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Drug–polymer interactions and their effect on thermoresponsive poly(*N*-isopropylacrylamide) drug delivery systems

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Abstract

Potential interactions between model drugs (benzoates, diltiazem, cyanocobalamin, dextrans) and a thermoresponsive poly(*N*-isopropylacrylamide) (PNIPA) hydrogel and corresponding linear polymer were investigated. The influence of the drugs on the equilibrium swelling level of the hydrogel was examined and drug–hydrogel binding isotherms were established where appropriate. Differential scanning calorimetry (DSC) was used to investigate the influence of the drugs on the lower critical solution temperature (LCST) of the linear polymer solution. Phase solubility studies were preformed to investigate binding. Drug–polymer co-precipitated blends were also prepared and analysed by X-ray diffraction (XRD), thermal analysis and Fourier transform infrared (FT-IR) spectroscopy. Hydrophobic binding was apparent between PNIPA and the aromatic ring/ester side chain of the unionised benzoate. The effect of this binding on hydrogel swelling was clarified in terms of the influence of the binding on the LCST of the system. The drug release rates of the benzoates from the hydrogel were shown to be dependent on drug binding properties. Ionisation of the benzoate prevented such hydrophobic binding, with a weaker salting out effect apparent with sodium benzoate. Significant interactions between diltiazem, cyanocobalamin (Vitamin B12) or the dextrans and PNIPA were not apparent. High concentrations of the hydrophilic drugs did, however, interfere with the magnitude of hydrogel equilibrium swelling. Hydrophobic binding, the salting out effect and the influence of the drugs on hydrogel swelling under non-sink conditions were therefore shown to be important effects which depended on the chemical nature of the drug present.

Keywords: Drug–hydrogel interactions; Thermoresponsive poly(*N*-isopropylacrylamide); Binding Isotherm; Hydrophobic binding; Hydrogel swelling

1. Introduction

Hydrogels are swellable systems that are potentially useful in the design of drug-delivery devices (Lee and Kim, 1991; Shah et al., 1991; Gutowska et al., 1992; amEnde et al., 1995; Ichikawa and Fukumori, 1997; Peppas and Wright, 1998; Qui and Park, 2001; Coughlan et al., 2004). Smart hydrogels, such as thermoresponsive poly(*N*-isopropylacrylamide) (PNIPA)-based hydrogels, have particularly been used to modulate drug release (Gutowska et al., 1992; Ichikawa and Fukumori, 1997; Coughlan et al., 2004). Our previous drug release study from a PNIPA hydrogel (Coughlan et al., 2004) revealed the importance of drug colligative properties when attempting to control the release rate from these systems. Physicochemical properties of the drug such as drug size and solubility were of major importance in the successful ability to turn on and off drug release by modulating the external temperature. The same study (Coughlan et al., 2004) suggested that the chemical nature of the loaded drug influenced the swelling of, and release kinetics from, the PNIPA hydrogel. In particular, the chemical nature of the benzoate drug series [benzoic acid (BA), methyl *p*-hydroxybenzoate (MHB) and propyl *p*-hydroxybenzoate (PHB)] slowed the swelling rate of the hydrogel to a greater degree than that of diltiazem base (DB). The study mentioned does not, however, examine the type or nature of the interactions present between the drugs and the hydrogel. The ability to understand, quantify and predict the observed swelling effects and concomitant effect on release kinetics caused by the loaded drug is desirable. In the case of temperature responsive drug delivery systems, drug–polymer interactions would have implications for the rate of drug release below the lower critical solution temperature (LCST), the magnitude of drug pulse on switching temperature above the LCST and therefore the ability to successfully control drug release characteristics (Coughlan et al., 2004).

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While drug-related factors such as solute size, solubility and loading have been shown to influence the release rate from swellable devices (Coughlan et al., 2004; Shah et al., 1991; Lee and Kim, 1991), the presence and effect of potential drug–polymer interactions on hydrogel swelling and drug release kinetics are not well researched. A limited number of studies on hydrogels (amEnde et al., 1995; Peppas and Wright, 1998; Alvarez-Lorenzo and Concheiro, 2002) have suggested the presence of ionic interactions between the loaded drug used and the polymer chains, therefore influencing the release rate from such systems. For example, amEnde et al. (1995) showed that that the cationic solute oxyprenolol strongly interacted with ionic hydrogels and showed increased hindrance in solute transport of the drug from an anionic solute at high pH. Other studies (Yu and Grainger, 1995; Wu et al., 2005) mention possible hydrophobic binding between the substituents used, although the implication for hydrogel swelling and drug release was not apparent.

The influence of salts (Horne et al., 1971; Eeckman et al., 2001; Saito et al., 2001) and surfactants (Schild and Tirrell, 1991; Kokufuta et al., 1993; Eeckman et al., 2001; Saito et al., 2001) on thermoresponsive polymers has been reported. Flocculation or a 'salting out' effect was shown on the addition of electrolytes, although some salts induced a 'salting in' process, e.g. tetra-alkyl ammonium salts. The 'salting out' process resulted in a decrease in the LCST of thermosensitive polymers while the 'salting in' process increased the transition temperature (Eeckman et al., 2001; Saito et al., 2001). The inorganic ions were not thought to be adsorbed into the polymer network but still affected the phase transition behaviour by disruption of hydrogen bonding (Saito et al., 2001). It was suggested that an increase in the hydrophobic character of PNIPA chains resulted, which consequently lowered the phase transition. Eeckman et al. (2001) showed that both the valence and size of the anion played an important role in the salting out process in respect of PNIPA polymers. The influence of surfactants on the LCST of linear PNIPA-polymers (Schild and Tirrell, 1991; Eeckman et al., 2001; Saito et al., 2001) and crosslinked PNIPA hydrogels (Kokufuta et al., 1993) were also reported. Surfactants caused a decrease or increase in the LCST of PNIPA depending on the hydrophobic chain length and the surfactant concentration (Schild and Tirrell, 1991). In contrast to the salting effect, it was suggested that these surfactants might bind to the polymer (Schild and Tirrell, 1991; Kokufuta et al., 1993; Eeckman et al., 2001; Saito et al., 2001), thereby altering the hydrophilic/hydrophobic balance of the polymer/hydrogel.

Binding between a drug and a hydrogel can potentially alter the drug release characteristics from the hydrogel in two ways. Binding to the hydrogel could directly slow the release rate of the drug due to interactions with the polymer chains, similar to the effect shown by amEnde et al. (1995). Secondly, the binding may affect swelling characteristics and therefore mesh size of the hydrogel, which in turn would have implications for concomitant release rate from that system (Coughlan et al., 2004). The present work therefore examines potential interactions between the model drugs used in our release study (benzoates, diltiazem, cyanocobalamin (Vitamin B12), dextrans) (Coughlan et al., 2004) and poly(*N*-isopropylacrylamide). The type of interactions present between the drugs and the polymeric system were examined. The underlying mechanism involved in any drug–polymer binding was investigated with the aim of understanding and being able to predict the drug release kinetics from these hydrogels.

2. Experimental

2.1. Materials

The various molecular weight dextrans [MW 4300 (D4), 10200 (D10), 43000 (D40), 68800 (D70)], benzoic acid (BA), sodium benzoate (NaB), methyl 4-hydroxybenzoate (MHB), propyl 4-hydroxybenzoate (PHB), cyanocobalamin/vitamin B12 (VB12) (all from Sigma–Aldrich), diltiazem HCl (DHCl) (Seloc PCAS) and diltiazem base (DB) (Elan) were used as received. All other chemicals were of reagent grade as previously described (Coughlan et al., 2004).

2.2. Polymer synthesis

A thermoresponsive PNIPA hydrogel (PNIPA-H) containing 1.15 mol% methylene bisacrylamide as crosslinker was synthesised in aqueous media as previously described (Coughlan et al., 2004). A linear polymer (PNIPA-L) was synthesised in an identical manner to the hydrogel but without the crosslinker. After the polymerisation process, the solution obtained was heated above 50 °C, the precipitated polymer collected by filtration, washed with water at 50 °C and then dissolved in cold water at room temperature. This process was repeated several times to purify the polymer. The collected polymer was then dried in a vacuum oven at 50 °C for 48 h.

2.3. Gel permeation chromatography

The molecular weight of PNIPA-L was determined by gel permeation chromatography, using a method similar to that described by David et al. (2003). Solutions of the polymer and polystyrene standards (2 mg/ml) were prepared in DMF and 100 μl was injected into a system consisting of a Waters Styragel® HR column, a Waters 510 pump and a Waters 410 Differential Refractometer (elution rate 1 ml/min). The internal temperature was set at 40 °C. Millennium® 2010 software was used to integrate the peaks. Samples were injected in triplicate and the elution time compared with a calibration curve to obtain an estimate of the polymer molecular weight.

2.4. Preparation of polymer/drug co-precipitate blends

A solvent casting method (Nair et al., 2001) was used to prepare blends of PNIPA-L and the model drugs. Solutions (10%, w/v) of the appropriate ratios of drug/polymer were prepared in water or ethanol. This solution was poured into a Petri dish and dried under vacuum at room temperature.

2.5. Glass transition temperature (T_g)

All differential scanning calorimetry (DSC) analysis was performed using a Mettler Toledo 821^e DSC. The glass transition temperatures (T_g) of PNIPA-L, PNIPA-H and the various drug/polymer co-precipitated blends were determined using 5–10 mg dried samples run in an open pan, i.e. a hermetically sealed aluminium pan with three vent holes pierced in the lid. All samples were initially heated to 180 °C at 10 °C/min, cooled to 25 °C at 20 °C/min followed by heating to 260 °C at 10 °C/min. The first heating cycle was to remove all residual moisture/solvent and erase the effect of previous thermal history. Each sample was analysed in duplicate and the glass transition temperature was taken as the midpoint of the inflection. The T_g of the drugs were determined (where appropriate) by melting the drug, followed by quench cooling in liquid nitrogen before scanning from −40 to 260 °C at 10 °C/min.

2.6. Equilibrium swelling studies

Equilibrium swelling of PNIPA-H at 25 °C was examined in phosphate buffer pH 6.8 (isotonic) (PB) unless otherwise specified. The ability of the hydrogel (PNIPA-H) to swell in the presence of the various drug solutions was examined. The dried hydrogels, previously weighed, were allowed to swell for 96 h in 10 ml of various concentrations of drug solution at 25 °C. Equilibrium swelling of PNIPA-H was shown in a preliminary experiment to be complete within 96 h.

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

$$\mathrm{SR}\ \frac{W_s - W_d}{W_d} \quad (1)$$

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

A plot of swelling ratio versus drug concentration was used to examine the influence of the drug on the ability of the hydrogel to swell.

2.7. Lower critical solution temperature (LCST)

The LCST of PNIPA-L (1.4% (w/v) solution) was determined by DSC using an aqueous sample (30–40 mg by weight) run in a sealed pan under nitrogen purge at a heating rate of 2 °C/min. Samples were scanned in the range of 25–40 °C. The phase transition was taken as the maximum of the endothermic transition peak (Eeckman et al., 2001). The LCST of a swollen sample of PNIPA-H was also determined by DSC at a heating rate of 2 °C/min.

The effect of the drug substances on the transition temperature was investigated using a similar method by scanning the polymer solution (1.4% (w/v) PNIPA-L solution at 2 °C/min) in the presence of various concentrations of the model drug. Samples (previously stored at 4 °C) were maintained at 10 °C for 5 min and then heated at a rate of 2 °C/min.

2.8. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of PNIPA-L/drug co-precipitate blends were obtained with a Nicolet Magna-IR 560 spectrometer using Zinc Selenide (ZnSe) salt plates (Specac Ltd., UK). Thin films of the blends were obtained by placing two to three drops of the polymer/drug solution prepared in Section 2.4 onto the plates. The plates were then dried under vacuum at room temperature for 48 h. The spectra were obtained by averaging 64 scans in the spectral range 4000–650 cm^{-1}.

2.9. X-ray diffraction

X-ray powder diffraction patterns (XRD) of the pure drugs, polymer (PNIPA-L) and drug–polymer co-precipitate blends were obtained using a Siemens D500 Diffractometer. Samples were evaluated from 5–35 2θ degrees, using a 1.00° dispersion split; a 1.00° scatter split and a 0.15° receiving split. The Cu anode X-ray tube was operated at 40 kV and 30 mA and diffraction patterns were recorded using nickel filtered Kα radiation.

Table 1
Melting and glass transition temperatures of PNIPA-L, PNIPA-H and the various model drugs

	Melting temperature, T_m[a] (°C)	Glass transition temperature, T_g (°C)	T_g/T_m
Hydrogel (PNIPA-H)	–	143.10 ± 1.60	–
Polymer (PNIPA-L)	–	140.88 ± 0.18	–
Sodium benzoate (NaB)	435.26 ± 0.33	ND	–
Benzoic acid (BA)	122.36 ± 0.01	ND	–
Methyl *p*-hydroxybenzoate (MHB)	125.66 ± 0.12	ND	–
Propyl *p*-hydroxybenzoate (PHB)	95.82 ± 0.39	ND	–
Diltiazem HCl (DHCl)	212.48 ± 0.12	102.38 ± 0.12	0.48
Diltiazem base (DB)	87.17 ± 1.73	28.17 ± 0.10	0.32
Vitamin B12 (VB12)	232.64 ± 1.04	85.88 ± 1.24	0.37
Dextran MW 4000 (D4)	–	177.74 ± 2.98	–
Dextran MW 10,000 (D10)	–	209.22 ± 0.22	–
Dextran MW 40,000 (D40)	–	218.99 ± 0.17	–
Dextran MW 70,000 (D70)	–	221.40 ± 0.58	–

ND = not detectable.
[a] Melting points (T_m) determined by DSC in an open pan at a heating rate of 10 °C/min and were taken as the average onset of melting of three samples.

2.10. Binding studies

2.10.1. Binding isotherm

Binding between the benzoates and PNIPA-H was investigated by the use of binding isotherms (Connors, 1987). Various drug solutions were prepared in either PB or 0.01M HCl (isotonic) (4 ml, $n = 3$) and their absorbance was measured at the appropriate wavelength (Coughlan et al., 2004). PNIPA-H was previously dried in a vacuum oven for 48 h and added (100 mg) to the drug solution maintained at 37 °C (>LCST). After equilibration at 37 °C for 24 h, the samples were filtered through a 0.45 μm filter and the absorbance of each solution was determined.

2.10.2. Phase solubility study

The type of phase solubility diagram between two interacting components was determined by complexation solubility analysis using the method of Higuchi and Connors (1965) and the overhead stirrer solubility method of Corrigan and Stanley (1981). Studies were carried out at 25 °C (<LCST).

3. Results and discussion

The model drugs used can be divided into four groups: benzoates, diltiazem, cyanocobalamin (Vitamin B12) and the dextrans. The melting and glass transition temperatures of the model drugs are shown in Table 1. Interactions between PNIPA and the drugs were examined using two distinct polymeric systems: a crosslinked PNIPA hydrogel (PNIPA-H) and a non-crosslinked linear PNIPA polymer [PNIPA-L, molecular weight $1.30\,(\pm 0.21) \times 10^6$ Da]. The glass transition temperatures of PNIPA-L and PNIPA-H are also given in Table 1. Studies have previously shown (Schild, 1992) that linear PNIPA chains and the corresponding lightly crosslinked PNIPA gels have similar thermoresponsive characteristics.

The influence of various concentrations of each drug in solution on the equilibrium swelling level of PNIPA-H at 25 °C (<LCST) was examined. Drug–hydrogel binding was also investigated where appropriate using a dried hydrogel at 37 °C (>LCST). Additional studies were carried out on the linear polymer. Due to the ability of PNIPA-L to dissolve below the LCST, studies were performed using both an aqueous drug-polymer solution and a drug polymer blend formed by co-precipitation. Differential scanning calorimetry (DSC) studies were used to investigate the influence of the drugs on the LCST of a polymer solution. Phase solubility studies were preformed at 25 °C (<LCST) to investigate binding. Drug–polymer co-precipitated blends were also prepared and were analysed by X-ray diffraction (XRD), thermal analysis and Fourier transform infrared (FT-IR) spectroscopy.

3.1. Benzoate–PNIPA interactions

3.1.1. Hydrogel (PNIPA-H)

3.1.1.1. Equilibrium swelling. The non-ionic hydrogel (PNIPA-H) was insoluble in aqueous media at all temperatures, whether swollen (<LCST) or collapsed (>LCST). The equilibrium swelling ratio (SR, Eq. (1)) of PNIPA-H at 25 °C was 9.81 ± 0.55 in phosphate buffer pH 6.8 (PB) and 12.95 ± 0.35 in an isotonic 0.01 M HCl solution; the difference between the swelling levels attributed to differences in salting out effectiveness of the solutions. The equilibrium swelling ratios of PNIPA-H in various concentrations of the benzoate solutions in PB at 25 °C (<LCST) are shown in Fig. 1. Fig. 1a also shows the equilibrium SR of PNIPA-H in various concentrations of BA at 25 °C in 0.01 M HCl (isotonic). The concentration of benzoate that caused a decrease in swelling by 50% was defined by a parameter, $k_{H50\%}$ (Table 2). A second parameter, $k_{H100\%}$, was derived as the molar quantity of drug required to inhibit hydrogel swelling. The presence of BA in the PB did not affect

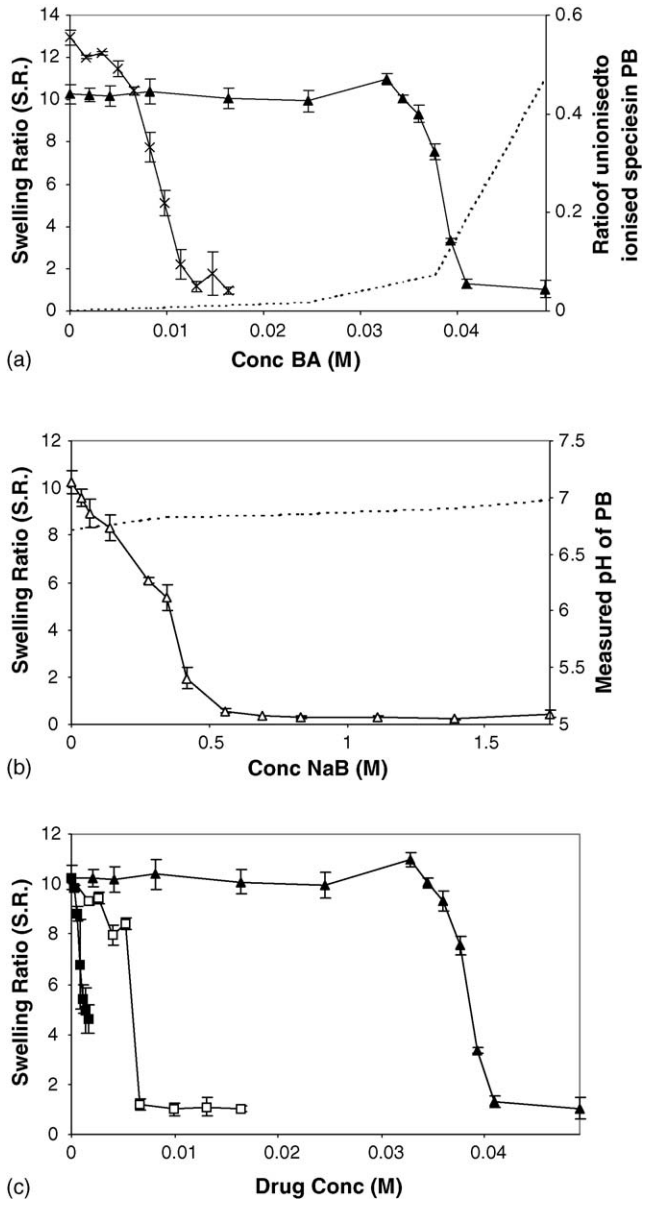

Fig. 1. Equilibrium swelling ratios ($n = 3$; mean ± S.D.) of PNIPA-H at 25 °C in various concentrations of (a) BA in PB (▲) and in 0.01 M HCl (×); (b) NaB (△) in PB; (c) BA (▲), MHB (□) and PHB (■) in PB. Also shown as dashed lines in (a) is the ratio of unionised to ionised species of BA in PB and in (b) is the measured pH of the PB solution.

Table 2
Drug concentration required to reduce swelling of PNIPA-H at 25 °C in PB by 50% of equilibrium value ($k_{H50\%}$) and binding constants calculated at 37 °C (k_b)

	Hydrogel (PNIPA-H)				Polymer (PNIPA-L)
	$k_{H50\%}$ at 25 °C		$k_{H100\%}$ (mol drug/mol NIPA in dry disc)	Binding constant (k_b) at 37 °C ($\times 10^2$ M^{-1}) (Eq. (2))	$k_{L100\%}$[a] (mol drug/mol NIPA)
	g/l	mol/l ($\times 10^2$ M)			
NaB	50.839 ± 1.149	35.280 ± 0.797	15.97	–	4.94
BA	4.721 ± 0.004	3.867 ± 0.004	1.75	ND	0.49
	1.093 ± 0.050[b]	0.895 ± 0.041[b]	0.41[b]	0.54[b]	–
MHB	0.894 ± 0.005	0.588 ± 0.003	0.27	3.53	0.10
				2.63[b]	–
PHB	0.230 ± 0.070	0.128 ± 0.039	0.06	9.20	0.09
				33.89[b]	–
DHCl	348.120 ± 20.517	77.188 ± 4.549	34.94	–	–
D10	57.244 ± 4.711	0.561 ± 0.046	0.25	–	–
D40	58.178 ± 8.949	0.135 ± 0.021	0.06	–	–
D70	62.445 ± 6.112	0.091 ± 0.009	0.04	–	–

$k_{H100\%}$ values estimate the number of moles of model drug per mole of NIPA required to inhibit swelling. Also given are the estimated number of moles of benzoate per mole of NIPA (PNIPA-L) required to reduce the LCST to 25 °C ($k_{L100\%}$). ND = not detectable.
[a] Data based on slopes of plots as shown in Fig. 5.
[b] Experiment performed in 0.01 M HCl.

the equilibrium swelling of PNIPA-H up to a concentration of ~0.035 M. Above this value, a sharp drop in swelling occurred which was shown to correspond to a drop in the measured pH of the buffer towards the pK_a of the acid (pK_a BA = 4.20; Martin, 1993). The pH drop resulted in an increase in the proportion of unionised species in the solution as calculated by the Henderson–Hasselbalch equation (Martin, 1993) and shown by the dashed line in Fig. 1a. In 0.01 M HCl, where BA was almost completely unionised, there was a significant decrease in equilibrium swelling at all concentrations of BA. In the concentration range studied, the effect of BA on swelling was therefore due to the unionised BA species.

The influence of the presence of NaB on the equilibrium swelling of PNIPA-H in PB at 25 °C is shown in Fig. 1b. NaB is ~40 times more soluble than BA in PB (Coughlan et al., 2004). The measured pH was ~6.8 and the weak acid present was in the ionised form in solution. At higher concentrations, there was a gradual decrease in the ability of the hydrogel to swell with the $k_{H50\%}$ value being ~10 times greater than that of BA. The measured pH of the buffer is also shown in Fig. 1b and a slight increase in pH was seen with increasing concentration of NaB. The upward shift in pH was caused by the presence of free sodium ions acting as a strong base. The benzoic anion and the sodium cation remained the dominant species present. The presence of the benzoic anion may have a "salting out effect" on the thermoresponsive polymer, similar to the salting out effect shown by Saito et al. (2001) and Eeckman et al. (2001) with various electrolytes.

The benzoate esters, although esterified at the acid group of BA, also contain a *para* hydroxyl group and pK_a values of 8.37 and 8.36 have been reported for MHB and PHB respectively (Büchi et al., 1971). MHB and PHB were, however, essentially unionised at pH 6.8 and the presence of these compounds was shown to have no effect on the measured pH. There was a decrease in the $k_{H50\%}$ value in PB as the length of the side chain group increased (Fig. 1c and Table 2).

The unionised forms of the benzoates as well as the hydrophobicity of the ester side chain were therefore significant factors in causing the observed, sharp collapse in equilibrium swelling at a temperature below the LCST of the polymer. A more gradual deswelling was seen with the ionised form of benzoic acid at higher concentrations.

3.1.1.2. Binding isotherm. To investigate potential adsorption or binding of the benzoates to PNIPA, spectroscopic studies were performed between solutions of the benzoates and PNIPA-H at 37 °C, a temperature above the LCST of the hydrogel. At this temperature, minimal swelling of the hydrogel occurred (Coughlan et al., 2004). Binding curves between the benzoates and PNIPA-H in 0.01 M HCl are shown in Fig. 2. Using this technique, binding was observed for MHB and PHB in PB while binding for all three benzoates in 0.01 M HCl was apparent (Fig. 2). Binding between BA and PNIPA was therefore seen in the unionised form in 0.01 M HCl and binding increased with increasing hydrophobicity of the ester side chain. The data presented in Fig. 2 was fitted by the Langmuir isotherm (Eq. (2))

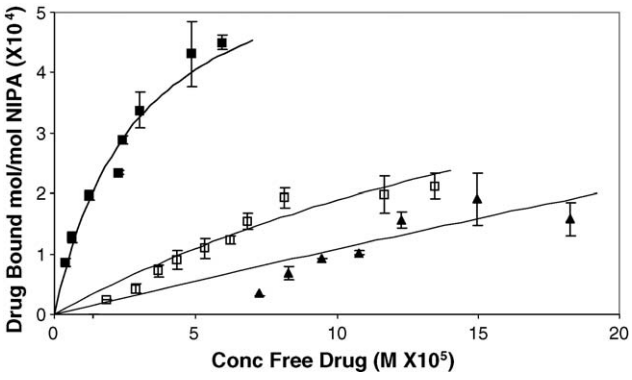

Fig. 2. Binding curve plot between the benzoates and PNIPA-H at 37 °C in 0.01 M HCl (n = 3; mean ± S.D.) [BA (▲), MHB (□), PHB (■)].

and the average values for the binding constant (k_b) are given in Table 2:

$$y = \frac{y_{max} k_b x}{1 + k_b x} \quad (2)$$

where y is the mole of drug bound per mole polymer, x the molar concentration of free drug, y_{max} the maximum possible number of moles of drug bound per mole of polymer and k_b is the constant.

From this study, the benzoates appear to bind by hydrophobic interactions between the aromatic ring/side chain ester and the hydrophobic groups of PNIPA. Based on the values in Table 2 in PB (for MHB and PHB) and in 0.01 M HCl (for BA) (representing unionised form in each case), there was a linear relationship ($R^2 = 0.9962$) between the binding constant (k_b) and the constant $k_{H50\%}$, consistent with drug–polymer binding being the cause of the drug dependent swelling profiles seen in Fig. 1a and c. As the level of binding (k_b) increased, there was a corresponding decrease in the molar amount required to affect the hydrogel swelling ($k_{H50\%}$). The binding observed therefore lowered equilibrium swelling of the hydrogel at a given temperature.

3.1.2. Linear polymer (PNIPA-L)

The reason that benzoate/PNIPA binding caused the observed swelling profiles (Fig. 1) was further investigated using the linear polymer. The LCST of the linear polymer, as determined by DSC analysis, decreased from 34.09 ± 0.06 °C in water to 29.95 ± 0.04 °C in PB due to the effect of the buffer salts on the LCST of PNIPA (Eeckman et al., 2001; Saito et al., 2001). Blends of the benzoates and polymer were prepared and were analysed by XRD, thermal analysis and FT-IR.

3.1.2.1. X-ray analysis. PNIPA-L was amorphous in nature while benzoic acid and its esters were highly crystalline solids as shown by the sharp peaks in the XRD pattern. Sodium benzoate showed less intense peaks. XRD of the drug–polymer co-precipitate blends revealed an inhibitory effect of the polymer on drug crystallisation for each of the benzoates (data not shown). No crystalline peaks were observed at drug concentrations up to 20% w/w for NaB, 40% (w/w) for BA and 30% (w/w) for MHB and PHB. PNIPA-L prevented recrystallisation of BA at lower concentrations than the salt form (NaB), consistent with the presence of greater BA–polymer interactions (Yoshioka et al., 1995).

3.1.2.2. Glass transition temperature. Interactions between co-precipitated drug and polymer can influence the glass transition temperature (T_g) of the polymer–drug blend (Nair et al., 2001). The influence of the benzoates on the T_g of the benzoate–PNIPA co-precipitated blends was examined. The T_g of the pure benzoates could not be determined experimentally due to rapid recrystallisation of the drug. The T_g of the polymer may be expected to decrease in the presence of a small molecule due to the plasticising effect of the drug on the polymer (Okhamafe and York, 1989). The change in glass transition temperature of the fully amorphous blends as a function of molar percentage of drug present is presented in Fig. 3. Previous studies on

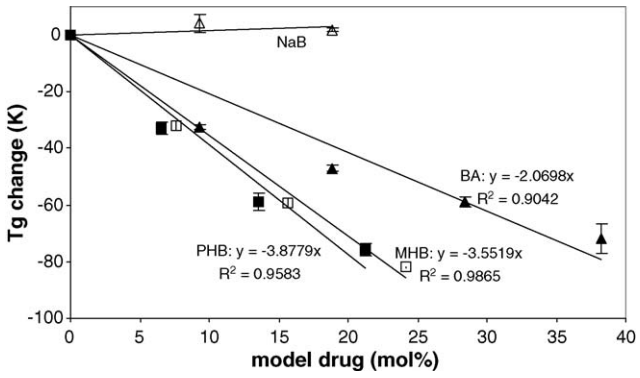

Fig. 3. Effect of drug type on glass transition temperature change of amorphous drug/polymer co-precipitated blends [NaB (△), BA (▲), MHB (□) and PHB (■)].

non-thermoresponsive polymers (Okhamafe and York, 1989; Gutierrez-Roca and McGinity, 1994; Nair et al., 2001) have suggested a correlation between decreased drug molecular size and increased plasticising effect on the polymer. The opposite effect occurred in the present study as the plasticising effect increased as the molecular size of the benzoate series increased, quantified by the slope of the lines in Fig. 3. More specifically, as the hydrophobicity of the series increased, the T_g decreased to a greater extent. The differences observed in depression of the T_g of the blends followed the same pattern as was shown with the binding constants (Table 2).

The T_g of the NaB–polymer blend did not change from that of the polymer (Fig. 3). Immiscibility of NaB with the polymer may have resulted in a lack of interactions. The ionic form of BA was shown to prevent binding interactions with the hydrogel. Crystalline peaks in the XRD were also seen at 20% (w/w) NaB in the co-precipitated blend, a lower value than BA and consistent with a lack of binding in the ionised form (Yoshioka et al., 1995).

3.1.2.3. FT-IR spectroscopy. Drug–polymer interactions in the solid state can be revealed using FT-IR by examination of wavelength shifts in the characteristic peak positions of either the drug or the polymer. Regions of the spectra where the peaks do not overlap are potentially useful. The FT-IR spectra obtained of the benzoates (Gangakhedkar et al., 1974; Bakker et al., 2003) and of PNIPA-L (Gupta et al., 2000; Liu et al., 2004) were consistent with those presented in the literature. The characteristic peak position of the benzoates with increasing concentration of PNIPA was examined. A peak at 707 cm^{-1} in the spectrum of BA and NaB was attributed to phenyl C–H out of plane bending (Gangakhedkar et al., 1974) and there was no significant peak overlap in this region with the spectrum of PNIPA-L. An increase in this peak position with increasing concentration of PNIPA in the blend was seen in the BA spectra (Fig. 4a), suggesting the presence of hydrophobic interactions between the aromatic ring of BA and the polymer. The presence of high PNIPA concentrations did not appear to saturate the effect.

A peak at ~849 cm^{-1} in the spectra of MHB and PHB were also attributed to aromatic C–H out of plane bending. An increase in the position of these peaks was seen with increasing

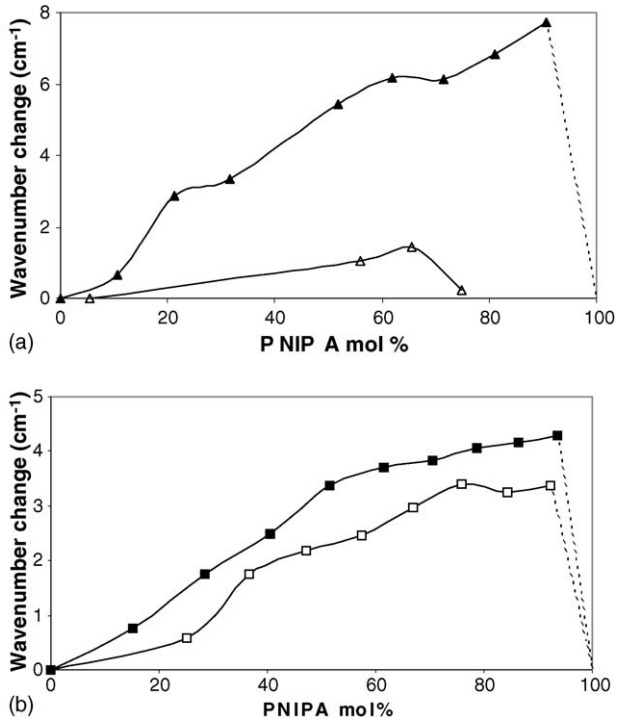

Fig. 4. FT-IR wavelength shifts of benzoate/polymer co-precipitated blends with increasing concentration of PNIPA-L: (a) wavelength change at 707 cm^{-1} in spectrum of blends containing BA (▲) and NaB (△) (b) wavelength change at 849 cm^{-1} in spectrum of blends containing MHB (□) and PHB (■). Both peaks assigned to aromatic C–H out of plane bending.

concentration of PNIPA in the blend (Fig. 4b), again suggesting the presence of hydrophobic interactions between the aromatic ring of the esters and the polymer. The aromatic peak shifts shown were stronger for BA, suggesting that the effect may be only secondary in the binding process of MHB and PHB where the side chain ester contributed to the binding. The peak shift observed at the aromatic ring site began to level off at approximately 50 and 70 mol% PNIPA in the cases of PHB and MHB, suggesting approximate 1:1 and 0.5:1 benzoate:NIPA binding ratios, respectively. There were no major shifts detectable in the C–H stretching peaks around 3000 cm^{-1}, with significant peak overlap in this region with the functional groups of PNIPA. A peak at ~771 cm^{-1} in the spectra of MHB and PHB was present and may be attributed to –COO– bending with ring deformation (Bakker et al., 2003). The presence of PNIPA also caused a weak upward peak shift at this position for MHB, possibly due to binding with the polymer amide group.

The polymer groups involved in the binding were in turn examined by the characteristic polymer (PNIPA) peak positions with increasing concentration of drug. The presence of BA caused an increase in the peak position of the hydrogen-bonded NH stretch peak at 3299 cm^{-1}, while a slight decrease was observed with NaB (data not shown), suggesting differences in bonding interactions with the acid and its salt form. Comparisons with the esters could not be made due to peak overlap in this region due to the *para* hydroxy group stretching. There was significant peak overlap in the area of the PNIPA backbone carbons (2971, 2933 and 2875 cm^{-1}; Gupta et al., 2000) and the PNIPA isopropyl group (1387 and 1367 cm^{-1}; Liu et al., 2004). These peaks were relatively weak and the functional groups common making definite conclusions difficult using this technique as to the polymer groups of PNIPA involved in the hydrophobic binding mentioned.

Binding of the benzoates studied therefore appears to involve mainly non-polar dispersion forces of the aromatic ring and the side chain ester with the hydrophobic groups of the polymer. There may also be some hydrogen bonding present. Ionisation of the acid appears to prevent the hydrophobic binding, consistent with the studies on the hydrogel.

3.1.2.4. Phase transition temperature. The influence of various concentrations of the benzoates on the LCST of a solution of PNIPA-L in PB is shown in Fig. 5. The decrease in phase transition temperature was quantified by the magnitude of the slope of the fitted lines in Fig. 5. The slopes were obtained by a best-fit line through the origin value. The value for BA was calculated for the downward portion of the graph as shown in Fig. 5a. The number of moles of benzoate per mole of NIPA required to decrease the LCST to 25 °C was calculated ($k_{L100\%}$) and shown in Table 2.

The presence of BA in PB up to a concentration of ~0.035 M did not have an effect on the LCST of the polymer solution as shown in Fig. 5a. Above this concentration, the measured pH of the solution approached the pK_a of BA (pK_a = 4.2), similar to the pH change shown in Fig. 1a. The appearance of significant concentrations of the unionised form of BA caused a decrease in the LCST of the polymer. The same pattern was shown in

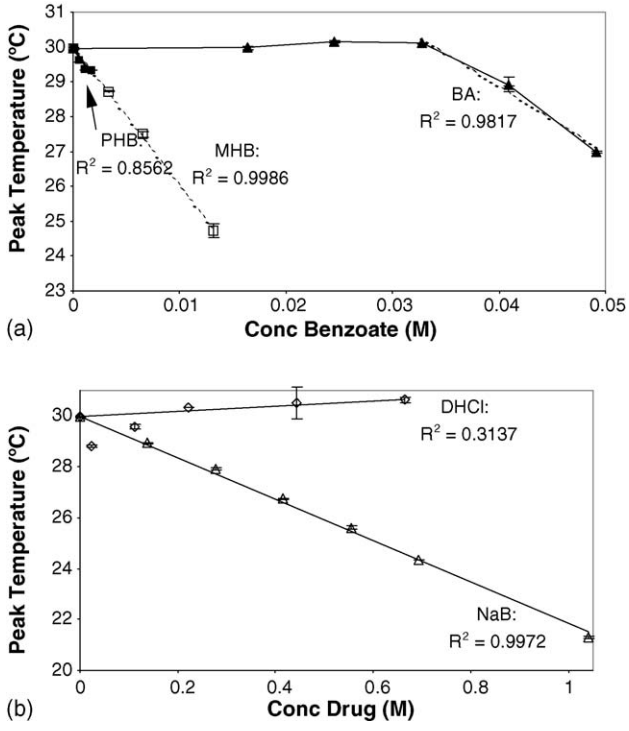

Fig. 5. Plot of endotherm peak value obtained by DSC of a 1.4% (w/w) solution of PNIPA-L containing various concentrations of (a) BA (▲), MHB (□), PHB (■) and (b) NaB (△), DHCl (◊).

Fig. 1a for equilibrium swelling of the hydrogel (PNIPA-H). Binding of the unionised benzoates to the hydrogel was shown to be the cause of the swelling pattern. Binding of the unionised form of BA to PNIPA was shown here to lower the LCST of the polymer. Swelling at 25 °C was therefore affected when the LCST was lowered sufficiently near this temperature.

Binding of the ionised form of BA did not appear to be significant in the concentration range studied. The presence of NaB (Fig. 5b) in the same concentration range as BA also did not affect the LCST, as this species exists in the ionised form at pH 6.8. At high concentrations, however, NaB caused a gradual decrease in the LCST of the polymer. The magnitude of the decrease in LCST, as measured by the $k_{L100\%}$ values, was ~10 times lower than the downward portion shown by BA. Since significant binding was not apparent, the effect seen on the LCST can be attributed to the large benzoic anion disrupting the hydrogen bonding between the polymer and water molecules. Such a salting out effect required higher concentrations to be present, resulting in an increase in the hydrophobic nature of the polymer and a decrease in LCST, as shown previously (Schild and Tirrell, 1991; Eeckman et al., 2001) with various salts and small surfactants. Such effects may explain the swelling pattern of the hydrogel (PNIPA-H) seen in Fig. 1b. An approximate 10-fold difference was also seen with the $k_{H100\%}$ values between BA and NaB (Table 2). The salting out effect lowered the phase transition temperature of the polymer chains and therefore the equilibrium swelling level of the hydrogel at a particular temperature.

Increasing the hydrophobic nature of the ester side chain resulted in a sharper decrease in the LCST caused by greater binding affinity (Table 2). From the FT-IR data, the benzoates appeared to associate with hydrophobic segments of the polymer, resulting in a more hydrophobic system and therefore causing the LCST to occur at a lower temperature. Comparisons can also be made between the hydrogel $k_{H100\%}$ values, which measure the number of moles required to theoretically prevent swelling of PNIPA-H at 25 °C, and the $k_{L100\%}$ values, which are the number of moles necessary to decrease the LCST of PNIPA-L to 25 °C. As the hydrophobicity of the ester series decreased, both the $k_{H100\%}$ and the $k_{L100\%}$ values also decreased, in line with the decrease in the binding constant in Table 2. The $k_{H100\%}$ and the $k_{L100\%}$ values for BA and the esters were of the same order between the two systems, indicating that the effect of binding on the LCST caused the swelling patterns seen in Fig. 1.

The magnitude of the endotherm integral values of PNIPA-L in the presence NaB and diltiazem HCl (DHCl), as measured by the area under the DSC curve, is shown in Fig. 6. A decrease in the integral value indicates a decrease in the magnitude of the measured phase transition of PNIPA-L in the presence of these compounds. A statistically significant decrease ($p < 0.05$) in the magnitude of the integral value was seen with NaB (Fig. 6). At the high concentrations, NaB may therefore interfere with the H-bonding ability of PNIPA in solution. Comparisons of the $k_{H100\%}$ and $k_{L100\%}$ values for NaB indicated that the salting out effect on the LCST may not be the only contributing factor to the swelling pattern. The very high concentrations of NaB may also have prevented solvation of the polymer chains due to competition for hydrogen bonding with the water molecules present.

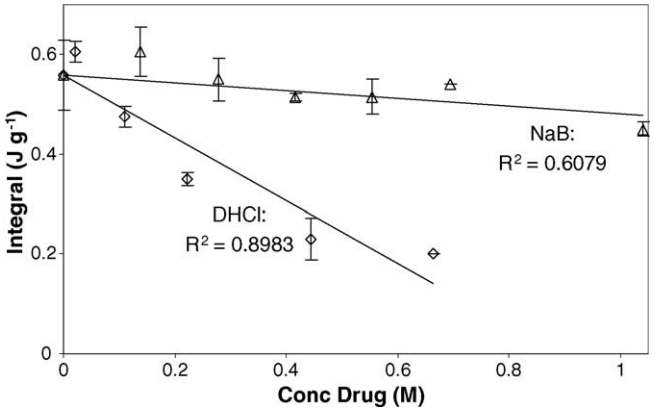

Fig. 6. Effect of NaB (△) and DHCl (◊) on the magnitude of the LCST, as measured by the area under the DSC curve.

3.1.2.5. Phase solubility study. The type of phase solubility diagram obtained due to binding between the benzoates and PNIPA-L was determined by complexation solubility analysis (Higuchi and Connors, 1965). Type B phase diagrams as described by Higuchi and Connors (1965) were observed in the present study (Fig. 7a–c), which indicated the presence of insoluble complexes. Binding of the benzoate to the polymer lowered the LCST of the polymer towards the temperature of the experiment (25 °C), resulting in precipitation of the insoluble complex. The formation of an insoluble complex between the unionised benzoates and the linear polymer is consistent with the swelling study (Fig. 1c). The formation of an insoluble complex with the polymer chains preventing swelling of the hydrogel.

An attempt was made to characterise the complexation phenomenon based on the phase solubility diagrams. Stoichiometric ratios can be estimated for Type B diagrams as described by Higuchi and Lach (1954). The benzoate:NIPA stoichiometric ratios obtained were in the range of 3:1 (BA and MHB) to 5:1 for PHB. Whether the stoichiometry is a valid reflection of the chemistry depends upon whether one or several complexes are formed; if more than one complex is contributing to the solubility behaviour at any region of the diagram, the stoichiometry may reflect contributions from all of the complexes (Higuchi and Connors, 1965).

3.2. Other model drugs

The influence of the other model drugs (DHCl, DB, VB12, dextrans) on the equilibrium swelling of PNIPA-H is shown in Fig. 8. The presence of DHCl (Fig. 8a) did not affect the swelling of PNIPA-H until relatively high concentrations (>0.4 M), giving a $k_{H50\%}$ value of 0.77 M (Table 2). There was a small decrease in the equilibrium swelling of the hydrogel in DB and VB12 (Fig. 8b). A $k_{H50\%}$ value was, however, not detectable. A drop in pH was also observed with increasing concentration of DHCl, although for both DHCl and DB the pH remained below the pK_a

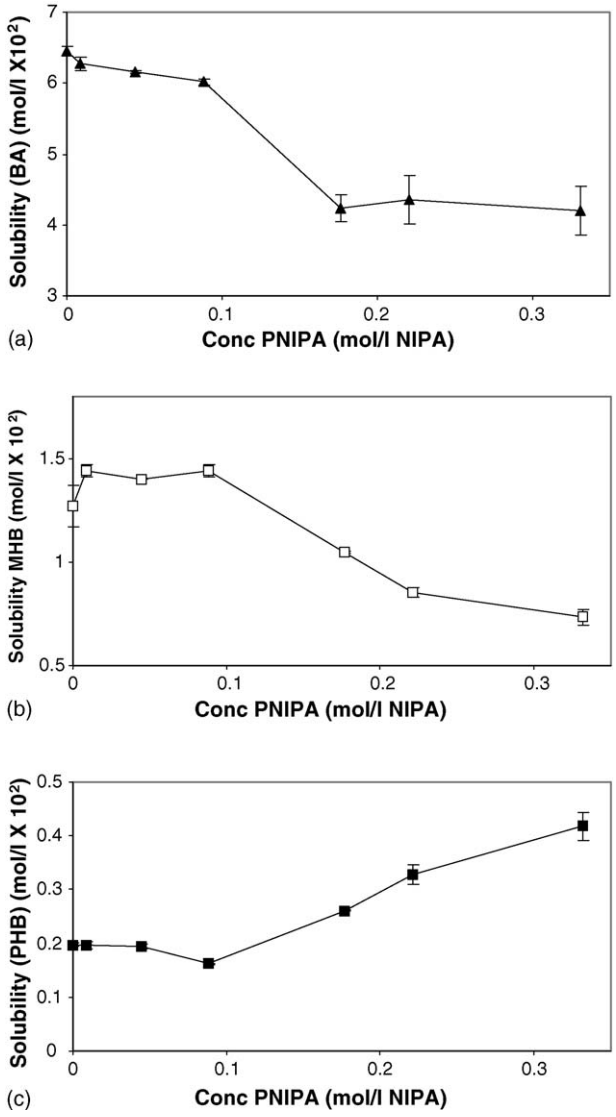

Fig. 7. Phase diagrams showing the effect of PNIPA-L on the apparent solubility of (a) BA, (b) MHB and (c) PHB at 25 °C in PB ($n = 3$, mean ± S.D.).

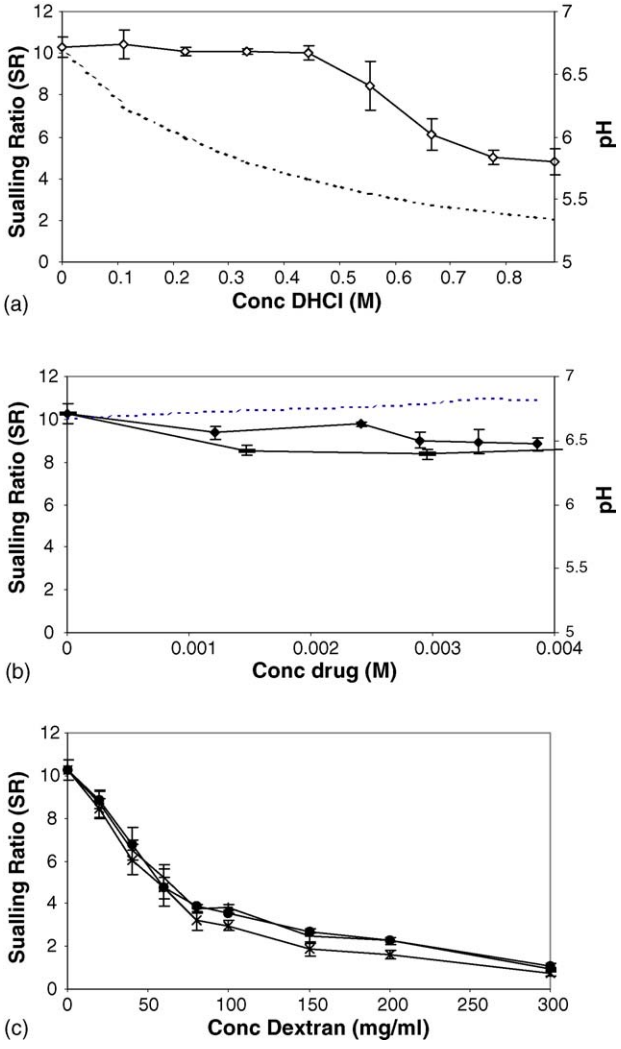

Fig. 8. Equilibrium swelling ratios in PB at 25 °C ($n = 3$; mean ± S.D.) of PNIPA-H in various concentrations of (a) DHCl (◊); (b) DB (♦), VB12 (−); (c) dextrans [D10 (×), D40 (◯) or D70 (+)]. Also shown as dashed lines in (a) and (b) are the measured pH values of DHCl and DB, respectively in PB.

of the base ($pK_a = 8.91$). The ionised species therefore dominated. The equilibrium swelling of the hydrogel exponentially decreased with increasing concentration of the dextrans, with no molecular weight difference in the swelling depression when analysed on a weight basis (Fig. 8c).

The inhibitory effect of PNIPA-L on diltiazem crystallization, as shown by XRD, was apparent only at drug concentrations ≤20% (w/w) for both DB and DHCl; values less than those observed with the unionised benzoates. Only one glass transition temperature was observed in the co-precipitated blends for both DHCl and DB and these values matched the predictions of the Gordon–Taylor equation in the amorphous range (Gordon and Taylor, 1952; Tajber et al., 2005), suggesting a lack of significant diltiazem-polymer interactions (Nair et al., 2001; Tajber et al., 2005). There was also no evidence of significant interactions on measuring the glass transition temperature of the VB12 or dextran blends. The FT-IR data of DB, VB12 and the four dextran blends showed little evidence of interactions between the components. The PNIPA-L peaks assigned to hydrogen-bonded N–H stretch (3299 and 3073 cm^{-1}) shifted to lower wavenumber in the presence of DHCl, indicating possible interactions between DHCl and PNIPA-L. No hydrogen bonding interactions were detected between PNIPA-L and the dextrans by FT-IR.

In contrast to the influence of the benzoates on the position of the LCST of PNIPA-L (Fig. 5), these model drugs did not significantly influence the position of the LCST. In addition, neither DB nor VB12 affected the magnitude of the LCST, as detected by the area under the DSC curve. The highly soluble DHCl (Fig. 6) and the dextrans, however, decreased the magnitude of the phase transition. The presence of high concentrations of the hydrophilic compounds diminished the hydrogen-bonding ability of PNIPA-L with water, decreasing the magnitude of the LCST. The magnitude of the decrease in the LCST was independent of the dextran molecular weight on a weight basis. In the crosslinked system, hydrogen bonding is necessary for hydrogel swelling. Equilibrium swelling at 25 °C was therefore affected,

with PNIPA-H unable to hydrogen bond and swell in the presence of high concentrations of the dextrans (Fig. 8c).

The effect of DHCl on the LCST of PNIPA is compared with the effect of NaB on the LCST (Fig. 6) and on hydrogel equilibrium swelling at 25 °C (Figs. 1b and 8a). When the fit shown in Fig. 6 was extrapolated to a zero integral value, a concentration of ~0.89 M DHCl was obtained, which is the concentration of DHCl which prevented detection of any phase transition. The hydrogen bonding ability of the polymer is therefore diminished at this concentration and is consistent with the $k_{H50\%}$ value of 0.77 M DHCl for the hydrogel. In the case of NaB, both the size of the phase transition (Fig. 6) and the temperature at which it took place (Fig. 5b) decreased with increasing concentration of salt added. The decrease in the position of the LCST was attributed to a salting out effect caused by the benzoate anion. The decrease in the magnitude of the transition may have been due to competition between the polymer and the high concentrations of drug for the available water molecules. Both of these factors resulted in the swelling profile observed (Fig. 1b). In the present case of DHCl, the salt had a greater effect than NaB on the magnitude of the phase transition, as shown by the greater slope (Fig. 6). A salting out effect was not apparent, as shown by the effect of DHCl on the position of the LCST of PNIPA-L (Fig. 5b). The decrease in the magnitude of the transition also appears to have been due to competition between the polymer and the drug for the available water molecules. Swelling of PNIPA-H in the presence of DHCl was unaffected up until relatively high concentrations as shown in Fig. 8a. The effect of very high concentrations of DHCl on the swelling of PNIPA-H can therefore be attributed to competition between the drug and the polymer for hydrogen bonding to the available water molecules.

The types of interactions found between the drugs examined and PNIPA can be divided into three types. The first and most significant type was hydrophobic binding, evident between the unionised benzoates and PNIPA. The benzoate anion did not show a significant binding tendency. The binding was hydrophobic in nature and occurred between the carbon backbone/isopropyl side chain of PNIPA and the aromatic ring/ester side chain of the benzoates. Binding increased with increasing hydrophobicity of the ester side chain. The consequence of the observed binding was to increase the hydrophobicity of the PNIPA, resulting in a decrease in the LCST of both the polymer and the hydrogel. Precipitation of the polymer, or shrinkage of the hydrogel, therefore occurred at a particular drug concentration when the extent of binding was sufficient to decrease the LCST to the temperature of the experiment.

Our previous drug release study from PNIPA-H1 (Coughlan et al., 2004) revealed a difference in the pattern of swelling and drug release within the hydrophobic drug series. In particular the benzoates were shown to depress the swelling rate of the hydrogel to a greater extent than DB, shown in the current study to be due to differences in drug-polymer interactions. There was a direct linear relationship ($R^2 = 0.9999$) between the hydrogel swelling rate constants at 25 °C (k_{s1}, Coughlan et al., 2004), obtained in the presence of the loaded benzoates, and the $k_{H50\%}$ values in PB, confirming that it was the drug–hydrogel binding in our previous study that controlled the rate of hydrogel swelling. In addition the $k_{H50\%}$ values were also linearly proportional ($R^2 = 0.9309$) to the benzoate release rate constants at 25 °C (k_d, Coughlan et al., 2004) from the same study. The linear relationships confirmed that the hydrogel swelling rates and concomitant drug release rates were dependant on drug binding properties. The release rate of DB observed in the previous study (Table 3 from Coughlan et al., 2004) deviated from that expected of a drug of its size and solubility when compared to the benzoate series, due to differences in drug–hydrogel interactions.

Examination of the literature on benzoates revealed studies from the 1950s to 1970s where they were shown to bind to other substances. The benzoates were shown to bind to non-ionic surfactants (Goodhart and Martin, 1962; Patel and Romanowski, 1970; Blanchard et al., 1977), thus decreasing the preservative activity of the benzoate. Patel and Romanowski (1970) showed that the magnitude of this inactivation increased from MHB to PHB, due to higher binding of PHB. The benzoates were also shown to bind to other compounds such as caffeine (Higuchi and Zuck, 1953) and a plastic nylon, capran polyamide (Patel and Nagabhushan, 1970). The later study is particularly relevant as binding was shown between the esters and the plastic polyamide. The thermoresponsive polymer used in our investigations is also a polyamide derivative.

The second type of drug–hydrogel interaction was a ‘salting out’ effect that occurred with PNIPA in the presence of NaB. Previous studies (Eeckman et al., 2001) have shown that both the valence and size of the anion play an important role in the salting out process in respect of PNIPA polymers, with the effect of the cation not being significant. The benzoate anion appeared to cause this salting out effect. The salting out process on the LCST of the polymer was relatively weak compared to the effect of direct hydrophobic binding on the LCST due to the unionised benzoate. While the binding effect of the unionised benzoates significantly influenced hydrogel swelling and drug release, the salting out effect of NaB did not depress the rate of swelling of the hydrogel (Coughlan et al., 2004). The swelling rate actually increased due to the osmotic effect of this small hydrophilic drug.

While aqueous salt solutions are thought to alter the water structure in the polymer hydration sheath causing the salting out effect, a third type of ‘indirect’ interaction also occurred in the drug–hydrogel system. Relatively high concentrations of the highly water-soluble model drugs (NaB, DHCl and the dextrans) prevented swelling of the hydrogel, probably by competition with the hydrogel for the available water molecules. The magnitude of the phase transition as detected by DSC was also weakened as a result of the decrease in available water molecules. An osmotic effect related increase in swelling rate generally occurred with the hydrophilic drugs, depending on drug size and loading (Coughlan et al., 2004). The effect of high drug concentrations on equilibrium hydrogel swelling would be significant in the case of high drug loadings and/or where non-sink conditions exist.

The physicochemical characteristics of the chosen drug were therefore shown to have a significant bearing on the swelling of, and drug release from, swellable systems. Our previous study

(Coughlan et al., 2004) revealed the importance of drug colligative properties when attempting to control the release rate from these hydrogels. Knowledge of the physicochemical properties of the drug such as drug size and solubility were of major importance in the successful ability to turn on and off release by modulating the external temperature (Coughlan et al., 2004). The present study revealed that the effect of drug–hydrogel interactions on hydrogel swelling and concomitant drug release rates is critical. The studies mentioned can reveal the underlying mechanism of interaction, including whether or not direct binding between the drug and polymer has occurred. A combination of the techniques described would be necessary to investigate potential interactions between a hydrogel and a drug. Simple equilibrium swelling studies can indicate the presence of interactions, which can be investigated in more detail by thermal or spectroscopic analysis on both the hydrogel and the corresponding linear polymer. The advantage of examining such interactions using the linear polymer in conjunction with the hydrogel was apparent. The linear polymer can conveniently and accurately be used to predict and quantify a series of drug–polymer interactions, which can then be extrapolated to the hydrogel system and therefore used to help predict their effect on hydrogel swelling and drug release.

4. Conclusions

Potential interactions between model drugs and poly(*N*-isopropylacrylamide) in both the linear and crosslinked form were examined. The most significant interaction observed was between the unionised benzoates and PNIPA. The net effect of binding was that a relatively low drug concentration (<0.01 M in unionised form) affected the equilibrium swelling level of the hydrogel. Such drug–hydrogel interactions were shown to be the cause of the lower than expected hydrogel swelling rates in our previous study (Coughlan et al., 2004), with the concomitant drug release rates from the hydrogel also dependant on drug binding properties. It was possible to obtain further insight into the nature of the binding phenomenon using the corresponding linear polymer of PNIPA by spectroscopic and thermal analysis of drug–polymer solutions and of drug–polymer co-precipitate blends. The binding was shown to be of the hydrophobic type between the unionised form of the benzoates and the polymer. Such binding influenced the position of the LCST of the polymer and therefore the equilibrium swelling and swelling rate of the corresponding lightly crosslinked hydrogel. Ionisation of the benzoate prevented hydrophobic binding, with a weaker salting out effect apparent with sodium benzoate. Direct interactions between the other model drugs and the hydrogel did not appear to be significant. The importance of investigating any drug–hydrogel interactions was therefore shown when attempting to use these smart thermoresponsive hydrogels as drug delivery systems. The techniques described can reveal the nature of the interactions in an attempt to predict their effect on swelling of, and drug release from, the hydrogel. Studies using the corresponding linear polymer of a lightly crosslinked hydrogel were shown to be useful in predicting and quantifying such interactions.

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