ENZYMATIC SYNTHESIS AND EVALUATION OF BIODEGRADABLE POLYESTERS FOR MICROPARTICULATE DRUG DELIVERY

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Thesis is being submitted in partial fulfillment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

School of Pharmacy and Biomolecular Sciences

January 2011

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Dedication

This work is solely dedicated to **Farhana Khan**

With acknowledgement that *"I am who I am just because of You"*

Statement of source declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of higher education. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature.

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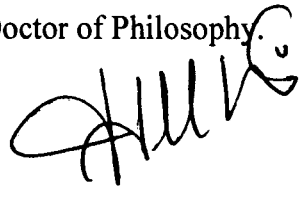


Abid Iftikhar

Acknowledgements

I would like to acknowledge the supervision of Dr Gillian A. Hutcheon, Dr Elsie Gaskell and Dr Christopher Rostron for their help, motivation and guidance throughout the tenure of this project. Especially, regular meetings to keep track of progress and their academic evaluation of the project and feedback were of enormous importance for the on-time completion of this project.

A project only achieves its successful completion after passing through the troughs and crests and facing the calamities of nature and this work is no different. I cannot go ahead without acknowledging and admiring the way, Dr Gillian Hutcheon, supported and helped me, when it was most required – Dr Gillian your "said and unsaid" support made a difference in my life! I sincerely thank my friend Dr Elsie Gaskell who has been guiding and supporting in the true sense. I also owe my gratitude for Gill Broadhurst and Geremy Burgess in the International Student Office for their exemplary help and support during the more difficult times of the project.

I wish to express my esteem gratitude and thanks to Dr Peter Wheeler, Dean, Faculty of Science and Dr Jim Ford Director, School of Pharmacy and Biomolecular Sciences and Institute of Health Research (IHR), LJMU for funding these studies, when it was most required. Also, thanks to Lyn Rostron and Imtiaz ur Rehman for their cooperation.

Finally, I have to say a BIG Thank-You to all my family. I would most notably like to dedicate all my efforts to Farhana Khan, who gave me the confidence to survive the last seven years, and I am thankful for her for remaining with me at every stage. For my parents, whom it was a difficult decision to let me go abroad for higher studies and both my brothers for their silent but unshakeable support.

For those I haven't mentioned personally, thank you for making this expedition a success and turning my dream into reality.

Abstract

Controlled drug delivery via biodegradable polymeric carriers is acknowledged as a means to deliver drugs at desired rates to the target sites in the human body with reduced drug toxicity and longer drug half lives. Although a few polymers have federal drug agency (FDA) approval for such applications, their use is limited as various drugs have individual properties and require different polymeric carriers for optimal encapsulation and release. The aim of the project was to develop a versatile polymeric system with adaptable properties which can be readily tuned to the characteristics of the drug for better encapsulation and improved control over the drug release.

Poly(glycerol adipate) (PGA) and poly(glycerol adipate-co-pentadecalactone) (PGA-co-PDL) were synthesised via enzyme catalysed esterification, using glycerol, divinyl adipate and ω-pentadecalactone as monomers. The evaluation of these polymers for drug delivery applications has previously been reported. In this work incremental changes were introduced in these polymers to alter their physicochemical properties. Various reaction parameters were investigated to understand their effects on the polymer synthesis and a set of reaction conditions were obtained which were applied for the reproducible synthesis of a polyester library in a good yield at a useful scale of 100g.

Functionalised and non-functionalised FDA approved monomeric units were used to impart different physicochemical properties to the polymers, such as, molecular weight, hydrophilicity, melting temperature (T_m), glass transition temperature (T_g) and degradability. It was observed that functionalised monomers such as glycerol, sorbitol and dithiothreitol altered the polymer properties, such as their physical state at room temperature, solubility, hydrophilicity, T_m and T_g values. Additionally these functionalised monomers also provide a site of attachment for post-synthetic modifications of the polymer backbone. Stearic acid and ibuprofen were successfully conjugated to the backbone hydroxyl (-OH) groups, altering the hydrophobicity of the polymer and also considerably slowing the drug release.

The synthesised polymers were formulated into microparticles and evaluated for the encapsulation of drugs via an oil-in-water (o/w) emulsion solvent evaporation technique. Four drugs from different therapeutic classes bearing a wide range of chemistries and properties were encapsulated, namely indomethacin, ibuprofen, rifampicin and levofloxacin (listed in order of increasing water solubilities). It was

observed that minor changes in polymer chemistry, such as the incorporation of glycerol in place of 1,3-propanediol or altering the ratio of monomers, did not show any significant effect on drug encapsulation and release. However, altering the polymer properties by using monomers such as triethylene glycol, polyethylene glycol (PEG) or stearic acid resulted in a considerable improvement in the encapsulation of poorly water-soluble drugs (ibuprofen, indomethacin) and watersoluble drug (levofloxacin). However, the rifampicin, failed to be encapsulated in any of the synthesised polymers via this o/w solvent emulsion technique. The resulting microparticle sizes ranged from 5 to 10μm, which is within the range required for pulmonary drug delivery.

The developed polymeric system was stable under storage in dry conditions and low temperatures with a good shelf life (longer than one year), but degraded completely within six weeks under simulated physiological conditions as required for a degradable drug delivery system.

The results obtained in this study were compared with the most commonly used FDA approved polymer, poly(lactic acid-co-glycolic acid) (PLGA) and it was observed that most polymers in the library exhibited comparable encapsulation efficiency but with better control over the release of the drugs from microparticles. Depending on the polymer and drug chemistry, the drug release profiles showed an initial burst followed by steady release.

In conclusion, a biodegradable polyester-based drug delivery system was developed, which is capable of controlled delivery of three of the drugs tested. Hence, it is potentially a good alternative to the currently available FDA approved polymers.

List of Abbreviations

API	Active pharmaceutical ingredient
BP	British Pharmacopoeia
CAL B	Candida *antarctica* lipase B
$CDCl_3$	Deuterated chloroform
CL	ε-Caprolactone
CPMP	Committee for proprietary medicinal products
Da	Dalton
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO-d_6	Deuterated dimethylsulfoxide
DSC	Differential scanning calorimetery
DVA	Divinyl adipate
FDA	Federal drug agency
FTIR	Fourier transform infra red spectroscopy
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
Ibu	Ibuprofen
ICH	International conference on harmonisation
Indo	Indomethacin
kDa	Kilodalton
Levo	Levofloxacin
lipase CA	Lipase *Candida antarctica*
lipase PC	Lipase *Pseudomonas cepacia*
MEK	Methy ethyl ketone
MeOH	Methanol
mg/ml	Milligram per millilitre
ml	Millilitre
µl	Microlitre
mm	Millimetre
µm	Micrometer
M_n	Number average molecular weight
M_w	Weight average molecular weight
Mw	Molecular weight
NMR	Nuclear magnetic resonance
NSAID	Non steroidal anti inflammatory drugs
o/w	Oil-in-water

List of Abbreviations *(continued)*

PBS	Phosphate buffer saline
PDI	Polydispersity index
PDL	ω-Pentadecalactone
PS	Polystyrene
θ	Contact angle
RH	Relative humidity
RI	Refractive index
Rif	Rifampicin
Rpm	Revolutions per minute
SLF	Simulated lung fluid
TEG	Triethyleneglycol
T_g	Glass transition temperature
THF	Tetrahydrofuran
T_m	Melting temperature
T_c	Crystallisation temperature
UV	Ultraviolet
w/o/o	Water-in-oil-in-oil
w/o/w	Water-in-oil-in-water
w/v	Weight/volume
w/w	Weight/weight

List of Polymer Abbreviations

PLA	Poly(lactic acid)
PCL	Poly(caprolactone)
PLGA	Poly(lactic acid-co-glycolic acid)
PVA	Poly(vinyl alcohol)
PEG	Poly(ethylene glycol)
PGA	Poly(glycerol adipate)
PGA-co-PDL	Poly(glycerol adipate-co-ω-pentadecalactone)
PEGme	Methoxypoly(ethylene glycol)
PPA	Poly(1,3-propanediol adipate)
PBA	Poly(1,4-butanediol adipate)
PTEGA	Poly(triethyleneglycol adipate)
PGA-co-CL	Poly(glycerol adipate-co-ε-caprolactone)
PPA-co-CL	Poly(1,3-propanediol adipate-co-ε-caprolactone)
PBA-co-CL	Poly(1,4-butanediol adipate-co-ε-caprolactone)
PPA-co-PDL	Poly(1,3-propanediol adipate-co-ω-pentadecalactone)
PBA-co-PDL	Poly(1,4-butanediol adipate-co-ω-pentadecalactone)
PTEGA-co-PDL	Poly(triethyleneglycol adipate-co-ω-pentadecalactone)
PSA-co-PDL	Poly(sorbitol adipate-co-ω-pentadecalactone)
PPTA	Poly(1,3-propanedithiol adipate)
PDTTA	Poly(dithiothreitol adipate)
PPTA-co-PDL	Poly(1,3-propanedithiol adipate-co-ω-pentadecalactone)
PDTTA-co-PDL	Poly(dithiothreitol adipate-co-ω-pentadecalactone)
PGA-co-PEG	Poly(glycerol adipate-co-polyethyleneglycol)
PGA-co-PEGme	Poly(glycerol adipate-co-methoxypolyethyleneglycol)
PPA-co-PEG	Poly(1,3-propanediol adipate-co-polyethyleneglycol)
PPA-co-PEGme	Poly(1,3-propanediol adipate-co-methoxypolyethyleneglycol)
PBA-co-PEG	Poly(1,4-butanediol adipate-co-polyethyleneglycol)
PBA-co-PEGme	Poly(1,4-butanediol adipate-co-methoxypolyethyleneglycol)
PGA-co-CL-co-PEG	Poly(glycerol adipate-co-ε-caprolactone-co-polyethyleneglycol)
PPA-co-CL-co-PEGme	Poly(1,3-propanediol adipate-co-ε-caprolactone-co-methoxypolyethyleneglycol)
PGA-co-PDL-co-PEG	Poly(glycerol adipate-co-ω-pentadecalactone-co-polyethyleneglycol)
PGA-co-PDL-co-PEGme	Poly(glycerol adipate-co-ω-pentadecalactone-co-methoxypolyethyleneglycol)
PGA-co-CL-co-PEGme	Poly(glycerol adipate-co-ε-caprolactone-co-methoxypolyethylene glycol)
PPA-co-CL-co-PEG	Poly(1,3-propanediol adipate-co-ε-caprolactone-co-polyethyleneglycol)
PPA-co-PDL-co-PEGme	Poly(1,3-propanediol adipate-co-ω-pentadecalactone-co-methoxypolyethyleneglycol)
PPA-co-PDL-co-PEG	Poly(1,3-propanediol adipate-co-ω-pentadecalactone-co-polyethyleneglycol)
PGA-co-PDL-C18	Poly(glycerol adipate-co-ω-pentadecalactone)-conjugated-stearic acid
PGA-co-PDL-Ibu	Poly(glycerol adipate-co-ω-pentadecalactone)-conjugated-ibuprofen

General Introduction

1.0 BACKGROUND

In pharmaceutical and scientific research, the development of drug delivery systems is an area of interest for researchers around the globe. The reason behind this growing interest lies in the fact that by the end of the last century, the use of antibiotics and other drugs increased several fold, which resulted in an increase in various unwanted side effects such as drug toxicity and the development of drug-resistance. To meet these challenges researchers put more effort into the development of either novel drugs or new drug delivery strategies. There are multiple extensive stages in the development of a new drug molecule, including the clinical trials requirements for the legal or FDA approval before marketing (Carpenter 2002). Hence, this process suffers the drawback of being very tedious and expensive, and so does not always meet the rising challenges and issues described above. Therefore, researchers have focused on the development of alternative drug delivery strategies to overcome issues such as drug toxicity by controlling the release of drugs in the human body. Such approaches involve the design, synthesis and application of materials that deliver the drugs to a targeted site in a safe and efficient manner. This made drug delivery research a broader scientific area, covering various stages of drug delivery, such as the synthesis of new materials, encapsulation and loading of therapeutic agents, targeted delivery and controlled drug release as well as, degradation and elimination of drug carriers from the human body.

1.1 DRUG DELIVERY

Drug delivery covers a broad and very significant area of research. It is defined as a method or process to administer drugs or pharmaceutical compounds in humans or animals to achieve a therapeutic effect. The most common routes of administration of therapeutic agents are oral, intravenous, intramuscular, inhalation and spinal. The main advantages of delivering drugs via these routes are to use a simple method to achieve

the targeted level of therapeutic agent in the subject's body and to get a fast response. Although these methods have been used in practice for a long time, there are some problems associated with these approaches, such as:

- Reduced efficacy of drugs because of partial degradation or shorter half lives
- Drug induced toxicity (by administration of higher doses of drug to overcome the drug loses in the human body)
- Increased costs and patient compliance issues

To solve the inherent disadvantages of classical drug delivery methods, targeted and controlled drug delivery approaches were developed and adapted with many benefits (Langer 1990), such as:

- Targeted delivery of the drug in the amount required to prevent overdosing and to diminish the drug toxicity and increase the drug efficacy
- Delivery of drugs without *in vivo* degradation
- Sustained release of a drug to keep a minimum therapeutic/blood drug level for longer durations without the need for repeated administrations
- Control of the release rates for reduced dosing frequency and increased patient compliance

Controlled drug delivery was first introduced in 1949, when the Wurster technique was developed for the coating of edible tablets (Wurster 1957). Further advances were then made by the development of new methods, such as coacervation (liquid encapsulation technique) (De Jong 1949), microencapsulation (Veis & Aranyi 1960) and pharmaceutical implants (Lilla & Vistnes 1976). Drug targeting was developed in the 1980s by the successful development of transdermal patches and this lead to the development of targeted (site specific) delivery of drugs in the 1990s. Out of all these techniques, microencapsulation received the most recognition and application in the

pharmaceutical industry. Nowadays more than 65% of all current drugs are delivered using some form of micro-encapsulation (Whateley 1992).

Polymeric drug encapsulation or entrapment works by surrounding or suspending the drug molecules in a polymer matrix, and the resulting particles are known as core-shell structures where the core consists of the drug and the shell is made up of polymer (Figure 1.1). This way the polymeric shell interacts with the surrounding physiological environment, while preventing unnecessary exposure of the drug.

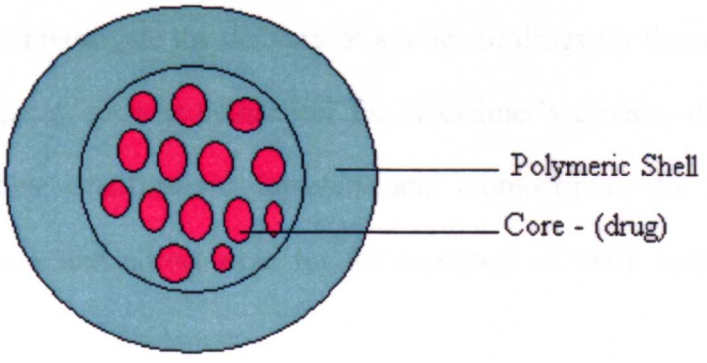


Figure 1.1: *Schematic of a drug loaded microparticle*

Different types of polymers can be used as the matrix for drug encapsulation and entrapment in micro/nanoparticles but biodegradable polymers are generally preferred for such applications.

1.1.1 Polymeric drug delivery

The use of biodegradable polymers in drug delivery applications is advantageous because of their biocompatibility, biodegradability and non-toxicity (Jain 2000). One of the world's first successful, commercial polymer-based controlled drug delivery devices was Ocusert® for the treatment of glaucoma. Ocusert® delivers pilocarpine continuously for 1 week with less side effects than the directly administered drug

(Armaly & Rao 1973). In addition, the anticancer agent doxorubicin was successfully delivered using *N*-(2-hydroxypropyl)methacrylamide polymer, resulting in reduced cardiac toxicity and side effects such as alopecia and nausea, by altering its pharmacokinetics (Zheng et al ; Patil et al 1991; Kline et al 1998).

Applications of polymeric drug delivery systems are not limited to small drug molecules; tetracycline was incorporated into ethylene-vinyl acetate copolymer and other different polymers for the treatment of periodontal disease (Kenawy et al 2002). Successful applications of these polymer-based drug delivery systems opened the door for researchers to investigate the delivery of a range of drugs for the treatment of more complicated diseases, such as bethanecol for Alzheimer's disease, diphosphonates to prevent heart valve calcification, dopamine and bromocriptine for the treatment of Parkinson's disease and nitrosoureas for the treatment of brain tumours (Levy et al 1985).

Many researchers used similar strategies for the delivery of larger and more complicated molecules such as proteins. Initially it was thought that very large molecules could not diffuse out of the polymeric system in a controlled fashion but different animal studies showed that proteins such as insulin (Hu et al 2006) and peptides can be delivered via polymers. The first successful demonstration of this principal was the FDA approval of the Lupron Depot®. This is a poly lactic acid-co-glycolic acid (PLGA) based system for the delivery of leuprolide acetate to treat prostate cancer (Farokhzad & Langer 2006). Applications of these polymers became more widespread and reached the extent where they were used for the delivery of lysozyme (Chang-Moon Lee 2005), genes (Bivas-Benita et al 2004; Convertine et al 2009), DNA (Bivas-Benita et al 2004; Bilati et al 2005), vaccines and antigens (Kirby et al 2008). Hepatitis B surface antigens were also delivered using PLGA (Carpenter 2002). This approach was further exploited by Hershfield et al (1993) to reduce the immunogenic response to certain therapeutic

enzymes by the attachment of polyethylene glycol (PEG) to the enzymes, for example, PEG-uricase, PEG-asparaginase and PEG-adenosine deaminase were all prepared. Some polymers, such as amphiphilic polymers, respond to different physiological signals such as temperature or pH. Hence, these materials received attention for drug encapsulation or release upon an external trigger, which led to improved targeting and controlled delivery of therapeutics (Schmaljohann 2006).

In the last decade, researchers around the globe have attempted the controlled delivery of various drugs, such as ibuprofen, flurbiprofen, cloricromene, diclofenac diethyl ammonium, piroxicam, methylprednisolone, cyclosporine A, orzolamide, pilocarpine, metipronolol, amikacin sulphate, betaxolol chlorohydrate, progesterone, carteolol, hydrocortisone, ganciclovir, gatifloxacin, acyclovir and betamethasone phosphate (Nagarwal et al). Drug molecules have a wide range of physicochemical properties, such as water solubility, hydrophilicity, molecular weight, molecular size and charge. Therefore, depending on the application, their encapsulation in a polymer matrix requires the use of different polymers and various specialised techniques to control the morphology and properties of the resulting microparticles.

1.1.2 Formulation and encapsulation of microparticles

The advantage of using microparticles for the targeted delivery of therapeutics includes their ability to reduce drug diffusion from the targeted site. For example, the controlled release of insulin (Liu et al 2007) and protein delivery for bone growth (Kempen et al 2008). Protecting the sensitive drug substances from the external environment results in increased halflives of the drug molecules. It is well established that for such applications microparticles of sizes ranging from 10-200μm are suitable (Anderson & Shive 1997) because smaller particles would either diffuse away from the site or can be

taken away from the targeted site by phagocytes. The precise properties of polymeric microparticles are usually modified according to the nature of their application. This can be achieved by varying certain variables, such as the:

1. ***Well-defined core-shell architecture:*** This allows the encapsulation of both freely and poorly water soluble drugs and therapeutic agents that are otherwise difficult to administer to the body. The polymeric matrix provides colloidal stability to the whole microparticle structure.
2. ***Size range*** (< 150nm or 1-200μm): Depending upon the application the particles can be formulated in various size ranges. For example for intravenous delivery, particles of about 200nm are formulated to move freely in blood vessels, while for pulmonary delivery particles of sizes greater than 10μm are used.
3. ***Surface area:*** By controlling the size and morphology of microparticles the surface area can be changed which helps to control the rate of drug release.
4. ***Targeting:*** This is also known as the enhanced retention and permeation (EPR) effect which provides some level of selectivity in drug targeting from the size and surface properties (charges) of particles.

1.1.3 Techniques for microparticle preparation

Different techniques can be employed for the encapsulation of therapeutics into biomaterials depending on the nature of the drug and polymer. Table 1.1 summarises the most commonly employed techniques for microencapsulation which can be used depending on the type of polymer, physical state of the matrix and the desired particle sizes. These techniques can be used for encapsulation of either liquid or solid therapeutic agents in the microparticle cores.

Table 1.1

Common techniques for the encapsulation of drugs in polymeric matrices (Gosh 2006)

Physico-mechanical processes are commonly used commercially because of the lower cost and less time consuming factors. For example, spray drying (Figure 1.2a) is already being used for fragrances, oils and flavours while fluid bed coating is the most common technique in the pharmaceutical industry.

Solvent evaporation or solvent emulsion methods are extensively used, along with other physicochemical methods, in drug encapsulation and microparticulate formation studies. Based on the application, the solubility of the drugs and the core material, the solvent evaporation method can be further categorised into the following procedures:

a) Oil-in-water emulsion (o/w)

b) Water-in-oil-in-water emulsion (w/o/w)

c) Water-in-oil-in-oil emulsion (w/o/o)

d) Interfacial disposition

e) Solvent enhanced dispersion

f) Nanoprecipitation

g) Salting-out

The most common emulsion system used for the encapsulation of hydrophobic drugs is oil-in-water (o/w) (Figure 1.2b), with the microspheres being produced by the emulsion solvent evaporation method. Various surfactants, such as Eudragit® and PVA, can be used for improved encapsulation and to stabilise the core-shell architecture. Drug loading is achieved by dissolving the polymer and the drug in an organic solvent followed by subsequent dilution in water and then evaporation of the solvent.

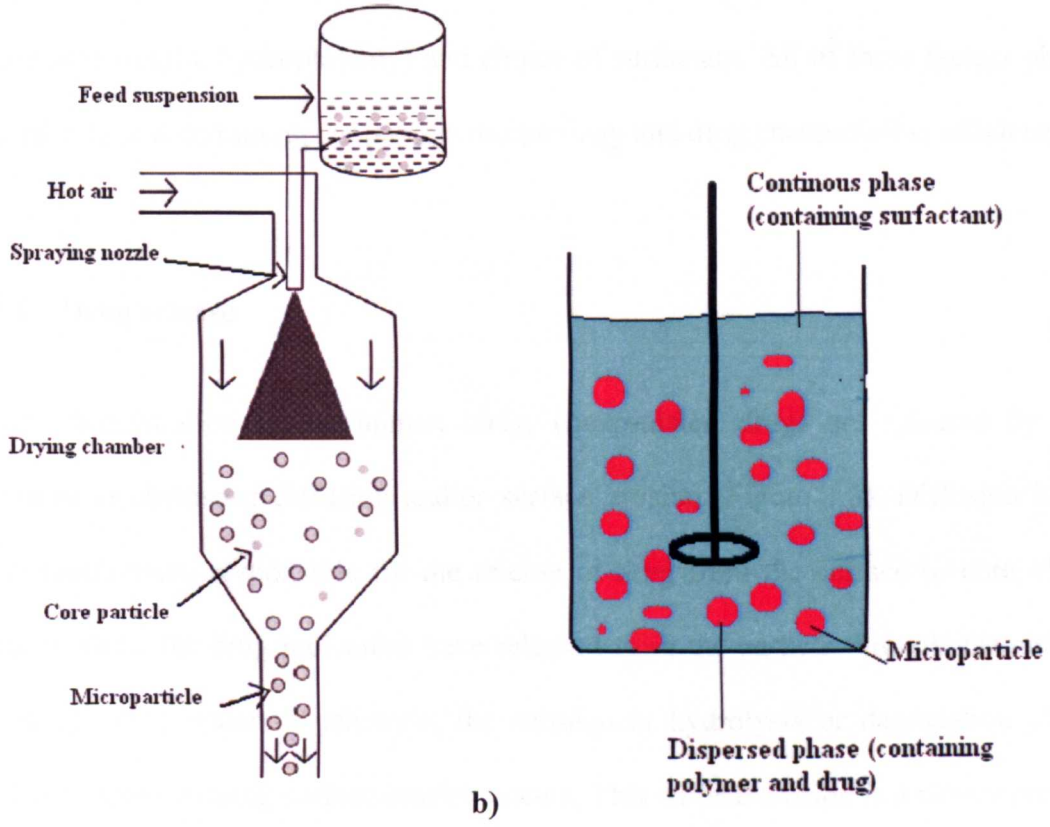


Figure 1.2: *Schematic illustration of a) spray drying technique b) oil-in-water emulsion solvent technique.*

Solvent emulsion is not limited to the encapsulation of small drug molecules, but can also be used for enzyme and protein encapsulation in microparticles. Hydrophilic drugs, proteins and enzymes, being more soluble in water, cannot be effectively encapsulated in polymers via the o/w solvent-emulsion method. To overcome this problem, a double emulsion system, w/o/w solvent-emulsion or spray-drying technique (Figure 1.2a) is usually used.

Factors effecting particle morphology and encapsulation

There are a number of formulation parameters which can affect morphology (size, shape, surface texture) and drug encapsulation in microparticles. These parameters do not only include experimental parameters such as mixing speed, type of solvent and polymer/drug concentration but also cover the effect of polymer/drug chemistry (molecular weight, hydrophilicity) and choice of surfactant. All of these factors play a pivotal role in determining the particle morphology and drug encapsulation efficiency.

1.1.4 Drug release

After administration to the human body, encapsulated drugs are released by two common mechanisms; diffusion and/or surface erosion (Figure 1.3). Diffusion is the early mechanism, responsible for the release of drug from the surface or core of the particle. Once the drug molecules have released from the particle through channels or pores, allowing water to penetrate, the subsequent hydrolysis or degradation of the polymer matrix causing surface erosion occurs. This surface erosion is a slower process and results in further release of the drug at a steady rate over a longer period. To control the release of the drug various physical and chemical properties of both the drug and polymer can be considered; for example their hydrophobic character, lipophilicity, molecular weight, size, pharmacokinetics and biodistribution.

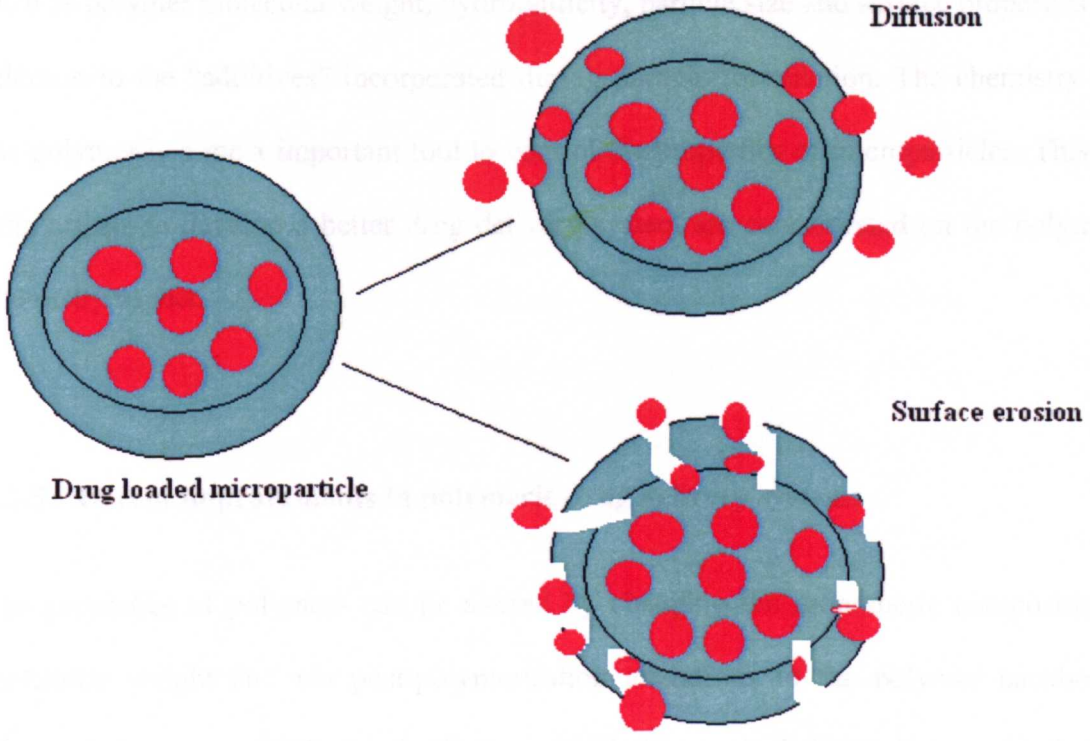


Figure 1.3: *Schematic mechanism of drug release from microparticles.*

FDA approved polymers, poly lactic acid (PLA) and poly lactic-co-glycolic acid (PLGA), are well studied and commonly used in microencapsulation as both polymers are biocompatible and degrade quite easily and quickly (Jain 2000). Another FDA-approved biodegradable polymer used for drug delivery applications is polycaprolactone (PCL), which is quite stable and has comparatively slower degradation rates (Sinha et al 2004). Hence this polymer is also used in controlled release drug delivery applications. Depending on drug chemistry, the drug loading is often low for these FDA-approved polymers (Govender et al 1999). Apart from the low drug loading and encapsulation efficiencies, control over the release of drugs from these polymers is not consistent, as drug release occurs rapidly (known as burst release) with PLA and PLGA while it is much slower with PCL.

A major challenge for researchers in this area is to control the burst release of drugs from microparticles. The drug release from microparticles depends on multiple factors

such as polymer molecular weight, hydrophilicity, particle size and surface properties in addition to the "additives" incorporated during particle formulation. The chemistry of the polymer is a most important tool to control the properties of microparticles. This is why efforts to develop a better drug delivery system are concentrated on the polymer chemistry aspect.

1.1.5 Recent improvements in polymeric drug delivery systems

The properties of polymers can be altered by changing the monomeric composition, molecular weight and via post-polymerisation alterations to the polymer backbone (Pasut & Veronese 2007; Li & Wallace 2008; Noga et al 2008; Kobayashi 2009; Kobayashi 2010). Although FDA approved polyesters have proven useful due to their biodegradability and drug loading, their applications are limited because these polymers lack free functional groups for post-synthetic modifications (Pasut & Veronese 2007).

If the physicochemical properties of the polymers could be tuned for different drugs to encourage better drug-polymer interactions, this would result in an increase in the range of possible applications. Control over drug delivery rates and prolonged bioavailability in the human body can provide added advantages in terms of improved patient compliance, reduced frequency of drug administration, improved consistency of blood drug levels and increased safety of high-potency drugs (Lordi 1986).

Some synthetic polymers are being used in the pharmaceutical industry for packaging, tablet coatings, tablet/syrup formulations as well as for drug delivery (Grover et al 2000). Synthetic polymers are ideal and well established tools for drug delivery because of their versatile composition and compatibility with drugs. The main requirement for polymeric materials for drug delivery applications is that they improve efficacy, are biocompatible, non-toxic and non-immunogenic, result in higher drug loading, slower

drug release and most importantly are able to degrade (biodegradable) and/or are excreted completely by the body without being deposited at any site within the body. Polymeric materials bearing these properties have an improved potential for controlled and targeted delivery of drugs either in the form of microspheres/nanoparticles (Jain 2000; Jhunjhunwala et al 2009), matrices or membranes (Du et al) along with other formulations that can be administered via different routes including parenteral, implantation, pulmonary, oral, inserts and transdermal (Brannon-Peppas 1995; Youn et al 2008).

Some drug formulations based on a limited number of polymeric carriers have received FDA approval while others are still in various stages of clinical trials (Middleton & Tipton 2000; Guerin et al 2004; Distel et al 2005). Basic research into the development of novel polymers and techniques for the development of drug delivery systems is ongoing and aimed at the synthesis of novel biodegradable polymers for improved drug delivery.

1.2 BIODEGRADABLE POLYMERS

Recognition of the important role and use of biodegradable polymers in drug delivery applications has influenced the successful design and synthesis of a range of novel polymeric materials (Greenwald et al 2003; Farokhzad & Langer 2006; Li & Wallace 2008). Some of these are FDA-approved for drug delivery applications and have been successfully marketed (Table 1.2).

Table 1.2

Some pharmaceutical products based on biodegradable polymers marketed in Europe & USA

Commercial product	Polymer	Drug	Manufacturer
Trelstar™ Depot	PLGA	Triptorelin	Pfizer
Decapeptyl® SR	PLA or PLGA	Triptorelin	Ipsen-Beaufour
Decapeptyl®	PLGA	Triptorelin	Ferring
Suprecur® MP	PLGA	Buserelin	Aventis
Nutropin Depot®	PLGA	Growth hormone	Genentech
Sandostatin® LAR	PLGA-glucose	Octreotide	Novartis
Somatuline® LA	PLGA	Lancreotide	Ipsen-Beaufour
Arestin®	PLGA	Minocycline	OraPharma

FDA approval of polymer-based drug delivery systems opened the door for further research into the design and synthesis of new polymeric materials for the development of novel drug delivery systems (Kobayashi 2009). More recently novel biodegradable polymers have been synthesised and evaluated as carriers for improved controlled delivery of drugs (Dutt & Khuller 2001; Jhunjhunwala et al 2009) or as modes of targeted delivery of genes (Bivas-Benita et al 2004) or proteins/enzymes (Dutt & Khuller 2001; Otsuka et al 2003).

There are a number of classes of polymer, currently in use for drug delivery applications, including polyesters, polycarbonates, polyurethane, polyethylene and poly(methyl methacrylate). However, polyesters have received the most attention for drug delivery applications because of their versatility in composition, architecture, compatibility and degradability (Kobayashi 2009; Kobayashi 2010).

1.2.1 Biodegradable polyesters

Among the different classes of polymers, polyesters are widely studied and used as biodegradable drug carriers for controlled drug delivery applications. All polymers approved by FDA are polyesters, such as PLA, PCL and PLGA, and these are the most commonly studied and used polymers for drug delivery applications. Polyesters are versatile biomedical materials used for a range of medical applications such as surgical sutures (Wang et al 2002), absorbable bone plates (Eppley & Reilly 1997), artificial skin (Schmolka 1972), tissue scaffolds (Agrawal & Ray 2001) and carriers of drugs, such as antitumor drugs, antibiotics and hormones (Dorati et al 2007) (Table 1.2). Most of the polyester-based biomaterials currently in use are linear and non-functional, such as PCL, PLA and PLGA (Figure 1.4).

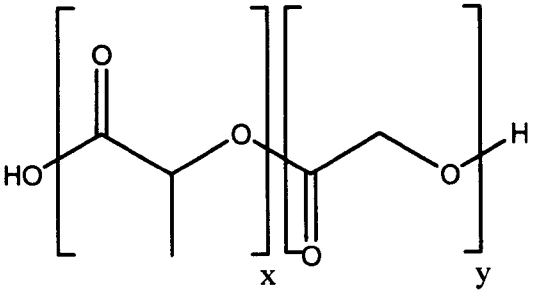


Figure 1.4: *Chemical structure of the FDA approved biodegradable polyester, PLGA poly(lactic acid-co-glycolic acid).*

The incorporation of monomers bearing active functional groups such as carboxylic acids, alcohols, amides and other reactive functionalities can affect existing properties or impart new properties to polyesters including altered biodegradability (Bruggeman et al 2008), increased water solubility (Sumerlin et al 2001), as well as providing sites for further reactions to give hyper-branched polymers (HBPs) (Saha & Ramakrishnan 2009). These properties are useful to widen the applications of polyester-based drug delivery systems, by improving drug loading of hydrophobic and/or hydrophilic drugs,

increasing the rate of degradation and altering drug-release profiles (Anderson & Shive 1997; Bivas-Benita et al 2004; Doan & Olivier 2009).

In addition, by incorporating functional monomers such as polyols into aliphatic polyesters, functional linear- or hyper-branched polymers can be prepared. These polymers are useful for specific biological activities and can be made responsive to environmental stimuli (Wang et al 2002) by altering the polymer's physicochemical properties.

Functionalities in polymers can be introduced by either the addition of free functional groups on the polymer backbone or by altering the type of linkage between monomeric units. For example, the effect of adding functionality into a polymer backbone was observed when polyoxoesters were compared to the corresponding polythioesters. Polythioesters (Figure 1.5) have very different properties compared to their corresponding oxoesters. For example, polythioesters have melting points that are 30-70°C higher compared to their corresponding oxoesters but have similar solubility in organic solvents (Buhrer & Elias 1970). This approach can be successfully utilised to control the properties of the polymeric materials for improved drug delivery.

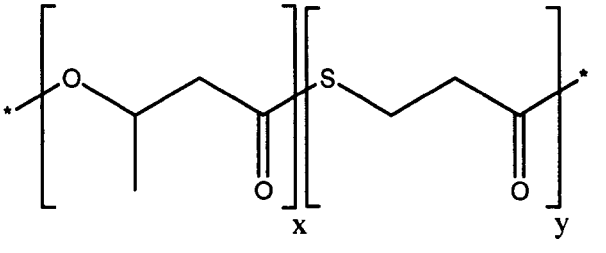


Figure 1.5*: Chemical structure of an example of a polythioester, Poly(3-hydroxybutyrate-co-3-mercaptopropionate) poly(3HB-co-3MP).*

*****: *polymer chain consisting of same repeating units*

1.2.2 Polyester synthesis

For the past two decades, synthetic chemists have been exploring efficient pathways for producing aliphatic polyesters with functional backbone groups (Bhattacharyya et al 1998; Kumar et al 2002; Uyama & Kobayashi 2002; Noga et al 2008).

Conventional synthesis of linear polyesters with active backbone functionalities often involves tedious and difficult multi-step reactions, which may result in the formation of polymer networks. Additionally, the chemical synthesis of biodegradable polymers commonly involves the use of heavy metal-based catalysts such as the oxides of zinc and tin which have been used in the preparation of PLA (Distel et al 2005; Chandure et al 2008). If the resulting materials are to be used for biomedical applications, complex purification steps usually are necessary to remove any contaminant metal catalysts from the final product (Hyon et al 1997). Strongly acidic catalysts used to catalyse condensation reactions tend to promote discoloration and hydrolysis of the reaction products can also occur if the catalyst is not neutralised and separated from the product (Chaudhary et al 1997).

To overcome the challenges and problems of chemical synthesis, a new biocatalytic approach was developed in which metal-based catalysts were replaced by enzymes. Biocatalysis is an alternative approach to the synthesis of both functionalised and non-functionalised linear polyesters. The main advantages of using enzymes as catalysts over conventional approaches are their stereospecificity (enantioselectivity and regioselectivity) and requirement of mild reaction conditions (such as low temperatures and shorter reaction times).

Enzymatic polymerisation often provides an environmentally favourable process, where the starting materials and products are within the natural material cycle (Uyama & Kobayashi 2002). So far, *in vitro* synthesis of not only biopolymers but also non-natural synthetic polymers through enzymatic catalysis has been achieved (Kobayashi et al

1997 ; Uyama & Kobayashi 2002). Additionally, enzymatic polymer synthesis is used for the synthesis of higher molecular weight materials with lower dispersity (Taylor 1997).

Researchers have exploited various classes of enzymes for biotransformation, including, oxidoreductases, transferases, hydrolases, ligases, lyases and isomerases. Notably, out of the above six classes of enzymes, only the first three have shown good potential for polymerisation (Kobayashi 2010). Lipases are hydrolases, and have shown very high potential for polyester synthesis due to its ability to polymerise a large number of substrates (including lactones, esters, carbonates, anhydrides and alcohols) compared to other enzymes (Kobayashi et al 2006).

Effect of various reaction parameters on polymerisation

The intrinsic specificity, stereo-selectivity (Margolin et al 1987) and regioselectivity (Cesti et al 1985; Therisod & Klibanov 1986; Rich et al 1995; Danieli et al 1997) of enzymes depend on different reaction parameters (Sakurai et al 1988) such as substrate, lipase type, enzyme preparation, reaction medium, time and temperature (Hu et al 2006), hence, affecting the overall quality of the resultant polymer. Alterations in the reaction parameters can lead to an overall reduction in the polymerisation rate, the polymer quality (molecular weight) (Chaudhary et al 1997) and polymer degradation.

Lipase Catalysis

Lipases are good catalysts for the esterification of low molecular mass substrates under mild reaction conditions (Therisod & Klibanov 1986) and have also been extensively investigated for the preparation of chiral compounds (Klibanov 1990; Santaniello et al 1992). Lipase derived from *Candida antarctica* has high catalytic activity towards the ring opening polymerisation of lactones (Uyama et al 1997; Kobayashi et al 1998b;

Kobayashi et al 1998a), polycondensation of divinyl esters and glycols (Distel et al 2005), diacids and diols (Uyama et al 1999) and polycarbonate synthesis from diethyl carbonates (Matsumura et al 1999). This lipase is available commercially under the brand name of Novozyme-435. It is an immobilized preparation of a triacylglycerol hydrolase derived from *C. antarctica* fraction B (CAL B). The gene coding for lipase was transferred from *C. antarctica* to the host organism *Aspergillus oryzae*. The enzyme produced by this host organism is further immobilized onto a macroporous acrylic resin. The fermentation extract comprises approximately 10% (w/w) of the total weight of the immobilized resin.

Lipase-catalysed polymerisation provides an environmentally friendly pathway to polyester synthesis due to the non-toxic nature of the enzyme and the mild reaction conditions required. Because of the enantioselectivity of lipases, these enzymes are extensively used for the synthesis of specific optically active isomers. The active sites of lipases contain serine residues and lipase-catalysed polyesterification reactions proceed via an acyl-enzyme intermediate (Uyama & Kobayashi 2002) (Figure 1.6). *C. antarctica* B lipase showed the highest catalytic activity, accepts a wide range of substrates (Sivalingam & Madras 2003) and is often preferred over the other enzymes for vinyl ester transesterification reactions as it is stable in the presence of the acetaldehyde by-product tautomerised from vinyl–OH (Weber & Faber 1997).

Figure 1.6: *Proposed reaction mechanism for lipase catalysed polymerisation of a diester and diol (Uyama et al 1995)*

Activated monomers for lipase catalysed esterification

For lipase-catalysed esterification, divinyl carboxylates/esters and glycols were shown to be effective monomers (Uyama & Kobayashi 1994; Uyama et al 1999; Uyama et al 2000) as their polycondensation took place under mild conditions to produce polyesters with molecular weight of higher than 10,000Da. However, polymer formation was not observed with adipic acid or diethyl adipate under similar reaction conditions (Uyama et al 2000). The use of divinyl adipate (DVA) as the diester substrate provided an irreversible system for performing the polytransesterification reaction because the reaction by-product, vinyl alcohol, rapidly tautomerizes to acetaldehyde, which results in the continuous shifting of the equilibrium in the forward direction (Chaudhary et al 1997) (Figure 1.7).

Figure 1.7: *Lipase catalysed polymerisation of divinyl adipate and propanediol (Uyama et al 1999)*

The reaction of substrates with free –OH groups such as glycerol, is dependent on the relative reaction rate of primary and secondary groups and it is well-known that primary hydroxyls are more reactive (Kline et al 1998). Hence, by using glycerol, polymerisation takes place only via the terminal primary hydroxyl groups, leaving the secondary hydroxyl group free on the polymer backbone. This can lead to the synthesis of functionalised polymers, with altered physicochemical properties, such as hydrophilicity and solubility as compared to the non-functionalised equivalent (Figure 1.8).

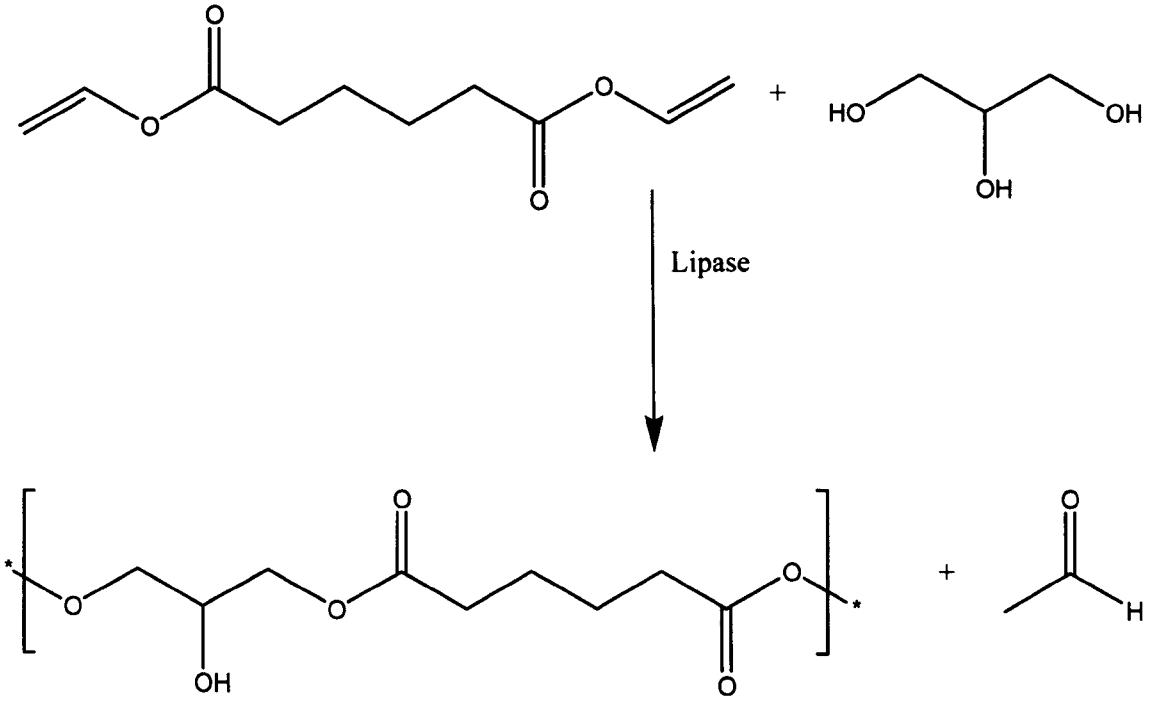


Figure 1.8: *Pendant hydroxyl groups obtained on polymer backbone by esterification of divinyl adipate with glycerol*

Functionalised polymers can be very useful in drug delivery applications either because of their altered physicochemical properties as compared to non-functionalised equivalents or by providing a site for post-synthetic modifications of the polymer backbone, such as the attachment of chemical moieties or drug molecules.

1.2.3 Functionalised polymers

For the synthesis of functionalised polymers, a number of monomers bearing free functional groups, such as hydroxyl, amine or carboxylate can be used (Kobayashi & Uyama 2005). By introducing new monomers into the polymer backbone, not only can new functional groups be introduced, but also the number of functional groups can be varied. For example glycerol can be used to introduce one pendant hydroxyl group on the polymer backbone, but if a sugar is used as a monomer instead of glycerol, it will introduce a higher number of secondary hydroxyl groups and may also make the polymer more biocompatible (Kulshrestha et al 2005). Chemically sugars are polyols in nature and have many free hydroxyl groups, which make polymers more hydrophilic and water-soluble. In addition, upon degradation some sugar-based polymers may enter the metabolic pathway without causing a sudden increase in blood sugar levels and also result in less toxic degradation products (Patil et al 1991; Rich et al 1995; Park et al 2000; Kim et al 2003; Hu et al 2006). Various sugar molecules have been used for polymer synthesis including xylitol (Bruggeman et al 2008), sorbitol (Fu et al 2003; Distel et al 2005; Kobayashi 2009), sucrose (Park et al 2001) and a disaccharide (Miura et al 2004) via chemical and enzymatic routes.

An alternate approach to modify the properties of the polyester backbone is the introduction of cyclic lactones as monomers by enzyme-catalysed ring-opening polymerisation.

Ring opening polymerisation

The synthesis of lactone-containing polymers can be achieved chemically or enzymatically. As the chemical catalytic routes bears the drawbacks of using of toxic catalysts in multi-step reactions, hence, enzymes are preferred for catalysing single-step ring-opening polymerisation under mild conditions (Figure 1.9). Lactones can afford the

polymerisation to homo- (same repeating units) (Figure 1.9) or co-polymers (different repeating units) (Figure 1.10), hence increasing the usefulness of such monomers several fold.

Various research groups have reported the ring opening polymerisation of lactones via chemical catalysis with higher molecular weights and better reaction rates (Chaudhary et al 1998; Kobayashi 1999, 2009). Nonetheless, enzyme catalysis is developing as an alternative synthetic route due to the nature of the polymer application and the advantages it has over the chemical catalysis, as discussed earlier. *C. antarctica* B (Novozyme 435) showed the highest catalytic activity for ring opening polymerisations (Sivalingam & Madras 2003) and is believed to proceed via a similar acyl-enzyme reaction intermediate as for polyester synthesis (Chaudhary et al 1998; Kobayashi 1999).

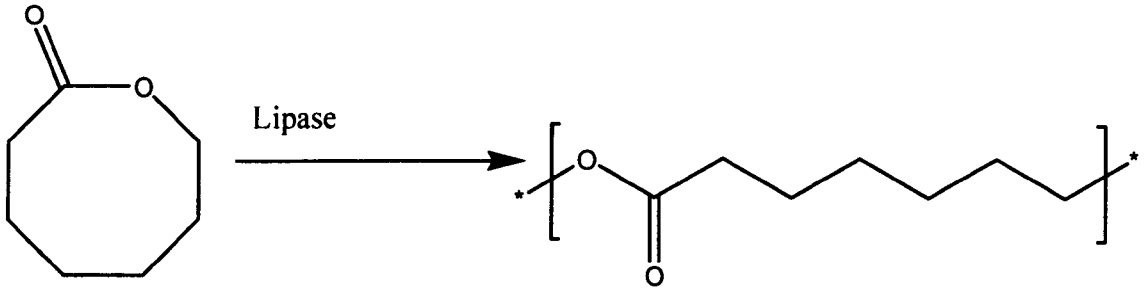


Figure 1.9: *Lipase catalysed ring opening polymerisation of lactone to a homopolymer.*

The reactivity of lactones of various ring sizes has been extensively studied for lipase catalysed synthesis of various homo- and copolymers. It was observed that when using conventional chemical catalysts, the large-sized lactones exhibited lower polymerizability than smaller ring lactones due to their lower ring strain. However, using enzymes resulted in the greater polymerisation of large ring lactones compared to smaller lactones (Chaudhary et al 1998). Another advantage of using alkanoic lactones as monomers is that varying the ring size can be used to control the polymer's hydrophobicity – the larger the ring the more hydrophobic the polymer.

Lipase catalysis is able simultaneously to perform ring-opening and condensation polymerisation (Figure 1.10) in the same pot, leading to a copolymer. Chemical synthesis, however, is devoid of such versatility. This property of the lipase-catalysed synthesis can afford polymers with the same repeating units (homo-polymer) or with varying monomeric units (copolymers). Polymer properties can therefore be tailored by using monomers possessing different chemistries and properties.

Figure 1.10: *Simultaneous ring opening and condensation polymerisation involving a lactone to a copolymer (Kobayashi 2009)*

An alternate approach to alter the backbone chemistry is to introduce different functional groups within the polyester backbone, such as replacing the oxygen in polyoxoesters by sulphur to produce polythioesters.

Polythioesters

Higher melting points, lower aqueous solubilities and higher heat stabilities along with the ability to degrade quickly are important properties which make polythioesters good

candidates for drug delivery applications as compared to their equivalent polyoxoesters. For example, poly(ε-thiocaprolactone) had a 45°C higher melting point than the FDA-approved corresponding oxoester PCL (Kato et al 2005).

Because a direct reaction of diacids as monomers is not possible for the synthesis of polythioesters, acid chlorides and phenol esters are usually used (Buhrer & Elias 1970). The drawbacks with the chemical synthesis of polythioesters are the same as for oxoesters. During the last 2 decades different chemical approaches have been adapted for the synthesis of polythioesters including, interfacial polycondensation (Podkoscielny & Wdowicka 1985), free radical, anionic and cationic ring opening polymerisation (Sanda et al 1999) and ring opening polycondensation (Kato et al 2005). These are too complex for the commercial synthesis of these polymers and perhaps this is why these polymers have not yet been studied for drug delivery applications.

To overcome these synthetic problems, scientists have explored the use of enzymes to catalyse thioesterification reactions (Weber et al 1999; Weber et al 2006) via the direct enzyme-catalysed polycondensation of mercaptoalkanoic acids, dithiols and ring-opening polymerisation of cyclic thioesters (Kato et al 2005, 2006, 2007), which was not possible by chemical catalysis. Kato and co-workers reported the lipase (*C. antarctica*) catalysed synthesis of an aliphatic polythioester with a molecular weight of 34 kDa, which had a 20°C higher melting point than the corresponding oxoester (Kato et al 2005).

As discussed earlier, to be efficient drug carriers polymers are required to bear specific physical and chemical properties. The most important properties are their biocompatibility and lack of immunogenicity. Most of the monomers available do not yet have FDA approval to be consumed in pharmaceutical products; hence research is mainly focused on those monomers which have already been used for either

pharmaceutical applications or as food components, such as poly(ethylene glycol) (PEG).

PEG copolymers

PEG, as a monomer, not only makes polymers more hydrophilic, but also is an FDA-approved polymer for pharmaceutical use. After its first introduction in the 1970s, the attachment of polyethylene glycol to enzyme molecules (process called as PEGylation) became a good strategy for modifying enzymes, without affecting activity (Abuchowski et al 1977). Since then a number of research groups have focussed their work on the utilisation of PEG in drug delivery systems. PEGylation was initially aimed at the preparation of drug/protein conjugates with modified pharmacokinetic profiles (Harris 2001; Jeong et al 2008).

Because PEG is a diol, it can be polymerised with diacids or esters into polyesters. Various research groups have reported PEG copolymerisation with lactones, acids, esters, dioxane and carbonates (Delgado C 1992; Veronese 2001; He et al 2003; Dong & Feng 2004; Chang-Moon Lee 2005; Parrish 2005; Veronese & Pasut 2005; Ghahremankhani et al 2007; Pasut & Veronese 2007; Ganji & Abdekhodaie 2008; Gong et al 2009; Hu et al 2009). The applications of PEG to alter the chemistry of the polymers increased several fold by using functionalised PEGs. This gave researchers an easy route to change polymer properties by exploiting the functional groups for conjugating, attaching or cross linking with various chemical moieties (Jeong et al 2000; Greenwald et al 2003; Chang-Moon Lee 2005). As PEG has terminal hydroxyl groups, it can react either by chemical catalysis (Cho et al 1999; Dong & Feng 2004; Zhou et al 2004), enzyme catalysis (Delgado C 1992; Kumar et al 2002; He et al 2003) or via catalyst-free synthesis (Lin et al 2005), to form a part of a polyester chain.

Many proteins and small drug molecules can easily pass through the kidneys, leading to shorter half lives or can trigger the immune system which results in the generation of

neutralising antibodies. PEGylation also gives a promising solution to these problems (Harris & Chess 2003).

1.2.4 Post-Synthetic modifications in polymers

The presence of free functional groups on the polymer backbone can be used for grafting or conjugating various chemical moieties to modify the properties of the polymers, hence widening their applications. Enzymes are not good catalysts for the modification of polyesters, as they may cause enzymatic degradation of the polymers. The various chemical approaches available also have the potential to degrade the labile ester linkages, via hydrolysis or by the use of heavy metal catalysts (Noga et al 2008). Hence, to overcome this problem, various coupling agents can be used, such as dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), and diisopropylcarbodiimide (DIC) amongst others (Moore & Stupp 1990). Kolhe and co-workers attached various chemical groups and drug molecules, such as ibuprofen, onto polymer pendant hydroxyl groups using DCC (Kolhe et al 2004). This is a good example of modifying polymers to increase the drug payload. This approach not only gives improved control over the release of the drug but also helps to increase the solubility of the drug molecules (Kolhe et al 2004; Namazi et al 2005). Conjugation of drugs to polymers was first introduced in the 1970s when Ringsdorf (1975) attached a range of drug molecules onto a water-soluble polymeric carrier. Based on a similar approach various products have now been developed, such as conjugates of doxorubicin, camptothecin, cyclodextrin, paclitaxel and galactosamine as summarised by Li & Wallace (2008). Pasut and Veronese (2007) have also summarised a list of polymer-drug conjugates which are marketed for clinical trials.

The different approaches discussed above provide routes to develop biodegradable polymeric systems bearing broad chemistries which can be used for controlled delivery of drugs.

1.2.5 Stability and degradation of polymeric drug carrier

Degradability of polymers is another pivotal property for the effective use of these materials as drug carriers and polymers intended for drug delivery should degrade completely under physiological conditions. Formulation developers can alter the polymer chemistry to control the biodegradation rate according to the specific drug delivery applications. For example, PLGA is rapidly biodegradable, which makes it useful in vaccine delivery applications (Wang et al 1990).

Biodegradable polyesters usually degrade by the random hydrolysis of the backbone chain (Kulkarni et al 2007) into diacid and diol units rapidly via "self-catalysis" (Figure 1.11). Some polyesters such as polycaprolactone (PCL) need an external catalyst or an initiator for degradation, resulting in a relatively slower rate of degradation. Hence, PCL is mainly used in tissue scaffolds, but also has uses as drug delivery devices which need very long half lives, for example as sutures marketed under the brand name Monocryl® (Bezwada et al 1995).

Synthetic polyesters have an advantage over other polymers because of the potential in varying their stereochemistry and copolymer ratio, which can allow the degradation rate of the polymers to be tailored to match the application.

Figure 1.11: *Scheme of hyd*

The degradation behaviour of polymeric particles also has been studied widely *in vitro* by either exposing polymeric micro-spheres to an aqueous environment (Schliecker et al 2003; Distel et al 2005; Dorati et al 2007; Fehling et al 2007), different pH buffers (Fehling et al 2007; Partini & Pantani 2007), high temperatures (thermolysis or pyrolysis) (Nur et al 2007), UV and daylight (photolysis) (Feller et al 2007) or enzymatic degradation (Zhao et al 2007). Depending on various chemical properties of the polymer backbone and the presence/absence of functionalities, polymers degrade to different extents under these extreme conditions.

Degradation of polyesters is advantageous for certain drug delivery applications; however this may also result in decreased shelf lives of the materials. The storage of polymers under different environmental conditions namely temperature, light and humidity has the potential to alter the physical and chemical properties of the polymers, leading to a decreased shelf life. Nonetheless, the literature available on the stability and degradation of polymers under storage/shelf conditions, addressing the natural degradation pathway and degradation rates during storage is scarce (Göpferich 1996; Delgado et al 1998).

1.3 AIM OF THE PROJECT

Few polymers have FDA approval and hence commercial applications. Different drugs bear different properties hence, the same polymeric carrier will not always be suitable for the delivery of different types of drugs. Therefore, rather than developing a new system for each drug, it would be easier to design a system which possesses adaptable physicochemical properties making it feasible to "tune-up" the polymeric carrier according to the drug chemistry.

This project aims to develop a library of novel polyesters for potential use in pulmonary drug delivery and investigate the effect of small incremental changes on the encapsulation and release of various drugs from microparticles. The strategy to develop such a system is divided into the following three stages:

a) Polyester Synthesis:

The development of a lipase-catalysed synthetic method is required which can be used for the synthesis of a library of functionalised polyesters under mild reaction conditions and shorter reaction durations. Hence, a library of polyesters with varying physical and chemical properties will be synthesised, characterised and then investigated for improved encapsulation and slower release of drugs of varying chemistry.

b) Drug encapsulation and release

A detailed exploration of microparticle formulation and encapsulation is required to understand and improve the drug load of various therapeutic agents into stable microparticles of desired sizes. One potential advantage of novel polymers would be

their use for pulmonary drug delivery. The release profile of the drugs from polymeric entities will supply the rationale for altered polymer chemistry and would feedback into the polymer synthesis to design polymers having the desired properties.

c) Stability and degradation of polyesters and microparticles

Obtained polyesters should be investigated for their ability to degrade *in vitro* under the simulated physiological (pulmonary) environment. Although the polymers are required to be biodegradable, at the same time they must be stable under normal storage conditions. Measured stability and degradation profiles of the synthesised polymers will feedback into polymer synthesis for the development of stable biodegradable polyesters.

Synthesis and Characterisation of a Library of Polyesters

2.0 BACKGROUND

Polymer chemistry is one of the variables which can be altered to develop materials with the desired physicochemical properties for drug delivery applications.

The presence of active functional groups like carboxylic acids, alcohols, amides and other reactive functionalities impart properties to polyesters including altered biodegradability (Oh et al 2007) and increased water solubility (Sumerlin et al 2001), as well as providing sites for further polymerisations or post-synthetic modifications. Although most polymers have reactive end groups, functionalised polymers can be obtained by synthesising polymers with functional groups distributed along the backbone. For example, sugar molecules have a higher number of free –OH groups, hence many researchers have used these monomers for the synthesis of functional polymers. Some of the commonly used sugar monomers are xylitol (Bruggeman et al 2008), sorbitol (Uyama et al 2000; Fu et al 2003), sucrose (Park et al 2001) and disaccharides and these have been reacted via both chemical and enzymatic routes (Miura et al 2004). Regioselective enzyme catalysis is a useful way of polymerising monomers bearing varying chemistries.

For enzyme-catalysed esterification, divinyl carboxylates and glycols were shown to be effective monomers (Uyama & Kobayashi 1994; Uyama et al 1999; Uyama et al 2000) and the polycondensation reaction took place under mild conditions to produce polyesters with molecular weights greater than 10,000Da, as reported by Kline et al (1998a) for the synthesis of polyglycerol adipate (PGA). Based on the same strategy Namekawa et al (2000) reported the synthesis of polyglycerol adipate-co-pentadecalactone (PGA-co-PDL) by the combined condensation and ring-opening

polymerisation of diacid, diol and lactone monomers, leading to the synthesis of lactone-containing polymers in single-step reactions, which would otherwise require a multi-step chemical reaction (Kumar et al 2000; Namekawa et al 2000; Ceccorulli et al 2005).

In addition to the ring-opening polymerisation of lactones, diacids, diols, esters and their derivatives are very good candidates for lipase-catalysed polymerisation reactions. This makes the application of lipase-catalysed reactions even wider and polyesters with required properties can be synthesised. For example, polyethylene glycol (PEG) is a diol that can be polymerised with diacids or esters into polyesters (Dong & Feng 2004; Ghahremankhani et al 2007). Its presence in the polymer chain imparts certain characteristics to the polymer, such as improved targeted and controlled delivery of therapeutics (Otsuka et al 2003). Lipase catalysis has also shown potential for the synthesis of polythioesters. However, many of these published studies only demonstrate the successful synthesis of such biomaterials on a very small scale, providing proof of principal that these materials can be prepared rather than making them in useful quantities.

Post-synthetic modification of polymers widens the applications of these materials, as various properties can be altered by conjugating new chemical moieties onto the polymer backbone. Various coupling agents, such as dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (Moore & Stupp 1990) can be used for the attachment of molecules to the pendant functional groups on the polymer backbone.

Recently, several papers outlined new routes to develop biodegradable polymeric systems with varying chemistries which have the potential to be used for a broad range of controlled drug-delivery applications (Kobayashi et al 1998; Kobayashi 1999; Uyama et al 1999; Namekawa et al 2000; Kim & Dordick 2001; Kim et al 2003). However, there is a need for the scaling up of these new polymerisation reactions to a useful scale to enable the application of these materials to drug delivery.

2.1 AIMS OF THE STUDY

The aim of this chapter was to extend the studies of Kallinteri et al (2005) and Thompson et al (2006) on PGA and PGA-co-PDL and develop a polyester library with incremental changes in the chemistry of the polymers to obtain materials of different molecular weight, hydrophilicity and melting temperatures by using different functionalised and non-functionalised monomers in varying ratios. The obtained polymers would then be assessed for drug encapsulation and release.

The first objective was to study the effect of various polymerisation reaction parameters, such as the quantity of enzyme used, solvent type and volume, substrate selection; monomer feed ratio and reaction duration, on the enzyme-catalysed polymerisation of divinyl adipate and glycerol and to obtain a good yield of poly(glycerol adipate) (PGA), of the desired molecular weight (8-10 kDa) in useful quantity (~10-100g).

Subsequently the optimal reaction conditions were applied to the synthesis of a library of biodegradable polyesters and polyester-co-lactones for potential use in colloidal drug delivery systems. Monomer type, ratio of lactone (monomer), incorporation of PEG units, type of ester linkage (oxoester or thioester) and increasing the number of pendant –OH functional groups were explored to produce polymers of varying chemistry and functionality. These were later exploited for post-synthetic modifications by the attachment of stearic acid and ibuprofen to the polymer backbone via DCC coupling reactions. The chemical structure and physical properties of the synthesized polymers were studied via nuclear magnetic resonance

(NMR), infrared spectroscopy (IR), gel permeation chromatography (GPC), differential scanning calorimetery (DSC), and contact angle measurements.

2.2 EXPERIMENTAL

2.2.1 Materials

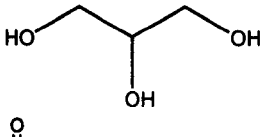
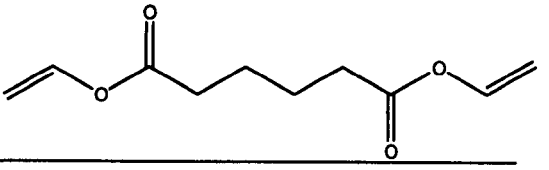
Novozyme 435 (a lipase derived from *Candida antarctica* and immobilised on an acrylic macroporous resin) was purchased from Bio Catalytics, USA and stored over P_2O_5 at 5°C prior to use. Divinyl adipate (DVA) was purchased from Flurochem, UK and used as received. Glycerol, pentadecalactone, caprolactone, 1,3-propanediol, 1,4-butanediol, sorbitol, dithiothreitol, 1,3-propanedithiol, triethyleneglycol, polyethylene glycol 1500Da (PEG_{1500}), methoxy-polyethylene glycol 2000Da ($PEGme_{2000}$) and stearic acid were purchased from Sigma-Aldrich, UK. Ibuprofen was obtained from (BDH). Poly(lactic acid-co-glycolic acid) (PLGA) was obtained from PURAC Biomaterials, The Netherlands. Tetrahydrofuran (THF), dichloromethane (DCM), chloroform, methanol (MeOH), dimethylformamide (DMF) and polystyrene standards were purchased from (Fischer, UK) and deuterated dimethyl sulphoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were obtained from CIL, USA. All glassware was thoroughly dried prior to use.

2.2.2 Polymer synthesis

The effects of different experimental parameters on the synthesis of the simplest polymer in the library, poly(glycerol adipate) (PGA) were studied. A series of enzyme-catalysed polymerisation reactions based on those developed by Kobayashi et al (1999) were carried out adopting standard procedures from Kallinteri et al (2005) for the lipase-catalysed polycondensation of glycerol and DVA (Table 2.1).

Table 2.1

Monomers used to synthesise PGA

Monomer	Molecular weight (g/mol)	Structure
Glycerol	92.09	
Divinyl adipate (DVA)	198.22	

2.2.2.1 Synthesis of PGA

DVA (9.9 g, 0.05 mol) and glycerol (4.6 g, 0.05 mol) were added to a clean, dried three-neck 250 ml round bottom flask, followed by THF (15 ml). The flask was immersed in a water bath maintained at 50°C, to the level of liquid in the flask and allowed to equilibrate for a minimum of 10 minutes. A stirring rod fitted with a Teflon paddle (15x52 mm, Sigma) was placed in the flask, with the stirrer paddle held just above the bottom of the flask (approximately 2-3 mm) to limit crushing of the enzyme support. A mechanical overhead stirrer (Heidolph RZRI stirrer) was secured over the water bath with the stirring rod held in place with a Quickfit thermometer adaptor. The system was also fitted with an open top condenser (to enable release of the acetaldehyde produced as a by-product of the reaction) and the free open neck of the flask stoppered. To such a set-up, 0.5 g (2.5% w/v) of Novozyme 435 was added via the available flask neck and the residual resin washed from the sides of the glassware with an additional 5 ml THF (making the total volume of THF used 20 ml). Stirring commenced at 2000 rpm (setting 6 on the Heidolph RZRI stirrer) and the reaction was allowed to proceed for 24 hours.

After 24 hours stirring was halted. THF was used as the processing solvent (unless stated otherwise). THF (100 ml) was added to the flask, washing any residual polymer off the stirring paddle. Additional THF (50 ml) was added if the suspension was very viscous. The contents of the flask were then vacuum-filtered by standard Buchner filtration through 2 layers of GF/A (Whatman) filters to remove the residual immobilised enzyme and stop any further reaction. The filtrate was poured into a round bottom flask and the solvent removed via rotary evaporation at 80°C (Laborota 4000, Heidolph Instruments attached to Rotavac plug and pump). The flask was then heated to 100°C for 30 minutes using a hotplate.

The resultant viscous polymer sample was transferred, without being cooled down, into a jar and stored overnight in the vacuum oven (60°C) to remove any traces of solvent, then sealed and stored over silica gel in the refrigerator.

2.2.2.2 Effect of reaction conditions on PGA synthesis

To study the effect of various reaction parameters on the polymer synthesis, a series of experiments were performed altering some of the reaction conditions of the standard procedure (2.2.2.1), as summarised in table 2.2.

Table 2.2

Variation in reaction parameters

Reaction Parameters	Changes/Variations
Heating medium	Water bath, hotplate
Stirring mode	Overhead stirrer, magnetic stirrer
Solvent moisture	Hydrous THF*, anhydrous THF
Solvent volume	5, 20, 25, 50 ml
Solvent evaporation	Allowed or restricted
Enzyme concentration	0.34 , 1.34 , 2.5, 3.4 % (w/w)
Reaction Duration	0 - 35 hours

*: Used without drying.

2.2.2.3 Synthesis of the polymer library

A library of novel polymers (list of polymer abbreviations, page xii) with varying chemistries was synthesised (Table 2.3) using the following general method.

General procedure for the synthesis of a library of polyesters

0.05 Molar equivalents of each monomer were added to a round bottom flask, followed by THF (25 ml). The flask was immersed in a water bath maintained at 50°C and equipped with a stirrer paddle. The system was also fitted with an open top condenser. To such a set-up, 1g (3.4% w/v) of Novozyme 435 was added. Stirring

commenced at 2000 rpm and the reaction was allowed to proceed for 24 hours (48 hours for polythioesters).

After 24 hours stirring was halted. THF was used as the processing solvent (unless stated otherwise). The contents of the flask were then vacuum-filtered by standard Buchner filtration through 2 layers of GF/A (Whatman) filters to remove the residual immobilised enzyme and stop any further reaction. The solvent was removed via rotary evaporation. The resultant polymer sample was transferred into a jar and stored overnight in the vacuum oven (60°C) to remove any traces of solvent, then sealed and stored in refrigerator over silica gel.

Purification/washing of the copolymers

To pre-melted copolymer (10 g) in a round bottom flask, MeOH (100 ml) was added and the resulting solution was agitated thoroughly to dissolve unreacted monomers and small molecular weight fragments of polymers. Copolymers insoluble in MeOH were filtered via vacuum filtration using a Buchner filtration. The obtained product was air dried and stored in an air tight container in the refrigerator over 3Å molecular sieves until required.

Table 2.3

Categories of polymers synthesised based on monomeric compositions and chemical classification

Class and Type of polymer		Functionalised*	Non-functionalised*
Polyoxoesters	*Straight chain*	PGA	PPA PBA PTEGA
	Co-lactone	PGA-co-CL PGA-co-PDL	PPA-co-CL PBA-co-CL PPA-co-PDL PBA-co-PDL PTEGA-co-PDL
	Polyol	PSA-co-PDL	-
Polythioester	*Straight chain*	PDTTA	PPTA
	Co-lactone	PDTTA-co-PDL	PPTA-co-PDL
PEG-copolymers	*Straight chain*	PGA-PEG	PPA-PEG PBA-PEG
	Co-lactone	PGA-co-CL-PEG PGA-co-PDL-PEG	PPA-co-CL-PEG PPA-co-PDL-PEG
PEGme-copolymers	*Straight chain*	PGA-PEGme	PPA-PEGme PBA-PEGme
	Co-lactone	PGA-co-CL-PEGme PGA-co-PDL-PEGme	PPA-co-CL-PEGme PPA-co-PDL-PEGme

*: see list of polymer name abbreviations

2.2.2.4 Polymer processing and purification

To ensure that the products were free from any residual monomers and the purification procedure was capable of completely removing unreacted/unattached PEG and small molecular weight fragments, two different purification processes were compared using a physical mixture of monomer (PEG) and polymer (PGA-co-

PDL). Two different solvents, MeOH and water, were tested for their purification abilities via the following procedure.

a) Controlled washing with water:

PGA-co-PDL (13.5 kDa, 0.5 g) was physically mixed with 0.1 g of PEG (1500 Da) in a 100 ml round bottom flasks. Distilled water (8.0 ml) was added and the contents of the flask agitated thoroughly to ensure complete dissolution of soluble contents. This solution was filtered through a 0.8 μm pore size filter (Whatman) to remove the undissolved polymer. The polymer residue was air dried overnight at room temperature and the filtrate containing PEG was freeze dried. Both solid residues were analysed via GPC for qualitative analysis and purity.

b) Controlled washing with methanol:

PGA-co-PDL (13.5 kDa), (0.5g) was physically mixed with either 0.1g of PEG (1500 Da) or PEG-me (2000 Da) in a 100 ml round bottom flask. MeOH (8.0 ml) was added to each round bottom flask and the contents of the flasks were agitated thoroughly to ensure complete dissolution of soluble contents. This solution was filtered through a 0.8μm pore size filter (Whatman) to remove the insoluble polymer. The polymer residue was air dried overnight at room temperature. MeOH from the filtrates containing PEG and PEG-me was removed under vacuum to obtain the solid dried PEG and PEG-me. The PEG, PEGme and polymer residues were analysed via GPC for qualitative analysis and purity.

2.2.3 Post-Synthetic modifications of polymers

2.2.3.1 Polymer-ibuprofen conjugation

In a closed 100 ml round bottom flask, 10 ml of DCC (2.5 mmol, 0.0515 g/ml in DMF) and 10 ml of ibuprofen in DMF (1.25 mmol for 50% and 2.50 mmol for 100% conjugation) were added at room temperature. The reaction mixture was mixed on a magnetic stirrer for 15 minutes. 20 ml of PGA-co-PDL (2.75 mmol, 0.061 g/ml in DMF) was slowly added to the solution while stirring. The round bottom flask was fitted with a drying tube and the reaction mixture stirred at room temperature for 24 hours. After 24 hours, the reaction mixture was filtered through a Whatman filter (GF/A) via vacuum filtration, to remove the by-product, dicyclohexylurea, which was discarded. The filtrate containing the conjugated product underwent rotary evaporation for the complete removal of solvent at 200rpm and 100°C. The obtained waxy solid product was collected and unreacted ibuprofen was removed before analysis using the procedure discussed in following section.

2.2.3.2 Polymer-stearic acid conjugation

For the conjugation of stearic acid to PGA-co-PDL, the same procedure was adopted as described under 2.2.3.1.

Purification of conjugated products

The conjugated material was dissolved in 25 ml DCM to give a clear solution, followed by addition of 20 ml MeOH (as a non-solvent). The solution was left to evaporate over a hot plate until the total volume reduced by half and it became turbid. The solution was allowed to cool to room temperature and filtered using vacuum filtration. The purified product was collected on the filter paper. The

resulting solid, white, powdery material was stored in sealed jars. The drug-polymer conjugate was analysed using GPC, FTIR, DSC and NMR (section 2.2.4).

2.2.4 Analytical procedures

Monomers and polymers were analysed by gel permeation chromatography (GPC), differential scanning calorimetry (DSC), contact angle measurement, nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy while the moisture/water content in the monomers was determined by a Karl Fischer titration.

2.2.4.1 Molecular weight analysis (Gel Permeation Chromatography(GPC))

Gel permeation chromatography (GPC) was used for the determination of molecular weight and molecular weight distribution. A Viscotek GPC system, TDA Model 300 coupled to a gpcMAX integrated solvent and sample delivery module (degasser, pump and auto-sampler) was used with OmniSEC 4.2 software. The system was fitted with two ViscoGEL GMHHR-N columns (also stored in the detector oven at 40°C), using THF (or chloroform for polythioesters) as the eluent with a flow rate of 1 ml/min. The RI detector alignment and instrument sensitivity parameters were previously calibrated using a range of polystyrene standards with different molecular weights. Polystyrene standards and polymer samples were prepared at 10 mg/ml in THF in dry 7 ml glass vials. The solutions were mixed for an hour on a roller-mixer (SRT1, Stuart) to allow the polymers to fully dissolve. The samples were then filtered (0.2 μm PTFE syringe filters, Whatman) into 2.0 ml glass vials. PTFE septa (Sigma) were used in the vial lids to avoid solvent evaporation and clogging up of the injecting needle. 100 μl of polymer solution in THF was injected in all cases. Blank THF samples were run prior to the other samples. Three injections were taken from each vial. Once completed, the peaks were manually identified and the

retention volumes used to determine the molecular weight using a polystyrene standards calibration graph.

2.2.4.2 Thermal analysis (Differential Scanning Calorimetery (DSC))

A TA instrument DSC 2910 calorimeter previously calibrated with an indium standard was used for thermal analysis. Samples weighing 5-10 mg were sealed in aluminium pans, at 25°C and then heated at a rate of 10°C/minute to 90°C followed by cooling to -25°C at the same rate. The same cycle was repeated to observe the changes in thermal properties upon heating/cooling. The melting temperatures (T_m) were obtained in both heating and cooling cycles, while glass transition temperatures (T_g) were calculated as the mid-point of the heat capacity change.

2.2.4.3 Hydrophilic character measurement (Contact angle measurement)

The contact angle measurement technique was used for the measurement of the hydrophilic character of solid polymers. Thin polymer films were placed on the standard stage of the previously-calibrated instrument, Attension (Theta Lite, CAM-101) by KSV instruments. The instrument was linked with a computer and controlled by the Attension one software. A very small aliquot of water (6-10 μl) was dropped on the polymer film via a pre-calibrated syringe needle and photographs of the dropping water droplet were taken until the droplet stabilised on the surface via a built-in digital camera at 16ms frame interval for 60ms. The circular model of drop profile fitting method was applied on these images to measure the contact angle.

2.2.4.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer operated via XWIN-NMR v3.5 and expressed in parts per million (δ) from internal tetramethylsilane. The samples were prepared in 7 ml clear vials at a concentration of

~100 mg/ml in high quality deuterated solvent by dissolving small quantities (approximately 100 mg) of the material in 1 ml chloroform-d containing 0.05% tetramethylsilane. The resulting solutions were then transferred into NMR sample tubes (Wilwad 5 mm NMR tubes, Goss Scientific Instruments Ltd.) and capped. Glycerol, however was dissolved in DMSO-d6 containing 0.05% tetramethylsilane. The Bruker automated program for ^{1}H-NMR (PROTON16) and ^{13}C-NMR were used. The data was collected at 320K using standard Bruker pulse sequences and micro-programs. The ^{1}H spectra were integrated to show the relative ratio of protons.

2.2.4.5 Fourier Transform Infra Red (FTIR) Spectroscopy

FTIR spectra were collected using a Perkin Elmer Spectrum BX spectrometer fitted with a PIKE Technologies MIRacle sampling accessory and using Spectrum v5.0.1 for data storage and processing. The sample was placed directly on the ATR stage and scanned over the range 4000cm^{-1} to 600cm^{-1} with 1cm^{-1} resolution. Each measurement was an average of 16 scans.

2.2.4.6 Karl Fischer Titration

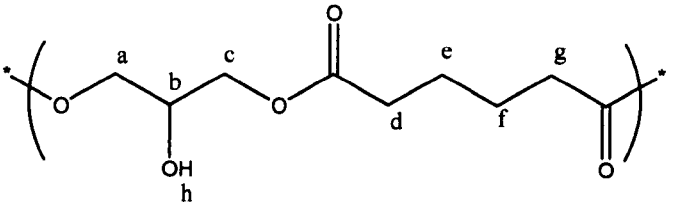
Water content was determined using a Karl Fischer titrator (701 KFT Titrino, 703 Ti Stand Metrohm). The titration vessel was filled with hydranal solvent in order to sufficiently cover the electrode. A syringe was used to transfer 10 µl of distilled water, of known weight, into the vessel and the mixture was stirred and titrated to the amperometric end-point using KF reagent (hydranal composite 5K). The water equivalence factor (F) was obtained in mg/ml and the procedure was repeated with a known weight of monomer/solvent sample. The titer volume (V) of water of the sample was determined and the water content was calculated by multiplying the F with the V value and expressed as percentage of weight of water (% w/w) present in the sample.

2.2.5 Characterisation data

2.2.5.1 Functionalised and non-functionalised linear polyesters

Poly(glycerol adipate) (PGA) – 1:1

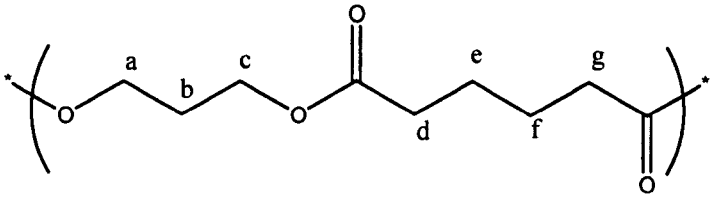
PGA was synthesised by the general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g) in 1:1 ratio and Novozyme (1.0 g).



Pale yellow viscous liquid, monomer composition calculated (1:1), $\theta = 50.10 \pm 5.36$, Mw: 10171Da, IR υ max: 3439.6, 2949.0, 2869.5, 1729.8, 1456.5, 1380.5, 1165.6, 1061.5, 906.7, 753.6, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.69 (s, 4H, H-e, f), 2.39 (s, 4H, H-d, g), 4.18 (m, 4H, H-a, c), 5.2 (s, H, H-h).

Poly(1,3-propanediol adipate) (PPA) – 1:1

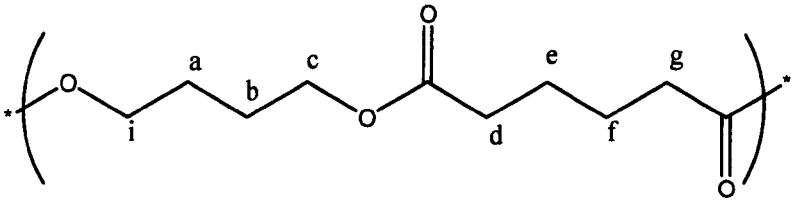
PPA was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g).



Pale yellowish viscous liquid, monomer composition calculated (1:1), Mw: 5387 Da, IR υ max: 2943.1, 2874.4, 1718.4, 1476.3, 1467.4, 1416.8, 1372.4, 1255.0, 1162.4, 1138.7, 1054.5, 1027.5, 946.9, 731.7 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.70 (t, 4H, H-e, f), 1.90 (q, 2H, H-b), 2.35 (t, 4H, H-d, g), 4.18 (t, 4H, H-a, c).

Poly(1,4-butanediol adipate) (PBA) – 1:1

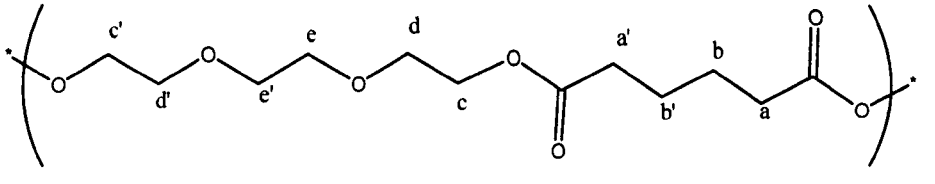
PBA was synthesised by general procedure via the reaction of 1,4-butanediol (0.05 mol, 4.50 g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g).



Pale yellow viscous liquid, monomer composition calculated (1:1), Mw: 7369 Da, IR υ max: 2953.55, 2872.44, 1725.48, 1460.94, 1417.72, 1397.91, 1367.67, 1254.94, 1158.41, 1063.18, 956.52, 908.35, 733.63 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.70 (m, 8H, H-e, f, i, c), 2.37 (t, 4H, H-d, g), 3.68 (t, H-b), 4.19 (t, 2H, H-a).

Poly(triethyleneglycol adipate) (PTEGA) – 1:1

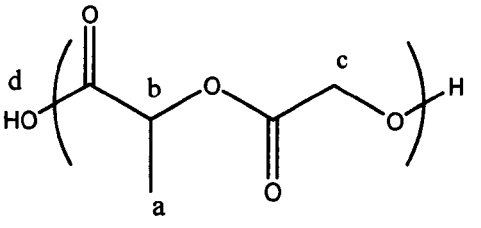
PTEGA was synthesised by general procedure via the reaction of triethylene glycol (0.05 mol, 7.50 g), divinyl adipate (0.05 mol, 9.91 g) in 1:1 ratio and Novozyme (1.0 g).



Pale yellow highly viscous liquid, monomer composition calculated (1:1), Mw: 8785 Da, IR υ max: 2867, 1727, 1453, 1382, 1349, 1242, 1172, 1123, 1064, 948, 860 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.66 (q, 4H, H-b,b'), 1.85 (t, 0.7H, terminal group), 2.35 (t, 4H, H-a, a'), 3.65 (s)-3.69 (t) (9H, H-d, e, d', e'), 4.23 (t, 4H, H-c, c')

Poly(lactic acid-co-glycolic acid) (PLGA) – 1:1

Sample received from PURAC biomaterials.



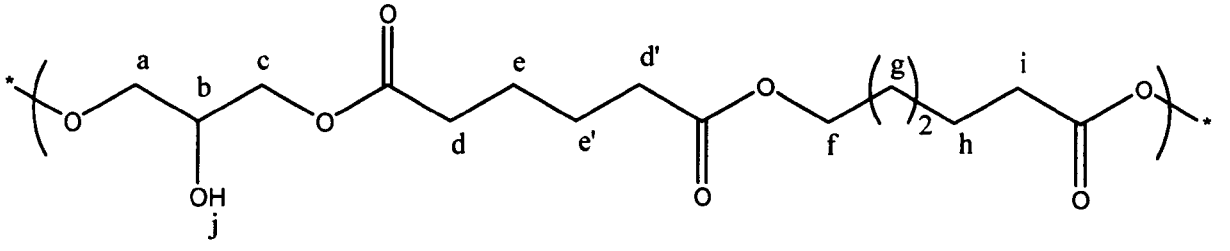
White crystalline solid, monomer composition calculated (1:1), Mw: 9500 Da, θ = 75.81 ± 1.34, T_m = 34.28°C, IR υ max: 3517.27, 2946.66, 1744.43, 1450.74, 1421.98, 1381.58, 1269.63, 1166.05, 1128.70, 1083.77, 845.04, 746.04 cm^{-1}, ^1H-NMR (δ_H $CDCl_3$, 300 MHz): 1.65 (d, 3H, H-a), 4.7 (s, 2H, H-c), 5.25 (q, 1H, H-b).

2.2.5.2 Functionalised and non-functionalised linear polyesters via ring-opening copolymerisation of lactones

a) Co-caprolactone

Poly(glycerol adipate-co-ε-caprolactone) (PGA-co-CL) – 1:1:1

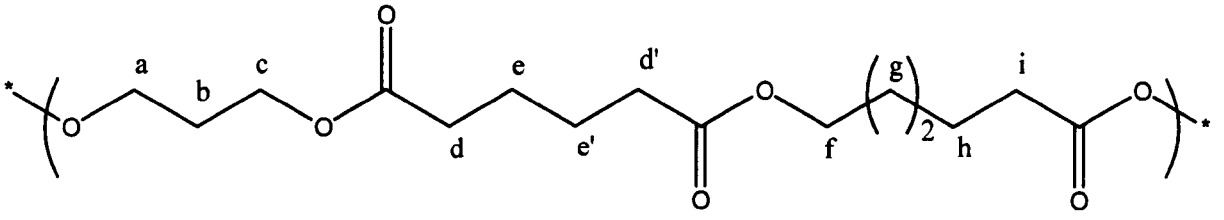
PGA-co-CL was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71 g) and Novozyme (1.0 g).



Highly viscous liquid, monomer composition calculated (1:1:0.7), Mw: 1855 Da, IR υ max: 3446.5, 2937.9, 2865.9, 1726.0, 1456.5, 1387.4, 1162.8, 1050.8, 734.7 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.40 (m)-1.7 (q) (8H, H-e, e', g, h), 2.38 (m, 4H, H-d, d', i), 3.70 (m, 3H, H- f), 4.04 (m, 4H, H-a, b, c), 5.2 (s, H, H-j).

Poly(1,3-propanediol adipate-co-ε-caprolactone) (PPA-co-CL) – 1:1:1

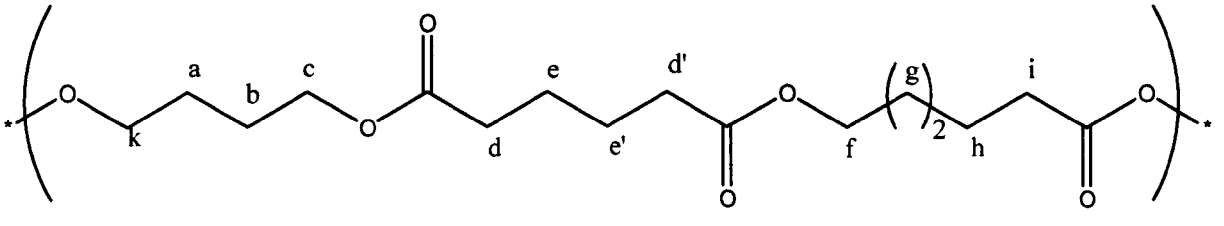
PPA-co-CL was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71) and Novozyme (1.0 g).



Viscous liquid, monomer composition calculated (1:1:0.45), Mw: 4901Da, IR υ max: 2938.0, 2865.9, 1725.6, 1456.5, 1418.9, 1390.1, 1360.5, 1159.5, 1042.8, 734.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.40 (m, 1.5H, H-g), 1.70 (m, 6H, H-e, e', h), 1.85 (m) – 1.9 (q) (3H, H-b, f), 2.35 (m, 4H, H-d, d'), 4.05 (t)-4.18 (t) (4H, H-a, c).

Poly(1,4-butanediol adipate-co-ε-caprolactone) (PBA-co-CL) – 1:1:1

PBA-co-CL was synthesised by general procedure via the reaction of 1,4-butanediol (0.05 mol, 4.50 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71g) and Novozyme (1.0 g).

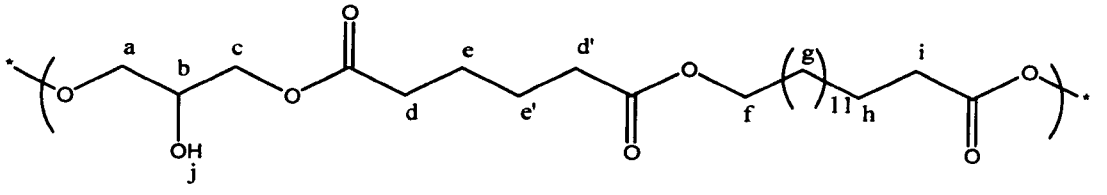


Viscous liquid, monomer composition calculated (1:1:0.40), Mw: 18388Da, IR υ max: 2953.3, 2870.9, 1725.1, 1458.5, 1417.9, 1395.1, 1367.5, 1150.1, 1061.5, 734.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.40 (m, 1.5H, H-g), 1.70 (m, 9H, H-e, e', h, k, c, f), 2.35 (m, 4H, H-d, d', i), 3.75 (t, H-b), 4.1 (m, 4H, H-a).

b) Co-Pentadecalactone:

Poly(glycerol adipate-co-ω-pentadecalactone) (PGA-co-PDL)

PGA-co-PDL was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g). The amount of ω-pentadecalactone used was (0.025 mol, 16.01 g) for 1:1:0.5 ratio, (0.05 mol, 12.02 g) for 1:1:1 ratio and (0.075 mol, 18.03 g) for 1:1:1.5 ratio.



a) 1:1:0.5

Soft solid, monomer composition calculated (1:1:1), θ = 70.02 ± 1.2, Mw: 15600 Da, T_m = 38°C, T_c = 24°C, IR υ max: 3448.8, 2915.3, 2848.0, 1729.0, 1463.3, 1365.9, 1162.3, 1058.8, 720 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30 (s, 19H, H-g), 1.65 (m, 10H, H-e, e', h), 2.35 (m, 8H, H-d, d', i), 4.05 (q)-4.18 (m) (7H, H-a, b, c, f), 5.2 (s, H, H-j).

b) 1:1:1

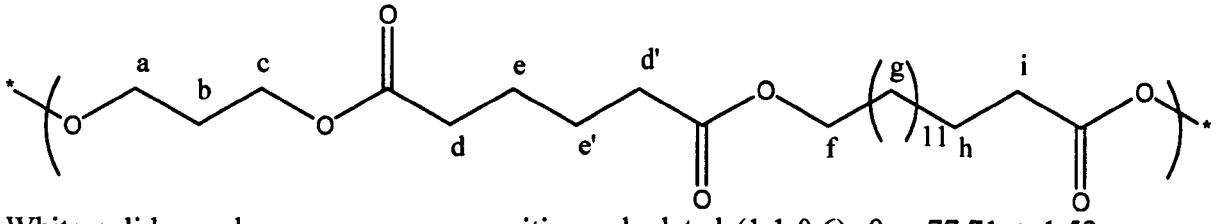
White solid powder, monomer composition calculated (1:1:1), θ = 65.65 ± 2.36, Mw: 17560 Da, T_m = 42°C, IR υ max: 3451.2, 2915.3, 2847.8, 1729.5, 1463.2, 1463.2, 1365.9, 1160.8, 1040.0, 719.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.34 (s, 22H, H-g), 1.65 (m, 8H, H-e, e', h), 2.32 (m, 6H, H-d, d', i), 4.05 (q)-4.18 (m) (6H, H-a, b, c, f), 5.2 (s, H, H-j).

c) 1:1:1.5

White solid powder, monomer composition calculated (1:1:1.2), Mw: 29149 Da, T_g = 36°C, T_m = 52°C T_c = 50°C, IR υ max: 3454.7, 2914.7, 2847.5, 1729.7, 1462.9, 1366.0, 1162.5, 957.6, 719.6 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30 (s, 25H, H-g), 1.68 (m, 9H, H-e, e', h), 2.32 (m, 6H, H-d, d', i), 4.05 (q)-4.18 (m) (6H, H-a, b, c, f), 5.2 (s, H, H-j).

Poly(1,3-propanediol adipate-co-ω-pentadecalactone) (PPA-co-PDL) – 1:1:1

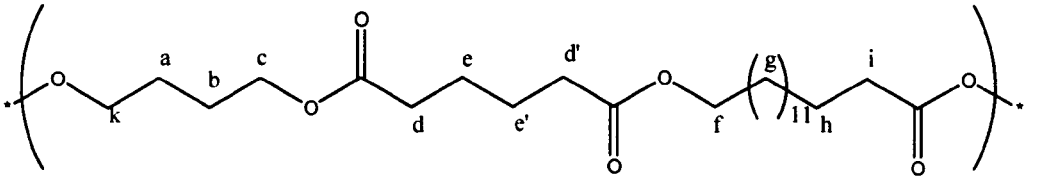
PPA-co-PDL was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) and Novozyme (1.0 g).



White solid powder, monomer composition calculated (1:1:0.6), θ = 77.71 ± 1.52, Mw: 8566 Da, T_g = 45°C, T_m = 56°C T_c = 36°C, IR υ max: 2916.3, 2848.1, 1728.2, 1463.0, 1414.4, 1365.3, 1255.4, 1158.5, 1052.4, 953.5, 730.5 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30 (s, 15H, H-g), 1.65 (m, 6H, H-e, e', h), 1.9 (q, 1.2H, H-b), 2.30 (m, 4.3H, H-d, d', i), 4.05 (t)-4.18 (t) (4H, H-a, c).

Poly(1,4-butanediol adipate-co-ω-pentadecalactone) (PBA-co-PDL) – 1:1:1

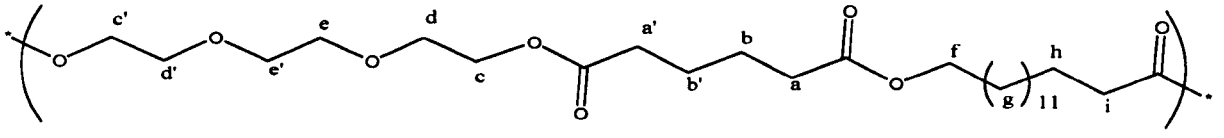
PBA-co-PDL was synthesised by general procedure via the reaction of 1,4-butanediol (0.05 mol, 4.50 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) and Novozyme (1.0 g).



White solid, monomer composition calculated (1:1:0.5), Mw: 15960Da, IR υ max: 2916.25, 2848.71, 1728.30, 1464.03, 1415.42, 1398.01, 1366.64, 1256.61, 1160.98, 1064.44, 956.21, 722.01 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30 (s, 14H, H-g), 1.65 (m, 9H, H-e, e', h, k, c), 2.30 (m, 4H, H-d, d', i), 3.7 (t, H-b), 4.05-4.17 (m, 4H, H-a, c).

Poly(triethylene glycol adipate-co-ω-pentadecalactone) (PTEGA-co-PDL)

PTEGA-co-PDL was synthesised by general procedure via the reaction of triethylene glycol (0.05 mol, 7.50g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g). The amount of ω-pentadecalactone used was (0.025 mol, 16.01 g) for 1:1:0.5 ratio, (0.05 mol, 12.02 g) for 1:1:1 ratio and (0.075 mol, 18.03 g) for 1:1:1.5 ratio.



a) 1:1:0.5

Soft wax like solid, monomer composition calculated (1:1:0.6), Mw: 7740 Da, IR υ max: 3445, 2919, 2850, 1729, 1456, 1417, 1349, 1242, 1172 1125, 1028, 953, 861, 720 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.26 (s, 10H, H-g), 1.65 (m, 6H, H-e, e', h), 2.37 (m, 5H, H-d, d', i), 3.65 (t)-3.7 (t) (8H, H-b, b', c, c'), 4.05 (t, H, H-f), 4.25 (t, 4H, H-a, a').

b) 1:1:1

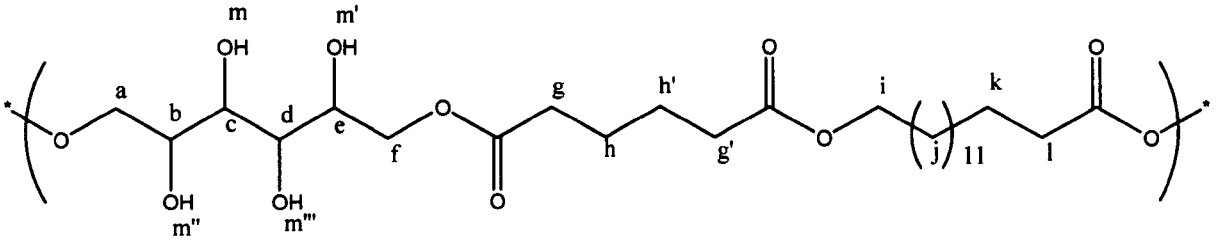
White amorphous powder, monomer composition calculated (1:1:1), Mw: 23801 Da, T_m = 49.816°C, T_c = 39.86°C, IR υ max: 2915, 2848, 1729, 1462, 1415, 1349, 1287, 1254, 1168 1128, 956, 919, 865, 720 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.26 (s, 22H, H-g), 1.62 (m, 8H, H-e, e', h), 2.36 (m, 6H, H-d, d', i), 3.62 (t)-3.68 (t) (8H, H-b, b', c, c'), 4.05 (t, 2H, H-f), 4.24 (t, 4H, H-a, a').

c) 1:1:1.5

White amorphous powder, monomer composition calculated (1:1:1.5), Mw: 17413Da, T_g= 49.32°C, T_m = 61.18°C, T_c = 49.84°C, IR υ max: 2915, 2848, 1729, 1462, 1415, 1349, 1287, 1254, 1168, 1128, 956, 919, 865, 720 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.26 (s, 34H, H-g), 1.65 (m, 11H, H-e, e', h), 2.34 (m, 7H, H-d, d', i), 3.63 (t)-3.7 (t) (8H, H-b, b', c, c'), 4.05 (t, H, H-f), 4.22 (t, 4H, H-a, a').

Poly(sorbitol adipate-co-ω-pentadecalactone) (PSA-co-PDL) – 1:1:1

PSA-co-PDL was synthesised by general procedure via the reaction of sorbitol (0.05 mol, 9.10 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) and Novozyme (1.0 g). The solvent, acetone (50 ml) was used in this reaction instead of THF at 90°C.

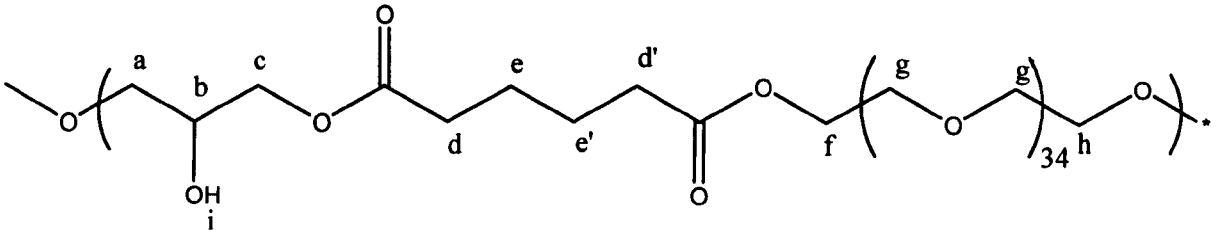


White solid, monomer composition calculated (1:1:0.5), Mw: 3258 Da (Chloroform GPC), IR υ max: 3417.73, 2916.45, 2848.60, 1729.84, 1462.04, 1367.28, 1335.00, 1219.66, 1172.33, 1093.29, 956.29 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30-1.38 (d, 24H, H-j, a, f), 1.65 (m, 7H, H-g, g', l), 2.18 (s, H, H-m, m', m'', m'''), 2.30 (m, 4.0H, H-h, h', k), 4.05 (t)-4.18 (t) (3H, H-b, c, d, e).

2.2.5.3 Functionalised and non-functionalised linear PEG-copolymers

Poly(glycerol adipate–co–PEG) (PEG-PGA-PEG) – 1:1:0.01

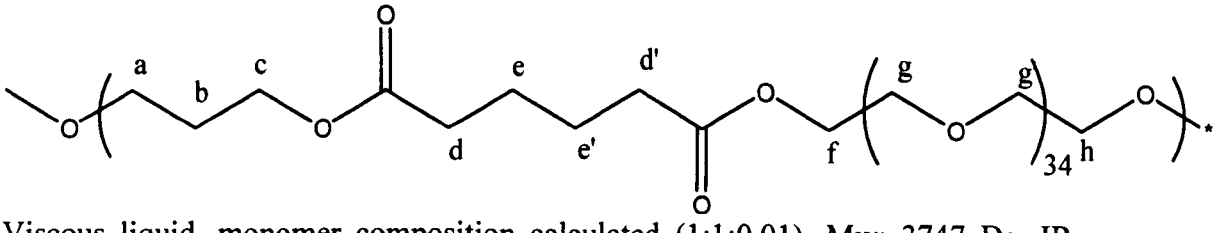
PEG-PGA-PEG was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g) in 1:1 ratio, polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).



Pale yellow viscous liquid, monomer composition calculated (1:1:0.04), Mw: 3836 Da, IR υ max: 3439.4, 2946.5, 2870.7, 1728.0, 1456.5, 1418.9, 1380.5, 1165.9, 1132.9, 1052.0, 940.6, 753.5, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.68 (m, 4H, H-e, e'), 2.38 (m, 4H, H-d, d'), 3.65 (s, 6.4H, H-f, g, h), 4.08 (m) – 4.18 (m) (4H, H-a, b, c), 5.1 (s, H, H-i).

Poly(1,3-propanediol adipate – co – PEG) (PEG-PPA-PEG) – 1:1:0.01

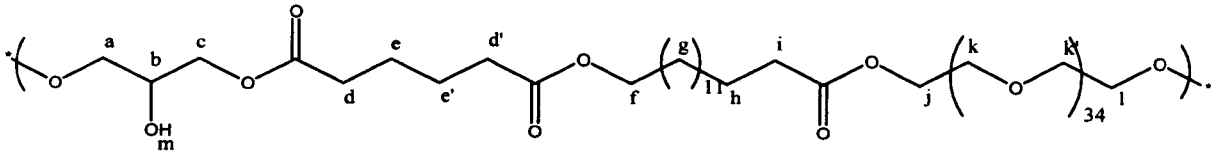
PEG-PPA-PEG was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).



Viscous liquid, monomer composition calculated (1:1:0.01), Mw: 3747 Da, IR υ max: 2943.1, 2873.0, 1718.4, 1476.2, 1476.2, 1416.9, 1372.6, 1254.9, 1163.4, 1055.0, 1027.4, 947.1, 731.6 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.65 (q, 4H, H-e, e'), 1.96 (q, 2H, H-b), 2.35 (q, 4H, H-d, d'), 3.64 (s, 1.5H, H-f,g,h), 4.18(t,4H,H-a,c).

Poly(glycerol adipate-co-ω-pentadecalactone)–co–PEG (PEG-PGA-co-PDL-PEG) – 1:1:1:0.01

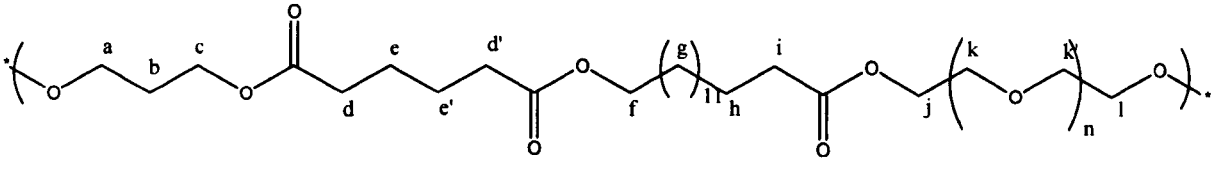
PEG-PGA-co-PDL-PEG was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g), polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).



White solid powder, monomer composition calculated (1:1:0.8:0.02), Mw: 8566 Da, T_g = 40°C, T_m = 52°C T_c = 41.5°C, T_m (2nd heat cycle) = 53°C, IR υ max: 3447.0, 2915.7, 2848.4, 1730.7, 1463.8, 1417.0, 1242.9, 1164.8, 720.1 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.28 (s, 21.5H, H-g), 1.65 (m, 8H, H-e, e', h), 2.38 (m, 6H, H-d, d', i), 3.64 (s, 2.6H, H-j, k, l), 4.05 (m)-4.18 (m) (5H, H-a, b, c, f), 5.1 (s, H, H-m).

Poly(1,3-propanediol adipate-co-ω-pentadecalactone)–co–PEG (PEG-PPA-co-PDL-PEG) – 1:1:1:0.01

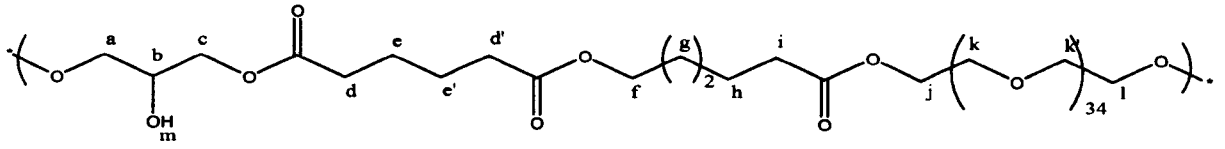
PEG-PPA-co-PDL-PEG was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g), polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).



White solid powder, monomer composition calculated (1:1:1.4:0.018), Mw: 6649 Da, T_g = 41°C, T_m = 53°C T_c = 43.8°C, T_m (2nd heat cycle) = 55.5°C, IR υ max: 2916.0, 2848.5, 1729.5, 1463.6, 1415.3, 1256.1, 1160.9, 1052.0, 953.5, 721.0 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.26 (s, 28H, H-g), 1.64 (m, 10.4H, H-e, e', h), 1.96 (q, 2H, H-b), 2.35 (m, 7.6H, H-d, d', i), 3.64 (s. 2.5H, H-j, k, l), 4.05 (t) – 4.16 (t) (7H, H-a, c, f).

Poly(glycerol adipate-co-ε-caprolactone)–co–PEG (PEG-PGA-co-CL-PEG) – 1:1:1:0.01

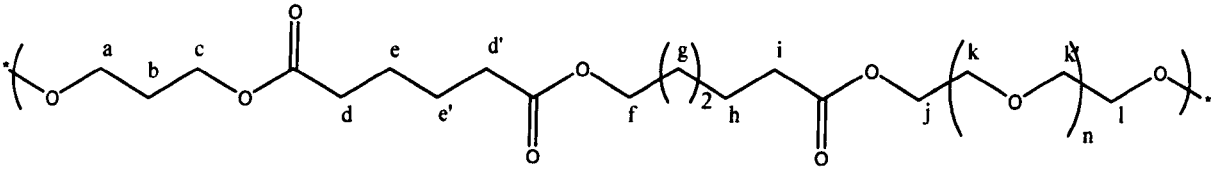
PEG-PGA-co-CL-PEG was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71 g), polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).



Highly viscous solid, monomer composition calculated (1:1:1:0.02), Mw: 3075 Da, IR υ max: 3447.0, 2938.1, 2865.8, 1727.8, 1456.5, 1418.9, 1387.3, 1162.5, 1052.5, 911.0, 734.8 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.40 (m, 2H, H-g), 1.64 (m, 8H, H-e, e', h), 2.38 (m, 6H, H-d, d', i), 3.64 (s, 3H, H-j, k, l), 4.06 (m)-4.18 (m) (7H, H-a, b, c, f), 5.1 (s, H, H-m).

Poly(1,3-propanediol adipate-co-ε-caprolactone)–co–PEG (PEG-PPA-co-CL-PEG) – 1:1:1:0.01

PEG-PPA-co-CL-PEG was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71 g), polyethylene glycol (0.0006 mol, 1 g) and Novozyme (1.0 g).

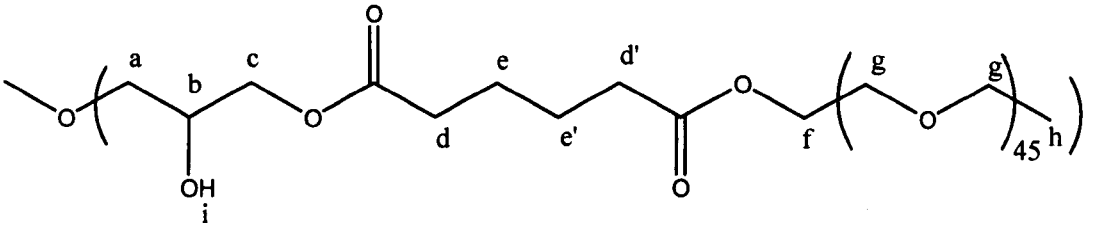


Very soft solid, monomer composition calculated (1:1:1.2:0.017), Mw: 5057 Da, IR υ max: 29415.6, 2865.7, 1726.0, 1456.5, 1358.5, 1160.1, 1060.8, 963.6, 738.1 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.4 (m, 2.2H, H-g), 1.64 (m, 9H, H-e, e', h), 1.98 (q, 2H, H-b), 2.36 (m, 7H, H-d, d', i), 3.64 (s, 2.3H, H-j, k, l), 4.05 (t)-4.18 (t) (6H, H-a, b, c, f).

2.2.5.4 Functionalised and non-functionalised linear PEGme-copolymers

Poly(glycerol adipate–co–PEGme) (PGA-PEGme) – 1:1:0.01

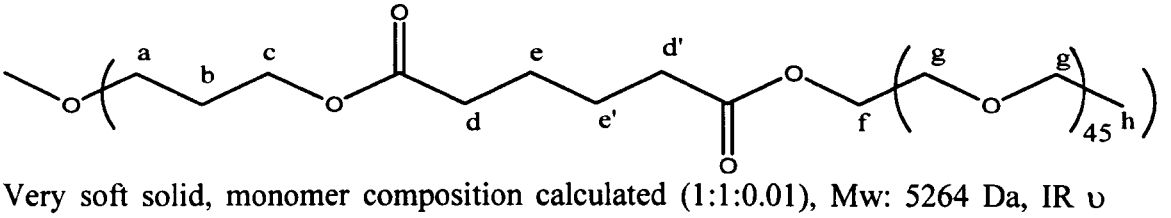
PEGme-PGA was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g) in 1:1 ratio, methoxy polyethylene glycol (0.0005 mol, 1 g) and Novozyme (1.0 g).



Pale yellow viscous liquid, monomer composition calculated (1:1:0.02), Mw: 9982 Da, IR υ max: 3413.5, 2948.0, 2870.7, 2358.6, 1730.2, 1456.5, 1418.9, 1381.2, 1165.9, 1134.0, 1061.9, 906.7, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.65 (t, 4H, H-e, e'), 2.38 (t, 4H, H-d, d'), 3.64 (s, 4.6H, H-f, g), 4.18 (m, 4H, H-a, b, c), 5.1 (s, H, H-i).

Poly(1,3-propanediol adipate–co–PEGme) (PPA-PEGme) – 1:1:0.01

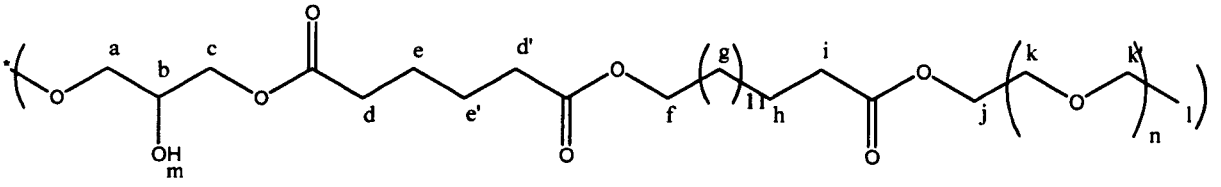
PEGme-PPA was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), methoxy polyethylene glycol (0.0005 mol, 1 g) and Novozyme (1.0 g).



Very soft solid, monomer composition calculated (1:1:0.01), Mw: 5264 Da, IR υ max: 2902.6, 2943.2, 2357.0, 1718.6, 1476.3, 1416.8, 1372.6, 1255.3, 1164.1, 1055.0, 1027.6, 946.7, 731.8 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.65 (q, 4H, H-e, e'), 1.98 (q, 2H, H-b), 2.36 (q, 4H, H-d, d'), 3.64 (s, 2H, H-f,g), 4.15 (t, 4H, H-a, c).

Poly(glycerol adipate-co-ω-pentadecalactone)–PEGme (PGA-co-PDL-PEGme)

PEGme-PGA-co-PDL was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) in 1:1:1 ratio and Novozyme (1.0 g). The amount of methoxy polyethylene glycol used was (0.00025 mol, 0.5 g) for 1:1:1:0.005, (0.0005 mol, 1 g) for 1:1:1:0.01 and (0.0010 mol, 2 g) for 1:1:1:0.02.



1:1:1:0.005

White solid powder, monomer composition calculated (1:1:1.5:0.01), Mw: 3775 Da, T_g = 36°C, T_m = 53°C T_c = 43.4°C, T_m (2nd heat cycle) = 51°C, IR υ max: 3449.8, 2916.9, 2849.1, 1729.8, 1463.8, 1414.8, 1367.0, 1243.0, 1162.6, 720.2 cm^{-1}, ^1H-NMR (δ_H $CDCl_3$, 300 MHz): 1.28 (s, 28H, H-g), 1.65 (m, 10H, H-e, e', h), 2.35 (m, 10H, H-d, d', i), 3.64 (s, 1.5H, H-j, k), 4.04 (m)-4.18 (m) (7H, H-a, b, c, f), 5.2 (s, H, H-m).

1:1:1:0.010

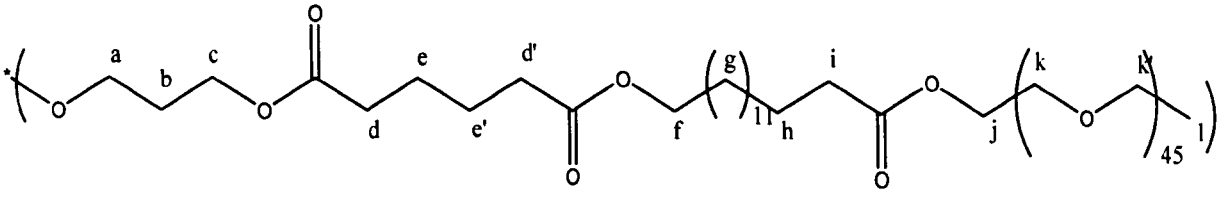
White solid powder, monomer composition calculated (1:1:1.4:0.01), Mw: 4729 Da, T_g = 36°C, T_m = 55°C T_c = 42°C, T_m (2nd heat cycle) = 52°C, IR υ max: 3449.4, 2917.0, 2849.3, 1729.8, 1463.8, 1414.9, 1367.0, 1288.1, 1162.1, 720.2 cm^{-1}, ^1H-NMR (δ_H $CDCl_3$, 300 MHz): 1.28 (s, 27H, H-g), 1.65 (m, 10H, H-e, e', h), 2.35 (m, 8H, H-d, d', i), 3.64 (s, 2H, H-j, k), 4.04 (m)-4.18 (m) (7H, H-a, b, c, f), 5.2 (s, H, H-m).

1:1:1:0.020

White solid powder, monomer composition calculated (1:1:1.3:0.02), Mw: 6478 Da, = 34°C, T_m = 55°C T_c = 43.7°C, T_m (2nd heat cycle) = 52°C, IR υ max: 3448.3, 2917.1, 2849.3, 1729.9, 1463.9, 1414.9, 1366.9, 1243.1, 1162.4, 720.3 cm^{-1}, ^1H-NMR (δ_H $CDCl_3$, 300 MHz): 1.28 (s, 26H, H-g), 1.65 (m, 10H, H-e, e', h), 2.35 (m, 7H, H-d, d', i), 3.64 (s, 3.5H, H-j, k), 4.04 (m)-4.18 (m) (7H, H-a, b, c, f), 5.2 (s, H, H-m).

Poly(1,3-propanediol adipate-co-ω-pentadecalactone)–PEGme (PPA-co-PDL-PEGme) – 1:1:1:0.01

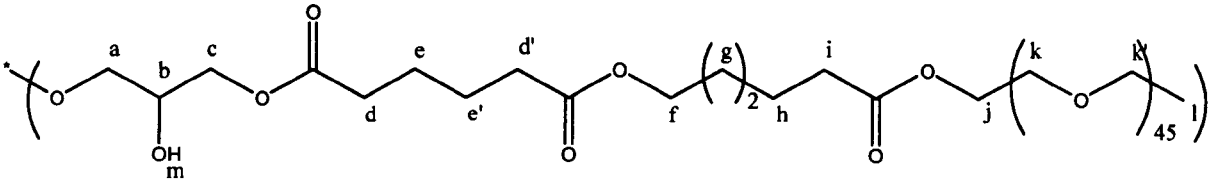
PEGme-PPA-co-PDL was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g), methoxy polyethylene glycol (0.0005 mol, 1 g) and Novozyme (1.0 g).



White soft solid, monomer composition calculated (1:1:.0.5:0.006), Mw: 9595 Da, T_g = 40°C, T_m = 55°C T_c = 41.8°C, T_m (2nd heat cycle) = 53°C, IR υ max: 2916.2, 2848.6, 1729.6, 1463.7, 1415.4, 1365.6, 1255.9, 1160.1, 1052.1, 953.5 cm^{-1}, ^1H-NMR (δ_H $CDCl_3$, 300 MHz): 1.28 (s, 14H, H-g), 1.65 (m, 5.5H, H-e, e', h), 1.96 (q, H, H-b), 2.34 (m, 4H, H-d, d', i), 3.64 (s, 1.2H, H-j, k), 4.04 (t)-4.16 (t) (4H, H-a, c, f).

Poly(glycerol adipate-co-ε-caprolactone)–PEGme (PGA-co-CL-PEGme) – 1:1:1:0.01

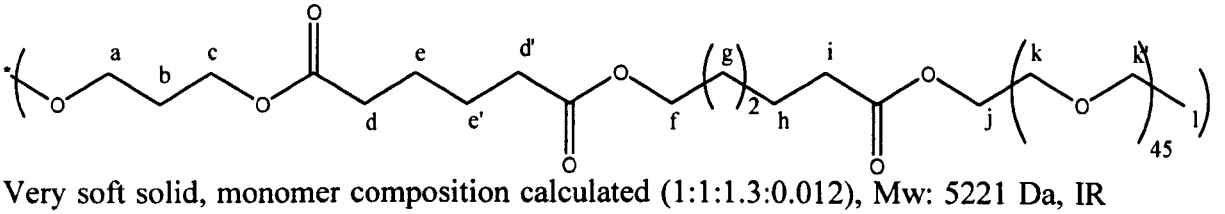
PEGme-PGA-co-CL was synthesised by general procedure via the reaction of glycerol (0.05 mol, 4.60 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71g), methoxy polyethylene glycol (0.0005 mol, 1 g) and Novozyme (1.0 g).



Wax like solid, monomer composition calculated (1:1:1.2:0.016), Mw: 6017 Da, IR υ max: 3447.1, 292.5, 2866.0, 2357.1, 1729.2, 1456.5, 1418.9, 1161.0, 1062.4, 963.3 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.40 (m, 2H, H-g), 1.64 (m, 9H, H-e, e', h), 2.38 (m, 7H, H-d, d', i), 3.64 (s, 3H, H-j, k), 4.05 (t)-4.18 (m) (7H, H-a, b, c, f), 5.1 (s, H, H-m).

Poly(1,3-propanediol adipate-co-ε-caprolactone)–PEGme (PPA-co-CL-PEGme) – 1:1:1:0.01

PEGme-PPA-co-CL was synthesised by general procedure via the reaction of 1,3-propanediol (0.05 mol, 3.80 g), divinyl adipate (0.05 mol, 9.91 g), ε-caprolactone (0.05 mol, 5.71 g), methoxy polyethylene glycol (0.0005 mol, 1 g) and Novozyme (1.0 g).

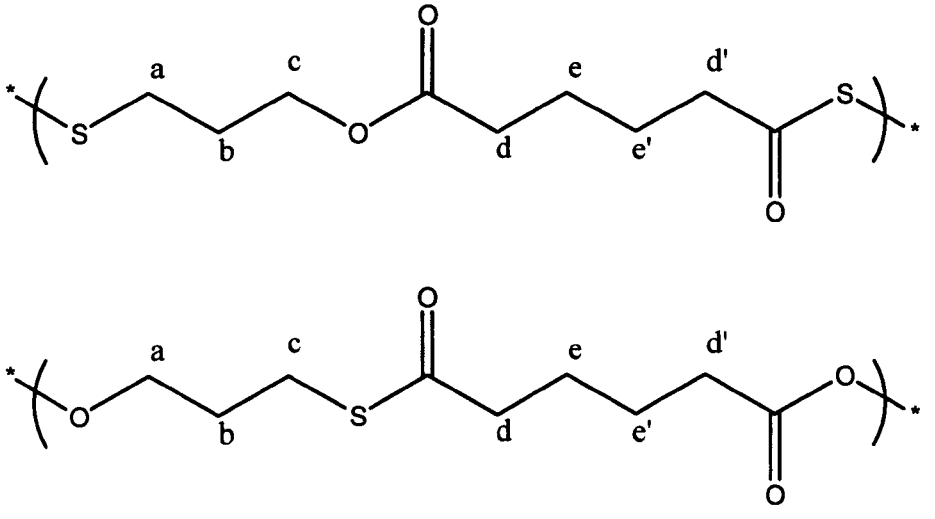


Very soft solid, monomer composition calculated (1:1:1.3:0.012), Mw: 5221 Da, IR υ max: 2941.5, 2865.6, 2358.6, 1726.2, 1456.5, 1419.0, 1358.7, 1159.5, 1060.6, 963.8, 909.0, 736.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.4 (m, 2.4H, H-g), 1.65 (m, 9.5H, H-e, e', h), 1.96 (q, 2H, H-b), 2.35 (m, 7H, H-d, d', i), 3.64 (s, 2.2H, H-j, k), 4.04 (t)-4.18 (t) (6.6H, H-a, c, f).

2.2.5.5 Functionalised and non-functionalised polythioester

Poly(1,3-propanedithiol adipate) (PPTA) – 1:1

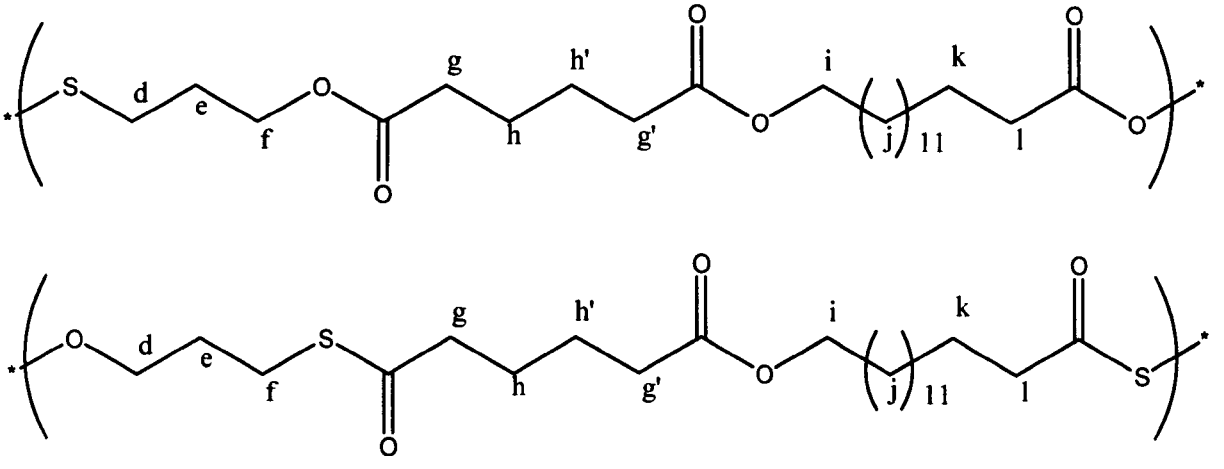
PPTA was synthesised by general procedure via the reaction of 1,3-propanedithiol (0.05 mol, 5.4 g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g). This reaction was solvent free, and drying tube with molecular sieves was used.



Both structures, presented above were confirmed. Transparent viscous liquid, monomer composition calculated (1:1), Mw: 2000 Da, IR υ max: 2927.1, 1729.9, 1710.3, 1644.9, 1442.0, 1412.0, 1373.1, 1139.9, 1055.0, 1004.5, 948.0, 843.2, 757.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.70 (m, 4H, H-e, e'), 1.90 (m, 2H, H-b), 2.35 (m, 2H, H-d'), 2.60 (m, 2H, H-d), 2.75 (m) – 2.95 (m) (4H, H-a, c).

Poly(1,3-propanedithiol adipate-co-ω-pentadecalactone) (PPTA-co-PDL) – 5:1:1

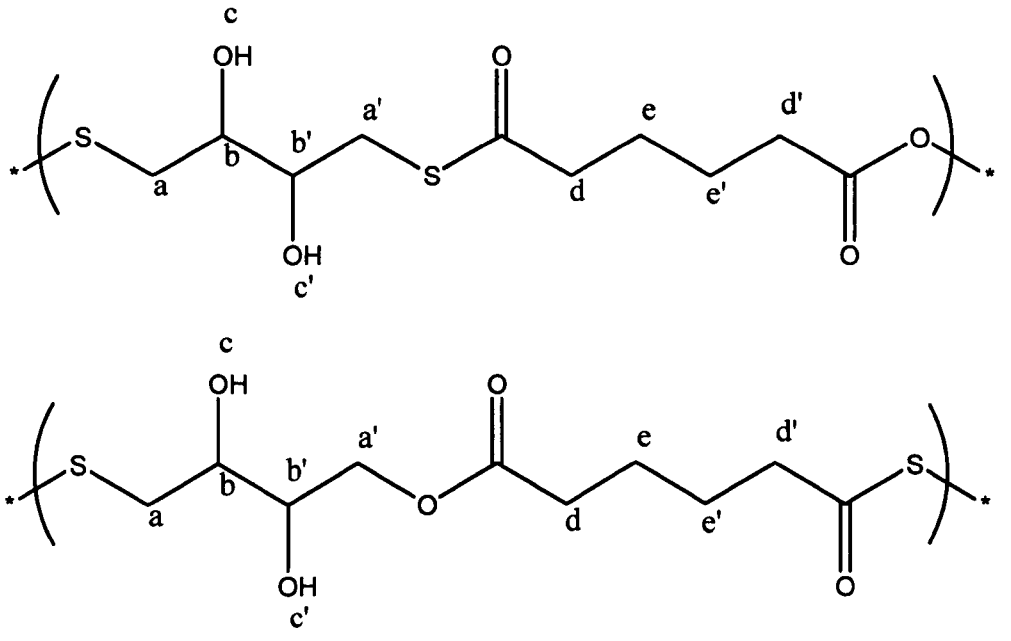
PPTA-co-PDL was synthesised by general procedure via the reaction of 1,3-propanedithiol (0.25 mol, 27 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) and Novozyme (1.0 g). This reaction was performed in bulk (solvent free) at 120°C for 96 hours on a hotplate, and drying tube with molecular sieves was used.



Both structures, presented above were confirmed. Pale yellowish solid, monomer composition calculated (2:1:1), Mw: 2262 Da, IR υ max: 2916.71, 2848.20, 1729.71, 1688.36, 1384.06, 1290.15, 1243.50, 1164.22, 1065.16, 719.19 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30-1.38 (m, 10H, H-j), 1.65 (m, 4H, H-g, g', l), 1.95 (m, 2H, H-e), 2.30 (m, 2.0H, H-I, d), 2.60 (m, 2H, H-h,h', k), 4.1 (t)-4.21 (t) (3H, H- d, f).

Poly(dithiothreitol adipate) (PDTTA) – 1:1

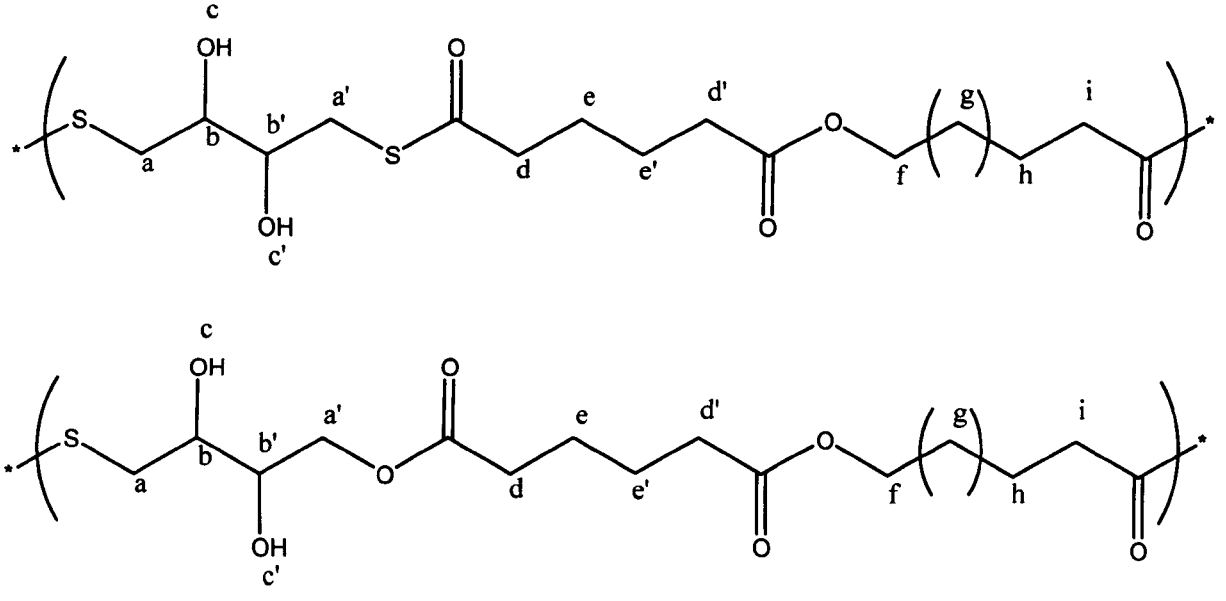
PDTTA was synthesised by general procedure via the reaction of dithiothreitol (0.05 mol, 7.71 g), divinyl adipate (0.05 mol, 9.91 g) and Novozyme (1.0 g).



Both structures, presented above were confirmed. Transparent viscous liquid, Mw: 300 Da, IR υ max: 3417.60, 2927.1, 1735.15, 1644.38, 1413.56, 1385.96, 1293.0, 1135.90, 1047.04, 947.40 cm^{-1}. NMR not obtained because of the poor solubility of polymer.

Poly(dithiothreitol adipate-co-ω-pentadecalactone) (PDTTA-co-PDL) – 0.5:1:1

PDTTA-co-PDL was synthesised by the general procedure via the reaction of dithiothreitol (0.025 mol, 3.85 g), divinyl adipate (0.05 mol, 9.91 g), ω-pentadecalactone (0.05 mol, 12.02 g) and Novozyme (1.0 g). This reaction was performed in bulk (solvent free) at 110°C for 48 hours on a hotplate, and a drying tube with molecular sieves was used.

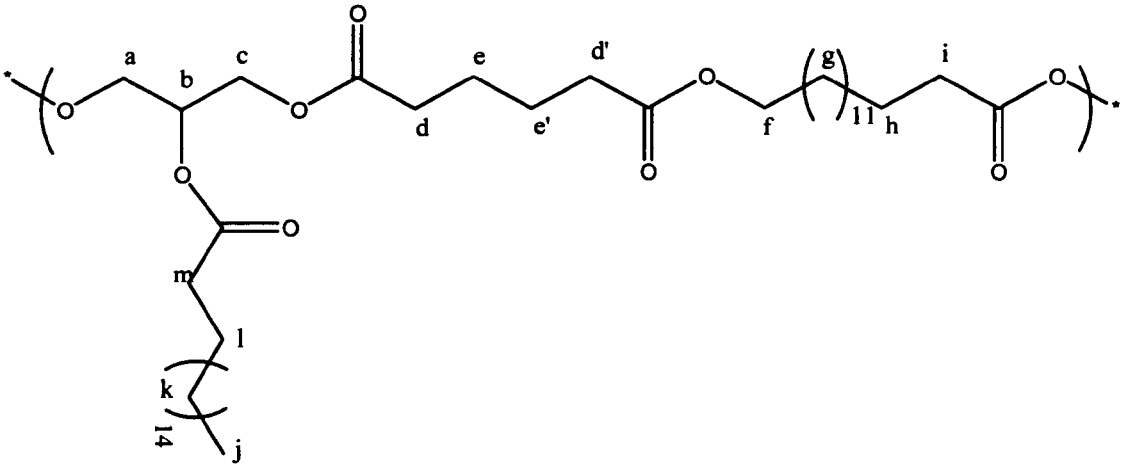


Both structures, presented above were confirmed. Pale yellowish soft rubber-like solid, monomer composition calculated (1:1:0.5), Mw: 10144 Da, IR υ max: 3442.49, 2922.57, 2354.38, 2127.08, 1727.00, 1644.45, 1455.30, 1415.10, 1384.46, 1135.5, 1061.67, 752.19 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 1.30-1.38 (m, 8H, H-g), 1.65 (m, 12H, H-d, d', i), 2.35 (m, 10H, H-a, a', f), 2.70 (m, 2H, H-c, c'), 2.80 (m, 9H, H-e, e', h), 3.65 (m, 2H, H-b, b').

2.2.5.6 DCC-catalysed coupling of stearic acid to PGA-co-PDL

Poly(glycerol adipate-co-ω-pentadecalactone)-conjugated stearic acid (PGA-co-PDL-C18)

PGA-co-PDL-C18 was synthesised by general procedure (2.2.3.1) via the reaction of PGA-co-PDL (2.75 mmol, 1.22g) and stearic acid (1.35 mmol, 0.35g for 50% and 2.75 mmol, 0.71g for 100%), using DCC as catalyst.



a) 50%

Soft white solid, % conjugated 50% (calculated), Mw: 16555 Da, T_g = 47°C, T_m = 64°C, T_m (second heat cycle) = 48°C, T_c = 18°C, IR υ max: 3470.9, 2918.5, 2846.5, 2115.8, 1730.9, 1655.16, 1517.8, 1408.1, 1384.9, 1257.3, 1168.5, 1088.2, 890.2, 657.4 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 0.88 (t, 3H, H-j), 1.30 (s, 74H, H-g, k), 1.65 (m, 16H, H-e, e', l, h), 2.35 (m, 10H, H-d, d', i, m), 4.05 (q)-4.18 (m) (5H, H-a, b, c, f).

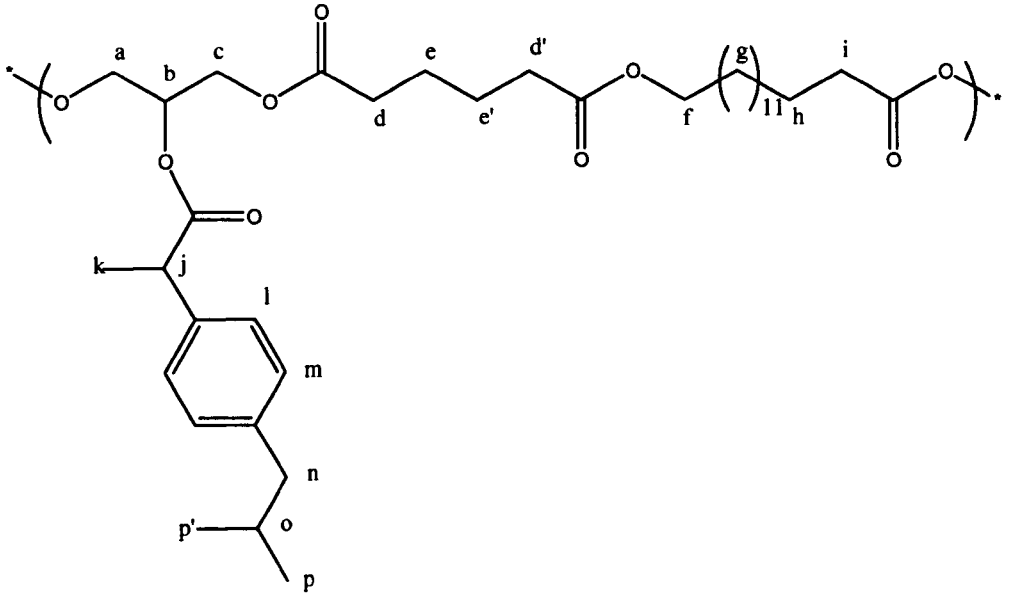
b) 100%

White solid powder, % conjugated 33.33% (calculated), Mw: 18476 Da, T_g = 42°C, T_m = 56°C, T_m (second cycle) = 58°C, T_c = 23°C, IR υ max: 2916.16, 2846.35, 1730.85, 1655.48, 1517.19, 1452.94, 1384.98, 1220.33, 1168.24, 1082.80, 726.80 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 0.88 (t, 3H, H-j), 1.30 (s, 119H, H-g, k), 1.68 (m, 30H, H-e, e', l, h), 2.32 (m, 23H, H-d, d', i, m), 4.05 (q)-4.18 (m) (9H, H-a, b, c, f).

2.2.5.7 DCC catalysed coupling of ibuprofen to PGA-co-PDL

Poly(glycerol adipate-co-ω-pentadecalactone)-conjugated ibuprofen (PGA-co-PDL-Ibu)

PGA-co-PDL-Ibu was synthesised by general procedure via the reaction of PGA-co-PDL (1.22g) and ibuprofen (0.282 g for 50% and 0.565 g for 100%) using DCC as catalyst.



a) 50%

Soft white solid, % conjugated 50% (calculated), Mw: 18219 Da, T_m = 42°C, T_m (second heat cycle) = 40°C, T_c = 34°C, IR υ max: 3488.8, 3289.0, 2921.4, 2849.4, 2116.1, 1665.3, 1535.6, 1384.0, 1227.7, 1088.1, 1062.8, 891.1, 657.9 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 0.89 (t, 6H, H-p, p'), 1.30 (s, 47H, H-g), 1.49 (d, 4H, H-k), 1.65 (m, 35H, H-e, e', h), 2.35 (m, 12H, H-d, d', I, n), 3.69 (m, 1H. H-j), 4.05 (q)-4.18 (m) (9H, H-a, b, c, f), 7.2 (d, 4H, H-l, l', m, m').

b) 100%

White solid powder, % conjugated 16.66% (calculated), Mw: 17413 Da, T_g = 36°C, T_m = 43°C, T_m (second cycle) = 38°C, T_c = 31°C, IR υ max: 3473.2, 2915.6, 2848.0, 1729.9, 1649.9, 1452.6, 1365.7, 1163.9, 1077.5, 921.8, 720.8 cm^{-1}, ^{1}H-NMR (δ_H $CDCl_3$, 300 MHz): 0.89 (t, 6H, H-p, p'), 1.30 (s, 138H, H-g), 1.49 (d, 4H, H-k), 1.65 (m, 57H, H-e, e', h), 2.35 (m, 42H, H-d, d', i, n), 3.69 (m, 1H. H-j), 4.05 (q)-4.18 (m) (37H, H-a, b, c, f), 7.2 (d, 1H, H-l, l', m, m').

2.3 RESULTS AND DISCUSSIONS

2.3.1 Polymer synthesis

2.3.1.1 Poly(glycerol adipate) (PGA)

Poly (glycerol adipate) (PGA) has previously been synthesised by the condensation of divinyl adipate (DVA) and glycerol, using the lipase from *C. antarctica* (Kobayashi et al 2006). This type of polymer has also recently shown great potential for new drug delivery applications (Kallinteri et al 2005; Puri et al 2008). In the literature, reports of PGA and related polymers' syntheses tend to be on a very small scale (less than 1 gram) for the synthesis of combinatorial libraries of polymers, mainly to show that enzymes can be used for the synthesis of a variety of different polymers (Namekawa et al 2000; Kim & Dordick 2001). Here, the synthesis of PGA was studied on a larger scale (100g) to obtain a useful yield for application to drug delivery studies. By altering the monomers used, derivatives of this polymer can be designed and synthesised for use in effective encapsulation and attachment of drug molecules.

PGA was synthesised successfully at 65-70% yield. Although this product was not purified to remove oligomers, the spectral and chromatographic data indicates that the product was free of any un-reacted monomers.

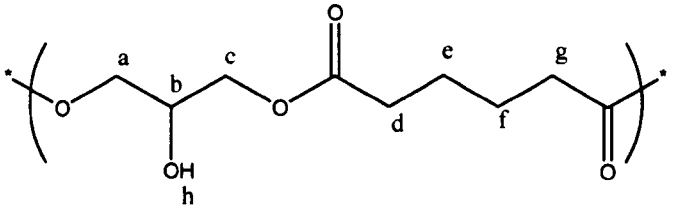


Figure 2.1: *Chemical structure of PGA*

PGA obtained was a viscous liquid at room temperature and was used without any further processing. The chemical structure of poly(glycerol adipate) is given in figure 2.1 which is supported by the characterisation data of PGA as summarised in section 2.2.5.1.

a) Molecular weight analysis:

Polymer molecular weight was determined by gel permeation chromatography (GPC). For determination of the molecular weight via GPC, two common methods can be used, namely, Conventional calibration and Universal calibration methods.

Chaudhary et al (1997) used the Universal Calibration method, which applies a relationship between the hydrodynamic volume and the retention time for GPC molecular weight determination and proved that by using polystyrene or PEG-based standards the materials' molecular weights were overestimated. Therefore, they applied two methods for calculating the absolute molecular weight including MALDI-MS and GPCV (Chaudhary et al 1997).

The GPC instrument used in this investigation was equipped with a refractive index detector along with low angle light scattering laser and intrinsic viscosity detectors which in theory would enable absolute polymer molecular weights to be obtained. However, due to the nature of the polymer (PGA), no reliable and reproducible response was recorded, because PGA has a relatively low molecular weight and low refractive index increment (dn/dc) values, hence, the GPC accurate mass detectors (or triple detectors) did not produce reliable or reproducible results. This problem was addressed by using the refractive index response versus time plot to enable a calculation of the molecular weight based on the more standard method of molecular

weight versus polystyrene standards (conventional calibration method). This also allowed a direct comparison of the polymer molecular weights obtained here with those cited in literature.

With the conventional calibration method, retention volumes of known polymer standards, for example polystyrene or PEG, are used for plotting a calibration curve. This curve is then used for the determination of the molecular weight of the polymer, by using the straight line equation, obtained from the standard curve. However, this method only gives an accurate mass if the material being analysed is the same as that used to generate the calibration graph. Other research groups commonly use polystyrene standards for calibration purposes for the analysis of PGA and related polymers (Kline et al 1998b; Thompson et al 2006).

The molecular weights in the present work were determined versus polystyrene standards. Thus from a series of 6 molecular weight polystyrenes (Mw = 650 to 50000Da) a calibration curve was obtained and the Mw for these samples were determined. The molecular weight of PGA was 10500Da (versus polystyrene). Based on the suggested structure it was proposed that the polymer chain contains 55 units (as one unit has a molecular weight of 190Da).

A representative GPC chromatogram of PGA is shown in figure 2.2a, which illustrates that the polymer is free of any residual monomers. The peak corresponding to the polymer is quite broad, hence, implying that the polymer is not mono-disperse in nature (PDI >1). Polydispersity index (PDI) is ratio of weight average molecular weight (M_w) to number average molecular weight (M_n). PDI is considered an important indicator of reaction completion, if the PDI is 1, all the chains in the

polymer have the same number of repeat units and equal molecular weights indicating the product is mono-dispersed. It was observed that for enzyme reactions PDI values normally increase with the degree of polymerisation as reported earlier by McCabe & Taylor (2004).

Hence, as expected, the synthesised material contains fragments of various chain lengths, with different molecular weights and number of repeat units. The quoted molecular weight (10500Da) was determined using the retention volume at peak maximum. Hence, this does not give the absolute molecular weight of the polymer sample. Since no absolute M_w (weight average molecular weight) and M_n (number average molecular weights) were obtained, no poly dispersity index (PDI) values were calculated.

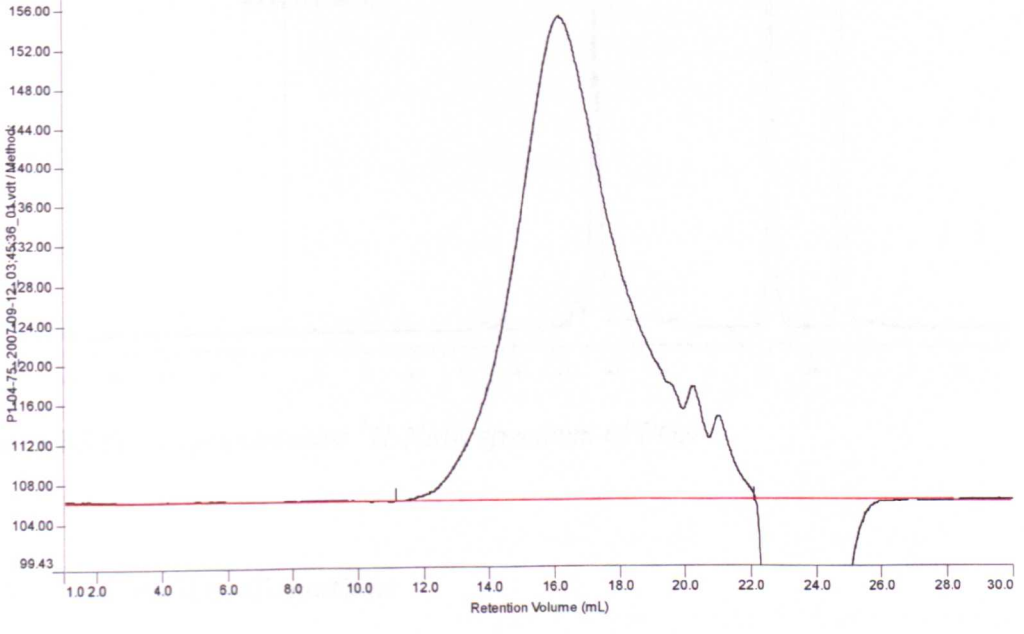


Figure 2.2 (a): *Representative GPC chromatogram of PGA*

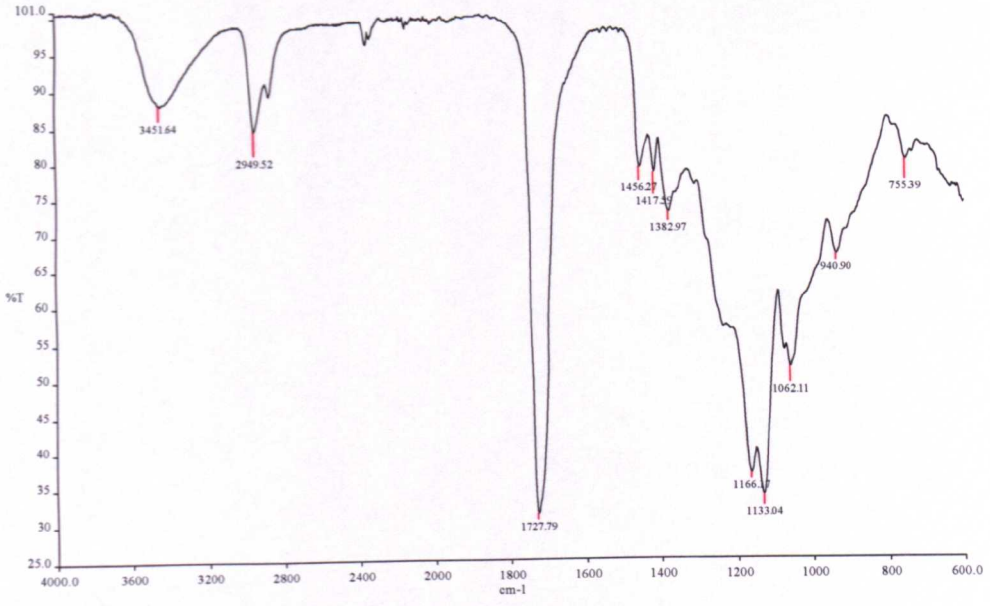


Figure 2.2 (b): *Representative FTIR spectrum of PGA*

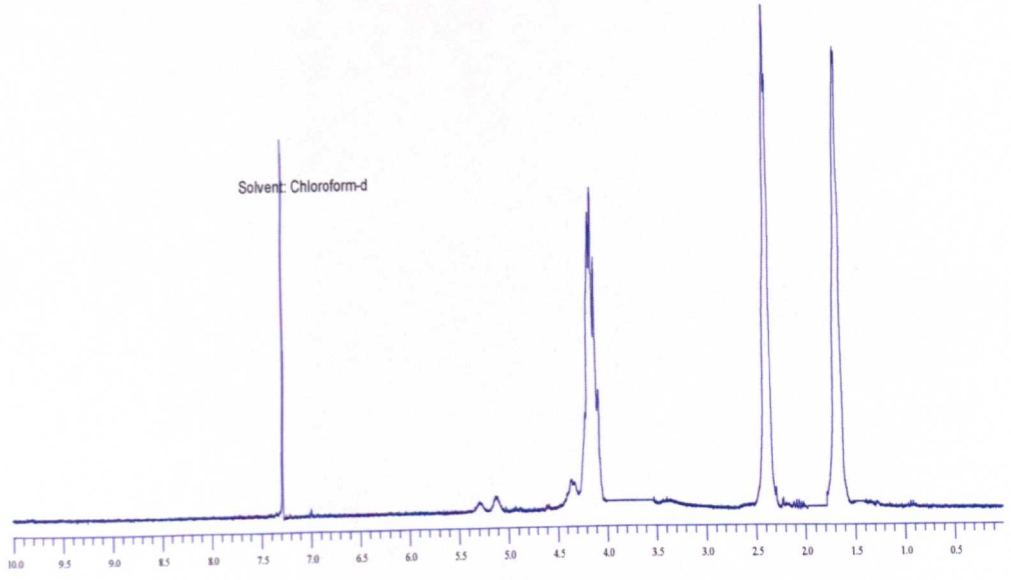


Figure 2.2 (c): *Representative ^{1}H-NMR spectrum of PGA*

b) Structural confirmation:

Structural elucidation was carried out by FTIR and ^{1}H NMR techniques. FTIR was also utilised for end group analysis of the polymer, which enabled confirmation of the reaction completion or product formation. The FTIR spectrum (Figure 2.2b) of the backbone polyester (PGA) clearly indicates the hydroxyl group at 3450 cm^{-1} and the large carbonyl peak at 1727 cm^{-1}. Further, the disappearance of bands related to

terminal vinyl groups of DVA at 1650 cm^{-1} confirms the complete consumption of monomers during the polymerisation.

The polymer was characterised via the proton assignments and integration of the ^1H-NMR spectrum (Figure 2.2c). It was observed that there was no evidence of any vinyl groups, which can be confirmed by the disappearance of signals at δ4.5, 4.8 and 7.3 corresponding to terminal vinyl protons, from the starting material (DVA).

The ^1H-NMR integration method (Kolhe et al 2004) was used to calculate the actual monomer ratio in the polymer. For DVA, the methylene ($-CH_2$) group, corresponding to 4 protons (d, g at 2.39 ppm) was considered as standard, and the variable multiplet at 4.18 ppm, corresponding to 4 $-CH_2$ protons (a, c) of glycerol were used for the calculation.

For PGA:

a) Number of $-CH_2$ protons (2.39 ppm) = 4protons = **1 molecule of DVA**

b) Number of $-CH_2$ protons (4.18 ppm) = 4protons = **1 molecule of Glycerol**

= 1 molecule of DVA + 1 molecule of glycerol

c) Ratio between DVA and Glycerol = 1:1 (DVA: glycerol)

For PGA synthesis, monomers were added in an equimolar ratio. Because DVA can only attach to glycerol a 1:1 ratio of monomers was expected. The above calculation supports the assumption that the monomer feed ratio and actual monomer ratio in the polymer were the same.

Although this reaction was previously utilised by Kallinteri et al. (2005) for the preparation of PGA for drug delivery studies, a detailed study of the effect of reaction parameters on the polymerisation reaction was not carried out. Hence, once the reaction was successfully performed on the larger scale (~100g) with good product yield, the effects of different reaction parameters were studied on the polymerisation of PGA. However, this was done on a slightly smaller scale of 10-15g, because it was not cost effective when large amounts of the polymer were not required.

2.3.1.2 Effect of reaction conditions on polymer synthesis

Reaction conditions were varied to study the effect on the molecular weight of PGA obtained.

Two different **heating media**, a metallic heating mantel fixed on a hot plate and a water bath, were compared to study the effect of the heating source on the polymerisation reaction. No difference in polymer molecular weight was observed between these, as similar molecular weight polymers were obtained (Table 2.5). The use of a hot plate as the heating medium for the polymerisation reaction was found to be a viable alternative option to the water bath for PGA synthesis. Both heating media provided the same temperature but the added advantage of using a hot plate was the water-free environment, as no water vapours were present around the reaction setup, hence minimising hydrolysis of polymer due to the introduction of moisture. However, one considerable drawback of using the hotplate is the incomplete immersion of the reaction contents in the heating mantle, which resulted in an uneven heating of the viscous reaction mixture. Thus the water bath was

selected as the heating medium for all future reactions for consistency of heating during polymer synthesis.

Table 2.5

Effect of heating medium on PGA molecular weight

Heating medium	Molecular weight (Da)
Water bath	9500 Da
Hot plate	8700 Da

Two different **stirring modes**, using a magnetic stirrer and an overhead stirrer, were investigated using a hotplate as the heating medium. It was observed that magnetic stirring failed to ensure proper mixing of the reactants resulting in the production of lower molecular weight polymers (Table 2.6). This can also be observed from the GPC traces shown in figure 2.3, which indicate the presence of a residual amount of monomer and small oligomers. This is probably due to the relatively small size of the magnetic bar (as compared to a Teflon paddle) and its inability to rotate and mix the increasingly viscous reaction mixture. Additionally, the magnetic stirrer may crush the enzyme support, resulting in loss of enzyme activity leading to less polymerisation and hence an overall reduction in molecular weight. All these factors result in an incomplete reaction, hence, unreacted monomers and very small molecular weight fragments can be seen at the tail of GPC chromatogram of the magnetically stirred polymer sample (Figure 2.3). At high enzyme loading, the enzyme beads become viscous and settle down or 'sink' in the reaction medium, resulting in the generation of two phases of medium with different

enzyme concentrations. Therefore, strong mixing is required to ensure an equal distribution of enzyme throughout the medium. This is why the polymer synthesised via magnetic stirring had a considerably lower molecular weight with a broader peak as compared to the overhead stirrer produced material. The overhead stirrer is a powerful means of stirring, and so improves the enzyme diffusion while in an increasingly viscous medium.

Table 2.6

The effect of different modes of stirring on molecular weights of PGA

Stirring mode	Molecular weight (Da)
Magnetic stirrer	3500 Da
Over head stirrer	8700 Da

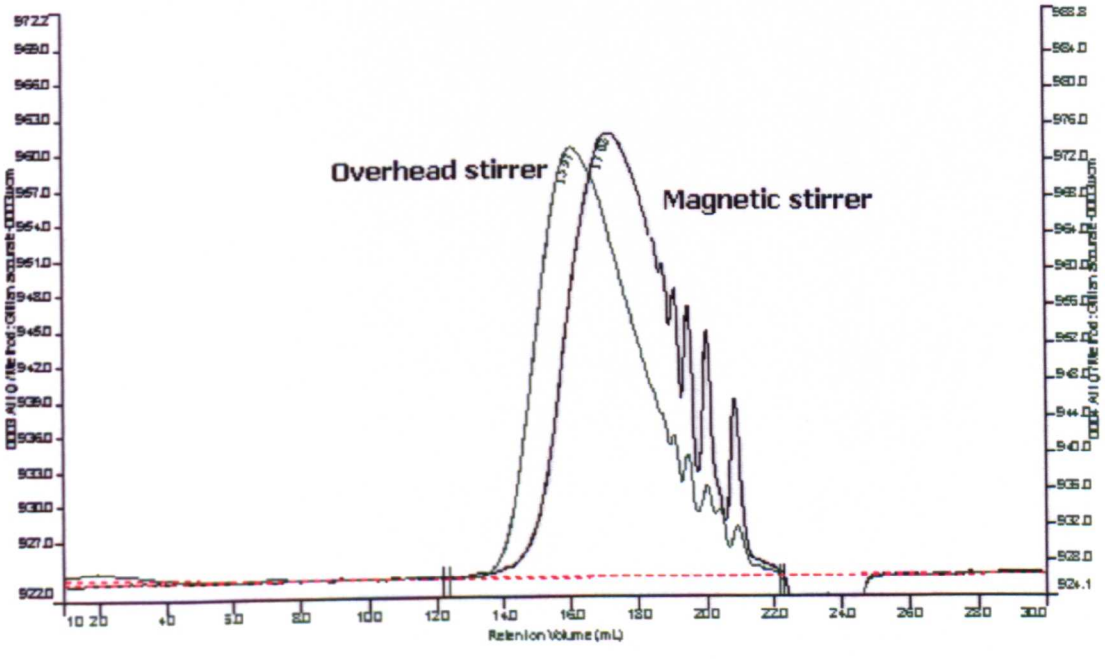


Figure 2.3: *GPC chromatogram of PGA synthesised using an overhead stirrer (green line) and magnetic stirrer (black line) for mixing of the reaction.*

Moisture content is one of the most important factors playing a vital role in polyester synthesis by maintaining the hydrolysis/condensation equilibrium. Hence, the moisture content of all the reactants was determined prior to use. The amount of moisture measured using Karl Fischer analysis was often different from the water content stated on the manufacturer's specifications. This may affect the enzyme activity, reaction equilibrium and hence the polymer molecular weight. A summary of these results are provided in table 2.7, which indicates that the amount of moisture in the reaction was higher than expected.

Table 2.7

Percent moisture content of reactants specified in the manufacturer's certificate of analysis and determined with a Karl Fischer titrator

Monomers	**% Moisture**	
	Measured	*Manufacturer's specifications*
DVA	0.5	N/A
Glycerol	0.9	0.1
Novozyme 435	0.2	N/A
Tetrahydrofuran	Not obtained	0.05
Tetrahydrofuran anhydrous	0.5	0.002

Traces of water associated with the enzyme are essential for optimum enzyme activity but also have the potential to hydrolyse the diester monomer and polyester end groups. The polymerisation reactions depend on simultaneous polymerisation, degradation and enzyme deactivation. Throughout these reactions only the moisture

content of Novozyme 435 was kept constant by placing it over activated silica and 3 Å molecular sieves. All other reactants were used without any further steps to reduce moisture content. However, in the polymerisation reaction the solvent constitutes the largest proportion. Therefore it has the potential to alter the reaction behaviour or product quality by introducing moisture into the reaction medium. To assess these two different types of THF, hydrous and anhydrous were studied. The molecular weights of polymers obtained are given in table 2.8, indicating that when anhydrous THF was used, a polymer with considerably lower molecular weight was produced.

Table 2.8

Effect of solvent moisture on molecular weight of PGA

Solvent type	**Molecular weight (Da)**
Hydrous THF	10171
Anhydrous THF	5733

As discussed earlier, some moisture is required for optimal enzyme activity (Chaudhary et al 1998), the results obtained further support the hypothesis that very low moisture contents led to decreased enzyme activity, hence, incomplete reaction. PGA synthesised in the anhydrous THF contained a higher number of small molecular weight fragments and un-reacted monomers (Figure 2.4), further proving that decreased enzyme activity resulted in incomplete reaction even after 24 hours. Additionally, this observation further confirms that decreasing moisture results in decreased enzyme (Novozyme 435) activity which is in accordance with the investigations done by Chaudhary et al (1998).

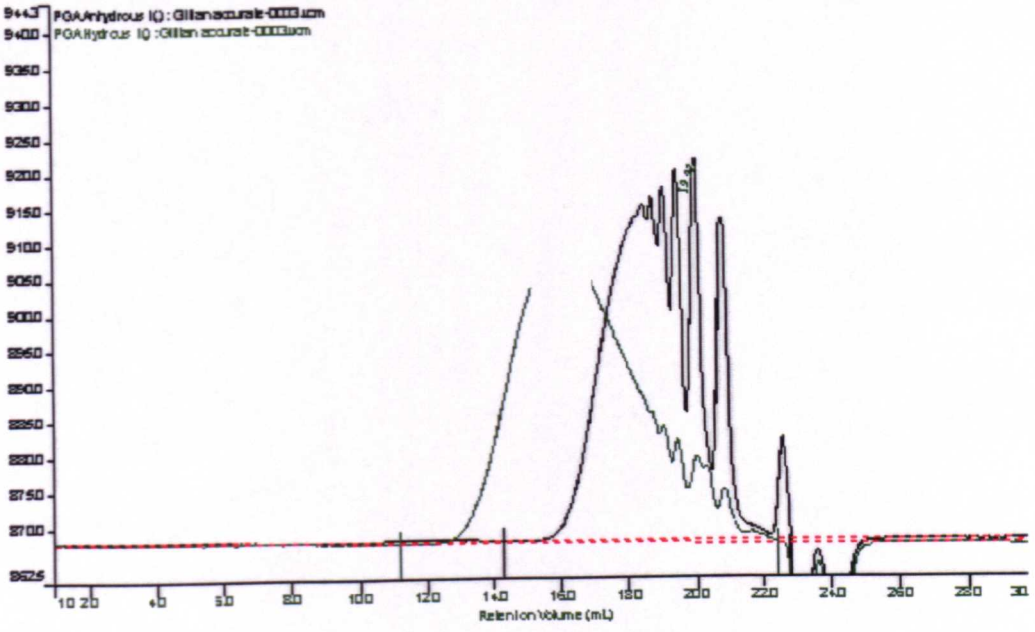


Figure 2.4: *GPC chromatogram of PGA synthesised in hydrous (green line) and anhydrous THF (black line).*

It is also worth mentioning here that immobilised enzymes (such as Novozyme-435) have water molecules trapped near the active sites, but in the case of pure enzymes (CAL-B), the situation will be other way around, as the enzyme support would not be there to keep hold of moisture thus result in better enzyme activity (Billie et al 2000). This further highlights the advantage of using immobilized enzyme for these reactions. Novozyme 435 requires a certain amount of moisture from its acrylic resin support for catalytic activity (Chaudhary et al, 1998). Addition of anhydrous solvent leaches moisture away from the acrylic resin resulting in a decreased ability of the enzyme to catalyse polymer synthesis. Moreover, it was observed that the acrylic resin starts deteriorating and passes through the filter, resulting in low molecular weight equivalent peaks in the GPC chromatograms. This reaction requires a balance between the water content required for enzyme activity and that which would cause hydrolysis of the products. Anhydrous solvents, whilst minimising hydrolysis also

result in decreased enzyme activity, hence leading to the synthesis of low molecular weight polymers.

Degradation of the enzyme support also affects the quality of the PGA polymer, as under anhydrous conditions the polymer product changed from a characteristic transparent, yellowish colour and viscous appearance to an opaque, brownish and very low viscosity liquid. Moreover the longer the enzymatic residue (along with fine acrylic resin) remains in the polymer, the more polymer degradation occurs. Figures 2.5 show the effect of decreasing moisture on the Novozyme 435 particles.

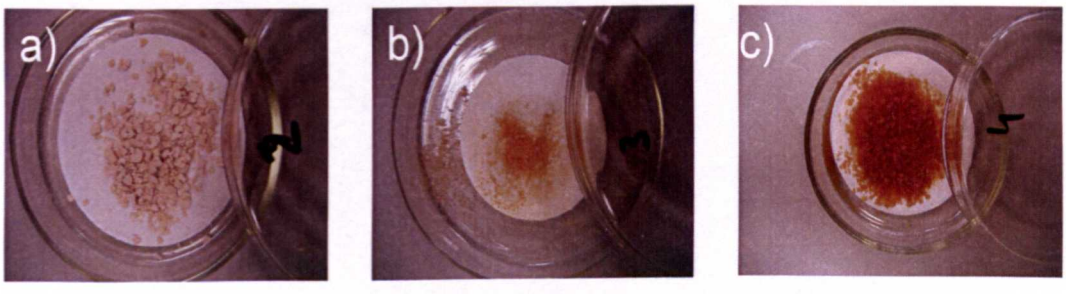


Figure 2.5: *Effect of volume of anhydrous THF on enzyme support integrity after 24hours: (a) 20 ml, (b) 10 ml and (c) 5 ml.*

The quantity of enzyme used in anhydrous media was further increased 10 times (to 5g) which resulted in an increase in molecular weight from 6500 Da to 9500 Da. This further supports the theory that a decrease in enzyme activity occurred in the presence of anhydrous solvent which resulted in a polymer of lower molecular weight being obtained. Increasing the amount of active enzyme resulted in a higher molecular weight polymer being formed. Hence, it may be concluded that in such reactions, where moisture content leads to decreased enzyme activity, addition of more enzyme will favour the reaction.

Solvent volume plays a crucial role in polymer synthesis in terms of provision of appropriate space for monomers to polymerise. Less solvent will reduce the freedom of monomers to form long straight chains of polymer, resulting in increased cross linking. Excessive solvent will result in increased distances between monomers and cause synthesis of many small chains of polymers, with low molecular weight.

A series of reactions were designed to investigate the effect of solvent volume on PGA synthesis. Molecular weights obtained throughout this series of experiments (Table 2.9) illustrated that there was no significant difference observed in the molecular weights of the polymers obtained. A lower volume of solvent appeared to result in a lower molecular weight than that obtained using a larger volume of solvent. Literature studies indicate that high molecular weight polymers can be obtained by solvent-free synthesis (Apurva et al 1997), but the results obtained with the published methods were not reproducible and either no polymer or very low molecular weight polymers were synthesised. All such reported reactions were carried out on very small scales, and the reason for not reproducing the results at large scale may lie behind the fact that not enough moisture for enzyme activity was available in such reactions or mixing was more difficult. Based on the results obtained, for any further reaction the solvent volume was set at 20-25 ml.

Table 2.9

Molecular weights of PGA obtained using different volumes of solvent (THF)

Solvent (THF) volume (ml)	Molecular weight (Da)
5	9782
20	10009
25	13363
50	12413

Further to the studies related to solvent volume, it was observed, during some reactions carried out by undergraduate project students, that when solvent evaporated from the reaction mixture via improperly closed joints of the reaction flask, this effected the polymer molecular weight. Hence, a series of reactions under controlled solvent evaporation were carried out to investigate this observation.

A series of experiments was designed to study the effect of solvent evaporation on PGA synthesis. An increase in evaporation was directly proportional to the considerable increase in molecular weight observed (Table 2.10). These results indicated that if solvent was allowed to evaporate to some extent from the reaction medium, it affected the molecular weight and physical properties of the polymer, possibly by facilitating cross linking, when solvent volume decreased.

Table 2.10

Molecular weight of PGA obtained under sealed and open reaction systems.

Reactor Status	Molecular weight (Da)
Closed/Evaporation limited	9000
Open/Evaporation allowed	14000

Further investigations were carried out, by allowing the solvent to evaporate over 24 hours of reaction then more THF was then added to the same reaction medium and left for a further 24 hours, before stopping the reaction. It was observed that solvent evaporation resulted in a slight increase in molecular weight of the polymer but if more solvent was added to the same reaction system, the molecular weight started to decrease slightly presumably via hydrolysis (Table 2.11).

Table 2.11

Change in molecular weight of PGA with addition and removal of THF

Solvent status in reactor	Molecular weight (Da)
Solvent evaporated	13417
Additional solvent added	12208

These results provided valuable information on the real-time control of molecular weight whilst proving the requirement for strict control over the reaction conditions

to get reproducible polymerisation that results in polymers with the same molecular weight and properties.

The next step was to study the effect of the concentration of enzyme on PGA synthesis. It is already well established that enzyme concentration affects the molecular weight and hence polymer properties (Chaudhary et al 1995). In a series of experiments various amounts of enzyme were used and the results obtained indicated that the molecular weight of the polymers increased considerably with increased concentration of enzyme (Table 2.12). These results are in accordance with the studies done by Uyama et al (1995), who also reported an increase in polymer molecular weight and yield by increasing the amount of lipase. To achieve the desired molecular weight of 10-12 kDa, the enzyme concentration was kept to 3.4% w/w Novozyme 435 for PGA synthesis in all further reactions.

Table 2.12

Effect of enzyme concentration on PGA molecular weight

Enzyme concentration *(% w/w monomers)*	**Molecular weight (Da)**
0.34%	5733
1.3%	6609
3.4%	9468

The effect of reaction time on polymer synthesis was studied in the final series of experiments by removing a small amount of reaction matrix at specific time intervals. This was then dissolved in 2 ml of THF and filtered through 0.45μm membrane filters to a GPC vial. Molecular weight analysis was performed at specific time intervals from 1 to 40 hours. It was observed that polymer molecular weight increased as a function of time until 24 hours, followed by a sharp decrease thereafter (Figure 2.6). These results support the fact that increasing time has an effect on polymer properties (molecular weight) as reported earlier by Kumar & Gross (2000). The corresponding GPC traces are shown in figure 2.7. Hence, 24 hours reaction durations were selected for all of the subsequent reactions. It is worth noting that, in enzyme catalysed polymerisation, the reaction duration allows conversion of monomers to polymer but at the same time enzyme activity can reduce over time, hence leading to a slower increase in molecular weight, the hydrolysis reaction can then lead to an overall decrease in polymer molecular weight (MacDonald et al 1995).

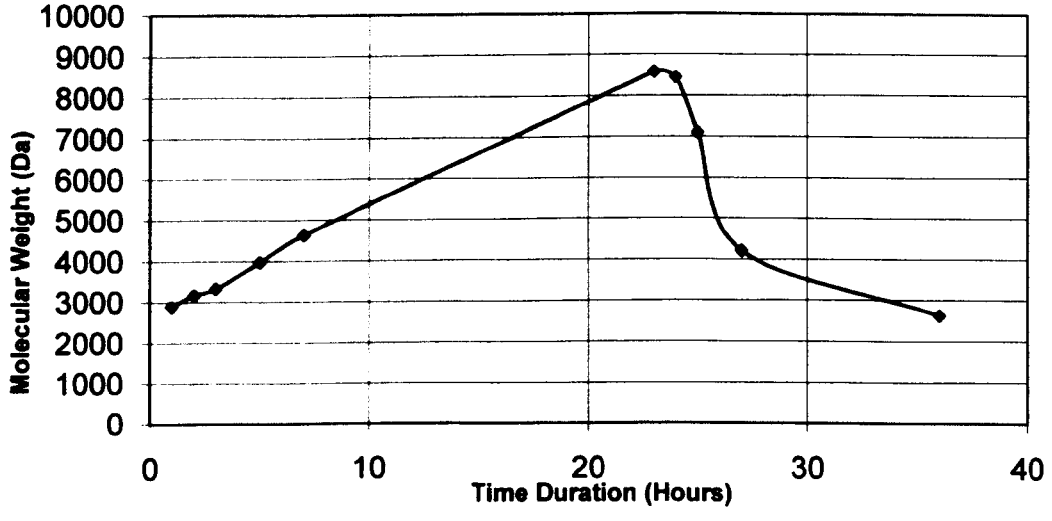


Figure 2.6: *Change in molecular weight of PGA with respect to reaction duration*

Polyesterification reactions occur in three stages, the first stage is characterised by very rapid transesterification, in the second stage the polymerisation and chain growth proceeds to a high molecular weight product, while in the third stage, the polymerisation rate decreases to limit the molecular weight of the polymer and chain growth. After the third stage the polymer starts degrading via hydrolysis (Billie et al 2000), which can also be seen in figure 2.7. Side reactions including hydrolysis and deactivation of biocatalysts also cause low molecular weight products. The increasing chain length causes a decrease in enzyme specificity and diffusional resistance to enzyme, which is also a hindrance to obtaining greater molecular weight of the product (Billie et al 2000).

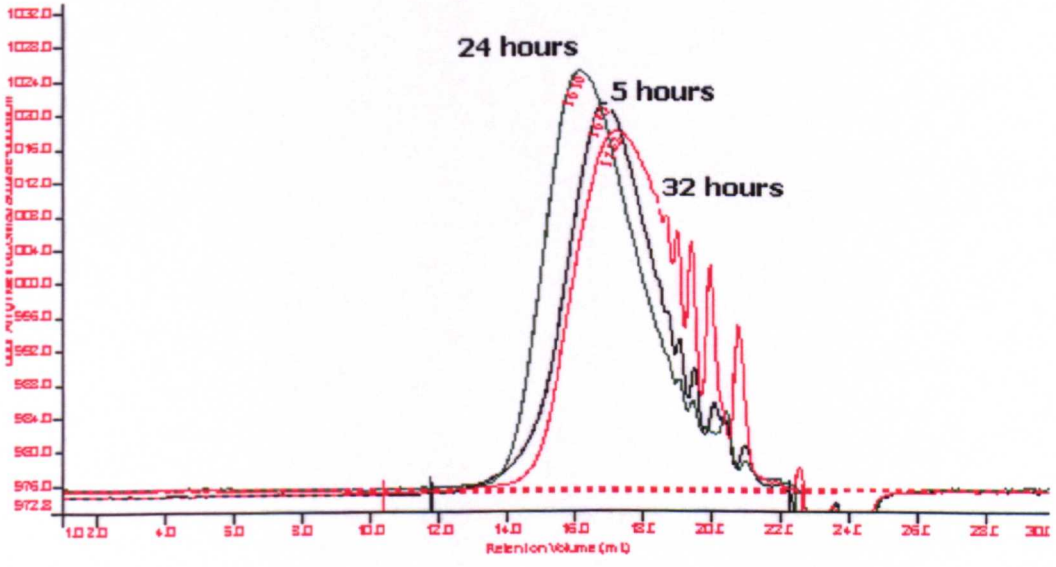


Figure 2.7: *Overlaid GPC chromatograms of PGA obtained after various reaction durations. Black: 5 hours, Green: 24 hours, Red: 32 hours*

In addition to hydrolysis, the hydroxyl groups of the added diol were involved in transesterification reactions which caused scissions of the high molecular weight polyester, hence the extent of transesterification was proportional to the reduction in average molecular weight of the polyester, as also confirmed by the findings of

McCabe & Taylor (2004). Kumar and Gross (2000) proposed that with an increase in molecular weight, the available number of hydroxyl groups decreases, resulting in transesterification rates slowing down also.

It is established that the first 30 minutes of transesterification is very important, but because of the kinetics and energetic status of these reactions, the system cannot remain isothermal, until temperature controlling measures are in place (Apurva et al 1998). Apurva et al observed a 20-25°C increase in temperature during this phase due to insufficient heat transfer, when DVA and 1,4-butanediol reacted in a water bath with the help of a magnetic stirrer (Apurva et al 1997). To solve the problem Billie and co-workers designed a isothermal reactor, but temperature changes were observed in the initial part of the reaction, which was dependent on enzyme concentration (Billie et al 2000). Hence, for the uniformity of reaction conditions, a 24 hour reaction duration was opted for all following reactions.

Once all the reaction parameters were studied for PGA synthesis, the following reaction conditions were set as optimal parameters; A water bath was used to maintain the temperature at 50°C; 25ml solvent (normally THF) was added to the reaction mixture in a properly sealed reaction vessel with the enzyme concentration of 3.4%(w/v) and the reaction was left stirring via an overhead stirrer (Teflon paddle) for 24 hours.

A series of experiments were then conducted using identical reaction conditions, for the evaluation of reproducibility of the synthesis of PGA of a desired molecular weight of approximately 10 kDa. Three PGA syntheses were carried out on different days, under the same reaction conditions and the results obtained are summarised in

table 2.13. These results indicate that polymers synthesised using optimised reaction conditions were reproducible.

Table 2.13

Reproducibility of PGA synthesis under identical reaction conditions

Experiment (month)	Molecular weight (Da)
1	8952
2	10009
3	10210
Average	**9723**

Once optimal reaction conditions were obtained, polymerisation reactions using various other monomers were performed under the same reaction conditions to produce polyester-co-lactones and PEG-copolymers. As some of the products were solid at room temperature, purification procedures were applied for the removal of unreacted monomers (if any) and small chain fragments or oligomers. Because monomers with different solubilities were used, the need for a standard procedure was highlighted, which enabled the removal of a broad range of impurities, monomers, fragments and oligomers. The following section describes the development of the purification procedure.

2.3.2 Polymer processing and purification

To obtain a useful product it was necessary to remove unreacted monomers and small molecular weight fragments from the crude material. Both water and methanol are good solvents for the monomers used as well as the small molecular weight polymer fragments formed, and did not dissolve co-polymers such as PGA-co-PDL. They can therefore be used to purify these polymer samples once polymerisation is terminated.

The ability of MeOH and water to effectively remove any residual unreacted monomers and oligomers was evaluated. A physical mixture of monomer (PEG) and polymer (PGA-co-PDL) was prepared to mimic the original unpurified sample. The overlaid GPC chromatogram (Figure 2.8) of the physical mixture of polymer, PEG and unpurified polymer sample shows that although peaks corresponding to both analytes eluted at different retention volumes, both peaks overlapped each other hence, it is a possibility that small concentrations of residual unreacted PEG were not detected by GPC (Figure 2.8). Therefore, washing of such polymers is very important to ensure a pure and monomer-free product.

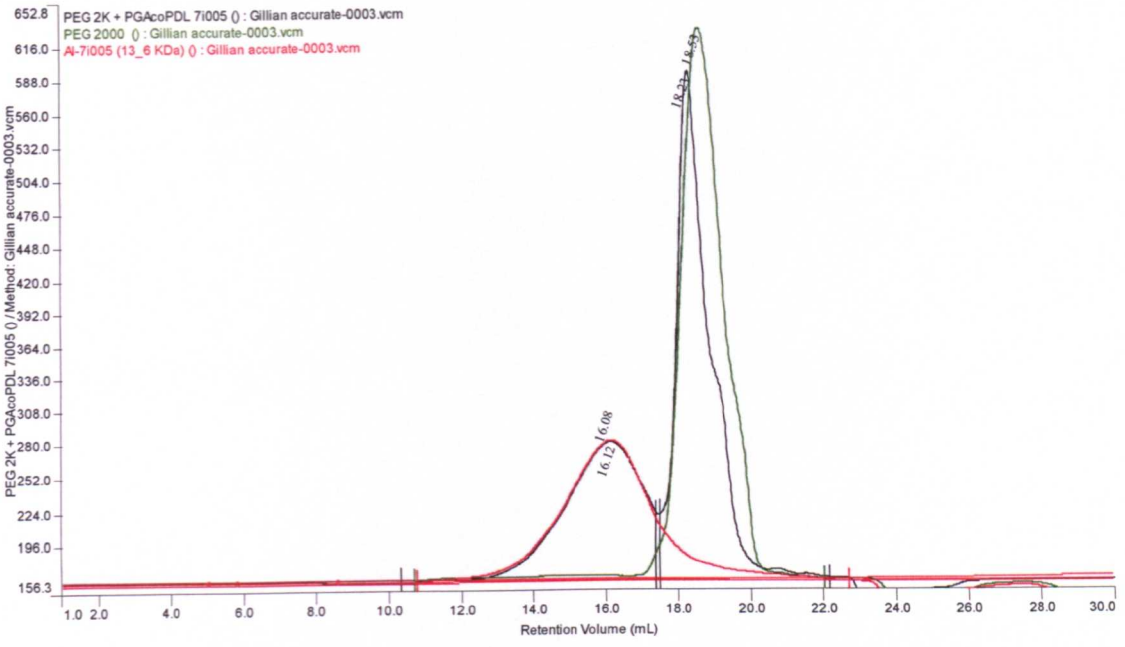


Figure 2.8: *GPC chromatogram of a physical mixture of PEG and PGA-co-PDL (black line) overlapped with chromatograms of pure PEG (green line) and unpurified PGA-co-PDL-PEG sample (red line)*

A physical mixture of PGA-co-PDL with PEG or PEGme was added to water and mixed thoroughly. Analysis of the filtrate and residue clearly indicated that all of the PEG passed into the filtrate leaving pure, clean polymer on the filter paper as a residue. Chromatographic analysis (Figure 2.9) confirmed that there were no traces of unreacted PEG and PEGme in the polymer samples.

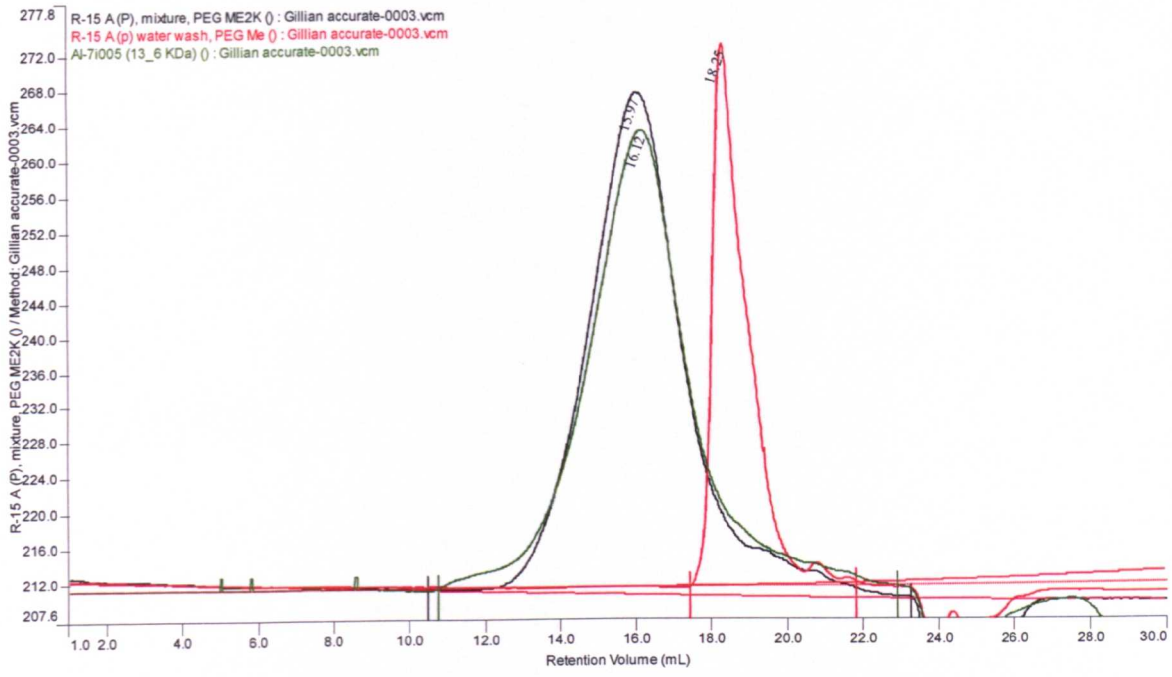


Figure 2.9: *GPC chromatogram of PEGme in filtrate (red line), PGA-co-PDL (green line) and PGA-co-PDL-PEG (black line) washed in water*

Since previous studies confirmed that water is a major source of degradation of these polymers via hydrolysis, efforts were made to replace water with another suitable solvent to avoid polymer degradation. Dong and Feng (Dong & Feng 2004) and Tefani et al (2006) reported the use of MeOH for the removal of unreacted PEG. Hence, MeOH was used as the purification solvent. It was observed that all PEG is removed from the polymer-PEG mixture, suggesting that MeOH can be used instead of water for the removal of PEG. Methanol is also a better choice than water because of the improved solubility of the monomers and small oligomeric fragments in methanol, while the efficacy of water is limited to the removal of only unreacted PEG from the polymer. The fronting of the PEG peak in methanolic filtrates is because of the removal of small molecular weight fragments of the polymers, which results in a lowering of the PDI values (expressed by peak narrowing) of polymer peaks as shown in figure 2.10.

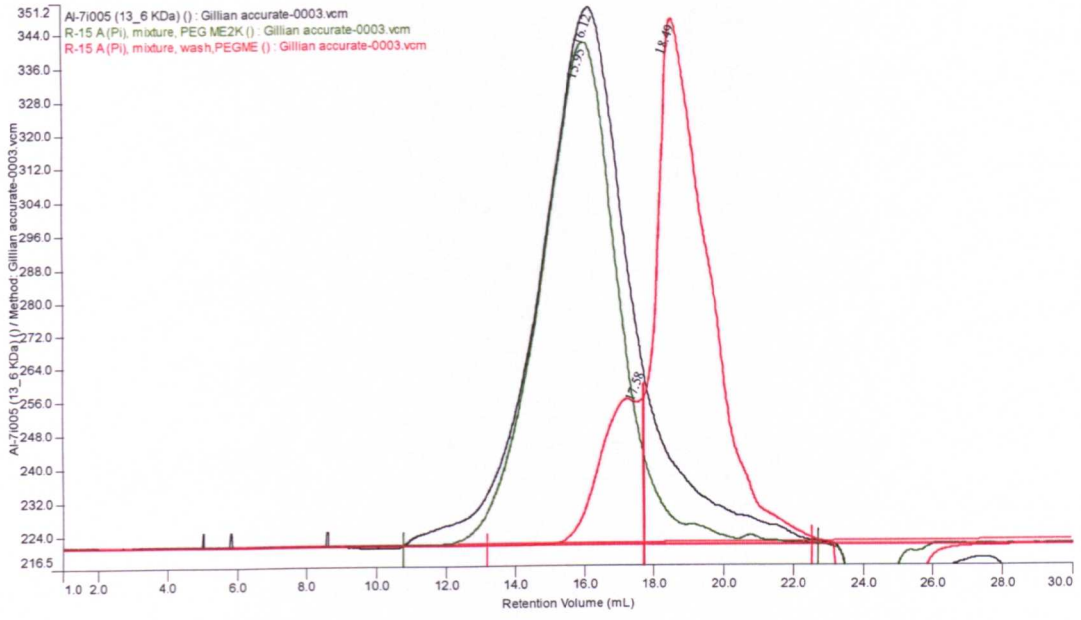


Figure 2.10: *GPC chromatogram of PEGme in wash (red line), PGA-co-PDL-PEGme (black line) and PGA-co-PDL-PEGme after washing in MeOH (green line)*

Hence it was additionally confirmed that water can only remove PEG but not polymer (PGA-co-PDL), because of its insolubility in water. The effectiveness of MeOH for not only the removal of PEG but also oligomeric fragments is evident in the analysis of filtrate samples (Figure 2.11), where it is clearly evident that although water is a good solvent to remove PEG, it failed to remove small molecular weight fragments from the polymer matrix.

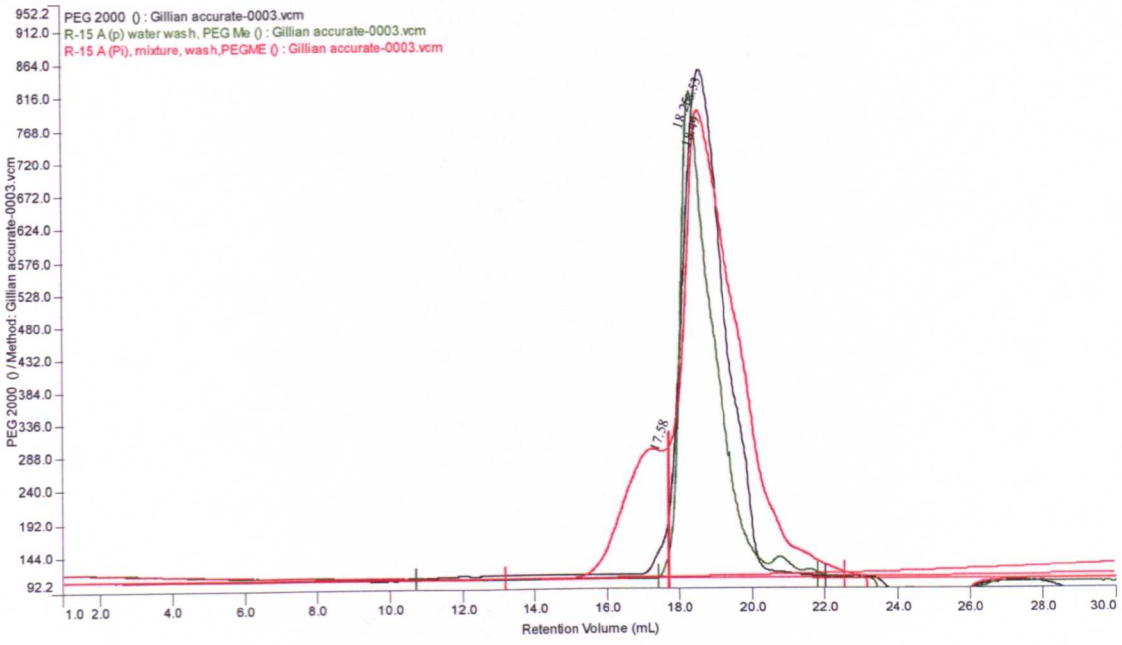


Figure 2.11: *GPC chromatogram of pure PEGme (black), PEG(me) in water wash (green) and PEGme in MeOH wash (red)*

Purification or re-crystallisation of polymers is required to ensure that polymer samples are free from unreacted monomers or low molecular weight fragments. All solid polymers that underwent re-crystallisation or re-precipitation from MeOH had decreased polydispersity and increased peak symmetry because of the removal of low molecular weight moieties and removal of unreacted monomers. Liquid polymers could not undergo the recrystallising/purification process because of their viscous nature and solubility in MeOH and other organic solvents. Hence, PGA was used without any further purification.

2.3.3 Development of a polymer library

Of all the polymers synthesised, PGA has the simplest structure, therefore it was selected as a model polymer to study the effect of varying different reaction parameters on polyester synthesis. This helped determine optimal conditions and allow control over some properties of the polymer obtained. However, the nature of this polymer was not very useful for drug delivery unless further modified (Kallinteri et al 2005). Hence, a library of more complex functionalised polyesters was synthesised, as discussed in following sections.

2.3.3.1 Functionalised and non-functionalised linear polyester synthesis

The divinyl ester monomer (DVA) was enzymatically polymerised with terminal diols, including glycerol, 1,3-propanediol and 1,4-butanediol. The same reaction conditions were used for the synthesis of all polymers with and without pendant hydroxyl groups on the polymer backbone. Lipase from *C. antarctica* (lipase CA) was used in a non-aqueous medium (THF). In most cases, the monomers were quantitatively consumed but the product was not obtained in high yield (60-65%). This may be due to the formation of a significant proportion of methanol-soluble oligomers which were removed during the purification/processing stage. The completion of the polymerisation was confirmed with the consumption of all monomers, as no bands corresponding to terminal vinyl group appeared in the corresponding FTIR and NMR spectra (section 2.3.1.1). However, hydrolysis of the vinyl esters may have taken place during the polymerisation. Lipases regioselectively catalyse the esterification of primary alcohols (Kobayashi 2009), hence, from FTIR, a decrease in the band corresponding to the –OH group in case of glycerol and the complete disappearance of the –OH bands from the diol monomers confirmed the

consumption of the monomers. The practically observed ratio of monomer units was also the same as the experimental ratio in which the monomers were used. The molecular weight of PGA was 10 kDa with 50 repeating units. While the molecular weights of non-functionalised, hydrophobic polymers based on 1,3-propanediol (PPA) and 1,4-butanediol (PBA) were in the range of 5.3-7.3 kDa (Table 2.14).

Based on the same reaction a novel non-functionalised hydrophilic polymer, PTEGA, was synthesised from the esterification of triethylene glycol and DVA. FTIR analysis of PTEGA also showed complete disappearance of the bands corresponding to –OH and vinyl groups and the molecular weight was confirmed as 8.7 kDa. This illustrates the successful use of the enzyme catalysed reaction for the synthesis of a range of versatile polymers. Although the synthesis of PBA via enzyme catalysis was previously reported by Apurva et al (1998), and the synthesis of PGA, PPA and related polymers have been reported by Kobayashi et al (2006), generally on the micro-scale. Using the optimal conditions studied, all these polymers were synthesized at useful scale (up to 100g) via one-step reactions. Application of the same reaction method for the synthesis of the novel polymer, PTEGA, indicates the versatility of this reaction. The summarised results of the polymer synthesis are tabulated in table 2.14. All of these polymers (at room temperature) were very soft solids or highly viscous liquids. The reason for this physical appearance lies in the fact that the polymeric units have shorter carbon chain lengths (9-10 carbon chains). Further purification of these polymers was not possible, because of the solubility of the high molecular weight and low molecular weight fragments of the polymer in same solvents.

Table 2.14

Lipase catalysed functionalised and non-functionalised linear polyester synthesis

Polymer	Functional group	Monomer ratio		Mw (Da)	Physical state	T_g (°C)	T_m (°C)	Contact angle (θ)	Yield (%)
		Feed ratio	Calculated ratio						
PGA	-OH	1:1	1:1	10171	Viscous	-	-	50.10 ± 5.36	55-65
PPA	---	1:1	1:1	5387	Viscous	-	-	-	55-65
PBA	---	1:1	1:1	7369	Soft Solid	-	-	-	55-65
PTEGA	---	1:1	1:1	8785	Viscous	-	-	-	65-75
PLGA*	---	1:1	1:1	9500	Solid	-	34.28	75.81 ± 1.34	-

* : PLGA was obtained from PURAC biomaterials.

- : Not available because of the viscous state of the polymer

PLGA, of a comparable molecular weight and of high purity (monodisperse) was purchased as a solid polymer at room temperature. Even though it had shorter chain length, it was probably solid because it had been purified. PLGA is an FDA-approved polymer and has been extensively studied (Jain 2000).

The PLGA chain consisted of 65 repeat units, while the hydrophilic polymer, PGA had 50 repeating units. On the other hand both PPA and PBA consisted of 40 repeating units and PTEGA was made up of 35 repeating units. Polymer properties, such as hydrophilicity and degradation can be altered by controlling the repeating units (Oster et al 2004) and number of pendant functional groups per chain (Bhattacharyya et al 1998). These contribute to the polymer properties and behaviour during drug delivery applications, and hence can be further exploited for the synthesis of polymers with desired properties.

The properties of all the synthesised polymers were compared against PLGA. The hydrophilicity of PGA was compared to PLGA. The contact angle (θ) on the surface of these polymers showed that, as expected, PGA has a smaller contact angle (50.10±5.36) than PLGA (75.81±1.34) due to the presence of free –OH groups making PGA more hydrophilic than PLGA. Hence, glycerol was selected in further polymerisations to both impart hydrophilic character to the material and afford pendant hydroxyl groups.

Neither PGA, PPA, PBA nor PTEGA showed any measurable thermal properties and T_g, T_m and T_c values were not obtained over the range of -25°C to 150°C. These polymers are very viscous at room temperature and did not solidify even at -25°C nor did they boil/evaporate at 100°C. However, the equivalent PLGA polymer, a solid at room temperature, has a T_m of 34.28°C. A polymer with a low glass transition temperature (T_g) and melting point (T_m) can be used effectively in drug delivery

applications as it can melt and release the drugs at body temperature. However, at the same time storing it at room temperature can decrease its shelf life by increasing degradation, resulting in a loss of important properties.

Polymer properties can be altered by incorporating different monomers which can help to make the polymers useful in drug delivery applications. Hence, PGA, PPA and PBA were copolymerised with lactones and PEG in an attempt to obtain materials with relatively higher molecular weights and solid states with higher T_g and T_m values. These polymers are discussed in the following sections.

2.3.3.2 Functionalised and non-functionalised linear polyesters via ring-opening copolymerisation of lactones

Thompson et al (2006), reported the large scale synthesis of PGA-co-PDL and its subsequent investigation for ibuprofen delivery, while Gaskell et al (2006) reported the application of the same polymer for pulmonary delivery of chymotrypsin. In this work variations of PGA-co-PDL were synthesised altering the properties to make the polymer more hydrophilic or more functionalised to study the effect of this on the quality and type of drug encapsulation.

Polymers equivalent to PGA-co-PDL, and PBA-co-PDL were synthesized with varying carbon chain lengths by introducing caprolactone (seven carbon members) instead of pentadecalactone (a 15 carbon chain) or by introducing triethylene glycol instead of glycerol to obtain PGA-co-PCL and PTEGA-co-PDL. The ratios of the repeating units were varied to alter the hydrophobicity of the polymer (PGA-co-PDL 1:1:1 and PGA-co-PDL 1:1:1.2). The numbers of pendant functional groups were altered by using non-functionalised polymers such as PPA-co-PDL, PBA-co-PDL or by using sorbitol, a sugar, as a monomer (PSA-co-PDL).

All the resulting polymers were obtained in good yields of about 60% (Table 2.15). Because of their solid states co-PDL polymers showed narrow molecular weight dispersities in GPC and were free of smaller molecular weight fragments.

The ^{1}H-NMR integration method (Kolhe et al 2004) was used to calculate the actual monomer ratio in the polymer. For DVA, the methylene (-CH_2) group, corresponding to 4 protons (d, d' at 2.32ppm) was considered as standard, and the variable multiplet at 4.18ppm, corresponding to 4 –CH_2 protons (a, c) were used for glycerol, while the sum of all protons at 1.34, 1.65, 2.32 and 4.05ppm were used to calculate the ratio of PDL in polymer. These polymers are complex in nature and have a broad distribution of molecular weight and chain lengths, therefore protons signals overlap each other. This decreases the accuracy of the one dimensional ^{1}H-NMR calculation to determine the exact monomeric ratio but does provide an estimate of the polymer composition, which can be used to explain and predict polymer properties.

For PGA-co-PDL:

a) Number of –CH_2 protons (2.32ppm) = 4protons = **1 molecule of DVA**

b) Number of –CH_2 protons (4.18ppm) = 4protons = **1 molecule of glycerol**

= 1 molecule of DVA + 1 molecule of glycerol

c) Number of –CH_2 protons (1.34, 1.65, 2.32 and 4.05ppm) = 28protons

= 1 molecule of PDL

d) Ratio between DVA and glycerol = 1:1:1 (glycerol:DVA:PDL)

For PGA-co-PDL synthesis, glycerol and DVA were added in equimolar ratio, while the amount of PDL was varied. Because DVA can react with glycerol or PDL, there are multiple possibilities of attachment of monomers expected, leading to a random polymer.

The NMR calculation supports the assumption that because monomer feed ratio and actual monomer ratio is not same, the polymer would be "random" in nature. Under consistent reaction conditions and assuming that monomers do not have similar reactivity, the polymers were presumed to be most likely random in nature as previously established by Namekawa et al (2000), which was then further confirmed from the ^{13}C-NMR spectrum of polymer, where by the peak corresponding to carbonyl groups (δ173-174ppm) was split in six peaks, corresponding to six possible ways the three monomers can join.

Two lactones were selected for copolymerisation, a seven-membered ring, ε-caprolactone (CL) and a fifteen membered ring, ω-pentadecalactone (PDL). The results presented in the table 2.15 showed that co-PDL polymers yielded products with a higher lactone ratio than co-CL polymers. For instance, a 1:1:0.7 ratio of monomers in PGA-co-CL was achieved when monomers were mixed in ratio of 1:1:1. On the other hand the corresponding 1:1:1 ratio of monomers in PGA-co-PDL was achieved from the mixing of 1:1:1 ratio of monomers. The details of the results are summarised in table 2.15.

Additionally, because of the small ring size, co-CL polymers did not result in high molecular weight polymers, hence causing all co-CL polymers to form viscous products at room temperature. On the other hand PGA-co-PDL based materials yielded solid products with high molecular weights. It is already established that the control imparted to lipase-catalysed ring-opening polymerisation reactions is not good and the molecular weight range and PDI of the products are very high (Kobayashi et al 2001).

Table 2.15

Lipase catalysed functionalised and non-functionalised linear polyester synthesis via ring-opening copolymerisation of lactones

Polymer	Functional group	Monomer ratio		Mw	Physical state	T_g	T_m	Contact angle
		Feed ratio	Calculated ratio	(Da)		(°C)	(°C)	(θ)
Co-Caprolactone								
PGA-co-CL	-OH	1:1:1	1:1:0.70	1855	Viscous	-	-	-
PPA-co-CL	---	1:1:1	1:1:0.45	4901	Viscous	-	-	-
PBA-co-CL	---	1:1:1	1:1:0.40	18388	Viscous	-	-	-
Co-Pentadecalactone								
PGA-co-PDL	-OH	1:1:0.5	1:1:1	15600	Soft solid	-	38.15	70.02 ± 1.20
PGA-co-PDL	-OH	1:1:1	1:1:1	17560	Solid	-	42.31	65.65 ± 2.36
PGA-co-PDL	-OH	1:1:1.5	1:1:1.2	29149	Solid	36.11	52.34	-
PPA-co-PDL	---	1:1:1	1:1:0.6	8566	Solid	45.10	56.25	77.71±1.52
PBA-co-PDL	---	1:1:1	1:1:0.5	15960	Solid	-	-	-
PTEGA-co-PDL	---	1:1:0.5	1:1:0.6	7740	Soft solid	-	-	-
PTEGA-co-PDL	---	1:1:1	1:1:1	23801	Solid	-	49.81	-
PTEGA-co-PDL	---	1:1:1.5	1:1:1.5	17413	Solid	49.32	61.18	-
PSA-co-PDL	-OH	1:1:1	1:1:0.5	3258	Solid	-	-	-

- : Not available because of the viscous state of the polymer

When the amount of added lactone was varied (0.5, 1 and 1.5moles) relative to the other monomers, it was observed that the ratio of monomers in the product was near to 1:1:1, regardless of the amount of added lactone. These results suggest that monomers are linked in equimolar ratios giving an A-B-C-C-C-A-B-A-B-A-B-C type polymer. However, this change in the monomer ratio also resulted in a difference in the physical properties of polymers obtained. For instance, all polymers were solid at room temperature but increases in the lactone ratio from 1:1:1(17.5 kDa) to 1:1:1.2 (29 kDa) caused an increase in T_m from 42.31 °C to 52.34°C. The same trend was observed for the contact angle of these polymers, as θ for 1:1:0.5 was 70.02±1.20 while for 1:1:1 the observed θ was 65.65±2.36. Hence, suggesting that molecular weight is the not only difference but these polymers also have different chemistries, which cannot be explained by the ^{1}H- or ^{13}C-NMR analysis.

The obtained results confirm that functionalised polymers (with pendant hydroxyl groups) were successfully synthesised possessing the desired properties of higher molecular weight, higher T_m, lower T_g values and in the solid state at room temperature. The corresponding non-functionalised (no pendant –OH) polymers, PPA-co-PDL and PBA-co-PDL, were also synthesised. These polymers also have the desired material properties; higher molecular weight, higher hydrophobicity, and higher T_m and lower T_g values, that are required for drug delivery applications as discussed in chapter 1.

One more important thing worth noting here is that PGA-co-PDL 1:1:1 and PPA-co-PDL 1:1:1, which differ only in the presence of –OH groups also exhibited different physical properties. A higher hydrophobicity (contact angle) and a 14°C higher T_m of the PPA-co-PDL was observed while the lactone ratio and molecular weight is lower than that of PGA-co-PDL. This signifies the effect of pendant hydroxyl groups on

the polymer properties. Based on a similar reaction, PTEGA-co-PDL was synthesised as a non-functionalised hydrophilic polymer. It was observed that PTEGA-co-PDL (1:1:1) and the corresponding PGA-co-PDL (1:1:1) have similar T_m values which may be considered an effect of the higher molecular weight.

Sorbitol was used as a monomer to obtain hydrophilic polymers with more pendant hydroxyl groups. The synthesis of such polymer products was more complex as these more hydrophilic polymers were not soluble in non-polar organic solvents. Additionally, the presence of higher numbers of –OH groups (four hydroxyl groups per unit) on the polymer backbone caused hydrogen-bonding between polymer chains which made them insoluble in polar organic solvents.

The polymer in which the lactone ratio was less than expected (1:1:0.5), can be explained by the facts that lipase have more tendency to polymerise at primary hydroxyl groups (regioselectivity) resulting in a higher ratio of sugar in the polymer. Additionally, a higher number of –OH groups made the medium more hydrophilic leading to a smaller molecular weight (3.2 kDa) polymer. A similar effect of more hydrophilic monomers on polymer molecular weight was also observed when PEG was used as a monomer (discussed in following section).

The results obtained in this section further illustrated that hydrophilic copolymers with and without functional groups were successfully synthesised via the same reaction scheme, involving both ring-opening and polycondensation polymerisation.

Following this, attempts were made to synthesise block copolymers of these co-lactones with PEG. These types of polymers have hydrophilic and hydrophobic segments (are amphipathic) and may afford better drug loading.

2.3.3.3 Functionalised and non-functionalised linear PEG-copolymers

The importance of using PEG block copolymers in drug delivery is already recognised by materials scientists (Harris 2001; Dong & Feng 2004). Many researchers have focussed their work on the synthesis of PEG copolymers with either PLGA (Ghahremankhani et al 2007) or polycaprolactone (PCL) as they are FDA-approved biomaterials (Yuan et al 2000). In terms of improving the properties of polymers investigated here, PEG was used as a monomer for the synthesis of block copolymers.

Although, lipase-catalysed PEG copolymer synthesis by ring-opening polymerisation of lactones has previously been reported (He et al 2003), the synthesis of PEG copolymers via simultaneous ring opening and polycondensation has not been reported before. As PEGs have terminal hydroxyl groups, lipase catalysis was explored for the copolymerisation with monomers such as glycerol, DVA and PDL for the synthesis of different novel polymers as listed in table 2.16 and 2.17. The physical states of the resulting polymers were generally similar; short chain copolymers were viscous liquids or soft solids at room temperature with molecular weights in the range of 3-5 kDa.

The ^{1}H-NMR integration method (Kolhe et al 2004) was used to calculate the actual monomer ratio in the polymer. In addition to the designated peaks of DVA, glycerol and PDL, the peaks at 3.6ppm, corresponding to CH_2 protons of PEG, were used to calculate the ratio of PEG in polymer.

For PGA-co-PDL-PEG:

a) Number of $-CH_2$ protons (2.39ppm) = 4protons = **1 molecule of DVA**

b) Number of $-CH_2$ protons (4.18ppm) = 4protons = **1 molecule of glycerol**

= 1 molecule of DVA + 1 molecule of glycerol

c) Number of $-CH_2$ protons (1.3ppm) = 24protons = **0.84 molecule of PDL**

d) Number of $-CH_2$ protons (3.64ppm) = 2.6protons = **0.02 molecule of PEG**

e) Ratio between DVA and glycerol = 1:1:0.8:0.02 (glycerol:DVA:PDL:PEG)

For PGA-co-PDL synthesis, glycerol, DVA and PDL were added in an equimolar ratio while the amount of PEG was varied. Because DVA can attach to glycerol, PDL or PEG, hence there are multiple possibilities for the attachment of monomers to form a random polymer. Based on NMR calculations it is most likely that PEG and PEGme copolymers were "random" in nature.

Work carried out at Boehringer Ingelheim (Germany) suggests that PEG-based copolymers for drug delivery applications must have 5-15% PEG in the polymer chain while for medical devices PEG should be less or equal to 5%. The synthesised polymers have PEG contents in accordance with these guidelines. The results shown in the following table (2.16) also confirms the findings of He et al (2003) that increasing the molar feed ratio of lactone/PEG increased the molecular weights of the product. This is why all the PEG and PEGme copolymers gave higher PEG contents in polymers. PGA-co-PDL-PEGme copolymers are solid at room temperature, although they have lower molecular weights than corresponding colactones. However, they have higher T_m values, but lower T_g values, which makes these polymers useful candidates for drug delivery applications.

All PGA copolymers of PEG and PEGme exhibited higher PEG contents (Tables 2.16 and 2.17) compared to PPA based PEG copolymers which showed lower PEG and PEGme contents. These observations were attributed to the fact that increasing hydrophobic contents in the reaction mixture lead to the occurrence of a biphasic reaction medium (where one phase consists of polar PEG and triol contents and the other phase consists of non polar diol and lactone contents), as indicated in the previous studies (Poojari & Clarson 2010). This is why the reaction of PEG and PEGme with glycerol yielded polymers with a higher %PEG as compared to the diol (1,3-propanediol)-based polymers.

However, no significant differences in T_m and T_g values were noted between PGA and PPA based PEG/PEGme copolymers. But further results obtained by using other monomers such as PDL, helped to conclude that the thermal properties could be altered, not only by the polymer molecular weight but also by changing the chemistry of the polymers. For example, PGA-co-PDL-PEGme (4.7 kDa) has the same T_m (55°C) as compared to PPA-co-PDL-PEGme (9.5 kDa). The difference of 4.8 kDa in the molecular weight of two polymers is compensated by the difference of 8.6% PEGme content, resulting in the polymers with same melting point. PGA-co-PDL-PEG polymers have similar T_m values but very high T_g values (40°C) which is higher than required for drug delivery applications to the human body. From the combined NMR calculation and the molecular weights of the PEG copolymers, it was observed that one polymer chain typically consists of one PEG molecule along with 5-12 repeating units of the PGA or PGA-co-PDL. The obtained triblock polymer ratio was close to that of the monomer feed ratio. On the other hand, diblock polymers exhibited greater differences from the monomer feed ratio, which altered the polymer properties. These observations were in accordance with studies by He et al (2003).

Table 2.16

Lipase catalysed functionalised and non-functionalised PEG copolymers

Polymer	Functional group	Monomer ratio		PEG content	Mw	Physical state	T_g	T_m	Contact angle
		Feed ratio	Calculated ratio	(%)	(Da)		(°C)	(°C)	(θ)
PGA-PEG	-OH	1:1:0.01	1:1:0.04	2.3	3836	Viscous	-	-	-
PPA-PEG	---	1:1:0.01	1:1:0.01	7.4	3747	Viscous	-	-	-
PGA-co-PDL-PEG	-OH	1:1:1:0.01	1:1:0.8:0.02	7.0	8566	Solid	40.01	52.50	-
PPA-co-PDL-PEG	---	1:1:1:0.01	1:1:1.4:0.02	5.4	6649	Solid	41.48	53.23	-
PGA-co-CL-PEG	-OH	1:1:1:0.01	1:1:1:0.02	8.6	3075	Viscous	-	-	-
PPA-co-CL-PEG	---	1:1:1:0.01	1:1:1.2:0.02	8.5	5057	Soft solid	-	-	-

- : Not available because of the viscous state of the polymer

Table 2.17

Lipase catalysed functionalised and non-functionalised PEGme copolymers

Polymer	Functional group	Monomer ratio		PEG content	Mw	Physical state	T_g	T_m	Contact angle
		Feed ratio	Calculated ratio	(%)	(Da)		(°C)	(°C)	(θ)
PGA-PEGme	-OH	1:1:0.01	1:1:0.02	16.6	9982	Viscous	-	-	-
PPA-PEGme	---	1:1:0.01	1:1:0.01	9.7	5264	Soft Solid	-	-	-
PGA-co-PDL-PEGme	-OH	1:1:1:0.005	1:1:1.5:0.01	3.4	3775	Solid	36.33	53.74	-
PGA-co-PDL-PEGme	-OH	1:1:1:0.010	1:1:1.4:0.01	3.6	4729	Solid	36.21	55.15	-
PGA-co-PDL-PEGme	-OH	1:1:1:0.020	1:1:1.3:0.02	7.2	6478	Solid	-	55.20	-
PPA-co-PDL-PEGme	---	1:1:1:0.01	1:1:0.5:0.01	8.8	9595	Solid	40.18	55.63	-
PGA-co-CL-PEGme	-OH	1:1:1:0.01	1:1:1.2:0.02	10.5	6017	Soft solid	-	-	-
PPA-co-CL-PEGme	---	1:1:1:0.01	1:1:1.3:0.01	5.6	5221	Soft solid	-	-	-

- : Not available because of the viscous state of the polymer

The presence of PEG and PEGme in polymer chains not only imparts some beneficial characters for drug delivery applications, but also helps the polymer to degrade quicker at specific environmental conditions (temperatures above T_g), via hydrolysis. The details of effects of PEG on drug encapsulation and release are discussed in chapter 3.

2.3.3.4 Functionalised and non-functionalised linear polythioesters

Polythioesters are a relatively new class of polymers and have not yet been fully explored for drug delivery applications. Polythioesters have many advantages over the corresponding oxoesters, for instance, higher melting points, high reactivity and quicker degradation (discussed in section 2.0). The previously reported syntheses of polythioesters are by "biological procedures" or biosynthesis, using microorganisms (Kawada et al 2003), by interfacial polycondensation (Podkoscielny & Rudz 1993) and by lipase catalysis (Podkoscielny & Wdowicka 1985; Kato et al 2006). These reactions were generally performed on a 0.2mmol scale (~0.03g), hence, in this work polythioesters were synthesised on a useful scale (~15-20g) to investigate their potential in drug delivery applications.

Polythioesters were synthesised using 1,3-propanedithiol and dithiothreitol as monomers with divinyl adipate and pentadecalactone. These reactions were performed in solvent-free environments because the higher reaction temperatures (120°C) would result in complete evaporation of the solvent. The use of enzyme catalysis proved a successful approach for the direct esterification of non-protected thiol groups. Removal of the by-product, water, was carried out by fitting a drying tube on the reaction flask.

Table 2.18

Lipase catalysed functionalised and non-functionalised linear polythioester

Polymer	Functional group	Monomer ratio		Mw	Physical state	T_g	T_m	Contact angle
		Feed ratio	Calculated ratio	(Da)		(°C)	(°C)	(θ)
PPTA	---	1:1	1:1	2000	Viscous	-	-	-
PDTTA	-OH	1:1	1:1	300	Viscous	-	-	-
PPTA-co-PDL	---	5:1:1	2:1:1	2262	Solid	-	-	-
PDTTA-co-PDL	-OH	1:2:2	1:1:0.5	10144	Soft solid	-	-	-

- : Not available because of the viscous state of the polymer

An additional aspect is the lower specificity of lipase towards thioesters (Öhrner et al 1996), which makes this reaction proceed at a slower rate. This is why a higher feed of thiol, longer reaction duration (48 hours) and higher temperatures (120°C) are required for such reactions. This is considered to be the main reason for the low molecular weights of the obtained products (Table 2.18). Hence, these polymers were viscous or soft solids at room temperature. It is well established that solvents also effect the polymerisation in different ways depending upon the monomers used and product properties (for example solubility, molecular weight and chain length of polymer) (Namekawa et al 2000). Hence, a decreased solubility of polymer in the reaction medium will result in chain scission and low molecular weight products (McCabe & Taylor 2004), which can be attributed to the low molecular weight of the polythioesters.

The PDTTA-co-PDL obtained was a solid at room temperature but in the form of a rubber-like material, not a solid crystalline powder like the corresponding oxoesters. Cross linking of polymer chains may have occurred - dithiothreitol has both terminal thiol and pendant hydroxyl groups and can react at either site. As the reactivity of thiols via lipase catalysis is lower than that of hydroxyl groups, not all monomers may have been consumed in the reaction. The FTIR spectrum of PDTTA-co-PDL has two separate bands corresponding to carbonyl groups, one from the thioester link and a second from the oxoester. This suggested that the polymer has two different types of ester linkages (shown in characterisation data, 2.2.5). The same observations were reported by Hedfors et al (2005)where only 70% of thiol was polymerised with caprolactone.

The NMR interpretation is more complex as two polymeric structures are possible for the same product; with the sulphur adjacent to carbonyl group and the oxygen

adjacent to carbonyl group, as represented in the characterisation data in section 2.2.5.5. Although polythioesters and the respective colactones were synthesised successfully via lipase catalysed reactions, the obtained polymers were not crystalline, hence may have limited use in drug delivery applications. Further explorations of the properties of these polymers are discussed in detail in chapter 3. Another reason for synthesising polythioesters was their predicted quicker degradation rate. The detailed studies of the degradation of these polythioesters are discussed in chapter 4.

2.3.4 Post-synthetic modification of the PGA-co-PDL

Many researchers have worked on the development of an improved controlled and targeted drug delivery system. A new strategy to increase the blood circulation time of drugs was developed by Liu et al (1999) who conjugated drugs to PEG molecules. Conjugation of drugs to various chemical moieties can not only increase their blood circulation time but also ensure the targeted delivery of the drug by minimising the exposure to the physiological environment, hence preventing any side reactions of the drug. In the present work, ibuprofen and stearic acid were conjugated via terminal carboxylic acid groups to the free hydroxyl groups on polymer backbones via DCC assisted conjugation reactions. Stearic acid, being a component of fatty acids, is biocompatible and has low toxicity. Stearic acid consists of an eighteen carbon-long chain, hence it was attached onto polymer backbone to make it more hydrophobic. Either stearic acid or ibuprofen molecules were attached to 50% or 100% of the available hydroxyl groups on the polymer backbone.

Previously researchers used an excess of carboxylic acid with the intention to react all –OH groups present (Kolhe et al 2004). To study the effect of conjugation on polymer properties, different amounts of ibuprofen and stearic acid were attached. Hence, in two separate experiments 50% and 75% of available –OH groups of polymer were conjugated. The average molar ratio of ibuprofen/stearic acid to polymer used was calculated on the basis of average molecular weight and average number of free hydroxyl groups on the polymer backbone.

Purification of the products was achieved by washing with a mixture of solvents, which lead to a product of broad molecular weight dispersity. This problem could be addressed in the future by using a dialysis membrane of specific molecular weight cut-off.

Successful conjugation was confirmed by the disappearance, or considerable decrease in intensity, of the –OH band (around 3400cm^{-1}) in the FTIR data of the product (section 2.5.5), while a considerable increase in molecular weight further confirmed the successful ibuprofen/stearic acid-polymer conjugation. Although polymers were purified, GPC chromatograms confirmed the presence of some unreacted ibuprofen or stearic acid in the conjugated polymer samples. Additionally the broad GPC peaks suggested the high polydispersity of the product molecular weight, which suggests that either conjugation had not completely taken place or perhaps, during the process, polymer chains had broken down (to create lower molecular fragments) or cross-linking had occurred between the –OH group of polymer backbone and the terminal group of the polymer chain, which could create longer or cross-linked polymeric chains.

The ^{1}H-NMR integration method (Kolhe et al 2004) was used to estimate the conjugation ratio. For stearic acid, the terminal methyl group, corresponding to 3 protons (j, at 0.88ppm) was considered as standard, and the variable singlet at 1.30ppm, corresponding to 22 $-CH_2$ protons (g) of PGA-co-PDL-C18 or PGA-co-PDL-ibu were used for the calculation. As 3 protons correspond to 1 stearic acid molecule, 28 protons were subtracted from the total number of protons at 1.30ppm, leaving behind the only protons corresponding to "g" protons of polymer. As one molecule of polymer contains 22 "g" protons, all obtained "g" protons were divided by 22 to get the polymer molecules in conjugated product. A similar procedure was followed with ibuprofen. In case of ibuprofen 6 protons corresponding to two methyl groups (p, p') at 0.89ppm were considered as the standard and compared against the $-CH_2$ protons ("g") at 1.30ppm. A sample calculation is shown below, while all the results are summarised in table 2.19.

For 50% stearic acid:

a) Number of $-CH_3$ protons (0.88ppm) = 3protons = **1 molecule of stearic acid**

b) Number of $-CH_2$ protons (1.30ppm) = 74protons = 28H of stearic acid + 46 H of polymer = **1 molecule of stearic acid + 2 molecules of polymer**

c) Ratio between stearic acid and polymer = 1:2 (stearic acid: polymer) or **50% stearic acid.**

Previously, Thompson et al (2008), reported the ibuprofen conjugation of ibuprofen acid chloride to PGA-co-PDL in feed ratios of 100% and 50%, but only 12.6% and 6.8% drug-conjugation was achieved whereas, through DCC coupling reaction, in the present work, 33% and 50% drug conjugation is reported. However, a non-linear relationship between drug conjugation and molecular weight was observed in both

cases. The DCC coupling proved to be a more efficient technique for drug conjugation because during ibuprofen acid chloride synthesis, the polymer is directly exposed to acid, which has a high potential to degrade the polymer's ester groups. The T_m values were considerably lower for DCC coupled ibuprofen-polymer conjugates as compared to acid chloride synthesis, which indicates that the presence of ibuprofen induced strain at ester linkages and weakened the interchain forces (hydrogen bonding) of the polymer backbone.

On the other hand, an increase in the T_m values of DCC-coupled stearic acid-polymer conjugates indicates a stabilisation of giant polymer structures, and T_m increases with increasing stearic acid content.

In conclusion, the successful conjugation of ibuprofen and stearic acid to PGA-co-PDL took place and conjugated products were obtained with various percentages of the conjugated species. The obtained products may have undergone cross-linking which resulted in higher molecular weights and polydispersity of the products. Inclusion of chemical moieties (stearic acid and ibuprofen) not only increased the molecular weight of the products but also altered the physical properties of the polymer (for example T_m). These products were further explored for their use in drug delivery applications (as discussed in chapter 3, Drug Encapsulation and Release).

Table 2.19

DCC coupling of stearic acid and ibuprofen to PGA-co-PDL

Polymer	Conjugate	% conjugation		Mw	Physical state	T_g	T_m
		Theoretical	Practical	(Da)		(°C)	(°C)
PGA-co-PDL	--	0	0	8000	Solid	-	-
PGA-co-PDL	Stearic acid	50	50	18476	Solid	47.33	64.19
PGA-co-PDL	Stearic acid	100	33	16555	Solid	42.41	56.10
PGA-co-PDL	Ibuprofen	50	50	18219	Solid	-	42.53
PGA-co-PDL	Ibuprofen	100	17	17413	Solid	36.71	43.11

2.4 CONCLUSION

An enzyme-catalysed esterification method was developed. This method was successfully used for the synthesis of a library of polyesters by incorporating a variety of monomers to afford incremental changes in chemistry, molecular weight, hydrophilicity and functionalisation of the polymer backbone. It was observed that small alterations not only in experimental factors and reaction variables but also in monomer chemistry play a crucial role to obtain materials having the desired properties. The reactions were successful and products were obtained on a larger scale with higher yields than previously reported. Hence, the materials produced can be effectively use for investigations of the relation between polymer and drug chemistry for the applications to encapsulate range of drugs.

These polymers may have better potential as drug carriers either by encapsulating a range of drugs possessing different physicochemical properties or by releasing the conjugated drug from the polymer backbone. This hypothesis is discussed in the next chapter (Drug Encapsulation and Release).

Drug Encapsulation and Release

3.0 BACKGROUND

A polymeric microparticulate system with maximal drug loading and high encapsulation would result in a reduced quantity of the carrier (polymer) being required for the administration of a sufficient amount of drug to the target site. This, in turn, would prevent overdosing and would lead to reduced drug toxicity while controlled drug release from microparticles is required to maintain the minimum drug level in blood. Drug release depends on multiple factors, including polymer molecular weight, hydrophilicity, particle sizes and surface properties in addition to "additives" in particle formulation.

A number of techniques for drug encapsulation have been discussed in chapter 1. One of the most common techniques is the emulsion solvent evaporation method. A number of experimental parameters in this technique can affect the encapsulation efficiencies, as listed in table 3.1;

Table 3.1

Factors affecting microparticulate formulation using the emulsion solvent evaporation technique

1. Solubility of polymer in dispersed phase (organic solvent)
2. Solubility of drug in continuous phase (aqueous phase)
3. Miscibility of continuous and dispersed phases
4. Ratio of dispersed phase to continuous phase
5. Viscosity of emulsion (polymer concentration and molecular weight)
6. Interaction between drug and polymer
7. Solvent removal rate (mixing speed a boiling point of organic solvent)
8. Surfactant/emulsifier (type and concentration)

These experimental parameters will affect the rate of solidification of microparticles and result in the alteration of encapsulation efficiencies. Apart from controlling the mechanical parameters (mixing speed, boiling point and concentration), polymer/drug chemistry and surfactant choice play important roles in determining the drug loading and encapsulation efficiencies. Hence, researchers in this field have altered the polymer chemistry to address the control and improvement in drug encapsulation efficiencies (Watts et al 1990; Whateley 1992; Jain 2000; Kumar et al 2006; Zhengxing et al 2009). The chemical properties of polymers can be altered by various parameters, such as molecular weight and hydrophilicity. The hydrophilicity of polymers can be influenced by the type and ratio of monomers in a polymer chain which can be used not only to control the encapsulation efficiencies but also alter the release rate of a drug from the microparticulate formulation (Wei et al 2004). The molecular weight of polymers can be controlled during synthesis or via post-synthetic modifications, as discussed earlier in chapter 1.

Researchers have explored various approaches to control drug encapsulation and release, such as altering the monomeric composition or the use of various polymers or additional materials as additives or excipients during the microparticle formation process (Watts et al 1990; Whateley 1992). For example, PEG has been used not only as a surfactant but also for its properties as a channelling agent to enhance the release of drugs from microparticles (Arida et al 1999). Another example of such an additive is Labrafil® (a non-ionic amphiphilic PEG derivative), which has been studied for controlling the release of ibuprofen from PLGA microparticles (Fernández-Carballido et al 2004). Controlling the release of the active pharmaceutical ingredient (API) is the main application of polymer-based drug delivery systems. Hence, along with improving the encapsulation efficiencies, efforts were made by researchers to control the release of API (Whateley 1992). However, FDA approved polymers can be used for only one type

of drug (freely or poorly water soluble) at one time. For example, PLGA is good for the encapsulation of poorly water soluble drug but also provides burst releases of the drug. However, water soluble drugs showed lower encapsulation efficiencies (Ito et al 2008).

Hence, there is a need to study the effect of both formulation and chemical parameters in the development of any new polymeric drug delivery system which can ideally be used for a range of drugs possessing different physicochemical properties. In this work, the polymers synthesised previously (chapter 2) were used to encapsulate drugs from two different classes (antibiotics and NSAIDs), bearing different aqueous solubilities and chemistries. Experiments were designed to study the effects of a number of formulation parameters and the varying polymer chemistry on the encapsulation of these different drug compounds.

3.1 AIM OF THE STUDY

The aim was to investigate the effects of altering the hydrophilicity of drugs and polymer chemistry on encapsulation efficiencies and to assess the release profiles of the drugs.

This was achieved by studying the effects of particle formulation parameters on particle size, surface properties and drug encapsulation using a model polymer, PGA-co-PDL, and a model hydrophobic, poorly water soluble drug, ibuprofen. In light of the obtained results, the encapsulation of four drugs, bearing different properties (molecular weight, hydrophilicity and water solubility), were then evaluated with a full range of polymers of varying physicochemical properties (chapter 2). The drugs selected for this study (in order of increasing water solubility) were indomethacin, ibuprofen, levofloxacin and rifampicin.

3.2 EXPERIMENTAL

3.2.1 Materials

Copolymer samples PGA (10.1 kDa), PBA (7.4 kDa), PGA-co-CL (1:1:0.7, 17.8 kDa), PBA-co-CL (1:1:0.4, 18.4 kDa), PGA-co-PDL (1:1:1 (17.5 kDa and 8 kDa), PGA-co-PDL (1:1:1.2 (29.1 kDa)), PPA-co-PDL (1:1:0.6, 8.5 kDa), PBA-co-PDL (1:1:0.5, 15.9 kDa), PTEGA-co-PDL (1:1:1 (23.8 kDa) and 1:1:1.5 (17.4 kDa)), PGA-co-PDL-PEG (1:1:0.8:0.02, 8.5 kDa), PGA-co-PDL-PEGme (1:1:1.5:0.01, (3.7 kDa), PGA-co-PDL-PEGme (1:1:1.4:0.01, (4.7 kDa)), PGA-co-PDL-PEGme (1:1:1.3:0.02 (6.5 kDa)), PLGA (50:50, 9.5 kDa), PPTA-co-PDL (2:1:1, 2.3 kDa), PDTTA-co-PDL (1:1:0.5, 10.1 kDa), PSA-co-PDL (1:1:0.5, 3.3 kDa), PGA-co-PDL-C18 (33% (16.5 kDa) and 50% (18.5 kDa)) and PGA-co-PDL-Ibu (50% (18.2 kDa) and 17% (17.4 kDa)) were previously synthesised (chapter 2). Poly(vinyl alcohol) (PVA, 9-10 kDa Mw, 80% hydrolysed), ibuprofen (Ibu), levofloxacin (Levo), indomethacin (Indo), rifampicin (Rif), trifluoroacetic acid, triethylamine and phosphoric acid were purchased from Sigma, UK. Acetonitrile (HPLC grade), ethyl acetate, butyl acetate, MEK, anisole, acetic acid, acetone, DMSO, DCM and THF were purchased from Fischer, UK.

3.2.2 Analysis and characterisation

3.2.2.1 Analytical method development and validation

Three analytical techniques were employed for the quantitative analysis of drugs in the microparticulate formulations and for the drug-release studies. Published analytical methods were followed for the use of ultraviolet (UV) spectrophotometric and high performance liquid chromatographic (HPLC) methods. For quantitative gel permeation chromatography (GPC) a new analytical method was developed and validated. The quantitative results of drug analyses of all three analytical techniques were compared using ibuprofen as the model drug.

3.2.2.1.1 GPC

The use of GPC in quantitative drug analysis is relatively new, as it is more commonly used for molecular weight analysis. Few research groups have previously reported the use of GPC for quantitative analysis (Márquez-Ruiz et al 1996). Chromatographic conditions used were the same as for the polymer analysis (chapter 2, section 2.2.4). Sample preparation for method development and validation is summarised below.

Analytical method development and validation:

A series of ibuprofen concentrations ranging from 0.1 mg/ml to 5.0 mg/ml in THF were prepared via serial dilutions. These standard solutions were analysed and a standard curve for ibuprofen was plotted to allow calculation of the ibuprofen content in samples. Peak areas for each solution were obtained using refractive index (RI) detector responses and plotted against the ibuprofen concentration (Figure 3.1). The obtained regression equation was used to calculate the amount of ibuprofen in the samples.

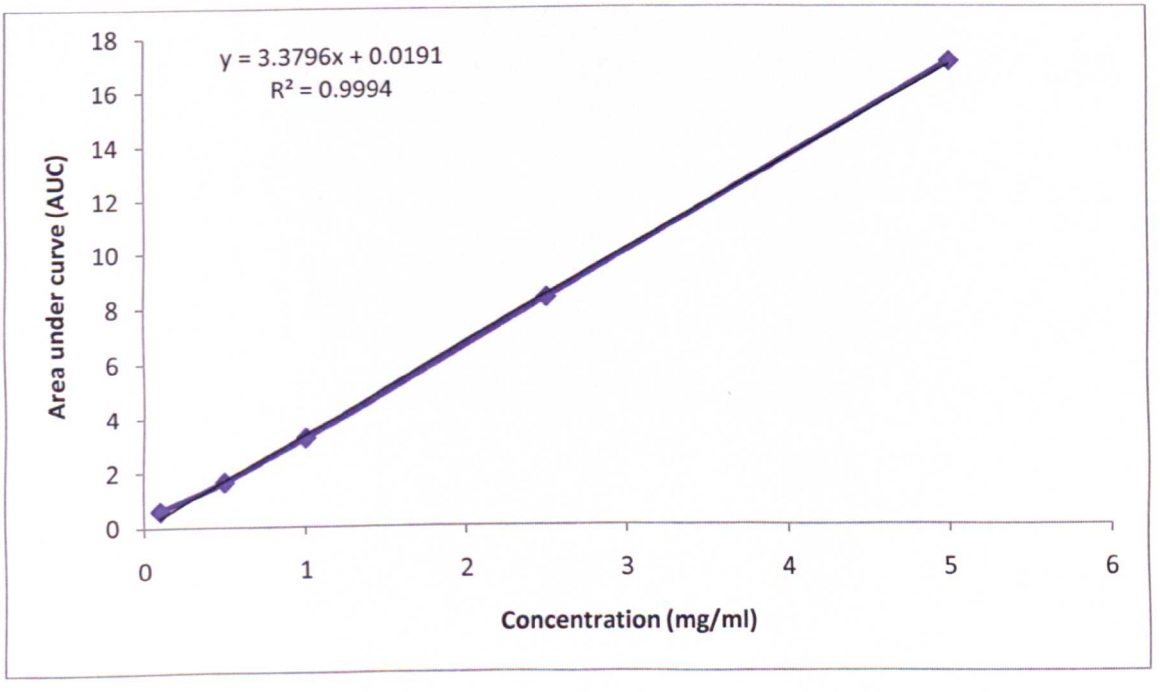


Figure 3.1: *Standard curve of ibuprofen concentrations against the area under curve (AUC) from the refractive index (RI) detector using GPC (n=3)*

To validate this developed method, an internal standard (polystyrene 29.3 kDa), was added to all the ibuprofen standard solutions at concentrations of 2.5 mg/ml. It was observed that addition of the polystyrene did not cause an effect on the quantitative recovery of ibuprofen and no change in the linearity of the ibuprofen standard curve was noted (Figure 3.2).

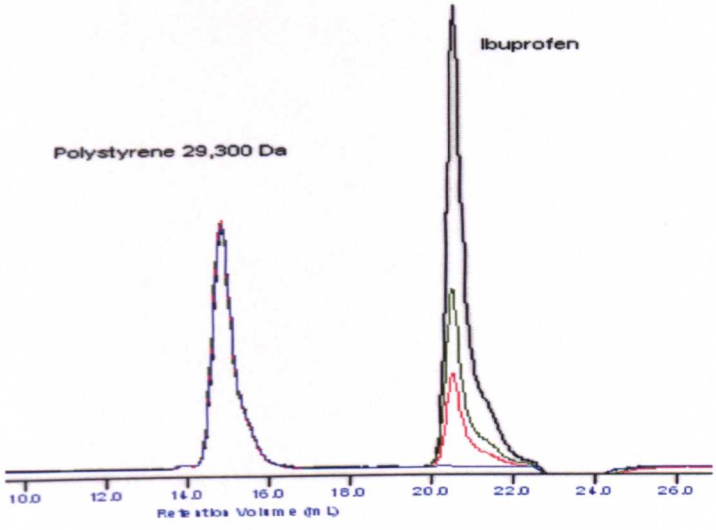


Figure 3.2: *GPC chromatogram of internal standard (Polystyrene 29.3kDa) with varying concentrations of ibuprofen*

3.2.2.1.2 UV-visible spectrophotometry

For the quantification of ibuprofen encapsulated in the microparticulate formulations by UV spectrophotometry, the absorbance of the samples was monitored at the optimum wavelength for ibuprofen, 273nm, using a UV-Vis spectrophotometer (Lambda 40, Perkin Elmer, run via UV WinLab software). Quartz UV cuvettes (Spectrosil®, Sigma-Aldrich) were used. The concentration of the drug was calculated using a calibration curve, as obtained by the following procedure.

Analytical method:

The UV spectrophotometric assay method for the quantification of ibuprofen was adopted from Thompson et al. (2007). A standard curve was obtained by analysing the standard ibuprofen solutions of concentrations ranging from 0.1 mg/ml to 5.0 mg/ml in THF (prepared via serial dilutions). The absorbances of the ibuprofen solutions were plotted against the ibuprofen concentration (Figure 3.3) and the obtained regression equation was used to calculate the amount of ibuprofen in the samples.

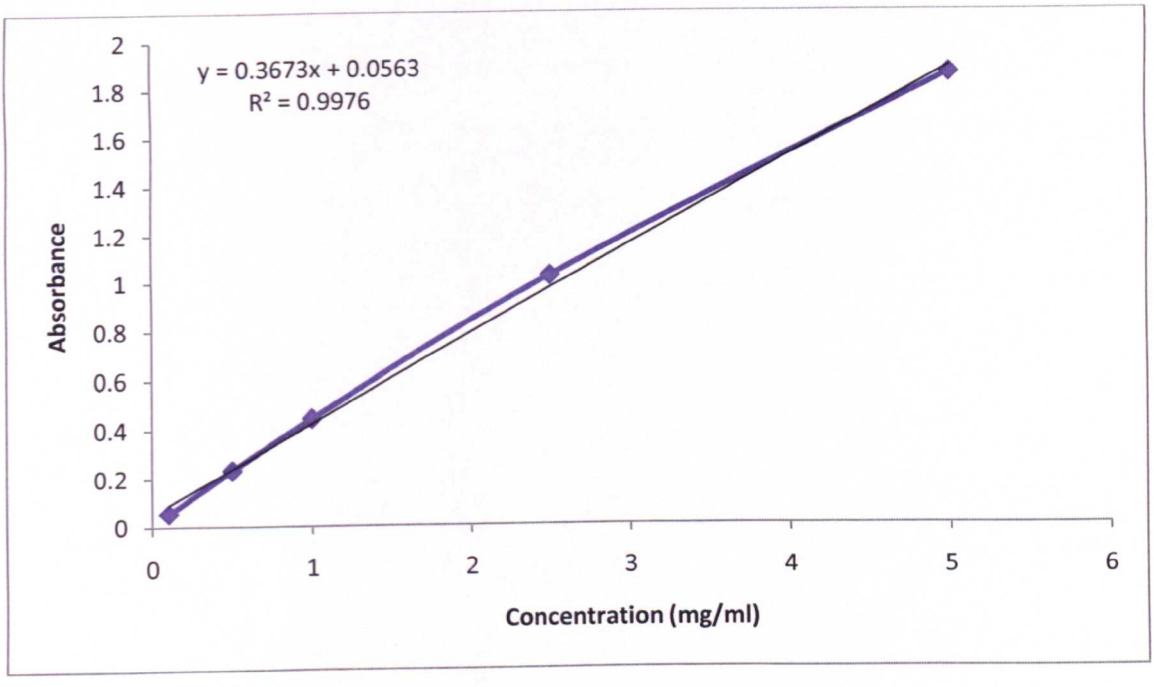


Figure 3.3: *Standard curve of ibuprofen concentrations against absorbance values at 273nm using UV spectrophotometery (n=3)*

3.2.2.1.3 HPLC

Analytical method

For quantitative analysis of drugs in the microparticulate formulations, a UV-HPLC assay technique was also used. An HP 1090 HPLC system, equipped with PDA detector, an auto sampler and a Rheodyne® sample injector (with a variable volume (1-100µl) sample loop) was used. A reversed-phase 150mm × 4.6mm Waters, RP-C18 (5 µm) column was used at ambient temperature. All samples were dissolved in the

appropriate solvent (as described in the methodology below), and filtered through 0.45μm filters (Whatman) into 2.5ml sample vials. Vials were capped with PTFE seals and placed in the autosampler. The mobile phases flow rate was 1.0ml/min, while the sample volume was set at 10μl. Different mobile phases were used depending on the drug to be analysed, as described below.

i) Ibuprofen and indomethacin

For quantification of ibuprofen and indomethacin, the analytical method was adopted from Farrington and Regan (2007). The mobile phase for ibuprofen and indomethacin consisted of acetonitrile:water:trifluoroacetic acid (80:20:0.1), which was mixed and sonicated before filtering through 0.45μm nylon filters (Sigma). The wavelength selected for the assay was 220nm.

ii) Levofloxacin

For quantification of levofloxacin, the analytical method was adopted from Gao et al (2007). The mobile phase for levofloxacin consisted of acetonitrile:water:phosphoric acid:triethylamine (14:86:0.6:0.3), which was mixed and sonicated before filtering through 0.45μm nylon membrane filters (Sigma). The wavelength selected for the assay was 293nm.

iii) Rifampicin

For quantification of rifampicin, the analytical method was adopted from Kumar et al (2004). The mobile phase for rifampicin consisted of acetonitrile:0.05M KH_2PO_4 (45:55), which was mixed and sonicated before filtering through 0.45μm nylon filters (Sigma). The wavelength selected for the assay was 254nm.

Standard curve

A standard curve was prepared for each drug by using standard drug solutions with concentrations ranging from 0.1 mg/ml to 5.0 mg/ml. The detector response was recorded for peak areas of standard solutions and plotted against the drug concentrations. A typical standard curve for ibuprofen is shown in figure 3.4. The obtained regression equation for each drug was used to calculate the amount of drug in the samples.

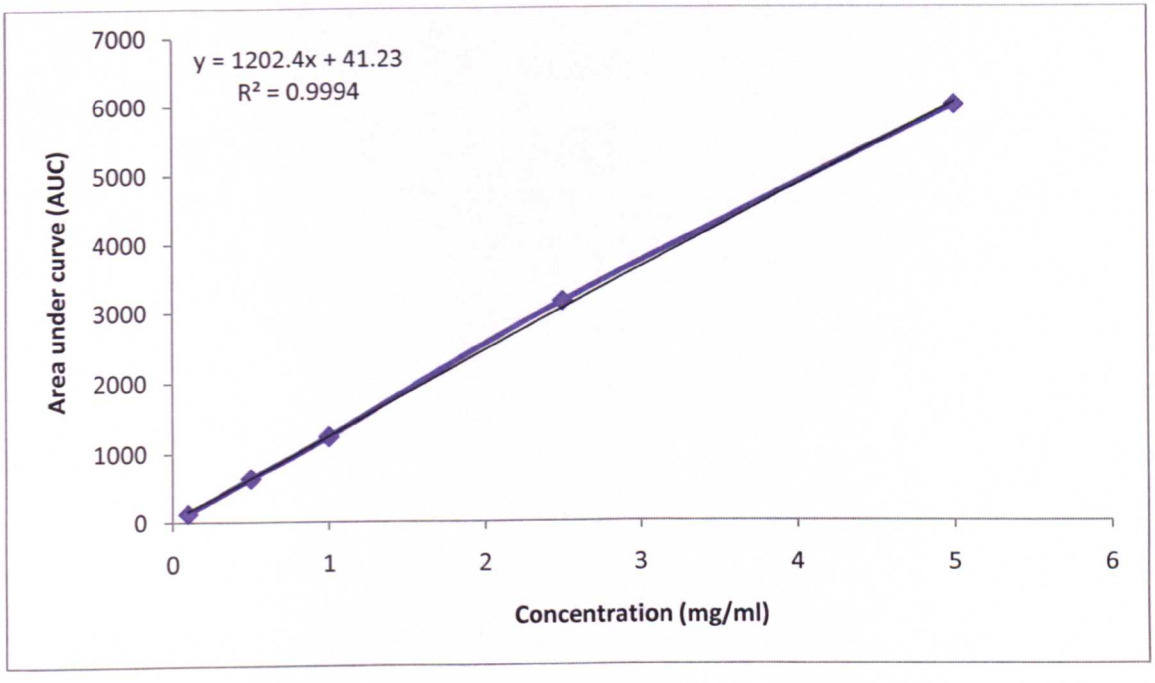


Figure 3.4: *Standard curve of ibuprofen concentrations against area under curve using UV detector (set at 220nm) in HPLC*

3.2.2.2 Microparticle characterisation using SEM

Particles were analysed for particle size, shape and surface morphology by scanning electron microscopy (SEM) using the following procedure.

Particles were analysed by using a JSM Jeol 840 Scanning Electron Microscope. Aluminium stubs (13mm diameter) were layered with a carbon tab (Agar scientific) and a sample of particles (5-10mg) was deposited on the surface and air dried. An atomic

layer of gold was deposited onto the particle-containing stubs using the Polaron E 5000 Gold Sputter Coater. Images were taken using a Ronatech Image Capture System, operated via PRISM software. The obtained images were used for approximate particle size determination by drawing calibrated lines across 10 random particles in each SEM image. The obtained data was used to calculate mean and standard deviations in particle sizes.

3.2.3 Microparticle formulation and drug encapsulation

Ibuprofen was used as a model drug to study the effect of various encapsulation parameters on drug encapsulation in colloidal particles via a modified single emulsion-solvent evaporation technique (Watts et al 1990), a water-in-oil-in-water solvent evaporation technique and a spray drying technique. Different experimental parameters were assessed to optimise the ibuprofen encapsulation efficiency and particle properties (size, shape, surface morphology).

3.2.3.1 General method

3.2.3.1.1 Oil-in-water (o/w) solvent evaporation for poorly water soluble drugs

The following standard method used for particle preparation was adopted from Gaskell et al. (2008) for the encapsulation of the poorly water soluble drugs, ibuprofen and indomethacin. Parameters in the adapted method were varied (Table 3.2). A Silverson mixer (L4 RT mixer), set to varying speeds, was used to mix 160ml of aqueous phase containing 0.2% poly(vinyl alcohol) (PVA) in a 250ml beaker. The temperature was maintained between 22-28°C. Polymer (60 mg/ml) and drug (6 mg/ml) were dissolved

in 5 ml of dichloromethane (DCM) and added drop-wise to the stirring aqueous phase. The resultant oil-in-water (o/w) emulsion was further stirred for 3 hours using the Silverson mixer at the chosen speed. Particles were collected by vacuum filtration (Millipore) through 0.45μm pore size filter papers (Whatman) and washed with 2-3ml of water, while on the filter paper. The obtained particles were analysed and characterised for drug encapsulation and particle morphology.

3.2.3.1.2 Water-in-oil-in-water (w/o/w) solvent evaporation for water soluble drugs

Drug-loaded microparticles were prepared by a ($W_1/O/W_2$) emulsion solvent evaporation method adapted from Blanco Preito et al (1994) for the encapsulation of water soluble drugs, levofloxacin and rifampicin. A 60mg sample of drug dissolved in 1.0 ml of distilled water (W_1) and 500mg of polymer dissolved in 5ml of DCM (O) were mixed and stirred using a YellowLine (DI 25 basic) probe homogeniser, set at 8000 RPM for 15 seconds to form a W_1/O emulsion (inner emulsion) at ambient temperature. The resultant inner emulsion was added to 160 ml of a 0.2% aqueous poly(vinyl alcohol) (PVA) solution (W_2), and further stirred using a Silverson mixer (3000 RPM) for 3 hours to allow complete solvent evaporation. Particles were collected by vacuum filtration (Millipore) through 0.45μm pore size filter papers (Whatman). Particles were washed with 2-3ml of water, while on the filter paper. The obtained particles were analysed and characterised for drug encapsulation and particle morphology.

3.2.3.1.3 Spray drying

The spray drying procedure for microparticle preparation was adapted from Rattes and Oliveira (2007). Polymer, 2.5%(w/v) was dispersed in the organic phase (DCM) without addition of any surfactants or stabiliser. The resulting dispersion was then fed to the spray dryer (Buchi-290) under the following conditions: aspiration rate 100%, inlet

gas temperature 68-70°C, outlet temperature 51-53°C, pump at 30% and drying air flow rate of 50mm. The spray dried microparticles were collected and stored until further analysis. The obtained particles were analysed and characterised for particle morphology, without any further processing or washing.

3.2.3.2 Variation of encapsulation parameters

To study the effect of various formulation parameters on ibuprofen encapsulation efficiency, particle size and surface properties, a series of experiments were performed altering the formulation parameters from the standard procedure (section 3.2.3.1.1) as shown in table 3.2. The obtained microparticles were analysed by GPC (for drug content) and SEM (for particle morphology), using the standard procedures described in section 3.2.2.

Table 3.2

Variation in microparticulate formulation parameters

Formulation parameters	**Variations**
Mixing speed (RPM)	1000, 2000, 3000
Polymer concentration (mg/ml)	20, 60, 100, 200
Drug concentration (mg/ml)	6, 12
Solvent	Class II : DCM, THF Class III : ethyl acetate, butyl acetate, methyl ethyl ketone, anisole, acetic acid, acetone, DMSO
Polymer molecular weight (Da)	2400, 14400, 17205, 30306
Polymer chemistry	PGA, PBA, PGA-co-PDL, PBA-co-PDL PGA-co-CL, PBA-co-CL
Encapsulation procedure	o/w, spray drying

3.2.4 Drug encapsulation

Four different drugs (listed in table 3.3) were encapsulated into polymers having different properties. The drugs and polymers listed in table 3.3 differ in their water solubility, hydrophilicity and molecular weights. All drug loaded particles were formulated using the general method as described above in section 3.2.3.1, unless otherwise stated. Once formulated, all preparations underwent analysis via HPLC (for drug content) and SEM (for particle morphology) using standard procedures as described in section 3.2.2.

Table 3.3

Properties of polymers and drugs used for drug loaded microparticle formulation

Drug name	***Class***	***Solubility In water (mg/ml)***	***Mol wt (Da)***	***Polymer (monomer ratios)***	***Class***
Indomethacin	*NSAID*	*0.035*	*357.8*	PGA-co-PDL (1:1:1) PGA-co-PDL (1:1:1.2) PPA-co-PDL (1:1:0.6) PBA-co-PDL (1:1:0.5)	*Polyester*
Ibuprofen	*NSAID*	*0.06*	*206.3*	PTEGA-co-PDL (1:1:1) PTEGA-co-PDL (1:1:1.5)	*Polyester*
Levofloxacin	*Quinolone Antibacterial*	*25.00*	*361.4*	PPTA-co-PDL (1:0.5:0.5) PDTTA-co-PDL (1:1:0.5)	*Polythioes-ter*
				PSA-co-PDL (1:1:0.5)	*Polyol*
Rifampicin	*Macromol-ecule anti-biotic*	*1.30*	*822.9*	PGA-co-PDL-PEG (1:1:0.8:0.02) PGA-co-PDL-PEGme (1:1:1.5:0.01) PGA-co-PDL-PEGme (1:1:1.4:0.01) PGA-co-PDL-PEGme (1:1:1.3:0.02)	*PEG copolymers*
				PGA-co-PDL-C18 (33%) PGA-coPDL-C18 (50%)	*Conjugated stearic acid*
				PGA-co-PDL-Ibu (17%) PGA-co-PDL-Ibu (50%)	*Conjugated ibuprofen*

3.2.5 Drug release from microparticulate formulations

The release of the drugs from the particles was studied by analysing three batches of particles of each formulation. A 10mg sample of drug-loaded particles was placed in 1.5ml clean and dry Eppendorf tubes followed by the addition of 1ml of PBS (pH 7.4) at 37°C. The resultant suspensions were mixed, on an orbital shaker at 100rpm (Stuart scientific) and at 37°C in an incubator (Stuart scientific). Samples were periodically collected at 0 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 48 h and filtered through 0.45μm membrane filters (Whatman). The supernatants were then collected and stored in 2ml sealed vials at 4°C until further analysis by HPLC as described in section 3.2.2.1.

3.3 RESULTS AND DISCUSSION

3.3.1 Comparison of analytical techniques for drug quantification

The ability of polymers with different chemical properties to encapsulate drugs with different water solubilities was explored. The encapsulated drugs were quantified by a variety of analytical techniques, as reported by various research groups, including UV spectrophotometry (Thompson et al 2007), HPLC (Farrington and Regan 2007) and a relatively new technique, quantitative GPC. GPC is generally used for qualitative purposes and sometimes for the quantitative estimation of low molecular weight polymers (Márquez-Ruiz et al 1996). Here, a new method was developed and validated for the quantitative estimation of ibuprofen via GPC. UV spectrophotometry is the most commonly employed technique for such applications due to the fact that most linear polymers lack chromophore groups and hence would not absorb UV or visible radiation. Thus UV spectrophotometry is considered the simplest, most cost effective and time efficient technique. However, during the current studies it was observed that although the polymers utilised in these studies interfere with the absorbance over the entire UV wavelength range (figure 3.5). The drug loaded PGA-co-PDL particles, when compared against ibuprofen, showed an increased absorbance at all wavelengths and hence baseline drift. The same observations were noted when blank PGA-co-PDL particles were compared against pure solvent (THF).

Keeping in view these observations, a set of experiments were designed to compare the three analytical techniques: UV, HPLC and GPC for the quantification of ibuprofen in two drug loaded PGA-co-PDL particle formulations. The same set of samples were analysed by each of the three techniques to keep sample variation to minimum.

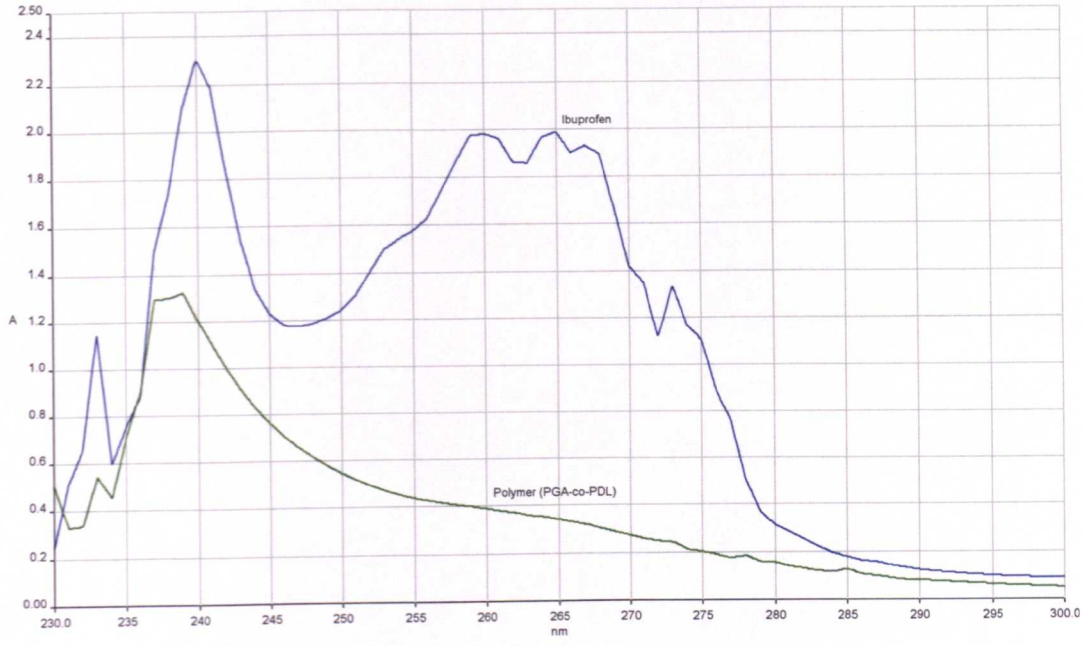


Figure 3.5: *UV spectra of THF solutions of a) ibuprofen (blue line) and b) PGA-co-PDL (green line) representing the interference from polymer throughout the UV range.*

The results obtained (Table 3.4) further support the fact that the amount of ibuprofen determined by UV spectrophotometry is considerably higher than that obtained by HPLC and GPC.

Table 3.4

Comparison of quantitative ibuprofen analysis by using various analytical techniques in two different drug-loaded particle formulations

Polymer	% Encapsulated Ibuprofen					
	UV		HPLC		GPC	
PGA-co-PDL (1:1:1)	82.46	±5.31	53.60	±2.74	49.50	±0.21
PGA-co-PDL (1:1:1.5)	106.45	±3.89	56.77	±2.23	58.10	±4.21

The main reason for this observation is the separation of components of analyte in both chromatographic techniques. In both HPLC and GPC, two separate peaks were

observed; one corresponding to ibuprofen (the main analyte) and a second peak corresponding to the polymer in the sample. The HPLC was equipped with a UV detector (set at 220nm) while the GPC was equipped with RI detector. In both cases the detection of separate peaks for the polymer highlighted the importance of the use of precise and robust analytical techniques. The results summarised in table 3.4, show that there is a variation of around 30-40% in the results obtained by UV spectrophotometer as compared to both chromatographic techniques. However, variations between the two chromatographic techniques are within their error limits and are not statistically different (P value for PGA-co-PDL 1:1:1 is 0.06 and P value for PGA-co-PDL 1:1:1.5 is 0.65). Hence, the UV spectrophotometer was not used further for quantitative analysis in this project. HPLC was preferred over GPC because of time and cost-effectiveness. No previous literature was found, directly comparing these techniques for drug quantification from microparticles as it appears researchers simply use one of the techniques. However, there are cases in the literature where it is likely that the amount of the drug in a particle formulation is over estimated (Thompson et al 2007). To further support the above findings the data obtained here were compared to a similar study by Thompson et al. (2007), the details of which are discussed in section 3.3.3.3a.

3.3.2 Investigations of the effects of encapsulation parameters

Although the interfacial deposition method is reported as a method for particle formation with PGA and related polymers (Kallinteri et al 2005), this technique suffers the drawback of requiring stable emulsion formation and in the current studies microparticles could not be formulated with this technology. Hence the method of oil-in-water emulsion solvent evaporation was selected for the preparation of polymeric particles and to study the effect of varying chemistry and other parameters on

encapsulation efficiency and particle size. During initial studies it was observed that the o/w technique was not suitable for the encapsulation of drugs in the viscous polymers (such as PGA), hence PGA-co-PDL, a solid polymer at room temperature, was chosen for the further development and exploration of these drug delivery systems. It was observed that, under the formulation parameters described in the general method, the emulsion-solvent evaporation technique produced distinct particles. Encapsulation of a model hydrophobic drug, ibuprofen was investigated in the various polymers. As the properties of the particles prepared are dependent on the chosen methodology, different parameters, settings and procedures were altered systematically to study the effects on the results obtained for the various materials.

Previous research on PGA-co-PDL investigated different parameters of the single-emulsion solvent evaporation system and the effects on particle size and morphology as well as its potential for encapsulating ibuprofen (Thompson et al 2006). The research presented here aims to extend the previous work done by Thompson et al. (2006) utilising a range of polymers, synthesised specifically for this study, in addition to the assessment of the particle preparation parameters to achieve increased ibuprofen encapsulation for therapeutic delivery.

Particles were prepared from all polymers (listed in table 3.3) using the solvent emulsion technique described in section 3.2.3.1.1. PVA is a commonly-used emulsifier that provides short term stabilisation of oil-in-water (o/w) emulsions. The concentration of PVA affects the particle size: with increasing concentrations, generally smaller particles are produced. The viscosity change of the emulsion with higher PVA concentrations contributes to the stability of the emulsion formed. 0.2 % (w/v) PVA was found to give a more even distribution of particle size via the single emulsion-solvent evaporation technique (Bolourtchain et al, 2005). Hence this concentration of PVA was kept constant in all the drug encapsulation work in this study.

Experimental parameters such as mixing speed, polymer concentration, solvent choice, polymer chemistry and molecular weight were varied to study their effect on ibuprofen encapsulation efficiency, morphology and particle size.

3.3.2.1 Effect of speed

Increasing the mixing speed of the emulsion from 1000 to 3000 RPM resulted in a decrease in encapsulation efficiency from 66.5% to 55.0% (Table 3.5). The size of the particles also ranged from 16.3 μm to 1.2 μm (Figure 3.6). These results further support the previous studies done by Bilati et al (2005), where it was established that an increase in speed caused smaller particles to form.

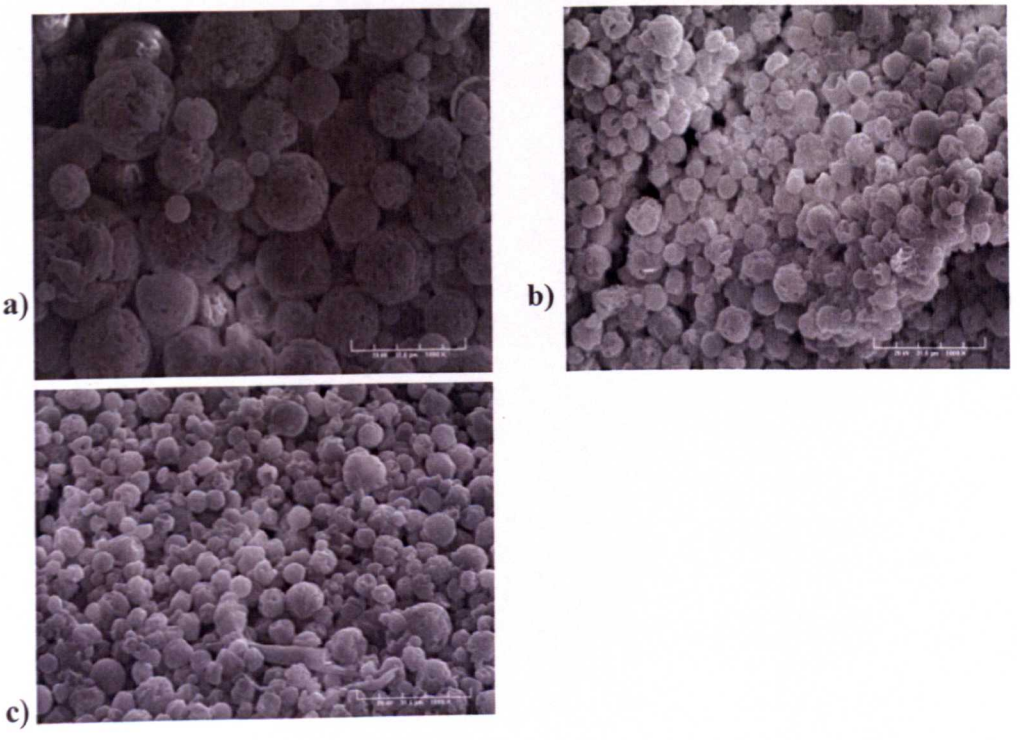


Figure 3.6: *SEM images of ibuprofen loaded PGA-co-PDL particles using a polymer concentration of 60 mg/ml (x1000, measuring bar = 31.6 μm) prepared at a) 1000 rpm b) 2000 rpm c) 3000 rpm*

This phenomenon can be explained by the fact that increasing the mixing speed of the emulsion resulted in the production of smaller oil droplets loaded with ibuprofen and

polymer which upon solvent evaporation formed smaller sized particles. As the particle size decreased, with increasing mixing speed, the amount of ibuprofen loaded in those particles also decreased leading to decreased encapsulation efficiency.

Table 3.5

Effect of mixing speed on ibuprofen encapsulation: Mean encapsulation efficiencies and particle sizes tabulated (n=3).

Mixing Speed (rpm)	**Encapsulation Efficiency (%)**	**Particle Size (μm)**
1000	66.5 ± 5.1	16.3 ± 5.2
2000	60.9 ± 4.5	1.5 ± 0.8
3000	55.0 ± 1.25	1.2 ± 1.0

Emulsifications at 2000 rpm yielded particles with a greater encapsulation efficiency compared to those prepared at 3000 rpm, with no notable change in particle size. Further decreasing of the mixing speed to 1000 rpm increased the encapsulation efficiency by a further 5.6%. However, this reduction in speed also influenced the particle size significantly, increasing it from 1.5 ± 0.8μm at 2000rpm to 16.3 ± 5.2μm at 1000rpm. This is due to the fact that at lower speed the shear experienced by the emulsion was not strong enough to create particles of smaller sizes. However, this also resulted in the entrapment of higher amounts of drug in the polymer matrix causing an increase in encapsulation efficiency. These results are supported by previous studies by Arida et al (1999), where it was established that ibuprofen encapsulation at higher speeds resulted in decreased drug encapsulation.

3.3.2.2 Effect of oil phase

Pharmaceutical grade III solvents are less toxic than class II solvents; hence, any residual class II solvent will be more harmful than class III solvents (Committee for Proprietary Medicinal Products 1998). Class III solvents, anisole, acetic acid, acetone, methyl ethyl ketone (MEK), ethyl acetate and butyl acetate were compared with the routinely used class II solvents dichloromethane (DCM) and tetrahydrofuran (THF). The results indicate that butyl acetate produced particles with higher encapsulation efficiency (53.1%) than any other class III solvent and also produced submicron sized particles. MEK and ethyl acetate (class III) produced particles with good encapsulation efficiencies, hence proving that they are better alternatives for pharmaceutical preparations (Table 3.6). However, particles prepared with ethyl acetate were very large (15.7 μm) compared to the 1.0 μm particles, prepared from other solvents.

Table 3.6

Effect of solvent on ibuprofen encapsulation. Mean encapsulation efficiencies and particle sizes are shown (n=3).

Solvent	Encapsulation Efficiency (%)	Particle Size (μm)
Class II Solvents		
DCM	55.0 ± 1.25	1.2 ± 1.0
THF	No particles	-
Class III Solvents		
Acetone	No particles	-
Acetic acid	No particles	-
Anisole	No particles	-
Butyl acetate	53.1 ± 3.45	0.9 ± 0.8
DMSO	No particles	-
Ethyl acetate	43.1 ± 4.10	15.7 ± 5.5
Methyl ethyl ketone	43.3 ± 3.60	1.0 ± 0.6

The conventionally used dichloromethane (DCM), a class II solvent, was substituted with different class III solvents including butyl acetate and methyl ethyl ketone (MEK).

This may result in a significant reduction in the toxic potential of the delivery system. MEK produced particles of equivalent size to DCM but with lower encapsulation efficiencies; however butyl acetate showed increased encapsulation efficiencies (of approximately 10% as compared to MEK) within particles of a similar size. On the other hand, THF, anisole, acetic acid, acetone, and DMSO did not form microparticles. The reason could be explained on the basis of their higher boiling points, high density and miscibility with water, which required a longer time for complete evaporation, and resulted in an unstable emulsion formation. Hence, these solvents were incompatible with the method used in this study.

3.3.2.3 Effect of polymer molecular weight

Particles were not obtained and hence no ibuprofen encapsulated with the lower molecular weight PGA-co-PDL (2.5 kDa) due to its liquid state. Increasing the molecular weight of this polymer resulted in the formation of ibuprofen encapsulated particles with increasing encapsulation efficiencies from 55.0% (for 14.4 kDa) to 60.3% (for 30.3 kDa), (Table 3.7).

Table 3.7

Effect of polymer molecular weight on ibuprofen encapsulation: Mean encapsulation efficiencies and particle sizes are shown (n=3).

Molecular Weight (Da)	Encapsulation efficiency (%)	Particle Size (μm)
2400	No particles	-
14435	55.0 ± 1.25	1.2 ± 1.0
17205	55.5 ± 2.1	1.8 ± 1.4
30306	60.3 ± 2.55	1.4 ± 1.1

However, no major change in particle size was observed and all particles obtained were in the range of 1.5 μm ± 0.3 μm (Figure 3.7). It is well established that increasing the

polymer molecular weight and polydispersity causes an increase in the viscosity of the emulsion formed which results in the higher encapsulation of drugs (Ramkissoon-Ganorkar et al 1999; Bilati et al 2005).

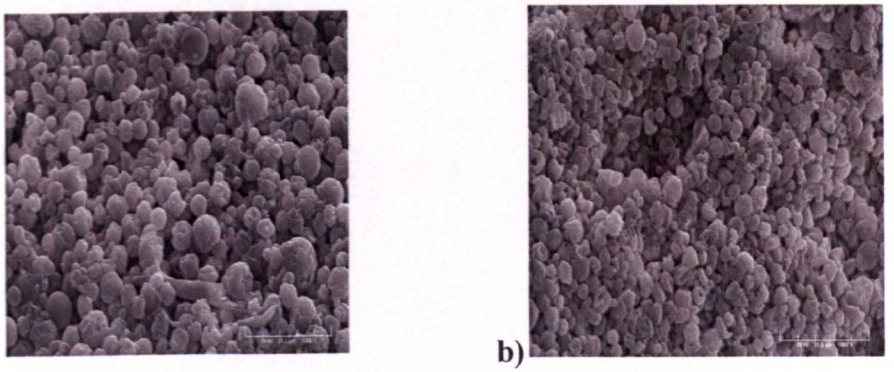


Figure 3.7: *SEM images of ibuprofen-loaded PGA-co-PDL particles with different molecular weight (x1000, measuring bar = 31.6μm) a) 14 kDa b) 30 kDa*

3.3.2.4 Effect of polymer and drug concentration

Increasing the polymer (14kDa) concentration from 20 mg/ml to 200 mg/ml caused an increase in viscosity of the emulsion system. This resulted in the oil-in-water emulsion droplets containing higher amounts of polymer hence an observed increase in particle size from 1.2 μm to 18.2 μm (Figure 3.8). Both higher polymer molecular weight and higher polymer concentration in the oil phase can increase the encapsulation efficiency, due to the increased viscosity of the emulsion, which in turn reduces the drug diffusion to the aqueous medium (Bilati et al 2005; Zhengxing et al 2009).

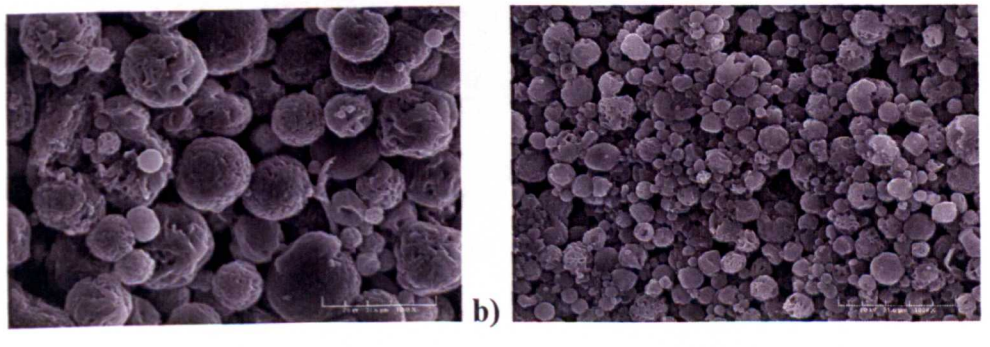


Figure 3.8: *SEM images of Ibuprofen loaded PGA-co-PDL particles with different polymer concentration (x1000, measuring bar = 31.6 μm) a) 200 mg/ml b) 60 mg/ml*

An increased polymer content in the emulsion resulted in a significant decrease of drug (ibuprofen) content in the resulting particles which led to a decrease in encapsulation efficiency from 55.0% to 24.8% (P value <0.005) (table 3.8). No particles were formed at polymer concentrations below 60 mg/ml due to no stable emulsion forming.

Table 3.8

Effect of polymer concentration on ibuprofen encapsulation: Mean encapsulation efficiencies and particle sizes are shown (n=3).

Polymer Concentration (mg/ml)	**Encapsulation Efficiency (%)**	**Particle Size (μm)**
20	No particles	-
60	55.0 ± 1.25	1.2 ± 1.0
100	41.4 ± 2.1	1.1 ± 0.9
200	24.8 ± 2.6	18.2 ± 4.2

Increasing the drug (ibuprofen) concentration from 6 mg/ml to 12 mg/ml resulted in a considerable increase in drug encapsulation in the microparticles (Table 3.9). This was due to the presence of a higher amount of drug in the emulsion and led to an increase in particle size from 1.2μm to 5.5μm. The presence of a higher amount of drug in an o/w emulsion can increase the encapsulation efficiency, because a more hydrophobic drug would associate in the polymer and consequently reduce the drug diffusion to the aqueous medium (Bilati et al 2005; Zhengxing et al 2009).

Table 3.9

Effect of drug concentration on ibuprofen encapsulation (while keeping the polymer concentration constant at 100 mg/ml): Mean encapsulation efficiencies and particle sizes tabulated (n=3).

Drug Concentration (mg/ml)	**Encapsulation Efficiency (%)**	**Particle Size (μm)**
6.0	41.4 ± 2.1	1.1 ± 0.9
12.0	53.6 ± 2.7	5.5 ± 2.5

3.3.2.5 Effect of polymer chemistry

The polymers PGA, PBA, PGA-co-CL, PBA-co-CL and PCL were unable to form particles using this solvent-evaporation technique because of their lower molecular weights and their liquid state at room temperature, which resulted in the formation of unstable emulsions. Upon solvent evaporation, preparations containing PGA, PBA, PGA-co-CL, PBA-co-CL and P-CL, appeared as uniform milky suspensions and no particles could be collected via filtration or by centrifugation. The lack of discrete solid particles from these materials under the given preparative methodology is due to their physical properties and/or low molecular weight. With the solid materials, micron size particles were obtained with variable geometry and surface properties, depending on the polymer used and the exact experimental parameters. Only solid polymers, PGA-co-PDL, PPA-co-PDL and PBA-co-PDL, formed stable emulsions that upon filtration yielded white powdery products with clear filtrates. The ibuprofen-loaded particles formed were visualised by SEM (Figure 3.9). It was observed that functionalised polymer, PGA-co-PDL, has a rough surface, in comparison to non-functionalised hydrophobic polymer, PBA-co-PDL. The same observation was noticed in case of

PLGA (discussed later, figure 3.11) which might be due to the effect of polymer solvent interactions.

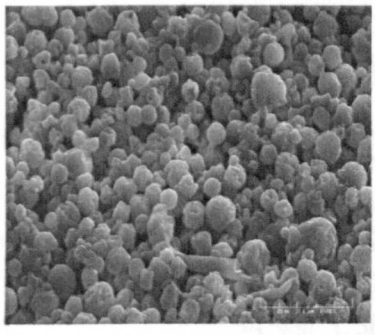
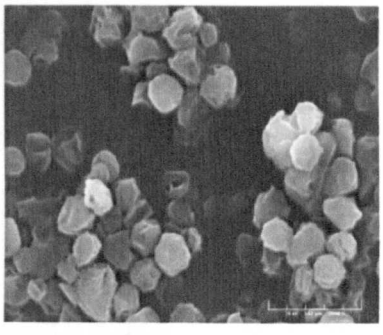
a)  b) 

Figure 3.9: *SEM images of Ibuprofen loaded particles with different polymers (x1000, measuring bar = 31.6 μm) a) PGA-co-PDL b) PBA-co-PDL*

The ibuprofen-encapsulated microparticles had similar encapsulation efficiency values, regardless of the polymer used (Table 3.10). The sizes of the particles were also very similar. Although it is well established that polymer chemistry plays a very important role in determining drug encapsulation and particle morphology, it was observed that, given the differences in the polymer chemistries of these three materials, there was no significant effect on the encapsulation efficiency (P value $\geq$ 0.5). This observation indicates that the presence of –OH groups on the polymer backbone did not produce any difference in the encapsulation efficiency of ibuprofen.

Table 3.10

Effect of polymer chemistry on ibuprofen encapsulation: Mean encapsulation efficiencies and particle sizes are shown (n=3).

Polymer	Encapsulation efficiency (%)	Particle Size (μm)
PGA	No particles	-
PBA	No particles	-
PGA-co-PDL	55.0 ± 1.25	1.2 ± 1.0
PPA-co-PDL	54.0 ± 1.50	1.4 ± 1.2
PBA-co-PDL	54.9 ± 1.50	1.1 ± 1.2
PGA-co-CL	No particles	-
PBA-co-CL	No particles	-
PCL	No particles	-

From the SEM images (Figure 3.9), it was evident that non-agglomerated particle formation was only achieved with ω-pentadecalactone-based copolymers (such as PGA-co-PDL and PBA-co-PDL) which are solid at room temperature. These polymers were off-white waxy solids at room temperature with molecular weights of 14.4 kDa and 15.9 kDa respectively. Under the applied methodology the influence of polymer chemistry on the formation of particles was evident. Only the materials that were solid at room temperature with higher molecular weights and minimal amounts of low molecular weight impurities produced discrete non-agglomerated particles. Whereas, liquid polymers could not formulate into microparticles, not only because of their liquid nature but also because of the fact that the added emulsifier was in very low quantity (0.2%) which did not aid the formation of particles from the liquid polymer. However, by evaluating other surfactants and by increasing their content, discrete particle formation was possible, such as that reported by Katare et al (2005), where PLA, a water soluble viscous polymer, and an oil soluble surfactant were used to produce discrete particles.

3.3.2.6 Spray drying

To evaluate the effect of a different encapsulation technique, spray drying was used for the blank microparticle preparation and ibuprofen encapsulation using PGA-co-PDL and PGA-co-PDL conjugated with stearic acid (PGA-co-PDL-C18). It was observed in the preliminary studies that blank PGA-co-PDL particles were in the range of 1μm diameter and were agglomerated. This is in concordance with previous research by Corrigan et al (2002) where additives such as lactose were used to improve particle preparations. In the current study, however, 0.2% PVA was used in o/w and w/o/w emulsion as surfactant but no external additives were used in spray drying and the ability of the polymeric materials alone was investigated for particle preparation (Figure 3.10).

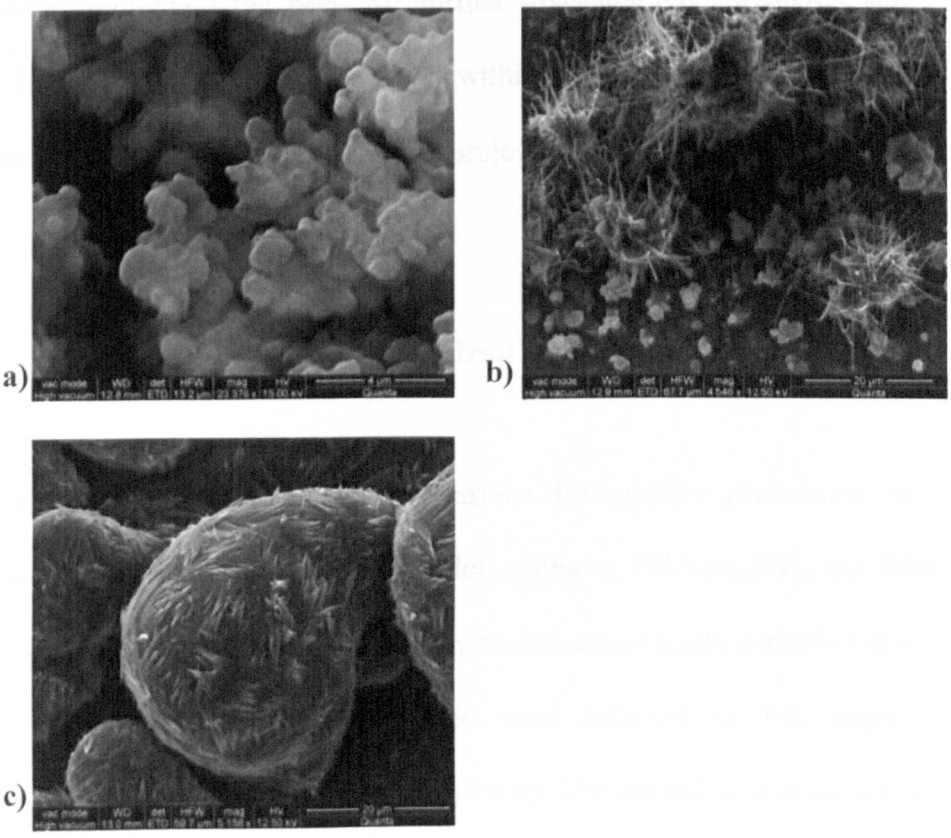


Figure 3.10: *SEM images of microparticles of a) PGA-co-PDL (x23376, magnification bar = 4μm) b) PGA-co-PDL-C18(100%) (x4546, magnification bar = 20μm) and c) ibuprofen loaded PGA-co-PDL (x5156, magnification bar = 20μm) obtained by spray drying.*

The stearic acid-conjugated polymer gave particles of a larger diameter (20μm) with a "spiky" surface (Figure 3.10b). It is believed that this is solely due to the physicochemical properties of stearic acid. Figure 3.10 shows the crystallisation of ibuprofen on the surface of particles obtained by spray drying. This is due to the fact that during the spray drying process all drug remained associated with the particles (either encapsulated or adsorbed on the surface), whereas in the solvent emulsion system the excess drug was removed with the continuous phase. The particles obtained by the spray drying technique would be expected to cause a larger initial burst release of drug compared to the particles obtained by o/w and w/o/w technique. Hence, it would be required for particles formulated with spray drying techniques to be washed with an appropriate solvent for the removal of surface drugs. These studies showed potential but also highlighted the need for further investigations, involving spray drying for the effective encapsulation of drugs within the library of polymers studied. This was, however, outside the scope of this project.

3.3.3 Particle preparation and drug encapsulation

After studying the effects of various formulation parameters on a model drug, ibuprofen, encapsulation in a "model" polymer, PGA-co-PDL, the following conditions were selected for further evaluation of drug encapsulation studies in different polymers. Polymer and drug concentrations were selected as 100 mg/ml and 12 mg/ml respectively with 0.2% surfactant (PVA). The emulsion was mixed at a speed of 3000 rpm using DCM as the organic solvent (oil). The resultant particles were analysed for drug content and release profile using HPLC. GPC was not used for drug quantification in drug-release samples, because of the column sensitivity towards aqueous solutions.

The optimal obtained parameters were then used for the encapsulation of various drugs in the library of polymers synthesised previously (listed in table 3.3) to evaluate the effects of altering polymer chemistry on drug encapsulation and release.

3.3.3.1 Polymer selection of drug encapsulation studies

The entire library of polymers was screened for potential for drug encapsulation. As outlined in section 3.3.2.5 the liquid polymers were unable to form microparticles via the solvent emulsion evaporation techniques (oil-in-water and water-in-oil-in-water). Hence only polymers that were solid at room temperature were carried forward for drug encapsulation studies. The list of the solid polymers assessed is summarised in table 3.3.

Even though the oil-in-water emulsion solvent evaporation technique was the main technique used in this work, its efficiency was limited to only hydrophobic (poorly water-soluble) drugs. This is why for the encapsulation of hydrophilic (water-soluble) drugs other techniques such as water-in-oil-in-water (w/o/w) and spray-drying were used. Another limitation for the use of the o/w technique was the limited solubility of some polymers to only in high boiling point solvents. For example, PSA-co-PDL is only soluble in chloroform which has a boiling point of 61°C. This makes evaporation of chloroform slower and incomplete from the emulsion at room temperature, and a longer duration for solvent evaporation will result in the encapsulated drug being released back into the continuous phase. An alternative approach of heating the solvent to facilitate the evaporation could not be applied because of the low melting points of the polymers. These factors made emulsion solvent evaporation technique a less efficient procedure for the formation of discrete microparticles. As an alternative, a spray drying technique was investigated. Table 3.11 summarises the results of microparticle preparation using various polymers.

Table 3.11

Data for microparticulate formulations prepared using various polymers and techniques

Polymer	Physical state at room temperature	Technique used	Particle size (μm)	Surface
PGA	Viscous	o/w	N/A	N/A
PGA	Viscous	Spray drying	N/A	N/A
PPA	Viscous	o/w	N/A	N/A
PBA	Soft solid	o/w	N/A	N/A
PTEGA	Viscous	o/w	N/A	N/A
PLGA	Solid	o/w	3-5	Smooth
PGA-co-CL (1:1:0.7)	Viscous	o/w	N/A	N/A
PPA-co-CL (1:1:0.45)	Viscous	o/w	N/A	N/A
PBA-co-CL (1:1:0.40)	Viscous	o/w	N/A	N/A
PGA-co-PDL (1:1:0.5)	Soft solid	o/w	N/A	N/A
PGA-co-PDL (1:1:1)	Solid	o/w	4-6	Rough
PGA-co-PDL (1:1:1)	Solid	w/o/w	5-10	Rough
PGA-co-PDL (1:1:1)	Solid	Spray drying	>2	Smooth
PGA-co-PDL (1:1:1.2)	Solid	o/w	8-12	Rough
PPA-co-PDL (1:1:0.6)	Solid	o/w	1-4	Smooth
PBA-co-PDL (1:1:0.5)	Solid	o/w	1-4	Smooth
PTEGA-co-PDL(1:1:0.5)	Solid	o/w	3-5	Smooth
PTEGA-co-PDL (1:1:1)	Solid	o/w	3-5	Smooth
PTEGA-co-PDL(1:1:1.5)	Solid	o/w	5-10	Smooth
PSA-co-PDL (1:1:0.5)	Solid	o/w	N/A	N/A

* N/A = No particles formed

Table 3.11 *(continued)*

Polymer	Physical state at room temperature	Technique used	Particle size (μm)	Surface
PGA-PEG	Viscous	o/w	N/A	N/A
PGA-PEGme	Viscous	o/w	N/A	N/A
PPA-PEG	Viscous	o/w	N/A	N/A
PGA-co-CL-PEG	Viscous	o/w	N/A	N/A
PGA-co-PDL-PEG	Solid	o/w	3-5	Smooth
PGA-co-PDL-PEGme				
1:1:1.5:0.01	Solid	o/w	5-8	Porous
1:1:1.4:0.01	Solid	o/w	5-8	Porous
1:1:1.3:0.02	Solid	o/w	5-8	Porous
PPTA	Viscous	o/w	N/A	N/A
PDTTA	Viscous	o/w	N/A	N/A
PPTA-co-PDL	Soft solid	o/w	N/A	N/A
PDTTA-co-PDL	Soft solid	o/w	N/A	N/A
PGA-co-PDL-C18 (50%)	Solid	o/w	3-5	Spiky
PGA-co-PDL-C18 (33%)	Solid	o/w	3-5	Spiky
PGA-co-PDL-Ibu (50%)	Solid	o/w	3-5	Smooth
PGA-co-PDL-Ibu (17%)	Solid	o/w	3-5	Smooth

* N/A = No particles formed

Table 3.11 summarises the results of particle formulation studies with all the synthesised polymers. It was observed that all the viscous and very soft solid polymers failed to formulate any microparticles via the o/w solvent evaporation technique. The chemistry of the polymers did not affect the particle sizes considerably, as all particles were in the range of 5-10μm in diameter. However the surface properties of the polymers varied as a result of altering the monomers present in the polymer. For example, the presence of stearic acid in the polymer caused a spiky surface whereas incorporation of PEG caused smooth but porous surfaces. The details of these effects are discussed in following section.

3.3.3.3 Effect of polymer and drug chemistry on drug encapsulation

Polymers with varying chemistries were evaluated for encapsulation of ibuprofen, a model hydrophobic drug. The ability of these polymers was also evaluated for the encapsulation of other drugs with different water solubilities (Table 3.3). The hypothesis for these studies was that more hydrophobic polymers will encapsulate higher amounts of poorly water soluble drugs and less hydrophobic polymer will encapsulate more of the water soluble drugs.

The results of the drug encapsulation in "model" polymer, PGA-co-PDL, were compared with the other members of the library of polyesters, as summarised in the following sections.

a) Comparison with PLGA:

PLGA is an FDA-approved and commercialised polymer for drug delivery applications, (as discussed in chapter 1). Hence, the encapsulation of drugs in PLGA was compared with that in the PGA-co-PDL. The results obtained in this study for the encapsulation and release of ibuprofen from PGA-co-PDL confirms the previous findings of

Thompson et al (2007), however the encapsulation efficiency they reported was 67%, which is 12% higher than that reported in current studies. This difference can be explained on the basis of the use of UV spectrophotometry as the analytical method adopted by Thompson et al (2007), which overestimates the ibuprofen concentration, as previously discussed in section 3.3.1.

Table 3.12 summarises the drug encapsulation efficiencies of three drugs, ibuprofen, indomethacin and levofloxacin in PLGA and PGA-co-PDL. The data for drug encapsulation in PGA-co-PDL confirmed that as the relative aqueous solubility of the drugs increased a significant increase in drug encapsulation was observed (P value 0.0114), and as the water solubility of drugs increased (from ibuprofen to levofloxacin) a more significant decrease in encapsulation efficiencies was recorded (P value 0.0001). The highly water soluble drug rifampicin failed to encapsulate in these polymers using the applied method because of its high water solubility.

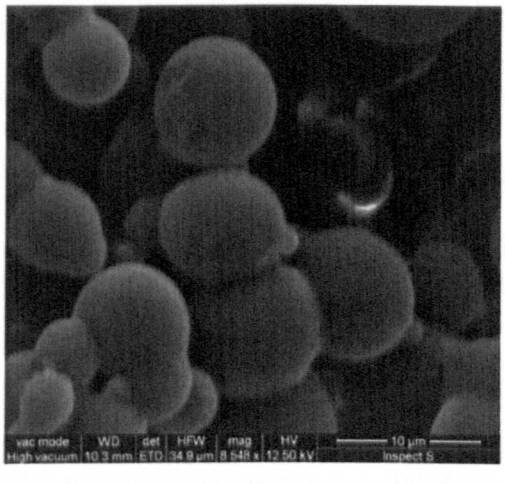


Figure 3.11: *SEM image of ibuprofen loaded PLGA microparticles (x8458, measuring bar = 10µm).*

When the ibuprofen encapsulation results for PGA-co-PDL were compared with PLGA, it was found that for ibuprofen encapsulation there is no significant difference (P value 0.6765) as compared to PGA-co-PDL, but a very significant increase was observed for

indomethacin encapsulation in PLGA (P value 0.0031) which can be explained by the higher hydrophobicity of PLGA. Although there was no difference in the particle sizes observed (Figure 3.11), the surface of PLGA particles were smoother compared to those of PGA-co-PDL.

Previous studies confirm the encapsulation efficiencies of ibuprofen in high molecular weight (34 kDa) PLGA were in the range of 46-52% using 1% PEG-300 (Labarafil®) (Fernández-Carballido et al 2004). The results obtained in the present work are supported by the previous research work, where a high encapsulation efficiency was obtained for ibuprofen (about 60-75%) and for indomethacin (80-90%) in the very hydrophobic polymer, ethyl cellulose (Bodmeier & Chen 1989). This confirms that a more hydrophobic polymer will encapsulate a higher amount of hydrophobic drug.

In this study, the encapsulation of levofloxacin (a water soluble drug) was significantly less in PLGA (9.5 kDa) as compared to PGA-co-PDL(17.5 kDa). Although other studies have also reported the encapsulation of levofloxacin in PLGA, they used higher surfactant concentrations (≥1%) (Gupta et al) compared to the 0.2%(w/v) PVA used in the present studies. Similarly, it has been reported for ciprofloxacin (water solubility 35 mg/ml) that with PLGA (15 kDa) and using 1%(w/v) PVA surfactant, the encapsulation efficiencies achievable were up to 42% (Jeong et al 2008).

Table 3.12

Encapsulation efficiencies of various drugs in PGA-co-PDL and PLGA

Polymer	**Drug encapsulation (%EE)**			
	Indomethacin (o/w)	*Ibuprofen (o/w)*	*Levofloxacin (w/o/w)*	*Rifampicin (w/o/w)*
PGA-co-PDL	62.99±2.44	53.6±2.74	12.59±3.10	0.00
PLGA	79.04±3.6	54.62±2.82	8.77±4.35	0.00

b) Effect of polymer composition:

No significant difference of ibuprofen encapsulation was observed between non-functionalised (hydrophobic) polymers, PPA-co-PDL, PBA-co-PDL and the functionalised (hydrophilic) polymer, PGA-co-PDL (Table 3.10). Hence, in a bid to alter the chemical properties of polymers in terms of hydrophobicity, the type of monomers and their amounts were varied in the polymer backbone.

A novel non-functionalised (hydrophilic) polymer, PTEGA-co-PDL was synthesized and compared with the model polymer, PGA-co-PDL, for the encapsulation of the hydrophobic drugs (indomethacin and ibuprofen). As illustrated in table 3.13, PGA-co-PDL (1:1:1.2), containing an increased lactone content within the polymer, showed a slight but not significant increase in encapsulation efficiencies. When the data for PGA-co-PDL (1:1:1) were compared with PTEGA-co-PDL (1:1:1) (a polymer with no pendant hydroxyl groups) it was observed that an extremely significant increase (P value 0.0003) in ibuprofen encapsulation and a significant increase (P value 0.0107) in indomethacin encapsulation was achieved, under similar formulation parameters.

Table 3.13

Comparison of encapsulation efficiencies of various ratios of PGA-co-PDL and PTEGA-co-PDL with different drugs

Polymer	**Drug encapsulation (%EE)**			
	Indomethacin (o/w)	*Ibuprofen (o/w)*	*Levofloxacin (w/o/w)*	*Rifampicin (w/o/w)*
PGA-co-PDL (1:1:1)	62.99±2.44	53.6±2.74	12.59±3.10	0.00
PGA-co-PDL (1:1:1.2)	66.36±6.19	56.77±2.23	-	-
PTEGA-co-PDL (1:1:1)	73.50±3.21	74.31±1.43	0.00	0.00
PTEGA-co-PDL (1:1:1.5)	74.00±3.15	70.38±5.92	-	-

PTEGA-co-PDL (1:1:1) is devoid of pendant hydroxyl groups but is a hydrophilic polymer, yet encapsulation of the hydrophilic drug, levofloxacin was not observed in this polymer. This was contrary to what was observed for PGA-co-PDL (1:1:1), where the encapsulation efficiency of levofloxacin was 12.59±3.10%. Also, the increase in the lactone content in triethyleneglycol-based polymers (PTEGA-co-PDL) from 1:1:1 to 1:1:1.5 did not significantly alter the encapsulation efficiencies. Similar trends were observed when comparing the encapsulation of the two drugs in PGA-co-PDL (1:1:1.5) and PTEGA-co-PDL (1:1:1.5).

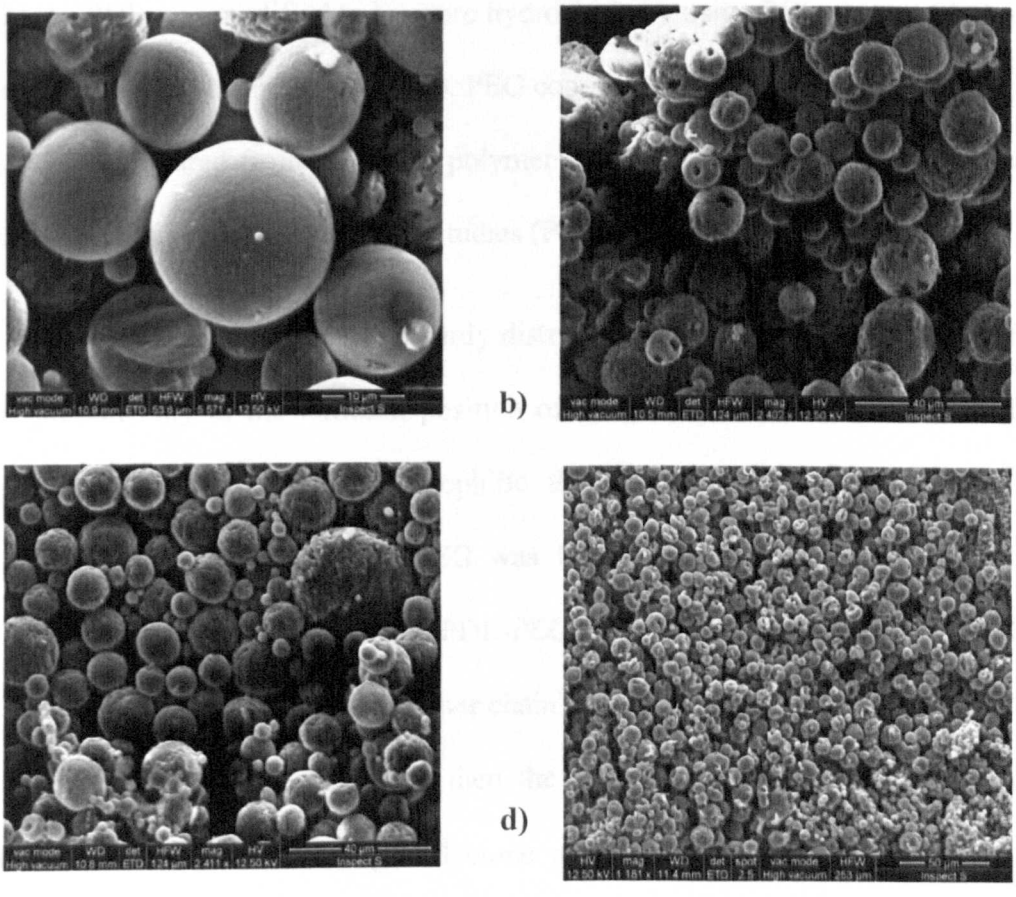


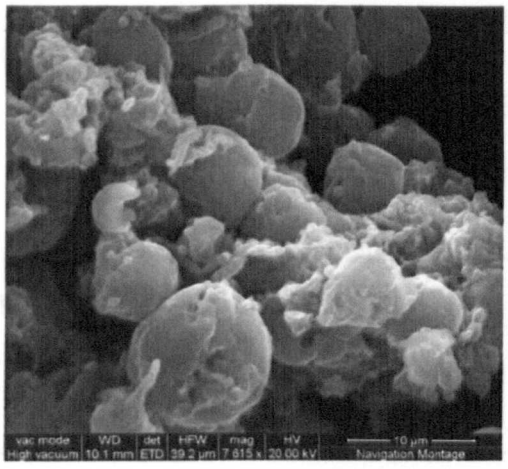
Figure 3.12: *SEM images of drug loaded microparticles of PGA-co-PDL (1:1:1) with a) ibuprofen (x 5571, measuring bar = 10μm) b) levofloxacin (x2422, measuring bar = 40μm) c) rifampicin (x 2411, measuring bar = 40μm) and of PTEGA-co-PDL (1:1:1) with d) indomethacin (x1181, measuring bar = 50μm).*

c) Comparison of PEG copolymers:

Many research groups have used PEG as a surfactant or additive while formulating microparticles. The purpose of introducing PEG as part of polymer backbone was to synthesise a polymer which could be formulated into microparticles without the addition of any external additive or surfactant and also to increase the drug encapsulation and to control the drug release. However, the PEG-copolymers synthesised here failed to form stable emulsions on their own. Hence, 0.2% PVA had to be added as a surfactant to formulate microparticles.

PGA-co-PDL was modified to be more hydrophilic by using PEG_{1500} and $PEGme_{2000}$ as additional monomers in the synthesis. PEG copolymer-based microparticles were bigger than those prepared from PEG-free polymers (as summarised in table 3.11 and figure 3.13), which is supported by earlier studies (Peracchia et al 1997).

In this current work PEG was randomly distributed in the polymer chain while PEGme was present only at the terminal position of the polymers. Hence, theoretically PEG copolymers should be more hydrophilic than PEGme copolymers but the actual monomer ratio in PGA-co-PDL-PEG was 1:1:0.8:0.02 (8.5kDa, 3-4 PEG units per polymer chain) while for PGA-co-PDL-PEGme the monomer ratio was 1:1:1.4:0.01 (4.7kDa, 1-2 PEGme units per polymer chain) (Table 2.16). This indicates that PEGme-copolymers are more hydrophobic then the equivalent PEG-copolymers, because of lower PEGme content and higher lactone content in the polymer backbone. It was previously established that incorporation of PEG into polymeric microparticles usually causes an increase in drug loading with an increased rate of drug release from these formulations (Huang & Chung 2001). PEG also acts as a channelling agent (Arida et al 1999) hence can increase the rate of drug release.

a) 

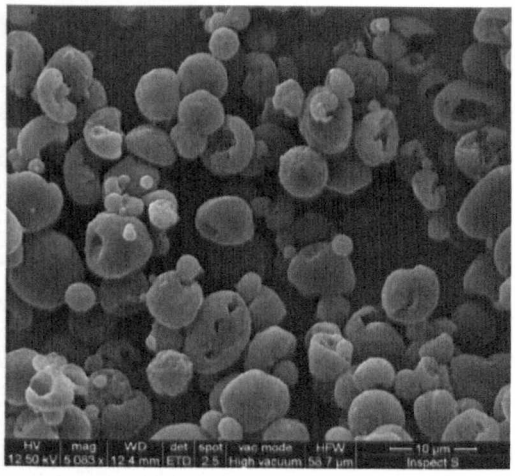
b) 

Figure 3.13: *SEM images of drug loaded microparticles of PGA-co-PDL-PEGme with a) ibuprofen (x7615) and b) indomethacin (x5063)*

The effect of changing polymer chemistry was evident in the drug encapsulation results, where a significant increase in ibuprofen encapsulation was observed for PGA-co-PDL-PEG (P value 0.0814) but no significant increase in ibuprofen encapsulation (P value 0.9345) was recorded for PEGme copolymer (Table3.14). The same observations were noted for indomethacin encapsulation where the PEG copolymer showed a significant (P value 0.0165) increase in indomethacin encapsulation whereas the PEGme based copolymer did not encapsulate significantly more indomethacin (P value 0.5631) compared to PGA-co-PDL. In further support of this, the levofloxacin encapsulation results clearly indicate that the PEG copolymer, being more hydrophobic, failed to encapsulate levofloxacin. On the other hand the PEGme copolymer with a more hydrophilic character showed equivalent levofloxacin encapsulation to PGA-co-PDL (P value 0.8011). Table 3.14 summarise these results.

Table 3.14

Comparison of drug encapsulation efficiencies of PGA-co-PDL-PEG and PGA-co-PDL-PEGme with PGA-co-PDL

Polymer	Drug encapsulation (%EE)			
	Indomethacin (o/w)	*Ibuprofen (o/w)*	*Levofloxacin (w/o/w)*	*Rifampicin (w/o/w)*
PGA-co-PDL (1:1:1)	62.99±2.44	53.6±2.74	12.59±3.10	0.00
PGA-co-PDL-PEG	76.14±5.19	64.04±3.82	0.00	0.00
PGA-co-PDL-PEGme	64.45±3.19	53.74±0.42	13.21±2.51	0.00

The similar drug encapsulation values were observed in polymers with slightly varied polymer compositions (Table 3.15). All ibuprofen encapsulation results were similar (P value 0.1224 – 0.9345), hence indicating that slight variations in monomer ratios (PEG/lactone content) does not considerably affect the encapsulation efficiencies.

Table 3.15

Comparison of ibuprofen encapsulation efficiencies of various ratios of PEGme-copolymers with PGA-co-PDL

Polymer	Monomer ratio	Drug encapsulation (%EE) *Ibuprofen*
PGA-co-PDL	1:1:1	53.6±2.74
PGA-co-PDL-PEGme	1:1:1.5:0.01	49.14±2.85
PGA-co-PDL-PEGme	1:1:1.4:0.01	53.74±0.42
PGA-co-PDL-PEGme	1:1:1.3:0.02	52.87±2.36

d) Comparison of post synthetic modification in polymer backbone:

A long-chain non-polar molecule, stearic acid, was conjugated to the pendant hydroxyl groups of PGA-co-PDL. Based on the theoretically-available numbers of hydroxyl groups, 50% and 100% hydroxyl groups should be conjugated. However, only 33% and 50% conjugation was achieved. As a non polar C18 chain was added to the polymer structure, this made the polymer more hydrophobic. Tabulated results (Table 3.16) show that increasing the stearic acid ratio (from 33% to 50%) caused a considerable increase in ibuprofen encapsulation, hence further supporting the hypothesis that more hydrophobic polymers will encapsulate more hydrophobic drugs. However, it was observed that conjugating the polymer with 33% stearic acid resulted in considerably less ibuprofen being encapsulated than with PGA-co-PDL alone. The reason for this observation might lie in the fact that after the conjugation the polymer molecular weight dispersity increased considerably, resulting in polymer chains of various lengths. This alteration in polymer chemistry may have had the potential to initiate cross linking of polymer chains, which may lead to altered drug-polymer interactions as compared to straight chain polymers, leading to different encapsulation efficiencies.

Table 3.16

Comparison of drug encapsulation efficiencies of ibuprofen and levofloxacin in stearic acid conjugated PGA-co-PDL with stearic acid-free PGA-co-PDL

C18 conjugated Polymer	*(% conjugation)*	**Drug encapsulation (%EE)** *Ibuprofen*
PGA-co-PDL (1:1:1)	0	53.6±2.74
PGA-co-PDL-C18	33	44.64±2.47
PGA-co-PDL-C18	50	61.99±1.23

These results indicate that by post-synthetically modifying the polymer, the drug encapsulation efficiencies could be altered. SEM images of stearic acid conjugated polymeric particles highlights the different surface properties, as compared to PGA-co-PDL particles (Figure 3.14). The same observations were recorded when particles were prepared via the spray drying technique (Figure 3.10).

On the other hand, when ibuprofen was conjugated to PGA-co-PDL, and formulated into microparticles, the surface of the particles was smooth. The particle sizes were very similar to the stearic acid-conjugated polymer (3-5μm). These results further indicate that the small alterations in polymer chemistry can not only affect the encapsulation efficiencies but also the particle morphology.

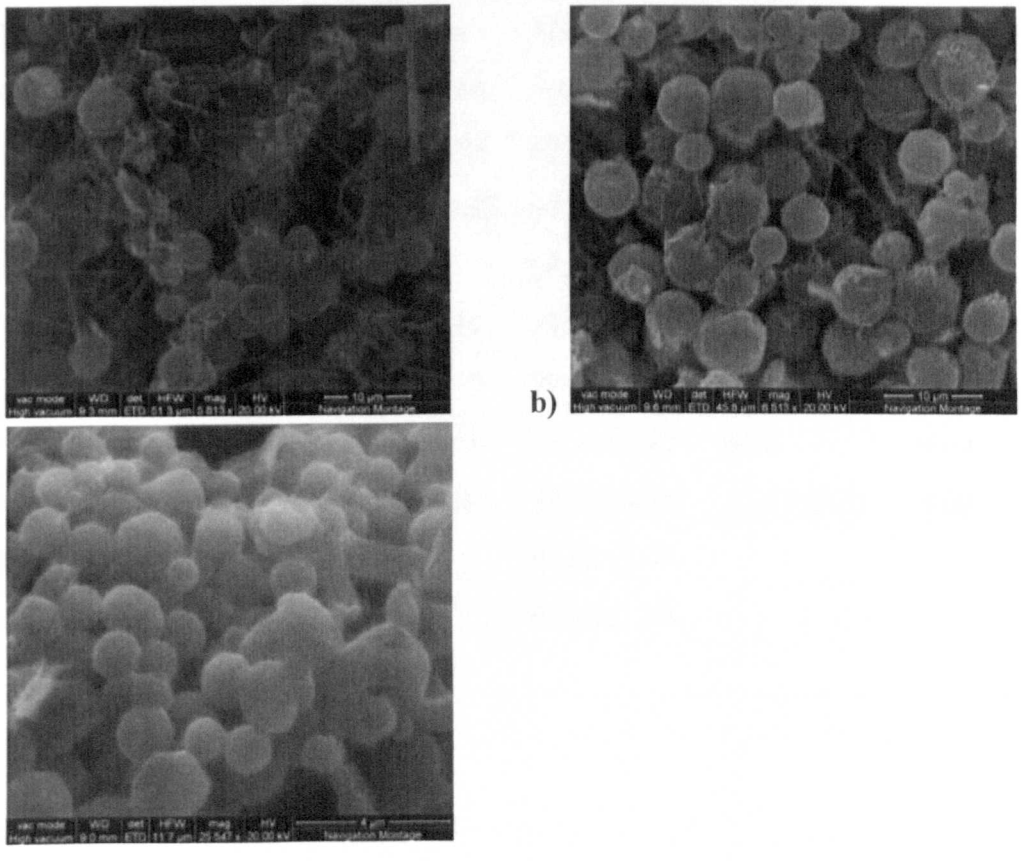


Figure 3.14: *SEM images of microparticles of PGA-co-PDL-C18 (100%) a) blank (x5813, magnification bar = 10μm) and b) with encapsulated ibuprofen (x6513, magnification bar = 10μm) and c) PGA-co-PDL-Ibu (100%) (x25547, magnification bar = 4μm)*

In summary, polymers with varying chemistries have been used to attempt the encapsulation of four different drugs. Encapsulation of freely and poorly water-soluble drugs was achieved to different levels, except for rifampicin which was not encapsulated in any of the polymers (Table 3.17). The drug-loaded formulations obtained were then assessed further to obtain drug release profiles.

Table 3.17

Encapsulation efficiencies of four drugs in different polymers (bearing different chemistries)

Polymer and monomer ratios	**Encapsulation efficiency (%)**			
	Indomethacin	*Ibuprofen*	*Levofloxacin*	*Rifampicin*
PLGA (50:50)	79.04±3.6	54.62±2.82	8.77±4.35	0.00
PGA-co-PDL (1:1:1)	62.99±2.44	53.6±2.74	12.59±3.10	0.00
PGA-co-PDL (1:1:1.2)	66.36±6.19	56.77±2.23	-	-
PPA-co-PDL (1:1:0.6)	56.04 ± 3.53	54.0 ± 1.50	-	-
PBA-co-PDL (1:1:0.5)	-	54.9 ± 1.50		
PTEGA-co-PDL (1:1:1)	73.50±3.21	74.31±1.43	0.00	0.00
PTEGA-co-PDL (1:1:1.5)	74.00±3.15	70.38±5.92	-	-
PGA-co-PDL-PEG	76.14±5.19	64.04±3.82	0.00	0.00
PGA-co-PDL-PEGme	64.45±3.19	53.74±0.42	13.21±2.51	0.00
PGA-co-PDL-C18 (33%)	-	44.64±2.47	-	-
PGA-co-PDL-C18 (50%)	-	61.99±1.23	-	-

3.3.4 Drug release profiles

Drug release studies are an important part of determining the potential of a new drug delivery system. All drug-loaded formulations (table 3.17) underwent drug release studies and the results are discussed below.

3.3.4.1 Release of encapsulated drugs

a) Ibuprofen release

The release of encapsulated ibuprofen was studied over 24 hours. It was observed that over this time ibuprofen release was not complete (100%) in any of the formulations (Figure 3.15). In PGA-co-PDL (1:1:1) the maximum drug released in 24 hours was about 80%. Making the polymer more hydrophobic reduced the drug release from these formulations considerably (see the curves for PLGA and PPA-co-PDL, figure 3.15). It is well established that not only the chemistry but also the particle size affects the drug release, that is, smaller particles release more drug due to a larger surface area (Dawes et al 2009). All three above-mentioned formulations had similar particle sizes (about 5μm) and %EE (about 53%), hence the differences in the release profiles observed were because of the polymer chemistry. The greatest amount of the drug was released in the first two hours for PLGA and PPA-co-PDL, but for the PGA-co-PDL, the maximum amount of the drug was released in 6 hours followed by release of the drug at very slow rate, supporting the zero-order release profile of ibuprofen from these polymers. Similar to PGA-co-PDL, the drug release from ethylene glycol containing polymers, that is, PTEGA-co-PDL, PEG and PEGme copolymers showed a zero-order release profile but maximum drug released in 24 hours did not exceed 30% of total encapsulated drug. According to the supposition, hydrophilic polymers should release drugs at a faster rate because of effective interactions with water. But the observations made here were

contrary to supposition. There are two possible reasons behind this phenomenon, as discussed below;

1) It was previously established that microparticles obtained from PEG copolymers may have non-homogeneity (Peracchia et al 1997). The hydrophilic entities such as ethylene glycol monomers (TEG, PEG, PEGme) occupy the outer shell region of the particles while the hydrophobic part (PGA-co-PDL) constitutes the inner core of microparticles. According to this hypothesis, the hydrophobic drug would dissolve in the hydrophobic part of the polymer; hence, it is assumed that ibuprofen would lie in the centre of the microparticles. This will make the drug release considerably slower compared to that from a particle made up of a homogeneous non-polar polymer (such as PLGA or PGA-co-PDL). However, the presence of PEG does not affect the cumulative drug release as compared to the other polymers which is in accordance with previous studies (Peracchia et al 1997). This is why PEG copolymers are good candidates for drug delivery systems which can markedly reduce the rate of drug release.

2) The other reason behind the slower drug release is the mechanism of drug release. Typically the drug initially releases from microparticles via diffusion-controlled mechanisms followed by a combination of polymer degradation and diffusion mechanisms. Various monomers such as PEG or polyethylene oxide (PEO) have the ability to swell in aqueous media by absorbing water, leading to slower water penetration into microparticles, which results in slower drug release (Jeong et al 2000). On the other hand, hydrophobic polymers can only go through diffusion and degradation or surface erosion at relatively higher rates – giving higher amounts of drug release.

These results suggest that the introduction of hydrophilic monomers has a profound effect on the percentage drug released and the way the drug releases from these formulations (Figure 3.15). The difference in molecular weight of the polymer also effects the drug release profile, as described earlier by Ramkissoon-Ganorkar et al (1999) and Kim (1998).

The initial burst release of drug may be the result of washing of the drug from the surface, as there was no specific procedure followed to "wash-out" the surface-bound drug by the use of specific chemicals or drug removing agents, such as a sodium carbonate solution. Leo et al (2000) have demonstrated that using a sodium carbonate solution to wash drug-loaded microparticles removed the ibuprofen from the particles' surface resulting in no burst release. In this work, however, particles were washed with distilled water but due to poor ibuprofen solubility in water, the surface drug may not have been washed away completely, as is confirmed by the results plotted in figure 3.15.

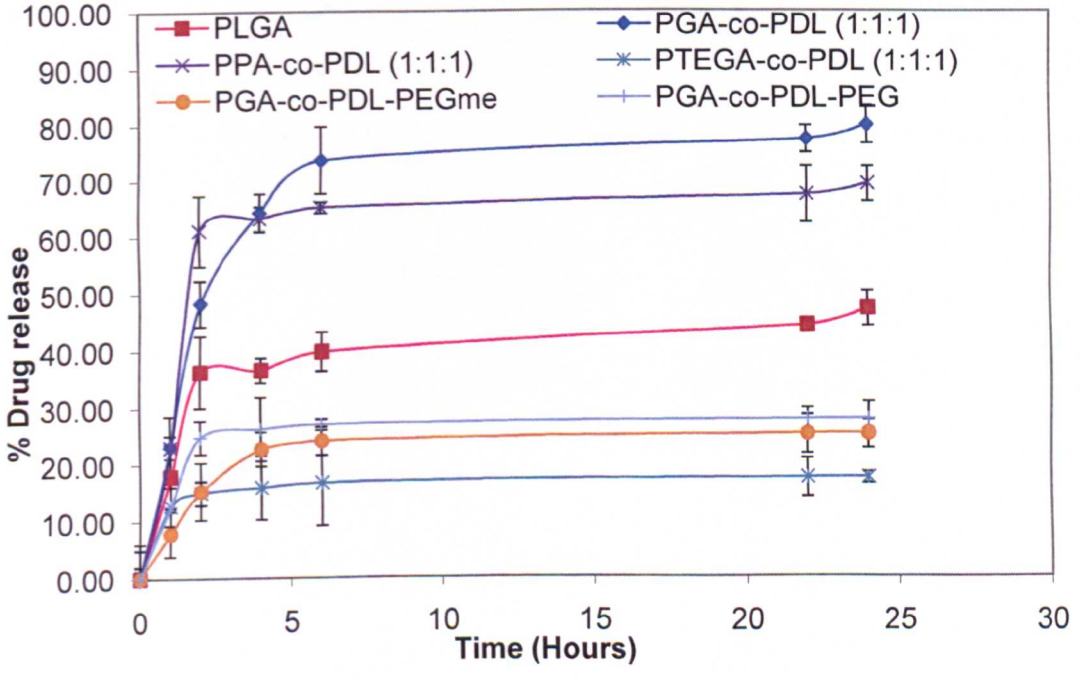


Figure 3.15: *Release profiles of encapsulated ibuprofen from various polymeric formulations (n=3).*

Additionally, when polymers underwent post-synthetic modifications by conjugation to stearic acid – a very non-polar unit, this also resulted in the release of very low amounts of ibuprofen from these formulations as compared to the non-conjugated polymer (Figure 3.16). The 33% conjugated polymer was expected to release a lesser amount of drug than 50% conjugated material. This was not observed, (Figure 3.16). This phenomenon may be explained by the actual composition of the polymers. As it has been established that longer polymeric chains of high molecular weight interact together, causing slower release of drugs from particles (Yang & Hon 2009), hence, the obtained release profiles were in accordance with this explanation. Increasing the number of stearic acid chains on polymeric molecules may have resulted in increased intramolecular interaction. This leads to more drug-polymer interaction and a reduced chance of surface erosion or degradation. Hence, this results in a slower release of the drug from particles (Figure 3.16). These results indicate that post-synthetic modification is a good alternative way of controlling the drug release from microparticles.

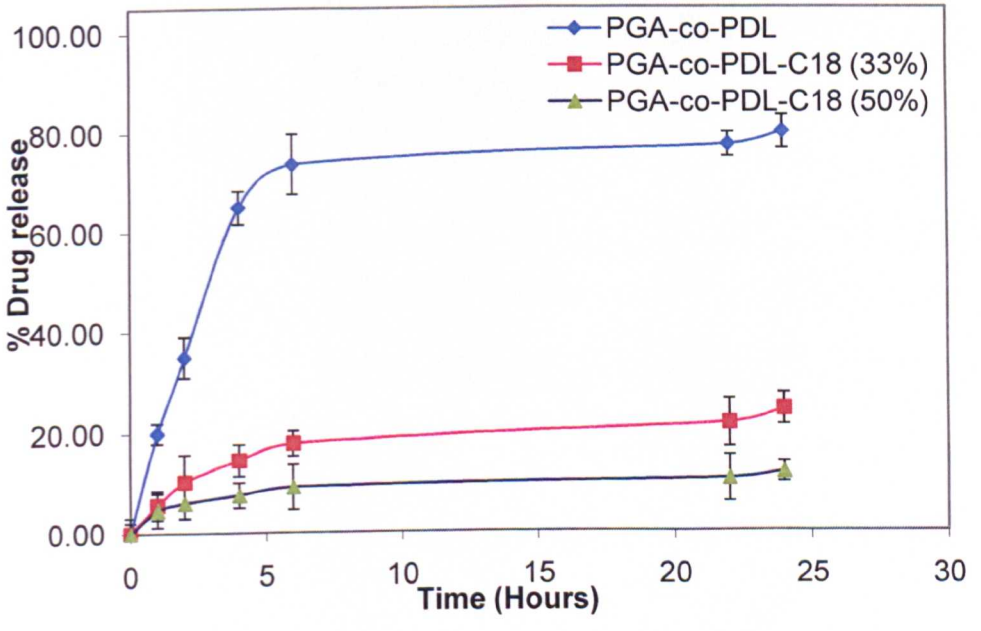


Figure 3.16: *Comparison of release profiles of ibuprofen from particles prepared from PGA-co-PDL with and without conjugated stearic acid (n=3)*

b) Indomethacin release

The release of a relatively less water soluble drug indomethacin followed the same release pattern as that of ibuprofen (Figure 3.17). For PLGA, PGA-co-PDL (1:1:1) and PPA-co-PDL, after an initial burst release, the drug release followed a zero order pattern. In the first 24 hours the maximum drug release was 75% from PGA-co-PDL but increasing the hydrophobicity of the polymer resulted in decreased drug release as can be seen in the profiles for PLGA and PPA-co-PDL (Figure 3.17). This is in accordance with previous studies where the rate of indomethacin release was considerably slower than that of ibuprofen from the hydrophobic polymer ethyl cellulose (Bodmeier & Chen 1989). However, using hydrophilic polymers like Eudragit® made the drug release rate considerably faster. Ethylene glycol containing polymers followed the initial slower release pattern (Figure 3.17) because of the reasons discussed in previous section.

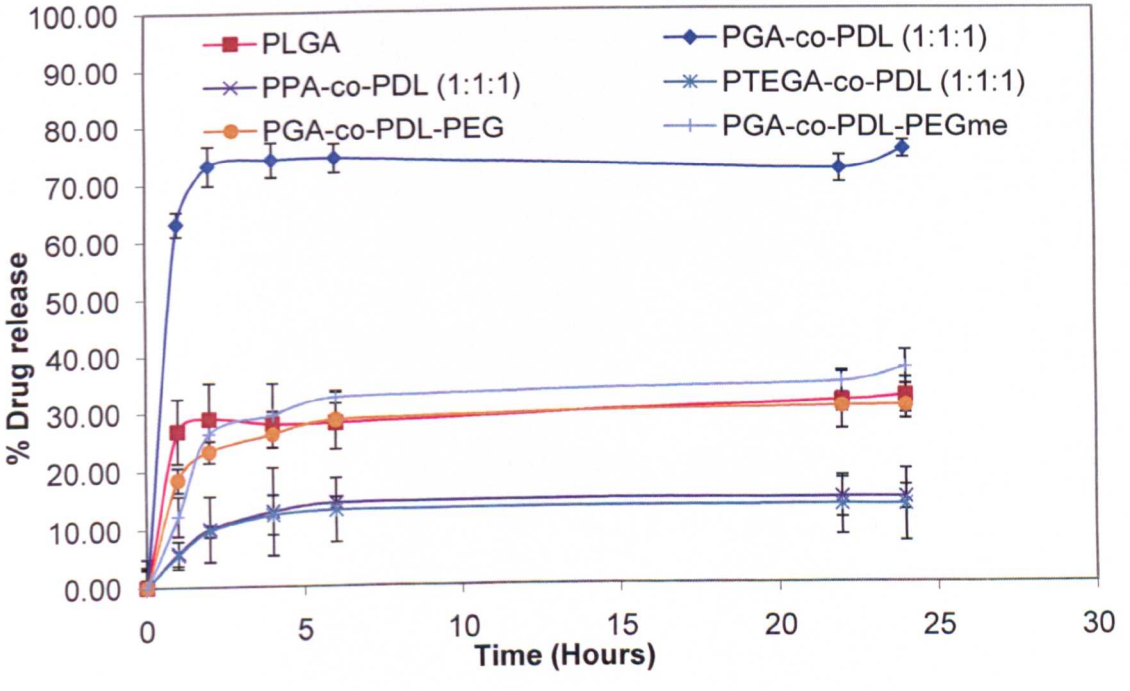


Figure 3.17: *Release profiles of encapsulated indomethacin from various polymeric formulations (n=3)*

Other parameters which can affect drug release from these polymers are the drug loading and drug partition coefficient (polymer/water). It is well established that higher drug loading also results in higher drug release rate (Huang & Chung 2001), but it was apparent from figure 3.17 that the polymer/drug chemistry and polymer-drug interactions, played more dominating roles in determining the drug release rather than total drug loading.

c) Levofloxacin release

The release of the more, water soluble, drug levofloxacin followed a different pattern as compared to indomethacin and ibuprofen. Instead of a very quick release in the first 1-2 hours (as observed in case of ibuprofen and indomethacin), the drug release was slower, reaching a maximum level after 6 hours from PGA-co-PDL and 100% release was not achieved even after 24 hours. Based on the assumption that hydrophilic drugs might release quicker from these polymeric formulations, these results suggest that some of the drug is encapsulated inside the polymeric matrix and would take longer to be released from the particles. One thing which is evident from figure 3.18 is the considerably higher release of the drug from ethylene glycol-containing polymers than PLGA and PGA-co-PDL.

These results are the opposite to what was observed with the hydrophobic drugs, indomethacin and ibuprofen. Ethylene glycol units, especially PEG are already well known for their polymeric coating and water solubility properties, which affect the drug release. The results presented in figure 3.18 are in accordance with the previous studies (Jeong et al 2008) where it was observed that ciprofloxacin (a water soluble drug) was released from the hydrophobic polymer, PLGA, microparticles in 14 days but only 15-60% of the encapsulated drug was released from microparticles in one day (Dillen et al

2004; Jhunjhunwala et al 2009). The mechanism of drug release would be more diffusion-controlled from hydrophilic polymers while for hydrophobic polymers surface erosion/degradation-controlled mechanism followed by diffusion would be predominant.

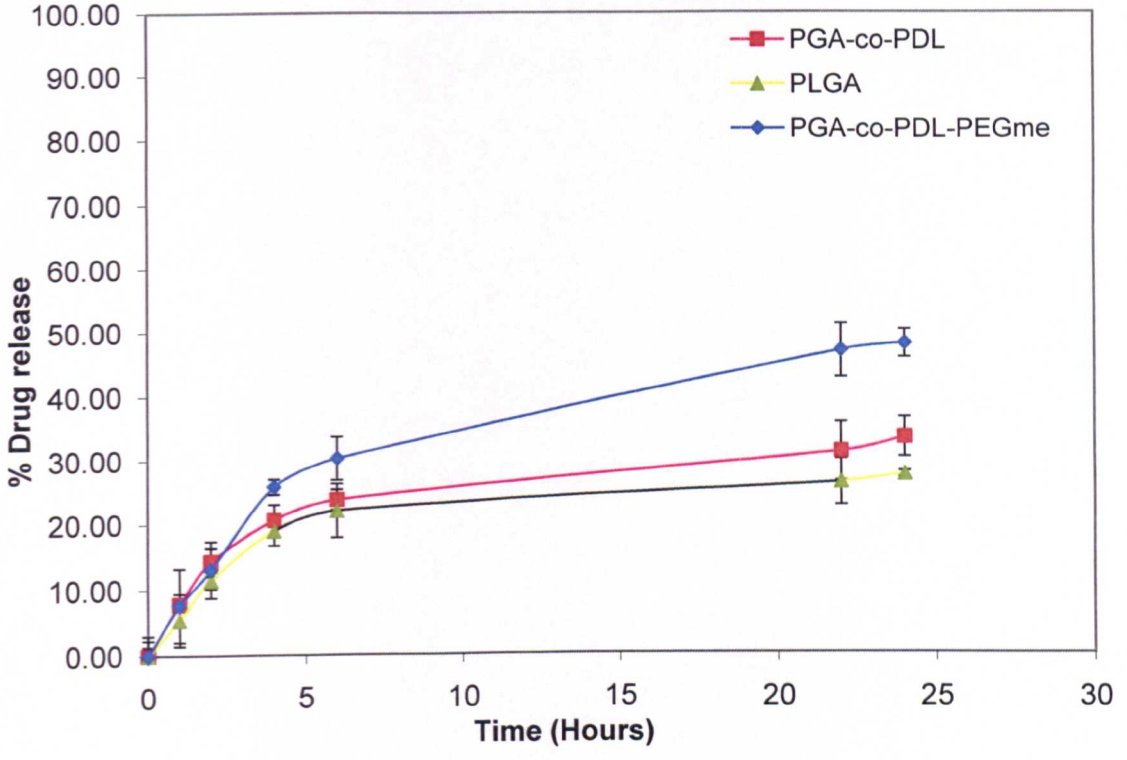


Figure 3.18: *Release profiles of encapsulated levofloxacin from various polymeric formulations (n=3)*

Another observation made in the present work was the absence of a burst release – which can be explained by the fact that during the particle formulation steps the water soluble drugs were "washed-out" by the aqueous continuous phase, leading to a drug-free particle surface. Hence, these results support the fact that the rate of water soluble drug-release is considerably higher from hydrophilic polymers.

d) Rifampicin release

Rifampicin was not successfully encapsulated in any of the microparticle formulations, via o/w or w/o/w techniques, probably because of its large molecular size and water solubility. However, some other groups have reported the encapsulation of rifampicin in PLA using w/o/w emulsion technique (Manca et al 2008) by using a considerably higher amount of PVA (4%) as the surfactant or by using the Shirasu porous glass (SPG) membrane technique (Makino et al 2004; Doan & Olivier 2009).

A spray drying technique was also reported as successfully encapsulating rifampicin into poly-(DL-lactide) using halothane as the solvent (Bain et al 1999), but as discussed earlier, ibuprofen containing microparticles obtained using the spray drying technique were agglomerated and were not suitable for these studies. Hence the release profile of ibuprofen loaded microparticles, obtained from spray drying was not studied. Further exploration into the parameters for spray drying may well have proven this technique to be useful for improved rifampicin loading into these materials.

3.3.4.2 Release of conjugated drugs

In an attempt to slow down the release of drugs from the microparticulate formulations, the model drug, ibuprofen was conjugated directly to the polymer backbone prior to particle formulation. Previous studies have suggested that conjugation of drugs to the polymeric backbone considerably decreases drug-induced toxicity and ensures the targeted delivery of drugs (Hu et al 2009). In this case the drug can only be released by the hydrolysis of weak ester bonds between ibuprofen and the polymer. As the polymer itself is a polyester and degrades via hydrolysis of the labile ester link, the proposed route of drug release was via polymeric degradation. These polymers are biodegradable

under simulated physiological conditions, but may take 6-8 weeks; so drug release would only be completed when all polymer-drug ester bonds are broken.

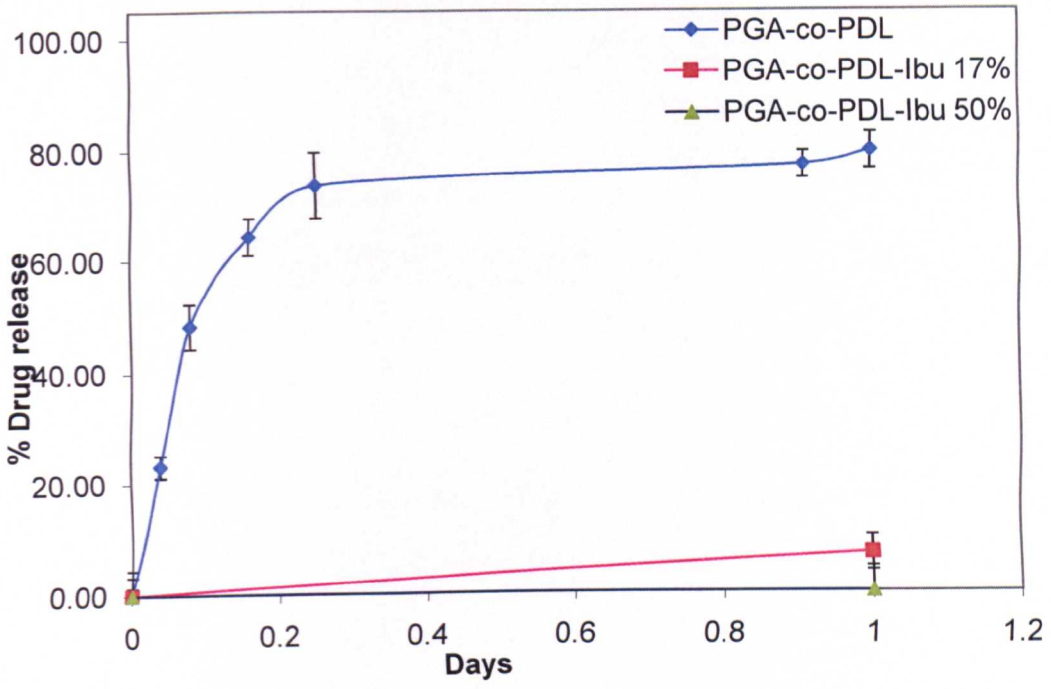


Figure 3.19: *Comparison of ibuprofen release from conjugated and encapsulated polymeric formulations in first 24 hours*

Figure 3.19, shows the marked difference between drug release from encapsulated and conjugated formulations over an initial period of 24 hours. It is noted that about 80% of the drug was released from the encapsulated formulations within 24 hours while in the same duration less than 10% of the drug was released from conjugated species. Even after 35 days the drug release from conjugated species did not exceed 30% (Figure 3.20), which confirms the fact that drug release can be markedly slow when conjugating to the polymer backbone. The difference between the release profiles of 17% and 50% conjugated ibuprofen is because of the fact that the 50% conjugated product contains 50% ibuprofen conjugated to the polymer backbone and hence there are 50% free –OH groups on the polymeric backbone while 17% conjugated product only had 17% conjugated ibuprofen, hence 83% of the –OH groups were free, which makes the

polymer relatively more hydrophilic and more prone to degradation via hydrolysis, resulting in higher drug release (polymer degradation is discussed in detail in chapter 4).

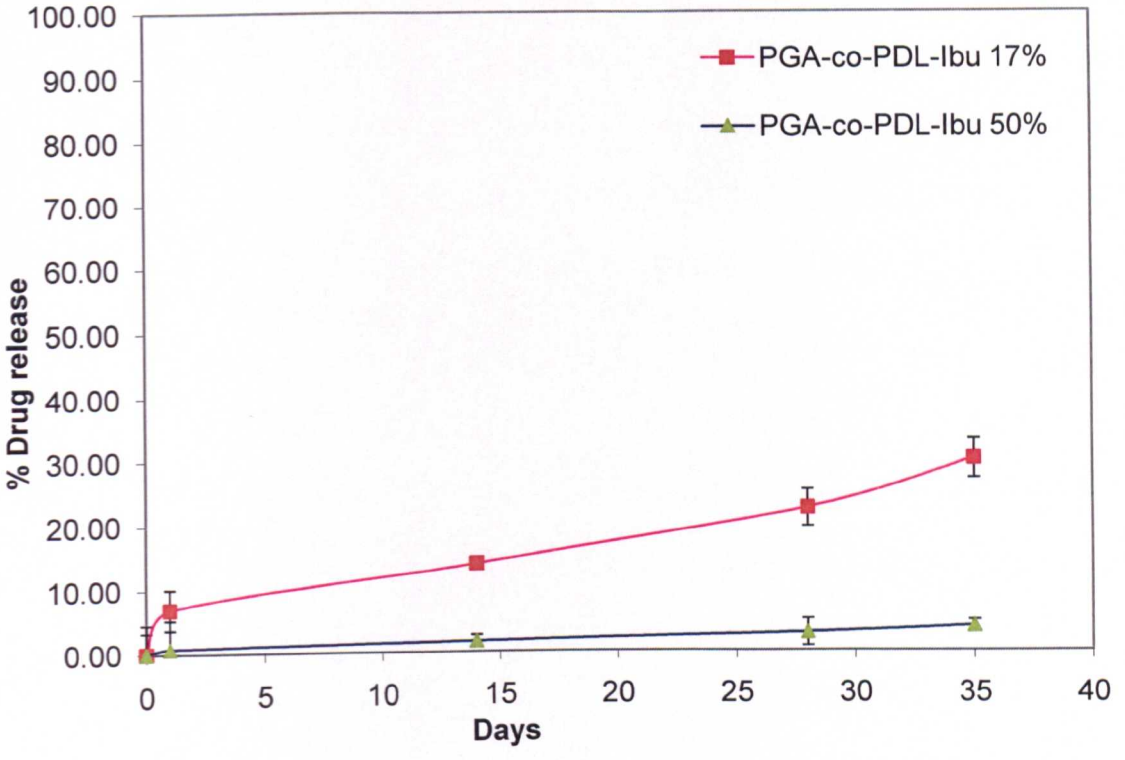


Figure 3.20: *Comparison of ibuprofen release from conjugated polymeric formulations in 36 days*

In summary, the chemistry of polymers has proved to be an important factor to control the drug release from microparticulate formulations. The properties of the synthesised functionalised polymers can be varied by post-synthetic modifications to alter the drug release.

3.4 CONCLUSION

These investigations indicate that several of the polymeric materials assessed demonstrated useful encapsulation efficiencies, comparable to the FDA-approved PLGA, but also showed a better control over the release of the drugs. Hence, synthesised polyesters such as PGA-co-PDL and PTEGA-co-PDL have potential as improved colloidal drug carriers for drugs. Additionally, the control over the various particle characteristics could enable tailored preparation and delivery of a variety of different therapeutic compounds. The similarity between particles formed from PGA-co-PDL and PBA-co-PDL contradicted our initial assumption that the absence of free hydroxyl groups in PBA-co-PDL would favour the encapsulation of lipophilic drugs like ibuprofen. The study indicates that rather than slightly altering the polymer chemistry, particle properties and drug encapsulation can be altered by modification of experimental parameters. Use of class III solvents is possible instead of class II solvents while not compromising particles sizes and encapsulation efficiencies. Mixing speed, polymers' molecular weights and concentrations can be varied to tailor the particles of desired size, properties and drug loads. Additionally these polymers have successfully encapsulated different drugs having various water-solubilities. However, the very water-soluble drug rifampicin failed to encapsulate in these polymers via the current o/w or w/o/w emulsion techniques. The drug release profiles indicated the slow release from these polymers, and it can be concluded that by varying the monomeric compositions of the polymers, the drug release profiles and the extent of the drug released can be markedly controlled. The strategy to slow down the release of the drugs by conjugating the drug onto polymeric backbone was satisfactory and showed that the release of the drug can be slowed down. There is need for further exploration of the water soluble drug rifampicin conjugation to polymer, as the drug failed to encapsulate in any of the synthesised polymers. These studies are helpful for the prediction of the type of

polymer required and influence of drug properties in the aim to deliver the required amount of drug at desired release rate.

Stability and Degradation Studies

4.0 BACKGROUND

The stability of pharmaceutical formulations primarily depends on the physico-chemical properties of the raw materials, including the excipients, container system, environmental and storage factors and their effect on the product. The stability of biodegradable polymers is a prime issue to be considered after synthesising new materials for potential use in drug delivery applications. The degradation rate of polymers is affected by different parameters such as polymer nature, polymer composition, molecular weight, hydrophilicity and physical state (viscous liquid, crystalline or amorphous) (Dorati et al 2007). Ideally a polymer should exhibit good storage stability, yet for successful utilisation in drug delivery applications, these polymers also need to demonstrate complete degradation *in vivo*. Extensive studies have been carried out to investigate the degradation of polymers upon exposure to simulated physiological environments (buffers and simulated fluids) (Wang et al 1990; Anderson & Shive 1997; Eppley & Reilly 1997; Schliecker et al 2003; Meng et al 2006; Lei et al 2007; Zhao et al 2007; Chandure et al 2008). For polyesters the preferred route of degradation, under such conditions, is mainly the hydrolysis of labile ester linkages, however this depends on monomeric composition, blending of polymeric materials and even the configuration of the monomeric units within a polymer chain (Zamora et al 2006). Keeping in view the effect of polymer chemistry on degradation, many research groups have altered the biodegradability of polymers via insertion of chemical moieties near the ester linkage to vary the hydrolytic susceptibility of the material (Albertsson & Eklund 1995). Zamora (2006) investigated the dependence of degradation on the monomeric configuration in polyester chains. The effect of chemistry on polymer degradation was further explored by a few research groups who discovered that the degradation profiles of polymers could be modified by synthesising copolymers, instead of homopolymers (Dorati et al 2007; Lei et al 2007; Zhao et al 2007; Chandure et al

2008; Moeller 2008). Homopolymers degrade at a constant rate, but with copolymers some fragments will degrade more slowly than the others, leading to an increase in the overall shelf lives of these materials (Huang et al 2004). Hence, the stability of polymers can be successfully modified by varying the monomer ratios in a copolymer (Lenglet et al 2009). These properties widen the range of applications for these materials in everyday life by enabling tailoring for specific functions, along with improved use in drug delivery. Modified polymeric materials have been used to develop numerous biodegradable and biocompatible biomaterials (He et al 2003; Dorati et al 2007; Zhao et al 2007; Gong et al 2009). Gaskell et al (2006) and Thompson et al (2007) have evaluated the use of one such polymer, PGA-co-PDL for pulmonary drug delivery applications.

Although various procedures are in place for studying polymer degradation; enzymatic, thermal, soil degradation etc., for biomaterials intended for use as drug carriers, studies of their degradation under physiological conditions is of greater importance. Previous studies demonstrated that the degradability of polymeric materials influences the release of drugs from polymeric microparticulate systems (Wang et al 1990). Different polymers degrade to different extents, hence affecting the release of the associated drugs. Degradation of polymers proceeds via two possible pathways, that is, either by surface erosion or by random chain scission. Depending upon polymer chemistry, either of these two pathways can cause polymer degradation. For instance, during hydrolytic degradation, random chain scission is the preferred route of degradation. Highly hydrophilic polymers will absorb more water and hydrolyse more quickly than more lipophilic polymers, where water penetration into the matrix is minimal, causing the least random chain scission (Göpferich 1996).

The effects of extreme storage conditions have been studied on different polymers for drug delivery and it was observed that, when employed at their extremes, such conditions promote their degradation. Hence, the regulatory requirements for the approval of biomaterials for colloidal drug delivery applications are: complete stability profiles; extensive characterisation; estimation of shelf life; determination of degradation products and the identification of any toxic by-products (Committee for Proprietary Medicinal Products 1998; Matthews 1999; Burgess et al 2004).

A desirable polymer-based drug delivery system will have good storage stability but will degrade easily under physiological conditions, leading to effective release of the therapeutics. Hence, a good understanding of the stability and degradation of any new material is required prior to the effective applications of such polymers in drug delivery technologies.

4.1 AIM OF THE STUDY

The aim of this part of the project was to study the effects of various storage factors, including light, temperature and humidity on the stability of the polymeric materials, as outlined by ICH guidelines. In the present work differences in the physical and chemical properties of four novel polymers on stability were investigated under various storage conditions. PGA and PGA-co-PDL possesses hydrophilic characteristics because of the pendant functional hydroxyl groups on the polymer backbone. Both polymers were compared to their more hydrophobic equivalents, PBA and PBA-co-PDL, respectively.

These polymers are required to be stable with shelf lives of approximately one year but with good biodegradability under physiological conditions. Hence, *in vitro* degradation studies of these polymers were also carried out under simulated physiological conditions. Since one potential application of these materials is microparticulate pulmonary drug delivery, simulated lung fluid (SLF) and PBS buffer were used at body temperature for the *in vitro* degradation studies. These studies were designed to provide an understanding of the effect of polymer chemistry on the *in vitro* degradation of these materials. The data obtained will be useful for the estimation of the storage shelf life and half life of these materials in the human body.

4.2 EXPERIMENTAL

4.2.1 Materials

Poly(glycerol adipate) (PGA) (P1a: 6609 Da and P1b: 8904 Da), poly(glycerol adipate-co-pentadecalactone) (PGA-co-PDL) (P2: 14431 Da), poly(1,4-butanediol adipate) (PBA) (P3: 7369 Da), poly(1,4-butanediol adipate-co-pentadecalactone) (PBA-co-PDL) (P4: 15960 Da), poly(dithiotheritol adipate-co-pentadecalactone) (PDTAA-co-PDL, 6500Da) were previously synthesised and characterised (section 2.2) and PGA-co-PDL microparticles (formulated in chapter 3). Deuterated chloroform ($CDCl_3$) was purchased from CIL, USA. Potassium acetate, magnesium chloride hexahydrate, sodium chloride, potassium chloride, sodium phosphate dibasic anhydrous, sodium sulphate anhydrous, calcium chloride dihydrate, sodium acetate trihydrate, sodium bicarbonate, sodium citrate dihydrate and tetrahydrofuran were purchased from Fischer Scientific. 3Å molecular sieves and indicator blue silica gel were obtained from Sigma-Aldrich and phosphate buffered saline (PBS) pH 7.4 tablets were purchased from Oxoid.

4.2.2 Methods

4.2.2.1 Storage stability under simulated environmental conditions

Approximately 2-3g of each polymer were placed in 5 ml screw-top vials that were capped and sealed with parafilm. These vials were then stored in 500 ml closed containers at either 4°C±2°C (in refrigerator), 25°C±2°C (in dark cabinets at room temperature) or 40°C±2°C (in oven). At each temperature the humidity in the containers was controlled at either 0%±5%, 25%±5% or 75%±5% relative humidity (RH) using saturated salt solutions (Brien 1948; Greenspan 1977). Temperature and humidity values were monitored using a Therma-Hydrometer (E.T.I, model 8703). A representative picture of a typical setup is shown in figure 4.1. A brief summary of the conditions applied to generate a controlled temperature and humidity environment is shown in

table 4.1. During the preliminary studies the effect of light and the effect of direct exposure to atmospheric humidity were studied by using clear/transparent and amber coloured vials and using open or closed vials within the containers.

Figure 4.1: *A typical setup used to study polymer stability under simulated environmental conditions.*

All polymer samples were analysed immediately after synthesis, prior to storage under simulated environmental conditions. Once equilibrated at the indicated conditions, small quantities (10-15 mg) were removed for analysis at 20 day intervals for 3 months then at 2 monthly intervals until 6 months. All samples were analysed for physical and chemical changes. Structural changes in the stability of samples of the polymers were studied using FTIR, while changes in molecular weight were recorded via GPC. Detailed analytical methods for these techniques are described under section 2.3.

Table 4.1

Storage conditions used to maintain temperature and percent relative humidity

%RH	Temperature	Procedure
0%± 5%	4°C±2°C	Pre-activated indicator silica gel mixed with 3A° molecular sieves.
0%± 5%	25°C±2°C	Pre-activated indicator silica gel mixed with 3A° molecular sieves.
0%± 5%	40°C±2°C	Pre-activated indicator silica gel mixed with 3A° molecular sieves.
25%± 5%	4°C±2°C	Sealed container containing saturated solution of potassium acetate (CH_3COOK) which produces humidity of 23.28%±0.34% at 4°C.
25%± 5%	25°C±2°C	Sealed container containing saturated solution of potassium acetate (CH_3COOK) which produces humidity of 22.51%±0.32% at 25°C.
25%± 5%	40°C±2°C	Sealed container containing saturated solution of potassium acetate (CH_3COOK) which produces humidity of 21.50%±0.30% at 25°C.
75%± 5%	0°C±2°C	Sealed container containing saturated solution of sodium chloride (NaCl), which produces humidity of 75.51%±0.34% at 4°C.
75%± 5%	25°C±2°C	Sealed container containing saturated solution of sodium chloride (NaCl), which produces humidity of 75.29%±0.12% at 25°C.
75%± 5%	40°C±2°C	Sealed container containing saturated solution of sodium chloride (NaCl), which produces humidity of 74.68%±0.13% at 40°C.

4.2.2.2 *In vitro* degradation in buffer

One tablet of PBS buffer (containing 150mM anhydrous sodium phosphate (dibasic) and 0.9 (w/v%) sodium chloride) was dissolved in 100 ml distilled water at room temperature in a 150 ml beaker using a magnetic stirrer until the contents were completely dissolved. The recorded pH of the resulting solution was 7.2. Samples of 15-20 mg of PGA-co-PDL (powder), PGA-co-PDL (microparticles) and PDTTA-co-PDL (powder) were dispersed separately in 5 ml of PBS buffer in six screw-top vials. The vials were gently continuously agitated with an orbital shaker (IKA, KS-130 basic) at 100 RPM in an environmental incubator (Stuart, SI60) at 37°C for six weeks. One of the vials was removed weekly and the polymer sample recovered by filtration. The recovered polymeric mass was dried between 2 layers of filter paper. Dried samples were dissolved in 2 ml of THF and analysed by GPC (section 2.2.4).

4.2.2.3 *In vitro* degradation in simulated lung fluid

Simulated lung fluid (SLF) was prepared using the method published by Gamble (1967), by dissolving specific amounts of various inorganic salts in one litre of distilled water, as summarised in table 4.2.

Table 4.2

Contents of the simulated lung interstitial fluid: listed in the order of addition.

Order	Salt	Quantity (g/L)
1	Magnesium chloride hexahydrate	0.2033
2	Sodium chloride	6.0193
3	Potassium chloride	0.2982
4	Sodium phosphate dibasic anhydrous	0.1420
5	Sodium sulphate anhydrous	0.0710
6	Calcium chloride dihydrate	0.3676
7	Sodium acetate trihydrate	0.9526
8	Sodium bicarbonate	2.6043
9	Sodium citrate dihydrate	0.0970

All salts were dissolved in the listed order to prevent precipitation, as it is known that precipitation may occur by the addition of components 6-9. The starting volume of the distilled water was 95% (950 ml) of the desired final volume (1 litre). The final pH of the simulated lung interstitial fluid solution was measured at 7.4.

A sample of 15-20 mg of PGA-co-PDL (powder), PGA-co-PDL (microparticles) and PDTTA-co-PDL (powder) were dispersed separately in 5 ml of simulated lung interstitial fluid in six screw-top vials. Vials were agitated gently with the shaker at 100 RPM, stored in an environmental incubator at 37°C for six weeks. Sampling and analysis was carried out as described under section 4.2.2.2.

4.3 RESULTS AND DISCUSSION

4.3.1 Storage stability

It is well known that polyesters degrade in a wide variety of environmental conditions, hence reducing their shelf life (Fujimaki 1998; Khan et al 1999). These polymers also degrade readily upon treatment with extreme conditions such as very high temperatures (thermal degradation) (Yusuf Nur 2007), exposure to enzymes (enzymatic degradation) (Zhao et al 2007), under UV and natural light (photodegradation) (Feller et al 2007) and upon exposure to moisture (hydrolysis) (Dorati et al 2007). These factors all contribute to the degradation of polymers, which can be indicated by a decrease in molecular weight, change in crystallinity, pH of the solution, formation of additional functional groups and changes in terminal group chemistry. Various techniques can be used to determine changes in polymers upon degradation, such as GPC, IR, NMR, DSC and viscometry. Factors responsible for polymer degradation fall into two major categories: chemical factors (hydrophilicity, molecular weight, physical state and polarity of the molecule) and storage factors (temperature, humidity and light).

4.3.1.1 Effect of polymer chemistry

The effect of polymer chemistry on the stability of polymers synthesized during this project was investigated by varying the monomer composition, molecular weight, free – OH groups and carbon chain lengths. Details of the polymers studied and their properties are summarised in table 4.3.

Table 4.3

Physical and chemical properties of polymers used in the stability studies

Polymer	Mol wt (Da)	State (at room temperature)	free –OH	Carbon chain length
PGA	8904	Viscous liquid	Yes	9
PGA-co-PDL	14431	Waxy solid	Yes	21
PBA	7369	Soft solid	No	9
PBA-co-PDL	15960	Waxy solid	No	21

4.3.1.1.1 Effect of pendant –OH groups

The polymers tested were synthesised using either glycerol (one free –OH group per repeat unit) or 1,4-butanediol (no free –OH group), thus altering the hydrophilic nature of the polymers. PBA and PBA-co-PDL polymers were waxy solids at room temperature that lacked pendant free –OH groups. They are less hydrophilic than the corresponding PGA and PGA-co-PDL and have a lower affinity towards moisture, which should make them less affected by humidity and temperature. On the other hand, PGA is a viscous polymer while PGA-co-PDL is a waxy solid. They both have pendant free –OH groups on the backbone which makes these polymers more hydrophilic, hence more prone to degradation by increased temperature and humidity. The change in molecular weight of these polymers after storage for six months under moderate conditions (25°C and 25%RH) is shown in figure 4.2.

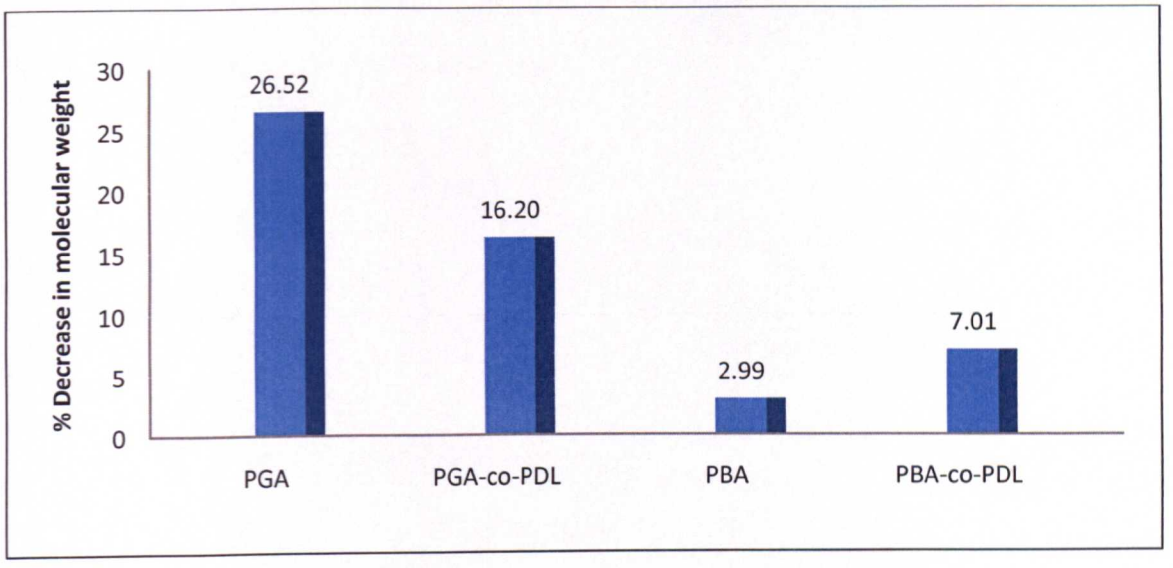


Figure 4.2: *Degradation of polymers after storage at 25% RH in 25°C for six months*

PGA and PBA differ only in the presence of free hydroxyl groups throughout the polymer backbone, and as expected the results (Figure 4.2) indicate the greater stability of PBA compared to PGA. These results suggest the innate involvement of the pendant hydroxyl groups in the degradation of the polymers. The difference in stability of the two polymers however, may also partly be due to their physical state. PBA, a solid polymer, is more stable than the viscous PGA. Similarly comparing PGA-co-PDL and PBA-co-PDL degradation it is noted that, under the same environmental conditions, the pendant hydroxyl group-containing polymer (PGA-co-PDL) degraded more compared to the PBA-co-PDL. In this case both polymers were waxy solids at room temperature.

The difference in degradation between PGA and PBA (23.53%) is much greater than the difference between the co-lactone polymers PGA-co-PDL and PBA-co-PDL (9.19%). Only after placing the PBA-co-PDL in the most extreme conditions tested (75% RH and 40°C) was a slight increase in the intensity of the hydroxyl band (at 3500cm^{-1}) from the FTIR spectra observed (Figure 4.3). This observation is in accordance with the above

results, indicating that this polymer is quite stable at ambient environmental conditions and resists degradation even under extreme conditions.

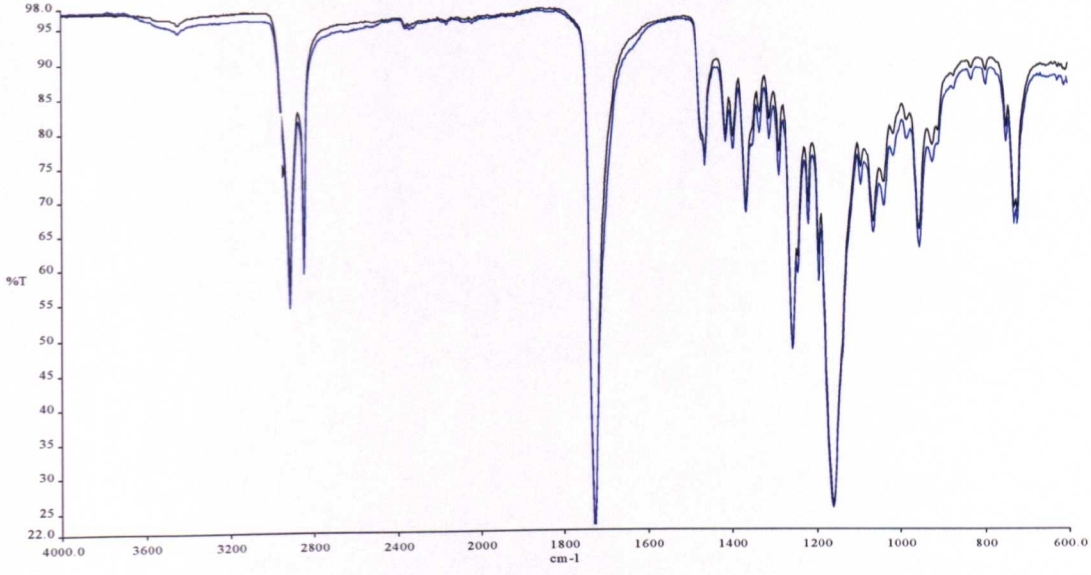


Figure 4.3: *FTIR spectra of PBA-co-PDL before (black line) and after (blue line) storage at 40°C and 75%RH for six months*

Contrary to this, the FTIR spectrum for PGA-co-PDL shows a considerable increase in hydroxyl band intensity signifying the greater extent of polymer degradation (Figure 4.4). These hydroxyl groups come from the end groups of the by-products (corresponding alcohol and acid) following hydrolysis.

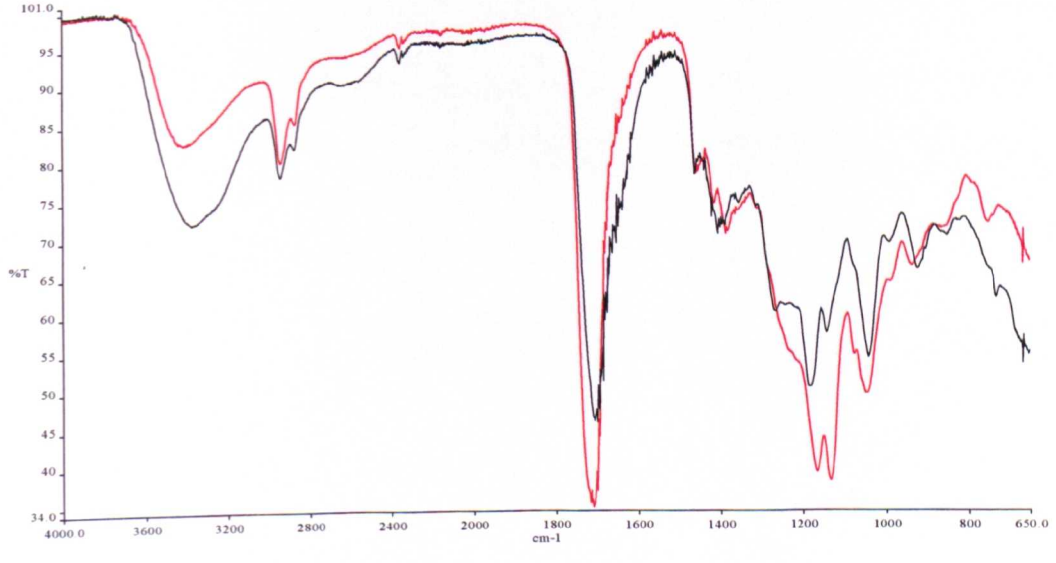


Figure 4.4: *FTIR spectra of PGA-co-PDL before (red line) and after (black line) storage at 75% RH at 40°C for six months*

This phenomenon can be explained on the basis of the number of free –OH groups per 100-carbon-chain length of the polymer. Given the random distribution of monomeric units in the polymer, per 100-carbon-chain of PGA there would be 11 free –OH groups in its back bone, whereas PGA-co-PDL would have only 5 free –OH groups. The presence of less –OH groups on the backbone of the polymers made these polymers less hydrophilic and more stable than the polymers with a higher number of free –OH groups. This hypothesis can be backed up further by the results obtained from comparing PBA and PBA-co-PDL where less degradation was observed in the absence of –OH groups on the backbone.

This illustrates that the presence of hydrophilic functional moieties on polymer backbones make polymers more hydrophilic and hence more prone to degradation even at ambient storage conditions. Their degradation is accelerated under higher temperature and humidity storage conditions compared with those polymers which lack this functionality.

4.3.1.1.2 Effect of carbon chain length and molecular weight

PGA-co-PDL and PBA-co-PDL have an extra 15 carbon chain of PDL in the repeating unit within their structure, compared to PGA and PBA. This results in a higher molecular weight of a repeat unit and a more non-polar polymer backbone. While comparing degradation of PGA with PGA-co-PDL, it was observed that PGA-co-PDL was relatively more stable than PGA over all temperature and humidity ranges. The results obtained by both groups of polymers PGA/PGA-co-PDL and PBA/PBA-co-PDL suggesting that higher molecular weight polymers are more stable than lower molecular weight polymers. This is supported by the work of Wang et al (1990). However, with the current polymers the influence of other chemical properties, such as pendant –OH groups and presence of non-polar chains cannot be excluded. Therefore, to investigate

this, the effect of molecular weight on the stability of polymers with the same chemistry was studied. Two samples of PGA (P1a, 8904 Da) and (P1b, 6609 Da) were tested. It was observed that under the same conditions the polymer with the lower molecular weight degraded 80% in 3 months while the higher molecular weight PGA degraded only 60% compared to its starting molecular weight, so a small change in molecular weight can have a big effect on stability and degradation rates. Wang et al (1990) reported the faster degradation of lower molecular weight polymers compared to higher molecular weight materials, along with a decrease in their viscosity. In addition to molecular weight, increased polydispersity also results in faster degradation.

Based on these observations, higher molecular weight polymers with no hydrophilic functional moieties on the polymer backbone have longer shelf lives and may be more suitable for drug delivery formulations in terms of enhanced stability. These results have direct implications in the design of controlled release formulations, as it is previously reported that polymer chain length and polydispersity have an impact on drug release profiles (Ramkissoon-Ganorkar et al 1999); the details of these factors have already been discussed in chapter 3 (Drug encapsulation and release).

4.3.1.2 Effect of storage factors

Storage factors, such as light, temperature and humidity play a major role in polymer degradation. This is why many pharmaceutical formulations are stored in specialised packaging for an improved shelf life of the products. For example, some tablet formulations come in special aluminium strips or with an adsorbent silica bag in the container. Some dosage formulations such as syrups or suspensions come in amber coloured vials to prevent the contents from the deteriorating effect of light. According to the British Pharmacopoeia (BP 2010) standard, 90% of the radiation, at any wavelength between 290-450nm (UV radiation), should be absorbed by the amber glass containers.

Keeping this in mind, the effect of these environmental factors were studied on a selection of polymers synthesised (in chapter 2), in accordance with the published ICH guidelines (Matthews 1999) .

4.3.1.2.1 Effect of light

PGA was selected for preliminary studies on the effect of light on polymer stability. PGA (molecular weight 8904Da) was stored in two different types of sealed containers; clear vials and amber-coloured vials. Initial results demonstrated that the polymer degraded more quickly upon exposure to light. Amber-coloured vials restricted the penetration of light to polymers, and these samples remained more stable. Figure 4.5 summarises the effect of container type on the molecular weight of PGA at 40°C under ambient humidity. After six months PGA stored in the amber vial had a higher molecular weight than the sample stored in the clear vial. This may be indicative of photodegradation of these polymers. Photodegradation of polymers can be reduced by blending polymers with other materials or by using fillers to make composites (Li et al 2008), or alternatively photolysis of such polymers can easily be prevented by storing polymers in amber- or dark-coloured containers (Feller et al 2007).

Light irradiation of polymers modifies their physical and chemical properties, for example inducing colour change and degradation. PGA and related polyesters have weak ester bonds which are labile to specific environmental conditions. Hence these materials are expected to break down via the hydrolysis of these ester bonds. Increased temperature (thermolysis) and exposure to light (photolysis) favours the hydrolysis of ester bonds, resulting in an increased degradation rate. The degraded material was analysed by GPC and FTIR to give an indication of the chemistry of the degraded polymer samples.

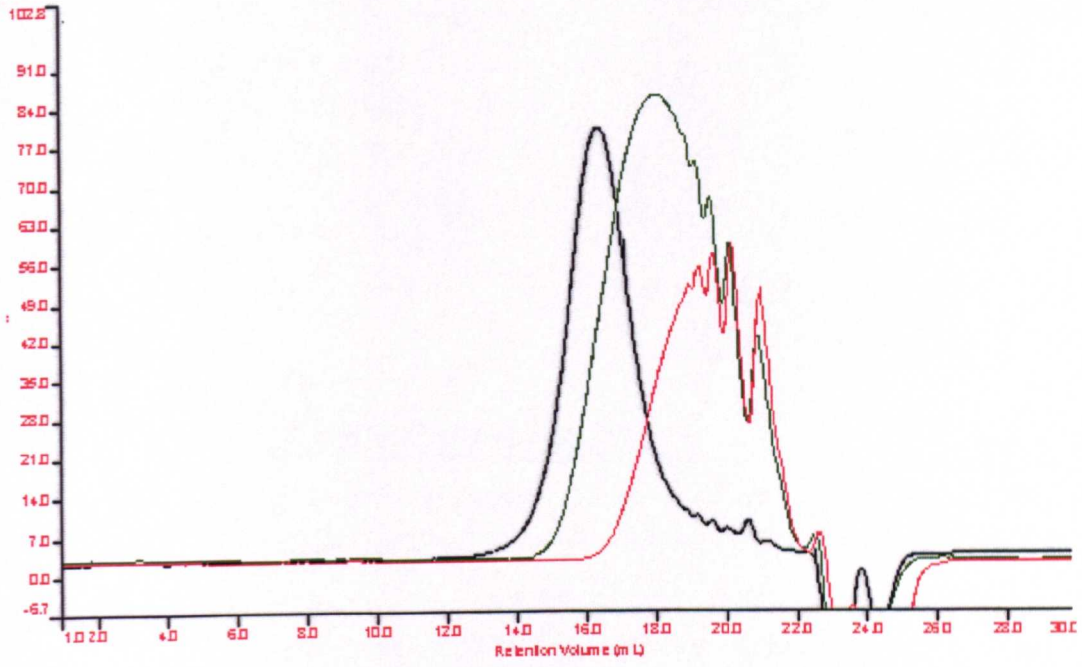


Figure 4.5: *GPC chromatogram of PGA prior to storage (black line) and after 6 months at 40°C under ambient humidity in a closed amber vial (green line) and clear vial (red line)*

To understand the qualitative nature of the degradative products, GPC traces of the original PGA and the degraded polymer samples were overlaid along with the GPC traces of the monomeric units, glycerol and adipic acid. Figure 4.6 very clearly shows that the degraded polymer contains fragments of molecular weight equivalent to glycerol, adipic acid and short oligomers of these. This indicates that, upon degradation, PGA converts to its non-toxic, biodegradable monomeric units, which can easily be excreted through the kidneys or enter one of the biochemical metabolic pathways (Stefani et al 2006).

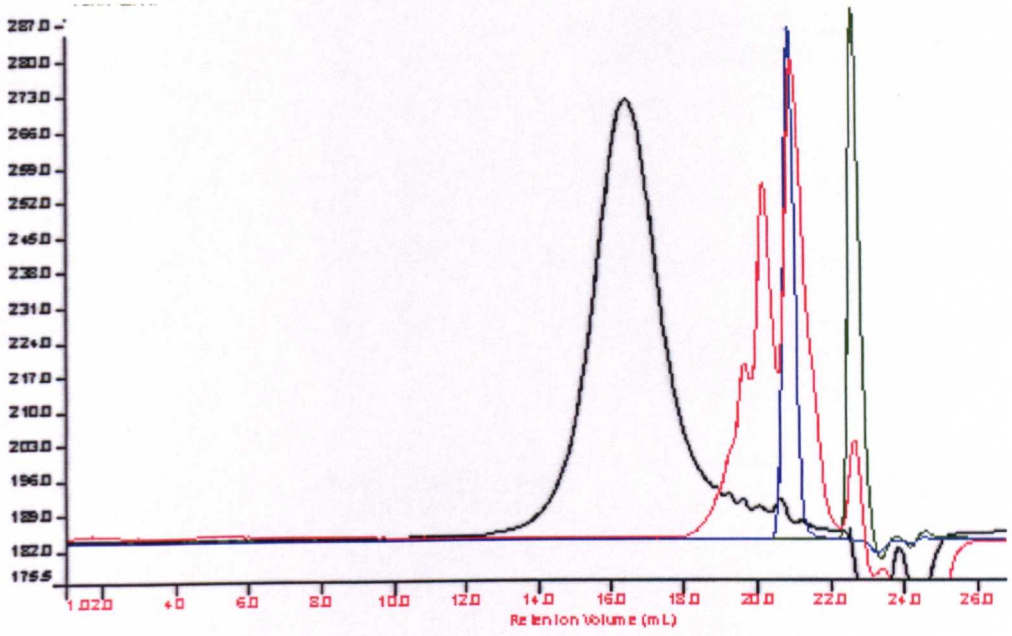


Figure 4.6: *GPC chromatogram of PGA before degradation (black) and the after degradation at 40℃ and 75%RH stored in clear open vial (red) is overlapped with DVA (blue) and Glycerol (green)*

This data indicates that there are two major steps in the degradation of the polymer

1) *Random polymer chain scission;* which results in low molecular weight polymer fragments and can easily be indicated by shifts in the retention volume of the polymer in the GPC traces (broadening of the GPC peaks).
2) *Conversion to monomeric units;* polymer chain scission ends with the reversion of the polymer back to its monomeric units (development of discrete peaks at the tails of the main peak).

4.3.1.2.2 Effect of temperature

Infrared spectroscopy has previously been adopted for hydrolytic degradation studies of aliphatic polyesters in an alkaline environment (Partini & Pantani 2007). Rosu et al (2009) also used FTIR to study the effect of UV light on the photodegradation of polymers by monitoring the degradation products. FTIR spectra of the polymer samples, stored at different temperatures for 6 months, correlated with the GPC results.

Figure 4.7 shows an increase in intensity of the hydroxyl band for PGA samples placed at 40°C for 6 months, while samples stored at 4°C and room temperature did not show any increase in hydroxyl groups. It clearly demonstrates that chain scission by hydrolysis of ester bonds results in the generation of more –OH groups, thus leading to an increased intensity of the hydroxyl band in the FTIR spectrum.

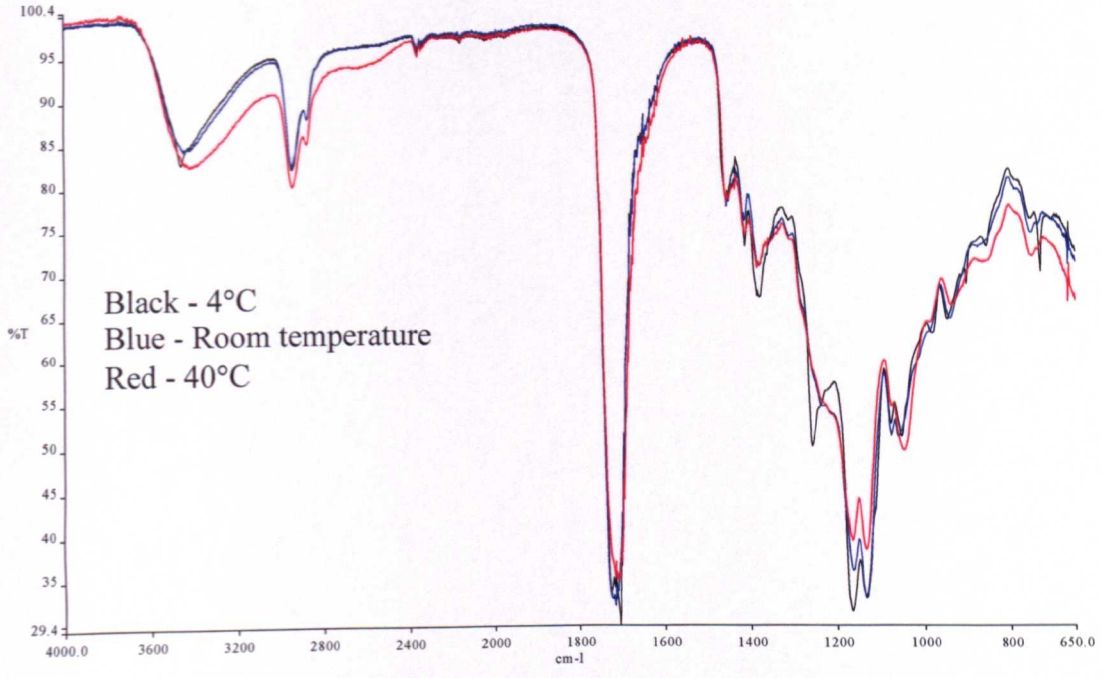


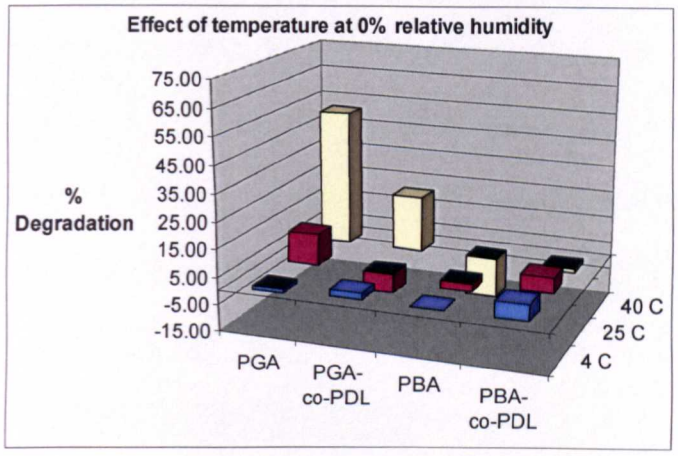
Figure 4.7: FTIR *spectra showing polymer degradation at different temperatures*

Polymers stored for 6 months at all temperatures degraded and a decrease in molecular weight was observed, as exhibited in figure 4.8. It is clear that all polymers remain most stable at 4°C, as the change in the molecular weight of the polymers was less than 5% but increasing the storage temperature to 25°C (room temperature) resulted in a decrease in molecular weight of up to 15%. This decrease in molecular weight was even more considerable when polymers were stored at 40°C and a maximum degradation of 51% was observed with PGA. PGA-co-PDL remained more stable than PGA in terms of percentage decrease in molecular weight due to its higher molecular weight, carbon chain length and solid state. At 0% RH, the molecular weight of PGA-co-PDL decreased by 25% at 40°C while at 4°C and 25°C it remained stable. A 10% variation in

molecular weight of PBA was observed at 4°C and 25°C and surprisingly a 7% increase in molecular weight of PBA was observed at 40°C in the first 3 months and that increase reached 15% over 6 months. The reason for this increase might be because in its original form PBA is a white powdery solid and storage at 40°C resulted in the polymer melting and hence its physical form changed, from a white powder to a wax-like solid. This might have lead to some intermolecular rearrangements leading to an increase in molecular weight. However, no previous literature was found to support the increase in molecular weight observed with this polymer. Further studies are required to understand this phenomenon and to obtain a more detailed explanation of the observed increase in molecular weight.

No major physical changes were observed in the polymers stored at 4°C and 25°C, but at 40°C the powdery appearance of the solid polymers was transformed to a waxy layer, as a result of slow melting, with a colour change from white to pale yellow, which is an indication of polymer degradation (Rosu et al 2009). Hence, if these materials were used for particle preparation and drug encapsulation, the microparticles would not be stable at 40°C due to the physical changes and melting which would result in the deterioration of particles after 60 days. Increasing the temperature (Figure 4.8) resulted in a proportional decrease of the molecular weight, indicating that thermolysis of ester bonds, causing random chain scissions, had occurred and hence an increased poly dispersity index (PDI) of all the polymers tested. PDI is an indicator of polymer degradation, however it is also a cause of accelerated polymer degradation (Partini & Pantani 2007). The more rapid degradation of polymers at 40°C assures the polymer degradation in human bodies at physiological temperature (37°C) and very high humidity.

a)



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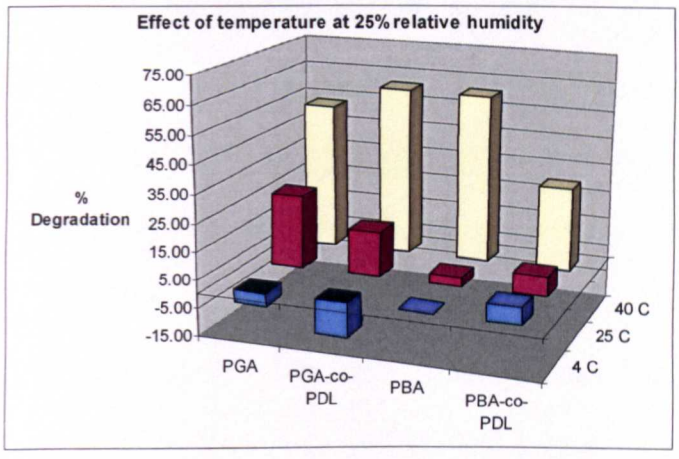

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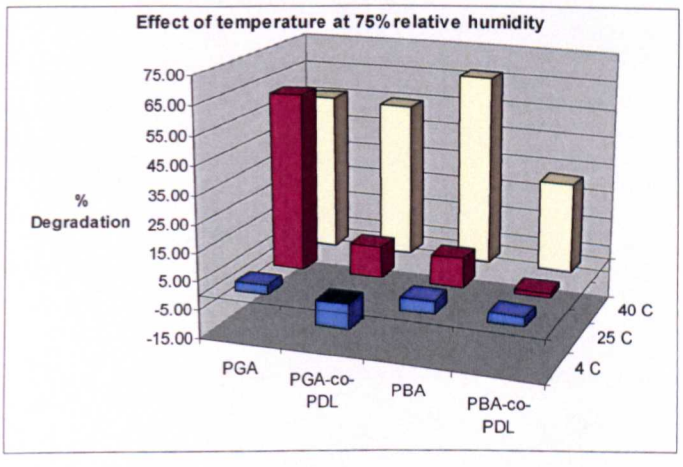

Figure 4.8: *Polymer degradation at various temperatures, when stored in various humidity environments: a) 0%RH, b)25%RH and c) 75%RH*

As discussed in section 4.3.1.1, PBA-co-PDL, was more stable than PGA-co-PDL with a molecular weight decrease of only 5-17% over 6 months across the entire temperature range studied (Figure 4.10). This is because of the lack of functional groups, low hydrophilic character and higher molecular weight. Polymers stored near or above their T_g degrade much faster (Zamora et al 2006) via chain breaking (Feller et al 2007). This is because at or near its T_g, polymer molecules have enough mobility to allow structural reorganisation which cause increased polymer degradation (Royal & Torkelson 1992). This further explains the higher degradation of PGA (the viscous polymer) as compared to the corresponding co-lactone (PGA-co-PDL). Figure 4.9 indicates the degradation of PGA by showing the increased presence of lower molecular weight fragments with increasing storage temperatures. It is evident from the increasing PDI of the GPC traces with increased storage temperature that PGA degraded to lower molecular fragments, while an overall shift of the peak towards a lower molecular weight indicates that the polymer's integrity decreased by chain scission under these environmental conditions over the monitored duration of time.

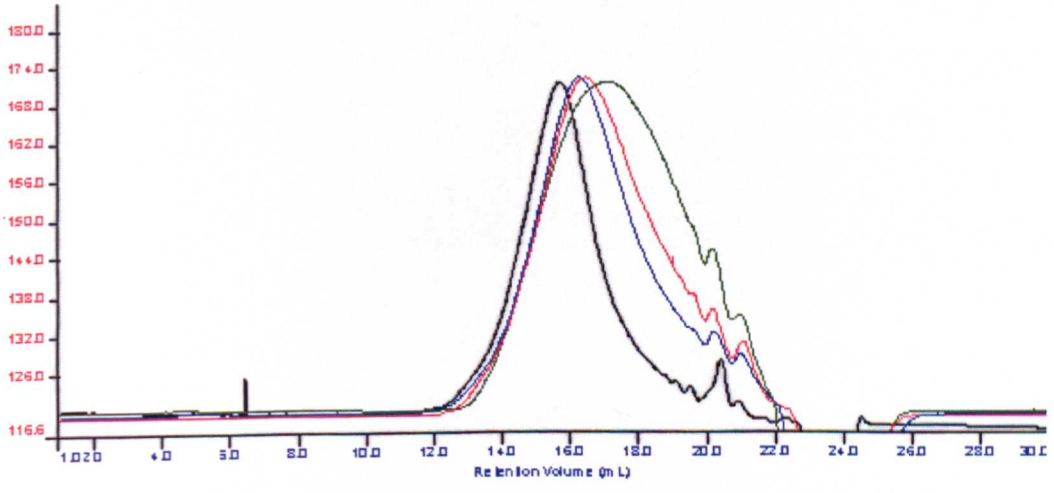

Figure 4.9: *GPC chromatogram of PGA before (black line) and after storage for six months at 25%RH and varying temperatures: 4 °C (blue line), 25 °C (red line) and 40 °C (green line).*

The deteriorating effect of temperature on polymer degradation when coupled with high humidity increased several fold. Although similar degradation trends were observed at different temperatures when stored in higher humidity environments, the degradation of polymers was more rapid and deteriorative. These observations are discussed in the next section.

4.3.1.2.3 Effect of humidity

The effect of humidity on the polymers tested is summarised in Figure 4.10. The effect of increasing humidity on degradation was obvious for all polymers. For instance at 0°C and 0%RH, PGA and PBA degraded by only 3-5%, PBA-co-PDL by 7-8% and a 10-15% variation in molecular weight was observed in PGA-co-PDL. The storage of polymers at higher humidity increased the polymer degradation several fold, as shown in figure 4.10.

All polymers (except PBA-co-PDL) degraded under increased humidity and their molecular weights decreased by 50-65%. PBA-co-PDL again exhibited a more stable nature, degrading by only 25%. These trends indicated that higher temperatures keep polymers in a liquid state, hence intermolecular distances remain large enough to allow penetration of moisture. This then facilitates the cleavage of the polymer chains by hydrolysis of labile ester bonds along with thermolysis. The data obtained at 4°C (Figure 4.10) further supports the assumption that at lower temperatures there is not enough intermolecular space for moisture to penetrate the polymer, hence a smaller surface area of polymer is exposed to moisture, leading to the hydrolysis of a smaller number of ester bonds and resulting in less polymer degradation. Hydrolysis of polymers usually proceeds with random chain scissions and depends on monomer structure, polymer molecular weight and copolymer ratio (Wang et al 1990). It is assumed that hydrolysis of these ester bonds proceeds linearly with time and thus

suggests that the controlling mechanism is a chemical reaction rather than water diffusion, as described by Partini & Pantani (2007).

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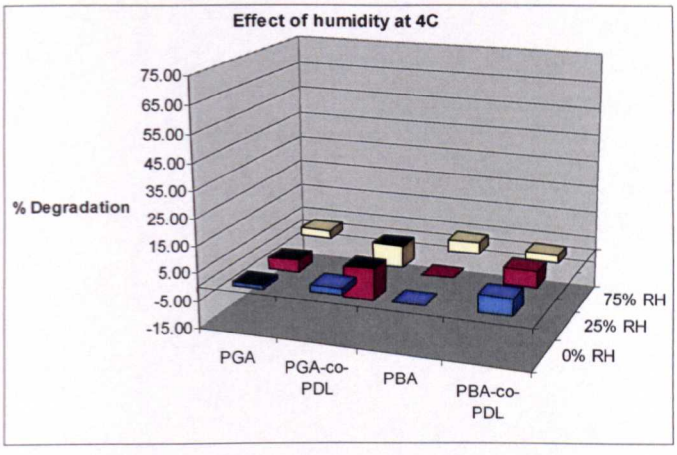

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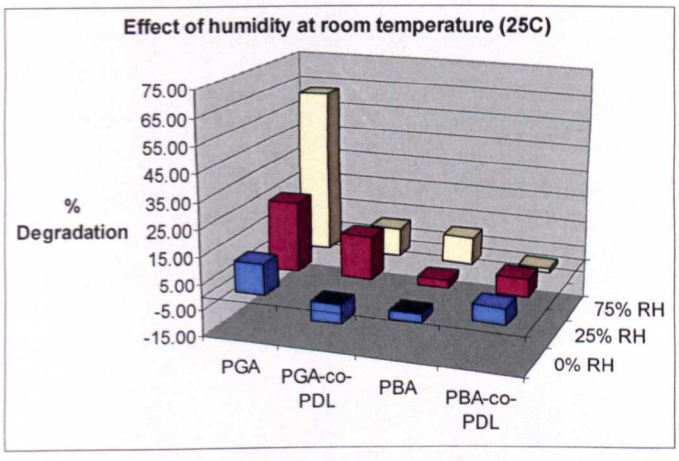

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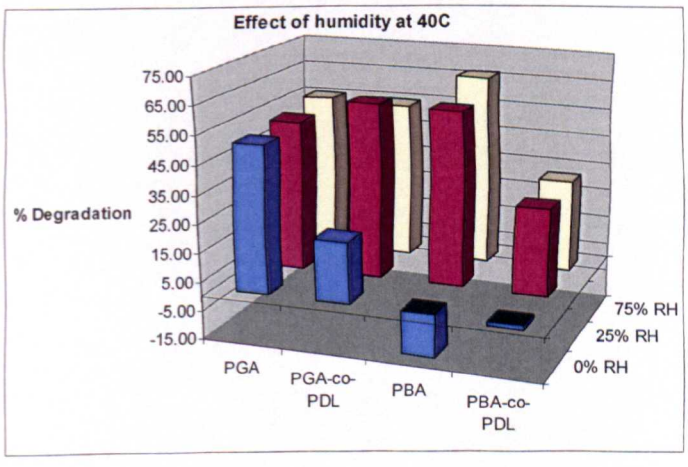


Figure 4.10: *Polymer degradation at different humidity, when stored at various temperatures; a) at 4°C, b) at 25°C and c) at 40°C*

Table 4.10 further illustrates that humidity plays a facilitating role in the deterioration of the polymer chains into smaller molecular weight fragments via hydrolysis. All vials were sealed and capped and stored in their final packaging, hence there was a reduced effect of environmental humidity. These results raised a question that if a slight increase in humidity can degrade polymers to such a level, what would be the extent and timescale for the degradation, if the polymers were directly exposed to the environmental conditions. A small experiment was conducted to study the effect of direct exposure to humidity.

Effect of direct exposure to humidity (at 40°C)

The presence of labile ester bonds makes polyesters very susceptible to humidity-induced degradation (hydrolysis) along with thermolysis of the polymer backbone at higher temperatures. Exposure of PGA to moisture and high temperature facilitated degradation of the polymer into smaller molecular weight fragments and to its monomeric units as shown in figures 4.11.

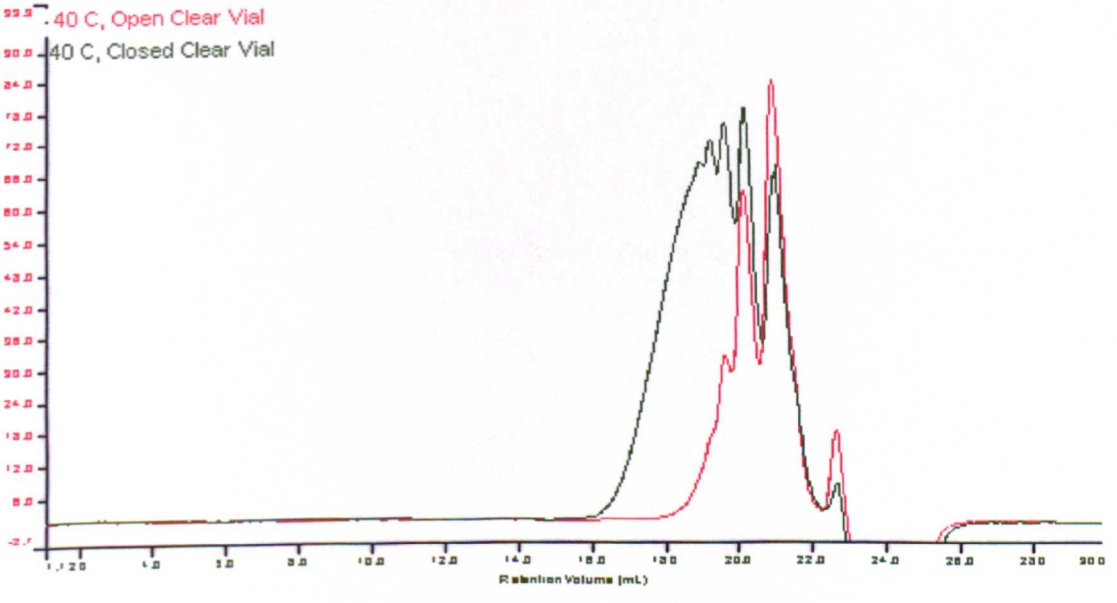


Figure 4.11: *GPC chromatogram showing the effect of humidity on stability of the PGA when stored in open (red line) and in closed vials (green line) for six months at 40°C and 75% RH.*

The effect of humidity was observed in samples stored with an opened top at 40°C and 75% RH where complete degradation of the polymer occurred after 6 months (Figure 4.11). A major change in the polymers' physical properties was also observed as a decrease from initial high viscosity state to a very low-viscosity liquid (Table 4.4). This further confirms that these polymers are labile to moisture and would be easily biodegradable. While under similar environmental conditions only a 55% decrease in polymer molecular weight was observed in closed vials (Figure 4.10), emphasising the reduced effect these conditions have in sealed vials. These results clearly indicate the deteriorative effects of humidity on polymers, even stored in closed vials. Table 4.4 summarises the changes in the physical properties of PGA. The molecular weight of PGA decreased from 6609 to 933 Da within two months, followed by complete degradation within 4 months, when exposed directly to 75% RH and 40°C.

Table 4.4

Effect of humidity on physical properties of PGA (at 40°C and 75%RH)

Duration	Physical Properties
	Closed Vial
2 months	Low viscosity
4 months	Very low viscous liquid, free flowing
6 months	Very low viscous liquid, free flowing
	Open Vial
2 months	Very low viscosity
4 months	Two phases: pale yellow liquid, white solid layer
6 months	Two phases: pale yellow liquid, white solid layer

The order of stability at near ambient conditions (25°C and 75% RH) ranging from most stable to least stable is PBA-co-PDL > PBA > PGA-co-PDL > PGA (Figure 4.12).

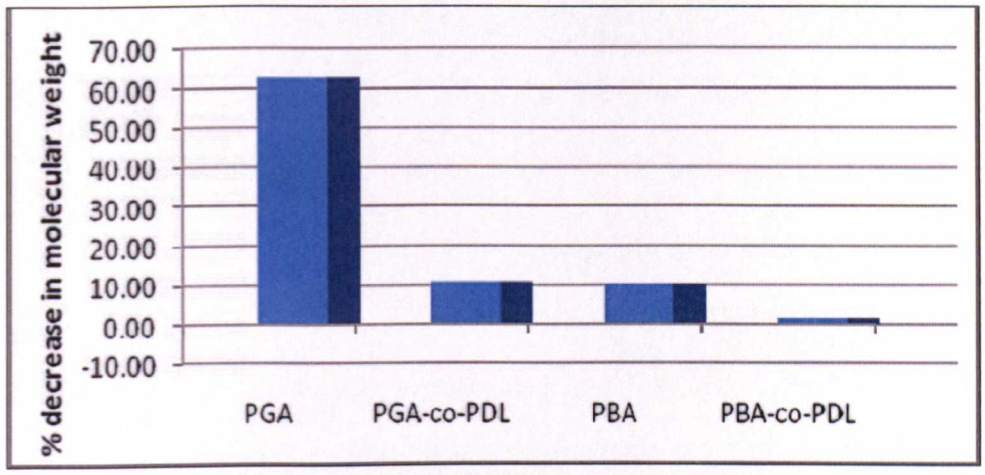


Figure 4.12: *Percentage decrease in molecular weight of the polymer at 25°C and 75% RH after six months*

This further confirms that apart from the influence of crystallinity, more hydrophilic polymers degrade more rapidly (Dorati et al 2007), while a decrease in hydrophilicity results in reduced interactions with aqueous media, resulting in slower degradation (Park et al 1992).

4.3.1.3 Shelf life estimation

The stability data obtained from the analysis of polymer samples at regular intervals were used to plot stability curves, according to ICH and pharmacopoeial guidelines. Extension of the plots can be used to assess and estimate the shelf life of the polymers.

a) Poly(glycerol adipate) (PGA)

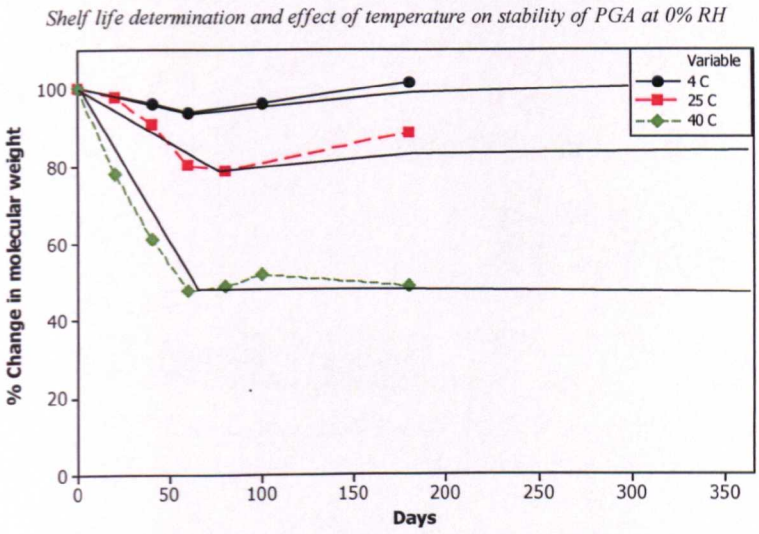
Figure 4.13 summarises the effect of simulated storage conditions and environmental factors on PGA, and clearly indicates that 4°C is the best storage condition for this polymer, with a maximum decrease of molecular weight of only 4%. Hence, storage of PGA at higher temperature is not advisable as it can lead to a 10-60% reduction in molecular weight over 6 months. Extended graphs for shelf life determination (Figure

4.13) show that at 4°C (at all humidity ranges tested) PGA can remain stable for more than 12 months while retaining to 90% of its molecular weight. It is also evident that polymer degradation reached its maximum within 60 days at higher temperature and humidity.

b) Poly(glycerol adipate-co-pentadecalactone) (PGA-co-PDL)

The percentage decrease in molecular weight of PGA-co-PDL, under different environmental conditions is presented in figure 4.14. This information enables the selection of suitable storage conditions for this particular type of polymer. It is clear that storage at 4°C and room temperature caused up to 20% variation in molecular weight of the polymer under all relative humidities tested, whilst approximately a 60% decrease in molecular weight was observed at 40°C at 25% and 75% RH only. Extended plots of the stability data (Figure 4.14), showed that PGA-co-PDL degraded by 25% within 6 months and this may extend to 30-35% by the end of 12 months at 0% RH over all temperature ranges. At 25% RH the polymer remained in a similar range of 30% degradation at both room temperature and 4°C, but at 40°C, the polymer had already degraded by more than 60%. Hence the PGA-co-PDL should be stored at either 4°C or room temperature, over drying sieves to avoid any physical and compositional changes.

a)



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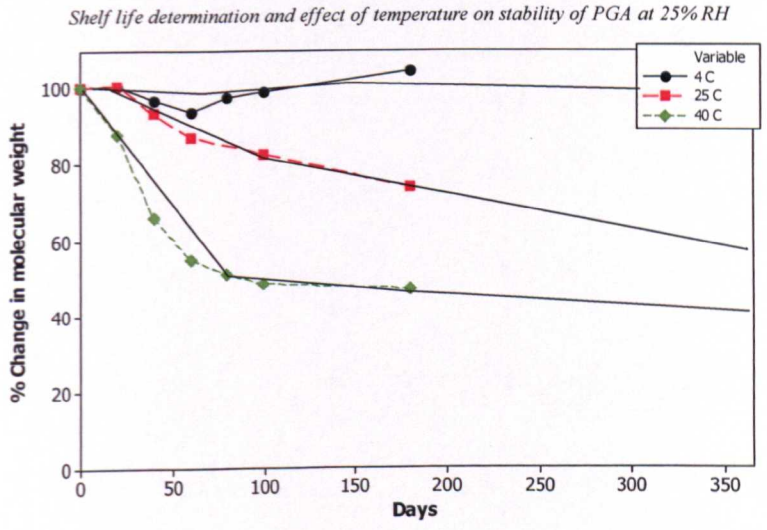

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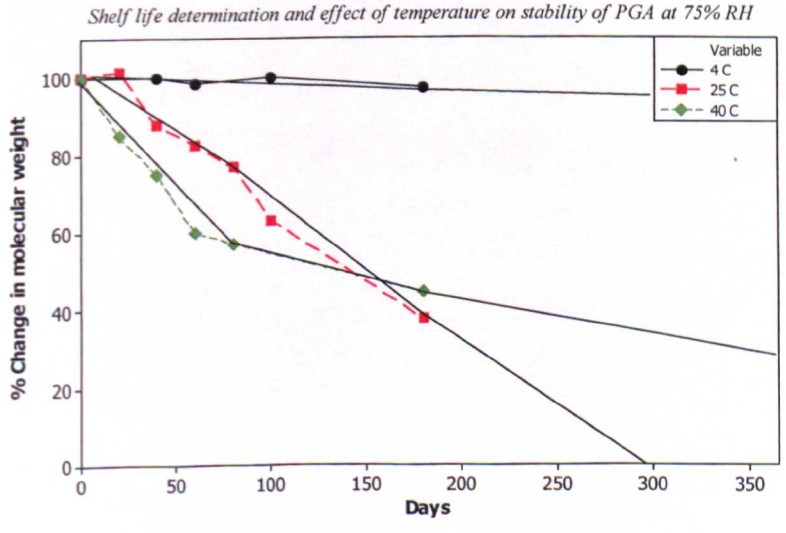
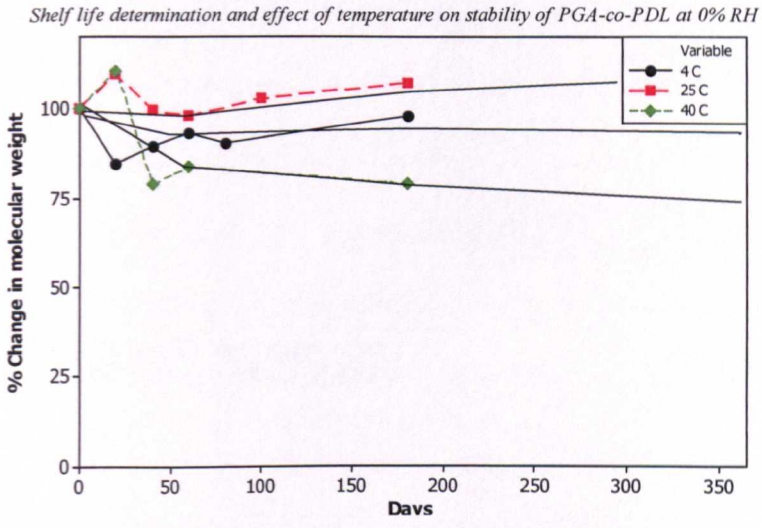


Figure 4.13: *Shelf life determination of PGA via extrapolation of decrease in molecular weight curves up to a year, under various storage conditions*

a)



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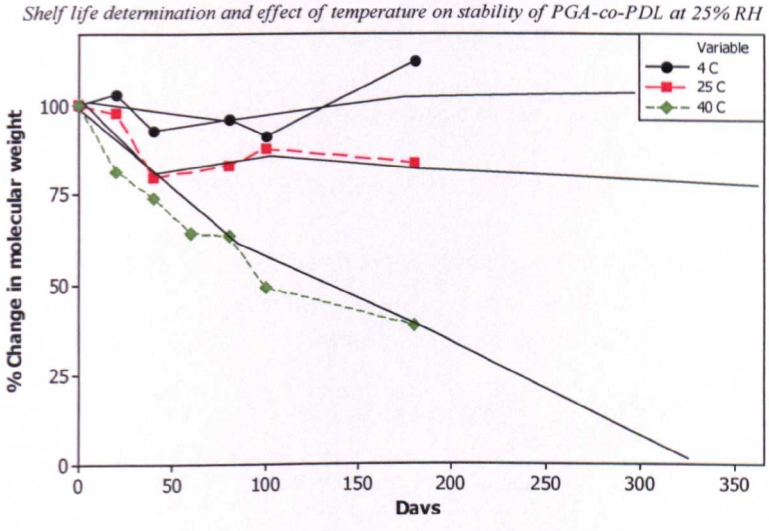

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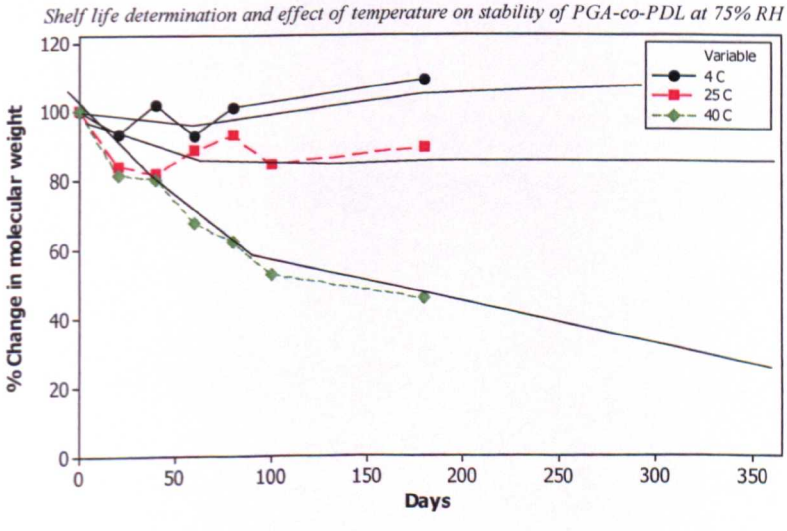


Figure 4.14: *Shelf life determination of PGA-co-PDL via extrapolation of decrease in molecular weight curves up to a year, under various storage conditions*

c) Poly(1,4-butanediol adipate) (PBA)

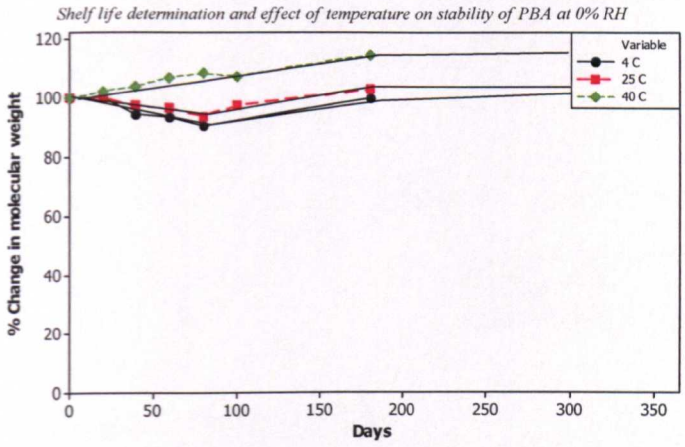
Figure 4.15, summarises the effects of humidity and temperature on the molecular weight of PBA. PBA can be stored at 4°C and room temperature without any major decrease (<5%) in molecular weight for 12 months, while storage at higher temperatures (≥40°C) is not advisable because of compositional changes in the polymer. Polymer samples stored at 40°C degraded by 60% within 6 months of their storage, hence storage of the polymers at 4°C or room temperature over drying sieves is advised.

d) Poly(1,4-butanediol adipate-co-pentadecalactone) (PBA-co-PDL)

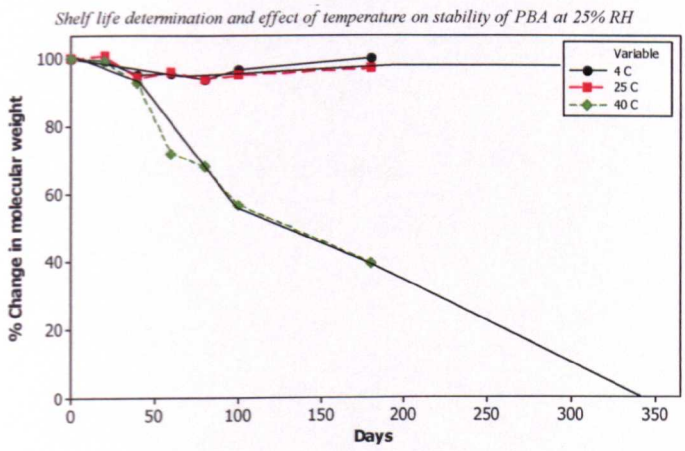
Figure 4.16, identifies the best conditions for the storage of the PBA-co-PDL copolymer. It is clear that room temperature or 4°C with ambient humidity is also the better option to store PBA-co-PDL. Furthermore, melting of the polymer at 40°C introduces another limitation on the storage of the polymer; hence storage at room temperature or below is essential for longer shelf life (nearly 2 years). From the data shown it can be observed that within 12 months the molecular weight of the polymer may decrease only by 20% at room temperature and 4°C, thus confirming that this polymer can stay stable for longer than 1 year, if stored appropriately.

In conclusion, 4°C or room temperatures (25°C) under dry conditions are the best storage conditions for all these polymers.

a)



b)



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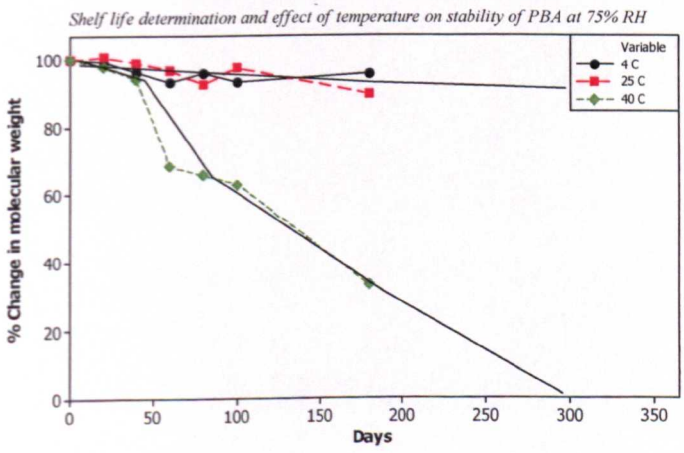
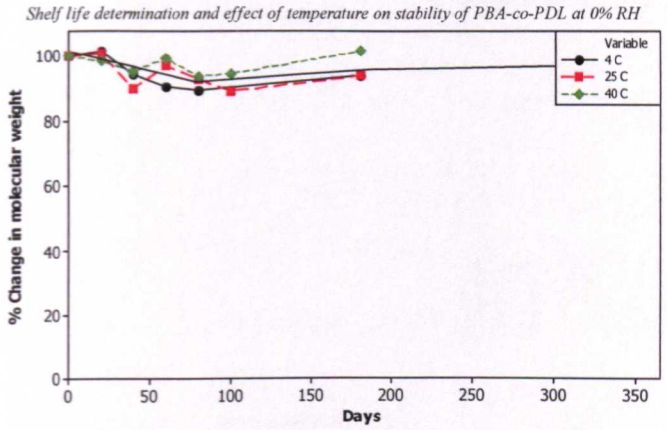


Figure 4.15: *Shelf life determination of PBA via extrapolation of decrease in molecular weight curves up to a year, under various storage conditions*

a)



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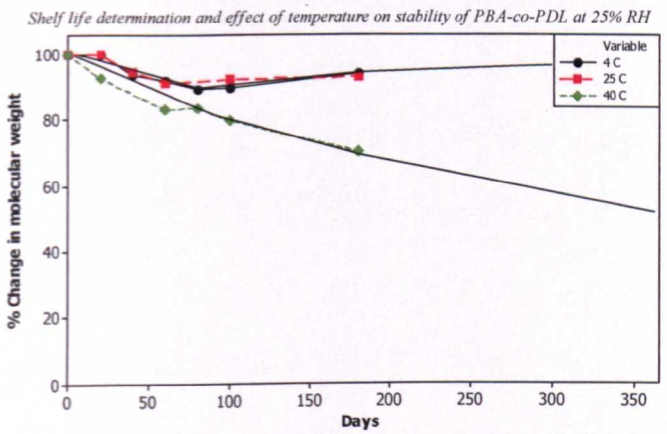

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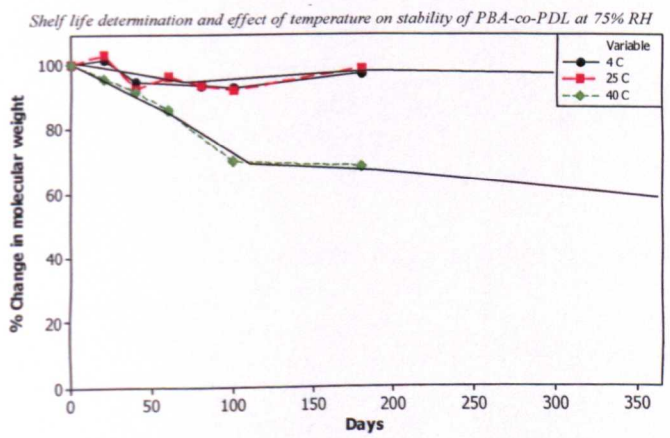

Figure 4.16: *Shelf life determination of PBA-co-PDL via extrapolation of decrease in molecular weight curves up to a year, under various storage conditions*

4.3.2 *In vitro* degradation

Along with a longer shelf life, another much desired property of the polymers for drug delivery applications is biodegradability. PGA-co-PDL was previously investigated for pulmonary drug and protein delivery by Gaskell et al (2008) and Thompson et al (2007). Regardless of the route of administration the polymeric matrix should degrade completely into non-toxic components and be excreted by the body via the physiological pathways. As the human body provides a range of physiological conditions, from very acidic (gastric juices) to neutral (blood) to very alkaline (intestinal juices), this highlights the importance of *in vitro* degradation studies under simulated physiological conditions. Phosphate-buffered saline (PBS), pH 7.4, is commonly considered the medium of choice for *in vitro* degradation studies, and most researchers report biodegradation in this buffer (Wang et al 1990). This buffer contains 150 mM sodium phosphate (dibasic) and 0.9% (w/v) sodium chloride. Although the final pH of this buffer is 7.4, equal to lung pH, the ionic strength and concentrations do not represent lung fluids. Gamble's solution is considered as the true representative of interstitial lung fluids, pleural fluid or simulated lung fluid (SLF) (Gamble 1967). The composition of SLF is summarised in table 4.2. As there are higher numbers of ions and higher ionic strength (with the same pH of 7.3-7.6, as pleural fluid) it was presumed that depending on polymer chemistry these materials would degrade to different extents. This is why there was a need to study the comparative polymer degradation in PBS and SLF, both of which provide 100% relative humidity (aqueous environment), pH 7.4±0.1 and physiological temperature (37°C). The results of polymer degradation under these conditions are discussed below.

4.3.2.1 Effect of polymer chemistry in simulated physiological fluids

For the *in vitro* degradation studies, the oxoester-containing PGA-co-PDL and the thioester-containing PDTTA-co-PDL were compared. Both polymers were in direct contact with the aqueous media (SLF and PBS), hence, all labile ester bonds on the polymer backbone had the potential to degrade via hydrolysis in a short time. It is well established that the thioester link is more readily hydrolysable than the oxoester, because of its high reactivity due to the polarity of the bonds (Figure 4.17) and delocalisation of electrons. Thioesters show high reactivity in base and enzyme catalysed hydrolysis (Aarsman & Den Bosch 1979).

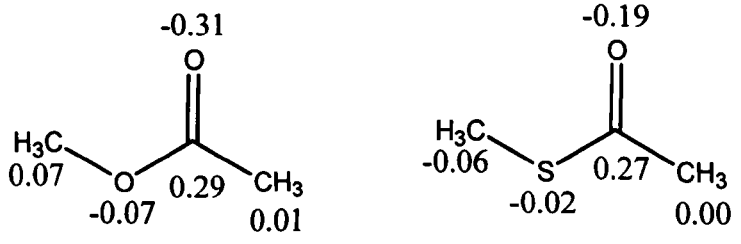

Figure 4.17: *Bond polarities in oxoester (left) and thioester (right)*

Figures 4.18 and 4.19 summarise the results of the *in vitro* degradation of PGA-co-PDL (powder) and PDTTA-co-PDL (powder) in two different physiological fluids. PDTTA-co-PDL degraded more rapidly and to a greater extent than PGA-co-PDL. This is in accordance with the hypothesis (chapter 2, section 2.3.3.4) that thioesters can degrade more quickly and more completely *in vitro* and, hence, are potentially more suitable candidates for drug delivery applications. Moreover, the degradation of thioester-based polymers is in accordance with observations reported by Aarsman & Den Bosch (1979) that the hydrolysis rate of thioesters is 2-5 times higher than that of the corresponding oxoesters.

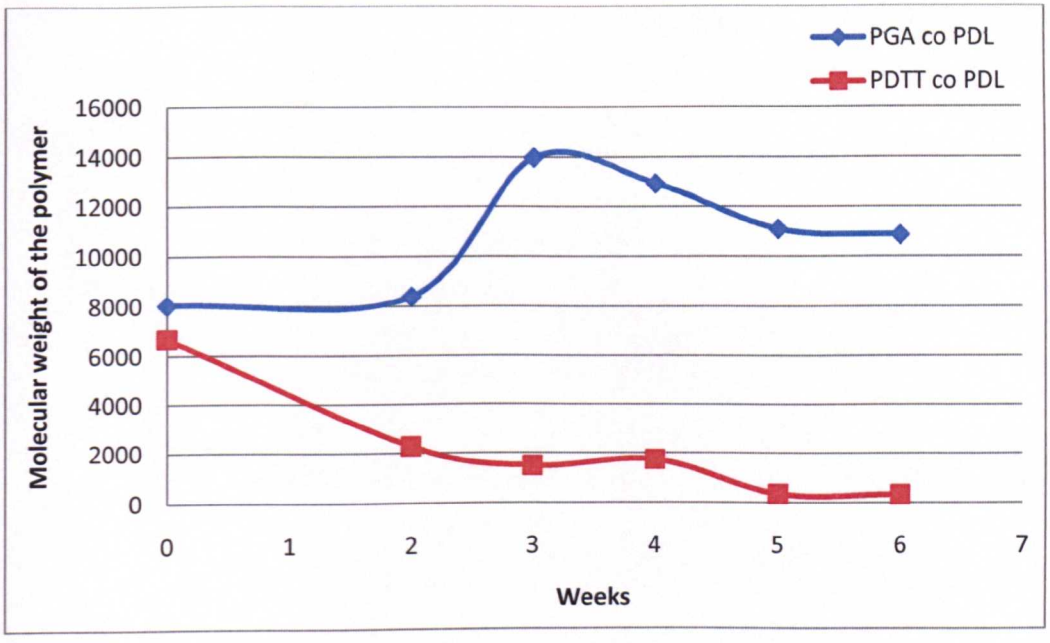

Figure 4.18: *In vitro degradation profile of PGA-co-PDL and PDTTA-co-PDL at 37℃ in SLF buffer during six weeks*

It is clear from figures 4.18 and 4.19 that the molecular weight of the PDTTA-co-PDL continuously decreased but the PGA-co-PDL did not show a consistent trend in the change of molecular weight. However, this non-consistent trend can be explained by the fact that both SLF and PBS contain various ions, but the ionic strength is higher in SLF than in PBS (although the pH is the same). In such cases there is a possibility of interaction between metal ions and electron rich functional groups which may lead to chelation where highly electropositive metal ions (from buffers) can form a complex or chelate with electronegative oxygen atoms in the carbonyl groups of the oxoester-based polymer.

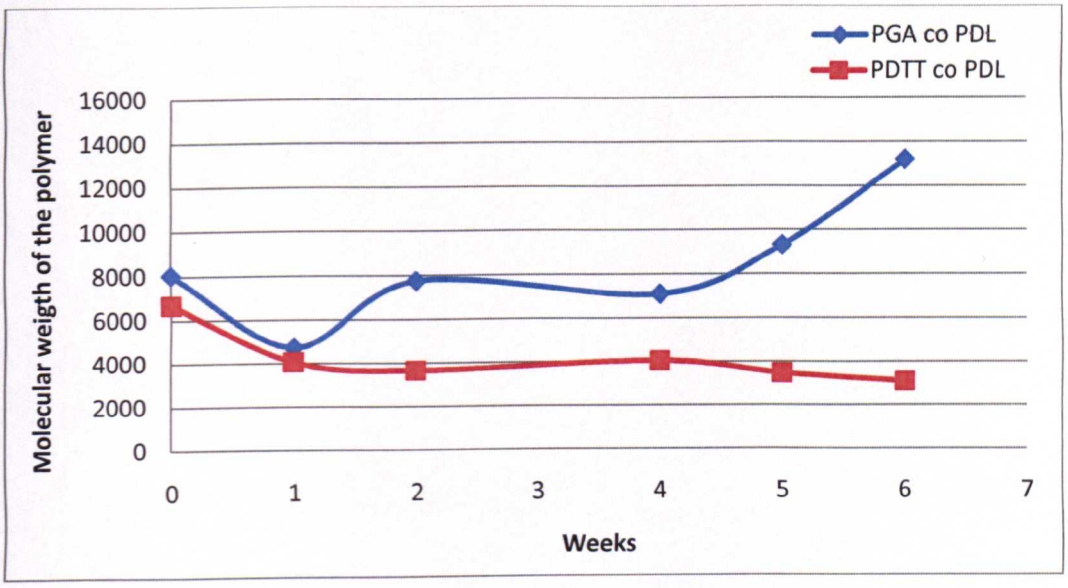

Figure 4.19: *In vitro degradation profile of PGA-co-PDL and PDTTA-co-PDL at 37℃ in PBS buffer during six weeks*

Figure 4.17 illustrate the electron densities around the carbonyl group, as oxygen is more electron rich than the sulphur in the corresponding thioesters. Hence, it provides a base for a hypothesis that the proposed metal-polymer chelation (which would result in higher molecular weight products) and polymer degradation via hydrolysis (resulting in decrease in polymer molecular weight) would proceed at the same time. An example in support of this assumption is the work done by Chung et al (2004), where $TiCl_4$, a Lewis acid, chelates with the ester carbonyl moiety in non-aqueous media. This hypothesis may explain the varying trends in molecular weight of oxoester-based polymer, but thorough investigations are required to confirm any such interaction in polymers discussed in presented work.

The implication of these results can further be seen from the GPC chromatograms of both polymers after six months in PBS and SLF (Figure 4.20 and 4.21). It is evident that peaks corresponding to PGA-co-PDL after six months storage are broader when compared to the starting polymers in both fluids indicating an increase in polydispersity. Shifting of the peaks towards the right hand side indicates an increase in molecular weight of the polymer after six months (Figure 4.20).

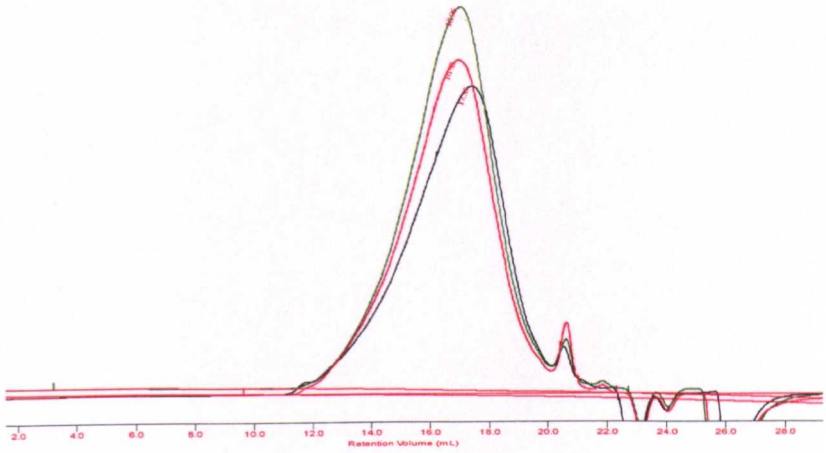

Figure 4.20: *GPC chromatogram of PGA-co-PDL (black) after six weeks storage in PBS (green) and in SLF (red) at 37℃*

On the other hand, PDTTA-co-PDL peaks moved towards the right hand side (higher retention volume) along with considerable peak broadening. This indicates higher degradation in both fluids, but in SLF the degradation is much greater than in PBS (Figure 4.21). These results prove that thioesters are more biodegradable than oxoesters. They also suggest that for *in vitro* degradation studies of materials for pulmonary drug delivery, the use of PBS can give misleading trends and observations. Hence, for pulmonary applications, SLF should be used instead of PBS as the simulated physiological fluid. While for other applications, such as oral delivery or intravenous delivery, the actual simulated fluid should be use instead of a model buffer, PBS.

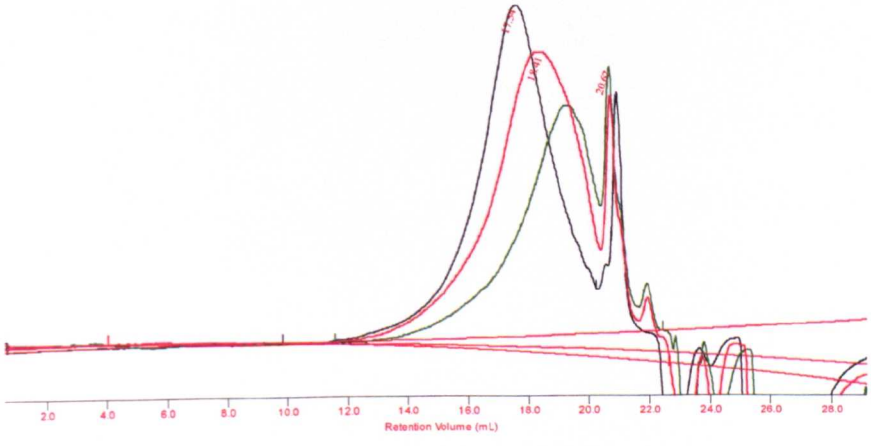

Figure 4.21: *GPC chromatogram of PDTTA-co-PDL (black) after six weeks storage in PBS (red) and in SLF (green) at 37℃*

4.3.2.2 Effect of surface area/particle sizes

Another very important factor which usually is often neglected while studying *in vitro* polymer degradation is the final dosage form of the biomaterial. Depending upon application, these materials are usually formulated either as microparticles or nanoparticles, but researchers often only use the raw polymeric material for degradation studies (Gopferich & Langer 1993). This is a source of misleading data on the biodegradability of polymeric materials, because after formulating into micro- and nanoparticles the surface area of these materials increases several fold. Hence, this could lead to further increases in the rate and extent of degradation. Based on this hypothesis, PGA-co-PDL matrix and microparticles were subjected to degradation studies under SLF and PBS conditions. The obtained results confirmed the hypothesis that smaller particles with a larger surface area; degrade more quickly and to a greater extent.

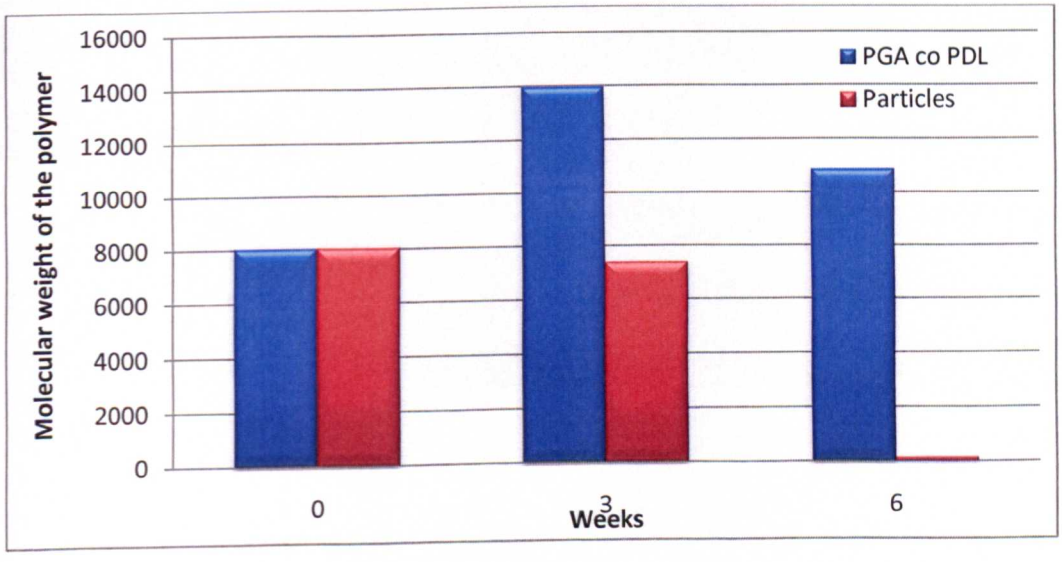

Figure 4.22: *In vitro degradation profile of PGA-co-PDL and its microparticles at 37℃ in SLF during six months*

Figures 4.22 and 4.23 represent the degradation of two forms of the PGA-co-PDL material in the two simulated fluids. It is very interesting to see that PGA-co-PDL, which exhibited an inconsistent degradation trend in its normal matrix form, completely degraded within six weeks when formulated into microparticles. This proves that PGA-co-PDL (oxoester) can completely degrade in its final dosage forms, hence making it a good candidate for drug delivery applications.

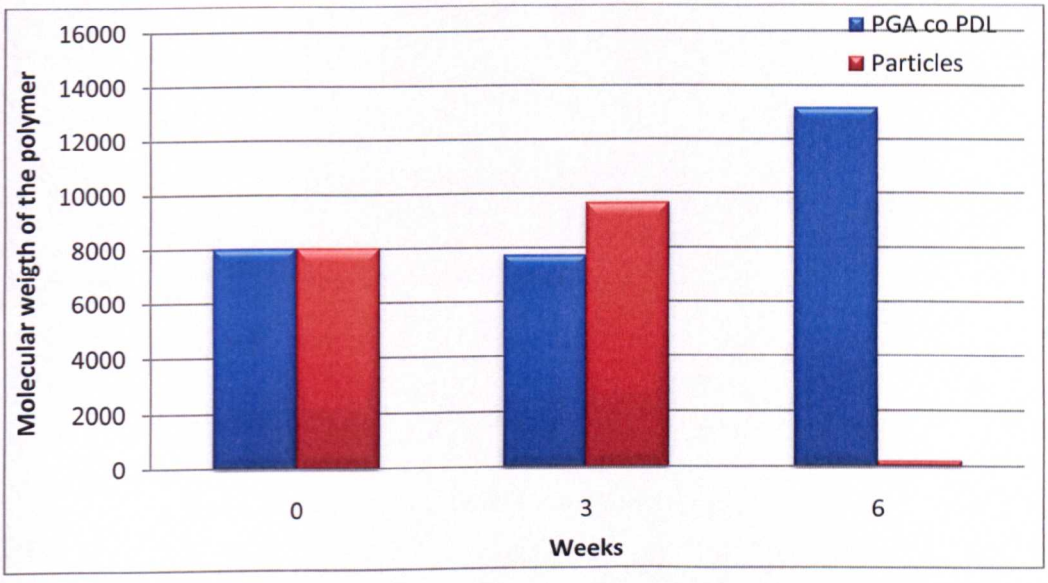

Figure 4.23: *In vitro degradation profile of PGA-co-PDL and its microparticles at 37℃ in PBS during six months*

One more important point to note here is the complete degradation of the micro-particles in both fluids. This further helps to conclude that by increasing the surface area, hydrolysis is promoted and this is why the polymer degrades completely, instead of increasing in molecular weight. The GPC chromatograms of microparticle degradation (Figure 4.24) further prove the degradation of the particles in two simulated physiological fluids.

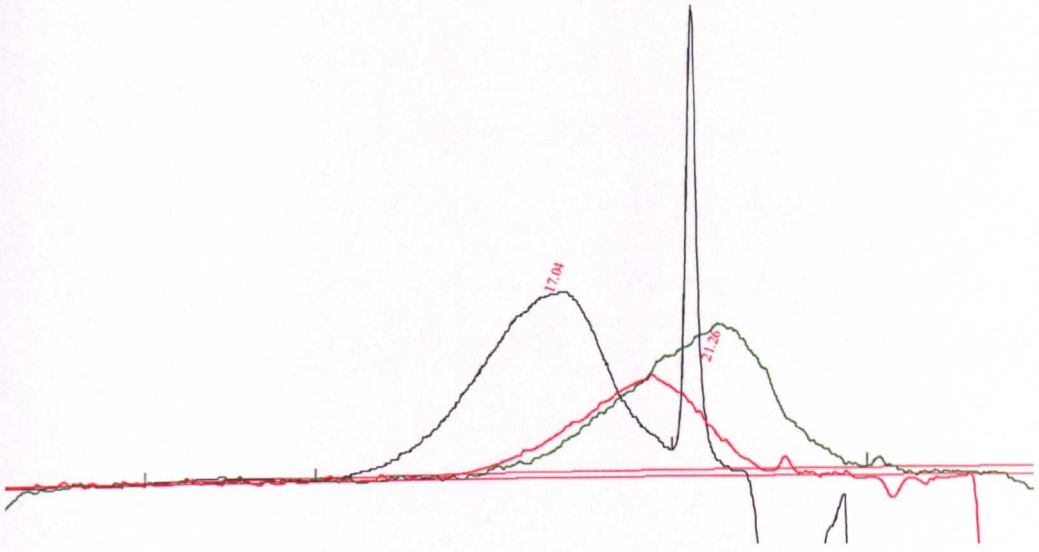

Figure 4.24: *GPC chromatogram of PGA-co-PDL microparticles (black) after six weeks storage in PBS (green) and in SLF (red) at 37°C*

The results discussed in this chapter provide an understanding of the *in vitro* degradation of polymers bearing varying chemistry and physical forms under different simulated physiological fluids. The hydrolytic degradation of these polymers proceed predominantly via random chain scissions (Wang et al 1990). The other major step which is involved in later stages of hydrolytic degradation is conversion back to monomeric units; a schematic representation of this is given in chapter 1 (Figure 1.9). However, the GPC traces of polymer degradation did not show any small molecular fragments or monomeric peaks, instead the whole peak had shifted to a lower molecular weight scale along with peak broadening (indicated by an increase in PDI). Hence, it was concluded that the predominant step in this polymer degradation is random chain scission.

An additional aspect of polymer degradation under *in vitro* conditions is the biodegradability of these polymers. The polymers are potential drug carriers and their complete biodegradation under physiological conditions is a prime concern for formulation developers, hence polymers with varying biodegradation rates are synthesised for specific drug delivery applications. For example, PLGA is rapidly biodegradable, which makes it useful in vaccine delivery applications (Wang et al 1990), and it degrades rapidly via "self-catalysis" while polycaprolactone (PCL), another FDA-approved biomaterial, needs an external catalyst or initiator for degradation, resulting in relatively slower degradation. Hence, PCL is mainly used in tissue scaffolds, but also has uses as drug delivery devices (for example as sutures, marketed under the brand name Monocryl®) (Bezwada et al 1995).

The results obtained for PGA-co-PDL indicated its slower degradation (over 6 weeks) compared to PLGA under *in vitro* conditions. Under similar conditions to those in this study, Lu et al (1999) reported that 94% PLGA (matrix) degraded in 4 weeks, while data obtained in the current work showed that no significant decrease in PGA-co-PDL molecular weight was observed. Hence, it can be concluded that although PGA-co-PDL is biodegradable like PCL, it does not degrade as rapidly as PLGA in its matrix form. The degradation of PGA-co-PDL can be altered not only by formulating it into various particle sizes but also by changing the monomer composition, molecular weight and copolymer ratio. In addition, if the type of ester link is changed from oxoester to thioester, it would increase the degradation rate, hence providing a solution for delayed degradation in the lung environment. PBS should be avoided for use as a simulated fluid for pulmonary drug delivery applications, because pH is not the only factor/criteria for a fluid to be representative of physiological environment. The presence of different ions and ionic strengths should be considered while formulating a simulated fluid. For degradation studies the final dosage forms should be used instead of raw material.

These results not only help to understand the various physical and chemical factors affecting *in vitro* degradation behaviour but also inform the design of biomaterials with desired degradation rates. These results indicate that polymer degradation ends in monomeric species which are non toxic in nature and can easily be excreted via various physiological pathways.

4.4 CONCLUSION

In conclusion, these results clearly indicate that these polymers can be stable if stored under appropriate conditions but are also biodegradable. Their degradation is affected by both their chemistry (hydrophilicity, molecular weight, carbon chain length, functional groups and type of ester link) and storage factors (temperature, humidity and light).

a) Recommended storage conditions

To achieve stable polymers with long shelf lives, they should be stored in dark containers at temperatures near or below 4°C, under drying conditions. Although all the polymers studied under the suggested conditions will remain stable for more than 1 year, they need to be re-analysed at 6 monthly intervals, to check their stability and degradation indications. For drug delivery applications polymers with longer shelf-lives (4-5 years) are desired, hence, there is a need for stability testing of such polymers for longer durations.

b) Chemistry of the polymers

Depending upon the application of these biomaterials, the degradation and stability of the polymers can be controlled by altering the chain length, presence of functional groups, molecular weight and the type of hydrolysable ester link.

c) *In vitro* degradation

These polymers are biodegradable under simulated physiological conditions. However, the *in vitro* degradation studies for pulmonary applications should be carried out in SLF rather than commonly used simulated physiological fluid, PBS, using the final dosage form of the biomaterials.

Although only five polymers of varying chemistries were studied here, the degradation processes and trends are applicable for many other polymer-based drug delivery systems studied in this research. The obtained data provide an understanding of the degradation process and enables selection of appropriate chemical parameters for the development of stable and biodegradable polymers. These studies also help draw basic guidelines for recommended storage conditions and shelf lives of the synthesised library of polyesters. This information can be used to achieve a longer shelf life and increased stability of the particles prepared from these polymers. This should provide better control of shelf life and *in vitro* degradation of the formulations. Based on these results, these particles can be further explored for *in vivo* applications.

5.0 General conclusion and future work

The aim of developing an effective polymeric drug delivery system via the enzyme catalysed synthesis of a series of polymers, possessing a range of physicochemical properties, was successfully achieved and subsequently investigated for drug delivery and degradation *in vitro*.

To develop effective candidates for drug delivery applications, novel polymeric materials were synthesised using simple, biocompatible and FDA-approved monomeric units and formulated into stable microparticles containing a range of drugs of various solubilities and molecular sizes. The devised polymeric systems released the drugs at various rates depending on the polymer/drug chemistry and interactions. Hence, drug release was varied by altering a range of physicochemical properties of the polymeric materials including molecular weight, melting temperature, hydrophilicity, availability of free functional groups and post-synthetic modifications.

The range of synthesised polymers showed varying rates of release of poorly water-soluble drugs from microparticles as compared to PLGA-based systems, depending on the hydrophilicity of polymers, indicating the successful development of new polymeric drug delivery systems compared to existing FDA-approved materials. The materials' physical properties played a vital role in controlling drug encapsulation and drug release. A polymer with a lower T_g value (near physiological temperature) released drugs at higher rates. However, making the polymer more hydrophobic, by attaching a non-polar chemical moiety to the backbone, resulted in considerably slower drug release. These two factors, T_g and hydrophilicity, might therefore be "tuned" to control the drug release at a required rate.

The developed polymeric systems can not only encapsulate drugs with a range of water solubilities. However, drugs with very high water solubilities could not be encapsulated via solvent emulsion techniques. To solve this issue, the use of alternative techniques such as spray drying, interfacial deposition and/or conjugation to the functionalised polymer backbone could be explored in future.

Altering the polymer chemistry by introducing monomers, such as PEG or thioesters was also shown to affect the drug encapsulation and rate of release. There is a need for further studies to exploit the use of such monomers for the improvement of microparticle formulation. For example, the synthesis of copolymers with increased PEG content may enable the formulation of particles via the solvent emulsion technique without requiring an external surfactant or emulsion stabilizer. Although, polythioesters were synthesised successfully and showed improved degradation rates and better thermo-chemical properties, these materials failed to encapsulate drugs. However, because of the much improved physicochemical properties there is a need to investigate these materials further for successful microparticle formulation, either via spray drying, interfacical deposition or by altering the chemistry of the polymer backbone to afford better mechanical strength.

PBS is the most commonly used buffer for *in vitro* drug-release studies, hence it was used to obtain drug-release profiles. However, because the polymer degradation rates were different in PBS and SLF, this indicates the need for the use of SLF as a "physiological fluid", especially for pulmonary drug delivery studies. SLF would be a better and more realistic physiological fluid because it contains the same ionic strength as the lung environment. The difference of true ionic strength between PBS and SLF can affect the polymer-medium interaction and can give realistic drug release profiles.

Overall these studies have been explorative in nature for the development of novel biodegradable polyesters with longer shelf-lives for a broad range of colloidal drug-delivery applications. The devised systems showed promising results in *in vitro* environments and hence, need to be further investigated *in vivo*.

Suggestions for future work

Based on the observations in this project, further work could be carried out to obtain a better understanding of different variables for improvement in the developed drug-delivery system. It was observed that small incremental changes in polymer chemistry did not show significant effects on drug encapsulation and release. Hence, there is a need for altering the polymer properties by evaluating a range of new monomers to extend the library of polymers, which can be tailored according to the application. In addition, to further prove and understand the efficacy of the developed polymeric system, these polymers should be evaluated for the delivery of a range of therapeutic agents, such as drugs, enzymes and genes. The obtained feedback can then be used to understand and improve the design of the drug delivery system by tuning the polymer-drug chemistry. To achieve these aims, some of the suggested studies are as follows;

1. Synthesis of polymers with new pendant functional groups such as amine and carboxylate for attachment of a range of drug molecules. This can be achieved by use of functionalised monomers via enzyme catalysis.
2. Synthesis of PEG copolymers with higher PEG content to assist the drug loaded microparticulate formulation.
3. Synthesis of polythioesters with better physico-mechanical properties, such as higher T_m and crystallinity. This can be achieved by either using higher lactone contents during synthesis or by conjugating non-polar chemical moieties to the functionalised polymer backbone.

4. Use of various other techniques, such as spray-drying and interfacial disposition for the encapsulation of freely water-soluble drugs into microparticles. This also allows the use of viscous polymers for drug delivery applications.
5. Use of better particle characterisation techniques, such as;
    a. Zetasizer: for surface charges, zeta potential measurement
    b. Laser diffraction particle sizer: for actual particle sizes and fine particle fraction measurement
    c. Transmission electron microscopy
    d. Confocal microscopy
6. Use of SLF for release studies in pulmonary drug delivery, instead of using PBS.
7. Detailed qualitative analysis of polymer degradation to estimate the toxicity of the by-products.

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7.0 Supporting studies associated with this project:

Publications

1 *A. Iftikhar, E. Gaskell, G. A. Hutcheon and C. Rostron, The Stability of Biodegradable Polyesters and Polyester-co-lactones utilised for drug delivery*, Journal of pharmacy and pharmacology, 60 (1), (2008), page A-58.

2 *A. Iftikhar, E. Gaskell and G. A. Hutcheon, Biodegradable Polyesters for drug delivery*, United Kingdom Society for Biomaterials (UKSB), 2008, page 56, ISBN: 978-0-9559548-0-1.

Oral Presentations

3 *Development of New Colloidal Drug Delivery System*, Royal Society of Chemistry (RSC) Biomaterials Chemistry Group 4th Annual Meeting, 15 January **2009**, SCI, 12-14 Belgrave Square, London, **UK.**

4 *Colloidal drug delivery: Evaluation of biodegradable polymers*, Faculty of science research seminar, 22 May **2008**, Liverpool John Moores University, **UK.**

5 *Evaluation of novel polyester microspheres for ibuprofen delivery*, Institute for Health Research Annual Conference (IHRAC 2008), 9 May **2008**, Liverpool John Moores University, Liverpool, **UK.**

Poster Presentations

6 *Synthesis and Characterisation of PEG-copolymers for Drug Delivery Applications,* 42nd International Union of Pure and Applied Chemistry (IUPAC) Congress, 2-7 August **2009**, SECC, Glasgow, **UK.**

7 *The Stability of Biodegradable Polyesters and Polyester-co-lactones utilised for drug delivery*, British Pharmaceutical Conference (BPC 2008), 7-9 September **2008**, Manchester, **UK.**

8 *Biodegradable Polyesters for Drug Encapsulation*, 7th Annual United Kingdom Society of Biomaterials (UKSB) conference and Postgraduate day, 26-27 June **2008**, University of Liverpool, **UK.**

9 *Evaluation of Novel Polyester Microspheres for Ibuprofen Delivery*, Royal Society of Chemistry (RSC) Biomaterials Chemistry Group 3rd Annual Meeting, 15 January **2008**, University of Manchester, **UK.**

10 *Optimisation of Synthesis of Biodegradable Polyesters for Drug Delivery*, Post Graduate Researchers in science medicine conference 2007 (PRISM 2007), 28 June **2007**, University of Chester, **UK.**