J Mater Sci (2007) 42:9845–9854
DOI 10.1007/s10853-007-1814-4

The effect of salts and pH buffered solutions on the phase transition temperature and swelling of thermoresponsive pseudogels based on *N*-isopropylacrylamide

Luke M. Geever · Michael J. D. Nugent · Clement L. Higginbotham

Received: 7 December 2006 / Accepted: 30 April 2007 / Published online: 21 August 2007

Abstract The temperature sensitive nature of poly(*N*-isopropylacrylamide) makes it an attractive candidate for controlled drug delivery devices. A series of temperature responsive poly (*N*-isopropylacrylamide)-polyvinyl pyrrolidinone random copolymers were produced by free radical polymerisation using 1-hydroxycyclohexylphenyketone as a UV-light sensitive initiator. The chemical structure of the xerogels was characterised by means of Fourier transform infrared spectroscopy (FTIR). The copolymers possess a lower critical solution temperature (LCST) in pure water, but the transition temperature may be affected by the addition of various cosolutes. The LCST of the pseudogels (physically cross-linked gels) was investigated in distilled water and a variety of salt and pH buffer solutions, using modulated differential scanning calorimetry (MDSC) and rheological analysis. The pH buffer solutions prepared mimic the variety of conditions encountered by drug delivery systems administered orally. The pH effects on the LCSTs of the temperature sensitive gels appear not obvious; while the salts used to prepare the pH buffer solutions have a more notable effect ('salting out effect') on the phase transition temperature. All swelling studies were carried out on the hydrogels at 37°C in distilled water, pH buffer 1.2 and pH buffer 6.8. The swelling/dissociation behaviour of the gels is found to be highly dependent on the pH buffer solution used, as the salts incorporated in preparing the pH buffer solutions lowers the phase transition of the copolymers to below the test temperature of 37°C, thus making them less soluble.

L. M. Geever · M. J. D. Nugent · C. L. Higginbotham (✉)
Centre for Biopolymer and Biomolecular Research,
Athlone Institute of Technology, Dublin Rd, Athlone, Co,
Westmeath, Ireland
e-mail: chigginbotham@ait.ie

L. M. Geever
e-mail: lgeever@ait.ie

M. J. D. Nugent
e-mail: mnugent@ait.ie

Introduction

Controlled release of therapeutic agents remains one of the biggest challenges in drug delivery. This is particularly the case in the effective delivery of proteins and peptide therapeutics because of their large molecular weights and unique three-dimensional structures. Repeated administration of a drug so as to maintain drug concentration within the therapeutic window may cause serious side effects, which in many cases necessitates the patient to stop taking medication. With conventional dosage forms, high peak blood concentrations may be reached soon after administration with possible adverse effects related to the transiently high concentration [1, 2]. Polymers have gained in importance in the pharmaceutical industry as both drug encapsulants and vehicles of drug carriage. Polymers employed to delay drug dissolution aim to slow the rate at which drug molecules are exposed to water from the aqueous environment surrounding the drug delivery system. Monolithic (matrix) devices are possibly the most common of the devices for controlling the release of drugs. This is because they are relatively easy to fabricate, compared to reservoir devices, and there is not the danger of an accidental high dosage that could result from the rupture of the membrane of the reservoir device [3].

Of particular interest to the current investigation is the use of intelligent polymers, which are capable of

undergoing sharp physical or chemical modifications in response to external stimuli such as temperature or pH. Hydrogels are one such class of intelligent or smart material [4]. Nguyen et al. presented a review on UV curable hydrogels that may be used as biomaterials in medical applications [5]. Photopolymerisation is currently being used for an increased number of biomedical applications due to its ability to rapidly convert liquid monomer into a crosslinked network and also because no organic solvents are involved during the polymerisation process [4]. Hydrogels are becoming increasingly important materials for pharmaceutical applications. They are used in a variety of applications including diagnostic, therapeutic, and implantable devices, particularly in controlled release drug delivery systems, where they have been studied extensively [1, 3–15].

Poly (*N*-isopropylacrylamide) (PNIPAAm) is one of the most investigated of the negative temperature sensitive hydrogels. It has been reported that aqueous PNIPAAm water solutions and hydrogels exhibit a Lower Critical Solution Temperature (LCST) in pure water at 32°C [16–20]. Below phase transition temperature, PNIPAAm is extremely soluble in water, however as the temperature is increased above its LCST, it becomes hydrophobic and precipitates out from the aqueous solution [17, 18]. Most papers report on the LCSTs of their polymers in de-ionised water. Few studies have been carried out to investigate the salt and pH effects on the LCST. It is fundamentally important to investigate such effects as blood and other body fluids are not salt free, and variations on the variety and concentration of salts as well as pH exist [17]. In this work we investigated the reversed temperature-dependent solubility of PNIPAAm based pseudogels in distilled water and pH buffered solutions. Our concept consists of controlling the dissociation time of UV cured pseudogels at physiological temperature. The rate at which these hydrogels dissociate is dependent on the LCST, which can be greatly affected by the salts present in the pH buffered fluids.

Experimental details

Synthesis of polymers

The hydrogels investigated in this work were prepared by free-radical polymerisation using ultra violet light according to the procedure reported earlier [21]. The monomers used were 1-vinyl-2-pyrrolidinone (NVP, Lancaster synthesis) and *N*-isopropylacrylamide (NIPAAm, TCI Europe). To initiate the reactions, 1-hydroxycyclohexyl-phenylketone (Irgacure® 184, Ciba speciality chemicals) was used as a UV-light sensitive initiator at 3 wt% of the total monomer weight. Table 1 lists the hydrogel name and composition of the xerogels produced. As NIPAAm monomer is a solid, PNIPAAm could not be synthesised by UV polymerisation using this procedure. Therefore, all tests on PNIPAAm homopolymer were carried out on Poly(*N*-isopropylacrylamide) (Polysciences Inc).

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was carried out on the rectangular samples that had being exposed to atmospheric conditions for a minimum of 7 days, using a Nicolet Avator 360 FTIR, with a 32 scan per sample cycle.

Preparation of aqueous salt and pH buffered solutions

A number of salts were dissolved in distilled water at between 0 and 5 wt% (in the concentration range from 0 M to 2 M) to investigate their influence on the LCST of thermosensitive polymer and copolymers. The solutes used included potassium chloride (KCl), potassium biphthalate ($C_8H_5O_4K$), potassium phosphate monobasic (KH_2PO_4) and boric acid (H_3BO_3). KCl, $C_8H_5O_4K$, KH_2PO_4 and H_3BO_3 were also used to prepare the following pH buffered solutions: pH buffer 1.2, pH buffer 4, pH buffer 6.8 and pH buffer 10. Hydrochloric acid and sodium hydroxide were used to adjust ionic strength of the solutions to 0.2 M. The pH of all the solutions was determined using a Thermo Orion model 420A pH meter.

Preparation of aqueous polymer and copolymer solutions

Homogeneous solutions of each of the hydrogels were prepared, by weighing appropriate amounts of the xerogel and distilled water, aqueous salt solutions or pH buffered solutions ranging from pH 1.2 to pH 10, leaving these mixtures at room temperature for a period of hours/days.

Table 1 Name and composition of hydrogels containing NVP, NIPAAm and distilled water in their feed ratios

Hydrogel Name	NVP (wt%)	NIPAAm (wt%)	Distilled water (wt%)
A1 (L1)	15	65	20
A2	15	60	25
A3	15	55	30
A4 (L2)	20	65	15
A5	20	60	20
A6	20	55	25
A7	25	55	20
A8 (L3)	30	60	10

Irgacure 184 was used as a UV-light sensitive initiator at 3 wt% of the total monomer weight for each of these gels

Aqueous polymer and copolymer solutions of 1, 1.5, 2, 2.5, 3 and 5 wt% were prepared for subsequent use in calorimetric and parallel plate rheometry measurements.

Phase transition determination

Modulated differential scanning calorimetry

The DSC method was among the techniques used for examination of the phase transition phenomenon exhibited by these thermosensitive gels. The analyses were preformed using a DSC 2920 Modulated DSC (TA Instruments) containing a refrigerator cooling system. Samples of between 8 and 10 mg were transferred by syringe and weighed out using a Sartorius scales capable of being read to five decimal places. Aluminum pans were crimped before testing, with an empty crimped aluminum pan being used as the reference cell. Calorimetry scans were carried out from 20°C to 55°C for each of the aqueous solutions. All DSC measurements were carried out at a scanning rate of 1°C/min under nitrogen atmosphere. Calibration was preformed using indium as standard. For the study of phase separation and phase separation kinetics, not the absolute value, but the changes of the specific heat capacity as a function of temperature and time are important. For this reason, the MDSC curves are shifted vertically for clarity.

Rheological analysis

Rheological measurements were performed using an Advanced Rheometer AR1000 (TA instruments) fitted with a peltier temperature control. It is a versatile research-grade rheometer designed for rapid characterisation of mobile and viscous liquids. The geometry used was a 6 cm diameter parallel steel plate and the instrument was calibrated for inertia and mapped before use. Aqueous polymer and copolymer solutions of 1.5 cm^3 were pipetted onto the peltier plate and the dynamic mechanical properties of the aqueous polymer solutions was measured in terms of polymer concentration and temperature. The tests were performed in oscillation mode at a low frequency of 5.81 rad/s, using a temperature ramp rate of 1°C/min and a sample gap of 0.8 mm.

Swelling studies

The swelling characteristics of the gels were investigated in triplicate at 37°C in distilled water, pH buffer 1.2 and pH 6.8. The preparation of the pH buffer solutions is detailed in section 2.3. Samples of the cured polymer with a mass of 1.1 ± 0.35 g were placed in a petri dish; the petri dish was filled with the appropriate solution and placed in a fan oven at the required temperature. Petri dish lids and Petri Seal (Diversified Biotech Ltd) were placed on the petri dishes while in the oven to prevent evaporation. Periodically, excess polymer solution was removed at predetermined time intervals by pouring the solution through a Buchner funnel. The samples were then blotted free of surface liquid with filter paper, and the wet weight of the gel sample was measured using a Sartorius scales at room temperature. The samples were re-submerged in fresh solutions and returned to the oven. The percentage that the hydrogels swelled was calculated using the formula:

$$\text{Swelling } (\%) = W_t/W_0 \times 100$$

where W_t is the mass of the gel at a predetermined time and W_0 is the dry mass of the gel. The process was continued until the sample appeared to have dissolved or for up to 120 h.

Results and discussion

Preparation and characterisation

The hydrogels were photopolymerised using Irgacure® 184 as a photoinitiator. Exposure to a UV light source produces free radicals by decomposition of the photoinitiator, which initiates polymerisation of the monomer [5]. All samples were cured on a silicone moulding, and prior to use dried for at least 24 h in a vacuum oven. The copolymers were synthesised to have their own distinctive phase transition temperatures by alternating the feed ratio, using the hydrophobic NIPAAm monomer and hydrophilic NVP monomer. This ability to shift the LCST of PVP/PNIPAAm copolymers provides excellent flexibility in tailoring transitions for specific uses. It was found that NIPAAm monomer could be dissolved more readily in aqueous mixtures of NVP and distilled water, than in pure liquid NVP alone. This allowed greater freedom in LCST control when compared with PVP/PNIPAAm copolymers prepared in the absence of distilled water. The xerogels were transparent and glass like in appearance after photopolymerisation. Hydrogels A1(L1) and A4(L2) were chosen for further investigation as they both have phase transition temperatures near body temperature, which is important especially for drug delivery applications [21].

The physically crosslinked PVP/PNIPAAm copolymers were characterised using Fourier transform infrared spectroscopy (FTIR). As reported previously, the disappearance of the characteristic NVP and NIPAAm monomer peaks in the PVP/PNIPAAm copolymer spectra indicate that the polymerisation reaction has taken place [22]. The FTIR spectra of hydrogel A1(L1) is shown in Fig. 1. The addition of small amounts of distilled water to the monomeric mixture was found to have little affect on the spectra of the xerogels. Characteristic peaks for the synthesised

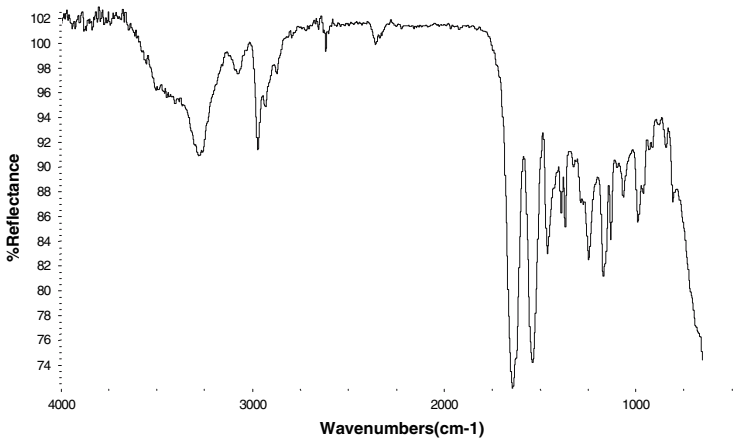

Fig. 1 FTIR spectra of physically crosslinked hydrogel A1(L1)

copolymers were observed at 1,641 cm^{-1} for C=O, at 1,540–1,538 cm^{-1} for NH, and at 1,386 cm^{-1} and 1,366 cm^{-1} representing the double band for the isopropyl group. These values are in good agreement with work carried out by other authors [17, 23, 24].

Phase transition determination

Aqueous solutions of PNIPAAm homopolymer have a phase transition temperature of about 32°C in distilled water [15–20], which can be determined by both DSC analysis, giving the endothermic transition peak [25] and by cloud point measurement, giving the cloud point value [18]. The calorimetric method measures the heat resulting mainly from the breaking down of hydrogen bonds between water and polymer [26], while the latter method visualises the clouding of the solution due to the precipitation of the polymer, when phase separation occurs [18]. The phase transition behaviour may also be detected using parallel plate rheometry, by measuring the viscosity of the gels under constant shear, as temperature is gradually increased but fewer studies have been done using this technique. Calorimetric and cloud point measurements were carried out on aqueous PNIPAAm homopolymer in a previous work [22] and were found to be in good agreement with the literature [18, 22, 26–29]. As it has been extensively reported, it provides an ideal reference material for comparison with obtained results.

Rheological analysis

The phase transition temperature of PNIPAAm homopolymer and PNIPAAm/PVP copolymers was investigated using rheological analysis. This technique can be used to detect any changes as the polymer solution is being heated and since the stress exerted by the polymer chains is measured continually, more information can be obtained [30]. Initially, the temperature dependent viscosity of aqueous PNIPAAm solutions was studied at varying polymer concentrations. The viscosity curve for aqueous 3 wt% PNIPAAm homopolymer as a function of temperature, at a shear rate of 5.81 s^{-1} is shown in Fig. 2, with LCST onset and peak maximum values of 27.9°C and 32°C recorded, respectively. Both LCST onset and peak maximum values are taken, as these values may vary by a number of degrees Celsius. Viscosity values for the aqueous polymer solutions (1–5 wt%) were very low below the transition temperature, ranging from 1.84×10^{-3} to

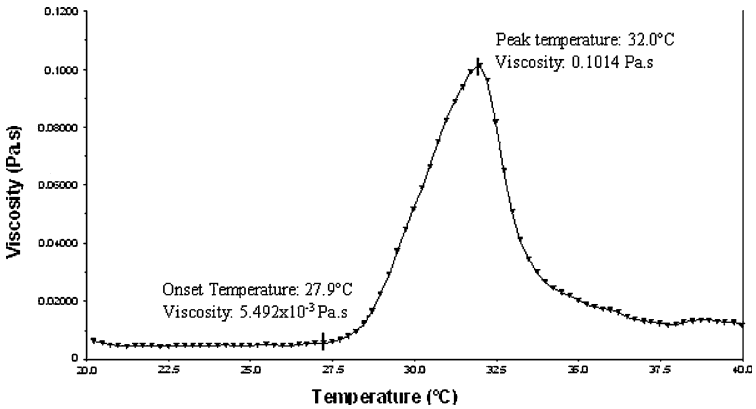

Fig. 2 Rheological scan carried out on aqueous poly (N-isopropylacrylamide) at 3 wt%

5.5×10^{-3} Pa.s. This is a result of the dilute solutions being made up of primarily water. At the onset of the LCST there is a sharp increase in the viscosity, with the peak maximum LCST values recorded also showing obvious dependence on concentration. After the peak maximum is reached there is an apparent decrease in the viscosity of the dilute systems. This is typical behaviour for each of the concentrations studied, corresponding to the region associated with the phase transition of the homopolymer.

Tam et al. has previously reported on the phase separation behaviour of PNIPAAm using rheometry and divided the thermosensitive viscosity curve into three regions, namely region I (before onset), region II (onset to peak) and region III (after peak) [30]. In this work, similar rheological trends are observed, although there is slight difference in the behaviour of the dilute polymer solution below LCST onset (region I), possibly due to the greater sensitivity of the equipment employed herein. Yang et al. carried out viscometry analysis on PNIPAAm homopolymer and also divided his findings in to three similar regions, a coiled region I, a transition region II and a globule region III [31]. Park et al. states that as the temperature increases, hydrophobic interactions among hydrophobic sections become strengthened, while hydrogen bonding becomes weaker [4]. A detailed review by Graziano et al. on the temperature-induced coil to globule transition of PNIPAAm in dilute aqueous solutions provides greater understanding of this complex transition [32].

It is commonly accepted that thermoreversible gelation occurs at the LCST of aqueous PNIPAAm solutions containing relatively high polymer concentrations [33, 34]. Therefore, analysis of aqueous solutions containing no higher than 5 wt% polymer are reported herein, as at concentrations of 10 wt% and 15 wt% the gels are seen to undergo thermoreversible gelation at the phase transition temperature, and as result no defined peak maximum value can be obtained using this technique. The effect of polymer concentration on aqueous solutions of poly(*N*-isopropylacrylamide) has been previously reported using modulated DSC and cloud point measurement [22]. These results are compared with current rheological findings in Fig. 3. At higher polymer concentrations, the demixing curves measured by both turbidity and calorimetry (onset value) coincides. This is due to the fact that at sufficiently high polymer concentrations, cloud point temperature is largely independent of the polymer concentration. However, as found previously for both PNIPAAm and other polymers, the cloud point temperature increases at low polymer concentrations [18]. This is evident as the cloud point curve starts to deviate from the MDSC peak onset signal at polymer concentrations lower than 3 wt%. Like cloud point measurement, rheological peak maximum and onset values are also found to increase at low polymer concentrations. The literature which quotes phase transition temperature as peak maximum of the endotherm, usually report LCST at 32°C [26–29], which is in good agreement with peak maximum values recorded. Rheological peak maximum values above 2 wt% concentrations are quite consistent with calorimetric peak values but onset results are approximately 1°C lower than that of the DSC technique, for each of the concentrations tested. The rheological onset and peak maximum values are noted to differ by over 3.5°C, and the viscosity curves are seen to be at least 1°C broader than the MDSC curves, for each polymer concentration. With these thermosensitive polymers, it is believed that a small fraction of the gel begins to undergo its phase transition at the onset temperature, while the bulk undergoes the transition at the peak maximum value.

The investigation of the phase transition temperature of A1(L1) and A4(L2) using this dynamic rheological technique proved unsuccessful. Results suggest that evaporation is a vital limiting factor in testing thermosensitive polymers with higher LCST values, using this procedure. The effect of pH buffer solution on the LCST of aqueous PNIPAAm homopolymer was also analysed. While in many cases there was an increase in viscosity using this technique, corresponding to the phase transition temperature as detected by modulated DSC, the reproducibility was inconsistent. Some flocculation and eventual coagulation occurred as the solution was further heated and

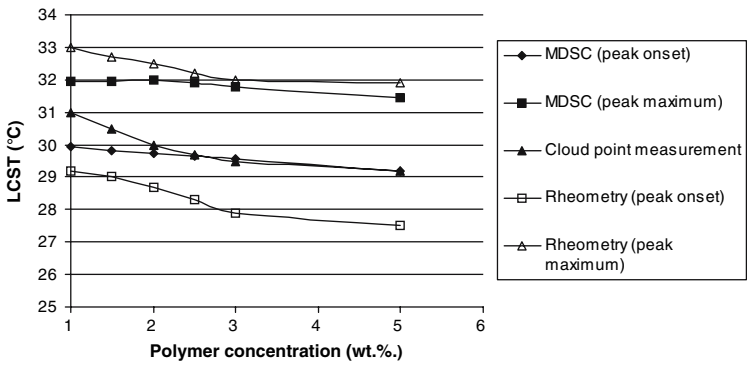

Fig. 3 LCST of aqueous poly(N-isopropylacrylamide) homopolymer solutions at varying polymer concentrations established by calorimetry, dynamic rheological analysis and cloud point measurement

sheared, above the LCST. This behaviour is thought to be as a result of the aqueous polymer solutions undergoing thermoreversible gelation, initiated due to salts incorporated in the buffer solutions binding to the polymer and lowering the LCST of the system. It can be concluded that rheological analysis using this procedure is more suitable for the characterisation of the transition temperature of thermosensitive polymers at relatively low temperatures and low polymer concentrations, in a salt free environment.

Modulated differential scanning calorimetry

In many of its applications, PNIPAAm is not swollen in distilled water alone but in the presence of aqueous solutions containing various co-solutes. Many of these co-solutes may interfere and perturb the LCST if they bind to the polymer or substantially change the water structure [18]. Therefore, it is extremely important to investigate the effect of co-solutes on the phase transition temperature, especially in applications such as oral drug delivery, as a variety of environments are encountered in the GI tract.

The phase transition behaviour of PNIPAAm homopolymer and PNIPAAm/PVP copolymers in a number of salt and pH buffer environments was analysed using modulated DSC, as it provides greater information than simple cloud point measurement and as rheological experiments did not yield desired results. The greater sensitivity of modulated DSC in phase transition temperature analysis, when compared with conventional DSC, is detailed in a previous work with particular emphasis on the roll of the reversing heat flow signal [22]. In literature to date, it is important to note that authors have differed in their interpretation of the phase transition endotherm. Otake et al. [20] defines the phase transition temperature as the onset of the transition endotherm (the interaction of the baseline and the leading edge of the endotherm), while Schild et al. [26] defines it as the temperature at the peak of the thermogram. This should always be taken into consideration, when analysing transition temperatures by calorimetry as peak and onset values may differ by a number of degrees Celsius. In this work, both peak onset and peak maximum values were recorded.

Effect of salts on LCST. Although temperature-induced phase transition is a common occurrence particularly in the biological systems, studies on the effects of water-soluble additives such as salt have remained sparse [17]. Those who have carried out studies on the effect of salts on thermosensitive polymers have found that the LCST may be increased (known as the 'salting-in' effect) or decreased (known as the 'salting-out' effect) depending on the chemical structure of the added salt [17, 35–38]. 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 presence of salts [38].

In this work each of the salts added; KCl, H_3BO_3, KH_2PO_4 and $C_8H_5O_4K$ was found to exert a 'salting out' effect. The effect of salt concentration on the phase transition endotherm is illustrated in Fig. 4, using H_3BO_3 A1(L1) salt solutions as an example. As can be seen, the endotherms of the aqueous A1(L1) solutions shift to the left with increasing H_3BO_3 salt incorporation indicating a decrease in LCST but still regain similar shape and transition half width at each salt concentration, which is comparable with the original copolymer [21]. This is the case for each of the salts analysed with the exception of copolymer solutions containing higher percentages of KH_2PO_4, which reduces the LCST to near ambient temperature and results in slightly broader endotherms. The addition of each of the salts at increasing concentrations also resulted in a decrease in phase transition temperature for the aqueous PNIPAAm solutions but again was found to have little effect on the size or transition half width of the endotherm, when compared with that of the homopolymer containing no incorporated salt [22].

It should be noted that all of the aqueous polymer and copolymer solutions were made up at 3wt% before the addition of the salts, as it is important to keep polymer concentration constant as the transition temperature and transition enthalpy (ΔH) have been found to be concentration dependent [21, 22]. As expected, the ΔH was found to be positive for each of the pseudogels tested, further proving that the phase transition is an endothermic reaction, as exothermic reactions have a negative transition enthalpy. Feil et al. suggest that a higher LCST leads to a reduced heat of phase separation due to the smaller amount

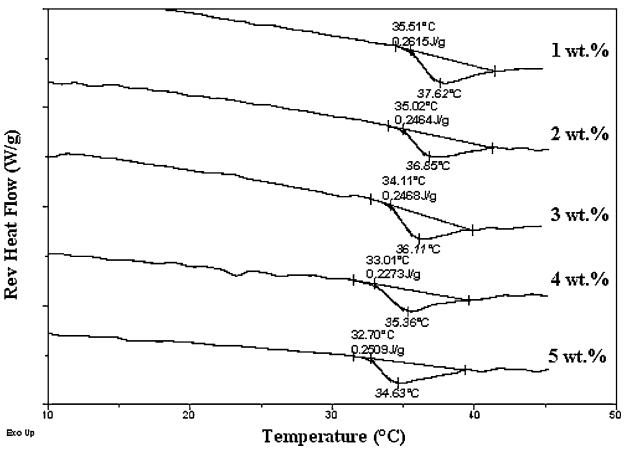

Fig. 4 Modulated DSC thermograms showing the effect of H_3BO_3 salt concentration (1–5 wt%) on the phase transition temperature behaviour of aqueous A1(L1) copolymer solutions

of structured water at higher temperatures [29]. This theory is supported by the literature, which examines changes in the enthalpy of phase transition for linear PNIPAAm homopolymer and copolymers containing increasing hydrophilic component [21, 22]. This trend is however not observed herein, with little difference in ΔH values for either the homopolymer or copolymer, despite the increasing salt concentration and decreasing LCST characteristic of both. For example, an average transition enthalpy value of about 0.25 J/g (as illustrated for H_3BO_3 in Fig. 4) was found for each of the aqueous copolymer salt solutions at each concentration, thus showing no obvious change or trend in ΔH despite the apparent differences in LCST. Others reporting on the effect of salt on the phase transition temperature have only reported the LCST values and not the ΔH associated with the transition [17, 35–38].

While the heat of phase separation and the overall endotherm size or transition half width are not greatly affected by addition of the salts, it has been established that their incorporation has an obvious and pronounced effect on the phase transition temperature. The calorimetric peak maximum results clearly show that addition of small amounts of the salts to both PNIPAAm and PVP/PNIPAAm aqueous solutions induce significant decreases in the phase transition temperature, as summarised in Figs. 5 and 6.

The salts incorporated bring about almost identical decrease in onset temperatures, as they do to the peak maximum values (highlighted by the uniform endotherm sizes and transition widths, with the exception of KH_2PO_4 at high salt concentrations), so only peak maximum values will be discussed from this point onwards. Based on the results for aqueous homopolymer and copolymer solutions,

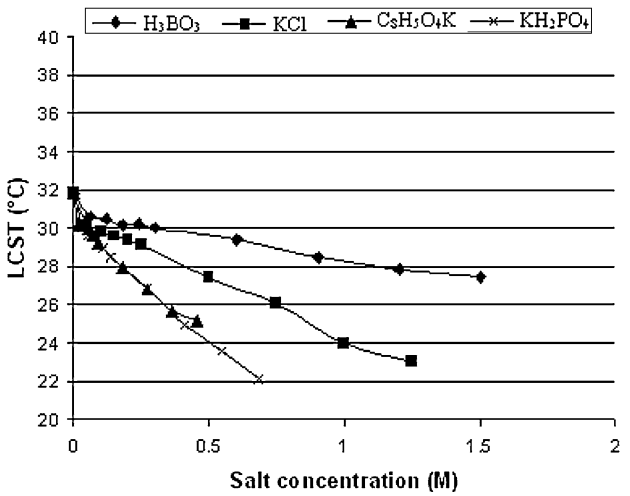

Fig. 5 The effect of a number of salts at varying concentrations on the LCST (peak maximum value) of aqueous PNIPAAm homopolymer as determined by modulated DSC

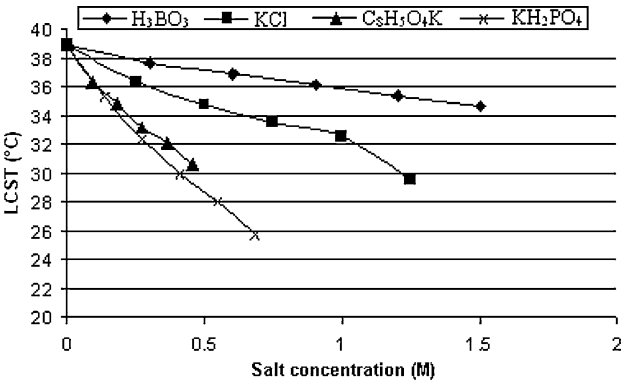

Fig. 6 The effect of a number of salts at varying concentrations on the LCST (peak maximum value) of aqueous A1(L1) copolymer as determined by modulated DSC

the 'salting out' effectiveness of each of the salt types added corresponds to the following order: H_3BO_3 < $C_8H_5O_4K$ < KCl < KH_2PO_4. When compared, the salts have a slightly stronger 'salting out' effect on the copolymer than on the homopolymer. This is possibly due to the comonomer (NVP) incorporated being hydrophilic in nature when polymerised, thus the hydrogen bonding among the hydrophilic chains becomes dominant, resulting in a stronger tendency for the polymers to associate and decrease their LCSTs [17]. Eeckman et al. states that 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 [37].

Effect of pH buffer on LCST. It is fundamentally important to investigate the effects of the pH buffered solutions on the LCST, as blood and other body fluids are not salt free, and variations on the variety and concentration of salts as well as pH in the human body exist [17]. The LCSTs of the homopolymer in distilled water and a number of pH buffer solutions are shown in Fig. 7, and similar trends are exhibited by copolymer A1(L1) as illustrated in Fig. 8. A decrease in the transition temperature was observed for each of the pseudogels in all of the buffer solutions, when comparison is made with their transition temperatures in distilled water. At a glance, it would appear that pH has a significant effect on the LCST of the temperature sensitive polymers, but this observation neglects to take in to account the 'salting out' effect that salts incorporated in the buffer solutions have on the LCST. Even when this is studied more closely (bearing in mind that KCl is used in the preparation of pH 1.2 buffer, $C_8H_5O_4K$ is used in the preparation of pH 4 buffer, KH_2PO_4 is used in the preparation of pH 6.8 buffer, and

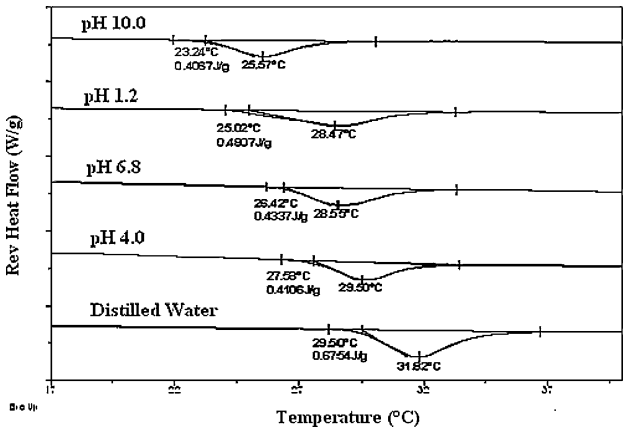

Fig. 7 Modulated DSC thermograms showing the effect of pH buffer solutions on the LCST of aqueous poly(*N*-isopropylacrylamide) homopolymer

H_3BO_3 and KCl are used in the preparation of pH 10 buffer), the decreases in LCST caused by each of the buffer solutions does not follow the 'salting out' effectiveness of the salts as found earlier. However, one should take into consideration that different concentrations of the salts are required to make up each buffer solution to a specific pH, and that the effect of the salt on the LCST has been found to be highly concentration dependent. Also, the use of either 0.2 M HCl or 0.2 M NaOH is necessary to prepare the buffer solutions, and these salts have been previously found to cause a 'salting out' effect on thermoresponsive polymers based on PNIPAAm in a detailed study conducted by Eeckman et al [37]. In said report it is recorded that NaOH has a more profound effect on the LCST than HCl. This may help to explain why pH 10 buffer solutions have the greatest negative effect on the LCST, as it uses the greatest volume of 0.2 M NaOH in its preparation. All of the other pH buffer solutions have similar effects on the transition temperature of the

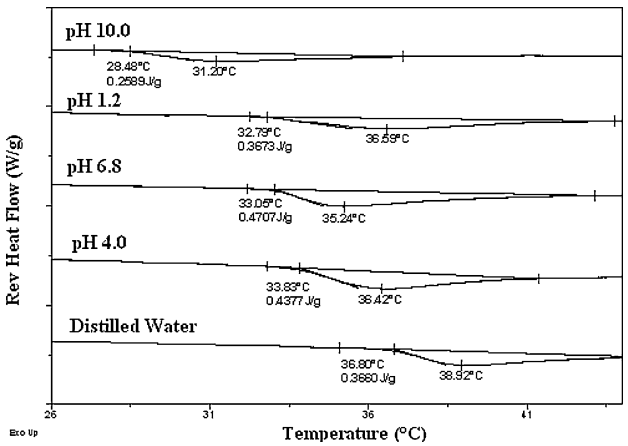

Fig. 8 Modulated DSC thermograms showing the effect of pH buffer solutions on the LCST of aqueous A1(L1) copolymer

homopolymer and copolymer and again there appears to be no particular trend in transition enthalpies. The difference in endotherm shape and width between the aqueous homopolymer and copolymer solutions has been reported previously [21].

In summary, the pH effects on the LCSTs of the temperature sensitive gels appear not obvious; while the salts used in preparation of the pH buffer solutions have a more notable effect on the LCST. Other works also support this view. Jones et al. states that the variation of pH of the aqueous solution of PNIPAAm has no significant effect on the LCST of the homopolymer [39]. Liu et al. studied the effect of salt and pH on the LCST of thermosensitive polymers and stated that incorporated salt had an obvious effect on the LCST, while effects of pH on the phase transition behaviours were negligible [17]. This behaviour is of particular importance to the current investigation, as LCST is one of the most important parameters for temperature-sensitive hydrogels used in drug delivery applications.

Swelling studies

In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between the polymer chains. Narasimhan et al. states that when an uncrosslinked, amorphous, glassy polymer is brought into contact with a thermodynamically compatible solvent, the latter dissociates into the polymer, and when the solvent concentration in the swollen polymer reaches a critical value, chain displacement begins to dominate and the polymer is eventually dissolved [40]. This behaviour has been studied using UV cured PVP pseudogels swelled in distilled water at various temperatures and a reduction in dissolution time with increasing temperature is noted [41]. This is typical dissolution behaviour for an analogous linear polymer in solution. Polymers with LCST however, decrease their water-solubility as the temperature increases and are called negative temperature-sensitive hydrogels. The swelling behaviour of these types of temperature sensitive gels in distilled water at a number of different temperatures has also been investigated previously. The negative temperature sensitive gels synthesised were found to exhibit a LCST in distilled water, below which they are water soluble and above which they become slightly less water soluble, significantly less water soluble, or water insoluble, as the test temperature is further increased above the LCST [21].

The swelling and dissociation behaviour of the gels was investigated at 37°C using pH buffer 1.2 and pH buffer 6.8 and is compared with swelling experiments carried out in distilled water. Clearly this behaviour is greatly affected by the nature of the aqueous environment as illustrated in

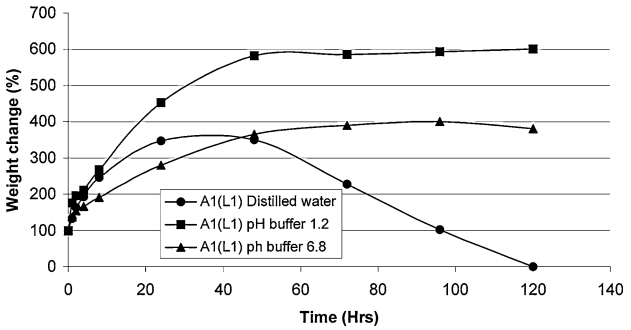

Fig. 9 Swelling behaviour of hydrogel A1(L1) in pH buffer 1.2, pH buffer 6.8 and distilled water at 37°C

Fig. 9. The gel reached its maximum swollen weight between 24 h and 48 h in distilled water and the dissolution phase was complete within 120 h. Onset and peak maximum MDSC values of 36.93°C and 38.92°C respectively have been recorded for the pseudogel in distilled water, so the bulk of the gel has not undergone the transition at 37°C and this explains why the gel has fully dissolved after 120 h. As mentioned earlier, it is believed that a small fraction of the gel begins to undergo its phase transition at the onset temperature, while the bulk undergoes the transition at the peak value. Modulated DSC results confirm that in pH buffer solutions the gels have lower LCSTs than in distilled water mainly due to the 'salting out' effect, with onset and peak maximum transition temperatures of less than 37°C recorded in both cases. As the swelling temperature was therefore greater than the LCSTs, the gels become less soluble, and instead of dissolving attained a relatively constant weight between 48 and 120 h. Also there appears obvious difference in the swelling ratio of the gels swollen in the higher pH when compared with those samples swollen in pH 6.8 and distilled water. The hydrophilic and hydrophobic balance of polymer side groups in PNIPAAm hydrogels, i.e., –CONH– is hydrophilic and $–CH(CH_3)_2–$ is hydrophobic [29, 42], are responsible for the interesting negative temperature sensitive swelling behaviour. Any molecules that can form hydrogen bonds to each other can alternatively form hydrogen bonds to water molecules [11, 43]. Because of this competition with water molecules, the hydrogen bonds formed between two molecules dissolved in water are relatively weak. It is generally believed that the phase transition behaviour of PNIPAAm based hydrogels in aqueous solutions is strongly related to the destabilisation of hydrogen bonds between water molecules and amide groups with increasing temperature, probably induced by the presence of the hydrophobic isopropyl group and backbone chain [16, 38]. It should be noted that very similar trends were exhibited by both gels A1(L1) and A4(L2), and as a result the negative temperature sensitive behaviour was described using only A1(L1) throughout the report for clarity sake.

Conclusion

We have synthesised a series of physically crosslinked random copolymers, containing NVP and NIPAAm at different monomeric concentrations, by photopolymerisation. The FTIR spectra of PVP-PNIPAAm complexes indicate that successful polymerisation of each of the monomers has taken place. The phase transition temperature of the gels was determined by calorimetry. By alternating the feed ratio, using the hydrophobic NIPAAm monomer and hydrophilic NVP monomer, copolymers were synthesised to have their own distinctive phase transition temperatures. The pH effects on the LCSTs of the temperature sensitive gels appear not obvious; while the salts used to prepare the pH buffer solutions are found to exert a 'salting out effect' on the phase transition temperature. This behaviour is of particular importance to the current investigation, as LCST is one of the most important parameters for temperature-sensitive hydrogels used in drug delivery applications. The swelling experiments conducted in pH buffer 1.2 and pH buffer 6.8 at 37°C show that the swelling/dissociation behaviour is highly dependent on the pH buffer solution used. With this in mind, it is hoped that the solubility properties of the thermosensitive hydrogels can be used as a means of controlling release in orally delivered drug devices. Further work is currently underway in order to investigate the effect of incorporation of a number of different drugs into the gels, with the hope achieving release of the drug at a predicted rate in given media, as a function of temperature.

Acknowledgements This study was supported in parts by grants from both Enterprise Ireland and the Athlone Institute of Technology research and development fund.

References

1. Wise DL (2001) Handbook of Pharmaceutical Controlled Release Technology. Marcel Dekker Inc., New York-Basel
2. Alderborn G, Aulton M (2002) Pharmaceutics, the science of dosage form design Ed., Elsevier Science, pp 397–441
3. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM (1999) Chem Rev 99:3181–3198
4. Park K, Qui Y (2001) Adv Drug Deliv Rev 53:321–339
5. Nguyen KT, West JL (2002) Biomaterials 23:4307–4314
6. Kishida A, Ikada Y (2002) Hydrogels for biomedical and pharmaceutical applications. In: Dumitriu S (ed) Polymeric biomaterials, 2nd ed., pp 133–45
7. Peppas NA, Bures P, Leobandung W, Ichikawa H (2000) Eur J Pharm Biopharm 50:27–46
8. Ravichandran P, Shantha KL, Panduranga Rao K (1997) Inter J Pharm 154:89–94

9. Kopeček J (2003) Eur J Pharmaceutical Sci 20:1–16
10. Bromberg LE, Ron ES (1998) Adv Drug Deliv Rev 31:197–221
11. LaPorte RJ (1997) Hydrophilic polymer coatings for medical devices. Technomic Pub. Co. Inc
12. Chilkoti A, Dreher M, Meyer D, Raucher D (2002) Adv Drug Deliv Rev 54:613–630
13. Grass M, Colombo I, Lapasin R (2000) J Controlled Release 68:97–113
14. Murata Y, Sasaki N, Miyamoto E, Kawashima S (2000) Eur J Pharmaceutics Biopharm 50:221–226
15. Bokias G, Staikos G, Iliopoulos I (2000) Polymer 41:7399–7405
16. Costa R (2002) Polymer 43:5879–5885
17. Liu XM, Wang LI, Wang L, Haung J, He C (2004) Biomaterials 25:5659–5666
18. Schild HG (1992) Prog Polym Sci 17:163–249
19. Deshmukh MV, Vaidya AA, Kulkarni MG, Rajamohonan PR, Ganapathy S (2000) Polymer 41:7951–7960
20. Otake K, Inomata H, Konno M, Saito S (1990) Macromolecules 23:283–289
21. Geever LM, Devine DM, Nugent MJD, Kennedy JE, Lyons JG, Hanley A, Higginbotham CL (2006) Eur Polymer J 42:2540
22. Geever LM, Devine DM, Nugent MJD, Kennedy JE, Lyons JG, Higginbotham CL (2006) Eur Polymer J 42:69–80
23. Ju HK, Kim SY, Kim SJ, Lee YM (2002) J Appl Polymer Sci 83:1128–1139
24. Ebril C, Kazancioğlu E, Uyanik N (2004) Eur Polymer J 40:1145–1154
25. Heskins M, Guillet JE (1969) J Macromol 2:1441
26. Schild HG, Muthukumar M, Tirrell DA (1991) Macromolecules 24:948–952
27. Kubota K, Fujishige S, Ando I (1990) J Phys Chem 94: 5154–5158
28. Boutris C, Chatzi E (1997) Polymer 38:2567–2570
29. Feil H, Bae YH, Feijen J, Kim SW (1993) Macromolecules 26:2496–2500
30. Tam KC, Wu XY, Pelton RH (1992) Polymer 33:436–438
31. Yang H, Cheng R, Wang Z (2003) Polymer 44:7175–7180
32. Graziano G (2000) Int J Biol Macromol 27:89–97
33. Han CK, Bae YH (1998) Polymer 39:2809–2814
34. Ruel-Gariépy E, Leroux JC (2004) Eur J Pharm Biopharm 58:409–426
35. Benrebouh A, Avoce D, Zhu XX (2001) Polymer 42:4031–4038
36. Gan LH, Roshan Deen G, Loh XJ, Gan YY (2001) Polymer 42:65–69
37. Eeckman F, Amighi K, Moës AJ (2001) Int J Pharm 222: 259–270
38. Eeckman F, Moës AJ, Amighi K (2002) Int J Pharm 241:113–125
39. Jones MS (1999) Eur Polymer J 35:795–801
40. Narasimhan B, Peppas NA (1996) Macromolecules 29:3283–3291
41. Devine DM, Higginbotham CL (2003) Polymer 44:7851–7860
42. Zhang XZ, Zhuo RX (2000) Eur Polymer J 36:643–645
43. Devine DM, Geever LM, Higginbotham CL (2005) J Mater Sci 40:3429–3436