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Biodegradation Studies of Rosin-Based Polymers

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ABSTRACT

This study was designed to investigate two rosin-based polymers (R-1 and R-2) for their in vitro and in vivo biodegradation behavior. The in vitro hydrolytic degradation was carried out in buffer solutions of pH 4.4, 7.4, and 10.4 at 37°C. Enzymatic degradation was studied using enzymes lipase, pancreatine, and pectinase. Free films of the two polymers were subcutaneously implanted in rabbits for the in vivo biodegradation. The extent of degradation was determined quantitatively by weight loss and was followed qualitatively by scanning electron microscopy. The extent and the rate of degradation was better in vivo than in vitro. The polymers showed poor enzymatic degradation and a highly pH-dependent hydrolytic degradation.

Key Words: Rosin polymers; Biodegradation; In vivo; Free films.

INTRODUCTION

Biodegradable polymers are of considerable interest to environmental, industrial, and academic researchers.[1] An important impetus for the early studies of biodegradable polymers was on their ecological value.[2] Interest in biodegradable polymers for biomedical applications also greatly increased when Schmitt and Polistina introduced Poly(glycolic acid) as suture material in 1967.[3] Biodegradation is an event that takes place through the action of enzymes and/or chemical decomposition associated with living organisms (bacteria, fungi, etc.) or their secretion products.[4] For biomedical and pharmaceutical uses biodegradable polymers should undergo degradation in a physiological environment.[5,6] The most widely investigated biodegradable polymers are aliphatic polyesters based on lactic acid and glycolic acid.[7,8] The copolymers of these two attracted much attention because the biodegradation rate is easily controlled by altering the composition and they have been shown to be biocompatible.[9,10] Biodegradable polymers can provide sustained drug release accompanied by degradation in tissue, thus avoiding

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removal once inserted into the body in the form of implants.[11] While it is possible to implant and remove drug-containing devices or polymeric matrices surgically, the requirement for such intervention could diminish patient acceptance of the product. This has lead to considerable development of interest in the biodegradable polymers with potential drug delivery applications. It is expected that a degradable polymer should elicit a desirable host response without any side effects, like carcinogenicity, toxicity, immunogenicity, or inflammation.[12]

Rosin derivatives and salts are polymeric biomaterials and have been extensively evaluated for their pharmaceutical applications.[13,14] Rosin being of natural origin, rosin-based polymers are expected to be environment-friendly, biodegradable, and biocompatible.[15] In the present communication free films of two new rosin-based polymers are studied for the rate of degradation by subcutaneous implantation in rabbits as a function of time. The results of the in vivo degradation are compared with those observed in vitro. Degradable rosin polymers may provide economically acceptable alternatives to the existing range of biodegradable polymers.

MATERIALS AND METHODS

Materials

Rosin N grade (Swastik Acids and Chemicals, Nagpur, India), Maleic anhydride (S. D. Fine Chemicals, Mumbai, India), Fumaric acid (S. D. Fine Chemicals), Glycerol (Qualligen Laboratories, Mumbai, India), Castor oil (Apex Laboratories, Mumbai, India), Isopropyl alcohol (S. D. Fine Chemicals), Potassium nitrate (S. D. Fine Chemicals), Potassium carbonate (S. D. Fine Chemicals), Potassium hydrogen phthalate (E. Merck, Mumbai, India), Potassium dihydrogen phosphate (E. Merck). Dipotassium hydrogen orthophosphate (E. Merck), Lipase (Novo Nordisk, Bangalore, India), Pancreatine (Novo Nordisk), Pectinase (Novo Nordisk).

Methods

Polymers

Rosin-based polymers (R-1 and R-2) used in the study were synthesized and characterized for film-forming property as reported earlier.[16] Briefly, R-1 was synthesized by reacting the ingredients (rosin: 85%; fumaric acid: 2.5%; maleic anhydride: 2.5%; glycerol: 10%) in a glass reactor at 160°C (1 hr), 265°C (1 hr), 250°C (1 hr), 225°C (1 hr), 210°C (1 hr), and 200°C (4 hr) consecutively. R-2 was synthesized by reacting the ingredients (rosin: 60%; fumaric acid: 10%; glycerol: 10%; castor oil: 20%) in a glass reactor at 225°C (1 hr), 210°C (2 hr), 200°C (2 hr), 200°C (2 hr), and 190°C (2 hr), consecutively. Free films of R-1 and R-2 were characterized for surface morphology (SEM), water vapor transmission rate (WVTR), and mechanical properties (tensile strength, percent elongation, and modulus of elasticity).[16] Films of polymers R-1 and R-2, prepared on a mercury substrate by solvent evaporation technique (30% w/v solution in dichloromethane) were tested in the present study for in vitro and in vivo biodegradation. The molecular weights of the initial films and degraded samples were determined using gel permeation chromatography (GPC) (Perkin Elmer, series-10, Newton Conter, MA) equipped with a refractive index detector (Perkin Elmer, LC-25). Samples were eluted through a PL gel 3 μm mixed column at a flow rate of 1 mL/min using tetrahydrofuran as solvent. The data are based on polystyrene (Polysciences) as a reference standard.

Hydrolytic Degradation[6,17]

The hydrolytic degradation of R-1 and R-2 was evaluated by placing rectangular samples of free films (2 cm × 1 cm × 0.5 mm) into 10 mL buffer solutions of pH 4.4, 7.4, and 10.4, with 0.3% sodium azide to prevent microbial growth (1) at 37°C on a rotating shaker. The buffer was changed for all the samples every 8 h for the first day, every day for the first week, and weekly thereafter in order to keep the pH relatively constant. Films were taken out of the buffer (if possible) at 30, 60, 90, 120, 150, and 180 d and dried at room temperature. The hydrolysis was monitored by change in molecular weight (Mw), and weight loss of the sample and morphology were determined using scanning electron microscope (Stereo Scan 250-MK-III, Cambridge, England).

Enzymatic Degradation[18,19]

Accurately weighed polymeric films of above dimensions were placed in capped tubes containing enzyme solutions (0.1% pH in 7.4 PBS) with 0.3% sodium azide to prevent microbial growth[1] at

37°C. The enzymes used for the study are Lipase (LIP), Pancreatine (PAN), and Pectinase (PEC). The enzyme solutions were refreshed frequently (thrice weekly). After 30, 60, 90, 120, 150, and 180 d of incubation the samples were removed (if possible) from the solutions, washed with distilled water, and dried at room temperature. The extent of degradation was determined quantitatively by change in Mw and weight loss and was followed qualitatively by scanning electron micrographs (SEM).

In Vivo Degradation[20]

Free films with dimensions 2 cm × 1 cm × 0.5 mm were implanted on the backs of albino rabbits (2–3 kg). Surgical procedures were performed under local anesthesia (Lignocaine hydrochloride; 2% w/v). The rabbits were shaved and betadine solution (10%) applied. A 10 mg/kg dose of tetracycline was given at the time of surgery. An incision (approx. 2.5 cm long) was made laterally about the midportion of the back. Pocket was formed subcutaneously around each incision and the free films were inserted. Each incision was closed by intermittent sutures with surgical nylon thread, 0.5 cm apart. Animals were maintained in rabbit holders during the surgical procedures. Implanted free films were removed at the end of 30, 60, 90, 120, 150, and 180 d and analyzed for Mw, % weight loss, and surface topography (SEM). Animal study protocols were approved by the Institute Animal Ethics Committee.

RESULTS AND DISCUSSION

The objective of this study was to provide an evaluation of the degradation behavior of the free films of rosin-based polymers (R-1 and R-2) both in vitro and in vivo. According to Gopferich, the polymer character is extensively modified after 1% degradation and in most cases, polymer integrity is completely lost after 10% degradation assuming random reaction.[21] Specifically, in the present communication the molecular weight, % weight loss of free films, and the surface morphological changes were monitored to understand the extent of degradation.

Hydrolytic Degradation

The percent weight loss of the free films of R-1 and R-2 expressing the hydrolytic degradation is shown in Fig. 1 and Fig. 2 respectively. Readings expressed are average of six determinations. Degradation is extremely slow at pH 4.4, which may be due to the acid resistant property of the two polymers. At 6 months, the maximum percent weight loss was 7.35 ± 0.22 and 6.31 ± 0.27 for R-1 and R-2, respectively. Increase in pH did not result in any significant degradation of R-1 films. The alkaline hydrolytic medium was changed frequently in the in vitro study as described previously to approximate a site that would not experience a build-up of low pH degradation or leachable products that could cause additional acid catalyzed degradation.[22] The free

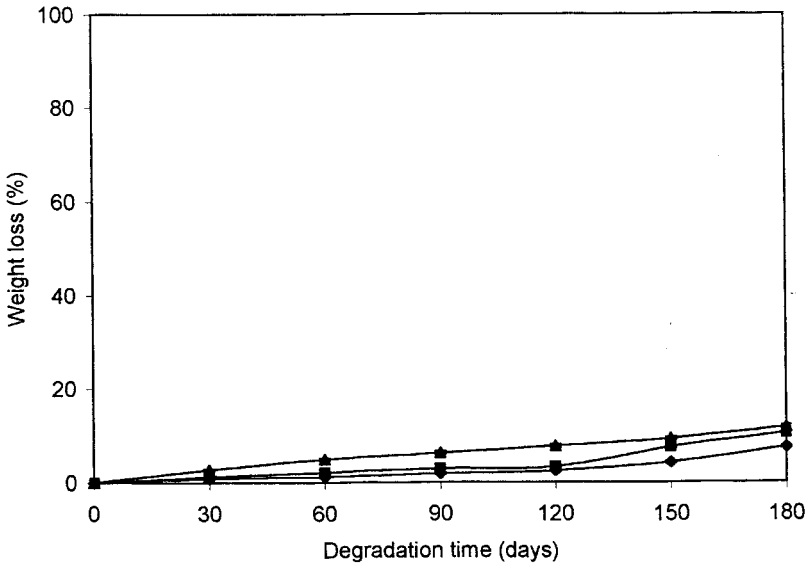

Figure 1. Hydrolytic degradation of free films of R-1 at pH (◆) 4.4; (■) 7.4; (▲) 10.4.

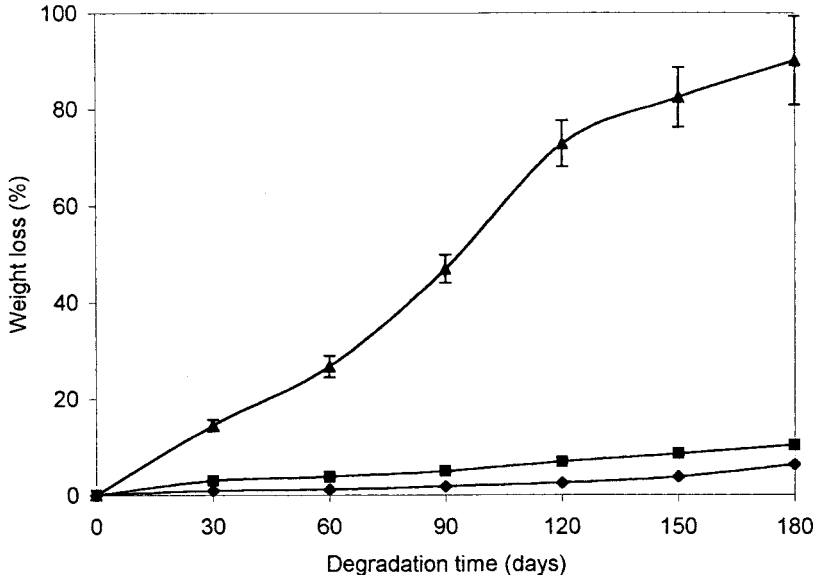

Figure 2. Hydrolytic degradation of free films of R-2 at pH (◆) 4.4; (■) 7.4; (▲) 10.4.

Table 1. Changes in molecular weight values of R-1 films after in vitro and in vivo degradation.

Polymer R-1 (Mw : 1652) time	In vivo	In vitro					
		Hydrolytic			Enzymatic		
		4.4	7.4	10.4	LIP	PAN	PEC
1 month	1617	1630	1626	1580	1639	1624	1640
3 months	1547	1610	1579	1500	1512	1580	1617
6 months	1424	1600	1520	1460	1530	1570	1592

Measured weight average molecular weight values are means of three determinations.

films or R-2 showed enhancement of degradation rate as the hydrolytic medium became more alkaline. At 6 months, a significant weight loss ($90.15 \pm 9.21\%$) was observed by R-2 free films in pH 10.4. This is possibly due to the solubility of the polymer and its degradation products at higher pH as well as due to the expected rise in the hydrolysis rate. It is well acknowledged that the rate of hydrolysis increases with increasing pH.[23] It is obvious from this study that a relationship between pH value and degradation rate of polymer R-2 exists. Table 1 and Table 2 summarize the results of molecular weight changes for R-1 and R-2 films after 1, 3, and 6 months of hydrolytic degradation. The overall change in Mw of samples shows maximum decrease of around 12% at 6 months in pH 10.4. Scanning electron micrographs of free films of the two polymers and the films degraded in pH 10.4 at the end of 6 months are shown in Fig. 6 (a, b) and Fig. 7 (a, b). Bulk mechanism of degradation is evident from the scanning electron micrographs of the degraded films compared with the free films.

Enzymatic Degradation

The percent weight loss of the free films of R-1 and R-2 expressing the enzymatic degradation is shown in Fig. 3 and Fig. 4, respectively. The enzymes selected in the present study were lipase, pancreatine, and pectinase, which belong to the esterases type of enzymes. No significant degradative activity was detected with any of the enzymes in respect to R-1 and R-2. At 6 months, the maximum percent weight loss was between 10–15% with free films of R-1 and between 7–12% with free films of R-2. The molecular

Table 2. Changes in molecular weight values of R-2 films after in vitro and in vivo degradation.

Polymer R-1 (Mw : 3817) time	In vivo	In vitro					
		Hydrolytic			Enzymatic		
		4.4	7.4	10.4	LIP	PAN	PEC
1 month	2642	3796	3760	3410	3782	3770	3790
3 months	1927	3750	3687	3148	3716	3708	3756
6 months	1475	3690	3590	2920	3610	3634	3700

Measured weight average molecular weight values are means of three measurements.

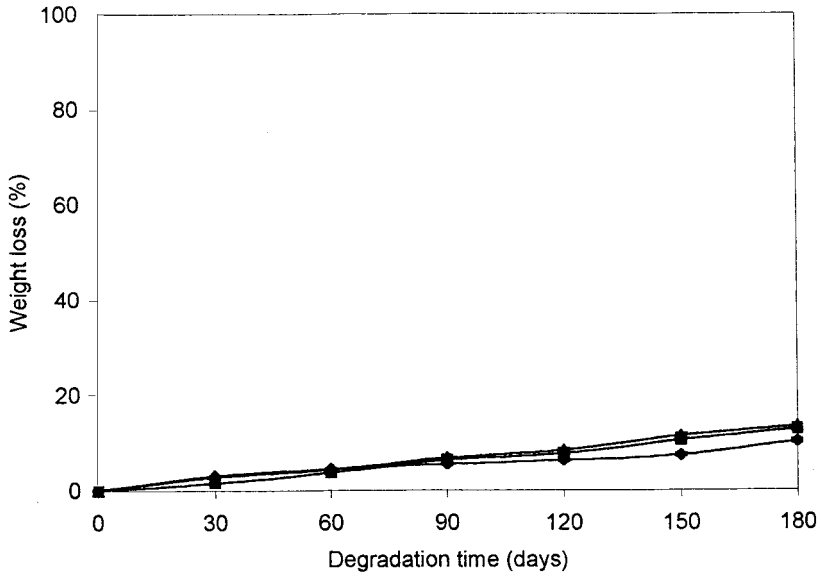

Figure 3. Enzymatic degradation of free films of R-1 by enzyme (◆) lipase; (■) pancreatine; (▲) pectinase.

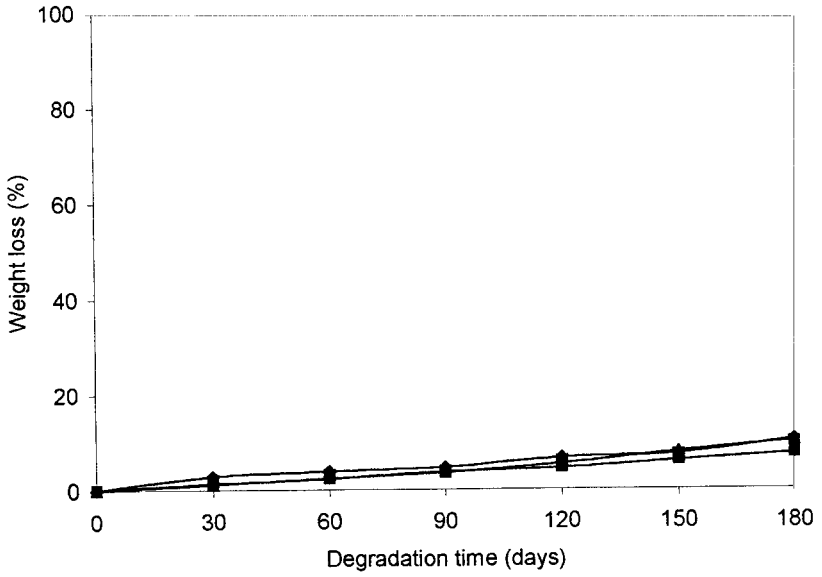

Figure 4. Enzymatic degradation of free films of R-2 by enzyme (◆) lipase; (■) pancreatine; (▲) pectinase.

weight changes shown in Table 1 and Table 2 for R-1 and R-2 films after enzymatic degradation reveal maximum decrease of around 6–7% with enzyme lipase. The loss is in agreement with the poor rate of enzymatic degradation as revealed by % weight loss. The amount of degradation is small but could have significant sequelae if reproduced in vivo.[18] The scanning electron micrographs of the enzymatic degradation of free films at the end of 6 months are shown in Fig. 6 (a,c) and Fig. 7 (a,c).

In Vivo Degradation

Free films were implanted subdermally in rabbits for a maximum period of 6 months to evaluate the in vivo degradation. No acute inflammation, abscess formation, or necrosis was observed in tissue surrounding the implanted materials. The weight loss of the free films is shown in Fig. 5. The percent weight loss at the end of 6 months was 17.05 ± 0.50 and 86.87 ± 9.12 for R-1 and R-2, respectively. A pronounced drop in the weight was observed by free films of R-2. Representative SEMs of R-1 and R-2 are shown in Fig. 6 (a,d) and Fig. 7 (a,d), respectively. The microphotographs show bulk erosion of the film surfaces. More pitting and irregularities are seen in the R-2 degraded film. The results of molecular weight changes for free films following 1, 3, and 6 months of subcutaneous implantation in rabbits are shown in Table 1 and Table 2. Polymer R-2 shows a pronounced loss of 30, 49, and 61% at 1 month, 3 months, and 6 months, respectively. The decrease of molecular weight is less than 15% at 6 months for R-1 films implanted subdermally.

In both in vitro and in vivo degradation studies polymer R-1 did not show any significant degradation characteristics. Polymer R-2 showed a pH-dependent rate of degradation, which is partly attributed to its solubility in alkaline medium. However, at physiological pH and temperature, that is, around pH 7.4 and 37°C, it did not show any significant degradation. Hence compared to the in vitro physiological environment, polymer R-2 showed a much rapid and improved in vivo rate of degradation. The faster degradation in vivo may be due in part to the foreign body response.[24,25] This response results in the accumulation of cells such as macrophages around the foreign body leading to a walling off of the region. Free radicals, acidic products, or enzymes produced by these cells during the foreign body response may accelerate degradation.

CONCLUSION

Degradation of free films of rosin-based polymers, R-1 and R-2, was studied in vitro and in vivo. Polymer R-1 did not show any significant degradation in the tests employed. Degradation was faster in vivo than in vitro by free films of polymer R-2.

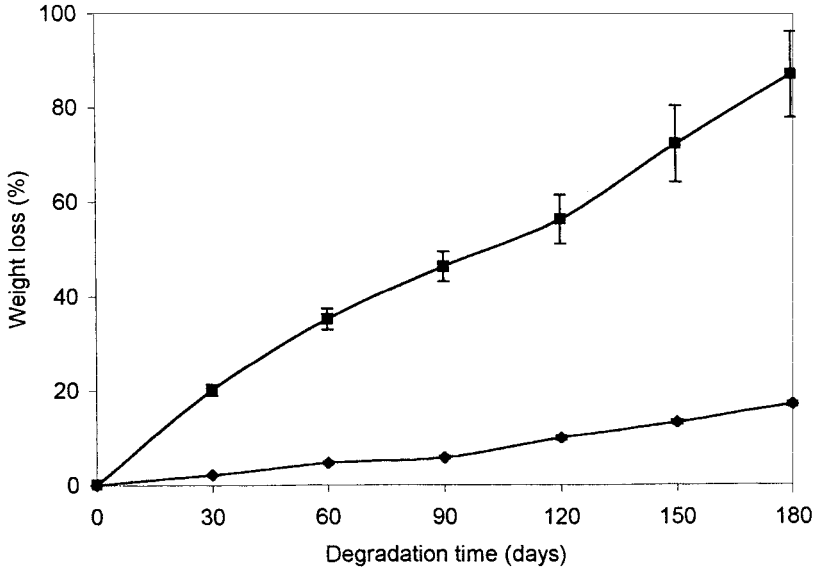

Figure 5. In vivo degradation of free films (◆) R-1; (■) R-2.

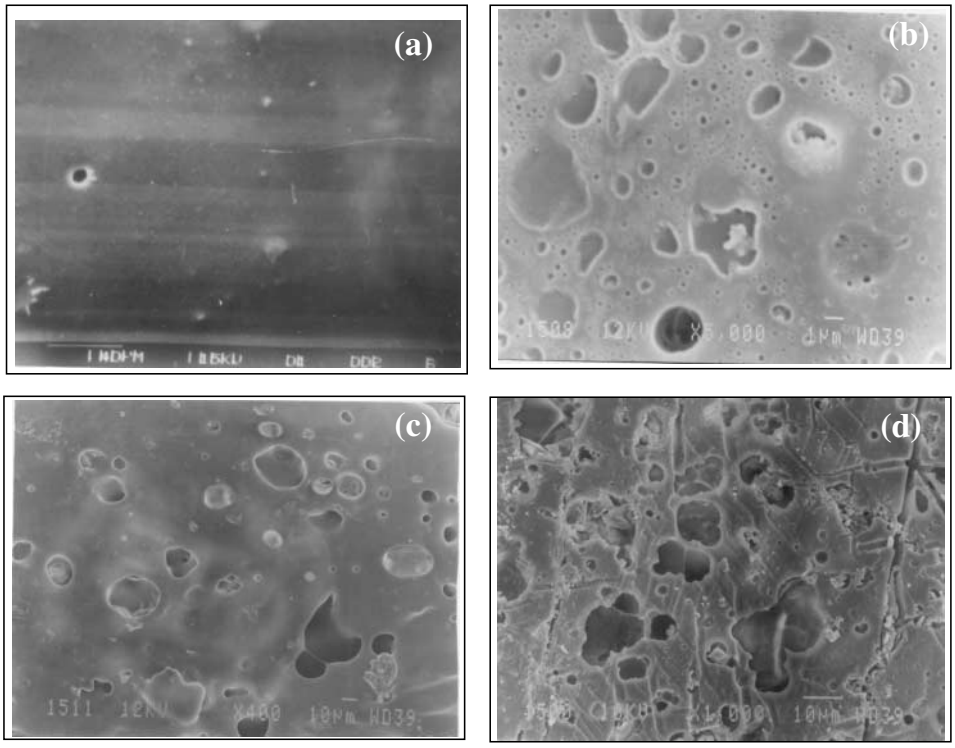

Figure 6. Scanning electron micrographs of (a) R1 free film, (b) R1 film degraded at pH 10.4, (c) R1 film degraded by enzyme pancreatine, (d) R1 film degraded in vivo.

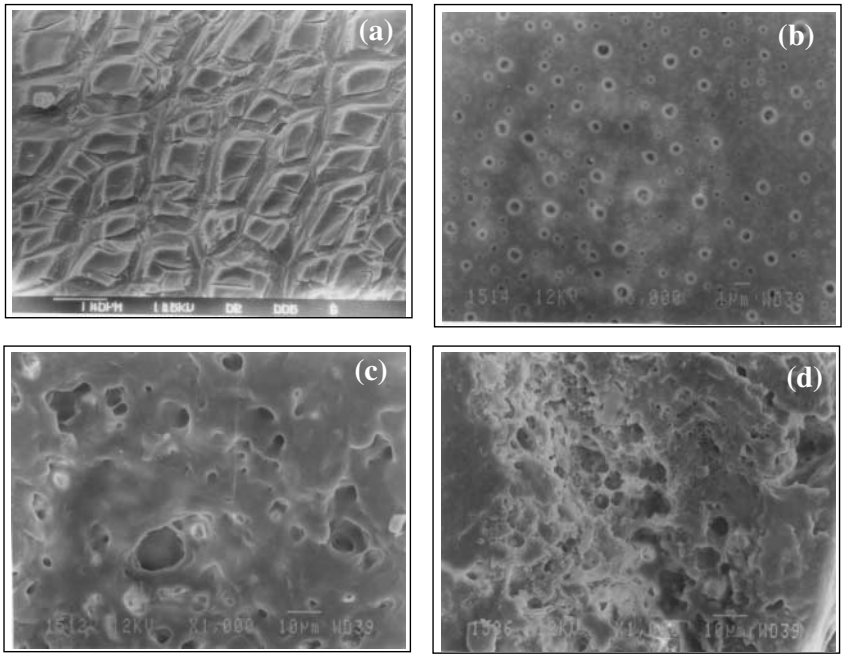

Figure 7. Scanning electron micrographs of (a) R2 free film, (b) R2 film degraded at pH 10.4, (c) R2 film degraded by enzyme pancreatine, (d) R2 film degraded in vivo.

With polymer R-2 the degradation rate was significantly affected by the pH of the hydrolytic medium. The degradation of both polymers did not show any significant effect on enzymes. Polymer R-2 degraded nearly 80–90% in terms of weight loss by 6 months, in vivo. The present study thus highlights the degradation behavior of two new polymers that may potentially be useful in various drug delivery applications.

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