Rheology and Drug Release Properties of Bioresorbable Hydrogels Prepared from Polylactide/Poly(ethylene glycol) Block Copolymers

Suming Li, Abdelslam El Ghzaoui, Emilie Dewinck*

Centre de Recherche sur les Biopolymères Artificiels, Faculté de Pharmacie, 15 Avenue Charles Flahault, 34060 Montpellier, France
E-mail: lisuming@univ-montp1.fr

Summary: Ring-opening polymerization of L(D)-lactide was realized in the presence of poly(ethylene glycol) (PEG), yielding PLLA/PEG and PDLA/PEG block copolymers. Bioresorbable hydrogels were prepared from aqueous solutions containing both copolymers due to interactions and stereocomplexation between PLLA and PDLA blocks. The rheological properties of the hydrogels were investigated under various conditions by changing copolymer concentration, temperature, time and frequency. The hydrogels constitute a dynamic and evolutive system because of continuous formation/destruction of crosslinks and degradation. Drug release studies were performed on hydrogel systems containing bovine serum albumin (BSA). The release profiles appear almost constant with little burst effect. The release rate depends not only on gelation conditions such as time and temperature, but also on factors such as drug load, as well as molar mass and concentration of the copolymers.

Keywords: bioresorbable; bovine serum albumin; hydrogel; poly(ethylene glycol); polylactide; rheology; stereocomplex

Introduction

Aliphatic polyesters such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(ε-caprolactone) (PCL) have been investigated worldwide as biomaterials due to their biocompatibility and degradability.[1-3] These polymers are very atractive biomaterials for temporary therapeutic applications such as sutures, osteosynthetic devices, sustained drug delivery systems (DDS), and scaffolds in tissue engineering.

Hydrogels present growing interest for applications as DDS because of their excellent biocompatibility due to the presence of large amounts of water.[4-6] Bioactive molecules can be physically entrapped in a hydrogel or chemically attached to the polymeric network. Hydrogels are usually formed by a hydrophilic polymer matrix crosslinked chemically through covalent bonds or physically through hydrogen bonds, crystillized domains or

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hydrophobic interactions. They are particularly interesting for the release of poorly soluble drugs, proteins, genes or nucleic acids as the drugs can be protected from hostile environments, e.g. the presence of enzymes, cells or low pH in the stomach.[7]

Among the various gel systems, degradable, injectable and thermosensitive hydrogels appear the most promising. Kim et al. reported hydrogels prepared from triblock copolymers containing both poly(ethylene glycol) (PEG) and poly(lactide-co-glycolide) (PLGA) blocks.[8-10] A sol-gel transition was observed which depends on the concentration and composition of the copolymers. Hennink et al. prepared a self-assembled hydrogel from enantiomeric PLA oligomers grafted to dextran. The hydrogel was formed due to the stereocomplexation of poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) blocks.[11-13] Later on, Grijpma and Feijen reported formation of a hydrogel by stereocomplexation of water-soluble PLLA-PEG-PLLA and PDLA-PEG-PDLA copolymers.[14]

In a series of articles, we reported the synthesis, characterization and stereocomplex-induced gelation of PLLA/PEG and PDLA/PEG copolymers.[15-17] The copolymers were synthesized by ring opening polymerization of L(D)-lactide in the presence of mono- or dihydroxyl PEG, using zinc metal as catalyst. Hydrogels were obtained from aqueous solutions containing both PLLA/PEG and PDLA/PEG block copolymers due to stereocomplexation occurring between PLLA and PDLA blocks. In this paper, we wish to report on the rheological properties and drug release behaviors of these bioresorbable hydrogels.

Experimental Section

L-lactide and D-lactide were obtained from Purac and recrystallized from acetone. Dihydroxyl PEG with molar masses of 10000, 12000 and 20000g/mol and monomethoxy poly(ethylene glycol) (mPEG) with molar mass of 5000g/mol were supplied by Fluka. Zinc powder was purchased from Merck. Bovine serum albumin (BSA, Fraction V, pH 7.0) was supplied by Across Organics. Copolymers were synthesized by ring opening polymerization of L(D)-lactide in the presence of mono- or dihydroxyl PEG by using zinc metal as catalyst, as previously reported.[15]

Predetermined amounts of PLLA/PEG and PDLA/PEG copolymers were mixed in 2 ml of distilled water. The aqueous solutions were then centrifuged to yield a homogeneous fluid. Gelation was then allowed to proceed at predetermined temperatures for various periods of time. BSA-containing hydrogels were prepared under similar conditions, BSA being mixed in the aqueous solution before gelation.

2 ml of BSA-containing hydrogel samples were immersed at 37°C in 4 ml of phosphate buffered saline (PBS). The release was regularly monitored by U.V. at 277 nm, using calibration curves obtained from standard solutions. 2 ml of buffer solution were taken out from the release medium, and placed in a 20 mm x 10 mm square quartz cell for each measurement. 2 ml of new PBS solution were then added to the release medium in order to maintain the same volume.

^{1}H Nuclear magnetic resonance (NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 250 MHz by using DMSO-d_6 as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

Rheological properties were determined on a Carri-Med CSL2 Rheometer of TA Instruments. For all the experiments, a cone-plate measuring geometry was used (steel, 4 cm diameter with an angle of 2 degrees, gap 56 μm). A solvent trap was used to prevent water evaporation. Measurements were realized in the linear viscoelastic range.

The release of BSA was monitored by a Lambda 15 Perkin Elmer UV-Vis spectrophotometer.

Circular dichroism (CD) spectra were registered with a Jobin Yvon CD6 instrument. The double monochromator and cell compartment were flushed with nitrogen at a flow rate of 9 l/min. A quartz cell with l = 0.1 cm was used. The CD spectra were obtained from five scans with an integration time of 0.3 seconds.

Results and Discussion

PLA-PEG-PLA triblock copolymers were synthesized by ring opening polymerization of L(D)-lactide in the presence of PEG with molar masses of 10000, 12000 or 20000. Similarly, PLLA-PEG and PDLA-PEG diblock copolymers were synthesized by using mPEG5000 as initiator. Non-toxic Zn powder was used as catalyst instead of stannous octoate which can be more or less cytotoxic.[18,19] Table 1 presents the molecular characteristics of the triblock and diblock copolymers used in this work. The molar ratio of ethylene oxide/lactyl (EO/LA) repeating units was in the range of 4 to 11 for the water solubility of the copolymers.

Table 1. PLA/PEG block copolymers obtained by ring opening polymerization of L(D)-lactide in the presence of PEG or mPEG.

Acronym	Structure	Initiator	Monomer	EO/LA [a]	$\overline{DP}_{PEG}$ [b]	$\overline{DP}_{PLA}$ [c]	$\overline{M}_n$ [d]
1L	$L_{19}EO_{227}L_{19}$	PEG10000	L-lactide	6.1	227	38	12700
1D	$D_{20}EO_{227}D_{20}$	PEG10000	D-lactide	5.6	227	40	13700
2L	$L_{20}EO_{273}L_{20}$	PEG12000	L-lactide	6.8	273	40	14900
2D	$D_{19}EO_{273}D_{19}$	PEG12000	D-lactide	7.3	273	38	14700
3L	$L_{21}EO_{454}L_{21}$	PEG20000	L-lactide	11.0	454	42	23000
3D	$D_{22}EO_{454}D_{22}$	PEG20000	D-lactide	10.5	454	44	23100
4L	$L_{28}EO_{113}$	mPEG5000	L-lactide	4.1	113	28	7000
4D	$D_{27}EO_{113}$	mPEG5000	D-lactide	4.2	113	27	6900

a) calculated from the integration of NMR bands belonging to PEG blocks at 3.6 ppm and to PLA blocks at 5.19 ppm.

b) $\overline{DP}_{PEO} = \overline{M}_{n\,PEG} / 44$

c) $\overline{DP}_{PLA} = \overline{DP}_{PEO} / (EO/LA)$

$\overline{M}_n = \overline{M}_{n\,PEG} + \overline{DP}_{PLA} \bullet 72$

Stereocomplexation is a well known phenomenon for optically active PLA stereocopolymers.[20-24] Stereocomplex can be obtained from co-precipitation of PLLA and PDLA in solution,[20,21] or through cooling from a melt of both polymers.[22] In the case of enantiomeric PLA-PEG-PLA copolymers, stereocomplex was obtained by co-precipitation or solution casting from homogeneous solutions.[23,24]

The phenomenon of stereocomplexation between PLLA and PDLA blocks was used to prepare hydrogels from aqueous solutions containing equal amounts of PLLA/PEG and PDLA/PEG copolymers. Figure 1 shows the evolution of storage modulus (G') and loss modulus (G") of a 14% 1L/1D solution as a function of time at 25°C and at a frequency of 1 Hz. Initially, G" was higher than G', the solution behaving as a viscoelastic solution. Both G' and G" slightly decreased at first and remained constant during the first 60 min. Beyond, the moduli increased continuously, G' increasing faster than G". A crossover point was observed at 7 h. After that, G' became higher than G" and a hydrogel was formed.

Figure 2 shows the changes of storage and loss moduli of the 14% 1L/1D sample as a function of frequency at t = 0 and t = 24h. Both moduli increased with increasing frequency. At t = 0, the storage modulus G' was higher than the loss modulus G", and both moduli increased almost linearly with frequency, which is characteristic of a viscoelastic liquid-like state. In contrast, at t = 24h, G' became higher than G", and both moduli tended towards a plateau at high frequency, which can be assigned to formation of a tridimensional network.

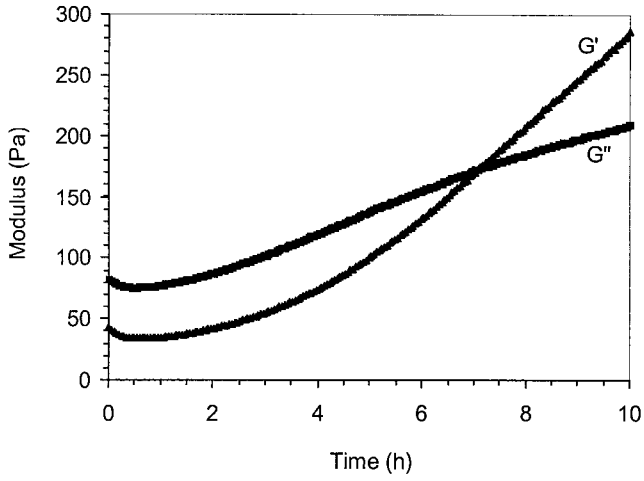

Figure 1. Time-dependent changes of storage modulus (G') and viscous modulus (G") of a 14% 1L/1D sample at 25°C and at 1Hz.

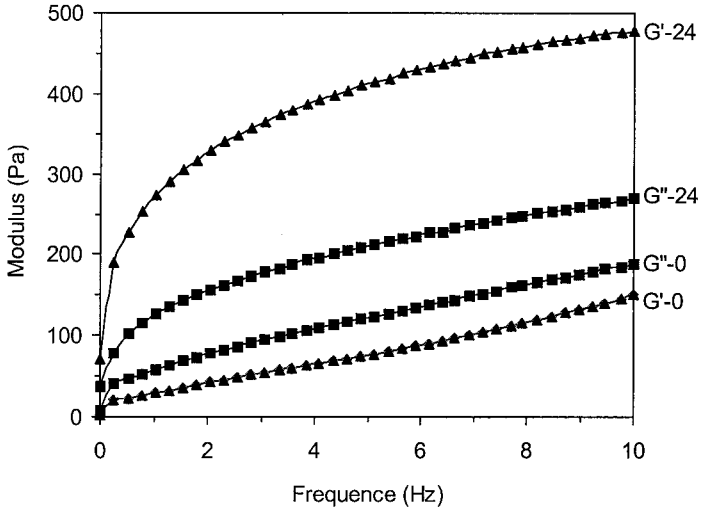

Figure 2. Changes of storage modulus (G') and loss modulus (G") of a 14% 1L/1D solution as a function of frequency at t = 0 and t = 24h at 25°C.

Rheological property changes of the 14% 1L/1D sample were followed for longer periods of time up to 336 h. Figure 3 presents the evolution of G' at 25°C as a function of frequency at

different time intervals. The G' value was initially rather low. After 24 h, a large increase was observed for the whole frequency range. At 1 Hz, for example, G' increased from initial 29 Pa to 274 Pa at t = 24 h. Afterwards, G' continued to increase but at a slower rate. After 336 h, G' at 1 Hz reached 507 Pa. These findings show that the hydrogel became more and more consistent as a function of time. A constant increase was also observed for G". At 1 Hz, G" increased from initial 58 Pa to 126 Pa at t = 24 h, and to 268 Pa after 336 h. From 24 h to 336 h, G" remained lower than G', the system behaving as a hydrogel.

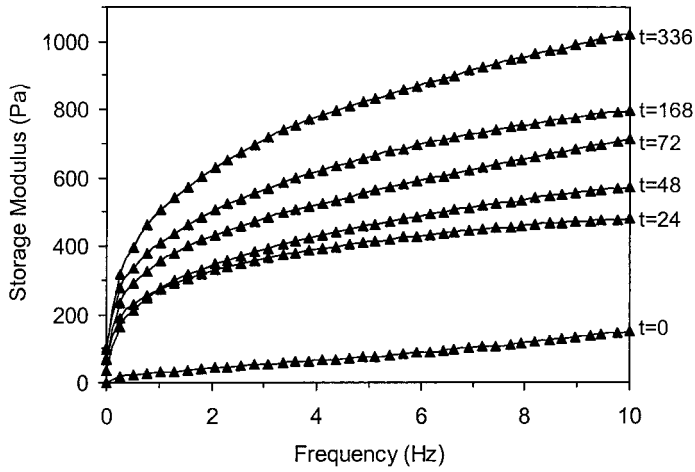

Figure 3. Storage modulus (G') changes of a 14% 1L/1D sample as a function of frequency at t = 0, 24, 48, 72, 168 and 336 h at 25°C.

Hydrogel formation depends also on the molar masses of the copolymers. In the case of PEG10000-derived 1L/1D, hydrogels were obtained at a concentration of 14%, while for PEG20000-derived 3L/3D samples, hydrogels were formed at a concentration of 8%. The higher the molar masse, the lower the concentration at which hydrogels can be obtained.

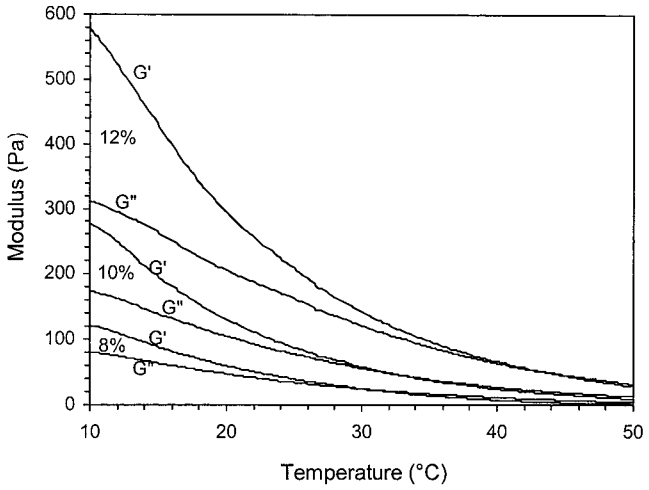

Figure 4. Temperature-dependent modulus changes of 2L/2D samples with concentrations of 8%, 10% and 12% at 1 Hz.

Modulus changes as a function of temperature were followed for 3L/3D samples of 8%, 10% and 12%, as shown in Figure 4. The temperature was increased from 10°C to 50°C at a heating rate of 1°C/min. The modulus values appeared higher for higher concentrations. Both G' and G" decreased with increasing temperature, G' decreasing faster than G". The modulus decrease could be assigned to the destruction of crosslinks at higher temperatures. A gel-sol transition was obtained at 31°C, 33° and 43°C for 8%, 10% and 12% samples, respectively. Therefore, the higher the concentration, the higher the G' and G" values, and the higher the gel-sol transition temperature. The thermo-versibility of the system was examined by following the modulus changes during heating and cooling processes. Data show that the gelation process is reversible although G' and G" values were not exactly the same during cooling and heating processes.

Comparisons were made between 3L/3D samples and 3L ones in order to elucidate the occurrence of L/D interactions and stereocomplexation. Figure 5 shows comparatively the time-dependent modulus changes of 8% 3L/3D and 3L samples. Both moduli of 3L were lower than those of 3L/3D. The 3L sample remained a viscous solution during the 10 h scan, although G' and G" slowly increased. In the case of 3L/3D, it was initially a hydrogel as G'

was slightly higher than G". Both moduli increased with time, G' increasing more rapidly than G".

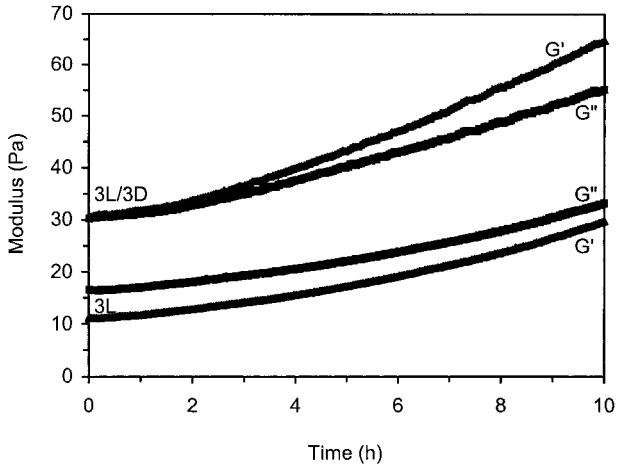

Figure 5. Time-dependent modulus changes of 8% 3L and 3L/3D samples at 25°C and at 1Hz.

In the case of 4L/4D copolymers with a diblock structure and lower molar masses, hydrogels were formed only for concentrations higher than 16%. Figure 6 shows the modulus changes of a 20% 4L/4D sample as a function of frequency at 37°C and at t = 0, 48 and 168 h. The sample was initially in the state of a gel, G' being largely higher than G". After 48 h, both moduli strongly increased, indicating that the gelation process continued at 37°C and the gel became much more consistent. After 168 h, the moduli slightly decreased as compared to values at 48 h, which can be assigned to the partial degradation of the copolymers. In fact, the hydrogel is a dynamic and evolutive system, gelation and degradation occurring simultaneously. Degradation was enhanced at 37°C.

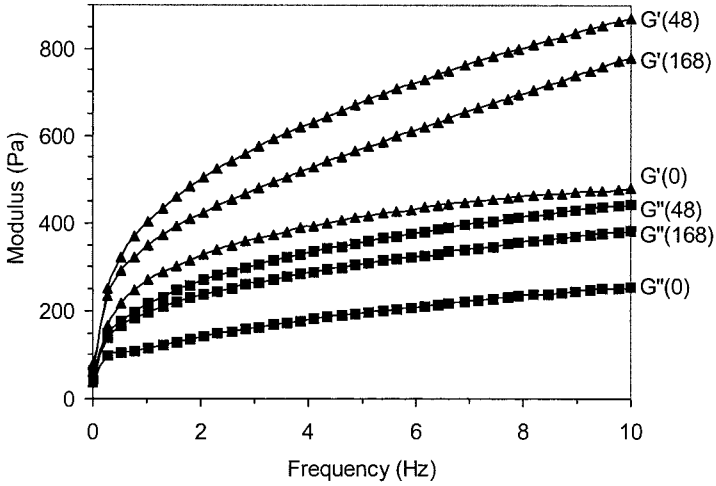

Figure 6. Modulus changes of a 20% 4L/4D sample as a function of frequency at 37°C and at t = 0, 48 and 168 h.

Bovine serum albumine (BSA) was retained as a model drug for release studies. The protein was mixed with the copolymer solution before gel formation. Various BSA-containing hydrogels were prepared under different gelation conditions in order to elucidate the drug release behaviors. Figure 7 shows the BSA release profiles of 20% 2L/2D hydrogels obtained after 90 h at 37°C or after 24 h at 50°C. The release rate appeared almost constant and there was almost no burst release in both cases. On the other hand, BSA release appeared faster from the hydrogel obtained at 37°C than from the one obtained at 50°C, even though the gelation time was longer for the former than for the latter. Nearly 38% and 24% of BSA were released after 360 h, respectively. The difference can be assigned to the fact gelation at 50°C led to much more consistent hydrogel structure than at 37°C, which disfavored drug diffusion.

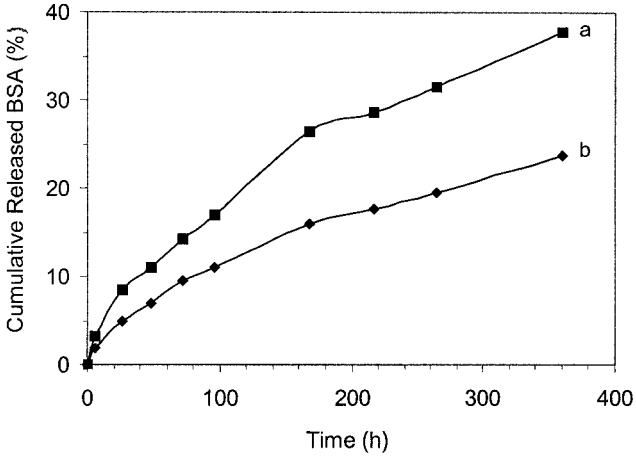

Figure 7. Drug release profiles of 20% 2L/2D hydrogels containing *c.a.* 40 mg of BSA: a) gelation for 90 h at 37°C, b) gelation for 24 h at 50°C.

Circular dichroism (CD) was used to determine whether BSA molecules were denatured after the gelation procedure. Figure 8 shows the CD spectra of original BSA and of BSA released from the hydrogel obtained at 50°C. The two spectra appeared almost identical, indicating that the gelation procedure at 50°C did not denature BSA proteins.

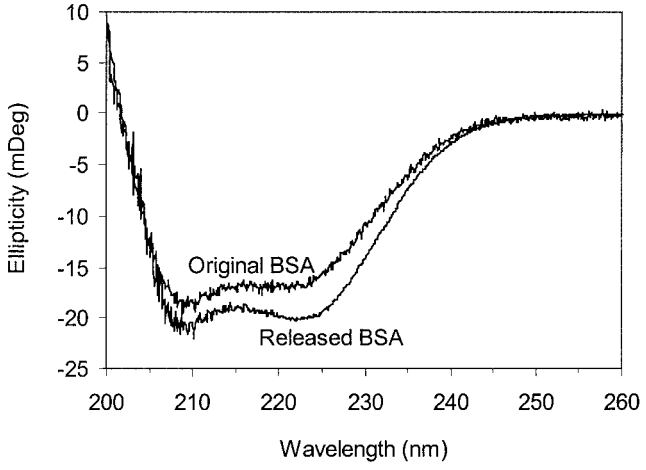

Figure 8. Circular dichroism (CD) spectra of original BSA and of BSA released from the hydrogel obtained at 50°C.

The influence of drug loading on the release behavior was examined by introducing various amounts of BSA in the hydrogel. Figure 9 shows drug release profiles of 20% 2L/2D hydrogels containing *c.a.* 10, 20, 40 and 80 mg of BSA after 24 hours' gelation at 50°C. The release rate decreased for increasing drug amount for gels containing up to 40 mg of BSA. This finding can be assigned to the fact that BSA molecules are molecularly dispersed inside hydrogels with low drug loading and can easily diffuse out, while agglomerates are formed in high drug loading systems. However, the release rate increased when the drug load was further increased to 80 mg, which could be explained by the percolation phenomenon previously described in literature.[25]

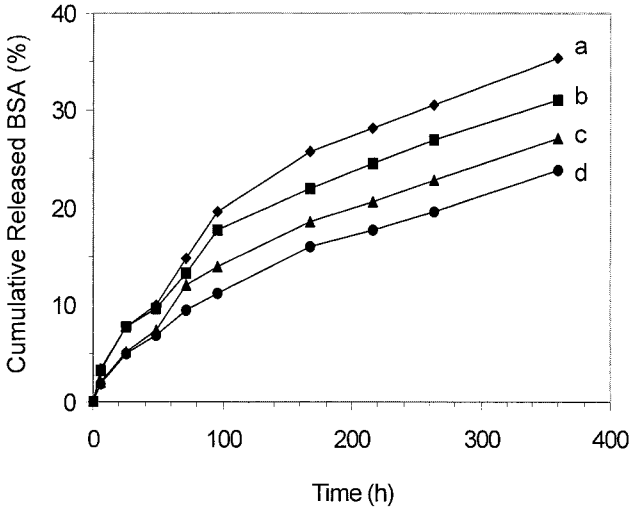

Figure 9. Drug release profiles of 20% 2L/2D hydrogels containing *c.a.* 10 (a), 20 (b), 40 (d) and 80 mg (c) of BSA (gelation for 24 h at 50°C).

Figure 10 shows comparatively the release profiles of 20% 2L/2D and 4L/4D hydrogels containing *c.a.* 40 mg of BSA after 24 hours' gelation at 50°C. It is worth to note that 2L and 2D triblock copolymers possess higher molar masses than 4L and 4D diblock ones. The release rate from 4L/4D gel appeared higher than that from 2L/2D gel, 48% and 24% of BSA being released after 360 h, respectively. The difference can be attributed to the higher molar masses of 2L/2D system which disfavored the diffusion of BSA molecules.

The influence of copolymer concentration on the drug release behavior was also examined. Figure 11 presents the release profiles of 4L/4D hydrogels containing *c.a.* 40 mg of BSA after 24 hours' gelation at 50°C, the copolymer concentration of the gel systems varying from 15% to 30%. Similar release curves were observed for different concentrations with almost constant release rate and little burst. Nevertheless, the release rate decreased with increasing copolymer concentration, which can be assigned to the fact that protein diffusion is enhanced with low concentrations.

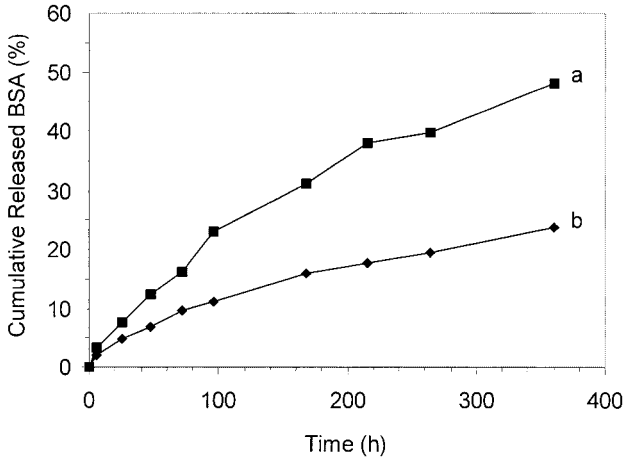

Figure 10. Drug release profiles of 20% 4L/4D (a) and 2L/2D (b) hydrogels containing *c.a.* 40 mg of BSA (gelation for 24h at 50°C).

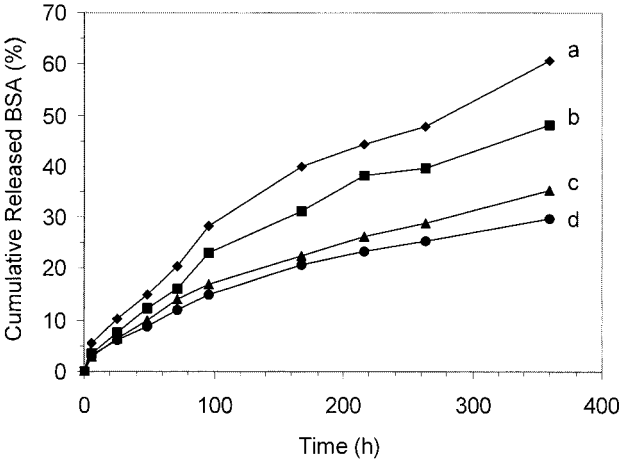

Figure 11. Drug release profiles of 15 (a), 20 (b), 25 (c) and 30% (d) 4L/4D hydrogels containing *c.a.* 40 mg of BSA (gelation for 24 h at 50°C).

Conclusion

Bioresorbable hydrogels were prepared from aqueous solutions containing both PLLA/PEG and PDLA/PEG block copolymers due to interactions and stereocomplexation between PLLA and PDLA blocks. Rheological studies showed that both storage and loss moduli depend not only on the polymer properties such as the molar mass and EO/LA ratio, but also on the factors such as the concentration, temperature, time and frequency. The gelation process is time- and temperature-dependent and the hydrogel is a dynamic and evolutive system because of continuous formation/destruction of crosslinks and degradation. Drug release studies show that the release rate can be adjusted by changing the gelation conditions and factors such as drug load, polymer concentration and molar masses.

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