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Synthesis and biodegradation of copolyesters from citric acid and glycerol

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Summary

Seven samples of cross-linked co-polyesters of citric acid and glycerol from seven different mole ratios of the reactants have been synthesised as initially insoluble amorphous solids which become soluble in water within 8–10 days due to partial hydrolysis of the cross-links. They have been characterised by their IR spectra, glass transition temperature and swelling behaviour. The acid to glycerol mole ratios 0.83 and 0.88 produce maximum cross-link density. Microbial degradation of the polymer samples in aqueous suspension has been studied using the fungus Aspergillus niger and the bacterium E. coli. All the polymer samples are degraded by Aspergillus niger and E. coli and the more cross-linked products have been found to be more degradable. The possible use of these cross-linked co-polyesters as matrices for controlled release of drugs has been illustrated.

Introduction

Recently[1-6] there has been an increasing interest for the development of biodegradable polymer matrices for controlled and sustained release of drugs, because such biodegradable carriers have the advantage of eliminating the necessity of their surgical removal. Many of the existing biodegradable carriers are linear polyesters[4-6] such as poly (lactic acid), poly(glycolicacid) and their co-polymers which undergo hydrolytic cleavage of their backbone chains as the drug is released. A cross linked polymer with a three dimensional network is expected to have a better control over the release of the drug from its molecular domain. A common prerequisite for all such polymer matrices is that they must biodegrade to non-toxic products to avoid adverse body reactions. Since citric acid has three carboxyl and one hydroxyl functions and glycerol has three hydroxyl functions and they are known to be benign to body, we have attempted to synthesise sufficiently cross linked copolyesters from citric acid and glycerol designed to degrade under controlled biological conditions. This paper reports their synthesis, characterization and their microbial degradation, and illustrates their drug release behaviour in vitro.

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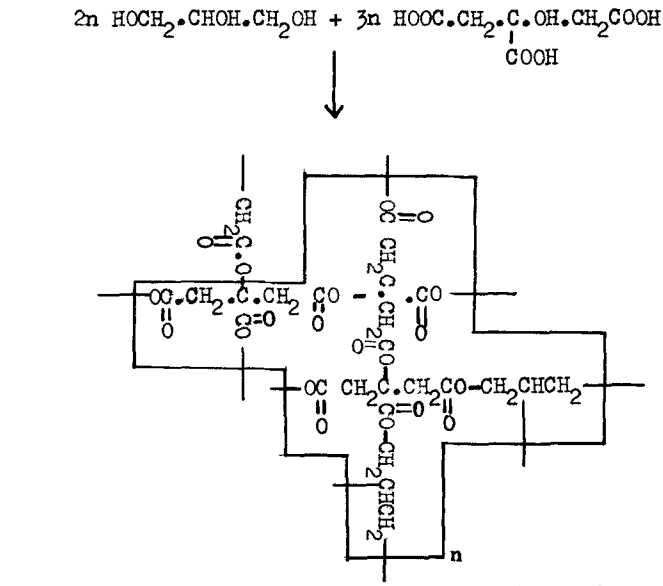
Experimental

Materials : Citric acid, glycerol and p-toluene sulfonic acid(catalyst) were all E. Merck analytical reagent grade products. Glycerol was dried by bubling dry nitrogen gas through it at 120°C.

Synthesis : Citric acid and dry glycerol in desired mole ratios and p-toluene sulfonic acid(approximately 0.8% of total weight) were taken in a 250 ml R.B. flask which was connected to a Dean Stark apparatus for eliminating the by-product water aziotropically with benzene. The reaction mixture was heated at 170°C under nitrogen atmosphere for 12 hrs. When elimination of water subsided, the mixture was heated for an additional 4 hrs under the same condition. The solid polymer was then collected from the reaction vessel and purified by leaching several times with boiling ethanol. Seven different mole ratios (shown in Table I) of citric acid and glycerol were used to obtain seven polymer samples (I-VII) in 85-95% yield as amorphous solids which were insoluble in water and in other common organic solvents. They were dried in a vacuum oven at 40°C and stored in a vacuum desiccator.

If the reaction was stoichiometric the expected structure of the polymer would be as follows.



Characterization : Since the polymers obtained were initially insoluble in water and in common organic solvents, their characterization by their molecular weights was not possible. They were characterised by their IR spectra, glass transition temperature (T_g) and swelling behaviour. The dried polymer samples were chilled and powdered and their IR spectral analysis was carried out on KBr pellets using a Perkin Elmer IR spectrophotometer. The T_g of the polymer samples was measured using a Perkin Elmer Differential Scanning Calorimeter (DSC II) at a heating rate of 20°/min. Equilibrium swelling measurement in dry and purified ethylene carbonate was done by a gravimetric method[7] taking proper care so that no solvent loss takes place during measurement.

Fungal Degradation : Fungal degradation of the polymers was studied using the fungus Aspergillus niger. The basal salt solution was prepared following Czapex Dox Broth and spore suspension according to a standard method[8] in microbiology. The polymer samples were used as the sole source of carbon and were taken in 250 ml Pyrex conical flasks(0.1 g in each case) in triplicate along with an empty flask (blank control). To each of the flasks including the control one 12 ml basal salt solution was added and the polymer suspensions were appropriately autoclaved. The content of each flask was inoculated with 1 ml spore suspension asceptically and the flasks were then stored loosely capped with cotton plug at 31-34°C for 14 days. All the solutions were then filtered and the fungus grown was dried in a vacuum oven at 40°C and weighed.

Bacterial Degradation : Bacterial degradation of the polymer samples was studied using the bacterium E. coli. The high phosphate mineral salt medium [K_2HPO_4, 10.5g; KH_2PO_4,4.5g; $MgSO_4.7H_2O$, 0.102g, $(NH_4)_2SO_4$, 1g; Na-citrate 0.47g made up to 1 litre] and the bacterial inoculum were prepared following standard methods and the polymer samples were used as the sole source of carbon. Each of the polymer samples (0.15g) was taken in a 100 ml Pyrex conical flask in quadruplicate in which 15 ml mineral salt medium was added and the polymer suspensions were appropriately autoclaved. Three samples of each set were inoculated with 1 ml of bacterial inoculum asceptically and incubated at 37°C for 45 hrs. The growth of the bacteria in the inoculated flasks of each set was then measured by a turbidimetric method[9] in which absorbance of the medium at a standard wave length, 440 nm, was recorded taking the uninoculated sample of the corresponding set as reference.

Drug Incorporation and Release : An antihypertensive drug Methyl dopa (3,4-dihydroxy phenyl, 2-methyl alanine) was incorporated in suitably cut slices of the polymer samples by swelling them in a dry ethylene carbonate solution of the drug. The fully swollen polymer slices were then dried in a vacuum oven to a constant weight at 100°C yielding dry translucent amorphous polymer matrices with the drug uniformly dispersed through them. By this simplest procedure only about 5% drug was incorporated. A study of the drug release in vitro was performed by placing a drug loaded polymer slice (6 mm x 4 mm x 3 mm) from sample III in 250 ml phosphate buffer of pH 7.4 at 37°C. After each 24 hrs an aliquot portion of the buffer solution was taken out and its absorbance at 280 nm was measured on a Hitachi spectrophotometer and was then returned to the release medium. Concentrations of the drug released in the buffer solutions were determined from a standard curve constructed by measuring the absorbance at 280 nm of the original drug in the buffer solution at concentrations from 0 to 60 x 10^{-6}moles $litre^{-1}$.

Results and Discussion

Synthesis : In about 4 hrs after the start of the reaction the polymer started separating as a floating solid which grew in size with time upto 12 hrs. All the polymer samples were initially insoluble in water and in all common organic solvents and were therefore, sufficiently cross linked. However, the initially insoluble polymers, if kept suspended in water, become soluble in it within 8-10 days probably due to partial hydrolysis of the cross-links.

Characterization : In the IR spectra of all the copolymers the C = O stretching frequency shifted from 1700 cm^{-1} to 1730 cm^{-1} and a band due

to $C(=O)OR$ appeared at 1190 cm^{-1}. However, the broad band around 3400 cm^{-1} in the original acid due to $C(=O)OH$ and C-OH was found to be much smaller in each of the copolymers. All these indicate the formation of ester bonds. The smaller broad band around 3400 cm^{-1} in the polymer samples is probably due to some unreacted carboxyl and hydroxyl groups.

Table I. Characterization and Biodegradation of Polymers from Citric acid and glycerol

Polymer	Acid/glycerol mole ratio	% of equilibrium swelling	T_g	Weight of the fungi grown in 14 days(mg)	O.D. at 440 nm of the bacteria containing medium after 45 hrs
I	0.75	182	16°C	8.92	0.296
II	0.83	167	21°C	9.11	0.468
III	0.88	169	22°C	9.05	0.457
IV	0.90	190	14°C	8.45	0.286
V	0.95	193	12°C	8.40	0.250
VI	1.10	202	9°C	8.17	0.216
VII	1.50	208	6°C	7.70	0.198

T_g of the polymer samples is shown in Table I. As seen from this table, the samples II and III from acid to glycerol mole ratio 0.83 and 0.88 respectively have the highest T_g. A lower or higher mole ratio of the monomers lowers the T_g which attains a value of 6°C when the mole ratio is 1.50 (the stoichiometric amounts). In a series of chemically similar cross linked polymers the changes in T_g give an estimate[10] of the changes in cross link densities, the two quantities being related linearly. The appreciably low T_g of the polymer(VII) from the stoichiometric amounts of the two monomers and the highest T_g of the polymers from the acid to glycerol mole ratio 0.83 and 0.88 indicate that not all the condensable functional groups in the monomers react completely. It may be inferred from these T_g values that the tertiary carboxyl and hydroxyl of the acid and the secondary hydroxyl of glycerol which are located on positions not very favourable sterically, do not react completely. That there is some amount of unreacted carboxyl and hydroxyl groups in the polymers is supported also by their IR spectra.

Equilibrium swelling of the polymers in dry ethylene carbonate is presented in Table I which shows that swelling is minimum of the polymers II and III prepared from the monomers in the mole ratio 0.83 and 0.88 respectively. When the mole ratio of the monomers is below or above these values, the polymers obtained swell more and the extent of swelling increases with increasing mole ratio of the monomers. In a given solvent at a particular temperature the extent of swelling for a series of chemically similar cross linked polymers is inversely proportional[11] to the cross link density in the network. So these results indicate that the polymers II and III have the highest cross link density which is less in polymers obtained from lower or higher mole ratios of the monomers. The results are in line with those from T_g measurements.

Degradation by Aspergillus niger : All the polymer samples I-VII are capable of supporting the growth of the fungus Aspergillus niger. The results of the degradation of the polymers measured in terms of the weight of the fungus grown are included in Table I. The growth of the

fungus is relatively large in the samples I,II and III and is less in the samples VI and VII. The retarded growth of the fungus and therefore, less fungal degradation in the samples VI and VII is probably because these samples have more unreacted functional groups, majority of which are carboxyl and, therefore, more acidic. The less unreacted carboxyl groups in the cross linked samples II and III and more unreacted hydroxyl functions in the sample I are probably more helpful for the growth of the fungus.

Degradation by E. coli : The method[9] used depends on the fact that,since there is no other carbon source, the bacteria can grow in the medium only at the expense of the polymer and the scattering of light by the medium increases with their increasing population. Thus with bacterial growth,the transmitance through the medium will decrease and absorbance increase and, therefore, absorbance of the medium at a standard wave length, say 440 nm, will provide a measure of the growth of the bacteria vis-a-vis the degradation of the polymers. Table I includes the results of the degradation of the polymers by the bacterium E. coli, expressed in terms of the absorbances of the media at 440 nm after 45 hrs. It shows that all the polymer samples I-VII are used up by the bacteria, the growth of the bacteria being maximum in II and III where the cross link density is highest. The other samples I and IV-VII with less cross links support the growth of the bacteria to much less extent. Very large absorbances of II and III clearly indicate that the cross link densities in the polymers may have some role in providing a favourable condition for the growth of the bacteria.

Drug Release Profile : To illustrate the pattern of drug release from citric acid-glycerol copolymer the release of Methyl dopa from a slice (6 mm x 4 mm x 3 mm) of sample III in phosphate buffer of pH 7.4 at 37°C is shown in Fig.1. The curve A and B show respectively the total release and release rate with time. After an initial burst a constant release rate was obtained upto 6 days after which the rate slowed down. Release was over in 8 days when the polymer slice eroded completely. The zero-order release kinetics suggests drug release by surface erosion and that diffusional release is minimum.

The results show that citric acid-glycerol copolymers do biodegrade under different conditions and suggest that the polymers can be employed as bioerodable matrices for controlled release of drugs at least for short periods. Currently, work is underway in this laboratory to increase the percentage of drug in the matrix by using other methods of drug incorporation, to extend the types of drugs released from the matrix and to examine the synthesis of novel copolymers of citric acid with other naturally occurring polyols.

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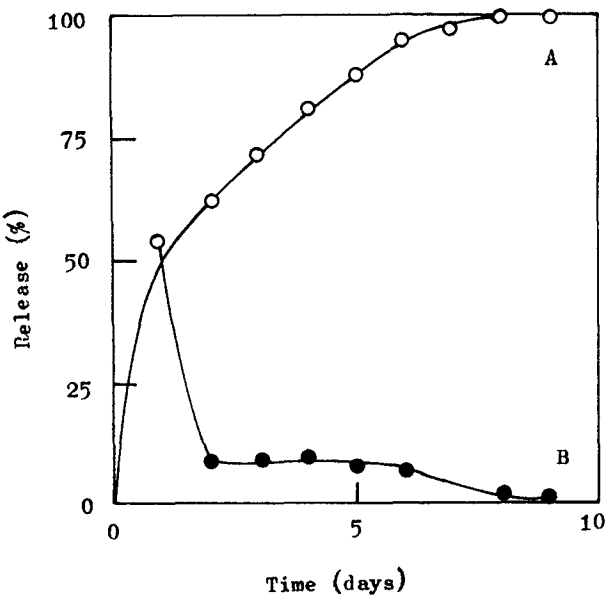

Fig.1. Release of Methyl dopa from a slice (6 mm x 4 mm x 3 mm) of the Polymer sample III in phosphate buffer of pH 7.4 at 37°C.
A : Total release(%) and B : Release rate (%/day).

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