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Solution behaviour of poly(*N*-acryloyl-L-leucine) and its copolymers with *N*-isopropylacrylamide

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A vinyl polymer carrying COOH groups and the groups (amido and isopropyl) present in the temperature-sensitive poly(*N*-isopropylacrylamide) (pNIPAAm) was synthesized along with two of its copolymers incorporating NIPAAm units. The protonation of COO^- groups, studied in aqueous media over a range of temperatures (25–35°C) that included the lower critical solution temperature of pNIPAAm, showed polyelectrolyte behaviour with a decreasing pattern of the basicity constant ($\log K$) and enthalpy ($-\Delta H^\circ$) changes in relation to the degree of protonation (α). Beyond a critical α value ($\alpha = 0.4$), the $\log K/\alpha$ slope drastically changed and the reduced viscosity (η/C) sharply decreased. Under these conditions the Fourier transform infra-red spectra revealed a strong band at $1625\,cm^{-1}$ presumably reflecting the interaction of the amido groups of several monomer units. For the copolymers the decreasing pattern of $\log K$ values decreased with increasing NIPAAm content, and beyond the critical α, the $\log K/\alpha$ slope increased again, showing a greater endothermic effect superimposed on the protonation of the COO^- group. The phenomenon was attributed to hydrophobic forces between isopropyl groups outweighing the repulsive electrostatic interactions of the ionized polymer. At higher temperatures, the $-\Delta H^\circ$ values decreased in a sensitive manner and the critical α shifted to a lower value because of enhanced hydrophobic interactions. A reduced amount of structured water molecules on the polymer was revealed by the lower entropy changes (ΔS°). A hydroxo-complex species of $Cu(OH)_2L_2^{2-}$ (L^- is the repeating unit of the polymer) stoichiometry was revealed at low pH with a linear decrease in stability constants ($\log \beta$) in relation to pH. The pH range for a single complex species was consistent with the electronic spectra. © 1997 Elsevier Science Ltd.

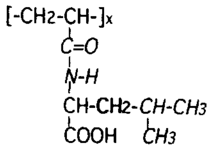
(Keywords: protonation thermodynamics; temperature-sensitive polymers; hydrophobic interactions)

INTRODUCTION

In previous papers we reported the synthesis and protonation thermodynamics of vinyl poly(carboxyl acid)s carrying amido and isopropyl groups in the well known temperature-sensitive poly(*N*-isopropylacrylamide) (pNIPAAm)[1,2]. The acrylic and methacrylic acid derivatives, containing L-valine residues, showed polyelectrolyte behaviour. The basicity constants ($\log K$) for the protonation of carboxylate groups decreased linearly with the degree of protonation α up to a critical α value, beyond which $\log K$ increased. Calorimetric data revealed sharp endothermicity superimposed on the protonation of COO^- groups. The data were explained in terms of hydrophobic interactions outweighing electrostatic repulsions above the critical α value. The whole protonation process was entropy-driven and led to collapse of the macromolecular coil and release of a large number of water molecules structured around the polymer.

These polymers were studied as multiple stimuli-responsive polymers in view of their application in 'chemical valve' systems[1,3–5]. These systems consist of porous substrates on to which stimuli-responsive polymer chains have been graft-polymerized. Membrane porosity changes in response to environmental conditions by virtue of changes in polymer chain conformation. Examples of valve function include systems in which the extended graft–polymer chains are brought back to collapsed coil conformation by changes in pH[1,6], temperature[1,4] and redox reaction[5,7]. Ionizable carboxyl groups made them responsive to pH, whereas isopropyl and amido groups improved temperature responsivity as in the case of uncharged pNIPAAm[8]. Aqueous solutions of the latter have a lower critical solution temperature (LCST) of 32°C. Many polymer solutions characterized by strong hydrogen bonding between polymer and solvent have a LCST. As temperature increases, hydrogen bonds weaken and the polymer collapses with a separation of phase. It is possible to increase or decrease the LCST of polymers by incorporating hydrophilic or hydrophobic comonomers, respectively[4]. Charges have a major effect because they sharply increase LCST for random charge distributions along the polymer[9,10]. Copolymers of NIPAAm with acrylic/methacrylic acid derivatives showed peculiar features. Any increase in NIPAAm content in copolymers increased the magnitude of the enthalpy changes and enhanced temperature sensitivity[2].

In this paper we report the solution behaviour for the protonation of the carboxylate group in poly(*N*-acryloyl-L-leucine) (PAL):


PAL

and its copolymers with NIPAAm. The presence of a further methylene group in the polymer side chain increases the distance between isopropyl and amido groups and weakens the critical hydrophilic–hydrophobic balance of pNIPAAm[11,12]. We evaluated the magnitude of the phenomenon at different temperatures, mainly on the basis of potentiometric, viscometric and calorimetric data. We also reported the formation of a copper(II) complex by evaluating the stability constants ($\log\beta$) and comparing them with previously studied (meth)acrylate polymers with L-valine residues[1].

EXPERIMENTAL

Spectroscopic measurements

Proton nuclear magnetic resonance (n.m.r.) spectra for all samples (20 mg dissolved in 0.5 ml deuterated dimethylsulfoxide) were recorded on a Bruker AC200 spectrometer (200 MHz) using tetramethylsilane as the internal reference. Fourier transform infra-red (*FT* i.r.) spectra were recorded on a Perkin-Elmer M1800 spectrophotometer following a previously reported procedure[13]. The u.v.–visible electronic spectra of the Cu(II) complex species were recorded on a Pharmacia LKB-Biochrom 4060 spectrophotometer. The pH of the aqueous solution was changed stepwise (by adding 0.1 M HCl) and checked with a PHM-84 Radiometer potentiometer equipped with a glass electrode.The polymer/Cu(II) molar ratios ranged from 2 to 3.

Viscometric measurements

Viscometric data were obtained with an AVS 310 Schott-Gerate viscometer. The intrinsic viscosity of PAL and its copolymers was measured at 30°C in dimethylformamide and dimethyl sulfoxide (DMSO), respectively (30 mg of compound dissolved in 15 ml of solvent). Viscometric titration data were measured at different temperatures (25, 30 and 35°C) in 0.1 M NaCl. Polymer solutions (50 mg of polymer dissolved in 25 ml 0.1 M NaCl with a large excess of NaOH) were titrated stepwise with 0.1 M HCl. The titration was stopped when the solution became cloudy. At the desired titration point, the degree of protonation α was calculated from the $\log K$ value by means of the FITH program[14].

Potentiometric measurements

Potentiometric titrations of the monomer, polymer and copolymers were carried out in aqueous solutions at two ionic strengths in a glass cell thermostated at the three temperatures (25, 30 and 35°C) using a previously described procedure[2,14]. A digital PHM-84 Radiometer potentiometer, equipped with glass and reference electrodes, was connected to an Olivetti M20 computer together with a Metrohm Multidosimat piston burette. Titrations were performed by stepwise addition of standard 0.1 M HCl to about 100 ml of 0.01 or 0.1 M NaCl containing a weighed amount of solid material (monomer, about 50 mg; polymer, about 40 mg; copolymer, 40–170 mg, depending on the content of COOH groups) and a known amount of standard NaOH solution. To avoid CO_2 contamination the surface of the solution was kept under a nitrogen stream. In the case of polymer and copolymers, the end-point of the titration curve was used to determine the excess of NaOH equivalents from which the amount of COOH groups was calculated. The basicity constants were evaluated by the APPARK program[14], and the stability constants with Cu(II) ions with the SUPERFIT program[15]. The potentiometric data for the complex system were obtained at 25°C using aqueous solutions (0.1 M NaCl) of polymer/Cu(II), at molar ratios of 3 and 2, containing a known amount of NaOH that was titrated with standard 0.1 M HCl.

Calorimetric measurements

Calorimetric titration data for the polymer and copolymers were obtained as previously reported with a Tronac calorimeter (model 1250) operating in the isothermal mode[2,14]. A 25-ml stainless-steel reaction vessel was used throughout and the water-bath was thermostated to the desired temperature of 25, 30 or 35°C by means of a Tronac PTC-40 precision temperature controller. The reaction vessel was filled with 25 ml of aqueous solution (0.01 or 0.1 M NaCl) containing a weighed amount of polymer (about 40 mg) or copolymer (about 50 mg) and a measured volume of standard NaOH. Standard 0.1 M HCl titrant was delivered through a Gilmont burette at a constant burette delivery rate of 0.1 ml min^{-1}. A North Star CCP930 computer automatically controlled the experiments via the THERMAL program[14]. Calibrations of the instrument were made at each temperature before each run. The FITH program was used to evaluate $-\Delta H^\circ$ values, and entropy changes were calculated simply as $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$.

Synthesis of monomer

The *N*-acryloyl-L-leucine (AL) was obtained by a procedure similar to one previously reported[1,2,16]. Proton n.m.r. and i.r. spectra, together with elemental analysis and potentiometric titration, revealed a product of high purity.

Synthesis of polymer

The PAL was obtained by radical polymerization of AL initiated with azobis(isobutyronitrile) (AIBN) at 65°C, in two different solvents: DMSO and water. In 25 ml of DMSO we dissolved 1.08 g of AL and 17 mg of recrystallized AIBN. The mixture was purged with nitrogen and allowed to react in a thermostated water-bath for 2 days. The polymer was precipitated in water and washed repeatedly with freshly distilled water. Theoretical and experimental values for $C_9H_{15}NO_3 \cdot 0.75H_2O$ were in substantial agreement: C, 54.39 and 54.24; H, 8.36 and 7.50; N, 7.04 and 6.71, respectively. We dissolved 1.05 g of AL, 0.37 g of NaOH and 18 mg of AIBN in 60 ml of water. The mixture was allowed to react under nitrogen for 2 days. The polymer was precipitated with a concentrated HCl solution, filtered by suction and washed with distilled water. The sample retained one water molecule per repeating unit of PAL, as revealed by elemental analysis. The spectroscopic data (i.r. and ^{1}H n.m.r. in DMSO-d_6) of both syntheses were similar and consistent with the proposed structure.

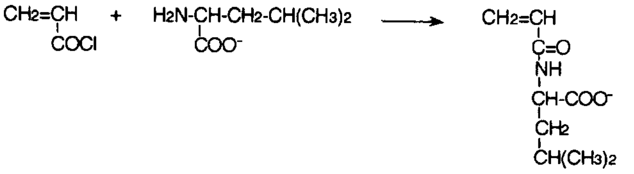
Copolymers

Two copolymers (*co*-A and *co*-B) with different AL/NIPAAm monomer compositions were prepared by radical polymerization in DMSO. The following feed compositions were used: *co*-A (30 ml DMSO): 0.501 g AL, 0.313 g NIPAAm and 16 mg AIBN; *co*-B (40 ml DMSO): 0.503 g AL, 3.108 g NIPAAm and 16 mg AIBN. Both mixtures were purged with nitrogen and allowed to react overnight at 60°C. At the end of the reaction *co*-A and *co*-B were recovered as fine white powders by precipitation in diethyl ether and water, respectively. The potentiometric titration of carboxyl groups, together with ^{1}H n.m.r. spectra, reflected the different comonomer incorporation levels.

RESULTS

Synthesis and characterization

Monomer and polymer. AL was obtained by the general acrylation reaction of acryloyl chloride with L-leucine dissolved in aqueous solution and containing two equivalents of NaOH[1,16].



A white solid crystallized from water after the addition of concentrated HCl. Its ^{1}H n.m.r. spectrum is reported in *Figure 1* along with the assignments of proton resonances. The strong i.r. bands for the COOH (1730 cm^{-1}), amide I (1660 cm^{-1}), amide II (1550 cm^{-1}) and CH_3 (1460 cm^{-1}) groups were clearly evident. The monomer was freely soluble in many solvents such as DMSO, dioxane and water, and did not appear to be very sensitive to atmospheric moisture.

In aqueous media, radical polymerization of AL, in the form of a sodium salt, caused a white powder to precipitate out on addition of strong acid. The complete disappearance of the vinyl double bond (1630 and 980 cm^{-1}) in PAL was confirmed by the ^{1}H n.m.r. spectrum. The latter showed broad lines for backbone and side-chain resonances, consistent with the presence of a slowly tumbling macromolecular species in solution. In fact, high-molecular-weight polymers were obtained, as shown by viscosity measurements. Intrinsic viscosities were 0.49 dl g^{-1} (PAL obtained from water) and 0.60 dl g^{-1} (PAL obtained from DMSO). They were white powders, amorphous under X-ray examination, without a definite melting point. Elemental analysis always revealed residual bound water molecules and this was consistent with the titration assays of carboxyl groups. PAL was soluble in aqueous media only at pH >4.5, and it dissolved in most organic solvents.

Copolymers. Two copolymers with NIPAAm were obtained by radical polymerization in DMSO, with NIPAAm/AL molar ratios of 1 (*co*-A) and 10 (*co*-B). The relative comonomer content, checked by ^{1}H n.m.r.

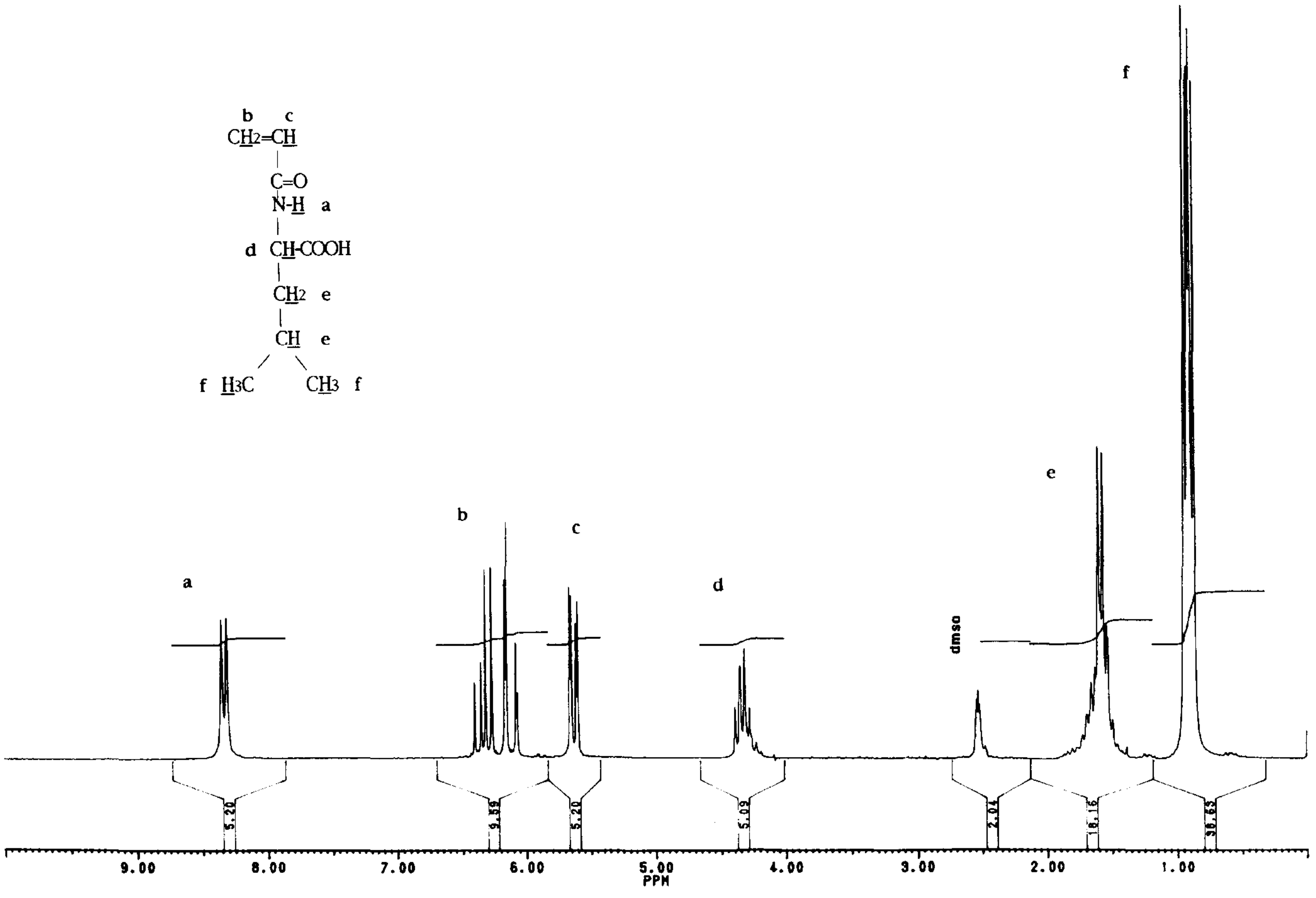


Figure 1 Proton n.m.r. spectrum of AL in DMSO-d_6 with the corresponding assignments of proton signals

spectra and titration assays of carboxyl groups, reflected a lower number of AL units in the copolymers than expected. *Co*-A revealed 49 wt% of titrated AL units compared to the 62 wt% calculated for the feed monomer mixture. A similar trend was observed for *co*-B (titrated 10 wt%; calculated 14 wt%). Like previously studied copolymers, having closer calculated and experimental numbers of acidic comonomer units[2,17], the proton resonances of the NIPAAm methyl groups were located at a higher magnetic field. *Figure 2* shows the range of significant proton resonances for the two copolymers overlapping the methyl group resonances of PAL. As for simple PAL, broad lines of the ^{1}H n.m.r. spectra were obtained, in agreement with the presence of high macromolecular species in solution. The intrinsic viscosities of both copolymers were in the range 0.6–0.8 dl g^{-1}. Both copolymers were soluble in most organic solvents and in aqueous media at pH > 4.

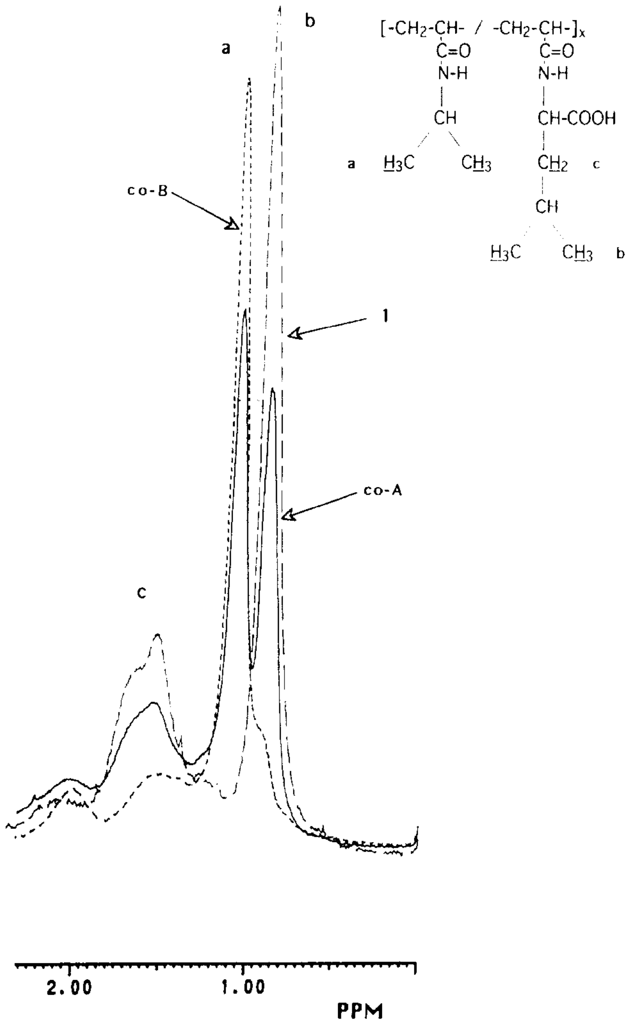


Figure 2 Proton n.m.r. spectra (in DMSO-d_6) in the range of methyl resonances of PAL (**1**) and two of its copolymers with different amounts of NIPAAm (*co*-A, 51 wt%; *co*-B, 88 wt%)

Thermodynamics of protonation

The protonation behaviour of PAL and poly(AL-*co*-NIPAAm)s was studied in aqueous media at different temperatures (25, 30 and 35°C) by viscometry, potentiometry and solution calorimetry. Infra-red spectra of PAL in aqueous solution at different pHs supported the thermodynamic data[13,18].

Figure 3 shows the free energy changes ($-\Delta G^0$) and viscometric titration data (η/C) for the protonation of the COO$^-$ group of PAL showing polyelectrolyte behaviour. The $-\Delta G^0$, related to the basicity constant ($\log K$) by the relation[19]:

$$-\Delta G^0 = 2.303\,RT\,\log K$$

showed a linear decreasing pattern in the low range of degree of protonation α. At values of $\alpha > 0.36$, changes in $-\Delta G^0/\alpha$ slopes occurred at the three temperatures, the free energy changes becoming flatter in relation to α. The effect of temperature was to increase the basicity of COO$^-$ groups at 30°C and above, following a pattern similar to that already observed for poly(*N*-acryloyl-L-valine) (PAV) and its copolymers with NIPAAm[2]. Conformational properties confirmed the lower degree of solvation of the polymer, which had lower reduced viscosities (η_{sp}/C) at higher temperatures in the low range of α values. In all cases, however, the ionized macromolecules first coiled slowly due to reduced electrostatic repulsion between negatively charged groups, followed by a sharp drop in viscosity above the critical α. In this condition, calorimetric titration data

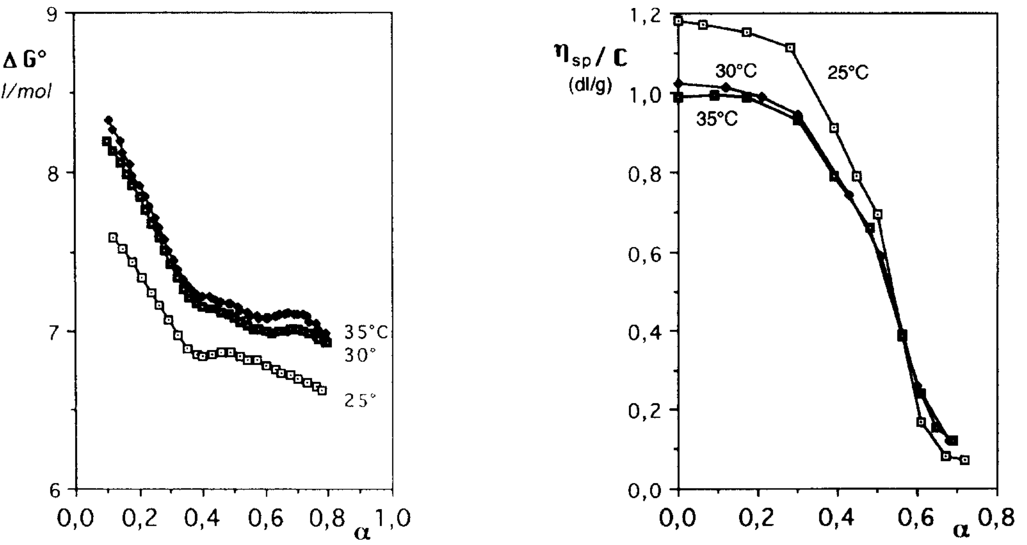


Figure 3 Free energy changes ($-\Delta G^0$) and reduced viscosity (η/C) in relation to α for the protonation of COO$^-$ groups in PAL in 0.1 M NaCl at three different temperatures (25, 30 and 35°C)

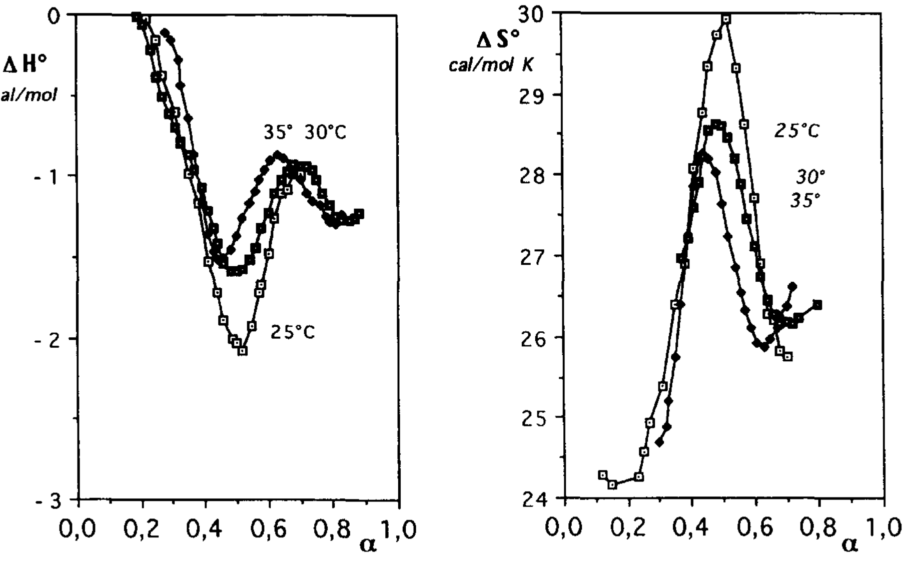


Figure 4 Enthalpy ($-\Delta H^{\circ}$) and entropy (ΔS°) changes in relation to α for the protonation of PAL in 0.1 M NaCl at three different temperatures (25, 30 and 35°C)

Table 1 Henderson–Hasselbalch parameters for the protonation of monomer, polymer and copolymers at different temperatures and ionic strengths

Compound	μ (mol dm^{-3})	T (°C)	α Range	$\log K^{\circ\,a}$	n^a
AL	0.1	25	0.10–0.80	3.676(3)	1.00
PAL	0.1	25	0.12–0.38	4.85	1.92
		30	0.10–0.36	4.98	2.06
		35	0.11–0.36	4.88	2.18
	0.01	25	0.11–0.44	5.45	2.00
co-A	0.1	25	0.11–0.45	4.65	1.70
	0.01	25	0.11–0.43	4.82	2.06
co-B	0.1	25	0.14–0.44	4.06	1.37
	0.01	25	0.12–0.46	3.98	1.50

[a] $\log K = \log K^{\circ} + (n-1)\log(1-\alpha)/\alpha$ (standard deviation in parentheses)

Poly(AL-*co*-NIPAAm): *co*-A, 51 wt% of NIPAAm; *co*-B, 88 wt% of NIPAAm

(*Figure 4*) revealed sharp endothermic peaks with temperature dependent $-\Delta H^{\circ}$ values. Any increase in temperature decreased the endothermic peak in a sensitive manner and this reflected lower ΔS° values.

Among the previously studied polymers of this series[1,2,17], the thermodynamic results showed that the normal polyelectrolyte behaviour of PAL was limited to a narrow range of α values. The basicity constants reported in *Table 1* followed the modified Henderson–Hasselbalch equation[19,20]:

$$\log K = \log K^{\circ} + (n-1)\log(1-\alpha)/\alpha$$

Any extrapolation to $\alpha = 1$ of the linear decreasing trend of $\log K$ in relation to α reached the intrinsic equilibrium constant of the hypothetical uncharged polymer that is close to the $\log K$ of the corresponding low-molecular-weight monomer (AL). This means that beyond the critical α, the protonation process not only involves electrostatic repulsions, but also further intramolecular interactions. Infra-red spectra in aqueous solution at pH 5.0, 6.5 and 9.0 showed the characteristic bands of the partially and completely ionized form of the polymer PAL, respectively. *Table 2* summarizes the main wavenumbers of the *FT*i.r. spectra along with their assignments. The spectrum at pH 9.0 shows the characteristic absorption frequencies of the asymmetric and symmetric C–O stretching of COO$^-$ at 1578 and 1400 cm^{-1}, respectively[13,18]. The amide I band appears as a shoulder at 1660 cm^{-1}. On decreasing the pH to 6.5, corresponding to $\alpha = 0.25$, a strong band at 1700 cm^{-1}, due to the C=O stretching of the COOH group, emerged clearly. Beyond the critical α value (pH 5.0, $\alpha = 0.6$) a strong band at 1625 cm^{-1} appeared in the spectrum[13].

Table 2 Main wavenumbers[a] observed for PAL in solution at different pH, and their assignments

Wavenumber in cm^{-1} at pH			
5.0	6.5	9.0	Assignment
1700 (s)	1700 (vs)		C=O stretch of COOH group
1650 (vs)	1650 (vs)	1660 (sh)	H-bonded amide C=O stretch
1578 (vs)	1560 (vs)	1578 (vs)	Asym.stretch. COO$^-$ group
1395 (s)	1396 (s)	1400 (s)	Sym. stretch. COO$^-$ group

[a] s = strong; vs = very strong; sh = shoulder

At the critical α value and lower ionic strengths, charges led to major competition between electrostatic and hydrophobic forces. A sensitive shift to a higher critical α was observed for PAL in 0.01 M NaCl (*Table 1*). Low salt concentrations reflected greater $\log K$ values, due to lower screening effects between charged carboxylate anions. This increased the electrostatic potential in the environment of the polyion. A similar trend was observed for the enthalpy ($-\Delta H^{\circ}$) changes[17]. These data indicated that the contribution of electrostatic repulsion was greater than the hydrophobic forces, shifting polymer collapse to a higher critical α.

Unlike PAL, the protonation behaviour of its copolymers with NIPAAm showed thermodynamic results similar to those previously reported for other related polymers[17]. *Figure 5* shows that beyond the critical α, the free energy changes of poly(AL-*co*-NIPAAm) increased upon protonation of the COO$^-$ groups, with a shift to greater basicities at higher temperatures. Above the critical α, the $-\Delta G^{\circ}$ term therefore increased for a wide range of α due to the close proximity of the carboxylate anions being protonated[2]. The effect of ionic strength was lower for the copolymers than for PAL because the charged group is shielded by the uncharged NIPAAm

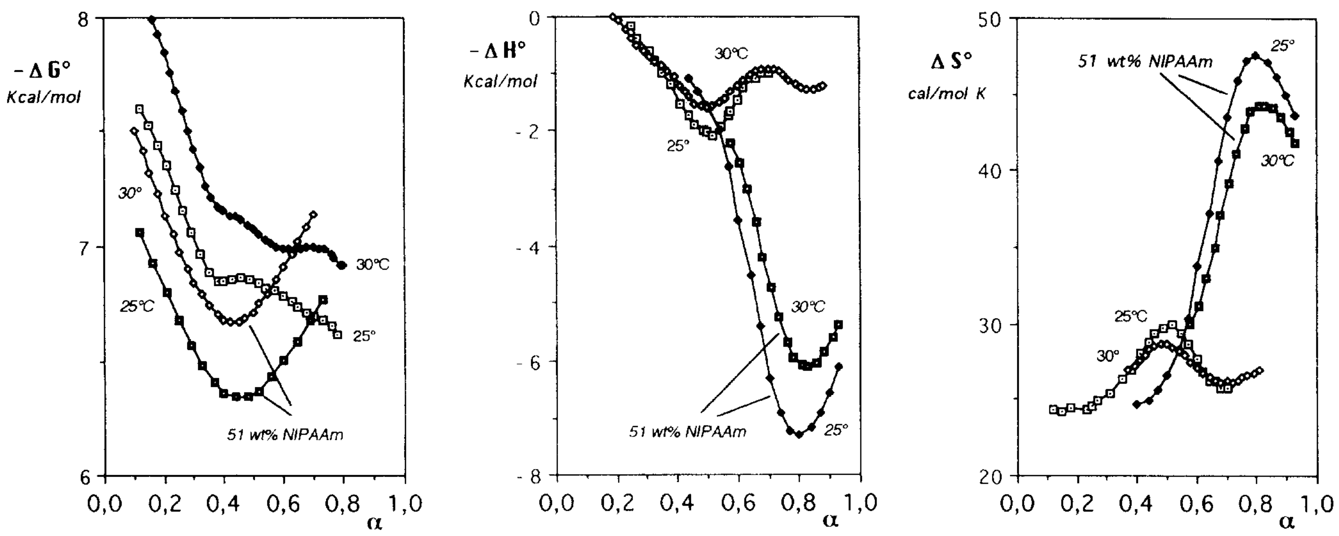


Figure 5 Thermodynamic functions ($-\Delta G^0$, $-\Delta H^0$ and ΔS^0) in relation to α for the protonation of COO^- groups in PAL and a copolymer with 51 wt% of NIPAAm (*co*-A) in 0.1 M NaCl at two different temperatures (25 and 30°C)

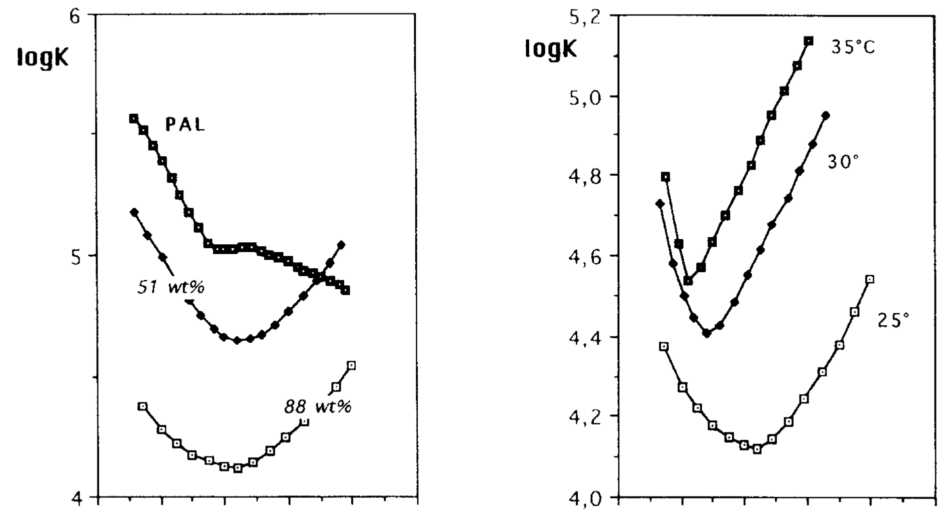


Figure 6 Basicity constants (log K) in relation to α for PAL and two copolymers with 51 wt% (*co*-A) and 88 wt% (*co*-B) of NIPAAm in 0.1 M NaCl at 25°C. Effect of temperature (°C) on log K for *co*-B (0.1 M NaCl)

moiety (*Table 1*). The hydrophobic interaction, which outweighed the electrostatic repulsion, was revealed by greater endothermicity superimposed on the protonation of the simple PAL.

Figure 6 shows the effect of content and temperature on copolymers incorporating higher amounts of uncharged temperature-sensitive NIPAAm. Any increase in NIPAAm content acted as a shield and decreased electrostatic interactions, whereas temperature increased hydrophobic interactions. This resulted in a decreasing critical α value and greater log K values.

Copper(II) complexes

The ability of PAL to form complex species with Cu(II) ion was studied to observe even more drastic conformational changes in a different range of pH[1,21].

Figure 7 shows the trend of stability constants (log β) and the wavelength of maximum absorption (λ_{max}) in relation to pH for PAL and two previously described polymers: PAV and poly(*N*-methacryloyl-L-valine) (PMAV)[1,2]. It is interesting to note the closer complexing behaviour in the low range of pH where the protonation behaviour showed significant differences. The calculated stability constants were consistent with $Cu(OH)_2L_2^{2-}$ (L^- is the repeating unit of the polymer) stoichiometry. The formation of a negatively charged complex species produced a decreasing pattern of log β in relation to pH[1,20,22]. The decrease was linear up to about pH 7, and λ_{max} confirmed the presence of the same complex species. Above this pH, λ_{max} shifted to higher energies with subtle changes in log β/pH slopes, indicative of other complex species.

DISCUSSION

PAL is a polyelectrolyte, which, besides the amido and isopropyl groups, has an ionizable COOH in the side chain. Its conformation changes as a result of changes in pH and temperature due to hydrophobic interactions[2,17]. Unlike pNIPAAm, which is a non-ionic polymer with a cloudy point at 32°C, PAL has charges which give it a higher critical solution temperature[9,10]. As is well known,

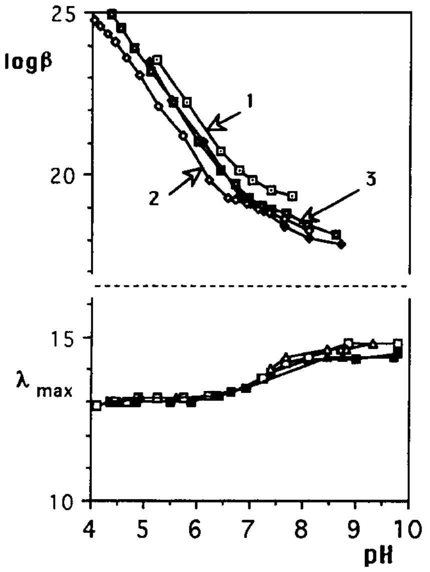


Figure 7 Stability constants (log β) and maximum absorption wavelength (λ_{max}, KK/$\lambda_{max} \times 10^4$) in relation to pH for the $Cu(OH)_2L_2^{2-}$ complex species (L^- is the repeating unit of the polymer) in 0.1 M NaCl at 25°C: (1) Cu(II)/PAV (3/1 molar ratio); (2) Cu(II)/PMAV (3/1 molar ratio); (3) Cu(II)/PAL (3/1 and 2/1 molar ratios)

the LCST is caused by a critical balance between hydrophilic and hydrophobic domains in the polymer side groups that trigger the formation of a layer of highly organized water molecules around the macromolecular chain. As the temperature increases, hydrophobic interactions are enhanced because of the weakening of hydrogen bonds between water molecules and the amido group of the polymer. This phenomenon is observed clearly in pNIPAAm, which contains amido and isopropyl groups chosen to balance the hydrophobic–hydrophilic quality in a critical manner[11,12,23]. The presence of charged groups showed that electrostatic and hydrophobic forces competed at a critical α, with hydrophobicity dominating at higher temperatures. The temperature-induced phase separation was mainly driven by this interaction, caused by reduced structuring of water molecules around polymer side groups.

The protonation thermodynamics of charged polymers, structurally related to pNIPAAm, has shown that any increase in the distance between amido and isopropyl groups increases hydrophobic contributions, shifting polymer collapse to lower α. PAL behaves differently from the previously reported PAV, because of the presence of an additional methylene group in the side chain[2,17]. The log K values are affected by temperature in a sensitive manner[2]. The small shift to greater basicities of COO^- groups at higher temperatures (30 and 35°C) has been attributed to the lower hydration of PAL and its increased hydrophobic character[24]. The enthalpy changes showed similar features with a lower endothermic effect and protonation revealed an entropy-driven process with smaller ΔS^0 at higher temperatures (*Figures 4* and *5*). Like previous results for polymers with L-valine residues[2], these findings are consistent with the LCST phenomenon according to which lower hydration of the polymer at higher temperatures releases fewer water molecules structured around the chain[12,23]. Temperature sensitivity was less, but pH changes revealed greater conformational characteristics. Reduced viscosity decreased very smoothly in relation to α up to the critical α value, beyond which subtle changes in the small coil were attributed to intramolecular interactions between hydrophilic and hydrophobic groups of several monomeric units[13]. Infra-red spectra at pH 5.0, corresponding to $\alpha = 0.60$, as calculated by the log K value, revealed the strong band at 1625 cm^{-1}, already found in vinyl polymers with a larger hydrophobic moiety[13]. This band lies in the region of the amide I vibrational mode where amido groups are involved in strong hydrogen bonds, implying other mechanisms in addition to simple hydration[25]. Besides hydrophobic interactions leading to increased endothermic effects, the amido groups of several monomeric units probably interact.

The presence of NIPAAm units in the copolymer facilitated the hydrophobic interaction (*Figure 5*) because this led to stronger enthalpy changes and a new increase in log K values beyond the critical α. The peculiar arrangement of the isopropyl groups in NIPAAm forced the macromolecular coil of the copolymers into close contact. Micelle-like behaviour is known to be induced by hydrophobic forces which bring the polar COO^- groups close together, making protonation easier[2,17] (*Figure 6*). Once again, the whole protonation process was entropy-driven because of the large number of water molecules released from the NIPAAm moiety. This number decreased with increasing temperature.

Although their protonation behaviour was different, the vinyl polymers carrying the COOH group showed similar complex formation with the Cu(II) ion. This is because Cu(II) is anchored to two adjacent COO^- groups of the α-amino acid residues, irrespective of the hydrophobic size of the lateral chain or the presence of methyl groups on the polymer backbone[1,21,22].

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