Polymerization Kinetics, Biodegradation and pH-Responsive Behavior of Poly[(Citric Acid-co-PEG-400)-Ran-(Glycerol)]

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Abstract: Poly[(citric acid-co-PEG-400)-ran-(glycerol)] was synthesized in a vacuum reaction vessel in presence of p-toluene sulphonic acid as catalyst. Synthesized co-polyester was characterized by its glass transition temperature (T_g), FTIR spectrum, elemental analysis and equilibrium swelling behaviors in water and in ethanol. Polymerization kinetics of the said copolymer was figured out. Biodegradation nature was monitored following simple soil burial test and microbial degradation study using the bacterium *Bacillus subtilis* inoculums. Hydrolytic degradation study reveals that the polymer remains almost intact in acid buffer solution of pH 1.2 but was gradually degraded in phosphate buffer solution of pH 7.4 at 37^0C. Because of having such pH responsive behavior, this co-polyester could be an ideal future candidate for the site targeted drug delivery medium.

Keywords: Poly[(citric acid-co-PEG-400)-ran-(glycerol)], Biodegradable polymer, Microbial degradation, Hydrolytic degradation, pH responsive behavior.

1. INTRODUCTION

Biodegradable polymers have made tremendous advances over the past 30 years in drug delivery system due to their surface and bulk properties [1-6]. The US Food and Drug Administration (FDA) first approved biodegradable product, Dexon® was a suture introduced in 1970 by Davis and Geck [7, 8]. After two decades in the 1990s, there has been a revolution of biodegradable polymer in the pharmaceutical industry. These improvements contribute to make medical treatment more efficient and to minimize side effects and other types of inconveniences for patients [9,10]. The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity and flow controlling agents in liquids, suspensions and emulsions. Polymers can be used as film coatings to disguise/mask the unpleasant taste of a drug, to enhance drug stability and to modify drug release characteristics [11, 12]. Biodegradable polymers have also been drawn much attention to the researchers because of their known biocompatibility and biodegradability to be used as implants to perform expected long term service. A good number of researchers and academicians have exerted their effort relentlessly to develop non-toxic and suitable biodegradable polymers to be undergone slow hydrolytic and microbial degradation releasing the impregnated materials at controlled rates [13-15].

One of the foremost beauties of synthetic biodegradable polymers is their desired modification considering environmental aspects. Our present research is aimed to develop pH responsive marketable polymers particularly designed to degrade under controlled biological conditions. The monomers of a synthetic biodegradable polymer for medical application should be non-toxic which enhance the possibility of the polymer to be non-toxic [16]. Citric acid, PEG-400 and glycerol have been using in pharmaceutical industries as various excipients [17]. Selecting these monomers we have tried to synthesize biodegradable pH responsive Poly[(citric acid-co-PEG-400)-ran-(glycerol)] (PCPG) which could be a future candidate for site targeted drug delivery. In this article we will report its synthesis, characterization, biodegradation and pH-responsive behavior.

2. EXPERIMENTAL

2.1. Materials

Citric acid, Glycerol, polyethylene glycol-400 and p-toluene sulphonic acid (catalyst) all were analytical reagent grade chemicals from either BDH-chemicals Ltd. England or E. Merck Germany and were used as such.

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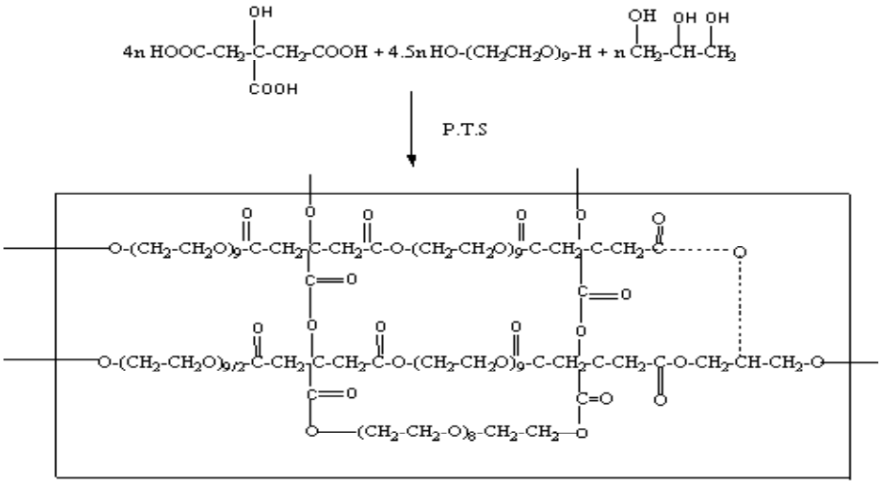
2.2. Synthesis of the Polymer

Citric acid, Glycerol, poly-ethylene glycol-400 (in acid to alcohol stoichiometric proportion) and p-toluene sulphonic acid as catalyst (approximately 0.4% of total weight) were taken in a 50 ml beaker which was placed in a reaction vessel under vacuum for eliminating water as by product. The reaction mixture was heated at 170-180^{0}c for 4-5 hours, when the elimination of water subsided, the mixture was heated for an additional hour under the same condition. The solid polymer was then collected from the reaction vessel. Three different types of polymers (I, II, III) from citric acid and polyethylene glycol-400 along with glycerol (15, 20 and 25% based on hydroxyl groups) were obtained; they were insoluble in water and in other common organic solvents. They were purified by leaching several times with boiling ethanol and then dried in a vacuum oven at 60^{0}c and stored in a vacuum desiccator.



The probable structure of the copolymer would be as follows:

2.3. Polymerization Kinetics

Kinetics of catalyzed and non catalyzed polymerization was studied. The required amount of citric acid, PEG-400 and Glycerol in -COOH to –OH stoichiometry was taken in a 50 ml beaker (0.4 mole% P-toluene sulphonic acid was used as catalyst for catalyzed reactions). The mixer of citric acid, PEG-400 and Glycerol was mixed with the stirrer. The reaction flask was connected to a vacuum pump for eliminating the byproduct water. The reaction mixture was then heated with a heating mantle at constant temperature. The constant temperature was maintained until bubbling starting first. Thus the varying time with the range of 0 to 120 minutes with an interval of 15 minutes, a number of reactions for 0, 30, 45, 60, 75, 90, 105, 120 minutes duration was carried out. The product in each of the reactions was separately dissolved in afixed volume (100 ml) of absolute alcohol. The un-reacted acid in each of the cases was titrated with 0.1N alcoholic KOH solution using phenolphaliene solution as an indicator and the degree of polymerizations was considered as an indication of progression of polymerization.

2.4. Characterization

Since the co-polymers obtained were initially insoluble in water and in common organic solvents, their characterization by their molecular weights was not possible. They were characterized by their equilibrium swelling behavior in water and ethanol, glass transition temperature (T_g), FTIR-spectrum, elemental analyses, hydrolytic degradation, microbial degradation and soil degradation tests. Equilibrium swelling was measured gravimetrically carefully avoiding any weight loss during the measurement. The elemental analysis for C and H was carried out by standard procedure at C.D.R.I. Lucknow, India.

2.4.1. FTIR-Spectrum

The polymer sample was powered cryogenically and its IR spectrum on KBr pellet was recorded by an IR Spectrometer (Model: IR Prestige21, Shimadzu, Japan).

2.4.2. Determination of T_g

The thermal degradation characteristics of the polymer were determined by thermogravimetry (TG) using a thermal analyzer SDT2960 simultaneous DSC-TGA (USA) instrument. Measurements were carried

out under nitrogen atmosphere, in the temperature range from 25 to 600 °C, at a heating rate of 20 °C min^{-1}. T_g values were taken at the onset of change of slope in the heat capacity versus temperature plot and were determined by extrapolating both the slope to the point of intersection.

2.4.3. Hydrolytic Degradation Test

The Experiment was carried out in buffer solution of pH 1.2 (gastric pH) and in a phosphate buffer solution of pH 7.4 (average pH of the human intestine) at 37^0C (human body temperature). The samples were cut into suitable slices and weighed. The, slices were then placed in 200 ml of the buffer solution at 37^0C. After suitable time interval an aliquot portion of the solvent was taken out, its absorbance was measured and was then returned to the hydrolyzing release medium. An acid is known to absorb near 200 nm as its corresponding ester chromphore. So, an increase in the absorbance of the polyester solution as a function of time at respective wavelength would indicate the degradation of the ester bond. However, no visual or change of pH of polymer immersed pH 1.2 buffer solution was observed indicating its non-degrading nature in acid media.

2.4.4. Microbial Degradation Test

Bacterial degradation of the polymer was studied using the bacterium *Bacillus subtilis*. The salt medium (culture medium: NaCl, 5 gm; K_2HPO_4, 1 gm; $NH_4H_2PO_4$, 1 gm; $MgSO_4$. $7H_2O$ 0.2 gm; made up to 1 litre with distilled water) and the bacterial inoculum was prepared following the standard methods, and the polymer was used as the sole source of carbon. In each of flasks (50 ml), 20 ml of the basal salt solution was added, and in two of them was added 0.2 gm of the polymer sample each. The four flasks with their contents were approximately autoclaved. Then the two flasks, one with the medium and the other with medium and the polymer were inoculated aseptically with 0.1 ml of the inoculum. A number of such sets were taken together to match with the experiment and incubated at 37^0c. The growth of bacteria in the incubated flasks of each set was followed by turbidmetric method in which the measurements of the intensity of the transmitted light attenuated by scattering by the dispersed bacteria in the medium were recorded at a standard wavelength 440 nm. The un-inoculated medium of the corresponding set was used as the reference for all the flasks of the same set and their O.D. were found out, namely R_1 (O.D. of the polymer suspension). R_2 (O.D. of inoculum) and R_3 (O.D. of polymer suspension + inoculum + grown up bacteria). Then the O.D. due to the grown up bacteria was calculated from R_3- (R_1 + R_2) relation.

2.4.5. Soil Degradation Test

Soil degradation test was carried out according to the procedure used by potts *et al.* For this purpose a number of beakers (250 ml) were taken and filled with gray color soil from the garden. The soils in the beakers were blended properly and each of the polymer sample weighing around 0.431 gm was placed in the mid of each of the beakers at a depth of 2 inches. The soils in the beakers were kept constantly wet with water. The laboratory temperature was around $30\text{-}35^0$c during the day-time and $25\text{-}30^0$c in the night-time. The beakers were numbered for each of the samples to have its own identity.

Then after regular intervals (15 days) one of the beakers was taken out and the polymer sample placed in it was found out, washed gently with water to remove soil adhered on its surface and then dried at 60^0c under vacuum until constant weight. Weight loss of the polymer in the soil with respect to time was recorded as a mark of its degradation. Three sets were taken and their average results were chosen for the purpose.

3. RESULTS AND DISCUSSION

All the polymers synthesized from citric acid, glycerol and polyethylene glycol-400 were solid masses, insoluble in water and in common organic solvents, and were, therefore, expected to be sufficiently cross linked.

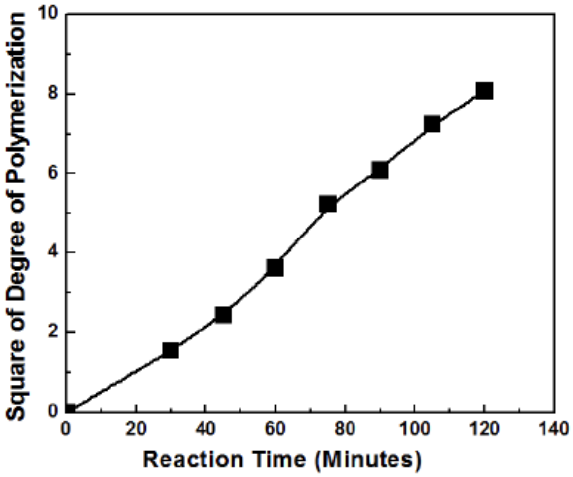
3.1. Polymerization Kinetics

It is clear from the Figure **1** and Figure **2** that the degree of polymerization in acid catalyzed esterification directly proportional to t ½, i. e. degree of polymerization in catalyzed esterification is square time greater than the same in non-catalyzed esterification.

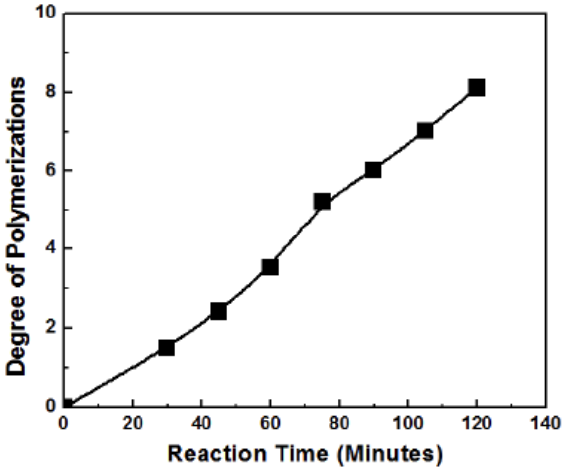
The result obtained in the kinetics study of condensation reactions time in non catalyzed esterification's reaction of citric acid, PEG-400 and Glycerol (% based on hydroxyl group) is in conformity with the results obtained by others [18-21].

3.2. Equilibrium Swelling Behavior

Equilibrium swelling values of the polymers I, II & III in water and ethanol at the ambient temperature are



(**1**): Non-catalyzed reaction kinetics



(**2**): Catalyzed reaction kinetics

Figures 1 and 2: Reaction kinetics of non-catalyzed and catalyzed condensation polymerization of poly[(citric acid-co-PEG-400)-ran-(glycerol)] at 150^{0}C.

shown in Figure **3** and Figure **4**. As can be seen from these figures, the equilibrium swelling values of sample-III in each of the two solvents is the lowest. When the higher percentage of PEG-400 than 75% (% bases on hydroxyl groups), the polymers obtained swell more in each of the two solvents.

The equilibrium swelling values are in the order III < II < I. In a given solvent at a fixed temperature, the extent of swelling for a series of chemically similar cross linked polymers is inversely proportional to the cross-link density in the network. So the swelling values indicate that the sample-III has the highest crosslink density. Again for each polymer sample the equilibrium swelling values in the two solvents are in the order: Water > ethanol. This is probably because of the better solvent property of ethanol than water.

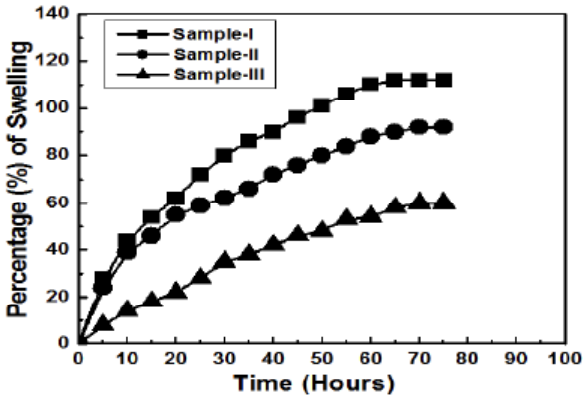
From the above discussion the sample-III seems to have higher crosslink density than other two, therefore, it is selected for subsequent study.

3.2. Elemental Analysis

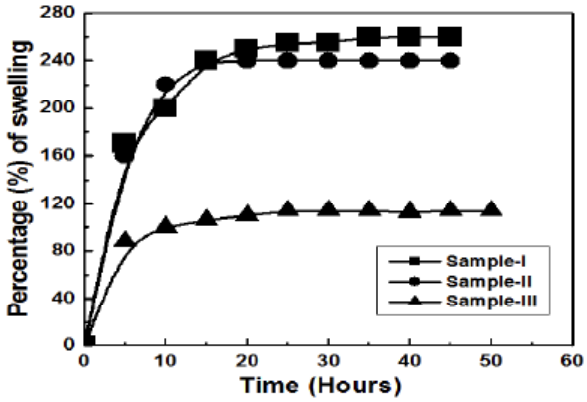
From elemental analysis it is found for sample-III: C = 51.40 %; H = 7.50 % and calculated for expected structure C = 51.61 %; H = 7.28 %. It is seen that the percentage composition of the sample obtained by calculation matches with the same obtained by analysis. The product is accordingly confirmed.

3.3. FTIR Characterization

The broad band representing the -OH group at the region 3100-3400 cm^{-1} in the spectrum of the diol is almost absent in the spectrum of the polymer (Figure **1**). The >C=0 stretching frequency of the



(**3**): Swelling in ethanol



(**4**): Swelling in water

Figures 3 and 4: Swelling behavior of the polymer samples in ethanol and in water.

spectra of citric acid shifted to 1730cm^{-1} and a new band representing the ester linkage appeared at 1275 cm^{-1} in the spectrum of the polymer (Figure **5**). All these indicate the reaction between-OH and -COOH groups forming ester linkages.

3.4. Thermo-gravimetric Analysis (TGA)

Figure **2** shows the TG/DTA curve of the copolymer. The decomposition process consists of three regions. Owing to the initial breakdown of the complex and spontaneous combustion, the first weight loss region is observed at 25–250 ºC for the co-polyester which indicates the evaporation of absorbed water. With the liberation of H_2O and CO_2 providing an oxidizing environment for the combustion of the organic components. The spontaneous combustion is caused from different ions in the sample. The second weight loss region observed at 250-500 ºC. It was ascribed to dehydration of -OH group in the polyester structure that lead to two degradation systems involving both inter and intra-molecular transfer reaction, the oxidation of complexes. The third weight loss region in the temperature range 500– 600 ºC which is believed to be due to the formation of corresponding phase. Above 600 ºC there is no weight loss. From this study, it is seen that the TGA curve is steady, demonstrating the absolute volatility of water, organic compound and the completion of crystallization route. From Figure **6** it is shown that the glass transition temperature of the sample is 54.3 ºC.

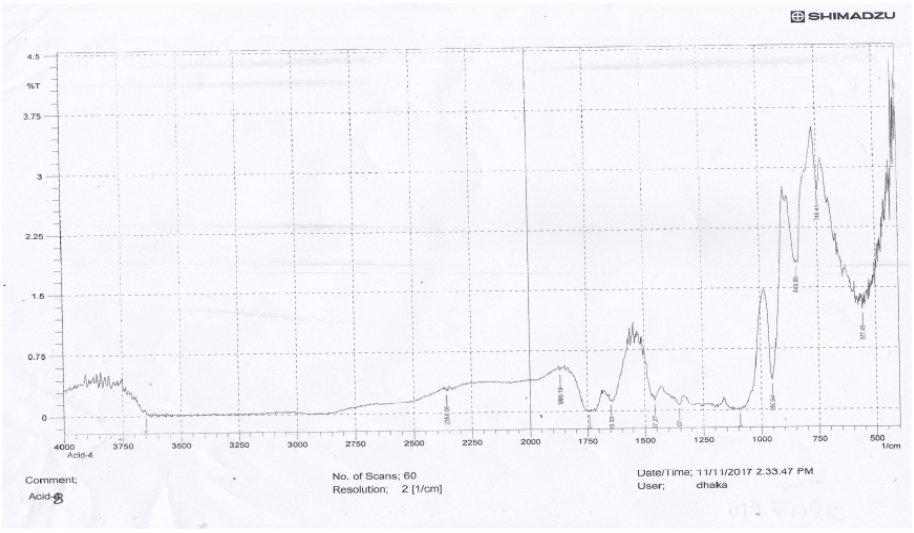


Figure 5: IR-spectrum of poly[(citric acid-co-PEG-400)-ran-(glycerol)]

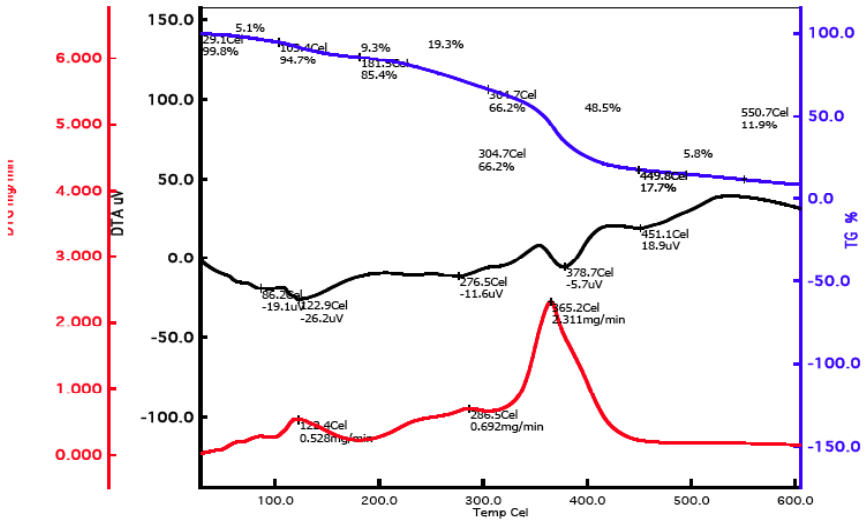


Figure 6: TGA/DTA curve of poly[(citric acid-co-PEG-400)-ran-(glycerol)].

3.5. Hydrolytic Degradation

Hydrolytic degradation of the said polymer is presented in Figure **7** and in Figure **8**. The in-vitro degradation by hydrolysis of ester bonds in the network structure of the polymer samples was expected to produce carboxylate and hydroxyl chromophores as end groups. The carboxylate (coo-) chromophores are known to absorb near 200 nm as ester chromphore. Hence, as the ester bonds are converted into carboxylate groups by hydrolytic degradation and the degraded parts go into solution, a gradual increase in the absorbance of the medium is expected. The absorbance at this λ_{max} (220 nm) goes on increasing continuously up-to 20 days indicating a gradual erosion of the polymer samples by hydrolytic cleavage of ester bonds. It is evident from the Figure **8** that the increase in absorbance at 220 nm was somewhat irregular after 20 days. This may be due to the formation of fungus in the dissolution medium.

3.6. Microbial Degradation

The method used for microbial degradation test depends on the fact that since there is no other carbon source; the bacteria can grow in the medium only at the expense to the polymer and the scattering of light by the medium increases with their increasing population. Thus with the bacterial growth, the transmittance through the medium will decrease and absorbance will increase and, therefore, the absorbance of the medium at a standard wave length (400 nm) with time will provide a measure of the growth of the bacteria vis-a vis the degradation of the polyester sample-III. It is seen from Figure **4** that the growth of *Bacillus sbutilis* here increases rapidly up to 3 days and then the growth become stationary. The rapid increase in the population of the bacteria in the medium up to 3 days may be attributed to the fact that the bacteria multiply at a very rapid rate for a short period of time. But this rate of multiplication of bacteria is not maintained indefinitely owing to the accumulation of toxic metabolic waste product and many of the cells die. The growth rate also increases as the culture ages. A phase is thus reached when the population of the living organisms (bacteria) remains constant *i.e*. the death rate of the organisms equals the rate of their multiplication. Such a phase is known as the maximum stationary phase. As is evident from Figure **9** that after 3 days the stationary growth of the bacteria *Bacillus Subtilis*. In the culture medium containing the polymer sample-III as the only source of carbon indicate and thereby the biodegradability of the polyester sample-III is manifested.

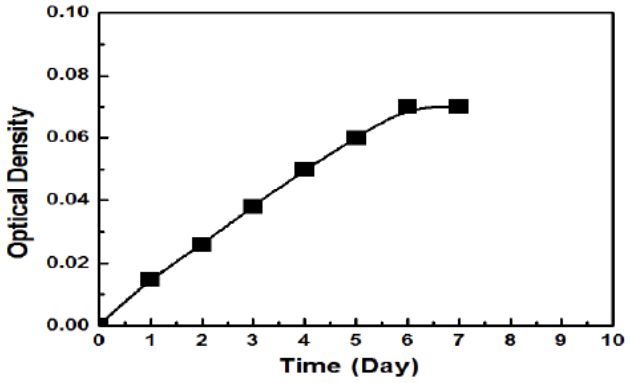


Figure 9: Microbial degradation of poly[(citric acid-co-PEG-400)-ran-(glycerol)] by bactarium *Bacillus subtilis*.

3.7. Soil Burial Test

It is seen from the Figure **10** that the polymer samples lose weight gradually in soil. After 90 days, the

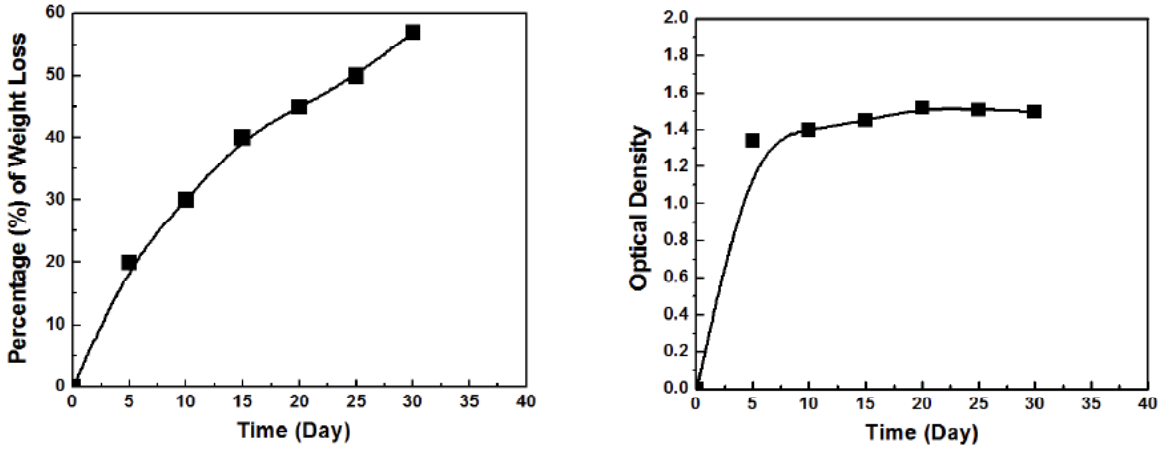


Figure 7 and 8: Hydrolytic degradation of poly[(citric acid-co-PEG-400)-ran-(glycerol)] in phosphate buffer of pH 7.4 at 37^0C.

polymer samples mixed completely with the soil, which indicated the soil degradation of the sample under investigation.

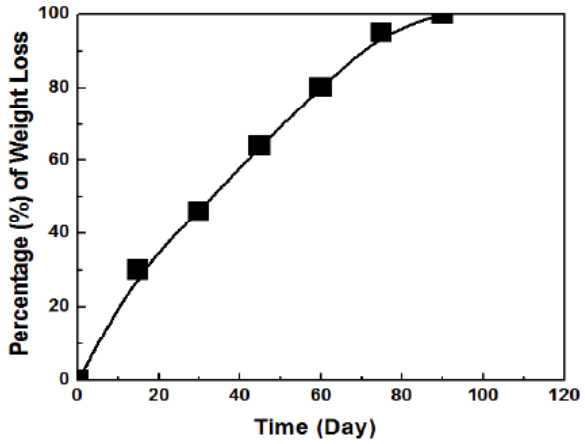


Figure 10: Soil degradation of poly[(citric acid-co-PEG-400)-ran-(glycerol)] at normal weathering condition

CONCLUSION

From the in-vitro degradation studies of the copolymer (hydrolytic, microbial and soil degradation tests), it is clear enough that the copolymers of citric acid and PEG-400 along with glycerol are biodegradable. This co-polymer has a unique property to degrade in basic media but can withstand in acid media. Keeping this in view, it is expected that the samples may be used as carriers for site targeted drug delivery. However, pharmaceutical compatibility of the polymer is yet to be performed.

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