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Synthesis, characterisation and solution behaviour of thermo- and pH-responsive polymers bearing L-leucine residues in the side chains

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Abstract

Polymers capable of responding to both temperature and pH changes were synthesised by radical copolymerisation of *N*-isopropylacrylamide (NIPAAm) and *N*-methacryloyl-L-leucine (MALEU) in different NIPAAm/MALEU molar ratios (15:1, 10:1, 2:1 and 1:1). The polymers were characterised by Size Exclusion Chromatography, acid–base titrations, FT-IR and ^{1}H-NMR analyses. Their solution behaviour was studied in 0.1 M NaCl and in citrate buffers (pH 4.0, 4.5, 5.0 and 6.0). At pH 4.0 and 4.5 the cloud point temperature (T_{CP}) of copolymers was depressed proportionally to their MALEU content. In contrast, at pH 5.0 and 6.0 the phase transition temperature increased linearly. The behaviour of the copolymers with NIPAAm/MALEU ratios 15:1 and 10:1 was investigated also between pH 3 and 11 in 0.1 M NaCl. In both cases a sharp increase in T_{CP} was observed around pH 4–5. The T_{CP} vs. pH curve was linearised and the acidity constants were determined by the linearisation procedure. Furthermore, it was possible to demonstrate that the phase transition temperature increases linearly with the ionic content of the polymers. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Intelligent polymers; Stimuli-responsive polymers; Thermosensitive polymers

1. Introduction

Intelligent polymers are soluble, surface-coated or cross-linked polymeric materials capable of undergoing sharp physical or chemical modifications in response to external stimuli such as temperature, pH, ions or other chemical species, electric or magnetic fields. For instance, thermo-sensitive polymers precipitate from their solutions above a temperature dependent on the polymer concentration, the minimum of the solubility curve being known as the lower critical solution temperature (LCST). This behaviour is observed in many solutions characterised by strong hydrogen bonds and is therefore particularly relevant to aqueous media. Thermo-sensitive polymers generally present in their structure both hydrophilic and hydrophobic moieties. Below the LCST they adopt an extended random coil conformation, while as the temperature increases the hydrogen bonds weaken, with concomitant release of hydration water, and the hydrophobic interactions tend to overcome the hydrophilic ones leading to a coil-to-globule transition and finally to phase separation [1,2].

This behaviour has attracted a great deal of attention both from a theoretical and a practical viewpoint. In fact, a correct modelling of this phenomenon may be of great help in investigating key aspects of the living organisms such as protein folding and DNA packing. On the other hand, many applications have also been designed for these polymers: for instance, they are being studied in drug delivery [3–5], solute separation [6,7] and solvent extraction [8]; furthermore, they can be grafted onto membranes to be used as chemical valves [9–12] or employed in the preparation of "chemomechanical systems", i.e. systems able to transform chemical energy into mechanical work [9,13].

Poly(*N*-isopropylacrylamide) (pNIPAAm) is the most popular polymer studied in this respect since it exhibits a sharp, thermoreversible phase transition around 32°C [14]. An interesting feature common to other thermosensitive polymers lies in the possibility of tuning the LCST by adding cosolvents [15,16], salts [17], surfactants [18] or polyelectrolytes [19] to the pNIPAAm solutions, or by incorporating comonomers with variable degree of hydrophilicity [1,20,21]; in fact, increasing or decreasing the hydrophilic content of a copolymer will usually result in an increase or decrease, respectively, of its LCST. Furthermore, polymerising *N*-isopropylacrylamide (NIPAAm) with

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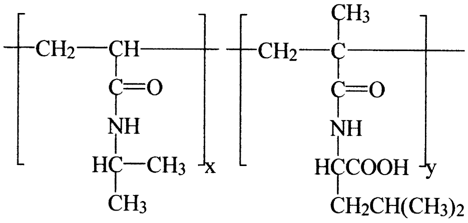
weakly ionisable comonomers allows to obtain intelligent polymers capable of responding to both temperature and pH variations; interestingly, owing to the variation of their degree of ionisation with pH, systems with pH-dependent LCST [22,23] are obtained.

In this connection we thought it interesting to investigate the solution behaviour of NIPAAm copolymers bearing α-aminoacidic residues in the side chains. In principle, varying the amino acid structure and content, polymers with a wide range of properties can be synthesised. Furthermore, owing to the presence of asymmetric carbon atoms, such systems can be employed also in applications where chiral recognition is required, such as in temperature-responsive chromatography of chiral molecules [24,25].

Previous thermodynamical investigations on the solution behaviour of copolymers with *N*-acryloyl-L-valine [26], *N*-methacryloyl-L-valine [26] and *N*-acryloyl-L-leucine [27,28] have revealed the occurrence of an entropy-driven coil-to-globule transition associated with the release of a large number of water molecules structured around the polymer. At a fixed temperature the transition has been shown to take place at a critical degree of protonation corresponding to the point where the attractive hydrophobic interactions outweigh the repulsive electrostatic forces and ultimately lead to chain collapse. However, so far no systematic determination of the phase transition temperatures has been undertaken on this class of polymers.

The aim of this work is to report on the synthesis and properties of a series of NIPAAm/MALEU copolymers obtained by radical polymerisation of NIPAAm with *N*-methacryloyl-L-leucine (MALEU) and having the following general structure:



These polymers were first characterised by Size Exclusion Chromatography (SEC), acid–base titrations, FT-IR and ^{1}H-NMR analyses. Then their cloud point curves in 0.1 M NaCl were determined. Finally, the influence of the pH on the transition temperatures was investigated and a linear relationship with the degree of ionisation was established.

2. Experimental

2.1. Materials

All solvents employed were ACS grade purchased from Antibioticos Co., and were used without purification. Isopropylamine (99.5% purity), acryloyl chloride (97%), L-leucine (LEU; 99%), methacryloyl chloride (97%), ammonium peroxo-disulphate (APS; 98%) and triethylamine (TEA; 99%) were purchased from Fluka Co. and used as received, except TEA which was distilled under reduced pressure. NIPAAm (97%) was from Aldrich Co. and was recrystallised from a toluene/hexane mixture (85 vol% hexane, 15 vol% toluene) before use. Citrate buffers were prepared mixing 0.1 M disodium citrate with either 0.1 M HCl (buffers with pH 4.0 and 4.5) or 0.1 M NaOH (buffers with pH 5.0 and 6.0) in the proper ratios and adding NaCl so as to have a 0.1 M salt solution. In turn, the disodium citrate solution was prepared neutralising anhydrous citric acid (from Fluka Co.; 99.5% purity) with 1 M NaOH.

2.2. Spectroscopic characterisations

^{1}H-NMR spectra were recorded in D_2O on a 270 MHz Bruker spectrometer using trimethylsilylpropionic acid as internal reference. Since pMALEU and copolymers were insoluble as free acids, their spectra were run on their sodium salts. FT-IR spectra were recorded on a Jasco 5300 FT-IR spectrophotometer by casting from methanol on ZnSe windows.

2.3. Acid–base titrations

Potentiometric titrations were carried out in aqueous solutions (0.1 M NaCl) at 25°C with an apparatus previously described [26]. A digital PHM-84 Radiometer potentiometer, equipped with a pHG211 High pH glass electrode (Radiometer) and a Ref201 reference electrode (Radiometer), and a Metrohm Multidosimat piston burette connected to the M20 Olivetti computer were used for controlling and recording potentiometric data.

For each experiment the thermostated glass cell was filled with ca. 100 ml of 0.1 M NaCl in which a weighed amount of solid compound (pMALEU: about 54 mg; copolymers: 60–300 mg depending on the MALEU content) and a measured quantity of standardised 0.1 M NaOH solution were dispersed by magnetic stirring under nitrogen stream, to avoid CO_2 contamination. Titrations were made with standardised 0.1 M HCl solution and back-titrated with 0.1 M NaOH. The equilibrium condition was reached within the 300 s we programmed for each step of titrant addition. Both forward and backward titrations were found reproducible.

2.4. SEC experiments

The molecular characterisation of polymers was performed by a multidetector SEC system consisting of an Alliance 2690 separation module (pump, injector and degasser), a differential refractometer (DRI) from Waters (Milford, MA, USA) and an additional multi-angle laser light scattering (MALS) Dawn DSP-F photometer from

Table 1
Feed compositions and reaction yields

Polymer[a]	NIPAAm	MALEU	APS[b]	TEA[c]	Yield
pNIPAAm	5.04 g (44.5 mmol)	0	25.2 mg	0.62 ml (4.5 mmol)	4.58 g (91%)
NIP15-*co*-MAL1	5.60 g (49.5 mmol)	0.66 g (3.3 mmol)	31.0 mg	0.51 ml (3.6 mmol)	4.50 g (72%)
NIP10-*co*-MAL1	6.10 g (53.9 mmol)	1.07 g (5.39 mmol)	35.9 mg	0.83 ml (5.9 mmol)	5.75 g (86%)
NIP2-*co*-MAL1	2.36 g (20.8 mmol)	2.08 g (10.4 mmol)	22.3 mg	1.60 ml (11.5 mmol)	3.33 g (75%)
NIP1-*co*-MAL1	1.20 g (10.6 mmol)	2.12 g (10.6 mmol)	16.6 mg	1.63 ml (11.7 mmol)	2.75 g (83%)
pMALEU	0	5.50 g (27.6 mmol)	27.5 mg	4.23 ml (30.3 mmol)	3.85 g (70%)

[a] NIP*x*-*co*-MAL*y* indicates the copolymer obtained reacting *x* moles of NIPAAm with *y* moles of MALEU.

[b] 0.5 wt% by weight compared to the sum of NIPAAm and MALEU.

[c] 10 mol% excess compared to MALEU. In the case of pNIPAAm: 10 mol% compared to NIPAAm.

Wyatt (S. Barbara, CA, USA). The description and performance of this SEC-MALS system was described in detail elsewhere [29–31].

Two different mobile phases were employed, namely Tris buffer pH 8.09 (0.1 M Tris + 0.2 M NaCl) for pMALEU and copolymers, and *N*,*N*-dimethylformamide (DMF) + 0.05 M LiBr for pNIPAAm. The experimental conditions in the first case were 0.6 ml/min flow rate, 25°C temperature and two TSK-Gel columns (G6000PW and G5000PW) from TosoHaas (Stuttgart, Germany). In the DMF mobile phase they were 0.8 ml/min, 25°C and three Styragel columns (HR5, HR4, HR3) from Waters.

The wavelength of the laser of the MALS detector was 632.8 nm. The light scattering signal was detected simultaneously at 15 scattering angles ranging, in aqueous mobile phase, from 14.5 to 151.3°. It is well known that from the intensity of the scattering to zero angle the MALS detector measures the molar mass of the polymer. Furthermore, from the angular variation of the scattering, it measures the root mean square radius, $\langle s^2\rangle_z^{1/2}$. The calibration constant of the MALS detector was calculated using toluene as standard assuming a Rayleigh factor of $1.406 \times 10^{-5}\ \mathrm{cm}^{-1}$. The photodiodes angular normalisation was made by measuring the scattering intensity of a bovine serum albumin (BSA) globular protein in the aqueous mobile phase and a very narrow low molar mass polystyrene standard ($M = 10.9$ kg/mol, $D < 1.03$) in DMF.

The differential refractive index increment, d*n*/d*c*, of polymers with respect to the mobile phases was measured by a KMX-16 differential refractometer from LDC Milton Roy (Riviera Beach, FL, USA).

2.5. Cloud point determinations

Test tubes 10 mm in diameter containing a solution of the polymer in the proper aqueous medium were immersed in a water bath and heated at 0.5°C/min. The cloud point was determined as the temperature at which the first opaqueness appeared in the solution. Cloud point measurements as a function of pH were carried out by preparing a number of polymer solutions in 0.2 M NaCl, neutralising for each solution a different fraction of carboxy groups with NaOH, adding the proper amount of water so as to obtain a 1 wt% polymer solution in 0.1 M NaCl and finally measuring the resulting pH and cloud point values.

2.6. Synthesis of MALEU

The reaction was carried out in a four-necked flask equipped with a mechanical stirrer and a thermometer; the temperature was maintained at 0°C by dipping the flask into a solid carbon dioxide/acetone mixture. First, LEU (43.9 g; 332 mmol) was dissolved in 1 M NaOH (332 ml); then methacryloyl chloride (30 ml; 301 mmol) and 10 M NaOH (30.1 ml) were added dropwise under vigorous stirring. After the completion of the addition, the cooling bath was removed, the reaction mixture was left at room temperature for 4 h, diluted with distilled water (300 ml) and its pH lowered up to pH 5–6 with 1 M HCl. The aqueous solution was extracted with chloroform (6 × 200 ml), each time checking for pH and adding HCl in order to keep the pH around 5–6. The organic phase was dried over sodium sulphate and the solvent was evaporated in vacuo at 30°C. The white powder thus obtained was washed with petroleum ether 40–70°C and dried in vacuo up to constant weight. Yield: 49.8 g (83%).

FT-IR (cm^{-1}): 3325 (ν(N–H)); 2961 and 2874 (ν(C–H)); 1726 (ν(COOH)); 1657 (amide I); 1611 (ν(C=C)); 1535 (amide II); 1387 and 1372 (ν(C–H) of isopropyl group); 1204 (ν(C–OH)). ^{1}H-NMR in $CDCl_3$ (ppm with respect to TMS): 0.98 (d, 6H, CH_3); 1.48–1.85 (m, 3H, CH–CH_2); 1.93 (s, 3H, CH2=CHCH_3); 4.60–4.92 (m, 1H, C*H*–NH); 5.46 and 5.79 (s, 2H, CH_2=C); 6.43 (broad d; 1H, NH); 10.97 (s, 1H, COOH). Elemental analysis, Calculated: C, 60.28%; H, 8.60%; N, 7.03%. Found: C, 60.39%; H, 8.57%; N, 6.98%.

2.7. Synthesis of polymers

All the polymers were prepared by the following general procedure. The amounts of reagents employed in each polymerisation are reported in Table 1. The reaction was carried out in water, at 25°C, under inert atmosphere obtained by repeatedly flushing nitrogen, using APS (0.5 wt% compared to the sum of the monomers) as initiator and TEA as activator. Since TEA was employed also to dissolve MALEU, on the overall a 10 mol% excess of TEA over MALEU was used except with pNIPAAm where TEA was 10 mol% with respect to NIPAAm. The monomers concentration was 15 wt% compared to water. First MALEU was dissolved in water by addition of TEA, then NIPAAm and APS were added and the mixture was allowed to react for 24 h, finally the pH was lowered with 1 M HCl till pH 2. Polymers NIP2-*co*-MAL1, NIP1-*co*-MAL1 and pMALEU precipitated out and were repeatedly washed with warm water prior to drying. In contrast, pNIPAAm, NIP15-*co*-MAL1 and NIP10-*co*-MAL1 were soluble at $T <$ 15°C; therefore, they were ultrafiltered in cold water and freeze-dried. All the polymers were finally extracted in a Soxhlet apparatus with diethyl ether over calcium hydride and dried in vacuo up to constant weight. The reaction yields are reported in Table 1.

FT-IR of pMALEU (cm^{-1}): 3350 (ν(N–H)); 2961 (ν(C–H)); 1726 (ν(COOH)); 1640 (amide I); 1532 (amide II); 1389 and 1370 (ν(C–H) of isopropyl group); 1200 (ν(C–OH)). FT-IR of pNIPAAm (cm^{-1}): 3434, 3304 and 3075 (ν(N–H)); 2973, 2934 and 2876 (ν(C–H)); 1649 (amide I); 1543 (amide II); 1460 (ν(C–H); 1387 and 1368 (ν(C–H) of isopropyl group).

The FT-IR spectra of the copolymers exhibit the typical bands of the homopolymers with relative intensities in qualitative agreement with the copolymer composition.

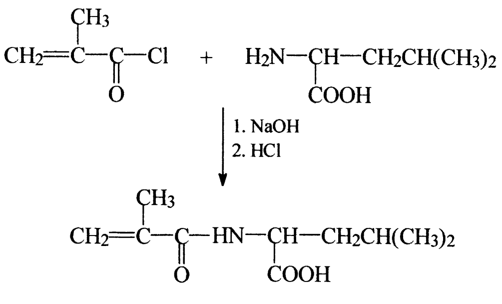
^{1}H-NMR of pMALEU (ppm): 0.73–1.26 (m, 9H, CH_3); 1.26–1.69 (m, 3H, CH–CH_2); 1.69–2.14 (m, 2H, C–CH_2); 4.12–4.49 (m, 1H, C*H*–NH). ^{1}H-NMR of pNIPAAm (ppm): 0.95–1.27 (m, 6H, CH_3); 1.33–1.84 (m, 2H, CH_2); 1.84–2.24 (m, 1H, C*H*–CH_2); 3.75–4.04 (m, 1H, C*H*–NH).

The ^{1}H-NMR spectra of copolymers show the peaks of the homopolymers with relative intensities in agreement with the copolymer composition.

3. Results and discussion

3.1. Synthesis and characterisation of MALEU

MALEU was synthesised by *N*-acylation of L-leucine with methacryloyl chloride as shown in the following scheme:



$$CH_2{=}\overset{\displaystyle CH_3}{\overset{|}{C}}{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}Cl + H_2N{-}\underset{\displaystyle COOH}{\underset{|}{CH}}{-}CH_2CH(CH_3)_2 \xrightarrow[\text{2. HCl}]{\text{1. NaOH}} CH_2{=}\overset{\displaystyle CH_3}{\overset{|}{C}}{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}HN{-}\underset{\displaystyle COOH}{\underset{|}{CH}}{-}CH_2CH(CH_3)_2$$

A part of sodium hydroxide was added immediately to L-leucine to neutralise the amino acid, while the other part was added dropwise along with methacryloyl chloride to neutralise the evolved HCl. This prevented the presence of a large excess of hydroxide ions thus reducing the hydrolysis rate of acyl chloride and ensuring good yields (83%).

The FT-IR and ^{1}H-NMR spectra (see Section 2) are consistent with the proposed structure while the potentiometric titration results indicate an analytical grade purity.

The DSC thermogram exhibits a melting peak with a maximum at 72°C, immediately followed by an exothermic peak with a minimum at 107°C. The latter is ascribed to polymerisation of MALEU. In fact if this is incubated under nitrogen atmosphere at 100°C for 4 h, a glassy material is obtained that FT-IR analysis reveals to be a mixture of pMALEU and unreacted monomer. Finally, at the concentration of 10 mg/ml, this monomer is soluble in many common organic solvents including chloroform, toluene, diethyl ether, ethyl acetate, methanol, *N,N*-dimethylformamide and dimethylsulphoxide, while it is insoluble in *n*-heptane and in aqueous media at pH <5–6.

3.2. Synthesis of polymers

Four NIPAAm/MALEU copolymers with molar ratios 15:1, 10:1, 2:1 and 1:1 (see Table 1) were synthesised. For comparison purposes the corresponding homopolymers pNIPAAm and pMALEU were also prepared. All syntheses were carried out at 25°C, in water, by radical polymerisation initiated by APS (0.5 wt% compared to the sum of the monomers) in the presence of TEA acting as accelerator. TEA was employed also to neutralise MALEU and therefore on the whole a 10 mol% excess of TEA over MALEU was used. The concentration of the monomers was in all cases 15 wt% with respect to the water content. After 24 h the polymers were isolated as free acids by addition of HCl up to pH 2. The NIPAAm-rich polymers (pNIPAAm, NIP15-*co*-MAL1 and NIP10-*co*-MAL1), which were soluble in water at $T <$ 10–15°C, were purified by ultrafiltration, whereas the others, which precipitated out on HCl addition and did not dissolve even at 4°C, were washed with warm water. In all cases the purification procedure was completed by exhaustive extraction with diethyl ether in a Soxhlet apparatus.

The composition of copolymers was determined by potentiometric titration of the carboxy groups. From Table 2 it is apparent that the NIPAAm content is very close to the feed composition, even though generally slightly lower. Only in the case of NIP2-*co*-MAL1 the difference is somewhat more pronounced (copolymer: 49.7 wt%; feed: 53.2 wt%). Probably during the precipitation and washing procedure some of the NIPAAm-rich fractions of the copolymer, which are more water-soluble, were lost. This did not occur to an appreciable extent in the NIP1-*co*-MAL1 sample, where the MALEU amount is much higher, nor in the ultrafiltered copolymers.

The FT-IR and ^{1}H-NMR spectra of homopolymers (see Section 2) are in agreement with the expected structures

Table 2
Polymer composition and d*n*/d*c* values

Polymer	NIPAAm (wt%)		NIPAAm/MALEU (molar ratio)		d*n*/d*c* (ml/g)	
	Feed[a] (%)	Titration[b] (%)	Feed[a]	Titration[b]	Exptl[c]	Calcd[d]
pNIPAAm	–	–	–	–	0.072[e]	–
pNIPAAm	–	–	–	–	0.174	–
pMALEU	–	–	–	–	0.195	–
NIP1-*co*-MAL1	36.2	36.1	1.00	0.99	0.184	0.185
NIP2-*co*-MAL1	53.2	49.7	2.00	1.74	0.179	0.182
NIP10-*co*-MAL1	85.0	83.8	10.0	9.10	0.172	0.176
NIP15-*co*-MAL1	89.5	89.4	15.0	14.8	0.175	0.175

[a] Composition of the reaction feed.
[b] Composition of copolymers as determined by acid–base titrations.
[c] Experimental value of d*n*/d*c* in Tris buffer pH 8.09.
[d] d*n*/d*c* calculated from copolymer composition and from d*n*/d*c* values of homopolymers assuming additivity of polarisability [32].
[e] Measured in DMF + 0.05 M LiBr.

while the copolymers exhibit the bands of both homopolymers, their relative intensities confirming the composition determined titrimetrically. Unfortunately, owing to the high molecular weight of the samples, their ^{1}H-NMR spectra exhibited broad bands that did not allow to gather indications on the copolymer microstructure.

3.3. Molecular characterisation

The d*n*/d*c* data of homopolymers and copolymers are reported in Table 2. Fortunately, the d*n*/d*c* values of both homopolymers are high, ensuring good instrumental sensitivity. Furthermore, their difference (≈ 10%), though meaningful, is not large. This is important in connection with the molecular characterisation of copolymers. In fact, it is well known that for copolymers the MALS technique furnishes only apparent values of the weight-average molar mass ($\bar{M}_w$) and of the gyration radius $\langle s^2\rangle_z^{1/2}$, hereafter denoted in short as gyration radius (R_g), unless the chemical composition is homogeneous or the homopolymers have the same d*n*/d*c* value. Therefore, in our specific case, taking into account the low difference in d*n*/d*c*, the apparent $\bar{M}_w$ and R_g values of the copolymers can be considered very close to the true values. Table 2 reports a comparison between the experimental d*n*/d*c* values of copolymers and those calculated from the d*n*/d*c* of the homopolymers on the basis of the average chemical composition of copolymers assuming additivity of polarisability [32]. The differences between the experimental and calculated values are within the experimental uncertainty, which indirectly confirms the average composition of copolymers.

Fractionation of pNIPAAm on TSK columns with an aqueous mobile phase presented many problems. In fact, pNIPAAm adsorbed on the column packing and polymer elution was not regular, in contrast with the behaviour of pMALEU and copolymers. We tried to optimise the experimental conditions (pH, ionic strength, and type of column) but in any case elution was unsatisfactory so that pNIPAAm was finally characterised using DMF + 0.05 M LiBr as the mobile phase, where only a small extent of aggregates was noticed.

Table 3 summarises the molar mass averages, dispersity index *D* and dimension of the macromolecules obtained by the SEC-MALS system. The results of the pNIPAAm characterisation were obtained in DMF. Obviously, any comparison of the macromolecular dimensions between pNIPAAm and the other polymers is not meaningful. The molar mass distribution (MMD) of homopolymers is quite similar. In contrast, the molar mass of copolymers regularly increases with the NIPAAm/MALEU molar ratio (Table 3 and Fig. 1).

Table 3
Molecular characterisation data obtained from SEC-MALS experiments

Polymer	$\bar{M}_n \times 10^{-3}$ (g/mol)	$\bar{M}_w \times 10^{-3}$ (g/mol)	$\bar{M}_z \times 10^{-3}$ (g/mol)	$\langle s^2\rangle_z^{1/2}$ (nm)	$\bar{M}_w/\bar{M}_n$
pNIPAAm[a]	222.8	495.5	910.0	47.2	2.22
pMALEU[b]	288.0	539.1	821.5	44.1	1.87
NIP1-*co*-MAL1[b]	277.3	525.5	843.6	53.5	1.90
NIP2-*co*-MAL1[b]	361.9	722.0	1192.0	66.7	1.99
NIP10-*co*-MAL1[b]	576.6	1080.0	1896.0	78.6	1.87
NIP15-*co*-MAL1[b]	665.4	1211.0	2041.0	75.9	1.82

[a] Mobile phase: DMF + 0.05 M LiBr.
[b] Mobile phase: Tris buffer pH 8.09.

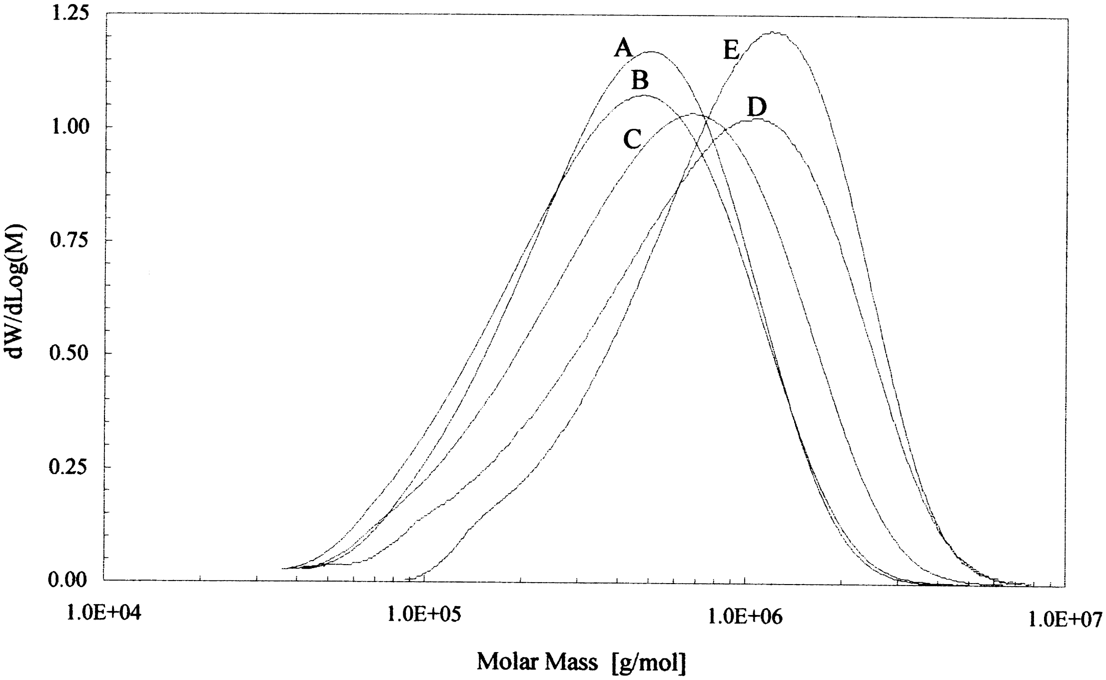


Fig. 1. Molecular mass distribution of: (A) pMALEU; (B) NIP1-*co*-MAL1; (C) NIP2-*co*-MAL1; (D) NIP10-*co*-MAL1; and (E) NIP15-*co*-MAL1.

The introduction of NIPAAm units on the pMALEU chains substantially changes their conformation. In fact the exponent of the $R_g = f(M)$ power law for pMALEU is relatively high ($\alpha = 0.63$), which indicates that pMALEU macromolecules behave as semi-stiff chains in aqueous solution. When the NIPAAm/MALEU ratio increases in copolymers two concomitant effects appear. The first one is clearly observable up to the 2:1 ratio, where R_g increases while the exponent of the $R_g = f(M)$ power law does not change appreciably ($\alpha \geq 0.6$). At constant molar mass NIP1-*co*-MAL1 exhibits systematically higher R_g values than pMALEU. This means that the NIPAAm-*co*-MALEU chains are less compact compared to the pMALEU chains. The second effect is clearly observable when the NIPAAm units are largely predominant in the copolymer (10:1 and 15:1 ratios). Since the mobile phase is a poor solvent for pNIPAAm at 25°C, the macromolecules assume a more compact conformation and tend to aggregate. In fact, the exponent of the $R_g = f(M)$ power law decreases from 0.61 to 0.52 passing from NIP1-*co*-MAL1 to NIP15-*co*-MAL1.

3.4. Solubility curves in 0.1 M NaCl

The solution behaviour of polymers was investigated by the optical method, heating the solutions at 0.5°C/min and considering as cloud point the temperature (T_{CP}) where the

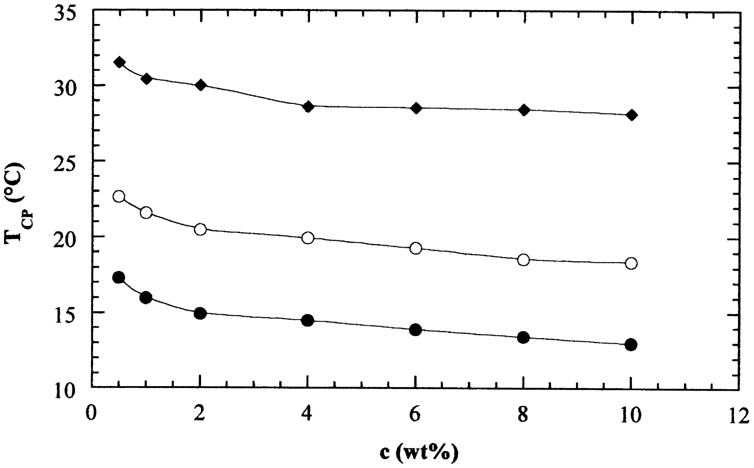


Fig. 2. Solubility curves in 0.1 M NaCl of pNIPAAm (◆), NIP15-*co*-MAL1 (○) and NIP10-*co*-MAL1 (●).

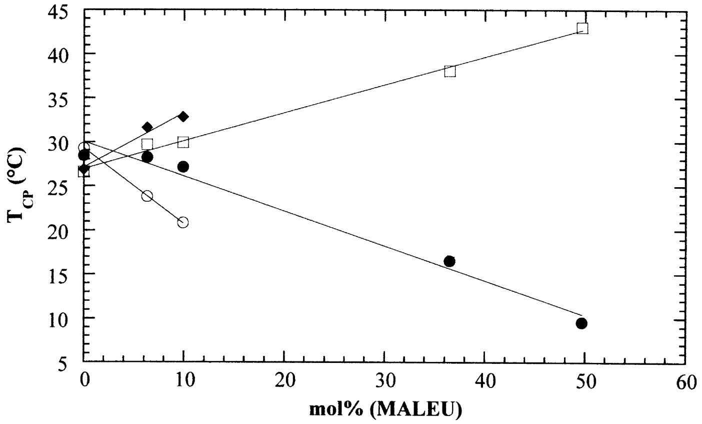


Fig. 3. Cloud point temperature as a function of the MALEU content in citrate buffers pH 4.0 (○), 4.5 (●), 5.0 (□) and 6.0 (◆).

first opaqueness appeared. First, the existence of a cloud point (CP) at the concentration of 1 wt% in 0.1 M NaCl was assessed. In the temperature range investigated ($4 < T < 60$°C), only the NIPAAm-rich samples (pNIPAAm, NIP15-*co*-MAL1 and NIP10-*co*-MAL1) exhibited a CP whose temperature decreased increasing the MALEU content. In contrast, the other polymers turned out to be insoluble because the amount of the hydrophobic MALEU residues depressed the T_{CP} below 4°C. As a consequence, only the solubility curves of the above three polymers could be determined (Fig. 2). It may be noticed that the T_{CP} decreases with concentration and no minimum is observed in the range investigated ($0.5 \leq c \leq 10$ wt%). Consistent with literature data, the concentration dependence for pNIPAAm is rather weak, except at low concentrations [33]. The curves of copolymers exhibit almost the same pattern but are translated towards lower temperatures according to the MALEU content. This behaviour can be explained considering that the amount of MALEU does not exceed 10 mol% so that it is probably high enough to affect largely intrachain interactions, causing a definite reduction in T_{CP}, but does not perturb interchain interactions to such an extent that the concentration dependence of the phase transition temperature is appreciably modified.

3.5. Solution behaviour in citrate buffers

The degree of ionisation is known to affect extensively the solution behaviour of thermosensitive polymers [1,23]. Therefore a knowledge of the acidity constants can be of great help to interpret the pH dependence of T_{CP}. An accurate evaluation of the NIPAAm/MALEU constants is in progress and will be published in a forthcoming paper. In previously studied copolymers of NIPAAm with *N*-acryloyl-L-leucine, *N*-acryloyl-L-valine and *N*-methacryloyl-L-valine the acidity constants have been shown to be *apparent*, since their value, in the range 4–5 [26,28], depends on the degree of ionisation of the polymer. Such dependence has been described by the modified Henderson–Hasselbach equation [34,35]:

$$pK = pK^\circ + (n-1)\log\frac{\alpha}{1-\alpha} \qquad (1)$$

where α is the degree of ionisation and n a parameter whose value is *one* for polymers possessing a *real* constant pK° independent of α, while it deviates more from unity when the acid–base behaviour of the polymer is more apparent.

Since the structure of the NIPAAm/MALEU copolymers is similar to that of the above studied polymers, we assumed that they also possessed similar acidity constants and therefore we performed our investigations on 1 wt% polymer solutions in citrate buffers with pH 4.0, 4.5, 5.0 and 6.0, where the degree of ionisation was expected to change more sharply. The plot of the phase transition temperatures vs. the polymer composition is shown in Fig. 3. Interestingly, in all cases a linear dependence of T_{CP} on the MALEU content may be noticed. A similar trend has been reported, even if over a narrower range of compositions, for other ionically modified NIPAAm copolymers [1,23]. At pH 4.0 and 4.5 T_{CP} decreases linearly with the MALEU percentage, the slope being less negative at the higher pH. Passing from pH 4.5 to 5.0 the slope becomes suddenly positive, so that at pH 5.0 and 6.0 the phase separation temperature increases with the MALEU content. Interestingly, the highest change in slope occurs between pH 4.5 and 5.0, where it may be noticed that all polymers except pMALEU exhibit a CP. In contrast, pMALEU, NIP2-*co*-MAL1 and NIP1-*co*-MAL1 samples do not dissolve at pH 4.0 whereas at pH 6.0 they dissolve but do not precipitate on heating. The observed behaviour can be explained supposing that at pH 4.0 most carboxy groups are unionised so that the MALEU residues behave as hydrophobic units and cause depression of the phase transition temperatures. By raising the pH increasingly more carboxy functions undergo ionisation, the macromolecular chains expand because of the electrostatic repulsion exerted by the carboxylate ions; and consequently chain collapse becomes more difficult, when the MALEU content is higher resulting in less negative slopes. A sharp increase in the degree of ionisation is likely to occur around

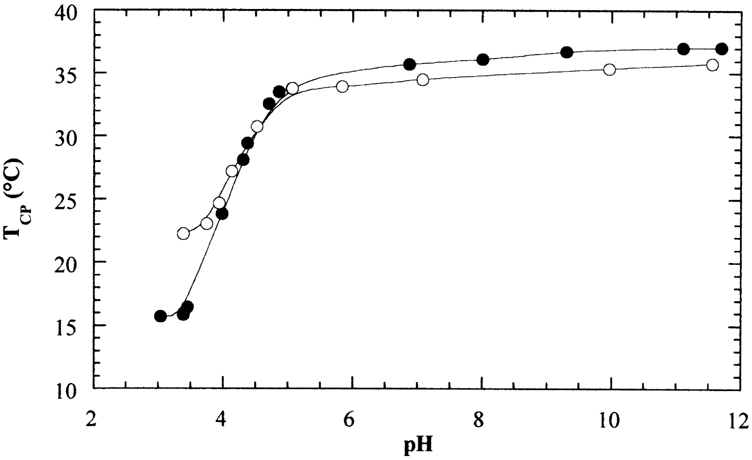


Fig. 4. Cloud point temperature as a function of the pH in 0.1 M NaCl for NIP15-*co*-MAL1 (○) and NIP10-*co*-MAL1 (●).

pH 4.5–5.0 where electrostatic repulsion becomes particularly strong and prevail over the hydrophobic effect exerted by unionised MALEU units so that the transition temperature is raised proportionally to the MALEU percentage. As regards the behaviour of NIP2-*co*-MAL1 and NIP1-*co*-MAL1, at pH 4.5 and 5.0 the fraction of ionised units is high enough to ensure solubilisation of the polymers but also low enough to allow the hydrophobic forces to prevail at a certain temperature resulting in phase separation. In contrast, at pH 6.0 the high electrostatic repulsion exerted by the elevated fraction of ionised units forces the polymers in chain-extended conformations so that chain collapse is prevented and no CP can be observed.

3.6. pH-dependent solution behaviour in 0.1 M NaCl

It may be noticed from Fig. 3 that the phase transition temperature of the NIPAAm solutions (i.e. with MALEU mol% = 0) is not constant but changes from 29.5°C at pH 4.0 to 26.5°C at pH 5.0. In practice, the temperature decreases by increasing the ionic strength of the buffers, which is comprised between 0.30 (pH 5.0) and 0.21 M (pH 4.0). On the other hand, it is well known that the cloud point temperature of NIPAAm solutions is affected by the type and concentration of added salts [17,36]. In order to suppress this influence and to span a wider range of pH values, the solution behaviour of NIP15-*co*-MAL1 and NIP10-*co*-MAL1 was investigated between pH 3.5 and pH 11.5 in 0.1 M NaCl at the polymer concentration of 1 wt%. To this purpose a number of polymer solutions in 0.2 M NaCl were first prepared, then for each solution a different fraction of carboxy groups was neutralised with NaOH and the proper amount of water was added so as to obtain a 1 wt% solution in 0.1 M NaCl; finally the pH and T_{CP} values were determined. The plot of T_{CP} s vs. pH is reported in Fig. 4. Consistent with the behaviour observed in citrate buffers, in both cases a sharp increase in the phase transition temperature occurs around pH 4–5, confirming that an abrupt change in the degree of ionisation takes place in that interval of pH. Owing to its lower MALEU content, NIP15-*co*-MAL1 exhibits a less marked dependence on pH. Interestingly, at pH ≥8, where the polymers are expected to be completely ionised, they show a CP at about the same temperature ($T_{CP} \approx$ 35–36°C) whereas at pH ≈ 3–3.5 their CP is observed at 22 and 16°C, respectively. Considering that the T_{CP} of pNIPAAm is located at 30°C, it may be concluded that the MALEU residues are much more effective in depressing the transition temperature under the unionised, hydrophobic form than in raising it through electrostatic repulsion when they are ionised.

3.7. Dependence of T_{CP} on the degree of ionisation

In an attempt to investigate more quantitatively the influence exerted by the degree of ionisation on the T_{CP} of NIP15-*co*-MAL1 and NIP10-*co*-MAL1, as a first, even if rough, approximation we assumed the validity of the Henderson–Hasselbach equation:

$$\mathrm{pH}_{CP} = \mathrm{p}K + \log\frac{\alpha_{CP}}{1-\alpha_{CP}} \tag{2}$$

where pH_{CP} and α_{CP} are the pH of the solution and the degree of ionisation of the macromolecule at the cloud point, respectively; pK the acidity constant that for simplicity was supposed to be independent of both α_{CP} and temperature. Assuming pK = 3.85, which is the constant of the MALEU monomer [11], from the knowledge of experimental pH_{CP}'s approximate α_{CP} values were calculated through Eq. (2). A plot of T_{CP} vs. α_{CP} was finally constructed from which, in spite of the rough approximations employed, a good linear relationship was immediately apparent for both polymers. The pK values were then refined iteratively so as to achieve the best linear fitting to the experimental points and turned out to be 4.01 for NIP10-*co*-MAL1 and 4.13 for NIP15-*co*-MAL1. Interestingly, the 10:1 copolymer with *N*-acryloyl-L-leucine has been found

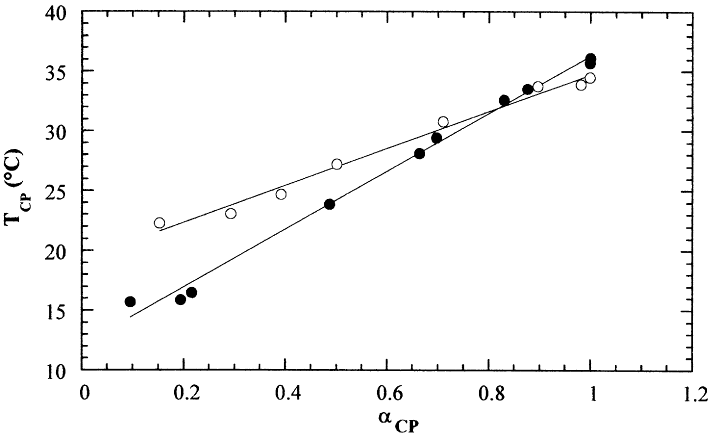


Fig. 5. Dependence of the cloud point temperature on the degree of ionisation at the cloud point in 0.1 M NaCl for NIP15-*co*-MAL1 (○) and NIP10-*co*-MAL1 (●).

to possess an intermediate acidity constant, its pK_0 value in Eq. (1) being 4.06 at 25°C [28]. This is not unexpected owing to the similar structure of the polymers. Furthermore, preliminary results of classical studies based on acid–base titrations of NIPAAm/MALEU copolymers indicate pK values close to those determined by the above procedure from cloud point measurements.

The optimised plot is shown in Fig. 5. The intercept represents the T_{CP} of completely unionised copolymer, while the slope is a measure of the influence exerted by carboxylate ions on T_{CP}. These features can be more easily discussed on the basis of Fig. 6, where the plot of T_{CP} vs. the fraction f_{CP} of ionised units over the sum of all units is reported. The equations of linear fittings are:

T_{CP} (°C) = 19.22 + 244.9·f_{CP} (NIP15-*co*-MAL1),

T_{CP} (°C) = 12.09 + 244.0·f_{CP} (NIP10-*co*-MAL1).

In both cases lower T_{CP} values compared to pNIPAAm (T_{CP} = 30.4°C) are observed in the absence of ionisation (i.e. for $f_{CP} = 0$). The T_{CP} depression turns out to be proportional to the MALEU content so that a lower intercept is observed with NIP10-*co*-MAL1. On the other hand the same slope is calculated for both polymers, which indicates that in both cases ionised units exert the same influence on T_{CP}.Apparently, the linearisation achieved by Eq. (2) using a single pK value is in contrast with the well-documented dependence of the acidity constants on the degree of ionisation observed for previously studied polymers with similar structure. This may be a consequence of their low MALEU content, which, in the case of random copolymers, can make unlikely the interactions between ionised units along the macromolecular chain, reducing deviations from the Henderson–Hasselbach equation. This is probably the case of the 10:1 copolymer with *N*-acryloyl-L-valine for which the *n* value in Eq. (1) is 1.14 [26]. However the same might be untrue for the NIPAAm/MALEU polymers, since higher *n* values have been observed in the corresponding copolymers with *N*-acryloyl-L-leucine and *N*-methacryloyl-L-valine. Furthermore, the constants determined for the

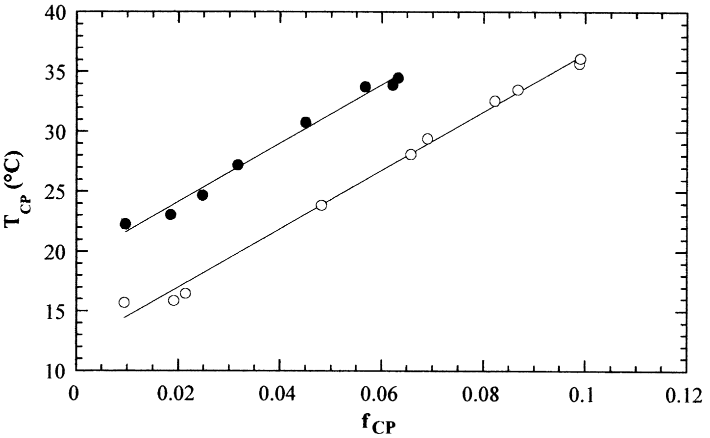


Fig. 6. Dependence of the cloud point temperature on the fraction of ionised units at the cloud point in 0.1 M NaCl for NIP15-*co*-MAL1 (●) and NIP10-*co*-MAL1 (○).

above polymers by acid–base titrations exhibited a clear dependence on temperature, while as a matter of fact, under the above assumptions, linearisation over an even wider range of temperatures was obtained with a single p*K* value. Therefore the linear dependence of T_{CP} on α_{CP} seems to be more strictly related to the coil-to-globule collapse, where the chains experience a subtle interplay of temperature-dependent attractive and repulsive interactions. Also in view of the assumptions introduced, further studies are necessary to ascertain this unexpected behaviour in other systems. On the other hand, it is worth noting that these results can be useful also from a practical viewpoint as they suggest that NIPAAm/MALEU copolymers with the desired phase separation temperature can be easily produced by neutralisation of the proper fraction of MALEU residues.

4. Conclusions

The aqueous solutions of the copolymers synthesised exhibit a cloud point at temperatures whose value is strongly dependent on polymer composition and pH. In particular:

(a) The pH being the same, T_{CP} changes linearly with the MALEU content. At pH less than about 4.5 T_{CP} is depressed compared to pNIPAAm, while the reverse happens at pH greater than about 5.
(b) The composition being the same, a sudden increase of the phase transition temperature occurs between pH 4 and 5. This behaviour is ascribed to the sharp variation of the degree of ionisation taking place in that interval of pH.
(c) For copolymers with less MALEU content (<10 mol%), a linear dependence of T_{CP} on the degree of ionisation at the cloud point is observed. Interestingly, this relationship was discovered by assuming the validity of the Henderson–Hasselbach equation where a *single* acidity constant independent of both the degree of ionisation and the temperature was employed.

Furthermore, it is worth stressing that acidity constants with values similar to those determined form classical acid–base titrations can be calculated by a procedure based on cloud point determinations, at least for the copolymers with low MALEU content.

Point (c) suggests that the acid–base behaviour of NIPAAm/MALEU copolymers at the cloud point is different from that exhibited in their random coil, uncollapsed state, where a clear dependence of p*K* on the degree of ionisation and temperature is noticed. Further studies are needed to ascertain if polymers with higher MALEU contents and/or with other acidic comonomers exhibit the same properties. In that case, from the practical viewpoint it would be possible to prepare systems with the desired T_{CP} simply by a proper choice of the composition and degree of neutralisation of the polymer, while from the theoretical viewpoint the above findings might prove useful in modelling the chain collapse of thermosensitive polyelectrolytes.

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