



Citric Acid Based Biodegradable Polyester Elastomers and their Characterization

KEYWORDS

Citric acid; itaconic acid; diethylene glycol; 1, 4 – butanediol; elastomer; polyester.

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ABSTRACT Biodegradable polymers are widely used in medical field such as tissue engineering, drug delivery and gene therapy. In tissue engineering technique, the synthetic biodegradable polymers are used as scaffolds which possess the property of communication with the host tissue and optimum mechanical stability. The present study focussed on the synthesis of two biodegradable elastomers in which citric acid, itaconic acid, diethyleneglycol(P4)/1,4-butanediol(P9) are used as monomers. The synthesized pre-polymers were characterized by FTIR and ¹NMR spectra. Thermal properties of the post-polymer were studied by TGA and DSC, mechanical, swelling and degradation analysis to evaluate elastomeric and biodegradable properties. The results show that both polyesters are elastic and biodegradable. Polymers P4 and P9 displayed an initial modulus of 2.124 and 0.75 MPa and elongation at break between 36.8% and 90 % respectively. This study consistently proved that these elastomers can be used for scaffold formation in future after proving the non toxic nature by cytotoxicity studies.

INTRODUCTION:

Synthetic biodegradable elastomers play an important role in emerging technologies like tissue engineering, drug delivery and gene therapy. Recently biodegradable polymers with elastomeric properties have received much attention due to their use in engineering of soft tissues like blood vessel, heart valves, cartilage, tendon and bladder which possess elastic properties. Most of the biodegradable elastomers developed so far, require costly and complex synthetic procedures which translate into higher manufacturing cost that hinder their commercial and clinical use (Yang et al, 2006; Jeong et al, 2004). Synthetic polymers with less cost of production is essential to meet out the increased demand for tissue engineering. The properties of the scaffolds used for tissue engineering should resemble the extracellular matrix of the tissue or organ to replace and to provide similar properties like communication, swelling and mechanical stability. It has been found that citric acid acts as a prime monomer for the synthesis of poly (1,8-octanediolcitrate) (POC), poly(1,2-propanediol-sabacate) citrate (PPSC) and poly(octamethylenemaleate) citrate (POMC) (Wang et al, 2002; Lijuan Lei et al, 2007; Gyawali et al, 2010). These polymers have been shown highly potential in the application of scaffold formation. Herein, we report the synthesis and characterization of two biodegradable elastomers, P4 made of citric acid, itaconic acid, diethyleneglycol and P9 made of citric acid, itaconic acid, 1,4-butanediol. All the monomers selected for the synthesis of these polymers were already used in the preparation of other biodegradable polymers so that the cytotoxicity was expected to be low.

MATERIALS AND METHODS:

All the chemicals used for the synthesis were purchased from Sigma Aldrich and Biomaterials Bangalore, India.

Synthesis of pre-Polymers

The pre-polymers were synthesized by melt condensation reaction without using any catalyst. Equimolar amounts of acids (citric acid+itaconic acid) and diol (diethylene glycol/1,4-butane diol) were added to a 250 ml three-necked round-bottom flask fitted with an inlet and outlet adapter. The mixture was heated up to 160-165°C with constant stirring for 15 minutes followed by heating at 140-145°C for 3 hrs under the flow of nitrogen. The pre-polymers thus obtained were treated with 1,4-dioxane (20% w/w solution) in order to remove any unreacted monomers and oligomers and used for film preparation. The pre-polymer solutions in 1,4-dioxane were

poured into a clean Teflon petri dishes and kept in an air oven maintained at 80°C for 24 hrs for further esterification. The films thus formed were subjected to mechanical and thermal analysis. The pre-polymers were purified by drop-wise precipitation in de-ionized water followed by freeze-drying (Trans et al, 2010).

Polymer characterization:

Fourier transform infrared (FTIR) spectral analysis

Fourier transform infrared spectra (FTIR) for the pre-polymers were obtained using ABB MB 3000 FT-IR spectrometer at 27°C. 5% pre-polymer solution were prepared in dichloromethane by solution casting technique over KBr crystal and dried for 12 hrs in vacuum hood before being used to obtain the spectra.

Nuclear magnetic resonance (NMR) spectral analysis

The pre-polymers for NMR spectral analysis were purified twice as mentioned earlier and dissolved in CDCl₃ in 5mm outside diameter tube. ¹H NMR spectra for pre-polymers were recorded using BRUKER AV III 500 MHz FT NMR Spectrometer. The chemical shift in ppm for ¹H NMR Spectra were obtained relative to TMS 0.00 ppm as internal reference.

Solubility test

Solubility of the pre-polymers was tested in different solvents.

Thermal analysis: *Thermo gravimetric analysis (TGA) and Differential scanning calorimetry (DSC)*

TGA thermo grams of the post-polymers were obtained at a scanning speed of 10°C min⁻¹ at the range of 30°C to 800°C under the flow of nitrogen gas by using TGA Q 500 V 20.10 Build 36. Differential scanning calorimetric (DSC) thermo grams were recorded in the range of -70 °C to 150 °C using DSC Q 200 V23.10 Build 79 at a heating rate of 10°C min⁻¹ under nitrogen gas.

Mechanical properties

The mechanical property of polyester films were measured using the UTM equipped with 500N load cell. The dog bone shaped polymer film strips were cut according to ASTM standard (45x5x0.2mm, length x width x thickness) and pulled at a strained rate of 10 mm min⁻¹. Values obtained were used to construct stress strain curve. Young's modulus were calculated from the initial slope of the curve.

Swelling property

The % swelling and the sol content of the polymers were measured in DMSO. Cylindrical disc of about 7cm were cut using cork borer from un-purified cross linked polymer film. The discs were pre-weighed to know the initial mass (W_0) and suspended in DMSO. The films were removed from DMSO at different time intervals blotted dry with filter paper and weighed (W_1). The discs were suspended in de-ionized water for 24 hrs to exchange DMSO and lyophilized for 74 hrs. The dried samples were weighed to find the dry mass (W_2). The swelling percentage was calculated using the formula (Tanodekaew et al, 2006).

$$\text{Swelling (\%)} = [(W_1 - W_2) / W_2] \times 100$$

The sol-gel fraction was calculated using the formula

$$\text{Sol\%} = [(W_0 - W_2) / W_2] \times 100$$

In vitro degradation

Hydrolytic degradation is examined by measuring the percentage degradation in 0.1M NaOH. Sodium hydroxide was chosen to find out the degradation in a relatively short period of time. Disc-shaped polyester films were weighed and placed in a tube containing 15ml of 0.1M NaOH. These tubes were incubated at 37°C for pre-determined times. After incubation the films were washed with water and vacuum dried for 1 week. The loss in mass percentage was calculated by comparing the initial mass (W_i) with the mass measured at a given point of time (W_t), using the formula $[(W_0 - W_t) / W_0] \times 100$.

Results and discussion

The pre-polymer P4 and P9 were prepared through initial melt condensation reaction between citric acid, itaconic acid, diethyleneglycol/1,4-butanediol. Both the pre-polymers were soluble in 1,4-dioxane, di-methylsulphoxide, tetrahydrofuran, chloroform, dichloromethane and acetone.

Polymer characterization

The FTIR spectrums of the synthesized pre-polymers (Fig 1a and 1b) show an intense absorption band around 1733cm^{-1} proves the presence of carbonyl stretching vibrations ($\text{C}=\text{O}$) of ester groups and thus confirmed the formation polyesters. (Song and sung, 1995, Pasupuleti et al, 2011, Lei et al, 2007). The bands centered around 2951 , 2961cm^{-1} assigned to methylene ($-\text{CH}_2-$) group of acids and diols (Brioude et al, 2007). Broad band around 3404 , 3474cm^{-1} was assigned to hydrogen bonded hydroxyl and carboxyl groups (Xie et al, 2000, Shaker et al, 2010). The absorption band around 1636 , 1638cm^{-1} shows the incorporation of ($\text{C}=\text{C}$) contributed by itaconic acid in the polyester (Shaker et al, 2010, Gyawali et al, 2010, Kaihara et al, 2008).

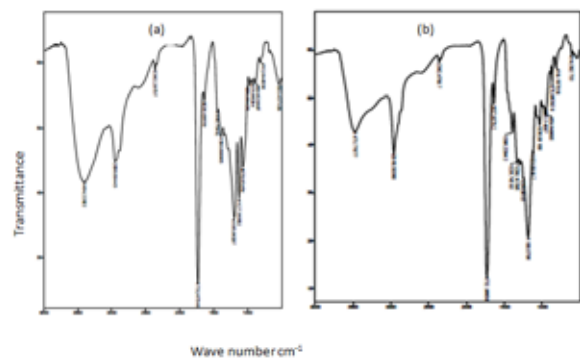


Figure 1: FTIR spectra of P4 (a) and P9 (b)

The ^1H NMR Spectra of the synthesized pre-polymer (Fig 2a and 2b) confirms the resonance of various hydrogens in the polymer back-bone. The peaks located around 2.8 ppm and

4.1 ppm were assigned to $-\text{CH}_2-$ and alcoholic $-\text{OH}$ -group of citric acid (Yangetal, 2006, Djordjevic et al, 2009, Pasupuleti et al, 2011). The peaks located around 1.2 and 1.7 ppm were assigned to $-\text{CH}_2-$ of acids and diols. The peak at 3.6 ppm was assigned to $-\text{OCH}_2-\text{CH}_2-$ of diol (Yang et al, 2006). The presence of alkene peak around 6.4 and 7.2 shows the presence of itaconic acid incorporated in the pre-polymer (Tran et al, 2010).

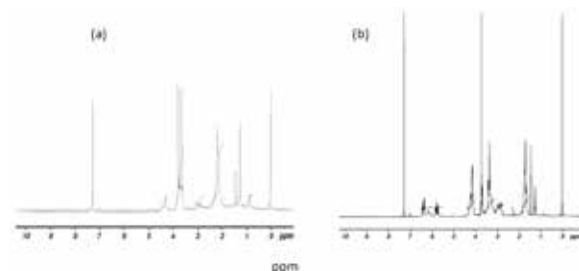


Figure 2: ^1H NMR spectra of P4 (a) and P9 (b)

The mechanical properties of the post-polymers were evaluated. The tensile test on the polymer films show the young's modulus (E) of the polymer film P4 and P9 were 2.124, 0.75 MPa and elongation at break between 36.8 %, 90 % respectively. The mechanical properties of the polymer P4 and P9 are different because of difference in diol monomer used.

Thermal Analysis

The thermal studies show that the synthesized polymers were stable. Fig 3a and 3b reveals the DSC of polymer P4 and P9. The T_g values of polymers P4 and P9 were 10.15°C and 4.27°C which reveals the elastomeric feature.

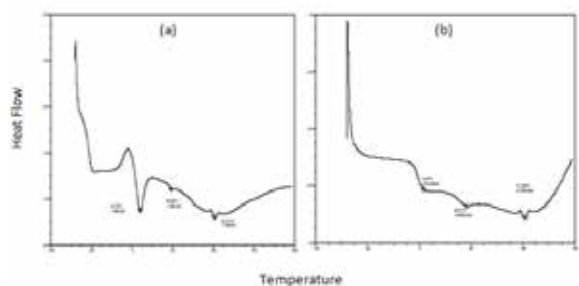


Figure 3: DSC of Polymer P4 (a) and P9 (b)

Swelling Analysis

The equilibrium swelling percentage of the polymers P4 and P9 are 110% and 84%. The sol content for polymer P4 and P9 were 13.3% and 6.7% respectively. The low sol content indicates the successful incorporation of cross link network during post polymerization. The pre-polymers are soluble in DMSO whereas the post-polymers are not, since intermolecular forces and strong hydrogen bonding exist in the post-polymers. This was in agreement with the presence of hydrogen bonded hydroxyl and carboxylic groups as evidenced by FTIR analysis (Djordjevic et al, 2009). The higher swelling for polymer P4 was due to the weakening of intermolecular forces and breaking of hydrogen bonds.

In vitro degradation

The degradation of polymers was confirmed with 0.1M NaOH. Fig 4 shows that polymer P4 degraded at the level of 98% in 6hrs whereas P9 at the level of 100% in 36hrs. This could be due to different monomers of the elastomers.

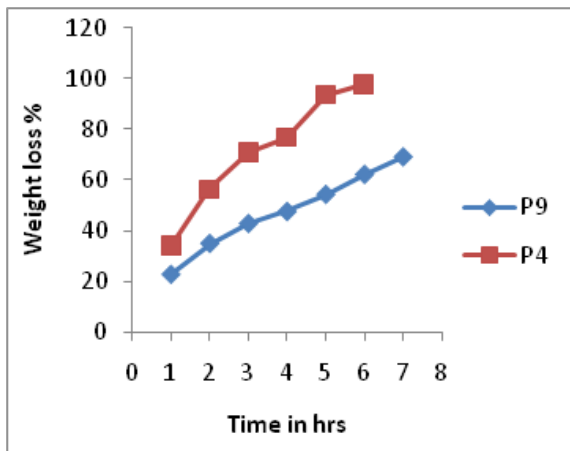


Figure 4: Degradation of P4 and P9.

Conclusion

The biodegradable polyester P4 and P9 were synthesized by melt condensation reaction without using any catalyst. The results of DSC show that the synthesized polymers are elastic in nature. The difference in swelling % and degradation indicates that the choice of monomers (diols) influence the physical properties.

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