Materials Science-Poland, Vol. 27, No. 1, 2009

Syntheses and characterization of pH-sensitive hydrogel from poly(γ-glutamic) acid

B. YAO[1, 2], C. YANG[1], K. ZHANG[1], C. NI[1], H. SONG[2], Z. NI[1], M. CHEN[1*]

[1]School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, P. R. China

[2]School of Chemical Engineering, Nanjing University of Science and Technology,
Nanjing 210094, P. R. China

Novel pH-sensitive hydrogels were prepared through crosslinking of poly(γ-glutamic) acid (γ-PGA) by using 1,4-butanediol diglycidyl ether as a cross-linker, tetraethylammonium bromide as a catalyst, and reacting in DMSO. The dependence of the swelling ratio of the hydrogels on pH values, ionic strength and cross-linking degree was investigated. It was found that the pH-sensitive range was clearly extended through introducing multiple hydrogen bonds to the hydrogel network during the preparation. The swelling ratios of γ-PGA hydrogels increased with the increase in pH of the aqueous solution from 2 to 9. The swelling of the γ-PGA hydrogels was firstly controlled by the ionization of carboxyl groups in the hydrogels within the pH range from 2 to 5, and then controlled by breaking of the multiple hydrogen bonds in the hydrogels within the pH range from 5 to 9. The swelling ratios of γ-PGA hydrogels were also strongly dependent on the ionic strength of the medium and cross-linking degree of the hydrogels. Increasing the ionic strength and the crosslinking degree resulted in a decrease in the swelling ratio of the hydrogels.

Key words: *poly(γ-glutamic) acid; pH-sensitive hydrogel; crosslinking; swelling*

1. Introduction

Biodegradable polymers are widely used in various fields such as suture materials, bone fixation materials, environmentally friendly materials and drug delivery systems. Recently, further applications of biodegradable polymers have been desired in many situations, such as stimuli-responsible drug delivery systems and tissue engineering, etc. [1–6].

Poly(γ-glutamic) acid (γ-PGA) is a kind of water soluble polyamide which has been extensively investigated due to its biodegradable and biocompatible properties, which are highly desirable for applications [7–16].

---

[*] Corresponding author, e-mail: mqchen@sytu.edu.cn

It has been reported that γ-PGA hydrogels can be prepared by many methods such as cross-linking following γ irradiation [17, 18] and esterification [19, 20]. In the reported literature, the crosslinked γ-PGA hydrogels were pH sensitive, and their swelling ratios increased with the increase of pH value of the medium, however, the swelling ratio changed little for pH values higher than 5. This was because the pH sensitivity depended only on ionization of carboxyl groups, which were completely ionized at pH = 5.1 [19]. A further decrease in pH had little effect on the swelling. It is evident that due to intermolecular hydrogen bonds, linear γ-PGA had apparent pH sensitivity for pH values higher than 7.4 [20]. Unfortunately, the intermolecular hydrogen bonds were broken during the preparation of crosslinked γ-PGA hydrogels when hydrophobic groups were introduced into the hydrogels [19, 20]. In order to extend the pH sensitivity range in hydrogels, and thereby enlarge their fields of application, we attempted to introduce multiple hydrogen bonds to γ-PGA networks during the hydrogel preparation. Thus, in this study, cross-linked three-dimensional networks of γ-PGA hydrogels were prepared using 1,4-butanediol diglycidyl ether (BDDGE) as a cross-linker, and tetraethylammonium bromide as a catalyst. The esterification reaction took place between the pendent carboxyl groups of γ-PGA and epoxy groups of BDDGE. After the reaction, the hydroxyl groups and ether bonds would be incorporated into the γ-PGA networks. The newly formed hydroxyl and ether groups would form intramolecular multiple hydrogen bonds which functioned as pH sensitive groups. Therefore, the swelling of the novel γ-PGA hydrogel was controlled firstly by ionization of carboxyl groups at a pH between 2 and 5, and then subsequently controlled by breaking the multiple hydrogen bonds at pH between 5 and 9.

2. Experimental

γ-PGA ($M_w = 3.15\times10^5$) was kindly donated by Meiji Seika Kaisya, Ltd. (Tokyo, Japan) and was used without further purification. 1,4-Butanediol diglycidyl ether (BDDGE, Ciba Chemical) and *tetra*-ethylammonium bromide (99.5%, Jiangyin Chengnan Chemical, Jiangyin, China) were used as received. The typical preparation of γ-PGA hydrogel was as follows: 0.2 g of γ-PGA, 16 mg of cross-linker (1,4 butanediol diglicydyl ether, hereafter referred to as BDDGE) and 8 mg of tetraethylammonium bromide were dissolved in dimethyl sulfoxide (DMSO) and placed in a reaction tube. The sealed tube was immersed in a shaking water bath (80 rpm) and kept at 60 °C for 48 h. After gelation, the hydrogels were extracted from the tube and cut into pieces. The γ-PGA hydrogels were washed by immersion in de-ionized water for 5 days: the deionized water was replaced every day to remove the unreacted compounds and linear polymers. Each hydrogel, in the form of a cube, was placed in an excess of deionized water at room temperature. In order to reach swelling equilibrium, the hydrogels were immersed in deionized water for one day. The weight of the swelling hydrogel was determined gravimetrically after eliminating the surface water of the hydrogel with filter paper. The swelling ratios of the hydrogels (Q) were calculated from the following equation:

$$Q = \frac{W_s - W_d}{W_d} \qquad (1)$$

where W_s is the weight of the equilibrium swollen hydrogel, and W_d is the weight of the dried hydrogel, respectively.

The γ-PGA hydrogels were immersed in deionized water until the equilibrium swelling had been attained, then the swollen hydrogels were placed in various buffer solutions with pH values ranging between 2 and 10, and in aqueous salt solutions with various sodium chloride and calcium chloride concentrations. KH_2PO_4 buffer and NaOH/HCl solutions were used for adjusting the pH (3.0–9.0), the ionic strength was adjusted with NaCl to 0.1 M. Samples were removed from the aqueous solutions at specific times, and weighted quickly, after having been wiped with filter paper in order to remove excess water from the hydrogel surface. The swelling ratios were calculated from Eq. (1).

3. Results and discussion

3.1. Syntheses of γ-PGA hydrogels

Figure 1 shows the cross-linking scheme of γ-PGA with BDDGE as the cross-linker. The reaction takes place between the pendent carboxyl groups of γ-PGA and the epoxy groups of BDDGE in the presence of tetraethylammonium bromide as catalyst.

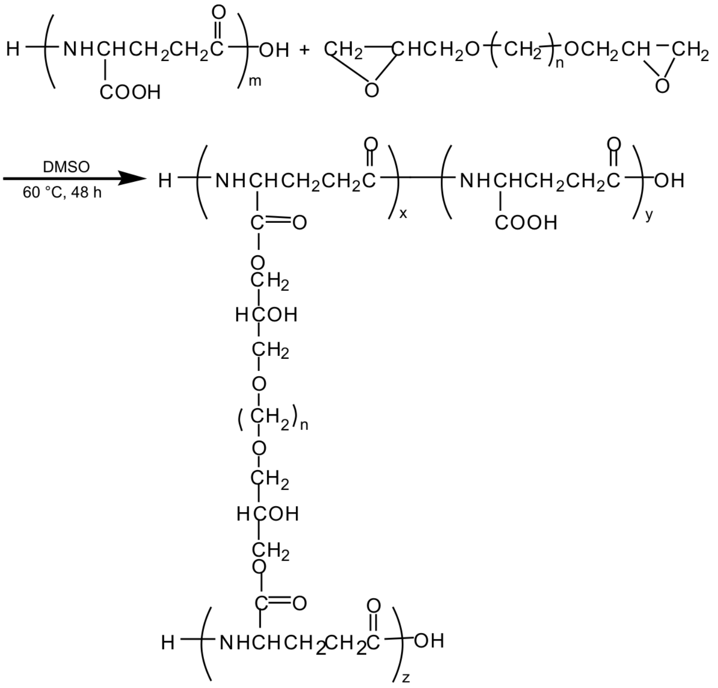

Fig. 1. The cross-linking process of γ-PGA hydrogel using BDDGE as a cross-linker

Table 1. Conditions of synthesis of γ-PGA hydrogel[a]

No.	γ-PGA		BDDGE		BDDGE/COOH molar ratio	Yield [%]
	[mg]	[mmol][d]	[mg]	[mmol]		
1[b]	201	1.56	3	0.015	0.009	–
2	204	1.58	7	0.035	0.022	65
3	198	1.53	10	0.050	0.033	74
4	197	1.53	16	0.079	0.052	84
5	202	1.57	26	0.129	0.082	86
6	196	1.52	39	0.193	0.127	83
7	197	1.53	79	0.612	0.400	89
8	200	1.55	159	0.787	0.507	86
9[c]	199	1.54	203	1.005	0.652	–
10	195	1.51	16	0.079	0.052	69
11[b]	200	1.55	16	0.079	0.052	–
12[b]	193	1.50	15	0.074	0.050	–

[a] Reactions were carried out in DMSO at 60 °C for 48h with tetraethylammonium bromide (4 wt. % of γ-PGA) as a catalyst. The volume of DMSO was fixed at 1 cm^3, except No. 10 (2 cm^3), No. 11 (4 cm^3) and No 12. (6 cm^3).

[b] No gel was obtained.

[c] The gel cracked under swelling in deionized water.

[d] The repeated structure units in the polymer.

In Table 1, the reaction conditions applied for the preparation of γ-PGA hydrogels have been listed. γ-PGA hydrogels can be prepared when the concentration of γ-PGA and the concentration of the cross-linker are higher than their critical values. A similar phenomenon was also reported by Gonzales et al. [24] in the preparation of γ-PGA hydrogel using dihalogenoalkane as a cross-linker. In their study, the critical concentration of γ-PGA was 10 wt. %. In the present study, at the γ-PGA concentration below 15 wt. %, no γ-PGA hydrogels were formed. The concentration of γ-PGA was too low for effective cross-linking, thus no three-dimensional network hydrogel was formed. Additionally, if the molar ratio of BDDGE to [COOH] is below 0.022, then, also, no hydrogel is formed (e.g., No. 1 of Table 1).

It is also found that the mechanical property of γ-PGA hydrogels is related to their cross-linking degree. If the BDDGE content is low (No. 2), γ-PGA hydrogel formed is soft and it is difficult to maintain its shape in the swelling state. Increasing the content of BDDGE will enhance the mechanical strength of the γ-PGA hydrogels. However, if the BDDGE content is high (No. 8), the swelling ratios of γ-PGA hydrogels will be very low and can easily be cracked.

3.2. Swelling behaviour of γ-PGA hydrogels

3.2.1. Effect of the cross-linker content on the swelling ratio of the hydrogels

The cross-linker content has an important effect on the structure of a three-dimensional network (Fig 2). When the cross-linker concentration (the molar ratio of

BDDGE to the repeated structural unit of γ-PGA) is varied from 0.022 to 0.575, the swelling ratio of the hydrogels decreases from 800 to 50. The swelling ratios of the γ-PGA hydrogels decreased with the increase of the concentration of BDDGE, and then remained nearly constant when the concentration of BDDGE exceeded 0.5. This agrees with common observations about hydrogels. Additionally, the –COOH group of γ-PGA hydrogels is the hydrophilic group. The cross-linking reaction between the epoxy group and –COOH group will make the free residual –COOH groups decrease. This might be another reason for the decrease in the swelling ratio of γ-PGA hydrogel with the increase of cross-linking degree.

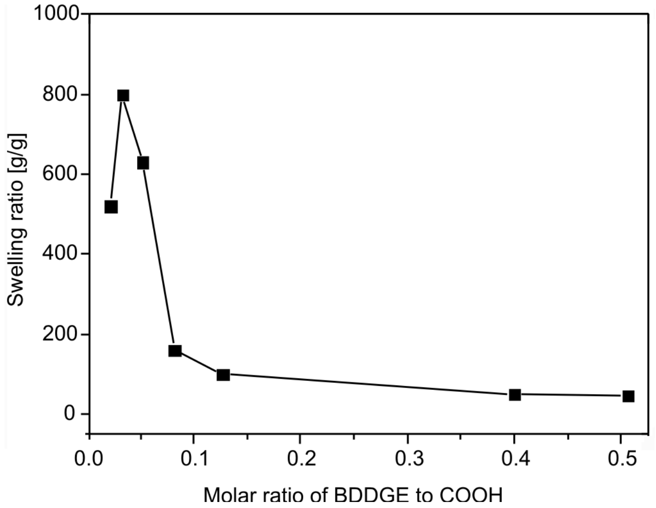

Fig. 2. The plot of the equilibrium swelling ratio of γ-PGA hydrogels in deionized water in function of the molar ratio of BDDGE to the repeated structural unit of γ-PGA. The conditions of hydrogel preparation are indicated in Table 1(from BDDGE/COOH, molar ratio: 0.022 to 0.507)

It was unexpectedly found that the swelling ratio of hydrogel No. 2 has a lower swelling ratio than that of hydrogel No. 3, even though the crosslinking content in No. 2 (0.022) is lower than in No. 3 (0.033). This abnormal result is explained by the fact that a part of the swollen hydrogel is lost in the medium, since the hydrogel is too soft in the low crosslinking state.

3.2.2. Effect of pH on the swelling behaviour of γ-PGA hydrogels

Swelling or contraction in response to pH change is a typical phenomenon of polyelectrolyte hydrogels. The effect of pH value on the swelling ratio of γ-PGA hydrogels is shown in Fig. 3. In this study, the pH values in aqueous solutions are varied from 2.0 to 10.0. It is found that the swelling ratio of γ-PGA hydrogel increases with the increase of the pH value.

The swelling ratio of the γ-PGA hydrogel is about 8 at a pH = 2.0, and sharply increases to 36 at pH = 3.0 (Fig. 3). According to Gonzales et al. [24], the pH corresponding to ca. 50% ionization of γ-PGA was around 2.1. The reason for the low swelling ratio at low pH values could be attributed to the protonation of carboxyl groups which causes greater interaction of the carboxyl groups and hence causes the shrinkage of the γ-PGA hydrogel. Conversely, carboxyl groups will be ionized at high pH values, which results in a higher swelling ratio of the hydrogels. This is due to the ionic repulsion of ionized carboxyl groups. So, the swelling ratio of hydrogel increases with increasing pH.

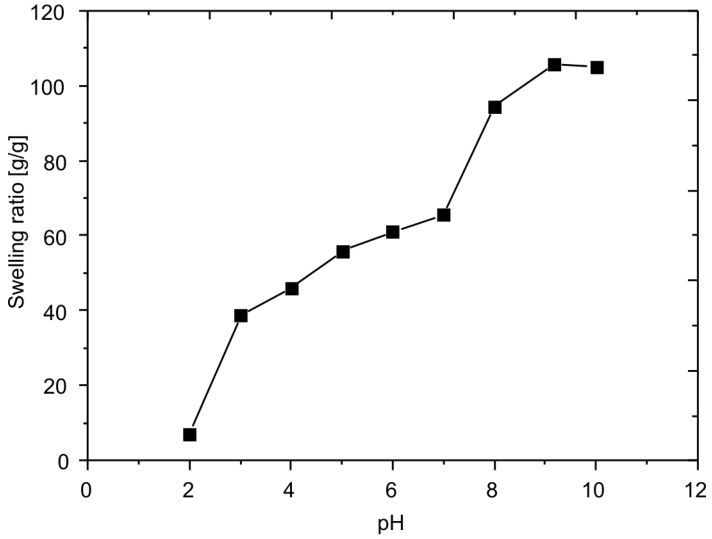

Fig. 3. Dependence of swelling ratio of γ-PGA hydrogel (BDDGE/COOH, molar ratio 0.052, sample 4 in Table 1) in function of pH at a constant ionic strength (0.1 M)

Gonzales et al. [24] and Akashi et al. [25] observed the swelling ratios of γ-PGA hydrogels and γ-PGA propyl hydrogels increased with increasing pH in the 2–5 pH range, but changed little when pH exceeded 5. This phenomenon was attributed to the 100% ionization of γ-PGA at pH = 5.1 [24]. However, in the present study, the swelling ratios of the obtained γ-PGA hydrogels continue to increase from 60 to 105 when the pH changes from 5 to 9. This suggests that the swelling mechanism of γ-PGA hydrogels prepared in the present study may be different from that of the γ-PGA hydrogels prepared by Gonzales et al. [24] and Akashi et al. [20].

Note that linear γ-PGA is insoluble in ultra-pure water, whereas it is soluble in an alkaline solution (pH > 7.4) [20]. This implies that linear γ-PGA can form intermolecular hydrogen bonds. If these multiple hydrogen bonds do not break after the cross-linking of the γ-PGA, they will influence the swelling of γ-PGA hydrogels in aqueous solution. For the γ-PGA hydrogels prepared by Gonzales et al. [24] and Akashi et al. [20], the multiple hydrogen bonds in γ-PGA were broken due to the incorporation of hydrophobic groups into the gels, therefore the swelling of the obtained γ-PGA only controlled by the ionization of carboxyl groups within the 2–5 pH range. The swelling ratio was no longer obviously affected by pH at pH > 5. However, in this study, some hydroxyl groups and ether bonds are introduced to the γ-PGA network during the cross-linking reaction. As a result, multiple hydrogen bonds in the γ-PGA will form. The breaking of multiple hydrogen bonds is governed by the pH value in the 7.4–9 range. Consequently, we can observe that the swelling ratio clearly increases as pH rises from 7 to 9. Thus the swelling is controlled initially by the ionization of carboxylic groups and then controlled by the breaking of multiple hydrogen bonds. These novel γ-PGA hydrogels have a significantly broader pH-sensitive range, which will enlarge their fields of application.

3.2.3. Effect of ionic strength on the swelling behaviour of γ-PGA hydrogels

It is known that the swelling ratios of polyelectrolyte hydrogels, such as γ-PGA hydrogel, are dramatically dependent on the ionic strength of the medium.

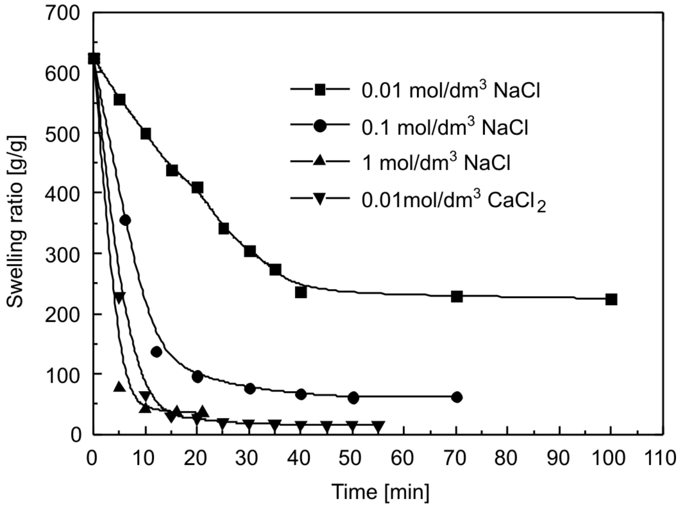

Fig. 4. The contraction curves of γ-PGA hydrogel (BDDGE/COOH, molar ratio 0.052 in Table 1) in various electrolyte solutions

Figure 4 shows the contraction behaviour of γ-PGA hydrogels in salt solutions. When the equilibrium swollen γ-PGA hydrogels are transferred into salt solutions, the γ-PGA hydrogels shrunk due to the ionic screening effect. In the case of NaCl solution, both the contraction velocity and contraction ratio of γ-PGA hydrogels increase with the increase in the NaCl concentration. Figure 4 also indicates that at the same ionic concentration (0.0l mol/dm^3), the equilibrium swelling γ-PGA hydrogels contract more quickly in $CaCl_2$ solution than in NaCl solution and the contraction ratio in $CaCl_2$ solution is higher than that in NaCl solution. When polyelectrolyte hydrogels are immersed in water, an osmotic pressure is established. The osmotic pressure is the main driving force for the swelling of polyelectrolyte hydrogel. In the present study, the γ-PGA hydrogels adsorb a lot of water in deionized water due to the exerting osmotic pressure of counter ions. When the equilibrium swelling γ-PGA hydrogels are put into salt solutions, the external ions of the γ-PGA hydrogels will diffuse into the hydrogels owing to different salt concentration between the exterior and the interior of the hydrogel, which causes the decrease in the osmotic pressure, and the contraction of γ-PGA hydrogels. Another reason is that when ionic strength increases, the hydrophobic interaction of polymer chains becomes stronger, which makes the hydrogel shrink. The diffusion degree and velocity of ions both increase with the increase in the salt concentration. This may be the reason why the contraction velocity and contraction ratio of γ-PGA hydrogel increase with the increase in the salt concentration. Strong effects are observed by using $CaCl_2$ solution. In this case, the swollen γ-PGA hydrogel exhibits more dramatic contraction than in NaCl solution with the same molar salt concentration. It can be attributed to a difference of ionic strength in NaCl and $CaCl_2$ solutions. Additionally, the ionic interaction between the Ca^{2+} ions and the carboxyl groups inside the hydrogel also had an important role in the shrinkage behaviour.

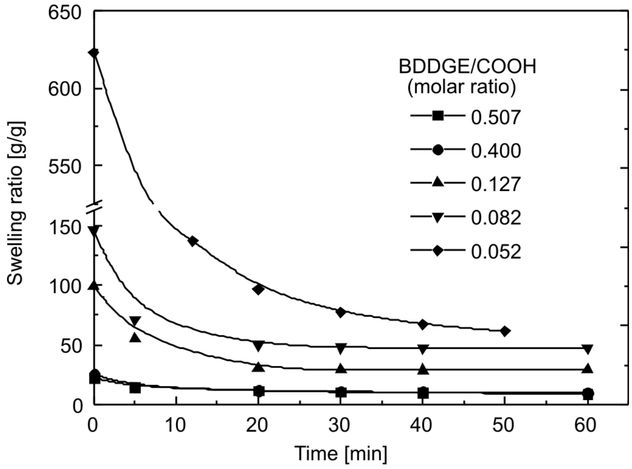

Fig. 5. The contraction curves of γ-PGA hydrogel with various crosslinking degrees in NaCl solution (0.1 mol/dm^3)

The contraction curves of γ-PGA hydrogels with various cross-linking degrees in NaCl solution are plotted in Fig. 5. The γ-PGA hydrogels are swollen in deionized water and subsequently soaked in the 0.1mol/dm^3 NaCl solution. Figure 5 shows that the swelling ratio of γ-PGA hydrogels with low cross-linking degree (No. 4) sharply decreases from 630 to 60 after being soaked in the 0.1mol/dm^3 NaCl solution for 50 min. However, the swelling ratio of the hydrogel (No. 8) with higher cross-linking degree just changes slightly from 22 to 10 during the same period of time. This can be attributed to the difference in the number of residual carboxyl groups in the γ-PGA hydrogels with various cross-linking degrees. It is acceptable that the γ-PGA hydrogels with lower cross-linking degree have more residual carboxyl groups. So, the external ions have a more noticeable effect on the swelling behaviour of γ-PGA hydrogels.

4. Conclusion

pH-sensitive hydrogels based on poly(γ-glutamic) acid (γ-PGA) were successfully synthesized by the reaction between the pendent carboxyl groups of γ-PGA and the epoxy groups of BDDGE in the presence of tetraethylammonium bromide as a catalyst in DMSO. The swelling ratio of γ-PGA hydrogel is strongly dependent on the pH value and salt concentration of the swelling medium. The pH-sensitive range of the hydrogels has clearly been extended up to pH = 9. This is because multiple hydrogen bonds are formed during the preparation of the hydrogels. This will enlarge the range of possible applications of hydrogels as pH-sensitive materials.

Acknowledgement

This work was financially supported by the National Nature Science Foundation of China, Project No. 50443012, 20671043.

References

[1] ENGELBERG I.,, KOHN J., Biomaterials, 12 (1991), 292.
[2] TOMIHATA K., IKADA Y., Biomaterials, 18 (1997),189.
[3] STUBBE B., MARIS B., DEN MOOTER G.V., DE SMEDT S.C., DEMEESTER J., J. Contr. Release, 75 (2001), 103.
[4] KIKUCHI A., OKANO T., Adv. Drug. Deliv. Rev., 54 (2002), 53.
[5] HOFFMAN A.S., Adv. Drug. Deliv. Rev., 43 (2002), 3.
[6] QIUY., PARK K., Adv. Drug. Deliv. Rev., 53 (2001), 321.
[7] TROY F.A., J. Biol. Chem., 248 (1973), 305.
[8] KUBOTA H., NAMBU Y., ENDO T., J. Polym. Sci. Part. A: Polym Chem, 34 (1996),1347.
[9] TACHABOONYAKIAT W., SERIZAWA T., ENDO T., AKASHI M., Polym J., 32 (2000), 481.
[10] SERIZAWA T., GOTO H., KISHIDA A., ENDO T., AKASHI M. J., Polym. Sci. Part A: Polym. Chem., 36 (1999), 801.
[11] SHIMOKURI T., KANEKO T., SERIZAWA T., AKASHI M., Macromol Biosci., 4 (2004), 407.
[12] MORILLO M., DE.ILARDUYA A.M., GUERRA S.M., Macromo1ecules, 34 (2001),7868.
[13] MORILLO M., DE.ILARDUYA A.M., ALLA A., GUERRA S.M., Macromo1ecules, 36 (2003), 7567.
[14] Melis J., Morillo M., de Ilarduya A. M., Alla A., Guerra S. M., Polymer, 42 (2001), 9319
[15] KUBOTA H., NAMBU Y., ENDO T., J. Polym. Sci. Part A: Polym. Chem., 31 (1993), 2877.
[16] KUBOTA H., NAMBU Y., ENDO T., J. Polym. Sci. Part A: Polym Chem., 33 (1995), 85.
[17] CHOI H.J., KUNIOKA M., Radiat Phys. Chem., 46 (1995), 175.
[18] CHOI H.J., YANG R, KUNIOKA M., J. App. Polym. Sci., 58 (1995), 807.
[19] GONZALES D., FAN K., SEVOIAN M., J. Polym. Sci. Part A: Polym. Chem., 34 (1996), 2019.
[20] SHIMOKURI T., KANEKO T., AKASHI M., J. Polym. Sci. Part A: Polym. Chem., 42 (2004), 4492.

Received 1 July 2008
Revised 2 February 2009