Biodegradation of Gelatin-g-Poly(ethyl Acrylate) Copolymers. II

G. SUDESH KUMAR,* V. KALPAGAM, and U. S. NANDI,†
Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore-560 012, India

Synopsis

Gelatin-*g*-poly(ethyl acrylate) copolymers were prepared in an aqueous medium using $K_2S_2O_8$ initiator. Three copolymer samples with grafting efficiencies of 33.3%, 61.0%, and 84.0% were tested for their microbial susceptibility in a synthetic medium with pure cultures of *Pseudomonas aeruginosa, Bacillus subtilis,* and *Serratia marcescens.* The percent weight losses were recorded over 6 weeks of incubation period in nitrogen-free and nitrogen-rich media. The relationship between [log (rate)] during the first week of the test period and the composition of grafted samples showed a linear behavior. Pure cultures were more effective than the mixed inoculum, although there was no essential difference in the agressivity of different bacterial stains. Growth–time curves and pH measurement also complement these observations.

INTRODUCTION

Considerable interest has been focussed on the synthesis and development of biodegradable polymers for specialized applications, such as controlled-release drug formulations, insecticide and pesticide carriers, and nontoxic surgical sutures and implant materials.[1,2] It should be apparent that the development of biodegradable systems should progress in response to these increasingly clearer definitions of needs for materials with special characteristics.

Natural polymers, like carbohydrates and proteins, although biodegradable, cannot be fabricated easily because they decompose on heating before they melt. On the other hand, most of the synthetic polymers are resistant to enzymatic attack.[3] To overcome this apparent paradox among the existing polyers, several synthetic approaches were formulated and tried with fair success.[1] We have initiated a program on the modification of proteins and their degradative behavior in bacteriological and physiological environment.[4] Earlier, we have reported the bacterial degradation of some gelatin graft copolymers by mixed bacterial inoculum[5] and preliminary results with single cultures.[6] Further ramifications of this investigation are presented here.

* Present address: Department of Chemistry, Bowling Green State University, Bowling Green, Ohio 43403.
† Present address: Room No. 5201, Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201.

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EXPERIMENTAL

Materials

Gelatin, bacteriological (BDH) ($\overline{M}_n$ = 90,000) was used in this investigation. Monomers were purified as reported earlier.[7] $K_2S_2O_8$ (E. Merck) was used as the initiator. The bacterial cultures were obtained from the culture collections of Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, and routinely maintained on nutrient agar slants.[8]

Preparation of Gelatin Graft Copolymers. Gelatin (10 g) was added to 200 mL of water and heated to 40°C in a thermostat under nitrogen till it dissolved. Distilled ethyl acrylate (10 mL) was added to the aqueous solution and the grafting sites were initiated on the protein backbone by the addition of $K_2S_2O_8$ (0.54 g). All the grafting experiments were carried out at required temperatures with constant stirring. The products were collected by filtration and purified as shown in the flow diagram (Fig. 1).

Analysis of Graft Copolymers. Grafted side chains were removed from the protein backbone by the procedure given by Rao et al.[9] The percent grafting and the efficiency of grafting were calculated as reported earlier.[5] The protein content of the graft copolymers was also calculated from total nitrogen content of grafted samples.

Film Preparation. The dry polymer was hot-pressed into film of uniform thickness (2 mm) by compression molding technique at a pressure of 100 kg/cm^2. The dye was maintained at 145–150°C. These films were cut into

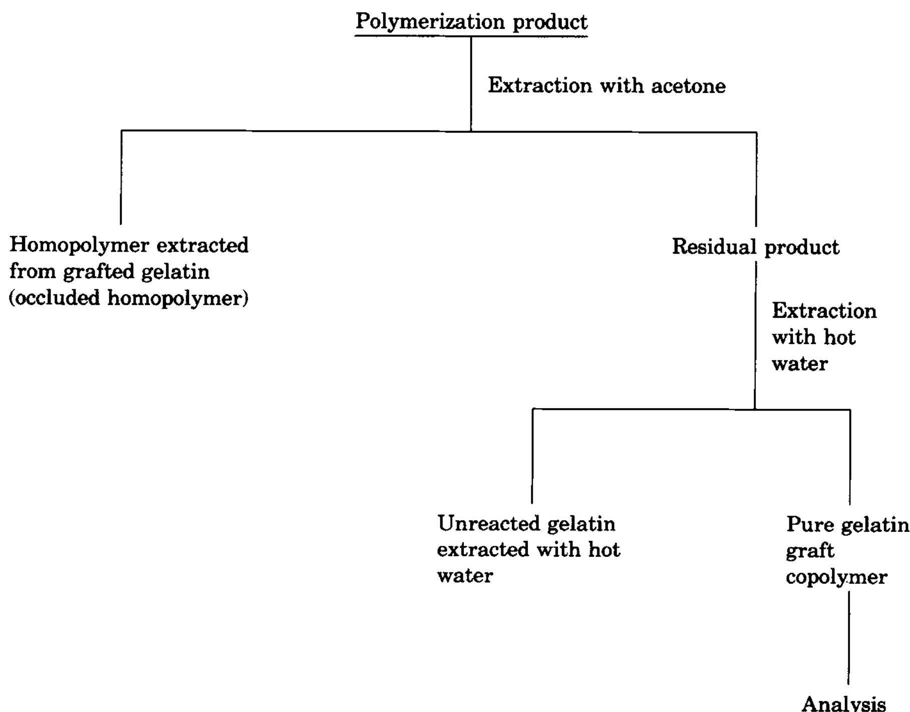

Fig. 1 Flow Diagram of Purification of Gelatin Graft Copolymers by Alternate Solvent Extraction Method.

dumbbell-shaped tensile pieces (6 mm × 8 cm), and tensile strength and percent elongation were measured on a Instron Universal Testing Machine.

Microbiological Testing

The detailed microbiological testing procedures were reported in our earlier publications.[5,6,10,11] In the present investigation bacterial stains, *Pseudomonas aeruginosa, Bacillus subtilis,* and *Serratia marcescens* were used as test organisms. The growth of the bacteria was followed by recording absorbance of the culture medium every 24 h in a Bausch and Lamb Spectronic 20 Calorimeter at 600 nm. pH measurements were carried out periodically on Beckmann digital pH meter.

RESULTS AND DISCUSSION

The detailed data pertaining to the grafting experiments is presented earlier.[6] Evidence for grafting was obtained by IR analysis. Pure gelatin has amide absorption centered around 1660 cm^{-1}. Pure poly(ethyl acrylate) has strong absorption due to ester carbonyl group centered around 1725 cm^{-1}. The IR spectra of the grafted samples showed characteristic absorption bands due to amide groups of gelatin (1660 cm^{-1}) and ester carbonyl groups of poly(ethyl acrylate).

All the graft copolymers were practically insoluble in almost all of organic solvents and hence could not be cast into films by solution casting. The systmatic and intense investigation of physicochemical and rheological properties is beyond the scope of this investigation. However, few general observations were made regarding their film-forming ability, which is of technological importance. The data are presented in Table I. Though these samples exhibited high initial strengths and elongations, their strength was found to decrease rapidly on exposure to moist environment. However, the rapid decrease in strength should not adversely affect the mulch film, since high strength is important only during application. These materials may have applications as degradable plastic mulches.

The presence of limited numbers of —CH(OH) groups, which are responsible for the generation of free-radical sites on gelatin backbone, restricts the selection of graft copolymer samples with widely varying compositions to be tested for microbial susceptibility.[12] This is in contrast to the possibility of a wider selection of grafted samples having carbohydrate molecule as their backbone with "bristling" hydroxyl groups. To investigate the effect of grafting efficiency and the number of grafting sites on the extent of biodegradation, three samples with grafting efficiencies of 33.3% (8.5% N),

TABLE I
Mechanical Behavior of Gelatin-*g*-Poly(ethyl Acrylate) Samples

Sample	Nitrogen (%)	Efficiency of grafting (%)	Tensile strength (psi)	Elongation (%)
GEA	8.5	33.3	1350	120
GEB	7.1	60.9	1020	290
GEC	5.6	84.0	950	350

60.9% (7.1% N), and 84.1% (5.6% N) designated as GEA, GEB, and GEC, respectively, were selected for testing bacterial susceptibility (Table I).

The selection of an appropriate organism in performing the biodegradability test is somewhat dependent upon the objectives of the experiment.[13] In some cases, it is desirable to use a pure culture when the ability of a specific organism to degrade a polymer is in question. If the objective of the study is to determine the precursor to biological attack from all sources, some sort of mixed inoculum is generally preferred. If the end use of the polymer is known, an inoculum derived from the environment in which the polymer may be expected to be exposed is an appropriate choice if the end use is not known; inocula prepared from soil, humus, domestic sewage, or a mixture of known organisms have all proved effective. We have selected *Pseudomonas aeruginosa, Bacillus subtilis,* and *Serratia marcescenes* as test organisms keeping in view their high proteolytic activity and relative abundance in Indian soil.

As in our earlier reports,[5,6] we have followed the percent weight loss as the objective criterion to follow biodegradation. The percent weight loss vs. time-plots are shown in Figures 2–4 obtained with *Pseudomonas* sp., *Bacillus*

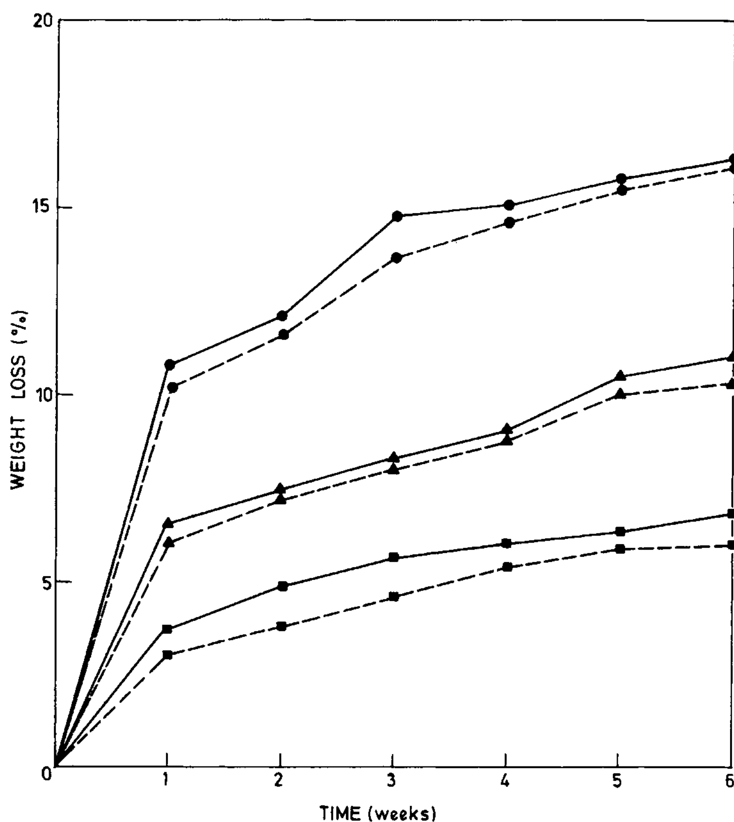

Fig. 2. Weight loss–time curves of gelatin-*g*-poly(ethyl acrylate) samples incubated with *Pseudomonas aeruginosa* in nitrogen-rich (---) and nitrogen-free (——) media: (●) GEA; (▲) GEB; (■) GEC.

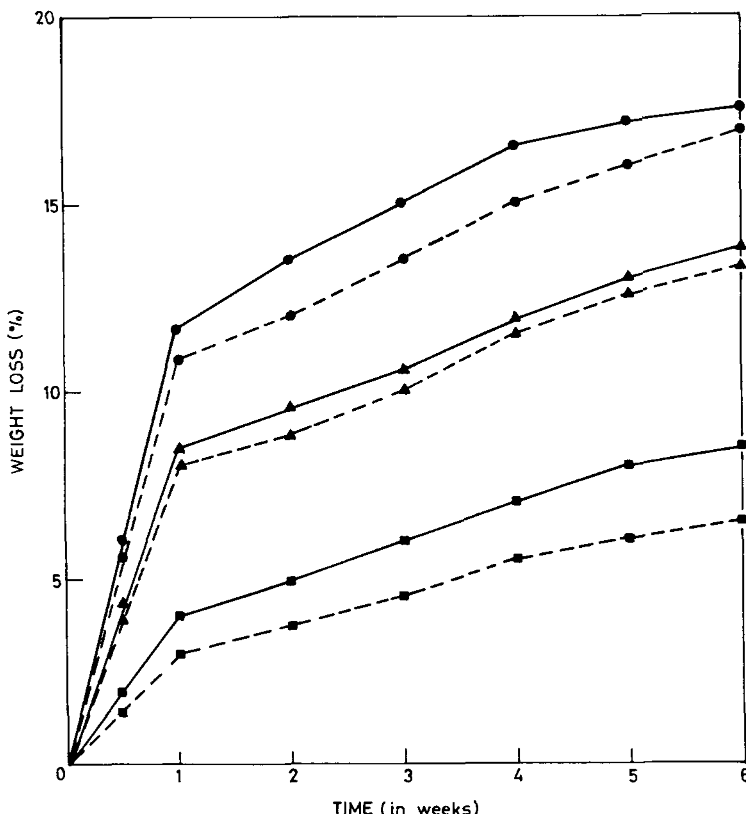

Fig. 3. Weight loss–time curves of gelatin-*g*-poly(ethyl acrylate) samples incubated with *Bacillus subtilis* in nitrogen-rich (—) and nitrogen-free (——) media: (●) GEA; (▲) GEB; (■) GEC.

sp., and *Serratia* sp., respectively. These figures show that the extent of degradation is in the following order: GEA > GEB > GEC. From Table I it is also clear that the efficiency of grafting and the number of grafting sites per molecule are in the following order: GEC > GEB > GEA. These results support our earlier observations that more the number of grafting sites and the efficiency of grafting, the slower will be the rate of biodegradation. Weight loss was assumed to be due to utilization of the gelatin position of the copolymer because of the relatively short incubation period used in this study and the well-known ability of gelatin to biodegrade.

Figures 5–7 illustrate the rates of degradation log(rate) of these copolymer samples during the first week of the test period plotted against the percentage of grafted chains in the above samples. It is evident from the above plots that the relationship between [log(rate)] and percentage of grafted PMA is almost linear (during the first week). The noted deviations are understandable considering the fact that microbial attack involves a large element of chance due to local accleration and inhibitions. The first-order dependence of the rates indicates the unimolecular nature of degradation. The unimolecular nature, may, in turn, point out the enzymatic degradation of the samples. However, it cannot be taken as in index for the same. The

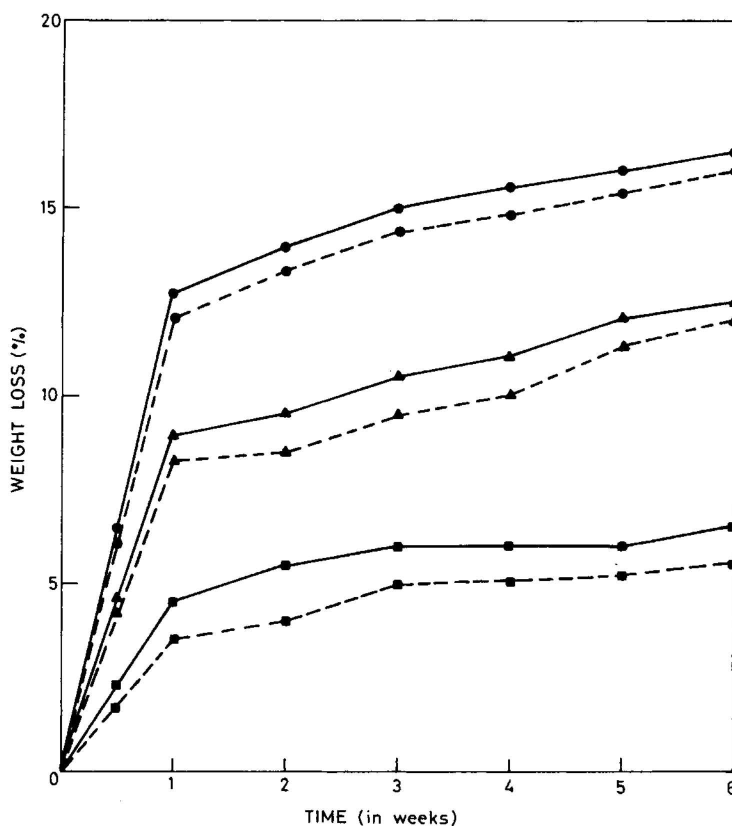

Fig. 4. Weight loss–time curves of gelatin-*g*-poly(ethyl acrylate) samples incubated with *Serratia marcescens* in nitrogen-rich (- - -) and nitrogen-free (——) media: (●)GEA; (▲) GEB; (■) GEC.

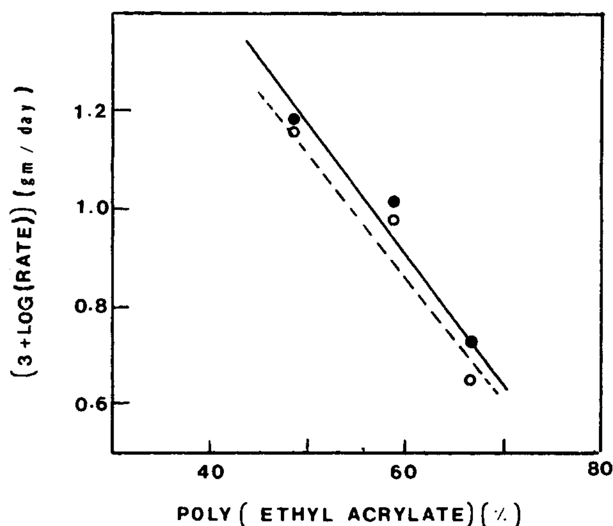

Fig. 5. Rate of biodegradation–percent of PMA of gelatin-*g*-poly(ethyl acrylate) samples (in the first week) incubated with mixed bacterial inoculum in nitrogen-free (●——●) and nitrogen-rich (○- - -○) media.

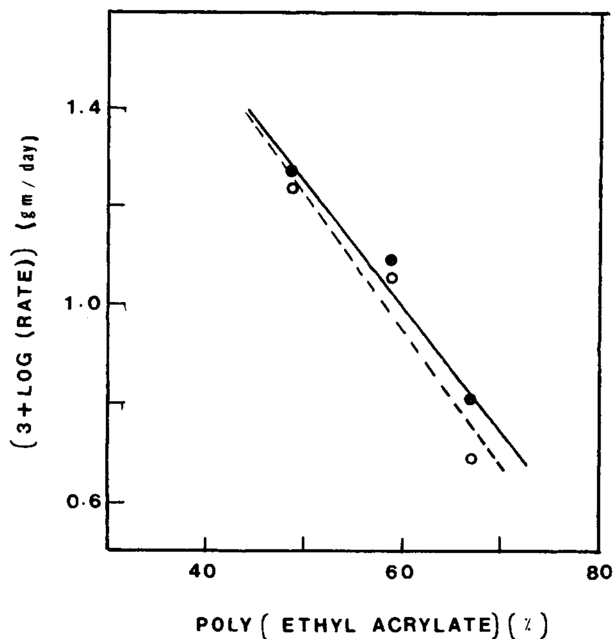

Fig. 6. Rate of biodegradation–percentage of poly(methyl acrylate) of gelatin-*g*-poly(ethyl acrylate) samples in the first week, incubated with *Serratia marcescens* in nitrogen-free (●——●) and nitrogen-rich (○– – –○) media.

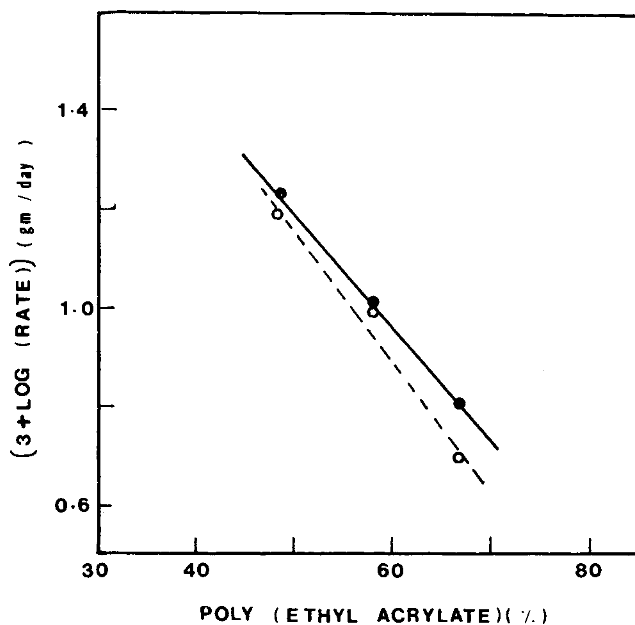

Fig. 7. Rate of biodegradation–percentage of poly(methyl acrylate) of gelatin-*g*-poly (ethyl acrylate) samples in the first week incubated with *Bacillus subtilis* in nitrogen-free (●——●) and nitrogen-rich (○– – –○) media.

extrapolation of these plots seem to indicate the possibility of the rate becoming negligible, when the percentage of synthetic polymer is approximately 95%. This is in full conformity with the reported stability of carbon chain polymers to bacterial attack, especially of those having side groups on alternate carbon atoms, as in the case with poly(ethyl acrylate). These side chains are supposed to prevent β-oxidation of the carbon-chain backbone.[14]

In fact, it is expected that such plots can be used to predict, with a reasonable degree of accuracy, the rate of degradation of a graft polymer sample of known composition under given conditions. But the rate of degradation appears to be considerably influenced by other factors resulting in substantially low rates of degradation. The long range objective can be set for the development of a predictive scheme that would allow the properties of yet unsynthesized polymers to be predicted.

Comparison of the molecular weights of the grafted branches, before and after testing, did not show any detectable difference, indicating that grafted PEA branches were not attacked. Growth tests coupled with enrichment techniques carried out over a long period might offer the bacterial strains the possibility of adapting themselves and may provide more affirmative answer to biodegradability.

The growth of bacteria, monitored by reading the absorbance at 600 nm showed a sharp increase during the first week (Figs. 8–10). The lag period, being less in a nitrogen-free medium as compared to a nitrogen-rich medium, indicates a better attack on the polymer in the absence of an added nitrogen source. Growth of bacteria in an adequate liquid medium is characteristically exponential which can be seen in all the plots. The sustaining and uninhibited growth observed with these single cultures might be responsible for greater losses in weights of the samples especially during the later half of the test period. This is in sharp contrast to the behavior of mixed inoculum, where the optical density started falling off after 3 weeks. The absence of antibiosis may be responsible for this difference. However,

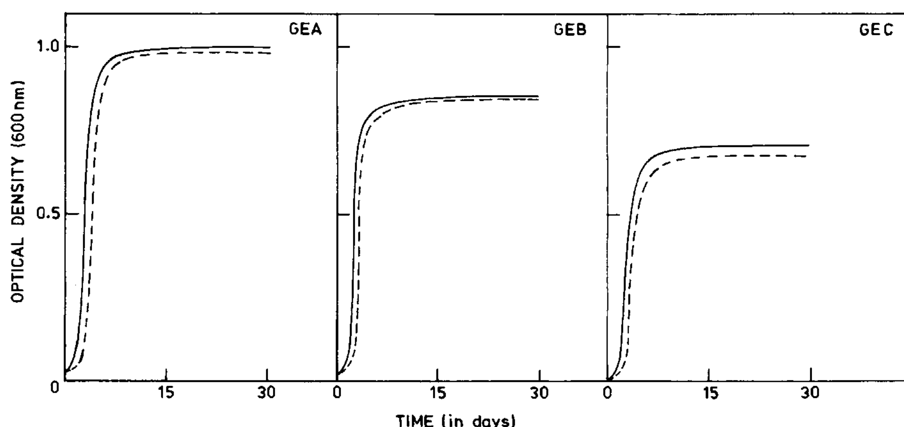

Fig. 8. Optical density of the culture medium at 600 nm vs. time plots of gelatin-*g*-poly (ethyl acrylate) samples incubated with *Serratia marcescens* in nitrogen-rich (---) and nitrogen-free (——) media.

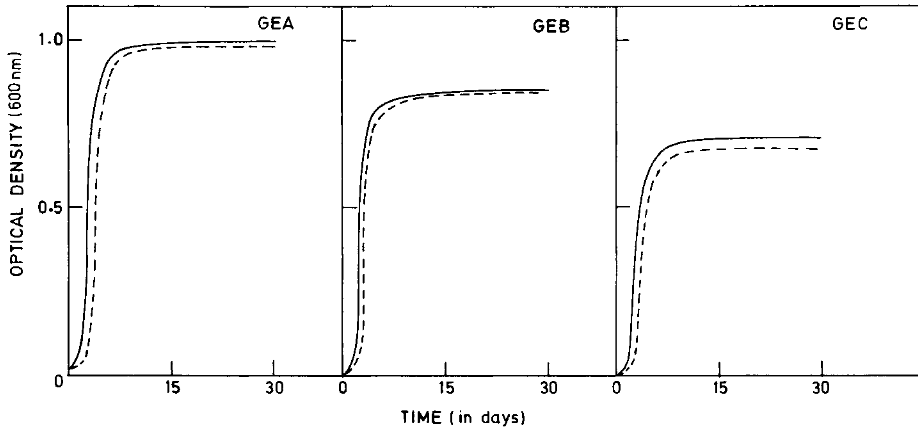

Fig. 9. Optical density of the culture medium at 600 nm vs. time plots of gelatin-*g*-poly(ethyl acrylate) samples incubated with *Bacillus subtilis* in nitrogen-rich (---) and nitrogen-free media (——).

there was no essential difference in the aggressivity of the different test organisms. When the extent of growth and the weight loss caused by the different species were compared, no essential differences were observed as all showed a similar grade of growth and gave similar weight losses.

The pH of the culture media remained stationary at 7.6, 7.3, and 7.8 in the case of *Pseudomonas, Bacillus,* and *Serratia,* respectively. It is known that for each microorganism there is a set of conditions that is optional for its growth, and any departure from these conditions adversely affects the growth to a lesser or greater degree. A greater increase in pH of the medium (8.9) inoculated with the mixed inoculum might be responsible for slower rates of degradation observed in weight loss–time curves. This extremity in pH may also explain the decrease in optical density of the culture medium incubated with the mixed bacterial inoculum. This also supports Hueck's apprehensions that the number of biological parameters, e.g., exoenzymes,

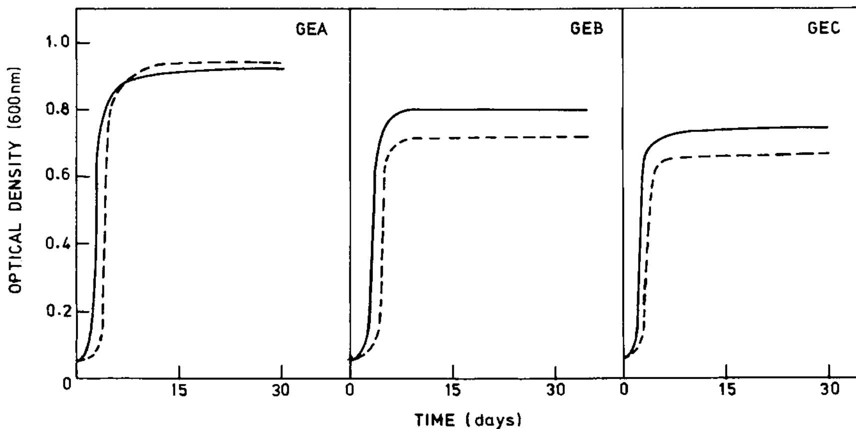

Fig. 10. Optical density of the culture medium (at 600 nm) vs. time plots of gelatin-*g*-poly(ethyl acrylate) samples incubated with *Pseudomonas aeruginosa* in nitrogen-rich (---) and nitrogen-free (——) media.

TABLE II
Total Nitrogen Content of Gelatin-Ethyl Acrylate Copolymer Samples Incubated with *Pseudomonas Aeruginosa*, before and after Testing

Sample	Initial nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GEA	8.53	5.5	5.36	4.22	4.12
GEB	7.15	4.8	5.15	3.20	3.67
GEC	5.60	3.78	3.70	3.36	3.21

TABLE III
Total Nitrogen Content of Gelatin-Ethyl Acrylate Samples Incubated with *Bacillus Subtilis*, before and after Testing

Sample	Initial nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GEA	8.53	6.0	5.82	3.8	3.7
GEB	7.15	4.7	4.91	3.4	3.58
GEC	5.6	3.8	3.6	2.9	2.8

change in pH of the medium, secretion of acids or bases, intercellular endogenous oxidation process, etc., outnumber the individual interspecies differences among most of the organisms.[15]

The total nitrogen content of GEA, GEB, and GEC before and after testing with the bacterial strains is presented in Tables II–IV. The considerable loss in the nitrogen content of the samples after the incubation period positively indicates the utilization of the gelatin portion of the molecule by these bacterial strains. But the differences in microbial activity in nitrogen-rich and nitrogen-free media, as reflected in weight loss–time curves and optical density time curves, could not be observed.

The observed phenomenon of rapid weight loss during the first week and concurrent cooperative changes such as exponential increase in bacterial population and pH of the culture medium, though seemingly independent strategies, "superimpose" well and indicate rapid and easy hydrolysis of protein followed by slow but steady utilization. The change in pH of the medium or the well-known phenomenon of catabolite repression may explain the retarded rate of degradation after the first week.

TABLE IV
Total Nitrogen Content of Gelatin-Ethyl Acrylate Copolymer Samples Incubated with *Serratia Marcescens*, before and after Testing

Sample	Initial nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GEA	8.53	5.89	5.68	4.44	4.23
GEB	7.15	5.98	5.23	3.18	3.20
GEC	5.60	4.11	4.32	3.01	2.98

CONCLUSIONS

The gelatin–ethyl acrylate graft copolymers were molded into films, and they do show desirable mechanical behavior. Pure cultures of *Bacillus* sp., *Pseudomonas* sp., and *Serratia* sp. exhibit no differences in aggressivities. The relationship between the [log(rate)] and percentage of grafted side chains is indicative of enzymatic degradation. The growth–time curves and pH measurements supplement the results obtained from weight loss measurements. The lower rates of degradation observed with mixed inoculum are due to either antibiosis or extremities of pH in the culture medium.

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References

1. G. Sudesh Kumar, V. Kalpagam, and U. S. Nandi, *J. Macromol. Sci. Rev.*, **120**, *Macromol. Chem. C* **22**(2), 225 (1982).

2. R. L. Kronenthal, in *Polymers in Surgery and Medicine,* R. L. Kronenthal, Z. Oser, and E. Marlin, Eds., Plenum, New York, 1975, p. 119.

3. J. E. Potts, *Aspects of Degradation and Stabilization of Polymers,* H. H. G. Jellinek, Eds., Elsevier, New York, 1978, p. 653.

4. G. Sudesh Kumar, Ph.D. thesis, Indian Institute of Science, Bangalore, India, August 1981.

5. G. Sudesh Kumar, V. Kalpagam, U. S. Nandi, and V. N. Vasantharajan, *J. Appl. Polym. Sci.,* **26**, 3633 (1981).

6. G. Sudesh Kumar, V. Kalpagam, U. S. Nandi, and V. N. Vasantharajan, *J. Polym. Sci., Polym. Chem. Ed.,* **19**(5), 1266 (1981).

7. U. S. Nandi, G. Sudesh Kumar, and P. C. Bhaduri, *Indian J. Chem.,* **20A**, 759 (1981).

8. ASTM Standards ASTM-D-2676, 1970, Part 26, p. 758.

9. K. P. Rao, K. T. Joseph, and Y. Nayudamma, *Leather Sci* (Madras), **16** (12), 401 (1969).

10. G. Sudesh Kumar, V. Kalpagam, U. S. Nandi, and V. N. Vasantharajan, *Polym. Bull.,* **8**(1), 33 (1982).

11. G. Sudesh Kumar, V. Kalpagam, and U. S. Nandi, IUPAC 28th Macromolecular Symposium at University of Massachusetts, Amherst, Mass., July 12–16, 1982.

12. J. E. Eastoe and A. A. Leach, *The Science and Technology of Gelatin,* A. G. Ward and A. Courts, Eds., Academic, New York, 1977, p. 73.

13. J. L. Osmon and R. E. Klausmeier, in *Biodelerioration Investigation Techniques,* A. H. Walters, Ed., Applied Science, London, 1977, p. 77.

14. L. Taylor, CHEM TECH September (1979), p. 542.

15. H. J. Hueck, in *Proceedings of Degradability of Polymers and Plastics Conference,* Institute of Electrical Engineering, London, 1973, p. 11/1.

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