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Poly(α-alkyl γ-glutamate)s of microbial origin: I. Ester derivatization of poly(γ-glutamic acid) and thermal degradation

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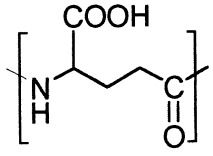
Abstract

A series of poly(α-alkyl γ-glutamate)s (PAAG-*n* with $n = 1–10$) were prepared from poly(γ-glutamic acid) (PGGA) of microbial origin by a two-steps synthesis procedure. The poly(α-ethyl γ-glutamate) was firstly obtained by esterification of PGGA and then transesterified with the selected alkanol to obtain the corresponding PAAG-*n*. The modification reactions were found to occur without alteration of the original enantiomeric composition but with a significant reduction in molecular weight. The thermal degradation of PAAG-*n* was examined by thermogravimetric analysis combined with IR and NMR spectroscopy. At low temperatures (<300°C) decomposition was found to take place by depolymerization with releasing of the corresponding alkyl pyroglutamate. Decarboxylation of PAAG-*n* to an unsaturated polyamide seemed to be the main process occurring at high temperatures (400–450°C).

Keywords: Poly(γ-glutamic acid); Poly(α-alkyl γ-glutamate)s; Thermal degradation

1. Introduction

Poly(γ-glutamic acid) (PGGA) is a biosynthetic polymer consisting of a nylon 4 main chain bearing a carboxyl group attached to the γ-carbon atom of every repeating unit. The naturally produced polymers may have molecular weights of up to millions and usually contain nearly equal amounts of D- and L-units. In laboratory fermentation cultures, the D/L ratio may be partially controlled so that polymers with varying degree of stereoregularity may be yielded [1–3]. An industrial process using *Bacillus subtilis* F-201 has been recently established for the production of racemic PGGA on a large scale [4]. The research interest presently renewed for this biopolymer is due to its potential as a biocompatible material with biodegradable properties. In fact, PGGA is known to be degraded by proteases at a rate that is depending on the degree of esterification and length of the alkyl side chain [5].



PGGA is highly hydrophilic, readily soluble in water and its physical behavior is largely affected by the presence of humidity. Esterification of PGGA is usually considered to be the most adequate method to modify the polymer in order to render materials with suitable processing and handling properties. With regards to their potential applications in the biomedical field, a good thermal stability compatible with a fair biodegradability is a combination of properties highly desired for these PGGA derivatives.

The synthesis and properties of a number of poly(γ-glutamate)s derived from natural PGGA have been described so far. The preparation procedures used by different authors [6–11] are slight variations in the application of the reaction originally developed by Bocchi et al. [12] for the esterification of aminoacids in polar aprotic solvents. Essentially, the method consists of treating the sodium salt of PGGA with alkyl or aryl halides in the suitable solvent and under the appropriate conditions of time and temperature. Poly(γ-glutamate)s with alkyl side chains ranging from methyl up to dodecyl, in addition to the benzyl derivative, have been thus prepared from PGGA attaining conversions between 0.9 and 1.0. The reliability of the method and the accomplishment of the esterification reaction are however depending on the size of the alkyl side group, with better results being seemingly reached for short esters. Thus, Shah et al. [6] reported that esterification of PGGA to poly(α-dodecyl γ-glutamate) had to be carried out in successive

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Table 1
Esterification of PGGA to PAAG-2

PGGA			Reaction conditions					PAAG-2				
	η (dl g^{-1})[a] (DMSO)	D/L[b]	Solvent	t (days)	T (°C)	C (%)[c]	Yield (%)		η (dl g^{-1})[a]		Mv[d]	D/L[b]
									DMS	DCA		
PG (DL)GA	1.77	59:41	NMP	1	60	100	88	PAA(DL)G-2	0.70	1.32	88,000	60:40
	0.86[e]	56:44	DMSO	7	45	>98	92		0.81	1.41	96,000	52:48
				11	45	>98			0.52	0.82	46,000	52:48
				13	45	>98			0.48	0.75	41,000	52:48
PG(D)GA	5.00	89:11	NMP	1	60	100	80	PAA(D)G-2	1.40	2.36	192,000	88:12
	1.70[e]	89:11	NMP	1	60	100	88		0.92	1.64	118,000	88:12
	0.30[e]	85:15	DMSO	11	45	100			0.38	0.57	28,000	87:13

[a] Intrinsic viscosity measured at 25 ± 0.1°C in dimethyl sulfoxide (DMSO) or dichloroacetic acid (DCA).
[b] Enantiomeric ratio determined by HPLC.
[c] Conversion degree measured by ^{1}H-NMR.
[d] Viscosity-average molecular weight estimated by using the Mark–Howink–Sakurada equation [24] $\eta = 2.9 \times 10^{-4} Mv^{0.74}$ established for poly(γ-methyl α,L-glutamate) in DCA.
[e] PGGA samples subjected to microwave irradiation for molecular weight reduction.

steps using *n*-dodecyl iodide in hexamethylphosphoramide in order to obtain conversions higher than 95%. The shortcoming of the esterification reaction carried out in solution is that the initially solubilized PGGA becomes insoluble as esterification proceeds. This fact restricts the accessibility of the partially modified polymer to the nucleophylic attack by the alkyl halide and prevents from reaching complete conversions. The chemical synthesis of optically pure PGGA and some of their esters, i.e. poly(γ-glutamate)s, was reported long time ago by Hungarian researchers [13–16] and recently revisited by Sanda et al. [17].

In this paper, we wish to report on a synthetic route that may be alternatively used for the systematic preparation of PGGA esters, as well as on the thermal degradation of these compounds. This new method of synthesis is based on the transesterification of the poly(α-ethyl γ-glutamate) with alkanols. By this means, a series of poly(α-alkyl γ-glutamate)s, henceforth abbreviated as PAAG-*n* with *n* standing for the number of carbon atoms in the alkyl side chain, are prepared for *n* ranging between 1 and 10. The method is of general application yielding good results regardless the length of the alkyl side chain. The stability to heating of poly(γ-glutamate)s are comparatively examined and the molecular mechanism involved in the thermal degradation is investigated.

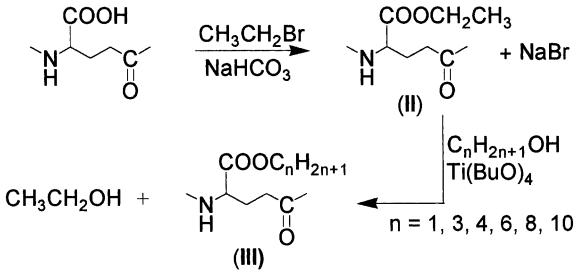
2. Experimental

2.1. Materials

All chemicals were obtained commercially from either Aldrich or Merck. They were analytical grade or higher, and used without further purification. Solvents to be used under anhydrous conditions were dried by standard methods. Alcohols for transesterification reaction were of analytical grade and used as received. Two poly(γ-glutamic acid)s differing in the enantiomeric composition were used in this work. PG(DL)GA with a nearly racemic D/L ratio and a weight average molecular weight of about 4×10^5 was kindly supplied by Dr Kubota of Meiji (Japan). PG(D)GA with a highly enriched D-composition and a weight average molecular weight of about 10^6 was prepared specifically for the purpose of this work by fermentation of *B. licheniformis*, as reported in detail elsewhere [18]. For evaluating the influence of the polymer size on the polymer modification reactions, some original samples were degraded by irradiation with microwaves according to the method developed by us for reducing the molecular weight of polypeptides [19]. The main characteristics of all the PGGA samples used in this work are shown in Table 1.

2.2. Esterification and transesterification reactions

Poly(α-ethyl γ-glutamate) (PAAG-2) was obtained by either of the two methods described in the literature [6–11]. Method A: to a solution of PGGA (5 g, 38.8 mmol) in dimethyl sulfoxide (DMSO) (125 ml) containing 6.5 g (77.6 mmol) of $NaHCO_3$, ethyl bromide (22 ml, 294 mmol) was added dropwise under vigorous stirring. The mixture was left to react for 5 days at 45°C and then precipitated with cool water. The partially ethylated PGGA was recovered by filtration, washed with water and then subjected to further esterification for 3 days under the same reaction conditions used in the first stage. Method B: 2 g (15.5 mmol) of PGGA suspended in *N*-methyl pyrrolidone (NMP) (200 ml) were left overnight under stirring at 80°C. The mixture was cooled down to 60°C and added with $NaHCO_3$ (5.25 g, 62.5 mmol). Then ethyl bromide (6 ml, 80 mmol) was slowly added for 2 h and left to react for a period of time between 20 and 30 h. After removing the NaBr precipitate, the reaction solution was poured into



Scheme 1.

acidified cool water (1.5 l, pH 1.5) to precipitate the polymer, which was then separated by filtration. In both cases, the PGGA-2 was obtained as a white powder that was repeatedly washed with cool water, ethyl ether and finally dried under vacuum at 50°C.

PGGA-*n* for $n = 1$–10 (except for $n = 2$) were obtained according to the following general procedure: PGGA-2 (*x* moles) were suspended in (10–50) *x* moles of the alcohol of choice containing 5 mol% of $Ti(OBu)_4$ at a temperature of 180°C and magnetically stirred under a nitrogen atmosphere. In the case that lower boiling point alcohols were used, reactions were conducted in a pressurized vessel. The advancement of the reaction was monitored by comparing the areas of the appropriate signal in the ^{1}H-NMR spectra. Once the reaction was finished the final solution was poured into methanol or ether and the mixture left to cool down to room temperature. The precipitated polymer was separated from the supernatant solution by decantation. Purification was accomplished by dissolving the product either in chloroform or in a mixture of chloroform–trifluoroacetic acid ($CHCl_3$–TFA) and reprecipitating it with methanol or ethanol.

2.3. Measurements

Enantiomeric compositions of PGGA and PAAG-*n* were determined according to the method developed by Cromwick and Gross [20]. The hydrolyzed polymer was made to react with the Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, Pierce, Rockford, IL) to form the diastereoisomeric dipeptides which were then analyzed by HPLC. Viscosities were measured at 25.0 ± 0.1°C using an Ubbelohde viscometer. FT-IR spectra were recorded on a Perkin–Elmer FT-2000 instrument from films prepared by casting from chloroform solution. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AMX-300 NMR spectrometer with samples dissolved in DMSO-d_6, $CDCl_3$ or $CDCl_3$–TFA mixtures and using TMS as reference. DSC thermograms were recorded in a Pyris I Perkin–Elmer calorimeter at a heating rate of 10°C min^{-1} under a nitrogen atmosphere.

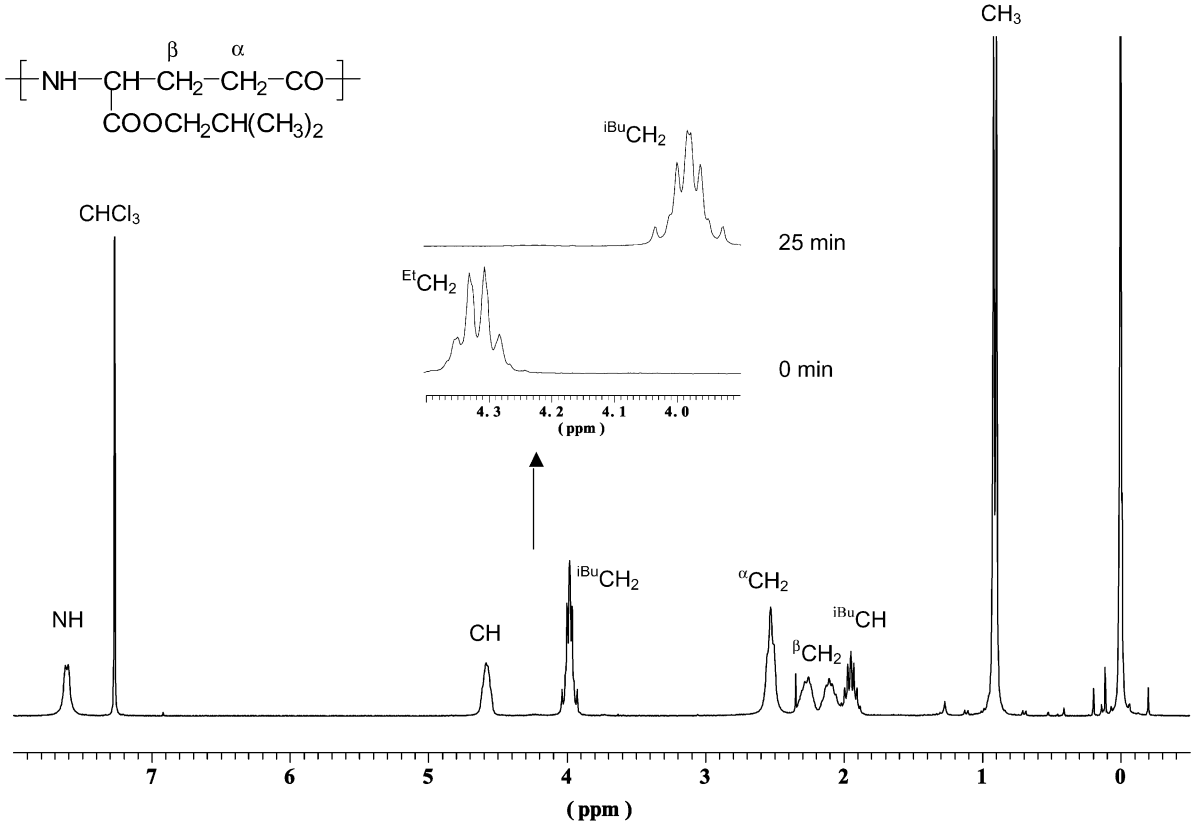


Fig. 1. Evolution of the transesterification reaction of PAA(DL)G-2 with isobutanol followed by ^{1}H-NMR. The inset shows that full replacement of the ethyl methylene protons (~4.3 ppm) by the butyl methylene protons (~4.0 ppm) happened after 25 min of reaction.

Table 2
Transesterification of PAA(DL)G-2 with alcohols

Polymer	t (min)[a]	C (%)[b]	Yield (%)	η (dl g^{-1})[c]		Mv[d]	D/L[e]	T_m
				DMSO	DCA			
PAA(DL)G-2	–	–	–	0.81	1.41	96,000	58:42	261
PAA(DL)G-1	85	100	85	0.39	0.9	50,000		228
PAA(DL)G-3	70	100	91	0.26	0.65	38,000	60:40	–
PAA(DL)G-4	20	100	59	0.43	0.94	55,000	–	236
PAA(DL)G-4[f]	25	100	84	0.37	0.81	45,000	58:42	260
PAA(DL)G-6[f]	40	100	88	–	–	–	–	230
PAA(DL)G-8[f]	45	>98	78	–	–	–	–	168
PAA(DL)G-10[f]	50	>98	73	–	–	–	55:45	–

[a] Reaction time at 180°C.
[b] Conversion degree measured by ^{1}H-NMR.
[c] Intrinsic viscosity measured at 25 ± 0.1°C measured in dimethyl sulfoxide (DMSO) or dichloroacetic acid (DCA).
[d] Viscosity-average molecular weight estimated by using the Mark–Howink–Sakurada equation [24] $\eta = 2.9 \times 10^{-4}$ Mv$^{0.74}$ established for poly(γ-methyl α,L-glutamate) in dichloroacetic acid (DCA).
[e] Determined by HPLC.
[f] Non-soluble in the solvents used for viscosity measurements.

Thermogravimetric analyses were performed with a Perkin–Elmer TGA-6 thermobalance under flowing nitrogen.

3. Results and discussion

3.1. Preparation and characterization of poly(α-alkyl γ-glutamate)s

Esterification of PGGA to PAAG-*n* was performed in two stages, as indicated in Scheme 1 (reaction scheme for the preparation of PAAG-*n* by esterification in two steps). The ethyl ester of PGGA (PAAG-2) was obtained in first place by treating the polyacid with ethyl bromide using the two methods available in the literature for the synthesis of this compound. These methods essentially differ to each other in the solvent and temperature used for reaction: DMSO at 45°C in method A and NMP at 60°C in method B. Two PGGA, one almost racemic, PG(DL)GA, and the other highly enriched in D-units, PG(D)GA, were used for esterification. Reaction conditions and characteristics of the resulting PAAG-2 are compared in Table 1. In both methods, results were found to be essentially independent of both molecular weight and enantiomeric composition of the polymer and no significant alteration of the D/L ratio was observed to occur. Although the conversion attained by the two methods was nearly complete, PAAG-2 obtained in NMP distinguished in showing no traces of unreacted carboxilic groups. Comparison of viscosities for PGGA and PAAG-2 (no strictly comparable) revealed that a considerable reduction in molecular weight took place upon esterification. Such a reduction is though to be due to uncontrolled hydrolysis of the polyamide chain, a process that appears to take place much easier for high molecular weight species and, according to expectations, to increase considerably with reaction time and temperature.

The transesterification reaction was carried out at 180°C with PAAG-2 suspended in a large excess of the alcohol of choice and in the presence of $Ti(BuO)_4$ as catalyst. The advance of the reaction was followed by ^{1}H-NMR by comparing the areas of the CH_2 signal of the ethyl leaving group with a conveniently selected signal of the replacing alkyl side group. The evolution of transesterification of PAAG-2 with isobutanol is shown in Fig. 1. The conversion was considered to reach 100% when the ethyl CH_2 signal completely disappeared. A good advantage offered by this reaction is that the polymer passes into solution as transesterification proceeds. Thus, the reaction mixture becomes homogenized when a certain degree of conversion is reached making easier the subsequent attack by the replacing alcohol (which is the reaction solvent) and possible to attain almost total conversion. The results obtained in the transesterification step are shown in Table 2 for the whole set of alcohols examined in this work. Conversions attained between 98 and 100%, the larger alcohols being less efficient as substituting reagents. Purification of the products was feasible by simple precipitation from the reaction mixture by addition of methanol. The purity of PAAG-*n* thus obtained was assessed by IR, ^{1}H and ^{13}C-NMR spectroscopy, as illustrated in Fig. 2 for the case of PAAG-6. Similarly to what happened in the esterification stage, a certain decreasing in viscosity was found to accompany the transesterification reaction whereas no appreciable change in the enantiomeric composition was detected.

It can be concluded from these results that the characteristics of the final poly(α-alkyl γ-glutamate)s obtained by transesterification do not differ significantly from those obtained by the single-step esterification methods described in the literature. The same can be said about yields, as far as short alkyl groups are concerned. Since the solubility of the polymer increases with the advancement of transesterification, almost complete conversions are attained regardless

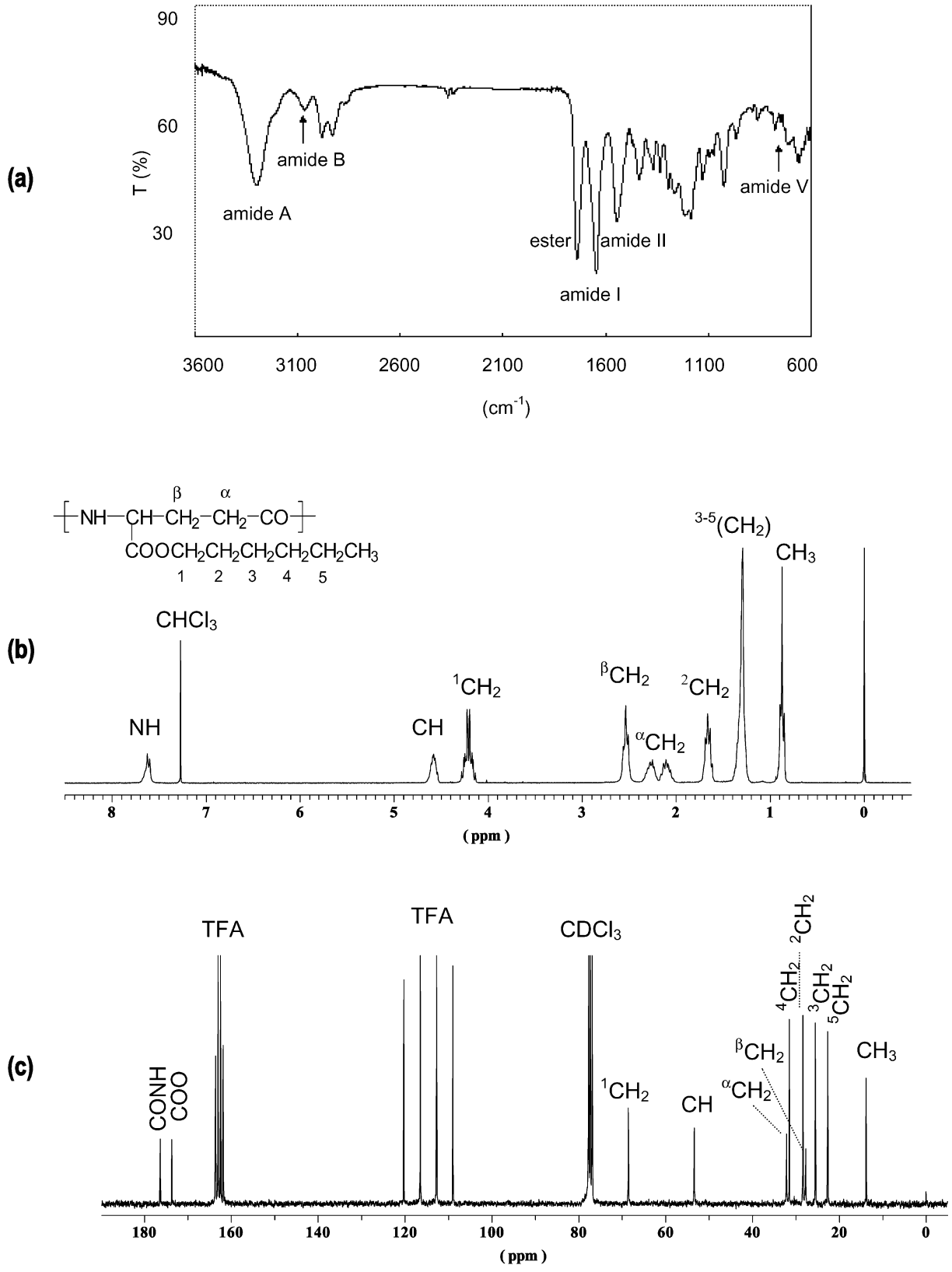


Fig. 2. Infrared (a), ^{1}H-NMR (b) and ^{13}C-NMR (c) spectra of PAAG-6.

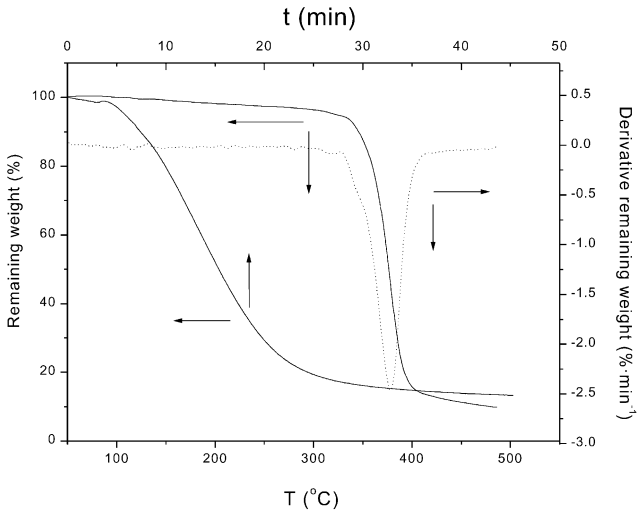


Fig. 3. TGA analysis of PAA(DL)G-4. Trace A: weight loss at a heating rate of 10°C min^{-1} Trace A* (dotted line): derivative curve of trace A. Trace B: weight loss as a function of time at a fixed heating temperature of 275°C.

the length of the alkylating alcohol. As a result, yields obtained for PAAG-*n* significantly improve for longer alkyl groups with regards to the single-step method. For instance, PAAG-10 is obtained with an overall yield of 65% whereas a value of 50% is reported for this polyglutamate when prepared by direct esterification [9]. Such differences are expected to be more accentuated as the length of the alkyl group increases. Evidence for this was obtained by an exploratory essay in which PG(DL)GA was subjected to conversion to PAA(DL)G-18 by the two methods. Whereas a complete conversion was attained by transesterification of PAAG-2 with 1-octadecanol with a overall yield of 60%, direct esterification with octadecyl bromide failed to afford conversions higher than 20%. A further advantage of the two-steps esterification method is the possibility of using alcohols instead of alkyl halides as alkylation reagents, a feature that expands the esterification options and makes more reliable the purification of the resulting PAAG-*n*. In principle any short PAAG-*n* could be selected as precursor

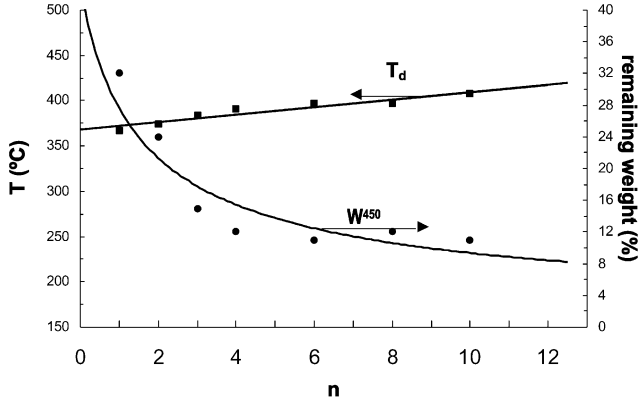


Fig. 4. Decomposition temperature (T_d) and remaining weight at 450°C (W^{450}) for PAAG-*n* as a function of *n*.

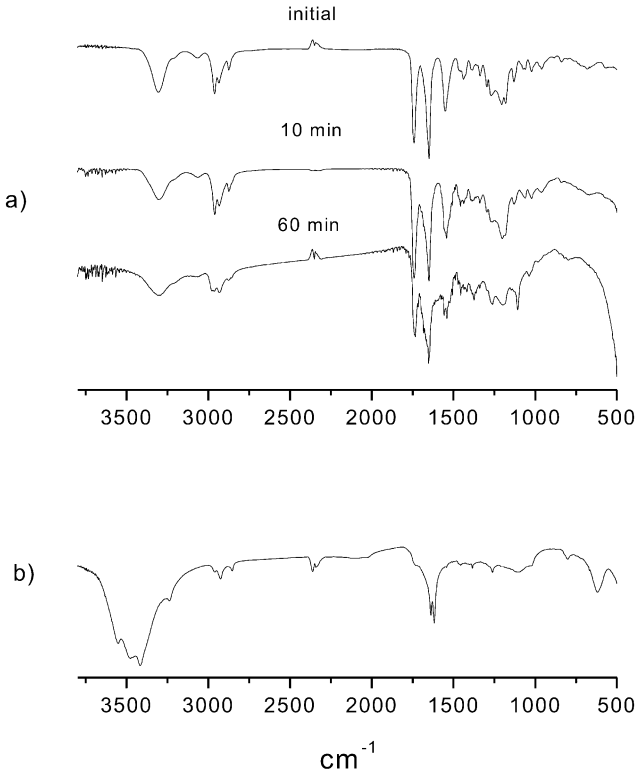


Fig. 5. (a) Evolution of the infrared spectra of PAA(DL)G-4 upon heating at 275°C for the indicated periods of time. (b) Infrared spectrum of the remaining PAA(DL)G-4 after heating at 450°C.

for transesterification, our preference for PAAG-2 obeying exclusively to reliability and simplicity reasons.

3.2. Thermal degradation

PAAG-*n* with $n \leq 6$ are semicrystalline polymers showing DSC traces with melting peaks that tend to decrease in both temperature and enthalpy as *n* increases. Thermal decomposition of PAAG-*n* is a property transcendent for technical processing of these compounds. TGA curves of PAAG-4, in both the isothermal and dynamical modes, are represented in Fig. 3 to illustrate the general behavior observed for the whole series. Dynamic TGA curves revealed that thermal degradation in PAAG-*n* happens well above T_m with decomposition onsets usually appearing around 300°C. Apparently, decomposition takes place in a single step with a maximum rate at a temperature (T_d) located between 350 and 400°C. The residue left at 450°C (W^{450}) decreases from ~30% down to ~10% of the initial mass as *n* increases from 1 up to 10. T_d and W^{450} for the whole PAAG-*n* series are plotted against *n* in Fig. 4, which clearly shows the trends displayed by these parameters with the variation in size of the alkyl side chain. On the other hand, isothermal TGA curves showed that, despite the apparent stability displayed by the dynamical TGA trace below 300°C, heating of the polymer at temperatures

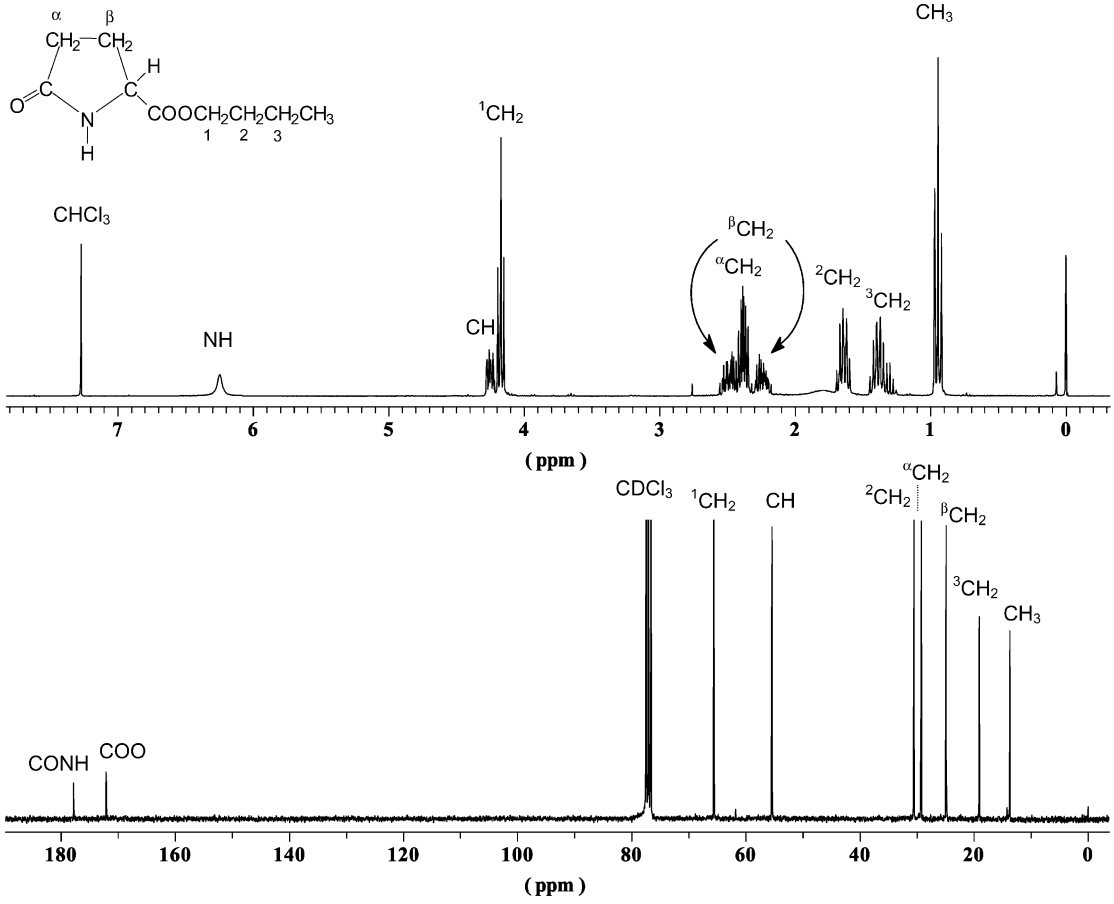
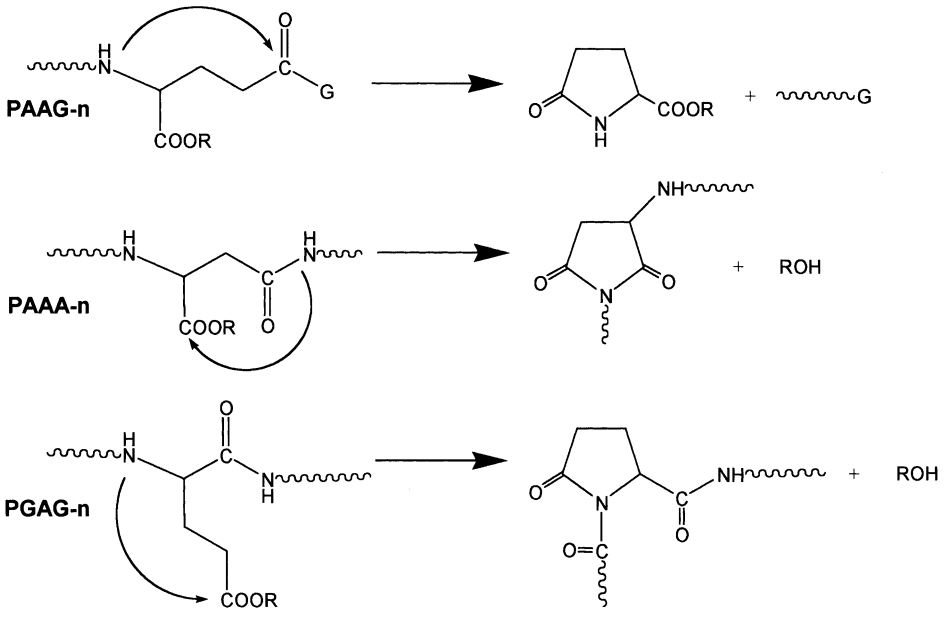


Fig. 6. ^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of the condensate yielded at the thermal degradation of PAA(DL)G-4 at 275°C.

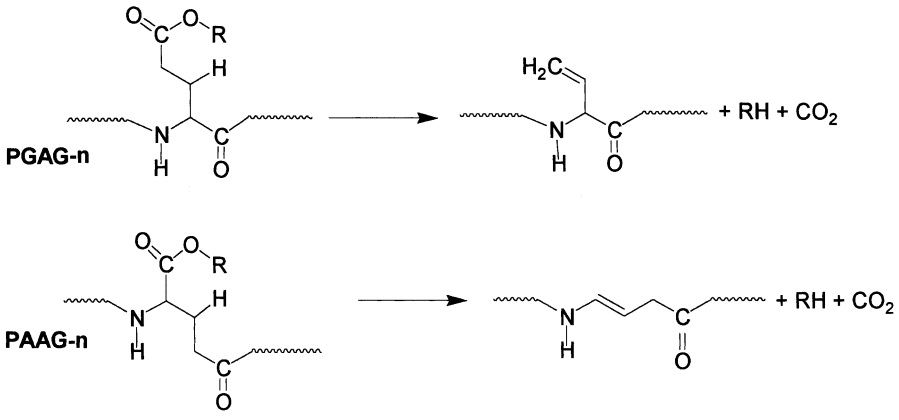
between 250 and 300°C for long periods of time implies a substantial weight loss.

It can be reasonably expected from TGA results that different molecular mechanisms must be operating in the low and the high temperature thermal decomposition of PAAG-*n*. To investigate the nature of these mechanisms, the products resulting from the thermal decomposition of PAAG-4 were analyzed by IR and NMR spectroscopy. A sample of this polymer heated at 275°C showed weight losses of about 25 and 75% after 10 and 60 min of treatment, respectively. The IR spectra of the sample before and after heating are compared in Fig. 5a, which proves that no significant changes have happened in the chemical structure of the polymer after treatment for 10 min. The spectra taken after heating for 60 min continues to be essentially the initial one although some changes indicative of side chain loss are observed. The NMR spectra of the condensed gases that were generated upon heating are shown in Fig. 6. Both ^{1}H and ^{13}C-NMR spectra evidenced that *n*-butyl pyroglutamate is the only product present in the condensate. Similar results were obtained when PAAG-10 was subjected to a similar investigation but using a heating temperature of 290°C in the isothermal treatment. It can be concluded therefore that the thermal decomposition of poly(α-alkyl γ-glutamate)s at temperatures below T_d takes place mainly by depolymerization of the chain with production of the corresponding *n*-alkyl-pyroglutamates.

A comparison of the decomposition mechanisms occurring at low temperatures (below 300°C) in poly(α-peptide)s, poly(β-peptide)s and poly(γ-peptide)s with regard to their chemical structures is depicted in Scheme 2 (scheme of the thermal degradation at low temperatures of poly(α-alkyl γ-glutamate)s (PAAG-*n*), poly(α-alkyl β-aspartate)s (PAAA-*n*) and poly(γ-alkyl α-glutamate)s (PGAG-*n*)). In the three cases, the nucleophylic attack of the NH onto the CO ester carbon is intramolecular and leads to formation of a five-membered amide ring. According to Kojima et al. [21], the thermal decomposition of poly(α-glutamate)s involves the scission of the ester side group with formation of a *N*-acyl pyrrolidone and releasing of the corresponding alkanol. The same process is known to occur in poly(β-aspartate)s with formation in this case of stable cyclic aspartimide [22]. In the case of poly(γ-glutamate)s, the occurrence of such a mechanism would lead to either a six-membered cyclimide or a three-membered amide ring, both structures being energetically disfavored. On the contrary, a stable five-membered 5-alcoxycarbonyl-2-pyrrolidone will be produced if the nucleophilic substitution reaction takes place on the



Scheme 2.



Scheme 3.

ester main chain. The process should happen preferentially at the chain ends in order to account for the observe releasing of the *n*-alkyl-pyroglutamate. A similar mechanism was proposed for the thermal decomposition of nylon 4 with concomitant generation of 2-pyrrolidone [23].

When PAAG-4 was further heated above 400°C, the remaining residue amounts less than 10% of the original sample weight. The IR spectrum of this residue is shown in Fig. 5b. This spectrum does not contain ester bands indicating that the alkoxycarbonyl side chain has completely disappeared. In fact, the most characteristic absorption of this spectrum is a couple of bands at 1638 and 1618 cm^{-1} in addition to a broad peak appearing around 3250 cm^{-1}. According to antecedents reported on the thermal degradation of poly(γ-methyl α,L-glutamate) above 300°C, such spectra could be interpreted as arising from the unsaturated polyamide that is formed upon decarboxylation of PAAG-4. The decomposition process would be therefore the same for poly(α-glutamate)s and poly(γ-glutamate)s, as schematically indicated in Scheme 3 (compared schemes of the thermal degradation of PGAG-*n* and PAAG-*n* at high temperatures). In the former case, the process results in the formation of a vinyl side group, whereas in the latter, the double bond becomes forming part of the polyamide backbone. In both cases, the mechanism would imply the occurrence of an intermediate cyclic conformer, which may be anticipated to be arranged more easily in poly(α-glutamate)s due to the flexibility of the side chain. This is in agreement with the lower temperatures that are observed to be required for the decomposition of these compounds.

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References

[1] Nitecki DE, Goodman JW. In: Weinstein B, editor. Chemistry and biochemistry of amino acids, peptides and proteins. New York: Marcel Dekker, 1971. p. 87.
[2] Troy FA. Peptide antibiotics biosynthesis and functions. New York: Walter de Gruyter, 1982 p. 49.
[3] Gross RA. In: Kaplan DL, editor. Biopolymers from renewable resources. Berlin: Springer, 1998. p. 195.
[4] Tanaka T, Hiruta O, Futamura T, Uotani K, Satoh A, Taniguchi M, Oi S. Biosci Biotech Biochem 1993;57:2148.
[5] Kubota H, Fukuda Y, Takebe H, Endo T. USA Patent 5,118,784, 1994.
[6] Shah DT, McCarthy SP, Gross RA. ACS Polym Prep 1993:488.
[7] Gross RA, McCarthy SP, Shah DT. US Patent 5,378,807, 1995.
[8] Kubota H, Nambu Y, Endo T. J Polym Sci, Polym Chem 1993;31:2877.
[9] Kubota H, Nambu Y, Endo T. J Polym Sci, Polym Chem 1995;33:85.
[10] Borbély M, Nagasaki J, Borbély J, Fan K, Bhogle A, Sevoian M. Polym Bull 1994;32:127.
[11] Gonzales D, Fan K, Sevoian M. J Polym Sci, Polym Chem 1996;34:2019.
[12] Bocchi V, Canasti G, Dossena A, Marchelli R. Synthesis 1979:961.
[13] Ivanovics G, Erdos LZZ. Immunitatforsch 1937;90:5.
[14] Kovacs J, Kovacs HN, Ballina R. J Am Chem Soc 1963;85:839.
[15] Kovacs J, Schmit GN, Jonhson B. Can J Chem 1969;47:3690.
[16] Katjar M, Bruckner V. Acta Chim (Budapest) Acad Sci Hung 1969;62:191.
[17] Sanda F, Fujiyama T, Endo T. J Polym Sci, Polym Chem 2001;39:732.
[18] Pérez-Camero G, Congregado F, Bou J, Muñoz-Guerra S. Biotech Bioengng 1999;63:109.
[19] García-Alvarez M, López-Carrasquero F, Morillo M, Muñoz-Guerra S. J Polym Sci, Polym Phys 1997;35:2379.
[20] Cromwick AM, Gross RA. Int J Biol Macromol 1995;17:259.
[21] Kojima T, Kurotu T, Kawaguchi T. Macromolecules 1986;19: 1281.
[22] Muñoz-Guerra S, López-Carrasquero F, Fernández-Santín JM, Muñoz-Guerra S. In: Salamone JC, editor. Polymeric materials encyclopedia, vol. 6. Boca Raton: CRC Press, 1996. p. 4694.
[23] Sekiguchi H, Tsourkas P, Carriere F, Audebert R. Eur Polym J 1974;10:1985.
[24] Tanaka S. PhD Thesis, Kyoto University, 1972.