Synthesis, conjugation and evaluation of some novel polymers and their micro particles for sustained release drug formulations

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Abstract: To prepare and evaluate three novels functionalized polymers (PGA, PGA-co-caprolactone & PGA-co-pentadecalactone) for the development of nanoparticles which were further used in the development of a novel polymeric prodrug using Ibuprofen as a model drug. The Ibuprofen-polymer prodrug was developed by coupling the drug to one of the three prepared polyester polymers via ester linkage. A hydrolytic enzyme was used to prepare two polymer monomers, glycerol and polyvinyl adipate, which are non toxic, ester linked biological monomers. The polymers and their prodrug were characterized using NMR, GPC, UV-spectrophotometer and DSC. *In vitro* drug release study of Ibuprofen-polymer conjugate was performed in phosphate buffer PH 7.4 using a roller (Stuart STR 1) placed in an incubator (Stuart SI 60) and the temperature was kept constant at 37 ± 1ºC. Among the three polymers, glycerol-adipate-co-pentadecalactone was observed to give a burst release following slow release in the medium. These characteristics suggest that these polymers can be successfully used in sustained release drug formulations.

Keywords: Polymer synthesis, ibuprofen, conjugation, encapsulation, *in vitro* release patterns.

INTRODUCTION

Polymer is derived from two Greek words, *poly* means many and *meros* meaning part, so polymer is large molecule consisting of many small parts. Polymers may be natural or synthetic in nature. The natural polymers include albumin and gelatin etc. and synthetic polymers include poly-lactic acid and ethyl cellulose etc (Jain JA, 2000; Giunchedi P and Conte U, 1995). The polymer of choice in microencapsulation of drug and proteins are the synthetic polymers because of good biocompatibility. These polymers are also non toxic in nature and biodegradable; their degradation occurs within the body and these biodegradable products can be easily eliminated from the body (Watts PJ, 1990). The most commonly used polymer in the process of microencapsulation is poly lactic acid (PLA), hydrophobic in nature having very small capability of wetting, therefore, having low capability of water uptake and degradation (Juni *et al*., 1985). The other commonly used polymer in the process of microencapsulation is PLGA, poly (lactic-co-glycolic acid), which is an amorphous co-polymer of Lactic acid and glycolic acid. Its degradation is faster than the two polymers do separately when it is in amorphous form (Jain *et al*., 2000). The aliphatic polymer, poly caprolactone (PCL), which is semi-crystalline, is also widely used in controlled release dosage forms (Sinha VR, 2004; Endelberg I and Kohan J, 1991; Pitt CG, 1990). However, PCL have comparatively low melting point as that of PLA and PLGA, so it is in a semisolid form at room temperature. The degradation of PCL occurs via an autocatalytic bulk degradation process (Yoo HS, 2000; Endelberg I and Kohan J, 1991). PLA and PLGA produce an acidic environment on degradation except PLA which does not produce acidic environment on degradation. However, the degradation process of PLA is very slow because of its low affinity for water; therefore, it is used commonly in controlled release drug delivery systems that require control release over extended periods of up to one year (Endelberg I and Kohan J, 1991). The release of drug can be controlled by fine tuning drug-polymer interactions. Drug delivery systems of a novel polymeric material with variable properties can be effectively used in a wide variety of applications. Coupling or conjugation is a chemical linkage between a chemically active agent and backbone of a suitable polymer so as to control drug release rate. The delivery of cytotoxics is made by drug-polymer conjugates, by making use of water soluble polymers such as polyethylene glycol (Khandare J and Minko T, 2006; Duncan R, 2003; Brigger *et al*., 2002; Putnam D and Kopecec J, 1995). We also have synthesized many polymers in our laboratories in UK and Pakistan and used the first three polymers in sustained release particles. These polymers showed very good results in these formulations which can be investigated further for the use in future C.R. formulations.

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MATERIALS AND METHODS

Materials

Polyvinyl alcohol (PVA), Pentadecalactone, Glycerol and

Table 1: Formulation of (PGA)

Glycerol (moles)	DVA (moles)	Novozyme (gm)	Solvent (ml)	GPC (kDa)
0.05 mol 4.60 gm	0.05 mol 9.91 gm	0.5 gm	20 ml	11800.0

Note: Here half of the quantities were used.

Table 2: Formulation of (PGA-co-caprolactone)

Glycerol (moles)	DVA moles)	Caprolact One (moles)	Novozy Me(gm)	Solvent (ml)	GPC (kDa)
0.104 mol 11.98 gm	0.104 mol 25.81 gm	0.104 mol 14.85 gm	1.0 gm	20 ml	43438.5

Table 3: Formulation of (PGA-co-pentadecalactone)

Glycerol (moles)	DVA (moles)	Pentadecalactone (moles)	Novozyme(gm)	Solvent (ml)	GPC (kDa)
0.104 mol 9.55 gm	0.104 mol 20.50 gm	0.104 mol 24.90 gm	1.0 gm	20 ml	13137.0

Table 4: Formulation of the conjugation of Ibuprofen with polymer PGA-co-PDL

Ibuprofen (moles)	DCM dry	Polymer (moles)	DMAP (moles)	GPC (kDa)
11mmole 2.266 gm	150+50 ml	11 mmole 5.0 gm	1.0 mmole 0.12 gm	15059.0

Novozyme 435 obtained from Sigma Aldrich Chemicals (USA), Divinyl Adipate was obtained from Flurochem (UK), and Tetrahydrofuran (THF) was obtained from BDH (UK), Phosphate buffered saline tablets were obtained from Oxoid (UK), Ibuprofen (Flurochem, UK)

Polymer synthesis

In laboratory three polymers were synthesized by taking help and modification of the known procedures.

1-PGA (Poly glycolate)

As shown in table 1, the first polymer (PGA) was synthesized in laboratory with the help of enzyme catalyzed procedure. A two necked round bottom flask (250ml) having a central stirrer guide and an open top condenser were taken in a water bath and the temperature of the water bath was kept constant at 50ºC. Equimolar proportions of divinyl adipate DVA and glycerol was taken in the flask. 15ml of Tetrahydrofuran THF was taken and added to the flask as a solvent. The solution was equilibrated to 50ºC for 20 minutes. 1 gm of Novozyme [enzyme complex in 5 ml THF (15+5 = 20 ml THF)] was added to the solution and the solution was allowed to react for 24 hours. The stirrer in the flask was maintained at a constant stirring speed of 2000 rpm. As a result a viscous liquid was obtained and was poured into 100 ml of dichloromethane/THF and the resultant enzyme was separated by filtration and the solvent was removed by a rotary evaporator, the temperature of which was maintained at constant temperature of 80ºC but was increased up to 100ºC for 30 min in order to denature any free enzyme present.

The other two polymers were synthesized by adopting the same procedure with some additions.

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2-PGA-co-caprolactone

As given in table 2, the second polymer (PGA-co-CL) was synthesized by adopting the same procedure as for (PGA), but with the addition of caprolactone.

3-PGA-co-pentadecalactone

As shown in table 3, the third polymer (PGA-co-PDL) was also synthesized by the same procedure which was used for PGA with the addition of pentadecalactone.

Polymers purification

Each polymer was taken and was added to 100 ml of DCM and 250 ml of methanol. This solution was placed in a plate and was heated until the polymer was dissolved and DCM was removed. After cooling the polymer was collected by filtration and was washed with sufficient amount of methanol. The polymer collected was dried in open air and was stored at room temperature.

Characterization of polymer

The polymers were characterized by Gel Permeation Chromatography (GPC), Differential Scanning Calorimeter (DSC) and NMR spectroscopy. The results for (GPC) were obtained using a viscotek system employing Omni SEC 3 software, TDA Model 300 at a constant flow rate of 1 ml/min, using THF as an eluent. For NMR spectrum a Bruker Avance 300 MHz spectrometer operating via XWING-NMR v 3.5 was used. The results of DSC were obtained using a TA instrument Q 1000 Differential Scanning Calorimeter.

Coupling/Conjugation
Only one polymer (PGA-co-PDL) was selected for conjugation studies with the active drug. The resultant polymer-drug conjugate showed higher molecular weight. For conjugation process Ibuprofen and all the other ingredients given in table 4, were taken in a 250 ml flask and were stirred with the help of a magnetic stirrer at a constant temperature of 4ºC for three hours (table 4).

Microsphere/Particle preparation
For microsphere preparation Emulsion Solvent Evaporation method (ESE) was used. Polyvinyl alcohol (PVA) and distilled water was mixed in a 160 ml beaker to form an aqueous solution. In another 10 ml beaker polymer solution was prepared by dissolving the polymer in dichloromethane. The aqueous and the polymer solutions were mixed together to form an emulsion.

Scanning Electron Microscopy (SEM)
The synthesized microspheres were mounted on aluminum stubs and then were coated with gold-palladium mixture using a sputter coater. Samples were taken and were scanned using a Jeol JSM-840 scanning electron microscope. Micrographs of each sample were taken at various magnification ranges in order to study morphology and to determine the particle size and mean diameter.

Encapsulation
For encapsulation purpose 300 mg of polymer and 30 mg of drug (P: D ratio of 10:1) were weighed with the help of electrical balance and dissolved in 5 ml DCM (organic phase). The mixer which was to be used was first rinsed with distilled water and was then immersed in 160 ml of 0.2% PVA solution. This mixture was stirred for 2 hours until the complete evaporation of the organic phase. The remaining solution was filtered through membrane filter and the absorbance of Ibuprofen was measured at 273nm with the help of UV-visible spectrophotometer. The particles which remained on the filter were scrapped off and dried overnight under vacuum. For the release studies four different particles were selected and prepared.

a. Blank particles of the polymer (PGA-co-PDL) and their coupling studies
PGA-co-Pentadecalactone = (1)
PGA-co-Pentadecalactone + Ibuprofen (conjugate) = (2)
These two particles were further encapsulated with Ibuprofen and are given below.

b. Ibuprofen encapsulated particles
1. Ibuprofen + 1 = (3)
2. Ibuprofen + 2 = (4)

Release studies of Ibuprofen from prepared particles
On the basis of encapsulation efficiencies and drug loading capacities, the polymer was selected for the release study. For the release studies, a roller (Stuart STR 1) placed in an incubator (Stuart S1 60) maintained at constant temperature of 37ºC. A solution of the particles was prepared in phosphate buffer PH 7.4 by taking 25 mg of the particles in 25 ml of phosphate buffer. Samples were collected after every hour for the first 6 hours and then every 2 hours for 24-48 hours intervals and were analyzed using UV-Spectrophotometer (Nicolet E300) at 273 nm.

Preparation of UV samples
From the release mixture, 1.5 ml was taken in an eppendorf tube and centrifuged to separate the particles. The supernatant fluid was separated and was transferred to another eppendorf tube and was analyzed using UV spectrophotometer. The remaining particles at the bottom were re suspended with buffer and were then poured back into the mixture for the purpose to maintain the release environment same throughout the intended period.

Method for drug release analysis
The supernatants were collected throughout the experiments and were analyzed using UV Spectro photometer (Nicolet E300) at 273 nm. Ibuprofen concentration in the supernatants was calculated from the absorbance shown by UV Spectrophotometer and was plotted against time. Quartz curvets were used for the drug release analysis.

RESULTS

Polymer synthesis
The synthesized polymers in laboratory were analyzed by GPC and (Viscotek, VE 2001), NMR (Bruker 300) and DSC and it was observed that polymerization had taken place. The molecular weights of the three polymers are given as under:

1. PGA = 11800.0
2. PGA-co- caprolactone = 43438.5
3. PGA-co-pentadecalactone = 13137.0
4. Polymer+ Ibuprofen conjugate = 15059.0

It was concluded from the above information that polymerization had taken place.

Particle preparation
For the particle preparation the experimental conditions were optimized using PGA-co-Pentadecalactone as a test polymer where as the other two polymers mentioned above were also prepared and analyzed. The conjugated particles of Ibuprofen with polymer were prepared at a D: P ratio of 10:1 and were analyzed.

Scanning Electron Microscopy
Scanning Electron Microscope (Joel, JSM-840 Japan) was used for the analysis of all the prepared blank particles of PGA-co-PDL and its coupling with Ibuprofen (1 & 2) and the particles which were encapsulated with Ibuprofen (3 & 4). The pictures of the particles are shown below:

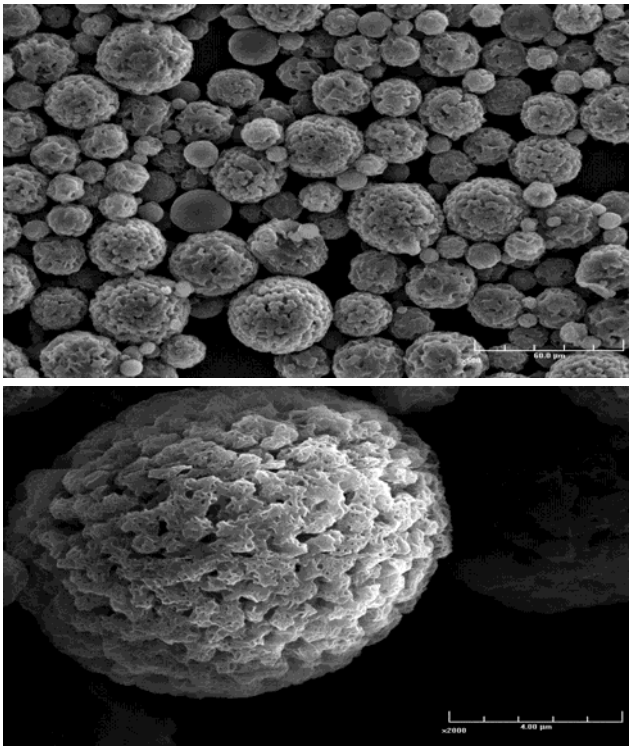

Fig. 1: Scanning electron micrographs of microspheres produced from Polymer PGA-co-PDL taken at ×500 and × 2000 magnifications and the scale bar represent 60µm and 4 µm

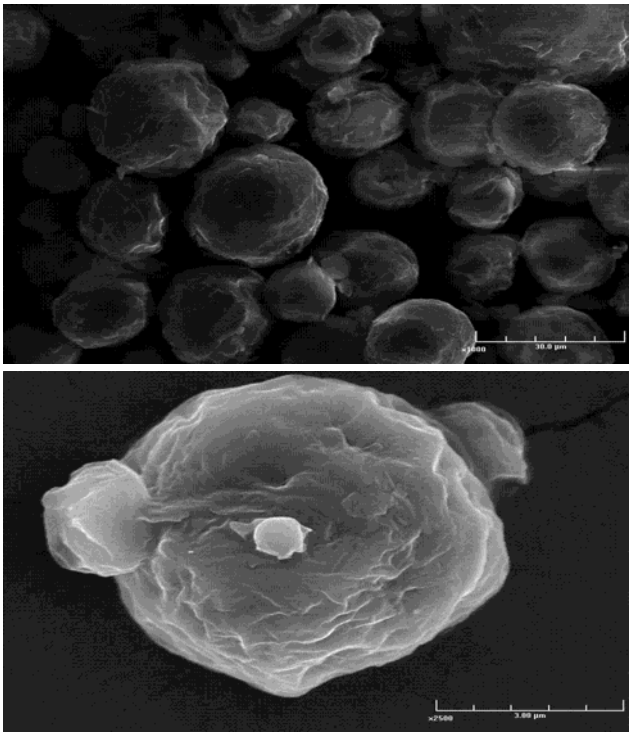

Fig. 2: Scanning electron micrographs of microspheres produced from Polymer PGA-co-PDL+ Ibuprofen conjugate taken at ×1000 & ×2500 magnifications and the scale bar represent 30µm & 3 µm

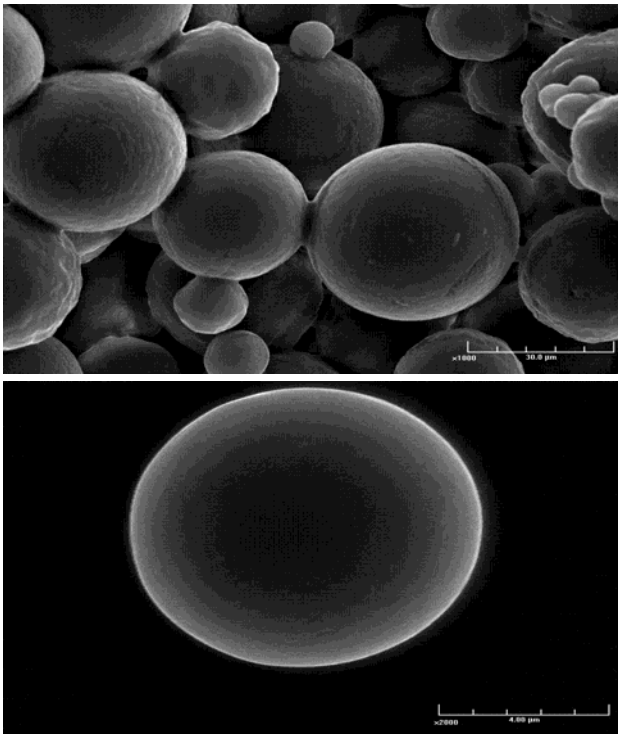

Fig. 3: Scanning electron micrographs of microspheres produced from Polymer PGA-co-PDL+ Ibuprofen encap sulated taken at ×1000 & ×2000 magnifications and the scale bar represent 30µm & 4 µm

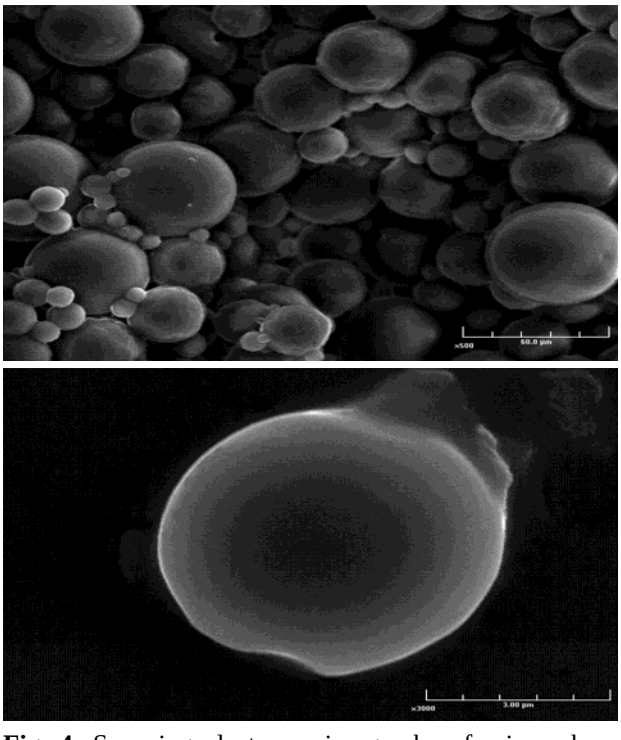

Fig. 4: Scanning electron micrographs of microspheres produced from Polymer PGA-co-PDL+ Ibuprofen + Ibuprofen encapsulated taken at ×500 & ×3000 magnifications and the scale bar represent 60µm and 3 µm.

NMR (Nuclear Magnetic Resonance)

For NMR studies, Bruker Avance 300 MHz spectrometer operating via XWING-NMR v 3.5 was used.

As shown in fig. 5a and 5b, it could be observed that new peaks have appeared at approximately 0.81 ppm, 4.29 ppm, 5.23 ppm and 7.10 ppm in 100% conjugate spectra which were not observed in the spectra for PGA-co-Pentadecalactone. From these observations it can be concluded that conjugation was successful because the appearance of new peaks are associated with Ibuprofen conjugation to the -OH group of polymer. From these spectra when the integration numbers were used to calculate the percentage of –OH groups taken by the conjugation, it was concluded that the process of conjugation was incomplete. In conjugation the percentage of –OH groups was calculated by dividing the integration number of peak by 0.81 ppm. This low efficiency of conjugation could be due to the degradation of the material during in proper storage and during the conjugation synthesis process.

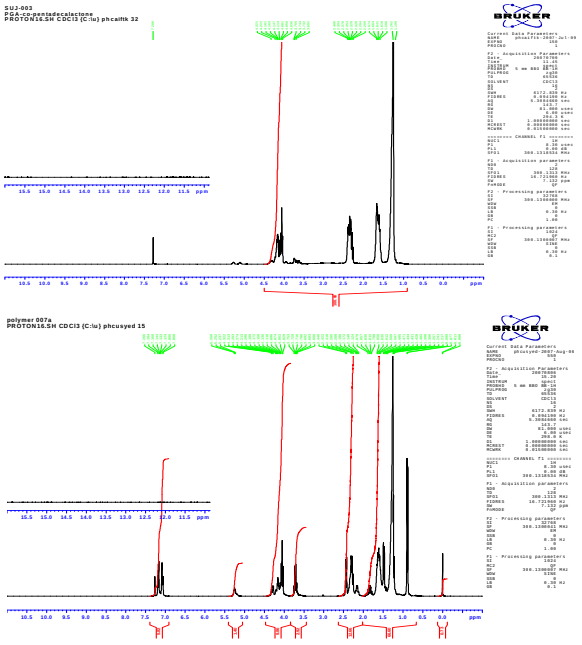

Fig. 5: (a) NMR spectra of PGA-co - pentadecalactone (b) Co-PDL+ Ibuprofen Conjugate.

DSC thermo grams

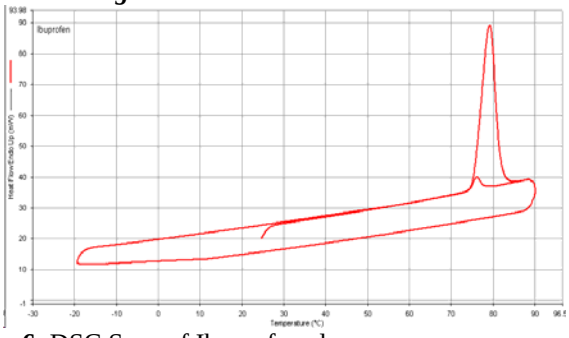

Fig. 6: DSC Scan of Ibuprofen alone.

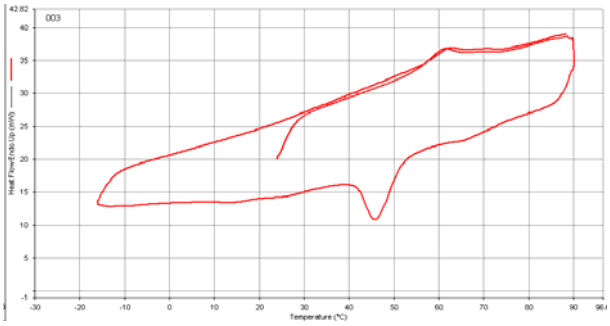

Fig. 7: DSC Scan of Polymer PGA-co-PDL

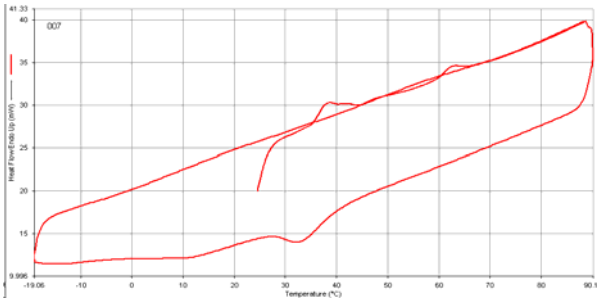

Fig. 8: DSC Scan of Polymer PGA-co-PDL + Ibuprofen conjugate.

Release studies

For all four particles, release studies were carried out. These investigative studies showed that the particles with conjugated Ibuprofen could be used for sustained release drug formulations. The drug release results of the performed studies are given below:

Release study for particle 3

It was observed that there was an initial burst release followed by a period of sustained release from PGA-Co-PDL + Ibuprofen conjugate for 24 hours but no release from PGA-Co-PDL alone.

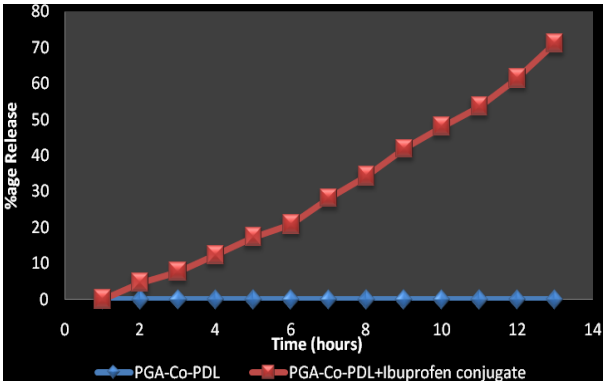

Fig. 9: *In vitro* release of PGA-co-Pentadecalactone and PGA-co- Pentadecalactone + Ibuprofen conjugate (D:P 10:1)

DISCUSSION

Polymer synthesis

In an attempt to make more versatile drug carriers, a

range of novel polymers were synthesized. The main repeating unit of these polymers consisted of glycerol and adipic acid. In the current study, copolymerization of two biological monomers was effected by using a hydrolytic enzyme to produce two polyesters. For this polymer rization reaction the hydrolytic enzyme chosen had a regio-selectivity for primary hydroxyl groups. From previous studies (Kline BJ *et al*., 1998), it could be observed that there are very little or no errors found using the supported enzymes. Linear polyester with one free secondary hydroxyl group per polymer repeat unit was obtained when using glycerol as one of the monomer. To achieve proper polymerization, mechanical stirrers were used to facilitate continuous mixing of the reagents and optimum temperature for polymerization was maintained using water baths. The hydrolytic enzyme was removed by simple filtration to limit any degradation on storage.

Release mechanism

It was observed that there was an initial burst release followed by a period of sustained release.

The burst release may be due to the presence of non-bonded Ibuprofen or too low molecular weight monomers which were solubilized in contact with the buffer (Oh *et al*., 1999) but the DSC studies showed no evidence of non bonded drug. The slow release followed by burst release could also be due to the chemical cleavage of the polymer back bone. As ester linkages were slowly hydrolyzed more monomers were formed which were then solubilized when they reach a low enough molecular weight (Oh *et al*., 1999). Samples were taken at specific time intervals for a period of 24 hours where as in one case for 264 hours where the sustain release remains constant almost for the whole period.

CONCLUSION

A range of novel substituted functionalized polymers were synthesized adopting enzyme-catalyzed synthesis method. These polymers were composed of non toxic biological monomers and can be synthesized in good yield (More than 80% yield in each case) and suitable quantities. The procedure adopted gives the flexibility of synthesizing a variety of polymer backbone molecular weights together with the incorporation of various amounts of drug substituent's through a subsequent modification. These synthesized polymers are capable of self-assemble into well defined particles of small size and high homogeneity having the high efficient ability to entrap the drug. The conjugated Ibuprofen with the polymer PGA-co-pentadecalactone showed good release patterns. Ibuprofen showed a burst release with all formulations/samples, declining with time. The characterization studies of the conjugated polymers strongly suggest that these polymers could be further investigated and could be used successfully in the development of nanoparticulate drug delivery systems.

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