ELSEVIER

International Journal of Pharmaceutics 222 (2001) 259–270

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Effect of some physiological and non-physiological compounds on the phase transition temperature of thermoresponsive polymers intended for oral controlled-drug delivery

Frederic Eeckman, Karim Amighi *, André J. Moës

Laboratory of Pharmaceutics and Biopharmaceutics, Free University of Brussels, Campus Plaine, CP-207, 1050 Brussels, Belgium

Received 8 January 2001; received in revised form 10 April 2001; accepted 27 April 2001

Abstract

Poly-*N*-isopropylacrylamide (PNIPAAm) thermosensibility makes this polymer a very attractive candidate for controlled drug delivery systems. The polymer possesses a lower critical solution temperature (LCST) which was found to be around 32 °C in pure water, but which can be affected by the medium composition, i.e. presence of salts or surfactants. The knowledge of the effects of such substances on the LCST is very important while using PNIPAAm as a controlled drug delivery agent. The influence of a number of physiological and non-physiological salts and surfactants has been studied. The results obtained show that the addition of salts provokes an important decrease of the LCST of the polymer (salting out effect). A strong influence of the valence and of the size of the anions of the halide group was found. As to the surfactants, according to their type and concentration, a decrease or an increase of the LCST or even no effect at all were found. The effect of the GI secretions on the PNIPAAm phase separation temperature is also discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Poly-*N*-isopropylacrylamide; Thermoresponsive polymers; Salts; Surfactant; Controlled-drug delivery; Gastro-intestinal secretions

1. Introduction

A major problem associated with the oral controlled release of drugs is the sensitivity of the dosage form properties to the variations of pH and transit in the human gastrointestinal (GI) tract (Moës, 1989). Consequently in some cases, the drug targeting to some specific areas of the GI tract, like the colon is difficult. Several innovative approaches have been considered to overcome those difficulties and among these, the use of stimuli-sensitive materials is promising. Different kinds of stimuli such as temperature (Cammas et al., 1992; Yoshida et al., 1992; Serres et al., 1996; Ichikawa and Fukumori, 1997; Kono et al., 1999; Ichikawa and Fukumori, 2000; Peppas et al.,

* Corresponding author. Tel.: +32-2-650-5252; fax: +32-2-650-5269.

E-mail address: kamighi@ulb.ac.be (K. Amighi).

0378-5173/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0378-5173(01)00716-5

2000), pH (Okatata et al., 1982; Dong et al., 1992; Kim et al., 1994; Brazel and Peppas, 1996; Serres et al., 1996), pressure (Tanaka et al., 1998) or electric fields (Eisenberg and Grodzinsky, 1984) can be used in order to provoke the drug release.

Thermosensitive polymers such as Poly-*N*-isopropylacrylamide (PNIPAAm) and its copolymers have recently been proposed for the delivery and selective release of drugs (Okano et al., 1990; Yoshida et al., 1992; Kiolchob et al., 1998; Kono et al., 1999). PNIPAAm is a water soluble polymer that precipitates out of water when the solution is heated above its cloud point of about 32 °C (Heskins and Guillet, 1968; Kubota et al., 1990; Wu and Zhou, 1995). It therefore exhibits an inverse solubility curve with a Lower critical solution temperature (LCST), contrary to most polymers, which exhibits an Upper critical solution temperature (UCST) in organic solvents. This inverse behavior is neither novel nor unique to PNIPAAm since most non-ionic water-soluble polymers that owe their solubility to hydrogen bonding (with water) also exhibit similar behavior. However, unlike poly(acrylamide) or poly(ethylene oxide) (Ataman and Boucher, 1982; Ataman, 1987; Plate et al., 1999), which have LCST values near or above the boiling point of water, the PNIPAAm LCST is close to the physiological temperature which makes it a very attractive candidate in the field of controlled-drug delivery. Moreover, the transition is found to be reversible and rather abrupt (Wu and Zhou, 1997). Finally, the LCST of PNIPAAm that is about 32 °C in water can be easily modified by co-polymerization (Ringsdorf et al., 1992; Feil et al., 1993) with hydrophilic (increase) or hydrophobic (decrease) monomers, or by addition of salts (Schild and Tirrell, 1990) or surfactants (Schild and Tirrell, 1991) to the aqueous polymer solutions.

The practical applications of PNIPAAm and its copolymers can be found in comprehensive review papers published by Schild (Schild, 1992) and Ichikawa (Ichikawa and Fukumori, 1997). It is the relatively recent recognition of the transition of PNIPAAm as a potential thermosensitive shrinking device that prompted a surge of interest in the polymer. However, the need of a certain environmental temperature change to obtain significant variations of the polymer properties is an important limiting factor for the potential in vivo applications of thermosensible polymers.

The aim of this work is to use PNIPAAm and its copolymers to prepare oral dosage forms having release properties which should be at the same time therapeutically adequate at the physiological temperature and independent of the components present in the GI fluids. With this in view, the influence of some physiological and other non-physiological compounds like salt ions and surfactants on the LCST of aqueous PNIPAAm solutions had first to be investigated in order to evaluate and better understand their effects on the behavior of the thermosensitive polymer. With this knowledge, it will then be possible to predict the potential effect of the GI secretions on the LCST of PNIPAAm, and in further studies, of copolymers with a LCST of 37 °C, or slightly higher.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAAm) monomer, sodium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Aldrich (Belgium). All other materials used were of analytical reagent grade.

2.2. Polymer preparation

PNIPAAm was prepared by free radical polymerization in water, at room temperature for 5 h under magnetic stirring. Sodium persulfate was used as an initiator (0.6 mol.%) and *N*,*N*,*N*′,*N*′-tetraethylmethylenediamine (TEMED) (2.1 mol.%) as an accelerator. The monomer solution (0.5 M) was bubbled with nitrogen for 20 min prior to polymerization in order to remove the remaining oxygen. After the polymerization process, the solution was heated above 32 °C and the precipitated polymer was collected by filtration, washed with hot water and dissolved in cold water. The process was repeated several times in order to complete the polymer purification.

The molecular weight (M_w) of PNIPAAm was determined by viscometry measurements using the Mark–Houwink–Sakurada equation. The measurements were performed in THF at 27 °C using an Ubbelhode viscometer (O_c type). The coefficients a and k are known to be equal to 0.65 and 0.00959, respectively in these conditions (Schild, 1992).

The M_w was then found to be around 1×10^6 for those samples synthesized in water.

2.3. Preparation of PNIPAAm solutions

Various amounts of salts and surfactants were added to aqueous PNIPAAm solutions. The aqueous polymer solutions had a mass fraction of 1.4%. Fatty acids were firstly neutralized in alkali medium (sodium hydroxide) in order to obtain the ionized fatty acid sodium salt. After treatment, the ionized surfactant solutions were added to PNIPAAm aqueous solutions.

2.4. Transmittance measurements

The phase transition phenomenon was examined by performing transmittance measurements of the polymer solutions (in duplicate) at 500 nm, using a Shimadzu 160 spectrophotometer (Shimadzu Corp., Japan). The temperature of the PNIPAAm solutions, containing various amounts of salts and surfactants, was raised by 0.1 °C steps, using a Cell positioner with a Peltier temperature controller (Shimadzu CPS-240A). The cloud point value was determined as the abscissa of the inflexion point of the transmittance versus temperature curves.

2.5. Differential scanning calorimetry (DSC)

The DSC method was also used in the study of the phase transition phenomenon of PNIPAAm. In this case, the phase separation temperature was taken as the maximum of the endothermic transition peak (average of two measurements). DSC analysis was performed using a Perkin Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with a intracooler-2 cooling system (Perkin Elmer Instruments, USA). Aluminum sealed pans containing the various PNIPAAm solutions were heated at a scanning rate of 2 °C/min, using nitrogen as blanket gas. Calibration was performed using cyclohexane and indium as standards.

3. Results and discussions

As already stated, PNIPAAm has a lower critical solution temperature of about 32 °C (Heskins and Guillet, 1968; Kubota et al., 1990; Wu and Zhou, 1995). The LCST values of PNIPAAm solutions were determined by both DSC analysis, giving the endothermic transition peak (Fig. 1) and by spectrophotometric analysis, giving the cloud point value (Fig. 2). The thermal analysis method allows the evaluation of the heat necessary to break hydrogen bonds between water and polymer, while the photometric method visualizes the clouding of the solution due to the precipitation of the polymer, when the phase separation occurs. The mean phase transition values of PNIPAAm in pure water were found to be about 32.2 ($\pm$0.1) and 32.7 °C ($\pm$0.1 °C), respectively for DSC and transmittance measurements.

From the calorimetric measurements of the endotherm associated with the LCST of PNIPAAm in pure water, thermodynamic parameters can be obtained. We found heats of transition of about 5 kJ/mol of repeating units. This value is similar to that reported by Fujishe et al., 1989 and is consistent with the loss of about one hydrogen bond per repeating unit upon phase separation. This was to be expected, as proposed (Fujishe et al., 1989), if the LCST phenomenon involves a coil-globule transition with subsequent aggregation.

3.1. Effect of salt ions

Generally, the addition of electrolytes provokes flocculation of the aqueous colloidal dispersions. This phenomenon is called the salting out effect. A similar effect can be observed with thermosensitive polymers for which a decrease of the transition temperature is obtained in presence of small amounts of salts. There exist, however, some salts, mainly tetra-alkylamonium salts, which induce a

salting in process (Horne et al., 1971), i.e. an increase of the transition temperature when the solute is added to the polymer solution.

The clouding phenomenon is known to arise from the sudden removal of the protective hydration layer of the molecules, thereby exposing the hydrophobic character of the polymer and causing its precipitation (Horne et al., 1971; Ataman, 1987). The cloud point temperature depends on the type of salt involved and on the concentration used.

The effects of the salt type and concentration on the LCST of aqueous PNIPAAm solutions are shown in Figs. 3 and 4. The results, obtained from DSC analysis, show that the addition of small amounts (0.05–1 mol/l) of salt ions to aqueous PNIPAAm solutions induces a significant decrease of the transition temperature.

As can be seen, the valence as well as the concentration of the electrolyte play a role in the phenomenon. The salts of trivalent phosphate ion have the greatest effect on the reduction of the PNIPAAm LCST. The salts of divalent and trivalent anions (monohydrogen phosphates, sulfates, ...) are more effective in this respect than the salts of monovalent anions. On the other hand, acetate salts are more effective than halides (except fluoride).

As examples, a NaCl concentration of 1 mol/l leads to a decrease of the PNIPAAm LCST of about 12 °C while a Na_2SO_4 concentration of only 0.2 mol/l leads to a decrease of about 10 °C (Figs. 3 and 4).

The classification of anions with respect to the salting out effectiveness of aqueous PNIPAAm solutions was found to be: $PO_4^{3-} > HPO_4^{2-} > SO_4^{2-} > OH^- > H_2PO_4^- > HCO_3^- > F^- > CH_3COO^- > Cl^- > Br^- > I^-$, on a molar concentration basis.

The found sequence is in accordance with the so-called Hoffmeister series (Ataman, 1987), which ranks the tendency of various salts to perturb the denaturation temperature of proteins. When the molecular weight of the salts is chosen instead of the molar concentration, the following ranking is found: $OH^- > F^- > PO_4^{3-} > SO_4^{2-}$

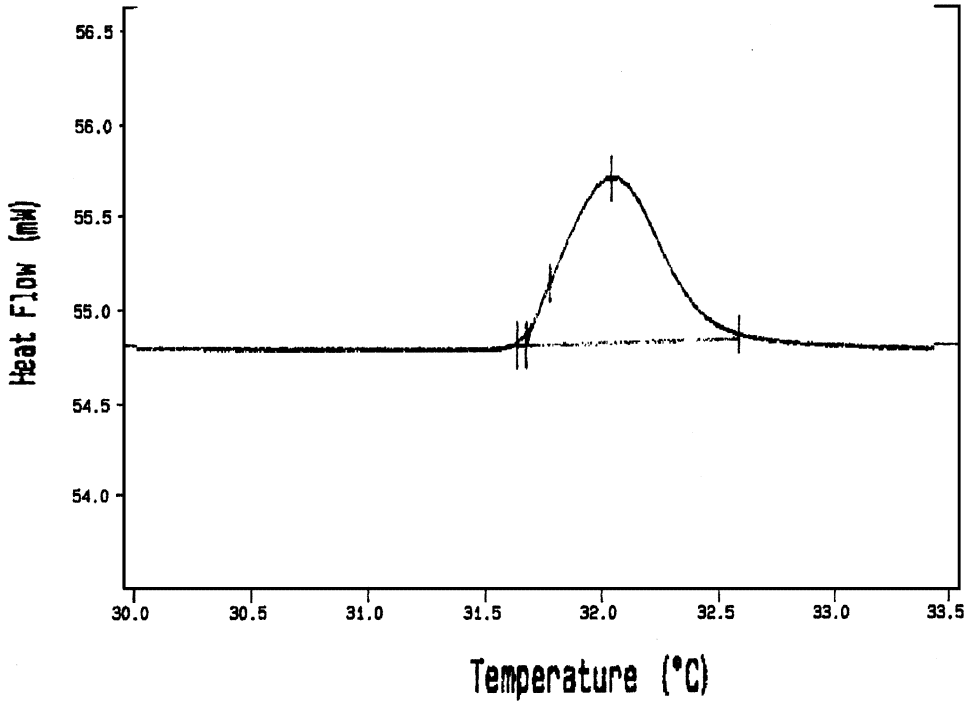


Fig. 1. DSC thermogram of a 1.4% w/w PNIPAAm aqueous solution (heating rate 2 °C/min).

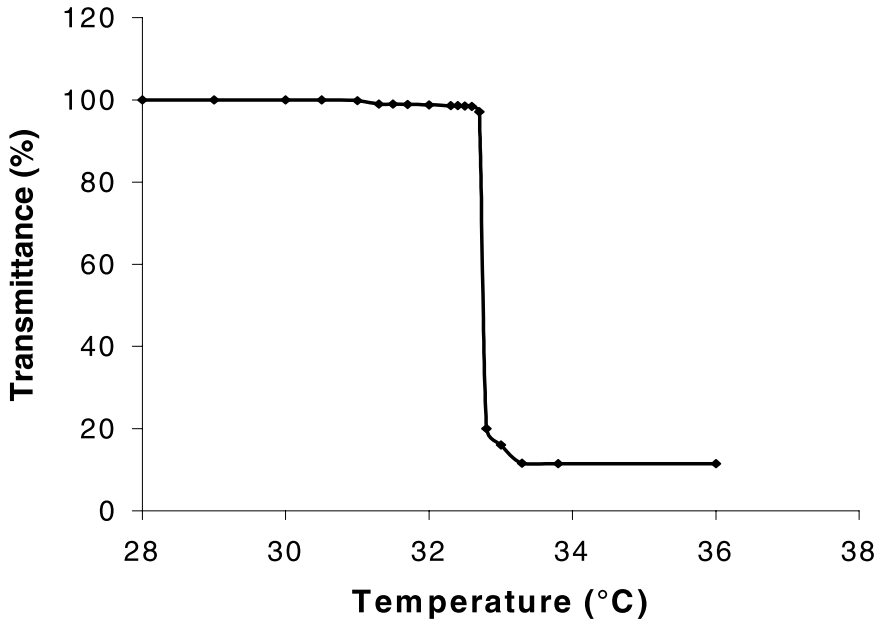


Fig. 2. Transmittance of a 1.4% PNIPAAm aqueous solution at 500 nm. From 28 to 36 °C by 0.1 °C steps.

$> HPO_4^{2-} > H_2PO_4^- > HCO_3^- > CH_3COO^- > Cl^- > Br^- > I^-$.

From the first ranking obtained it can be seen that the valence of the anion plays an important role in the salting out process. From the curves of the halide series (Fig. 3), it can be seen that the size of the anion also plays a role in the salting out process, $F^- > Cl^- > Br^- > I^-$. On the other hand, the effect of the cation type seems to be insignificant as the decreasing effects of salts containing the same anion (NaCl and KCl, Fig. 3; Na_2HPO_4 and K_2HPO_4, Fig. 4) on the LCST of PNIPAAm are quite similar.

The observed salting out process for PNIPAAm aqueous salt solutions can be explained as a combination of several effects, i.e. changes of the water structure in the polymer hydration sheath and changes of the interactions between the polymer and the solvent, due to the presence of salts.

It is known that the addition of electrolytes to water changes the normal hydrogen bonded water structure. According to the model of Frank and When (Ataman, 1987) for aqueous salt solutions, water consists of three regions: region A is the region that is permanently associated with the ion. Region B consists of water molecules partially ordered by the electric field of region A. In region C the structure of water is normal. The extent of region B is a measure of the ability of the ion to destroy water structure. Depending on whether the ion is a structure breaker or not, the normal hydrogen bonded structure of water is destroyed or retained. This is the reason why the phase transition phenomenon of aqueous PNIPAAm salt solutions is associated with changes in the normal structure of water induced by electrolytes.

When dissolved in water, PNIPAAm is surrounded by an extended so called hydration sheath where water has lost its normal structure. The addition of salts to aqueous PNIPAAm solutions changes the properties of the hydration layer and could cause a disruption of the highly oriented water molecules which surround the polymer. This results in an increase of the hydrophobic character of the PNIPAAm chains, which consequently lowers the polymer transition temperature. According to the type and the concentration of ions, the hydration sheath will be more or less destructured (Ataman, 1987) (in the case of salting out ions) or strengthened (in the

case of salting in ions). All salts used in this study brought about a decrease of the LCST.

3.2. Effect of surfactants

The set of surfactants used in this study can be divided into two classes. The first one (Table 1) is composed of molecules whose hydrophobic and hydrophilic parts are well separated. It consists mainly of sodium salts of fatty acids with a hydrophobic aliphatic carbon chain and an hydrophilic anion head, i.e. micelle-forming anionic surfactants. The evaluated surfactants of this class are caproic acid (C6), caprylic acid (C8), capric acid (C10), undecylenic acid (C11), lauric acid (C12) and myristic acid (C14) in the form of sodium salt, and sodium dodecyl sulfate (SDS).

The second class is composed of molecules whose hydrophilic and hydrophobic parts are not well distinct. The evaluated surfactants of this class are the sodium salts of glycocholic acid, and galacturonic acid and polysorbate 80.

Fig. 5 shows the effect of the surfactants of the first class on the LCST of 1.4% w/w aqueous PNIPAAm solutions. It can be observed that the effect of surfactants of the first class on the PNIPAAm solution properties is highly dependent on the length of the carbon tail. The results obtained show that the sodium salts of fatty acids with a long hydrocarbon aliphatic tail having a number of carbon atoms greater than or equal to eight, and SDS lead to an increase of the LCST at sufficiently high concentrations. Moreover, with the exception of SDS and of the myristic acid sodium salt, a certain decrease of the LCST at small concentrations is observed. This is in accordance with the results reported by Schild (Schild and Tirrell, 1991).

Such a behavior might be qualitatively explained as being the outcome of two antagonistic phenomena, i.e. a salting out effect due to the anionic character of the free surfactant molecules, on the one hand, and a conversion of the originally hydrophobic polymer into a more hydrophilic complex by bonding of the long aliphatic hydrocarbon chains of the surfactants with the polymer hydrophobic parts, on the other hand. When the later phenomenon prevails, the polymer is partially covered by surfactant molecules turning their hydrophilic head to the outside. It then tends to behave as an ionized hydrophilic polymer and to remain soluble in water for higher temperatures.

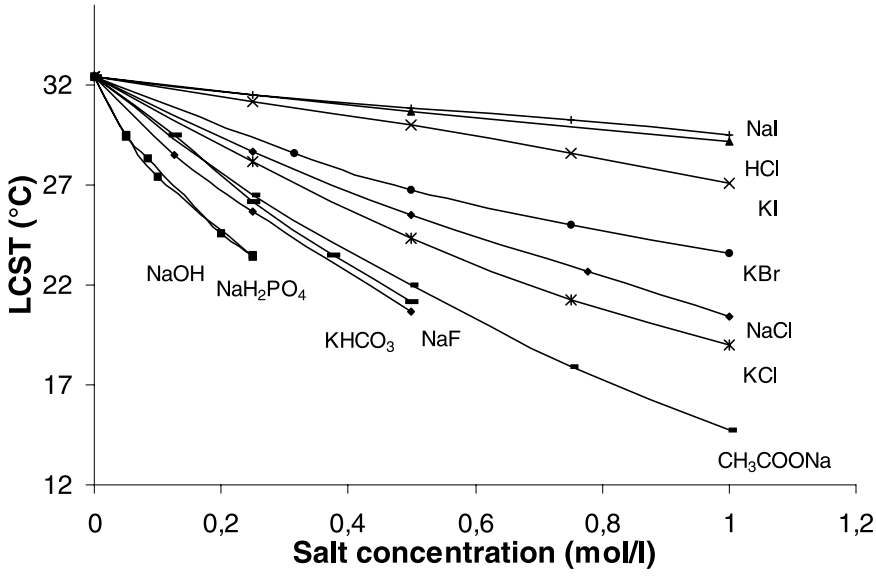


Fig. 3. Influence of the concentration of monovalent salts on the LCST of a 1.4% w/w PNIPAAm solution. Results from DSC Analysis (mean of two measures).

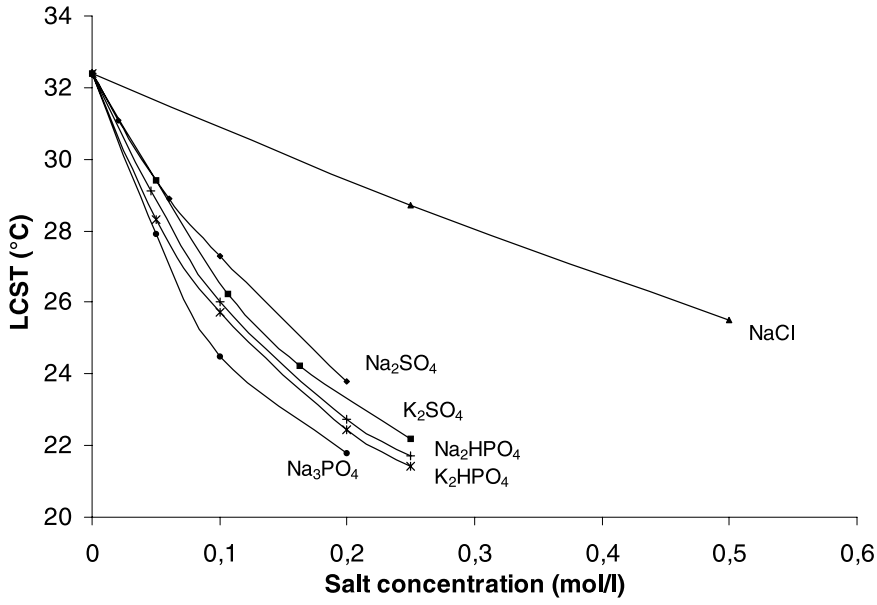


Fig. 4. Influence of the concentration of bi and trivalent salts on the LCST of a 1.4% w/w PNIPAAm solution. Results from DSC analysis (mean of two measures).

The accepted model of the complexes formed from the association of non-ionic polymer and anionic surfactant molecules, proposed by Cabane (Cabane and Duplessix, 1982), established that isolated polymer chains bind a number of miniature surfactant micelles along the chain contour. The concentration at which the formation of the attached micelles occurs is called the critical aggregation concentration (cac). It was found that the cac is lower than the critical micellar concentration (cmc) for the surfactants that induce an increase of the LCST, and as the chain length of the surfactant decreases, the gap between the cac and the cmc narrows and may become indistinguishable for the shortest hydrocarbons chains lengths.

A lowering of the LCST is observed at low surfactant concentrations ($C = 6, 8, 10, 11$ and 12) because those concentrations are under the cac (and the cmc) of these surfactants. The free surfactants behave thus as salts and lead to a decrease of the LCST. No initial decrease of the LCST was found for SDS and myristic acid sodium salt probably because of their very low cac. Schild and Tirrell, (1991) have showed that the cac value corresponds to the surfactant concentration at which the PNIPAAm LCST reaches its minimum value (before being increased). In this work, we found cac values of around 0.35, 0.050 and 0.005 mol/l for C8, C10 and C12, the cmc given in literature (Mukerjee and Mysels, 1971) being equal to 0.36, 0.103, and 0.0030 mol/l, respectively. We can thus see that the gap between the cac and the cmc narrows when the chain length decreases.

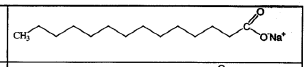
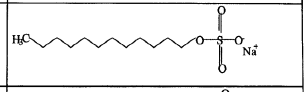
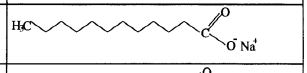
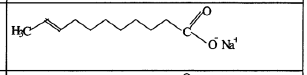
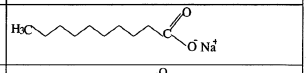
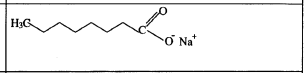
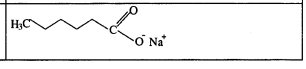
The plateau value of the LCST observed in Fig. 5 for caprylic acid (C8), capric acid (C10), undecylenic acid (C11) and lauric acid (C12) may be due to the saturation of the polymer by surfactants. The slightly decrease of the LCST observed for higher surfactant concentrations may be due to the free micelles formed in solution, which behave in the manner of simple salts, reducing the LCST.

Fig. 6 shows the effect of the surfactants of the second class. Except for the sodium salt of galacturonic acid, nearly no effect of the surfactants on

the LCST of PNIPAAm was observed. The sodium salt of the galacturonic acid, which in fact is not a true surfactant, lowers the LCST because of its smaller molecular size, that makes it behaves like a salt. While ionized at the pH of the experiment (pH = 7, pK_a = 4.4), the glycocholic acid sodium salt does not act as a salt, probably due to its large size.

It appears thus that surfactants with a very simple structure, i.e. with a well distinct separation of the hydrophobic and hydrophilic parts of the molecule, lead to an increase or a decrease of the LCST depending on the hydrophobic chain length and the surfactant concentration. But when the structure becomes more complicated, i.e. when no distinct separation between the hydrophilic and the hydrophobic parts of the molecule, the effect on the LCST becomes non-existent. Due to their larger size and more complicated structure, the surfactants of the second class are supposed to be unable to form micromicelles around the polymer chain, unlike those of the first class who do form micromicelles, nor to act as 'normal' salts (except for the smallest one).

Table 1
Surfactants of the first class studied

Myristic acid sodium salt C=14	
Sodium dodecyl sulfate C = 12	
Lauric acid sodium salt C = 12	
Undecylenic acid sodium salt. C = 11	
Capric acid sodium salt C = 10	
Caprylic acid sodium salt C = 8	
Caproic acid sodium salt C = 6	

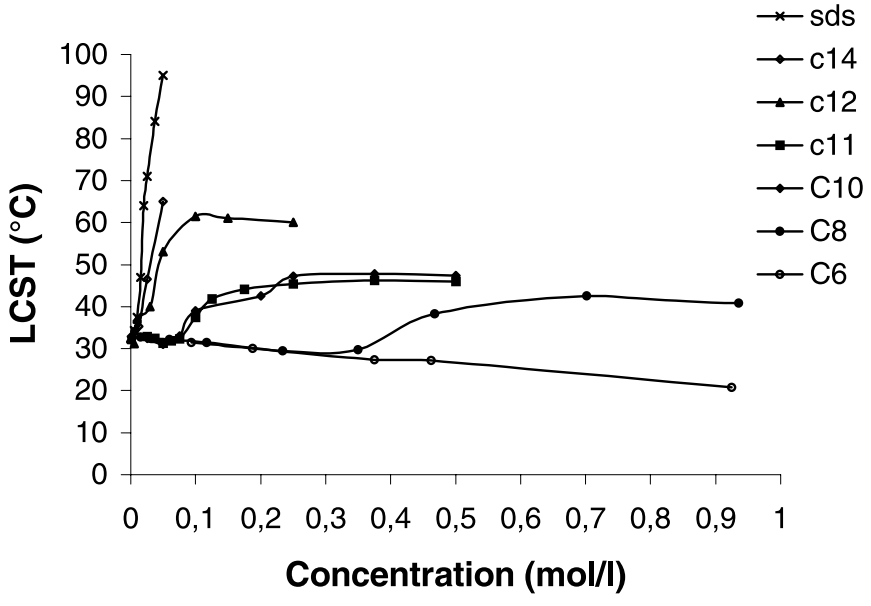


Fig. 5. Influence of the concentration of surfactants of the first class on the LCST of a 1.4% w/w PNIPAAm aqueous solution. Results from UV–visible analysis (mean of two measures).

3.3. Influence of saliva and gastro-intestinal secretions

Before considering the preparation of oral controlled-release dosage forms based on the use of thermosensitive polymers, it would be useful to evaluate the effect of the major components present in the GI fluids, and more particularly of salts, on the LCST of PNIPAAm. This effect was estimated by considering the experimental results given above and the data from the literature (Geigy Scientific Tables 1, 1981) on the theoretical composition of each undiluted GI secretion. The theoretical effect on the LCST was considered as a concentration weighted sum of the individual effects of the components present in each undiluted GI secretion. The data from the literature give the total amounts of salt ions present in the saliva and the GI secretions, i.e. gastric juice, pancreatic juice, hepatic juice and intestinal secretions (Table 2).

Considering the total saliva secretions (which include the parotid saliva, the submandibular saliva, the sublingual saliva and the labial saliva) (Table 2), and summing the effects on the LCST of each anion, it could be estimated that there is nearly no effect of saliva on the LCST of PNIPAAm; only a slight decrease of 1 °C is to be expected. In the case of the gastric juice, a decrease of about 2.5 °C of the LCST might be expected. This effect might mainly be due to HCl and NaCl present in this secretion (0.5 and 2 °C, respectively). A more important decreasing effect, of about 2–3.5 °C, is expected for the pancreatic secretions (Table 2). The inconstant concentration of HCO_3^- and Cl^-, whose concentration sum is always equal to ~ 0.150 mol/l, is due to the fact that when the bicarbonate concentration increases (due to incipient secretin-mediated stimulation), a corresponding decrease of Cl^- occurs, whereas the Na^+ concentration remains almost unchanged (Geigy Scientific Tables 1, 1981), leading thus to a decrease comprised between 2 and 3.5 °C. Finally, the salting out effects of the bile secretion and of the intestinal juices, with their important NaCl contents are evaluated at 1.5 and 2 °C, respectively.

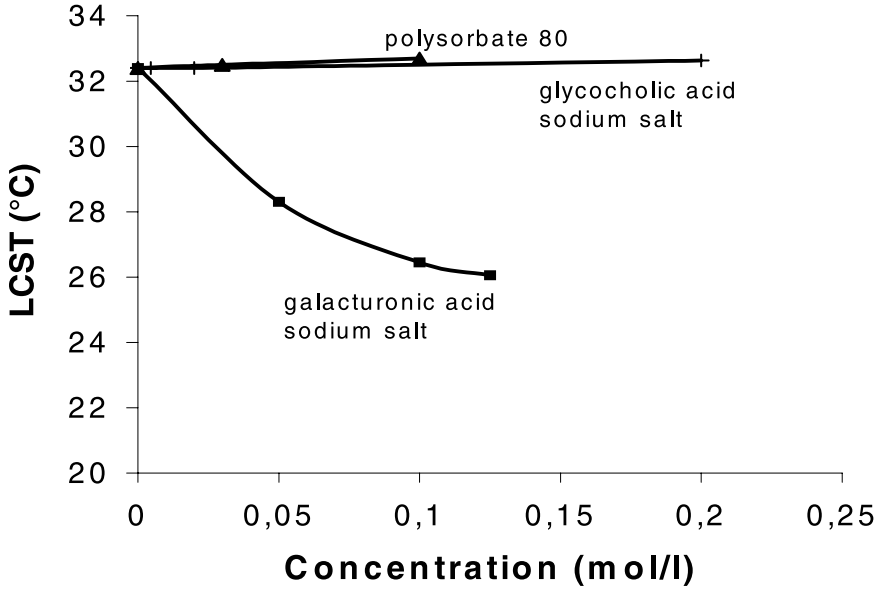


Fig. 6. Influence of the concentration of surfactants of the second class on the LCST of a 1.4% w/w PNIPAAm aqueous solution. Results from UV–visible analysis (mean of two measures).

Table 2
Decreasing effect of physiological compounds on the L.C.S.T of PNIPAAm aqueous solutions

	Cl^- (mol/l)	HCO_3^- (mol/l)	Na^+ (mol/l)	H^+ (mol/l)	Phosphates (mol/l)	K^+ (mol/l)	Ca^{2+} (mol/l)	Phospholipids (mol/l)	Decreasing effect on the L.C.S.T. (°C)
Stimulated saliva	0.045	0.022	0.0075	–	0.005	0.0023	0.002	–	~1
Parietal secretions	0.170	–	–	0.148	–	0.016	–	–	~0.5
Non-parietal secretions	0.132	0.025	0.138	–	–	0.01	0.001	–	~2
Pancreatic juice	0.135	0.140	0.138	–	–	0.007	–	–	~3.5/2
Hepatic bile	0.117	0.030	0.158	–	–	0.006	0.005	0.005	~1.5
Gallbladder bile	0.066	0.045	0.264	–	–	0.020	0.017	0.065	~1.8
Intestinal secretions jejunum	0.135	0.008	0.142	–	–	0.004	–	–	~2
Ileum	0.125	0.003	0.140	–	–	0.005	–	–	~2

Since the aim is to develop oral dosage forms presenting a drug release profile practically unaffected by the GI secretions, the effects estimated above would surely be undesirable as they might affect the constancy of drug release rates in vivo. Nevertheless since the foregoing considerations are valid for the undiluted secretions and since they are actually heavily diluted in the GI tract, we can reasonably assume that their effects on the LCST of PNIPAAm will remain reasonably small (less than 1 °C). Moreover, while using such an ion sensitive polymer patients should be advised to take it between meals rather than immediately before or after eating. So avoiding undesired effects of substances contained in the food.

4. Conclusions

The set of salts studied induce a decrease of the LCST of PNIPAAm. However, in presence of surfactants, the behavior of the polymeric solutions depends on the surfactant type and on the hydrocarbon chain length. The knowledge of the influence of those compounds allows us to assume that only a slight effect of saliva and of the GI secretions on the LCST of PNIPAAm might be expected.

Further work has to be undertaken in order to prepare PNIPAAm and copolymers formulations and to optimize them with drug release kinetics independent of the physiological GI conditions.

Acknowledgements

Financial support from the Région Wallonne (convention no. 14403) is gratefully acknowledged.

References

Ataman, M., Boucher, E.A., 1982. Properties of aqueous salt solutions of poly(ethyleneoxyde). J. Polym. Sci. Polym. Phys. Ed. 20, 1585–1592.

Ataman, M., 1987. Properties of aqueous salt solutions of poly(ethylene oxide) cloud point, theta temperature. Colloid Polm. Sci. 265, 19–25.

Brazel, C.S., Peppas, N.A., 1996. Pulsatile local delivery of thrombolytic and antithrombotic agent using poly (N-isopropylacrylamide-co-methacrylic acid) hydrogels. J. Control Rel. 39, 57–64.

Cabane, B., Duplessix, R., 1982. Organisation of surfactant micelles adsorbed on a polymer molecule in water: a neutron scattering study. J. Phys. 43, 1529–1535.

Cammas, S., Suzuki, K., Sone, C., Sakurai, Y., Kataoka, K., Okano, T., 1992. Thermo-responsive polymer nanoparticles with a core shell micelle structure as site specific drug carriers. J. Control Rel. 19, 121–130.

Dong, L.C., Yan, Q., Hoffman, A.S., 1992. Controlled release of amylase from a thermal and pH-sensitive macroporous hydrogel. J. Control Rel. 19, 171–178.

Eisenberg, S.R., Grodzinsky, A.J., 1984. Electrically modulated membranes permeability. J. Memb. Sci. 19, 173–194.

Feil, H., Bae, Y.H., Feijen, J., Kim, S.W., 1993. Effect of comonomer hydrophilicity and ionisation on the lower critical solution temperature of N-isopropylacrylamide copolymers. Macromolecules 26, 2496–2500.

Fujishe, S., Kubota, K., Ando, I., 1989. Phase transition of aqueous solutions of poly(N-isopropylacrylamide) and poly(N-isopropylmethacrylamide). J. Phys. Chem. 93, 3311–3313.

Geigy Scientific Tables 1, 1981. Unit of measurement-body fluids-composition of the body nutrition, 8th ed.

Heskins, M., Guillet, J.-E., 1968. Solution properties of poly(N-isopropylacrylamide). J. Macromol. Sci. Chem. A2 (8), 1441–1455 December.

Horne, R.A., Almeida, J.P., Day, A.F., Yu, N.-T., 1971. Macromolecule hydration and the effect of solutes on the cloud point of aqueous solutions of polyvinyl–methyl–ether: a possible model for protein denaturation and temperature control in homeothermic animals. J. Colloid Surfaces Sci. 35, 1 January.

Ichikawa, H., Fukumori, Y., 2000. A novel positively thermosensitive controlled release microcapsule with membrane of nano-sized poly(N-isopropylacrylamide) gel dispersed in ethylcellulose matrix. J. Control Rel. 63, 107–119.

Ichikawa, H., Fukumori, Y., 1997. New applications of acrylic polymers for thermosensitive drug release. S.T.P. Pharma. Sci. 7 (6), 529–545.

Kim, Y.H., Bae, Y.H., Kim, S.W., 1994. pH/T° sensitive polymers for macromolecular drug loading on release. J. Control Rel. 28, 143–152.

Kiolchob, T., Kimura, S., Imanishi, Y., 1998. Thermoresponsive release from poly(glucone)-block-poly(sar) microcapsules with surface-grafting of poly(N-isopropylacrylamide). J. Control Rel. 50, 205–214.

Kono, K., Henmi, A., Yamashita, H., Hayashi, H., Takagishi, T., 1999. Improvement of temperature-sensitivity of poly(N-isopropylacrylamide)-modified liposomes. J. Control Rel. 59, 63–75.

Kubota, K., Fujishige, S., Ando, I., 1990. Single chain transition of poly(N-isopropylacrylamide) in water. J. Phys. Chem. 94, 5154–5158.

Moës, A.J., 1989. Recherche et développement de formes orales à libération contrôlées. J. Pharm. Belg. 44 (1), 60–70.

Mukerjee, P., Mysels, K.J., 1971. Critical micelles concentration of aqueous surfactant systems, National Standard Reference Data System — Nat. Bur. Stand. (US) 36.

Okano, T., Bae, Y.H., Jacobs, H., Kim, S.W., 1990. Thermally on-off switching polymers for drug permeation and release. J. Control Rel. 11, 255–265.

Okatata, J., Noguchi, H., Seki, T., 1982. Functional capsule membranes 26. Permeability control of polymer grafted capsule membranes responding to ambiant pH changes. Macromolecules 20, 15–21.

Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.

Plate, N.A., Lebedeva, T.L., Valuev, L.I., 1999. Lower critical solution temperature of N-alkyl-substituted polyacrylamides. Polym. J. 31 (1), 21–27.

Ringsdorf, H., Simon, J., Winnik, F.M., 1992. Hydrophobically-modified poly(N-isopropylacrylamides) in water: a look by fluorescence techniques at the heat-induced phase transition. Macromolecules 25, 7306–7312.

Schild, H.G., 1992. Poly(N-isopropylacrylamide): experiment, theory and application. Prog. Polym. Sci. 17, 163–249.

Schild, H.G., Tirrell, D.A., 1991. Interaction of poly(N-isopropylacrylamide) with sodium n-alkyl sulfates in aqueous solution. Langmuir 7, 665–671.

Schild, H.G., Tirrell, D.A., 1990. Microcalorimetric detection of lower critical solution temperature in aqueous polymer solution. J. Phys. Chem. 94, 4352–4356.

Serres, A., Baudys, M., Kim, S.W., 1996. Temperature and pH-sensitive polymers for human calcitonin delivery. Pharm. Res. 13, 196–201.

Tanaka, N., Takemura, M., Konno, T., Kunugi, S., 1998. Pressure effect on the chain shrinkage of poly(vinylpyrrolidone) induced by complexation with a hydrophobic compound in aqueoussolution. Macromolecules 31, 8840–8844.

Wu, C., Zhou, S., 1995. Thermodynamically stable globule state of a single poly(N-isopropylacrylamide) chain in water. Macromolecules 28, 5388–5390.

Wu, C., Zhou, S., 1997. Volume phase of transition of swollen gels: discontinuous or continuous? Macromolecules 30, 574–576.

Yoshida, R., Sakai, K., Okano, T., Sakurai, Y., 1992. Drug release profiles in the shrinking process of thermoresponsive poly(N-isopropylacrylamide-co-alkylmethacrylate) gels. Ind. Eng. Chem. Res. 31, 2339–2345.