International Journal of Pharmaceutics 366 (2009) 21–30

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Thermosensitivity and release from poly *N*-isopropylacrylamide–polylactide copolymers

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ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 20 August 2008
Accepted 23 August 2008
Available online 3 September 2008

Keywords:
Thermoresponsive-biodegradable hydrogels
Indomethacin
Poly-lactic acid
N-isopropylacrylamide
LCST

ABSTRACT

A series of thermoresponsive-co-biodegradable polymers, containing varying molar ratios of *N*-isopropylacrylamide (NIPA) and poly-lactic acid diacrylate macromer (PLAM) were prepared and characterised. Chemical structures were confirmed by nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR). The hydrogels were thermoresponsive, exhibiting an increase in the lower critical solution temperature (LCST) the higher the percent of PLAM present. Swelling properties were dependant on both temperature and PLAM content. The degradation behaviour of the three-dimensional polymeric networks formed was dependent on both structural (mesh size, molecular weight distribution, composition) and environmental parameters (temperature). Swelling and in vitro biodegradation-induced morphological structural changes were examined using scanning electron microscopy (SEM). A greater rate of degradation and disruption to the porous network could be seen with increasing lactide content. Degradation was faster below the LCST, demonstrated by FTIR, pH decrease and acid release, consistent with the increased hydrophilicity of the network. The release profiles of the model drug indomethacin (IDM), from these thermoresponsive-co-biodegradable polymers, were found to be dependant on copolymer composition, drug loading and temperature, more rapid release occurring below the LCST.

1. Introduction

Temperature sensitive polymers, particularly poly (*N*-isopropylacrylamide) (PNPA) and its copolymers are interesting materials for pharmaceutical and biomedical drug delivery applications. Such smart polymers undergo rapid reversible swelling changes in response to external stimuli such as temperature and or/pH (Hoffman, 1987; Schild, 1992; Kost and Langer, 1991). By undergoing large volume changes in response to external environment stimuli, they exhibit swelling-dependent drug release.

Biodegradable polymers offer further advantages in that there will be no need to remove residual biomaterial from the implantation site, the drug release profiles have greater versatility and are useful in a wider range of applications. Hydrogels composed of poly (L-lactic acid) (PLA) and dextran monomers have been reported in recent years and demonstrated thermoresponsive behaviour and hydrolytic/enzymatic degradation (Huang et al., 2004). More recently Perez et al. (2008) evaluated thermosensitive pNIPA hydrogels cross-linked with a biodegradable pseudo-peptide, the dimethacrylate of tyrosine–lysine–tyrosine.

The physical and mechanical properties of hydrogels are also dependent upon the chemical composition. Improved biocompatibility and flexibility by controlling network properties can be achieved by chemical modification of the hydrogel (Yoshida et al., 1993; Dong and Hoffman, 1990). Such modifications include addition of monomer(s) (Okano et al., 1990; Beltran et al., 1991; Feil et al., 1992), cross-linking (Wu et al., 2005; Coughlan and Corrigan, 2008) and the introduction of biodegradable hydrophilic and hydrophobic segments (Huang et al., 2004; Huang and Lowe, 2005; Bromberg, 1997; Ju et al., 2001).

Therefore a promising strategy for designing novel drug delivery systems is to employ polymers with the dual functionalities of thermoresponsiveness and degradability. In the present work, a series of thermoresponsive-co-biodegradable hydrogel networks were synthesised composed of PNPA and poly-lactic acid diacrylate macromer (PLAM) of varying molar ratios. While the synthesis of one such system was reported by Huang et al. (2004) their use for drug delivery was not evaluated. The rationale for incorporating PLAM into the three dimensional hydrogel networks arises from the hydrolytic degradation properties of PLA and its ability to control drug delivery (Fitzgerald and Corrigan, 1996). Furthermore the addition of the hydrophobic characteristics of PLAM could modify the lower critical solution temperature (LCST) and thus both the swelling properties and the mechanical strength of the device, leading to versatile release profiles and the incorporation of a wider

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doi:10.1016/j.ijpharm.2008.08.036

range of compounds. The chemical structures of the hydrogels were characterised using NMR, Fourier transform infrared (FTIR), and gel permeation chromatography (GPC). The influence of temperature on polymer swelling and degradation were investigated. Finally the controlled release of a model drug, indomethacin (IDM) was examined as a function of both hydrogel composition and temperature.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPA) (Aldrich, U.K.) was recrystallised from hexane and dried under vacuum at room temperature for 48 h. PLA (MW 2000) was purchased from Boehringer Ingelheim (England). *N*,*N*,*N*,*N* Tetramethylenediamine (TEMED); *N*,*N* methylene bicacrylamide (MBA); 2,2-azobis(2-methylpropionitrile) (AIBN); 2-aminoethanol, acryloyl chloride and triethylamine (ET_3N) were obtained from Aldrich (U.K.) and used as received. 1-Hydroxybenzotrazole (HOBt) was received from FLUKA (U.K.). Indomethacin was received from Sigma (U.K.). All chemicals were used as received unless otherwise stated.

2.2. Polymer preparation

Polymer synthesis consisted of three steps. Firstly PLA was chemically modified to enable incorporation of unsaturated vinyl groups, as described by Lowe et al. (2003). Briefly, PLA was modified to a diacrylate macromer by conversion of –COOH end group of PLA to –OH by coupling of 2-aminoethanol in the presence of a strong desolvating agent. These –OH groups were then converted to acrylate units using acryloyl chloride.

Copolymers were synthesised in a cylindrical tube (internal diameter 15 mm) in DMF at 70 °C by solution polymerisation for 4 h using TEMED (9% mol) as accelerator, AIBN (4% mol) as the initiator and MBA (4%mol) as the cross-linker. A range of copolymers were synthesised by varying the molar ratio of NIPA to PLAM. Details are given in Table 1, in which PNPL signifies the *N*-isopropylacrylamide–poly-lactide copolymer. L (linear) and C (cross-linked) signify systems prepared in the absence and in the presence of MBA, respectively. The resulting composites were washed in DMF and water, sliced into discs and cleaned by repeatedly swelling in water/ethanol (1:1) mixture and then dried in a vacuum oven at 50 °C for 48 h. The chemical structure was confirmed using NMR and FTIR.

Linear polymers were synthesised in a similar manner to the hydrogels but without the cross-linker (BMA). After the solution polymerisation process, the polymer was collected by precipitation in dichloromethane. The polymer was collected by filtration washed in hot water and then dissolved in cold water. This process was repeated several times to purify the polymer. The collected polymer was dried in a vacuum oven at 50 °C for 48 h.

2.3. Nuclear magnetic resonance

The chemical structures of the polymers were determined by NMR. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity spectrophotometer at 400.130 MHz. The ^{1}H NMR acquisitions were performed under the following parameter settings: SW 20.5524 ppm, TD 32,768 and TE 300.0 K. The sample concentration in the deuterated chloroform was ~25% (w/v). All chemical shifts were reported in parts per million (ppm). The central chloroform-d1 resonance was set at 77.23 ppm in the ^{13}C NMR analysis. The peak of chloroform-d1 at 7.27 ppm in the ^{1}H NMR analysis was used as the reference line.

2.4. Fourier transform infrared

FTIR spectra were recorded on a Nicolet Magna-IR 560 spectrometer. Spectra were obtained by averaging 64 scans in the spectral range 4000–400 cm^{-1}. PLA, PLA diol and PLA macromer were prepared by grinding a calculated amount of the sample (2 mg) with 220 mg of KBr in a mortar and pestle followed by compression in a 13-mm diameter punch and die set under 739 MPa for 5 min (Zhang et al., 2000).

FTIR spectra of the linear polymers were obtained by casting a thin film on zinc selenide (ZnSe) salt discs from a THF solution (1%, w/v) and further dried in a vacuum oven for 24 h at room temperature to remove any residual solvent (Huang et al., 2004).

FTIR spectra of the contents of the degradation medium after swelling studies were obtained using ZnSe salt plates (Specac Limited, U.K.). A sample of 0.1 ml of the media was applied to the plates. Plates were then dried under vacuum at room temperature for 24 h.

Discs composed of physical mixtures of the polymers were prepared as follows. PLA was dissolved in dichloromethane using sonification (ultra turex T25 mixer) at 8000 rpm for 5 min. PNPA was then dissolved in 5 ml of an acetone:dichloromethane mix (1:1). Both solutions were then combined using the ultra turex mixer for a further 5 min running at 8000 rpm. The solution was then poured onto a Teflon plate and evaporated under vacuum for 3 days. The resultant films were scraped off the plate, ground with a mortor and pestle and compressed into cylindrical 13 mm diameter discs (PerkinElmer punch and die set).

2.5. Gel permeation chromatography

The molecular weights of the linear polymer systems were determined by gel permeation chromatography. Samples were evaluated against a series of polystyrene standards (Aldrich). Solutions of the polymers were prepared in DMF and 50 μl was injected into the system consisting of a Waters Styragel® HR column, a Waters 510 pump and a Waters 410 Differential Refractometer (elution rate 1 ml/min). Millennium® 2010 software was employed to

Table 1
Comparison of properties of *N*-isopropylacrylamide–poly-lactic acid-based polymer networks (mean ± S.D.)

	Molar ratio	(w/w) ratio	Molecular weight ($\times 10^4$)	Glass transition temperature (°C)	Phase transition temperature (°C)
PNPA–L[a]	100	100	2.01 ± 0.20	111 ± 0.52	28.4 ± 0.20
PNPL–L1	93–7	84–16	1.52 ± 0.14	107 ± 0.46	28.5 ± 0.15
PNPL–L2	86–14	76–24	1.09 ± 0.08	90.6 ± 0.58	29.5 ± 0.25
PNPL–L3	72–28	56–44	0.87 ± 0.17	87.0 ± 0.97	32.5 ± 0.25
PNPA–C[a]	100	100	–	122 ± 1.23	28.4 ± 0.18
PNPL–C1	93–7	84–16	–	117 ± 0.84	29.1 ± 0.20
PNPL–C2	86–14	76–24	–	111 ± 0.72	30.3 ± 0.27
PNPL–C3	72–28	56–44	–	103 ± 0.54	31.5 ± 0.14

[a] L (linear) and C (cross-linked) signify systems composed in the absence and in the presence of MBA, respectively.

integrate the peaks. Samples were injected in triplicate and elution time compared with a calibration curve.

2.6. Acid–base titrations

Acid–base titrations were conducted on the linear polymers to confirm incorporation of lactic acid into the polymer backbone. Similarly acid base titrations were preformed on the degradation medium following swelling studies to calculate the amount of LA present (Caulfield et al., 2003; Chiellini et al., 2002) from $pH = -\log[H^+]$, where $[H^+]$ is the concentration of acid in the degradation medium.

2.7. Differential scanning calorimetry (DSC)

The LCST of the linear polymers (0.5%, w/v) was determined using an aqueous sample (30–40 mg by weight). The samples were run in a sealed aluminium crucible under a nitrogen purge at 2 °C/min unless otherwise stated. Samples were analysed in the range of 20–50 °C. The transition temperature has been defined in previous studies as either the temperature of onset (Otake et al., 1990) or the peak temperature (Eeckman et al., 2001). In the current work the phase transition temperature was defined as the maximum of the endothermic transition peak. Evaluation was preformed using STAR©software version 3.0. Each experiment was undertaken in triplicate.

The hydrogel networks were similarly analysed using a solid sample (30–40 mg).

2.8. Scanning electron microscopy (SEM)

The structure/morphology of the hydrogel systems were visualised using scanning electron microscopy (Coughlan and Corrigan, 2008). The freshly polymerised hydrogels were allowed to equilibrate for 48 h at specified temperature (20 °C and 37 °C) in an excess of phosphate buffer (PB) before flash freezing using liquid nitrogen SEM was also carried out on the hydrogel systems to investigate the morphological effect of degradation over time. The swollen hydrogels, after 2 months incubation in PB at 20 °C and 37 °C were removed from the media and immediately frozen in liquid nitrogen to retain the swollen structure. All samples were dried overnight using a Virtis Bench Top 6K Freeze Dryer. Samples were fractured, mounted on aluminium stubs and sputter coated with gold for examination using SEM (Hitachi S-35500) Coughlan and Corrigan, 2008).

Approximate pore size in the SEM scans were measured using Imagetool for windows® version 3.0. Twenty measurements were obtained at random on $n = 3$ SEM images (magnification, 1500×) (PNPL–C systems).

2.9. Swelling studies

Swelling studies were preformed in phosphate buffer 7.4 (isotonic) (Wade, 1980). To determine the swelling ratio the dry disc was placed in an excess of the swelling medium at a specific temperature and the disc was allowed to swell to equilibrium. Swelling studies over time were preformed in a similar manner. The swollen hydrogel was briefly removed from the medium at specific time intervals and blotted with filter paper to remove any excess of surface moisture before weighing as previously described by Coughlan et al. (2004). Experiments were undertaken in triplicate ($n = 3$).

The phase transition temperature (LCST) was determined by decreasing the temperature of the media at daily intervals by 5° to allow equilibrium swelling at the particular temperature. The LCST was defined as the temperature at which the hydrogel showed a significant increase in swelling from the baseline value.

In other experiments the pH of the medium was monitored (Olewnik et al., 2007). A decrease in pH signified degradation of the PLA component.

The swelling of the hydrogels was then examined using the swelling ratio equation (Gutowska et al., 1992):

$$\text{S.R.} = \frac{W_s - W_d}{W_d} \quad (1)$$

where W_s is the weight of hydrogel at time t and W_d the weight of the dry disc.

2.10. Drug release studies

Drug release studies were conducted using indomethacin as a model drug. The discs were loaded with 10% (w/w) drug by sorption from ethanol solutions over 24 h and then dried under vacuum at room temperature to remove solvent. Release experiments ($n = 3$) were carried out under sink conditions in 900 ml phosphate buffer solution pH 7.4 in a dissolution bath, Sotax AT7 (USP paddle method) at 50 rpm below (20 °C) and above (37 °C) the LCST. Drug release was analysed by UV spectroscopy at 318 nm.

2.11. Statistical methods

Statistical significance between two parameters was evaluated by an independent two-sample t-test (0.05 probability level) using Minitab® statistical software, version 13.32. SCIENTIST® for Windows™, version 2.1 (Micromath Inc.) was used to fit swelling and release profiles to model equations. The coefficient of determination (CD) provided a measure of the goodness of fit of the mathematical model described.

3. Results and discussion

3.1. Characterisation of polymers

Characterisation of PLA, PLA diol and PLA diacrylate macromer were carried out using FTIR spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR). The spectroscopy results were consistent with the successful synthesis of each step from the PLA to the acrylate macromer and were similar to those of Huang et al. (2004).

Both linear and cross-linked polymers were synthesised for comparison (Otake et al., 1990; Schild, 1992), using different molar ratios of *N*-isopropylacrylamide and PLAM. Their compositions are summarised in Table 1.

The IR spectra confirmed that the chemical structure of the hydrogels was consistent with that shown in Fig. 1. PNPA was observed in the IR spectra of all hydrogels (data not shown) by the presence of two absorption bands of the symmetric $-CH(CH_3)_2$ group at 1388 cm^{-1} and 1370 cm^{-1}. In the copolymeric systems additional absorption bands were present at 1760 cm^{-1} (CO stretching), 1215 cm^{-1} (C–O–C asymmetric stretching), 1200 cm^{-1} (O–CO stretching), 1117 cm^{-1} [CH(CH3)═O stretching] and 1050 cm^{-1} (C–OCO stretching), indicating successful incorporation of PLAM into the backbone of the hydrogel systems. The higher the amount of PLAM incorporated into the backbone of the hydrogels, the stronger the intensities of the observed peaks.

The FTIR spectra of the chemically modified PNPL–L2 and the physical mixture of PNPA and PLAM (86–14), manufactured by

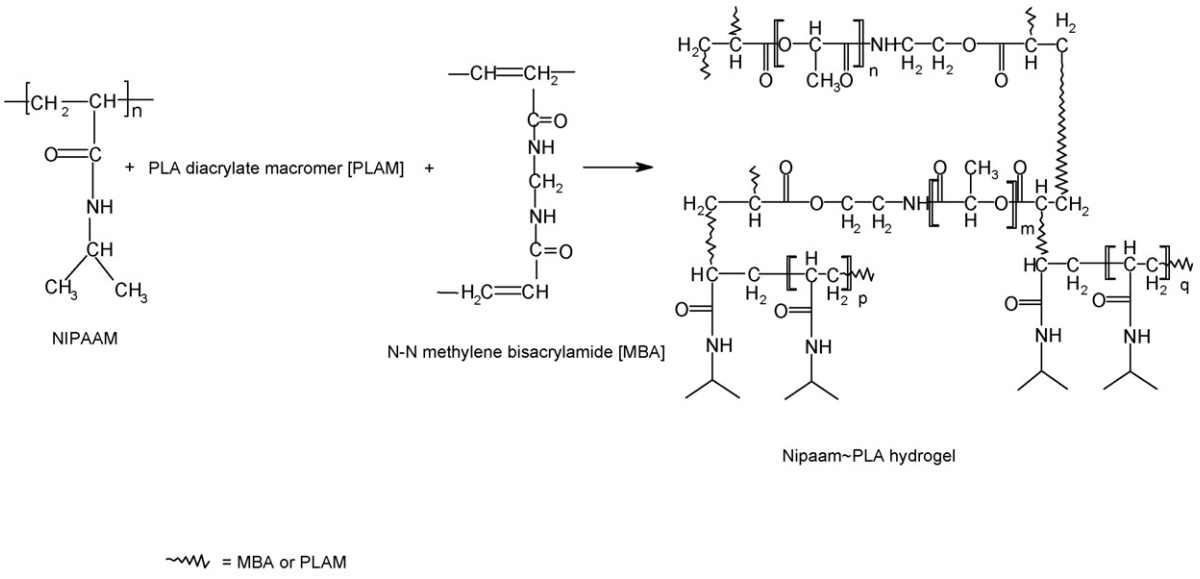

Fig. 1. Schematic diagram of reaction forming the cross-linked hydrogel networks, where ~~~ symbolizes the cross-linking agent; PLAM or MBA in the hydrogel networks.

solvent evaporation, were also examined. A decrease of the C=C bands at 1638, 1407 and 810 cm^{-1} in PNP2 after polymerisation of PLAM and NIPA were consisted with cross-linking. In the physical mixture, the double bonds are still present indicating PLAM was not chemically incorporated into the backbone of the hydrogel.

The ^{1}H NMR and ^{13}C NMR spectra of PNPA-L were consistent with the results obtained by Tokuhiro et al. (1991). The peak assignments of PLA diacrylate macromer and the PNPL–L2 polymer network, determined by comparison with the corresponding original polymers and from the literature, are listed below.

PLA diacrylate macromer ($CDCL_3$):

^{1}H NMR ($CDCl_3$): δ 6.70–5.75 (m, H9, H10 and H11); δ 5.19 (m, H1 and H5); δ 4.3 (m, H3); δ 3.7 (m, H8); δ 3.4 (m, H7); δ 1.5 (d, H2, H4 and H6).

^{13}C NMR ($CDCl_3$): δ 132.2 and 127.6 (CH=CH_2, C14 and C15, respectively); δ 169.18(–C=O, C9–C13); δ 68.55 (–CH, C1 and C5); δ 68.30 (–CH, C3); δ 62.3 (–CH_2, CH8); δ 42.6 (–CH_2, CH7); δ 16.19 (–CH_3, C2, C4 and C6).

The ^{1}H NMR of the PLA diacrylate macromer shows small peaks for the protons of the acrylate group in the region of δ 5.75–6.70 ppm that are not found in the diol indicating successful incorporation of the acrylate groups into the PLA acrylate macromer. In addition the ^{13}C NMR displayed chemical shifts at δ 127.6 ppm and δ 132.2 ppm, which are characteristic of two carbon atoms of a double bond.

PNPL–L2 ($CDCl_3$):

^{1}H NMR ($CDCl_3$): δ 1.14 (CH_3 on isopropyl group of PNPA), δ 1.50 (CH_2 on PNPA), δ 1.58 (CH_3 on PLAM), δ 1.91(−CH on PNPA), δ 3.97 (NH on PNPA), δ 5.16 (−CH on PLAM).

^{13}C NMR ($CDCl_3$): δ 16.12 (CH3 on PLAM), δ 22.5 (methyl carbon on the isopropyl group of PNPA), δ 68 (methylene carbon of PLAM), δ 169 (carboxyl carbon).

The ^{1}H NMR of PNPL–L2 showed a decrease in the proton signals from 5.6 to 6.2 (H_2C=CH–) indicative of the integration of PLAM into the backbone of the polymer. The double bonds (H_2C=CH–) in the ^{13}C NMR were also significantly decreased in the spectrum at 125 ppm and 130 ppm (data not shown). The absence of residual acid confirmed the successful incorporation of PLAM into the backbone of the polymer.

3.2. Thermoresponsive properties

The relationship between swelling ratio and temperature for the four systems is shown in Fig. 2. This thermoresponsive study demonstrated that the swelling ratios of all the hydrogels were dependent on hydrogel composition. A distinct phase transition around 30 °C was observed for PNPA–C, PNPL–C1 and PNPL–C2 polymeric systems. All the hydrogels at this temperature experienced a substantial volume collapse and became turbid and white. However in the case of PNPL–C3, there was a significant increase in the switch temperature. The degree of change in the equilibrium-swelling ratio with temperature became less sharp as the molar portion of PLAM increased.

The phase transition temperatures (LCSTs) of the PNIPAAM-co-PLAM gels, were determined in phosphate buffer 7.4 by DSC analysis (Table 1). A linear increase in the endotherm peak temperature, from 28.4 °C to 31.5 °C, was observed as the percent of PLAM increased. This increase in peak temperature suggests that PLAM increased the overall hydrophilicity of the systems as shown by the increase in the LCSTs. Schild (1992), in a review of NIPA polymers, discussed whether hydrogen bonding or hydrophobic effects influence the LCST. The increase in the LCST could be explained in terms of hydrogen bonding since ester groups along the PLAM chain are polar or weakly charged. This promotes polymer–water

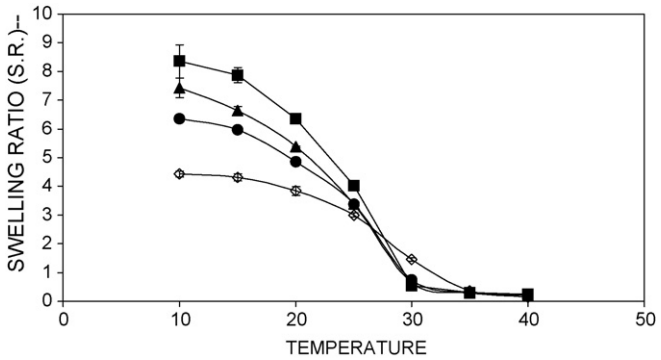

Fig. 2. Thermoresponsive properties of hydrogels (PNPA–C (■), PNPL–C1 (▲), PNPL–C2 (●), PNPL–C3 (◊) determined by measuring swelling ratios as a function of temperature from 10 to 50 °C in PBS solution.

Table 2
Swelling rate constant k_s ($S = k_s t^{0.5}$) (±S.D.) at 20 °C and 37 °C with the associated coefficient of determination (CD) for *N*-isopropylacrylamide (NIPA)–poly-lactic acid-based polymers

Polymer system	20 °C		37 °C	
	$k_{s20} \times 10^2$ ($min^{-0.5}$)	CD	$k_{s37} \times 10^2$ ($min^{-0.5}$)	CD
PNPA–C	17.4 ± 0.28	0.9893	1.34 ± 0.05	0.9840
PNPA–C1	19.2 ± 0.38	0.9843	1.88 ± 0.04	0.9843
PNPA–C2	14.9 ± 0.23	0.9899	2.52 ± 0.05	0.9779
PNPA–C3	14.6 ± 0.32	0.9815	3.47 ± 0.09	0.9922

interactions rather than polymer–polymer interactions, leading to a reduced driving force for phase transition and an increase in the LCST (Inoue et al., 1997; Sousa et al., 2005; Feil et al., 1992, 1993; Huglin et al., 1997; Beltran et al., 1991; Peppas and Khare, 1993). Han et al., 2003 showed an increase in the LCST with increasing the molar ratio of the cross-linker tetra ethylene glycol dimethylacrylate. This was attributed to a combination of increased hydrophilicity of the hydrogel and an increased stiffness of the hydrogel, which may prevent hydrophobic interactions. An apparent increase in the LCSTs with the introduction of MBA into the network was seen in the present study (Table 1), while the higher the PLAM content the larger the increase in the LCST also.

The effect of the molecular weight on the LCST in aqueous solution has been explored by numerous authors (Furyk et al., 2006). Altering the molar ratio of reactants in the current work, led to a significant difference ($p < 0.05$) in molecular weight of the batches of polymers (Table 1). There was an observed decrease in molecular weight as the amount of PLAM increased. The current work is also consistent with the finding that an increase in the MW leads to a decrease in the LCST of thermoresponsive polymers.

The equilibrium-swelling ratio of the hydrogel systems was investigated below and above the LCST at 20 °C and 37 °C, respectively. At 20 °C, the hydrogels expanded from 5.8 to 8 times their volume depending on the amount of PLAM incorporated into the backbone of the polymer network. As the PLAM component increased a more compact structure and suppression of swelling was observed. Increasing the amount of hydrophobic PLAM decreased the swelling ratio linearly ($R^2 = 0.923$).

In contrast above the LCST (37 °C), due to the hydrophobic nature of PNIPAAM, the equilibrium-swelling ratio was significantly smaller than at 20 °C. The magnitude of swelling change between the two temperatures decreased with increasing the percent of PLAM. The incorporation of PLAM therefore influenced the equilibrium swelling at both temperatures. An upward trend was seen in the swelling ratio with increasing PLAM content at 37 °C.

3.3. Swelling kinetics of hydrogels

The incorporation of different percentages of PLAM not only influenced equilibrium swelling at a particular temperature, but also affected the rate of swelling at that temperature. The swelling ratios (S) versus time (t) profiles of the hydrogel discs were examined at 20 °C and 37 °C (Fig. 3). At both temperatures the swelling profiles, up to 60% of equilibrium swelling, were found to be approximately proportional to the square root of time ($S = k_s t^{0.5}$) where k_s is a constant (Table 2) (Crank, 1975; Coughlan et al., 2004; Coughlan and Corrigan, 2008). The rate constants ranged from 13- to 4-fold higher at the lower temperature, the difference decreasing as the PLAM content increased, as was the case with the equilibrium swelling. However at 20 °C the swelling rate was greater for PNPL–C1 than for PNPA, while further addition of PLAM led to a decrease in the rate of swelling, probably reflecting the hydrophobic nature of PLAM. Previous studies have also shown that swelling decreased with an increase in the incorporation of a hydrophobic moiety (Zhang et al., 1999; Inoue et al., 1997; Lee and Yeh, 2005). All hydrogels with PLAM incorporated into the backbone continued to take up water at slow rates without reaching a true equilibrium, consistent with a gradual degradation of the PLAM present in the backbone of the hydrogel network.

Above the LCST (37 °C), the extent of swelling was considerably less than below the LCST. The PNPA gel had reached equilibrium within 4 h. However the PNPL gels swelled to a greater extent, the degree of swelling increasing as the proportion of PLAM increased. These swelling profiles were more sustained. Similar observations have been reported by Huang et al. (2004) for thermoresponsive-co-biodegradable triblock polymers consisting of PNPA, PLAM and dextran. The slow extended sluggish swelling was attributed to an increase in the hydrophilicity of the networks as PLAM underwent hydrolytic degradation.

3.4. Evidence for polymer degradation

At 20 °C, below the LCST, the PNPL systems displayed a continual uptake in water for the first 15 days of immersion in PB. The higher the PLAM content the lower the increase in the swelling ratio in the 1–2 days swelling period. After a period of 1–2 months a decline in the swelling ratio was observed (not shown), the effect being more pronounced the greater the amount of PLA macromer incorporated into the hydrogel network. Since hydrogels are highly hydrophilic at this temperature, abundant water could easily access and hydrolyse the ester bonds. Thus it was expected that ongoing cleavage of these cross-links within the gel would systematically decrease the cross-linking density thereby increasing the swelling ratio. After a period the network cannot sustain itself and a decline in mass is observed. The percent polymer mass loss after 2 months at both temperatures is given in Table 3.

Interestingly PNPA also showed a decline in mass (2% above and 5% below the LCST) after 2 months. Previous studies by Torchilin et al. (1977) noted slow degradation of cross-linked polyvinylpyrrolidone (PVP) hydrogels by hydrolysis of the cross-linker (BIS) and degradation was sensitive to the concentration BIS employed.

In contrast at 37 °C, above the LCST, the degradation of the four gels was much slower than at 20 °C. In the case of PNPL–C2 and PNPL–C3, a slow gradual uptake of media was seen after 10 days. This was followed by a decline in the swelling ratio. No significant difference over a 2-month period was observed for PNPA–C or PNPL–C1 polymer systems at this temperature. PEG/PLA polymeric systems have previously shown mass loss from chemically cross-linked networks (Metters et al., 1999).

At the end of the swelling studies (60 days), the gels were removed, reweighed and dried. Mass losses of 19.8%, 28.2% and 37.0% (w/w) were observed for PNPL–C1, PNPL–C2 and PNPL–C3, respectively at 20 °C (Table 3). At 37 °C, a slower rate of degradation led to mass losses of 9.71%, 18.2% and 26.4% for PNPL–C1 and PNPL–C2 and PNPL–C3, respectively. The molar ratios (weight) used to synthesise the gels are presented in Table 1 (PNPL–C1 (84–16, w/w), PNPL–C2 (76–24, w/w), and PNPL–C3 (56–44, w/w)). A greater mass loss was observed below the LCST than the weight of PLAM present in PNPL–C1 and PNPL–C2 systems indicating that PNPA chains are also being cleaved in the degradation process. Huang et al. (2004) previously reported an increase in the rate of degradation below the LCST with respect to that above the LCST. This phenomenon has been attributed to an increase in the water uptake, thus leading to an increase in the rate of hydrolytic degradation and indicating that temperature can affect degradable hydrogels in a complex manner; directly through degradation kinetics and indirectly through the swelling behaviour.

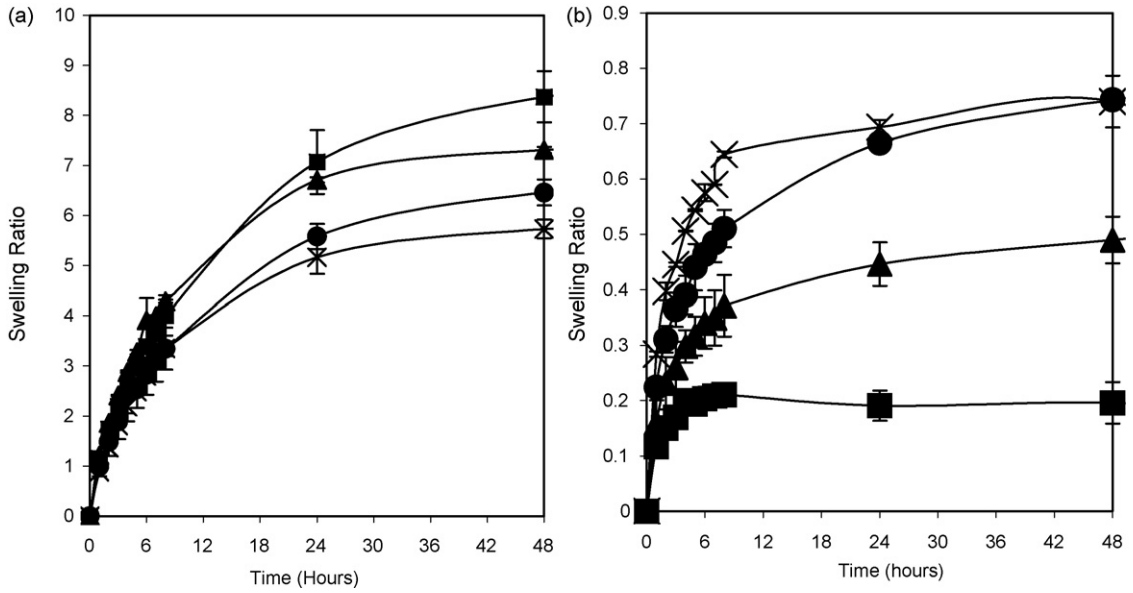

Fig. 3. Swelling kinetics of PNPA–C (■), PNPL–C1 (▲), PNPL–C2 (●) and PNPL–C3 (×) over time at (a) 20 °C and (b) 37 °C in PB.

Table 3
The [COOH] present in the degradation media and the mass loss observed after 2 months for each hydrogel system both below (20 °C) and above (37 °C) the LCST. In addition the ratio of the [COOH] is displayed between systems on changing the temperature

Gel	[COOH] in degradation media of gels at 20 °C [mol/L] (±S.D.)	[COOH] in degradation media of gels at 37 °C [mol/L] (±S.D.)	$[COOH]_{20°C}/[COOH]_{37°C}$	% Mass loss at 20 °C (±S.D.)	% Mass loss at 37 °C (±S.D.)
PNPL–C11	0.0195 ± 0.086	0.0066 ± 0.0026	2.95	19.8 ± 2.5	9.71 ± 3.0
PNPL–C2	0.0291 ± 0.097	0.0141 ± 0.0015	2.06	28.2 ± 1.5	18.2 ± 5.2
PNPL–C3	0.0333 ± 0.099	0.0191 ± 0.0018	1.74	37.0 ± 4.2	26.4 ± 4.7

The pH of the media was also monitored during swelling studies of the gels at 20 °C. PLA polymers degrade in aqueous media to acidic oligomers and ultimately lactic acid. Fig. 4 shows the change in pH of each of the hydrogel systems as a function of time, the decrease in pH is consistent with degradation of PLAM. The higher the percent of PLAM in the polymer disc, the greater the drop in pH over time. Simultaneously swelling increased as the pH decreased indicating disruption to the porous network, leading to a higher degree of porosity. The concentration of liberated acid in the aqueous medium was calculated as a function of time. The early time release of LA into the swelling media increased, the larger the PLAM content of the gel. The limiting rates of degradation of each system approximated zero order kinetics, giving values of 0.27 mg/day, 0.33 mg/day, and 0.37 mg/day and R^2 values of 0.9577, 0.9482 and 0.9011 for PNPL–C1, PNPL–C2 and PNPL–C3, respectively.

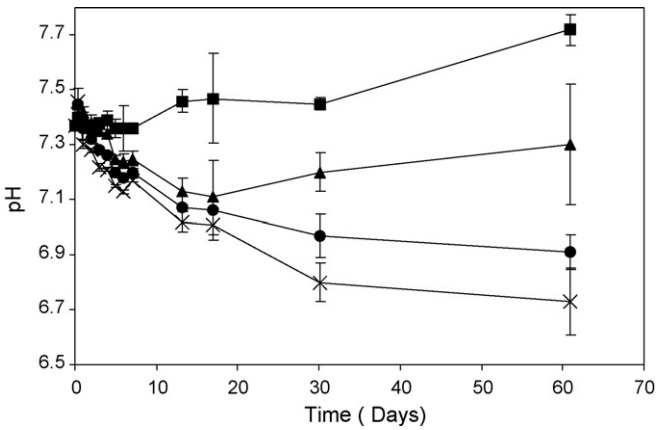

Fig. 4. (a): Degradation behaviour of hydrogels series: PNPA–C (■), PNPL–C1 (▲), PNPL–C2 (●), and PNPL–C3 (×) as a function of pH at 20 °C.

SEM analysis provided information on the morphology of the polymers both before after degradation. While freeze-drying under vacuum may affect the shrinkage of the pores to some degree, all polymeric systems (before and after degradation) were investigated under the same sets of conditions so the relative trends observed are relevant and reflect the effect of composition on pore morphology. Freshly made polymeric systems, which had been allowed to equilibrate, below the LCST, for 48 h and the corresponding degraded polymeric systems are shown in Fig. 5. In the case of the non-hydrolysed polymer samples, the influence of the hydrophobic PLAM below the LCST on the pore size can be seen in the SEMs in Fig. 5(i) where at similar magnification (500×), differences in morphology, induced by PLAM were evident. A trend towards a decrease in pore size with increasing the percent of PLAM was observed. PNPA–C swells to a greater extent at this temperature than all other hydrogels (Fig. 2) resulting in a greater pore size.

The effect of incorporating PLAM on the morphology after 2 months is shown in Fig. 5(ii). The higher the amount of PLAM present, the greater the porosity and loss in the structural integrity within the hydrogel, consistent with hydrolytic degradation of PLAM resulting in larger pore size. Similarly a greater difference is seen between the systems of higher PLAM content when comparing SEMs performed before and after degradation, indicating the hydrolytic cleavage of PLAM.

The hydrogel macropore size estimates determined by SEM, before and after 2 months degradation are summarised in Table 4. A trend is evident indicating that the higher the content of PLAM

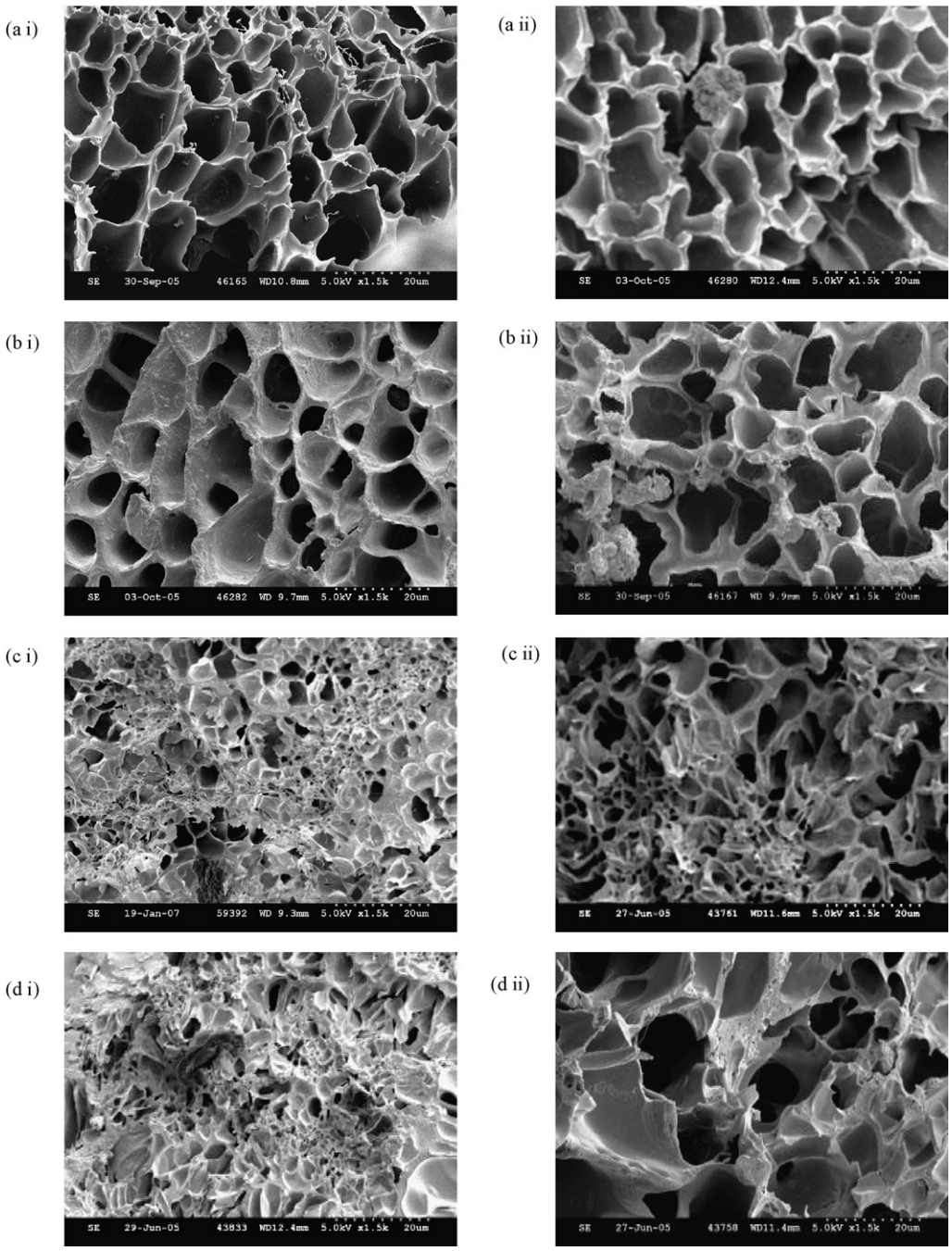

Fig. 5. SEM images (500×) of (a) PNPA–C, (b) PNPL–C1 (c) PNPL–C2 and (d) PNPL–C3 before (i) and after (ii) degradation studies undertaken at 20 °C.

the larger the pore size as a result of hydrolytic degradation. The results are in rank order with the pH studies. An increase in the pore size after degradation was observed, the greater the percent PLAM incorporated into the hydrogel systems.

FTIR analysis was also conducted on the swelling medium of the hydrogel systems in order to detect the possible presence of lactic acid and/or PNPA fragments in the swelling medium reflecting hydrolysis of the copolymers. At 20 °C, evidence for the presence

Table 4
Calculated pore size (SEM average diameter) of *N*-isopropylacrylamide (NIPA)–poly-lactic acid (PLA)-based polymers before and after degradation at 20 °C

Hydrogel	Molar PLA (%)	Observed pore size (μm) before degradation (±S.D.)	Observed pore size (μm) after 60 days (±S.D.)	Change in pore size due to degradation
PNPA–C	0	8.52 ± 1.70	9.80 ± 1.05	1.28
PNPL–C1	7	5.79 ± 1.01	7.71 ± 1.58	1.92
PNPL–C2	14	3.94 ± 0.98	6.19 ± 1.16	2.25
PNPL–C3	28	2.82 ± 0.68	8.91 ± 2.46	6.09

of PNPA was detected in all PB media solutions by the presence of the amide 1 and 2 bands at 1670 cm^{-1} and 1540 cm^{-1}, respectively. Similarly peaks at 1388 cm^{-1} and 1371 cm^{-1} were characteristic of the isopropyl groups. Thus it is possible that PLAM undergoes hydrolysis, leading to scission of NIPAAM units in the copolymeric systems and their release into the PBS solution. PNPA also revealed NIPAAM fragments in the degradation medium, which could be the result of the cross-linker degrading (Torchilin et al., 1977), consistent with the mass loss observed above.

For the two component hydrogels, PNPL–C1, PNPL–C2 and PNPL–C3, the C=O stretching band at 1720 cm^{-1} was detected for all systems suggesting the presence of the carboxylic acid in the aqueous medium and indicative of hydrolytic cleavage of the ester bond of the PLAM component in the three dimensional hydrogel systems. As the percent of PLAM incorporated into the hydrogel backbone increased the peak intensity became stronger at 1720 cm^{-1}. Similarly IR peaks at 1638 cm^{-1} and 1411 cm^{-1} were attributed to the C–O asymmetric stretching and C–O symmetric stretching of the carboxylate, respectively. They were stronger in intensity as the ratio of PLAM incorporated into the backbone increased. The IR peak 1160 cm^{-1} (O–CO stretching) was weakly present in the 85–15 PNPL system and increased in intensity the more PLAM present. An extra peak at 1117 cm^{-1} ($CH(CH_3)$=O stretching) also was apparent in the media of the polymeric systems with higher amounts of PLAM present.

At 37 °C, the presence of PNPA within the degradation media was not clearly visible, however overlapping of PLA and PNPA is possible in the lower region of the spectrum (1100–600 cm^{-1}). The spectra showed three strong absorption bands at 1080 cm^{-1}, 988 cm^{-1}, and 863 cm^{-1} which can be assigned to the –CO– stretch, the –C–C– stretch and the –C–COO– stretching of PLA, respectively (Kister et al., 1995). The collapsed hydrophobic state of the gel at this temperature may lead to physical entrapment of PNPA within the hydrogel once the bonds have been cleaved. The absorption spectrum of the swelling medium is characteristic of that of PLA with the –CH– deformation bands visible in the region of 1450–1350 cm^{-1} and the carboxylic group in the region of 1800–1700 cm^{-1}. The increase in absorption was noted in all systems of the gels at 20 °C consistent with degradation occurs faster below the LCST.

After 2 months acid base titrations were preformed on the degradation media to calculate the concentration of acid present. The results are summarised in Table 3. A trend is evident whereby the higher the percent of PLAM incorporated into the gel, the faster degradation occurred. Also degradation was faster below the LCST due to the hydrophilic state of the polymer. The effect of the temperature on the rate of hydrolysis can be seen where a 2.9-, 2.0- and 1.7-fold increase in the amount of PLA in the degradation medium was present at 20 °C for PNPL–C1, PNPL–C2 and PNPL–C3, respectively relative to the amount at 37 °C (Table 3). At 37 °C, a combined effect of a larger swelling ratio and increasing PLAM content in the gel led to a decrease in the $[COOH]_{20°C}/[COOH]_{37°C}$ ratio with increasing PLAM%. These results indicate that both temperature and water absorption are important in modifying degradation behaviour.

The FTIR study and acid–base titrations supported the swelling and mass loss studies indicating temperature can affect degradable hydrogels in a complex manner (Table 3). The impact of network structure and comonomer chemistry on the macroscopic physical properties plays an important role during degradation.

3.5. IDM release from PNPL copolymers

The release of IDM from the four PNPL systems was investigated above (37 °C) and below (20 °C) the LCST and the fraction released over time at both temperatures is shown in Fig. 6. Con-

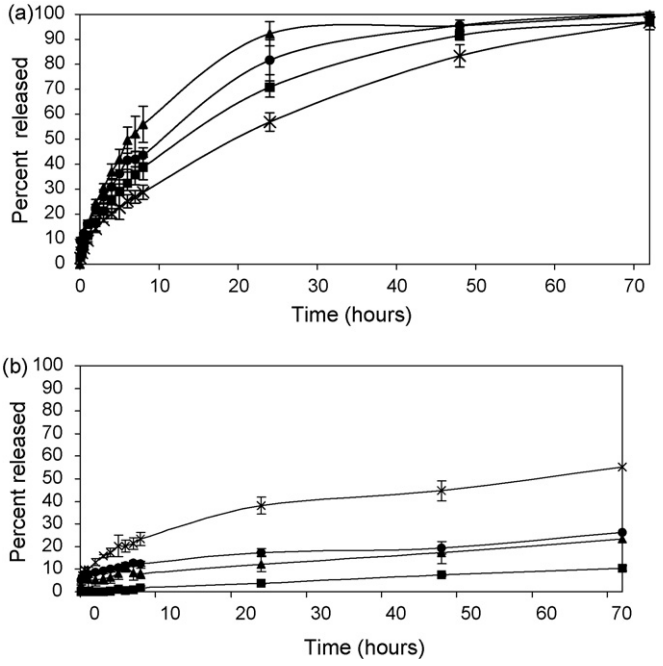

Fig. 6. Fraction released over time of IDM from hydrogels containing various percentage of PLAM at (a) 20 °C and (b) 37 °C [PNPA–C (■), PNPL–C1 (▲), PNPL–C2 (●) and PNPL–C3 (×)].

trolled release of IDM was achieved at both temperatures with the reduced swelling at 37 °C significantly slowing the release of the drug. In order to explore the drug release mechanism, the profiles were fitted to the following equation

$$F = k_{dh} t^{0.5} \quad (2)$$

where F is the fraction of drug released at time t and k_{dh} is the Higuchi release rate constant for matrix diffusion controlled release (Higuchi, 1961, 1962). The data were also fitted to the following equation

$$F = k_{dp} t^{n} \quad (3)$$

where k_{dp} is the release rate constant and n is the diffusional-release exponent (Ritger and Peppas, 1987). The parameter estimates are shown in Table 5.

At 37 °C, above the LCST, the release rate of IDM from the hydrogels increased as the percent of PLAM increased, consistent with the pattern of swelling above the LCST and suggesting less resistance to diffusion with increased swelling. Drug release was incomplete over the study period. This is a result of the collapsed state of the gel at 37 °C whereby the drug is largely trapped within the polymer network, therefore limiting drug release at this temperature. The morphological change as a result of polymer degradation also suggest that the release of entrapped drug was agumented by the polymer degradation, the effect being greater the greater the PLAM content. Polymer degradation leads to a greater mesh size and an increase in solute diffusion, which in turn would lead to a reduction in any polymer–drug interactions. Hydrophobic binding between model drugs and PNIPAAM has previously been reported (Coughlan and Corrigan, 2006). PNPLC-3 had released ~50% after 3 days which can be attributed to the faster and more extensive formation of a loose 3D-network structure in this PNPL gel. After 3 days, drug release from the remaining copolymer systems had levelled off at ~10–20%, depending on composition.

In contrast at 20 °C drug release was much faster and complete release was obtained within the 3-day study period. The drug

Table 5
Release rate constants (k_{dh}) of IDM from various hydrogels at 20 °C and 37 °C calculated using Eq. (2). The diffusional exponent and release rate constants (k_{dp}) were also estimated using Eq. (3). Also shown are the associated CD.

20 °C	$F = k_{dh}{}^{0.5}$		$F = k_{dp}t^n$			
	k_{dh} ($min^{-0.5}$) (×10^2)	CD	k_{dp} (min^{-n}) (×10)	$n \pm$ S.D.	CD	k_{dh20}:k_{dh37}
PNPA–C	1.36 ± 0.12	0.6693	0.946 ± 0.03	0.601 ± 0.005	0.9997	5.0:1
PNPL–C1	2.53 ± 0.04	0.9742	1.602 ± 0.22	0.581 ± 0.065	0.9935	8.3:1
PNPL–C2	2.11 ± 0.05	0.9515	2.653 ± 0.43	0.460 ± 0.055	0.9920	3.3:1
PNPL–C3	1.39 ± 0.15	0.7594	0.868 ± 0.05	0.575 ± 0.037	0.9984	1.5:1
37 °C	k_{dh} ($min^{-0.5}$) (×10^2)	CD	k_{dp} (min^{-n}) (×10)	$n \pm$ S.D.	CD	
PNPA–C	0.272 ± 0.06	0.4724	0.005 ± .0002	0.897 ± 0.244	0.9913	
PNPL–C1	0.304 ± 0.04	0.8155	1.091 ± 0.27	0.353 ± 0.090	0.9571	
PNPL–C2	0.408 ± 0.08	0.5968	2.115 ± 0.25	0.278 ± 0.017	0.9815	
PNPL–C3	0.912 ± 0.03	0.9375	2.161 ± 0.17	0.385 ± 0.010	0.9967	

release rate from PNPL–C1 was larger than that of PNPA, but on further increasing the PLAM content the drug release rate declined. This trend is the same as that observed for polymer swelling rate (Table 2) and underlines the importance of swelling rate in determining drug release below the LCST.

The ability to thermally control the release of the low molecular weight model drug, IDM, was most successful with PNPL–C1 and the least thermal control was observed with PNPL–C3. The ratio of the release rate constants (k_{dh}) at 20 °C and 37 °C (k_{dh20}:k_{dh37}), indicative of the ability of the gel to thermally control the release of drug, were 5, 8.3, 3.3, and 1.5 for PNPA, PNPL–C1, PNPL–C2, and PNPL–C3, respectively (Table 5). The limited ability to thermally control the release of IDM from PNPL–C3 can be attributed to a smaller net difference in the swelling kinetics between the two temperatures.

Zhang and Chu (2002) also observed relatively slower release of IDM from PLAM, which they attributed to hydrophobic interactions and slow pore formation. Inoue et al. (1997) also found that IDM release rate decreased as the concentration of hydrophobic oligomers of methyl methacrylate (OMMA) increased in poly acrylic acid (PAAc) hydrogels. This was attributed to increased hydrophobic interactions with increasing hydrophobic content. Coughlan and Corrigan (2008) investigated the release of BA from a series of poly (*N*-isopropylacrylamide) matrices with various percentages of cross-linker. The presence of BA strongly influenced the swelling rate and therefore the effective mesh size of the hydrogel systems. The resulting release rates from the BA-loaded hydrogels were of a similar order of magnitude and proportional to the square root of time.

As expected the data gave a better fit when the diffusional coefficient was allowed to vary, using Eq. (3) (Table 5). At 20 °C the PNPL copolymer systems gave values of n close to 0.5 (range 0.46–0.58). Similar values were reported for benzoic acid release from a series of PNPA polymers, with increasing cross-linker content (Coughlan and Corrigan, 2008). At 37 °C the n values for the PNPL copolymers were <0.5 (range 0.28–0.39) reflecting the incomplete release of entrapped drug.

The pore morphology present in the polymer matrix will influence porosity and tortuosity and hence release rate. A plot of the relationship between the pore size and release rate from the hydrogel series revealed that a larger mesh size/swelling ratio facilitates a faster drug release rate. The importance of polymer composition, such as cross-linking content, in controlling the mesh/pore size and therefore the rate of drug release from other thermoresponsive systems has been previously demonstrated (Canal and Peppas, 1989; AmEnde et al., 1995).

4. Conclusions

A series of thermoresponsive-co-biodegradable hydrogel systems have been prepared and characterised comprised of PNIPA as a thermoresponsive unit and PLAM as a hydrolytically degradable hydrophobic unit. Thermal analysis revealed an increase in the lower critical solution temperature as the percent of PLAM increased. Similarly the phase transition became less sharp as the molar ratio of NIPAAM decreased and the proportion of PLAM increased.

All hydrogels swelled to a greater extent at 20 °C than at 37 °C and their dynamic swelling profiles were significantly different at these two temperatures. Swelling kinetics below the LCST were dependent on the amount of hydrophobic PLAM present. Above the LCST, the higher the PLAM content the greater the extent of swelling.

The hydrogels containing PLAM were hydrolytically degradable due to the cleavage of the ester bond into the carboxylic acid. Acid release and FTIR analysis indicated that the rate of degradation was faster the higher the percent of PLAM present. Also SEM analysis revealed a significant increase in porosity following degradation, the effect increasing the higher the molar ratio of PLAM. Hydrolytic degradation of the polymer was also faster at 20 °C for all the PNPL systems due to the high hydrophilicity and swelling of the networks at that temperature.

The release of IDM was much greater below the LCST, the limited swelling at 37 °C significantly slowing the release of the drug. At 37 °C the release rate of IDM from the hydrogels increased as the percent of PLAM in the hydrogel increased, consistent with the pattern of swelling above the LCST and suggesting less resistance to diffusion with increased swelling. In addition morphological change as a result of polymer degradation also led to the slow release of entrapped drug, the effect being greater the greater the PLAM content. Polymer degradation also lead to a greater mesh size and an increase in solute diffusion. In contrast at 20 °C drug release was much faster, complete release was obtained and the drug release rate followed the same trend as that observed for polymer swelling rate underling the importance of swelling rate in determining drug release below the LCST. Higher swelling resulted in a larger mesh size, which facilitates a faster drug release rate.

Acknowledgements

The authors wish to thank John O'Brian, Department of Chemistry, Trinity College Dublin for his technical assistance in Nuclear magnetic resonance, and Neal Leddy for his assistance in scanning

electron microscopy, Centre for Microscopy and Analysis, Trinity College Dublin.

References

AmEnde, M.T., Hariharan, D., Peppas, N.A., 1995. Factors influencing drug and protein transport and release from ionic hydrogels. React. Polym. 25, 127–137.

Beltran, S., Baker, J.P., Hooper, H.H., Blanch, H.W., Prausnitz, J.M., 1991. Swelling equilibria for weakly ionizable, temperature-sensitive hydrogels. Macromolecules 24, 549–551.

Bromberg, L., 1997. Zein–poly(*N*-isopropylacrylamide) conjugates. J. Phys. Chem. B 101, 504–507.

Canal, T., Peppas, N.A., 1989. Correlation between mesh size and equilibrium degree of swelling of polymeric networks. J. Biomed. Mater. Res. 23, 1183–1193.

Crank, J., 1975. The Mathematics of Diffusion, 2nd edition. Clarendon Press, Oxford.

Caulfield, M.J., Hao, X., Qiao, G.G., Solomon, D.H., 2003. Degradation on polyacrylamides. Part I. Linear polyacrylamide. Polymer 44, 1331–1337.

Chiellini, F., Petrucci, F., Ranucci, E., Solaro, R., 2002. Bioerodible hydrogels based on 2-hydroxyethyl methacrylate: synthesis and characterization. J. Appl. Polym. Sci. 85, 2729–2741.

Coughlan, D.C., Corrigan, O.I., 2006. Drug–polymer interactions and their effect on thermoresponsive poly(*N*-isopropylacrylamide) drug delivery systems. Int. J. Pharm. 313, 163–174.

Coughlan, D.C., Corrigan, O.I., 2008. Release kinetics of benzoic acid and its sodium salt from a sereis of poly(*N*-isopropylacrylamide) matrices with various percentage crosslinking. J. Pharm. Sci. 97, 318–330.

Coughlan, D.C., Quilty, F.P., Corrigan, O.I., 2004. Effect of drug physicochemical properties on swelling/deswelling kinetics and pulsatile drug release from thermoresponsive poly(*N*-isopropylacrylamide) hydrogels. J. Control. Release 98, 97–114.

Dong, L.C., Hoffman, A.S., 1990. Synthesis and application of thermally reversible hetrogels for drug delivery. J. Control. Release 13, 21–31.

Eeckman, F., Amighi, K., Moes, A.J., 2001. Effect of some physiological and non-physiological compounds on the phase transition temperature of thermoresponsive polymers intended for oral controlled-drug delivery. Int. J. Pharm. 222, 259–270.

Feil, H., Bae, Y.H., Feijen, J., Kim, S.W., 1992. Mutual influence of pH and temperature on the swelling of ionizable and thermosensitive hydrogels. Macromolecules 25, 5528–5530.

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

Fitzgerald, J.F., Corrigan, O.I., 1996. Investigation of the mechanisms governing the release of levamisole from poly-lactide-co-glycolide delivery systems. J. Control. Release 42, 125–132.

Furyk, S., Zhang, Y., Ortiz-Acosta, D., Cremer, P.S., Bergbreiter, D.E., 2006. Effects of end group polarity and molecular weight on the lower critical solution temperature of poly(*N*-isopropylacrylamide). J. Polym. Sci. A: Polym. Chem. 44, 1492–1501.

Gutowska, A., Bae, Y.H., Feijen, J., Kim, S.W., 1992. Heparin release from thermosensitive hydrogels. J. Control. Release 22, 95–104.

Han, M.-H., Kim, J.-W., Kim, J., Ko, J.Y., Magda, J.J., Han, I.S., 2003. Temperature-dependent transparency of poly(HPMA-co-DMA) hydrogels: effect of synthesis parameters. Polymer 44, 4541–4546.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50, 874–875.

Higuchi, W.I., 1962. Analysis of data on the medicament release from ointments. J. Pharm. Sci. 51, 802–804.

Hoffman, A.S., 1987. Applications of thermally reversible polymers and hydrogels in therapeutics and diagnostics. J. Control. Release 6, 297–305.

Huang, X., Lowe, T.L., 2005. Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs. Biomacromolecules 6, 2131–2139.

Huang, X., Nayak, B.R., Lowe, T.L., 2004. Synthesis and characterization of novel thermoresponsive-co-biodegradable hydrogels composed of *N*-isopropylacrylamide, poly(L-lactic acid), and dextran. J. Polym. Sci. A: Polym. Chem. 42, 5054–5066.

Huglin, M.B., Liu, Y., Velada, J., 1997. Thermoreversible swelling behaviour of hydrogels based on *N*-isopropylacrylamide with acidic comonomers. Polymer 38, 5785–5791.

Inoue, T., Chen, G., Nakamae, K., Hoffman, A.S., 1997. A hydrophobically-modified bioadhesive polyelectrolyte hydrogel for drug delivery. J. Control. Release 49, 167–176.

Ju, H.K., Kim, S.Y., Lee, Y.M., 2001. pH/temperature-responsive behaviors of semi-IPN and comb-type graft hydrogels composed of alginate and poly(*N*-isopropylacrylamide). Polymer 42, 6851–6857.

Kister, G., Cassanas, G., Vert, M., Pauvert, B., Térol, A., 1995. Vibrational analysis of poly(L-lactic acid). J. Raman Spectrosc. 26, 307–311.

Kost, J., Langer, R., 1991. Responsive polymeric delivery systems. Adv. Drug Deliv. Rev. 6, 19–50.

Lee, W.-F., Yeh, Y.-C., 2005. Studies on preparation and properties of NIPAAm/hydrophobic monomer copolymeric hydrogels. Eur. Polym. J. 41, 2488–2495.

Lowe, T.L., Nayak, B.R., Huang, X., 2003. Engineering biodegradable-co-responsive hydrogels for drug delivery. Proc. Int. Symp. Control. Rel. Bioact. Mater. 30, 707–708.

Metters, A.T., Anseth, K.S., Bowman, C.N., 1999. Annual International Conference of the IEEE Engineering in Medicine and Biology – Proceedings 2, pp. 727.

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

Olewnik, E., Czerwinski, W., Nowaczyk, J., 2007. Hydrolytic degradation of copolymers based on L-lactic acid and bis-2-hydroxyethyl terephthalate. Polym. Degrad. Stab. 92, 24–31.

Otake, K., Inomata, H., Konno, M., Saito, S., 1990. Thermal analysis of the volume phase transition with *N*-isopropylacrylamide gels. Macromolecules 23, 283–289.

Peppas, N.A., Khare, A.R., 1993. Preparation, structure and diffusional behavior of hydrogels in controlled release. Adv. Drug Deliv. Rev. 11, 1–35.

Perez, P., Gallardo, A., Corrigan, O.I., San Roman, J., 2008. Thermosensitivity of *N*-isopropylacrylamide hydrogels cross-linked with degradable cross-linker. J. Biomater. Sci. Polym. Edn. 19, 769–783.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Control. Release 5, 37–42.

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

Sousa, R.G., Prior-Cabanillas, A., Quijada-Garrido, I., Barrales-Rienda, J.M., 2005. Dependence of copolymer composition, swelling history, and drug concentration on the loading of diltiazem hydrochloride (DIL.HCl) into poly[(*N*-isopropylacrylamide)-co-(methacrylic acid)] hydrogels and its release behaviour from hydrogel slabs. J. Control. Release 102, 595–606.

Tokuhiro, T., Amiya, T., Mamada, A., Tanaka, T., 1991. NMR study of poly(*N*-isopropylacrylamide) gels near phase transition. Macromolecules 24, 2936–2943.

Torchilin, V.P., Tischenko, E.G., Smirnov, V.N., Chazov, E.I., 1977. Immobilization of enzymes on slowly soluble carriers. J. Biomed. Mater. Res. 11, 223–235.

Wade, A. (Ed.), 1980. Pharmaceutical Handbook, Society of Great Britain. The Pharmaceutical press, London, U.K..

Wu, J., Liu, S.Q., Heng, P.W.S., Yang, Y.Y., 2005. Evaluating protein release from and their interactions with, thermosensitive poly(*N*-isopropylacrylamide) hydrogels. J. Control. Release 102, 361–372.

Yoshida, R., Sakai, K., Okano, T., Sakurai, Y., 1993. Pulsatile drug delivery systems using hydrogels. Adv. Drug Deliv. Rev. 11, 85–108.

Zhang, Y., Chu, C.-C., 2002. In vitro release behaviour of insulin from biodegradable hybrid hydrogel networks of polysaccharide and synthetic biodegradable polyester. J. Biomater. Appl. 16, 305–325.

Zhang, Y., Won, C.Y., Chu, C.C., 1999. Synthesis and characterization of biodegradable network hydrogels having both hydrophobic and hydrophilic components with controlled swelling behavior. J. Polym. Sci. A: Polym. Chem. 37, 4554–4569.

Zhang, Y., Won, C.Y., Chu, C.C., 2000. Synthesis and characterization of biodegradable hydrophobic–hydrophilic hydrogel networks with a controlled swelling property. J. Polym. Sci. A: Polym. Chem. 38, 2392–2404.