Chemically Modified Fibrin–Gelatin Composites: Preparation and Characterization

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ABSTRACT: The crude fibrin which is hitherto discarded as waste in slaughterhouses was recovered and purified. Using this fibrin, fibrin–gelatin composites were prepared and crosslinked with either basic chromium sulfate or glutaraldehyde. These composites were also graft-copolymerized with polyhydroxyethyl methacrylate using hydrogen peroxide–ferrous ammonium sulfate initiation technique. The graft copolymers were characterized for their moisture content, water absorption capacity, tensile strength, infrared spectroscopy, scanning electron microscopy, and thermogravimetric profile. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1109–1115, 1998

Key words: fibrin; gelatin; hydroxyethyl methacrylate; graft copolymerization; wound dressing

INTRODUCTION

Biomaterials are macromolecules which are prepared from biological origin and used for various clinical and industrial purposes. There are certain characteristics that should be consistently important for the biomaterials,[1] i.e., the material should be a nonirritant and nontoxic; it should not be antigenic; it should not be carcinogenic; and it should be sterilizable and adequately available for widespread utilization. Fibrin and gelatin seem to offer most of these characteristics to serve as biomaterials.

Fibrin is a good hemostatic and wound-dressing material that can be made in the form of sponge, film, powder, fibrin glue, etc.[2–4] Concentrated solution of fibrinogen, when resolubilized and mixed with thrombin, is used as fibrin glue or fibrin adhesive to adhere tissues together.

In the present study the crude fibrin which is hitherto discarded as waste in slaughterhouses is used as a raw material for the preparation of pure fibrin. This is used to make a composite with gelatin.

Gelatin is a degraded form of collagen which is a connective tissue protein present in most of the vertebrates.[5] Gelatin is well known for its wound-healing properties. Sinha and colleagues[6] treated burns with gelatin sheets. They opined that this biological dressing prevented secondary infection and fluid loss due to exudation. Yannas and associates[7] mentioned a process for preparing gelatin films from animal products for the purpose of wound-healing. Eckmayer[8] reported the favorable properties of gelatin-based biomaterials for use as hemostatic and wound-healing agents. Marois and coworkers[9] indicated that carbodiimide-crosslinked gelatin could be used as a new coating for porous polyester arterial prostheses. By comparative study of the potentiality of hydrogels composed of gelatin and poly(L-glutamic acid) (PLGA) as a biological glue for soft tissues with a conventional fibrin glue, Otani and coworkers[10] used water-soluble carbodiimides to crosslink the aqueous mixture of gelatin and PLGA.

In the present study, fibrin–gelatin composites were prepared and crosslinked. The crosslinked fibrin–gelatin (FG) composites were later grafted

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with polyhydroxyethyl methacrylate (PHEMA). Grafting of proteins with selected polymers will improve functionality, thermal stability, and other physicochemical properties of the products.

EXPERIMENTAL

Materials

Gelatin (MBD Gelatin, Bombay, India) was pharmaceutical grade. Crude fibrin was collected from the local slaughterhouse (Madras, India). Hydroxyethyl methacrylate (HEMA) was obtained from Fluka, Switzerland. All other reagents used were of analytical grade.

Purification of Fibrin

Fibrin was purified as described in our earlier article.[11] Briefly, the crude fibrin that was separated from fresh blood by churning was collected from the slaughterhouse and washed thoroughly under running water to remove the blood clots. Later the fibrin was treated with 0.5*M* sodium acetate solution and 30% hydrogen peroxide solution. The fibrin was then washed thoroughly under running water and ground to paste by a mixer. This paste contained 60% solid.

Preparation of Gelatin Solution

Gelatin, 10 g, was dissolved in 100 mL of water at 55°C in a water bath.

Preparation of FG

To 250 g of fibrin paste, 13.2 mL of gelatin solution and 4.4 mL of ethylene glycol solution were added and mixed well. Then the mixed contents were cast into a film and dried at 55–60°C in a hot-air oven. The dried film was removed, stored in a polythene cover, and denoted as FG.

Glutaraldehyde Crosslinking of Fibrin–Gelatin Composite

To 250 g of fibrin paste, 13.2 mL of gelatin solution, 4.4 mL of ethylene glycol solution, and 6.6 mL of 25% glutaraldehyde solution were added and the contents were mixed well at a pH of 7.4. The mixed contents were cast into a film and dried at 55–60°C in a hot-air oven. The dried film was removed, stored in a polythene cover, and denoted as FG-G.

Chromium Crosslinking of Fibrin–Gelatin Composite

To 250 g of fibrin paste, 13.2 mL of gelatin solution, 4.4 mL of ethylene glycol solution, and 9.5 mL of basic chromium sulfate solution (0.3% w/v) were added and mixed well at a pH of 3.0. The mixed contents were cast into a film and dried at 55–60°C in a hot-air oven. The dried film was removed, stored in a polythene cover, and denoted as FG-C.

Grafting of FG with PHEMA

About 5 cm × 5 cm FG was treated with 20 mL of 30% hydrogen peroxide solution for 30 min. Later, the hydrogen peroxide solution was decanted. A 1.8-m*M* concentration of ferrous ammonium sulfate solution (0.314 g in 100 mL of water) and 5 mL of HEMA were added to the film. The film was shaken in a mechanical shaker for about 1 h at 55°C. The grafted film was first washed with water and extracted with acetone to remove poly-HEMA homopolymer, then dried in a hot-air oven at 55°C. The dried film was removed, stored in a polythene cover, and denoted as HEFG.

Grafting of FG-G with PHEMA

The procedure described above for the preparation of HEFG was followed again, except that FG-G was used instead of FG. This was denoted as HEFG-G.

Grafting of FG-C with PHEMA

The procedure described above for preparing HEFG was followed again, except that FG-C was used instead of FG. This was denoted as HEFG-C.

Characterization

The analyses of the products prepared (i.e., FG, FG-G, FG-C, HEFG, HEFG-G, and HEFG-C) were carried out to study moisture content, water absorption capacity, tensile strength, infrared spectrum, morphology, scanning electron microscopy, and thermal decomposition pattern.

Moisture Content

About 2–4 g of the sample was taken in a china dish and dried at 100–105°C for 5 h. Then it was cooled in a desiccator and weighed to a constant weight. Loss of weight was reported as moisture content.

Water Absorption Capacity

Water absorption capacity was estimated by the method of Rao and colleagues.[12] The water absorption capacity of FG, FG-G, HEFG, HEFG-G, and HEFG-C were determined by swelling small pieces of each sample of known weight in distilled water at room temperature. The swollen weights of the samples were determined by first blotting the samples with filter paper, then accurately weighing the sample. The weights of the swollen pieces were recorded every 1, 2, and 3 h, and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula

$$E_s = \frac{W_s - W_o}{W_o} \times 100$$

where W_s is the weight of the sample (moist) at a given time, W_o is the initial weight of the sample, and E_s is the percent of swelling at a given time.

Tensile Strength

Two dumbbell-shaped specimens, 4 mm wide and 10 mm long, were punched out of the prepared films. Mechanical properties such as tensile strength and percentage of strain at break were measured using an Istron 4501 tensile testing system (according to Vogel[13]) at an extension rate of 5 mm/min.

Infrared Spectroscopy

To provide proof of grafting, the infrared (IR) spectra of FG composite and graft copolymer of FG composite were taken in a Nicolet Impact 400 Fourier Transform Infrared Spectrometer, using a 500-mg KBr pellet containing 2–6 mg of the sample.

Scanning Electron Microscopy

Scanning electron micrographs were taken both for FG and FG-PHEMA graft copolymer using a JSM 5300 Scanning Microscope.

Table I Moisture Content

Sample Number	Sample	Percentage of Moisture
1	FG	14.6
2	FG-G	14.2
3	FG-C	13.7
4	HEFG	10.4
5	HEFG-G	8.1
6	HEFG-C	7.3

Thermogravimetric Analysis (TGA)

TGA was carried out using a Seiko SSC 5200 H in nitrogen atmosphere (80 mL/min) at a heating rate of 10°C/min. Primary weight change of the samples as a function of temperature was recorded using this study.

RESULTS AND DISCUSSION

Today, combinations of synthetic and natural polymers are finding increased applications in the medical field. However, neither natural materials nor synthetic macromolecules alone can meet all the complex demands of the biomaterials. A meaningful interaction between the areas of synthetic and natural polymers has recently become well-recognized. Keeping these factors in view, PHEMA was grafted onto FG with the aim of improving physicochemical and thermal properties.

In this study, grafting of PHEMA onto FG composite was established using hydrogen peroxide–ferrous ammonium sulfate initiation technique.

Moisture Content

The moisture content of the films FG, FG-G, FG-C, HEMA, HEFG-G, and HEFG-C are given in Table I. The moisture content varied between 7.3 and 14.6%. Films with this range of moisture content were flexible and behaved like skin. This property enables the films to cover contours of a wound when applied. This is an essential property for all wound-dressing materials.

Water Absorption Capacity

Previously, collagen sheets, chemically modified gelatin sheets, and amniotic membranes[14] were used as wound-dressing materials. Sometimes, however, the sheets did not adhere to the wound

Table II Water Absorption Capacity

Sample Number	Sample	Percentage Swelling Time 1 h	2 h	4 h	24 h
1	FG	307	281	277	244
2	FG-G	294	265	252	240
3	FG-C	294	249	248	228
4	HEFG	303	310	311	311
5	HEFG-G	277	284	286	289
6	HEFG-C	210	227	230	238

properly and the absorption was poor. To overcome this problem, chemically modified FG composites were graft-copolymerized with PHEMA. The water absorption capacities of FG, FG-G, FG-C, HEFG, HEFG-G, and HEFG-C appear in Table II. HEFG-G shows better water absorption capacity than the crosslinked natural material (FG-G and FG-C). The water absorption capacities of crosslinked FG composites are less than the uncrosslinked composite. This may be due to the blocking of hydrophilic groups on the backbone by the crosslinking agents. According to this study, HEFG (the graft copolymer prepared without crosslinking HEFG) shows higher water-absorption capacity.

IR Spectroscopy

The IR spectra of FG composite and the graft copolymer of FG-PHEMA are shown in Figures 1 and 2. The IR spectrum of FG composite shows the characteristic amide absorption bands around 1660 and 1550 cm^{-1} (Fig. 1), whereas the spectrum of FG graft copolymer shows the characteristic absorption bands of the ester carbonyl group of PHEMA at 1720 cm^{-1} (Fig. 2) in addition to the amide absorption bands (Fig. 1). These results clearly indicate the grafting of polymer chains onto FG composites.

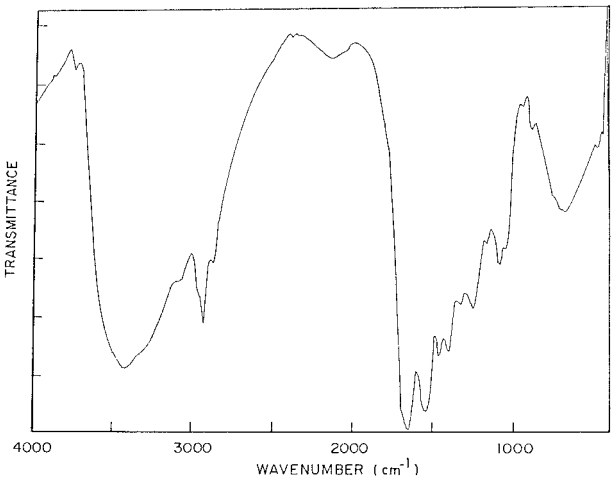

Figure 1 FTIR spectrum of FG.

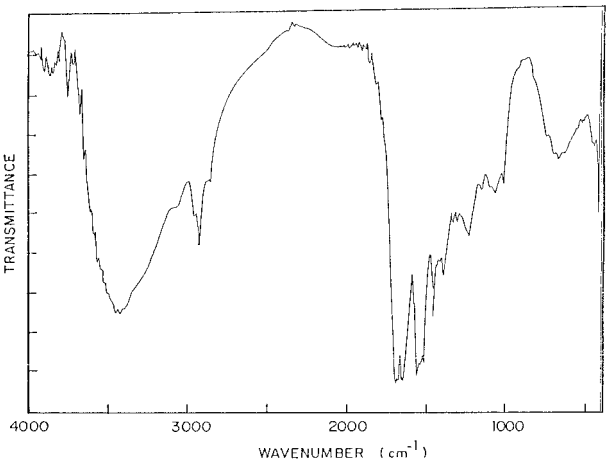

Figure 2 FTIR spectrum of FG-PHEMA.

Scanning Electron Microscopy (SEM)

Figures 3 and 4 show the scanning electron micrographs of FG and FG grafted with PHEMA, respectively. It can be seen from Figure 3 that the morphological structure of FG is expressed in the form of a fibrous network. On the other hand, the wonderful network of fibrin seen in the SEM of FG (Fig. 3) was completely coated by PHEMA molecules after the composite was grafted (Fig. 4). The marked difference in the surface morphol-

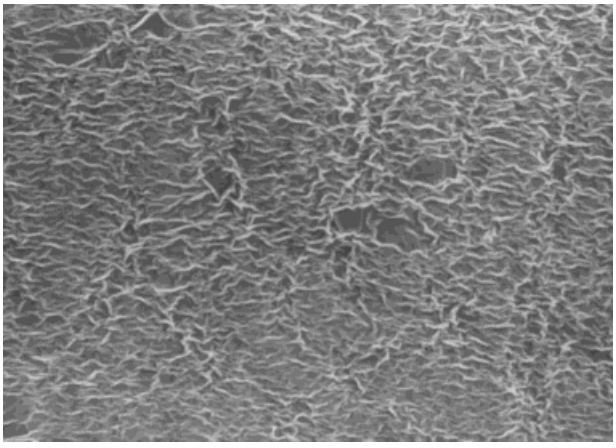

Figure 3 Scanning electron micrograph of FG (×2,000).

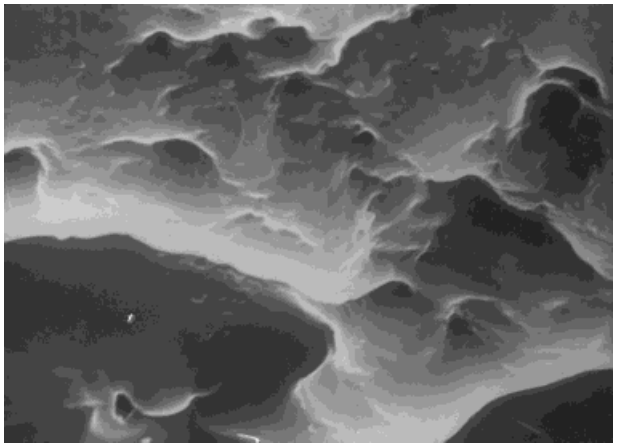

Figure 4 Scanning electron micrograph of FG-PHEMA (×2,000).

ogy of protein before and after coating with polymer confirms grafting of PHEMA onto FG.

Tensile Strength

The stress–strain curves of FG, FG-G, and FG-C are given in Figure 5. As seen from this figure and Table III, the samples show tensile strength

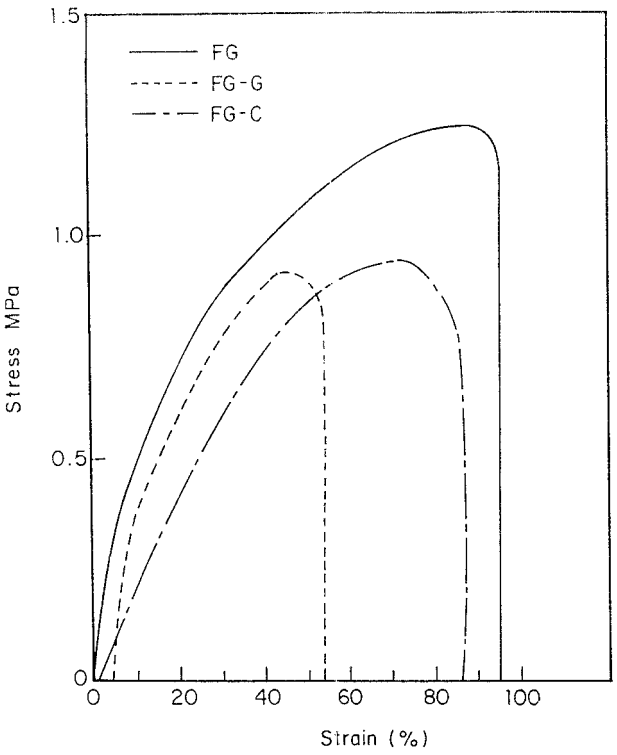

Figure 5 Stress–strain curves of FG, FG-G, and FG-C.

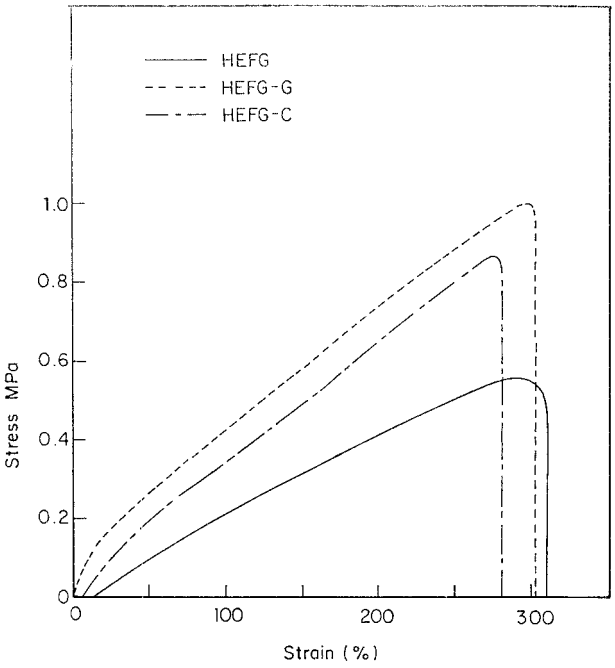

Figure 6 Stress–strain curves of HEFG, HEFG-G, and HEFG-C.

(σ max) of 1.25, 0.926, and 0.941 MPa for FG, FG-G, and FG-C, respectively. This may be due to the following facts:

1. Gelatin has an open helical structure and thus has more functional groups exposed to make bridges with basic chromium sulphate and glutaraldehyde molecules.
2. Whereas the fibrin molecule has the subunit surface structure of $(\alpha\beta\gamma)_2$, fibrin will not have as many functional groups as gelatin to crosslink with basic chromium sulphate and glutaraldehyde. Because these two proteins are chemically and structurally different, the extent of crosslinking also differs. The crosslinking agents interact more with gelatin than with fibrin. This results in the reduction of adhesion between fibrin and gelatin, and may be the reason for the lower tensile strength in the crosslinked FGs than in the FG itself. The extension at maximum stress was greater in FG than in FG-G or FG-C. This shows that both the tensile strength and extension at break values are greater for FG than for FG-G or FG-C.

The stress–strain curves of HEFG, HEFG-G, and HEFG-C appear in Figure 6. As seen from

Table III Tensile Strength

Sample Number	Sample	Tensile Strength (N/mm^2)	Percentage Strain at Break
1	FG	1.250	89.58
2	FG-G	0.9296	44.94
3	FG-C	0.9471	74.73
4	HEFG	0.5561	299.6
5	HEFG-G	1.038	302.70
6	HEFG-C	0.8789	278.6

this figure and Table III, the samples show tensile strength (σ max) of 0.5561, 1.038, and 0.8789 MPa for HEFG, HEFG-G, and HEFG-C, respectively. The extension at break value for HEFG-G is more than that of HEFG or HEFG-C. The increase in tensile strength and extension at break for the glutaraldehyde-crosslinked HEFG may be attributed to the bridging property of the dialdehyde through its functional groups (—CHO) at both ends. This crosslinker, apart from bridging the fibrin and gelatin molecules, is believed to bridge the proteins and poly-HEMA as well. From these studies it is understood that FG and HEFG-G seem to offer better mechanical properties that are ideal for a wound-dressing material. However, for better shelf life, the latter may be preferred.

TGA

Figures 7 and 8 show the thermal decomposition pattern of the fibrin–gelatin matrix and its grafted form with PHEMA, respectively. In thermogravimetry, the losses of weight due to evolution of water, carbon monoxide, carbon dioxide, and evaporation of other pyrolysis products are collectively measured as percentage of original weight. In this investigation, the FG composite and its PHEMA-grafted form were heated separately at steadily increasing temperature from 30 to 600°C in an atmosphere of nitrogen (80 mL/min). It can be seen from the thermogram of FG (Fig. 7) that the major portion of protein composite was decomposed at temperatures between 190 and 425°C. The weight loss at 425°C was 60%, with a thermal decomposition peak at 300°C where the loss was observed to be 30%. It is also understood from the thermogram that the FG decomposition is a single-stage process, in spite of the two different proteins, which may be due to their backbone structural similarity.

On the other hand, the PHEMA-grafted FG

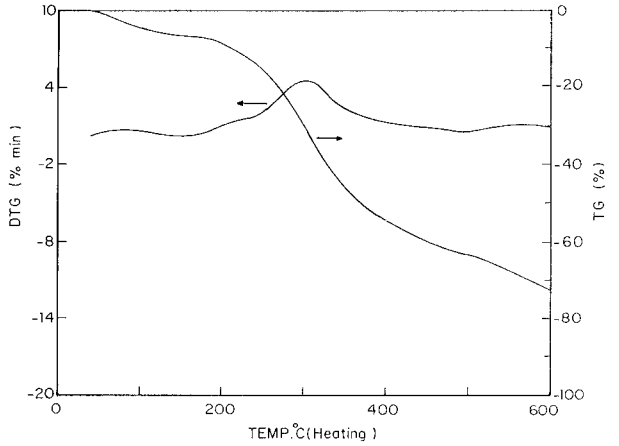

Figure 7 Thermogram of FG.

showed a two-stage thermal decomposition at 200–365 and 400–470°C (Fig. 8). The first decomposition stage (200–365°C) denotes the pyrolysis of protein moiety of FG-PHEMA as seen in the case of FG (Fig. 7); however, a minor shift (about 5–10°C) observed in the protein decomposition of the protein (FG) portion of FG-PHEMA may be due to the interaction of the former with the polymer upon grafting. This result is in good agreement with the earlier report on grafting[15]; it is also seen in the faster decomposition of the protein in free form compared with that of the grafted one, as reported previously by Sastry and colleagues.[15] As seen from the results (Figs. 7 and 8), the percent weight losses at 300°C for free FG and PHEMA-grafted FG were 30 and 20, respectively. Similarly, at 360°C, the loss was 57% and 40%, respectively, for FG and FG-HEMA. The second stage of pyrolysis starts at about 400°C, as evidenced by both DTG and TGA curves, and com-

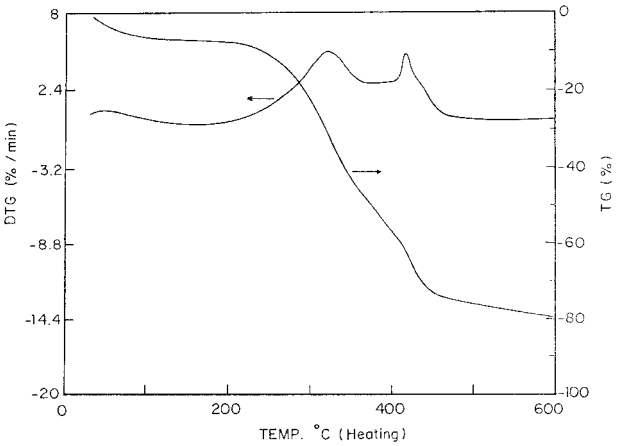

Figure 8 Thermogram of FG-PHEMA.

pletes almost at 470°C with a loss of 74%. These results clearly indicate that the process of grafting improves the material's thermal stability which, in turn, reflects its improved mechanical properties as evidenced by tensile strength and other parameters (Fig. 6).

CONCLUSIONS

In conclusion, HEFG-G offers better water absorption capacity and tensile strength properties. Even though HEFG has shown better water absorption capacity than HEFG-G, it is not crosslinked, whereas HEFG-G is crosslinked with glutaraldehyde and therefore can be stored for a longer time, and the probability of bactericidal attack on HEFG-G is less. Moreover, the improved thermal stability observed in the crosslinked FG-PHEMA indirectly indicates some degree of resistance to biodegradation. Based on these studies, HEFG-G has better wound-dressing properties.

REFERENCES

1. B. Phariss, *J. Am. Leath. Chem. Assoc.,* **75,** 474 (1980).
2. K. Laki, *Fibrinogen,* Marcel Dekker, Inc., New York, 1968.
3. S. Bergel, *Deut. Med. Wochschr.,* **35,** 663 (1909).
4. A. Plenk, *Arch. Klin. Chir.,* **198,** 402 (1940).
5. A. G. Ward and A. Courts, *The Science and Technology of Gelatin,* Academic Press, New York, 1977.
6. R. N. Sinha, D. K. Varma, and P. Madan, *J. Plastic and Reconstructive Surgery,* **5,** 56 (1972).
7. I. V. Yannas, J. F. Barke, P. L. Gordon, C. Huang, and R. H. Rubenstein, *J. Biomed. Mater. Res.,* **14,** 107 (1980).
8. Z. Eckmayer, Recycling of Animal Byproducts: Report on the Third Mission to Madras, submitted to C.L.R.I., Adyar, Madras, India, 1983.
9. Y. Marois, N. Chakfe, D. Xiaoyan, M. Mardis, T. How, M. W. King, and R. Guidoin, *Biomaterials,* **16,** 1131 (1995).
10. Y. Otani, Y. Tabata, and Y. Ikada, *J. Biomed. Mater. Res.,* **31,** 157 (1996).
11. P. Ravindra Babu, T. P. Sastry, C. Rose, and N. Muralidhara Rao, *J. Appl. Polym. Sci.,* to appear.
12. K. P. Rao, K. T. Joseph, and Y. Nayudamma, *Leath. Sci.,* **16,** 401 (1969).
13. H. G. Vogel, *Biochem. Biophys. Acta.,* **252,** 580 (1971).
14. T. P. Sastry and K. Panduranga Rao, *J. Bioactive and Compatible Polymers,* **5,** 430 (1990).
15. T. P. Sastry, M. Vijayalakshmi, M. N. Nazer, S. Gomathinayagam, C. Rose, and N. Muralidhara Rao, *J. Macromol. Sci. Pure Appl. Chem.,* **A34**(5), 915 (1997).