RESEARCH ARTICLE

Stability of Polyanhydrides as Potential Drug Delivery Systems – Effect of Molecular Weight

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Manuscript Info

Abstract

Controlled release of a variety of therapeutic agents is possible through the use of biodegradable polymeric drug delivery systems. Understanding factors that control degradation will be critical to the future development of these materials. The aim of this study was to gain a greater insight into the role of initial molecular weight on the degradation characteristics of polyanhydrides in nonaqueous solvents. Copolymers of poly 1,3-bis(*p*-carboxyphenoxy) propane (CPP) and sebasic acid (SA) with a molecular ratio of 20:80 were synthesised by melt polycondensation of mixed anhydrides of diacids and acetic acid using the method described by Domb and Langer. A series of copolymers were synthesised with molecular weights of 78000, 52000, 34000 and 26000.

Static light scattering and gel permeation chromatography (GPC) were used to assess the effect of polymer initial molecular weight on the degradation characteristics on polyanhydride copolymer composed of -bis(*p*-carboxyphenoxy) propane (CPP) and sebasic acid (SA) in organic solvent. The degradation profile characteristically displays an increase in the rate of M_W reduction with increase of the polymer initial molecular weight. The degradation profiles for polymers with higher initial molecular weight is biphasic and after an initial burst the degradation rate of polymers follows first order kinetics.

Introduction

Biodegradable polyanhydrides have been used in the fabrication of controlled drug delivery devices (Leong et al., 1985; Reboucas et al., 2012). Such devices may exhibit surface erosion resulting in zero-order release of incorporated materials. It is the hydrolytic instability of the anhydride linkage which renders polyanhydrides attractive as biodegradable drug-carrier matrices. The water liable anhydride linkage provides the basis for using a variety of backbones and yet ensures biodegradability (Rosen et al., 1988). Polyanhydride degradation is due to the anhydride linkages, as a result of their reactivity toward nucleophilies.

Polyanhydrides based on a variety of aromatic and aliphatic dicarboxylic acids have been developed as bioerodable carrier matrices for controlled delivery application (Paradise, 2012; Carrillo-Conde at al., 2012; Cavez-Santoscoy et al., 2012; Huntimer et al., 2012). Polyanhydrides are sufficiently hydrolytically labile to produce heterogeneous erosion at rates suitable for controlled release, without requiring any additives (Rosen et al., 1983; Ron et al., 1991; Lasen-Deschamps et al., 2012). This group of biodegradable polymers has demonstrated zero-order drug release over several days, a relatively rapid biodegradation, biocompatibility when implanted in the tissue, its degradation products are easily metabolised by the body and are non toxic, and non-immunogenic (Tamada and Langer, 1993; Cuebas et al., 1992; Laurencin et al., 1990).

Polyanhydrides have been shown to possess physical properties that enable device fabrication. They can be designed with relatively low melting points, usually below 100ºC, and are soluble in common organic solvents.

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They are flexible enough before and during degradation so that they do not crumble or fragment during use. They are easy to manufacture at a reasonable cost and are hydrophobic enough so that the drug is released in a predictable and controlled manner (Dogan and Acar, 2013; Domb and Maniar, 1993).

The molecular weight of polymer is an important factor in the degradation process and plays an important role in the overall degradation pattern. It has been reported that the erosion profiles, such as water uptake and the loss of mass, in a polymer with a relatively narrow molecular weight distribution is at first small and then increases sharply, as some critical degree of degradation is achieved. In a polymer with a broader molecular weight distribution this transition is less dramatic (Livshits and Zaikov, 1992).

It has been reported that the erosion profile of polyanhydride devices characteristically displays an induction period during which the erosion rate is relatively slow with initial decrease in molecular weight (D'Emanuele et al., 1992) The induction period in p(CPP:SA) is a period during which the polymer molecular weight decreases rapidly to below a value of approximately 5000. The length of this period depends on the initial molecular weight of polymer, higher initial molecular weight leading to a longer induction period. This molecular weight dependence induction period has been observed during the erosion of the other biodegradable polymers. It has been reported that the erosion profile of copolymers of L-lactic acid, DL-hydroxyisocaproic acid (Fukuzaki et al., 1990), copolymers of hydroxybutyrate-hydroxyvalerate (Holland et al., 1987), polyesters (Wand et al., 1990), and poly(ε-caprolactone) (Pitt, 1990) depend strongly on the molecular weight. In all of these biodegradable polymers a parabolic degradation profile changes into an S-type profile (due to a lag period) as the molecular weight is increased (Lee and Chu, 2007; Torres et al., 2006;, Ben-Shabat et al., 2003; Bedell et al., 2001). It may speculated that the lag period is due to the time required for the polymer molecular weight todecrease below a certain level (Ouimet et al., 2012).

The erosion of polyanhydrides in different media has been studied previously by monitoring changes of the molecular weight of the polymer using gel permeation chromatography (GPC) and viscosity measurements (Domb and Langer, 1989). In the present study we have examined changes in the weight average molecular weight (M_W) of p(CPP:SA) 20:80 polymer during degradation using both GPC and static light scattering techniques.

Light scattering is a valuable method for the study and characterization of polymers, and allows the determination of the weight average molecular weight, shape, and dimensions of the particles in the solutions, and also is a suitable method for the determination of thermodynamic quantities such as viral coefficients, chemical potentials, preferential adsorption coefficients and excess free energies of mixing. Static light scattering allows instantaneous measurements with no significant perturbation of the system, and it permits representative sampling of polydispersed samples because of the large number of particles involved (Katime et al., 1989).

Experimental
Materials
Sebacic acid (99%, Aldrich) was recrystallised in ethanol, p-Hydroxy benzoeic acid (99%, Aldrich) was reccrystallised in water/aceton2 2:1 (v/v), 1,3-Dibromopropane (99%, Aldrich) and freshly opened samples of acetic anhydride (99%, Aldrich) were used to prepare of CPP monomers, CPP pre-polymers and SA pre-polymers.
Copolymers of poly[1,3-bis(p-carboxyphenoxy)propane-sebasic acid] (CPP-SA) with a molar ratio of 20:80 were synthesised by melt polycondensation of mixed anhydrides of diacids and acetic acid using the method describes by Domb and Langer (Domb and Langer, 1989). CPP pre-polymer was mixed with SA pre-polymer in a reaction kettle equipped with a nitrogen inlet and a magnetic stirrer. The reaction kettle was immersed in an oil bath and the reaction temperature maintained at 180°C. After pre-polymer had melted completely, the system was evacuated and the melt polycondensation byproduct (acetic anhydride) was boiled off and trapped using a liquid nitrogen trap. At 15 minute intervals the reaction kettle contents were purged with dry nitrogen gas for 30 seconds to effect mixing of the melt. Polymerization was allowed to continue for 90 minutes, after which the reaction kettle was cooled and the vacuum removed. The polymer was removed from the sample tube and stored at -18°C under dry nitrogen gas. A series of copolymers were synthesised with molecular weights of 78000, 52000, 34000 and 26000, as determined by GPC. HPLC grade chloroform (BDH, 99.8%) was used as solvent in this study. Before use it was vacuum filtered using a 0.45 μm Millipore filter, and degassed ultrasonically (Ney 300 ultrasonic bath).

Methods
Light Scattering Measurements: Static light scattering (intensity light scattering) was carried out using a Malvern PCS100 photometer with vertically polarised incident light of wavelength 488 nm supplied by an Innova 2 Watt argon ion laser.

The laser beam was directed onto the scattering cell by a primary lens. A variable width aperture controlled the intensity of the beam, and beam intensity was adjusted to give a maximum photon count rate of 150 K. an optically designed glass sample cell (Malvern PCS10, Burchard cell) was centrally placed into a water bath thermostated at

25°C by a temperature controller (Malvern Instruments Ltd.). The water in the water bath, was clarified for 15 minutes prior to each experiment by recirculation through a 0.45 μm Millipore filter.

Laser light was focused in the center of the sample cell, and the scattered light was collected by a photomultiplier tube which was mounted on a rotating motorized turntable. The intensities of scattered light were measured at angles between 45°, 90° and 135°. The system was configured to make 100 measurements of photon count rate for each selected measurement angle, each of 0.1 second duration. A mean count rate at each angle for each sample was calculated by a software program supplied by the manufacturers.

Five concentrations of polymer (0.5, 0.75, 1.0, 1.25, 1.50% w/v) were prepared by dissolving in chloroform and filtering twice under positive pressure through 0.22 μm Millipore filters, into the Burchard sample cell. Then the sample cell was placed in the water bath and left to equilibrate for 10 minutes.

If the ratio of light scattered at 45° and 135° was more than 1.10, it is indicate of contamination with dust and large particles, and the solution was reclarified.

Before each test sample measurement, the scattering from a clean benzene sample (previously filtered through a 0,1 μm filter) was measured at 90°. This provides an internal standard for the spectrometer and allowed the test data to be automatically normalized.

The light scattering results were obtained as the ratio of count rate of sample to count rate of benzene at 90° and the molecular weight was calculated. The basis for the analysis of the light scattering data was the Rayleigh-Gans-Debye equation:

$$S_{90} = I - I_s = KcM_w \qquad (eq. 1)$$

Where I is intensity of light scattered from the polymer solution at a scattering angle of 90° relative to that from benzene; I_s is the corresponding value for pure solvent, c is the concentration (in g cm^{-3}) and K is obtained by:

$$K = (\square \ \square^{\square}/N_A \square^{\square} \square \square \ (n_B^2 /R_B) (dn/dc)^2 \qquad (eq. 2)$$

Where N_A is Avogadro's constant, n_B and R_B are the refractive index and Rayleigh ratio of benzene respectively, and dn/dc is the refractive index increment which was measured using an Abbe 60/ED precision refractometer (Bellingham and Stanley, Ltd). The measurements were carried out at intervals of one hour for the first hours and then periodically up to 33 hours of degradation. All experiments repeated for three times.

Gel Permeation Chromatography Measurements: A GPC system consisting of a pump (Waters-Millipore 510), Rheodyne syringe injector valve (model 7010) with a 100 μl loop and a differential refractometer (Waters-Millipore 410) was used in this study. A 0.3×3 cm PL gel column with a particle size of 5μm was used in this study (Polymer Laboratories Ltd.). The column was consisted of 5 μm beads of a cross-linked styrene-divinyl benzene copolymer with mixed pore volumes. A 0.3×3 cm column with identical packing was fitted above the main column. An in-line filter containing a 5 μg element (Waters-Millipore) was used prior to the pre-column, for protection of pre- and main columns. The samples were eluted with chloroform through the column at a flow rate of 1 ml min^{-1}. Toluene was used as an internal marker. The molecular weight of the polymers was determined relative to polystyrene standards (Polymer Laboratories Ltd.). All experiments repeated for three times. Data were analyzed using commercial software (Millennium 2010, Waters-Millipore).

Results and Discussion

The concentration dependence of the light scattering intensity, S_{90}, for polymer solutions with different initial molecular weight are shown in figure 1. Weight average molecular weights were determined from the gradient of these linear plots using equation 1. Figure 2 shows the effect of initial molecular weight on the degradation of polymer determined by light scattering.

The GPC traces obtained for poly(CPP:SA) 20:80 with initial molecular weights of 76000, 52000, 34000 and 26000, injected at different time points (10, 90, 270, 330, 600 minutes after adding solvent), are shown in figure 3. Weight average molecular weights (M_W), were determined from these traces. Figure 4 shows the change of molecular weight of polymers with different initial molecular weight as a function of time.

Figure 4 (from GPC) and 2 (from light scattering) both indicate that the initial rate of decrease of molecular weight (during the first 2-3 hours) increased with an increase in the initial molecular weight of the polymers, i.e, the polymers with higher molecular weights had a faster depolymerisation rate during the first hours of study. These results are very similar to results of previous work which carried out in aqueous media (Whitaker-Brothers and Uhrich, 2004), (Whitaker-Brothers and Uhrich, 2006).

As seen from the GPC chromatogram in figure 3 there is a high molecular weight shoulder on polymer samples with an initial molecular weight greater than approximately 50000. This shoulder has been observed by other groups (Domb and Langer, 1987). The presence of the high molecular weight component may be responsible for the biphasic degradation profile observed for these polymers (Hou et al., 2007).

As it can seen from figure 5 after an initial burst the depolymerisation (molecular weight reduction) rate of polymer follows the first order equation:

$$InM_W = InM^0_W - Kt \qquad \text{(eq. 3)}$$

Where M^0_W is the original molecular weight, K is the first order depolymerisation rate constant, and t is the time during which depolymerisation occurred. As seen from figure 5 the burst effect is reduced as the initial molecular weight of polymer decreases. Other similar study using laser light scattering showed similar results as well (FU and Wu, 2001).
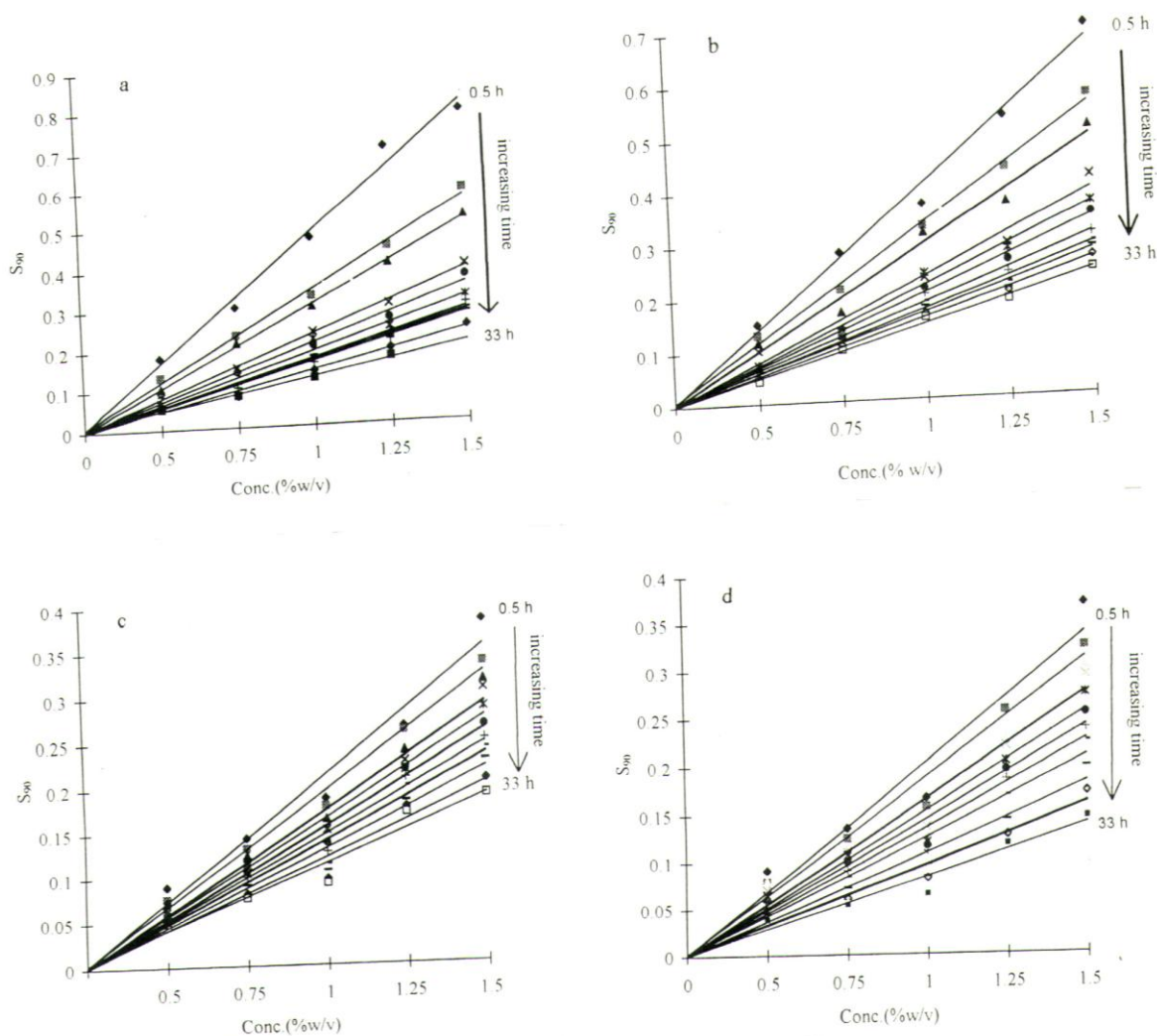
Fig.1. light scattering intensity, S90 against concentration for solutions of P(CPP:SA) 20:80 with initial molecular weights of 78000 (a), 52000 (b), 34000 (c), and 26000 (d) in chloroform as a function of time.
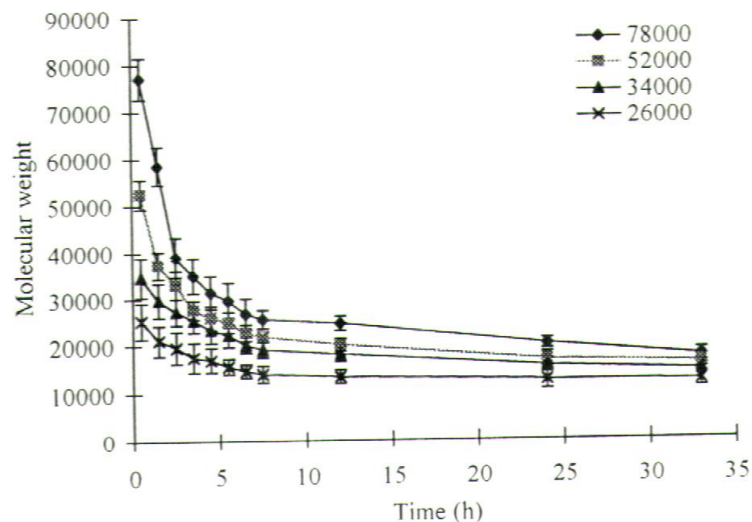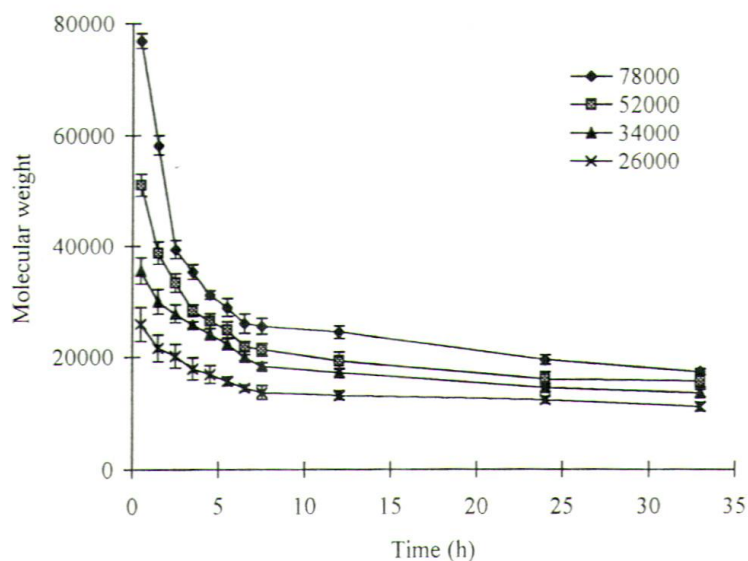
Fig.2. Changes of molecular weight (from static light scattering) of P(CPP:SA) 20:80 with different initial weight average molecular weights in chloroform at 25OC as a function of time after preparation.
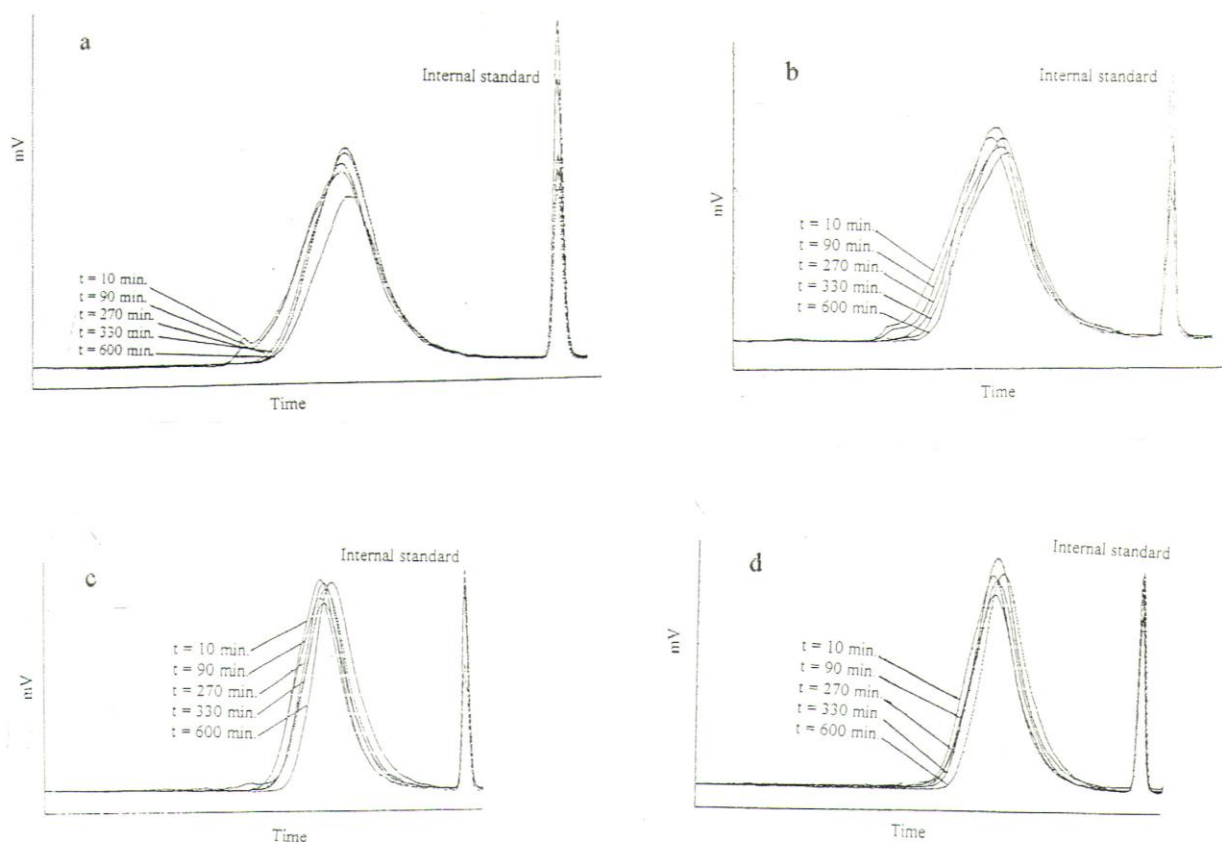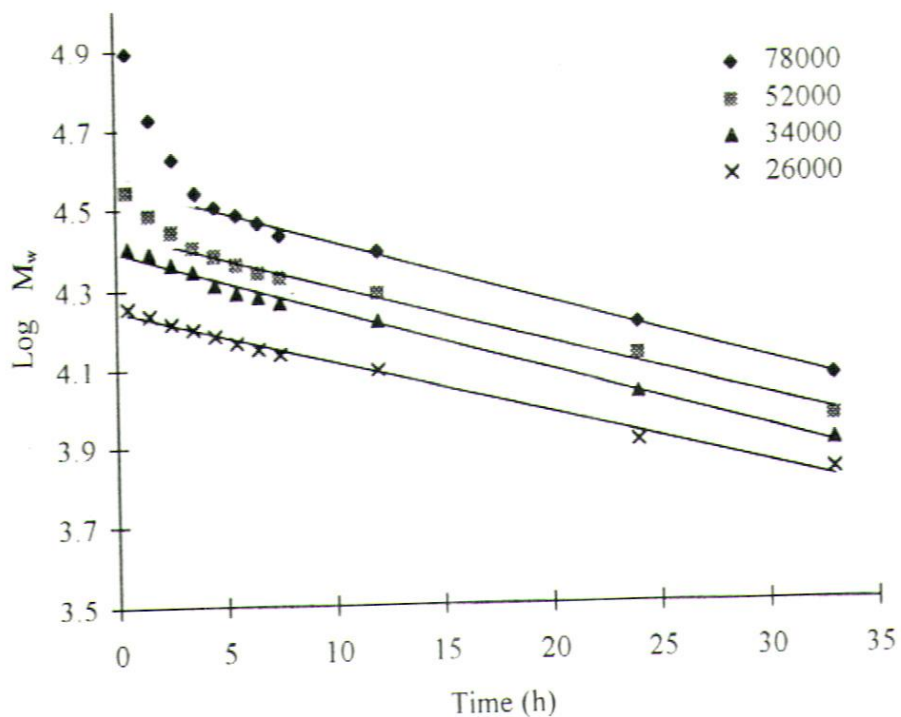

Fig.3 GPC chromatograms of P(CPP:SA) 20:80 with initial molecular weights of 78000 (a), 52000 (b), 34000 (c), and 26000 (d), degrading in chloroform, at the time intervals indicated.
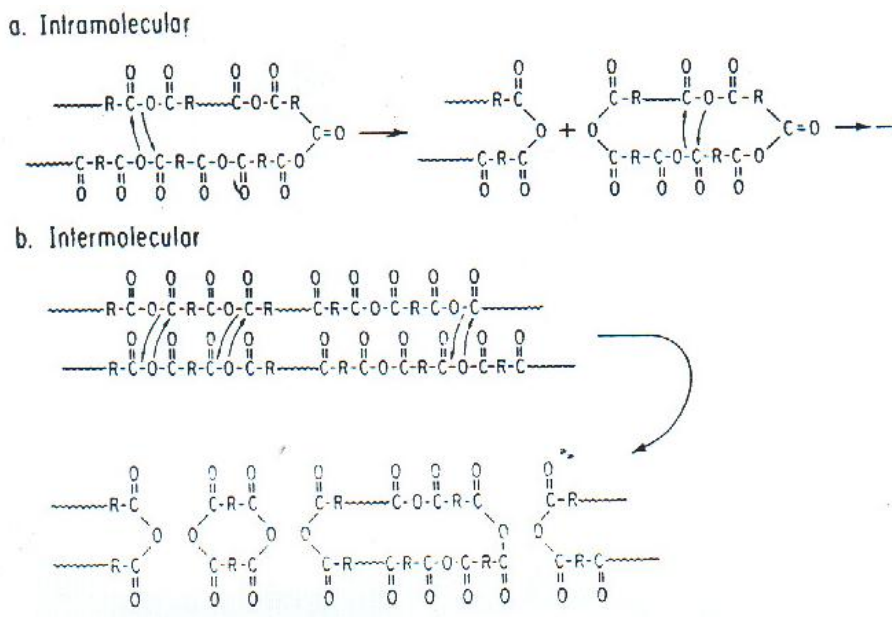
Fig.4 Changes of molecular weight (from GPC) of P(CPP:SA) 20:80 with different initial weight average molecular weights in chloroform at 25OC as a function of time after preparation.

Fig.5 Log of MW of P(CPP:SA) 20:80 against time for polymers with different initial MW, in chloroform as determined by static light scattering.

Fig. 6 Mechanism of the depolymerisation of polyanhydrides.

As it mentioned previously, polyanhydrides are hydrolytically unstable and degrade in the presence of moisture, however studies have shown that the reduction of molecular weight of polymer in organic solvents cannot be a hydrolytic process (Domb and Langer, 1989), (Akbari et al., 1996), (Akbari et al., 1998a), (Akbari et al., 1998b) (Dinarvand et al., 2005). Reversibility of molecular weight of depolymerised polymer in organic solvent, exhibition of no radioactivity of polymer when the polymer solution was mixed with tritiated water, and no increase in depolymerisation rate of polymer in the presence of increasing concentrations of water, suggest a self-depolymerisation process in organic solvents. Domb and Langer (Domb and Langer, 1989) suggested a self-depolymerisation via intermolecular and/or intramolecular anhydride interchange. As it can seen from figure 6, there is a possibility of reaction of the etheric oxygen of one anhydride bond with the carbonyl cabon of another anhydride bond. This process can happen intramolecularly (figure 6a), or it may happen between two molecules (figure 6b) in a chain at several points simultaneously. Since, there is a higher possibility of intermolecular or intramolecular anhydride interchange in longer polymer chains, and then the faster depolymerisation in polymer with higher initial molecular weight can be expected.

Conclusion

Gel permeation chromatography and static light scattering were used to follow the degradation of p(CPP:SA) 20:80 in chloroform and both methods produce similar results. In the case of high molecular weight polymers, molecular weight reduction followed a biphasic pattern. There was a relatively fast molecular weight reduction during the first few hours of the degradation process, after which the rate of molecular weight reduction decreased significantly. The initial phase was associated with depolymerisation of hight molecular weight fractions. A self depolymerisation process in organic solvent, via intermolecular and/or intramolecular anhydride interchange, has been suggested. This mechanism has been supported by the fact that the decrease in molecular weight is reversible and polymerisation of the depolymerised polymer yielded the high molecular weight polymers. Since, there is a higher possibility of intermolecular or intramolecular anhydride interchange in longer polymer chains, and then the faster depolymerisation with higher initial molecular weight can be expected. After the initial burst, the depolymerisation rate of polymer followed the first order kinetics.

In summary, it was revealed that the rate of depolymerisation of the polymers increased with an increase of the initial molecular weight of the polymer. Hence, the initial molecular weight of polymer has crucial role in the depolymerisation rate. This effect needs to be considered in the fabrication of delivery devices.

Future Work
As in this study only chloroform has been used as the solvent, and during polyanhydride drug delivery system fabrication different organic solvents will be used, so the influence of the other solvents on the degradation of these polymers can be done as future work.

Acknowledgements

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