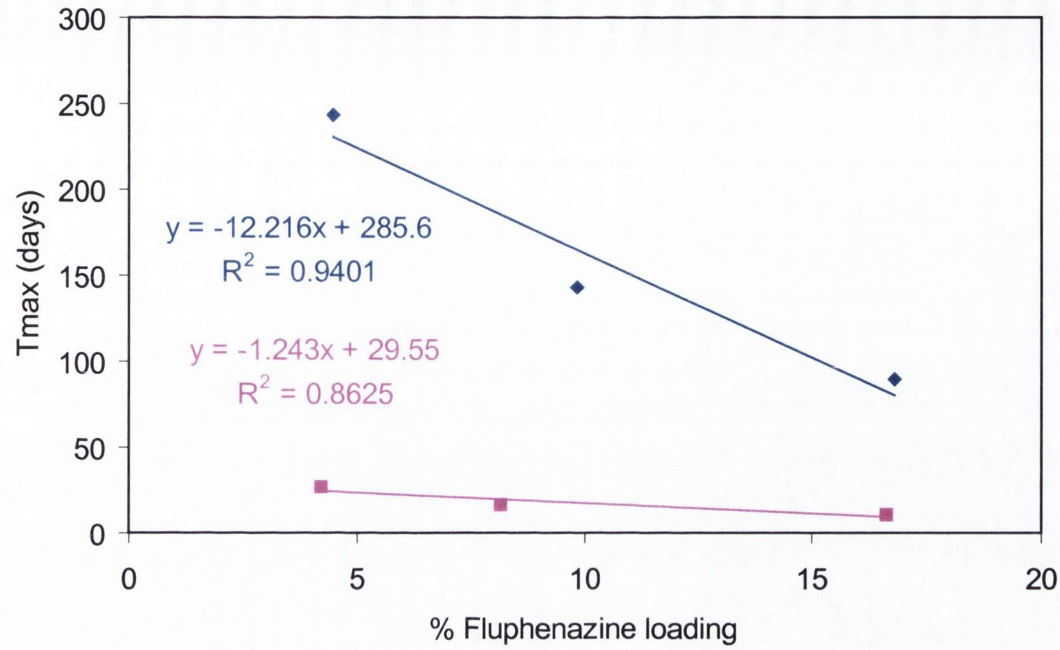


Figure 11.13 The effect of drug loading on the parameters for the dissolution of fluphenazine from PLA and PLGA microspheres, for ■ PLGA microspheres and ◆ PLA microspheres.



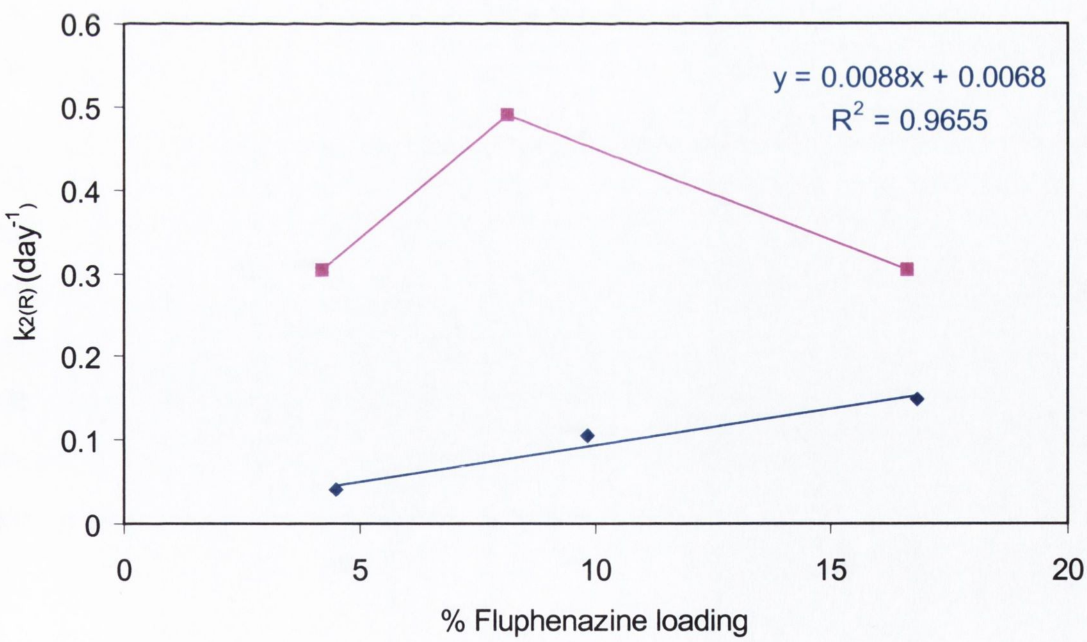


Figure 11.14 The effect of drug loading on the parameters for the degradation controlled release of fluphenazine from PLA and PLGA microspheres, for ■ PLGA microspheres and ◆ PLA microspheres.

11.10.4 Effect of polymer properties on the dissolution and degradation components of the release profile from PLA and PLGA microspheres.

The relationship between drug release and concurrent matrix properties was investigated using fluphenazine loaded microparticles using a range of polyesters that had been evaluated as drug free microparticles. Table 11.11 and 11.12 show the release and concurrent polymer degradation rates compared for a range of polymers.

Tables 11.11 and 11.12 show that the degradation components of the release profiles for a range of PLGA (RG504, RG503 and RG502) were quantitatively similar. Concurrent polymer degradation showed that the higher the molecular weight of the polymer, the faster the rate of polymer molecular weight reduction. This relationship was true for both the drug free and drug-loaded microspheres. The second phase of the degradation phase showed equivalent rate constants for the three polymers for both the drug free and drug loaded microspheres. It was observed that for the drug-loaded microspheres, the time *Tau* and the molecular weight Mp_1 were comparable for the three polymers. This is thought to be the explanation for the three polymers of different starting polymer molecular weight having similar rates of drug release.

The use of the more hydrophilic series polymer RG502H was found to release fluphenazine and degrade at a faster rate than the RG502 polymer. This is consistent with what was observed for the degradation of the drug free microspheres of this polymer. For PLA the dependence of the release profile on the polymer molecular weight is shown. The PLA (R104) degraded at a much faster rate than the PLA (R203). PLA (R104) showed a monophasic degradation process in the presence of Fluphenazine compared to the triphasic process demonstrated for PLA (R203).

11.11 Parameters for the degradation of PLA/PLGA microspheres.

Polymer	% w/w Fluphenazine	Mp	T_{lag} (days)	k_1 (day^{-1})	k_2 (day^{-1})	*Tau* (days)	Mp_1
RG504	0.00	42124	-	0.046	0.014	29.788	10833
RG504	8.17	27294	-	0.169	0.086	7.000	4776
RG503	0.00	25206	-	0.028	0.014	25.350	12342
RG503	9.25	18969	-	0.126	0.088	9.371	5835
RG502	0.00	12194	-	0.014	0.013	21.990	8983
RG502	8.73	10800	-	0.098	0.075	6.999	5426
RG502H	0.00	8474	-	N/A	0.019	0	-
RG502H	8.12	8369	-	N/A	0.100	-	-
R104	0.00	4654	-	0.017	0.002	4.688	3874
R104	9.13	4486	-	N/A	0.013	0.00	-
R203	0.00	19914	140.987	0.005	0.003	302.532	7401
R203	9.86	19580	16.659	0.016	0.007	91.271	4363

Table 11.12 Parameters for the release of fluphenazine from PLGA and PLA particles determined using Equation 10.1.

Polymer (% loading)	*k* (day^{-1})	*Tmax*	F_{BIN}	k_1 (day^{-1})	MSC	CD
RG504 (8.17)	0.491	15.836	0.170	5.112	6.056	0.998
RG503 (9.25)	0.454	17.767	0.149	3.843	5.226	0.996
RG502 (8.73)	0.446	18.065	0.135	4.229	4.895	0.995
RG502H (8.02)	0.5117	11.875	0.106	4.608	4.789	0.998
R104 (9.13)	0.154	39.820	0.139	4.600	3.936	0.989
R203 (9.86)	0.105	143.230	0.170	2.656	4.707	0.993

Basic drugs have been shown to exhibit a specific basic catalytic effect on polylactic (PLA) and poly lactic-glycolic (PLGA) polymer degradation (Maulding *et al.* 1986, Cha and Pitt 1989 and Lin *et al.* 1994). Cha and Pitt (1989) studied the factors responsible for the catalytic effect of a range of basic drugs (mepereridine, methadone, naltrexone and prometazine) and found that neither the drug pKa, polymer/water partition coefficient nor the drug induced reduction in the T_g were correlated with the catalytic effect, but with the drug concentration in the polymer. A more recent study by Delgado *et al.* (1996) showed that for PLA containing 17-47% methadone base the polymer molecular weight was the deciding factor. O'Donnell and McGinity (1998) demonstrated that the encapsulation of Thioridazine HCl (17.80%) also influenced the molecular weight of the polymer and catalysed the hydrolysis of PLGA. Thioridazine HCl was released in a triphasic manner where ca. 18% of the encapsulated drug was released in the first 24 hours, followed by a lag phase and then the onset of the degradation controlled release after 12-14 days.

In general it was noted through the literature that the release of biomolecules from PLA/PLGA matrices followed release profiles that correlated well with the degradation profiles shown in this work for PLA/PLGA drug free microspheres. Crotts and Park (1997) demonstrated that BSA was released from PLGA microspheres release in a triphasic manner. Where ca. 20% of the encapsulated protein was released in the first 24 hours, followed by a lag phase and then the onset of the degradation controlled release of BSA after 21 days. This profile correlates well with the degradation and erosion profile and parameters shown for PLGA in Chapter 7. Other studies with biomolecules e.g. glycoprotein gp120 also demonstrate a correlation with the degradation profile of PLGA microspheres (Batycky *et al.*1997) while in another investigation peptide release rate from PLGA microspheres showed an approximate linear relationship between polymer molecular weight and release (Mehta *et al.* 1996).

11.11 SUMMARY

The mechanism of degradation of polylactide and polylactide-co-glycolide is primarily dependent on the physicochemical characteristics of the polymer. In this study it was decisively shown that the particle size influenced the degradation of PLA and PLGA particles.

Polymer hydrolysis produces a degradation profile that contains distinct components of polymer degradation and polymer erosion. The concept of multiphasic degradation patterns which were influenced by the physicochemical characteristics of the polymer and dissolution conditions was introduced. The first phase in the profile was a polymer degradation controlled phase followed by a polymer erosion controlled phase. The application of mathematical models to the polymer degradation and erosion profiles allow the estimation of parameters that could be used to study the factors influencing polymer degradation and erosion. Comparison of two mathematical models to the polymer profiles established that the second phase of degradation is associated with the onset of mass loss. The application of mathematical models to the polymer degradation and erosion profiles could be used to the predict degradation and erosion rates for polymers of different molecular weights within a given polymer series.

Both of these underlying mechanisms of polymer degradation and erosion were also shown to influence other properties of the polymer matrix such as polymer thermal properties and morphology.

The underlying mechanism of PLA/PLGA degradation is polymer hydrolysis, a reaction that is influenced by acid/base conditions and incubation temperature. The degradation of PLGA was shown to undergo different polymer degradation profiles under the influence of acid/base conditions. This was shown through changes in polymer molecular weight, polymer erosion and microsphere morphology.

In this work the activation energy for PLGA hydrolysis was calculated. Rate constants calculated based on Phase 1, Phase 2 or based on polymer erosion (mass loss) using the appropriate mathematical models were used to calculate the activation energy for PLGA.

Three comparable values (Ea: 26.7, 28.5 and 23.62 kcal/mol) for the activation energy of PLGA were obtained which indicates that the underlying process of polymer degradation and erosion is hydrolysis. A lower activation energy (Ea: 8.8 kcal/mol) was calculated for the induction phase in the degradation phase which suggests that this is a dissolution controlled phase.

The influence of polylactide and polylactide-co-glycolide physicochemical characteristics on the release of a model drug Fluphenazine was investigated. The presence of Fluphenazine was shown to influence the physicochemical characteristics of the microspheres and the rate of polymer degradation. The release of Fluphenazine showed a burst effect followed by a sigmoidal release profile. This consists of a surface diffusion controlled release, a lag phase and a phase where release is controlled by polymer degradation. A model previously developed by Corrigan *et al.* (1998) to describe the release from polymer discs was used to depict the release from nano- and microspheres. Concurrent polymer degradation was also studied. The relative difference between PLA and PLGA $Tmax_{(r)}$ of the release profile was directly comparable to the relative difference between PLA and PLGA *Tmax* erosion. The rate of erosion for Fluphenazine loaded PLA and PLGA microspheres was 3 and 6 times respectively compared to the drug free microspheres. A comparison of the concurrent drug release profile from both PLA and PLGA microspheres indicated that drug release was occurring at a faster rate than the concurrent polymer erosion. The time to reach T*max* for erosion was shorter than the corresponding T*max* for the release profile. This is attributed to a faster loss of polymer material to create the porous matrix after which drug release proceeds. The use of the various models to describe Fluphenazine release, polymer degradation and polymer erosion demonstrated their utility for the elucidation of the factors controlling the release mechanism from PLA/PLGA particulate systems.

The influence of the microenvironment of the degradation properties and release profile was also examined through studies on the effects of lactic/glycolic acid and the uses of alternative dissolution techniques. These properties have important implications for the performance of these devices as controlled release systems *in vivo*.

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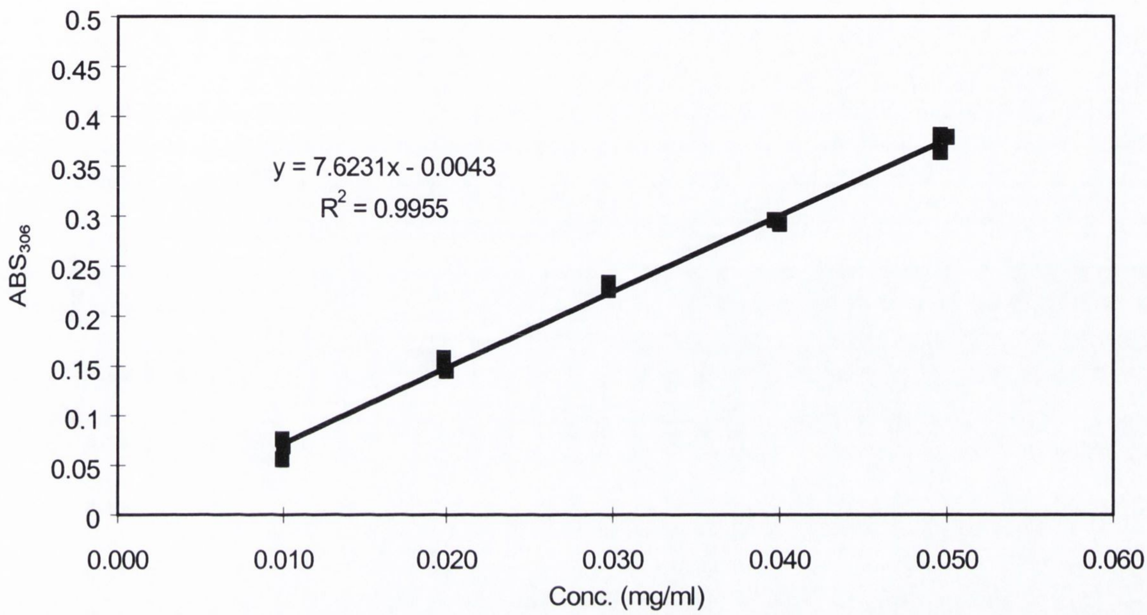
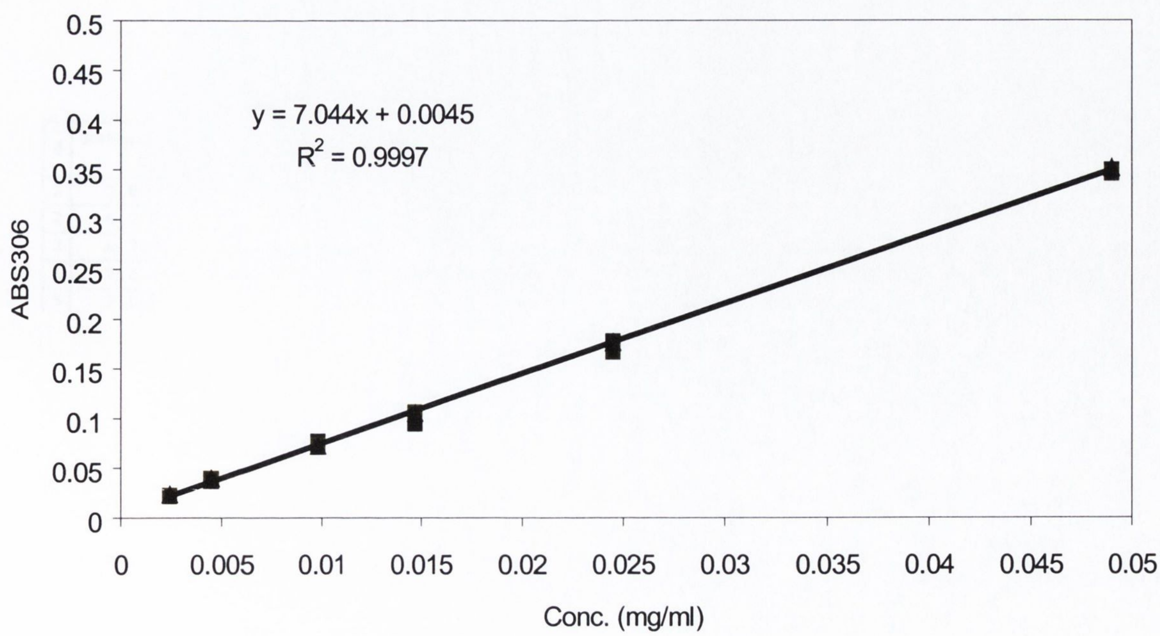
Appendix

APPENDIX I

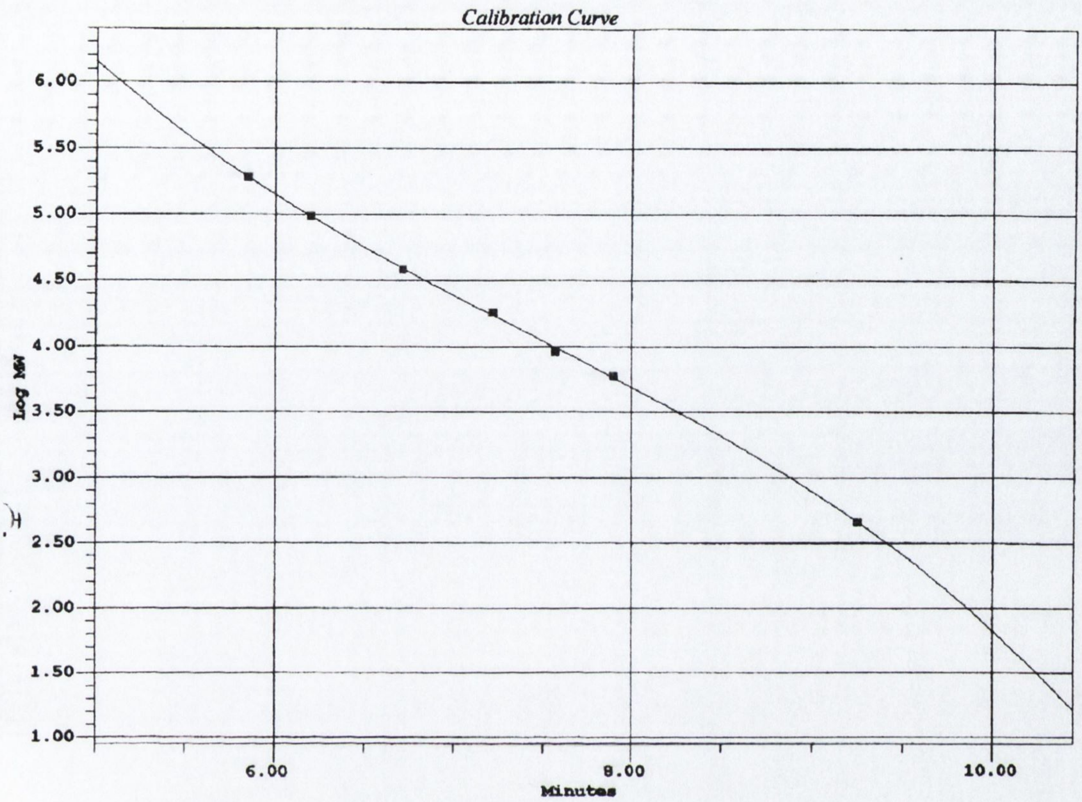
Calibration curve for Fluphenazine in phosphate buffered saline



Calibration curve for Fluphenazine in 0.1N HCl



APPENDIX II



Calibration Information

Processing Method	CAL4_500to190000	System	GPC_SYS
Channel	410	Date	09-MAY-96
Type	GPC	Manual Coeffs	No
Data Origin		Order	3
A	20.821102	B	-5.396498
C	0.635604	D	-0.028590
E	0.000000	F	0.000000
R	0.999777	R^2	0.999554
Standard Error	0.058060	Vo	5.000 ml,min
Vt	10.450 ml,min	Valid	Yes
Mo		b	
Slope		K	1.000000000 dl/g
alpha	0.000000		

Narrow Standard table

#	Retention Time (min)	Elution Volume (ml)	Mol Wt (Daltons)	Log Mol Wt	Intrinsic Visc (dl/g)	K (dl/g)
1	5.850	5.850	190000	5.278754	1.000000	1.000000000
2	6.200	6.200	96400	4.984077	1.000000	1.000000000
3	6.717	6.717	37900	4.578639	1.000000	1.000000000
4	7.217	7.217	18100	4.257679	1.000000	1.000000000
5	7.567	7.567	9100	3.959041	1.000000	1.000000000

Narrow Standard table

#	Retention Time (min)	Elution Volume (ml)	Mol Wt (Daltons)	Log Mol Wt	Intrinsic Visc (dl/g)	K (dl/g)
6	7.900	7.900	5970	3.775974	1.000000	1.000000000
7	9.250	9.250	456	2.658965	1.000000	1.000000000

Narrow Standard table

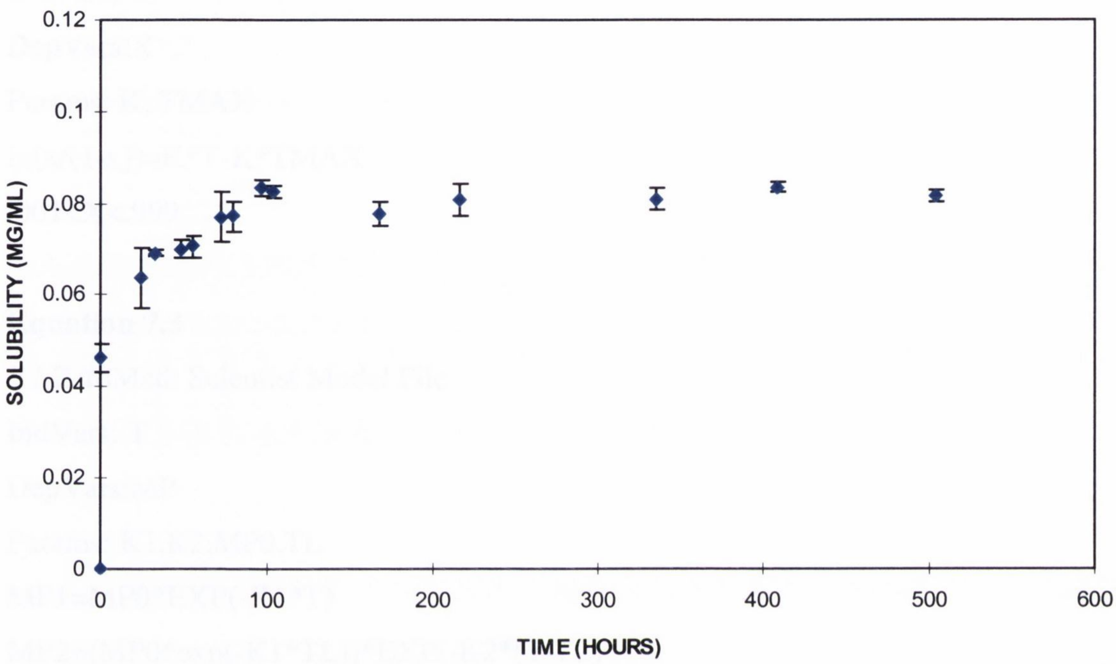
#	alpha	Hydrodynamic Volume (dl)	Log(MolWt[n])	Calculated Weight (Daltons)	% Residual	Manual	Ignore
1	0.000000	190000	5.278754	190423	-0.222	No	No
2	0.000000	96400	4.984077	95843	0.582	No	No
3	0.000000	37900	4.578639	38528	-1.629	No	No
4	0.000000	18100	4.257679	17110	5.786	No	No
5	0.000000	9100	3.959041	9833	-7.458	No	No
6	0.000000	5970	3.775974	5763	3.587	No	No
7	0.000000	456	2.658965	457	-0.114	No	No

Narrow Standard table

#	Ignore [n]	Standard Type
1	No	Narrow
2	No	Narrow
3	No	Narrow
4	No	Narrow
5	No	Narrow
6	No	Narrow
7	No	Narrow

APPENDIX III

Solubility of Fluphenazine in phosphate buffer pH 7.4



APPENDIX IV

Equation 3.23

```
// MicroMath Scientist Model File
IndVars: T
DepVars:X
Params: K, TMAX
ln(x/(1-x))=K*T-K*TMAX
.001<X<.999
```

Equation 7.3

```
// MicroMath Scientist Model File
IndVars: T
DepVars:MP
Params: K1,K2,MP0,TL
MP1=MP0*EXP(-K1*T)
MP2=(MP0*exp(-K1*TL))*EXP(-K2*(T-TL)
MP=MP1*(1-FLAG)+MP2*FLAG
FLAG=UNIT(T-TL)
```

Equation 8.1

```
// MicroMath Scientist Model File
IndVars: T
DepVars:MP1
Params: K1,MP0
MP1=MP0*EXP(-K1*T)
```

Equation 8.2

```
// MicroMath Scientist Model File
IndVars: TLAPS
DepVars:Y
Params: K1,K2,MP0,TL,TLAG
T=TLAPS-TLAG
MP1=MP0*EXP(-K1*T)
```

```
MP2=(MP0*EXP(-K1*TL))*EXP(-K2*(T-TL)
MP=MP1*(1-FLAG)+MP2*FLAG
FLAG=UNIT(T-TL)
Y=IFLEZERO(TLAPS-TLAG,MP0,MP)
```

Equation 10.1

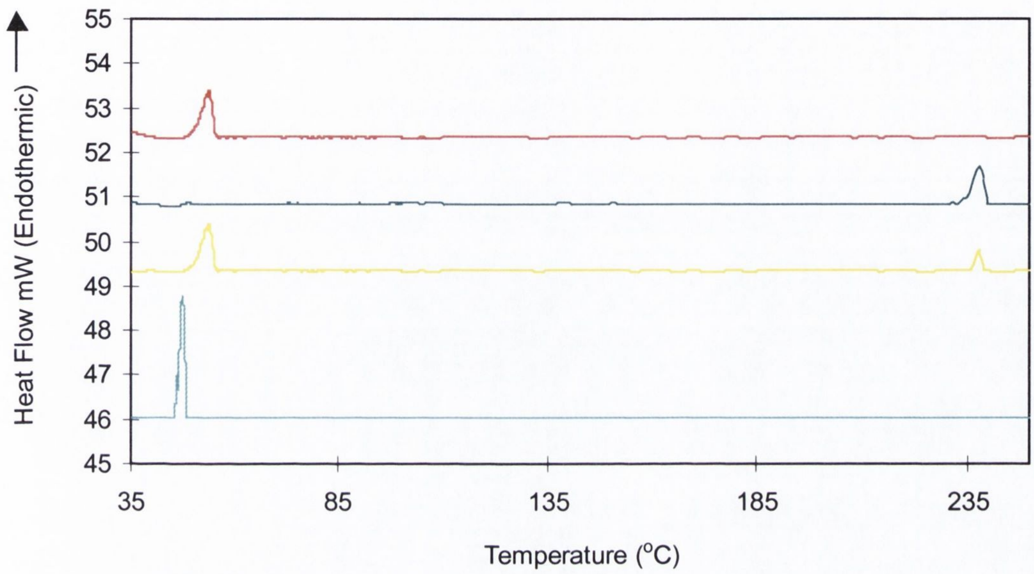
```
// MicroMath Scientist Model File
IndVars: T
DepVars: FTOT
Params:K,TMAX,FBIN,K1
FB=FBIN*(1-EXP(-K1*T))
1=FBIN+F
LN(X/(1-X))=K*T-K*TMAX
FDEG=F*X
FTOT=FDEG+FB
//constraints
0.0<X<1
```

Equation 11.1

```
// MicroMath Scientist Model File
IndVars: TLAPS
DepVars:Y
Params:K3,TLAG
T=TLAPS-TLAG
X^0.333=1-K2*(T)
XTOT=1*(X^0.333)
y=IFLEZERO(TLAPS-TLAG,0.95,XTOT)
```

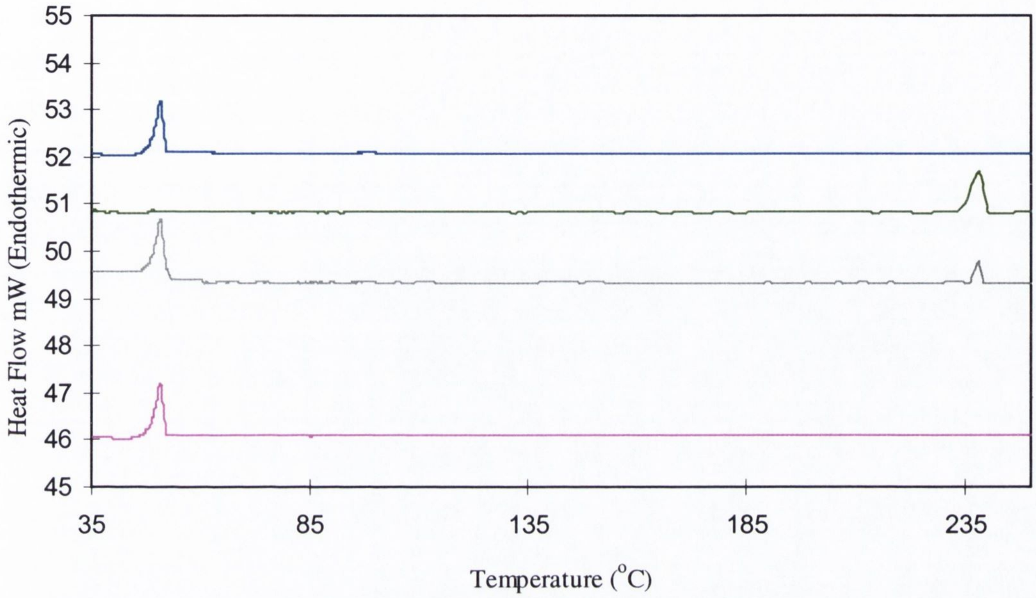
APPENDIX V

DSC thermograms of PLA polymer, Fluphenazine HCl, Fluphenazine HCl:PLA physical mix and 9.86% Fluphenazine HCl loaded PLA microspheres



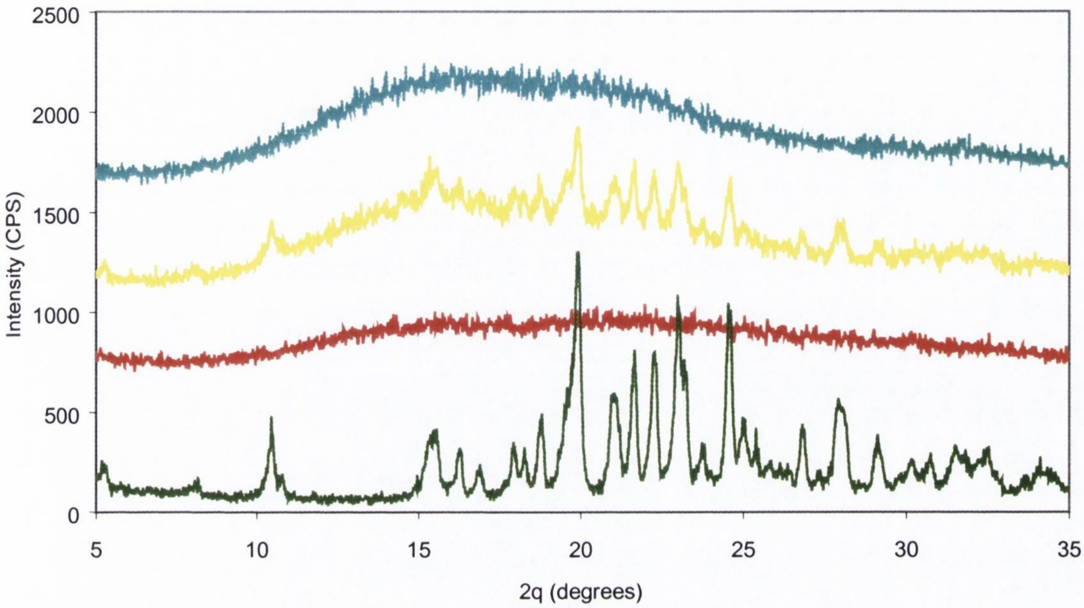
----- PLA polymer, ----- Fluphenazine HCl, ----- 10% Fluphenazine HCl:PLA physical mix and -----9.86% Fluphenazine HCl loaded PLA microspheres

DSC thermograms of PLGA polymer, Fluphenazine HCl, 10% Fluphenazine HCl:PLGA physical mix and 9.86% Fluphenazine HCl loaded PLGA microspheres



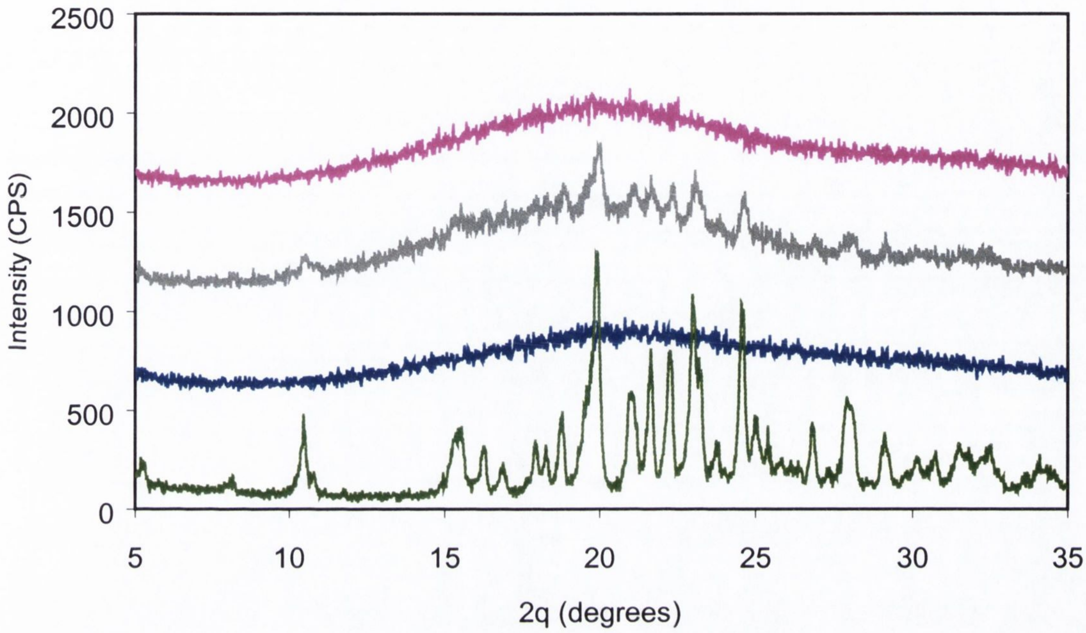
----- PLGA polymer, ----- Fluphenazine HCl, ----- 10% Fluphenazine HCl:PLA physical mix and ----- 9.86% Fluphenazine HCl loaded PLA microspheres

XRD pattern of PLA(R203) polymer, Fluphenazine HCl, 10% Fluphenazine HCl:PLA physical mix and 9.86% Fluphenazine HCl loaded PLA microspheres



----- PLA polymer, ----- Fluphenazine HCl, ----- 10% Fluphenazine HCl:PLA physical mix and ----9.86% Fluphenazine HCl loaded PLA microspheres

XRD pattern of PLGA polymer, Fluphenazine HCl, 10% Fluphenazine HCl:PLGA physical mix and 9.86% Fluphenazine HCl loaded PLGA microspheres



----- PLGA polymer, ----- Fluphenazine HCl, ----- 10% Fluphenazine HCl:PLA physical mix and ----- 9.86% Fluphenazine HCl loaded PLA microspheres