Hydrolytic and enzymatic degradations of physically crosslinked hydrogels prepared from PLA/PEO/PLA triblock copolymers

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Degradable copolymers were synthesized by ring opening polymerization of lactide in the presence of poly(ethylene glycol) (PEG), using CaH_2 as a biocompatible initiator. The resulting PLA/PEO/PLA triblock copolymers were dissolved in a biocompatible solvent, namely tetraglycol. Physically crosslinked hydrogels were then prepared by introducing small amounts of water into the thus obtained solutions. Hydrolytic degradation of the highly swollen hydrogels was realized in 0.13 M pH = 7.4 phosphate buffer, while the enzymatic degradation was carried out in 0.05 M pH = 8.6 Tris buffer containing a PLA-degrading enzyme, proteinase K. In both cases, degradation was initially very fast with dramatic weight loss. The LA/EO ratio of the remaining material increased rapidly, in agreement with the release of PEO-rich segments. In a second phase, the degradation rate slowed down. The presence of proteinase K strongly accelerated the degradation rate of the hydrogels, indicating that the enzyme was able to penetrate inside and attack the PLA domains which constituted nanometric nodes in the gel network.
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1. Introduction

Hydrogels are composed of polymeric materials which can absorb large amounts of water and thus swell in aqueous media. In the swollen state, the properties of hydrogels strongly depend on the water content which can exceed 100% in the case of superabsorbants [1–4]. Degradable and biocompatible hydrogels are promising biomaterials for a number of pharamaceutical applications, in particular in protein and peptide delivery systems [5–7]. Bioactive molecules can be physically entrapped in the hydrogel or chemically attached to the polymer chains. In the case of physical entrapment, the hydrogel is loaded with a drug to form supersaturated solutions or suspensions of drug particles. Depending on the structure of the hydrogel matrix, the drug can be released through different mechanisms [3–5]. The most common mechanism is passive diffusion driven by the concentration gradient of the drug, and usually followed by a combination of both diffusion and degradation driven release. In contrast to implants or injectable microspheres, hydrogels do not require complicated fabrication processes which may denature protein drugs. When highly swollen, hydrogels can be injected through small trocars.

Basically, two types of hydrogels can be distinguished: physical hydrogels and chemical ones, depending on the nature of crosslinks. In a previous paper [8], we reported the release characteristics of physically entrapped proteins from degradable hydrogels of the PLA/PEO/PLA triblock copolymer-type. In these hydrogels, PEO blocks constitute the hydrophilic and swollen part, PLA blocks constituting nanometric nodes in the gel network [9]. The particularity of these gels is that they were phase separated by addition of water into an organic solution of the copolymers. The phase separation method led to a much higher degree of swelling than similar hydrogels prepared by swelling from the dry state.

The *in vitro* degradation of the PLA/PEO/PLA triblock copolymers in solid state has been studied in detail [10]. Dramatic compositional and morphological changes were observed during degradation. In this work, we considered the hydrolytic degradation of the hydrogels derived from PLA/PEO/PLA triblock copolymers. For the sake of comparison, the enzymatic degradation of these hydrogels was also considered in the presence of proteinase K, an enzyme which is known to affect PLA degradation. The results obtained from various analytical techniques are reported herein.

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2. Experimental

2.1. Materials

D,L-lactide (Purac) was recrystallized from acetone. PEG with molar masses 8000 and 20,000 (Fluka) were dried under vacuum prior to use. CaH_2 (40 mesh size) and tetraglycol (polyethylene glycol monotetrahydrofurfuryl ether) were used as received from Aldrich. Sodium azide was obtained from Merck. Trizma base, Trizma/HCl and proteinase K in the form of lyophilized powder (13 units/mg) were supplied by Sigma.

2.2. Polymerization

The triblock copolymers were synthesized by ring opening polymerization of D,L-lactide in the presence of PEG8000 or PEG20000, using CaH_2 as co-initiator [11, 12]. Predetermined amounts of D,L-lactide and of PEG were first introduced into a flask, the lactyl/ethylene oxide or LA/EO mole ratio being 2.5/1 for PEG8000 and 4/1 PEG20000, respectively. The selected catalyst, CaH_2 (in 1/1 mole ratio with respect to hydroxyl endgroups of PEG), was then added. After degassing, the flask was sealed under vaccum and the polymerization was allowed to proceed under gentle stirring at 140 °C. After five days, the product was recovered by dissolution in $CHCl_3$, followed by filtration on sintered glass filter and precipitation in ethanol. Finally, the product was dried under reduced pressure until constant weight.

2.3. Preparation of hydrogels

Four hundred milligrams of polymer were dissolved in tetraglycol at 40 °C in a 5 ml vial. After cooling to room temperature, 2 ml of bidistilled water were added into the polymer solution which was allowed to swell during two days. The resulting disk-shaped gel was then recovered and immersed in 10 ml of bidistilled water to remove the solvent. The aqueous solution was changed every 12 h for a period of two days. Thereafter, the hydrogel was taken out and gently wiped. It was weighed when no further weight change was detected.

2.4. Hydrolytic degradation

Hydrogel disks were prepared according to the procedure described above. They were then placed in vials filled with 10 ml of 0.13 M pH = 7.4 phosphate buffer and incubated at 37 °C. After predetermined time intervals, the hydrogel samples were removed from the buffer and gently rinsed with three portions of distilled water. They were dried under vacuum up to constant weight and then subjected to various analyses. The soluble degradation products were recovered by freeze-drying the solution, followed by extraction with warm $CDCl_3$ or DMSO-D_6 for proton nuclear magnetic resonance (^{1}H NMR) analysis.

2.5. Enzymatic degradation

Gel disks were immersed in vials filled with 3 ml of 0.05 M pH = 8.6 Tris buffer containing 0.8 mg of proteinase K. The buffer solutions were freshly exchanged every 24 h. At appropriate time intervals, the gels were collected from the vials and rinsed with distilled water. After vacuum drying up to constant weight, the samples were weighed and subjected to various analyses. The soluble degradation products were recovered as described above.

2.6. Measurements

^{1}H NMR spectra were recorded at 30 °C with a Bruker spectrometer operating at 360 MHz. $CDCl_3$ or DMSO-d_6 were used as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane (TMS) as an internal reference. Fourrier transformed infrared (FTIR) spectra were recorded on a Perkin Elmer 1760 spectrometer. Thin films were cast from chloroform solutions onto a NaCl plate.

3. Results and discussion

3.1. Characterization of the copolymers

The copolymers are composed of a PEO central block bearing PLA sequences at both ends. They are identified in this paper by the acronym $PLA_x/PEO_y/PLA_x$ where $x = \overline{DP}_{PLA}$ and $y = \overline{DP}_{PEO}$ represent the mean number average degree of polymerization of PLA and PEO blocks, respectively. Table I presents the characteristics of the two copolymers considered in this work.

The LA/EO ratio in the copolymers was determined from the integration areas of the resonance due to PEO blocks at 3.6 ppm (-O-CH_2-CH_2, singlet) and to PLA blocks at 5.19 ppm (-CH, quartet) as previously described in literature [11–14]. The number average molar mass, $\overline{M}_n$, was calculated according to the following equation:

$$\overline{M}_n = \overline{DP}_{PEO} \times 44 + \overline{DP}_{PLA} \times 2 \times 72 \qquad (1)$$

where

$$\overline{DP}_{PEO} = \overline{M}_{n\,PEG} / 44, \quad \overline{DP}_{PLA} = \overline{DP}_{PEO} \times (LA/EO)/2.$$

As shown in Table I, the LA/EO molar ratio was close to that of the corresponding feed in the case of PEG8000,

TABLE I $PLA_x/PEO_y/PLA_x$ triblock copolymers obtained from polymerization of D,L-lactide in the presence of PEG8000 and PEG20000 using CaH_2 as co-initiator

Copolymer PLA/PEO/PLA	LA/EO in feed	LA/EO in product[a]	$\overline{DP}_{PEO}$[b]	$\overline{DP}_{PLA}$[b]	$\overline{M}_n$[c]	Yield (%)
$PLA_{264}/PEO_{182}/PLA_{264}$	2.5	2.9	182	264	45 750	80
$PLA_{410}/PEO_{454}/PLA_{410}$	4	1.8	454	410	78 930	35

[a]Determined by using the integration ratio of resonances due to PEO blocks at 3.6 ppm and to PLA blocks at 5.19 ppm in the ^{1}H NMR spectra.
[b]$\overline{DP}_{PEO} = \overline{M}_{n\,PEO}/44$, $\overline{DP}_{PLA} = \overline{DP}_{PEO} \times (LA/EO)/2$.
[c]$\overline{M}_n = 44 \times \overline{DP}_{PEO} + 2 \times 72 \times \overline{DP}_{PLA}$.

which suggested a good conversion of monomer. However, when PEG20000 was used, the final LA/EO molar ratio was lower than that of the feed. This means that the conversion ratio of lactide was relatively low, probably because of the lack of reaction sites or endgroups in the case of PEG of high molar mass.

3.2. Preparation of hydrogels

Hydrogels were prepared by slowly adding small amounts of distilled water over tetraglycol solutions of the triblock copolymers. After water absorption and swelling during two days, a consistent and highly swollen hydrogel was formed. Tetraglycol was selected as the solvent for the preparation of hydrogels because of its low toxicity which allowed it to be used in a number of medications formulated as injectable solutions [15, 16]. Even though, tetraglycol was carefully eliminated by washing with distilled water, ^{1}H NMR analysis of the washed gels showed that the amount of residual tetraglycol was negligible.

The degree of swelling (DS) of the hydrogels was deduced from the weights of the swollen gel and the dry polymer. The DS value was 630% for $PLA_{410}/PEO_{454}/PLA_{410}$, i.e. three times higher than that obtained for $PLA_{264}/PEO_{182}/PLA_{264}$ (200%). This means that the amount of water absorbed in the hydrogel was correlated with the PEO content or LA/EO ratio of the triblock copolymer. The higher the PEO content or the lower the LA/EO ratio, the higher the DS of the hydrogel.

3.3. Hydrolytic degradation

The hydrolytic degradation of both hydrogels was carried out in a pH = 7.4 phosphate buffer at 37 °C. Fig. 1 shows the weight loss profiles. During the first three days, a small weight loss (less than 10%) was observed in both cases. Afterwards, weight loss increased rapidly to reach 61.6% after seven days and 70% after fifteen days in the case of $PLA_{410}/PEO_{454}/PLA_{410}$. Beyond fifteen days, weight loss appeared to level off. In the case of $PLA_{264}/PEO_{182}/PLA_{264}$, weight loss rapidly reached 49.1% after ten days. Thereafter, weight loss continued to slowly increase to reach 76.8% at the end of the 30 days' period.

^{1}H NMR analysis was used to detect changes in LA/EO ratio (Fig. 2). In the case of $PLA_{410}/PEO_{454}/PLA_{410}$, the LA/EO ratio increased slightly from 1.8 to 2.5 during the first three days. From three to ten days, the ratio increased quickly to reach 27.9. Thereafter, it remained almost constant. In contrast, for $PLA_{264}/PEO_{182}/PLA_{264}$, the LA/EO ratio increased slowly and continuously: from 2.9 to 11.8 within 26 days. A slight decrease of LA/EO ratio was observed from 26 to 31 days.

It is of interest to compare weight losses and LA/EO ratio changes during degradation. PLA blocks degraded to form soluble products, including PLA oligomers and PEO blocks with short PLA segments, thus leading to weight loss and LA/EO ratio changes. The fact the LA/EO ratio strongly increased suggested that the release of PEO-rich segments was predominant. In the case of $PLA_{410}/PEO_{454}/PLA_{410}$, the profile of weight loss was similar to that of LA/EO ratio change, indicating that the rapid weight loss observed during the first stage was due to the release of water soluble PEO-rich products. Beyond ten days, little changes of weight loss and LA/EO ratio were observed because of the slow degradation of the PLA-rich residual hydrogel. In the case of $PLA_{264}/PEO_{182}/PLA_{264}$ which had a higher LA/EO ratio before degradation, both weight loss and LA/EO ratio increases were slower than those of $PLA_{410}/PEO_{454}/PLA_{410}$. The difference could be assigned to the higher LA/EO ratio and lower molar mass of PEO segments of $PLA_{264}/PEO_{182}/PLA_{264}$. The dissolution of degradation products should be favored in the gel derived from $PLA_{410}/PEO_{454}/PLA_{410}$ because it contained longer hydrophilic PEO chains which should help to dissolve longer PLA segments.

^{1}H NMR analysis was also used to monitor the appearance of degradation products dissolved in the buffer solution. Fig. 3 shows the spectrum of the filtrate corresponding to the soluble degradation products of $PLA_{264}/PEO_{182}/PLA_{264}$ after 22 days. The most intense signal located at 3.5 ppm belongs to methylene protons of

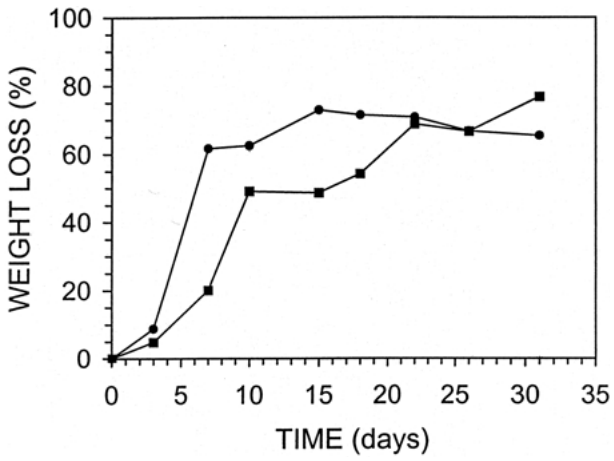

Figure 1 Weight loss profiles of hydrogels issued from $PLA_{410}/PEO_{454}/PLA_{410}$ (●) and $PLA_{264}/PEO_{182}/PLA_{264}$ (■) during hydrolytic degradation.

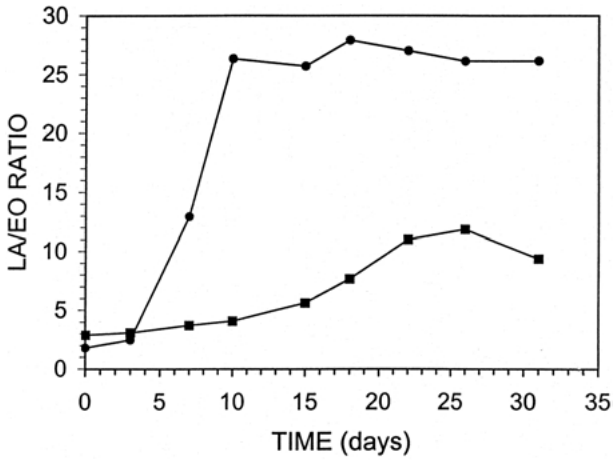

Figure 2 LA/EO ratio changes of hydrogels issued from $PLA_{410}/PEO_{454}/PLA_{410}$ (●) and $PLA_{264}/PEO_{182}/PLA_{264}$ (■) during hydrolytic degradation.

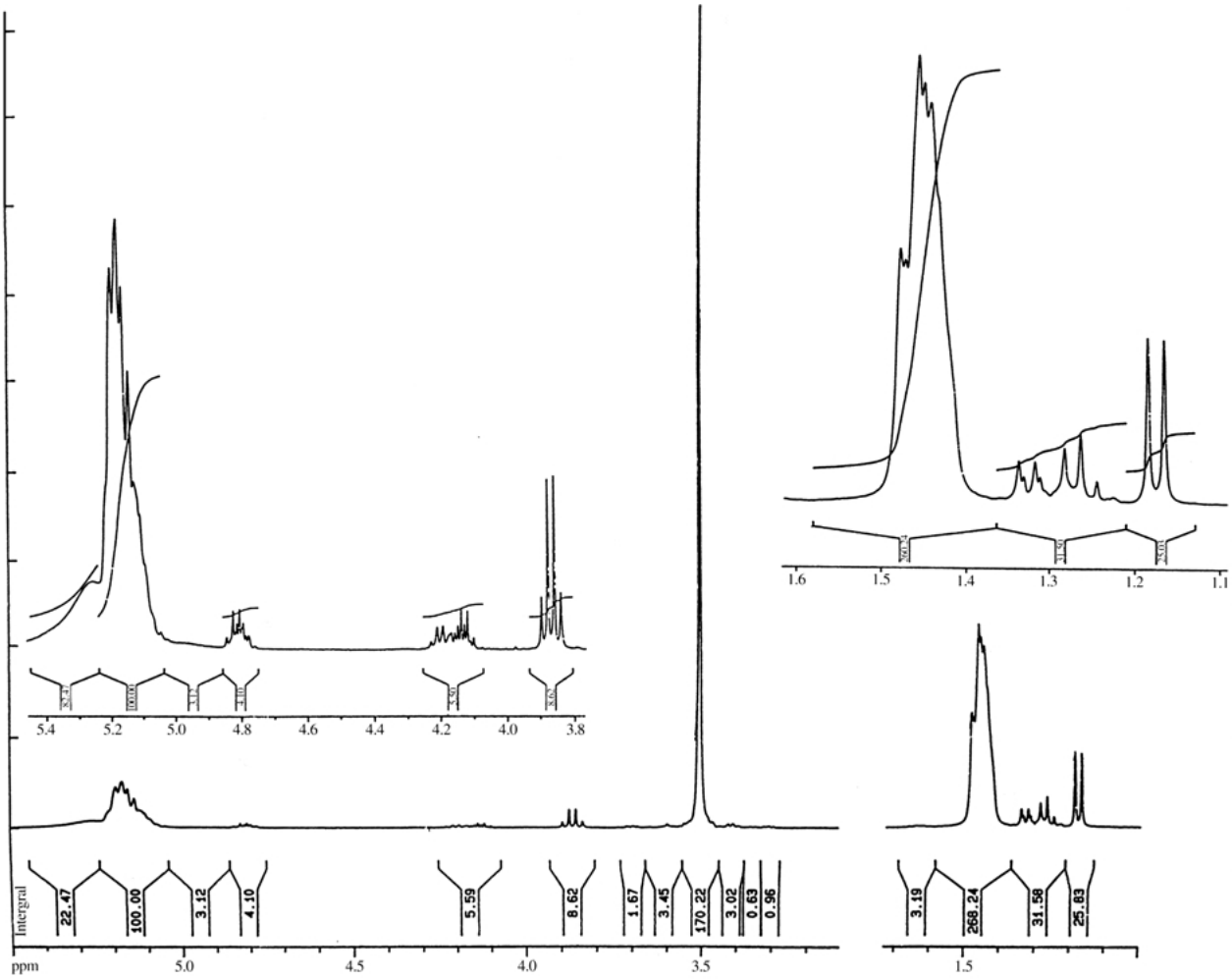

Figure 3 ^{1}H NMR spectrum of the filtrate corresponding to the soluble degradation products of $PLA_{264}/PEO_{182}/PLA_{264}$ after 22 days hydrolytic degradation.

PEO blocks, in agreement with the presence of soluble compounds with a high PEO content. The signals in the 5.2–5.1 ppm and 1.5–1.4 ppm ranges corresponding to methine and methyl protons of PLA segments either free or attached to PEO blocks. Moreover, one can clearly distinguish a quartet at 3.87 ppm and a doublet at 1.18 ppm which were assigned to methine and methyl protons of lactate, respectively. In comparison with the chemical shift values of lactic acid (4.03 and 1.23 ppm for methine and methyl protons) [17], an upfield shift was observed for lactate, in agreement with the higher electron-repulsive effect of lactate in comparison with its carboxylic acid form, i.e. lactic acid. Methine protons of lactate exhibited a larger shift (0.16 ppm) than methyl ones (0.05 ppm).

The signals in the 4.9–4.7 ppm range were assigned to methine protons in carboxylated lactyl end units of dimer and higher oligomers, while the signals in the 4.3–4.1 ppm range were assigned to methine protons in hydroxylated lactyl end units and the α-methylene protons of PLA-linking EO units [11]. Similarly, the signals in the 1.4–1.2 ppm range were assigned to methyl protons of free dimer and higher oligomers as well as hydroxylated lactyl end units. These assignments were made possible by the use of DMSO-d_6 known as a resolution-enhancing solvent for NMR analysis of PLA polymers [17].

IR analysis provided qualitative information on composition changes of the copolymers during degradation. The IR spectrum of $PLA_{264}/PEO_{182}/PLA_{264}$ showed the carbonyl stretching band at 1756 cm^{-1} and the C-H stretching bands at 2994 and 2943 cm^{-1} typical of PLA blocks, and the C-H stretching band at 2876 cm^{-1} typical of PEO blocks (Fig. 4). After ten days degradation, the relative intensity of the band at 2876 cm^{-1} decreased, suggesting a decrease in PEO content in the residual hydrogel. In contrast, the IR spectrum of the filtrate showed that the intensity of the absorption band of PEO blocks, at 2876 cm^{-1} increased with respect to the carbonyl stretching band at 1756 cm^{-1} due to PLA blocks, in agreement with the high PEO content in the soluble degradation products. Similar features were observed for $PLA_{410}/PEO_{454}/PLA_{410}$ (Fig. 5). The relative intensity of the absorption band of PEO blocks at 2880 cm^{-1} was higher than in the case of $PLA_{264}/PEO_{182}/PLA_{264}$, indicating a higher PEO content in the filtrate of $PLA_{410}/PEO_{454}/PLA_{410}$.

The degradation of hydrogels issued from PLA/PEO/PLA triblock copolymers appeared to be faster than that of films or plates from similar copolymers as evidenced by weight loss and LA/EO ratio data [10, 18]. This finding can be assigned to the swollen state of the hydrogels which facilitated degradation and release of soluble segments.

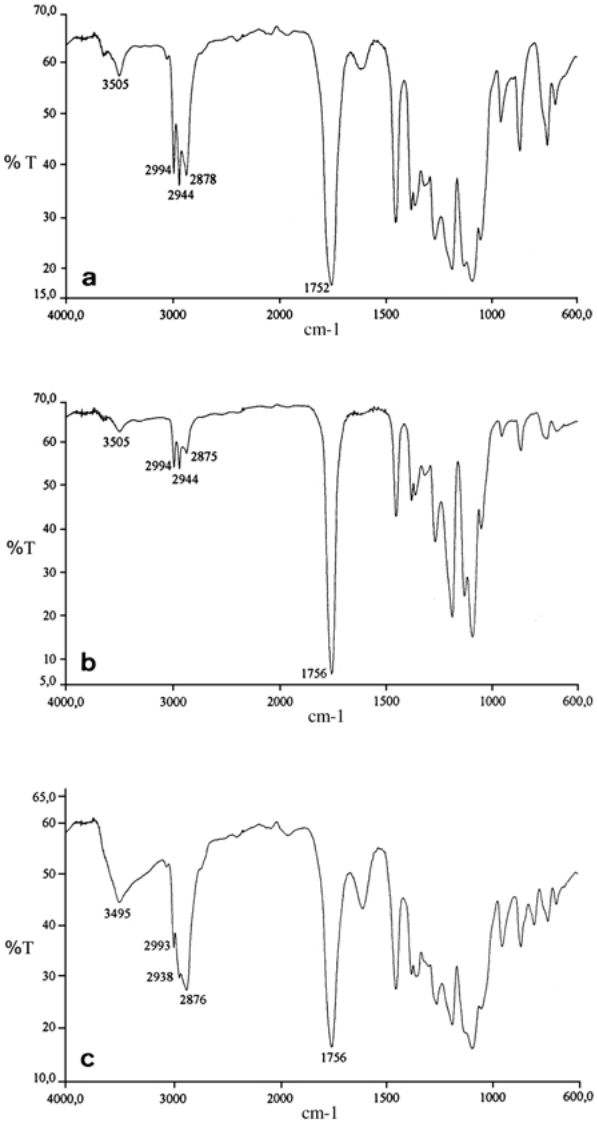

Figure 4 IR spectra of the initial copolymer (a), residual hydrogel (b) and filtrate (c) of $PLA_{264}/PEO_{182}/PLA_{264}$ after 10 days hydrolytic degradation.

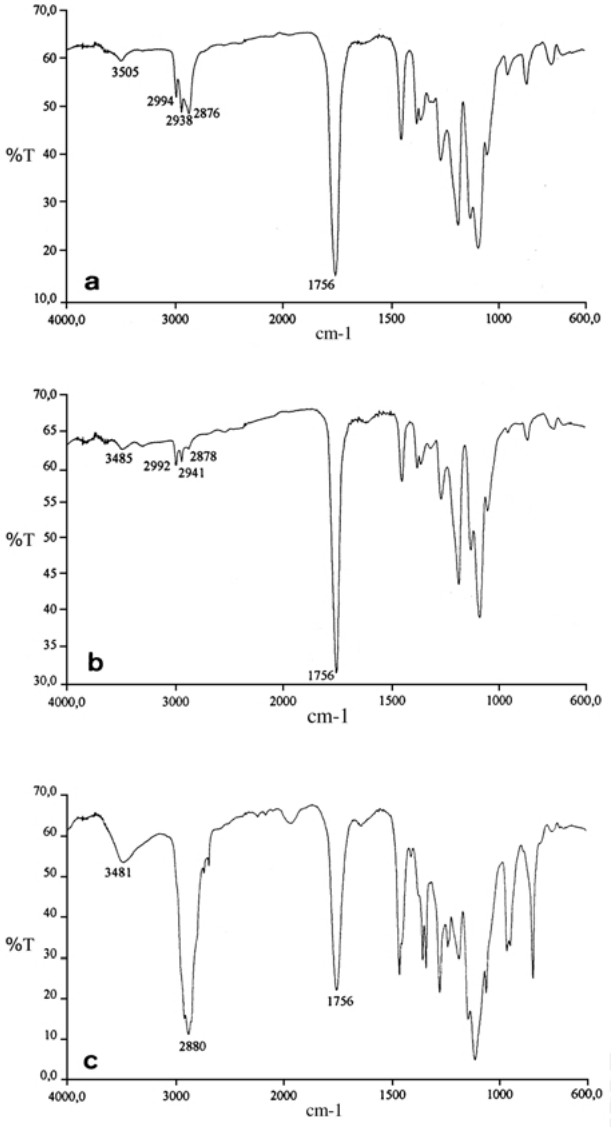

Figure 5 IR spectra of the initial copolymer (a), residual hydrogel (b) and filtrate (c) of $PLA_{410}/PEO_{454}/PLA_{410}$ after 10 days hydrolytic degradation.

3.4. Enzymatic degradation

The enzymatic degradation of the hydrogel issued from $PLA_{264}/PEO_{182}/PLA_{264}$ was studied using proteinase K, an enzyme capable of degrading high molar mass PLA [19]. Fig. 6 shows the weight loss profile of the hydrogel during enzymatic degradation, in comparison with that of the hydrolytic degradation. A much faster weight loss was observed in the former case. After 80 h in the presence of proteinase K, the hydrogel lost 56.9% of its initial material, in contrast to a weight loss value of 5% for the hydrolytic degradation. Therefore, the presence of proteinase K accelerated the degradation of the hydrogel as in the case of PLA polymers [19]. This finding suggests that the enzyme could penetrate the gel network in which PLA blocks constitute the nodes, in agreement with the fact that rather large proteins like fibrinogen and albumin could leach out from similar hydrogels [8].

The recovered hydrogel remnants and the corresponding filtrates were both analyzed by ^{1}H NMR. The LA/EO ratio in the residual hydrogel was found to increase as in the case of the hydrolytic degradation. After three days for example, the LA/EO ratio in the residual hydrogel increased to 3.1, i.e. equal to the value found for the hydrolytic degradation. This was in agreement with the fact that proteinase K attacked PLA segments, but the release of PEO-rich blocks was predominant. Actually, after only 6 h of enzymatic degradation, signals corresponding to PEO blocks, lactate and lactylactate were observed on the ^{1}H NMR spectrum of the filtrate.

4. Conclusion

The physically crosslinked hydrogels prepared from PLA/PEO/PLA triblock copolymers presented interesting degradation properties. The hydrolytic degradation was initially very fast with dramatic weight loss. LA/EO ratio of the remaining material also rapidly increased, in agreement with the release of PEO-rich segments. Beyond, the degradation rate slowed down because of the high PLA content in the residual

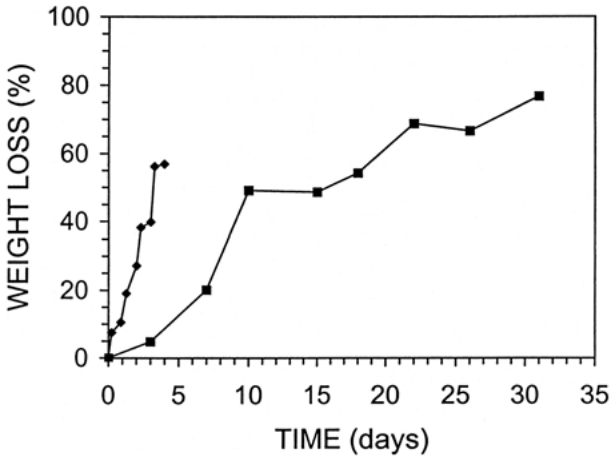

Figure 6 Weight loss profiles of hydrogels issued from $PLA_{264}/PEO_{182}/PLA_{264}$ during hydrolytic degradation (■) and enzymatic degradation (◆).

hydrogel. The presence of proteinase K strongly accelerated the degradation rate of the hydrogels, indicating that the enzyme is able to penetrate inside and attack the PLA domains which constitute the nodes in the gel network.

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