Drug release mechanisms from bioerodible polymers for stent application

Alexis, Frank

2005

Alexis, F. (2005). Drug release mechanisms from bioerodible polymers for stent application.
Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/5048>

<https://doi.org/10.32657/10356/5048>

Nanyang Technological University

Downloaded on 26 Apr 2023 16:42:37 SGT

a35622

Drug Release Mechanisms From Bioerodible Polymers For Stent Application

Frank Alexis

School of Materials Engineering

A thesis submitted to the Nanyang Technological University
in fulfillment of the requirement for the degree of the
Doctor of Philosophy

2005

Acknowledgements

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude to his supervisor, Professor Subbu Venkatraman and Professor Freddy Boey, for their supervision and assistance. Professor Subbu insight and guidance have insured that this thesis has progressed fruitfully.

Thanks very much for Doctor Santosh Kumar Rath for his strong guidance and assistance.

I express my sincere thanks to Prof. Gan Leong Huat, Deputy Head (Chemistry) of National Institute of Education, for his kind help and advice during the course of the use of $^1\text{H-NMR}$ in his division.

Also thanks for Professor Marc Abadie for his constant moral support.

Aknowledgments are also extended all technicians in School of Materials Engineering: Mdm Irene Heng, Mdm Wang Lee Chin, Mdm Toh Swee Sing, Mr Samsudin Bin Sarim, and Nelson Ng.

Thanks very much to my parents, siblings and friends for the encouragement and support.

Table of Contents

TABLE OF CONTENTS

Acknowledgements	i
Table of Contents	ii
Abstract	vii
List of Figures	ix
List of Tables	xiii

Chapter 1	Introduction	Page
1.1	Background	1
1.2	Objectives and Scopes	4
1.2.1	Problem statement and objectives	4
1.2.2	Project methodology	6
Chapter 2	Literature Survey	7
2.1	Polymers in Controlled Release Technology	7
2.1.1	Localized delivery	9
2.1.2	Sustained systemic delivery examples	10
2.1.3	Combinations with localized delivery	12

Table of Contents

2.2	Thrombosis and Restenosis	13
2.2.1	Introduction	13
2.2.2	Overview of stents	15
2.2.3	Drugs used to prevent heart disease	17
2.3	“Model” Drugs	26
2.3.1	Lidocaine (base)	26
2.3.2	Lidocaine hydrochloride (salt)	27
2.4	Drug Release Mechanisms in Biodegradable Polymers	27
2.4.1	Effects of degradation in drug release mechanisms	27
2.4.2	Drug release kinetics review	30
2.4.3	Burst release	35
2.5	Other Factors Affecting Drug Releases	36
2.5.1	Drug solubility	36
2.5.2	Drug loading	37
2.5.3	Effect of drug chemistry	39
2.5.4	Effect of polymer average molecular weight (M_w)	43
2.5.5	Effect of degree of crystallinity of polymer	44
2.5.6	Effect of composition	44
2.5.7	Effect of addition of plasticizer	45
2.5.8	Effect of gamma-irradiation	45
2.5.9	Effect of mechanical stress	45
2.5.10	Effect of shape	46
2.6	Mechanisms of Biodegradation	47
2.6.1	Introduction	47
2.6.2	Effect of drug chemistry	48

Table of Contents

2.6.3	Effect of size of the matrix	50
2.6.4	Effect of polymer crystallinity	53
2.6.5	Effect of average molecular weight (M_w)	55
2.6.6	Effect of composition	57
2.6.7	Effect of pH	57
2.6.8	Effect of fluid flow	60
2.6.9	Effect of processing	60
2.6.10	Effect of temperature	62
2.6.11	Effect of strain	63
2.6.12	Effect of plasticizer	63
2.6.13	Effect of sterilization	64
2.6.14	Effect of enzymes	65
2.7	Biocompatibility and Bio-elimination	67
2.7.1	Biocompatibility	67
2.7.2	Bio-elimination	70
2.8	Mechanical properties	71
2.9	Biopolymer characteristics	73
2.9.1	PGA and PLGA	73
2.9.2	PLA	74
Chapter 3	Experimental Techniques	80
3.1	Materials	80
3.2	Preparation of Films and Drug-Incorporated Stents	81
3.3	Degradation Study	83
3.4	Thermal Analysis	84

Table of Contents

3.5	Size Exclusion Chromatography (SEC)	86
3.6	Scanning Electron Microscopy (SEM)	87
3.7	¹ H-NMR Studies	87
3.8	Monte Carlo Calculations	88
3.9	Drug Release Study	89
3.10	High Performance Liquid Chromatography (HPLC)	90
Chapter 4	Polymer Degradation	98
4.1	Introduction	98
4.2	Degradation of the Homopolymer	100
4.2.1	Water absorption, weight loss, and change in M_w	100
4.2.2	Thermal analysis	108
4.2.3	SEM analysis	111
4.3	Effect of Composition on Degradation Behavior	112
4.3.1	Water absorption, weight loss, and change of M_w	112
4.3.2	Visual examination	118
4.3.3	Nuclear Magnetic Resonance (¹ H-NMR) study	119
4.3.4	Application of Monte Carlo model	122
4.3.5	Thermal analysis	124
4.3.5	SEM analysis	126
4.4	Effect of Drug Chemistry	128
4.4.1	Water absorption of PdILGA and PILA	128
4.4.2	Weight loss of PdILGA and PILA	131
4.4.3	SEC analysis of PdILGA and PILA	134
4.4.4	Change of glass transition temperature (T_g)	138

Table of Contents

4.4.5	Change of the degree of crystallinity	141
4.4.6	SEM analysis	143
4.5	Summary	145
Chapter 5	Drug Release Mechanisms	148
5.1	Introduction	148
5.2	Effect of Type of Drug on Drug Release Mechanism	149
5.2.1	PdILGA	149
5.2.2	PILA IV= 4.37	153
5.3	Effect of Composition on Drug Release Mechanism	155
5.4	Effect of Crystallinity on Drug Release Mechanism	157
5.5	Effect of Glass Transition Temperature	159
5.6	Summary	160
Chapter 6	Drug Release Mechanisms From Stent	162
6.1	Introduction	162
6.2	Effect of Shape on Drug Release Mechanisms	164
6.3	Drug Release Mechanisms of Different Drugs From Stent	168
6.4	Summary	174
Chapter 7	Conclusion	176
Chapter 8	Recommendations	183
References		186
Appendix I		209
List of Publications		230

Abstract

ABSTRACT

PILA, PdlLA and PLGA copolymers were examined for the details of their degradation behaviour. All polymers exhibited a homogeneous mode of degradation, with uniform rates of degradation throughout the film. Crystallinity inhibited water absorption, and hence retarded degradation. Addition of GA units substantially increased degradation rates, and this effect overwhelms the effect due to hydrophilicity. The glycolide linkage was more susceptible to hydrolytic scission than lactide. We used ^1H -NMR to quantitatively confirm this. In spite of the faster degradation rate for the GA units, the overall scission was still a random process, as verified by the application of a Monte Carlo model to the degradation results. The details of the mechanistic study of different factors influencing the process of degradation as reported here, may have important implications in terms of selecting the right material for specific biomedical applications.

The effect of the chemical nature of the drug on matrix degradation and drug release behavior of degradable polymers was studied, using lidocaine as a model drug in base and salt forms. As matrices, a range of lactide/glycolide based polymers was studied, representing variation in composition, crystallinity and molecular weight. We showed in this study that the drug in the base form has a substantial effect on the release characteristics, through an accelerating effect on matrix degradation. Study of drug release from PdlLGA showed that lidocaine salt followed a triphasic release pattern, in contrast to the biphasic release of the lidocaine. However, PILA showed a

Abstract

different drug release pattern, with only a single diffusion phase exhibited for both lidocaine and lidocaine salt.

To examine the applicability of this work to stents, we evaluated the effect of the shape and chemical properties of drugs on release behavior of degradable polymers using two potent anti-restenotic drugs such as rapamycin and paclitaxel. New HPLC methods were developed to study the drug release of paclitaxel and rapamycin *in vitro*. Two amorphous polymers PdlLA and PdlLGA, were selected for slow and fast drug release studies. Release profiles of lidocaine from films and stents of PdlLGA and PdlLA showed that the diffusion coefficient should be independent on matrix thickness; D was constant for films and stent. Both stents and films of PdlLA and PdlLGA showed a common release pattern with the first phase of diffusion controlled release followed by acceleration of drug release due to the matrix break down. The data show that release profiles may be manipulated according to the needs of administration. These release profiles may have relevance to anti-restenotic effects and to local or systemic toxic effects.

List of Figures

LIST OF FIGURES

List of Figures			Page
1.	Figure 2.1	Mechanism of thrombosis	18
2.	Figure 2.2	Molecular structure of paclitaxel	20
4.	Figure 2.3	Molecular structure of rapamycin	24
5.	Figure 2.4	Molecular structure of lidocaine	26
6.	Figure 2.5	Biodegradable device designs	28
7.	Figure 2.6	Two common profiles of drug release	29
8.	Figure 2.7	Cycle of bio-elimination of lactide and glycol	71
9.	Figure 2.8	Cyclic dimer of PGA	73
10.	Figure 2.9	Poly (lactic _n -co-glycolic _m) acid	74
11.	Figure 2.10	Cyclic dimer d-lactide	76
12.	Figure 2.11	Cyclic dimer l-lactide	77
13.	Figure 2.12	Meso lactide	77
14.	Figure 2.13	Poly l-lactic acid	78
15.	Figure 2.14	Random chain scission of PLA	79
16.	Figure 3.1	Representative glass transition temperature thermogram	85
17.	Figure 3.2	Representative thermogram after recrystallization.	86
18.	Figure 3.3	Calibration curve of paclitaxel	91

List of Figures

19.	Figure 3.4	Overlaid chromatograms of paclitaxel	92
20.	Figure 3.5	Amount of paclitaxel monitored as a function of time	93
21.	Figure 3.6	Calibration curve of rapamycin	94
22.	Figure 3.7	Overlaid Chromatograms, showing the rapamycin peak	95
23.	Figure 3.8	A comparison of the intensity of the rapamycin peak	96
24.	Figure 4.1	Water absorption, weight loss, M_w and M_n of PILA 4.37	100
25.	Figure 4.2	Water absorption, weight loss, M_w and M_n of PILA 2.04	101
26.	Figure 4.3	Water absorption, weight loss, M_w and M_n of PdlLA	102
27.	Figure 4.4	Gel permeation chromatography (GPC) spectra of PdlLA	105
28.	Figure 4.5	First-order degradation rates of homopolymers	107
29.	Figure 4.6	Glass transition, melting point, and crystallinity of PILA	108
30.	Figure 4.7	Degree of crystallinity of PILA 4.37 and PILA 2.4	109
31.	Figure 4.8	Glass transition temperatures of homopolymers	110
32.	Figure 4.9	a. SEM of PILA 4.37 reference b. SEM of PILA 2.04 reference c. SEM of PdlLA 2.4 reference d. SEM of PILA 4.37 after 365 days immersion e. SEM of PILA 2.04 after 210 days immersion f. SEM of PdlLA 2.4 after 206 days immersion	111
33.	Figure 4.10	Water absorption, weight loss, M_w and M_n of copolymers	113
34.	Figure 4.11	Log M_w vs Immersion time for PILA and PLGA 8020	114
35.	Figure 4.12	Size Exclusion Chromatography (SEC) curves of PdlLGA	116
36.	Figure 4.13	Water absorption, weight loss, M_w and M_n of PdlLGA	117
37.	Figure 4.14	a. PdlLGA samples reference b. PdlLGA samples after 10 days immersion	118

List of Figures

38.	Figure 4.15	^1H -NMR spectra of PdILGA 53/47 0.84	119
39.	Figure 4.16	Percentages of lactide and glycolide content in PdILGA	121
40.	Figure 4.17	Monte Carlo plots for PLGA 8020 and PdILGA 5347	123
41.	Figure 4.18	Traces of DSC thermograms for PLGA 8020 4.8	124
42.	Figure 4.19	Melting point (T_m) and crystallinity for PLGA 8020 4.8	125
43.	Figure 4.19	a. SEM of PLGA 80/20 4.8 reference	126
		b. SEM of PLGA 80/20 4.8 after 210 days immersion	
44.	Figure 4.21	Water adsorption of PdILGA (with and without drug loading)	128
45.	Figure 4.22	Water adsorption of PILA (with and without drug)	131
46.	Figure 4.23	Weight loss of PdILGA (with and without drug)	132
47.	Figure 4.24	Weight loss of PILA (with and without)	133
48.	Figure 4.25	Change of average molecular weight (M_w) of PLGA	134
49.	Figure 4.26	Effect of drug on (M_w) of PILA	137
50.	Figure 4.27	Change in glass transition of PdILGA (with and without drug)	139
51.	Figure 4.28	Change in glass transition of PILA (with and without drug)	140
52.	Figure 4.29	Change of degree of crystallinity of PILA	141
53.	Figure 4.30	a. SEM of PdILGA film before immersion into buffer.	143
		b. SEM of the lidobase loaded-PdILGA film before immersion	
		c. SEM of the lidosalt loaded-PdILGA film before immersion	
		d. SEM of PdILGA film after 37 days of immersion into buffer	
		e. SEM of the lidobase loaded-PdILGA film after 30 days of immersion	
		f. SEM of the lidosalt loaded-PdILGA film after 23 days of immersion	

List of Figures

54.	Figure 4.31	a. SEM of PILA film before immersion b. SEM of the lidobase loaded-PILA film before immersion c. SEM of the lidosalt loaded-PILA film before immersion d. SEM of PILA film after 52 weeks of immersion e. SEM of the lidobase loaded-PILA film after 42 weeks of immersion f. SEM of the lidosalt loaded-PILA film after 52 weeks of immersion	144
55.	Figure 5.1	Fraction of drug released from films of PdlLGA 53/47	149
56.	Figure 5.2	Fraction of drug release from films of PILA	153
57.	Figure 5.3	Release of lidobase from PILA 4.37 and PLGA 4.8	155
58.	Figure 5.4	Release of lidobase from PdlLA and PILA	157
59.	Figure 6.1	Effect of shape on release profiles from PdlLGA matrix	164
60.	Figure 6.2	Effect of shape on release profiles of from PdlLA matrix	166
61.	Figure 6.3	Release profiles of rapamycin, paclitaxel and lidocaine from PdlLGA stents.	168
62.	Figure 6.4	Effect of release medium composition on Tg of PdlLGA	170
62.	Figure 6.5	Effect of medium on water absorption of PdlLGA	171
63.	Figure 6.6	Effect of drugs on release mechanism from PdlLA matrix	173

List of Tables

LIST OF TABLES

List of Tables			Page
1.	Table 2.1	Applications of burst effect	35
2.	Table 2.2	Blood flow values	70
3.	Table 3.1	Details of the polymers used in this study	91
4.	Table 4.1	First-order degradation constants of the polylactides	107
5.	Table 4.2	First-order degradation rates for PLGA 4.8 and PILA 4.37	115
6.	Table 4.3	First-order degradation constants of PdILGA drug loaded	136
7.	Table 4.4	First order degradation constants of PILA drug loaded	138
8.	Table 4.5	Summarize of First order degradation constants	145
9.	Table 4.6	Critical molecular weight of polymers	146
10.	Table 5.1	Polymers under investigation for drug release study	148
11.	Table 5.2	Drug release kinetics (lidobase and lidosalt) from PdILGA	152
12.	Table 5.3	Drug release kinetics (lidobase and lidosalt) from PILA	154
13.	Table 5.4	Release kinetics of lidobase from PILA and PLGA	156
14.	Table 5.5	Release kinetics of lidobase from PILA and PdILA	158
15.	Table 6.1	Drug release kinetics of lidocaine from PdILGA matrices	165
16.	Table 6.2	Drug release kinetics of lidocaine from PdILA matrices	167
17.	Table 6.3	Drug release kinetics of different drugs from PdILGA stent	169
18.	Table 6.4	Drug release kinetics of different drugs from PdILA stent	174

CHAPTER 1

INTRODUCTION

1.1 Background

The term biomaterials describe materials used for therapies in the human body [1]. Some biomaterials are designated to degrade slowly in the body: these biodegradable materials find several applications. Biodegradable polymers can be either natural or synthetic [2; 3]. In general, synthetic polymers offer greater advantages than natural materials in that they can be tailored to give a wider range of properties and have more predictable lot-to-lot uniformity than can materials from natural sources [2]. Generally, such polymers that lose its weight over time in the living body are called absorbable, resorbable or bioabsorbable polymer as well as biodegradable polymer, regardless of its degradation mode; enzymatic as well as non-enzymatic hydrolysis [4]. For biodegradable products to be useful, the break-down products must be non-toxic; also, the body must have a mechanism for eliminating the by products [5]. Homopolymers and copolymers based on L-lactic acid, D, L-lactic acid and glycolic acid have received interest in their medical and pharmaceutical field because of their biodegradability and toxicological safety. Poly (lactic-co-glycolic) acid (PLGA) is among the few synthetic polymers approved for human clinical use [1]. High molecular weight lactic/glycolic acid polymers are widely used in medical field as sutures. They can be processed into desired configuration and their physical, chemical, mechanical, and degradative properties can be engineered

to fit a particular need. The 'biocompatibility' of PLGA has also been demonstrated in many biological sites [6-8].

An exciting application for which biodegradable polymers offer tremendous potential is for drug delivery. *Controlled release* refers to the use of polymeric materials within which drugs are stored in reservoirs or integrated into the polymer matrices to allow sustained delivery of the drugs at a controlled rate [9]. If drug-release kinetics can be controlled, plasma concentrations of drug can be maintained in the therapeutically appropriate range and harmful side effects can be reduced. Sustained long-term administration can eliminate the discomfort associated with multiple injections and improve patient compliance [10]. Polymeric matrices can protect drugs having short in vivo half-lives and improve the bioavailability of drugs having low aqueous solubility. The drug and the matrix must be chemically compatible. Physical properties of the drug, including its molecular weight and solubility in biological fluids and in the polymer matrix, can profoundly affect the delivery rate from diffusion-controlled devices. Finally, the way in which the drug will be used, e.g., the organ that is targeted, the duration of treatment, and compliance considerations, directly affects the choice of a delivery system.

Another application of biodegradable polymers is as stents meant to keep a vessel in the body open. Stents have the potential to remain in situ for a predicted period of time keeping the vessel wall patent and then degrade to non-toxic substances. Colombo et al. [11] suggested that a biodegradable stent represents the first move toward a new concept of coronary stent. Agrawal et al. [12] evaluated a PLLA monofilament stent and suggested that some of the potential problems associated with metal stents can be alleviated by a bioabsorbable polymeric stent. Chapman et al. [13] investigated a polymeric self-expanding stent and they reported that the stent remained patent, and became endothelialized. Tamai et al. [14] reported initial and 6 month results of biodegradable PLLA

coronary stent in humans. Their preliminary experience suggested that coronary PLLA biodegradable stents are feasible, safe, and effective in humans. In addition, researchers have discussed the concept of incorporating drugs into stents [15]

To some, the ideal solution for restenosis is a more flexible [16; 17], non-permanent [18] and biodegradable stent, which can release certain actives therapeutics locally to prevent restenosis [19]. Therefore, the approach with the best chance of success is one that involves polymeric eluting stents for drug delivery, preferably locally. Local rather than systemic therapy is crucial to the success of this interventional treatment [19].

Two types of drug are needed [20-22]:

1. An anti-thrombotic agent to prevent the formation of a thrombus or clot in the vicinity of the vessel treated.
2. An anti-proliferative or anti-inflammatory agent that minimizes cell growth around the treated artery.

The eventual choice will depend on the following factors [15]:

1. Compatibility of the drug to the process conditions used in making the prototype.
2. The potency of the drug, and in a related sense, the required loading of the drug for efficient action over a 2-4 month time period related to pathology of restenosis.
3. The release characteristics of the drug from the polymer.

Chapter 1

At least one of the drugs needs to be incorporated into the polymer [23]. Natural biodegradable polymers like bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin, and hemoglobin have been studied for drug delivery. The use of these natural polymers is limited due to their higher costs and questionable purity [24].

The polymers selected for the parenteral administration must meet several requirements like biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties along with the ease of processing.

The lactide and glycolide family of polymers has been chosen for this study, as these polymers are commercially available, approved for application involving human body and approved by the FDA (Food and Drug and Administration), for several therapeutic applications, because of their biodegradability, biocompatibility, and the long history of safe use in humans [21].

1.2 Objectives and Scopes

1.2.1 Problem statements and objectives

Drug release from biodegradable polymer matrices is controlled by polymer erosion or diffusion through the polymer matrix or pores in the matrix. However, it remains difficult to predict drug release profiles, because these profiles are affected by many factors, including polymer average molecular mass (M_w), copolymer ratio, crystallinity, preparation and properties of the incorporated drug and so on.

Despite the fact that the drug hydrophilicity may have an impact on release kinetics of the matrix, its effect on drug release from PLA and PLGA matrices has not yet been fully explored in a

systematic way. Therefore it is desirable to characterize the effect of chemical properties of the drug on drug release from the matrices using a series of related compounds. Also, auto-catalysis by acidic degradation products, which are retained in the polymer matrix, is thought to accelerate degradation of PLG chains inside the matrix. In general, mechanistic details of the degradation process for these polymers are occasionally contradictory.

The purpose of this study has been to study the mechanisms of drug release and the degradation process of selected bioerodible polymers. Such mechanistic studies are critical in the research and formulation of controlled-release systems, as the studies help to select the optimum formulation for a given application. The focus is on drug delivery from polymer-drug formulations with an application in biodegradable coronary stents.

Keeping in view the above-discussed aspects, the overall objective of the current research is:

Study the mechanisms of drug release including both diffusion and degradation controlled release.

Related objectives include

- a. The rate and mechanism of biodegradation of selected polymers with and without incorporation of drug.
- b. Establish the overall feature of degradation:
 - Random or non-random chain scission?
 - Homogeneous or heterogenous matrix erosion?
- c. The factors that influence the relative contributions of bioerosion and diffusion to the overall drug release.
 - Effect of addition of drug in salt form

- Drug in base form
 - Matrix crystallinity, molecular weight and composition
- d. Preparation of drug-loaded stents from polymers with slow and fast drug release characteristics, with two potent antithrombotic drugs, paclitaxel and rapamycin with an objective to evaluate the release rates.

1.2.2 Project methodology

In order to achieve the above objectives, our methodology involved the following steps:

- a. Effect of the polymer and drug features on biodegradation rate and drug release mechanisms.
- Effect of copolymer composition (PLGA 80/20 IV=4.8 gr/dl and PLLA IV=4.37 gr/dl).
 - Effect of average molecular weight (M_w) (PLLA IV= 4.37 gr/dl and IV=2.04 gr/dl; PLGA 53/47 IV=0.47 gr/dl and IV=0.84 gr/dl).
 - Effect of crystallinity (PdLLA IV= 2.4 gr/dl completely amorphous and PLLA IV=2.4 gr/dl semi crystalline).
 - Effect of chemical properties of drug (salt and base).

Additionally, to evaluate the release of anti-restenotic drugs, we studied the following:

- b. Development of new methods for the study of drug release of paclitaxel and rapamycin, in vitro.
- c. Effect of shape on drug release rates (stent vs films).

CHAPTER 2

LITERATURE SURVEY

For the sake of convenience, the literature review section has been divided into the following sub sections:

- Polymers in Controlled Release Technology
- Overview of Stents
- Drugs Used to Prevent Heart Disease
- Drug Release Mechanisms from Biodegradable Polymer
- Matrices Mechanisms of Matrix Erosion
- Biocompatibility and Bio-elimination
- Mechanical Properties
- Characteristics of Biopolymers

2.1 Polymers in Controlled Release Technology

Conventionally, active agents are administered to a system by non-specific, periodic application. A constant release prevents the problems of cyclic variations in the drug concentrations in blood with

time and offers a maximum pharmacological efficiency at a minimum drug dose. For example, in the conventional practice of medicine, drugs are introduced at periodic intervals by ingestion of pills, liquids, or by injection. The drug introduced into the body gets distributed throughout much of the body by the natural physiological process, rather than being directed to a specific target organ. Certainly, a more desirable regime would be to release the active agent at a controlled rate that maintains its concentration in the system within optimum limits, and it would be even better to release agents directly to the target area of the system, if one exists or can be identified. In spite of considerable efforts, drug release rates often deviate from an ideal "infusion like" profile, generated by zero-order release kinetics [25]. This technology is not limited to medicine: controlled release has been used for pet flea collars, pesticides, anti-fouling agents, fertilizers, and fragrances [6,26].

A fascinating field is the development of drug delivery systems with a prolonged and controlled release of pharmaceutical drugs from a biodegradable carrier. The process can be described as a superposition of diffusion and the changing diffusion constant via reduction of the matrix molecular weight and formation of porous structures. The pores are formed as low- M_w degradation products leach out and are usually water-filled.

In the field of medical applications, contraception or fertility control has historically been one of the most publicized applications of biodegradable polymers. Similar formulations to deliver narcotic antagonists [27], fluorides for dental purposes, drugs to combat cancer and cardiac arrhythmia have been reported [25]. Administration of drugs to the ocular, nasal, buccal, rectal, and vaginal cavities presents the opportunity to deliver drugs directly to the site of action or, alternatively, to avoid hepatic first-pass metabolism [28].

In spite of extensive research into the use of biodegradable polymers for drug delivery, there are few commercial products. The first product was a gonadorelin analog used against prostate cancer,

breast cancer, endometriosis, uterine fibroma, and other indications. Other drugs used for the long-term delivery include leuprolide acetate, a peptide hormone analog [26; 29; 30].

Of drug delivery particular interest are formulations with drugs such as:

2.1.1 Localized delivery

One of the first clinically used controlled release polymer systems was the Ocusert, a reservoir system designed to improve therapy for glaucoma, one of the world's leading causes of blindness. The conventional treatment involved the use of pilocarpine eye drop four times a day. The eye drops often caused side effects, and patient compliance was poor. The Ocusert delivers pilocarpine (20 or 40 $\mu\text{g}/\text{hour}$) continuously for one week and controls intraocular pressure with lesser drug and fewer side effects. Despite these advantages, the Ocusert never achieved widespread use because of poor acceptance by older patients who were reluctant to adjust to this system [26].

Another example where localized delivery may be beneficial is the delivery of 5- fluorouracil (5-FU) and mitomycin C (MMC), two anti-metabolites for their beneficial effects on glaucoma filtration surgery, in patients with high risk of failure [31]. The therapy consists of a daily sub-conjunctival injection for up to 7 days, is very painful for the patient and increases the risk of side effects and infections. Thus, it would be useful to develop systems, which provide a localized and sustained release of the therapeutic agent over an extended period of time [32].

Tuberculosis still remains the cause of high mortality and morbidity, particularly in developing countries. It is curable to a large extent but has resurfaced due to emergence of multi-drug resistant strains and AIDS pandemic. Short term chemotherapy involves daily administration of four drugs namely isoniazid, rifampicin, pyrazinamide and ethambutol for 6-8 months and this has many disadvantages like toxicity, decreased bioavailability at the target sites and most importantly, non-compliance by the patient thus leading to drug resistance. The shortcomings of this route of chemotherapy can be circumvented by delivering a drug in a sustained manner; over a longer

period of time. Ain et al. [33] developed an oral formulation based on PLG micro-particles for delivery of anti-tuberculosis drugs. This class of polymers release drug in a controlled manner at therapeutic levels with minimal toxicity.

Ike et al. [34] developed adriamycin (ADR)-containing PILA microspheres for a slow-releasing and long acting delivery system. Anticancer agents generally harm not only tumour cells but also normal cells, causing unavoidable side effects. Therefore, reduction of the systemic drug concentration and enhancement of the local concentration in the target tumor are required to obtain the maximum pharmacological activity with minimal systemic side effects. The results show that the formulated ADR-microspheres followed an almost constant release and disappeared within 20 days. ADR-microspheres, administered in muscles of rabbits were biodegraded, releasing all of the ADR in less than 2 weeks, with the formation of scar tissues. Local administration of ADR-microspheres led to a high concentration of released ADR at the administration site related to a strong anticancer effect and a very low concentration of ADR in serum related to low systemic side effects. When ADR-microspheres were administered at a suitable dose, the drug was more effective against tumor cells, as compared against the administration of free ADR. The problem with traditional chemotherapy, which is done on an outpatient basis, is that the drugs disperse in the bloodstream in about 15 minutes. An outpatient chemotherapy session lasts one to two hours. If it takes an hour for the drugs to make it to the brain, such a small amount of medication reaches the tumor site that it does not make much of an impact. Also, the blood-brain barrier: the lining of the brain that protects it from poisons in the bloodstream and the skull are two major obstacles and tends to further lessen the amount of drug that makes it to the remaining cancer cells.

2.1.2 Sustained systemic delivery examples

Leuprolide acetate is useful in the treatment of hormone-dependent diseases in males and females. To eliminate the inconvenience of daily injection of the conventional parenteral solution and to

achieve greater efficiency, Okada et al. [29] developed one-month release injectable microspheres of the drug using PLGA. Single injection of this one-month depot provided sustained serum levels of the drug and suppressed serum gonadotropin and testosterone levels and growth of reproductive organs for over one month in male rats and dogs. Clinical studies confirmed the expected therapeutic efficacy in patients with advanced prostate cancer, uterine leiomyomata, and endometriosis upon chronic administration of this depot formulation.

The use of polymers to deliver contraceptive steroids has been widely studied. Norplant™, a subdermal reservoir implant composed of a non-degradable polymer matrix that releases the drug over a period 5 years. Progestasert™, an ethylene-vinyl acetate copolymer reservoir that contains a 3-day supply (38 mg) of the amount of progesterone; normally taken orally. It delivers progesterone locally at a rate of approximately 65 µg/day, lasts for over one year [26].

For certain clinical applications, a delivery system that can ensure continuous release of peptides for longer than 30 days would provide a convenient and efficacious system for delivery of these compounds. A 90-day duration provides for administration of only four times per year providing better patient compliance. This study showed that extended testosterone suppression profiles can be obtained by microspheres made from different polymers. In two cases, microsphere combinations produced castration, which persisted through the 90-day challenge dose. By combining microspheres at the proper ratio, effective therapies can be administered over a variety of intervals. Further, microspheres can be physically combined at any ratio to modulate the release [35].

For a long time, subcutaneous implantable drug pellets using nondegradable polymers have been used for long-term, continuous drug administration. Biodegradable implantable systems release the drug over a long period of time with simultaneous or subsequent degradation in the tissue of the polymer to harmless constituents, thus avoiding removal once the therapy is complete.

Subcutaneous tissue is essentially a sheet of areolar tissue lying directly underneath the skin. It is rich in fat, but poor in nerve network and hemoperfusion. Therefore, the subcutaneous tissue is an ideal location for implantation and prolonged drug administration because of its ready access to implantation, slow drug absorption, and low reactivity to the insertion of foreign materials. It is considered that the future application of these drug delivery systems will embrace two broad areas of therapeutic utility. The first is the delivery of the new class of biological mediators, peptides and proteins. One application concerns the potential utility of polymeric systems in engineering novel controlled-release profiles to augment the immunogenicity of molecules in the new vaccination strategies [36].

2.1.3 Combinations with localized delivery

In the last two decades, localized antibiotic therapy has emerged as an important approach to treating orthopedic infections. Osteomyelitis is an inflammatory bone disease caused by microbial infection of the bone medullary cavity, cortex and/or periosteum. One of the most common causes of osteomyelitis is post-operative sepsis following orthopaedic procedures. Currently, prolonged parenteral and oral antibiotic therapy for 4-6 weeks is necessary for treatment. Some disadvantages of prolonged parenteral therapy by intravenous and intramuscular antibiotic injections are the high cost of treatment, systemic toxicity, side effects and patient discomfort. In addition, because osteomyelitis is often associated with bone destruction and limited vascularity at the infected site, systemic therapy may fail to produce therapeutic tissue levels. Therefore, localized delivery of an antibiotic to the infected site was introduced in the seventies to overcome the difficulties. Sampath et al. [37] developed a PILA drug delivery system that provides local bactericidal concentrations of gentamicin sulfate for 2-3 weeks. Cylindrical, PILA implants containing gentamicin sulfate was obtained by compression of microcapsules prepared by a non-solvent-induced, coacervation process. Release profiles of different drug loadings monitored with time, followed a square-root-

time relationship and all batches of microcapsules and implants released greater than 80 % gentamicin sulfate within 3 weeks.

For the same application, Zhang X. et al. [38] developed and investigated controlled-release antibiotic (gentamicin) films, melt-extruded cylinders, and suspension-extruded/coated cylinders of PdlLA and poly (d,l-lactide-co- ϵ -caprolactone). The coated gentamicin/PdlLA cylinder with a 30 % drug loading appears to be a promising antibiotic delivery device for treatment of bone infection in orthopedic surgery. In addition, Price et al. [39] investigated a biodegradable (PLGA) implant coating with impregnated antibiotics (gentamicin) as an adjunct to current therapy of chronic osteomyelitis.

2.2 Thrombosis and Restenosis

2.2.1 Introduction

A constriction of the artery is commonly referred to as a stenosis. The important clinical consequence arising from the constriction is leading to the limitation of flow rate of the artery. Thrombosis is the formation of a blood clot, called a thrombus, inside a living blood vessel. The mechanisms of thrombosis are identical to the mechanisms of hemostasis, clotting system that protects the body from excessive blood loss. The usual corrective treatment for stenosis is balloon angioplasty with or without stent insertion. Following this procedure drugs are given systemically to prevent another clot. Angioplasty is a surgical repair of a blood vessel, during balloon angioplasty; obstructed blood vessel is repaired by dilating it with balloon catheter. Restenosis is the process of reclosing the artery, involving a complex interaction between endothelial cells, smooth muscle cells, platelets, monocytes, lymphocytes, and lipoproteins [40]. Many studies have documented that following angioplasty there is recurrence of a significant stenosis [10; 41-43]. Restenosis occurs in 20-50 % of patients within 6 months [10; 44]. There have been suggestions

that in-stent restenosis is a prolonged phenomenon, but such good evidence suggests that it is essentially complete, like restenosis after PTCA, by 6 months, with little worsening thereafter [44]. To prevent this recurrence, the use of drugs has been suggested.

Synergistic agents that when used individually, inhibit successfully the various aspects of the mechanism of restenosis, and ideally would not interfere with each other's actions, could lead to optimal therapeutic approaches. For example, the combining of an antithrombotic agent with an anti-proliferative agent would appear to be ideal [20; 22].

A large number of drugs were investigated for prevention of restenosis and could be categorized as anti-thrombotic, anti-platelet, anti-proliferative, anti-inflammatory, calcium antagonist, and lipid-lowering drugs. All these drugs are administered systemically. A wide variety of compounds inhibit the proliferative neointimal response in animal models of arterial injury when applied locally from endovascular stents or implantable drug depots. Agents such as heparin, dexamethasone, forskolin, colchicine, paclitaxel and other antineoplastic drugs, span the full range of molecular weight, conformation and molecular charge. These physicochemical properties determine their circulation through the vasculature and penetration into blood vessels from local devices [45]. The disappointing results obtained with the large number of drugs tested may be due in part to the use of suboptimal dosages in an effort to avoid systemic intolerance or difficulty in providing controlled administration of the drugs for an adequate period of time [41 ; 46].

Development of local delivery methods has been largely driven by dual goals of high local concentrations and low systemic exposure. Local drug delivery to the arterial wall allows the transfer of drug from point of release to the target tissue, following mechanisms of drug transport and binding such as diffusion, convection and partitioning in and around target tissues. As a result, there is increasing interest in less soluble, more hydrophobic compounds. Hydrophobic compounds

are relatively insoluble in the aqueous phase, and tissue partitioning so retention are achieved via binding to available hydrophobic elements on tissue sites. Unlike hydrophilic drugs, hydrophobic compounds can possibly remain in and around target arterial tissues for some time after application. The binding of these drugs to serum proteins raises their levels in solution, but hinders their distribution through tissues. The protein binding properties of hydrophobic compounds allow for beneficial effects on transvascular transport, deposition and distribution, and may enable prolonged effect and rationally guide local and systemic strategies for their administration [47].

2.2.2 Overview of stents

A stent may be defined as a wire mesh tube used to hold open a vessel that has been cleared of excess plaque, resulting in improved blood flow to the affected body muscle. A drug-eluting stent is a device releasing single or multiple bioactive agents that can deposit in or affect tissues adjacent to the stent [48].

Stent implantation, acting as a scaffolding device, helps to optimise the initial result of balloon angioplasty. In addition, stent implantation almost completely prevents abrupt vessel closure and has significantly reduced late restenosis by eliminating acute elastic vessel recoil and in particular chronic negative vessel remodelling [7; 49]. Although the overall prevalence of coexisting conditions and severity of disease remain constant, an increase in the use of stents, from 14.2 % to 58.7 % of all procedures, was associated with a significant decrease in the incidence of major cardiac events in one year. As expected, stent implantation, as compared with angioplasty, resulted in a larger luminal diameter immediately after the procedure and a lower rate of restenosis on angiography at 6.5 months, although there was no significant difference in mortality at either 30 days or 6 months [50]. Sermys et al. [42] showed that immediately after a self-expanding stainless-steel stent (Wallstent) implantation, there was an additional increase in the minimal luminal

diameter of the vessel and a decrease in the percentage of stenosis of the diameter. However, after three months slight but diffuse narrowing was observed in the artery containing the stent. The optimal stent size has not yet been defined, but if the diameter of a stent was too large for the recipient vessel (> 1.3 times larger); it may induce excessive intimal proliferation around those stent struts in intimate contact with the vessel wall.

The first biodegradable stent model was designed by Stack and Clark at Duke University. Many bioabsorbable polymers were examined, and a specialized form of PLA proved most suitable for further study. In vivo testing revealed that the stent was able to prevent significant collapse in the femoral arteries of dogs. The polymer degradation was nearly complete by 9 months, with minimal inflammatory response [12; 51]. The Higaki-Tamai [13] PILA self-expanding stent was the first one to be implanted in humans. Within 6 months study in human coronary arteries reported that repeat angioplasty for in-stent restenosis was performed easily and safely. The morphology of in-stent restenosis was similar to the metallic stents and histological findings suggest biocompatibility of PILA stent.

The Kyoto University bioabsorbable stent made of PGA was successfully deployed in canine coronary arteries, with subsequent endothelialization and partial degradation within the vessel in 2 months [12].

It has been estimated that the local drug delivery through a polymer stent can deliver a drug dose to coronary artery that is 10 times higher than systemic administration. Thus, coated stents have been successful at reducing restenosis. Therefore, leading to multiple advantages including resorption of the stents, the vessel wall can preserve its normal function and surgical procedures to remove the stent are avoided. It is interesting to note that the thin polymer coating may not be as efficient due to a limited amount of drug loading, thus making it difficult to maintain a sustained

release for long time [52]. However, Hähnel et al. [53] described successful study of a biodegradable stent coating based on a high molecular weight PILA, which can serve as a sustained release drug carrier. This study was designed to evaluate the local anti-proliferative potential of paclitaxel integrated into the stent coating drug carrier to inhibit vascular SMC growth in a cell culture model. Paclitaxel was found to significantly inhibit proliferation in a dose-dependent manner. Also, the National Heart Centre of Singapore, in January 2002, experimented with drug-coated Cypher stents; the stents being coated with sirolimus [54]. The Cypher stent has been clinically tested with over 200 patients in Europe and the US, and no re-blockage was reported even after six months [55].

2.2.3 Drugs used to prevent heart disease

Small compounds diffuse through tissues faster than larger ones, and charged molecules are highly soluble and thus rapidly diffuse into and out of tissues more readily than neutral hydrophobic compounds [45]. Hydrophilic compounds distribute freely but are also cleared rapidly. Hydrophobic drugs are insoluble in aqueous solutions; bind to fixed tissue elements, potentially prolonging tissue residence and biological effect [47]. Two classes of drugs are of interest in this study: anti-thrombotics / anti-coagulants that prevent clot formation, and anti-proliferatives that prevent neointimal cell proliferation.

a. Anti-thrombotics/ anti-coagulants

The anti-thrombotics prevent or minimise thrombosis, which is caused mainly by collagen or platelet aggregation inside a blood vessel (**Figure 2.1**) [40].

Figure 2.1 Mechanism of thrombosis [40]

Heparin is an anionic, sulfated glycosaminoglycan, strongly acidic and reacts with certain basic compounds with a consequent loss of pharmacological activity [56]. Heparin (HEP) is an anti-thrombotic compound that works by interfering with blood coagulation through a specific interaction with thrombin, a promoter of coagulation. Therefore, heparin has been shown to inhibit intravascular thrombosis effectively after vascular surgical procedures [46; 57], but clinically, the drug is often administered intravenously and its effects persist with a half-life of up to several hours [58]. When ingested orally, heparin is degraded to inactive oligomeric fragments while systemic administration is complicated by the need of continuous infusion and the potential for uncontrolled hemorrhage. In addition, subcutaneous administration is efficacious but is uncomfortable and associated with poor patient compliance [56]. It is clear that the mode of heparin delivery is critical to its potential, either by preventing platelet aggregation or by directly inhibiting smooth muscle cell proliferation [46]. However, De Scheerder et al. [59] reported that the heparin coating of metallic coronary stents decreases their thrombogenicity but does not improve late vessel patency and neointimal hyperplasia during the follow-up in a porcine coronary model.

Three unrelated drugs whose only common feature is their impact on the multiple arms of restenosis pathogenesis were shown to be at least partially effective blockers of restenosis: probucol, Transilat®, and abciximab [60].

Probucol has been shown to decrease retention by routine of its anti-adhesion, anti-platelet and anti-migration activity. Also, Transilat® was developed as an anti-allergic compound, but proved to be a potent anti-fibrotic, and anti-migration drug. In a preliminary small scale trial Transilat® was highly effective (57.7 % reduction of angiographic restenosis three month after directional coronary atherectomy) [60]. Abciximab is a monoclonal antibody to the glycoprotein IIb/IIIa receptor. The promising results indicate that the monoclonal platelet GP IIb/IIIa receptor antibody, when passively adsorbed to vessel wall and eluting from a cellulose polymer coated coronary stent significantly reduces platelet deposition [60]. Other authors have shown the potential effect of platelet GP IIb/IIIa on restenosis [61-62].

Other anticoagulants include warfarin or Hydroxy-3-(3 oxo-1-phenylbutyl) coumarin ($C_{19}H_{16}O_4$, FW = 308.3 g/mol). Warfarin is the most commonly used oral anticoagulant, because a single dose causes a stable anticoagulation as a result of the excellent oral absorption [63]. In addition, because of its ability to inhibit platelet aggregation, aspirin has been extensively investigated for potential therapeutic effects in the prevention of thrombosis [64; 65].

b. Anti-proliferatives

Paclitaxel [66-70] and rapamycin [66; 71-73] have recently generated interest as potent drugs to prevent clotting. In addition, Yang D et al. [74] patented (US Patent) a stent coating including an agent selected from the group consisting of paclitaxel, paclitaxel analogues, paclitaxel derivatives, and combinations thereof.

Paclitaxel ($C_{47}H_{51}NO_{14}$, FW= 853.9 g/mol) (**Figure 2.2**), a natural or semi-synthetic diterpene ($T_m = 223\text{ }^{\circ}\text{C}$ [75]), is an antineoplastic agent. Paclitaxel consists of a rigid taxane ring and a flexible side chain. It is an anti-tumor agent (has anti-tumor activity against ovarian, breast, lung and prostate cancer), acting as a promoter of tubulin polymerization. Paclitaxel occurs as a white to off-white crystalline powder, highly lipophilic and insoluble in water (0.01 mg/ml) and possesses no side groups that can be ionized in an acceptable pH range [47].

Figure 2.2 Molecular structure of paclitaxel

The poor solubility in aqueous solution, make paclitaxel an excellent candidate for sustained delivery from stents and prolonged deposition in atherosclerotic vessels. Values for paclitaxel solubility of 0.7 $\mu\text{g/ml}$, 6 $\mu\text{g/ml}$ and 30 $\mu\text{g/ml}$ have been reported. However, Liggins et al. [76] have reported an apparent solubility of paclitaxel of about 3.5 $\mu\text{g/ml}$ at 37 $^{\circ}\text{C}$ in water.

Paclitaxel exhibits a single melting endotherm at 223 $^{\circ}\text{C}$ and a degradation temperature (T_d) of $248.5 \pm 1\text{ }^{\circ}\text{C}$. Paclitaxel was stable for 96 days in relative humidities up to 94% at 25 $^{\circ}\text{C}$, and paclitaxel.2H₂O stored in a vacuum oven at 25 $^{\circ}\text{C}$ and 200 mmHg for several weeks showed no evidence of weight loss or dehydration [77].

Because of the nonlinearity of paclitaxel pharmacokinetics, relatively small changes in dose may lead to large changes in peak plasma concentrations and total drug exposure. In addition, paclitaxel has developed some dose safety concern including Neutropenia and hypersensitivity reactions [78].

Sollot et al. [79] found that taxol interferes both with platelet-derived growth factor-stimulated vascular smooth muscle cell (VSMC) migration and proliferation in the carotid artery after balloon dilatation and endothelial denudation injury. Baumbach et al. [80] reported that by using infusion parameters with a balloon support, drug concentrations in arterial wall were significantly higher for the lipophilic paclitaxel, as compared to the hydrophilic reviparin. The results demonstrated that the characteristics of the delivered drug contribute largely to the delivery efficiency. In addition, Nativ et al. [67] reported that a dose-dependent inhibitory effect of the drug on cell growth was evident. However, at the paclitaxel concentrations used, cell growth was only partially inhibited. These studies revealed a partial inhibition of cell growth in 2×10^{-8} M paclitaxel and a 30-fold increase in concentration had only a small additional effect on the degree of inhibition. Lovich and al. [45] quantified carrier-mediated transport for paclitaxel by determining, in the presence or absence of carrier proteins, the maximum concentration of drug in aqueous solution, the diffusivity in free solution, and the diffusivity in arterial tissues. When paclitaxel mixed in these solutions was applied to arteries in vitro and in vivo, drug was more abundant at the tissue interface, but protein carriers tended to retain drug in the lumen. In the absence of added proteins, paclitaxel reached a maximum solubility of 3.0 ± 0.7 $\mu\text{g/ml}$ in PBS, an effective diffusivity $D_0 = 76.6 \pm 6.1$ $\mu^2\text{g/s}$, and effective diffusivity in arterial media $D_{\text{art}} = 0.22 \pm 0.08$ $\mu^2\text{g/s}$. Compared with drugs that do not exhibit such binding, the effective diffusivity of paclitaxel is much lower than would be predicted from its molecular weight. For example, heparin is extremely hydrophilic and does not partition into elements of the arterial wall as does paclitaxel. The effective diffusivities of paclitaxel in arterial media are ~40 times slower than that of heparin (7.7 $\mu^2\text{g/s}$) despite the

smaller molecular weight of the former (854 Da) compared with that of the latter (14 kDa). Creel et al. reported that at the outer edge of the media, 0.45 mm from the lumen border, the paclitaxel partitioning increased gradually to peak within adventitia. The gradual decline in partitioning at the outer edge of the adventitia is likely due to the corresponding non-uniform radial thickness of this arterial layer. Demonstration of the beneficial effects of paclitaxel at retarding post-vascular interventional intimal hyperplasia may stem in part from the physical properties of this compound and its interaction with tissue elements. Hydrophobic drugs such as paclitaxel heavily partition into tissues, and drug binding significantly slows transport, leading to large localized concentrations adjacent to sites of application. The important role hydrophobicity plays in transarterial transport is further demonstrated by comparing the average deposition of paclitaxel to that of a very hydrophilic molecule, heparin. The average deposition for paclitaxel when compared with heparin is 19.4 fold higher after endovascular and 25.6 fold higher after perivascular applications. This increased deposition is the result of the ability of paclitaxel to bind to many more non-specific sites than heparin. The measured diffusivity of heparin in the arterial media was $7.73 \times 10^{-6} \text{ mm}^2/\text{s}$. The estimates of the effective diffusivity of paclitaxel in arteries ranged from 1.26×10^{-6} and $4.87 \times 10^{-6} \text{ mm}^2/\text{s}$. The diffusivity of heparin in arterial tissues is more than double that of paclitaxel, despite the larger size of the heparin molecule (14000 and 854 respectively).

Because of its anti-neoplastic nature [67; 79], paclitaxel has been proposed for local drug delivery to prevent restenosis. Site-specific delivery of drugs includes delivery to the region surrounding the vessel. For substances like paclitaxel, with the potential cardiotoxicity, this aspect has to be included in safety considerations.

Suh et al. [81] reported that paclitaxel-loaded poly (ethylene oxide)-poly (lactide/glycolide) nanospheres allowed the drug to release over 4 weeks. The short cellular residence time of paclitaxel suggests that paclitaxel needs to be formulated in a sustained-release dosage form in this system. The sustained drug release profile and cellular internalisation results suggest that

nanospheres loaded with paclitaxel may potentially be used as a local sustained drug delivery system for the prevention of restenosis. The intimal smooth muscle cell proliferation (SMC) that follows vascular injury in animal models reaches a maximum at 10-14 days after injury and ceases upon re-endothelialization.

Drachman et al. [82] determined the long-term effect of poly (lactide-co- ϵ -caprolactone) stent-based delivery of paclitaxel on the amount, rate, and composition of neointimal thickening after stent implantation. Stents, uncoated or coated with a thin layer of poly (lactide/caprolactone) copolymer alone or containing paclitaxel (200 pg), were implanted in the iliac arteries of rabbits. These authors report that the in vitro release profile followed first-order kinetics with almost 90 % drug released in about 2 months. However, about 36 % of paclitaxel is released in one day and should be considered as an important burst effect with possible risk of toxicity.

Rapamycin ($C_{51}H_{79}NO_{13}$; FW= 914.2 g/mol) is a carboxyl lactone-lactam macrolide (**Figure 2.3**). Rapamycin has been shown to have both anti-fungal and anti-neoplastic properties. It can dissolve in chloroform (5 mg/ml), methanol (25 mg/ml) and DMSO (25 mg/ml). Rapamycin is also soluble in ethanol, ether, acetone, propylene glycol, glycerine, polysorbate 80 and polyethylene glycol 400, and is substantially insoluble in water. Because of its remarkable solubilizing power, the solubilization approach focused on the benzyl alcohol as the primary solubilizer. Other aromatics with similar physicochemical properties such as benzoic acid would also be expected to possess similar solubilizing power toward rapamycin. Rapamycin contains no functional groups that are ionisable in the pH range of 1-10. The solubility in water was determined to be 2.6 μ g/ml [83].

Figure 2.3 Molecular structure of rapamycin

The antibiotic rapamycin is also a powerful antirejection agent, which acts by inhibiting cytokine production, T-cell growth, and clonal expansion. Its ability to inhibit the growth of vascular smooth muscle cells may be of value in cardiac treatment. Moreover, this inhibition appears to be a long-term effect [84].

Sirolimus when used on coated stents has been shown to be effective with a remarkable restenosis rate of 0 % [73; 85; 86]. These findings provoked considerable enthusiasm, but also some scepticism. The major criticism focused on the lack of data in complex lesions and on the lack of long-term data [87]. The first multicentre trial with stents coated with sirolimus was by Morice et al. [85] in a trial of 238 patients, restenosis of 50 % or more at 6 months was 0 and 27 % respectively, with sirolimus or normal stents after percutaneous revascularisation. Sousa et al. [73] studied the implantation of 18 mm sirolimus-eluting BX velocity stent (Cordis) in human trials. Zero percent of restenosis rate was achieved compared to more than 20 % with uncoated stent, Degertekin et al. [87] studied the value of sirolimus eluting stent implantation in patients with complex in-stent restenosis. Coated BX Velocity stents with $140\text{ }\mu\text{g sirolimus}/\text{cm}^2$ metal surface area in a slow release formulation (> 28 days). The results show that the implantation of the stent

in patients effectively prevents neointima formation and recurrent restenosis at four months of angiographic follow-up. Klugherz et al. [88] in a study of coated stents with a nonerodable polymer ($< 20 \mu\text{m}$ thick) incorporating sirolimus were implanted in iliac arteries of rabbits. A low ($64 \mu\text{g/stent}$) and high dose ($196 \mu\text{g/stent}$) coating of sirolimus were compared with polymer-coated and uncoated stents. The follow up period was 28 days and neointimal formation was reduced by 23 % with low-dose formulation and 45 % with the high-dose formulation, compared to control animals. Regar et al. [86] reported a sub analysis of the multicenter RAVEL trial to investigate the relationship between vessel size and late lumen loss after implantation of sirolimus-eluting stent (18 mm sirolimus-eluting Bx VELOCITY™ stent or a uncoated stent), because is believed that small vessels tend to have relatively more neointimal hyperplasia than larger vessels. Quantitative coronary analysis was performed at baseline and at 6-month follow-up. The results show that the sirolimus coated stent prevents neointimal growth and late lumen loss irrespective of the vessel size with a restenosis rate of 0 %, in contrast to uncoated stent.

Very few drugs have demonstrated an unequivocal reduction in the incidence of restenosis [41; 59], except rapamycin [73; 85; 86] and paclitaxel [66; 82]. The unsuccessful attempts to control restenosis by systemic pharmacological intervention has prompted many researchers to look for more promising therapeutic approaches such as local drug delivery [59]. The success of rapamycin and paclitaxel-coated stents in small studies, confirms the advantage of local vs systemic delivery of these agents.

Sirolimus and paclitaxel may be the first successful attempts at a drug-eluting stent but many other drugs (other immuno-suppressants, antineoplastic agents, vascular endothelial growth factors) are under evaluation. Nevertheless, despite great enthusiasm over drug-eluting stents, their average market penetration rate in Europe has only been 6 %, after regulatory approval. One of the most debated issues is the large increase in costs of the procedure [48]

2.3 “Model” Drugs

Of the drugs used in extended release products, 63 % may be clinically classified as weak bases, 15 % as weak acids and the remainder (22 %) as nonionizing compounds or as salts. Thus bases are far most common group and vast majority are incorporated as compared to the acid or the salt form. The most common salt forms used are hydrochlorides, sulphates and maleates [89].

In view of the extensive nature of most of the drugs mentioned above, we have used two “model” drugs for our initial studies. The drugs are described in this chapter. These are lidocaine (lidobase) and its salt form, lidocaine hydrochloride (lidosalt).

2.3.1 Lidocaine (base)

Lidocaine (Xylocaine, $C_{14}H_{22}N_2O$, FW=234.3 g/mol) is a widely used amide-type local anaesthetic (**Figure 2.4**). Because of considerable steric hindrance, the compound is extremely stable against hydrolysis. Lidocaine is a white, crystalline powder, practically insoluble in water, very soluble in alcohol and in methylene chloride and freely soluble in ether. Lidocaine exhibits a melting point, T_m , in between 66 and 70 °C [90].

Figure 2.4 Molecular structure of lidocaine

2.3.2 Lidocaine hydrochloride (salt)

Lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot H_2O, HCl$, FW=288.8 g/mol) is a white crystalline powder, very soluble in water and freely soluble in alcohol (pKa 7.86). Lidocaine hydrochloride exhibits a melting point T_m between 74 and 79 °C [91]. The drug, an amide-type local anesthetic, is also used as an antiarrhythmic agent [92].

2.4 Drug Release Mechanisms from Biodegradable Polymers

Bulk erosion leads to hydrolysis throughout the bulk of the polymer matrix and the mechanism of drug release involved, is a combination of diffusion and erosion [36].

Surface erosion leads to hydrolysis of the polymer matrix confined to the outer surface only, and the interior of the polymer matrix remains essentially unchanged. The rate of drug release remains constant with time, provided the devices maintain a constant surface geometry [36].

2.4.1 Effects of degradation on drug release mechanisms

Because kinetics of drug release and behaviour of the devices are significantly different in bulk erosion and surface erosion, they are discussed separately.

a. Effect of bulk erosion of matrix on drug release profiles

In bulk erosion, hydrolysis occurs throughout the bulk of the polymer, and in general, an analysis of kinetics of drug release is complex because it combines diffusion ($k_{dif.}$) and erosion ($k_{er.}$) (Figure 2.5).

For bulk eroding polymers, the drug profiles may resemble one of the following:

- Complete diffusion control (first-order drug release):

$k_{dif.} \gg k_{er.}$; leading to a square root of time dependence ($t^{1/2}$).

The effect of bulk erosion on diffusion release is clearly shown in **Figure 2.6**. During the early stages where little polymer erosion occurs, rate of drug release is typically first order and is determined by simple diffusion (A).

- Complete erosion control (zero-order drug release):

$k_{er.} \gg k_{dif.}$; leading to a linear time dependence (t) with no release until appreciable weight loss occurs.

- Combination of diffusion and erosion (bi-phasic):

Corresponding to a two-phases profile with a first phase dependent on the square root of time, followed by a second phase dependent of time. As the polymer begins to erode, release rate increases because it is determined by a combination of diffusion and polymer erosion (**Figure 2.6**).

Figure 2.5 Biodegradable device designs; (A) degradation controlled; (B) diffusion Controlled [93].

Figure 2.6 Two common profiles of drug release [93]

b. Effect of surface erosion on drug release profiles

For surface-eroding polymers, hydrolysis of the polymer is confined to the outer surface, and the interior of the matrix remains essentially unchanged. In order to have purely erosion-controlled systems, polymer erosion has to be faster than water uptake or drug diffusivity [36].

Furthermore, because release of the drug is a direct consequence of the erosion process, release rates are predictable if the erosion process of the polymer is understood. The release patterns can be divided into those that release drug at a slow zero or those that provide an initial rapid dose, followed by slow zero release of sustained component. Formulations that release drug at a slow zero order rate are more common than those containing a fast release component. For an ideal heterogeneous eroding system (negligible edge effects), the release kinetics will equal the degradation rate of the polymer. As the degradation boundary moves into the bulk, the drug is released into the surrounding media. As a result, the drug release kinetics is predictable, and zero order release kinetics will be obtained.

2.4.2 Drug release kinetics review

The physical erosion mechanisms may be characterized as heterogeneous or homogeneous.

a. Heterogeneous erosion only

For a simple slab of area A containing a uniformly dispersed drug at concentration C_0 , the amount of drug released at any time, t is [93]:

$$\frac{dM_t}{dt} = BC_0A \quad (2.1)$$

Where B is the surface degradation rate (cm/s).

Integrating, we get

$$M_t = BC_0At + K = G \times t + K \quad (2.2)$$

At $t = 0$, $M_0 = 0$; so $K = 0$

If $G = BC_0A = \text{zero order rate} = \text{constant}$, then

$$M_t = G \times t \quad \text{Zero order drug release} \quad (2.3)$$

b. Homogenous erosion

The release of drug from homogeneously eroding matrix is more complicated [94]. The Higuchi model (which assumes drug loading is greater than C_s , where C_s is the limit of solubility in the polymer) describes the release of the drug initially from the surface of the slab and progressively through a thicker drug-depleted layer. Using this model for a slab, the following expression *Eq.2.4* for drug release has been derived [93]:

$$dM_t = \frac{A}{2} \left(\frac{2PC_0}{t} \right)^{1/2} dt \quad (2.4)$$

Where M_t is drug released at time t , P is the permeability of the polymer, A is the surface area of both sides of the slab (cm^2), and C_0 is the initial concentration of the drug in the slab (mg/cm^3).

If P is constant, then

$$dM_t = C_1 t^{-1/2} dt$$

$$A \times \sqrt{\frac{PC_0}{2}} \quad (2.5)$$

Where $C_1 =$

Integrating **Eq. 2.5**,

$$\int_{M_0}^{M_t} dM_t = 2C_1 \int_0^t \frac{dt}{t^{1/2}} \quad (2.6)$$

So,

$$M_t - M_0 = 2C_1 t^{(1/2)}$$

Thus,

$$M_t = 2C_1 \sqrt{t} + M_0$$

at $t=0$ $M_t=0$, so $M_0=0$

So

$$M_t = 2C_1 \sqrt{t} \quad \text{First order drug release} \quad (2.7)$$

A plot of the integrated form of this equation along with diffusion release without erosion; is shown in **Figure 2.6**. The drug release without erosion; follows square root of time kinetics ($t^{1/2}$ kinetics-first-order).

The drug release data obtained from the in vitro release study was analyzed for the rate of release, using the Higuchi [93] drug release equation given below (which assumes drug loading $< C_s$, where C_s is the limit of solubility in the polymer):

$$\frac{M_t}{M_{\text{inf}}} = k \times t^{\frac{1}{2}} \quad (2.8)$$

(where M_t/M_{inf} is the fraction of drug released, t is the release time and k is a constant characteristic of the system)

or a linear relationship:

$$\frac{M_t}{M_{\text{inf}}} = k \times t \quad (2.9)$$

for the different stages, as explained further below.

The value of the diffusion coefficient in the case of C_s is greater than C_0 , D , has been calculated according to the relation:

$$k = 4(D / \pi l^2)^{1/2} \quad (2.10)$$

where, l = thickness of film or slab.

which is valid for release of less than 60 % of initial load, as applied to a monolithic device containing dissolved drug [95]. This equation is applied along with **Eq.2.8** to calculate D .

In the case of over-loading of drug inside the polymer matrix; C_0 is greater than C_s and D , has been calculated according to the relation:

$$k = A(2DC_s C_0)^{1/2} \quad (2.11)$$

The Higuchi model is the most widely used one to describe drug release from monolithic matrices. However, Hixson and Crowell cube-root equation, as well as Weibull distribution are also mathematical models evaluated considering lipophilic matrices [96].

If both erosion and diffusion occur at comparable rates, then the situation is more complex, as the permeability, P , increasing with time. This increase in permeability is attributed to the gradual loosening of the matrix by hydrolytic cleavage, based on assumption that the permeability of the matrix depends on the number of cleaved bonds, and if we assume that the permeability of the matrix is inversely proportional to bonds remaining [93]:

$$\frac{P}{P_0} = \frac{N_0}{N_0 - Z} \quad (2.12)$$

Where N_0 is the initial number of bonds, Z is the number of cleaved bonds and P_0 is the original permeability. If we assume simple first-order kinetics, where the rate of bond cleavage dZ/dt is proportional to the number of bonds remaining, then:

$$\frac{dZ}{dt} = K(N_0 - Z) \quad (2.13)$$

Where K is the first order rate constant. Then by integration:

$$K \times t = \ln \left(\frac{N_0}{N_0 - Z} \right) \quad (2.14)$$

Eq. 2.12 and 2.13 can be combined to yield:

$$P = P_0 \times \exp(Kt) \quad (2.15)$$

Substituting this expression into the Higuchi **Eq.2.4** yields:

$$\frac{dM_t}{dt} = \frac{A}{2} \times \sqrt{\frac{2P_0 C_0 \exp(Kt)}{t}} = C_4 \times \frac{1}{\sqrt{t}} \times \exp\left(\frac{Kt}{2}\right) \quad (2.16)$$

Where C_4 is a constant equal to $\frac{A}{2} \times \sqrt{2 P_0 C_0}$

The integral of **Eq.2.16** is an approximation as a series rather than a defined expression:

$$\begin{aligned} \int x^n \exp(ax) dx &= \frac{x^n \exp(ax)}{a} - \frac{n}{a} \int x^{n-1} \exp(ax) dx = \\ &\cong \frac{\exp(ax)}{a} \times \left[x^n - \frac{n \times x^{n-1}}{a} + \frac{n \times (n-1 \times x^{n-2})}{a^2} - \dots \frac{(-1)^n \times n!}{a^n} \right] \end{aligned}$$

With $a = K/2$ and $n = -1/2$

At the third order:

$$M_t = \frac{K}{2} \exp\left(\frac{Kt}{2}\right) \times \left[t^{-1/2} - \frac{t^{-3/2}}{K} + \frac{3t^{-5/2}}{K^2} \right] \quad (2.17)$$

Because of the polymer degradation, the permeability increases and the normal decline in release rate is slowed down, before reversing completely.

2.4.3 Burst release

In many of the controlled release formulations, immediately upon placement in the release medium, an initial large bolus of drug is released before the release rate reaches a stable profile. This phenomenon is typically referred to as “burst release”. Burst release leads to higher initial drug delivery and also reduces the effective lifetime of the device [97]. Narasimhan and Langer [98] in a mathematical analysis to describe the role of the burst effect showed that the burst release is controlled by the drug solubility in the release medium as well as by the diffusion coefficient of the drug in the matrix.

The burst effect can be viewed from two perspectives: it is often regarded as an undesirable consequence of creating long-term controlled release devices, or, in certain situations, considered may be desirable (**Table 2.1**).

Table 2.1 Applications of burst effect [97]

Favorable burst release situations	Negative burst release effects
-Wound treatment (burst release followed by a diminishing need for drug) -Encapsulated flavours -Targeted delivery -Pulsatile release	-Local and systemic toxicity (from high drug concentrations) -Short half-life of drugs in vivo -Wastage of drug, economically and therapeutically -Shortened release profile requires more frequent dosing

Burst release may be the optimal mechanism of delivery in some instances. One of the current difficulties with burst release is that it is unpredictable, and even when the burst is desired; the amount of burst cannot be significantly controlled. It has been shown that many drugs need to be administered at varying rates, and for some drugs used at the beginning of wound treatment, an initial burst release provides immediate relief followed by prolonged release to promote gradual healing. In several pulsatile delivery processes, burst release may also be a goal because the active agent can then be delivered rapidly upon changes in environmental conditions that trigger the release [97].

Researchers seek to avoid burst release, because the initial high release rates may lead to drug concentrations near or above the toxic level, *in vivo*. Any drug released during the burst stage may also be metabolized and excreted without being effectively utilized. Even if no harm is done during the burst release, this amount of drug is essentially wasted, and the ineffective drug usage may have therapeutic and economic effects [97].

2.5 Other Factors Affecting Drug Release

In this section, various system parameters, which are important in determining the controlled release profiles of drugs from various polymeric drug delivery systems are reviewed.

2.5.1 Drug solubility

The term solubility refers to solubilities in different media and varies between two extremes: polar solvents, such as water and nonpolar solvents, such as lipids. The name hydrophilia, or lipophobia, refers to solubility in water, and lipophilia, or hydrophobia, to that in lipids [99].

Drug solubility in a polymeric system is an important consideration from two points of view. First, the manufacture of some drug delivery systems is very much dependent on the drug solubility in the polymer. Second, drug solubility in the polymer plays an important role in drug release kinetics and mechanisms. Low drug solubility in polymer leads to recrystallization of the drug inside the matrix and often exhibits a burst release due to the large crystals close to the outer layer of the matrix and low homogeneity of the interface drug/polymer.

2.5.2 Drug loading

The drug content has a considerable effect on the rate and duration of drug release. Micro-granules having higher drug content possess a larger initial burst drug release than that having lower content. This is due to the smaller oligomer-drug ratio. However, this drug content effect disappears when the drug content reaches certain level. There is no significant release rate difference between the micro-granules having 15 % and 20 % drug content [100].

Similarly, Wada et al. [101] found that the release rate of drugs from the PILA ($M_w = 4.7$ kDa) microspheres was greatly affected by the initial loading. In the case of 20 % insulin loaded microspheres, the burst effect reached 50 percent of the initial amount of drug compared to about 20 percent for 10 % loaded microspheres. Only 70 percent of the total amount of drug was released after 15 days. Sampath et al. [37] investigated PILA microcapsules and cylindrical implants as localized antibiotic therapy. The release of gentamicin sulfate was strongly dependent on the loading (5, 10, 33, 50, 67 % w/w). The release profiles of microcapsules containing 50 and 67 % w/w gentamicin are similar to that of microcapsules containing 33 % w/w drug, while microcapsules with 20 % w/w drug had a release profile similar to those with 10 % w/w loading and complete drug release occurred within 3 weeks. Microcapsules with higher drug loading, e.g. 33 % w/w drug or greater, showed essentially complete release of gentamicin in 3 days. Implants loaded with drug showed the same pattern with a faster release rate for the higher loadings. Okada

et al. [29] investigated in vivo release mechanisms of PLGA (18.2 kDa) microspheres charged with different amounts of leuporelin (9-18 %). The release rates were consistent with the loading of the drug in the microspheres. The release rates for the microspheres charged with 15 and 18 % were rapid due to an increase in the number of aqueous channels formed by the hydrophilic drug. After 4 weeks, microspheres loaded with 15 and 18 % of drug, released 60 % of the initial loading, in contrast to 80 % for 9 and 11 % loading.

Gümüşderelioğlu and Deniz [32] investigated the effect of drug loading on PLGA 70:30 (IV = 0.6 dl/g) loaded with mitomycin-C on drug release. The results suggest that MMC release rates and total releasable fraction, significantly increased with increasing the initial drug loading (2:300; 1:300; 0.5:300 mg drug:polymer). The release rate and the amount of released drug decreases in the following order: high, medium, and low drug loading.

On the other hand Miyajima et al. [102] reported that the fraction of drug release from PLGA 70:30 ($M_w = 4.5$ kDa) rods containing 5 and 10 % of basic drug verapamil and found that the percent release rate from the rod with 5 %-loading was larger than that from the 10 %-loaded one. Higher drug content resulted in higher glass transition, smaller water absorption and weight loss. This relationship indicates that in the 10 %-loaded rod, a larger fraction of the polymer carboxyl residues are held by the interaction with the basic drug, slowing the release. The glass transition of the 10 % loaded rod is higher than that of 5 %-loaded rod, by at least 5 °C. By implication, the effect of drug content on the release has no significant effect on drug release behaviour. In another study, Miyajima et al. [103] investigated the effects of drug content (5, 8, 10, 15, 20 % w/w) on the release of papaverine (PAP) from PLGA (4 kDa). A drug-dissolved cylindrical matrix was prepared by heat compression melting. In the case where the rods contained more than 15 % of PAP, the drug precipitated out to form crystals during release. The release profiles of PLGA rods containing 5 to 10 % of PAP were very similar, characterized by a diffusion-controlled release. However, a slower PAP release was observed with increasing drug content. Increasing PAP content up to 10 %

resulted in a decrease of diffusion coefficient and a rise in T_g . Possible interactions between the polymer carboxyl residues and the drugs, resulting in an increase in matrix rigidity, was suggested as the possible reason.

2.5.3 Effect of drug chemistry

It has been reported that the physico-chemical properties of the incorporated drug in the polymer matrix are important factors in polymer degradation and drug release. As PLA and PLGA have terminal carboxylic residues, basic drugs are anticipated to interact with the polymer and affect their own release profiles from the polymer matrix. In such a scenario, two results are possible:

Drug release can be accelerated. Basic drugs behave as a base catalyst acting on the ester bonds in polymer chains; this behaviour enhances the polymer degradation and, hence, drug release [104-106].

Also, drug release can be suppressed. Basic drugs neutralize the polymer terminal carboxyl residues, so that the autocatalytic effect of acidic chain ends on polymer degradation is minimized [107].

Bodmeier et al. [107] while studying the system based on PdlLA loaded with caffeine (2.8 %), salicylic acid (6.7 %) and quinidine (12.5 to 22.5 %), reported different drug release patterns. High molecular weight (120 kDa) PdlLA was blended in films of low- M_w PdlLA (2 kDa) containing drugs. Transparent, crystal-free films were obtained for salicylic acid and caffeine; however, the films containing quinidine increased in transparency. Low- M_w PdlLA films containing salicylic acid, caffeine and quinidine showing homogeneous dissolution of the drug can be compared and it is clear that caffeine releases faster than salicylic acid, which in turn releases faster than quinidine. The faster drug release was attributed to a reduction of the glass transition temperature, and quinidine exhibits a pattern of release complicated by interactions from carboxyl groups generated from degradation of PdlLA.

On the other hand, Li et al. [104] examined the mechanism of hydrolytic degradation of PdlLA matrix in presence of caffeine (1, 2, 5 and 20 %), in order to elucidate the influence of this basic compound. Thick plates (1.5 mm) and films (0.3 mm) were used as drug carrier matrices. At low content (< 2 %), caffeine is dissolved in the matrix and acts catalytically to increase rate of degradation. At higher loadings, caffeine was partially crystallized, and hence some of it was unavailable for catalytic action. Thus the drug release was dependent on caffeine loading in a complicated manner. The low content matrices exhibited a diffusion phase for 11 days, and a degradation phase thereafter.

Miyajima et al. [102] reported the effects of the physico-chemical properties of drug on its release properties from a matrix of poly (lactic-co-glycolic acid) 70:30 (M_w 4.5 kDa). Five basic drugs (chlorpheniramine, papaverine, diltiazem, verapamil, Nicardipine) one acidic drug (naproxen) and two neutral drugs (testosterone and griseofulvin) were investigated from PLGA cylindrical matrix. Each PLGA rod was prepared using heat compression method loaded with 5 and/or 10 % drug. Basic drugs were found to show high PLGA/aqueous medium partition coefficients, implying a strong ionic interaction with the polymer. Incorporation of the various basic drugs raised the T_g of the PLGA rods from 30 °C to more than 41 °C, presumably due to interactions between the carboxylic group of the polymer and the drugs. This interaction kept these drugs dissolved in the matrix during the release studies. The interaction also shielded the polymer terminal carboxylic residues resulting in the slower matrix erosion. In contrast, acidic and neutral drugs had only weak interactions with PLGA. Naproxen and testosterone precipitated in the matrix as crystals within 1 day of immersion in the release medium. Thus, the PLGA rods containing these drugs transformed into a drug-dispersed matrix due to water penetration into the matrix. Comparing the release profiles, the acidic drug released faster than neutral drugs, and the results were consistent with the corresponding matrix glass transition temperature because acidic drug was found to have less interaction with the main chain of the polymer than neutral ones and thus increase the glass

transition temperature. However, the water absorption was shown only for the first day so at this time point the drug release of acidic and neutral drugs are about the same so it is difficult to really use the water absorption to explain a faster drug release of acidic drug. In addition, the weight loss was very similar for the matrices loaded with acidic and neutral drugs. Looking to the difficulties to explain the results, the authors concluded that the factor controlling the drug release on the drug-dispersed rods was the solubility of the drug in the polymer matrix due to the higher solubility of the neutral acidic drug. To compare the drug release rate, acidic drug release faster than basic drugs releasing faster than neutral drugs. However, it may be noted that a comparison with drug release behaviour of the base would be difficult because of the fact that acidic/neutral drugs undergo crystallization to form a dispersed drug-matrix, in contrast to dissolved drug-matrix in case of basic drugs.

In another study, Miyajima et al. [105] investigated the effect of drug physico-chemical properties on the release of basic drugs from cylindrical matrices of PILA (M_w 4 kDa). The drug release mechanism from the PILA rods containing chlorpheniramine, diltiazem, or verapamil was found to be identical to the mechanism for rods containing papaverine. The polymer-drug interaction shielded the terminal carboxyl residues on the hydrolyzed polymer, thereby resulting in a less hydrated matrix and consequent diminished drug diffusion. Chlorpheniramine released the fastest, followed by diltiazem and verapamil. The results were consistent with the weight loss and water absorption profiles and this was due to the stronger ionic interactions between the dissociated polymer terminal carboxyl residues and the basic drug ions. Water penetration is affected by the dissociation of the PILA terminal carboxyl residues, hence when the incorporated drug strongly interacts, consequent shielding of the carboxylic residues suppresses water penetration into the rod. The same authors [106] investigated PILA (4 kDa) matrices containing acidic (naproxen (NP)) or neutral drugs as testosterone (TES) (5-10 %). They expected that the acidic and neutral drugs would precipitate out as crystals transforming the matrix from a drug-dissolved to a drug-dispersed

type. PILA rods containing the drugs used in this study was reported to be transparent and it may be assumed that the matrix loses its crystallinity due to the preparation of the rods by heat compression. Therefore, totally amorphous PILA rods do not show crystallization upon 10 % drug loading. The glass transition temperature before immersion of TES-rod was 26 °C and T_g of NP-rod was 21 °C compared to 28 °C for the drug-free polymer. The release profiles of the acidic drugs consist of a slow first stage and faster second stage. The release was reported to be completed within 10-20 days for the acidic drug and more than 2 months for neutral drug. However, for all the drugs, the data show that after about 2 days, the acidic drugs are released to the extent of 50 % of initial loading, in contrast to 20 % for the neutral drug. No degradation data was shown to confirm the drug release mechanisms proposed.

A comparison of the results reported in two studies of Miyajima et al. [105; 106]; using PILA rods (M_w 4kDa) made by heat compression and loaded with basic [105] and acidic/neutral drugs [106], shows that the extent of release of the basic drug is about 40 % of the initial loading, whereas the acidic and neutral drugs are released to the extent of 50 and 20 %, respectively, within two days of release. Drug release rate of acidic drugs was faster than basic drugs whereas neutral drugs were found to release the slowest. The reasons were not very clear but it was suggested that it was due to differences of interactions of drug-polymer and solubility of the drug in the polymer matrix.

Sung et al. [108] have reported the drug release results from PLGA matrix of two different compositions; lactide:glycolide ratio of 75:25 and 50:50 (M_w of 40 kDa to 100 kDa). Results from their study show that the rate of drug release, and matrix hydration are functions of copolymer composition; with a lower lactide/glycolide ratio favoring faster drug release and matrix hydration.

A panel of four nalbuphine prodrugs with various ester chains were incorporated into PLGA based matrices by using solvent evaporation method. The results demonstrate that, within the 28 days of experiment time, the release of nalbuphine prodrug from those matrices is consistent with a diffusion mechanism. As drug solubility decreases, the duration of drug release increases. Again,

this study is carried out with implants made of 50 mg of drug and 100 mg of polymer corresponding to a dispersed drug-polymer matrix. The data shows that different nalbuphine prodrugs release with different rates. The author explained the differences of drug release rate by the differences of hydrophilicity of the drugs.

2.5.4 Effect of polymer average molecular weight (M_w)

A study [109] on the effect of the molecular weight of PdlLA on the release of theophylline from tablets (25 % loading) showed that the rate decreases as molecular weight increases. Five molecular weight grades (3.5, 42, 92, 138, and 153 kDa) were investigated. The release slowed down progressively as the molecular weight increased from 3.5 to 553 kDa. The difference in rate of release became less significant and reached a limiting asymptotic value as the molecular weight increased to 138 kDa. With the exception of 3.5 kDa M_w , all tablets containing the higher molecular weight polymers demonstrated a diffusion controlled release mechanism. Tablets containing 3.5 kDa PdLA rapidly disintegrated and released 100% drug within the first hour.

Liggins and Burt [110] investigated PILA microspheres having molecular weights of 2, 4, 10, 50 kDa loaded (about 30 %) with paclitaxel. Three milligrams of paclitaxel loaded microspheres were mixed into 50 ml of PBS. The procedure of measuring the in vitro paclitaxel was carried out at given time points and the microspheres were resuspended in fresh PBS to maintain sink conditions, which were taken to be paclitaxel concentration in PBS not exceeding 15 % of solubility. As the molecular weight increased from 2 to 50 kDa the amount of drug released from microspheres over 14 days decreased. Based on the total content, between 11 and 76 % of the total paclitaxel was released over the 14 days of the release study. However, based on a solubility value for paclitaxel in the release medium, it was suggested that concentrations near saturation were encountered in the first 3 days of the study, but would not affect the cumulative amount released in the course of the entire study due to the experimental procedure.

Wada et al. [101] investigated the effect of molecular weight on the drug release rate from PILA microspheres loaded with insulin (10 % loading) and doxorubicin hydrochloride (ADR)(2 % loading). The release rate was greatly affected by the molecular weight (3.4, 4.7, 10 kDa). It was found that the remaining ADR amounts were 28.3 and 34 % of the initial loading for the 7.2 and 10 kDa, respectively; whereas the same reaches a value of 100 % for microspheres of 3.4 kDa. As expected, the same effect of molecular weight was shown for insulin.

2.5.5 Effect of degree of crystallinity of the polymer

Hurrell and Cameron [111] reported that the initial morphology does affect the release profiles of theophylline from the matrices. This study was carried out by small pellets of PGA ($IV = 1.2 \text{ dl/g}$) and loaded with 5 % of theophylline. Samples with higher initial crystallinity release more drugs at the earlier stages of the degradation. This was suggested to be due to a partitioning of drug molecules to the surfaces and an increase in the concentration of drug in the amorphous phase, due to the exclusion of drug molecules from the crystals.

2.5.6 Effect of composition

Gümüşderelioglu and Deniz [32] investigated the effect of lactide (LA) and glycolide (GA) ratios on the mitomycin-C (MMC) release kinetics. LA:GA ratio was varied from 70:30 to 90:10, keeping all other parameters such as loading (1:300 mg), film thickness (150 μm) and the intrinsic viscosity of copolymers (0.6 dl/g), constant. Increasing glycolide content results in an impressive increase in the release rate and release amount. After 3 months, PLGA 90:10 released only 30 % of MMC, as compared to 70 % of release from PLGA 70:30. This is due to the fact that for PLGA copolymers, an increasing glycolide content increases polymer hydrophilicity, as well as degradation rate.

2.5.7 Effect of addition of plasticizer

Andreopoulos [112] investigated the effect of plasticization (of propylene glycol) on low-molecular weight PdlLA tablets. It was clear that the rate of delivery of salicylic acid for the plasticized samples is higher than that of the pure polymer, at least for a period of 150 hours. Interestingly, as release proceeds the delivery rate seems to reach a level independent of the initial plasticizer content. These variations in delivery rate might be very beneficial in cases of treatment where a fast delivery is necessary for the first 2 or 3 days and then a slower rate is recommended. A possible interpretation should consider leaching of plasticizer by the surrounding medium, so that the composition of tablets eventually becomes the same for all.

2.5.8 Effect of gamma-irradiation

Faisant et al. [113] reported that release of 5-fluorouracil (5-FU) from microparticles based on PLGA, depends significantly on the applied gamma-irradiation dose (4 to 33 kG). Release profiles were all biphasic: a rapid initial drug release phase was followed by a slower, approximately constant drug release phase. Only the initial rapid drug release was accelerated by gamma-irradiation; the subsequent zero-order phase was almost unaffected. This could be explained with the free volume theory of diffusion: exposure to the gamma-irradiation source results in random polymer chain scission with decrease in the average molecular weight. The consequent free volume increase with increasing gamma-irradiation dose, results in more pronounced effect on drug-release.

2.5.9 Effect of mechanical stress

Arm and Tencer [114] demonstrated that the rate of release of bovine albumin from PLGA 50:50 rods correlated with bending load. Cumulative drug release was significantly higher when a cyclic bending load was applied and increased with magnitude of the load. Highly significant differences

in the rates of release of drug for the three loading cases were observed at day 1, comparing unloaded specimens and specimens with 0.5 mm of deflection. With 1 mm of central deflection of the implant under load, release was complete after 4 days. The differences in release between the 0.5 and 1 mm deflection groups were significant up to 4 days, with the faster release observed in the more highly stressed implants. There were no differences measured between specimens subjected to the different loading regimes, and no differences were found on the average molecular weight with magnitude of deflection of the implant. However, pores on the surface of the polymer in regions of highest stress, were elongated into cracks, compared with pores in the low-stress region of the same implant, which were roughly circular. Presumably these pores were responsible for the faster drug release observed.

Edelman et al. [115] proposed the use of mechanical deformation to modulate protein release in polymer matrices. The results showed self-diffusion through pores, rupture of the polymer matrix as a result of sorption of water by the hydrophilic protein, and polymer biodegradation. Mechanical loading creates stresses on the implant surface, which are greatest on the outside layer. Pores which may be the result of protein dislodgement at the surface act as stress concentrators, from which cracks initiate and enhance the rate of release.

2.5.10 Effect of shape

Karasulu et al. [116] revealed statistically how various geometrical shapes such as triangle, cylinder and half-sphere affect the release rate of theophylline from erodible hydrogel based matrix tablets. The shape of the tablet changed the release profile of the drug. The release of theophylline out of erodible hydrogel matrix tablets was found out to be highest for triangular tablets and lowest for cylindrical tablets. The reason why tablets having the same weight and formulation, and pressed under the same pressure have got different release rates is explained by a different erosion

rate. No more explanations were provided but these results were consistent with many others studies.

2.6 Mechanisms of Biodegradation

2.6.1 Introduction

Most of the biodegradable polymers contain hydrolysable bonds and the most important degradation mechanism is, therefore, chemical degradation via hydrolysis or enzyme catalyzed hydrolysis. The latter effect is often referred to as biodegradation, meaning that the degradation is mediated at least partially by a biological system. The process of “degradation” involves chain scission during which polymer chains are cleaved to form oligomers and finally to form monomers. The term “Erosion” designates the loss of material owing to monomers and oligomers leaving the polymer following degradation [117]. Hydrolysis of the labile bonds causes scission of the polymer backbone, thereby forming low molecular weight, water-soluble fractions. Polymers undergoing this type of erosion process include poly (lactic acid), poly (glycolic acid) and their copolymers [117]. Degradation properties are of crucial importance in biomaterial selection and design. The rate of degradation may affect a range of processes, such as tissue growth and regeneration, drug release and response. In cases where polymers tend to degrade too slowly or too quickly for a specific application, one might choose to regulate the velocity of chemical degradation.

To classify degradable polymers, a distinction is made between surface (or heterogeneous) and bulk (or homogeneous) eroding materials. During an application, surface eroding polymers lose material only from the surface. Although reduced in dimension as a result of such erosion, the original geometric shape is retained. For bulk eroding polymers, degradation and erosion are not

confined to the surface of the device alone. Therefore, the size of the device remains constant for a considerable portion of time during its application [118]. In the case of polymers that degrade in bulk, the rate of water penetration into the matrix is faster than the rate of polymer degradation. The process is a homogeneous one in which degradation occurs at a uniform rate throughout the polymer matrix. In contrast, for polymers that undergo surface degradation, the rate of water penetration into the matrix is slower than the rate of polymer degradation.

There are several factors that influence the overall rate of degradation: pH [118-123] and copolymer composition [118; 123]. In general, polymer degradation is accelerated by greater hydrophilicity in the backbone or end groups [117; 118; 124-129], less crystallinity [130], lower average molecular weight (M_w) [131-133], and smaller size of the finished device [128; 134].

Many other factors influence the degradation of PLGAs including flow rate [135], processing [136; 137], sterilization [137-139], strain [140], presence of plasticizers [141], humidity [142], blends [126; 129], solvents [143], buffer concentration [121], porosity [135; 144], temperature [122; 127; 145] and ionic strength of the medium [120; 121]. The degradation kinetics is furthermore affected by additives, such as metal salt [146; 147], basic [104; 148] as well as acidic drugs.

2.6.2 Effect of drug chemistry

At the moment, the literature reflects contradictions about the role played by chemically reactive additives. A possible explanation is the existence of two opposite effects. First, basic compounds can catalyse ester linkage scission and thus speed up polymer degradation [104; 148]. On the other hand, they can neutralize carboxyl end groups [102; 105] and thus decrease the degradation rate, as a result of reduced acid catalysis.

Giunchedi et al. [148] investigated the degradation profiles of diazepam loaded microparticles of PLGA 50:50 ($M_w = 34$ kDa) by a new HPLC method. Diazepam (base) loaded particles (2 % w/w)

have different polymer degradation behaviour with respect to those prepared without the drug. The rate of degradation was faster for drug loaded microparticles. About 35 % of GA monomer was released from drug loaded PLGA microspheres in 20 days, in contrast to 8 % released from blank microspheres.

Li et al. [104] examined the mechanism of hydrolytic degradation of PdlLA thick plates in presence of caffeine (1, 2, 5 and 20 %), in order to elucidate the influence of this basic compound. For low contents ($< 2\%$), it is suggested that caffeine was molecularly dispersed in matrices and considerably accelerated the matrix degradation with respect to the caffeine-free device. In the early stages of degradation, the overall catalytic effect was larger for devices with low caffeine contents than for highly loaded ones where caffeine was in crystallized state and hence less available for basic catalysis. According to the data reported, caffeine played a rather complex role in the degradation of PdlLA matrix. During early stages, caffeine clearly catalyzed the hydrolysis: however, the lower loadings degrade the matrix faster. Accordingly, caffeine should have acted catalytically according to contents, a feature which was not observed experimentally. Therefore, the larger catalytic effect observed in the case of 1 and 2 % loading, as compared to 5 and 20 % drug-loading, was ascribed to differences in the morphology of the drug at different loadings. At higher loadings, the drug is in dispersed form in the polymer and needed some time to dissolve and catalyze the degradation. In conclusion, the authors reported that the presence of caffeine appeared rather complicated because of the contribution of a number of parameters such as base catalysis, neutralization of carboxyl groups, porosity, and drug morphology.

Zhang Y et al. [147] investigated the effects of encapsulated metal salts (10 % w/w) on PLGA 50:50 (42 kDa) on water absorption and degradation properties. Polymer films were characterized with respect to the kinetics of water uptake, morphology changes, degradation, and weight loss after hydration. It was found the degradation was strongly influenced by the presence and nature of

salts. The control films without incorporated salts showed an initial water absorption of about 2 % during the first day. This was followed by a lag period during which there was negligible additional water uptake. A second phase of water uptake began approximately around 7 days and continued. The effects of salts with low water solubility (magnesium hydroxide, magnesium carbonate, calcium carbonate, and zinc carbonate) show an important initial increase of water absorption on the first day compared to the control film. Water absorption profiles for films containing water-soluble salts (magnesium sulfate, zinc sulfate, and sodium chloride) are compared with the control film. As with sparingly soluble salts, the initial water absorption by soluble salt-containing films was an order of magnitude greater than observed with the blank control. Contrary to expectations, the increased uptake of water by encapsulated salt-containing films did not bring about an increase in the rate of degradation as assessed by SEC and DSC. The reduced degradation rates was speculated to be due to the alkaline properties of hydroxide and carbonates salts, expected to disrupt the autocatalytic degradation brought about by carboxylic acids groups generated during polyester hydrolysis.

2.6.3 Effect of size of the matrix: heterogeneous vs homogeneous degradation

The size of polymer samples has been recently recognized as an important factor that affects the degradation path. It was previously believed that devices fabricated using polyester materials degraded via homogeneous bulk erosion process [119; 134; 149; 150]. Recent studies have demonstrated evidence of an autocatalytic heterogeneous mechanism in large devices [104; 151-155].

The degradation has been shown to be heterogeneous in the case of large size devices (parallelepiped devices), the rate of degradation being greater inside than at the surface. This was observed both *in vitro* and *in vivo*. However, the model also suggested that devices with

dimensions smaller than the thickness of the outer layer should degrade less rapidly than larger ones. Basically, all the leachable oligomeric compounds formed within the matrix should be able to escape from it as soon as they become soluble.

Witt and Kissel [149] reported that the rates of PLGA 50:50 ($M_w = 29.3$ kDa) weight loss decreased with increasing surface to volume ratio from tablets (1.5 mm^{-1}) and rods (2.8 mm^{-1}) to films (7.3 mm^{-1}) and microspheres (38.2 mm^{-1}). PLGA exhibited an apparent constant rate of degradation of $0.041 \text{ (day}^{-1}\text{)}$, $0.093 \text{ (day}^{-1}\text{)}$, $0.115 \text{ (day}^{-1}\text{)}$, $0.1035 \text{ (day}^{-1}\text{)}$ for microspheres, films, rods and tablets, respectively. However, the average molecular weight decreases faster for microspheres compared to tablets.

Grizzi et al. [134] showed that the degradation rate of various devices derived from the same PDLA polymer depends very much on size. The hydrolytic degradation proceeds heterogeneously in the case of large size devices, the rate being greater inside than at the surface. Suggestions have been made that devices with dimensions smaller than the outer layer; should degrade less rapidly than the larger ones. The results shows that plates have a lower water absorption and weight loss for the first 10 days, followed by a rapid increase of water absorption and weight loss to complete the degradation after 15 weeks, in contrast to films reaching only a weight loss of 25 % after 30 weeks of immersion. These results are consistent with the decrease of the average molecular weight. The greater the thickness of the device; the faster the degradation. They proposed a value of 0.2-0.3 mm as a critical thickness above which the PLGA matrix is believed to undergo heterogeneous degradation. Li and Vert [153] have shown that round plates (2 mm thick) made of PDLA degraded more rapidly inside than at the surface. The intrusion of water triggers the polymer degradation chemically, leading to the creation of oligomers and monomers. Progressive degradation changes the microstructure of the bulk through the formation of pores, through which oligomers and monomers are released, leading to weight loss.

Lu et al. [156] demonstrated that thick films degrade faster than the responding thin films of same composition. Thickness levels were 5-10 μm for thin PLGA (75:25 M_w = 60 kDa; 50:50 M_w =35 kDa) films and 85 to 100 μm for thick films. Thick films degraded faster than thin ones with the same copolymer ratio over the 10-week period and thus a greater extent of water uptake. This was evident from the accelerated profiles of mass loss and drop in molecular weight. The faster degradation of thick films was suggested to be due to the greater extent of autocatalytic effect. The intermediate degradation products were trapped inside the film before their molecular weights decreased to a critical value of about 1.1 kDa and become soluble in water. The accumulation of carboxylic groups over time, in the center of the specimen led to faster internal degradation. This was reported to be less evident for thin films, as expected. All the PLGA films degraded by heterogeneous bulk degradation. This result was in disagreement with Grizzi's critical thickness [134].

Li et al. [151] investigated the hydrolytic degradation of PdlLA pellets (M_w =65 kDa). At week 3 the specimens revealed heterogeneous cross-sections after breaking. A whitish outer layer appeared, whereas the inner part was yellowish and transparent. At week 5, the inner part of specimens appeared as a very viscous liquid, whereas the outer layer was still rigid. At week 12 most of the inner viscous liquid disappeared, whereas the outer layer remained and appeared as flattened empty shell. The SEC analysis of the outer and inner parts of partially degraded specimens revealed that biodegradation was significantly larger in the inner part than at the surface. In another study, Li et al. [153] reported a heterogeneous degradation path of semicrystalline and amorphous PLA pellets. Amorphous PLA remained transparent for 18 weeks and then turned whitish. At week 40 cross sections appeared white from the surface to the centre for the semi-crystalline in contrast to PLA amorphous specimens exhibiting a heterogeneous degradation with a surface-centre differentiation. However, after 12 weeks there was little difference in molecular weight appeared between the surface and the centre for both PLA.

2.6.4 Effect of polymer crystallinity

Other important properties of the polymer matrix that depend upon the copolymer composition, such as the glass transition temperature and crystallinity, can have additional indirect effects on degradation rates.

Nakamura et al. [157] reported *in vivo* that the decrease in M_w for both amorphous ($M_w = 2.01 \times 10^5$ Da) and crystalline ($M_w = 1.56 \times 10^5$ Da) PILA, one month after implantation, was less than 20 % of original. By the third month, the decrease in molecular weight for the amorphous sample was 30 – 40 %, while that for crystalline PILA was 70 – 80 %. The amorphous sample retained over 90 % of its initial bending strength, whereas the crystalline PILA lost more than 50 % of its initial value, over the same period of time. The reason why the crystallized PILA showed a faster degradation rate may have been due to the high temperature processed (200 °C) during crystallization.

Tsuji et al. [143] investigated the effect of initial crystallinity on the long-term *in vitro* hydrolysis of PILA films ($M_n = 1.3 \times 10^6$). Polymer films after melting were either annealed or quenched to prepare films with different degrees of crystallinity (0, 2, 30, 45, and 54 %). Within a period of hydrolysis of 24 months, it was observed that the rate of decrease in molecular weight was higher for samples with high initial crystallinity. Melting and glass transition temperatures of the PILA films increased in the first 12 months of the study, while decreasing for the next 24 months. Weight loss results are consistent with the decrease in molecular weight: with PILA 54 losing 15 % after 36 months of incubation as against PILA amorphous, of only 7 % of mass. This finding has been ascribed to the highly microporous structure or increased surface area per unit weight of PILA films having a higher initial crystallinity, formed by removal of water-soluble oligomers. In a very similar study, Tsuji and Ikada [130] examined that PILA films with higher initial degree of crystallinity underwent faster hydrolysis. The PILA films ($M_w \sim 11 \times 10^5$ Da) were annealed at 100,

120 and 140 °C for 600 minutes each, prior to immersion in buffer. An additional consideration was done on the crystalline thicknesses. It was reported that the mass loss as well as the drop in molecular weight was more rapid for PILA films with higher initial degree of crystallinity. They have explained the findings in terms of the crystalline domains introducing defects into the amorphous matrix, thus facilitating the penetration of water, more easily. This suggestion was based on possible decreases of the density of effective tie chains with an increase of degree of crystallinity.

In contrast, Li et al. [152] reported the effect of crystallinity on the degradation of high-molecular weight PILA ($M_w = 130$ kDa). Specimens of similar size were processed by compression moulding and made either amorphous by quenching or semicrystalline by annealing. The mass of amorphous specimens remained unchanged during the first 18 weeks of study, and decreased by 4 and 49%, respectively, at the end of 31 and 110 weeks. No weight loss was detected for the semicrystalline samples until 7th week, after which the weight loss was linear until 31st week. A comparison within the 7th and 31st week showed that the weight loss was higher for the semicrystalline samples. However, the opposite was observed from week 40. At week 110, PLA semicrystalline had lost only 24.6 % of its initial mass compared to 50 % for the amorphous PLA. During the first 18 weeks both types of specimens absorbed water very slowly. Beyond week 18 the two sets of specimens behaved differently, the rate of water absorption increasing significantly for amorphous PLA up to week 31 (40 %), whereas it increased slowly for the semicrystalline sample during the same period. Beyond week 31, water absorption continued to increase slowly for both sets, attaining 105 % at week 110 for amorphous PLA. Water absorption for semicrystalline samples was smaller and reached only 28 % after 70 weeks. No additional data was shown for semicrystalline PLA due to brittleness. However, no significant difference in molecular weight drop was observed between the crystalline and amorphous samples, after the 40th week. In the case of initially amorphous PLA, the degradation of polymer chains, its effect on chain relaxation

allows chains to undergo crystallization in aqueous media. The derived crystalline structures appear to be very resistant to degradation. PLA amorphous crystallized during degradation, in contrast to PLA semicrystalline so may be a larger size and thicknesses of spherulites during crystallisation of PLA could explain his surprising degradation rate. Cai et al. [158] showed the relationship between enzymatic degradation of PLA as function of heat of fusion(crystallinity). The weight loss values are almost comparable below the heat of fusion of 20 J/g. This shows that the amorphous regions are easily accessible to enzymatic attack, as the limited extent of crystallinity (low heat of fusion) does not inhibit degradation. However, there is a sudden drop observed in the weight loss values beyond a heat of fusion of 20 J/g, which is indicative of a critical amount of crystallinity that inhibits degradation. In addition, Montaudo et al. [159] have reported that copolyesters exhibit faster rates of enzymatic degradation when the sample crystallinity decreases.

At the moment, it is clear that there are confusing reports on the effect of crystallinity on the degradation rate. Tsuji et al. [130; 143] are in disagreement with Li et al. [152] findings in vitro that the crystallinity decrease the degradation rate and Nakamura et al. [157] found a faster degradation rate in vivo of crystalline PLA in contrast to Montaudo et al. [159]. However, Nakamura et al. explained this disagreement due to the processing.

2.6.5 Effect of average molecular weight (M_w)

Wu and Wang [155] reported that molecular weight has a great influence on the biodegradation rate of PLGA. Polymers with higher molecular weights degrade faster than those with lower molecular weights after the initial period. In a study of PLGA samples with the same composition but varying weight average molecular weight, M_w , (166; 124; 66; 31, 10 kDa), they observed that the rate constant of degradation decreases from 0.0969 to 0.0472, in that order. High molecular weight polymers have longer polymer chains than their low molecular weight counterparts. It was

speculated that the longer the polymer chains, more likely are chances of it being attacked by water molecules: a required condition for polymer chain hydrolysis and consequent degradation. Degradation behaviors of microspheres from two samples of PdlLA (17 kDa and 41 kDa) were examined [131]. The degradation of the microspheres during the 53 days incubation period was greatly affected depending on the initial molecular weight of the starting sample. The lower molecular weight microspheres exhibited significant degradation with reduced glass transition temperature, while higher molecular weight microspheres did not show any detectable change in the degradation until 53 days. It was found that the immediate hydration inside lower M_w microspheres allowed the polymer morphology to change from the glassy to rubbery state by lowering the glass transition temperature. This led to a physical state more susceptible to degradation. On the other hand, the high M_w microspheres were in glassy state until they started to degrade.

Wang et al. [132] used PLGA 72/28 having molecular weights of 1.317 and 3.025 kDa respectively, to study the effect of molecular weight on biodegradation. These two oligomers have a similar hydration period of 6 days. But the oligomer having high molecular weight has a lower biodegradation rate.

In contrast, Cam et al. [133] investigated the effect of molecular weight on degradation for films made of PILA (300, 450, 650, and 3000 kDa), in alkaline medium (pH 11.8). There was an increase in crystallinity from 3 to 30 % for samples from high to low molecular weight, in that order. Throughout the degradation process, PILA samples of lower crystallinity lost weight more quickly, with little water absorption. On the other hand, PILA of 3000 kDa lost almost 100 % of initial weight after 175 days, in contrast to 50 % for PILA of 300 kDa. In conclusion, it is clear that the crystallinity dominates the molecular weight on the degradation rate.

2.6.6 Effect of composition

Wang N et al. [100] studied the effect of the composition on the biodegradation rate. Three different samples with different compositions, PLGA 46/54 ($M_n = 990$), PLGA 63/35 ($M_n = 912$) and PLGA 72/28 ($M_n = 1317$), were used in this experiment. The results show that an increase of glycolic acid residue in the oligomers, accelerates the rate of weight loss. Li [160] detailed a study of the degradation mechanism and the effects of composition on the degradation of PLA/PGA polymers. PLGA 75:25 ($M_w = 111$ kDa) exhibited faster degradation than PLGA 85:15 ($M_w = 112$ kDa) due to preferential degradation of GA units assigned by higher hydrophilicity compared to LA units. PLGA 75:25 exhibited a half-life of 10 weeks compared to 20 weeks for PLGA 85:25. Wu and Wang [155] have reported the effect of composition of polymer using 5 different compositions of LA:GA monomers. A series of polymers having similar molecular weights (12.62 kDa to 14.13 kDa) but different compositions were used in this set of experiments (PLGA 50:50; 65:35; 75:25; 85:15; and PILA). The polymers with higher lactic acid content have higher hydrophobicity and consequently, lower wettability. The absolute value of the biodegradation rate constants increases with increasing glycolic acid content. The first-order biodegradation rate, k , varies from 0.0222 to 0.0544 day^{-1} for PILA until PLGA 50:50, with decreasing lactide content.

2.6.7 Effect of pH

Wu and Wang [155] used PLGA for a set of experiments having an equal molar ratio of lactic to glycolic acid (50/50) ($M_w = 13$ kDa). The effect of pH on the rate of biodegradation/hydrolysis of the PLGA was not really obvious, within first two weeks of immersion. Different rates of degradation were apparent, depending on the pH, starting around the third week of incubation. It was observed that the rate of degradation increases in acidic pH (pH 5), but slows down when the medium is alkaline (pH 9.24). While the degradation in pH 9.24 reaches a plateau, in neutral (pH 7.4) and acidic media (pH 5.0) the polymer continues to degrade, with fastest rate recorded at pH

5. The result suggests that the PLGA in a basic medium may degrade slower than in an acidic one. In the first few weeks, the integrity of the matrix is retained, although the polymer chains might undergo hydrolytic scission, leading to lowering of molecular weight. Presumably, the hydroxide ions from the medium outside and the hydronium (H_3O^+) ions generated by the initial matrix hydrolysis; cannot diffuse in and out of the matrix freely, thus accounting for the non-obvious difference in the rate of degradation at the initial stage. In contrast, Makino et al. [121] have reported that microcapsules of PLGA exhibited rapid weight loss in alkaline media (pH 8-9). Trends in decrease of weight average molecular weight, M_w , indicate that such drops occur more readily in alkaline media of pH range 8-9. While there is no change in the distribution of molecular weight in neutral solutions, there is a broadening of the distribution in alkaline solutions. Apparently, depolymerization may have been accelerated by production of intermediate molecular weight fractions. In closely related studies, Wang et al. [132] have shown that the rate of degradation of PLGA is faster at pH 9.4 than at pH 7.4, indicating that base catalysis may be dominant in the alkaline range of pH 7.4-9.0. It is known that the hydrolysis of a carboxylic ester is an equilibrium reaction. The basic incubating medium favors shifting the equilibrium to the right because one of the hydrolysis products has a free carboxylic group, which is neutralized by the alkaline buffer and hence removed. In a study of 24 days of degradation of two samples of PdlLA (M_w of 25 and 95 kDa, respectively), Belbella et al. [122] reported that degradation of PdlLA ($M_w = 25$ kDa) appeared to be directly related to the pH value, whereas that for PdlLA ($M_w = 95$ kDa) was the highest at extremes of pH. PdlLA of M_w 25 kDa was more susceptible (than its high M_w counterpart) in acid medium, losing more than 75 % of initial value. Inversely, in basic medium (pH 10.1) the degradation of PdlLA (95 kDa) was much more pronounced. Thus, 102 days of study for the sample sees 50 % reduction in near neutral body pH (7.4), whereas degradation was almost complete in both acidic (pH 2.2) and basic (pH 10.1) media. Different behaviour between the two samples of PdlLA in acidic pH can be explained by the tail spreading of the chromatogram

of PdILA (25 kDa) towards very low molecular weights, which indicates the presence of water-soluble oligomers, possibly taking a significant part in catalyzing the process of hydrolysis. The fall of M_w and especially the faster mass loss of PdILA suggest that the polymer is degraded by chain cleavage. On the other hand, with a basic medium, the degradation of low M_w PdILA (25 kDa) is lower at early stages in contrast to the trend in acidic medium. This can be explained by the adverse effect of the carboxylate ions, which form a negatively charged screen hindering OH^- ions from penetrating into the polymer bulk. Thus the attack is concentrated on the surface at early stages, which is in agreement with the moderate decrease of M_w and mass loss. Holy et al. [161] studied the in vitro degradation of macroporous PLGA 75/25 for application in tissue engineering. The in vitro degradation behavior was followed at 37 °C, over a period of 30 weeks and at one of three different pHs. The degradation profile of foams maintained at pH 5, 6.4 and 7.4 was similar until week 16, after which foams maintained at pH 6.4 and 7.4 had comparable degradation patterns whereas foams maintained at pH 5.0 degraded faster. However, Makino et al. [121] observed a faster decrease of molecular weight of PdILA microcapsules in the highly alkaline buffer solution (pH 9.6) than in the highly acidic pH (1.6-3) and slightly alkaline buffer solution (pH 7.4). However, no significant changes were observed during the immersion in the slightly acidic buffer solutions (pH 5). Since the cleavage of ester bonds takes place by different mechanisms depending on the pH of the medium (acid- and base-catalyzed reactions), large quantities of low-molecular weight PdILA are produced in the highly alkaline solution by the de-esterification reaction, via production of polymers of intermediate molecular weights. It has been suggested that water molecules penetrate first into the amorphous zone of the semicrystalline material.

At the moment, one can reasonably conclude that both alkaline and strongly acidic media accelerate polymer degradation. The difference between slightly acidic and neutral media; however, is less pronounced due to auto-catalysis by the carboxylic end groups.

2.6.8 Effect of fluid flow

Agrawal et al. [135] studied the effect of fluid flow on the in vitro degradation of biodegradable scaffolds using PLGA 50:50 (IV=0.69 dl/g). These scaffolds were subjected to degradation in phosphate buffer solution (PBS) for up to 6 weeks under two test conditions: static and flow (250 μ l/min). The results showed that the lower the porosity and permeability of the scaffolds, the faster their rate of degradation. Additionally, fluid flow decreased the degradation rate significantly. Weight loss and average molecular weight decreased much faster in case of static conditions compared to flow conditions for all samples. Because the hydrolysis of PLA-PGA materials is catalyzed by the presence of a high concentration of carboxylic end-group, the degradation products PLA-PGA materials serve as catalysts for further degradation. If these degradation products are removed from the vicinity of the reaction in an expedient fashion, they will lead to a slower rate of degradation and mass loss. It is likely that the acidic byproducts are washed away rapidly under flow conditions, thus impeding the autocatalytic process.

2.6.9 Effect of processing

Lu et al. [144] reported a decreased in M_w and M_n and an increase in index of polydispersity relative to the values of the raw materials. Using the solvent casting technique in solution of chloroform, the samples were air-dried for 20h and subsequently placed under high vacuum for 24 h to remove any remaining solvent. Surprisingly, the films were soaked in distilled deionized water for 90 min., and then carefully lifted off the glass with a razor blade. The films subsequently were air- and vacuum-dried to obtain the initial samples. The thin (5-10 μ m) PLGA 75:25 films were of

$M_w = 62$ kDa (PI=14.8), and the thick PLGA 75:25 films (85-100 μm) were of $M_w = 58$ kDa (PI=6.4). After processing, the thin PLGA 50:50 films were of $M_w = 37$ kDa (PI=8.9), and the thick PLGA 50:50 samples were of $M_w = 34$ kDa (PI=4.5). However, the error was about ± 2 kDa for the molecular weight and ± 1 for the PI for all the polymers. For the same composition, the thin films had a lower (almost 50 %) M_n than the corresponding thick ones; indicating increased degradation of the thin films during sample preparation. Degradation occurred during film fabrication due to the sample's exposure to air and water.

During the preparation of microspheres, Park et al. [131] reported that hydrolysis occurs with the formation of oligomers. The process of solvent evaporation and the drying process permit the polymer to have relatively short exposure time with water; reducing chances of the process parameters affecting the degradation. The average molecular weight decreased from 17 kDa to 12 kDa, during the preparation of PdLA microspheres.

Weiler et al. [136] reported that PdLA was amorphous after processing (extrusion, injection molding). The solid extrusion was carried out at temperatures in the range of 105-170 $^{\circ}\text{C}$. Commonly used melt-processing leads usually to extensive material degradation. The melt-extrusion of poly (D-lactide) bars showed 40 % reduction in the viscosity-average molecular weight as a result of thermo-oxidative degradation. The molecular weight decreased from 280 kDa to 160 kDa. Mainil-Varlet et al. [162] studied the effect of in vivo and in vitro degradation on molecular and mechanical properties of various polylactides. The results show that the raw material had a significant decrease of M_w after injection molding from 202 kDa to 55 kDa respectively.

In contrast, Rothen-Weinhold et al. [137] reported that the effect of extrusion of PLGAs 75/25 and PLGAs 50/50 at 90 $^{\circ}\text{C}$ causes no significant decrease in weight-average molecular weight (less than 5 %). Surprisingly, for all polymers investigated, extrusion prior to gamma-sterilization appears to protect the polymer from molecular degradation.

2.6.10 Effect of temperature

Belbella et al. [122] reported that higher temperatures accelerated the degradation process of PdlLA ($M_w = 25$ kDa, $IV = 0.3$ dl/g; $M_w = 95$ kDa, $IV = 0.8$ dl/g). Results were based on the quantity of lactic acid released in the aqueous phase of the PdlLA nanosphere dispersion, after one month of conservation at three temperatures: -18 , $+4$ and 37 °C. At $pH = 7.4$, the degradation of the nanospheres is much faster at 37 °C than at $+4$ °C and -18 °C. Jamishidi et al. [163] studied a long-term degradation behavior of PILA fibers in PBS at 37 °C and 100 °C (30 °C higher than the T_g of PILA fibers). Tensile strength was reduced to half of the initial strength after 10 hours in PBS at 100 °C in contrast to no changes at 37 °C. Reed et al. studied the degradation mechanism of a series of PLGA. Six degradations studies were set up at 25 , 30 , 35 , 40 , 45 , and 50 °C. Temperature has a dramatic effect on the rate of mass loss. At 25 °C, PGA sutures show a weight loss of 10% after 12 weeks, in contrast to a weight loss of 90% after only 6 weeks of immersion at 50 °C. At higher temperature than the glass transition temperature of PGA ($T_g = 36$ °C), the rate of weight loss was drastic. Aso et al. [164] reported the effect of temperature (37 and 50 °C) on degradation rate of PdlLA microspheres and discs ($M_w = 300$ kDa) at $pH 12$. The degradation of discs and microspheres showed temperature dependence. At 37 °C the molecular weight change was negligible but appreciable weight loss was observed for one week (8%). This suggested that the degradation of PdlLA was restricted to the surface of the matrix. On the other hand, at 50 °C, the molecular weight decreased rapidly and indicated of a bulk process. In agreement, Hakkarainen et al. [127] reported a dramatic effect of temperature (37 and 60 °C) on the degradation rate PILA and PLGAs. At 60 °C the hydrolysis was several times faster. After 60 days 56% of PILA was lost compared to only 20% at 37 °C. The molecular weight decrease was consistent with weight loss results showing a remaining molecular weight of about 50% after 10 days at 37 °C, compared to 15% at 60 °C. The same pattern was shown for PLGAs.

2.6.11 Effect of strain

Miller and Williams [140] reported the effect of strain applied on poly (glycolic acid) suture. It was clear from both the in vivo and in vitro experiments that the rate of hydrolysis of degradable PGA suture was affected by the magnitude of the imposed strain.

In contrast, if the carrier is subjected to mechanical deformation, degradation rate has been shown to be unaffected [114]. Degradation rates of several polymers were investigated when subjected to cyclical mechanical stress. PLGA 50:50 subjected to high stress, medium stress and low stress shows that after 2 weeks the average molecular weight was lower than the implant initially. However, no significant decrease could be recorded, and all the average molecular weights were within 10 % error.

2.6.12 Effect of plasticizer

Kranz et al. [141] investigated the mechanical properties of PdLA and PLGA 50:50 films as a function of exposure time in buffer and correlate with the degradation/erosion of the polymer. Triethyl citrate (TEC), the water-soluble plasticizer, leached from the films, resulting in major differences with films with the more permanent water-insoluble acetyltributyl citrate (ATBC). A significant difference in M_w decrease was seen between plasticizer-free and plasticizer-containing PdLA films, but not for PLGA films. Plasticized PdLA films, which were above their glass transition temperature in the rubbery state, showed a faster decrease in M_w than plasticizer-free PdLA ones, which were in the glassy state. The plasticizer addition to the PLGA did not enhance the polymer degradation; the plasticizer-free PLGA was already in the rubbery state.

2.6.13 Effect of sterilization

The most frequent method used for destroying all forms of microbial life is steam sterilization, but this process cannot be used with delivery systems based on PLGA/PLA for two reasons. First, the heating would cause a deformation of the matrix, and second, the penetrating high pressure steam would initiate a hydrolysis of the polymer. Gases are not recommended either for sterilization of materials that are to be implanted in the body, because the residual vapor has been found to be mutagenic. Therefore, radiation sterilization is still considered to be the technique of choice to produce sterile drug-polymer-matrices for clinical uses. Several authors have examined the influence of γ -irradiation on the degradation of LA/GA polymers. Gamma-rays are known to induce structural changes, such as scission and cross-linking, in irradiated polymers. Such changes will in turn influence the drug release characteristics and the rate of biodegradation, when these polymers are used as drug carriers. Doses of 2.5 Mrad are generally accepted as being satisfactory for sterilizing pharmaceutical products. SEC analysis of the irradiated PLGA 75/25 and PLGA 50/50 shows that both M_n and M_w decreased. For high-molecular-weight polymers gamma-sterilization results in degradation of the M_w (25 %) [137]. Hausberger et al. [138] demonstrated a substantial effect of γ -irradiation on initial molecular weight distribution and onset of mass loss for PLGA. The first 1.5 Mrad of radiation produced the largest drop in molecular weight (14 %) and 26 % drop of M_n . Irradiation decreased M_n more drastically than M_w . Increasing the dose from 1.5 to 5.5 Mrad increased the degradation rate. On the other hand, the dispersity index increases with the dose of radiation from 1.7 to 2.3 at 1.5 and 5.5 Mrad, respectively. The time required to reach 50 % of the initial mass decreases with increasing irradiation dose. Thus, values were approximately at 8 weeks (0 Mrad), 7 weeks (1.5 Mrad), 6 weeks (2.5 and 3.5 Mrad), and 5 weeks (4.5 and 5.5 Mrad). Nugroho et al. [165] investigated the effect of γ -irradiation on degradation of PLLA. Melting point, glass transition temperature and number-average molecular weight decreased with increasing irradiation dose. At low doses, T_m , T_g and M_n decreased sharply at first, at a slower

rate. Up to 200 kG, PLA was predominantly degraded by random chain-scission. Above 200 kG, recombination reactions or partial crosslinking may occur in addition to chain-scission. Biodegradation of PLA was retarded with increasing dose due to the introduction of crosslinking during irradiation. Hooper KA et al. [139] studied the effect of ethylene oxide and γ -irradiation on selected tyrosine-derived polycarbonates and poly (L-lactic acid). Ethylene oxide exposure of PILA did not affect the molecular weight, surface exposition, mechanical properties, or in vitro degradation rate. However, upon irradiation at 10.6 Mrad, PILA retained only 29 % of its initial molecular weight. Irradiation of PILA induced significant losses in the Young's modulus, and % strain at break.

2.6.14 Effect of enzymes

Despite extensive investigation, controversial data are still found in literature, in so far as the degradation mechanism (hydrolytic vs enzymatic cleavage) are concerned.

The suggestion has been made several times that enzymes may be involved in the degradation processes of polymers. Authors generally base their discussion on in vitro loss of radioactivity of ^{14}C radio-labelled polymers and sometimes on the decrease of mechanical properties or the differences observed between in vivo and in vitro degradation rates. The polyesters were re-examined in the hope of a better understanding of specific structural configurations in a polymer that favour biodegradability [166]. Lu et al. [144] compared the in vivo and in vitro degradation of porous poly (dl-lactic-co-glycolic acid) foams. Significant difference in half-life was found between in vitro and in vivo degradation. The degradation time in vivo was significantly shorter due to the autocatalysis effect of the acidic degradation products; which were released from the polymer matrix but accumulated in the medium surrounding the implants. This effect was minimized for foam degradation in PBS by the frequent change of the medium. Li et al. [167], in studies on amorphous film from 1,d-lactide (10:90, 25:75, 40:60 l:d) showed a remarkable

dependence of l content on the weight loss rate. It was confirmed that proteinase K preferentially degrades l-lactyl units as opposed to d-lactyl ones. With only 10 % of l-lactyl units in its chains, PLA₁₀ showed an negligible enzymatic degradation, while degradation of PLA₄₀ and PLA₂₅ was much more pronounced. The higher water uptake ratio in the case of PLA₄₀ and PLA₂₅ could also have facilitated the enzymatic attack. These results are consistent with other studies from the same authors [159; 168]

In contrast, Hooper K.L. et al. [169] reported the similarity of the in vivo and in vitro degradation rates of each polymer, confirming the absence of enzymatic involvement in the degradation process. A comparison of molecular weight retention, water uptake, and mass loss in vivo with two commonly used in vitro systems of PBS and SBF they demonstrated that for PILA the in vivo results were better simulated in vitro using PBS. While incubation in PBS did not lead to noticeable change in the surface roughness, incubation in SBF resulted in blistering at the implant surface. Also, while pins incubated in PBS equilibrated within a short period of time at 20-30 % water uptake, pins incubated in SBF continued to swell. There were noticeable differences in mass loss. In spite of those differences, the rate of backbone degradation was identical. Furthermore, the curves of M_w retention in vivo and in vitro superimpose accurately. Thus, both PBS and SBF can be used to simulate the rate of backbone degradation of PILA in vivo. However, PBS seems to be the more appropriate incubation buffer for an accurate simulation of the in vivo water uptake and erosion behaviour. Leeslag et al. [170] investigated the in vitro and in vivo degradation of high molecular weight PILAs (M_n = 680-950 kDa). No significant differences between the in vivo and in vitro degradation were observed which excludes the additional effect of enzymes for 39 weeks.

2.7 Biocompatibility and Bio-elimination

2.7.1 Biocompatibility

An early definition of biocompatibility was: “the quality of not having toxic or injurious effects on biological systems”. More recently “biocompatibility” is regarded as “the ability of a material to perform with an appropriate host response in a specific application”[166]. As with other biomaterials, the basic design criteria for polymers used in the body call for compounds that are biocompatible (new definition), processible, sterilizable, and capable of controlled stability or degradation in response to biological conditions.

Poly (α -hydroxy acids) such as polylactide and polyglycolide and their copolymers have been the most applied because they were cleared by the FDA (Food and Drug and Administration) as suture materials, pins plates, screws and drug delivery devices over 25 years ago [166].

In general, PLA-PGA biomaterials have demonstrated satisfactory biocompatibility and absence of significant toxicity [7; 8; 171-175], although some reduction in cell proliferation has been reported [169; 176]. Therefore, from the present combination of literature, it is possible to state that poly (α -hydroxy acids) are polymers of generally good biocompatibility, regardless of shaping and site ofimplantation [157; 171; 174-178].

A critical aspect of the use of such polyesters is represented by their poor hydrophilic properties, which prevent cell adhesion [179]. The micrographs of pure PILA and the corresponding composites with ossein (95/5 w/w) (a biological hydroxyapatite) after treatment with plated osteoplasts shows the evidence of the attachment of osteoblasts and colonised all the samples forming a dense network. This phenomenon is more pronounced on the surfaces of composites, and it is suggested to be due to the presence of the hydrophilic component (ossein) enhancing the cell adhesion on the otherwise hydrophobic polyesters.

Van Sliedregt et al. [7] tested four poly (lactides), PILA (100, 240, 500 kDa), and a Pd,ILA (400 kDa) in vitro by using five cell types. Significant differences were observed for cell types and experimental settings. In general, the biocompatibility of PLAs was satisfactory. Some inhibitory effect on the proliferation of high monomer concentrations in culture media was seen. In the first, cells were directly exposed to the polylactides by culturing on PLA films. In the second, the long-term degradation of polylactide was simulated by exposure of cells to degradation products of artificially aged polylactides. The solution mimicking the long-term degradation was used as a basis for culture medium. Finally in the third experiment the cells were given different concentrations of the end-products of PLA degradation. Polylactides tested by culturing cells on films and by culturing cells with artificial ageing media revealed a satisfactory biocompatibility. In another study, Van Sliedregt et al. [180] used three kinds of epithelial cells as well as fibroblasts and osteosarcoma cells to test the biocompatibility of PILA. The different concentration of monomers revealed normal cell morphologies except for the 10 mg/ml concentrations, which showed larger fibroblasts. The degradation products of polylactide (100, 240, 500 kDa) are not expected to cause adverse reactions when implanted, since cell cultures with monomers concentrations up to 1mg/ml resulted in normal morphologies. Ignatius et al. investigated the toxicity of PdILA and PLGA 90:10 by the agar diffusion test and the filter test with L929 mouse fibroblasts. Both materials revealed no signs of cytotoxicity during the agar diffusions and filter tests. However, high concentrations (8-15 mg/ml) of the degradation products had a toxic influence on the cell culture system used.

Leenslag et al. [170] investigated three PILA samples ($M_v = 6.8 \times 10^5$; 9×10^5 , 9.5×10^5 kDa) in the back of rats. It was found that up to 39 weeks, the PILA implants were very well tolerated by the body. No signs of chronic inflammation were observed. The micrograph reveals a thin fibrous layer close to the PLA implant and a slightly increased cellular activity near the implant site. The present histological findings confirm the frequently reported biocompatibility of PILA. Pistner et

al. [8] studied PILA with three different molecular weight (inherent viscosity 4.9 dl/g, $M_w = 429$ kDa; inherent viscosity 2.8 dl/g, $M_w = 203$ kDa; inherent viscosity 1.8 dl/g, 120 kDa) implanted to dorsal muscle of rats. All three polylactides had incorporated well, forming a collagenous fibrous layer. Crystalline block PLA remained stable in form and structure over the whole observation period. Schakenraad et al. [175] in a study of tissue reactions toward biodegradable PILA implants were monitored by studying the activity pattern of seven enzymes as a function of time. Only an inflammatory reaction as a host response upon the traumatizing implantation of a biomaterial, followed by a wound healing process that is somewhat prolonged, possibly as a result of mechanical irritation of tissue by the implant. It is concluded that the PLA hollow fibers ($M_w = 1.59 \times 10^5$ Da) used in these implantation studies are completely tissue compatible, comparable with a normal wound-healing process and have a very low inflammatory potent. Martin et al. investigated the acidity near eroding polylactide-polyglycolide in vitro and in vivo in rabbit tibial bone chambers. Implants were fabricated from PLGA (IV=0.79 dl/g) and statistically significant differences (of the order of 0.2 pH units) were found between the control (unloaded) and experimental (PLGA bearing) samples. Other authors have reported similar patterns. Nakamura et al. [157] reported that the quicker the degradation of the implant (PdILA and PILA) proceeded, the stronger was the reaction of the surrounding tissues. Matsusue et al. [173] in a study of ultra-high-strength PILA rods ($M_w = 4 \times 10^5$ Da), reported no inflammatory or foreign reaction in the medullary cavity for 52 weeks. Drawn PILA shows no toxicity, and induces the improvement of a thick “interposed bone formation” in early stages.

In contrast, Hooper K.L. et al. [169] showed that the response to PILA fluctuated as a function of the degree of degradation, exhibiting an increase in the intensity of inflammation as the implant began to loose mass. This phenomenon may be associated with the leaching of residual monomer

or processing aids from the implant. The release of lactic acid, which has been shown to induce cell death in vitro, or changes in pH in the tissue surrounding the degrading PLLA implant.

The blood flow plays an important role in the biocompatibility of the polymers, since a high flow rate absorbs the release of monomers due to the degradation more efficiently [177].

Table 2.2 Blood flow values in different arteries

	Coronary arteries	Femoral arteries	Peroneal arteries
Blood flow (ml/min)	250	120	100

Finally, it appears that biocompatibility of polyesters depends primarily on factors other than the polymers themselves. The leaching of low molecular mass compounds either because of degradation or because of the presence of leachable impurity; is a major source of triggering inflammation. Synthetic polymers usually do not elicit any allergic response, except when monomers act as adjuvants or complex with protein to present antigen. Silicone polymers have been claimed to trigger allergic responses [4].

2.7.2 Bio-elimination

The L/G polymer chains are cleaved by hydrolysis to the monomeric acids and are eliminated from the body through the Krebs cycle, primarily as carbon dioxide and in urine. Glycolic acid is a component of several neutral mucopolysacchrides in animal tissues. It can be excreted unchanged by the kidneys or enter the tricarboxylic acid cycle and be eliminated as carbon dioxide and water as shown in Figure 10. In a bio-elimination study using ¹⁴C-labeled poly (lactic acid) implants; 36.8 % of the radioactivity had been lost from the implants after 168 days. Of this value, only 4.6

% was recovered in the urine, 2.8 % in the faeces, and less than 0.3 % was found in tissue. It was suggested that the elimination of lactic acid occurred mainly via respiration as carbon dioxide [21].

Figure 2.7 Cycle of bio-elimination of lactide and glycol [21]

2.8 Mechanical properties

Physical properties of polymeric materials depend on their molecular characteristics as well as ordered structure such as crystalline thickness, crystallinity, morphology, and degree of chain orientation. These physical properties are very important, because they reflect the highly ordered structure of materials and influence their mechanical properties and their change during hydrolysis. The mechanical characterization of PILA samples having different tacticity and molecular weights have evidenced relevant differences. More interesting tensile and flexural properties have been observed for both amorphous and crystalline PILA than in the case of PdILA.

The mechanical properties of amorphous biodegradable PdlLA polymers were described to be very dependent on the glass transition temperature, T_g [121].

Poly (lactic acid) has lower tensile strength, higher elongation, and a much more rapid degradation time compared to poly (glycolic acid). Basically, lactic acid polyesters have the characteristics of a glassy material with T_g in the 55-60°C range. As for all polymers, the physical and mechanical characteristics depend very much on processing history, on molecular weight, and on molecular weight distribution [6].

PILA is a relatively high-strength but brittle material (tensile strength 58 MPa, bending strength 120 MPa and Young Modulus 4000 MPa). PdlLA has a tensile strength of 50 MPa and Young modulus of about 3000 MPa [3]. Perego et al. [181] investigated the effect of molecular weight and crystallinity on PLA mechanical properties. As a confirmation, PdlLA samples showed no variation of impact strength due to their complete amorphous nature. The characterization data have shown more interesting tensile and flexural properties for both amorphous and crystalline PILA than in the case of PdlLA, probably due to the stereoregular nature of poly (1-lactic acid) chain. The increment of molecular weight has shown a marked effect mainly on the properties of crystalline PILA, which seems to reach a plateau at higher molecular weights, around $M_v = 55$ kDa. Engelberg et al. [182] tested three different samples each of PILA (50 kDa, 100 kDa, 300 kDa) and PdlLA (21 kDa, 107 kDa, 550 kDa). PILA's respectively have tensile strengths of 28, 50 and 48 Mpa and tensile modulus of 1200, 2700, and 3000 Mpa, whereas PdlLA's have tensile strengths of 29 and 35 MPa with equivalent tensile modulus of 1900, and 2400 MPa.

Duek et al. [183] investigated the in vitro degradation of PILA pins. Given the possible use of PILA in the fabrication of implants to replace metallic devices for the promotion of osteosynthesis, these rods were produced with two different degree of crystallinity. The data shows that crystalline PILA loses its mechanical bending properties more quickly than its amorphous counterpart. After 4 weeks, the samples of crystalline PILA rupture without elongation, which confirms the fragility of

the more crystalline substance, while the samples of PILA amorphous ruptured only after 8 weeks. The samples with higher amorphosity had higher resistance to bending than samples with higher crystallinity.

2.9 Biopolymer Characteristics

This is an overview more specific of PLA/PGA class of polymers in terms of molecular structure, solubility, biodegradation, biocompatibility and drug delivery characteristics.

2.9.1 Poly (glycolic) acid and poly (lactic-co-glycolic)acid

a. Molecular structure

Lactic/glycolic acid polymers are linear, hydrophobic polyesters. For the sake of clarity, polymer chains are identified in this text by using acronyms where 'n' is the percentage of lactic acid present in the monomer feed; whereas 'm' is the percentage of glycolic acid. LA and GA mean lactic and glycolic constitutive repeating units, respectively.

Poly (glycolic acid) is the simplest linear aliphatic polyester. PGA is highly crystalline (45-55 %), with a high melting point of about 225 °C and a glass transition temperature of about 35 °C [184].

Figure 2.8 Cyclic dimer of PGA

PGA was used in the development of the first totally synthetic absorbable suture by American Cyanamid. PGA sutures have been commercially available under the trade name DexonTM.

Copolymers of L-lactic and glycolic acid (**Figure 2.9**) are amorphous in the range of 25 % to 70 % glycolide, whereas copolymers of D,L-lactide and glycolide are amorphous in the range 0 to 70 % glycolide. The other compositions exhibit crystallinities between zero and the value of the pure homopolymer [21, 93].

Figure 2.9 Poly (lactic_n-co-glycolic_m) acid

However, its low solubility in common solvents has made the formulation of composites difficult and its use in drug delivery problematic [21].

b. Solubility

At less than 50 % of glycolide content, L/G copolymers are quite insoluble materials. Exotic solvents such as hexafluoroisopropanol are often used in analytical procedures as solvents for those polymers. PGA because of its high degree of crystallization; is not soluble in most organic solvents. With the exception of PGA, the polymers in this family are soluble in many common organic solvents and thus can be processed by a variety of thermal and solvent-based methods [21]. According to the solubility parameter concept or the principle of "dissolving the similar," the solvents with solubility parameter values close to that of a lactic/glycolic acid polymer will be suitable solvents for the polymer. Ethyl acetate, chloroform and methylene-chloride have solubility parameter values similar to the lactic/glycolic acid polymers.

The copolymers can dissolve in many organic solvents. Ethyl acetate and methylene-chloride (50 % solubility) are currently the solvents of first choice because they are less toxic. Polymer crystallinity plays an important role in the solubility of the polymers. The structural randomness of the polymer also has influence on the solubility of the polymer. On the other hand, amorphous polymers of d,l-lactide acid and copolymers of lactic acid and glycolic acid that have a low glycolic content are soluble in many organic solvents at ambient temperatures: hexafluoroisopropanol, chloroform, dioxane, tetrahydrofuran, ethylene dichloride, hexafluoroacetone sesquihydrate, acetonitrile, dimethylsulfoxide, and acetone (only d,l-lactic acid, homo and copolymers). These polymers are slightly soluble or insoluble in water, methanol, petroleum ether, ethylene glycol, heptane, and hexane [21].

c. Biodegradation

Rate of degradation of the polymeric materials has been shown to be an important consideration in regard to drug release.

Polyglycolic acid (PGA) has been known since 1954 to be a potentially low cost tough fiber forming polymer, but has one major limitation, i.e. hydrolytic instability. Many comonomers have been used in an attempt to increase the hydrolytic stability but have always produced materials with inferior properties to the homopolymer [184].

The lactide/glycolide carriers degrade predominantly by "bulk" hydrolytic process [21; 112; 121; 145; 148; 152; 167]. The degradation of PLGA involves, chain scissions of ester bond linkages in the polymer backbone by hydrolytic attack of water molecules. The higher the glycolic acid content of the PLGA oligomers, the faster the biodegradation rate [27; 125; 160; 185].

2.9.2 Poly (lactic acid) (PLA)

A broad spectrum of performance characteristics with the polylactides can be obtained by careful manipulation of four key variables: monomer stereochemistry, polymer chain linearity, and polymer molecular weight [3]

The differences of crystallinity of PdlLA and PILA have important effects. Since PdlLA is an amorphous polymer, it is usually considered for applications such as drug delivery, where it is important to have homogeneous dissolution of the active species within a monophasic matrix. The semi-crystalline PILA is preferred in applications where high mechanical strength and toughness are required [182].

a. Molecular structure

The presence of asymmetric carbon atoms generates structural particularities which make lactic acid-derived polymers rather special as compared with other polymers. Unlike glycolic acid, which contains only symmetric carbon atoms, lactic acid has one asymmetric carbon atom and is thus a chiral molecule that exists as two optical isomers or enantiomers, d-lactic acid and L-lactic acid (which are called **R** and **S** enantiomers according to the nomenclature of the International Union of Pure and Applied Chemistry [IUPAC]). When two lactic acid molecules combine to form the cyclic dimer lactide, three possible stereoisomers can be formed: d-lactide, which is composed of two d-lactic acid moieties (**Figure 2.10**), l-lactide consisting of two l-lactic acid moieties (**Figure 2.11**); and meso-lactide, made of a d-lactic acid moiety and an l-lactic acid moiety (**Figure 2.11** [21]).

Figure 2.10 Cyclic dimer d-lactide [2]

Figure 2.11 Cyclic dimer 1-lactide [2]

Figure 2.12 Meso lactide [2]

In addition, an equimolar mixture of d-lactide and l-lactide forms d,l-lactide. The lactide isomers give rise to four morphologically distinct polymers: poly (d-lactic acid) and poly (l-lactic acid), which are the two stereoregular polymers; and poly (meso-lactic acid) and poly (d,l-lactic acid), which are racemic polymers. The polymers derived solely from the optically active lactide or from the l-lactide are semicrystalline, while those derived from the optically inactive meso-lactide or d,l-lactide are amorphous [184]. In a computer-generated poly (d-or l- lactic acid) helical structure, ester groups in the polymer backbone are buried inside the helix, while hydrophobic methyl groups are oriented outside, suggesting that the unstable ester linkages are shielded from the bulk aqueous medium and have a limited accessibility [187].

Poly (l-lactic acid) (**Figure 2.13**) is much more frequently used than Poly (d-lactic acid) because its biodegradation product, L-lactic acid, is a naturally occurring stereoisomer and is easily metabolized by the body. Poly (d-lactic acid), and Poly (l-lactic acid) are semi-crystalline polymers

due to the high degree of regularity of their polymer chains. PLA is about 37 % crystalline, with a melting point of 175-178 °C and a glass-transition temperature of 60-65 °C [21].

Figure 2.13 Poly L-lactic acid

PdLA is amorphous, and as it has only one morphological phase, it does not have a high crystalline melting point, but undergoes a solid melt transition in the region of 130-150°C [21].

b. Solubility

The crystalline homopolymers of lactic acid also do not dissolve in most organic solvents. The homopolymer from (d,l), d, and l-lactide are quite soluble in halogenated hydrocarbons, ethyl acetate, THF and dioxane [21].

c. Biodegradation

Copolymers of l-lactide and d,l-lactide have been prepared to disrupt the crystallinity of l-lactide and accelerate the degradation process [21]. The heterogeneous [151-153; 160] and slow degradation of poly (lactic acid) is partially due to the steric effect because the alkyl group hinders the attack of water. Autocatalysis due to heterogeneous degradation path can be the source of many other phenomena such as size-dependence of the degradation rate, non-random chain cleavages, and degradation-induced morphology changes [6].

Hydrolytic degradation of poly-lactic acid (PLA) has been studied by various workers [133; 171; 186; 188]. It has been suggested that during hydrolytic degradation, PLA undergoes random chain scission [189]. As a result of the total degradation, carbonyl carbon-oxygen bond cleavage is more active or competes with the cleavage of a carbon-oxygen bond shown in **Figure 2.14** [94].

Figure 2.14 Random chain scission of **PLA**

CHAPTER 3

EXPERIMENTAL TECHNIQUES

3.1 Materials

Poly(1-lactide)(PILA) of two different molecular weights (intrinsic viscosity, IV= 4.37 and 2.04 dL/g), poly(d,l-lactide)(PdILA IV = 2.4 dL/g), poly(1-lactide-co-glycolide 80:20) (PLGA 8020, IV = 4.8 dL/g) and poly(d,l-lactide-co-glycolide 53:47) (PdILGA 5347 IV= 0.84) were purchased from Purac Far East Pte Ltd, Singapore. The polymers, for the sake of convenience, would be referred to as PILA 4.37, PILA 2.04, PdILA 2.4, PLGA and PdILGA, respectively. The numbers following the description of copolymers refer to the molar composition of lactide and glycolide units and the intrinsic viscosities. Thus, PdILGA 5347 0.84 is composed of 53 percent of d- and l-stereo isomers of lactide and 47 percent of glycolide and the polymer has an intrinsic viscosity of 0.84 dL/g. The intrinsic viscosity data of the polymers was provided by the supplier and the M_w was determined in our laboratory using size exclusion chromatography (SEC).

Table 3.1 summarizes details of the polymers used in this investigation, along with the data for intrinsic viscosity, provided by the supplier.

Table 3.1 Details of the polymers used in this study

Polymer	Intrinsic Viscosity (dL/g)*	Average Molecular Weight (M _w)(kDa)**
PdLGA 53/47	0.84	40
PILGA 80/20	4.8	910
PdLLA	2.4	313
PILA	2.04	300
PILA	4.37	810

- Data reported by supplier, ** Determined by SEC.
-

All polymers shown a Index of Polydispersity of about 1.5-1.8.

The drugs, lidocaine (both in base and salt forms, referred to as lidobase and lidosalt, respective
paclitaxel and rapamycin, were purchased from Sigma-Aldrich Pte Ltd., Singapore, Dabur
Limited, New Delhi, India and Hande Chemicals Company, China.

The buffer solution (pH 7.4) was prepared by dissolving ready-made buffer salt (HydriTM
Sigma-Aldrich Pte Ltd, Singapore) from individual sachets into 500 mL of deionised water.
Tetrahydrofuran (THF), methanol, acetonitrile, ethanol, dichloromethane (DCM), and water (all
HPLC grades) were purchased from Tedia Chemical Company, Fairfield, USA. Deutera
chloroform, CDCl₃ (99.8 atom % of Deuterium, containing 0.03 % (v/v) tetramethylsilane (TMS)
was used as the solvent for all quantitative ¹H-NMR studies. Benzophenone (Sigma-Aldrich Pte
Ltd, Singapore) was used as an internal standard.

3.2 Preparation of Polymer Films and the Drug-Incorporated Stents (2% weight)

Appropriate amount of the polymer was dissolved into dichloromethane (DCM) under stirring using a magnetic stirrer. Film casting was done from the resulting solution using a gravure rod of 25 mils (about 228.6 μm) thickness. The film was allowed to dry at room temperature for 24 hours. Subsequently, drying was continued in vacuum at 30 °C, for 3 more weeks. The above conditions achieved almost complete removal of solvent with minimal bubble formation. Twenty days of drying in vacuum oven at 30 °C yielded films with less than 1 percent of residual solvent, as determined by thermogravimetric analysis. This was considered acceptable for the in vitro investigations in order to avoid plasticizing effect of the solvent on the polymer matrix. The thickness of the dry films ($25 \pm 5 \mu\text{m}$) was determined with a thickness gauge. To eliminate the influence of polymer shape on the degradation process, all film samples had dimensions of 40 mm X 25 mm, during subsequent studies. For preparation of drug loaded film samples, a second step was followed by way of adding the solution of drug into the polymer solution and subsequent homogenization through overnight stirring. The casting and drying of drug loaded film was carried out using similar procedure, as described above.

Drug containing polymer films to be used subsequently for making the stents were casted using an Automatic Film Applicator (AG-2150; Gardner Germany), which was adjusted to provide an uniform thickness. Film thickness was about $120 \mu\text{m} \pm 20 \mu\text{m}$ for all the prototype stents. The film was allowed to dry under ambient conditions for 24 hours following which; the drying was continued in a vacuum oven at 30 °C, for three weeks. Polymer-drug films were cut into strips with the length of 4.5 cm and width 2 mm, scrolled onto a rod with an outer diameter of 2 mm and allowed to dry for 3 more days under vacuum, to form the helicoidal stents.

Incorporation of drugs did not interfere with the formation of homogeneous films and stents; the crystals were dissolved uniformly within the polymeric matrix. Transparent, crystal-free films were obtained at drug concentrations below 2 % (w/w). No cracks or imperfections were seen on the film surface prior to the dissolution study (**Figure 4.30** and **4.31**).

3.3 Degradation Study

All film samples (10-20 mg) were put into individual glass vials (of 60 ml capacity each). The films were completely submerged in 50 ml of buffer solution (pH 7.4) forming a dilute solution (more than 100 times the sample weight) to ensure a constant pH of the solution [199]. The vials with the films and buffer solution were capped and put into an incubator and maintained at $37 \pm 0.1^\circ\text{C}$. The films were removed at regular intervals and characterized with respect to water uptake and weight loss as follows:

Water uptake: Films samples were periodically recovered for determination of the amount of water absorption. In a typical test, the film surface was wiped with a clean and dry tissue paper to get rid of excess buffer solution adhering on the surface, weighed and later, dried to constant weight in order to determine the weight loss. Water uptake was calculated at each time point using the following equation:

$$\% \text{ Water Uptake} = 100 \times \frac{W_{\text{wet}} - W_0}{W_0} \quad (3.1)$$

W_{wet} is the weight of the wet film at each time point, during the course of the study and W_0 , the original dry weight of the film. Results presented are the average values obtained from the three samples for each film. All weights were measured with an accuracy of ± 0.01 mg.

Weightloss: Weight loss of the films during the *in vitro* study period was measured from changes in dry weight of the original film and that at each time point. For each such measurement, the film after removal from the buffer medium, was rinsed with distilled water, wiped with a tissue cloth and dried until constant weight. Once again, the values reported are the average of the three film samples. Following equation was used:

$$\% \text{ Weight Loss} = 100 \times \frac{W_0 - W_t}{W_0} \quad (3.2)$$

Where W_0 = starting dry weight; W_t = dry weight at time, 't'

3.4 Thermal Analysis

TA instruments modulated differential scanning calorimeter (DSC 2920), equipped with refrigerated cooling system (RCS) was used for measurement of the glass transition temperature (T_g) (**Figure 3.1**), melting temperature (T_m) and crystallization temperature (T_g) (**Figure 3.2**) of the samples before and during the *in vitro* study. The DSC data were obtained from a single heating profile of the films and thus reflect the thermal history imparted by the film preparation process. The history was assumed to be identical for each sample because an identical process was used to prepare films. Typical weight of samples was 5-10 mg. Samples were run in the modulated mode, under nitrogen flow, from -20 to 220 °C at a heating rate of 5 °C/min with an amplitude of modulation of ± 1 degree per 60 seconds.

Figure 3.1 A representative thermogram, showing the glass transition temperature

Samples were thoroughly dried to eliminate the effect of remaining moisture on thermal events.

The degree of crystallization (DOC) was calculated from the following relationship:

$$DOC(\%) = \frac{\Delta H_f - \Delta H_c}{\Delta H_{theo}} \quad (3.3)$$

Where, ΔH_f , ΔH_c , and $\Delta H_{f\ theo}$, refer respectively, to the enthalpy of melting, enthalpy of crystallization and enthalpy of melting for the 100percent crystalline sample of the same material (93.1 J/g for PILA [185; 191]). It was not possible to calculate the degree of crystallinity of PLGA 80/20, in absence of the enthalpy data for a 100 percent crystalline material, so an estimation was carried out for the original material using 80 and 20 % of the enthalpy of melting for the 100%

crystalline sample, respectively for PILA and PGA (185 J/g). Also the difference, $\Delta H_f - \Delta H_c$, representing the change in crystallinity has been plotted.

Figure 3.2 A representative thermogram showing the heat induced recrystallization and the melting events.

3.5 Size Exclusion Chromatography (SEC)

The weight and number average molecular weight (M_w and M_n) as well as molecular weight distribution (MWD) of the polymers were determined using an Agilent series 1100 liquid chromatography system. The equipment consisted of an isocratic pump, an online vacuum degasser, an auto-sampler, a thermostat column compartment and a refractive index detector. A mixed bed column (PLGel Mixed Bed C, 5 μ , 300 x 7.5 mm, Polymer Laboratories), along with a guard column (50 x 7.5 mm, 5 μ , Polymer Laboratories), was used. The mobile phase was a

mixture of tetrahydrofuran (THF) and dichloromethane (DCM) in the ratio of 80:20 (vol:vol) because the polymer is not very well soluble in THF. The flow rate was 1 ml/min and the temperature (both the column compartment and the flow cell of the refractive index detector) was maintained at $35 \pm 0.1^\circ\text{C}$. The typical solute concentration was 1 % (w/v). Samples were dissolved in the mobile phase, filtered through 0.22 μm disposable syringe filters into 2 ml glass vials and put into the auto-sampler tray. The typical injection volume was 20 μL . Molecular weights of samples were obtained relative to a calibration curve from polystyrene standards (molecular weight between 165 and 5000 kDa).

3.6 Scanning Electron Microscopy (SEM)

Surface morphology of the films was studied using SEM, for evidence of film degradation. SEM was performed on surface of the samples at different time points, for the samples from *in vitro* degradation. The samples were air and vacuum-dried for 3 weeks at 40°C , before observation with SEM. The individual films were gold coated (gas pressure 20 millibars, current 18 mA and coating time 75 seconds) and the SEM micrographs were observed at 10 kV (AS JEOL, model JSM-5410 VL).

3.7 ^1H -NMR Studies

The copolymer composition of PdlLGA 5347 0.84, before and after the *in vitro* degradation were determined from ^1H -NMR (Bruker Advance-400 MHz spectrometer). The sample solutions were prepared in appropriate concentrations by dissolving about 1 mg of the polymer in CDCl_3 . The composition of the copolymer remaining after hydrolytic degradation was calculated from

integration of peak areas of lactide (methine; $\delta = 5.2$ ppm) and glycolide (methylene; $\delta = 4.8$ ppm) protons, with reference to the benzophenone aromatic protons (7.5 - 8 ppm), as internal standards.

3.8 Monte Carlo Calculations

Methods for structure and molecular characterization of polymer materials undergoing degradation have been studied using different models. Random or nonrandom chain scission is one of the degradation processes considered. Monte Carlo procedure, which basically attempts a comparison of simulation results with experimentally determined molecular weights, allows detailed mechanistic description about such scission paths. This model is valid only for depolymerization reactions following chain scissions and giving rise to volatile products (monomer and/or oligomers). In the case of degradation occurring with chain scission, polymer samples undergo changes in degree of polymerization (DP) as well as weight loss. The degradation can be followed as a function of the degree of conversion, c , representing the weight loss:

$$c = 1 - \frac{w}{w_0} \quad (3.4)$$

where, W_0 = weight of the undegraded sample and W = weight of the degraded sample.

The Monte Carlo parameter R_m is calculated from the ratio of the number average degree of polymerization during degradation (X_n), say at a degree of conversion, c , and the degree of polymerization (X_{n0}) of original undegraded material using the relationship in equation 5.

$$R_m = (1 - c) \frac{X_{n0}}{X_n} \quad (3.5)$$

With $0 \leq c \leq 1$

The parameter, R_m , when plotted against degree of conversion, c , gives an indication of the characteristic degradation path followed by the system under the conditions of study. Random chain scission is characterized as a hyperbolic profile with a drop down once the polymer begins to register some weight loss [192]. Initial increase in R_m is because of polymer degradation without weight loss. The model provides important insight into the process of degradation for systems under investigation in this study. Monte Carlo calculations for PLGA 8020 4.8 and PdLLGA 5347 0.84, was carried out from number average molecular weight (M_n) (obtained from GPC), number average degree of polymerization (X_{n0} and X_n , respectively, for the original and degraded samples), and the degree of conversion, c , (calculated from weight loss during *in vitro* study and the original weight of samples).

3.9 Drug Release Study

Drug-containing matrices were weighed and placed inside 60 ml glass vials with 50 ml of phosphate buffer (pH 7.4). The vials in turn, were tightly capped to control evaporation and placed inside the incubator maintained at 37 ± 0.1 °C. Samples were removed from the vials at predetermined intervals for quantitative estimation of the amount of drug released. In a typical test, 1 ml of buffer solution containing the drug was removed and the vial was replenished with 1ml of fresh buffer solution, to maintain a constant volume of the release medium. The amount of drug released was quantitatively estimated using reverse phase chromatography on Agilent's liquid chromatography system. For each HPLC run, 20 μ l of sample from *in vitro* study was injected into the column. The resulting chromatogram peak for the drug was integrated and the quantity of drug calculated from the calibration curve. The resulting value was reported as a percentage of drug loading (taking into account the dilution factor in the total volume of the release medium). All

the drug release tests were carried out in triplicate and the results are presented as average to minimize experimental errors from drug loading, film preparation and quantification of drug release.

3.10 High Performance Liquid Chromatography (HPLC)

Detection and quantitative analysis of drugs was performed using Agilent's HPLC System, which consists of a quaternary pump with online vacuum degasser, Rheodyne manual injection port with 20 μ L injection loop and variable wavelength detector (VWD) with wavelength range of 190-600 nm. A 4.6 x 250mm ZORBAX Eclipse C-18 column (particle size: 5 μ m) was used. Release mediums and mobile phase combinations were selected to conduct the drug release study under the best optimized conditions.

Details of HPLC methods followed for each individual drug studied are as follows:

Paclitaxel: Reverse phase chromatography was run at room temperature with a mobile phase composition of 40:60 (vol:vol) water and acetonitrile, at a flow rate of 1.00 ml/min for detection and quantification of paclitaxel. The wavelength of detection was set at 227 nm. The retention time of the drug under these conditions was at 6.5 minutes. No binding of the drug onto the walls of the glass vials was observed using a 10:90 (v/v) mixture of methanol:water as the release medium. The calibration curve, which was made from injections of nine different concentrations, was linear ($r=0.9999$) in a range of 0.01156-23.12 μ g/mL (with a coefficient of correlation, $R^2=0.9999$). The sensitivity of the method reported herein is better than that reported by Dordunoo et al. [193] (mobile phase ACN:methanol:water 58:5:37, flow rate 1ml/min. $\lambda=232$ nm, injection volume: 20

μl , calibration with concentration range: 1-250 $\mu\text{g/ml}$). The amount of drug in the solutions (at time $t=0$) was quantified using the calibration curve in **Figure 3.3**, by injecting 20 μl of the sample.

Figure 3.3 Calibration curve for paclitaxel

In view of several reports of the property of paclitaxel adsorbing onto the walls of the contain we were interested in following the amount of drug over a period of time, from three different initial concentrations. Accordingly, the initial concentrations were made: $C_1 = 8.0 \mu\text{g/ml}$, $C_2 = 180.0 \mu\text{g/ml}$ and $C_3 = 542.0 \mu\text{g/ml}$, by appropriate dilution from the initial stock solution.

Figure 3.4 Overlaid chromatograms of paclitaxel at four different injection amounts

Subsequently, the quantification was done at the end of 3 days and 7 days respectively, to see if there was a fall in the concentrations. The results are presented in **Figure 3.5**. As shown in the figure, there is no change in the amount of drug (hence no adsorption onto the glass walls), within the seven days period of study. Apparently, we are not seeing the effect of adsorption of paclitaxel onto the walls of the glass vials, under the conditions of our experiment.

Figure 3.5 Amount of paclitaxel (from three different initial concentrations) monitored function of time.

Rapamycin: Reverse phase chromatography was run with a mobile phase composition of 90:10 (vol:vol) acetonitrile and dichloromethane, at a flow rate of 0.65 ml/min for rapamycin. The wavelength of detection was set at 278 nm. The retention time of rapamycin under these conditions was at 5.04 minutes. The calibration curve (**Figure 3.6**) was made with a lower limit of 6 ngr/ml, which had a coefficient of correlation of $R^2 = 0.99997$. Simamora et al. [83] have reported a linear calibration curve ($r = 0.998$), for the drug, in the range of 1-100 $\mu\text{l/ml}$.

Figure 3.6 Calibration curve for rapamycin

The immediate objective was to study the release kinetics of the drug *in vitro*. Although rapamycin has negligible solubility in water, it is easily soluble in ethanol. The stock solution of the drug was made in a 10:90 (v/v) mixture of ethanol and buffer (pH 7.4), which is the intended composition of the release medium for the drug from the drug-loaded polymer matrix. This composition was chosen to increase the solubility of rapamycin and to be able to study the drug release *in vitro* in a medium similar to buffer pH 7.4. The limit of solubility of a drug in a release medium is one of the major issues during attempts to quantify drug release [95]. This is because of the fact that the concentration of the hydrophobic drugs in polymer matrix is often much higher than the saturation concentration.

Figure 3.7 Overlaid Chromatograms, showing the rapamycin peak at four different injection of increasing amount of drug

We have attempted to repeat the method reported by Simamora et al. [83], with methanol/water (80:20) as the mobile phase at 1.00 ml/min. The drug elutes at 6.201 minutes under these conditions. However, it was observed that an increase in column pressure occurred from about 105 bars at the beginning of the run to about 200 bars, at the end of running the highest concentration, using the same column. In contrast, the column pressure for ACN/DCM (90:10) at 0.65 ml/min; was about 36 bars, throughout. Moreover, running any one concentration of the drug, under both mobile phase conditions, produces widely different intensity of absorption. **Figure 3.8** presents two runs for the highest concentration (0.5136 μg in 20 μl injection volume) using the same column: 'A' being the run with ACN/DCM (90:10) at 0.65 ml/min and 'B', with MeOH/Water (80:20) at 1.00ml/min. As seen from figure, the difference in peak intensity is convincing.

This, coupled with the fact that there is an increase in column pressure by about 100 bars, shows that the latter mobile phase was not strong enough in eluting the entire drug injected into the column. Presumably the mobile phase under conditions A had a better elution strength and resolution in comparison to B. The added advantage of this method is that is done at room temperature.

Figure 3.8 A comparison of the intensity of the rapamycin peak (same injected mass) under two different mobile phase conditions

Lidocaine: Reverse phase chromatography was run with a mobile phase composition of acetonitrile, at a flow rate of 0.80 ml/min for lidocaine. The wavelength of detection was set at 270 nm. The retention time of rapamycin under these conditions was at 4.2 minutes. A calibration

curve was created covering the range of concentrations up to 100 percent release of drugs from films and stents, prior to running actual sample from *in vitro* experiments.

CHAPTER 4

DEGRADATION MECHANISMS

4.1 Introduction

The process of “degradation”, in the case of polyesters, essentially describes hydrolytic chain scission, during which polymer chains are cleaved to form oligomers and finally to form monomers [118]. While classifying degradable polymers, a distinction is made between surface (or heterogeneous) and bulk (or homogeneous) eroding materials. In the case of surface-eroding polymers, the degradation occurs layer by layer, with the interior of the polymer sample being the last to undergo degradation. In a given polymer, both mechanisms could be operative, depending on the polymer characteristics. The systematic study of this dependence has not been literature reported.

Thus, the first objective of the present study was to compare the *in vitro* degradation aspects of a range of polyesters, covering poly (1-lactide)s, poly (d,l-lactide) and copolymers of polylactide with polyglycolide. Polymer samples were carefully selected to present a variation in composition, crystallinity and molecular weight. PILA (IV = 4.37) and PILA (IV = 2.04) are two homopolymers of the 1-lactide, with different molecular weights and appreciably different crystallinity. On the other hand, PdlLA (IV = 2.4), a random copolymer of d- and 1-lactides, is completely amorphous.

In addition, PLGA 8020 (IV = 4.8) and PdlLGA 5347 (IV = 0.84) are random copolymers composed of 1-lactide and glycolide units. **Table 4.1** summerizes the mechanistic degradation study methodology.

Table 4.1 Summerise of the degradation study methodology.

Parameters	PILA (4.37)	PILA (2.4)	PdlLA (2.04)	PILGA 80:20 (4.8)	PdlLGA 53:47 (0.84)
effects					
Mw	X	X			
Crystallinity		X	X		
Composition	X			X	X

The degradation study section has been divided into the following sub sections:

- Degradation of the homopolymer
- Degradation of copolymers
- Effect of drug nature

The results have been discussed in terms of water absorption, weight loss, molecular weight (M_w), glass transition temperature (T_g), degree of crystallinity, H-NMR, Monte Carlo Model, visual inspection and scanning electron micrographs.

4.2 Degradation of the Homopolymer

4.2.1 Water absorption, weight loss, and change in M_w

Figures 4.1 shows changes in the molecular weight (weight-average, M_w and number-average, M_n) of PILA 4.37 (as the percentage of original) with immersion time. Also plotted are the percent water uptake and weight loss over the same period of time.

Figure 4.1 Overlay of water absorption, weight loss, M_w and M_n of PILA 4.37 versus immersion time.

For PILA 4.37, the M_w and M_n both decreased to about 50 percent of original molecular weight over the period of study. However, the extent of water uptake and weight loss (of the order of 2 and 1 percent, respectively) was very low over the same period of time (**Figure 4.1**).

Figures 4.2 shows changes in the molecular weight (weight-average, M_w and number-average M_n) of PILA 2.04 (as the percentage of original) with immersion time. Also plotted are the percent water uptake and weight loss over the same period of time.

Figure 4.2 Overlay of water absorption, weight loss, M_w and M_n of PILA 2.04 versus immersion time

PILA 2.4 lost only about 22 percent of its initial M_w and there was hardly any appreciable water uptake, over a period of 300 days (**Figure 4.2**).

Figures 4.3 shows changes in the molecular weight (weight-average, M_w and number-average, M_n) of PdlLA 2.4 (as the percentage of original) with immersion time. Also plotted are the percent water uptake and weight loss over the same period of time.

Figure 4.3 Overlay of water absorption, weight loss, M_w and M_n of PdlLA (IV=2.4) versus immersion time

In contrast to the PILA polymers (**Figure 4.2**), PdlLA (**Figure 4.3**) followed a two-step water absorption behavior, with initial water absorption of less than 10 percent for 25 days. This was followed by a second phase characterized by a sharp increase in water absorption to form a plateau

for 200 more days, and a final phase with another increase in water absorption due to formation and leaching out of oligomeric products from the polymer film. The decrease in weight average molecular weight, M_w , showed two distinct phases. It is interesting to note how well the decrease of molecular weight correlated the water absorption data, during the first phase. The M_w started decreasing soon after immersion till about 50 days, followed by a lag phase for 100 days, when M_w remained stable. The second phase of drop in the M_w began at the end of 150 days (co-inciding with the increase in weight loss) and decreased until it reached an asymmetric value of 10 % of original at the end of 260 days. This good correlation between the water absorption, decrease of M_w and weight loss shows that in the first phase of swelling, the M_w decreases due to the ester hydrolysis and the production of carboxylic end groups, followed by a second decrease of M_w corresponding with the start of the weight loss. The later decrease of M_w is due to the breaking down of the films, increasing the release of substantial oligomers. However, it is not obvious that the water absorption increased significantly after the starting of weight loss but one can note an increase from day 125 and we could not measure the water absorption after 210 days due to brittleness.

In resume, the first decrease of M_w is due to the hydrolysis and the diffusion of the oligomers through the polymer film and the second decrease of the M_w is due to the start of breaking down of the matrix facilitating the release of oligomers.

The interesting observation here is that the onset of measurable weight loss occurs when the molecular weights have decreased to about 15 % of the initial value (roughly 50 kDa). Examining the M_w and M_n changes for each polymer decreased by the same amount during the initial stages of degradation, and thus the M_w distribution was constant in a range of 1.8-2.1. This was seen to

occur for all the three polymers, and is thought to be indicative of homogeneous degradation. For the PDLA 2.04 and PDLA 2.4, the M_n decreased at a faster rate than M_w , at around day 50. This indicates a broadening of the molecular weight distribution, but does not imply the onset of any heterogeneous degradation. The reason is that as lower- M_w fragments were produced, the M_n would necessarily affect more than the M_w . In addition, no bi-modal distributions were seen in the SEC traces for any of the polymers (**Figure 4.4**).

Although two regions of water absorption and two corresponding regions of M_w decrease have been found for PDLA in **Figure 4.3** (in contrast to Grizzi et. al. [134]), it must be pointed out that all the SEC traces obtained in this work did not show any bi-modality.

It may be noted that Li et al. [152] reported heterogeneous degradation for PDLA. Their results showed that a massive device degraded more rapidly inside than at the surface [104; 189; 190]. Possibly, size is the reason for the different degradation paths. Li and coworkers used plates of about 2.1 mm thickness. The lower the thickness, the easier for low molecular weight oligomers and fragments to leach out. Grizzi et al. [134], in an important study, showed that the degradation proceeds heterogeneously for cases of large size devices and that the rate of degradation was greater inside than at the surface. They concluded that the critical thickness above which the hydrolytic degradation of amorphous PLGA polymer became heterogeneous, was in the range of 0.2-0.3 mm.

Figure 4.4 shows SEC spectra of PdLLA films for different hydrolysis times.

Figure 4.4 SEC chromatograms of PdLLA films after hydrolysis for different times: 0 days (a); 128 days (b) and 275 days (c)

The molecular weight distribution of PdLLA film was relatively narrow and shifted to a lower value with hydrolysis for 275 days, without forming any specific multi-modal distribution. As the oligomers in the polymer film were subjected to hydrolysis, it can be seen that the chromatogram shows a uni-modal M_w distribution in agreement with their thinness, which favoured exchanges

with surrounding media and thus precluded heterogeneous degradation. Hence, it is thought that the PILAs and the PdlLA had undergone homegeneous degradation in the range of thickness studied for the films (25 μm). The heterogeneity of the auto-catalytic degradation as seen in by multi-modal SEC chromatograms, was explained by a difference of degradation rate in the inner matrix compared to the outer [120; 121; 131; 152; 153; 189; 190]. Li et al. [104] have found the appearance of bimodal distributions for plates in contrast to films, and this was in agreement with characteristics typical of the size dependence of degradation [134]. However, this was in disagreement with Lu et al. [156] reported the in vitro degradation of thin poly (dl-lactic-co-glycolic acid) films. PLGA films of copolymers ratios of 75:25 and 50:50 were manufactured with thickness levels of 10 μm thin and 100 μm “thick” and was incubated in buffer pH 7.4, at 37 °C. The SEC chromatograms of the degraded PLGA 50:50 films suggest that the degradation proceeded heterogeneously for both thin and “thick” films.

Figure 4.5 plots the semi-log plot of the M_w versus time of PILA 4.37, PILA 2.04 and PdlLA 2.4. The straight-line nature of the plots is representative of a first order degradation path and the slopes are indicative of the relative order of degradability of the matrices. A quantitative estimation of the relative rates of degradation for the three materials, PILA 4.37, PILA 2.04 and PdlLA 2.4, is obtained using linear regression of the data points over the period. It is worth to note that PdlLA does not show a single straight line but two phases.

Figure 4.5 Overlay of first-order degradation rates of PILA 4.37, PILA 2.04, and PdILA 2.4.

From the slope of the lines, it is clear that PdILA shown a much faster degradation rate than PILAs.

The pseudo-first order degradation constants (k) were obtained from the slope of the straight lines and are summarized in **Table 4.1** with the respective coefficients of correlation.

Table 4.1 Pseudo first-order degradation constants of the polylactides

First-order degradation const.	PILA IV=4.37	PILA IV=2.04	PdILA IV=2.4	
Period (day)	0-365	0-270	0-150	150-250
$k_{deg.} (day^{-1})$	0.0015	0.0009	0.0087	0.0088
R^2	0.9724	0.982	0.9752	0.9642

As is evident from the slopes in **Figure 4.5**, the relative degradability of the matrices followed in the order: PdlLA 2.4 > PILA 4.37 > PILA 2.04.

4.2.2 Thermal analysis

Figure 4.6 presents the melting temperature, percent crystallinity and the glass transition temperature for PILA 4.37 over a period of 320 days of immersion in buffer 7.4. PILA 4.37 showed a very small increase in crystallinity, followed by a slight decrease, over the 300 days, while both the glass transition as well as the melting temperatures remain constant. The constancy of glass transition in spite of a drop of about 50 % in M_n must be attributed to the fact that the critical M_n value has not yet been attained.

Figure 4.6 Overlay of glass transition, melting point, and degree of crystallinity of PILA 4.37 versus immersion time.

Figure 4.7 shows an overlay of the degree of crystallinity of PILA 4.37 and PILA 2.04, over a period of 290 and 210 days respectively. PILA 2.04 had a higher degree of crystallinity (50%) than PILA IV 4.37 (35 %). Cam et al. [133] reported a higher degree of crystallinity for PiLA of lower molecular weight. It was shown that PILA with M_w of 650 kDa had a initial crystallinity of 5 % only, as compared to 30 % for the PILA with an M_w of 300 kDa.

Figure 4.7 Overlay of degree of crystallinity of PILA 4.37 and PILA 2.4.

Clearly, the higher degrees of crystallinity (**Figure 4.7**), explained the practically negligible water uptake and weight loss of PILA 4.37 and PILA 2.04 (**Figures 4.1** and **4.2**) and the slower degradation rate, as compared to amorphous PdILA 2.4 (**Figure 4.3**). The results showed that in case of semi-crystalline polymers, the degree of crystallinity is a limiting factor working against

the matrix degradation. However, Tsuji et al. [130] reported that the decrease in PILA molecular weight was more rapid for PILA films with higher initial crystallinity. They have explained the findings in terms of the crystalline domains introducing defects into the amorphous matrix, thus facilitating the penetration of water, more easily. Our results clearly are in disagreement with Tsuji et al. [130], and in agreement with the results published by Li et al. [190].

Overlays in glass transition temperatures for PILA 4.37, PILA 2.04 and PdILA 2.4 are presented in **Figure 4.8**.

Figure 4.8 Glass Transition Temperatures of PILA 4.37, PILA 2.4 and PdILA 2.04 as function of immersion time

PILA 2.04 had a consistent T_g of about 65 °C over a period of 210 days of immersion, while 4.37 and PdILA 2.4 had relatively constant T_g s of about 62 and 46 °C, respectively.

4.2.3 SEM analysis

Polymer films (original films as well as those after in vitro degradation for different lengths of time) were observed under scanning electron microscope, for proof of surface and bulk erosion. **Figures 4.9a to 4.9c** present the micrographs of PILA 4.37, PILA 2.04 and PdILA 2.4 films before immersion in the buffer medium, respectively. On the other hand, **Figures 4.9d to 4.9f** present the micrographs of same batch of polymers films after being subjected to in vitro degradation, in the same order.

The morphological differences are quite obvious. The un-degraded PdILA film (**Figure 4.9c**), which was quite smooth, did develop a rough and porous surface after immersion time (**Figure 4.9f**). In contrast, the films from PILAs showed no marks of degradation yet. These SEM micrographs confirmed the results above of substantial degradation of PdILA compared to PILAs.

In summarize, one can note that the crystallinity is the major factor controlling the degradation. All polymers degraded by homogeneous path as shown from the uni-modal SEC chromatograms and in agreement with the study of the decrease of M_w , and M_n . PILA (IV=4.37) degraded faster than its lower molecular weight PILA (IV=2.04) due to a lower degree of crystallinity. In agreement, the amorphous PdILA degraded faster than semi-crystalline PILA of about the same molecular weight.

4.3 Degradation of Copolymers

4.3.1 Water absorption, weight loss, and change of M_w

Figure 4.10 presents overlaid graphs of percent remaining M_w and M_n along with percent water uptake and weight loss versus immersion time for PLGA 80/20 4.8.

The material degraded almost completely at the end of 300 days of immersion in buffer. The percent water uptake, after a long lag period of about 100 days (~5 % absorption), increased to about 10 % over 220 days. Subsequent percent water uptake was rapid, reaching up to 55 % on the 282nd day. Percent weight loss closely followed the trend in water uptake. A mere 7 percent at the end of 217 days, the percent weight loss sharply increased up to 84 percent at the end of 282nd day.

The M_w at the onset of rapid weight loss (we term it the critical M_w) was about 45 kDa, about 5% of original value.

Figure 4.10 Overlay of water absorption, weight loss, M_w and M_n of PLGA 80/20 4.8 versus immersion time

These results showed that the integrity of the matrix remained intact for 200 days (90 % remaining weight), until about 95 % of the original molecular weight degraded and leached out.

Comparison of the water absorption and degradation behavior for PLGA 4.8 (**Figure 4.10**) and PILA IV= 4.37 (**Figure 4.1**), demonstrated the important effect of the composition of the polymer. Almost no water absorption and weight loss was recorded for PILA 4.37 over 300 days. In contrast, PLGA 80/20 4.8 degraded almost completely at the end of 275 days, even at substantial overall water contents (50 %).

We believe that PLGA 80/20 film also undergone homogeneous degradation, as the SEC did not exhibit a bi- or multi-modal distribution during the degradation process. This is in contrast to the findings by Burg et al. [188], who in a study of PLGA 90/10; reported a heterogeneous degradation pattern with a faster degradation of the inner, with release of acidic degradation products only after sufficient degradation of the exterior. We believe again, as in the case of PILA discussed above, that the discrepancy could be due to the differences in sample dimensions in the two studies. Burg et al. [188] used films of thickness 0.8 mm (larger than the limit reported by Grizzi et al. [134], for the matrix to undergo homogeneous degradation), in contrast to films of about 25 μm used in this study. Interestingly, in a related study, Witt et al. [149] showed that the rate of PLGA erosion decreases with increasing surface to volume ratio from tablets and rods, to films and microspheres.

Figure 4.11 Overlay of log M_w vs immersion time for PILA 4.37 and PLGA 8020 4.8: Effect of composition on degradation kinetics

PLGA 80/20, in a manner similar to PdlLA and PILAs follows pseudo first-order degradation kinetics. A comparison of the slopes of the two curves shows that PLGA 4.8 degrades faster than PILA 4.37. Using the same procedure as described for polylactides, the relative rate of degradation for the PLGA 80/20 4.8 was calculated using the principle of linear regression. The results are tabulated in **Table 4.2** along with that for PILA 4.37, for ease of comparison.

Table 4.2 A comparison of first-order degradation rates for PLGA 4.8 and PILA 4.37

First-order degradation	PILA IV= 4.37	PLGA 80/20 IV=4.8
Period	0-365 days	0-239 days
k_{deg} (day ⁻¹)	0.0015	0.013
R^2	0.9724	0.98

The difference in rates might be attributed to (a) the inherently lower rate of degradation for lactide groups in comparison to glycolide units; and (b) the lower rate of water absorption of PILA of mostly to its higher crystallinity and relatively higher hydrophobicity. In any case, the exponential decrease of the average molecular weight with apparent first-order degradation (without the appearance of bimodality) indicated a random chain scission and a negligible effect of autocatalysis. Similar studies on the effect of composition on degradation had also been reported by Wang and Wu [5]. In a study [5] of homopolymers and copolymers of lactic and glycolic acid having a similar M_w 's of the order of 12 kDa, it was shown that PLGA 85:15 degraded two times faster as compared to PILA.

Similarly to PLGA 80/20, PdILGA 53/47 degradation study is detailed using $^1\text{H-NMR}$ chromatography and Monte Carlo Model to have more details on the degradation mechanisms.

Figure 4.12 presents SEC chromatograms of PdILGA films for different hydrolysis times.

Figure 4.12 Size exclusion chromatography (SEC) curves of PdILGA films after hydrolysis for different times: 0 days (a); 12 days (b) and 41 days (c)

Figure 4.12 shows the bell-shaped mono-modal SEC chromatograms of PdILGA films, the SEC traces shifted with hydrolysis for 41 days to lower M_w and are relatively narrow. As already mentioned for PdLLA, it is easy to see that the degradation process was homogeneous.

Figure 4.13 Overlay of water absorption, weight loss, M_w and M_n of PdILGA 53/47 versus immersion time

PdILGA 53/47 films started to lose substantial mass after about 35 days of immersion and completely degraded after 3 months. A three-phase water absorption and weight loss profile was recorded. It might be noted that it was quite difficult to measure the percent water uptake for samples after the 24th day. This was because the sample was quite fragile and it was difficult to maintain its integrity in order to measure the mass of the film with relative accuracy. The two-weeks degradation mechanism of a similar material was reported [161; 184]. As per the previous report, the changes in state of the matrix from glassy to rubbery were in good agreement with the water absorption and weight loss pattern following a homogeneous degradation pattern [191]. It is interesting to note that at anytime of the degradation, pattern of fall in M_n followed closely with that of M_w . This suggested that all the low molecular weight fragments (oligomers and monomers)

could easily diffuse out, with no build up of acidic products in the matrix and hence no acid catalysis. This behavior was typical of homogeneous degradation and was consistent with the mono-modal distribution of the SEC chromatograms (**Figure 4.12**). Similar reports of homogeneous degradation behavior for PdILGA 50/50 [151; 152] published.

4.3.2 Visual examination

Figure 4.14a and 4.14b present the visual changes of the PdILGA after immersion in buffer. This result was recorded for all polymers, which started to absorb a substantial amount of water.

The film transmittance drastically decreased with degradation time. Initially, PdILGA films were totally transparent (**Figure 4.14a**), and the specimens turned whitish after absorption of water for 10 days (**Figure 4.14b**).

Figure 4.14a

Figure 4.14b

Generally, poor transparency or opaqueness of crystalline films is thought to be due to the existence of light-scattering entities such as voids or aggregates of crystallite whose refractive index is different from that of the surrounding amorphous phase. However, PdILGA was totally amorphous during the degradation process so the decrease in transparency upon degradation might be attributed to increase of most likely, the generation of low-Mw compounds that dissolve in water

leading to microsphere separation that causes opacity. PdlLGA became whitish after only few days of immersion in contrast to the other polymer investigated: PdlLA, PdlLGA 80/20 and PILAs, in respective order. Light transmittance decreased with aging time much more rapidly for polymer films of with lower crystallinity.

4.3.3 Proton Nuclear Magnetic Resonance (^1H NMR) study

We have used ^1H -NMR to quantitatively confirm that the addition of GA units substantially increased degradation rates, over and above the effect due to reduced crystallinity. This was due to a faster hydrolysis rate for the glycolide units. In spite, the overall scission is still a random process, as verified by the application of a Monte Carlo model to the degradation results.

Figure 4.15 presents the ^1H -NMR spectra of PdlLGA 53/47; A: reference sample (unhydrolzed) and B: the sample after 50 days of immersion in buffer (pH 7.4).

Figure 4.15 ^1H -NMR spectra of PdlLGA 53/47 before immersion (A) and after 50 days immersion (B)

The solution spectra were recorded using CDCl_3 as solvent, which contains 0.3 % (w/w) of tetramethylsilane (TMS), used as internal reference. The NMR lines occurring between 7.5-8 ppm was due to the benzophenone protons used as internal standard. Benzophenone was chosen for this purpose because the chemical shifts of the aromatic protons were well separated from those of the polymers. The un-hydrolyzed PdLGA 53/47 0.84 and PdLGA 53/47 0.84 after 50 days of immersion yielded similar spectra showing three distinct chemical shifts; which corresponded to the methyl ($\delta = 1.6$ ppm, from lactide moiety), the methine ($\delta = 4.8$ ppm, from lactide moiety) and the methylene ($\delta = 5.2$ ppm, from glycolide moiety) protons. The composition of the copolymers was calculated from the integration of the peaks for the methylene and methine protons using the benzophenone protons as internal standard. The methylene proton signal accounts for the lactic acid units and the methine proton signal accounts for the glycolic units. It can be clearly seen from the integration of the NMR lines that the residual amount of lactic units decreased from $I_{\text{ref}} = 0.46$ to $I_{50\text{d}} = 0.34$, while the amount of glycolic units decreased from $I_{\text{ref}} = 0.82$ to $I_{50\text{d}} = 0.29$. The methine peak of the lactic unit decreased from $I_{\text{ref}} = 4.41$ to $I_{50\text{d}} = 3.29$. Interestingly, the two peaks representing the end groups ($\delta = 1.2$ ppm) were significantly reduced after 50 days. This could be explained by the fact that some of the oligomers and monomers formed from the degradation could have leached out from the film (**Figure 4.12**). These results showed the evidence of a preferential degradation of the glycolic as compared to the lactide linkages. This was due to the difference in steric hindrance in the two moieties, (Scheme -I)

Scheme I: The presence of the bulky methyl group on the α -carbon in lactide enhances steric crowding and reduces the partial positive charge of carbonyl carbon

Figure 4.16 presents the contents of the lactide and glycolide of the copolymer remaining after degradation, as determined by $^1\text{H-NMR}$.

Figure 4.16 Relative percentages of lactide and glycolide content in PdILGA 5347, as calculated from $^1\text{H-NMR}$

It can be clearly seen that the glycolic fragments/units degraded faster than the lactide. The lactide content, which was shown to be 55.4 % in the original sample (as against 53 % reported by the supplier), steadily increased to about 62 %, over a period of 41 days of degradation. The glycolide content, on the other hand, declined from about 45.6 % of the original value to about 38 %, over the same period. The results clearly showed a higher reactivity of the glycolic links towards hydrolytic degradation, as compared to the lactide linkages. Hakkarainen et al. [151] studied by gas chromatography-mass spectroscopy the degradation products of PLGAs and found that

glycolic acids units in the copolymers always degraded faster than the lactic acid units. As a result, PLGA 75/25 became PLGA 87/13 after 80 days of immersion. Giunchedi et al. [148] looked at monomer release rates, found that the GA monomer releases more than the LA monomer, leading to the same conclusion regarding differential glycolide scission rates. Kamei et al. [194] studied the degradation hydrolysis of PLGAs by an HPLC method and the oligomers of LA up to pentamer were detected. The glycolic units were found to decrease more rapidly than LA.

Chemically, the two ester links differ in the additional methyl side group on the α -carbon. The process of hydrolytic degradation involves nucleophilic attack of water molecules on the carbonyl carbon, which is electrophilic in nature because of the electron-withdrawing inductive effect of the carbonyl oxygen. The process of nucleophilic attack is aided or inhibited by several factors, the state of the electronic environment of the carbonyl carbon being the most important. A comparison of the electronic environments of the carbonyl carbons in lactide and glycolide units shows two important differences: first, the bulky methyl group on the α -carbon in the lactide adds to the steric crowding around the carbonyl carbon, thus hindering the approach of the nucleophile. Second, the methyl group being electron-donating in nature (hyperconjugation), the electrophilicity of the carbonyl carbon in the lactide unit is relatively reduced, by virtue of additional electron-density from the methyl group. Both these factors inhibit the extent of hydrolytic degradation of lactide link, in comparison to the glycolide. Thus for PLGA, a random copolymer, preferential hydrolysis of glycolic ester bonds is likely to result in the formation of lactide-rich oligomers.

4.3.4 Application of Monte Carlo model

For the two PLGA copolymers, it was also possible to perform Monte Carlo calculations in an attempt to confirm the randomness of scission events.

Figure 4.17 shows the overlaid Monte Carlo plots for PLGA 8020 and PdILGA 5347, the two polymers that exhibited measurably high weight loss. Using the SEC values for M_n and the weight loss values, we computed R_m and other parameters in *Eq. 3.5*.

Figure 4.17 Overlaid Monte Carlo plots for PLGA 8020 and PdILGA 5347

As seen from the **Figure 4.17**, both the copolymers showed patterns of random chain degradation. The hyperbolic profile is defined by three different parts, an increase, a dome and a decrease. The first part corresponds to the degradation of the polymer without weight loss, the dome corresponds to the “stabilization region” and the beginning of the weight loss, and the last part shows the decrease corresponding to the leaching out of oligomers. The maximum of the Monte Carlo curve applied to PLGA 80/20 was much higher than PdILGA 53/47. Again, the conclusion is that the overall hydrolytic scission process was random in nature, in spite of the higher reactivity of the

glycolide unit. This probably reflected the fact that the two polymers are random copolymers with very little content of blocks.

4.3.5 Thermal analysis

The thermal behaviour of PLGA 80/20 had unusual features. **Figure 4.18** presents the traces of DSC thermograms for PLGA 8020 4.8 showing the appearance of a crystallization peak with immersion time.

Figure 4.18 Traces of DSC thermograms for PLGA 8020 4.8 showing the appearance of the crystallization peak with immersion time

This peak appears due to one of two possible reasons: (a) the lower- M_w molecules, formerly part of the amorphous phase, had reorganized into crystalline domains, or (b) the preferential

generation of lactide-rich segments during degradation led to crystallization of these segments. For reasons to be described below, we favor the latter explanation. Due to the low enthalpy of melting for the reference and 12 weeks samples, it is not possible to evaluate the peak in this thermogram but the values are reported in **Figure 4.19**.

Figure 4.19 presents the overlay of the melting temperature (T_m) and the enthalpy differential of melting and recrystallization peaks ($\Delta H_f - \Delta H_c$), as a function of immersion time, for PLGA 8020.

Figure 4.19 Melting point (T_m) and crystallinity for PLGA 8020 4.8

There was an initial increase in DOC (up to about 200 days), followed by a decrease. The explanation for all the above observations is as follows. The recrystallization peak was due to the crystallization of lactide segments, which organize into crystalline regions upon heating beyond

their T_g . If lower- M_w fragments could lead to crystallization, we would have observed this for PILA 2.04. The total degree of crystallinity ($\Delta H_m + \Delta H_c$) obviously increased with time of immersion. The differential DOC ($\Delta H_m - \Delta H_c$) increased during the 0-200 day period because the amorphous region was slowly being depleted by preferential attack of the amorphous domains, and the leaching-out of the amorphous phase.

The decrease in differential DOC seen after 200 days was due to the water attacking the crystalline regions. Interestingly, the melting point was shifted upwards from 145 °C to reach a value of 165 °C at the end of the 30th week, followed by a decrease to 150 °C toward the end of the study. This may be explained in terms of preferential hydrolysis of glycolide linkages, which consolidated the remaining 1-lactide chains. Tsuji and Ikada [130] observed a similar pattern for PILA film and reported that the increase in T_m was due to the thickening of the PILA crystallites. The subsequent decrease in T_m is because of the reduced thickness of the crystallites with increasing extent of hydrolysis and the drop in molecular weight of the material.

4.3.6 SEM analysis

Figures 4.19a and **4.19b** present the SEM micrographs of initial PLGA 4.8 film before immersion and that after 30 weeks of immersion in the buffer, respectively.

Figure 4.19a

Figure 4.19b

As evident from the micrographs, the film before immersion was quite smooth, whereas the film after 30 weeks of immersion exhibits the formation of large holes (about 30 μm), characteristic of bulk erosion process. A comparison with the micrographs of PILA 4.37 (**Figure 4.9d**) showed the difference in the extent of degradation.

The results presented show that the degradation can be modulated by several factors. All polymer film investigated showed a homogeneous degradation path and a strong effect of crystallinity and GA content on the degradation rate.

Decreasing crystallinity and increasing the GA content, both increase degradation rates substantially. Effect on the drug loaded in polymer matrix is also a determinant factor for drug release studies.

The effects of basic, acidic and neutral drugs on degradation from PLA or PLGA matrices are fairly complex. Particularly for basic drugs, there is conflicting data with respect to the effects on degradation and release rates. In a second objective, the effect of the chemical nature of the drug on matrix degradation behavior of degradable polymers (PdlLGA 53:47 and PILA 4.37) was studied, using lidocaine as a model drug in base and salt forms.

Similarly to the degradation study of the polymer, the results have been discussed in terms of water absorption, weight loss, molecular weight (M_w), glass transition temperature (T_g), degree of crystallinity, and scanning electron micrographs.

4.4 Effect of Drug Chemistry

4.4.1 Water uptake of PdILGA and PILA, with and without drugs

Figure 4.21 presents the results of water uptake for PdILGA with and without drug loading, as a function of incubation time.

Figure 4.21 Water absorption profiles of PdILGA (with and without drug loading)

The water absorption profile for the polymer film without any drug was different from the profile of drug-loaded films (**Figure 4.21**).

Surprisingly, percent water absorption of PdILGA with lidobase was higher as compared to the film loaded with lidosalt and the drug-free sample. It was expected that the film with lidosalt would have a higher osmotic driving force for water uptake than the one with the lidobase. However, another factor appears to overwhelm the osmotic effect, as discussed below.

The water uptake of the drug-free PdILGA showed the following pattern:

- 0-2 days: Lag time before the beginning of water absorption, might be due to the time necessary for water diffusion into a glassy hydrophobic matrix
- 2-12 days: Water absorption increased to follow a diffusion pattern to about 10 % absorption.
12–20 days: Substantial increased in water absorption.
- 20-42 days: Water absorption appeared to attain a plateau of about 44 %.

PdILGA with lidosalt showed a tri-phasic pattern:

- 0-10 days: Water absorption was detected within one day of immersion in buffer and increased up to 28 % at the end of 10 days in what appeared to be a diffusion-controlled profile. This higher water absorption (compared to the drug-free film) was attributed to the greater osmotic pressure within the salt-drug filled film.
- 10-28 days: Water absorption attained a plateau at about 28 % for 18 days.
28-34 days: A very rapid increase of water absorption to about 62 % at the end of 34 days was due to the degradation of the polymer, leading to higher hydrophilicity.

PdILGA with lidobase shows a biphasic pattern:

- 0-4 days: Water absorption was detected within one day of immersion and increases rapidly to 40 %. The higher rate of water absorption was attributed to two factors: a relatively small osmotic effect, and a greater effect due to onset of degradation, which generates more -COOH end groups, which in turn increased hydrophilicity.
- 4-25 days: Water absorption attained a plateau at about 40 % for 21 days.
- 25-28 days: A very rapid increase to about 98 % at the end of 28 days was due to extensive degradation and leaching-out of oligomers that led to creation of pores, thus facilitating water uptake.

The results showed that the water absorption of drug-free PdILGA was lower than the water absorption of PdILGA loaded with lidosalt, which was lower than the water absorption of PdILGA loaded with base. The higher rate for the PdILGA with salt was evidently due to an osmotic effect, while the even higher absorption by the PdILGA with lidobase was due mostly to earlier onset of degradation, as will be reinforced by the molecular weight (M_w) results, below. The second step seen in the water absorption of the drug-free PdILGA and PLGA loaded with a salt drug was observed in an earlier study by us [195]; this was attributed to a transition from a glassy to rubbery phase.

Figure 4.22 presents the results of water uptake for PILA with and without drug loading, as a function of incubation time.

Figure 4.22 Water absorption of PILA (with and without drug) with time (days) immersion

On the other hand, PILA 4.37 (with and without drug loading) showed a different pattern (Figure 4.22): water absorption was very slow, with less than 4 % of water uptake after 300 days(immersion, for both drug-loaded and drug-free samples.

Apparently, the combination of very high molecular weight, high crystallinity and relatively hydrophobic nature of PILA matrix, prevented any appreciable water uptake, within the period of investigation in this study.

4.4.2 Weight loss of PdILGA and PILA, with and without drugs

Figure 4.23 presents the results of weight loss profiles (with and without drug) of PdILGA films as a function of incubation time.

Figure 4.23 Variation in percent weight loss profiles of PdILGA (with and without drug) with time (days) immersion

The percent mass loss for PdILGA (**Figure 4.23**) was characterized by an initial lag phase without appreciable detection of mass loss, followed by a sigmoid decay of the polymeric matrix, characteristic of a bulk degradation pattern [150; 197].

A typical two-phase pattern was shown: a slow weight loss (5 %), followed by an accelerated phase. In the second phase, the rate of weight loss for PdILGA loaded with lidobase was higher as compared to PdILGA alone or PdILGA loaded with lidosalt. The matrix loaded with lidobase started losing substantial mass as early as day 16, well before any significant loss was seen in case of PdILGA with lidosalt (day 26) or PdILGA alone (29 days). This difference is due to the effect of

base catalysis (catalytic hydrolysis of polyester linkage by lidobase) on degradation of the PdILGA.

Figure 4.24 presents the results of weight loss profiles (with and without drug) of PILA films as a function of incubation time.

Very little weight loss (**Figure 4.24**) was registered and virtually no difference was observed between the drug-loaded and drug-free samples of PILA 4.37.

Figure 4.24 Percent weight loss of PILA (with and without) with time (days) immersion

This was in agreement with the water absorption profiles of the films (**Figure 4.22**). Once again, this might be due to the very high molecular weight, high degree of crystallinity and hydrophobicity associated with PILA.

Tsuji and Ikada [130], in a related study involving high molecular weight PILA ($M_w = 10^6$ Da) films, reported that the mass of all PILA samples in their study, remained unchanged up to 12 months. Weight loss as a result of hydrolysis was observed only at the end of 18 months, whereas 36 % of original mass was still left at the end of 36 months. This is consistent with the data reported here.

4.4.3 SEC analysis of PdlLGA and PILA, with and without drugs

Figure 4.25 presents the change in weight average molecular weight of the samples of PdlLGA and PILA (with and without drug loading), as function of immersion time.

Figure 4.25 Change of average molecular weight (M_w) of PdlLGA with time (days) immersion

The fast decreased in the molecular weight for PdILGA (**Figure 4.25**), followed a well-known exponential pattern, representative of random chain scission by bulk degradation [104]. The onset of M_w decreases occurred almost immediately after immersion in buffer and attains a plateau level in about a month's time.

The matrix during this phase apparently undergone the so-called first-order degradation. At a M_w of about 15 kDa, the rate of degradation slowed down to reach a value of about 10 kDa for more than 100 days. As shown in **Figure 4.25**, the pattern of degradation of the matrix was different for drug-free and drug-loaded samples. Both drug-free and lidosalt-loaded samples of PdILGA had a similar degradation profile and continue to have higher molecular weight than PdILGA loaded with lidobase, over a period of one month. The greater effect of lidobase in the degradation rate was clearly because of the catalytic effect of the drug as a base. While most of the decrease in M_w (**Figure 4.23** and **4.25**) occurred continuously to the 23rd day for lidobase (40th day for lidosalt and drug-free PdILGA), the real threshold time for weight loss to start, was 20th day (28th day for lidosalt and drug-free matrix). The samples did not start any appreciable weight loss before this threshold time, because not enough leachable oligomers had been formed.

The effect of base on catalytic degradation was reported by Li et al. [104] for PdILA in the presence of caffeine (a tertiary amine). It was reported that for low amine contents (< 2 %), the process of degradation was considerably accelerated with respect to caffeine-free devices. In a similar sense, it has been found here that the dissolved lidobase accelerated hydrolytic degradation of PdILGA, to the extent that M_w -decrease is observed from the first day after immersion.

The first order degradation constants were calculated as above from a linear regression analysis of the semilog plot of $\ln M_w$ vs time and **Table 4.3** lists the constants for the first-order degradation rate of PdILGA with and without drugs, as obtained from the slope of the straight line.

Table 4.4 First order degradation constants of PILA 4.37: With and without drugs

First-order degradation	PILA	PILA with lidosalt	PILA with lidobase
Period (days)	0-365	0-300	0-300
Rate k (day^{-1})	0.0019	0.0055	0.0028
R^2	0.9606	0.9631	0.9774

These results were consistent with the results of water absorption and weight loss (**Figure 4.21** and **4.23**) showing the early break down of PdILGA matrix loaded with lidobase and were indicative of the relative importance of the base catalysis as compared to acceleration due merely to increased water absorption.

Under the same conditions as PLGA, the effect of the drug on the degradation rate of PILA has been carried out.

Figure 4.26 shows the decrease in weight average molecular weight (M_w) of PILA matrix with and without drugs.

Figure 4.26 Change in weight average molecular weight (M_w) of PILA 4.37 (with and without drug loading) with time of immersion

For drug-free PILA, there was only one slow phase of change in M_w over a period of 300 days of incubation. Once again, the determining factor appeared to be the high molecular weight, crystallinity and the relative hydrophobicity of the matrix. However, the changes in M_w for drug loaded samples are faster, both for lidosalt as well as for lidobase, compared to drug-free PILA. These results were in accordance with the water absorption (Figure 4.22 and 4.24) and weight loss results showing acceleration in degradation (for PLLA) caused mostly by the osmotic effect of drug on water absorption.

The rate constants (k) for the first-order degradation of PILA with and without drug were obtained from the slope of the straight lines from the semilog plot of $\ln M_w$ vs time and are summarized in **Table 4.4**.

Table 4.4 First order degradation constants of PILA 4.37: With and without drugs

First-order degradation	PILA	PILA with lidosalt	PILA with lidobase
Period (days)	0-365	0-300	0-300
Rate (day ⁻¹)	0.0019	0.0055	0.0028
R ²	0.9606	0.9631	0.9774

The degradation was the fastest for salt-containing film, in the case of PILA, especially after day 50. This was consistent with the water absorption rates, which appeared to increase for the salt-containing film after day 100.

4.4.4 Change of glass transition temperature (T_g) of the PdILGA and PILA, with and without drug

Figures 4.27 presents plots of the glass transition temperatures (T_g) of PdILGA with and without drug loading, as function of immersion time. All the films used in this study were dried to remove any absorbed buffer solution that would obscure the interpretation of T_g .

Figure 4.27 Change in glass transition of PdLLGA (with and without drug) with time (day) immersion

In contrast to the T_g of PdLLGA loaded with lidobase, the T_g for drug-free and lidosalt-loaded PdLLGA follow the same pattern (**Figure 4.27**). Before immersion in buffer, both drug-free and drug-loaded samples of PdLLGA had T_g s higher than the average body temperature ($T = 37^\circ\text{C}$). The T_g s of the drug-free and lidosalt loaded samples of PdLLGA started to drop after about 14 days of immersion. It might be assumed that the T_g drop was due to a decrease in M_n , to a value below the M_n -threshold for constancy of T_g . In contrast, T_g of PdLLGA loaded with lidobase was constant for 5 days at about 39°C followed by almost linear decrease to reach a value of $\sim 33^\circ\text{C}$ after 30 days immersion. The results might be explained on the basis of M_w -dependence of the T_g .

As shown by the Fox-Flory equation,

$$T_g = T_g^\infty - \frac{K}{M_w}$$

the glass transition temperature of a polymer is influenced by its molecular weight, until a certain value known as limit of glass transition temperature or persistent glass transition temperature; beyond this, effect of the molecular weight is not pronounced [196].

So the drop in T_g was consistent with the significant decrease in M_w of PdILGA loaded with lidobase. From **Figures 4.25**, we might infer that the critical molecular weight characterized by the beginning of the drop of the glass transition for this particular material might be between 40-50 kDa (M_w -value).

Figures 4.28 presents plots of the glass transition temperatures(T_g) of PILA with and without drug loading, as function of immersion time.

Figure 4.28 Change in glass transition of PILA (with and without drug) with time of (day)immersion

Figure 4.28 shows that the incorporation of drug has no effect on the original T_g of the PILA. T_g (about 63°C) was much higher than the body temperature and remains unchanged for 30 weeks. For drug loaded samples, the T_g started dropping after 300 days. Apparently, the decrease in T_g was a consequence of decrease of degree of crystallinity, allowing a higher free volume, for chain movement.

4.4.5 Change of the degree of crystallinity of the PILA with and without drug

Figure 4.29 represent the change in degree of crystallinity (DOC %) of PILA with and without drug versus immersion time.

Figure 4.29 Change of degree of crystallinity of PILA with time (day) of immersion

Interestingly, the DOC (%) of PILA showed different patterns for drug-free and drug-loaded samples. PILA free of drug had a very slight increase in the degree of crystallinity from 35 % (before immersion) to 40 % after about 300 days of immersion. On the other hand, samples of PILA loaded with lidosalt and lidobase showed significant increases in DOC for 210 days followed by a decrease. The DOC (%) of PILA loaded with lidobase, which was about 40 percent initially, increased to reach almost 70 percent after 210 days of immersion, followed by a decrease to reach 60 percent after 300 days. A similar pattern was observed for PILA loaded with lidosalt. This was consistent with the changes in the glass transition temperature seen in **Figure 4.28**. The increase of crystallinity of the polymer upon immersion is similar for drug-free and drug loaded matrices [130; 132; 152]. In one of these studies, PILA ($M_w = 300$ kDa) was reported to have shown an increase in DOC from 30 to 49 % in alkaline medium [132]. Li et al. [152] claimed that the ability of PILA to crystallize is related to the stereo-ordered isotactic chains composed of L-lactide repeating unit. Mainil-Varlet et al. [162] in an in vivo study reported that an initial degradation took place in the amorphous regions, since these domains were more accessible to the release medium. The second stage of degradation took place through a chain scission in the crystalline domains and was manifested by a decrease in the overall crystallinity of the matrix. The crystallinity of PILA samples in their study increased over the first 12 weeks of implantation and then decreased gradually until the end of the implantation period. The pattern observed here is similar to that reported by Mainil-Varlet et al. [162], and hence attributed to the initial degradation attack on the crystalline domains.

4.4.6 SEM analysis

The SEM micrographs of PdILGA film without any drug, PdILGA loaded with lidobase and lidosalt, before immersion into buffer, are presented in **Figures 4.30a-c**. Micrographs of the same set of films after immersion in buffer and taken out at different time points are presented in **Figure 4.30d** (PdILGA after 37 days of immersion), **Figure 4.30e** (PdILGA loaded with lidobase and immersed for 30 days) and **Figure 4.30f** (PdILGA with lidosalt immersed for 23 days).

Figure 4.30a

Figure 4.30b

Figure 4.30c

Figure 4.30d

Figure 4.30e

Figure 4.30f

There was appreciable erosion of the individual matrices as clear from the micrographs. The extent of erosion was more apparent in the case of drug-loaded samples and was visible in the form of pores and blisters on the sample surface. The degradation pattern was compatible with the pathway

of bulk degradation. It might be noted that the micrographs of the original films (before immersion) showed no evidence of drug on the surface. This is because of the lower drug loading (2 % w.r.t the matrix), which was within the limit of solubility of the drug in the matrix. As discussed in a separate chapter on the drug release profiles from these films, this explains the absence of the familiar burst effect. Loading the matrix beyond saturation also obscured results relating to drug release and degradation rates.

Figures 4.31a-c present the micrographs of PILA film without drug, PILA with lidobase, and PILA with lidosalt, respectively. The micrographs of the same set of films after 42 weeks of immersion into the buffer are presented in **Figures 4.31d-f**, respectively.

As seen from the micrographs, the surface morphology of the films was smooth and showed no evidence of crystals for the drug-loaded samples. Although there were signs of emerging surface roughness, no signs of surface/bulk erosion were apparent, even after 42 weeks of immersion.

These results were in accordance with the results of water absorption and weight loss discussed above (**Figure 4.22** and **4.24**). The findings also agreed well with previous observations obtained under similar conditions. [131-133; 156; 190].

4.5 Summary

In summary, the degradation of all polymers appears to occur homogeneously by random chain scission. However, the glycolic acid unit hydrolysis is faster than the lactic unit as shown with the NMR study. The study of PILAs with two different molecular weights clearly shows that the degree of crystallinity is a dominating factor compare to the molecular weight. The higher the molecular weight, the lower the crystallinity and thus the faster the degradation rate. In other hand, the composition is also affecting the degradation rate by a higher hydrophilicity and lower crystallinity with an increased of glycolic content leading to higher degradation rate. **Table 4,5** summarizes the degradation rates of all polymers.

First-order degradation const.	PdLGA 53/47	PLGA 80/20	PILA IV=4.37	PILA IV=2.04	PdILA	
Period (day)	0-40 days	0-239 days	0-365	0-300	0-150	150-250
k _{deg.} (day ⁻¹)	0.0184	0.013	0.0015	0.0009	0.0087	0.0088
R ²	0.981	0.98	0.9724	0.982	0.9752	0.9642

Table 4.5 Summarize of first-order degradation constant of all polymers.

The SEC chromatograms (no bimodality) as well as the profile of M_w decrease confirm homogeneity of the hydrolysis. Measurable weight loss (defined to be when the MW reaches 10 % of initial value) starts to occur when samples attain a certain M_w range (**Table 4.6**).

Table 4.6 Critical molecular weight of polymers

Critical molecular weight	PdILA	PLGA 80/20	PLGA 53/47
M_w (kDa)	41	45	17
M_n (kDa)	22	20	9

For PLGA 80/20, this onset of weight loss also coincides with attack on the crystalline regions, as evidenced by a drop in the degree of crystallinity. Finally, Monte Carlo calculations attest to the random nature of this hydrolytic cleavage.

In addition we clearly showed the effect of the drug loaded into polymers film on degradation rate. The degradation was strongly accelerated with polymer loaded with base drugs due to a base catalysis on the ester linkages. The polymer loaded with the salt drug degraded faster than the polymer free of drug due to a higher water absorption, resulting of the osmotic effect created by the salts but was slower than the polymer loaded with base drug. PdILGA loaded with lidobase showed only two of the three steps in the degradation process. Because of base catalysis, stages 1 and 2 are merged, i.e., the decrease in M_n was so rapid that the T_g appeared to decrease continuously.

In contrast, this behavior was not confirmed with the semi-crystalline PILA and may be due to a very little water absorption for the polymer free of drug and drug loaded during the period of

study. The degradation of PILA (with and without drugs) occurred through a very slow increase in hydration with little degradation. In the hydration stage, a small amount of water penetrated the polymer film, which resulted in a very slow degradation rate, starting from the amorphous regions and leading to an increase of crystallinity, before the hydrolytic attack on the crystalline sites. At a further degradation stage, the polymer chains continued to be cleaved and the molecular weight of the polymer decreased accompanied by a decrease in crystallinity and the glass transition temperature. However, there was no mass loss as not enough oligomers had been created.

CHAPTER 5

DRUG RELEASE MECHANISMS

5.1 Introduction

As both hydrophilic and hydrophobic drugs are widely used, it was of interest to carry out a systematic study of the effect of chemical nature of the drug on the drug release behavior.

To our knowledge, only one report has been published of a study of the release behavior of the same drug in different forms [197]. As the objective in this thesis was to isolate all factors affecting the drug release, tests were performed to evaluate the effect of different drug forms (base and salt), polymer matrices of different composition, molecular weight, crystallinity on the drug release characteristics. A summary of these variables is shown in **Table 5.1** below.

	Polymers	Drugs
Effect of drug type	PLGA 53:47 (IV=0.84g/dl) (amorphous) PILA (IV=4.37 g/dl) (semicrystalline)	Lido base and Lido salt
Effect of crystallinity	PdILA (IV=2.04 g/dl) (amorphous) PILA (IV=2.4 g/dl) (semicrystalline)	Lido base
Effect of M_w	PILA (IV=2.4 g/dl) (semicrystalline) PILA (IV=4.37 g/dl) (semicrystalline)	Lido base
Effect of composition	PLGA 80:20 (IV=4.8 g/dl) (semicrystalline) PILA (IV=4.37 g/dl) (semicrystalline)	Lido base

It should be noted that the effect of degree of crystallinity could not precisely be isolated from the due to different values of crystallinity of PLGA 80/20 and PILA.

The drug release data obtained from the in vitro release study was analyzed for the rate of release, using the Higuchi [198] drug release equation given below:

$$\frac{M_t}{M_{inf}} = k \times t^{\frac{1}{2}} \quad (5.1)$$

(where M_t/M_{inf} is the fraction of drug released, t is the release time and k is a constant characteristic of the system)

or a linear relationship:

$$\frac{M_t}{M_{inf}} = k \times t \quad (5.2)$$

for the different stages, as explained further below.

The value of the diffusion coefficient, D , has been calculated according to the relation:

$$k = 4(D / \pi l^2)^{1/2} \quad (5.3)$$

where, l = thickness of film or slab.

This is valid for release of less than 60 % of initial load, as applied to a monolithic device containing dissolved drug [198]. This equation is applied along with Eq. (5.1) to calculate D .

5.2 Effect of Type of Drug on Drug Release Mechanism

5.2.1 Poly (d,l-lactide-co-glycolide)

Figure 5.1 plots the drug release from PdlLGA as fraction of the total loading versus the immersion time in the release medium.

Figure 5.1 Fraction of drug released from films of PdILGA 53/47 IV=0.84 vs. immersion time.

It is clear that the drug release behavior of lidobase and lidosalt from the matrix were different. Release of lidobase followed a well-defined two-phase pattern after day one. On the other hand, the salt followed a tri-phasic pattern after a lag time of two days. No initial burst effect was observed, apparently because of a very small drug-loading (2 %). These results are well in agreement with the SEM micrographs of the PdILGA loaded with drugs (**Figures 5.2b** and **5.2c**). The initial lag phase might be due to the relatively slow diffusion rate through a glassy matrix. The lower (1 day) lag time for the PdILGA loaded with lidobase compared to 2 days for PdILGA loaded with lidosalt was because of a higher water absorption in the first day for the lidobase-loaded PdILGA, resulting in a swollen matrix. The lag phase in the case of lidobase, was followed by a stage of diffusion

controlled release starting from day 2 to day 12. On the other hand, lidosalt follows a stage of diffusion controlled release starting from day 3 to day 11. Diffusion controlled release from the PdILGA matrix loaded with lidobase was faster than PdILGA loaded with lidosalt due to higher water absorption with lidobase (**Figure 4.21**) and a faster decrease of T_g (**Figure 4.27**). At the end of day 12, the release of lidobase increased significantly: this coincided somewhat with the onset of significant weight loss, seen in **Fig. 4.23**. The release of lidobase was then linear to almost completion (near 100 %) at the end of 35 days. This apparent linear rate constant can be calculated from Eq. (5.2), and is shown to be $3.7 \% \text{ day}^{-1}$.

In contrast, a second phase of diffusion-controlled release for lidosalt took place between 12 and 30 days. The diffusion was slightly faster in this second phase than the first (day 2-11). This is consistent with the transition from a swollen glassy to a swollen rubbery state of PdILGA free of drug and PdILGA loaded with salt starting at day 16 (**Figure 4.27**) and a higher rate of water absorption starting at day 11 (**Figure 4.21**). Beyond 30 days of immersion, a significant extent of release of lidosalt was registered, reaching nearly 100 % at the end of 40 days. This third stage was similar to the degradation-controlled stage of the lidobase-loaded PLGA (with an apparent rate constant of $5.3 \% \text{ day}^{-1}$) and runs parallel to the weight loss phase seen around day 30 for the lidosalt (**Figure 4.23**). The drug release path in this final stage was most likely through the water channels created by the leached-out oligomers. This pattern of release using hydrophilic drugs has been reported [35, 195] and explained by our group [195].

As reported here, the release pattern of lidobase and lidosalt from the matrix of PdILGA shows:

- The drug as a salt could be sustained in the matrix for longer time than its base counterpart.
- The first stage of diffusion-controlled release (up to day 12) was similar for both drugs. However, the lidobase diffuses faster than lidosalt due likely to a higher water absorption rate (leading to a matrix with lower T_g).
- Only the lidosalt-loaded sample showed a second diffusion phase, and it occurred between days 12-30, with a slightly higher diffusion rate. This was due to diffusion through a rubbery phase. Degradation set in prior to the observation of this second diffusion phase for the lidobase-loaded matrix.

Table 5.2 presents the diffusion coefficients (D) of lidobase and lidosalt from PdILGA matrix, as obtained from plots of amount of drug release vs. square root of release time, t in PBS 7.4. The diffusion coefficient was not calculated for the last phase of release because that phase is degradation-controlled.

Table 5.2 Results from kinetics of drug release (lidobase and lidosalt) from PdILGA

Effect of drug	PdILGA with lidosalt		PdILGA with lidobase
Period (days)	3-11	12-31	1-12
Rate k (percent day-1)	$k_1 = 1.8$	$k_2 = 4.6$	$k_1 = 3.85$
Diffusion coef. (cm ² /s)	$D_1 = 4.6 \text{ E-}11$	$D_2 = 3.0 \text{ E-}10$	$D_1 = 1.59 \text{ E-}10$
R ²	$R_1^2 = 0.9721$	$R_2^2 = 0.9737$	$R_1^2 = 0.939$

These results were consistent with the water absorption (**Figure 4.21**), weight loss (**Figure 4.23**) and the glass transition temperature (**Figure 4.27**) data. The diffusion coefficients were also consistent with the diffusion coefficient values reported in a previous study of $D_1 = 2.42 \times 10^{-10} \text{ cm}^2/\text{sec}$ and $D_2 = 5.82 \times 10^{-10} \text{ cm}^2/\text{sec}$, for the two phases 1 and 2 respectively, for the release of metoclopramide hydrochloride from PdLGA 53/47 IV=0.47 dl/g [195].

5.2.2 Poly (I-Lactide) IV =4.37

Figure 5.2

Within the period of study of 260 days, the release of both lidobase and lidosalt was observed to follow a single diffusion pattern. Lidobase was released slightly faster than lidosalt and the drug release profile showed a first-order diffusion-control over 200 days. This is in good accordance with the trends in water absorption as shown (**Figure 4.22**), weight loss (**Figure 4.24**) and glass transition temperature (**Figure 4.28**). This result is in agreement with PdlLGA, where the base released faster than the salt due to a higher degradation rate of the drug-loaded matrix. However, PILA loaded with drug did not yet show significant level of matrix degradation. It is worth to note that from the graph it is not obvious that the salt release slower than the base due to an early burst discarded from the calculations. The drug release, therefore, was seen to be control by simple diffusion through the amorphous regions of the semi-crystalline polymer and the more soluble drug (salt) diffused faster than the hydrophobic base form.

As described above, the first-order rate constant (k) of the release process and the diffusion coefficient of the drugs from the matrix were calculated and have been summarized with their coefficients of correlation, in **Table 5.3**.

Table 5.3. Results of drug release kinetics of lidobase and lidosalt from PILA 4.37

Effect of drug	PILA IV 4.37 with lidosalt	PILA IV 4.37 with lidobase
Period (days)	7-260	1-266
Rate k (percent day ⁻¹)	$k = 0.055$	$k = 0.07$
Diffusion Coef (cm ² /s)	$D = 4.29 \text{ E-}14$	$D = 6.95 \text{ E-}14$
R^2	$R^2 = 0.9951$	$R^2 = 0.9712$

The magnitude of the diffusion coefficient as reported here reflects diffusion of a medium- M_w drug from a completely glassy matrix as described by Baker [198].

5.3 Effect of Composition on Drug Release Mechanism

Figure 5.3 plots the release of lidobase as fraction of total loading from matrices of PILA 4.37 and PLGA 80/20 4.8 versus the immersion time in the release medium.

Figure 5.3 Release of Lidobase from PILA 4.37 and PLGA 4.8 as a function of release time: effect of composition on release

Release of lidobase from PLGA 80/20 4.8 is characterized by a diffusion phase of 80 days. Similarly, PILA films exhibit a single diffusion controlled release of about 250 days in a drug release window of 8-10 %, after 50 days. Degradation-controlled release is not observed for either polymer in this time frame, thus demonstrating that the lidobase affects PLGAs with greater GA content

much more (**Fig.5.1**). It can also be due to the difference of crystallinity and lower glass transition leading to a higher water absorption and faster drug diffusion.

The first order drug release constant (k) and the diffusion coefficient (D) for the release of lidobase from PILA 4.37 and PLGA 4.8, is summarized in **Table 5.4**, with the respective coefficients of correlation.

Table 5.4. Results of release kinetics of lidobase fi-om PILA 4.37 and PLGA 4.8

Effect of composition	PILA IV 4.37	PLGA 80:20 IV 4.8
Period (days)	1-266	0-108
Rate, k (percent day ⁻¹)	$k= 0.07$	$k= 1.48$
Diffusion Coeff (cm ² /s)	$D= 6.95 \text{ E-}14$	$D= 3.11 \text{ E-}11$
R^2	$R^2= 0.9712$	$R^2= 0.9593$

It can be clearly seen that the effect of composition significantly affects the diffusion rate of the drug through the polymer film. This was due to higher hydrophilicity and lower crystallinity of PLGA 80/20 4.8 as compared to PILA 4.37. PLGA 4.8 exhibited an enthalpy of melting of about 10 J/g for this period of time compared to about 50 J/g for PILA 4.37. The magnitude of the diffusion coefficient for lidobase for PLGA 4.8 and PILA 4.37 respectively, was consistent with the results reported above.

5.4 Effect of Crystallinity on Drug Release Mechanism

Figure 5.4 presents the fraction of lidobase release as a function of time, from PILA 2.04 and PdILA 2.4. The results of kinetic analysis (rate constant, k , diffusion coefficient, D), along with the coefficients of correlation, calculated as per the description before, are presented in **Table 5.5**.

Figure 5.4 Release of lidobase from PdILA 2.4 and PILA 2.04, as a function of release time: Effect of matrix crystallinity on drug release behavior.

It is evident that crystallinity of the matrix does play an important role on the drug release profile, PdILA matrix exhibited a two-phase release pattern for lidobase over a period of 250 days. The first stage, which is a diffusion controlled profile lasting over 70 days, is followed by the second phase characterized by a faster release of the drug due to onset of the matrix degradation. The first phase sees the diffusion release of lidobase within a window of 5-17 % in 100 days, followed by a fast increase to about 94 % at the end of 241 days. The release profile of lidobase from PILA, in

contrast, followed a single diffusion pattern, delivering less than 10 % of original loading at the end of 213 days. The release results clearly establish the role of crystalline vs. amorphous domains in the matrix: an amorphous matrix absorbing water and the diffusion-controlled release of the drug being facilitated from a hydrated matrix.

Table 5.5 Results of kinetic analysis of the release of lidobase from PILA 2.04 and PdILA 2.4

Effect of crystallinity	PILA IV 2.04	PdILA IV 2.4
Period (days)	1-213	1-67
Rate, k (percent day ⁻¹)	k= 0.052	k= 1.38
Diffusion Coeff (cm ² /s)	D= 3.84 E-14	D= 2.7 E-11
R ²	R ² = 0.9525	R ² = 0.9548

The glass transition temperatures of both PdILA 2.4 and PILA 2.04 are much higher than the test temperature of 37 °C. Both the matrices were therefore in the glassy state. A higher value of diffusion coefficient could therefore only be explained on the basis of a lower degree of crystallinity for PdILA, leading to an earlier onset of the degradation phase (day 100).

It is interesting to note that the diffusion coefficient of the PILA 2.04 ($D_1= 3.84 \text{ E-14 cm}^2/\text{sec}$) is almost half that of PILA 4.37 ($D= 6.95 \text{ E-14 cm}^2/\text{sec}$). This may not be surprising especially when considered in light of the fact that PLLA 2.04 has distinctly higher degree of crystallinity as compared to PILA 4.37 (50 % vs. 40 %, respectively) and higher glass transition temperature. The slight reversal in trend noted for PdILA and PLGA 80/20 may reflect the different degrees of water absorption during the diffusion-controlled phase.

5.5 Effect of Glass Transition Temperature on Drug Release Mechanism

It is interesting to examine the diffusion-controlled stages of drug release for each of the polymers, in a little more detail. Shown below are the calculated values for the diffusion coefficients, using lidobase as the diffusant (**Table 5.6**):

Polymer	PILA 2.04	PILA 4.37	PdILA	PLGA 80/20	PLGA 53/47
D (cm²/s)	3.84 10 ⁻¹⁴	6.95 10 ⁻¹⁴	2.7 10 ⁻¹¹	3.11 10 ⁻¹¹	1.596 10 ⁻¹⁰
T_g* (°C)	70	62	48	50	40

* T_g's are measured for the dry polymers

A trend can be seen: In general, the magnitude of the diffusion coefficient correlated inversely with the magnitude of measured T_g. For PILA 4.37 and PILA 2.04, which absorb very little water (the measured T_g of the dry polymer should be substantially the same as the polymer immersed in buffer), the values of D reflect diffusion through a glassy phase. For PdILA and PLGA 80/20, which absorbed more water (and hence have T_g's lowered for the wet films), the D values represent diffusion through a swollen glassy polymer, or perhaps a rubbery polymer close to its T_g (at 37 °C). In the case of PLGA 53/47, which absorbs considerably more water, the wet polymer will have a T_g below 37 °C, and hence the measured D was representative of diffusion through a rubbery phase.

5.6 Summary

Lidobase shows a significant effect on drug release profile of PdILGA. Lidobase releases in two-phase profile in contrast to a distinct three-phase drug release path of lidosalt from PdILGA. Lidobase accelerated significantly the degradation by base catalysis leading to an early breakdown of the matrix compared to a more stable PdILGA loaded with lidosalt film. The reason of an additional diffusion phase of lidosalt releasing from PdILGA is believed to be due to a slower degradation rate and transition from glassy to totally amorphous matrix. The second diffusion phase is characterized by a swollen glassy/amorphous matrix leading to an increase in the diffusion of the drug through the water-filled pores. The drug was sustained for 2-3 weeks in a range of 10-20 % before breaking down and totally release after one month.

Semi-crystalline PILA shows a single diffusion controlled release path for both drugs due to its insignificant water absorption and degradation state. However, lidobase is releasing slightly faster than lidosalt. The drug was slowly sustained for more than 30 weeks at about 10%.

It is clear that one should seriously consider the effect of the chemical properties of the drug to explain the drug release mechanisms of a particular system using biodegradable polymers.

This study shows the controlling factor of the crystallinity and hydrophilicity on the drug release behavior. Semi-crystalline PILAs release slower than amorphous PdILA and in agreement PLGA 80/20 IV 4.8 releases faster than PILA IV 4.37 due to a higher hydrophilicity and lower crystallinity. The correlation of the glass transition temperature with the diffusion coefficient clearly shows the controlled diffusion of the drug by the polymer properties.

Thus for a short-term release requirement (weeks to month), we would advice to use amorphous polymer with high hydrophilicity. For a longer-term release requirement (1-6 month), a choice of an

amorphous polymer with high molecular weight could be adequate and thus for a long-term release, a semi-crystalline polymer with high degree of crystallinity or high molecular weight could be consider.

CHAPTER 6

DRUG RELEASE MECHANISM FROM STENTS

6.1 Introduction

Based on current interest on drug-eluting biodegradable stents and recent success in overcoming restenosis with incorporation of paclitaxel and rapamycin, we studied the drug release mechanisms *in vitro* of drug-incorporated biodegradable stents and compared the drug release behavior with lidocaine as the model drug.

Currently, paclitaxel presents two majors difficulties for *in vitro* studies. First, the limit of solubility of the drug in water makes it impossible to study the release properties in buffer (the universal release medium for *in vitro* studies), since the saturation concentration is reached within a few days of release. One solution would be to use a very large amount of medium, but this is grossly inconvenient, with the limit of detection as an added issue. The second major problem is the reported binding and the adsorption of paclitaxel onto the walls of the glass vials and plastic containers, resulting in a consequent lower drug concentration in the solubility medium. Almost all the published data on paclitaxel release report extraction of the drug into a more suitable solvent, before quantification. In addition, Martin et al. [199] report a value of 90 % extraction efficiency of taxol from plasma, while Mu et al. [75] report only 46 % from microspheres. The data obtained for analysis of the *in vitro* release was corrected accordingly to the extraction efficiency, clearly showing that extraction method leads to additional experimental errors. In the case of stent loaded

with paclitaxel procedure, the stents would be “sacrificed” at each time point to be assayed for residual paclitaxel, due to difficulties in assaying for paclitaxel in the release medium.

Similar to paclitaxel, rapamycin presents a major difficulty to study because of its limit of solubility in water and extremely low saturation concentration. At the same time, the release dose of rapamycin as well as paclitaxel effect on restenosis is also not very clear from the literature.

There are no reported in vitro release studies of rapamycin-incorporated stents release. Klugherz et al., [88] have reported on two doses in a coated stent: low (64 $\mu\text{g}/\text{stent}$) and high dose (196 $\mu\text{g}/\text{stent}$) coating of sirolimus, in a rabbit study. Reduction in neointimal formation was 23 % with the low-dose formulation and 45 % with the high-dose formulation compared to control animals, in a 28 days follow up period. It is difficult to assess whether the drug release kinetic is significant in restenosis prevention.

Keeping the above-discussed points in view, the objective in the present investigation is several folds:

- 1) Selection of two matrices based on previous work in our laboratory: PdlLGA 5347 0.84 and PdlLA 2.04 were selected in view of their relatively fast degradation rates.
- 2) Investigate and select a convenient method to precisely quantify paclitaxel and rapamycin drug release.
- 3) Using lidocaine base as a model drug, compare the release profiles of paclitaxel and rapamycin from these biodegradable matrices. What level of control can be achieved with respect to release of either drug?

- 4) How does release from a helical stent compare with release from a film of the same loading?
- 5) Suggest possible stent compositions for sustained release of either drug.

6.2 Comparison of Drug Release Mechanisms of Lidocaine from Stent and Film

Figure 6.1 plots the lidocaine release from PdLLGA stents and films as fraction of the total loading versus the immersion time in the release medium.

Figure 6.1 Effect of shape on release profiles of lidocaine from PdLLGA matrix

It is clear that the drug release behavior from stents and films was very similar. Release of lidocaine followed a well-defined two-phase pattern after one day of lag time. No initial burst effect was observed for both stents and films, apparently because the drug was dissolved in the matrix. The stage of diffusion controlled release lasted up to day 12 for both films and stents. At the end of day 12, the release of lidocaine increased significantly for stent ($k = 4.57\%$ /day, as compared to $k = 3.74\%$ /day, for films). This is because of the onset of base-catalyzed matrix degradation as shown in the previous chapter, and the extent of release is almost complete (near 100 %) in one month. Both the stents and films of PdlLGA loaded with lidocaine started to degrade (erosion controlled-release after day 12), as a result of homogeneous degradation (the thickness of both matrices are less than the so-called critical limit of $200\mu\text{m}$ [134]). The fact that both films and stents exhibited the same kinetics, in spite of thickness differences, attests to homogeneous degradation.

Table 6.1 presents the diffusion coefficients, D , of the drug from PdlLGA matrices in stent and film forms. The diffusion coefficient, D , was obtained from plots of fraction of drug release vs. square root of release time, t , in PBS 7.4, for the diffusion phase.

Table 6.1 Results from kinetics of drug release of lidocaine from PdlLGA films and stents.

Effect of Shape	PdlLGA stent	PdlLGA film
Period (days)	0-13	1-12
Rate k (percent day ⁻¹)	$k = 0.71$	$k = 3.35$
Diffusion coeff. (cm ² /s)	$D = 1.65 \text{ E-}10$	$D = 1.60 \text{ E-}10$
R^2	0.9253	0.969

Diffusion coefficients for PdLGA stent and film are comparable, as would be expected from a purely diffusion controlled release phase. In essence, this result shows that we can use simple matrix diffusion calculations to predict the amount of drug release in the initial phase.

Figure 6.2 plots the lidocaine release from PdILA stents and films as fraction of the total loading versus the immersion time in the release medium.

Figure 6.2 Effect of shape on release profiles of lidocaine from PdILA matrix

It is not very clear here, that the drug release profiles were very similar from stents and films, in the limited period of study. The limited period of study was considered sufficient to have an estimation of the drug release behavior due to the very slow drug release of PdILA. In addition the full drug release was done with the films. Drug release from PdILA stent and film was

characterized by a slow diffusion phase, and films reached near 100 % by the third phase of faster release at the end of 241 days, due to onset of the matrix degradation ($k=0.5\%$ / day). The second phase sees the diffusion release of lidocaine from films and stent within a window of 5-17 % until 100 days. However, lidocaine releases slightly faster from the stent within the first few days of the phase of diffusion.

Table 6.2 presents the diffusion coefficients, D , of the drug from PdlLA matrices in stent and film forms. The method of calculation of diffusion coefficient, D , has been discussed in previous chapters.

Table 6.2 Results from kinetics of drug release of lidocaine from PdlLA films and stents.

Effect of Shape	PdlLA stent	PdlLA film
Period (days)	16-118	1-67
Rate, k (percent day ⁻¹)	$k=0.1588$	$k=1.3828$
Diffusion coeff. (cm ² /s)	$D=8.25\text{ E-}12$	$D=2.715\text{ E-}11$
R^2	0.9225	0.9548

For the PdlLA, the D values were considerably lower than PLGA, as expected on the basis of its higher T_g (49 °C dry state). However, the diffusion coefficients were a bit different from film and stent with an error of $\pm 15\%$ (**Table 6.2**). This is consistent with the drug release profile **Figure 6.2**.

6.3 Drug Release Mechanisms of Different Drugs From Polymeric Stent

Figure 6.3 plots the release of lidocaine, rapamycin and pacliatxel from PdlLGA stents as fraction of the total loading versus the immersion time in the release medium.

Figure 6.3 Release profiles of rapamycin, paclitaxel and lidocaine as function of immersion time in buffer, for PdlLGA stents.

We compare the initial release profiles of all 3 drugs in **Figure 6.3**, from PdlLGA stents. All drugs show an initial diffusion phase, releasing about 10-20 % of drug. The duration of this phase is the same for the hydrophobic drugs paclitaxel and rapapmycin (20 days), whereas it is shorter for lidocaine (12 days). In an earlier study, we showed that lidocaine in base form, does catalyze the hydrolysis of PdlLGA, hence this behaviour is to be expected. All three drugs show substantially linear degradation-controlledregimes, leading eventually to complete release of drug. The data are tabulated in **Table 6.3** below:

Table 6.3 Results from kinetics of drug release (lidocaine, paclitaxel, and rapamycin) from PdILGA stent.

Effect of Drug	Paclitaxel	Rapamycin	Lidocaine
Period (days)	0-17	0-19	0-13
Rate k (percent day-1)	k= 2.09	k= 0.37	k= 0.71
Diffusion coeff. (cm ² /s)	D= 5.72 E-9	D= 4.48 E-11	D=1.60 E-10
R ²	0.9125	0.945	0.9253

For rapamycin and lidocaine, the calculated diffusion coefficients appear to follow the trend of molecular size: rapamycin (FW 914.2 g/mol) has larger molecular weight compared to lidobase (FW 234.3 g/mol. However, for paclitaxel (FW 853.9 g/mol) the calculated D value is higher than expected. Reasons for this anomaly are explored below.

In the erosion-controlled phase, the calculated rates (k= 4.6 %/day, lidobase), compare reasonably well with rapamycin and paclitaxel (k=8.3%/day; k= 6.6 %/day, respectively).

We believe the higher diffusion coefficient of paclitaxel is due to the release medium used (10/90 methanol/buffer). We believe that the methanol plasticizes the PdILGA. We measured the Tg’s of the PdILGA after immersion in both MeOH/buffer and buffer alone, to quantify this. The films were removed from the vials, washed with distilled water and wiped. The glass transition temperature was examined in the “wet state” to understand the plasticizing effect of methanol in the release medium. The values are plotted in **Figure 6.4**. PdILGA (in buffer) shows a decrease of

T_g from 42 °C to about 39 °C after one day, which subsequently remains stable for about 10 days. In contrast PdlLGA in buffer/methanol shows a sharp decrease of about 10 °C to reach a value of about 30 °C after one day of immersion, followed by slow decrease and stabilization at about 28 °C for 29 more days. There is clearly an effect of the methanol on the T_g of PdlLGA, which shows a distinct drop. Thus the higher than expected diffusion coefficient is in fact due to plasticization. However, this plasticization appears to have little effect on the erosion-controlled phase.

Figure 6.4 Effect of the composition of release medium on glass transition temperature of PdlLGA films

Figure 6.5 presents a plot of the water absorption of PdlLGA films in buffer and in buffer/methanol, as a function of immersion time.

The results show the same pattern of water uptake for PdLLGA films in buffer and buffer/methanol. Diffusion of buffer in the polymer matrix was followed by an acceleration of water absorption after about 20 days. However, the extent of water absorption by the polymer matrix in the first 11 days, was higher (about twice) in buffer/methanol due probably to methanol. After 12 days, the extent of water absorption was very similar in both the media.

Figure 6.5 Effect of release medium on water absorption for PdLLGA films

These results show the additional plasticizing effect of methanol until a certain amount of methanol from the medium has been absorbed within the first 11 days (about 1 %), assuming that methanol molecules diffuse in the polymer films at the same rate as water molecules. The higher water absorption in the initial period of immersion of PdLGA in buffer/methanol as compared to buffer alone explains the burst release of paclitaxel in buffer/methanol compared to common diffusion phase of lidocaine (**Figure 6.4**). Rapamycin does not exhibit such a higher diffusion rate in buffer/ethanol. This could be due to the fact that ethanol has no plasticizing effect than methanol. Plasticizers form weak secondary bonds between polymer chains and serve to spread the chains apart; increasing the flexibility of polymer matrix. The higher polarity of the methanol than ethanol could be one of the reasons for a higher plasticizing effect.

It may be noted that all the three drugs contain amine functions in their chemical structures (**Figures 2.3-2.5**). Rapamycin is characterized by a cyclic tertiary amine, paclitaxel has a secondary amine between a carboxyl and a phenyl group, whereas lidocaine has two amines: a secondary amine between a carboxyl group and a phenyl group, and a tertiary amine between two ethyl groups. Based on chemical structure, lidocaine is a much stronger base as compared with either rapamycin or paclitaxel. The effect of base-catalyzed erosion being a function of the strength of the base, the results should follow the order of basicity. This explains the trend in **Figure 6.3**, which indeed shows longer lag phases for PdLGA stents loaded with rapamycin and paclitaxel than lidocaine.

Figure 6.6 plots the drug release of lidocaine, rapamycin and paclitaxel from PdLGA stents as a function of total loading, versus the immersion time in the release medium.

Figure 6.6 Effect of drugs on release mechanism from Pd/LA matrix

The figure shows that the initial release profile of all the drugs followed a very slow diffusion phase (may be considered as lag time), the said duration being shortest for lidocaine (20 days), as compared with both paclitaxel and rapamycin (80 days). Both rapamycin and paclitaxel show a very slow diffusion release (less than 2 %), within the period of 80 days. For lidocaine, the initial lag phase of 20 days was followed by a diffusion release phase for three more months as seen in **Figure 6.6**. Release of paclitaxel increased by about 4 % in a second phase lasting about 15 days, following the initial lag phase of 80 days. Clearly, both paclitaxel and rapamycin released slower than lidocaine and the release profile for both drugs is similar with longer lag times. The difference in diffusion-controlled release of the three drugs might be due to the relative size and molecular weight of the drugs.

Table 6.4 presents the diffusion coefficients, D , (calculated as per the description above) of lidocaine, rapamycin, and paclitaxel from PdlLA matrix.

Table 6.4 Results from kinetics of drug release (lidobase, paclitaxel, and rapamycin) fi-om PdlLA stent

Effect of Drug	Paclitaxel	Rapamycin	Lidocaine	
Period (days)	1- 85	0-80	1-16	16-118
Rate, k (percent day-1)	$k_1= 0.088$	$k_1= 0.097$	$k_1= 0.1153$	$k_2= 0.1588$
Diffusion coef (cm^2/s)	$D_1= 2.53 \text{ E-}12$	$D_1= 3.07 \text{ E-}12$	$D_1= 4.35 \text{ E-}12$	$D= 8.25 \text{ E-}12$
R^2	0.9572	0.9314	0.9608	0.9225

The results, once again show that during the lag phase, rapamycin and paclitaxel diffuse slower than lidocaine from PdlLA stent. One can only compare the diffusion coefficient (D_1) of the drugs for the lag phase because of the limited period of study. Thus, the relative size and molecular weight of the drug does affect the release profile of the drug. It is clear that paclitaxel and rapamycin release slower than lidocaine from PdlLA stents and these results are consistent with **Figure 6.6** and the results from PdlLGA.

6.4 Summary

We have studied two amorphous polymers that could be potential candidates for a biodegradable, drug-releasing stent. Our survey of the literature did not show that there was a “desirable” release profile for rapamycin or paclitaxel; only high-dose and low-dose coated stents have been studied.

Agrawal et al. [12] have shown that the stent collapse pressure increases with decreasing stent diameter. In addition, in a study of epinephrine stimulated canine arteries, the maximal transluminal pressure lie in the range of 200-275 atm, and even with a factor of safety of two for the intraluminal pressures, the pressure to withstand is still much lower than the available mechanical data from all the polymers investigated so far. In addition, PdlLA have lower but similar mechanical properties as compared with PILA [182] due to its high glass transition temperature. This can, therefore also be used as a coating on metallic stents, or be a part of a multilayered stent. Eury RP [200], in a patent in 1994 disclosed a multilayered biodegradable stent, whereby one layer is ascribed to the structural requirement, whereas the outer and inner layers are designed to release drugs at predictable rates. Polymers such as poly (L-lactides), poly (glycolide), polyorthoesters or polyanhydrides have been claimed to be used as structural materials, and PdlLA and polycaprolactone have been used as drug-eluting layers. Hence, with a more advanced concept, drugs could be released from the best combination of matrices.

Nevertheless, it is reasonable to assume that controlling the release over a period of weeks to months would be a desirable feature compared to “dose-dumping” as may happen with coated stents. Certainly, the drug loading can be substantially increased in these stents because the overall mass of the stent is much higher compared to a coating. This might prolong the duration of delivery. We have studied the release characteristics of two potent anti-restenosis drugs, rapamycin and paclitaxel, from biodegradable stent matrices. As expected, this in vitro drug release study of a biodegradable stent shows similar profile than from films, indicating homogeneous degradation kinetics. This could be an important point to avoid embolization, however, one should note that the diameter of the vessel would be a determinant factor of success or not of the device. In addition, we have clearly shown that the burst effect for paclitaxel can be eliminated with judicious drug loading, thereby avoiding possible toxic effects. Whether the polymer/drug combinations reported

here may or not be the optimal formulation for the stent can only be decided by animal studies showing sustained suppression of neo-intimal hyperplasia consistent with minimal toxicity.

CHAPTER 7

CONCLUSION

In the literature, it is generally accepted that the rate of drug release from most bioerodible polymers can be both diffusion-controlled and degradation-controlled. The drug release profile is thus generally accepted to be biphasic, showing these two mechanisms. However, when polymer and drug variables are changed, the profile deviates from the expected biphasic, due to the combined (and sometimes opposing) effects of these variables on degradation and water absorption.

The process of “degradation”, for these polymers, essentially describes hydrolytic chain scission, during which polymer chains are cleaved to form oligomers and finally to form monomers. While classifying degradable polymers, a distinction is made between surface (or heterogeneous) and bulk (or homogeneous) eroding materials. In the case of polymers that degrade in bulk, the rate of water penetration into the matrix is faster than the rate of polymer degradation. The process is a homogeneous one in which degradation occurs at a uniform rate throughout the polymer matrix. Water enters the polymer bulk, which might be accompanied by swelling. Many factors influence the degradation of PLGAs including polymer composition, molecular weight, M_w the nature of incubating/hydrolyzing media such as pH, fluid flow, processing, sterilization, strain, presence of plasticizer, porosity, temperature, polydispersity, and ionic strength of the medium. The

degradation kinetics is furthermore affected by the presence of additives, such as metal salt, unreacted monomer, basic, and acidic drugs.

In general, polymer degradation is accelerated by greater hydrophilicity in the backbone or end groups, greater reactivity among hydrolytic groups in the backbone, less crystallinity, and larger finished size of the device. Though these factors have been reported, a study of the effects of these factors on the mechanistic details of hydrolysis was still lacking.

1. Effect of polymer type:

In our study, it was found that lidocaine release for most of the bioerodible polymers studied, the drug release was governed by common biphasic pattern in the period of one year. However, a single diffusion phase was shown for highly crystalline polymers such as PILAs. It was shown that the effect of crystallinity to be in contradiction to some of the earlier work, which showed an enhancement of release rate with higher initial crystallinity. We found the opposite effect in all the polymers studied to date. We believe this contradiction may be reconciled by the fact that we had intentionally kept the drug concentration well below saturation limits, and some of the earlier reports of enhanced release with increased crystallinity may be attributed solely to drug desolvation, leading to a leaching out of drug crystals. It was clearly shown that the glycolic acid content is an important factor controlling the degradation rate and thus the drug release rate.

2. Effect of drug chemistry

In our study, it was found that the effect of the chemistry of the drug on the matrix degradation dominates the release pattern, by influencing the rate of degradation as well as the rate of water absorption. Contrary to other reported work, no effect of complexation of carboxylic end groups by base was found, leading to slower release of the base drug. We believe that the reported complexation effects occur when dealing with low- M_w PILAs and copolymers, as the

concentration of end groups is fairly high in low-MW polymers. In our work, it was demonstrated that the presence of lidobase accelerates the hydrolysis of polyester links via a base-catalyzed reaction, and that this effect dominates any other complexation. In addition, it was showed that the main effect of the salt form (neutral) of the drug was on water absorption rate, due primarily to an osmotic effect. The enhanced water absorption enhanced the diffusion rate, and also contributes to an earlier onset of degradation (when compared to the drug-free polymer).

In summary, the effect of basic, acidic and neutral drugs on release from PLA or PLGA matrices is fairly complex. Particularly for basic drugs, there is conflicting data with respect to the effects on degradation and release rates. Currently, the literature reflects contradictions about the various roles played by the chemical nature of the drug, molecular weight, crystallinity, and composition of the polymer matrix. A possible rationalization of these contradictory reports is the existence of opposing effects.

A study of the effect of the chemistry of the drug on the matrix degradation showed that presence of lidobase accelerated the hydrolysis of polyester links via a base-catalyzed reaction. Drug-free matrices as well as matrices loaded with lidosalt showed similar pattern of degradation. However, polymer loaded with lidosalt degraded faster than the polymer free of drug due to higher water absorption, as a result of an osmotic effect.

3. Effect of geometry of the matrix

After the development of rapamycin and paclitaxel in vitro drug release method, we have quantified the release characteristics from biodegradable stent matrices. This in vitro study of a biodegradable stent shows that drug release is by a combination of diffusion and degradation control. Both film and stent shapes (in the thickness range studied here) show the same release profile, indicating homogeneous degradation kinetics. In addition, we have demonstrated that the

burst effect for paclitaxel release can be eliminated by judicious selection of polymer and drug combination. At the same time, we have shown sustained release for periods of 1 month (for PdlLGA) and possibly several months (using PdlLA).

We summarize the important findings in this study, as follows:

- Effect of polymer type:
 1. All the matrices exhibit homogeneous bulk erosion. This is primarily due to the size of the devices under study, which are films of thickness about 25 μm .
 2. Both crystallinity and matrix composition of the polymers play important roles in the process of matrix degradation. A high level of crystallinity delays the process of water absorption and matrix degradation, while copolymer composition enhances matrix degradation by way of increased water absorption and the faster hydrolysis rate of the GA unit.
 3. For PdlLGA copolymers, our ^1H -NMR data clearly shows preferential faster degradation of the glycolide moiety.
 4. For those PLGA polymers with a high lactide content, the preferential degradation of GA units leads to an interesting crystallization behaviour. For PLGA 80/20, the cleavage results in generation of lactide-rich polymers (or oligomers) that can crystallize upon heating beyond T_g . This also leads to an apparent increase in T_m for the degrading PLGA 80/20 polymer. This is not observed for PdlLGA 53/47, which does not possess crystallizable units.

5. The overall hydrolytic scission process is still random, as verified by the application of a Monte Carlo model to the degradation profile.
6. The PdlLGA 53/47 shows a biphasic pattern of release for the lidobase drug, with a slow diffusion phase followed by a faster degradation phase. The only other polymer that shows degradation-controlled release in the time period of the study (approximately 300 days) is PdlLA (IV=2.4), with the onset occurring around day 100. The lower crystallinity of this polymer permits easy ingress of water, followed by base catalysis in the hydrated amorphous regions of the polymer.
7. The crystallinity of the polymer matrix appears to be another major parameter that affects the drug release rate. For similar molecular weight, an amorphous matrix of PdlLA 2.4 releases lidobase at a much faster rate as compared to the crystalline matrix of PILA 2.04. Similarly, PILA 2.04 with a higher crystallinity releases the drug at a slower rate than PILA 4.37.

- Effect of drug chemistry:

1. In general, the addition of a water-soluble salt drug increases degradation rate of a PILA or PLGA matrix. The reason is enhanced water absorption due to a purely osmotic effect.
2. The addition of a base drug enhances the rate of degradation even further, due to a catalytic effect.
3. In high-GA content PdlLGA (PdlLGA 53/47), the effect of lidobase on the degradation rate is drastic, with significant weight loss being observed from day 10 as compared to around day 30 for drug-free PLGA of the same composition.
4. The drug release patterns and calculated diffusion coefficients reported here are consistent with the water absorption, weight loss and T_g data reported. Thus the data are explainable on the basis of diffusion through glassy, swollen glassy/rubbery and rubbery phases.

5. The PdlLGA 53/47 shows a biphasic pattern of release for the lidobase drug, with a slow diffusion phase followed by a faster degradation phase. In contrast, the lidosalt release from the same polymer is triphasic, with 2 distinct diffusion phases followed by a degradation phase. This striking difference illustrates well the extensive catalytic effect of the lidobase in precipitating an earlier onset of the degradation-controlled release phase.

Our studies have shown that the release rate of a drug from this class of polymers may be manipulated by crystallinity, glycolic acid content, and basicity of the drug.

In order of importance, the effects are: GA content > crystallinity > basicity of the drug

We have clearly delineated the conditions under which a uniphasic, biphasic and triphasic release pattern can be seen from a matrix that contains dissolved drug:

1. A uniphasic release profile is seen mostly for PILA's over a 3-6 month period; this is diffusion-controlled.
2. A biphasic region is seen for PdlLA, PLGA 80/20 and PdlLGA 53/47 over a three month period, and consist of diffusion and degradation controlled phases of a base drug.
3. A triphasic region is seen for a salt drug in a PdlLGA 53/47 matrix. This consists of two diffusion phases, and a degradation phase. This happens even though degradation sets in early for this system.

Mechanistically, we have observed only homogeneous degradation in all these systems. This is primarily due to the thicknesses we have used. We have demonstrated randomness of scission; however, we have found that GA units hydrolyse more readily than LA units.

With respect to drug release from stent matrices, a biphasic release (without a burst) effect can be achieved for both rapamycin and paclitaxel for PdlLGA and PdlLA. Such polymers may be useful either as coatings for stent or as the major constituent of a fully polymeric stent.

CHAPTER 8

RECOMMENDATION FOR FUTURE WORK

Based on the literature review and the limit of the results of this study, we summarize the future recommendations:

1. Effect of drug content on degradation and drug release rate

It will be interesting to understand the effect of the loading on the degradation and drug release rate. One can expect a burst release due to a higher loading of the drug but the effect of the amount of the drug on the degradation rate is still not very clear.

2. Effect of addition of plasticizers on degradation and drug release rate

Plasticizers are commonly used to manipulate the drug release rate but a few works have been done on their effect on degradation. It also will be interesting to study the effect of the chemistry of the plasticizers and loading on the drug release and degradation rate from a polymer.

3. Effect of mechanical stress and degradation and drug release rate

Most of the *in vitro* studies aimed to be the closest of the *in vivo* environment, thus it will be important to settle an experiment and do drug release and degradation studies with a mechanical stress based on the arterial mechanical stress applied to the stent. One can easily expect a faster

degradation and drug release rate due to mechanical stress but a quantification could be interesting to determine if it is significant or not. In the case of the rates are significantly increased, thus the experimental methods should be based on a closet requirements on in vivo environment.

4. Effect of hydrodynamics conditions on degradation and drug release rate

The effect of fluid flow on drug release and degradation rate is still not very clear. A clear disagreement is often reported on the increase or decrease of degradation rate of biodegradable matrices. In the case of a significant effect of the fluid on degradation and drug release rate, then like above the experimental methods should take into account this parameter.

5. Effect of processing on degradation and drug release rate

Processing has been shown to have a lot of effect on the degradation and thus on the drug release. From our data, we can confidently say that the processing does not affect the results due to the same processing for all the matrices but some precautions must be taken on the temperatures used for the processing, the storage and the mechanical stress applied on the matrices.

6. Study other materials like PdLA, poly orthoesters (POE) or polycaprolactone (PCL)

Investigations on PdLA might be interesting to compare the degradation rate, degradation path and drug release behavior of PdILA and PILA polymers already studied. The stereoisomers show an effect on the crystallinity due to differences on the arrangement of polymer chains and thus investigating a possible specific degradation on isomers could be another factor to consider for further formulation trials.

In addition none of our polymers are showing a zero order drug release profile, thus it might be interesting to investigate POE and PCL for their possible zero-order drug release behavior.

7. Try other concepts of stents like multilayered, film coated, etc... .

Many concepts of drug eluting stent have already been developed and patented but no one knows which one would fit the better requirements based due to the different drugs and *in vivo* environment reported. Hence, a systematic study of different concepts would be interesting.

8. Improve the paclitaxel drug release method

We developed the paclitaxel *in vitro* drug release method to overcome the problems of absorption and low drug solubility in the medium. Even if so far the method is the most convenient to use, we believe that with some more effort, the methanol plastization effect on hydrophilic polymer could may be overcome.

9. Study the degradation of the stent as a larger device compared to film

Based on our results the stent and the film shows a similar drug release profile and we could speculate on the homogeneous degradation of these two matrices. However, a additional study of the degradation rate could show if there is any differences and if it is significant or not.

10. Compare *In vitro* and *In vivo* degradation and drug release rate

In vivo studies will be the final step to understand the efficiency of the concepts and the requirements needed for the best formulation.

It will be important to compare the degradation and drug release rate in order to valid *in vitro* evaluation studies, and explain systematically the differences due to the environments. Once these studies are completed, one can roughly evaluate the *in vitro* data and extrapolate. It will be interesting to manipulate the *in vitro* experimental method and manipulate to be the closest of the *in vivo* environment.

References

REFERENCES

- [1] Griffith, L.G. (2000). Polymeric biomaterials. *Acta Materialia*, 48,263-377.
- [2] Amecke, B., Bendix, D., & Entenmann, C. (1992). Resorbable Polyesters: Composition, properties, applications. *Clinical Materials*, 10, 47-50.
- [3] Amecke, B., Bendix, D., & Entenmann, C. (1992). Synthetic resorbable polymers based on glycolide, lactides, and similar monomers. In D.L. Wise, D.J. Trantolo, D.E. Altobelli, M.J. Yaszemski, J.D. Gresser, & E.R. Schwartz (Eds.), *Encyclopedic handbook of biomaterials and bioengineering. Part A: Materials* (pp.977-1007). New York: Marcel Dekker.
- [4] Ikada, Y., Tsuji, H. (2000). Biodegradable polyesters for medical and ecological applications. *Macromolecular Rapid Communication*, 21, 117-132.
- [5] Wang, N., Wu, X.S. (1997). Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid oligomers: Part 11. Biodegradation and drug delivery application. *Journal of Biomaterials Science Polymer Edition*, 9, 75-87.
- [6] Vert, M., Schwarch, G., Coudane, J. (1995). Present and future of PLA polymers. *Pure Applied Chemistry*, A32,787-796.
- [7] Van Sliedregt, A., Radder, A.M., De Groot, K., Van Blitterswijk, C.A. (1992). In vitro biocompatibility testing of poly-lactides. Part I: proliferation of different cell types. *Journal of Materials Science: Materials and Medicine*, 3, 365-470.

References

- [8] Pistner, H., Gutwald, R., Ordnung, R., Reuther, J. (1993). Poly(l-lactide): A long-term degradation study in vivo. I. Biological results. *Biomaterials*, 14, 671-677.
- [9] Orloff, L.A., Domb, A.J., Teomim, D., Fishbein, I., Golomb, G (1997). Biodegradable implant strategies for inhibition of restenosis. *Advanced Drug Delivery Review*, 24, 3-9.
- [10] Bailey, S.R. (1997). Local drug delivery: Current applications. *Progress in Cardiovascular Disease*, 40, 183-204.
- [11] Colombo, A., Karvouni, E. (2000). Biodegradable stents. *Circulation*, 102, 371-373.
- [12] Agrawal, C.M., Haas, K.F., Leopold, D.A., Clark, H.G. (1992). Evaluation of poly (l-lactic acid) as a material for intravascular polymeric stents. *Biomaterials*, 13, 176-182.
- [13] Chapman, G.D., Gammon, R.S., Bauman, R.P., Howell, S., Clark, H., Mikat, E.M., Pamos, L., Buller, C.E., Stack, R.S. (1990). A biodegradable stent: initial experimental results. *Circulation Supplement III*, 82, 72.
- [14] Tamai, H., Igaki, K., Kyo, E., Kosuga, K., Kawashima, A., Matsui, S., Komori, H., Tsuji, T., Motohara, S., Uehata, H. (2000). Initial and 6-month results of biodegradable poly-l-lactic acid coronary stents in humans. *Circulation*, 102, 399-404.
- [15] De Feyter, J. (2000). The quest for ideal stent. *European Heart Journal*, 22, 1766-1768.
- [16] Schatz, R.A. (1989). A view of vascular stents. *Circulation*, 7, 445- 457.
- [17] Schatz, R.A., Baim, D.S., Leon, M., Ellis, S.G., Goldberg, S., Hirshfeld, J.W., Cleman M.W., Cabin, H.S., Walker, C., Stagg, J., Buchbinder, M., Teirstein, P.S., Topol, E.J. Savage, M., Perez, J.A., Curry, R.C., Whitworth, H., Sousa, J.E., Tio, F., Almagor, Y. Ponder, R., Penn, I.M., Leonard, B., Levine, S.L., Fish, R.D., Palmaz, J.C. (1991). Clinical experience with Palmaz-Schatz coronary stent. Initial results of a multicenter study *Circulation*, 83, 148-161.

References

- Murphy, J., Schwartz, R., Edwards, W.D., Camrud, A.R., Vlietstra, R.E., Holmes, D.R. (1992). Percutaneous polymeric stents in porcine coronary arteries. Initial experience with polyethylene terephthalate stents. *Circulation*, 86, 1596-1604.
- Markou, C.P., Lutostansky, E.M., Ku, D.N., Hanson, S.R. (1998). A novel method for efficient drug delivery. *Annals Biomedical Engineering*, 26, 502-511.
- Schomig, A., Neumann, F-J., Kastrati, A., Schuhlen, H., Blasini, R., Hadamitzki, M., Walter, H., Zitzmann-Roth, E-M., Richard, G., Alt, E., Schmitt, C., Ulm, K. (1996). A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *New English Journal Medicine*, 334, 1084-1089.
- Wu, X.S. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In D.L . Wise, D.J. Trantolo, D.E. Altobelli, M.J. Yaszemski, J.D. Gresser, R.D. Schwartz (Eds.), *Encyclopedic handbook of biomaterials and bioengineering. Part A: Materials* (pp. 1015-1054). New York: Marcel Dekker.
- Schneider, D., Dichek, D.A. (1997). Intravascular stent endothelialization. A goal worth pursuing? *Circulation*, 95, 308-310.
- Liistro, F., Colombo, A. (2001). Late acute thrombosis after paclitaxel eluting stent implantation. *Heart*, 86, 262-264.
- Nathan, A., Kohn, J. (1994). Amino acid derived polymers. In S.W. Shalaby (Eds.), *Biomedical polymers*. Munich, Vienna, New York Hanser Publishers.
- Paul, D.R. (1976). Polymers in controlled release technology. In D.R. Paul, F.W. Harris (Eds.), *Controlled release polymeric formulations* (pp.1-13). Washington DC: ACS symposium series 33.
- Langer, R. (1990). New methods of drug delivery. *Science*, 249, 1527-1533.

References

- [27] Lewis, D.H. (1990). Controlled release of bioactive agents from lactide/ glycolide polymers. In M. Chasin, R. Langer (Eds.), *Biodegradable polymers as drug carriers system* (pp.1-42).New York Marcel Dekker.
- [28] The Pharmaceutical Codex (1994), 12th Edn. In W. Lund (Eds.), *Biopharmaceutics and pharmacokinetic principles* (pp. 311-321).London: The Pharmaceutical Press.
- [29] Okada, H., Doken, Y., Ogawa, Y., Toguchi, H. (1994). Preparation of three-month depot injectable microspheres of leuporelin acetate using biodegradable polymers. *Pharmaceutical Research*, 11, 1143-1147.
- [30] Jain, R.A. (2000). The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide)(PLGA) devices. *Biomaterials*, 21,2475-2490.
- [31] Merkli, A., Heller, J., Tabatabay, C., Gurny, R. (1996). Purity and stability assessment of a semi-solid poly(ortho-ester) used in drug delivery systems. *Biomaterials*, 17, 897-902.
- [32] Gümüşderelioğlu, M., Deniz, G. (2000). Sustained release of mitomycin-C from poly (DL-lactide)/poly (dl-lactide-co-glycolide)films. *Journal Biomaterial Science Polymer Edition*, 11, 1039-1050.
- [33] Ain, Q., Sharma, S., Garg, S.K., Khuller, G.K (2002). Role of poly (dl-lactide-co-glycolide) in development of a sustained oral delivery system for antitubercular drugs. *International Journal Pharmaceutics*, 239, 37-46.
- [34] Ike, O., Shimizu, Y., Ikada, Y., Watanabe, S., Natsume, T., Wada, R., Hyon, S-H., Hitomi, S. (1991). Biodegradation and antitumour effect of adriamycin-containing poly (l-lactic acid) microspheres. *Biomaterials*, 12,757-762.
- [35] Burton, K.W., Shameem, M., Thanoo, B.C., DeLuca, P.P. (2000). Extended release peptide delivery systems through the use of PLGA microsphere combinations. *Journal Biomaterial Science Polymer Edition* ,7, 715-729.

References

- [36] Sinha, V.R., Khosla, L. (1980). Bioabsorbable polymers for implantable therapeutic systems. *Drug Development Industry Pharmaceutical*, 24, 1129-1138.
- [37] Sampath, S.S., Garvin, K., Robinson, D.H. (1992). Preparation and characterization of biodegradable poly (1-lactic acid) gentamicin delivery systems. *International Journal Pharmaceutics*, 78, 165-174.
- [38] Zhang, X., Wyss, U.P., Pichora, D., Goosen, M.F.A. (1994). Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties. *Journal Pharmacy Pharmacology*, 46, 718-724,
- [39] Price, J.S., Tencer, A.F., Arm, D.M., Bohach, G.A. (1996). Controlled release of antibiotics from coated orthopaedic implants. *Journal Biomedical Materials Research*, 30, 281-286
- [40] Wootton, D.M., Ku, D.N. (1999). Fluid mechanics of vascular systems, diseases, and thrombosis. In Annual Reviews (Eds.), *Annual review of biomedical engineering* (pp.300-329). Palo Alto: Annual Reviews.
- [41] Eccleston, D.S., Lincoff, A.M. (1997). Cathether-based drug delivery for restenosis. *Advanced Drug Delivery Review*, 24, 31-43.
- [42] Sermys, P.W., Strauss, B.H., Beat, K.J., Berthrand, M.E., Puel, J., Richards, A.F., Meter, B., Goy, J-J., Vogt, P., Kappenberger, L., Sigwart, U. (1991). Angiographic follow-up after placement of self-expanding coronary-artery stent. *New English Journal Medicine*, 324, 13-17.
- [43] Lowe, H.C., Oesterle, S.N., Khachigian, L.M. (2002). Coronary in-stent restenosis: Current status and future strategies. *Journal American College Cardiology*, 39, 183-193.
- [44] Gunn, J., Cumberland, D. (1999). Does stent design influence restenosis? *American Heart Journal*, 20, 1009-1013.

References

- [45] Lovich, M.A., Creel, C., Kristy, H., Hwang, C-W., Edelman, E.R. (2001). Carrier proteins determine local pharmacokinetics and arterial distribution of paclitaxel. *Journal Pharmaceutical Science*, 90, 1324-1335.
- [46] Golomb, G., Moscovitz, D., Fishbein, I., Mishaly, D. (1985). Perivascular polymeric controlled-release therapy. In D.L. Wise, D.J. Trantolo, D.E. Altobelli, M.J., Yaszemski, J.D. Gresser, E.R. Schwartz (Eds.), *Encyclopedic Handbook of Biomaterials and Bioengineering. Part B: Applications* (pp.1043-1056).New York Marcel Dekker.
- [47] Creel, C.J., Lovich, M.A., Edelman, E.R. (2000). Arterial paclitaxel distribution and deposition. *Circulation Research*, 86, 879-884.
- [48] Fattori, R., Piva, T. (2003). Drug-eluting stents in vascular intervention. *The Lancet*, 361, 247-249.
- [49] Sigwart, U., Puel, J., Mirkovitch, V., Joffre, F., Kappenberger, L. (1987). Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. *New English Journal Medicine*, 316, 701-706.
- [50] Jacobs, A.K. (1999). Coronary stents: Have they fulfilled their promise? *New English Journal Medicine*, 341, 2005-2006.
- [51] Tanguay, J.F., Zidar, J.P., Phillips, H.R., Stack, R.S. (1994). Current status of biodegradable stents. *Cardiovascular Clinical*, 12, 699-713.
- [52] Peng, T., Gibula, P., Yao, K-D., Goosen, M.F.A (1996). Role of polymers in improving the results of stenting in coronary arteries. *Biomaterials*, 17, 685-694.
- [53] Hahnei, I., Alt, E., Resh, A., Markl, B., Stemberger, A., Schomig, A. (1998). *Journal American College Cardiology*, 278A.
- [54] Julia, Ng. (2002, 28 Jan). *Channel New Asia*.

References

- [55] Fishbein, I., Chorny, M., Gati, I., Rabinovich, L., Golomb, G. (2000). Drug delivery systems for treatment of restenosis. In D.L. Wise (Eds), *Biomaterials and bioengineering handbook* (pp.313-336). New York: Marcel Dekker.
- [56] *AHFS Drug information* (1989). Heparin sodium (pp. 1394-1396). Bethesda: American Society of Health-System Pharmacists.
- [57] Ellis, S.G., Roubin, G.S., Wilentz, J., Douglas, J.S., King, S.B. (1989). Effect of 18- to 24-hour heparin administration for prevention of restenosis after uncomplicated coronary angioplasty. *American Heart Journal*, 117,777-782.
- [58] Gersh, B.J., Opie, L.H, (1992). Anti-thrombotic agents: Platelet inhibitors, anticoagulants, and fibrolytics. In H. Lionel, L.H. Opie, K. Chatterjee (Eds.), *Drugs for the heart, 4th Edn.* (pp.112-122). Philadelphia: Saunder WB Co.
- [59] De Scheerder, I., Wang, K., Wilczek, K., Meuleman, D., Van Amsterdam, R., Vogel, G., Piessens, J., Van de Werf, F. (1997). Experimental study of thrombosis and foreign body reaction induced by heparin-coated coronary stents. *Circulation*, 95, 1549-1553.
- [60] Schomig, A., Kastrati, A., Dirschinger, J., Mehilli, J., Schricke, U., Pache, J., Matinoff, S., Neumann, F.J., Schwaiger, M. (2000). Coronary stenting plus platelet glycoprotein IIb/IIIa blockade compared with tissue plasminogen activator in acute myocardial infarction. *New English Journal Medicine*, 343,385-391.
- [61] EPIC investigators (1994). Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. *New English Journal Medicine*, 330, 956-961.
- [62] Aggarwal, R.K., Ireland, D.C., Azrin, M.A., Ezekowitz, M.D., de Bono, D.P., Gershlick, A.H. (1996). Antithrombotic potential of polymer-coated stents eluting platelet glycoprotein IIb/IIIa receptor antibody. *Circulation*, 94, 3311-3317.

References

- [63] *AHFS Drug information* (1989). Warfarin sodium (pp.1386-1387). Bethesda: American Society of Health-System Pharmacists.
- [64] Leon, M., Baim, D.S., Popma, J.J., Gordon, P.C., Cutlit, D.E., Ho, K.K.L., Giambartolomei, A., Diver, D.J., Lasorda, D.M., Williams, D.O., Pocock, S.J., Kuntz, R.E. (1998). A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. *New English Journal Medicine*, 339, 1665-1671.
- [65] Makkar, R., Litvack, F., Kaul, S., Zeng, H., Eigler, N. (1997). High-shear overrides the antithrombotic effects of aspirin on stent thrombosis. *Journal American College Cardiology*, 354A.
- [66] Oberhoff, M., Herderg, C., Baumbach, A., Karsch, K.R. (2002). Stent-based antirestenotic coating (sirolimus/paclitaxel). *Catheter Cardiovascular Intervention*, 55, 404-408.
- [67] Nativ, O., Aronson, M., Medalia, O., Moldavsky, T., Sabo, E., Ringel, I., Kravtsov, V. (1997). Anti-neoplastic activity of paclitaxel on experimental superficial bladder cancer: In vivo and In vitro studies. *International Journal Cancer*, 70, 297-301.
- [68] Hwang, C-W., Wu, D., Edelman, E.R. (2001). Physiological transport forces govern drug distribution for stent-based delivery. *Circulation*, 104, 600-605.
- [69] Heller, P.F., Jenkins, G.M., Cheng, L., Leong, K., Shao, W., Brinker, J., Hope, E., Nater, C., Lakatta, E., Froehlich, J. (1995). The sustained release of galardin and taxol from gelatin chondroitin sulfate coarcevate films. *Materials Research Society Symposium Process*, 394, 55-60.
- [70] Heldman, A.W., Brinker, J.A. (1999). The right stuff (to the right place, at the right dose..). *Catheter Cardiovascular Intervention*, 47, 107-108.

References

- [71] Kahan, B.D., Camardo, J.S. (2001). Rapamycin: Clinical results and future opportunities. *Transplant*, 72, 1181-1193.
- [72] Marx, S.O., Marks, A.R. (2001). Bench to bedside. The development of rapamycin and its applications to stent restenosis. *Circulation*, 104, 852-855.
- [73] Sousa, J.E., Costa, M.A., Abizaid, A., Abizaid, A.S., Feres, F., Pinto, I.M.F., Seixas, A.C., Staico, R., Mattos, L.A., Sousa, A.G.M.R., Falotico, R., Jaeger, J., Popma, J.J., Serruys, P.W. (2001). Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries: a quantitative coronary angioplasty and three-dimensional intravascular ultrasound study. *Circulation*, 103, 192-195.
- [74] Yang, D., Stanslaski, J.L., Wang, L., Smith, S.R. (Jul. 2001). *United State Patent N° 6,258,121 B1. 10.*
- [75] Mu, L., Feng, S.S. (2001). Fabrication, characterization and in vitro release of paclitaxel (Taxol®) loaded poly (lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid/cholesterol emulsifiers. *Journal Controlled Release*, 76, 239-254.
- [76] Liggins, R.T., Hunter, W.L., Burt, H.M. (1997). Solid-state characterization of paclitaxel. *Journal Pharmaceutical Science*, 86, 1458-1463.
- [77] *AHFS Drug Information* (1989). Paclitaxel (pp.1075-1086). Bethesda: American Society of Health-System Pharmacists.
- [78] Rowinsky, E.K., Donehower, R.C. (1995). Drug therapy: Paclitaxel (Taxol). *New English Journal Medicine*, 332, 1002-1014.
- [79] Sollot, S.J., Cheng, L., Pauly, R.R., Jenkins, G.M., Monticone, R.E., Kuzuya, M., Froehlich, J.P., Crow, M.T., Lakatta, E.G., Rowinsky, E.K., Kinsella, J.L. (1995). Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat. *Journal Clinical Investigation*, 95, 1869-1876.

References

- [80] Baumbach, A., Herdeg, C., Kluge, M., Oberhoff, M., Lerch, M., Haase, K.K., Wolter, C., Schroder, S., Karsch, K.R. (1998). Local drug delivery: Impact of pressure, substance characteristics, and stenting on drug transfer into the arterial wall. *Catheter Cardiovascular Intervention*, 47, 102-106.
- [81] Suh, H., Jeong, B., Rathi, R., Kim, S.W. (1998). Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide) nanospheres. *Journal Biomedical Materials Science*, 42, 331-338.
- [82] Drachman, D.E., Edelman, E.R., Seifert, P., Groothuis, A.R., Bornstein, D.A., Kamath, K.R., Palasis, M., Yang, D., Nott, S.H., Rogers, C. (2000). Neointimal thickening after stent delivery of paclitaxel: Change in composition and arrest of growth over six months. *Journal American College Cardiology*, 36, 2325-2332.
- [83] Simamora, P., Alvarez, J.M., Yalkowsky, S.H. (2001). Solubilization of rapamycin, *International Journal Pharmacy*, 213, 25-29.
- [84] Burton, P.B.J., Yacoub, M.H., Barton, P.J.R. (1998). Rapamycin (Sirolimus) inhibits heart cell growth in vitro. *Pediatric Cardiology*, 19, 468-470.
- [85] Morice, M.C., Serruys, P.W., Sousa, J.E., et al., for the RAVEL study group (2002). A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *New English Journal Medicine*, 346, 1773-1780.
- [86] Regar, E., Laarman, G., Blanchard, D., Eltchaninoff, H., Sousa, J.E., Fajadet, J., Perin, M., Hayashi, B., Morice, M.C., Serruys, P.W. (2002). Sirolimus inhibits restenosis irrespective of the vessel size: a subanalysis of the multicenter RAVEL trial. *Journal American College Cardiology*, 58A.
- [87] Degertekin, M., Regar, E., Tanabe, K., Smits, P.C., van der Giessen, W.J., Carlier, S.G., de Feyter, P., Vos, J., Foley, D.P., Ligthart, J.M.R., Popma, J.J., Serruys, P.W. (2003).

References

- Sirolimus-eluting stent for treatment of complex in-stent restenosis. The first clinical experience. *Journal American College Cardiology*, 41, 184-189.
- [88] Klugherz, B., Llanos, G., Lieuallen, W. (2000). Stent-based delivery of sirolimus for the prevention of restenosis. *Journal American College Cardiology*, 35, 58.
- [89] Corrigan, O.I. (1997). The biopharmaceutic drug classification and drugs administered in extended release formulations. In D. Young, J.G. Devane, J. Butler (Eds.), *In vitro- in vivo correlations: Advances in experimental medicine and biology* (pp.111-128). New York Plenum press.
- [90] *British Pharmacopoeia* (2001). . Lidocaine (pp. 995-996). London: General Medical Council by Constable & Co.
- [91] *British Pharmacopoeia* (2001). Lidocaine hydrochloride (pp. 996-997). London: General Medical Council by Constable & Co.
- [92] *AHFS Drug Information* (1989). Lidocaine Hydrochloride (pp.1614-1617). Bethesda: American Society of Health-System Pharmacists.
- [93] Baker, R. (1987). Biodegradable systems. In R. Baker (Eds.), *Controlled release of biologically active agents* (pp. 85-131). New York: John Wiley.
- [94] Ron, E., Langer, R. (1992). Erodible systems. In A. Kydonieus (Eds.). *Treatise on controlled drug delivery: Fundamentals, optimization, applications* (pp.199-224). New York Marcel Dekker.
- [95] Baker, R. (1987). Diffusion-controlled systems. In R. Baker (Eds.), *Controlled release of biologically active agents* (pp. 38-83). New York: John Wiley.
- [96] Dredan, J., Antal, I., Rácz, I. (1996). Evaluation of mathematical models describing drug release from lipophilic matrices. *International Journal Pharmacy*, 145, 61-64.
- [97] Huang, X., Brazel, C.S. (2001). On the importance and mechanisms of burst release in matrix controlled drug delivery systems. *Journal ControlledRelease*, 73, 121-136.

References

- [98] Narasimham, B., Langer, R. (1997). Zero-order release of micro- and macromolecules from polymeric devices: The role of the burst effect. *Journal Controlled Release*, 47, 13-20.
- [99] Korolkovas, A. Theoretical aspects of drug action. In L.Z. Benet, N. Massoud, J.G. Gambertoglio (Eds.), *Essential of medical medicine*, 2nd Edn. New York: Raven Press.
- [100] Wang, N., Wu, X.S. (1998). Lactic-glycolic acid oligomers microgranules for aspirin delivery and stabilization. In I. Mc Culloch and S.W. Shalaby (Eds.), *Tailored polymeric materials for controlled delivery systems* (pp. 255-265). Washington DC: ACS symposium series 709.
- [101] Wada, R., Hyon, S-H., Ikada, Y. (1990). Lactic acid oligomer microspheres containing hydrophilic drugs. *Journal Pharmaceutical Science*, 79, 919-924.
- [102] Miyajima, M., Koshika, A., Okada, J., Kusai, A., Ikeda, M. (1998). The effects of drug physico-chemical properties on release from copoly (lactic/glycolic acid) matrix *International Journal Pharmaceutics*, 169, 255-263.
- [103] Miyajima, M., Koshika, A., Okada, J., Kusai, A., Ikeda, M. (1998). Factors influencing the diffusion-controlled release of papaverine from poly (L-lactic acid) matrix. *Journal Controlled Release*, 56, 85-94.
- [104] Li, S., Girod-Holland, S., Vert, M. (1996). Hydrolytic degradation of poly (D,L-lactic acid) in the presence of caffeine base. *Journal Controlled Release*, 40, 41-53.
- [105] Miyajima, M., Koshika, A., Okada, J., Ikeda, M. (1999). Mechanism of drug release from poly (L-lactic acid) matrix containing acidic or neutral drugs. *Journal Controlled Release*, 60, 199-209.
- [106] Miyajima, M., Koshika, A., Okada, J., Ikeda, M. (1999). Effect of polymer/basic drug interactions on the two-stage diffusion-controlled release from a poly (L-lactic acid) matrix, *Journal Controlled Release*, 61, 295-304.

References

- [107] Bodmeier, R., Oh, K.H., Chen, H. (1989). The effect of the addition of low molecular weight poly (DL-lactide) on drug release from biodegradable poly (dl-lactide) drug delivery systems. *International Journal Pharmaceutics*, 51, 1-8.
- [108] Sung, K.C., Han, R-Y., Hu, O.Y.P., Hsu, L-R. (1998). Controlled release of nalbuphine prodrugs from biodegradable polymeric matrices: influence of prodrug hydrphilicity and polymer composition. *International Journal Pharmaceutics*, 172, 17-25.
- [109] Omelczuk, M.O., McGinity, J.W. (1992). The influence of polymer glass transition temperature and molecular weight on drug release from tablets containing poly (dl-lactic acid). *Pharmaceutical Research*, 9, 26-32.
- [110] Liggins, R.T., Burt, H.M. (2001). Paclitaxel loaded poly (l-lactic acid) microspheres: properties of microspheres made with low molecular weight polymers. *International Journal Pharmaceutics*, 222, 19-33.
- [111] Hurrel, S., Cameron, R.E. (2002). The effect of initial polymer morphology on the degradation and drug release from polyglycolide. *Biomaterials*, 23,2401-2409.
- [112] Andreopoulos, A.G. (1994). Plasticization of biodegradable polymers for use in controlled release. *Clinical Materials*, 15, 89-92.
- [113] Faisant, N., Siepmann, J., Oury, P., Laffineur, V., Bruna, E., Haffner, J., Benoit, J.P. (2002). The effect of gamma-irradiation on drug release from bioerodible microparticles: A quantitative treatment. *International Journal Pharmaceutics*, 242, 281-284.
- [114] Arm, D.M., Tencer, A.F. (1997). Effects of cyclical mechanical stress on the controlled release of proteins from a biodegradable polymer implant. *Journal Biomedical Materials Science*, 35,433-441.
- [115] Edelman, E.R., Fiorino, A., Grodzinsky, A., Langer, R. (1992). Mechanical deformation of polymer matrix controlled release devices modulates drug release. *Journal Biomedical Materials Science*, 26, 1619-1631.

References

- [116] Karasulu, H.Y., Ertan, G., Kose, T. (2000). Modeling of theophylline release from different geometrical erodible tablets. *European Journal Pharmacy Biopharmaceutic*, 49, 177-182.
- [117] Siepmann, J., Gopferich, A. (2001). Mathematical modelling of bioerodible, polymeric drug delivery systems. *Advanced Drug Delivery Review*, 48, 229-247.
- [118] Gopferich A. (1996). Mechanisms of polymer degradation and erosion. *Biomaterials*, 17, 103-114.
- [119] Kenley, **R.A.**, Lee, M.O., Mahoney, T.R., Sanders, L.M. (1987). Poly (lactide-co-glycolide) decomposition kinetics in vivo and in vitro. *Macromolecules*, 20, 2398-2403.
- [120] Makino, K., Arakawa, M., Kondo, T. (1985). Preparation and in vitro degradation properties of polylactide microcapsules. *Chemistry Pharmaceutical Bulletin*, 33, 1195-1201.
- [121] Makino, K., Oshima, H., Kondo, T. (1986). Mechanism of hydrolytic degradation of poly (L-lactide) microcapsules: Effects of pH, ionic strength and buffer concentration. *Journal Microencapsulation*, 3, 203-212.
- [122] Belbella, A., Vauthier, C., Fessi, H., Devissaguet, J-P., Puisieux, F. (1996). In vitro degradation of nanospheres from poly (d,l-lactides) of different molecular weights and polydispersities. *International Journal Pharmaceutics*, 129, 95-102.
- [123] Von Burkersroda, D., Schedl, L., Gopferich, A. (2002). Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials*, 23, 4221-4231.
- [124] Chu C.C. (1981). An in-vitro study of the effect of buffer on the degradation of poly (glycolic) sutures. *Journal Biomedical Materials Science*, 15, 19-27.
- [125] Park, T.G. (1995). Degradation of poly (lactic-co-glycolic acid) microspheres: Effect of copolymer composition. *Biomaterials*, 16, 1123-1130.

References

- [126] Von Recum, H.A., Cleek, R.L., Eskin, S.G., Mikos, A.G. (1995). Degradation of polydispersed poly(L-lactic acid) to modulate lactic acid release . *Biomaterials*, 16, 441-447.
- [127] Hakkarainen, M., Albertsson, A-C., Karlsson, S. (1996). Weight losses and molecular weight changes correlated with the evolution of hydroxyacids in simulated in vivo degradation of homo- and copolymers of PLA and PGA. *Polymer Degradation Stability*, 52, 283-291.
- [128] Lu, L., Garcia, C.A., Mikos, A.G. (1999). In vitro degradation of thin poly(dl-lactic-co-glycolic acid) films. *Journal Biomedical Materials Science*, 46, 236-244.
- [129] Mauduit, J., Pérouse, E., Vert, M. (1996). Hydrolytic degradation of films prepared from blends of high and low molecular weight poly (dl-lactic acid)s. *Journal Biomedical Materials Science*, 30, 201-207.
- [130] Tsuji, H., Ikada, Y. (2000). Properties and morphology of poly (l-lactide). 4. Effects of structural parameters on long-term hydrolysis of poly (l-lactide) in phosphate-buffered solution. *Polymer Degradation Stability*, 67, 179-189.
- [131] Park, T.G. (1994). Degradation of poly (d,l-lactic acid) microspheres: Effect of molecular weight. *Journal Controlled Release*, 30, 161-173.
- [132] Wang, N., Qiu, J.S., Wu, X.S. (1998). Biodegradation of lactic-glycolic acid oligomers. In I. Mc Culloch and S.W. Shalaby (Eds.), *Tailored polymeric materials for controlled delivery systems* (pp.242-254). Washington DC: ACS symposium series 709.
- [133] Cam, D., Hyon, S-H., Ikada, Y. (1995). Degradation of high molecular weight poly (l-lactide) in alkaline medium. *Biomaterials*, 16, 833-843.
- [134] Grizzi, I., Garreau, H., Li, S., Vert, M. (1995). Hydrolytic degradation devices based on poly(DL-lactic acid) size dependence. *Biomaterials*, 16, 305-311.

References

- [135] Agrawal, C.M., McKinney, J.S., Lancot, D., Athanasiou, K.A. (2000). Effects of fluid flow on the in vitro degradation kinetics of biodegradable scaffolds for tissue engineering. *Biomaterials*, 21, 2443-2452.
- [136] Weiler, W., Gogolewski, S. (1996). Enhancement of the mechanical properties of polylactides by solid-state extrusion. *Biomaterials*, 17, 529-535.
- [137] Rothen-Weinhold, A., Besseghir, K., Gurny, R. (1997). Analysis of the influence of polymer characteristics and core loading on the in vivo release of a somatostatin analogue. *European Journal Pharmaceutical Science*, 5, 303-313.
- [138] Hausberger, A.G., Kenley, R.A., DeLuca, P.P. (1995). Gamma irradiation effects on molecular weight and in vitro degradation of poly (d,l-lactide-co-glycolide) microparticles. *Pharmaceutical Research*, 12, 851-855.
- [139] Hooper, K.A., Cox, J.D., Kohn, J. (1997). Comparison of the effect of ethylene oxide and γ -irradiation on selected tyrosine-derived polycarbonates and poly (l-lactic acid). *Journal Applied Polymer Science*, 63, 1499-1510.
- [140] Miller, N.D., Williams, D.F. (1984). The in vivo and in vitro degradation of poly (glycolic acid) suture material as a function of applied strain. *Biomaterials*, 5, 365-368.
- [141] Kranz, H., Ubrich, N., Maincent, P., Bodmeier, R. (2000). Physicomechanical properties of biodegradable poly (d,l-lactide) and poly (d,l-lactide-co-glycolide) films in the dry and wet state. *Journal Pharmaceutical Science*, 89, 1558-1566.
- [142] Starkweather, H.W., Avakian, P., Fontanella, J.J., Wintersgill, M.C. (1993). Internal motions in polylactide and related polymers. *Macromolecules*, 26, 5084-5087.
- [143] Tsuji, H., Mizumo, A., Ikada, Y. (2000). Properties and morphology of poly (l-lactide). Effect of initial crystallinity on long-term in vitro hydrolysis. High molecular weight poly(l-lactide) film in phosphate buffered solution. *Journal Applied Polymer Science*, 77, 1452-1464.

References

- [144] Lu, L., Peter, S.J., Lyman, M.D., Lai, H-L., Leite, S.M., Tamada, J.A., Uyama, S., Vacanti, J.P., Langer, R., Mikos, A.G. (2000). In vitro and in vivo degradation of porous poly (d,l-lactide-co-glycolide) foams. *Biomaterials*, 21, 1837-1845.
- [145] Lee, K.H., Won, C.Y., Chu, C.C., Gitsov, I. (1999). Hydrolysis of biodegradable polymers by superoxide ion. *Journal Polymer Science A: Polymer Chemistry*, 37, 3558-3567.
- [146] Webber, W.L., Lago, F., Christopher, T., Mathiowitz, E. (1998). Characterization of soluble, salt-loaded, degradable PLGA films and their release of tetracycline. *Journal Biomedical Materials Science*, 4, 18-29.
- [147] Zhang, Y., Zale, S., Sawyer, L., Bernstein, H. (1997). Effects of metal salts on poly (d,l-lactide-co-glycolide) polymer hydrolysis. *Journal Biomedical Materials Science*, 34, 531-538.
- [148] Giunchedi, P., Conti, B., Scalia, S., Conte, U. (1998). In vitro degradation study of polyester microspheres by a new HPLC method for monomer release determination. *Journal Controlled Release*, 56, 53-62.
- [149] Witt, C., Kissel, T. (2001). Morphological characterization of microspheres, films and implants prepared from poly(lactide-co-glycolide) and ABA triblock copolymers: is the erosion controlled by degradation, swelling or diffusion? *European Journal Pharmacy Biopharmaceutical Science*, 51, 171-181.
- [150] Schmitt, E.A., Flanagan, D.R., Linhardt, R.J. (1993). Degradation and release properties of pellets fabricated from three commercial poly (d,l-lactide-co-glycolide) biodegradable polymers. *Journal Pharmaceutical Science*, 82, 326-329.
- [151] Li, S., Garreau, H., Vert, M. (1990). Structure-property relationships in the case of the degradation of massive poly (α -hydroxy acids) in aqueous media. Part 1: poly (d,l-lactic acid). *Journal Materials Science: Materials Medicine*, 1, 123–130.

References

- [152] Li, S., Garreau, H., Vert, M. (1990). Structure-property relationships in the case of the degradation of massive poly (α -hydroxyl acids) in aqueous media. Part 3: Influence of the morphology of poly (L-lactic acid). *Journal Materials Science: Materials Medicine*, 1, 198-206.
- [153] Li, S., Vert, M. (1994). Morphological changes resulting from the hydrolytic degradation of stereocopolymers derived from l- and d,l-lactides. *Macromolecules*, 27, 3107-3110.
- [154] Ali, S.A.M., Doherty, P..J, Williams, D.F. (1993). Mechanisms of polymer degradation in implantable devices. 2. Poly (dl-lactic acid). *Journal Biomedical Materials Science*, 27, 1409-1419.
- [155] Wu, X.S. Wang N. (2001). Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers. Part 11: Biodegradation. *Journal Biomaterial Science Polymer Edition*, 12, 21-34.
- [156] Lu, L., Garcia, C.A., Mikos, A.G. (1999). In vitro degradation of thin poly(dl-lactic-co-glycolic acid) films. *Journal Biomedical Materials Science*, 46, 236-244.
- [157] Nakamura, T., Hitomi, S., Watanabe, S., Shimizu, Y., Jamshidi, K., Hyon, S-H., Ikada, I. (1989). Bioabsorption of polylactides with different molecular properties. . *Journal Biomedical Materials Science*, 23, 1115-1130.
- [158] Cai, H., Dave, V., Gross, R.A., McCarthy, S.P. (1996). Effect of physical aging, crystallinity, and orientation on the enzymatic degradation of poly (lactic acid). *Journal Polymer Science. Part B: Polymer Physics*, 34, 2701-2708.
- [159] Montaudo, G., Rizzarelli, P. (2000). Synthesis and enzymatic degradation of aliphatic copolyesters. *Polymer Degradation Stability*, 70, 305-314.
- [160] Li, S. (1999). Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. *Journal Biomedical Materials Science (Applied Biomaterials)*, 48, 342-353.

References

- [161] Holy, C.E., Dang, S.M., Davies, J.E., Shoichet, M.S. (1999). In vitro degradation of a novel poly (lactide-co-glycolide)75/25 foam. *Biomaterials*, 20, 1177-1185.
- [162] Mainil-Varlet, P., Curtis, R., Gogolewski, S. (1997). Effect of in vivo and in vitro degradation on molecular and mechanical properties of various low molecular weight polylactides. *Journal Biomedical Materials Science*, 36,360-380.
- [163] Jamshidi, K., Hyon, S-H., Nakamura, T., Ikada, Y., Shimizu, Y., Teramatsu, T. (1986). In vitro and in vivo degradation of poly-L-lactide fibers. In P. Christel, A. Meunier, A.J.C. Lee (Eds.), *Biological and biomechanical performance of biomaterials*. Amsterdam; New York Elseiver.
- [164] Aso, Y., Yoshioka, S., Po, A.L.W., Terao, T. (1994). Effect of temperature on mechanisms of drug release and matrix degradation of poly (d,l-lactide) microspheres. *Journal Controlled Release*, 31,33-39.
- [165] Nugroho, P., Mitomo, H., Yoshii, F., Kuma, T. (2001). degradation of poly (l-lactic acid) by γ -irradiation. *Polymer Degradation Stability*, 72, 337-343.
- [166] Vert, M., Li, S., Spenlehauer, G., Guerin, P. (1992). Bioresorbability and biocompatibility of aliphatic polyesters. *Journal Materials Science: Materials Medicine*, 3,432-446.
- [167] Li, S., Girard, A., Garreau, H., Vert, M. (2001). Enzymatic degradation of polylactide stereocopolymers with predominant D-lactyl contents. *Polymer Degradation Stability*, 71, 61-67.
- [168] Li, S., McCarthy, S. (1999). Influence of crystallinity and stereochemistry on the enzymatic degradation of poly (lactide). *Macromolecules*, 32,4454-4456.
- [169] Hooper, K.L., Macon, N.D., Kohn, J. (1998). Comparative histological evaluation of new tyrosine-derived polymers and poly (l-lactic acid) as a function of polymer degradation. *Journal Biomedical Materials Research*, 41,443-454.

References

- [170] Leeslag, J.W., Pennings, A.J., Bos, R.R.M., Rozema, F.R., Boering, G. (1987). Resorbable materials of poly (1-lactide). VII: In vivo and in vitro degradation. *Biomaterials*, 8, 311-314.
- [171] Anderson, J.M., Shive, M.S. (1997). Biodegradation and biocompatibility of PLA and PLGA microspheres. *Advanced Drug Delivery Review*, 28, 5-24.
- [172] Athanasiou, K.A., Niederauer, G.G., Agrawal, C.M. (1996). Sterilization, toxicity, biocompatibility and clinical application of polylactic acid/polyglycolic acid copolymers. *Biomaterials*, 17, 93-102.
- [173] Matsusue, Y., Yamamuro, T., Oka, M., Shikinami, Y., Hyon, S-H., Ikada, Y. (1992). In vitro and in vivo studies on bioabsorbable ultra-high-strength poly (1-lactide) rods. *Journal Biomedical Materials Research*, 26, 1553-1567.
- [174] Trantolo, D.J., Gresser, J.D., Wise, D.L., Lewandrowski, K-W., Bozic, K.J. (2000). Biocompatibility of self-reinforced poly (lactide-co-glycolide) implants. In D.L. Wise (Eds.), *Biomaterials and bioengineering handbook* (pp.619-640). New York: Marcel Dekker.
- [175] Schakenraad, J.M., Hardonk, M.J., Feijen, J., Molenaar, I., Nieuwenhuis, P. (1990). Enzymatic activity toward poly (1-lactic acid) implants. *Journal Biomedical Materials Research*, 24, 529-545.
- [176] Van Der Giessen, W.J., Lincoff, M., Schwartz, R.S., Heleen, M.M., Van Beusekom, M.M., Serruys, P.W., Holmes, D.R., Ellis, S.G., Topol, E.J. (1996). Marked inflammatory sequelae to implantation of biodegradable and non-biodegradable polymers in porcine coronary arteries. *Circulation*, 94, 690-1697.
- [177] Bostman, O., Hiervensalo, E., Mäkinen, J., Rokkanen, P. (1990). Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *Journal Bone Joint Surgery*, 72-B, 592-596.

References

- [196] Peyser, P. (1997). Glass transition temperature of polymers. In J. Brandrup, E.H. Immergut (Eds.). *Polymer Handbook; 3rd Edn.* (pp.VI209-VI483). New York John Wiley.
- [197] Gallagher, K.M., Corrigan, O.I. (2000). Mechanistic aspects of the release of levamisole hydrochloride from biodegradable polymers. *Journal Controlled Release*, 69, 261-272.
- [198] Baker, R. (1987). Diffusion-controlled systems. In R. Baker (Eds.), *Controlled Release of Biologically Active Agents* (pp. 39-83). New York John Wiley.
- [199] Martin, N., Catalin, J., Blachon, M.F., Durand, A. (1998). Assay of paclitaxel (taxol) in plasma and urine by high-performance liquid chromatography. *Journal Chromatography B*, 709, 281-288.
- [200] Eury, R.P. (1994). Multilayered biodegradable stent and method for its manufacture. *European Patent. N 0604022A1*.

Appendix I

APPENDIX I

Study of the Initial Stages of Drug Release from a Degradable Matrix of Poly(d,l-lactide-co-glycolide)

Frank Alexis, Santosh Kumar Rath, Freddy Boey and Subbu Venkatraman*

School of Materials Engineering, N4.1-1-30, Nanyang Technological University, Nanyang Avenue, Singapore 639798.

* To whom correspondence should be addressed (e-mail: assubbu@ntu.edu.sg)

Abstract

The initial stages of the in vitro degradation of and the drug release from a matrix made of poly(d,l-lactide-co-glycolide) was carried out in a phosphate buffer saline (pH 7.0) medium. It has been observed that substantial matrix degradation occurs at the end of two weeks of immersion. The drug release using films of the polymer shows a tri-phasic pattern, unlike the bi-phasic patterns usually seen. Mechanisms are proposed for each phase of release, based on results from weight loss, amount of water absorption and scanning electron microscopy. The

Appendix I

details of the structural changes and their effects on drug release may have implications for delivering potent drugs over a 2-week period.

Introduction

Applications in tissue engineering and drug delivery have primarily relied on materials that resorb or degrade in body fluids so that the device ultimately disappears with no ill effects. A wide variety of both solid and hydrogel type polymers have been developed and many serve dual applications in drug delivery and tissue engineering. In this respect, synthetic polymers [1-4] offer greater advantages than natural materials, because of their ability to be tailored to suit particular applications and better control over batch uniformity. Homopolymers and copolymers based on L-lactide, D-lactide and glycolide have received considerable research interest in view of their biodegradability and toxicological safety. Poly (lactide-co-glycolide)(PLGA) is among the few synthetic polymers approved for human clinical trials. The biocompatibility of PLGA has been demonstrated in many biological sites [5]. Another exciting use for which biodegradable polymers offer tremendous potential is as a basis for drug delivery, either as drug delivery system alone or in conjunction with a medical device [6,7]. The drugs can be incorporated either in dissolved or dispersed form within the polymer matrix, which subsequently degrades in the body releasing the drug in a controlled manner [8-10]. Various forms of controlled release devices including microspheres [11-15], rods [16], films [17] and nanoparticles [18] have been

Avvendix I

fabricated from PLGA to control the drug release with suitable modification in polymer composition, crystallinity and structure.

Qian et al. [16] studied millirods of PLGA 50150 (IV=0.65) for the local treatment of tumors giving a diffusion controlled drug release pattern. On the other hand, Eliaz and Kost [19] proposed an alternative approach using microspheres as an injectable drug delivery system. The formulation of PLGA 50150 (IV=0.38) containing a mixture of BSA (bovine albumin serum) and sp-55-R (human soluble p55 TNF receptor) has been shown to exhibit an erosion controlled drug release pattern with a burst effect within the first day. Singh et al. [20] prepared microparticles of PLGA 50150 (IV=0.2) with entrapped recombinant insulin-like growth factor (rhIGF-I) showing diffusion and erosion controlled drug release with a burst effect within the first day.

There is presently a lack of data on the early stages of drug release and a corresponding lack of understanding concerning the associated mechanism of release. Such details have become more important especially for the delivery of potent drugs with a narrow therapeutic window. Systems that deliver drugs over a period of 2-4 weeks (e.g., Nutropin® Depot for release of human growth hormone) are currently being marketed and developed. This paper reports on the details for the early stages (0-15 days) of drug release, and attempts to rationalize the observed release profiles.

The copolymer PLGA is the material of choice for such relatively shorter-duration applications, while the homopolymers (PLLA and PGA) are preferred for longer-term delivery of drugs. Hence, our current investigation was aimed at understanding the drug release mechanism from a

Appendix I

matrix of PLGA as well as the pathways of early degradation of PLGA as a matrix material. The particular polymer selected for this study is a copolymer of d- and l-lactide (53 percent of d,l-lactide) with glycolide, which will be referred to in subsequent text as PdlLGA. The drug selected for this study is metoclopramide monohydrochloride monohydrate (MTCPMH), an antipsychotic and antiemetic drug [21]. The in vitro degradation studies as well as the drug release have been studied using phosphate buffer saline (PBS, pH 7.0), as the release medium. The system has been characterized with respect to weight loss, water uptake and morphological changes, in order to explain the drug release and matrix degradation mechanisms.

Materials and Methods

Materials

Metoclopramide monohydrochloride monohydrate (MTCPMH, FW=336.3 g/mol), was purchased from Sigma-Aldrich (Singapore). Poly (d,l-lactide-co-glycolide) 53:47 (PdlLGA 53:47) with intrinsic viscosity of 0.47 dl/g and M_w of approx. of 40 kDa was purchased from Purac Far East Pte. Ltd., Singapore. Phosphate saline buffer (pH 7.0) (PBS) and methylene chloride were purchased from E. Merck, Germany. All the chemicals were used as received.

Preparation of polymer films and pellets

Drug-loaded polymer (10 wt %) films (thickness –0.2 mm) were used for studying the drug release characteristics, whereas pellets (thickness –1 mm) were used to study the in vitro degradation of the polymer matrix itself. Polymer films were prepared using the method of solution casting. Appropriate amount of the polymer was dissolved into methylene chloride

Appendix I

under stirring using a magnetic stirrer. The drug was dissolved into 2ml of a 50:50 (v/v) mixture of methanol and ethanol. The resulting solution of drug was added to the polymer solution in methylene chloride. The mixture was stirred for uniformity followed by sonication for 30 minutes. Film casting was done from the resulting solution using a gravure coating rod of 250 μm thickness. The wet film was allowed to dry at room temperature for 24 hours. Subsequently, drying was continued in vacuum at 40°C, for a week. The above conditions achieved almost complete removal of solvent with minimal bubble formation. Samples of 2 cm x 2.5 cm dimension were cut from the dried film for subsequent characterization.

Similarly, a 40-50 wt % of PdlLGA 53:47 in methylene chloride was poured into polytetrafluoroethylene (PTFE) mould with the solvent allowed to evaporate initially at room temperature, and subsequently thoroughly dried to constant weight at 40°C under vacuum for one week.

Degradation study

Two pellets were put into each of a total of thirteen individual glass vials (of 60ml capacity). The pellets were completely submerged in 50 ml of the buffer solution (pH 7.0). The vials with the pellets and buffer solution were capped and sealed and put into a constant temperature water bath, maintained at $37 \pm 0.1^\circ\text{C}$. Pellets were removed at pre-determined time intervals, and evaluated for water uptake and weight loss.

Water uptake: Pellet samples were periodically recovered for determination of the amount of water absorption. In a typical test, the pellet was wiped with a clean tissue paper to get rid of

Appendix I

excess buffer solution adhering on the surface, weighed and later, dried to constant weight in order to determine the weight loss. Water uptake was calculated at each time point using the following equation:

Where, W_{wet} and W_0 respectively, are weights of the wet and starting dry weight pellets as measured at time, t . Values obtained for duplicate samples were averaged. All weights were measured to an accuracy of (± 0.01 mg)

Weight loss: Polymer weight loss during film hydration was measured by the changes in dry weight after incubation for specified time periods. For such tests, two vials for each polymer composition were taken out of the water bath. The buffer was discarded and the residual pellets rinsed with distilled water three times and dried in a vacuum oven for 1 week at 40° C. Values obtained for duplicate samples were averaged. Percent weight loss was computed according to the following equation:

$$\% \text{ Weight Loss} = 100 \times \frac{W_0 - W_t}{W_0}$$

Where W_0 = starting dry weight; W_t = dry weight at time, ' t '

Thermal analysis: The glass transition temperature of the Pdl-LGA samples was measured using a Modulated Differential Scanning Calorimeter, MDSC (DSC 2920, TA Instruments, USA). Typical weight of samples was 5-10 mg. Samples were run in the modulated mode, under nitrogen flow, from -40 to 220°C at a heating rate of $10^\circ\text{C}/\text{min}$ with an amplitude of modulation of ± 1 degree per 60 seconds. The temperature at half-height of the first endothermic step change

Appendix I

in the reversible heat flow curve was accepted as the glass transition temperature. The melting point (T_m) was taken as the temperature corresponding to the maximum in the endothermic peak, and the ΔH_f and ΔH_c was taken as the area upper and under the same peak, respectively. For the percent crystallinity calculation, ΔH_f minus ΔH_c was divided by the theoretical 100 % crystalline ΔH_f^0 :

$$DOC(\%) = 100 \times \frac{\Delta H_f}{\Delta H_f^0}$$

Drug release study

Films were weighed and placed inside the baskets of a dissolution test apparatus. The dissolution test apparatus (Vankel 7000), which was supplied by Vankel, USA, comes equipped with six 100 ml vessels (with conversion kits) and each of the vessels can be simultaneously stirred by spindles at variable speeds. Each of the vessels was filled with 50 ml of the buffer solution (as the release medium) and the height of the spindles was so adjusted as to immerse the baskets containing the films in the buffer completely. The temperature of the vessels was maintained at $37 \pm 0.1^\circ\text{C}$ and the speed of rotation of the spindles was set at 50 rpm.

The quantitative measurement of the amount of drug released was done with a Shimadzu UV-250 1PC UV-VIS Spectrophotometer. A calibration curve was done as the concentration versus absorbance for the drug in buffer (wavelength of absorption, $\lambda = 270 \text{ nm}$). Samples of 2 ml each were drawn from the release medium at predetermined intervals and the absorbance of the drug

Appendix I

measured using the W-VIS Spectrophotometer, followed by putting back the sample into the release medium.

Scanning Electron Microscopy (SEM)

Surface morphology of the films was studied using SEM, for evidence of film and pellets degradation. SEM was performed on surface of the samples at 0, 7 and 15 days, for the samples from in vitro degradation as well as drug release tests. The samples were air and vacuum-dried for 1 week at 40° C, before observation with SEM.

The individual films were gold coated (gas pressure 20 millibar, current 18 mA and coating time 75 seconds) and the SEM micrographs were observed at 10 kV (AS JEOL, model JSM-5410 VL).

Results and Discussion

I. Degradation Study

Water uptake of PdILGA pellets

Figure 1 presents the results of water uptake of the PdILGA samples as a function of incubation time. Water absorption was detected within hours after immersion in buffer and increased for the first 4 days after an initial adsorption of 5 % (1 day). Subsequently, the rate of water absorption increased rapidly to day 7, then slowed until day 11 and finally increased once again to reach a maximum of 33 % at the end of second week. Thus, the water uptake of the PdILGA shows a three stage pattern. The first step is the absorption up to day four, followed by a second phase

Appendix I

where the amount of water absorption increases once again, and reaches a second plateau. The third stage begins on day 11, and continues to the day of the last measurements, day 14.

Weight loss of PdLLGA pellets

Figure 2 presents the weight loss with incubation time for the samples discussed in Figure 1. The pattern again is tri-phasic and similar to the water uptake profile in Figure 1, but with some differences. Weight loss is measured at the end of one day. This is followed by a continuous weight loss until day 7. There is a slower weight loss subsequently, until day 11 (similar to the second "knee" in the water uptake), before the next phase of weight loss starts to occur. The extent of weight loss at the end of the first stage was about 14%, which increased to 33% at the end of the third stage.

S. Li [22] has studied a PdLLA ($M_w=65000$ Da) containing caffeine contents of 2 and 20 %. The weight loss followed a tri-phasic profile: the 2 % blend has a slow weight loss to day 9 (3%) (Phase 1), followed by an acceleration for 2 days (20%), followed again by a lag phase of 2 days (Phase 2) and another acceleration until the blend completely break down and degrade (Phase3). The PdLLA-20 % caffeine blend has a first fast weight loss of 20 % within two days (Phase 1), followed by a lag phase of 5 days, another acceleration for two days (18%) (Phase 2), followed by a lag phase until day 20 (20%), and the final acceleration (Phase 3). No mechanistic explanation was forwarded for the observed behaviour. Although PdLLA has different properties from PdLLGA 53/47, they are both completely amorphous and of comparable molecular weight. Figure 3 presents a comparison of the results from Figure 1 and 2.

Appendix I

SEM analysis

The PdILGA pellets (original films as well as those after in vitro degradation for different lengths of time) were observed under scanning electron microscope, for proof of surface and bulk erosion. Figure 4 presents the micrograph of a dry PdILGA film before immersion in the buffer medium. On the other hand, Figures 5 and 6 present the micrographs of same batch of PdILGA films after subjecting to in vitro degradation for 7 and 14 days, respectively. The morphological differences are quite obvious. The original film, which is quite smooth (Figure 4), does develop into a rough terrain with distinct marks of surface erosion after one week of degradation (Figure 5). At the end of 14 days, the extent of erosion has been sufficient so as to leave a network of interconnected channels and pores (Figure 6).

This finding agrees well with previous observations [12,14,15] obtained under similar conditions. Many investigations [17,21-24] have indicated that the intrinsically amorphous PLGA undergoes bulk degradation in aqueous media. Explanation for the tri-phasic patterns observed has been attempted in a later section of this paper.

Thermal analysis

Figure 7 presents a plot of the glass transition temperatures (T_g) of the original PdILGA film and those obtained after immersion in the buffer. The T_g drops significantly, after four days of immersion. An important observation may be made here in connection with the results of drug release discussed in later parts of this manuscript. The T_g drops to less than 37°C (the body

Appendix I

temperature), after four days. This should have an influence on the rate and extent of drug release from the polymer matrix.

II. Drug Release Study

All the drug release tests were carried out in duplicate to control the homogeneity of the drug release and the results obtained from the samples were averaged.

Figure 8 plots the amount of drug release, as a fraction of the total loading, against the immersion time in the release medium. The results again evidently show that the release of drug from the matrix occurs in three phases. The initial burst (about 8%) is likely to be due to undissolved drug particles on the film surface. This initial burst is followed by three distinct release phases (stage 1: 1 to 4 days and stage 2: 4 to 11 days and stage 3: 11-15 days). The drug release data obtained from the in vitro release study was analyzed for the rate of release, using the Higuchi [25] drug release equation given below:

$$\frac{M_t}{M_{\text{inf}}} = k \times t^{\frac{1}{2}} \quad \text{Eq. 1}$$

(where M_t/M_{inf} is the fraction of drug released, t is the release time and k is a constant characteristic of the system)

or a linear relationship:

$$\frac{M_t}{M_{\text{inf}}} = k \times t \quad \text{Eq.2}$$

for the different stages, as explained further below.

Appendix I

Figures 9a, 9b and 9c are scanning electron micrographs of the drug loaded (10 wt %) PdLLGA films, before and after immersing into release medium for 7 and 14 days, respectively. Figure 9a shows a substantial amount of drug on the outside surface of the film, which accounts for the initial burst effect in Figure 8. Figure 9b shows that most of the drugs at the surface have been leached out to leave the pores observed, after immersion for 7 days. Finally, Figure 9c shows the presence of some drug crystals in the interior of the film, together with what appears to be fissures within the matrix, indicating that the matrix has degraded extensively.

III. Proposed Mechanisms of Drug Release

To date, most of the reported data on drug release from biodegradable polymers has been from films [26] or microspheres [11-15,27]. The release data show either a bi-phasic pattern (a diffusion phase followed by erosion) or a single diffusional phase (probably a combination of diffusion and erosion). The burst effect, which occurs typically in day 1, has usually not been considered as one of the phases of release.

Drug release from films

In a detailed study, Shah et. al. [26] examined testosterone release from cast and compressed films. The loading of testosterone was below its saturation limit in the PLGA, a 50/50 copolymer of M_w 18,000. Film thicknesses were approximately 0.4 mm for compressed films (which was a physical mixture of micronized polymer and drug), and about the same thickness for the cast films. Surprisingly, no difference in behaviour was observed between the cast and compressed films. The main conclusions reached by these investigators are given below:

Appendix I

- Up to day 8, the drug release was diffusion-controlled with a D value of about $2 \times 10^{-12} \text{ cm}^2/\text{s}$. From day 9 till exhaustion of drug, the profile was erosion-controlled.
- The drug release profile and the weight loss profile could be superposed on one another, indicating that the weight loss also followed an initial diffusional pattern, then an erosion-controlled pattern.

E. R. Edelman et. al. [27], described the design and development of a novel biodegradable system for perivascular delivery of heparin to the blood vessel wall with well-defined release kinetics. This system consisted of heparin-encapsulated poly (dl-lactide-co-glycolide) microspheres sequestered in an alginate gel. The heparin was encapsulated in P(dl-LGA) 50/50 ($M_w = 10^5 \text{ Da}$). Drug release of the microspheres (unencapsulated by alginate gel) was bi-phasic. An initial burst of 18 % in the first day was followed by a lag phase of 11 days, followed by a nearly constant release for an additional 69 days. The initial burst was explained by the dissolution of drug molecules close to the microsphere surface, and the subsequent lag phase of minimal release as an induction time before erosion of polymer matrix becomes sufficient to release macromolecules. No diffusional phase was observed. Although not explicitly noted by the authors, we believe that their data show a first order drug release lasting from day 14 to day 42, followed by the second first order drug release for another 28 days.

K. W. Burton et al. [12], studied leuprolide, an LHRH superagonist incorporated into Pdl-LGA 50/50 ($M_w = 10754 \text{ Da}$) microspheres. After a burst of nearly 20 %, a period of very gradual release through 21 days was noted during in vitro testing. After 28 days, the rate of leuprolide release increased. Release continued at a steady rate through 63 days before leveling at 70 days

Appendix I

with 95 % released. Although not explicitly stated, this release profile also appeared biphasic, with a diffusional phase (up to 28 days), followed by erosion-controlled release. The authors also contrasted this with a 50/50 copolymer with acid termination, for which the onset of erosion happens at 124 rather than 28 days. No mechanistic explanation was proposed for the observed patterns and the differences in behaviour.

The focus in this study has been on the first 15 days of release, where diffusional release may initially dominate, and where the details of water uptake and weight loss play a major role in the overall kinetics. Once erosion starts to dominate, release of drug is rapid, and follows no predictable pattern. The first 15 days are important for many applications, including the delivery of growth hormone [12], where the injectable microspheres deliver the hormone in 2 weeks.

Figure 10 represents the overlay of the amount of drug release (fraction of the total loading), without the burst stage. The latter data (i.e., data without the burst contribution) is used for all calculations.

Here, we propose an explanation of the drug release mechanism for this tri-phasic profile based on our data, including weight loss, water uptake, changes of glass transition, and scanning electron microscopy pictures.

As can be seen from the Figures 1 and 10, it does appear that the drug release profile more closely follows the water uptake profile, rather than the weight loss profile. This observation and others below show some points of divergence from the results of Shah et al. [26].

Appendix I

Tables 1 and 2 list the results of statistical regression analysis in fitting the data to a linear time dependence and a dependence to the square root of time. We note the following points:

- (a) The drug release kinetics in Stage 1 (to day 4) is better described by Higuchian kinetics (diffusion from a slab, Eq.1). The value of the diffusion coefficient, calculated according to:

$$k = A(2DC_sC_0)^{1/2}$$

is $2.42 \times 10^{-10} \text{ cm}^2/\text{sec}$, which is representative of diffusion through a (highly crosslinked) rubbery matrix, or from a polymer close to its T_g .

- (b) The second stage is also best described by Higuchian kinetics from a saturated slab, with an apparent D value of $532 \times 10^{-10} \text{ cm}^2/\text{sec}$. This is representative of diffusion from a rubbery matrix.

- (c) The third stage has an apparent diffusion coefficient ($45.8 \times 10^{-10} \text{ cm}^2/\text{sec}$) that is indicative of diffusion from a swollen or porous matrix.

- (d) The water uptake also appeared to have 3 stages, each stage more or less described by diffusional kinetics. However, water absorption in Stage 3 appeared to be increasing faster than driven purely by diffusion; the effect of erosion may be dominating in Stage 3 for water absorption.

- (e) The weight loss profile, in contrast, exhibited linear dependence on time, for up to 7 days. This was unexpected, and in contrast to the observations of Shah et al. [26].

- (f) The T_g of the polymer fell below the measurement temperature (37°C) in about 2-4 days, thus corroborating the value of the diffusion coefficient that results from the initial drug release.

Appendix I

The magnitude of the diffusion coefficient for drug release, in Stage 1, was consistent with diffusion of a medium-MW drug from a matrix close to its T_g somewhere between a completely rubbery and completely glassy matrix, as described by Baker [28]. The value obtained in this paper tallies closely with the values obtained by Miyajima et al. [29], who studied drug release from a low-MW PI-LA polymer that was amorphous to start with, but became crystalline after exposure to aqueous buffers. This low-MW PI-LA polymer had a T_g of about 39-42°C after drug incorporation.

After day 4, the measured T_g dropped below the temperature of measurement, due mostly to a drop in M_w of the polymer, since the amount of swelling by water would not be sufficient to account for the T_g drop at this stage (please note that our T_g measurements were made on the dried rather than the swollen polymer, and hence reflected a substantial decrease in M_n). Shah et al. [26], in contrast, observed $t^{1/2}$ kinetics through day 8 (for testosterone, FW=288 g/mol), but their calculated D was $\sim 10^{-12}$ cm²/sec, 2 orders of magnitude lower. Surprisingly, their reported T_g data appeared more consistent with the existence of a rubbery matrix, in apparent contradiction to their calculated D .

The Stage 3 diffusion coefficients obtained in Table 1 compare well to the 2nd-stage diffusion coefficients reported by Mujiyama et al. [29], from a PI-LA matrix, who attributed the high values (in their Stage 2) to diffusion through water-filled pores. This explanation is probably valid for the Stage 3 observed in this work.

Appendix I

Burton et al. [12] reported a so-called "tri-phasic" pattern of release from PLGA microsphere formulations. Two different PLGA formulations were used but the tri-phasic release was observed for only one of them, a 50/50 copolymer of $M_n=100,000$ (higher than reported here). In any case, the 3 phases in this instance consisted of a "burst" phase, followed by an induction or lag phase, with a "final" erosion-dominated phase. No calculations of apparent rates was given. Closer examination of their release profile showed a pattern very similar to the one observed in this report, but over a longer time period. The first "knee" was observed from day 15-30, and the second from day 48-56. However, the occurrence of the second knee was not noted by the authors, who instead stated that after the lag phase (to day 15), the release was nearly "constant" to day 70.

The disparity between the kinetics of weight loss and drug release in this paper needs elaboration. We surmise that in the initial stage (up to day 4), diffusion through the unswollen, slightly rigid rubbery matrix dominated the drug release kinetics, even though hydrolysis and weight loss were occurring. The build-up of oligomeric units increased the overall hydrophilicity enough by day 5 to dramatically increase water uptake (swelling), leading to a swollen matrix. The measured T_g of the dried films at this stage showed no further drop, but presumably the swollen matrix has an overall low T_g . Concurrent with the increased water uptake was an acceleration in drug release from day 4 to 11, with a higher diffusion coefficient. From around day 11, there was also a build-up of interconnected channels or pores due to leaching out of the oligomers. The pores are clearly seen in the SEM micrograph on day 14, but not in samples taken for day 7. Thus the three stages observed are due (in sequence) to the following events:

Appendix I

1. A decrease in T_g (due to degradation) to a value below 37°C , leading to a "rigid" rubbery matrix, close to its T_g .
2. A substantial swelling of this matrix from day 4-6 due to an increase in the overall hydrophilicity of the polymer, leading to faster diffusion in a rubbery matrix.
3. Erosion-related channel formation, leading to the third stage of release from day 12 onwards.

The T_g values and the SEM pictures on days 0, 7 and 14 are consistent with the above sequence of events.

Conclusion

There is substantial degradation in the early phase of drug release from a 53/47 PLGA copolymer. Drug release is however governed by diffusion, through 3 different phases: a transitional phase, where the phase is close to T_g , then a rubbery phase (swollen) and finally a porous matrix. Erosion-controlled release appears to set in only beyond day 11 for this system, even though degradation has occurred in that time period. In Stage 3, diffusion is probably through an erosion-induced porous matrix. These details may be important in designing systems that deliver potent drugs over a 2-week period.

References

[I] Amecke B, Bendix D, Entermann G. Synthetic resorbable polymers based on glycolide, lactides, and similar monomers. In: Ratner BD et al., editors. Biomaterials Science: an introduction to materials in medicine. San Diego: Academic Press 1996. p. 977-1007.

Appendix I

- [2] Domb AJ, Amselem S, Maniar M. Biodegradable polymers as drug carrier systems. In: Wise DL, Trantolo DJ, Altobelli DE, Yaszemski MJ, Gresser JD and Schwartz ER , editors. Encyclopedic handbook of biomaterials and bioengineering. New York: Marcel Dekker Inc 1995. p. 399-430.
- [3] Athanasiou **KA**, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical application of poly-lactic acid/Poly-glycolic acid copolymers. Biomaterials 1996; 17:93-102.
- [4] Avgoustakis K, Nixon JR. Biodegradable controlled release tablets: III. Effect of polymer characteristics on drug release from heterogeneous poly(lactide-co-glycolide)matrices. Int J Pharmacol 1993; 99:247-252.
- [5] Fukuzaki H, Yoshida M, Asano M, Kumakura M, Mashimo T, Yuasa H, Imai K, Yamanaka H. In vivo characteristics of low molecular weight copolymers composed of glycolic acid and various dl-hydroxy polyester biodegradable carriers for drug delivery systems. Biomaterials 1990; 11:441-445.
- [6] Wang N, Wu XS. Characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid oligomers: part II. Biodegradation and drug delivery application. J Biomater Sci Polym Edn 1997; 9:75-87.
- [7] Orloff LA, Domb AJ, Teomim D, Fishbein I, Golomb G. Biodegradable implant strategies for inhibition of restenosis, Adv Drug Deliv Rev 1997; 24: 3-9.
- [8] Markou CP, Lutostansky E M, Ku D.N, Hanson SR. A novel method for efficient drug delivery. Annals Biomed Engg 1998; 26:502-511.

Appendix I

- [9] Wu XS. Biomaterials in drug delivery systems. In: Wise DL, Trantolo DJ, Altobelli DE, Yaszemski MJ, Gresser JD and Schwartz ER, editors. Encyclopedic handbook of biomaterials and bioengineering. New York: Marcel Dekker Inc, 1995. p. 1201-1224.
- [10] Lewis DH, Controlled release of bioactive agents from lactidel glycolide polymers. Inc: Chasin M, Langer R, editors. Biodegradable Polymers as Drug Carriers System. New York: Marcel Dekker Inc.; 1990. p. 1-42.
- [11] Meinel L, Illi OE, Zapf J, Malfanti M, Merkle HP, Ganger B. Stabilizing insulin like growth factor-I in poly(d,l-lactide-co-glycolide) microspheres. J Controlled Rel 2001; 70:193-202.
- [12] Burton KW, Shameem M, Thanoo BC, DeLuca PP. Extended release peptide delivery system through the use of PLGA microsphere combinations. J Biomat Sci Polym Edn 2000; 11:715-729.
- [13] Blanco MD, Bernardo MV, Gómez C, Muñiz E, Teijón JM. Bupivacaine-loaded comatrix formed by albumin microspheres included in a poly (lactide-co-glycolide) film: in vivo biocompatibility and drug release studies. Biomaterials 1999; 20:1919-1924.
- [14] Park TG. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. Biomaterials 1995; 16:1123-1130.
- [15] Zhu JH, Shen ZR, Wu LT, Yang SL. In vitro degradation of poly lactide and poly(lactide-co-glycolide) microspheres. J Appl Polym Sci 1991; 43:2099-2106.
- [16] Qian F, Szymanski A, Gao J. Fabrication and characterization of controlled release poly(d,l-lactide-co-glycolide) microfilaments. J Biomed Mat Res 2001; 55:512-522.
- [17] Lu L, Garcia CA, AG Mikos. In vitro degradation of thin poly(dl-lactic-co-glycolic acid). J Biomed Mat Res 1999; 46:236-244.

Appendix I

- [18] Leroux JC, Gravel P, Balant L, Volet B, Anner BM, Allemann E, Doelker E, and Gurny R. Internalization of poly(d,l-lactic acid) nanoparticles by isolated human leukocytes and analysis of plasma protein onto the particles, J Biomed Mat Res 1994; 28:471-481.
- [19] Eliaz RE, Kost J. Characterization of a polymeric PLGA-injectable implant delivery system for the controlled of proteins. J Biomed Mat Res 2000; 50:388-396.
- [20] Singh M, Shirley B, Bajwa K, Samara E, Hora M, O'Hagan D. Controlled recombinant insulin-like growth factor from a novel formulation of polylactide-co-glycolide microparticles, J Controlled Rel 2001; 70: 21-28.
- [21] Mitchell AG. Polymorphism in metoclopramide hydrochloride and metoclopramide. J Pharm Pharmacol 1985;37:601-604.
- [22] Li S, Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. J Biomed Mater Res (Appl Biomat) 1999; 48:342-353.
- [23] Gopferich A, Mechanisms of polymer degradation and erosion. Biomaterials 1996; 17:103-114.
- [24] Kenley RA, Lee MO, Mahoney TR, Sanders LM. Poly(lactide-co-glycolide) decomposition kinetics in vivo and in vitro. Macromolecules 1997; 20:2398-2403.
- [25] E. Ron, R. Langer, Erodible systems. Kydonieus A, editor. Treatise on Controlled Drug Delivery: Fundamentals, Optimization, Applications. New York: Marcel Dekker Inc, 1992.p 199-224.
- [26] Shah SS, Cha Y, and Pitt CG. Poly (glycolic acid-co-dl-lactic acid): diffusion or degradation controlled drug delivery? J Controlled Rel 1992; 18:261-270.
- [27] Edelman ER, Nathan A, Katada M, Gates J, Karnovsky MJ, Perivascular graft heparin delivery using biodegradable polymer wraps. Biomaterials 2000; 21: 2279-2286.

Appendix I

[28] Baker, R.W.: Controlled Release of Biologically Active Agents, New York: John Wiley, 1987. p.31-32.

[29] Miyajima M, Koshika A, Okada J and Ikeda M: Effect of polymer/base drug interaction on the two-stage diffusion-controlled release from a poly(L-lactic acid) matrix. J Control Rel 1999; 61: 295-304.

List of Publications

LIST OF PUBLICATIONS

International Journals: Published articles

1. **Alexis** Frank, Santosh Kumar Rath, Freddy Boey and Subbu Venkatraman. "Study of the Initial Stages of Drug Release from a Degradable Matrix of Poly(d,l-lactide-co- glycolide", *Biomaterials*, 25, 813-821.
2. **Alexis** Frank, Santosh Kumar Rath, Boey FYC, Subbu Venkatraman. "In vitro study of release mechanisms of paclitaxel and rapamycin from drug-incorporated biodegradable stent ". *Journal of Controlled Release*, 98, 67-74.
3. **Alexis** Frank, Santosh Kumar Rath, Boey FYC, Subbu Venkatraman. "Controlled drug release of bioerodible polymers: Effect of drug type and composition ". *Journal of Controlled Release* (Accepted).

List of Publications

Research Papers Presented in International Conferences

1. **Alexis Frank, Santosh Kumar Rath, Freddy Boey and Subbu Venkatraman.** *"Factors Affecting the Drug Release Mechanisms of PLGA 53/47 and PLLA"*. International Conference of Materials Processing for Properties and Performance (MP3). Conrad Hotel, Singapore, 01 - 03 Aug 2002.

2. **Alexis Frank, Santosh Kumar Rath, Freddy Boey and Subbu Venkatraman,** *"Comparative Study of Drug Release from Matrices Made of PLLA and PLGA Copolymers"*; International Conference on Materials for Advanced Technologies; Suntec Singapore International Convention and Exhibition Center, Singapore; 7 Dec-12 Dec 2003.