Characterisation of polylactide microspheres

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For Dad, Mum and my darling Husband Babs
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Abstract

This thesis describes the preparation of poly d, l-lactide (PDLA) microspheres by the solvent evaporation method. Laser diffraction particle size analysis showed that the median diameter of the PDLA microspheres produced was relatively small (7–22 µm). The shape of the PDLA microspheres was observed by SEM to be spherical. Upon increasing the drug load within PDLA microspheres, drug particles appeared on the surface of the microspheres. The solvent evaporation process proved to be efficient, as the experimental drug loading was close to the theoretical drug loading, indicating little raw material was lost during microsphere preparation. The release profile of progesterone and estrone from the microspheres was characterised by two release phases; an initial quick burst of drug followed by a slower sustained release. In vitro release kinetics of progesterone and estrone loaded PDLA microspheres were identified as being dependent on the square root of time, indicating release was mainly diffusion controlled, although SEM revealed the release of the compounds from PDLA was also due to surface erosion. Progesterone plasticised PDLA at drug loads of 30% w/w and less, whilst estrone did not show any evidence of plasticisation. At 20% w/w progesterone loading, cold crystallisation of progesterone was observed indicating an amorphous form of the drug was present. Cold crystallisation was not observed for estrone, indicating only a crystalline form of drug was present. PDLA microspheres were evaluated for their in vitro degradation behaviour in ethanol phosphate and phosphate buffers (EPBS and PBS) using gel permeation chromatography (GPC). The mode of degradation of drug free PDLA microspheres incubated in PBS and EPBS were biphasic. GPC results suggest the rate of M_w change of progesterone and estrone PDLA microspheres incubated in EPBS were similar to each other. Therefore, it is deemed changing the drug from progesterone to estrone (and vice versa) had very little effect on the M_w change of PDLA during the 40 days of incubation in EPBS. Overall it was determined that PDLA degradation was homogeneous.
Abbreviations

ACL  Anterior cruciate ligament
Cp   Heat capacity
Cp’  In phase component
Cp”  Out phase component
D (v, 0.1) Diameter below which 10% of the volume of particles lie
D (v, 0.5) Volume median diameter
D (v, 0.9) Diameter below which 90% of the volume of particles lie
Da   Daltons
DSC  Differential scanning calorimetry
\( \varepsilon \) Extinction Coefficient
EPBS Ethanol: phosphate buffered solution
FTIR Fourier transform infrared spectroscopy
GA   Glycolic acid
GPC  Gel permeation chromatography
\( h \) Planck’s constant
I    Intensity transmitted
I₀   Intensity Incident
IA   Intraarterial
IV   Intravenous
IM   Intramuscular
K    Rate
Kda  Kilo Daltons
In   Natural logarithm
M    Mass
\( M \) Unreleased entrapped drug
\( M_i \) Molecular weight
MMD  Metastable molecular dispersion
MTDSC Modulated temperature differential scanning calorimetry
\( M_n \) Number average molecular weight
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<thead>
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<tr>
<td>$M_v$</td>
<td>Viscosity average molecular weight</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Weight average molecular weight</td>
</tr>
<tr>
<td>$n$</td>
<td>Mean</td>
</tr>
<tr>
<td>$n_1$</td>
<td>Number of moles</td>
</tr>
<tr>
<td>$[\eta]$</td>
<td>Intrinsic viscosity</td>
</tr>
<tr>
<td>$p$</td>
<td>Momentum</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered solution</td>
</tr>
<tr>
<td>PD</td>
<td>Polydispersity</td>
</tr>
<tr>
<td>PDA</td>
<td>Poly d, lactide</td>
</tr>
<tr>
<td>PDLA</td>
<td>Poly d, l-lactide</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly glycolic acid</td>
</tr>
<tr>
<td>PLA</td>
<td>Polylactide</td>
</tr>
<tr>
<td>PLGA</td>
<td>Polylactide co glycolide</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly l-lactide</td>
</tr>
<tr>
<td>PLLALL</td>
<td>Poly l - lactic acid co l - lysine</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly vinyl alcohol</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SS</td>
<td>Solid solution</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>$T_{g1}$</td>
<td>Glass transition temperature of fast cooled system</td>
</tr>
<tr>
<td>$T_{g2}$</td>
<td>Glass transition temperature of slow cooled system</td>
</tr>
<tr>
<td>TG</td>
<td>Thermogravimetry</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>$T_{gD}$</td>
<td>Dehydrated glass transition temperature</td>
</tr>
<tr>
<td>$T_{gH}$</td>
<td>Hydrated glass transition temperature</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrapuran</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melt transition temperature</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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V = Volts
VMD = Volume median diameter
\( \lambda \) = Wavelength
\( w_i \) = Weight of the \( i^{\text{th}} \) monomer unit
XRD = X-ray diffraction
XRPD = X-ray powder diffraction
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Chapter One

Introduction
1.1 Historical background of polylactide and its applications

Poly d, l-lactide (PDLA) is synthesised from lactic acid. Lactic acid (Figure 1.1) was first discovered by the Swedish chemist Carl Wilhelm Scheele in 1780, who found that lactic acid occurred in nature as a racemic form as well as an optically active acid, and was present in sour milk. Other scientists such as Berzelius and Braconnot found lactic acid to be present in fresh milk, ox meat, blood and fermented rice water (described by Holten et al, 1971).

![Figure 1.1 Structure of lactic acid.](image)

Polylactide was first described in the literature in 1913 (French patent) and was reported as a satisfactory material for use in biodegradable surgical sutures and implants in 1966 (Kulkarni et al, 1966). Kulkarni et al (1966) found the polymer to be non-toxic, non-tissue reactive and to degrade slowly when implanted in rats and guinea pigs, which showed it could be very useful as a material for surgical implants. Polylactide is easily prepared and undergoes simple hydrolysis, which forms natural occurring non-toxic metabolites (carbon dioxide in urine and respired air) via the Krebs cycle. Medical grades of the polylactide were made into rods and films which were developed to replace non-degradable stainless steel, Steinman pins (used to repair mandible and maxilla fractures of dogs and monkeys) and as sutures prepared from spun fibres of the polymer (Kulkarni et al, 1971). Polymers of lactide were amongst the first biodegradable materials to be used for controlled drug delivery and continue to be popular choices for the development of new delivery systems. For the structure of PDLA see Figure 1.2.
PDLA is relatively strong and has long-standing safe use. Acceptance of PDLA as surgical sutures has encouraged the application of PDLA for drug delivery.

![Figure 1.2 Structure of poly d, l-lactide.](image)

The first description of a biodegradable implant for delivery of narcotic antagonists such as cyclazocine is credited to Yolles et al (1973). They prepared delivery devices, which consisted of narcotic antagonists encased within polylactic acid (PLA) films, or drug-loaded polymer powders of PLA suspended in a dispersing agent for subcutaneous injection. In 1973, Jackanicz et al (1973) used poly l-lactide as a drug carrier for the contraceptive, d-norgestrel, where the drug was incorporated into poly l-lactide (PLLA) implantable films and implanted subdermally in rats. However, the polymer hydrolysed less rapidly than the rate at which the drug was released. So Anderson et al (1976) developed a deep intramuscular system, using PLLA with norethisterone (contraceptive steroid) coated with PDLA to provide zero order release over 90 days. Following earlier studies of PLA polymers, other hydroxyl acids were synthesised and investigated for their suitability as implant materials and carriers in drug delivery. Polyglycolic acid (PGA) (Figure 1.3) proved especially useful and was used in conjunction with PDLA to determine the release profile of progesterone.

![Figure 1.3 Structure of glycolic acid.](image)
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(Schindler et al, 1977). In 1977, Miller et al (1977) used PLLA in conjunction with PGA to make oral resorbable, implants as PLLA was found to be inflexible and have a half-life of 168 days (degradation). By addition of PGA, the newly formed copolymer became flexible and the half-life was reduced, by varying the PGA content.

In 1975 a narcotic antagonist (naltrexone) was dispersed in copolymer delivery systems of PDLA, PLLA and Polylactide co glycolide (PLGA). These were implanted in rats as rods and found to be useful in producing longer term controlled release of the narcotic antagonist for treatment of opiate addiction (Howes et al, 1975). Copolymers of PDLA and PLGA have also been used for the sustained release of the anti-malarial drug (Quinazoline) (Wise et al, 1976). Nakano et al (1981) prepared and evaluated the release of the local anaesthetics; butamben, tetracaine and dibucaine, and concluded that the encapsulation of local anaesthetics within PDLA microspheres may be applied to the sustained control of pain in pain clinics. Kwong et al (1986) studied the in vitro and in vivo release of insulin from PLLA micro beads and pellets. They found PLLA to be readily adapted and suitable for the continuous release of insulin. Andreas Stahelin (1999) made PDLA into fixation screws (Sysorb), which are used in anterior cruciate ligament (ACL) knee reconstruction. The torn ACL is removed from the knee and a tendon is removed from the patient's inner thigh or the third middle patella tendon, put in place of the torn ACL and secured with the fixation screws to the bone. The time frame of degradation is long enough to stabilize the tendon transplant during the one to two months healing process (Sulzer Technical Review, 1999). Following these early studies, different drugs have been incorporated into microparticles made from various biodegradable polymers and copolymers to give a wide range of degradation and release behaviours. Due to the amorphous nature of the copolymers of PDLA and PLGA and the small particle size of the carriers, degradation occurs within a matter of weeks rather than months depending on the polymer and copolymer ratios used.
1.2 Polymers

1.2.1 Introduction to polymers

PDLA is a polymer of repeating alternating chains of d and l-lactide. Polymers are materials that are used widely in everyday life, such as in clothing, household materials and appliances, car and aeroplane parts and as drug delivery agents. The study of structure and properties, i.e. materials science, can be applied to polymers in order to understand the relationship between the manufacturing process, the structures produced and the resulting chemical, physical and mechanical properties of the polymer.

<table>
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<th>Table 1.1 Polymer applications</th>
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<td><strong>Drug delivery agents &amp; Fibres</strong></td>
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<td><strong>Films/Packaging</strong></td>
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<tr>
<td><strong>Membranes</strong></td>
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<td><strong>Resins</strong></td>
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<tr>
<td><strong>Adhesives</strong></td>
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<tr>
<td><strong>Elastomers</strong></td>
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<td><strong>Coatings</strong></td>
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Polymers have several advantages over materials such as metals and ceramics: polymer processing costs are low, it is possible to prepare various molecular weight ranges, and properties such as transparency and toughness form unique combinations. Most polymers have useful features, such as elongation and impact strength, tensile strength and modulus, which make them more cost-effective than metals and ceramics. Polymers are made into a wide range of forms, such as fibres, membranes, films, filters and moulds (Table 1.1).

1.2.2. Definitions

Polymers are large molecules formed by joining a large number of small molecules, or monomers in a chain together. The small repeating units react together chemically to form long molecules. The repetition of the molecules can be linear, branched or interconnected. Homo-polymers are comprised of single repeating monomers and hetero-polymers are composed of several repeating monomers. Examples of homo-polymers are poly d, lactide (PDA), PLLA and PGA. Copolymers are the most common form of hetero-polymers. They are often formed by a sequence of two types of monomer units. Alternating copolymers can be simple alternating repeats of two monomers, e.g. \(-A-B-A-B-A-B-\), or random repeats of two monomers, e.g. \(-A-A-A-B-B-A-B-A-A\), where as block copolymers, include long sequences of one repeat unit, e.g. \(-B-B-B-B-B-B-A-A-A-B-B-B-B-A\). There are many forms of block copolymers, including AB and ABA where A and B each stand for a sequence of several hundred monomers. Examples of hetero-polymers are PDLA, Poly lactide-co-glycolide (PLAGA) and Poly (L-lactic acid-co-L-lysine) (PLLALL). There are three main classes of polymers: thermo-plastics, thermo-sets and elastomers. Examples of polymers that are typical of each class are listed in table 1.2.
Table 1.2 Major classes of polymers

| Crystallisable thermo-plastics | Polyacetal, polyamide, polycarbonate, polyethylene terephlate, polyethylene, polypropylene. |
| Glassy thermo-plastics | Poly d, l-lactide, polystyrene, poly (methyl methacrylate), poly (vinyl chloride), poly (vinyl acetate). |
| Thermo-sets | Epoxy, phenolic, polyester (unsaturated). |
| Elastomers | Ethylene-propylene copolymers, ethylene-vinyl acetate, polybutadiene, Styrene-butadiene rubber. |

1.2.2.1 Thermo-plastics

These polymers, referred to, as plastics are linear or branched, and can be reversibly melted or dissolved in a suitable solvent. In some cases thermoplastics are cross-linked (chemical bonds between molecules) to provide heat stability and to limit the flow and melting during their use. Examples of thermoplastics are: poly lactide, poly glycolide, polyacetal, polyamide, polyethylene, polystyrene, polyvinyl acetate and polymethyl methacrylate (Conti et al, 1992). Many amorphous thermoplastics are brittle therefore limiting their range of application. Toughening with rubber is well known to enhance fracture resistance and toughness. Thermoplastic enhanced with rubber are termed multiphase polymers.

1.2.2.2 Thermo-sets

These polymers have a three-dimensional network structure, being a single highly connected molecule, which imparts rigidity and intractability. Thermo-sets form rigid structures, but once set they do not melt or dissolve in suitable solvents. Thermo-sets have short chains between cross-links and exhibit glassy brittle behaviour. Examples of thermo-sets are: epoxy adhesives, phenolics and unsaturated polyesters.
1.2.2.3 Elastomers (rubbers)

Elastomers or rubbers are polymers with long flexible chains between the cross-links. Like the thermo-sets they cannot be melted. Elastomers are characterised by three-dimensional cross-linked network (chemical bonds between molecules), which has a well known property of being stretchable and being able contract back into its original shape and size. An example of cross-linking is vulcanisation of rubber, where sulphur molecules react with carbon double bonds to form the structure. Examples of elastomers are: polybutadiene, ethylene-propylene copolymers and styrene-butadiene rubber. Multi-phased polymers, combinations of thermo-plastics and elastomers, take advantage of the ease of fabrication of thermo-plastics and increased toughness of elastomers, providing engineered resins with high impact strength (Sawyer and Grubb, 1996).

1.3 Lactic acid

PDLA belongs to a family of aliphatic (straight-chained molecules) polyesters. PDLA is derived from lactic acid (Figure 1.1). Lactic acid contains an asymmetric carbon, i.e. four different groups are attached to a central carbon and therefore it exists as two optical isomers. The configurations of the two isomers are shown in figure 1.4. Polymers made of these lactic acids can also exist as either d or I or as a d, I racemic mix. The d and I forms are crystalline whilst the racemic mixture is amorphous (Wise et al, 1979). The d and I configurations are derived by analogy from the structures of (-) and (+) glyceraldehydes respectively and designate the relative configuration of the molecules. The plus and minus rotations specify the direction of rotation of the sodium D line (polarimetry) (Wise et al, 1979). In nature, during carbohydrate metabolism, lactic acid is present as an intermediate or as an end product. It is extensively distributed in all living things. It appears as L (+), D (-) or as a racemic mixture in various proportions and it has been found in all tissues, body fluids and excreta in many mammals.
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including man. In mammals, the L (+) is the form, which is metabolised in the body, whereas the D (-) isomer has only been detected in the faeces.

\[
\begin{array}{c|c}
\text{COOH} & \text{COOH} \\
\text{HO–C–H} & \text{H–C–OH} \\
\text{CH}_3 & \text{CH}_3 \\
\hline
\text{L (+)} & \text{D (-)} \\
\end{array}
\]

Figure 1.4 D and L configurations of lactic acid.

The concentration of lactide varies amongst body components and also varies from animal to animal (Holten, 1971). The D (-) isomer has also been isolated from micro-organisms in the form of bacteria cells and enzymes, in some cases a yield of both isomers (D and L) may occur in the same cell (Mahler and Cordes, 1966).

Under anaerobic conditions glucose may be converted into lactic acid in the aerobic cells of most higher plants and animals and aerobic microorganisms. Most aerobic organisms have the capacity to conserve energy from glucose by means of an anaerobic pathway (Figure 1.5), which is the preparatory step for further oxidation. Aerobic glycolysis yields carbon dioxide and water as end-products, as long as enough oxygen is provided. Under these conditions most tissues do not produce or accumulate lactic acid. However, oxygen deficiency will lead to a build up of lactic acid (anaerobic glycolysis). The lactic acid will diffuse through the plasma membrane to the surroundings as waste. During strenuous exercise there is a deficiency of oxygen, so the muscles are forced to work anaerobically in order to produce energy. The lactate (homo-lactic fermentation) (Figure 1.5) escapes from the cells into the blood, which carries it into the liver where it is rebuilt to glucose during recovery (Voet and Voet, 1990).
Figure 1.5 Overview of glucose metabolism (Reproduced from Voet and Voet, 1990).
1.3.1 Synthesis of poly lactide

Hydroxyl acids may be condensed to form polyesters. Lactic acid condensation occurs spontaneously in aqueous solutions of the acid with concentrations greater than 25% of lactic acid. Polyesters formed in aqueous solutions give rise to linear dimers (lactoyllactic acid), trimers (lactoyllactoyllactic acid) and higher polymers all of which have a general formula as shown in Figure 1.6.

![Figure 1.6 Lactic acid condensation.](image)

In aqueous systems cyclic dimers are not formed. Optically active lactic and racemic lactic acid six member cyclic dimers may be synthesised by removing free water and bound water from aqueous solution of the acid by vacuum distillation. Bimolecular cyclic esters of lactic acid are called dilactides. As a heterocyclic compound, it is often referred to as 3, 6 - dimethyl - 1, 4, - dioxane - 2, 5 - dione and is commonly known as lactide (Figure 1.7) (Wise et al, 1979).

![Figure 1.7 Optical isomers of lactide.](image)
The optical isomers of lactide have different melting properties (Table 1.3). From D (-) lactic acid, L (+) lactic acid, D (+) lactic acid and L (-) lactic acid (Figure 1.6) a variety of polymers can be derived: Poly l-lactic (PLLA), D (-) lactic acid, L (+) lactic acid, poly d, l-lactic acid (PDLA), Poly l-lactide (PLLA), D (-) lactide, L (+) lactide, poly d, l-lactide (PDLA) (Holland et al, 1986).

Table 1.3 Isomers of lactide (Holten, 1971).

<table>
<thead>
<tr>
<th>Isomer</th>
<th>L-lactide</th>
<th>D-lactide</th>
<th>Racemic</th>
<th>Meso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>95°C</td>
<td>95°C</td>
<td>120 - 128°C</td>
<td>41 - 42°C</td>
</tr>
</tbody>
</table>

Lactide homo and copolymers have been extensively studied. These polymers have been synthesised by two methods (Figure 1.8), which are as follows:

1. Direct polycondensation of lactic acid
2. Ring opening condensation of cyclic diesters of lactide

Figure 1.8 Synthesis of polylactide from straight chain and cyclic monomers of lactic acid (Wise et al, 1979).
Direct polycondensation produces low molecular weight polymers (<10,000 kDa) using antimony trioxide as a catalyst. These low molecular weight polymers are not suitable for many applications. Ring-opening condensation can produce intermediate molecular weights of 10,000 – 40,000, and high molecular weight polymers in excess of 40,000 using catalysts such as antimony, tin and cadmium (Kulkarni et al, 1971; Gilding and Reed, 1979; Fukuzaki et al, 1991; Kitchel and Wise, 1991). Polymers with a particular molecular weight can be manufactured by selection of the appropriate polymerisation conditions, i.e. the amount of catalyst and duration of the reaction (Marcotte and Goosen, 1989). Chain control agents such as lauryl alcohol can be used to control the molecular weight of lactide polymers (Gilding and Reed, 1979). Ring - opening condensation of the cyclic dimers is the preferred method. Polylactide is synthesised by ring-opening condensation of cyclic dimers carried out at 120°C for several hours, which produces a medical grade, commercially available material (Radomsky et al, 2000). At reaction temperatures below 120°C an acid catalyst is required to increase the rate of reaction, but above this temperature the rate - limiting step is water removal, so little benefit is gained by using a catalyst above 120°C.

1.4 Polymer morphology

The term “polymer morphology” refers to the size, shape, molecular weight distribution and physical form of a polymer. Techniques such as optical light scattering, scanning electron microscopy and gel permeation chromatography are applied to polymers to determine their shape, size and molecular weight, whilst techniques such as modulated temperature differential scanning calorimetry, x-ray powder diffraction and infra-red spectroscopy are used to determined their physical structures. These techniques will be discussed in detail in Chapter 2.
Polymers are considered to be either amorphous or crystalline, or they may not be completely one or the other. Some crystalline polymers are now correctly termed semi-crystalline, as their measured densities differ from those obtained from perfect materials (starting material). The degree of crystallinity may be measured by x-ray diffraction. X-ray diffraction shows these polymers are less than completely crystalline.

1.4.1 Amorphous polymers

Amorphous polymers exhibit no long-range order, i.e. molecules are not arranged in an orderly fashion, but in a random manner with the atoms or molecules packed in repeating patterns (homo-polymers or hetero-polymers), giving the compounds pharmaceutically interesting physicochemical characteristics. At low temperatures, these polymers are characterised by their high viscosity. On heating, the polymer passes through a reversible transition, termed the glass transition, during which the viscosity decreases by several magnitudes resulting in a liquid or a rubbery state.

The temperature at which this transition occurs is termed the glass transition temperature ($T_g$). $T_g$ is the temperature above, which a polymer is rubbery and can be elongated (above $T_g$ and below $T_m$) and below which the polymer behaves as a glass. $T_m$ is the temperature at which a polymer melts. Polymers in the rubbery state will usually have high permeability and are flexible. Thermal analysis of amorphous polymers shows only a glass transition temperature (Figure 1.9). The first-order crystallisation at $T_m$ is shown in conjunction with the discontinuity in volume/enthalpy at $T_g$ (Figure 1.9). The short dashed line indicates the behaviour of a system cooled at a slower rate than that corresponding to the solid line. The long dashed vertical line shows the $T_g$ values for the fast cooled ($T_{gi}$) and slowed cooled ($T_{g2}$) systems (Craig et al, 1999).
Figure 1.9  Schematic representation of the change in enthalpy or volume with temperature for a material undergoing glass transition or crystallisation (reproduced from Craig et al, 1999).

During the glass transition mechanical and electrical properties of the material will change, but the molecular structure will remain the same. The amorphous form may occur as result of three circumstances. First, the drug, excipient or delivery system (polymer) may have been prepared in an amorphous form to improve product performance characteristics. Secondly the drug delivery system may be partially amorphous at room temperature, making the dosage forms prepared with these delivery systems partially amorphous, examples of these drug delivery systems include poly d, l-lactide, polyvinyl pyrrolidone and polyethylene glycol. Thirdly, the amorphous form may be generated accidentally by grinding, drying and compression (Craig et al, 1999) and by super-cooling from melt, vapour condensation, precipitation from solution, lyophilization, or milling of crystals (Hancock and Zografi, 1997).
Amorphous polymers that have commercial importance include polymers, which are glassy or rubbery at room temperature. Amorphous thermo-plastics such as poly d, l-lactide, polystyrene, polymethyl methacrylate, poly [di (oxymethylene) oxy-1, 4-phenylene carbonyl-oxycarbonyl-1, 4-phenylene] and poly (oxyethylene oxycarbonyl-1, 5-naphthylene carbonyl etc. form brittle glasses when cooled from melt (Sawyer and Grubb, 1996). Due to their high internal energy, most amorphous compounds have a tendency to crystallise during manufacture and storage. Therefore knowledge of the crystallisation rate, \( T_g \), temperature and the effect of humidity on the physical stability of amorphous compounds is important in pre-formulation studies (Hancock and Zografi, 1997).

### 1.4.2 Crystalline polymers

Crystalline polymers exhibit long-range order, i.e. are in a highly organized state making them rigid and brittle, with the atoms or molecules packed in repeating patterns. On heating a crystalline solid, it will become liquid at a specific melting temperature. This temperature is known as a melting transition temperature (\( T_m \)) (Figure 1.9). The crystalline state is the lowest energy state available and hence is thermodynamically stable, so molecules or atoms will prefer to pack in this state when possible.

The differences between the formation of amorphous and crystalline systems is shown in Figure 1.9. In a crystalline system, cooling the temperature from the liquid-state to the \( T_m \) results in the transition to a crystalline form (assuming no super-cooling), which below \( T_m \) is the thermodynamically stable state with respect to non-crystalline forms. The exothermic crystallisation leads to sudden contraction of the system due to a decrease in free volume. As a result, both enthalpy and volume decrease at \( T_m \). In the case of amorphous systems (glass forming material) the cooling process is too fast for crystallisation to occur, which is either due to the large molecular size and shape of the compound or to the use of a rapid cooling rate. No discontinuity in enthalpy or volume is seen on cooling.
the material below $T_m$ and the system forms a super-cooled liquid. As the material is cooled further, a point is reached where the material becomes frozen (glassy state). At this point the bonding between molecules remains essentially the same as that of the liquid, but translational and rotational motions are greatly reduced, whilst vibrational motions taking place below $T_g$ are dominant. Slower cooling rates result in lower $T_g^2$ values as indicated in Figure 1.9.

Dissolution rates and bioavailability can be improved by the formation of amorphous rather than crystalline compounds from the same compound. For example, the bioavailability of a drug substance may be limited by the rate of dissolution of the drug in the gastrointestinal tract. If the drug is converted into an amorphous form, the higher molecular mobility and reduced density may increase the dissolution rate. Such an approach has been illustrated with novobiocin (Mullins and Macek, 1960), 9,3"-diacetylmidecamycin (MOM) (Sato et al, 1981) and indomethacin (Fukuoka et al, 1986). This improvement is critical for many poorly soluble drugs (Hancock and Zografi, 1997). The inclusion of glassy materials in formulations can also produce beneficial effects for processes such as freeze-drying. This procedure can alter the structure of protein-based drugs making them susceptible to chemical attack and shortening the shelf life of the product. It has been found that the addition of disaccharides causes the protein to become trapped in the sugar glass on freeze drying so that degradation is reduced.

Determination of the glass transition temperatures of pharmaceutical materials is important due to the many differences in behaviour above and below this temperature. Chemical stability decreases above $T_g$ and heating above $T_g$ may result in the collapse of freeze-dried products. Thus, it is important to fully characterise the behaviour of such systems and obtain an understanding of the nature of the glassy state. The increasing importance of the glassy state in pharmaceutical formulations has resulted in a number of reviews of this phenomenon (Hancock and Zografi, 1997; Craig et al, 1999). Some materials
exist in the amorphous state because the shape of the molecule does not allow regular packing in the crystalline form. Polymers in particular can have irregularities along their chain length that prevent crystallisation. For many other materials the structure can be either crystalline or amorphous depending on how the material was processed. It has also been suggested that there exists another type of structure between crystalline and amorphous, which is formed at the boundary between these two forms in semi-crystalline polymers.

1.5 Designing polymers for drug delivery systems

1.5.1 Polymer selection

Polymer selection is an important factor when designing drug delivery systems because the polymer is the major component in the final product, and generates the fundamental system behaviour. Polymers used for drug design may be separated into two classes, those that are in the glassy state \( T_g > 37^\circ C \) and those that are in the rubbery state \( T_g < 37^\circ C \) at body temperature, although they may not be completely one or the other. If a polymer is in the glassy state it will be tough and inflexible with low permeability, and drug release by leaching and erosion will be favoured relative to diffusion. The only possibility of achieving a constant rate of drug release from this class of polymer is by constant bioerosion of a device with a constant surface area, i.e. a slab, or by diffusion controlled release system such a microcapsule reservoir device where thin walls and increased surface to volume ratio compensate for the reduced permeability (Pitt et al, 1980). Poly lactic acid, \( T_g 57^\circ C \), formulated as microcapsules is an example of the latter. Polymers in the rubbery state will have high permeability, but lack sufficient mechanical strength and stability unless reinforced by cross links, crystalline domains or strong interactions between chains such as hydrogen bonds. Silicone rubber is an example of a non-degradable rubbery polymer \( T_g 120^\circ C \), which must be strengthened, by light cross-linking. If the polymer is to be biodegradable, this means the reinforcement must also be biodegradable. This
can present some problems if controlled release is by diffusion, since degradation of the cross-links may change the polymer morphology and hence its permeability. Changes in the dimensions and crystallinity of devices produced by biodegradation will also affect the rate of drug diffusion. These problems can be minimised if bioerosion occurs after the drug is depleted (Pitt et al, 1980).

1.5.2 Natural and synthetic polymers

Polymers used for drug delivery may be naturally occurring or synthetic materials (Table 1.4). From the early 1970s both naturally occurring and synthetic polymers, which were capable of enzymatic and non-enzymatic breakdown were investigated. For these polymers to be completely acceptable, the end products had to be non-toxic and excreted via normal physiological pathways and not cause any adverse effects. Polymers, which are non-biodegradable, required surgical removal, e.g. silicone rubber implants and polyethylene. Biodegradable polymers on the other hand required no surgical removal (Jalil and Nixon, 1992).

Table 1.4 Types of biodegradable and non-biodegradable polymers used as drug delivery systems.

<table>
<thead>
<tr>
<th>Biodegradable: Natural</th>
<th>Albumins, collagen, gelatine, polysaccharides, serum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodegradable: Synthetic</td>
<td>Aliphatic polyesters, polyamides, polycarbonates, polyurethanes.</td>
</tr>
<tr>
<td>Non-biodegradable</td>
<td>Polyethylene, silicone rubber.</td>
</tr>
</tbody>
</table>

A problem associated with some non-biodegradable implants is that they may leave residues, which could be hazardous to the body. To avoid surgical problems associated with non-biodegradable implants, micro and nano particles made from biodegradable synthetic and naturally occurring polymers have been made, which can be injected if the particles are less than 125 μm
(nanospheres/microspheres) (Ashardy, 1999). Biodegradable synthetic polymers are preferred over biodegradable natural occurring polymers because natural polymers lack batch-to-batch reproducibility, and purity often differs upon batch production (Pitt et al, 1980). The advantages of using synthetic biodegradable polymers are that they can be chemically modified, easily prepared economically in large quantities and are non-immunogenic. These attributes are essential for a drug carrier, which is to be administered parenterally (Roerdink and Kroon, 1989b). PDLA is an example of a synthetic biodegradable polymer it belongs to an aliphatic polyester group, examples of aliphatic polyester polymers and their physical properties are shown in Figure 1.10.

The permeability, biodegradability and mechanical properties may all be changed by logical but small changes in chemical structure of the subsistent or chains. The polyesters (Figure 1.10) provide a good illustration. On the basis of electronic effects and properties of monomeric esters, one can anticipate the rate of non-enzymatic hydrolysis of polymers of $\alpha$-hydroxyl acids, e.g., polyglycolic acid, will be greater than polymers of $\beta$, $\gamma$, $\delta$, and $\epsilon$-hydroxyl analogues. Aromatic esters will be less readily hydrolysed. Alkyl or aryl substituents adjacent to the ester group can retard hydrolysis by steric hindrance. The $T_g$ and $T_m$ provide useful information of the polymers, structure-morphology relationship. For instance polyglycolic acid is an aliphatic polyester, which is practically insoluble, has a high melting point and is semi-crystalline with a $T_g$ of 36°C. These properties represent the polarity and structural order of the polymer. Stereospecific introduction of a methyl group, as in poly d or l-lactic acid, has little effect on the $T_g$ and $T_m$. Non-stereospecific introduction of methyl group, as in the racemic poly d, l-lactic acid does not change the $T_g$ greatly, but completely eliminates the crystallinity, because of the random arrangement of chiral centres. Similarly, non-stereospecific introduction of two different alky groups will eliminate both the crystallinity and reduce the $T_g$. For example polydimethylethylglycolic acid is rubbery at 37°C, but if both alkyl groups are identical, as in polydimethylglycolic acid, the symmetry and crystallinity are restored (Pitt et al, 1980).
Figure 1.10 Chemical structures and relevant physical properties of some polyesters (Pitt et al, 1980).
1.6 Microencapsulation

Microencapsulation is a process where solids, liquids or gases inside one or more polymeric coatings are entrapped. Microencapsulation is useful for the manufacture of controlled release delivery formulations. Two major methods of encapsulation have evolved. Depending on the process, two types of structures are possible, reservoir (microcapsules) and monolithic (microspheres). In microcapsules a central core is surrounded by polymer matrix, whilst in microspheres the drug is evenly dispersed throughout the polymer matrix (Dash et al, 1997).

1.6.1 Microcapsules

Microcapsules are small particles having a well-defined core and a well-defined shell. The core can be liquid, solid or gas and the shell can be made from porous or nonporous polymers, but fats and waxes have also been used. The drug can be dispersed inside the microcapsule as solid particles with regular or irregular shapes, or as a solution, suspension and emulsion, or as a combination of an emulsion and suspension. At present there are no universal size ranges that particles must possess in order for them to be classed as microcapsules. However many people have classed capsules of less than 1 μm as nanocapsules and capsules larger than 1000 μm as macrocapsules. A wide range of core materials have been encapsulated in microcapsules and macrocapsules, including pharmaceuticals, agrochemicals, fragrances, live cells, active enzymes and flavours (Mathiowitz et al, 1999). Microcapsules can have a variety of structures. Some are spherical with a continuous core surrounded by a continuous shell, while others can have irregular geometries, which contain a number of small droplets of core material (Mathiowitz et al, 1999).
1.6.2 Microspheres

The term microspheres refers to small particles intended as carriers for drug or other therapeutic agents. Microspheres are monolithic, whereby the drug is dispersed homogeneously. In such systems, a drug in the crystalline state, or as a suspension, is trapped within the polymer matrix, which delays the release of the drug through the polymer matrix. If diffusion is not possible, then the polymer must erode before the drug is released. Hence careful investigation into polymers would deem it possible to develop and control systems with specific release profiles. Microspheres can vary from $<1 \mu m$ up to $100 \mu m$. The smaller particles are sometimes termed as nanospheres or nanoparticles. Particle size of microspheres is an important property since the route of administration will determine the choice of microsphere size (Figure 1.11) (Roerdink and Kroon, 1989a).

<table>
<thead>
<tr>
<th>Size (μm)</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of Administration</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation &amp; Embolization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.11 Size requirements of microspheres for various routes of administration (Deluca et al, 1993).
Microspheres intended for drug delivery can be prepared from a variety of natural and synthetic polymers materials (Table 1.5) and have different characteristics depending on the use to which they are put (Juni and Nakano, 1987). The choice of material will be dictated by factors such as the drug, the intended destination, disease condition to be treated and duration of action. For example, microspheres intended for delivering drugs to the bone marrow following intravenous administration need to be small and have appropriate surface characteristics that will exploit biological recognition processes. They should degrade rapidly at the desired site of action to release the entrapped drug (Davis and Illum, 1986). In contrast, microspheres for controlled release of hormones will be, for example, injected (implanted) into muscle tissue to release the entrapped drug slowly and uniformly over a prolonged period of time (Juni and Nakano, 1987). The choice of materials for microspheres will also be influenced by toxicity considerations that include immune responses. Microspheres intended for non-parenteral application (e.g. intranasal, oral) will not need to satisfy such rigorous constraints as those intended for parenteral use (Vert, 1986).

Table 1.5  Examples of Microsphere Systems for drug delivery.

<table>
<thead>
<tr>
<th>Delivery Matrix</th>
<th>Drug</th>
<th>Loading (%)</th>
<th>Size (μm)</th>
<th>Route</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>Chloramphenicol</td>
<td>5</td>
<td>20 - 40</td>
<td>Ocular</td>
<td>Shell, 1978</td>
</tr>
<tr>
<td>PDLA</td>
<td>Progesterone</td>
<td>10 - 50</td>
<td>100 - 130</td>
<td>-</td>
<td>Hill et al, 1998</td>
</tr>
<tr>
<td>PDLA</td>
<td>Leuprolide</td>
<td>13.4</td>
<td>52</td>
<td>SC</td>
<td>Woo et al, 2001</td>
</tr>
<tr>
<td>PLGA</td>
<td>Levonorgestrel</td>
<td>15 - 30</td>
<td>&lt;100</td>
<td>SC</td>
<td>Dinarvand et al, 2001</td>
</tr>
</tbody>
</table>
1.6.2.1 Therapeutic rationale of using microsphere systems for controlled drug delivery

The term “controlled drug delivery” as commonly used, embraces the technology by which therapeutic agents are introduced into the body and are made available to the target site of action at a uniform drug release rate, or provide an unvarying blood plasma concentration – “zero order” release. This means the drug is delivered at a constant rate so that the release and absorption of the drug from the dosage form is uniform and independent of the drug concentration (Evers, 1996). Controlled release delivery systems offer a number of advantages in treatment. Compounds with relative short biological half-life, instability, degradability in the gastro-intestinal tract and high toxicity, such as proteins, peptides and steroids are required to be administered as often as several times a day to achieve a therapeutic effect. Controlled release delivery systems for these compounds potentially offers a prolonged duration of delivery, enabling dosing to be reduced or less frequent intervals of dosing. Therefore the therapeutic agents' pharmacological activity is prolonged.

Controlled delivery may eliminate the peak (toxic) and trough (sub-therapeutic) effects which may be observed with frequent dosing of traditional parenteral (subcutaneous or intramuscularly) and oral systems. Widely varying blood plasma concentration levels can result in the development of undesirable adverse effects at peak blood plasma concentration levels, while periods of insufficient treatment may occur at trough levels (Figure 1.12). Therefore, controlled delivery systems, in theory, can result in better drug utilization by delivering the drug at a desired rate, resulting in a narrower range of blood plasma concentration levels. Moreover, parenteral controlled release delivery systems offer the advantage of improved patient compliance. The number of drug doses are reduced and the patient is not required to remember to take doses one or more times a day. Instead the patient receives a dose during a routine physician visit every four to
six weeks, depending on the formulation delivery design. Alternatively the patient could be trained to self-administer on routine basis if desired (Floy et al, 1993).

Figure 1.12 Schematic representation of therapeutic ranges.

Even though parental controlled release delivery systems offer several advantages, there are some limitations that must be considered in determining the therapeutic agent’s potential for these systems. A therapeutic agent with a low potency is not an optimal candidate for controlled delivery systems. The lower the potency, the greater the quantity required for a specific delivery period. Large quantities of drug are not compatible with reasonably sized injectable or implantable systems. Low potency will require high drug loadings levels, which may result in unacceptable or unpredictable release profiles and create problems with dose dumping.

Therapeutic agents with narrow therapeutic index must be carefully scrutinised if they are to be used with a parental controlled delivery system. For these agents, the dose at which the adverse effects or toxicity occurs may be very close to that producing the therapeutic effect. Therefore the controlled release system must perform reliably and reproducibly, to prevent over dosage or therapeutic failure.
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Delivery systems designed with biodegradable polymers such as PDLA, PLLA, PGA and PLGA are desirable because they do not need to be retrieved when the drug is depleted from the delivery system. However they do pose potential problems if there is need to terminate therapy (due to some adverse effect). In the time shortly after injection or implantation, little or no polymer degradation has taken place and the systems will be reasonably intact and may be retrieved. Microsphere systems will be more difficult to retrieve than solid matrix implants. Once degradation begins, the delivery system will become fragmented and smaller, making retrieval difficult if not impossible (Floy et al, 1993).

1.6.3 PLA microsphere preparation

PLA microspheres can be prepared by several different methods such as phase separation (interfacial phase deposition), emulsion solvent evaporation, emulsion solvent extraction, spray drying and the melt method.

1.6.3.1 Phase separation

Phase separation can be used to produce microspheres and is usually used for the encapsulation of water-soluble drugs (Ogawa et al, 1988; Okada, 1989). The drug is dissolved in water and then added to a solution of biodegradable polymer (PLA or PLGA) in organic solvent to form a water in oil emulsion. A second polymer (silicone oil), which is poorly miscible with the biodegradable polymer but miscible with the first organic solvent, is added whilst stirring. This causes precipitation of PLA or PLGA around the aqueous droplets or solid particles, which contain the drug. Hardening of the microspheres is accomplished by the addition of a non–solvent (heptane), from which the microspheres are isolated. The method has the disadvantage that a large volume of solvent is required and it may be difficult to completely remove the solvents from the microparticle system after manufacture. There are a number of process variables in this method, which must be adequately characterised and controlled for manufacture.
of a satisfactory final product. These include addition rates of solution and polymer solvents, polymer concentration, drug concentration, solvent volumes, mixing rates, mixing times, process temperature, vessel and stirrer geometries and isolation techniques. Some of these variables may have greater or lesser effects on the final product. These effects can be determined through experimentation (Floy et al, 1993).

**Figure 1.13** Schematic diagram of the preparation of microspheres by the phase separation technique.

### 1.6.3.2 Emulsion solvent evaporation

The most common technique for the preparation of PLA microspheres is the emulsion solvent evaporation process. The polymer is dissolved in an organic volatile solvent (e.g. dichloromethane, methylene chloride). The drug is either dissolved or suspended in the solution of polymer and volatile organic solvent,
then the solution or dispersion is emulsified in an aqueous medium to form microdroplets by stirring. The organic solvent then diffuses into the aqueous phase and evaporates at the water/air interface (Figure 1.14). Evaporation can take place at either atmospheric pressure or reduced pressure. The microdroplets solidify and solid, free flowing microspheres are obtained after complete organic solvent evaporation, filtration and drying. In some methods the process is interrupted before complete removal of organic solvent, and one that does not contain an emulsifier replaces the aqueous phase. This has been shown to reduce the formation of surface crystals (Benita et al., 1984). The solvent evaporation process is most suitable for the entrapment of hydrophobic drugs, as hydrophilic materials will tend to partition into the aqueous phase resulting in low drug loading. The advantage of this method is that a wide range of particle sizes can be achieved (100 μm to < 1 μm) by controlling the stirring rate (Bodmeier and McGinity, 1987).

1.6.3.3 Emulsion solvent extraction

The solvent extraction method is considered to be a modification of the emulsion solvent evaporation method. This process is hardly ever used to prepare PLA microspheres (Gupta et al., 1989; Conti et al., 1991), but has been used to prepare PGA microspheres (Kanke et al., 1986; Sato et al., 1988; Hazrati et al., 1989). The procedure comprises of an emulsification phase similar to emulsion solvent evaporation (Figure 1.14). Once the emulsification phase is complete, the emulsion is poured into a diluent phase that does not solubilise the polymer, but is miscible with the continuous and dispersed phases of the emulsion. As a result the solvent migrates from the polymer microdroplets into the diluent phase. The system is continuously stirred until extraction of the solvent from the microparticles is complete. The advantage of this method is that it is possible to obtain extraction of the solvent at low temperatures, without approaching the solvent boiling point (Conti et al., 1992).
Figure 1.14 Schematic diagram showing the preparation of microspheres by emulsion solvent evaporation and extraction techniques.

1.6.3.4 Spray-drying

The advantage of this technique is that both water-soluble and hydrophobic compounds can be encapsulated efficiently. In this approach, the drug is in aqueous solution or as a solid particle is dispersed in a solution of polymer. The mixture is pumped through the atomiser of a spray-drier into the drying chamber
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where a heated carrier gas dries the particles as they are carried to the jet separator for collection. Typical process variables are polymer concentration, drug concentration, volumes, atomiser characteristics, fluid pumping rate, inlet and outlet gas temperature and gas flow rate.

Figure 1.15 Schematic diagram showing the preparation of microspheres by spray-drying.

PDLA microspheres loaded with progesterone have been prepared by spray-drying (Bodmeier and Chen, 1988). Spray-dried progesterone is predominantly in the alpha form, but when combined with PDLA the beta form of progesterone predominates. Therefore spray-drying may modify core drugs, which are crystalline. PDLA has also been used to coat chloramphenicol particles, which yielded particle size ranges of $< 40 \mu m$, and these were used in an ophthalmic suspension (Shell, 1978). The advantages of the spray-drying process, is that the
procedure is performed under mild conditions, which yield large production batches of microspheres (Conti et al, 1991).

1.6.3.5 Melting method

In this process, the PLA and the drug are melted together above the polymer's glass transition temperature. The melt is cooled and poured into a solution of Tween 80 and emulsified at a temperature where the PLA – drug mixture is molten. After emulsification the particles are recovered by centrifugation and subsequent freeze-drying or by spray-drying. The advantage of this technique is that the process avoids the use of chlorinated solvents, eliminating the problems associated with solvent toxicity (Wichert and Rohdewald, 1990).

Figure 1.16 Schematic diagram showing the preparation of microspheres by the melting method (Wichert and Rohdewald, 1990).
1.7 Modes of controlled drug release

A drug can be included in a polymer delivery system by entrapment or by covalent attachment to the polymer matrix. The time required for the polymer to degrade and for the drug to be released, are the controlling factors of the delivery device. Polymer degradation can take place throughout the drug release process, during which only a portion of the drug is released, or after the drug has been depleted. Most biodegradable devices are designed to degrade after the drug they contain has been depleted. Depending on the polymer chosen (biodegradable or non-biodegradable) there are three primary mechanisms by which an active agent can be released from the polymer. Drug delivery may be controlled by swelling, diffusion and erosion. Only the latter involves biodegradation. Most delivery systems will act by a combination of the three mechanisms. Regardless of the approach selected, it is necessary to control the properties of the polymer. The most important of which are its biodegradability, permeability, biocompatibility and mechanical strength. Since these properties are often dependent on one another, their optimisation can present a challenging task in polymer design (Skiens et al, 1980).

1.7.1 Diffusion-controlled release systems

Two types of diffusion-controlled devices have been used in drug delivery. These are reservoir and monolithic devices. The drug component of either device may be dispersed or dissolved within the device. Examples of diffusion-controlled release systems are shown in Figures 1.17 and 1.18. Diffusion occurs when a drug, or active agent, passes through the polymer that forms the controlled release device. Drug diffusion can occur through the pores of the polymer matrix or by passing between polymer chains. Since the drug concentration in the device is much higher than that on the outside, the driving force for diffusion across the membrane will be constant with time.
1.7.1.1 Monolithic devices

When the polymer and the drug are mixed to form homogeneous systems, they are often referred to as a matrix system. The drug may be evenly dispersed or completely dissolved in the polymer matrix. Diffusion occurs when the drug or active agent passes through the polymer matrix into the external environment. As drug release progresses, the release rate normally decreases with the homogeneous system, because the drug has a longer distance to travel and hence requires a longer diffusion time to release (Brannon-Peppas, 1997).

Figure 1.17 Drug delivery from a typical monolithic system.
1.7.1.2 Reservoir devices

In this design, a reservoir whether solid drug, dilute solution or a very concentrated drug solution within a polymer matrix is surrounded by a film or membrane of rate controlling material. The polymer layer surrounding the reservoir effectively limits the release of drug from this reservoir device. Since the polymer coating is effectively uniform and of non-changing thickness, the diffusion rate of the drug or active agent can be kept fairly constant throughout the lifetime of the delivery system. Once the drug or active agent has been released into the external environment, one might assume that any structural control over the drug delivery has been relinquished, but this is not always the case. As with transdermal drug delivery, once the drug penetrates through the skin it has to pass through several layers of skin (stratum corneum, epidermis, dermis and then subcutaneous fat) all of which have additional diffusive and active transport steps (Brannon – Peppas, 1997).

Figure 1.18 Drug release from a reservoir delivery system.
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Drug delivery systems, which are diffusion controlled are fundamentally stable in a biological environment and do not change in size either through swelling or degradation. In systems such as these, the combination of drug and polymer matrices chosen must allow for the drug to diffuse through pores or between polymer chains upon introduction of the delivery system into the biological environment, without inducing any change in the polymer itself. It is also possible to have a combination of reservoir and monolithic device, which are often referred to as laminated matrices.

1.7.2 Swelling controlled systems

Drug delivery systems can be designed so that they are incapable of releasing their active agent until placed in the appropriate biological environment. Swelling controlled systems are initially dry, but when placed in the body, they will absorb water or biological fluids causing them to swell. The swelling increases the aqueous content within the formulation as well the polymer size, enabling the drug to diffuse through the swollen network into the external environment (Brannon-Peppas, 1997). Examples of these systems are shown in Figures 1.19 and 1.20 for reservoir and matrix systems respectively. Most materials used in swelling control are based on hydrogels, which are polymers that swell, without dissolving, when placed in water or other biological fluid. Examples of swelling hydrogels are polyacrylamide and collagen poly (hema) (Jeyanthi and Rao, 1990; Risbud and Bhonde, 2000). Hydrogels can absorb a lot of fluid and at equilibrium can comprise 60 – 90% fluid and only 10 – 40% polymer. Polymer swelling can be triggered by a number of environmental changes, such as pH, temperature or ionic strength. Polymer systems can either shrink or swell. These changes depend on the type of polymer used. For most polymers the structural changes are reversible and repeatable upon additional changes in the external environment. An example of structural changes is shown in Figure 1.19. With these types of systems, drug release is only achieved when the polymer swells.
Because many of the potentially useful hydrogels swell at high pH values and collapse at low pH values, the triggered drug delivery occurs upon an increase in the pH of the environment. Materials of this kind are ideal for oral delivery systems, where the drug is not released at low pH values in the stomach, but is accelerated at high pH values in the upper small intestine. An example of is poly ethyl or methyl acrylate- co - methacrylic acid, which swells and releases drug at pH 5.5 (Ranjha and Doeiker, 1999).

**Figure 1.19** Drug delivery from a reservoir swelling controlled release system.

**Figure 1.20** Drug delivery from a monolithic swelling controlled release system.
1.7.3 **Biodegradable controlled systems**

Erosion controlled systems are very different from the systems previously outlined in that the other systems are based on polymer systems that do not change their chemical structure beyond what occurs during swelling. Biodegradable controlled release systems break down within the body as a result of natural biological processes, eliminating the need for removal after the drug has been depleted. Most biodegradable systems are designed to degrade as a result of hydrolysis of polymer chains into smaller compounds. Polylactides, polyglycolides and their copolymers will eventually breakdown into lactic and glycolic acid, which will enter the Kreb’s cycle and further break down into carbon dioxide and water (Figure 1.5).

![Figure 1.21 Schematic representation of drug release from a bulk eroding biodegradable system.](image)

**Figure 1.21 Schematic representation of drug release from a bulk eroding biodegradable system.**

![Figure 1.22 Schematic representation of drug release from a surface eroding biodegradable system.](image)

**Figure 1.22 Schematic representation of drug release from a surface eroding biodegradable system.**
These end products are excreted in respired air, urine and faeces. Degradation may take place through bulk hydrolysis in which the polymer degrades in a fairly uniform manner throughout the matrix (Figure 1.21). For other degradable polymers such as polyanhydrides and polyorthoesters, the degradation only occurs on the surface of the polymer, which results in a release rate that is proportional to surface area of the delivery system (Figure 1.22).

1.8 Mechanism of polylactide degradation

The lactide polymer chains are cleaved by hydrolysis to monomeric acids and are eliminated from the body during carbohydrate metabolism as described by Kulkarni et al, 1966 and outlined in Section 1.1. The rate of hydrolysis of the polymer is dependent on significant changes in temperature and pH, or the presence of a catalyst. Very little difference is observed in the rate of degradation at different body sites, which is an advantageous with regard to drug delivery and formulation.

The role of enzymes in biodégradation of lactide polymer has been somewhat controversial. Most early literature concluded that bioerosion of lactide occurred by hydrolysis with no enzymatic involvement. Other researchers have suggested that enzymes play a significant role in the breakdown of lactide materials. Much of this speculation is based on the differences observed between in vivo and in vitro degradation rates (Lewis, 1990). Holland et al (1986) concluded that little enzyme involvement is expected in the early stages of biodégradation with polymers in the glassy state, whereas enzymes can play a significant role for polymers in the rubbery state.

As drug delivery systems, it is desirable to have polymers, which degrade by hydrolytic processes. The release mechanism of the drug from the polymer matrix will be determined by the design of the device and also by the degradability or erodability of the polymer. For systems in which the polymer
does not degrade, the release is limited by the diffusion of the drug through the polymer. The nature of degradation, heterogeneous or homogeneous, determines the release kinetics of the polymer.

1.8.1 Heterogeneous degradation

This occurs at the surface of the polymeric carrier (Figure 1.23) where it is interfaced with the physiological environment (water, biological fluid etc.). In this case the degradation rate is constant. The un-degraded carrier retains its chemical integrity during the process. Carriers, which have high surface to volume ratio, would undergo faster degradation compared to their lower ratio counterparts.

![Figure 1.23 Schematic representation of heterogeneous biodegradable polymer degradation with time.](image)

1.8.2 Homogeneous degradation

This involves random cleavage throughout the bulk of the polymeric carrier (Figure 1.24) when placed within the physiological environment (water, biological fluid etc.). In this case, the molecular weight of the polymer steadily decreases, whilst the polymer can remain in its original shape and retain its mass until the polymer has undergone significant degradation, i.e. as much as 90%, and reaches a critical molecular weight, at which time solubilization and mass loss commences.
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Figure 1.24 Schematic representation of homogeneous biodegradable polymer degradation (small black and white circles represent dispersed drug particles).

Biodegradation times vary depending on the molecular weight, the sequencing and cross-linking within the polymer backbone, as well as the surface area and porosity of the carrier. Lactide carriers degrade predominantly by homogeneous degradation (Deluca et al, 1993).

The degradative process may be described and quantified in several molecular weight terms. The most common of these terms are the number-average molecular weight ($M_n$) and the weight average molecular weight ($M_w$), represented by equations 1.1 and 1.2 respectively.

\[
M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum w_i}{\sum w_i/M_i}
\]

Equation 1.1

\[
M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum w_i M_i}{\sum w_i}
\]

Equation 1.2

Where $M_i$, $n_i$, and $w_i$ are molecular weight, number of moles, and weight of the $i^{th}$ monomer unit, respectively.
Both $M_n$ and $M_w$ are unique values for a given polymer. $M_n$ is most affected by the lower molecular weight polymer fractions, whereas $M_w$ is most affected by the higher fractions. Therefore $M_w$ is always greater than $M_n$, except in the rare or non-existent case of a totally monodisperse polymer. However, polymers are inevitably polydisperse, meaning several or many different molecular weight fractions exist. The extent of variability in molecular weight is determined by the polymer's polydispersity (PD) calculated by equation 1.3.

$$PD = \frac{M_w}{M_n}$$  \hspace{1cm} \text{Equation 1.3}$$

A relationship known as the Mark - Houwink equation, exits between the intrinsic viscosity, $\eta \text{[]}$ and the molecular weight, $M$ of a polymer, as shown in equation 1.4.

$$[\eta] = KM^a$$  \hspace{1cm} \text{Equation 1.4}$$

The constants $K$ and $a$ are determined for a given polymer in a specific solvent at a designated temperature. Typical values for $K$ range from 0.5 to $5 \times 10^{-4}$, and those for $a$ are commonly between 0.6 and 0.8. Knowing the value for $a$, one can calculate another average known as the viscosity-average molecular weight ($M_v$).

$$M_v = \left( \frac{\sum \eta_i M_i^{1+a}}{\sum \eta_i M_i} \right)^{1/a} = \left( \frac{\sum w_i M_i^a}{\sum w_i} \right)^{1/a}$$  \hspace{1cm} \text{Equation 1.5}$$

$M_v$ is always greater than $M_n$ and usually less than $M_w$, but can equal $M_w$ if the upper limit of $a$ is reached for random coil polymers (Copper, 1989). The bulk degradation process can be characterised by both the decrease in average molecular weight and the polymer mass as show in Figure 1.25.
The profile shows an induction period for both $M_w$ loss and mass loss (erosion or solubilization). The induction period for $M_w$ loss was short and is believed to be due to permeation of buffer medium into the matrix or the polymer backbone. $M_w$ loss occurred abruptly and was exponential with time. Mass loss was much slower and occurred after about 90% of the original polymer composition had degraded. This suggests that the molecular weight had to fall below a critical value before solubilization occurred (Kenely et al, 1987).

Figure 1.25 Degradation profile of poly (d, l-lactide - co - glycolide); 50:50 $M_n$ 28,800. Percent mass loss and $M_n$ as function of time in buffer (Kenely et al, 1987).
1.9 Scope of the thesis

There are many reasons why it is necessary to characterise polymeric materials. Forensic analysis, recycling and failure analysis of a component all require identification of a polymer type although they may not be concerned with the precise measurement of all its physical properties. The design of polymers for specific applications will often require knowledge of the physical properties of a material and the techniques used may be used in some instances for characterisation (Campbell et al, 2000). Hence the aim of this PhD project was to characterise poly d, l-lactide microspheres.

The general aim of this PhD was to entrap low molecular weight hydrophobic compounds within high molecular weight polylactide microspheres, to study the entrapment efficiency of the solvent evaporation process. The second aim was to determine how the entrapped drugs are released from the polymer and investigate the polymers' degradation characteristics in phosphate buffered solutions. The model compounds used for this research were the steroids, progesterone and estrone. Previous work has been conducted on progesterone and estrone loaded polylactide microspheres for studies of degradation and release, but little has been published on the effect the release media, i.e. the effect cosolvent might have on the glass transition temperature, surface and the rate of degradation of the microspheres. This was the focus of the work described in thesis.

The objectives of this investigation were to study the dissolution, stability and morphological properties of biodegradable PDLA microspheres containing progesterone and estrone. Further, how varying the drug loading of the chosen model compounds may have affected the polymers' glass transition temperature and release characteristics were also determined.
Chapter Two

Materials, Methods and Instrumentation
2.1 Materials

2.1.1 Poly d, l-lactide

PDLA with a molecular weight (M_w) of 99800±1600 (lot 46H0142), and polydispersity (PD) 1.52±0.012 and PDLA 103,000±1300(lot 47H0200) PD of 1.52± 0.012 were obtained from Sigma Chemicals (Poole, UK), in glass vials and stored at less than -20°C in airtight containers containing silica gel below until required.

A preliminary study was carried out on PDLA Mw 99,800 as received from supplier, and after being made into microspheres using modulated temperature differential scanning calorimetry (MTDSC) (Section 5.24). Particle size analysis and scanning electron microscopy (SEM) (Section 3.23) were also performed on prepared PLA microspheres.

Polylactide or polylactate (PLA) is the most widely investigated member of the hydroxy acids used for the preparation of polyester based biodegradable microspheres. However the optically active polymers of d- and l-lactide exist (Figure 2.1).

![Structural formulae of d, l, and d, l-lactide.](image)

Figure 2.1 Structural formulae of d, l, and d, l-lactide.
D-lactide and l-lactide are both crystalline polymers whilst d,l-lactide is amorphous. Drug loaded (progesterone and estrone) and drug free PDLA microspheres were characterised for PDLA degradation properties in phosphate buffered solution (PBS) (pH 7.4) and in a mixture of ethanol: phosphate buffered (EPBS) (chapters 5 and 6).

### 2.1.2 Progesterone

Progesterone (minimum 99%, lot number 18H1188) obtained from Sigma-Aldrich Chemicals (Poole, UK) was received in a plastic bottle and stored in a desiccator at room temperature until required. Progesterone was used to manufacture progesterone loaded PDLA microspheres, as a model drug to investigate the effectiveness of the solvent evaporation process, the interaction between drug and polymer, and to determine the release characteristics of the polymer (PDLA).

Progesterone is an ovarian hormone, produced by the corpus lumen after the ovary has released an egg. Progesterone is also produced by the placenta during pregnancy and is used to maintain pregnancy in women with history of recurrent miscarriage, to alleviate premenstrual abnormalities symptoms and postnatal depression and for birth control (contraceptive) (BNF 42, 2001).

![Figure 2.2 Structure of progesterone.](image)

Progesterone exists at room temperature as solid yellow–white crystals.
Chapter 2: Materials, methods and instrumentation

Progesterone has two polymorphic crystalline forms of equal physiological activity. The alpha form has an orthorhombic (prism) crystal structure, with melting points between 127°C - 131°C. The beta form has an orthorhombic (needle) structure, with a melting point of approximately 121°C (Merck Index 12th edition, 1996b). Progesterone is available from several pharmaceutical manufacturers (Table 2.1). Preparations are available for oral, vaginal, rectal, intramuscular or uterine administration. (ABPI Compendium of 1999 – 2000; BNF 42, 2001).

Table 2.1 Marketed progesterone preparations

<table>
<thead>
<tr>
<th>Pharmaceutical manufacturer</th>
<th>Product and its uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cilag AG</td>
<td>Micronor ®: Oral contraceptive.</td>
</tr>
<tr>
<td></td>
<td>Progestasert ®IUD: Sustained delivery device contraceptive.</td>
</tr>
<tr>
<td>Alza</td>
<td></td>
</tr>
<tr>
<td>Ferring</td>
<td>Gestrone ®: Intramuscular oily injection used to treat dysfunctional uterine bleeding.</td>
</tr>
<tr>
<td>Forest</td>
<td>Gestrol 50®: Intramuscular oily injection used to treat dysfunctional uterine bleeding.</td>
</tr>
<tr>
<td>Shire</td>
<td>Cyclogest ®: Pessaries intended for vaginal or rectal administration used to maintain pregnancy.</td>
</tr>
<tr>
<td>Wyeth laboratories</td>
<td>Crinone ®: vaginal gel used to treat symptoms of premenstrual tension and depression.</td>
</tr>
</tbody>
</table>

2.1.3 Estrone

Estrone (minimum 99%, lot number 19H0581) obtained from Sigma chemicals (Poole, UK) was received in glass vials and stored in a desiccator at room temperature until required. Estrone was used to manufacture estrone-loaded PDLA microspheres as a model drug to investigate the solvent evaporation process, the interaction between drug and polymer, and to determine the release
and degradation characteristics of the polymer (PDLA). Estrone is an ovarian hormone produced by developing follicles in the ovary. Estrone is used to alleviate premenstrual symptoms, correct symptoms associated with menopause such as hot flushes, insomnia and restlessness (BNF 42, 2001). Estrone is also used in combination with progesterone as an oral contraceptive (morning after pill) and may be used to treat some forms of cancers, such as breast and prostrate cancers (BNF 42, 2001). Estrone at room temperature exists as solid white crystals. Estrone exits in three polymorphic crystalline forms, with the polymorphic form obtained depending on the mode of crystallisation. Two of the three forms are orthorhombic (prism): form 1 is stable and melts at 259°C and form 2 is metastable and melts at 256°C (Merck Index, 1996a). The third form, form 3 is monoclinic and metastable and melts at 254°C (Both, 1983, Merck Index, 1996a). Preparations are available for oral and intramuscular administration (Table 2.2) (Martindale, 1999; ABPI compendium of 1999 - 2000).

![Structure of estrone](image)

**Figure 2.3** Structure of estrone.

<table>
<thead>
<tr>
<th>Table 2.2</th>
<th>Marketed estrone preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Manufacturer</td>
<td>Product and its uses</td>
</tr>
<tr>
<td>Dunhill</td>
<td><strong>Aquest®</strong>: An oily injectable suspension used for hormonal replacement therapy</td>
</tr>
<tr>
<td>Shire</td>
<td><strong>Hormonin®</strong>: Tablets for oral administration used for hormonal replacement therapy</td>
</tr>
</tbody>
</table>

Although oral administration of both these hormones results in loss of activity due
to the first pass effect of the liver it still remains the most popular route. At present typical therapy is moving towards intramuscular injections and suppositories. Frequent administration of progesterone and estrone are reported to have adverse effects such as irregular vaginal bleeding, abnormal liver function, headaches, irritability, and acne and weight gain. A sustained release dosage form may reduce the need for frequent administration, and the severity of adverse effects (BNF 42, 2001).

2.1.4 Other materials used

<table>
<thead>
<tr>
<th>Material</th>
<th>Lot Number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane (AnalaR 99.5%)</td>
<td>K2645119917</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Ethanol (HPLC grade 99%)</td>
<td>-</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Polyvinyl alcohol (Mw 22,000 98%)</td>
<td>K26522545838</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Silica Gel moisture indicator</td>
<td>K26344014919</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Tween 80</td>
<td>10035755</td>
<td>Lancaster synthesis (Morecambe, UK)</td>
</tr>
<tr>
<td>Tetrahydrofuran (HPLC grade 99.7%)</td>
<td>K281748035</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Water (deionised)</td>
<td>-</td>
<td>Laboratory Supply</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>-</td>
<td>BOC (Guilford, UK)</td>
</tr>
<tr>
<td>Helium</td>
<td>-</td>
<td>BOC (Guilford, UK)</td>
</tr>
<tr>
<td>Polystyrene standard Mw 580 – 377,400</td>
<td>14</td>
<td>Polymer laboratory Ltd (Shropshire, UK)</td>
</tr>
<tr>
<td>Sodium hydroxide, volumetric solution 1N (AnalaR)</td>
<td>90341183</td>
<td>BDH (Poole, UK)</td>
</tr>
<tr>
<td>Potassium di-hydrogen orthophosphate (AnalaR 99.5%)</td>
<td>A116725906</td>
<td>BDH (Poole, UK)</td>
</tr>
</tbody>
</table>
Chapter 2: Materials, methods and instrumentation

2.2 Sample preparation methods

2.2.1 PDLA microsphere preparation

The PDLA microspheres were prepared using the solvent evaporation method of Benita et al. (1984). This method was chosen because progesterone and estrone are hydrophobic drugs, therefore will partition in the organic phase, leading to a high drug yield within the microspheres. The microspheres were made with PDLA 

\[ M_w \ 99800 \ & 103,000 \]

containing 0, 10, 20, 30, 40 and 50% w/w progesterone and estrone, in three batches each.

Table 2.3 The mass ratio of progesterone and estrone to polylactide in prepared microspheres.

<table>
<thead>
<tr>
<th>Progesterone &amp; estrone ratio</th>
<th>Polylactide Ratio</th>
<th>Concentration Progesterone/Estrone (w/w)</th>
<th>Mass Progesterone/Estrone (mg)</th>
<th>Mass PDLA (mg)</th>
<th>Total Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>10</td>
<td>50</td>
<td>450</td>
<td>500</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>20</td>
<td>100</td>
<td>400</td>
<td>500</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>30</td>
<td>150</td>
<td>350</td>
<td>500</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>40</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
<td>250</td>
<td>250</td>
<td>500</td>
</tr>
</tbody>
</table>

The appropriate polymer and weights of drug were weighed separately in weighing boats and then placed into a 50 mL beaker. The total mass in each case was 500 mg (Table 2.3). Dichloromethane (10 mL) was measured in a graduated glass pipette and poured into the 50 mL beaker, which contained polymer or polymer and drug. A glass rod was used to ensure complete mixing. The dichloromethane solution was then poured into a 300 mL beaker containing...
Chapter 2: Materials, methods and instrumentation

125 mL of 2% w/v polyvinyl alcohol (PVA) in deionised water. The mixture was homogenised at a rate of 8000 rpm for 2 min using a homogeniser (Heidolph DIAX 60, LabPlant, Huddersfield, UK). The resulting emulsion was then stirred continuously with an overhead stirrer (Heidolph Type 741, LabPlant, Huddersfield, UK) at a rate of 500 rpm, at room temperature to allow for the evaporation of dichloromethane and formation of the microspheres. The solvent evaporation process was interrupted after 4 h and the preparation allowed to settle. Once the particles had settled, the aqueous phase was poured off and replaced with 100 mL deionised water, stirred and once again allowed to settle. The water was removed and replaced twice as this has been shown to reduce crystal formation (Benita et al, 1984). The solvent evaporation process was then continued for a further 16 h before the process was finally stopped. Once the process was complete, the formed microspheres were filtered through qualitative filter paper (Grade 6) with a fine pore size of 3 µm (Whatman ®, Maidstone, UK) under vacuum and washed with approximately 200 mL of deionised water. The microspheres were then dried in an oven set to 35°C for five days to remove any residual solvent, so as to limit the amount of plasticisation to the polymer (PDLA).

2.2.2 Yield of microspheres

The yield of dried PDLA microspheres was determined by expressing the weight of the microspheres collected before sieving as a percentage of the weight of the total mass of the starting raw materials. The microspheres were sieved using a 180 µm sieve and stored in a desiccator containing silica gel at room temperature to provide a dry atmosphere until characterisation was required. The microspheres were sieved to remove large and coarse particles.
2.2.3 Solubility studies of progesterone and estrone

Progesterone and estrone are very slightly soluble in aqueous medium; 0.1 and 0.03 mg/mL respectively (Merck index, 1996b; Dollery, 1999). To increase the solubility, mixtures of 10 – 60%v/v ethanol phosphate buffer (0.1M pH7.4) were prepared (Table 2.4). The media, which gave maximum solubility, was used to study the release behaviour of progesterone and estrone from PDLA microspheres. Excess progesterone and estrone (70 mg) were placed in 50 mL centrifuge tubes, each containing 30 mL of the dissolution medium (ethanol/phosphate buffer 0.1M pH7.4). The centrifuge tubes were screw capped to avoid evaporation and agitated (40 rpm) in a water bath (Grant Instruments, Cambridge, UK) equipped with a shaker set, to 37°C for five days to equilibrate. Once equilibrium had been achieved, the tubes were centrifuged at 10,000 rpm for 10 min. From the resulting suspension, 1 mL was taken and made up to 25 mL in a volumetric flask with dichloromethane/ethanol solution (2:23). The solution of dichloromethane / ethanol (2:23) was also used as a blank for the UV analysis.

Table 2.4 Showing the ratio of ethanol to phosphate 0.1M pH7.4 buffer used for solubility studies.

<table>
<thead>
<tr>
<th>Amount of model compound (mg)</th>
<th>Volume of ethanol (mL)</th>
<th>Volume of phosphate buffer (mL)</th>
<th>Total volume of ethanol/phosphate buffer (mL)</th>
<th>Ethanol content (%v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>3</td>
<td>27</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>24</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>70</td>
<td>9</td>
<td>21</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>70</td>
<td>12</td>
<td>18</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>70</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>70</td>
<td>18</td>
<td>12</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>
The drug concentration in each solution was determined by measuring the absorbance at the determined maximum wavelengths for progesterone and estrone by extrapolating the values from the appropriate calibration curves (Figures 2.6–2.9) obtained using ultra violet spectroscopy.

2.2.4 In vitro release studies of progesterone and estrone from PDLA microspheres

A 50:50 v/v release medium of ethanol phosphate buffered solution (EPBS) adjusted to pH 7.4 with 0.1M sodium hydroxide was chosen to mimic the pH of the blood. Drug loaded PDLA microspheres (10 mg) containing model compounds (progesterone and estrone) at 10, 20, 30, 40 and 50% w/w were placed in 50 mL centrifuge tubes, each containing 30 mL of release medium. The centrifuge tubes were screw capped and agitated (40 rpm) in a water bath (Grant Instruments, Barrington, Cambridge, UK) set at 37°C. 2 mL samples of clear supernatant (centrifuged at 10,000 rpm for 10 min before sampling using a J2–HA Centrifuge (Beckmann-RIIC Ltd, High Wycombe, UK) were removed at scheduled time points over a period of 6 weeks and analysed spectrophotometrically. For comparison, dissolution of free progesterone and estrone in the release medium was also determined.

2.2.5 In vitro degradation of drug free PDLA microspheres in phosphate buffered solutions

PDLA Microspheres with a median particle size of 7.7μm were prepared using solvent evaporation technique (Section 2.2.1). PDLA (5g) was dissolved in 100 mL dichloromethane. The solution was poured into 1.25 L of 2% w/v polyvinyl alcohol solution in water and homogenised for 2 min at 8000 rpm. The resulting emulsion was stirred continuously for 20 h with an overhead stirrer at 500 rpm. Once microspheres had formed, they were washed, filtered and dried for 5 days in an oven set to 37°C. 50 mg of drug free PDLA microspheres were incubated in...
50 mL centrifuge tubes. Each tube contained, 20 mL of the buffer medium (0.1M PBS pH 7.4 and in 0.1M EPBS pH 7.4) the tubes were agitated at 40 rpm in a water-bath at 37°C. At predetermined intervals (10 day intervals) the microspheres were isolated from PBS and EPBS and analysed. The surface morphology was observed by scanning electron microscopy (SEM) (Section 2.3.4) (Philips XL20, Philips Electron Optics, Eindhoven, Netherlands), particle size was measured by laser diffraction (Section 2.3.3) (Malvern Mastersizer SX, Malvern Instruments, Malvern, UK). Mass loss of the microspheres was determined gravimetrically from the sample weight (w₀) before incubation in media (EPBS and PBS) and the weight of dried samples (wᵢ) after various incubation times (% Mass loss was calculated as = 100 (w₀ . wᵢ)/w₀). Molecular weight loss was determined by gel permeation chromatography (GPC) (Section 2.3.6). Modulated temperature differential scanning calorimetry (MTDSC 2920, TA Instruments, Leatherhead, UK) studies (Section 2.3.8) were performed to monitor the glass transition change of PDLA upon degradation in PBS and EPBS when hydrated (TgH) and dehydrated (TgD). The pH of the release media, were also measured changes during the 100 day study.

2.3 Analytical Instrumentation

2.3.1 Thermogravimetric analysis

Thermogravimetric analysis is a thermal analysis technique for measuring the amount and rate of change in sample mass as a function of temperature and time. It is used to characterise any material that exhibits weight loss or phase changes as a result of decomposition, dehydration and oxidation. There are two modes used to investigate thermal stability behaviour in controlled atmospheres:

1. Dynamic, in which the temperature is increased at a linear rate.
2. Isothermal, in which the temperature is kept constant.
Although this technique is called thermogravimetry (TG) several manufacturers and users prefer to call the technique thermogravimetric analysis (TGA) to avoid confusion with glass transition temperature \( T_g \). In this thesis, TGA will be used for the same reason. In the polymer and pharmaceutical industries, TGA is used to study degradation, stability, moisture content, residual amount of solvent in finished products and raw materials.

The TGA 2950 (TA, Instruments Leatherhead, UK) used in these studies has five major components (Figure 2.4), which are as follows:

1. **Balance**: This provides precise measurement of the sample weight.
2. **Sample Loading bay**: This is a platform that pivots automatically, i.e. loads and unloads the sample from the TGA balance to the furnace area and vice versa.

![Figure 2.4 TGA 2950 (Reproduced from TA Instruments Operational Manual, 1994).](image)

1. Balance: This provides precise measurement of the sample weight.
2. Sample Loading bay: This is a platform that pivots automatically, i.e. loads and unloads the sample from the TGA balance to the furnace area and vice versa.
3. Furnace: The furnace consists of a furnace housing, gas heater, thermocouple and a furnace base that moves upwards (to close) and downwards (to open). The furnace controls the temperature and atmosphere (inert) in the heating jacket.

4. Cabinet: This section of the TGA contains the electrical and mechanical components of the instrument.

5. Heat exchanger: The heat exchanger removes heat from the furnace when cooling is needed. It consists of a fan which blows cool air to the radiator, a radiator which exchanges heat between water and air, a water reservoir, a pump that pushes water through the system, a temperature control switch which detects temperature conditions and a flow switch which regulates flows and detects lack of flow.

In these studies, a sample was placed in aluminium, hermetic differential scanning calorimetry (DSC) pan without a lid. The open pan was then placed on a platinum holder, which was connected to the balance by a hang down wire (Figure 2.5). The furnace was then raised around the sample and provided the controlled heating. The temperature of the sample was measured by a thermocouple, which hung directly above the sample pan. An inert gas (nitrogen) was purged through the balance and furnace using, flow rates of 40 mL/min and 60 mL/min.

The TGA 2950 operates on a null balance principle. The sample and tare pans hang from either side of the balance arm (Figure 2.5). The balance arm is maintained in a horizontal reference position by an optically active actuated servo loop. Positioned at the top centre of the balance arm is a flag, which when the balance is in a null position blocks out an equal amount of light (emitted by an LED) from reaching the two photodiodes.
When the sample weight decreases due to mass loss, the beam becomes unbalanced (triggering the balance arm and flag to rotate). This causes an unequal amount of light to hit the photodiodes. The unbalanced signal (error signal) is reduced to zero (null) by the control circuitry, which then returns the balance arm back to its original horizontal (null) position. Therefore, the change in current required to accomplish this task is directly proportional to the change in mass of the sample. The null position principle ensures the sample maintains a constant position in the furnace.

The TGA 2950 was calibrated for temperature and weight before the drug loaded PDLA microspheres were analysed (Section 3.2.4). The temperature signal was calibrated by comparing the observed melting temperature of indium to a literature value. Melting is not associated with a change in weight, so melting is determined by observing its effect on the derivative temperature signal. The weight signal calibration was performed, using standard reference weights, 100 mg and 1 mg, supplied by the manufacturer.
The sample can be placed directly on the platinum holder or in an aluminium hermetic DSC open pan, which is then placed on the platinum holder. All work was performed using aluminium hermetic DSC open pans. This eliminates contamination of the platinum holder. Before any work was carried out the platinum holder was flamed until red hot, to remove any contaminants, permitting the aluminium pans to have direct contact with the surface of the platinum holder. TGA was used to determine the amount of residual solvent present in the PDLA microspheres after the solvent evaporation process (i.e. after drying at 35°C) using nitrogen as the purge at a heating rate of 10°C min⁻¹.

2.3.2 Ultraviolet spectroscopy

Ultraviolet spectroscopy is the study of materials using their absorption of ultraviolet radiation. Ultraviolet (UV) light lies between visible light and X-ray. Visible and ultraviolet spectra of organic compounds are associated with transitions between electronic energy levels. The transitions are generally between a bonding or lone pair orbital and an unfilled non-bonding or antibonding orbital (Williams and Fleming, 1995). Measurements of wavelength of the absorbed light allows for the calculation of the transition energy, which is a measure of the separation of the orbital concerned.

Energy of electronic excitation

Energy is related to wavelength by equation 2.1.

\[ E \text{ (kJ mol}^{-1} \text{)} = \frac{1.19 \times 10^5}{\lambda \text{ (nm)}} \]  

Equation 2.1

When electrons in \( \sigma \)-bonds are excited (120 –200 nm range) they give rise to the highest energy separation. This range is known, as the vacuum ultraviolet, at this range air must be excluded from the instrument, because it makes measurement of the absorption difficult and gives uninformative spectra. Above 200 nm,
excitation of electrons from \( p \), \( d \), and \( \pi \)-orbitals and particularly \( \pi \)-conjugated systems gives rise to measurable and informative spectra. PDLA, progesterone and estrone all contain \( \pi \)-conjugates (C=O and C=C bonds), which make them measurable. It has been found that absorption intensities follow two empirical laws, which are as follows:

- **Lambert's law**
  This states that the fraction of the incident light absorbed is independent of the intensity of the source.

- **Beer's Law**
  This states absorption is proportional to the number of absorbing molecules. A combination of both laws, give rise to the Beer-Lambert equation (Equation 2.2).

\[
\log_{10} \left( \frac{l_0}{l} \right) = \varepsilon \cdot l \cdot c 
\]

Equation 2.2

\( l_0 \) and \( l \) are the intensities of the incident and transmitted light respectively. \( l \) is the path length of the absorbing solutions (cm), and \( c \) is the concentration (mol\(^{-1}\)L). \( \log_{10} (l_0/l) \) is called the absorbance or optical density. \( \varepsilon \) is the molar extinction coefficient (1000 cm\(^2\) mol\(^{-1}\)). The measurement of absorbance allows the concentration of the absorbing sample to be calculated.

An ultraviolet visible spectrometer 554 (Perkin Elmer, Beaconsfield, UK) was used to determine the concentration of progesterone and estrone in PDLA microspheres and the solubility of progesterone and estrone in EPBS mixtures. The instrument contains a deuterium lamp, which emits a monochromatic beam of light. The beam of light is split by a rotating chopper and passes through the sample and reference cells, and is reflected on to a photomultiplier, which converts the light into an electrical current. The electrical signal from the sample and reference cells are separated and were used to calculate the absorbance of
the sample. Before the instrument was used, a calibration was prepared to determine if there is a relationship between the absorbance and drug concentration (Beer-Lambert law) and to determine the wavelength of maximum absorbance ($\lambda_{\text{max}}$) for progesterone and estrone (Figures 2.6 – 2.9).

### 2.3.2.1 Progesterone and estrone calibration curves

Approximately 5 mg progesterone or estrone were dissolved in 20 mL dichloromethane and made up to 250 mL with ethanol to give a final concentration of 0.0200 mg/mL. This stock solution was used to produce a range of calibration concentrations (0.018, 0.016, 0.014, 0.012, 0.100, 0.008, 0.006, 0.004 and 0.002 mg/mL). A second solution of 20 mL dichloromethane made up to 250 mL with ethanol was used as a blank for the reference cell and for diluting the stock solutions. The solutions were analysed using 1cm path length quartz cells. The stock solutions of progesterone and estrone were analysed for their maximum absorbance. The maximum absorbance was determined manually by entering wavelength values throughout the region of the absorbance peak and recording the corresponding absorbance values. The progesterone stock solution exhibited a maximum absorbance at a wavelength of 242 nm, which approximated the literature value of 240 nm, whilst the estrone stock solution exhibited a maximum absorbance at a wavelength of 280 nm, which also approximated the literature value of 282 nm (Merck Index, 1996a; 1999b). Before measuring absorbances, both quartz cells were filled with blank solution of dichloromethane: ethanol (2:23) and the absorbance of the instrument was manually zeroed at 242 nm or 282 nm. The sample cell was then filled with progesterone or estrone solution and the absorbance measured (242 nm or 282 nm). For all UV analysis, each time the concentration of solution in the sample was changed, the sample cell was rinsed with the next concentration solution of progesterone or estrone several times, the outside of the sample cell was cleaned with tissue to rid any spills and finger prints before subsequent measurements.
Figure 2.6  UV calibration curve of progesterone in dichloromethane: ethanol (2:23) solution at 242 nm.

Figure 2.7  UV calibration curve of estrone dichloromethane: ethanol (2:23) solution at 280 nm.
Each of the solutions prepared were analysed by UV three times. Mean values were plotted against drug (progesterone and estrone) concentration (Figures 2.6 and 2.7). The data gave an acceptable linear regression through the origin with $R^2$ (correlation coefficient) of 0.9977 and 0.9983 for progesterone and estrone respectively. The calibration curves for progesterone and estrone showed the measured absorbance was related to the concentration of the progesterone and estrone (mg/mL).

\[
\text{Absorbance (Y)} = 54.45 \times \text{Concentration (X)} \quad \text{Equation 2.3}
\]
\[
\text{Absorbance (Y)} = 9.34 \times \text{Concentration (X)} \quad \text{Equation 2.4}
\]

Equations 2.3 and 2.4 were used to convert the obtained absorbance values into progesterone and estrone concentration values. The actual progesterone and estrone content in the PDLA micropsheres was determined by weighing 10 mg of drug loaded microspheres into a plastic weighing boat using Mettler M3 balance (4 decimal place). The microspheres were then transferred to a 25 mL volumetric flask and 2 mL of dichloromethane was added to the volumetric flask containing the microspheres. Once the microspheres had dissolved in dichloromethane, the solution was made up to 25 mL with ethanol, to precipitate the polymer (PDLA). The resulting suspension was centrifuged at 10,000 rpm for 10 min using a J2 – HA Centrifuge (Beckmann-RIILC Ltd, High wycombe, UK). From the resulting supernatant 1 mL was taken and diluted in 24mL of dichloromethane: ethanol solution (2:23). The absorbance of the supernatant was then measured at 242 or 282 nm and the solution of dichloromethane / ethanol (2:23) was also used as a blank for the UV analysis. The concentration of progesterone and estrone present in the microspheres was calculated by extrapolating the obtained values of absorbance from the calibration curve for progesterone and estrone.

The calibration for progesterone and estrone was repeated again using the same method outlined above, but the media was changed from dichloromethane: ethanol (2:23) to EPBS.
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1.2

Figure 2.8 UV calibration curve of progesterone in EPBS at 242 nm.

Figure 2.9 UV calibration curve of estrone solution in EPBS at 242 nm.
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The ethanol was replaced with EPBS (Figures 2.8 and 2.9) as this media would be used to study the in vitro release of progesterone and estrone from PDLA microspheres (Section 4.24). The data gave an acceptable linear regression through the origin with \( R^2 \) (correlation coefficient) of 0.9996 and 0.9931 for progesterone and estrone respectively (Figures 2.9 and 2.10). The calibration curves for progesterone and estrone showed the measured absorbance is related to the concentration of the progesterone and estrone (mg/mL).

Absorbance \( (Y) = 51.93 \times \) Concentration \( (X) \) \hspace{1cm} \text{Equation 2.5}
Absorbance \( (Y) = 9.20 \times \) Concentration \( (X) \) \hspace{1cm} \text{Equation 2.6}

The actual amount of progesterone and estrone released from PDLA microspheres during the in-vitro release study was determined by weighing 10 mg of drug loaded PDLA microspheres into a plastic weighing boat. 10 mg of PDLA microspheres containing progesterone and estrone contents of 10, 20, 30, 40 & 50% w/w was dispersed in 50 ml centrifuge tubes, each containing 30 ml EPBS. The centrifuge tubes were screw capped and agitated (40 rpm) in a water bath (Grant Instruments, Barrington, Cambridge, UK) set at 37°C. 2mL samples of clear supernatant (centrifuged at 10,000 rpm for ten minutes before sampling) was removed at scheduled time points and analysed spectrophotometrically (UV). From the resulting supernatant 1 mL was taken and diluted in 24 mL of dichloromethane: EPBS solution (2:23). The absorbance of the supernatant was then measured at 242 or 282 nm, with dichloromethane: EPBS solution (2:23) used as a blank. For comparison, dissolution of free progesterone and estrone in EPBS was carried out. The amount of progesterone and estrone released from the microspheres was calculated by extrapolating the obtained values of absorbance from the calibration curve for progesterone and estrone (Figures 2.8 and 2.9).
2.3.3 Particle size analysis

The Malvern particle sizer 2600c (Malvern Instruments Ltd, Malvern, UK) was used to analyse progesterone and estrone loaded PDLA microspheres. The operation of this instrument is based on the principle of Fraunhofer diffraction and uses radiation from low energy helium-neon laser (Malvern Instruments, 1987). When light is scattered by a particle, the pattern of light shows variations with angle (Figure 2.10).

![Figure 2.10 Schematic diagram showing the angle of light scattering of small and large microspheres.](image)

Small particles scattered at large angles, whilst large particles scatter at small angles. These angles are then converted to a particle size by a detector (Figure 2.11).

![Figure 2.11 Schematic of the main components of the Malvern particle sizer.](image)
The light from a low power helium-neon laser is used to form a collective and monochromatic beam of light, which is then focused on sample particles, suspended in liquid and agitated with a magnetic stirrer. The scattering pattern from the particles in the sample cell is, then focused by a Fourier transform lens on to a detector. The instrument software determines the particle size based on a system of equivalent spheres. This is a measurement based on volume. For analysis of PDLA microspheres, the instruments receiver lens had a focal length of 300 mm, which provided measurements in the range of 0.5 to 900 μm. Before measuring the sample, the laser beam was aligned on the detector to give a good intensity of un-deflected light. The sample cell was filled with 0.1% w/v Tween 80 in water and a background measurement was taken. For sample analysis 20 mg of PDLA microsphere were suspended in a 50 mL beaker containing 20 mL of aqueous solution of 0.1% w/v Tween 80, and sonicated (Transonic T890/H, Camlab, UK) for 45 min, before being added to the 15 mL sample cell, which was agitated by a magnetic stirrer. The concentration of the PDLA micropsheres was found to give obscuration values between 10 and 12%. The volume median diameter (VMD) D (v, 0.5) and span values were recorded and used to characterise the samples. The D (v, 0.5) value is the diameter of the sphere for which 50% of the total volume measured is below the size and 50% above. The span is a measure of width of the volume distribution and given by equation 2.7.

\[
\text{Span} = \frac{D (V, 0.9) - D (V, 0.1)}{D (v, 0.5)} \quad \text{Equation 2.7}
\]

D (v, 0.9) and D (v, 0.1) represent the diameters below which 90% and 10% of the volume is measured. Three measurements were taken for each suspension.
2.3.4 Scanning electron microscopy

Magnifying glasses were first used in ancient Rome, though the idea of seeing more by imaging with two glasses in succession appears to date back to 1590. From that time till the 1930s a microscope was effectively describable as an instrument in which light is focused by two glass mirrors to form enlarged images of a sample. The basic understanding of light microscope then shifted to the development of the electron microscope. At this point it became clear that microscopes were instruments in which radiation was focused by lenses. Before the 19th Century, development of the light microscope was slow because specimens were unavailable and the theory was still undeveloped. In 1801 the understanding of the wavelike character of light appeared and the interaction of these light beams could be explained in detail. Thereafter, designers rapidly improved the quality of the lenses and microscopes, and developed methods for sample preparation. In 1924 Louis de Brogile suggested if light was considered to have wavelike properties and could show particle like behaviour, then electrons, which are particles may also be treated as waves (Slayter and Slayter, 1992).

Microscopes are then defined as transmission instruments (light or electrons) in which radiation is focused by lenses to illuminate and form images of the specimen. This provides maps of specimen detail at resolutions superior to those obtainable by direct observation. Scanning electron microscopy (SEM) is a technique in which an image is formed using beams of electrons instead of light. (Watt, 1997).

The resolving power of high quality light microscopes are limited by the wavelength of imaging light to about 200 nm, whilst wavelengths associated with electron beams suggest resolving powers in the order of $10^{-3}$ nm should be possible, but other factors limit the resolving power of electron microscopes to levels currently approaching 0.1 nm.
Figure 2.12 Schematic diagram showing the main components of a scanning electron microscope.

In scanning electron microscopes (Figure 2.12), radiation from the source is focused to form a very fine probe. As the probe is moved frequently across the sample, an input transducer collects radiations leaving individual sample positions. The input transducer signal is transferred and amplified by an amplifier, leading to large intensities appearing on a monitor screen at positions related to those of the original sample (Slayter and Slayter, 1992).

In this study, the morphology of the PDLA microspheres was investigated using a Philips XL20 SEM (Philips Electron Optics, Eindhoven, Netherlands). A small amount of progesterone and estrone loaded PDLA microspheres was fixed on a metal stub using carbon impregnated double-sided sticky tape. Excess progesterone or estrone loaded PDLA microspheres was removed using compressed air. The sample was then sputter coated with gold for four minutes in an argon atmosphere using Emitech K550 sputter coater (Emitech) set at 30 mA. The gold coating promotes electron reflection at the surface instead of multiple scattering, which can take place, hence improving the image contrast.
2.3.5 X-ray Powder diffraction

X-ray diffraction is a versatile, non-destructive analytical technique for the identification and quantitative determination of various crystalline compounds known as phases, which are present in solid materials and powders. In this thesis X-ray diffraction will be referred to as X-ray powder diffraction (XPRD) as the raw materials and microspheres were in powder form (Philips analytical .com, 1999).

XPRD describes the interaction of X-rays with crystalline phases of a sample. The analysis of the sample is phase sensitive. The XPRD works by analysing the shape of the sample. A crystalline sample will have a regular three-dimensional (cubic, rhombic etc.) distribution of atoms in space. The atoms are arranged so that they form a series of parallel planes (Figure 2.13) separated from one another by a distance, d (Figure 2.14), which varies according to the nature of the material.

![Figure 2.13 Parallel planes of crystal lattices.](image)

![Figure 2.14 Diffraction patterns of crystals obtained by varying the theta angle.](image)
Braggs Law is given by the following equation:

\[ n\lambda = 2d \sin\theta \]  \hspace{1cm} \text{Equation 2.8}

When a monochrome X-ray beam with wavelength, \( \lambda \) is projected on to a crystalline material at an angle \( \theta \), diffraction occurs only when the distance travelled by the rays reflected from successive planes differs by a complete number \( n \) of wavelengths. By varying \( \theta \), the Bragg's law (Equation 2.8) conditions are satisfied by different \( d \) spacing in polycrystalline materials. Plotting the angular positions and intensities of the resultant diffraction peaks produces a pattern, which is characteristic of the sample. Amorphous materials, which are randomly ordered do not produce any diffraction at characteristic angles and are easily identified as diffuse halos.

Figure 2.15 Picture of the Brukker D8 Advance Diffractometer.
XPRD studies of the raw materials and model compound loaded PDLA microspheres were performed at Merck, Sharp and Dohme, Research Laboratory, Hoddesdon, UK using a Bruker D8 Advance Diffractometer (Figure 2.15). The X-ray radiation was CuK$_1$ ($\lambda = 1.5405$ Å) and CuK$_2$ ($\lambda = 1.54439$ Å) with a ratio of 0.5. The divergence and anti-scatter slits were set at 12 mm, the detector (receiving) slits was set at 0.2 mm. The data was collected over a range of 2θ, 7 to 35° using a step size of 0.02° and counting time of 1 s per step. All measurements were performed at 25°C.

2.3.6 Size exclusion chromatography

Size exclusion chromatography is a technique for separating molecules in solution based on their size and shape. The technique is often referred to as gel permeation chromatography (GPC) if organic solvents are used, or gel filtration if aqueous solvents are used. In these studies an organic solvent, tetrahydrofuran (THF) was used so the technique will be referred to as GPC. The stationary phase used for GPC, was porous particles of polystyrene divinyl benzene (Polymer Laboratory, Birmingham, UK) with a closely controlled pore size of 5 μm.

![Figure 2.16 Separation by size exclusion (Lindsay, 1992).](image)
In size exclusion chromatography there is no interaction between the solute and the surface of the stationary phase. Depending on the size and shape of the solute molecules, these may be able to diffuse through the pores of the stationary phase particles, whilst larger molecules will be excluded from the narrower parts of the porous stationary phase, but will be able to move more freely through the wider passages (Figure 2.16). If there are any extremely large solute molecules present they will be excluded from the pores, and will be rapidly eluted from the column, whilst the smaller molecules will penetrate the pores of the stationary phase much more easily and result in a longer retention time in the column.

In this study, the initial molecular weight of the raw material (PDLA), drug free PDLA microspheres and corresponding changes of the molecular weight of drug loaded PDLA microspheres in PBS and EPBS was monitored against time using GPC. The raw PDLA and microsphere samples were dissolved in THF to a final concentration of 5 mg/mL and were filtered through a 0.45 μm nylon filter (Fisher Scientific, Loughborough, UK) before being injected and eluted from two serial 5 μm PL-gel mixed-D (pore size) columns (Polymer Laboratories, Shropshire, UK) at a flow rate of 1 mL min⁻¹ (HPLC intelligent pump PU 980, Jasco, Essex, UK) and a temperature of 37°C (column heating jacket 7981 Jones Chromatography, Mid Glamorgan, UK). Calibration was carried out with monodisperse polystyrene standards with molecular weights ranging from 580 – 377,400 Kda (Polymer Laboratories, Shropshire, UK). The elution profiles were detected with a refractive index detector set to a sensitivity of 4 (Gilson Instruments, Luton, UK). The data was analysed with a PL-DCU and PL-calibre GPC software (Polymer Laboratories, Shropshire, UK).

2.3.7 Fourier transform infrared spectroscopy

The functional groups of the raw materials, polylactide, progesterone, estrone and progesterone and estrone loaded polylactide microspheres were analysed using an Avatar 360 FT-IR spectrometer (Nicolet Analytical Instruments,
Madison, USA) (Figure 2.17). Fourier transform infrared (FTIR) spectroscopy instruments measure light absorbed or light emitted from a sample. The measurement provides valuable chemical composition information. The key components of the Fourier transform system are the source, the interferometer and the detector.

Figure 2.17 A simple spectrometer layout (Nicolet Analytical Instruments User Manual, 1998).

FTIR was developed to overcome the problems associated with dispersive instruments. The main difficulty was the slow scanning process. A method for measuring all of the infrared frequencies simultaneously, rather than individually was required. To conquer this problem a simple optical device called an interferometer was developed (Figure 2.17). The interferometer produces a unique signal, which has all infrared frequencies encoded into it. The signal can
be measured very quickly, usually within a few seconds rather than several minutes.

Most interferometers have beam splitters, which split the incoming infrared beam and divide it into two optical beams. One beam reflects off a flat mirror, which is fixed, the other beam reflects off a flat mirror, which is on a mechanism that allows the mirror to move very short distances (few millimetres) away from the beam splitter. The two beams reflect off their individual mirrors and are recombined when they meet back at the beam splitter. Because the path that one beam travels is a fixed length and the other is constantly changing as the mirror moves, the signal which exits the interferometer is the result of the two beams interfering with each other. The resulting signal is called an interferogram (Figure 2.18), which has a unique property of every data point, which makes up the signal that comes from the source.

Figure 2.18 Schematic diagram of FTIR spectroscopy (Nicolet Analytical Instruments user manual, 1998).

The sample analysis process is as follows:
1. The source: Infrared energy is emitted from a glowing blackbody source. The beam passes through an opening, which controls the amount of energy presented to the sample.
2. The interferometer: The beam enters the interferometer where the spectral encoding takes place. The resulting interferogram leaves the interferometer.

3. The sample: The beam enters the sample compartment where it is transmitted through, or reflected off, the surface of the sample.

4. The detector: The beam finally passes to the detector for a final measurement (measure interferogram signal).

5. The computer: The measured signal is digitised and sent to the computer where the Fourier transformation takes place.

A relative scale for absorption intensity is needed, so a background spectrum must be measured. This is a measurement with no sample in the beam. This is compared to the measurement of the sample in the beam to determine the percentage transmittance. This results in a spectrum in which all the instrumental characteristics have been removed. Thus, all the spectrum features are due to the sample.

Before analysis was started the sample surface was cleaned with ethanol and a background spectrum was collected. Once background collection was completed PDLA microspheres were placed on the sample area and scanned. Three measurements were taken for each sample of raw material, polylactide acid, progesterone, estrone and progesterone and estrone loaded polylactide microspheres.

2.3.8 Modulated temperature differential scanning calorimetry

The original ‘Modulated DSC’ (MDSC) method was invented by Dr. M. Reading, then of ICI Paints, and was commercialised and patented by TA Instruments Ltd. (Reading et al., 1993). The technique was launched in the UK at the 10th International Confederation for Thermal Analysis Congress at Hatfield in August 1992 (Reading et al., 1992b). Subsequently, several other thermal analysis instrument manufacturers have released their own versions of the technique with
each manufacturer using a new nomenclature, such as Alternating DSC (Mettler-Toledo), Dynamic DSC (Perkin-Elmer) and Oscillating DSC (Seiko Instruments). To avoid confusion, it has been suggested that the generic name 'modulated temperature DSC' (MTDSC) be used to encompass all these instruments (Reading, 1997).

Modulated temperature differential scanning calorimetry (MTDSC) can be compared to its well-established forerunner differential scanning calorimetry (DSC). DSC is an analytical technique in which the difference in heat flow (power) between a sample and an inert reference is measured as a function of time and temperature as both the sample and reference are subject to a controlled environment of time, temperature, atmosphere and pressure.

There are two types of DSC instruments:

1. Power compensated DSC

In power compensated DSC, the sample and reference pans are heated by two separate heaters. The temperature difference between the sample and reference is kept close to zero whilst the power needed to maintain equal temperature is measured.

2. Heat flux DSC

In heat flux DSC the same heater heats both the sample and reference pans (Figure 2.19). The difference in temperature, \( \Delta T \) (between the sample and reference pans) is divided by thermal resistance of the cell, to give the heat change delta \( Q \). The rate of change of this heat change is equal to \( dQ/dt \), which is equal to power.
MTDSC is based on the heat flux DSC. The sample and reference pan sit on a thermoelectric disc (constantan) (Figure 2.19). The thermoelectric disc is the main means of heat transfer to and from the sample and reference. As heat is passed through the thermoelectric disks, the differential heat flow to the sample and reference is measured. The alumel and chromel wires are attached to the chromel disk to form thermocouples, which measure the sample temperature. An inert gas (nitrogen or helium) is passed through a hole in the heating block, and is preheated by circulating through the block, before entering the sample chamber. This removes moisture and oxygen leading to a uniform, stable thermal environment which gives rise to excellent baseline flatness and good sensitivity. In DSC, the cooling and heating rates obtained from the sample and reference are linear. The cooling and heating rates can be as fast as 200°C min⁻¹ to rates as slow as 0°C min⁻¹.
MTDSC is a technique, which also measures heat flow between a sample and an inert reference as a function of time and temperature. A different heating profile is applied to the sample and reference. A sinusoidal modulation is overlaid on a linear heating ramp to yield a heating profile in which an average sample temperature continuously increases with time, but not in a linear fashion. This results in two experiments run simultaneously on the material, one experiment with a linear heating rate and one at a sinusoidal heating rate (Figure 2.20). The rates for these two simultaneous experiments is dependent on three operating variables:

1. Underlying heating rate (range $0 - 10\degree C \text{ min}^{-1}$)
2. Period of modulation (range $10 - 100$ s)
3. Temperature amplitude of modulation (range $\pm 0.01 - 10\degree C$)

As outlined above, MTDSC differs from conventional DSC in that a sinusoidally varying heating profile is used instead of the usual linear programme. The application of this more complex heating program yields heat flow data that can be deconvoluted to produce extra information compared to conventional DSC (Reading et al., 1992a; Reading, 1993; Sauerbrunn et al., 1993b; Reading et al., 1994). In particular, the heat flow from processes, which are reversible over the time scale of the modulation can be separated from the heat flow due to processes which are not reversible on this time scale.

The ability to separate these two types of heat flow (Equations 2.9 and 2.10) has been found to produce a number of benefits; heat capacity can be measured with improved accuracy over conventional DSC measurements, overlapping transitions can be separated which aids in the identification of thermal processes and small glass transitions can be more easily detected.
DSC

Equation 2.9

\[
\frac{dq}{dt} = \text{Heat Capacity} + \text{Kinetic}
\]

MTDSC

Equation 2.10

\[
\frac{dq}{dt} = \text{Reversing heat flow} + \text{Non-reversing heat flow}
\]

Heat flow definitions

- **Total heat flow \((dq/dt)\)**: Sum of all thermal events in a sample.
- **Heat capacity \((C_p)\)**: The amount of heat required to raise the temperature of a sample by 1°C.
- **Reversing heat flow**: This is the heat capacity component of the total heat flow.
- **Non-reversing heat flow**: kinetic component of the total heat flow.

The main components of the reversing and non-reversing heat flow (Equations 2.9 and 2.10) are shown in Table 2.5.

Table 2.5 Main components of reversing and non-reversing heat flows.

<table>
<thead>
<tr>
<th>Reversing Heat flow</th>
<th>Non-reversing heat flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass transition</td>
<td>Evaporation</td>
</tr>
<tr>
<td>Melting</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>Relaxation at (T_g)</td>
</tr>
<tr>
<td></td>
<td>Cold crystallization</td>
</tr>
<tr>
<td></td>
<td>Melting</td>
</tr>
</tbody>
</table>
As the MTDSC technique has become more widely used, a diverse range of material types have been investigated including metals (Aubuchon et al., 1995; O'Reilly and Cantor, 1996), liquid crystalline samples (Roussel and Buisine, 1996), petroleum fuels (Zanier and Jäckle, 1996) and biopolymers (bacterial thermoplastics) (Cesàro et al., 1993). Studies in the food and pharmaceutical sciences are also increasing in number as the advantages of the technique are determined for an increasing variety of samples including polyethylene glycols (Craig, 1995), frozen sucrose solutions (Izzard et al., 1996; Thomas and Aubuchon, 1997), foodstuffs such as gelatin and pasta (Bell and Touma, 1996), proteins (Aldén and Magnusson, 1997), HPMC (McPhillips et al., 1998) and an amorphous drug, saquinavir (Royall et al., 1998). A review of MTDSC for pharmaceutical thermal analysis has been given recently by Coleman and Craig (1996).

2.3.8.1 Calibration of a MTDSC instrument

Before the MTDSC is used the equipment needs to be calibrated. There are three calibration steps necessary to produce accurate and precise MTDSC results, which are as follows:

1. Baseline calibration

This is used to eliminate any baseline slope, i.e. thermal imbalance. In an ideal empty cell the heat capacity should be zero. This is not always the case, since small irregularities in the cell produce a thermal imbalance on either the sample side or reference side. This causes small heat signals (positive or negative) to be measured when the empty cell is heated. To eliminate these irregularities, the size of the signal needs to be determined, because the thermal imbalance has been known to affect the heat capacity constant, K (Cp). In MTDSC the heat capacity is always positive, because heat capacity is calculated from amplitudes of modulated signals. The phase lag between the modulated heat flow and the
derived modulated temperature is used to determine if there is an imbalance towards the sample or reference side (TA Instruments, 1997). If the phase lag is positive, then the sample is bias and the measured heat capacity should be subtracted from all successive runs. And if the phase is negative then the bias is on the reference side and the heat capacity should be added to all successive runs. For the MTDSC 2920 instrument the phase was positive, hence the sample was bias.

2. Temperature calibration

The MTDSC is calibrated for temperature using standards such as indium, cyclohexanene, bismuth, tin and n-octadecane. All five were chosen as suitable temperature calibrants. The temperature of samples being tested ranged from 40 – 250°C so calibrants below and above the range were chosen (PDLA has a Tg of 45°C, progesterone melts at 121°C and estrone melts at 250°C).

3. Heat capacity calibration

The heat capacity calibration was performed with aluminium oxide. The literature heat capacity of aluminium oxide is divided by the measured heat of capacity of aluminium oxide to produce a heat capacity constant K (Cp). The heat capacity constant is entered into MTDSC software to adjust the heat flow.

The MTDSC analyses was made using TA Instruments MTDSC 2920 with a refrigerated cooling system (TA Instruments, Leatherhead, UK). The raw PDLA, progesterone, estrone and microsphere samples were analysed using a heating rate of 1°C min⁻¹, a modulation period of 40 s and modulation amplitude of ±0.5 °C. The MTDSC was equilibrated to −10°C and kept isothermal for 10 min before heating. All samples were placed in aluminium hermetic pans with lids (Perkin Elmer, Beaconsfield, UK) and crimped before they were placed in the MTDSC. Sample and reference pans were matched by mass to approximately 0.05 mg for
each experiment. Small sample masses between 1.5 to 5.5 mg were used to prevent thermal lag developing in the sample.
Chapter Three

Preparation of Progesterone and Estrone loaded PDLA Microspheres Formed by Solvent Evaporation
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

3.1 Introduction

The value of purified polyethylene and silicone rubber as implantable biocompatible polymers for sustained drug delivery is limited due to their non-biodegradability, because of their thrombus formation and encapsulation by collagenous fibrous tissues, which takes place around their surface when implanted in the body (Ikada, 1994). The disadvantage has been overcome by the development of polymers and copolymers of lactic and glycolic acids, which are biodegradable and yield the normal metabolites (carbon dioxide and water) of the metabolic pathway (Rak et al, 1985). Such polymers have extensive medical applications (Ikada and Tsuji, 1999). Conte et al (1998) have shown PLGA microspheres degrade faster than PDLA according to the higher hydrophobicity of the copolymer; the two monomers are released at a different rate in the case of PLGA (faster GA (glycolic acid), slower for PDLA (lactic acid)). PDLA was chosen for this investigation as it is amorphous and free from harmful tissue reaction following implantation, and degrades slowly in vitro and in vivo rendering it suitable for drug delivery (Schindler et al, 1977; Kulkami et al, 1971).

A promising approach to improve drug delivery and solubility, prolong drug release, and reduce dose, dosing intervals and drug toxicity or to achieve targetability is to bind a low molecular weight drug to a polymeric carriers (Giammona et al, 1998; Lovrek et al, 2000). This project began, by encapsulating hydrophobic compounds of low molecular weight (progesterone and estrone) and low aqueous solubility, in high molecular weight PDLA microspheres using a modification of the solvent evaporation technique, described previously by Benita et al (1984). The PDLA microspheres were loaded with hydrophobic compounds at 0, 10, 20, 30, 40 and 50% w/w concentrations. The PDLA microspheres were homogenised at 8000 rpm for 2 min before mixing at 500 rpm was continued (see Section 2.2.1). Hill et al, (1998) and Parikh et al, (1993) have respectively prepared progesterone (0 - 50%w/w) and estrone (5 - 25%w/w) PLA microspheres with sizes range of 100 - 130µm and 125 - 160µm. These
microspheres were prepared using the solvent evaporation technique described by Benita et al, 1984 but without homogenisation. The aim of this part of the study was to note if the inclusion of the homogenisation step would have any effect on the particle size of PDLA microspheres produced by the modified solvent evaporation technique.

The second aim of this study was to determine the concentration of progesterone or estrone at which the entrapped hydrophobic compounds may begin to appear on the outer surface of the microspheres, as determined by scanning electron microscopy (SEM). It has been suggested by Benita et al (1984) that, progesterone payloads between 35 - 68%w/w yielded PDLA microspheres with drug crystals on the surface of the microspheres, whilst Hill et al (1998) produced progesterone PLA micropsheres of 30 & 50%w/w with rough surfaces. Hill et al (1998) stated that the rough surface at 50%w/w progesterone was due the presence of progesterone crystals on the surface, due to the limited solubility of drug in the polymer. This investigation determined the precise concentration at which this phenomenon occurred for each model compound in question. The solvent evaporation technique required the use of a volatile organic solvent (dichloromethane) for the preparation of PDLA microspheres. Dichloromethane is known to be carcinogenic and have anaesthetic properties, so it is important for level of dichloromethane present within the PDLA microspheres to be kept to a minimum (Merck Index, 2001). Hence thermogravimetric analysis (TGA) was employed, to investigate removal of solvent molecules from the PDLA microspheres, since the removal of solvent would be accompanied by a loss of sample weight. The third aim of this study was to find the solubility of progesterone and estrone in a suitable release medium, which would be used later to study the release behaviour (Chapter 4) and degradation (Chapter 5 and 6) of PDLA micropsheres upon incubation in the release medium.
3.2 Results and discussion

3.2.1 Quantification of drug load and percentage yield of progesterone and estrone loaded PDLA microspheres.

Progesterone and estrone are poorly soluble in water, with solubility of 0.1 mg/mL and 0.03 mg/mL respectively (Dollery, 1999; Merck Index, 1996b). Equations 3.1 – 3.4 show the formulae for how theoretical and experimental drug load, % yield, incorporation efficiency were calculated.

Theoretical drug load (% w/w) =

\[
\text{Mass of model compound (g) } \times 100 \\
\text{Total mass (combined mass of the model compound and polymer) (g)}
\]

Equation 3.1

Mean yield (%) of microspheres =

\[
\text{Mass of microspheres achieved before sieving } \times 100 \\
\text{Total mass (combined masses of the model compound and the polymer) (g)}
\]

Equation 3.2

Mean experimental drug load =

\[
\text{Amount of drug determined by UV } \times 100 \\
\text{Amount of microspheres used}
\]

Equation 3.3

Mean incorporation efficiency =

\[
\text{Mean experimental drug load } \times 100 \\
\text{Theoretical drug load}
\]

Equation 3.4
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

Results of the theoretical drug load, experimental drug load, incorporation efficiency, percentage yield, particle size and mass loss of progesterone and estrone loaded PDLA microspheres are shown in Tables 3.1 and 3.2. The mean percentage yields of progesterone loaded PDLA microspheres, were found to be greater than 81% in all cases (Table 3.1), whilst the mean percentage yields of estrone loaded PDLA microspheres were found to be greater than 85% in all cases (Table 3.2). The experimental drug load was determined by using UV spectroscopy (Section 2.3.2).

The mean experimental progesterone and estrone loads achieved were found to be close to the theoretical drug loads, indicating the manufacturing process may have prevented the formation of free drug crystals. Benoit et al, (1985) reported free progesterone crystals are formed in the aqueous phase if the water soluble emulsifier (PVA) is not removed from the aqueous phase before dichloromethane evaporation was complete, which would lead to a low drug loads and low entrapment efficiencies. Since the mean experimental progesterone and estrone loads are close to theoretical load and incorporation efficiencies are high, it would seem that little or no crystals were formed during the microsphere manufacture. The surface of the PDLA microspheres was investigated experimentally by SEM to observe if this hypothesis of no surface drug crystals was correct (Section 3.2.3). In the case of progesterone loaded PDLA microspheres (10% and 20% w/w) (Table 3.1) and estrone loaded PDLA microspheres (10%, 20% and 30% w/w) (Table 3.2), the mean experimental drug loads achieved were found to be slightly higher than the theoretical drug loads. The deviations of drug loading efficiency from theoretical (100%) may be attributed to two causes: loss of polymer or drug. Positive deviations may be due to the separation of the pure polymer during the modified solvent evaporation process and the formation of empty PLA microspheres, which may be removed during the separation process. Negative deviations probably result from drug loss in the form of free crystal (progesterone or estrone) loss during the decantation and rinsing processes.

111
Table 3.1  Progesterone loading, incorporation efficiency and % yield of progesterone loaded PDLA microspheres (Mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Theoretical loading (% w/w)</th>
<th>Mean experimental loading (% w/w)</th>
<th>Mean incorporation efficiency (%)</th>
<th>Mean yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>94.1 ± 2.9</td>
</tr>
<tr>
<td>10</td>
<td>11.1 ± 0.3</td>
<td>110.8 ± 2.9</td>
<td>81.1 ± 2.1</td>
</tr>
<tr>
<td>20</td>
<td>21.3 ± 1.5</td>
<td>106.6 ± 7.7</td>
<td>95.3 ± 2.5</td>
</tr>
<tr>
<td>30</td>
<td>29.2 ± 1.0</td>
<td>97.3 ± 3.4</td>
<td>93.4 ± 1.3</td>
</tr>
<tr>
<td>40</td>
<td>39.9 ± 1.5</td>
<td>99.7 ± 3.4</td>
<td>97.2 ± 2.3</td>
</tr>
<tr>
<td>50</td>
<td>48.7 ± 0.5</td>
<td>97.3 ± 1.0</td>
<td>94.0 ± 2.1</td>
</tr>
</tbody>
</table>

The positive deviations were observed at 10 and 20% w/w progesterone loading (Table 3.1). As the amount of polymer in the formulation increases (Table 2.3), the experimental drug loading tends to increase slightly (Table 3.1 and 3.2), indicating, that more empty PDLA microspheres were formed or more polymer was lost at 10 and 20% w/w progesterone loading, while at 30, 40 and 50% w/w progesterone loading negative deviations were observed i.e. more drug was lost than polymer.
Table 3.2  Estrone loading, incorporation efficiency and percentage yield of estrone loaded PDLA microspheres (Mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Theoretical loading (% w/w)</th>
<th>Mean theoretical loading (% w/w)</th>
<th>Mean incorporation efficiency (%)</th>
<th>Mean yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12.1 ±0.3</td>
<td>121.2 ±9.4</td>
<td>84.6 ±2.6</td>
</tr>
<tr>
<td>20</td>
<td>22.1 ±1.3</td>
<td>110.5 ±5.5</td>
<td>88.2 ±1.6</td>
</tr>
<tr>
<td>30</td>
<td>33.0 ±3.0</td>
<td>110.0 ±9.9</td>
<td>90.2 ±2.8</td>
</tr>
<tr>
<td>40</td>
<td>38.8 ±1.2</td>
<td>97.2 ±2.9</td>
<td>93.9 ±4.1</td>
</tr>
<tr>
<td>50</td>
<td>47.2 ±1.1</td>
<td>94.5 ±2.8</td>
<td>94.5 ±2.8</td>
</tr>
</tbody>
</table>

Estrone loaded PDLA microspheres displayed a similar trend to progesterone loaded PDLA microspheres, with more polymer than drug being lost at 10, 20 and 30% w/w estrone loading, while at 40 and 50% w/w estrone loading more drug was lost than polymer (Table 3.2).

The deviations were confirmed by the mean incorporation efficiency values (Tables 3.1 and 3.2). All progesterone and estrone loaded microspheres had an incorporation efficiency >94.5%. At 10 and 20% w/w progesterone the incorporation efficiency was >100% at progesterone loadings greater than 20% w/w, the efficiency was <100%. Whilst for estrone loaded PDLA microspheres, at 10, 20 and 30% w/w the efficiency was >100% and loadings greater than 30% w/w, the efficiency was <100%.
The theoretical progesterone and estrone loadings were compared graphically to the mean experimental progesterone and estrone drug loadings to see if there was any correlation between the two drug loadings. With an increase in the drug content in the organic phase (dichloromethane), the drug content in the fully prepared microspheres increased. The superimposed lines of best fit shown in Figure 3.1 indicated this. The $R^2$ value for Progesterone and estrone was $>0.983$ and $>0.997$ respectively.

Figure 3.1 Comparison of the theoretical and mean experimental drug load (progesterone and estrone) in PDLA microspheres (Mean ± SD; $n = 9$).
3.2.2 Particle size of progesterone and estrone loaded PDLA microspheres

Particle size analysis showed most drug free PDLA microspheres, progesterone and estrone PDLA loaded microspheres had a mean VMD in the range of 7 to 22 µm (Table 3.3). The span value obtained for both progesterone and estrone (Table 3.3) was large, indicating the size distribution was wide. The results for particle size analysis therefore suggest the modified solvent evaporation technique produced small sized microspheres, which may be suitable for IV and subcutaneous delivery (Figure 1.11). Hill et al (1998) and Parikh et al (1993) produced progesterone and estrone loaded PDLA microspheres without homogenisation, having median particle size range 100 to 130µm and 125 to 160µm respectively. In this investigation the solvent evaporation technique with homogenisation yielded smaller PDLA microspheres. Although Hill et al (1998) produced microspheres having a larger VMD the span values were quite narrow (0.53±0.08) compared to the values shown in Table 3.3. Parikh et al (1993) did not perform particle size analysis by laser diffraction, but with the use of a microscope fitted with an ocular micrometer, therefore no span values were quoted. Overall the particle size data suggested the smaller the VMD of the microspheres produced by the modified solvent evaporation technique the greater the size distribution.

The VMD of progesterone PDLA microspheres was significantly increased with an increased drug loading, whilst the VMD of estrone PDLA microspheres was significantly decreased with an increased drug loading (Table 3.3). From 0 to 20%w/w drug load progesterone and estrone the VMD of the microspheres were not significantly different (p<0.05). Above 20%w/w drug load, both progesterone and estrone microspheres have significantly different VMD (p<0.05). This may be interpreted as estrone not being as miscible as progesterone in PDLA and hence smaller VMD was obtained for estrone PDLA microspheres. This hypothesis was further investigated with MTDSC (Section 4.2.2).
Table 3.3  Particle size distribution of progesterone and estrone PDLA microspheres (Mean ± SD; $n = 9$).

<table>
<thead>
<tr>
<th>Theoretical loading (% w/w)</th>
<th>Progesterone PDLA Volume median diameter ($\mu$m)</th>
<th>Span</th>
<th>Estrone PDLA Volume median diameter ($\mu$m)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.4 ± 1.5</td>
<td>4.3</td>
<td>10.4 ± 1.5</td>
<td>4.3</td>
</tr>
<tr>
<td>10</td>
<td>9.9 ± 1.1</td>
<td>7.1</td>
<td>9.7 ± 1.7</td>
<td>3.0</td>
</tr>
<tr>
<td>20</td>
<td>13.8 ± 2.2</td>
<td>5.7</td>
<td>12.8 ± 2.9</td>
<td>4.1</td>
</tr>
<tr>
<td>30</td>
<td>18.0 ± 5.1</td>
<td>4.4</td>
<td>7.0 ± 1.4</td>
<td>3.6</td>
</tr>
<tr>
<td>40</td>
<td>21.2 ± 4.7</td>
<td>5.1</td>
<td>6.8 ± 0.3</td>
<td>5.5</td>
</tr>
<tr>
<td>50</td>
<td>18.0 ± 6.2</td>
<td>5.3</td>
<td>6.8 ± 1.0</td>
<td>6.2</td>
</tr>
</tbody>
</table>
3.2.3 Scanning electron microscopy of progesterone and estrone loaded PDLA microspheres

Scanning electron micrographs (SEM) of drug free and drug-loaded microspheres revealed that these microspheres were spherical in morphology (Figures 3.2 to 3.12.). The spherical structures are comparable to the spherical PDLA microspheres produced by Benoit et al (1984); Bodmeier and McGinity, (1987); Parikh et al, (1993); Pradhan et al, (1994) and Hill et al, (1998). The surfaces of the microspheres with low drug loadings, i.e. 10% w/w and less were smooth with no pores (Figures 3.2, 3.3 and 3.8). As the progesterone and estrone loading was increased to greater than 10% w/w, the microspheres began to lose their smooth surface. This was observed particularly at drug loadings of 30% w/w and greater for progesterone PDLA microspheres (Figures 3.5, 3.6 and 3.7) and estrone PDLA microspheres (Figures 3.10, 3.11 and 3.12). Above the 30% w/w drug load, progesterone and estrone PDLA microspheres began to differ in surface and shape.

The estrone loaded PDLA microspheres retained their spherical shape and had a slightly smoother surface than progesterone loaded PDLA microspheres (above 30% w/w). However, the progesterone loaded PDLA microspheres were not at all smooth. Their spherical shape was lost and what looked like masses of aggregated drug was observed on the surface of progesterone loaded PDLA microspheres. The appearance of progesterone loaded PDLA microspheres at 30% w/w and greater was similar to the surface of the progesterone loaded PDLA obtained by Benoit et al (1984) at 68% w/w. Benoit et al (1984) stated the rough surface was due to the formation of progesterone crystals on the surface of the PDLA microspheres.

The drug particles of non-incorporated drug were observed on the surface of estrone PDLA microspheres at greater than 10% w/w load. Whilst progesterone loaded PDLA microspheres showed visual signs of drug crystals on the surface
of the PDLA microspheres at drug loadings of 30% w/w and greater. The results suggest that progesterone may be more miscible in PDLA than estrone, and hence little or no non-incorporated drug was detected on surface of the progesterone loaded PDLA microspheres. This agrees with the observations from the size analysis (Section 3.2.2). SEM studies suggest that at 10% w/w and less drug load, the model compounds are probably evenly dispersed in the PDLA microspheres.

The SEMs of PDLA microspheres suggest the 4 h interruption (isolation) step during microsphere preparation (Section 2.2.1) where the aqueous phase (PVA solution) was poured off and replaced with water to reduce the formation of crystals (Benita et al 1984), did not wash off drug crystals (10% w/w and greater for estrone and 30% w/w and greater for progesterone) on the microsphere surface. PDLA microspheres of this type would cause the mean experimental drug loadings to be close to the theoretical loading, with little loss of drug and / or polymer, as observed in Tables 3.1 and 3.2. The SEM results suggest a significant proportion of drug load may not have been incorporated inside the PDLA microspheres and this would have a pronounced effect on the release and profile kinetics. In the chapter 4, XRPD will be used to confirm the presence or absence of drug crystals on the surface PDLA microspheres.
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

Figure 3.2 SEM of drug free PDLA microspheres.
Figure 3.3  SEM of 10% w/w progesterone PDLA microspheres.
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

Figure 3.4 SEM of 20% w/w progesterone PDLA microspheres.
Figure 3.5  SEM of 30% w/w progesterone PDLA microspheres.
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

Figure 3.6  SEM of 40% w/w progesterone PDLA microspheres.
Figure 3.7 SEM of 50% w/w progesterone PDLA microspheres.
Figure 3.8 SEM of 10% w/w estrone PDLA microspheres.
Figure 3.9  SEM of 20% w/w estrone PDLA microspheres
Figure 3.10  SEM of 30% w/w estrone PDLA microspheres
Figure 3.11 SEM of 40% w/w estrone PDLA microspheres
Figure 3.12  SEM of 50% w/w estrone loaded PDLA microspheres
Chapter 3: Preparation of progesterone and estrone loaded PDLA microspheres

3.2.4 Thermogravimetric analysis of progesterone and estrone loaded PDLA microspheres

Thermogravimetric analysis (TGA) measurements of progesterone and estrone loaded PDLA microspheres on heating (20 – 120°C) produced mass losses of 1% and less (Table 3.4). The method for TGA is outlined in Section 2.3.1. This indicates that the amount of solvent or water present in the formed microspheres was low.

Table 3.4 Mass loss of progesterone and estrone loaded PDLA microsphere systems (Mean ± SD; n = 9).

<table>
<thead>
<tr>
<th>Theoretical Loading (% w/w)</th>
<th>Progesterone PDLA mean mass loss (%)</th>
<th>Estrone PDLA mean mass loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>10</td>
<td>0.9±0.2</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>20</td>
<td>0.9±0.2</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>30</td>
<td>1.0±0.2</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>40</td>
<td>0.9±0.2</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>50</td>
<td>1.0±0.1</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

Although the TGA results have shown the mass loss was quite small, it could not be determined whether the loss was due to residual dichloromethane or water. Progesterone loaded PDLA microspheres had a mean mass loss of 1% whilst estrone loaded PDLA microspheres had a mean mass loss of 0.3% (Table 3.4). The difference in % mass loss of progesterone and estrone loaded PDLA
microspheres, could be due to a difference of solubility of progesterone and estrone in aqueous media (Section 2.2.3). Since estrone is more hydrophobic than progesterone it will retain less moisture and hence have a lower mass loss on drying. Hill et al (1998) performed TGA on progesterone loaded PDLA microspheres and achieved a mass loss of 0.4 - 0.6% ±0.1. In this study, the % mass loss was slightly higher than the value obtained by Hill et al (1998). Which could be explained in terms of particle size of the microspheres. Hill et al (1998) produced microspheres with VMD of 100 - 130μm, whilst in this study; a VMD of 9.9 - 21μm was produced. Hence the larger the surface area the greater the % solvent loss and this was observed for progesterone PDLA microspheres.

3.2.5 Solubility studies of progesterone and estrone loaded PDLA microspheres

Before performing dissolution studies on progesterone and estrone loaded PDLA microspheres, a suitable dissolution media had to be identified. A common problem in dissolution testing of hydrophobic compounds, such as progesterone and estrone, is the attainment of sink conditions. Sink conditions are defined as maintaining a volume of dissolution media that is 5 to 10 times greater than the volume at the saturation point for the dosage being tested. An example would be mixing a certain amount of drug in a selected media; a point is eventually reached where no more drug dissolves into the liquid. This is the saturation point. To have sink conditions requires 5 to 10 times more volume than that at saturation so; there is no risk of drug being present that cannot dissolve into the media. Since progesterone and estrone are very poorly soluble in water (Section 2.2.3), co-solvents were chosen to provide sink conditions. This is appropriate so long as the drug release is diffusion-controlled and the matrix is insoluble in the co-solvent (Corrigan, 1991). Since PDLA is insoluble in ethanol (Beck et al, 1979a; 1979b) and the release of progesterone and estrone has proved to be diffusion-controlled, ethanol was selected as an appropriate co-solvent for this investigation.
From Table 3.5, it can be seen that progesterone has a higher solubility than estrone in the ethanol phosphate buffer solutions (EPBS). With solvent systems containing less than 40%v/v EPBS, progesterone solubility was <1.31 mg/mL. In 50 and 60%v/v EPBS the progesterone solubility was 2.29 and 2.25 mg/mL respectively. With solvent systems containing less than 40%v/v EPBS, the solubility of estrone was 0.3 mg/mL and less. In 50 and 60%v/v EPBS the estrone solubility was found to be 0.4 and 0.7 mg/mL respectively. A 50%v/v EPBS was chosen as suitable release media for future studies as the media contained equal amounts of ethanol and PBS.

Table 3.5 Solubility of progesterone and estrone in EPBS mixtures, pH 7.4 at 37°C (Mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Ethanol: Phosphate buffer pH 7.4 (% v/v)</th>
<th>Excess amount of drug (mg)</th>
<th>Progesterone solubility (mg/mL)</th>
<th>Estrone solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>70</td>
<td>0.01±0.004</td>
<td>0.12±0.006</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>0.03±0.002</td>
<td>0.17±0.001</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>0.15±0.010</td>
<td>0.19±0.003</td>
</tr>
<tr>
<td>40</td>
<td>70</td>
<td>1.31±0.300</td>
<td>0.27±0.011</td>
</tr>
<tr>
<td>50</td>
<td>70</td>
<td>2.29±0.230</td>
<td>0.39±0.005</td>
</tr>
<tr>
<td>60</td>
<td>70</td>
<td>2.25±0.250</td>
<td>0.69±0.010</td>
</tr>
</tbody>
</table>

3.3 Conclusion

The modified solvent evaporation method proved to be capable of encapsulating the hydrophobic compounds progesterone and estrone. The experimental drug loadings were close to the theoretical loading, indicating little raw material was lost during manufacture. The mean percentage incorporation efficiencies of drug loaded PDLA microspheres were all greater than 95% w/w, indicating that the
solvent evaporation method was efficient for drug encapsulation. The production method yielded PDLA microspheres having VMD in the range of 7 to 22 μm. When compared to the work of Hill et al (1998) and Parikh et al (1993), homogenisation reduced the median particle size of the PDLA microspheres to size potentially useful for IV, Inhalation and subcutaneous formulation although the size distribution was wide. Overall the modified solvent evaporation technique indicated that increased agitation during production decreased particle size. A trend in VMD and drug load was observed; as progesterone load increased, the VMD of microspheres increased, whilst as estrone load increased, the VMD of the microspheres decreased. Below 20%w/w drug load, microspheres of both had similar VMD above 20%w/w drug load; both microspheres had significantly different VMDs for each steroid.

SEM revealed that the modified solvent evaporation produced spherical microspheres, similar to structures described previously by Benoit et al, (1984); Bodmeier and McGinity, (1987); Parikh et al, (1993); Pradhan et al, (1994) and Hill et al, (1998). SEM was useful in providing direct visual evidence of the surface of the PDLA microspheres, revealing that progesterone was present on the surface of the PDLA microspheres (30% w/w and greater), whilst estrone was present on the surface of the PDLA microspheres at all drug loading concentrations. TGA produced small mass loses of 1% and less. TGA also indicated a possible relationship between VMD of progesterone PDLA microspheres and % mass loss. The larger the surface area of progesterone PDLA microspheres, the greater the % mass loss of residual solvent. The mass loss could either be water or residual dichloromethane the subsequent chapter (Section 4.2.3) was used to determine which solvent remained in the PDLA microspheres after manufacture.

Using the solvent evaporation process for microsphere production, a drug may be either dissolved or physically dispersed in the polymer matrix. If the drug is initially dispersed and remains in this form during the process, the drug will be
physically suspended in the polymer matrix. However if the drug is initially dissolved, three opportunities may occur: Firstly the drug may be finally dissolved in the polymer, leading to a solid solution (SS). Secondly, the drug may remain molecularly dispersed in the polymer, but with interactions between the drug molecules and the polymer chains too weak to lead to a stable state. This is a metastable molecular dispersion (MMD): interactions between the drug molecules are strong and the molecules will diffuse through the polymer network and crystallise. The rate of diffusion of molecules will depend on the matrix viscosity and it may be so slow that recrystallisation would take years. Thirdly, the drug may crystallise during the course of microsphere preparation; it will then be physically dispersed in the polymer matrix in the form of a crystalline dispersion. Because these three states will differ in terms of stability during ageing or in terms of drug release characteristics, it is essential to differentiate between them (Dubernet, 1995). If we apply Dubernet's theory to the data obtained from particle size and SEM for estrone and progesterone PDLA microspheres, it could be assumed that progesterone loaded PDLA microspheres existed as a SS, whilst estrone loaded PDLA microspheres existed as a MMD due to the drug solubility differences within the polymer PDLA. This will be explored in greater detail using MTDSC in chapter 4.
Chapter Four

Characterisation of progesterone and estrone loaded PDLA microspheres
4.1 Introduction

In Chapter 3, progesterone and estrone PDLA microspheres were prepared by the modified solvent evaporation technique. SEM indicated that when the drug load was increased above 0% w/w for estrone and 30% w/w for progesterone, the surface of the drug loaded PDLA microspheres became rough, and what seemed to be small drug crystals appeared on the surface of PDLA microspheres. The first aim of the study described in this chapter was to investigate the surface, and confirm if the particles that appeared on the surface of the PDLA microspheres were drug crystals. X-ray powder diffraction (XRPD) was employed. XRPD is a very useful tool for determining whether a compound is crystalline or amorphous. As mentioned in Section 1.4.2 and 1.4.1 both the model compounds (progesterone and estrone) are crystalline, whilst PDLA is amorphous. If drug crystals are present on the surface of the PDLA microspheres they should appear as Bragg's diffraction when analysed with XRPD, i.e. needle like spikes indicating signs of crystallinity, but if a diffuse halo appear when analysed with XRPD this will indicate the presence of an amorphous material which would suggest the drug is dispersed homogenously within PDLA, making the formulation completely amorphous.

The second aim of this study was to characterise the physical form of progesterone and estrone in the PDLA microspheres, upon increasing the drug load, using modulated temperature differential scanning calorimetry (MTDSC). This would give an indication as to the miscibility of the model compounds in PDLA, and also would show if the model compounds have a plasticising effect on the polymer. In Section 3.2.4 both model compounds were analysed by TGA and found to have mass losses of 1% or less, but it could not be determined whether the loss was due to removal of residual dichloromethane or water. Fourier transform infrared spectroscopy (FTIR) was employed to try and confirm the presence or absence of dichloromethane (which would appear as a sharp
chlorine function group) and water (which will appear as a broad hydroxyl functional group).

Progesterone and estrone have been formulated into drug loaded PDLA microsphere delivery system. The time required for the PDLA to degrade and for the drug to be released, are the controlling factors. Polymer degradation can take place throughout the drug release process, during which only a portion of the drug is released, or after the drug has been depleted. PDLA microspheres are designed to degrade throughout the drug release process by hydrolysis of the ester bonds (Senior and Radomsky, 2000). Drug delivery may be controlled by swelling, diffusion and erosion. Only the latter involves biodegradation, which is applicable to PDLA. Most delivery systems will act by a combination of the three mechanisms. The mechanism of drug release from PDLA is a combination of diffusion and erosion. As the drug on the surface of the microsphere diffuses away, exposed polymer hydrolyses and a greater matrix surface area is exposed. Drug may diffuse through the polymer to the surface. The loss of drug is more rapid than the loss of polymer and the device will pass through a very porous stage before it is totally eroded (Kitchell and Wise, 1991).

Drug release from microsphere delivery systems, has been described kinetically by either a square root of time relation (Jalsenjak et al, 1980) or by first order kinetics (Nixon and Walker, 1971). Adherence of kinetic data to both square root of time and first order kinetics has been reported (Benita and Donbrow, 1982) and data analysis using differential rates have made it possible to distinguish between the two (Schwartz et al, 1968). So the fourth aim of this study was to determine the release characteristics and kinetics of the model compounds from the PDLA microspheres incubated in EPBS, i.e. determine the length of time required for PDLA microspheres to release all of their drug content, and to explore if the release of these model compounds from PDLA was sustained, and dependent on drug loading concentration, diffusion and / or erosion.
4.2 Results and discussion

4.2.1 X-ray powder diffraction of progesterone and estrone loaded PDLA microspheres

X-ray powder diffraction (XRPD) was used to determine the polymorphic form of the estrone and progesterone loaded PDLA microspheres. The method is described in Section 2.3.5. The XRPD patterns of progesterone and estrone compounds confirmed the model compounds (progesterone and estrone) were crystalline (Figures 4.1 and 4.2 respectively). XRPD of PDLA microspheres without entrapped model compound showed that the polymer was amorphous (Figure 4.3). When the model compounds were incorporated into the PDLA microspheres there was a change in the pattern produced from the sample. XRPD patterns of progesterone loaded PDLA microspheres (Figure 4.3) showed that at 20% w/w progesterone loading and less, microspheres were all amorphous, this was shown as a diffuse halo, suggesting that progesterone was fully encapsulated and dispersed homogenously within PDLA microspheres at 20% w/w and less, as an amorphous formulation. This may also imply that progesterone has mutual miscibility in PDLA at 20% w/w and less, i.e. exists as a SS. Above 20% w/w progesterone loading, the PDLA microspheres begins to exhibit signs of crystallinity (i.e. Bragg's diffractions: needle-like spikes). At 40% w/w and 50% w/w (Figure 4.3) progesterone loadings the XRPD patterns become similar to the starting raw material progesterone (Figure 4.1), which is crystalline. This is an indication of loss of mutual miscibility of progesterone in PDLA, and the presence of progesterone on the surface of the PDLA microspheres. This suggests that above 20% w/w, progesterone exists as a MMD. XRPD patterns of estrone loaded PDLA microspheres (Figure 4.4) show all the PDLA microspheres have some form of crystallinity, although it was observed less at 10% and 20% w/w estrone. Above 30% w/w estrone loading the PDLA microspheres begin to show similar patterns to that of the starting raw material estrone (Figure 4.2), i.e. needle-like spikes. The results indicate estrone has no mutual miscibility with PDLA, hence the presence of crystallinity within the PDLA microspheres at low
(10% and 20% w/w) and high (above 30% w/w) estrone loadings, and the presence of estrone on the surface of the PDLA microspheres. These observations were further studied using MTDSC (Section 4.2.2).

Figure 4.1 XRPD pattern of pure progesterone.

Figure 4.2 XRPD pattern of pure estrone.
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.3 XRPD patterns of pure progesterone and progesterone loaded PDLA microspheres.

Figure 4.4 XRPD patterns of pure estrone and estrone loaded PDLA microspheres.
4.2.2 Modulated temperature differential scanning calorimetry of progesterone and estrone loaded PDLA microspheres

Modulated temperature differential scanning calorimetry (MTDSC) of the raw materials; progesterone and estrone were run as received (Figures 4.5 – 4.6) and showed endotherms with peak temperatures of $129.9 \pm 0.4^\circ C$ and $248.9 \pm 0.2^\circ C$ respectively (Mean $\pm$ SD; $n = 3$). The melting temperatures obtained for progesterone and estrone correspond well with melting ranges obtained from the Merck Index of 127 to 131°C (progesterone alpha form), 121°C (progesterone beta form) and 251 to 254°C (estrone) (Merck Index 12th edition, 1996b and 1996a respectively). The results thus suggest the raw material progesterone was supplied in the alpha form.

The PDLA (raw material) was analysed using MTDSG and produced total heat flow, heat capacity and phase lag information shown in Figure 4.7. For the PDLA sample the total heat flow shows an endotherm with an onset of $43.7 \pm 0.2^\circ C$. The transition can be identified as a glass transition of the polymer by the presence of a step change in the heat capacity signal, which had an onset of $44.5 \pm 0.2^\circ C$. The glass transition measured for PDLA was lower than many reported values, which typically range from 50 - 57°C (Benita et al, 1984; Benoit et al, 1984; Rak et al, 1985). However the underlying heating rate used in this study ($1^\circ C \text{ min}^{-1}$) was much slower than the usual $10 - 20^\circ C \text{ min}^{-1}$ used in conventional DSC experiments and this would cause a lower transition temperature to be measured. The glass transition temperature obtained for PDLA in this study ties in with glass transition temperature results of $44.4 \pm 0.1^\circ C$ obtained by Hill et al, (1998).

The phase lag signal, $\vartheta$, measures the lag between the modulated heat flow and the heating rate, and can be used to determine where there is a kinetic component to the heat capacity signal (Figure 4.7).
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.5  Total heat flow (MTDSC) of pure progesterone (raw material).

Figure 4.6  Total heat flow (MTDSC) of pure estrone (raw material).
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.7 Total heat flow, complex heat capacity and phase lag, $\theta$, measured by MTDSC for PDLA.

Figure 4.8 Results of phase correction of complex heat capacity (Kinetic & reversing heat capacity) for PDLA.
When this is observed, the phase lag is used to separate the measured complex heat capacity (Figure 4.8) into the “in phase” and “out of phase” components. The in phase, \( C_p' \), is termed the reversing heat capacity, whilst the out phase component, \( C_p'' \), is thought to relate to a kinetic process (Aubuchon and Gill, 1997). Figure 4.8 shows the phase corrected heat capacity for PDLA raw material. The reversing (in phase) heat capacity (Figure 4.8) was identical to the complex heat capacity in the glass transition region (Figure 4.7). Thus in this instance the complex heat capacity can be used directly for characterisation of the sample without the extra deconvolution step.

When the \( T_g \) of the initial (raw material) PDLA (Figure 4.7) was compared to the \( T_g \) of the finished empty PDLA microspheres (Figure 4.9 and Table 4.1) an increase in the \( T_g \) value was observed (From approximately 45 to 53°C), which indicated the solvent evaporation process may have caused the polymer PDLA to rearrange its order from a random form, to a much more stable and ordered form making it more rigid, with a resultant glass transition temperature. Dubernet, 1995 and Hill et al (1998) observed this in their studies, where they found the solvent evaporation technique modified the organisation of the polymer chains in the solid state leading to mechanical discrepancies in both the \( T_g \) event and crystallisation of PDLA.

Figures 4.9 – 4.14 show the total heat flow, complex heat capacity and phase lag for each of the progesterone and estrone PDLA microspheres preparations. The curves have been separated on the Y-axis in each figure to aid presentation. With an increase in progesterone loading there was a decrease in the \( T_g \) to an extent (30% w/w progesterone) (Table 4.1). The onset of the change in heat capacity was seen to decrease from 53 to 40°C, but as the progesterone loading increased above 30% w/w, the onset change in heat capacity was seen to increase from 40 to 51°C (Figure 4.10). This suggests progesterone 0 to 30% w/w has a plasticisation effect on PDLA i.e. the presence of progesterone made the polymer soften, possibly acting as an impurity to PDLA, hence lowering the \( T_g \) of PDLA.
Table 4.1  Glass transition temperatures of drug free PDLA, progesterone and estrone loaded PDLA microspheres (mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Theoretical drug load (%w/w)</th>
<th>Mean $T_g$ (°C) of progesterone PDLA microspheres</th>
<th>Mean $T_g$ (°C) of estrone PDLA microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53.3±0.5</td>
<td>53.3±0.5</td>
</tr>
<tr>
<td>10</td>
<td>49.4±0.4</td>
<td>53.6±0.1</td>
</tr>
<tr>
<td>20</td>
<td>41.2±0.5</td>
<td>53.4±0.2</td>
</tr>
<tr>
<td>30</td>
<td>39.9±0.5</td>
<td>53.4±0.3</td>
</tr>
<tr>
<td>40</td>
<td>50.2±1.1</td>
<td>52.8±0.4</td>
</tr>
<tr>
<td>50</td>
<td>51.4±1.1</td>
<td>52.9±0.2</td>
</tr>
</tbody>
</table>

Figure 4.9  Total heat flow (MTDSC) of progesterone PDLA microspheres.
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.10 Complex heat capacity measured by MTDSC of progesterone PDLA microspheres.

Figure 4.11 Phase lag measured by MTDSC of progesterone PDLA microspheres.
The MTDSC data for progesterone PDLA microspheres suggests that between 0 and 10% the drug loaded PDLA are present as a SS. With SS, drug molecules and polymers develop strong interaction between each other leading to plastisation of the polymer. The consequences are a lower $T_g$ and the absence of a fusion event of the drug. i.e. the lack of a progesterone recrystalisation peak (Dubernet, 1995), which was seen for progesterone loaded PLA 10%.

Exothermic peaks (Figure 4.9) were observed in the total heat flow which had an onset value of 65°C at drug loads between 20 and 40% w/w for progesterone loaded PDLA microspheres. This would suggest that above 20% w/w the progesterone loaded PLA are present as a MMD. For MMD, the drug remains in the molecular state only because the viscosity of the medium is so high that drug diffusion, and therefore crystallisation, is inhibited. In an ideal case, the drug molecules and the polymer do not interact with each other, leading to conserved polymer characteristics ($T_g$). As long as the polymer remains in the vitreous state, crystallisation will not occur in the few weeks or months following microsphere preparation. However as soon as the matrix is in the rubbery state, the viscosity breaks down which allows crystallisation to occur readily. The result suggests the 10% w/w progesterone PDLA microsphere formulation is present as a homogenously dispersed amorphous formulation (SS), i.e. progesterone is miscible in PDLA at this concentration, so we obtain a $T_g$ of just the polymer PDLA. As the concentration of progesterone is increased above 10% w/w, the formulation becomes partially amorphous, with the presence of some recrystallisation regions, which appeared as exothermic peaks (noted to appear as from 65°C upwards). This was noted to have occurred at progesterone concentrations of 20% w/w to 40% w/w. Also at progesterone concentrations of 20% w/w to 40% w/w, the presence of, melt transitions at 110°C and greater appeared, indicating the presence of the crystalline progesterone. At 50% w/w progesterone concentration the exothermic peak disappeared, indicating the lack of an amorphous form of drug and the presence of a crystalline form of progesterone within the PDLA microspheres (Figure 4.9).
Overall, the results suggest as the progesterone loading increases above 10% w/w the drug particles which were once presence as an amorphous drug form in the formulation revert back to their original crystalline form, therefore indicating there is a limit to the miscibility of progesterone in PDLA, which is exceeded at loadings of 20% w/w and above. It has been suggested by Benoît et al. (1996) that progesterone has little mutual miscibility with PDLA, this was based on evidence that 23%w/w progesterone loaded PDLA microspheres contained crystalline progesterone after heat treatment at 110°C.

Above 10% w/w progesterone loadings the sample shows melt transitions (different amorphous forms that crystallise on heating). The first is a broad peak, which began soon after the cold crystallisation exotherm and had a peak temperature of 112°C. The second has a peak temperature of 125°C. At 20 and 30% w/w a progesterone melt peak appeared at 112°C, 40% w/w progesterone had two melt peaks at 112°C and 125°C, whilst at 50% w/w progesterone, one melt peak at 129°C appeared (Figure 4.9). This suggests that at lower progesterone loads between 20% w/w and 30% w/w progesterone exists as the beta form, whilst at 40% w/w progesterone exists in both alpha and beta forms, and at 50% w/w progesterone exists in the alpha form. Thus the change in miscibility of progesterone in the polymer may be linked to the change in progesterone polymorphic form upon increase in progesterone concentration. This ties in with the work of by Benita et al., (1984), Benoît et al., (1984) and Hill et al, (1998), who found that the physical form of progesterone in microspheres changed with increased drug loading. Figures 4.11 and 4.14 show the phase lag signal for each of the experiments shown in Figures 4.9, 4.10, 4.12 and 4.13. It was seen that these peaks (Figures 4.11 and 4.14) were quite small when compared to the complex heat capacity and would have a negligible effect on $T_g$ (Figures 4.10 and 4.13) region. No baseline step changes in phase lag were observed (Figures 4.11 and 4.14) this indicated optimal thermal contact between PDLA (progesterone and estrone) microspheres and the sample pan during heating.
Estrone loaded PDLA microspheres (Figure 4.12) behave quite differently to progesterone (Figure 4.10), in that the T_g as determined from the complex heat capacity remained the same as the estrone drug loading increased. Increasing the estrone loading in the PDLA microspheres had little or no effect on the glass transition temperature of the polymer PDLA (Figure 4.12 and Table 4.1). Which indicates estrone has no plasticisation effect on polymer PDLA. This would also suggest estrone is not miscible with PDLA hence a T_g for PLA and a T_m for estrone was observed at all loadings (Figures 4.12 and 4.14). Estrone loaded PDLA microspheres showed no evidence of exothermic peaks (Figure 4.12) as seen in the total heat flow of progesterone loaded PDLA microspheres (Figure 4.9). This suggests the estrone formulation was never amorphous, or even partially amorphous. The presence of melt peaks (Figure 4.12) indicated estrone crystals were detected within the estrone loaded PDLA microspheres.
Figure 4.13 Complex heat capacity measured by MTDSC of estrone loaded PDLA microspheres.

Figure 4.14 Phase lag measured by MTDSC of estrone loaded PDLA microspheres.
Although it is indicated that estrone was not miscible with PDLA the results obtained for estrone suggest estrone was initially trapped as a MMD rather than a true SS as no plasticisation of the polymer PDLA occurred, (Dubernet, 1995). Benoit and Thies (1996) have found it is possible to trap a drug with little mutual miscibility within the polymer as a metastable molecular dispersion due to the nature of the solvent evaporation process. This phenomenon explains what occurred at progesterone loadings above 10%w/w and all estrone loadings, and was confirmed in the mean incorporation efficiency results for drug loaded PLA microspheres (Table 3.1 and 3.2).
4.2.3 Fourier transform infrared spectroscopy of progesterone and estrone loaded PDLA microspheres

Fourier transform infrared spectroscopy (FTIR) spectra of progesterone, estrone, PDLA and progesterone and estrone loaded PDLA microspheres are shown in Figures 4.15 to 4.21. Standard spectra of progesterone and estrone were received (Figures 4.15 and 4.16). The standard spectrum of progesterone did not show a sharp absorbance peak at 3400 cm\(^{-1}\), but estrone had an absorbance peak at 3400 cm\(^{-1}\), which is indicative of a hydroxyl group (confirming the presence of OH in estrone and its absence in progesterone).

![Figure 4.15 Standard FTIR spectrum of progesterone as received](image-url)
Comparing standard spectra of progesterone and estrone as received (Figures 4.15 and 4.16) to run spectra of progesterone and estrone (Figures 4.17 and 4.18), it was observed that both spectra were similar and no broad absorbance peaks were observed between 3700 – 3600 cm\(^{-1}\), which indicated the lack of free water in the raw materials before the modified solvent evaporation process. Structurally, progesterone and PDLA do not have hydroxyl groups present whilst estrone has one hydroxyl group present on carbon "3" atom (For structures of raw materials refer to Figures 2.2, 2.3 and 2.1 respectively) and this was confirmed in Figures 4.17 to 4.19 by the lack of absorbance between 3700 – 3600 cm\(^{-1}\).

![Standard FTIR spectrum of estrone as received.](image)

**Figure 4.16 Standard FTIR spectrum of estrone as received.**
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.17 FTIR spectrum of progesterone run as received.

<table>
<thead>
<tr>
<th>Wave number (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 – 2800</td>
<td>C-H aromatic stretch</td>
</tr>
<tr>
<td>2400 – 2300</td>
<td>C=C</td>
</tr>
<tr>
<td>1700 – 1650</td>
<td>C=O</td>
</tr>
<tr>
<td>1475 – 1350</td>
<td>C-CH₃</td>
</tr>
<tr>
<td>1400 – 1300</td>
<td>CO-CH₃</td>
</tr>
<tr>
<td>1200 – 1000</td>
<td>C-C stretch and of large number of H - present</td>
</tr>
</tbody>
</table>
Figure 4.18 FTIR spectrum of estrone run as received from supplier.

<table>
<thead>
<tr>
<th>Wave number (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500 – 3200</td>
<td>O-H stretch</td>
</tr>
<tr>
<td>3000 – 2800</td>
<td>CH aromatic stretch</td>
</tr>
<tr>
<td>1800 – 1700</td>
<td>C=O</td>
</tr>
<tr>
<td>1600 – 1550</td>
<td>C=C aromatic stretch and endocyclic bonding</td>
</tr>
<tr>
<td>1500 – 1300</td>
<td>Symmetric and asymmetric deformation vibrations of CH in CH$_3$</td>
</tr>
<tr>
<td>1200 – 1000</td>
<td>C-C stretch and of large number of H - present</td>
</tr>
</tbody>
</table>
Figure 4.19 FTIR spectrum of PDLA as received from supplier.

<table>
<thead>
<tr>
<th>Wave number (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 – 2900</td>
<td>CH aliphatic stretch</td>
</tr>
<tr>
<td>1800 – 1700</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1500 – 1300</td>
<td>Symmetric and asymmetric deformation vibrations of CH in CH$_3$</td>
</tr>
<tr>
<td>1250 – 1050</td>
<td>C-O and C-O-C stretching vibrations</td>
</tr>
</tbody>
</table>
PDLA has four main functional groups (Figures 2.1 and 4.19) CH stretch (aliphatic) between 3000 – 2900 cm\(^{-1}\), C=O (carbonyl) between 1800 – 1700 cm\(^{-1}\), C – O stretch between 1300 – 1000 cm\(^{-1}\) and a CH bend between 1200 – 1000 cm\(^{-1}\) (Rak et al, 1985). The spectrum of progesterone loaded PDLA microspheres (Figure 4.20) showed broad peaks between 3600 – 3200 cm\(^{-1}\), which is indicative of hydroxyl functional groups. PDLA and progesterone have no hydroxyl groups, but spectra of both materials showed signs of small, but broad peaks at transmission wavelengths of 3600 – 3200 cm\(^{-1}\) (Figures 4.19 and 4.17). The spectra (Figure 4.20), suggests the progesterone loaded PDLA microspheres had absorbed some water during the modified solvent evaporation process, as the individual raw materials (Figures 4.19 and 4.17) gave no absorbance between transmission wavelengths of 3600 – 3200 cm\(^{-1}\). As the progesterone concentration within the PDLA microspheres increased the intensity of the hydroxyl group increased (Figure 4.20). At transmission wavelengths between 3000 – 2800 cm\(^{-1}\) there are OH peaks present which are indicative of CH aromatic stretch from progesterone (Figure 4.17) and CH aliphatic stretch from PLA (Figure 4.19). The CH aliphatic stretch peaks begin to intensify and change form as the progesterone concentration increases. This is possibly due to the incorporation and interaction of progesterone with PDLA. Both PDLA and progesterone have carbonyl groups present giving peaks at wavelengths 1800 – 1600 cm\(^{-1}\) (Figures 4.17 and 4.19), but when PDLA and progesterone are formulated into microspheres (Figure 4.20) there appeared to be a mixture of carbonyl groups, i.e. there is possibly interaction between each other. There is no indication or presence of residual chlorine (C – Cl) from dichloromethane in the spectrum obtained for progesterone loaded PDLA microspheres. If residual solvent from microsphere preparation were present within the PDLA microspheres, a sharp intense peak would have appeared between 1000 – 800 cm\(^{-1}\). Figure 4.21 shows the spectra of estrone loaded PDLA microspheres. At wavelength 3450 cm\(^{-1}\) there is a strong sharp peak, which represents the OH group on carbon 3 in estrone.
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.20 FTIR spectra of PDLA microspheres loaded with varying concentrations of progesterone.

Figure 4.21 FTIR spectra of PDLA microspheres loaded with varying concentrations of estrone.
Between 3700 – 3600 cm⁻¹ there was a broad weak peak, which suggested the presence of OH from water. At wavelength 3000 cm⁻¹ there were peaks, indicative of CH aromatic stretch from estrone (Figure 4.18) and CH aliphatic stretch from PDLA (Figure 4.19). The CH aromatic stretch begun to intensify as estrone concentration increased within the microspheres. Between 1800 – 1700 cm⁻¹ there was a strong carbonyl peak, which was contributed by estrone and PDLA. There was also presence of a combination of carbon double bonds (C = C), which was contributed by estrone. Between 1200 – 1000 cm⁻¹ there was a combination of methyl (CH₃) bending. There was no indication or presence of chlorine (C – Cl) bonds in the spectra obtained for estrone loaded PDLA microspheres, which suggested the absence of residual dichloromethane.

From the FTIR spectra obtained for progesterone and estrone loaded PDLA microspheres (Figures 4.20 and 4.21), there is no evidence to suggest the presence of residual dichloromethane, but there is evidence to suggest the presence of water (broad weak OH between 3700 – 3600 cm⁻¹). The results from the FTIR study are in agreement with work performed by Passerini and Craig (2001) where a significant amount of water was retained in PLA and PLGA microspheres. The spectra show progesterone PDLA loaded microspheres had more of a broad peak at 3700 – 3600 cm⁻¹ (Figure 4.20) than estrone PDLA loaded microspheres (Figure 4.21). Hence this confirmed progesterone retained more water than estrone and during the encapsulation (modified solvent evaporation) process and drying (Section 2.2.1) period. This correlates with the TGA (Section 3.2.4) where progesterone loaded PDLA microspheres had a three fold mass loss on heating, compared to estrone loaded PLA microspheres. The results imply that not only is FTIR useful in identifying functional groups and the presence of water (free OH), but could also be used visually, to detect increasing amounts of drug load and moisture content.
4.2.4 In vitro release studies of progesterone and estrone loaded PDLA microspheres

A 1:1 ethanol phosphate buffer 50% v/v (EPBS) solution pH 7.4 was used as a release medium, since it provided sink conditions for progesterone and estrone in the in solubility studies (Section 3.2.5). Calibration curves of progesterone and estrone in EPBS were constructed to determine the amount of drug released from the PDLA microspheres during the in vitro release study (for calibration procedure and method see to Sections 2.3.2.1 and 2.2.4). Free progesterone and estrone were placed in the chosen release media to determine the time it took for free drugs to completely dissolve. Progesterone completely dissolved in the release media in less than 1 hour (Figure 4.22), whilst estrone completely dissolved in less than 3 hours (Figure 4.23). This correlated with the solubility studies (Section 3.2.5), which indicated that estrone was less soluble than progesterone and hence would require a greater length of time than progesterone to be released from the polymer PDLA. Figure 4.22 shows a comparison between the dissolution of free progesterone and the release profile of progesterone from PDLA microspheres. The free drug (10mg) dissolved within an hour, whilst the release of progesterone from the microspheres was significantly sustained. Figure 4.22 shows that all progesterone loaded PDLA microspheres followed a similar trend, an initial burst of release followed by a slower sustained release of progesterone. Complete release of progesterone from PDLA microspheres took approximately 1008 hours. One would expect PDLA microspheres with the highest progesterone load to release their contents quicker than PDLA microspheres with lower progesterone load, due to diffusion, but this was not the case, as release time did not seem to be dependent upon progesterone concentration. The initial burst was estimated to have lasted 7 hours (Figure 4.24: see vertical part of plot). Upon increasing the progesterone loading there was little difference in the percentage (24 to 32%) of progesterone released (Table 4.2) during the initial burst.
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Figure 4.22 Mean cumulative amount of progesterone released from 10 mg PDLA microspheres in EPBS (mean ± SD; n = 6).

Figure 4.23 Mean cumulative amount of estrone released from 10 mg PDLA microspheres in EPBS (mean ± SD; n = 6).
Figure 4.24 Mean cumulative percentage progesterone released from 10 mg PDLA microspheres in EPBS (mean ± SD; n = 6).

Table 4.2 Amount of progesterone released from PDLA micropsheres during the 7 hours initial burst (mean ± SD; n = 6).

<table>
<thead>
<tr>
<th>Progesterone loading (% w/w)</th>
<th>Duration of initial burst (hours)</th>
<th>Percentage of progesterone released (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
<td>26.0 ± 2.1</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>24.2 ± 1.0</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>31.0 ± 1.7</td>
</tr>
<tr>
<td>40</td>
<td>7</td>
<td>28.1 ± 1.1</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>31.5 ± 2.0</td>
</tr>
</tbody>
</table>

The overall ranking of rate of release of progesterone i.e. the rate at which the formulation released 100% of its drug content from PDLA was as follows 10% w/w >20% w/w >30% w/w >40% w/w >50% w/w (Figure 4.24) the release of progesterone from PDLA may be dependent on the polymorphic form of progesterone present within PDLA. As the progesterone loading increases the
formulation changes from that of amorphous to that of a semi crystalline (Section 4.2.2) and this could have implications on the dissolution rate of progesterone (Gennaro, 1990). Figure 4.24 shows all progesterone loaded PDLA microspheres released approximately 30% progesterone in 7 hours. The remaining 70% w/w progesterone was released, over a much slower time of 1000 hours. It appears that in EPBS, 30% of the progesterone, which was released during the initial burst phase, could represent the progesterone localised on the surface of the PDLA microspheres at 20% w/w to 50% w/w. The slower portion of the curve suggests that progesterone diffusion through the polymer (PDLA) matrix was quite slow and this portion of the curve could represent 70% of the progesterone encapsulated within the PDLA microspheres.

A comparison of the dissolution of free estrone to the release profile of estrone from PDLA microspheres is shown in Figure 4.23. The free estrone (10mg) dissolved within three hours whilst the release of estrone from the microspheres was significantly sustained. The release of estrone from PDLA microspheres was monitored over 2016 hours (Figure 4.23). The estrone loaded PDLA microspheres followed a similar trend to progesterone loaded PLA microspheres; an initial burst of drug release followed by a somewhat slower sustained release of estrone. The initial burst was estimated to have occurred in 8 hours, but complete release of estrone from PDLA microspheres was not achieved within 1000 hours, as for progesterone, so the experiment was terminated at 2016 hours. The percentages of estrone released during the initial burst, are shown in Table 4.3. The percentages of estrone released during the initial burst were not similar to each other moreover, that as the estrone loading increased the percentage of estrone released during the initial burst decreased. Overall the ranking of rate of release of estrone during the burst phase from the PDLA microspheres was as follows 10% w/w>20% w/w >30% w/w >40% w/w >50% w/w (Figure 4.25).
Figure 4.25 Mean cumulative percentage estrone released from 10 mg of PDLA microspheres in EPBS (mean ± SD; n = 6).

Figure 4.25 shows all estrone loaded PDLA microspheres released approximately 2 to 3% progesterone in approximately 8 hours. The remaining estrone was released, over a much slower period of time. The 2 to 3% of the estrone, which is released during the initial burst phase, could represent the estrone localised on the surface of the PLA microspheres as visualised by SEM (Figures 3.8 –3.12) and analysed by XRPD (Figure 4.4). The later portion of the curve suggests that estrone diffusion through the polymer (PDLA) matrix was slow and may thus represent the remaining portion of estrone entrapped within the PDLA microspheres.
Table 4.3  Amount of estrone released from PDLA micropsheres during the 8 hours initial burst (mean ± SD; n = 6).

<table>
<thead>
<tr>
<th>Estrone loading (% w/w)</th>
<th>Duration of initial burst (hours)</th>
<th>Percentage of estrone released (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

The results in Table 4.3 suggest that as the estrone concentration increased, the estrone molecules might have aggregated together within the PDLA microspheres (due to their hydrophobicity), hence making the release of estrone slow. The possible aggregating effect could act as a protector for PDLA hence decreasing the rate of degradation. This was investigated further (Sections 5.2.3 and 6.2.3) by gel permeation chromatography (GPC) and SEM.

In therapy, this burst effect could provide a loading dose for immediate therapeutic action for patients with osteoporosis or on menopause, whilst the much slower portion of release could serve as a maintenance dose. It is interesting to compare the results of this present study to the work of Beck et al. (1979) who employed an in vitro release medium (2:3 v/v, ethanol/water) to predict the in vivo release behaviour of progesterone from PLA microcapsules. This demonstrated an initial quick release of approximately 50%, whilst the remainder was released at a slower rate over several days. Gupta and Deluca (1989) also employed an in vitro release medium (1:1 v/v, ethanol/phosphate buffer) to predict the in vivo release of 10%w/w progesterone from PDLA microspheres. An initial quick release of over 80% progesterone occurred in ten hours, whilst the remaining 20% was released over three weeks. Parikh et al. (1993) evaluated the release of 10%w/w estrone from PLLA microspheres using...
an in vitro medium (1:1 v/v, methanol/phosphate buffer) where 50 to 65% of estrone was released in twelve hours, whilst the remaining 35 to 50% was released over 3 days at a slower rate. The authors explained the burst effect to be attributed to the drug on the surface of the microspheres and leaching of drug localised in the porous network of the PLA microsphere matrix.

Bodmeier and McGinity (1987) have explained the effect of drug loading on the release pattern in three distinguishable release phases. Where a lag time with no drug release is followed by an apparent burst effect of rapid drug release within a short period of time, followed again by a slow release phase. The lag time is due to the time required for the medium to penetrate the PDLA matrix. A long lag time will confirm the absence of free drug crystals on the surface which if available would cause rapid dissolution. In this study a lag time was not observed, i.e. a region of no drug release from progesterone and estrone loaded PDLA microspheres. This suggests the PDLA microspheres had drug on surface of the microspheres or that the matrix was porous (not detected by SEM), which allowed for the penetration of medium producing an initial burst effect. Overall, the results suggest the release of drug from PDLA microspheres is not dependent on drug concentration, but is possibly dependent on PDLA degradation. This was investigated by gel permeation chromatography (GPC) (Section 5.2.3).
4.2.4.1 In vitro release kinetics of progesterone and estrone loaded PDLA microspheres

Four models have been proposed to describe progesterone and estrone release kinetics from PDLA microspheres: Zero order, first order, square root of time and a cube root model (Equations 4.1 to 4.4) (Malamataris and Avgerinos, 1990). Zero order occurs when the release rate remains (Linear) with time constant. First order rate of reaction occurs when the release rate declines with time. A cube root model describes geometric disappearance of the delivery system (contracting geometry) and the square root model (Higuchi kinetics) describes drug release by diffusion, which is square root of time dependent.

Zero order \[100 - M = k_0 t\] \hspace{1cm} Equation 4.1

First order \[\ln M = k_1 t\] \hspace{1cm} Equation 4.2

Cube root \[3\sqrt{100 - 3\sqrt{M}} = k_2 t\] \hspace{1cm} Equation 4.3

Square root \[100 - M = k_3 \sqrt{t}\] \hspace{1cm} Equation 4.4

where \(M\), represents the percentage of unreleased drug, \(K_0, K_1, K_2\) and \(K_3\) are all rate constants of drug release and \(t\) represents time taken for drug release. The zero order (rate of release versus time) was found to be inapplicable to the data obtained for progesterone, since progesterone release was non-linear and the release rates were dependent on time (Figure 4.26). The release of progesterone from PDLA supports the first order model. The release rate of progesterone from PDLA decreased with time (Figure 4.27). The \(R^2\) values indicate a very good fit of data (Table 4.4). Figure 4.28 suggests that progesterone PDLA microsphere geometry contracts with time. The release of progesterone from PDLA microspheres increases with square of time as shown in figure 4.29. The data suggests the release of progesterone from PDLA is time dependent.
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.26 Rate as a function of time, for progesterone loaded PDLA microspheres, plotted according to the zero-order model.

Figure 4.27 In of % progesterone unreleased as a function of time, plotted according to a 1st order release model.
The release rates constants ($k$) and the corresponding correlation coefficients ($R^2$) for first order, square root and cube root plots are shown in Table 4.4. The correlation coefficients of the three models used for progesterone loaded PDLA microspheres adequately fit the release data, which is an indication of diffusion-controlled release. Since both first order and square root models are acceptably linear, and only one of them is correct, a more rigorous test based on the differential forms of their rate equations (Schwartz et al, 1968) was needed to elucidate the release mechanism.

![Graph showing cube root of % progesterone released as a function of time plotted according to a cube root model.](image)

**Figure 4.28** Cube root of % progesterone released as a function of time plotted according to a cube root model.
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Figure 4.29 Percentage progesterone released as a function of square root of time, plotted according to a square root model.

Table 4.4 Fit of in vitro dissolution results for progesterone loaded PDLA microspheres to the three kinetic models.

<table>
<thead>
<tr>
<th>Drug load (% w/w)</th>
<th>First order</th>
<th>Square root</th>
<th>Cube root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k'$ (h$^{-1}$)</td>
<td>$R^2$</td>
<td>$k'$ (h$^{-1}$)</td>
</tr>
<tr>
<td>10</td>
<td>-0.006</td>
<td>0.9657</td>
<td>3.372</td>
</tr>
<tr>
<td>20</td>
<td>-0.003</td>
<td>0.9755</td>
<td>3.006</td>
</tr>
<tr>
<td>30</td>
<td>-0.007</td>
<td>0.9254</td>
<td>3.169</td>
</tr>
<tr>
<td>40</td>
<td>-0.004</td>
<td>0.9797</td>
<td>2.968</td>
</tr>
<tr>
<td>50</td>
<td>-0.006</td>
<td>0.9903</td>
<td>3.251</td>
</tr>
</tbody>
</table>
For the Higuchi matrix mechanism, the rate of release is given by:

\[ \frac{dQ'}{dt} = \frac{(k_2S)^2}{2Q'} \]  
Equation 4.5

Where \( Q' \) is the percentage of drug released, \( S \) is the surface area of the microspheres and \( k_2 \) the release constant. Release follows Higuchi kinetics if the rate is inversely proportional to \( Q' \). The rate predicted by first - order kinetics is given by:

\[ \frac{dQ'}{dt} = k_1W_0 - k_1Q' \]  
Equation 4.6

Where \( W_0 \) is the initial amount of drug and \( k_1 \) is the first – order release constant. The release follows first – order kinetics if the rate is proportional to \( Q' \). It is possible to distinguish between the two mechanisms by plotting release rates, \( \frac{dQ'}{dt} \), as a function of \( Q' \) and \( 1/Q' \). For results of the two mechanisms see Table 4.5 (plots of the two mechanisms at 10% w/w progesterone are shown in figures 4.30 and 4.31).

Table 4.5  Comparison of correlation coefficients (R^2) for plots of release rate (dQ'/dt) as a function of drug released (Q') for progesterone loaded PDLA microspheres.

<table>
<thead>
<tr>
<th>Progesterone drug load (% w/w)</th>
<th>Rate vs. % released (Q') R^2 (First order kinetics)</th>
<th>Rate vs. reciprocal % released (1/Q') R^2 (Higuchi kinetics)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.6271</td>
<td>0.9857</td>
</tr>
<tr>
<td>20</td>
<td>0.6209</td>
<td>0.9633</td>
</tr>
<tr>
<td>30</td>
<td>0.6993</td>
<td>0.9497</td>
</tr>
<tr>
<td>40</td>
<td>0.6806</td>
<td>0.9371</td>
</tr>
<tr>
<td>50</td>
<td>0.7372</td>
<td>0.9209</td>
</tr>
</tbody>
</table>
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

Figure 4.30 Rate as a function of reciprocal percentage drug (10% progesterone) released (Higuchi kinetics).

Figure 4.31 Rate as a function of percentage drug (10% progesterone) released (First order kinetics).
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Only the plots of \( \frac{dQ'}{dt} \) versus \( 1/Q' \) were linear (Figure 4.30) for the five progesterone loading studied (Table 4.5). Which indicated that the release process is mainly due to diffusion, for progesterone loaded PDLA microspheres. The zero order model was also found to be inapplicable for estrone loaded PDLA microspheres since the plot obtained was non linear (Figure 4.32). The data suggested the release rate did not remain constant.

![Figure 4.32 Rate as a function of time, for 10 – 50% estrone loaded PDLA microspheres plotted according to the zero – order model](image)

The release data obtained for estrone loaded PDLA microspheres was applied to the first order model (Figure 4.33). The data obtained for estrone loaded PDLA microspheres showed the release rate decreased with time. But the correlation coefficient values were less than 0.9 (Table 4.6) hence this suggested a not so perfect fit was obtained and that the data did not fit the first order. Figure 4.34 showed the release data obtained for estrone loaded PLA microspheres being fitted to the cube root model. The data does not assume geometric contraction, as the \( R^2 \) value is less than 0.9.
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Figure 4.33 Natural logarithm of %-unreleased estrone as a function of time, plotted according to a first-order model.

Figure 4.34 Cube root of % estrone released as a function of time, plotted according to a cube root model.
Figure 4.35 Square root of % estrone released as a function of time, for 10 – 50% loaded microspheres, plotted according to the square root model.

Table 4.6 Fit of dissolution results to different kinetic models for estrone loaded PDLA microspheres.

<table>
<thead>
<tr>
<th>Drug load (% w/w)</th>
<th>First order</th>
<th>Square root</th>
<th>Cube root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k^1 (h^-1)</td>
<td>R^2</td>
<td>k^1 (h^-1)</td>
</tr>
<tr>
<td>10</td>
<td>-0.0002</td>
<td>0.7861</td>
<td>0.9525</td>
</tr>
<tr>
<td>20</td>
<td>-0.0002</td>
<td>0.7692</td>
<td>0.7330</td>
</tr>
<tr>
<td>30</td>
<td>-0.0001</td>
<td>0.7818</td>
<td>0.6103</td>
</tr>
<tr>
<td>40</td>
<td>-0.0001</td>
<td>0.7821</td>
<td>0.5556</td>
</tr>
<tr>
<td>50</td>
<td>-0.0001</td>
<td>0.7709</td>
<td>0.4623</td>
</tr>
</tbody>
</table>
Chapter 4: Characterisation of progesterone and estrone loaded polylactide microspheres

The release data (Table 4.6) obtained for estrone loaded PDLA microspheres did not fit three of the four models. The only model, which adequately fits the release data, is the square root model (Figure 4.35) as $R^2$ are above 0.9, which is an indication of diffusion - controlled release. Rigorous test based on the differential forms of their rate equations were not required as the first order model was not linear, whilst the square root models was linear. The cube root model for estrone loaded microspheres indicates that PDLA loaded with estrone did not assume geometric disappearance.

4.3 Conclusion

XPRD confirmed that both model drug compounds were crystalline and that PDLA was amorphous. The presence of progesterone on the surfaces of PDLA microspheres at 30% w/w and above was confirmed by XPRD. Estrone loaded PDLA microspheres on the other hand, showed signs of crystallinity at all estrone loadings, indicating estrone was not miscible with PDLA although it was efficiently encapsulated in the PDLA. MTDSC confirmed that progesterone did have a plasticisation effect on PDLA, whilst estrone on the other hand did not have a plasticisation effect on PDLA. Progesterone was miscible with PDLA to an extent i.e. 10% w/w. Above this progesterone loading exothermic peaks were observed, indicating crystalline regions and hence progesterone's immiscibility in PDLA. As stated earlier progesterone exists in two different forms; alpha and beta. The starting material (progesterone) before encapsulation was found to be in the alpha form, but as the progesterone loading increased above 30% within the microspheres, both the alpha and beta forms are present within. Estrone on the other hand did not plasticise PDLA, and did not change its physical form upon increasing estrone concentration within PDLA microspheres. Thus the plasticisation of the polymer PDLA only occurs with progesterone and indicates progesterone is only present as a true molecular dispersion at 10% w/w. FTIR spectra of progesterone and estrone loaded PDLA microspheres show no residual solvent (dichloromethane) was present but indicated the presence of
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water within drug loaded PDLA microspheres. In vitro release studies indicate progesterone and estrone are released from PDLA in two stages (biphasic), a fast phase known as the “initial burst” and slow continuous sustained phase. The release of progesterone and estrone from PDLA may have occurred by the dissolution media penetrating into the PDLA microspheres, dissolving the drug (creating pores) which then diffused out into the bulk solution. Also the penetrating dissolution media may hydrate the PDLA polymer causing it to swell and affect the diffusion and transfer of drug (this will be studied further in chapters 5 and 6 where degradation of PDLA micropsheres was monitored by SEM). In vitro release studies demonstrated progesterone loaded PDLA microspheres had a quicker release rate than estrone loaded PDLA microspheres in EPBS, which was due to the amorphous nature of the progesterone PDLA microspheres. As the presence of amorphous material will enhance the dissolution rate and this explains why the lower loadings appear better in vitro for progesterone.

The in vitro release kinetics of progesterone and estrone from PDLA microspheres was identified as being square root of time dependent. Which indicates that the mechanism of drug release is mainly by diffusion. The absence of zero order release suggested that the release rate changed with time. Furthermore, the partial fit to the first order and square root models of progesterone loaded PDLA microspheres constitutes evidence, that progesterone release may be due to the simultaneous operation of more than one release mechanism, whilst estrone loaded PDLA microspheres only fits the square root model suggesting only one release mechanism is occurring namely diffusion. Diffusion controlled release was evident in drug loaded PDLA formulations as a result of the linearity of the release rate as a function of the square root of time (Le Corre et al, 1997). The current investigation has shown that the release mechanisms of steroids from PDLA microspheres are rather complex and depend on many factors, which interfere with each other. Even if incorporated drugs are closely related as in the case of progesterone and estrone, from a
structural point of view, extrapolation from one drug to another should be taken with caution. Considering a particular drug, its release profile is influenced by the relative proportion between the amount of drug dissolved within the matrix as molecular dispersion and the amount of drug present as a particulate dispersion (Le Corre et al, 1997).
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Chapter Five

In vitro degradation of drug free PDLA microspheres in EPBS and PBS
5.1 Introduction

The purpose of this chapter was to study the degradation of PDLA microspheres in vitro. Although a number of studies have been directed towards determining the drug release kinetics from formulated microspheres (Jalil and Nixon, 1990), degradation has not been studied in detail. In general, the degradation rate of aliphatic polyesters has been determined by molecular weight and structure (Reed and Gliding, 1981). More hydrophobic and crystalline polymers exhibit slow degradation rates due to the low degree of hydration of the microspheres, and this is related to the water accessibility of hydrolytically unstable ester linkages (C-O-C) in the polymer backbone. PDLA is known to degrade into natural by-products of the body (Carbon dioxide and water) and is excreted as lactic acid (Section 1.3). It is believed that PDLA microspheres degrade homogeneously (Section 1.8.2) by bulk erosion (Kenely et al, 1987; Deluca et al, 1993). This is due to the large surface area of microspheres, which allows for a faster release of acidic degradation products. In vitro degradation work has been carried out on large (monolithic) devices such as films, rods and disks of PDLA (Li et al; 1990; Li and McCarthy, 1999; Tarvainen et al, 2002). Studies have shown these large devices degrade heterogeneously which leads to surface/centre differentiation, with faster degradation in the centre due to autocatalytic effect of the enclosed acid degradation products of PDLA. Therefore data obtained from small devices such as microspheres cannot be extrapolated from large devices such as films, rods and disks, as the mode of degradation is dependent on size, molecular weight and surface area (Shah, 1992).

The PDLA used in this study was a copolymer (50:50 molar ratio with a $M_w$ of 103,000) of two stereoisomers, D- and L-lactic acid. Due to the amorphous morphology, it degrades much faster than the homopolymer of D- or- L lactic acid, which have crystalline structures. The effect of PDLA molecular weight on the degradation has been examined in previous studies. One study reported that PDLA cylindrical devices having molecular weight range from 1500 to 3500
showed marked differences in degradation profiles (Fukuzaki et al., 1989). Another study showed lower molecular weight (17k) PDLA microspheres degrade faster than high molecular weight (41k) microspheres (Park 1993) and Ying and Shuli (2000) have also shown that the degradation rate of PDLA microspheres is dependent on molecular mass. Normally a decrease in molecular weight lowers the $T_g$. When dry and glassy polymeric microspheres having $T_g$ above the incubation temperature are placed in an aqueous media, water hydrations allow the $T_g$ to shift to the lower temperature (Shah et al., 1992). This is due to the plasticisation effect of water on the polymer. If the $T_g$ is lowered below the incubation temperature, the initial glassy microspheres become rubbery with the hydration, making the polymer chains segments more mobile. However, if the lowered $T_g$ is above the incubation temperature, the hydrated microspheres are still in the glassy state. Thus, it is anticipated that the molecular weight, by influencing the $T_g$ shift plays an important role in the overall degradation profile of PDLA.

In this study, two media were used to compare the hydrolytic degradation behaviour of drug free PDLA microspheres: 0.1 M phosphate buffered solution (PBS) and ethanol phosphate buffered solution (EPBS). The objective of this study was to determine whether PDLA microspheres undergo homogeneous or heterogeneous degradation and investigate the effect PBS and EPBS might have had on the degradation rate. The two media were chosen for the degradation study as EPBS was used during the release studies (Section 4.2.4), whilst PBS was used as a control. This should determine whether the inclusion of ethanol in EPBS affected the degradation rate of PDLA. For procedure and methods of analysis of in vitro degradation study refer to Section 2.2.5. The second aim of the study was to use MTDSC to study the degradation behaviour of PDLA microsphere as a decrease in molecular weight is associated with a decrease in $T_g$ (Shah, 1992). MTDSC was used to monitor the physical state of both the hydrated $T_g$H (analysed straight from incubation in PBS and EPBS without drying) and dehydrated $T_g$D (dried in an oven at 37°C for 5 days after incubation.
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in PBS and EPBS) PDLA microspheres and determine the morphological changes within the polymer. It is important to consider the physical state of the hydrated microparticles since water has been reported to plasticise the polymer; thereby decreasing the T_g. Studies by Craig and Passerini (2001) suggest that if water levels are not controlled the microspheres may inadvertently be introduced into the body in a liquid (rubbery) state, which may lead to microspheres not reaching their desired site of action. The T_g of PDLA microspheres has been correlated to the drug release rate (Aso et al, 1994). Hill et al (1998) have also suggested that the measurement of T_g may be an effective means of characterising the distribution and physical state of drug in PDLA.

5.2 Results and discussion

5.2.1 pH and percentage mass changes of drug free PDLA microspheres

The initial pH values of the release media PBS and EPBS was 7.4. By the end of the 100 days study the pH values of both media had decreased (Figure 5.1 and 5.2). The drug free PDLA microspheres incubated in PBS had on day 100 a pH value of approximately 7.4, whilst the drug free PDLA microspheres incubated in EPBS had a pH value of approximately 5.0 on day 100. The decrease in pH could indicate the onset of hydrolysis and or alcoholysis of PDLA. As described in Section 1.8, PDLA polymer chains are cleaved by hydrolysis, to form monomers of lactic acid. Since hydrolysis leads to acid production a fall in pH with time will occur. This phenomenon was observed by Grizzi et al (1995); Li and McCarthy (1997) and Ying and Shuli (2001) where the pH of the release media consistently decreased with time due to the formation and accumulation of acidic polymer degradation products. These previous studies found pH had a direct relationship with weight loss. As PDLA weight loss increased the pH decreased, indicating release of monomers of lactic acid into the medium.
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Figure 5.1 Changes in percentage mass loss and pH of PDLA microspheres incubated in PBS over 100 days (mean ± SD; n = 3).

Figure 5.2 Changes in percentage mass loss and pH of PDLA microspheres incubated in EPBS over 100 days (mean ± SD; n = 3).
In principle, solubilization of the hydrophobic polymer (PDLA) by a chemical reaction can take place at either a C=O group of the polymer or the polymer backbone (ester bond, C-O-C) (Figure 5.3). Clearly, when the chemical reaction is confined to the C=O group of the polymer, no backbone cleavage takes place and one of the reaction products is a hydrolytically stable, water-soluble polymer. We can also conceive reactions where both the backbone cleavage and side chain groups take place. In this case, the side chain group reaction might be the rapid process that leads to chain solubilisation and hence drug release, whereas the backbone cleavage might be the slow process that allows the ultimate metabolic elimination of the polymer from the body (Heller et al, 1978). PDLA has been reported to solubilise by an ionisation reaction of the carbonyl (C-O) groups (Deluca et al, 1993). Deluca has described the hydrolytic process in four stages:

1. Hydration, which disrupts hydrogen bonds and other hydrophobic force.
2. Initial cleavage of the covalent bonds randomly through the polymer.

Hydration is a nucleophilic addition on to a carbonyl group. An addition implies that two systems combine to form a single entity. The medium chosen, water (PBS) and Ethanol/water (EPBS) are both nucleophiles that donate protons. Ethanol is a strong nucleophile, whilst water is a weak nucleophile. Hence a 1:1 combination of ethanol and water (EPBS) should produce a nucleophile, which is stronger than water. Figure 5.3 shows the chemical reaction that PBS and EPBS have with PDLA.
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Figure 5.3  Solubilisation reaction mechanism for PDLA polymers.

Water acts by protonating the oxygen on the carbonyl group and results in the production of geminal diol (Two hydroxyl groups). Water is a very weak acid, i.e. it is a proton donor.

\[
\text{H}^+ + \text{OH}^- \rightarrow \text{OH}_2
\]

The H\(^+\) does not actually exist in water: H\(^+\) reacts with H\(_2\)O to form hydroxonium ion H\(_3\)O\(^+\).
Chapter 5: In vitro degradation of PDLA microspheres in phosphate buffer solutions

\[ \text{H}^+ + \text{OH}_2 \rightarrow \text{H}_3\text{O}^+ \]

The reaction mechanism generally involves the protonation of the acyl oxygen by the hydroxonium ion (H$_3$O$^+$), which accentuates the positive charge on the acyl carbon making it more attractive to a water molecule. The second step involves water attaching itself to the acyl carbon using a lone pair on the oxygen atom of water making the oxygen atom positively charged. The third and final step involves water pulling off a proton from the oxygen thus restoring the electrical neutrality and production of the geminal diol.

The reaction mechanism for ethanol (alcoholysis) involves the attachment of an alcohol molecule to an acyl carbon using the lone pair electrons from the ethanol's oxygen atom. This makes oxygen positive and displaces the pi electrons of the pi bond onto the acyl oxygen making the atom negatively charged. A second step involves an intramolecular proton exchange between the alcohol oxygen and the acyl oxygen, which results in formation of a hemi acetal. The products of hydrolysis and alcoholysis are soluble in their ionised state. As EPBS is a combination of ethanol and water, there is a possibility that hydrolysis and alcoholysis occur at the same time, which should lead to faster degradation of PDLA as observed in Figures 5.1 and 5.2.

Figures 5.1 and 5.2 also show the mass loss of PDLA over 100 days. By the end of the 100 day study the percentage mass losses of the drug free PDLA microspheres incubated in PBS and EPBS had decreased quite dramatically. Drug free PLA microspheres incubated in PBS had a total percentage mass loss of approximately 30 % (Figure 5.1), whilst the drug free PDLA microspheres incubated in EPBS had a total percentage mass loss of approximately 90 % (Figure 5.2) over 100 days. Thus the percentage mass loss PDLA microspheres incubated in EPBS occurs three times faster than in PBS. This indicated PDLA was solubilising in the degradation media, leading to mass loss. The mass loss is
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less in PBS as only one process of degradation is occurring (hydrolysis); whist in
EPBS the mass loss is due to two processes (hydrolysis and alcoholysis).

There seems to be a relationship between percentage mass loss and pH change
(Figures 5.1 and 5.2). As the percentage mass loss increases the pH decreases
(potentially noticeable in Figure 5.2). Therefore percentage mass loss is inversely
proportional to pH. Both release media are neutral solutions at the beginning of
the study. As the incubation progressed the pH of the EPBS becomes acidic,
suggesting the production of lactic acid from PDLA whilst the pH of the PBS
remains neutral. The studies indicated the pH of EPBS changed more rapidly
than PBS, which suggests the production of lactic acid is more rapid for drug free
PDLA microspheres incubated in EPBS. Overall the results for mass loss and pH
data suggested the drug free PDLA microspheres were hydrolysed and/or
alcoholised.

5.2.2 Scanning electron microscopy of drug free PDLA microspheres
incubated in PBS and EPBS.

Scanning electron micrograph (SEM) studies of the drug free PDLA microspheres
before incubation revealed that the microspheres were spherical and smooth
surfaces with no pores (Figure 5.4). Once the PDLA microspheres were
incubated in PBS, the surface began to change slightly. From day 10 onwards,
the microspheres began to lose their smooth surface, but maintained their
sphericity (Figure 5.5). When PDLA microspheres were incubated in PBS (pH
7.4) over 100 days the smooth surface changed very slightly, but sphericity was
maintained (Figures 5.5 to 5.14). Drug free PDLA microspheres incubated in
EPBS behaved quite differently to drug free PDLA microspheres incubated in
PBS. As soon as the drug free PDLA microspheres were incubated in EPBS the
surface became rough and sphericity was maintained for only 30 days (Figures
5.15 to 5.17). From day 40 to day 70 the drug free PDLA microspheres incubated
in EPBS were not spherical, (i.e. they lost their shape). Rather they comprised large clumps of aggregates with rough surfaces (Figures 5.18 to 5.21). From day 80 onwards the drug free PDLA microspheres incubated in EPBS remained as large clumps of aggregates with very rough surfaces, but large pores began to appear on the surface (Figures 5.22 to 5.24). The SEM studies indicated PDLA microspheres incubated in PBS and EPBS behaved very differently over the 100 days of study. The differences could be due to the different processes that occurred during degradation in PBS (hydrolysis) and EPBS (hydrolysis and alcoholysis). From the mass and pH change study (Section 5.2.1) it was determined the rate of PDLA degradation depended on the degradation medium. As EPBS lead to fast hydrolysis and alcoholysis of PDLA, this will have implications on the release rate of drug from PDLA.

The pH of the drug free PDLA microspheres incubated in EPBS decreased very rapidly and became acidic (Section 5.2.1). The acidity of the medium could be responsible for the rapid change in shape and appearance of PDLA microspheres incubated in EPBS. Although mass loss and pH studies suggest degradation is occurring in drug free PDLA microspheres incubated in PBS and EPBS, it was not seen for empty PDLA microspheres incubated in PBS by SEM. The differences were examined by GPC (Section 5.2.3) to monitor any change in molecular weight, to quantify the extent to which degradation of PDLA occurred.
Figure 5.4 SEM of empty PDLA microspheres prior to incubation in PBS
Figure 5.5  SEM of empty PDLA microspheres in PBS, day 10.
Figure 5.6  SEM of empty PDLA microspheres in PBS, day 20.
Figure 5.7  SEM of empty PDLA microspheres in PBS, day 30.
Figure 5.8  SEM of empty PDLA microspheres in PBS, day 40.
Figure 5.9 SEM of empty PDLA microspheres in PBS, day 50.
Figure 5.10 SEM of empty PDLA microspheres in PBS, day 60.
Figure 5.11  SEM of empty PDLA microspheres in PBS, day 70.
Figure 5.12  SEM of empty PDLA microspheres in PBS, day 80.
Figure 5.13  SEM of empty PDLA microspheres in PBS, day 90.
Figure 5.14  SEM of empty PDLA microspheres in PBS, day 100.
Figure 5.15  SEM of empty PDLA microspheres in EPBS, day 10.
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Figure 5.16  SEM of empty PDLA microspheres in EPBS, day 20.
Figure 5.17  SEM of empty PDLA microspheres in EPBS, day 30.
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Figure 5.18  SEM of empty PDLA microspheres in EPBS, day 40.
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Figure 5.19  SEM of empty PDLA microspheres in EPBS, day 50.
Figure 5.20 SEM of empty PDLA microspheres in EPBS, day 60.
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Figure 5.21 SEM of empty PDLA microspheres in EPBS, day 70.
Figure 5.22  SEM of empty PDLA microspheres in EPBS, day 80.
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Figure 5.23  SEM of empty PDLA microspheres in EPBS, day 90.
Figure 5.24  SEM of empty PDLA microspheres in EPBS, day 100.
5.2.3 Gel permeation chromatography of drug free PDLA microspheres incubated in PBS and EPBS

Sections 5.2.1 and 5.2.2 revealed the possible occurrence of degradation of drug free PDLA incubated PBS and EPBS. The degradative process of PDLA may be described and quantified in several molecular weight terms using gel permeation chromatography (Section 1.8.2). The most common of these terms being, weight average molecular weight \( M_w \), number-average molecular weight \( M_n \), viscosity average molecular weight \( M_v \) and polydispersity (PD) (For definition of terms refer to Section 1.8). Gel permeation chromatography (GPC) is capable of determining these terms. These terms will indicate what state the polymer was in during degradation and will determine the rate at which degradation has occurred. The initial average molecular weight \( M_w \) of drug free PDLA microspheres as determined by GPC was found to be 106,000 \( M_w \pm 12000 \), with a PD of 1.8 compared to the \( M_w \) and PD values obtained from Sigma-Aldrich for the raw material PLA 103,000 \( M_w \pm 1300 \) and 1.5 respectively. PD is a ratio used to represent the broadness of a molecular weight distribution. Polydispersity is the ratio of \( M_n \) to \( M_w \). If the polydispersity is equal to 1, then \( M_n \) equals \( M_w \) and the polymer is said to be monodisperse. In real life, polymers are not truly monodisperse, although polystyrenes (Standards used in GPC; Section 2.3.6) made anionically can come close to having a polydispersity of 1. As \( M_n \) changes with \( M_w \), the polydispersity changes. So PD will always be greater than 1. The results suggest the raw material PDLA had combination of varied molecular weights hence a PD of 1.5. The results obtained also suggest the modified solvent evaporation technique may have increased the PD of PDLA, as the PD increased from 1.5 to 1.8 before incubation in buffered media. The modified solvent evaporation technique exposed PDLA to water during microsphere preparation; therefore there is a possibility that the polymer might have degraded a little due to hydrolysis during preparation. Park (1994) made a similar observation in a study where it was found that solvent evaporation hydrolysed PDLA during microsphere preparation, increasing the PD value.
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Figure 5.25 Time dependent $M_w$, $M_n$, $M_v$ and PD changes of drug free PDLA microspheres incubated in 0.1M PBS at 37°C (mean ± SD; n = 3).

As seen in figure 5.25 the $M_w$ of the drug free PDLA microspheres incubated in PBS decreased with time. The initial $M_w$ of the drug free PDLA microspheres was 106,000 Daltons (Da). On day 100 of the study the $M_w$ had decreased to 50,000 (Da), i.e. less than half the initial molecular weight. The $M_v$ also changed as the molecular weight changed. This is an indication that the empty PDLA microspheres incubated in PBS were undergoing hydrolysis (cleavage of ester bonds) and that as the hydrolysis occurred the viscosity of the PDLA decreased. The PD was initially 1.8 (Figure 5.25), indicative of a fairly varied polymer chain length. The PD stayed at approximately 1.8 till day 40, after which the PD decreased gradually with time to 1.2. Which suggested the drug free PDLA microspheres were nearing the uniformity value of 1 as incubation time in PBS neared 100 days. Park et al (1993) and Chiellini et al (1986) reported that chains of hydrolytically degradable polymer degrade into shorter chains by the degradation, which makes the $M_w$ distribution of the polymer broader. In this
sample the value of PD ($M_w/M_n$) decreased with time of incubation, which suggested the $M_w$ distribution (Figure 5.25) became narrower. The phenomenon is considered to be due to the relatively wide distribution of their polymer chain length at the beginning of the degradation. That is PDLA composing the microspheres originally had a wide distribution of polymer chain length before degradation and as the chains became shorter, water-soluble monomers were produced which were easily released from the PDLA matrix, which led to a decrease in PD. Mogi et al (2000) performed similar work with PLGA polymers and likewise found the PD decreased with incubation time during degradation.

Drug free PDLA microspheres incubated in PBS showed an apparent biphasic degradation profile (Figure 5.25). An initial accelerated phase of 60 days followed by a slower phase between 60 and 100 days. The profile could be interpreted as rapid surface erosion and buffer media penetration into the PDLA microspheres followed by slow ester bond cleavage, i.e. hydrolysis of PDLA ester bonds.

The $M_w$ of the drug free PDLA microspheres incubated in EPBS also decreased with time (Figure 5.26). The initial $M_w$ of the drug free PDLA microspheres decreased from 106,000 Daltons (Da) to 3000 (Da) on day 100 of the study. The $M_v$ also decreased as the $M_w$ decreased. This is an indication that the PDLA microspheres incubated in EPBS underwent degradation (hydrolysis and alcoholysis) and that as the hydrolysis occurred the viscosity of the drug free PDLA microspheres incubated in EPBS decreased. The PD was initially 1.8 (Figure 5.26) and decreased to 1.2 on day 40, after which the PD stayed approximately constant up until the day 100 of the study. This suggests PDLA in EPBS was nearing uniformity as from the 40th day of the study. The drug free PDLA microspheres incubated in EPBS show an apparent biphasic degradation profile (Figure 5.26). An initial accelerated phase of 40 days followed by a final but slower accelerated phase between 40 and 100 days. The profile could be interpreted as rapid surface erosion and fast buffer media penetration into the PDLA microspheres followed by slow bond cleavage.
The GPC results suggest that when drug free PDLA microspheres are incubated for 100 day in PBS and EPBS degradation is biphasic. GPC results also suggest that as $M_w$ decreased faster than $M_n$, which indicated hydrolysis was mainly by backbone cleavage as suggested by Heller et al, (1978). Overall GPC results confirm that degradation of PDLA occurred during the 100 days of study. GPC results also suggest the two-buffered media hydrolyse PDLA differently, with EPBS degrading PDLA faster than PBS. This agrees with pH and mass studies where it was suggested that EPBS degraded PDLA by hydrolysis and alcoholysis, whilst PBS degraded PDLA by hydrolysis and could have implications on the rate of release of drug from drug loaded PDLA microspheres. As it was now clear PDLA incubated in PBS and EPBS degraded at different rates, to elucidate the possible reason for this a MTDSC study was undertaken to determine any changes associated with $T_g$ of PDLA, which would indicate the morphological state of PDLA during incubation in phosphate buffer solutions at 37°C.
5.2.4 Modulated temperature differential scanning calorimetry of drug free PDLA microspheres incubated in PBS and EPBS

Drug free PDLA microspheres were incubated in PBS and EPBS for 100 days. During the 100 day study the PDLA microspheres were analysed at 10 day intervals (six batches). The six batches were divided equally, one part dried in an oven for 5 days at 35°C before analysis by MTDSC (dehydrated T_gD), whilst the other part was analysed directly by MTDSC (hydrated T_gH). Drug free PDLA microspheres (1.5 – 4.5 mg) were placed in hermetically sealed pans and heated from -20°C to 140°C at heating rate of 1°C/min.

5.2.4.1 MTDSC of drug free PDLA microspheres incubated in PBS

The initial T_g of the drug free PDLA microspheres was approximately 55°C (Table 5.1 and Figure 5.27). By day 100 the hydrated drug free PDLA microspheres T_g had decreased to 42°C. The fall in T_g of the hydrated (Figures 5.27 and 5.28.) drug free PDLA microspheres became virtually constant from day 20, which could imply that there was a limit to the plasticising effect of PBS on drug free PDLA microspheres over 100 days. The dehydrated empty PDLA microspheres on the other hand showed a small decrease in T_g. The initial T_g was 55°C by the day 100 had decreased to 52°C (Table 5.1 and Figures 5.29 and 5.30). The T_g of the dehydrated empty PDLA microspheres became constant from day 20. The T_g of the dehydrated drug free PDLA microspheres did not revert back to the initial T_g of 55°C after drying for five days in an oven at 35°C. The fall in T_g may be an indication of the onset of PDLA degradation, as a decrease in T_g has been associated with a change in M_w. Park (1994) looked at dried PDLA microspheres after incubation in phosphate buffered medium. He discovered PDLA with different molecular weight have different T_gS, i.e. PDLA polymers with high M_w have high T_g values, whilst PDLA polymers with low Mw have low T_g values. Jamshidi et al (1988) have also observed this phenomenon, where a decrease in T_g is associated with a decrease in M_w, hence degradation.
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Figure 5.27 Total heat flow measured by MTDSC of TgH drug free PDLA microspheres over 100 days incubation in 0.1M PBS, pH 7.4 at 37°C.

Figure 5.28 Heat capacity measured by MTDSC for TgH drug free PDLA microspheres over 100 days incubation in 0.1M PBS, pH 7.4 at 37°C.
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Figure 5.29 Total heat flow measured by MTDSC $T_g$D drug free PDLA microspheres over 100 days incubation in 0.1M PBS, pH 7.4 at 37°C.

Figure 5.30 Heat capacity measured by MTDSC for $T_g$D drug free PDLA microspheres over 100 days incubation in 0.1M PBS, pH 7.4 at 37°C.
Chapter 5: In vitro degradation of PDLA microspheres in phosphate buffer solutions

Table 5.1  \(T_g\) of drug free PDLA microspheres incubated in PBS at 37°C (mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Days</th>
<th>PDLA microspheres incubated in PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(T_g) hydrated (°C)</td>
</tr>
<tr>
<td>0</td>
<td>55.33±0.42</td>
</tr>
<tr>
<td>10</td>
<td>51.42±0.56</td>
</tr>
<tr>
<td>20</td>
<td>40.88±0.04</td>
</tr>
<tr>
<td>30</td>
<td>40.99±0.26</td>
</tr>
<tr>
<td>40</td>
<td>40.81±0.33</td>
</tr>
<tr>
<td>60</td>
<td>40.61±0.38</td>
</tr>
<tr>
<td>70</td>
<td>40.48±0.67</td>
</tr>
<tr>
<td>80</td>
<td>42.50±0.37</td>
</tr>
<tr>
<td>90</td>
<td>42.71±0.34</td>
</tr>
<tr>
<td>100</td>
<td>41.30±1.01</td>
</tr>
</tbody>
</table>

The results show both the hydrated and dehydrated drug free PDLA microspheres incubated in PBS over 100 the days study were in the glassy state because the \(T_g\) temperature did not fall below 37°C (Table 5.1). In the glassy state, the mobility of the polymer chains are restricted, so high \(M_w\) PDLA microspheres would allow for slow diffusion of PBS into their structures, hence resulting in slow degradation of the polymer PDLA. This agrees with Section 5.2.3 where PDLA microspheres incubated in PBS were observed to have had a slow degradation rate, and lost half its \(M_w\) during the 100 days of incubation. The slow degradation of the drug free PDLA microspheres incubated in PBS was also confirmed in the SEM study (Section 5.2.2), where the PDLA microspheres incubated in PBS maintained their sphericity and smoothness. Hence MTDS further confirms PDLA was relatively stable in PBS over 100 days of incubation, as the polymer remained in the glassy state, i.e. \(T_g\) was above 37°C.
5.2.4.2 MTDSC of drug free PDLA microspheres in EPBS

The initial $T_g$ of the drug free PDLA microspheres prior to incubation in EPBS was determined to be approximately 55°C (Table 5.2). At the end of the 100 days study the drug free hydrated PDLA microspheres incubated in EPBS had a $T_g$ of 3.2°C, whilst the drug free dehydrated PDLA microspheres incubated in EPBS had $T_g$ of 22.3°C (Table 5.2).

Table 5.2 $T_g$ of drug free PDLA microspheres incubated in EPBS at 37°C (mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Days</th>
<th>PDLA microspheres incubated in EPBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_g$ hydrated (°C)</td>
</tr>
<tr>
<td>0</td>
<td>55.33±0.42</td>
</tr>
<tr>
<td>10</td>
<td>36.61±0.98</td>
</tr>
<tr>
<td>20</td>
<td>34.23±0.96</td>
</tr>
<tr>
<td>30</td>
<td>30.94±1.44</td>
</tr>
<tr>
<td>40</td>
<td>13.75±2.62</td>
</tr>
<tr>
<td>50</td>
<td>10.69±1.10</td>
</tr>
<tr>
<td>60</td>
<td>6.79±1.15</td>
</tr>
<tr>
<td>70</td>
<td>3.79±1.19</td>
</tr>
<tr>
<td>80</td>
<td>2.53±1.69</td>
</tr>
<tr>
<td>90</td>
<td>2.72±2.94</td>
</tr>
<tr>
<td>100</td>
<td>3.20±0.86</td>
</tr>
</tbody>
</table>

The results show (Table 5.2, Figures 5.31 to 5.34) the $T_g$ of the both the hydrated and dehydrated drug free PDLA microspheres decreased throughout the 100 day study, unlike the PDLA microspheres incubated in PBS, where the $T_g$ was virtually constant from the day 20 onwards.
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Figure 5.31 Total heat flow measured by MTDSC of T$_4$H drug free PDLA microspheres over 100 days incubation in 0.1M EPBS, pH 7.4 at 37°C.

Figure 5.32 Heat capacity measured by MTDSC for T$_4$H drug free PDLA microspheres over 100 days incubation in 0.1M EPBS, pH 7.4 at 37°C.
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Figure 5.33 Total heat flow measured by MTDSC of TgD drug free PDLA microspheres over 100 days incubation in 0.1M EPBS, pH 7.4 at 37°C.

Figure 5.34 Heat capacity measured by MTDSC for TgD drug free PDLA microspheres over 100 days incubation in 0.1M EPBS, pH 7.4 at 37°C in 0.1M EPBS, pH 7.7 at 37°C.
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The steady decrease in $T_g$ indicates rapid degradation of the polymer PDLA. Table 5.2 indicates that as from 10th day to the end of the 100th day study all the empty hydrated PDLA microspheres had glass transition temperatures below 37°C. Since the $T_g$ temperatures of all hydrated PDLA microspheres were below 37°C, this suggests the PDLA was in the rubbery state. In the rubbery state, the chains are less restricted, which would allow for easier diffusion of EPBS into their structures to cause fast degradation of the polymer chains, i.e. hydrolysis and alcoholsysis of the ester bonds. The $T_g$ of the dehydrated PDLA microspheres stayed above 50°C for 30 days after which the $T_g$ fell below 37°C (Table 5.2 and Figure 5.34). The results suggested that less than 30 days the dehydrated drug free PDLA microspheres were in the glassy state, but as from day 30 onwards the polymer was in the rubbery state. This confirmed the GPC studies (section 5.2.3), which indicated PDLA incubated in EPBS degraded faster than PDLA incubated in PBS.

5.3 Conclusion

The physical analysis (SEM, GPC and MTDSC) revealed the drug free PDLA microspheres incubated in PBS and EPBS degraded over the 100 days of the study. Although the rates of degradation were varied, the degradation was more pronounced for PDLA microspheres incubated in EPBS. Mass loss and pH studies indicated an increase in percentage mass loss was associated with a decrease in pH over the 100 days, which suggested the production of lactic acid from the empty PDLA microspheres. SEM confirmed that the drug free PDLA microspheres, prior to incubation in PBS and EPBS, were spherical and had smooth surfaces. Once the PDLA microspheres were incubated in phosphate buffered solutions their surfaces began to lose their smoothness, but maintained their spherical shape over the 100 days. Whilst the drug free PDLA microspheres incubated in EPBS lost both their spherical shape and smooth surface over the 100 days of the study.
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GPC studies indicate there may be more than one phase of degradation for PDLA microspheres. PDLA microspheres incubated in PBS and EPBS degraded biphasically. Work performed by Reich (1997) suggests several phases of degradation exist and there is some evidence of this in the present study. GPC studies also indicate that the mode is by surface erosion and diffusion of the release medium into the microspheres, which created pores within the microspheres and caused hydrolysis of the ester bonds. MTDSC confirmed the phosphate buffer solutions acted as a plasticiser during incubation. The MTDSC results showed there was a limit to the PDLA microsphere plasticisation in PBS over 100 days of the study ($T_g$ of approximately 40°C, table 5.1), whilst drug free PDLA microspheres incubated in EPBS showed signs of increasing plasticisation throughout the study (Table 5.2).

Hydrolytic degradation is characterised by a reduction in molecular weight, enhanced water uptake and ultimately, weight loss of the polymer. All these events occurred at a temperature, which was below or near the $T_g$ of PDLA. This in turn implied the morphological changes were likely to occur within the polymer whilst hydrolysis is occurring. This was confirmed by SEM of the degraded products, which showed the development of porosity (Figures 5.22 to 5.24) within the degrading the polyester. Although drug free PDLA microspheres incubated in PBS and EPBS degrade at different rates, the study showed the PDLA microspheres decreased in mass steadily throughout the 100 days of study, which implied these microspheres degraded homogenously. The results also implied that the addition of ethanol to the PBS increased the rate of degradation. Therefore we can assume the addition of ethanol to the PBS would have influenced the rate of release and degradation of the drug containing PDLA microspheres.
Chapter Six

In Vitro degradation of drug loaded PDLA microspheres in EPBS
Chapter 6: In vitro degradation of drug loaded PDLA microspheres incubated in EPBS

6.1 Introduction

In vitro degradation studies were performed on PDLA microspheres containing progesterone and estrone at concentrations of 10%, 30% and 50% w/w. This was performed to study whether the presence of drug increased or decreased polymer degradation rate when incubated in EPBS. It has been reported by various authors that the chemical interactions between the entrapped compounds and PDLA based polymers may have a strong effect on the polymer degradation and on the drug release (Vert et al, 1991; Brannon-Peppas, 1997 and Vert, 2000). Neutral drugs can either catalyse or retard polymer degradation rate: a hydrophobic drug tends to work against water uptake and thus decreases the degradation rate, while a hydrophilic drug has an opposite effect on water uptake and degradation rate (Vert et al, 1991). For acidic drugs a faster hydrolysis of ester bonds is found (Vert et al, 1991; Brannon-Peppas, 1997 and Vert, 2000). In the case of basic drugs, contradictory results have been observed: basic drugs have both accelerated (Maulding et al, 1986; Cha and Pitt, 1989) and suppressed (Bodmeier and Chen, 1989; Mauduit et al, 1993 and Miyajima et al, 1998) degradation rates of PDLA based polymers. As progesterone and estrone are both hydrophobic drugs we aim to determine which hypothesis relates to the drug release and degradation of progesterone and estrone loaded PDLA microspheres.

PDLA used in this study was a copolymer (50:50 molar ratio with a $M_w$ of 99,800) of D- and L-lactic acid. The degradation of drug loaded PDLA microspheres was monitored by GPC to determine the initial $M_w$ of the drug loaded PDLA microspheres prior to and during incubation in the EPBS over the 40 days of the study. This would give an indication of what occurred during in vitro release Section 4.2.4. The drug loaded PDLA microspheres were characterised for mass loss, changes in pH and visually by SEM for changes in shape and surface. The preparation methods (Section 2.2.1) and drug-release characterisation techniques (Section 2.2.5) were applied to the drug loaded PDLA microspheres.
6.2 Results and discussion

6.2.1 pH and percentage mass changes of drug loaded PDLA microspheres incubated in EPBS.

The initial pH values of the release medium EPBS was 7.4. By the end of study the pH of the medium EPBS that contained progesterone and estrone loaded PDLA microspheres incubated in EPBS had decreased (Figures 6.1 and 6.2).

![Graph showing changes in % mass loss and pH over time](image)

Figure 6.1 Changes in % mass loss and pH of medium for progesterone loaded PDLA microspheres incubated in EPBS over 40 days (mean ± SD; n = 3).

The percentage mass loss results showed both progesterone (Figure 6.1) and estrone (Figure 6.2) loaded PDLA microspheres gradually decreased in mass over 40 days. All drug loaded PDLA microspheres, lost at least half their initial mass by the end of the study.
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Figure 6.2 Changes in % mass loss and pH of medium for estrone loaded PDLA microspheres incubated in EPBS over 40 days (mean ± SD; n = 3).

By the 40th day, drug loaded PDLA microspheres with progesterone loading of 10, 30 and 50% w/w had lost 51, 51 and 69% mass loss, whilst drug loaded PDLA microspheres with estrone loadings of 10, 30 and 50% w/w had lost 46, 56 and 68% mass loss respectively. These results suggest there was little or no difference in percentage mass loss between progesterone loaded PDLA microspheres and estrone loaded PDLA microspheres. This indicates changing the drug from progesterone to estrone and vice versa, does not increase or decrease percentage mass loss i.e. both model compounds behaved the same when incorporated in PDLA microspheres, in terms of PDLA percentage mass loss. On days 10 and 20 of the study the percentage mass loss of progesterone loaded PDLA microspheres was in the following order 10% w/w > 30% w/w > 50% w/w. This suggested higher drug loads of progesterone retarded the polymer degradation rate on the 10th and 20th day. Hydrophobic drugs tend to work against water uptake and thus decrease the degradation rate (Vert et al, 1991). On days 30 and 40 the percentage mass loss order changed to 50% w/w > 30% w/w > 10% w/w. This suggested two processes had taken place; progesterone
within PDLA had begun to dissolve into the degradation medium and erosion had taken place. From day 10 to 30 the percentage mass loss of estrone loaded PDLA microspheres was in the following order 50% w/w was equal to 10% w/w. On the 40th day, 50% w/w estrone had a 70% mass loss, whilst 10% w/w had lost 46%. 30% w/w estrone had the least percentage mass loss on the 10th and 20th day, but on the 30th day the loss was 53%, which was higher than 50% w/w and 10% w/w drug loadings. No apparent trend was observed for estrone PDLA microspheres, as concentration of drug seemed to have no effect on the percentage mass loss. On the 40th day the order of percentage mass loss for estrone loaded PDLA microspheres was as follows 50% w/w > 30% w/w > 10% w/w, which was similar to progesterone PLA on the 40th day.

A decrease in pH was observed with a decrease in percentage mass loss (Figures 6.1 and 6.2). As described in section 5.2.1, a decrease in pH was associated with a decrease in mass, which indicated hydrolysis and alcoholysis had taken place, i.e. degradation of PDLA. Therefore it is assumed the same event that occurred in drug free PDLA microspheres, occurred in drug loaded PDLA microspheres. The pH value did not become slightly acidic on day 40 as with empty PDLA microspheres incubated in EPBS day 100, but remained neutral. This suggested the degradation of drug loaded PDLA microspheres might be slower than empty PDLA microspheres. By comparing results shown in Figures 6.1 and 6.2 for progesterone and estrone loaded PDLA microspheres with Figure 5.2 (drug free PDLA microspheres) it is seen that on day, 40 that drug free PDLA microspheres had a percentage mass loss of 70%, whilst drug loaded PDLA microspheres with progesterone loadings of 10, 30 and 50% w/w had lost 51, 51 and 69% mass and drug loaded PDLA microspheres with estrone loadings of 10, 30 and 50% w/w had lost 46, 56 and 68% mass. Overall the results suggest incorporation of drug at concentrations of 30% w/w and below retarded mass loss, whilst indicating drug loaded PDLA microspheres degraded during the 40 days of study.
6.2.2 Scanning electron micrographs of progesterone and estrone loaded PDLA microspheres.

Scanning electron micrographs of drug loaded PDLA microspheres prior to and during incubation in EPBS, over 40 days were observed (Figures 6.3 – 6.32). The SEM’s of the drug loaded PDLA microspheres prior to incubation revealed the PDLA microspheres were spherical and that the surface of the microspheres were not smooth, but rough with clumps of drug and or polymer on the surface (Figures 6.3, 6.8, 6.13, 6.18, 6.23 and 6.28).

After ten days of incubation in EPBS the surface of the 10% w/w progesterone loaded PDLA microspheres became rougher, but microspheres were still spherical (Figure 6.4). On day 20 the surface had appeared to become smooth and little pores began to appear on the surface (Figure 6.5). On day 30 the surface appeared slightly rough, with larger pores (Figure 6.6), and on day 40 the surface was rough with dimples (Figure 6.7). These dimples could be pores, about to form. After 10 days of incubation in EPBS the 30% w/w progesterone loaded PDLA microspheres had a large number of pores on the surface (Figure 6.9). On day 20 the surface was smoother with fewer pores, but larger pore sizes (Figure 6.8), on day 30 the surface appeared smooth with more pores (Figure 6.11) and on day 40 the surface was smooth with many of pores (Figure 6.12). After ten days of incubation in EPBS the 50% w/w progesterone loaded PDLA microspheres had a smooth surface, with no pores (Figure 6.14). On day 20, the surface was relatively smooth with a few clumps on the surface, but no pores were observed (Figure 6.15). On day 30, the surface became very smooth and the microspheres were spherical in shape (Figure 6.16) and on day 40 the surface was relatively smooth, and the microspheres had pores on the surface (Figure 6.17).
Figure 6.3 SEM of 10% w/w progesterone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.4  SEM of 10% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.5 SEM of 10% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 20.
Figure 6.6 SEM of 10% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 30.
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Figure 6.7  SEM of 10% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 40.
Figure 6.8 SEM of 30% w/w progesterone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.9  SEM of 30% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.10  SEM of 30% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 20.
Figure 6.11  SEM of 30% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 30.
Figure 6.12  SEM of 30% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 40.
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Figure 6.13 SEM of 50% w/w progesterone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.14  SEM of 50% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.15 SEM of 50% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 20.
Figure 6.16 SEM of 50% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 30.
Figure 6.17 SEM of 50% w/w progesterone loaded PDLA microspheres incubated in EPBS, day 40.
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After ten days of incubation in EPBS the 10% w/w estrone loaded PDLA microspheres were spherical with a rough surface (Figure 6.19). The surface also had aggregates of small microspheres on the surface of the larger microspheres. On day 20, the surface of the 10% w/w estrone loaded microspheres was smoother (Figure 6.20). On day 30 the surface of the microspheres was smooth, but fewer particles were seen on the surface (Figure 6.21). On day 40 the surface of the 10% w/w estrone microspheres was still smooth, but large cavities had appeared on their surface (Figure 6.22). After 10 days of incubation in EPBS, the 30% w/w estrone loaded PDLA microspheres were oval in shape, with many white particles on the surface (Figure 6.24). On day 20 the surface of the microspheres were smoother, with fewer particles on the surface (Figure 6.25). On day 30 the surface of the microspheres was not as smooth as day 20, and a few particles were seen on the surface. Small PDLA microspheres were also seen between the large PDLA microspheres (Figure 6.26). On day 40 the surface of the 30% w/w estrone PDLA microspheres had particles on the surface. These particles appeared to be flatter than those seen on day 30 (Figure 6.27). After ten days of incubation in EPBS, the 50% w/w estrone loaded PDLA microspheres appeared as slightly spherical aggregates with many white particles on their surface (Figure 6.29). On day 20, the surface had become smoother, with no particles on the surface of the microspheres (Figure 6.30). On day 30 the 50% w/w estrone microspheres appeared spherical and the surface was smoother than on day 20. Small microspheres were seen embedded on the large PDLA microspheres (Figure 6.31). On day 40 the surface of the 50% w/w estrone loaded PDLA microspheres was relatively smooth with small particles on the surface. These microspheres lost their sphericity and dimples appeared on the surface (Figure 6.32).
Figure 6.18 SEM of 10% w/w estrone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.19  SEM of 10% w/w estrone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.20  SEM of 10% w/w estrone loaded PDLA microspheres incubated in EPBS, day 20.
Figure 6.21  SEM of 10% w/w estrone loaded PDLA microspheres incubated in EPBS, day 30.
Figure 6.22 SEM of 10% w/w estrone loaded PDLA microspheres incubated in EPBS, day 40.
Figure 6.23 SEM of 30% w/w estrone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.24 SEM of 30% w/w estrone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.25  SEM of 30% w/w estrone loaded PDLA microspheres incubated in EPBS, day 20.
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Figure 6.26 SEM of 30% w/w estrone loaded PDLA microspheres incubated in EPBS, day 30.
Figure 6.27 SEM of 30% w/w estrone loaded PDLA microspheres incubated in EPBS, day 40.
Figure 6.28 SEM of 50% w/w estrone loaded PDLA microspheres prior to incubation in EPBS.
Figure 6.29  SEM of 50% w/w estrone loaded PDLA microspheres incubated in EPBS, day 10.
Figure 6.30  SEM of 50% w/w estrone loaded PDLA microspheres incubated in EPBS, day 20.
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Figure 6.31  SEM of 50% w/w estrone loaded PDLA microspheres incubated in EPBS, day 30.
Figure 6.32 SEM of 50% w/w estrone loaded PDLA microspheres incubated in EPBS, day 40.
The SEM results suggest progesterone and estrone loaded PDLA microspheres behave differently to each other when incubated in EPBS. The SEM results imply the release of progesterone from PDLA microspheres is by two processes, one being erosion of the surface, which lead to dissolution of surface crystals to reveal smooth surface and the other diffusion of the release medium into the PDLA microspheres. Once the medium (EPBS) diffuses into the PDLA microspheres, it begins to dissolve the progesterone and creates channels, which are seen as pores. These pores permit the release of the dissolved progesterone into the external medium. The SEM results suggest that the release of estrone from PDLA microspheres is by one process, which is erosion of the surface (dissolution of surface crystals to reveal smooth surface). No pores were seen for estrone loaded PLA microspheres during the 40 days study, so it is likely that no diffusion took place.

These SEM results also confirm the results of Chapter 4 where progesterone was suggested to be released by two processes which were taking place simultaneously hence created a much quicker drug release than estrone from PDLA microspheres.

6.2.3 Gel permeation chromatography of drug loaded PDLA microspheres incubated in EPBS.

As mentioned in Section 6.2.1 the pH value of the degradation media did not become slightly acidic on the day 40 with drug present (Figures 6.1 and 6.2) as with empty PDLA microspheres, but remained neutral. This suggests the degradation of drug loaded PDLA microspheres might be slower than empty PLA microspheres. This hypothesis was investigated with GPC (see Section 2.3.6 for GPC procedure). Drug loaded PDLA microspheres were monitored by GPC over 40 days. The initial average Mₘₜ of the drug loaded PDLA microspheres ranged from 95000 to 98000 Da (Table 6.1). The Mₘₜ was slightly less than the Mₘₜ of 99,800 provided by Sigma-Aldrich, which suggested that during the modified
solvent evaporation technique the drug loaded PDLA were exposed to water; therefore there is a possibility that the polymer might have degraded a little i.e. hydrolysed during preparation. By the end of the 40 days study the average $M_w$ of progesterone and estrone loaded PDLA microspheres had decreased to less than 25% of their initial values (Table 6.2), indicating degradation. When the % mass loss over 40 days (Figures 6.1 and 6.2) is compared to % $M_w$ loss over the 40 days (Figures 6.33 and 6.34), it was observed that % $M_w$ loss occurred faster than % mass loss. This suggests the large chains within the polymer have been hydrolysed into smaller chains, rapidly decreasing $M_w$.

### Table 6.1 Initial mean $M_w$ of drug loaded PDLA microspheres prior to incubation in EPBS (mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Drug load % w/w</th>
<th>Progesterone loaded PDLA</th>
<th>Estrone loaded PDLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>95,200 ± 2000</td>
<td>98,000 ± 3300</td>
</tr>
<tr>
<td>30</td>
<td>95,000 ± 2700</td>
<td>98,000 ± 1400</td>
</tr>
<tr>
<td>50</td>
<td>96,000 ± 6500</td>
<td>95,000 ± 3000</td>
</tr>
</tbody>
</table>

### Table 6.2 Final mean $M_w$ of drug loaded PLA microspheres after 40 days incubation in EPBS for (mean ± SD; n = 3).

<table>
<thead>
<tr>
<th>Drug load % w/w</th>
<th>Progesterone loaded PDLA</th>
<th>Estrone loaded PDLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20,650 ± 350</td>
<td>17,310 ± 80</td>
</tr>
<tr>
<td>30</td>
<td>20,770 ± 300</td>
<td>29,340 ± 180</td>
</tr>
<tr>
<td>50</td>
<td>21,400 ± 330</td>
<td>19,630 ± 1700</td>
</tr>
</tbody>
</table>

The rate of $M_w$ change of progesterone and estrone loaded PDLA microspheres were compared to each other (Figures 6.33 and 6.34).
Chapter 6: In vitro degradation of drug loaded PDLA microspheres incubated in EPBS

![Graph showing time-dependent Mₚ changes of progesterone loaded PDLA microspheres incubated in 0.1 M EPBS at 37°C (mean ± SD; n = 3).]

Figure 6.33  Time dependent Mₚ changes of progesterone loaded PDLA microspheres incubated in 0.1 M EPBS at 37°C (mean ± SD; n = 3).

![Graph showing time-dependent Mₚ changes of estrone loaded PDLA microspheres incubated in 0.1 M EPBS at 37°C (mean ± SD; n = 3).]

Figure 6.34  Time dependent Mₚ changes of estrone loaded PDLA microspheres incubated in 0.1 M EPBS at 37°C (mean ± SD; n = 3).
Chapter 6: In vitro degradation of drug loaded PDLA microspheres incubated in EPBS

The rate of $M_w$ change was calculated from the slope of the curves i.e. $M_w$/time. The rates of $M_w$ change (Table 6.3) for progesterone and estrone loaded PDLA microspheres over the 40 days of the study were very similar. Therefore it seems that changing the model drug from progesterone to estrone, and its drug loading had very little effect on $M_w$ change of PDLA during the 40 days of incubation in EPBS. But when the rate of $M_w$ change of empty PDLA incubated in EPBS, which was approximately 2400 d$^{-1}$ (Section 5.2.3) was compared to the $M_w$ change of progesterone and estrone loaded PDLA microspheres, it was observed that the rate of $M_w$ change was much less for progesterone and estrone loaded PDLA microspheres. This suggests that the encapsulated model drugs have retarded the $M_w$ change of PDLA to a certain extent when incubated in EPBS for 40 days due to their hydrophobic nature.

Table 6.3  Mean rates of $M_w$ degradation of progesterone and estrone loaded PDLA microspheres during incubation in EPBS.

<table>
<thead>
<tr>
<th>Drug load % w/w</th>
<th>Progesterone loaded PDLA</th>
<th>Estrone loaded PDLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K'$ (d$^{-1}$)</td>
<td>$K'$ (d$^{-1}$)</td>
</tr>
<tr>
<td>10</td>
<td>1820</td>
<td>1970</td>
</tr>
<tr>
<td>30</td>
<td>1780</td>
<td>1770</td>
</tr>
<tr>
<td>50</td>
<td>2002</td>
<td>1890</td>
</tr>
</tbody>
</table>

This agrees with the observations seen in % mass loss data (Section 6.2.1) where little or no difference in percentage mass loss between progesterone loaded PDLA microspheres and estrone loaded PDLA microspheres was noted. Therefore indicating changing the drug from progesterone to estrone and vice versa, did not increase or decrease percentage mass loss.
6.3 Conclusion

The results suggest that the drug loaded PDLA microspheres incubated in EPBS degraded over the 40 days of study. The pH of the medium decreased during the 40 days of study, which indicated PDLA degradation and possible production of lactic acid. The mass loss of the drug loaded PDLA microspheres increased with time. As for the drug free microspheres an increase in mass loss was associated by a decrease in pH. SEM confirmed that the surfaces of the progesterone and estrone loaded PDLA microspheres changed during the 40 days study. SEM also confirmed that the release of progesterone occurred by two processes, whilst the release of estrone apparently occurred by only one process. The two processes for progesterone loaded PDLA microspheres were surface erosion and diffusion, whilst the single process for estrone loaded PDLA microspheres was erosion. Mass loss and GPC studies indicated a decrease in $M_w$ and mass loss with time, which implied bulk degradation process had taken place during the 40 day study. GPC results suggest the rates of $M_w$ change (Table 6.3) for progesterone and estrone loaded PDLA microspheres over the 40 days of the study were very similar to each other. Thus changing the model drug from progesterone to estrone, and drug loading had very little effect on $M_w$ change of PDLA during the 40 days of incubation in EPBS.

The main aim of the study was to determine if the incorporation of the model compounds (progesterone and estrone) increased or decreased degradation of polymer, when compared to drug free PDLA microspheres incubated in EPBS. The results indicate that there was evidence to suggest a difference in degradation rates of PDLA.
General conclusions

The aim of the study was to prepare and characterise drug loaded PDLA microspheres. Chapter 3 described how progesterone and estrone were evaluated for incorporation into PDLA microspheres using a modified solvent evaporation process. The efficiency of the solvent evaporation process was evaluated by calculating drug loads and percentage yields after microspheres were prepared. The experimental drug loads were found to be similar to the theoretical drug loads, which indicated loss of raw material and drug during preparation was minimal.

The solvent evaporation process produced microspheres with VMD in the range of 7 to 22 μm. Compared to the work of Hill et al (1998) and Parikh et al, (1993) the modified process, with a homogenisation step produced much smaller PDLA microspheres. SEM electron microscopy revealed that the microspheres were spherical. The surfaces of drug free PDLA microspheres were found to be smooth with no pores, but upon incorporation and increase of drug load, drug particles appeared on the surface of the once smooth PDLA microspheres. The results suggested that increasing the drug loading had no effect on the particle size of the estrone PDLA microspheres, but an increase in particle size was observed for progesterone loaded PDLA microspheres up to 20% w/w.

The prepared PDLA microspheres were further analysed by TGA to determine the amount of residual moisture. The PDLA microspheres were found to have less than 1% moisture (progesterone 1% and estrone 0.3%). It was important that the moisture content was kept to a minimum as water plasticises and destabilises the polymer, whilst the solvent (dichloromethane) has anaesthetic properties and is toxic in large quantities. Solubility studies indicated progesterone was more soluble than estrone in the chosen release media, so this would have implications in release studies, as estrone would be released slowly from PDLA microspheres.
Chapter 4 used XRPD, MTDSC and FTIR to characterise the physical properties of the model compounds, polymer and microspheres. XRPD revealed that both model compounds gave Bragg's diffractions, which indicated the model compounds were crystalline, whilst the polymer PDLA gave no Bragg's diffraction, but appeared as diffuse halos, which indicated the polymer was amorphous. XRPD also confirmed that the particles present on the surface of progesterone loaded PDLA microspheres with progesterone concentrations above 10% w/w and estrone loaded PDLA microspheres at all loading were drug crystals. XRPD results suggested that at 10% w/w progesterone loading the PLA microspheres formulation was completely amorphous. This indicated that at 10% w/w progesterone, progesterone was miscible with PDLA and was homogeneously dispersed within the microspheres.

MTDSC analysis of the polymer PDLA, steroids and steroids loaded microspheres was performed to characterise the physical state of the polymer and model compounds before and after microsphere preparation. A sharp endotherm was observed for progesterone and estrone at 130°C and 250°C respectively, which corresponded to their melting phase transitions. PDLA did not show a melt transition, but showed a glass transition at 44°C. The MTDSC results for the raw materials reconfirmed the XRPD studies, indicating that the drug compounds were crystalline and the polymer was amorphous. Crystalline compounds show melt transitions, whilst amorphous show glass transitions.

The drug free PDLA microspheres (placebo) was observed to have one thermal event (53°C), which did not correspond to the $T_g$ of the raw material PDLA (44°C). One thermogram was observed for 10% w/w progesterone loaded PLA microspheres. The thermogram observed corresponded to the transition temperature of the placebo PDLA microspheres. But as the progesterone loading increased to 20% w/w and 30% w/w, three thermal events were detected. These events corresponded to the glass transition of the polymer, recrystallisation of progesterone and melt of progesterone. At 40% w/w progesterone, four thermal
events were observed. These events corresponded to the glass transition of the polymer, recrystallisation of progesterone and melts of alpha and beta progesterone. At 50% w/w progesterone, two thermal were observed, the glass transition of the polymer and melt of beta progesterone. The MTDSC results suggested progesterone plasticised the polymer PDLA. Because the onset of the \( T_g \) began to occur earlier, as the progesterone loading increased. Once the progesterone loading increased above 30% w/w, the PDLA did not plasticise any further, but the \( T_g \) reverted back to the initial \( T_g \) of drug free PDLA microspheres. Because progesterone loaded PDLA microspheres showed recrystallisation peaks, this suggests that the drug was in a partially non–crystalline state.

Estrone loaded PDLA microspheres were observed to exhibit two thermal events. The thermal events corresponded to the \( T_g \) of the polymer, and the \( T_m \) of estrone. No recrystallisation peaks were observed for estrone loaded PDLA microspheres. This suggested that at no point did estrone exist in the non–crystalline state. The MTDSC results showed estrone did not plasticise the polymer, indicating that estrone was not miscible with polymer PDLA, although it was entrapped within microspheres. Upon increasing the estrone loading, the \( T_g \) of the polymer did not change, but remained constant.

FTIR analysis was performed on PDLA, steroids and drug loaded microspheres. FTIR was performed in order to determine whether water or dichloromethane was present within the drug-loaded microspheres. Studies revealed the polymer and the raw materials had no water present prior to microspheres preparation. The FTIR spectra of the drug loaded PDLA microspheres showed no signs of dichloromethane being present within these microspheres, but revealed the presence of water. Hence the weight loss from TGA was assumed to be water. TGA revealed the presence of moisture in progesterone and estrone loaded PDLA microspheres to be 1 and 0.3 % respectively.
The in vitro release profile of progesterone and estrone loaded PDLA microspheres was determined by UV analysis. The model compounds were released in two stages, an initial accelerated phase, followed by a slower sustained phase. It has been suggested that the initial accelerated phase could represent the dissolution of drug crystals present on the surface of the PDLA microspheres. Whilst the slower sustained phase represents release of the drug entrapped within the PDLA microspheres. Progesterone was found to be more soluble than estrone in the chosen release media, so it was suggested that during in vitro release the rate at which estrone would be released would be much slower than progesterone and this was demonstrated to be the case.

Four kinetic models were applied to the release data for progesterone and estrone from PDLA microspheres. The zero order kinetic model was found to be inapplicable to the release of both model compounds since the release was non-linear. The in vitro release kinetics of progesterone and estrone from PDLA microspheres was identified as being square root of time dependent. This indicated that the mechanism of drug release was mainly by diffusion. The mode of diffusion for progesterone and estrone from PDLA could be interpreted as the release media eroding the surface and penetrating into the PDLA microspheres, hence dissolving the drug within the microspheres (creating pores) which then diffused out into the bulk solution. The penetrating release media may have hydrated the PDLA polymer causing it to swell and affect the diffusion and transfer of drug. Furthermore the partial fit of progesterone loaded PLA microspheres to the first order and square root models constitutes evidence, that progesterone release may be due to the simultaneous operation of more than release mechanism, whilst estrone loaded PDLA microspheres only fits the square root model suggesting only one release mechanism is occurring.

Chapter 5 described the degradation of the drug free PDLA microspheres incubated in the release media PBS 0.1 M pH7.4 and EPBS 0.1 M pH7.4. The objective was to determine whether the prepared PDLA microspheres underwent
homogeneous or heterogeneous degradation, and compare the effect the addition of ethanol might have had on PDLA microspheres. The pH and the mass loss of the empty PDLA microspheres incubated in PBS and EPBS were monitored for 100 days. The results revealed a relationship existed between pH and mass loss. As the pH decreased the mass loss increased. The decrease in pH was suggested to be due to the production of lactic acid from polylactide, i.e. hydrolysis of the ester bonds occurred during incubation, which in turn produced microspheres of lower molecular weights and mass. These hypotheses were confirmed in the mass loss and GPC results. The mass loss and pH changes of drug free PDLA microspheres incubated in EPBS and PBS were compared to each other. The effect of EPBS on the drug free PDLA microspheres was more pronounced than the drug free PDLA microspheres incubated in PBS. Microspheres incubated in EPBS had a total mass loss of 90%, while microspheres incubated in PBS had a total mass loss of 30%. The pH studies revealed that the pH of the release medium EPBS and PBS decreased during the 100 days of study. Both media started off as near neutral solution, by the end of the 100 days of study the EPBS medium had become acidic, whilst PBS remained neutral. Overall the results for mass loss and pH change suggest the drug free PDLA microspheres were degrading. The decrease in pH suggested the degrading drug free PDLA microspheres by-products (lactic acid) were solublising in the release medium, hence decreasing the pH of the release medium and making it acidic.

The drug free PDLA microspheres incubated in EPBS and PBS were visualised by SEM over the 100 days of study to determine what occurred on the surface of the microspheres during degradation. Prior to incubation, the drug free PDLA microspheres were spherical and smooth with no pores. The SEM studies indicated that when empty microspheres were incubated in PBS (pH 7.4) over 100 days the smooth surface changed very slightly, whilst sphericity was maintained (Figures 5.5 – 5.14). Drug free PDLA microspheres incubated in EPBS behaved quite differently to empty PDLA microspheres incubated in PBS.
As soon as the empty PDLA microspheres were incubated in EPBS the surface became rough, and sphericity was maintained for only 30 days (Figures 5.15 – 5.17). From day 40 to day 70 the empty PDLA microspheres incubated in EPBS appeared as large aggregates with very rough surfaces (Figures 5.18 – 5.21) and from day 80 onwards the microspheres incubated in EPBS developed large pores, which appeared on the surface of the large aggregates.

GPC analysis was performed on the drug free PDLA microspheres prior to incubation. The initial molecular weight was found to be 106,000 Da. After 100 days of incubation the microspheres incubated in EPBS had a molecular weight of 3000 Da, while microspheres incubated in PBS had a molecular weight of 50,000 Da. The results suggest there was a difference in the rate of degradation. This could account for the differences observed during SEM analysis. A decrease in average molecular weight change was associated with decrease in average molecular weight viscosity, which confirmed that the PDLA microspheres underwent degradation during the 100 days of study. The GPC results suggest that when drug free PDLA microspheres are incubated in PBS and EPBS over 100 days the mode of degradation was biphasic. The biphasic mode of degradation could be interpreted as rapid surface erosion and fast buffer penetration into the drug free PDLA microspheres, followed by slow bond cleavage. Although the mode of degradation was biphasic for drug free PDLA microspheres incubated in PBS and EPBS, the rates of degradation differed. The combined mass loss, pH, SEM and GPC studies suggest that during the 100 days of study, the PDLA microspheres incubated in EPBS and PBS steadily decreased in mass and degraded at different rates which implied that these PDLA degraded homogeneously.

The second objective of Chapter 5 was to compare the effect EPBS and PBS might have on the PDLA glass transition, i.e. to what extent did these media plasticise the polymer, and were the effects the same or did they differ. The initial $T_g$ of the empty PDLA microspheres prior to incubation was observed to be 55°C.
After 100 days of incubation in PBS and EPBS, the $T_g$ of hydrated drug free PDLA microspheres was 42°C and 3°C, whilst the $T_g$ of the dehydrated drug free PDLA microspheres was 51°C and 22 °C respectively. The MTDSC results suggest both media plasticised the polymer, but the extent of plasticisation varied. The MTDSC results for hydrated PDLA microspheres incubated in PBS suggest the polymer remained in the glassy state because the $T_g$ did not fall below 37°C, indicating the polymer had restricted mobility during the 100 days of study. MTDSC results for hydrated PDLA microspheres incubated in EPBS suggest the polymer did not remain in the glassy state, but became rubbery, since the $T_g$ fell below 37°C almost instantly, which indicated the polymer had lost its restricted mobility during the 100 days of study. Overall the MTDSC results suggested EPBS plasticised PDLA more dramatically than PBS, which accounts for the rapid rate of degradation of PDLA in EPBS. Thus the rapid degradation of PDLA in EPBS could be responsible for a rapid release of the drugs from PDLA during the in vitro release studies.

Chapter 6 described a detailed study of drug loaded PDLA microspheres incubated in EPBS for 40 days, to give an idea to what extent, degradation occurred during the vitro release study. The pH of the release medium decreased very slightly, which indicated the PDLA microspheres had began to degrade. The pH results also suggest varying the drug load had no effect on the pH of the release medium. Overall the mass loss studies indicated that the drug loaded PDLA microspheres lost at least 50% of their mass over 40 days, which is also evidence of degradation of PDLA. Percentage mass loss results suggest incorporation of model compounds did not increase or decrease the mass loss, when results were compared to the mass loss results of the drug free PDLA microspheres incubated in EPBS. SEM results confirmed release of progesterone from PDLA was by surface erosion and diffusion, whilst the release of estrone from PDLA was by surface erosion over the 40 days of study. GPC studies indicated that the drug loaded PDLA microspheres had decreased to less than 25% of their initial average molecular weight during the 40 days of study. When
General conclusions

Percentage mass was compared to Percentage $M_w$ loss, we observed the $M_w$ change occurred quicker than mass loss. GPC results also suggested changing the model compound from progesterone to estrone had little effect on the $M_w$ change of PDLA during the 40 days incubation period in EPBS.

Overall, this study aimed to develop a better understanding of the preparation of PDLA microspheres, interaction of the polymer and drugs and also the interaction of the microspheres with release media. The studies have indicated it was possible to prepare spherical microspheres with VMD of ranges 7 to 22\(\mu\)m, which had residual water content of 1\% or less. Studies proved the solvent evaporation process to be efficient in entrapping the model compounds, because little raw was lost during microsphere preparation. MTDSC studies implied progesterone plasticised the polymer PDLA, whilst estrone did not. MTDSC studies also implied both release medium plasticised the PDLA microspheres, but extent of plasticisation varied.
Chapter Eight

References
References


References


References


References


Mogi, T., Ohtake, N., Yoshida, M., Chimura, R., Kamaga, Y., Ando, S., Tsukamoto, T., Kakajima, T., Uenodan, H., Otsuka, M., Matsuda, Y., Ohshima,


Radomsky, M., Liu, L., Iwamoto, T., Sustained–release injectable products


References


References


References


References

