Polymer Bulletin 8, 33–37 (1982)

Polymer Bulletin

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Microbial Susceptibility of Condensation Products of Carboxy-Terminated Polybutadiene and Glycerol*

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Summary

Keeping in view the prospects of biodegradable polymers, a polymer was synthesized by the condensation of carboxy-terminated polybutadiene (CTPB) of $M_n \simeq 5000$ with glycerol and tested for its microbial susceptibility. The results of end group estimations and viscosity measurements indicated a quantitative reaction between the two reactants under experimental conditions. The clear-zone method was employed in this investigation to test biodegradability. Two strains of Serratia and three strains of Staphylococcus did show a clear zone surrounding the colony. However, the microbial growth was found to diminish after 4 or 5 days.

Introduction

Considerable interest is being focused on the development of biodegradable polymers for special applications such as controlled-release drug formulations, insecticide and pesticide carriers, as well as non-toxic surgical implant materials. The modification of synthetic polymers is one of the potential routes outlined for achieving biodegradability.

Copolymerization of a monomer (e.g. ethylene or styrene) with a monomer possessing polar functional groups offers a convenient route to enhance biodegradability. However, attempts to demonstrate the feasibility of this approach have been unsuccessful.[1,2] Huang et al.[3,4] have shown that N-substituted nylons exhibited higher biodegradability than the corresponding unsubstituted polymers.

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* Presented at the International Conference on "Structure-Property Relations of Rubber" held at IIT, Kharagpur (India) Dec. 28-31 (1980)

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0170-0839/82/0008/0033/$ 01.00

Another potential approach to render the synthetic polymers enzyme-susceptible would be to incorporate into the polymeric backbone naturally occurring linkages so that the water-borne enzyme system gains a 'foot-hold' on the polymer surface which, presumably, is a prerequisite of enzymatic action. Such a 'starter' might trigger an enzyme-induction mechanism leading to progressive splitting of C_2-units from the carbon chain.

Keeping in view the widespread distribution of ester linkages among fatty acid esters and the relatively non-specific action of esterases[5], triggering of inductive enzymes of the outlined model by the esterase-producing micro-organisms is not per se unthinkable. Hence, a logical attempt has been made to render the polybutadiene moiety biosusceptible through incorporation of ester linkages by condensation of CTPB with glycerol. Considering different assumptions and interpretations presented in the literature, we have pursued this approach on the basis of the hypothesis that a possible stimulation of biodegradation might proceed with the successive removal of C_2-units from one or both the ends, similar to the well-known β-oxidation of fatty acids.

Experimental

Materials: The carboxy-terminated polybutadiene used for this investigation was Hycar CTPB ($M_n \simeq 5000$) of Goodrich Chemical Company, USA and was purified as reported earlier[6]. All the solvents and chemicals used were pure Analar grade samples. The microbial cultures were obtained from the culture collections of Microbiology and Cell Biology Laboratory, Indian Institute of Science, and routinely maintained on nutrient agar slants.

Preparation of CTPB-Glycerol Condensation Product: The purified CTPB was dissolved in dry benzene and refluxed with excess thionyl chloride for 24 hours under a nitrogen atmosphere. The residual thionyl chloride and the solvent were separated by vacuum distillation. To the diacid chloride, anhydrous glycerol was added dropwise with constant stirring, while stripping off HCl from the reaction mixture by operating at reduced pressure. The reaction was carried out for an hour at 90°C under nitrogen and was stopped before extensive cross-linking took place. The resulting product was repeatedly dissolved in ether and reprecipitated in ethanol to free it from unreacted glycerol. The separated product was vacuum dried for 24 hours at 40°C.

End Group Estimations: The carboxy end groups in CTPB and in the condensation product (Gly-CTPB) were estimated in dry pyridine solvent by titrating against standard sodium methoxide solution. About 0.50 g of polymer was accurately weighed into a conical flask and 50 ml of pyridine and 10 drops of thymol blue indicator were added. The titration was carried out under nitrogen with stirring till the solution turned royal blue. A blank titration was carried out under similar conditions and the above value was corrected. The carboxy

equivalents in terms of equivalents per 100 g of the polymer (EPHR) were calculated. The process was again repeated with Gly-CTPB.

Viscosity Measurements: The intrinsic viscosities of CTPB and Gly-CTPB were measured in toluene at 30°C using an Ubbelode viscometer.

Microbiological Testing: For testing biodegradability, mineral salts-agar medium with the polymer in question as the sole source of carbon was employed[7]. The composition of the medium was $K_2H_2PO_4$, 0.7 g; KH_2PO_4, 0.7 g; $MgSO_4.7H_2O$, 0.7 g; NH_4NO_3, 1.0 g; NaCl 0.005 g; $FeSO_4$, 0.002 g; $ZnSO_4$, 0.002 g; $MnSO_4$, 0.001 g and 15 g of Agar in one litre of distilled water. The test medium was sterilized by autoclaving at 120°C for 15 min. The pH of the medium was adjusted to 6.5 by the addition of 0.01N NaOH solution. The mineral-salt agar was poured into sterilized petridishes and allowed to solidify. The ether solution of the polymer was poured onto the solidified agar surface and a thin layer was formed after total evaporation of the solvent. The plates were then inoculated with the test organisms in an inoculation chamber and incubated at 30°C.

Results and Discussion

The direct condensation reaction between glycerol and CTPB-diacid chloride was necessarily heterogeneous and was carried out with constant stirring with an excess of glycerol. The exact and complete 3:2 molar stoichiometric condensation between the diacid chloride and glycerol should result in a polyunsaturated triglyceride-like structure. However, because of the trifunctional nature of glycerol and difunctional nature of CTPB, the condensation reaction will proceed with extensive branching and an insoluble resin is expected. Therefore, the reaction was stopped well before the stage of cross-linking. The small amount of ether-insoluble products was filtered and removed. The results of end group estimations and viscosity measurements of both CTPB and Gly-CTPB are presented in Table 1.

Table 1

Polymer	EPHR	η
CTPB	0.0285	0.1746 dl/g
Gly-CTPB	0.0060	0.4606 dl/g

It can be seen from Table 1 that $[\eta]$ has increased by 2.6 times of its original value and the carboxy content has decreased by 4.6 times indicating that the reaction has taken place between these reactants under experimental conditions. The product was completely freed from unreacted glycerol before being tested for microbial susceptibility.

The clear-zone method, described by Fields et al.[8], was employed in this investigation to test the microbial action on CTPB and on Gly-CTPB. About 30 esterolytic bacterial strains whose esterate activity was confirmed earlier by the Nile-blue test[9] were inoculated. Pure CTPB was completely resistant to microbial attack. This is in full conformity with the investigations of Tsuchii et al.[10] that oligomers of polybutadiene up to a molecular weight of 500 were assimilated by some strains of Acienobacter and the higher oligomers were not assimilated. The stability of CTPB against enzymatic attack is also expected considering the resistance of natural rubber and other polyisoprenoids to biological oxidation. As mentioned earlier, there is no 'rubberase' in nature as one might expect for a natural polymer and this might be the reason for the non-biodegradability of polybutadiene rubbers.

From Table 2, it is evident that the condensation product promotes the growth of few bacterial strains. After inoculation and incubation, colonies of cells were observed growing on the polymer which indicates that the polymer could act as a source of carbon. Most of these bacterial strains were local isolations from dead silk worms and associated leaves and plants. Many of these were characterized only up to the level of genus in the Microbiology and Cell Biology Laboratory here. It is obvious that one strain of Serratia and three strains of Staphylococcus exhibited prominent clearing around the growth ring ranging from 10 to 30 mm in diameter. From this one can infer that the polymer in the zone was broken down into fragments that are soluble or have been metabolized. This is usually due to a secretion of extracellular enzymes which predigest or breakdown the polymers so that the fragments can be assimilated by the cells[11]. The microbial susceptibility of this polymer is not totally unexpected considering the presence of ester groups and its structural resemblance, polyunsaturated triglycerides which are abundantly found in vegetable oils. The bacterial growth was found to diminish after 4 or 5 days. This means that the source of carbon in these degradation experiments was glycerol which could have been released due to esterolysis. It may also be due to the utilization of low molecular weight oligomers.

Table 2

Results of microbiological tests of Gly-CTPB performed on an agar medium of mineral salts

Sl. No.	Bacterium	No. of strains tested	No. of effective strains
1.	Bacillus sp.	9	-
2.	Serratis sp.	9	2
3.	Staphylococcus sp.	5	3
4.	Micrococcus sp.	2	-
5.	Nocardia sp.	4	-
6.	Proteus vulgaris	1	-
7.	Mycobacterium sp.	1	-
8.	Unidentified Gram-Positive Rods	6	2

Though no definite conclusions can be drawn from these preliminary observations, it is reasonable to expect that the use of low molecular weight CTPB or increasing the hydrophilicity of CTPB by the functionalization of double bonds might improve the enzyme susceptibility of polymers. A combination of esterolytic species and the hydrocarbon-utilizing strains may also result in enhanced biodegradability of this condensation product.

REFERENCES

1. POTTS, J.E., CLENDINNING, R.A., ACKART, W.B., EPA-R2-72-046, U.S. Environmental Production Agency, Washington, (1972)

2. TAYLOR, L.J., CHEM TECH, 548 (1979)

3. HUANT, S.J., et al in Textile and Paper Chemistry and Technology, Author, J.C., ACS, Washington, D.C., Chap. 5 (1977)

4. HUANT, S.J., et al, in Proc. 3rd Internatl. Biodegradation Symposium, SHARPLEY, J.M., and KAPLAN, Eds., Appl. Science Publ., London, p. 731 (1976)

5. DIXON, M., WEBB, E.C., Enzymes, 2nd Ed., Academic Press, New York, p. 194 (1964)

6. McNEILL, I.C., ACHERMAN, L., GUPTA, S.N., J. Polym. Sci., Chem. Ed., 16(9), 2169 (1978)

7. "ASTM Standards Book" Amer. Soci. Philadelphis part 27-D-1924 (1970). p. 653

8. FIELDS, R.D., RODRIGUEZ, F., FINN, R.K., Amer. Chem. Soc., Polym, Prepr., 14, 1244 (1973)

9. NORRIS, J.R., RIBBONS, D.W., EDS., "Methods in Microbiology" 6A, Academic Press, New York p. 39 (1971)

10. TSUCHI, A., SUZUKI, R., TAKAHARA, Agri. Biol. Chem., 42(6), 1217 (1978)

11. PANTKE, M., Biodeterioration Investigation Techniques, WALTERS, A.H. Eds., Applied Science Publ. London, p. 63-64 (1977)

Received March 12, accepted after revision May 4, retyped July 15, 1982