Interactions in aromatic probe molecule loaded poly(N-isopropylacrylamide) hydrogels and implications for drug delivery

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Abstract

Small aromatic molecules are known to interact with poly(N-isopropylacrylamide) (PNIPA) based hydrogels, one of the most frequently employed polymers in temperature induced drug delivery systems. These interactions are poorly understood at the molecular level. In this article we investigate PNIPA both at the macroscopic and at the molecular level using measurements of swelling, differential scanning microcalorimetry (DSC), X-ray powder diffraction (XRD) and solid state ^{1}H NMR methods. The nature and the strength of the interactions affect the efficiency and kinetics of drug delivery. Phenols exert a major influence on PNIPA by reducing its phase transition temperature. The effect depends linearly on the phenol concentration, and is influenced also by the number of phenolic OH groups, as well as their relative positions. The strong interaction between phenol and the polymer that is detected by NMR hinders the crystallisation of phenol when the water is gradually evaporated. The aminoethyl phenol derivative dopamine has a much more limited effect, but in the opposite direction - the transition temperature increases slightly. The strong interaction observed among the dopamine molecules disables the polymer – dopamine interaction and favours crystallization of the dopamine when water is removed. These results reveal that embedding the drugs into polymer matrices for controlled delivery can alter the crystallinity of the stored molecules. As morphology is one of the crucial factors in delivery, this may compromise the rate and the efficiency of release.

Keywords responsive polymers, poly(N-isopropylacrylamide) gel, host-guest interactions, controlled drug delivery, phenols, dopamine

1. Introduction

Responsive hydrogels are among the most frequently proposed vehicles for targeted and controlled drug delivery. These smart stimuli-sensitive hydrogels change their physical properties in response to external physical (temperature, mechanical effect, electromagnetic radiation, electric or magnetic field) or chemical stimuli (solvent conditions: composition, dissolved species, pH, ionic strength) [1]. Their ability to store and release drugs puts them at the focus of interest as possible drug eluting systems. Furthermore, hydrogels can protect drug molecules from unfavourable conditions, such as the presence of enzymes or low pH [1]. Since temperature is a highly important parameter in the mammalian body, temperature-sensitive hydrogels have become the most investigated smart polymers [2-3]. The majority of thermosensitive hydrogels investigated in the past decades are synthetic polymers based on poly(N-isopropylacrylamide) (PNIPA) [2-4]. PNIPA hydrogels exhibit a non-linear volume phase transition (VPT) at a lower critical solution temperature (LCST) around 34 °C, which is close to the natural temperature of the human body [3,5]. On being triggered by stepwise temperature changes PNIPA can exhibit a pulsatile drug release profile [3,6-7].

In terms of kinetics and efficiency of controlled delivery, the nature and strength of the interaction between the drug molecule and the polymer chains are of vital importance. Chemical properties of the guest molecule and potential drug - polymer interactions are crucial in the swelling and release process, but these factors are poorly understood [8-9]. During the loading process free diffusion occurs into and out of the gel matrix, which is usually also related to the swelling of the gel [10-11]. The most common mechanism for discharge is diffusion. When a small molecule interacts with the polymer chains either reversibly or irreversibly, interactions in the gel matrix will determine the rate of release [10]. Interactions between the guest molecule and the gel network can inhibit release by binding of the drug to the polymer chains and/or by altering the swelling properties [8-9].

Molecules with phenolic OH can influence the swelling behaviour of the PNIPA in various ways [12-16]. While some molecules have only a slight effect or no effect at all, other guest molecules can change the transition temperature even at low additive concentrations [16, 17]. At a certain concentration (critical concentration) an abrupt collapse of PNIPA may occur already at or below room temperature. The temperature shift depends on the concentration and chemical structure of the additive. Several molecules and ions reduce the LCST [12-16]. Anionic (e.g., sodium dodecyl sulphate) and cationic surfactants (e.g., dodecyl trimethyl ammonium chloride) increase the LCST [18].

In our previous publications the influence of phenol, resorcinol and phloroglucinol, studied by scattering, calorimetric and later by NMR techniques were reported in detail (12-14, 19). It was found earlier that they exhibit an interaction with the polymer chains that depends on the number of the OH-groups [19]. Binding of phenol to PNIPA is mediated by hydrogen bonds between the amide group of the NIPA chain and the hydroxyl group of the phenol molecule [20]. The associative behaviour between phenol and PNIPA was also demonstrated by small-angle neutron scattering (SANS) and solid state NMR techniques [12-14]. By contrast, ^{1}H solid-state NMR results showed that dopamine does not associate with the polymer chains [15].

Here we report novel results that not only encompass all OH-substituted benzene molecules, together with another derivative 4-(2-aminoethyl)benzene-1,2-diol (i.e., dopamine), but also yield an interpretation based on the interactions between PNIPA hydrogels and these biomedically relevant molecules. Phenols are used widely as model molecules for several small aromatic drugs, including tyrosine [20]. Dopamine acts as a hormone and neurotransmitter in the human brain and nervous system. Abnormal levels of this molecule may result in Parkinson's disease and mental disorders [21]. The consequences of our findings on drug delivery vehicles will also be considered.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPA) (99%) and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED) (99%) were purchased from Fluka, *N,N'*-methylenebisacrylamide (BA) (99%), ammonium persulphate (APS) (99%) and dopamine hydrochloride (98 %) from Sigma-Aldrich, and phenol (99.5 %), resorcinol (99%), phloroglucinol (99%), catechol (99%), pyrogallol (99%) and hydroquinone (99%) from Merck. All chemicals were used without further purification. Some of the relevant physico-chemical properties of these aromatic probes, such as solubility in water and p*K*a, are listed in Table 1.

2.2. Synthesis of the polymer gel

PNIPA gel films with the molar ratio of [NIPA] / [BA] = 150 were synthesised by mixing 1 M aqueous solution of NIPA (18.75 mL) and 0.1 M solution of BA (1.225 mL) with water (4.9 mL) and TEMED (0.25 μL). After addition of APS (125 μL) to the mixture, polymerization took place at 20 °C within 24 hrs. The 2 mm thick gel films were dialyzed in double distilled water and cut into disks of 7 mm (for swelling experiments) and 17 mm (for

XRD and NMR techniques), then dried and stored above concentrated sulphuric acid. For calorimetric measurements dry gel disks were powdered (particle size 0.2-1 mm).

2.3. Swelling measurements

Swelling experiments were carried out by equilibrating dry disks in excess aqueous solutions with different initial concentrations (c_0) of the following compounds: phenol, resorcinol (1,2-dihydroxybenzene), phloroglucinol (1,3,5-trihydroxybenzene) and hydroquinone (1,4-dihydroxybenzene) (c_0 = 0-100 mM), catechol (1,2-dihydroxybenzene) (c_0 = 0-175 mM), pyrogallol (1,2,3-trihydroxybenzene) (c_0 = 0-175 mM) and dopamine hydrochloride (c_0 = 0-500 mM) for one week at 20.0 ± 0.2 °C. The dry gel/liquid ratio was 0.012. The equilibrium swelling ratio ($1/\varphi_e$) was determined from the mass balance as:

$$1/\varphi_e = \frac{m_{\mathrm{gel,dry}}/\rho_{\mathrm{gel,dry}} + (m_{\mathrm{gel,swollen}} - m_{\mathrm{gel,dry}})/\rho_{\mathrm{solution}}}{m_{\mathrm{gel,dry}}/\rho_{\mathrm{gel,dry}}}, \qquad (1)$$

where $m_{gel,dry}$ and $m_{gel,swollen}$ are the mass of the dry and the equilibrated gel disks, respectively. The density of the free liquid phase ($\rho_{solution}$) was taken as 1 g/mL, and that of the dry PNIPA gel ($m_{dry\ gel}$) is 1.115 g/cm^3 [13]. The reproducibility of the swelling degree of different batches was 1.5-3.5 %. The swelling degrees reported here are relative to that measured in pure water.

The aromatic guest uptake n_a (mmol/$g_{dry\ gel}$), was determined from the initial (c_0) and equilibrium molar concentrations (c_e):

$$n_a = \frac{c_0 V_0 - c_e V_e}{m_0}, \qquad (2)$$

where V_0 and V_e are the initial and equilibrium volume of the free liquid phase, respectively.

2.4. Differential scanning microcalorimetry (DSC)

DSC measurements were performed on a MicroDSCIII apparatus (SETARAM). Powdered PNIPA was used to reduce the swelling time. Approximately 10 mg powdered gel was incubated in 500 µL of aqueous solution of the different aromatic molecules for 2 hours, then heated from 10 to 40 °C at the scanning rate dT/dt = 0.02 °C/min. The values of the enthalpy ΔH were obtained in 25 mM solutions of the different guest molecules with a standard error of 5-10 %. At this concentration at 20 °C the gels are swollen in the aromatic solutions.

2.5. Solid state ^{1}H NMR spectroscopy

The dry gel samples were swollen in solutions of phenol (60 mM) and dopamine (1 M) in deuterated water for one week below LCST conditions. After reaching the equilibrium swelling ratio the gels were incubated above the LCST (25 °C for the phenol and 42 °C for the dopamine). The samples were regularly removed and placed into the NMR rotor to check the stability of tuning and matching conditions. In phenol solution the equilibrium state was reached after three weeks, while in dopamine solution it took only three days. NMR spectra were recorded on a Varian NMR system operating at ^{1}H at 600 MHz with a Chemagnetics 3.2 mm narrow bore triple-resonance T3 probe in double-resonance mode. The single pulse NMR measurements were carried out with 2.5 μs $\pi/2$ pulse and with 10 s repetition delay. For the combined rotation and multiple-pulse spectroscopy (CRAMPS) the wPMLG-5 sequences was used with the same pulse length and delay as for the single pulse spectra. The on-resonance position of the RF field lay outside the spectral response (-5 kHz from the centre of the proton spectra). The proton chemical shift resonances were referenced with a single pulse spectrum of a H_2O sample (4.8 ppm). Spinning speed was 10 kHz for both techniques.

2.6. X-ray powder diffraction (XRD)

For XRD measurements dry gel disks were equilibrated in phenol and dopamine solutions with the initial concentration of c_0 = 500 mM (1 week, 20.0 ± 0.2 °C). XRD spectra were obtained daily from the disks during the drying process at room temperature on a PANanalytical X'pert Pro MPD XRD powder diffractometer. [22] The XRD response of the loaded samples was corrected for the background signal of the glass sample holder and the signal of the gel equilibrated in pure water as well as in the identical state of drying. For comparison pure model drugs were freshly recrystallized from water and dried on the glass sample holder.

3. Results and discussion

3.1 Swelling measurements

Dry PNIPA gel disks were equilibrated in excess aqueous solutions of the OH-substituted phenols with different initial concentrations. All phenols reduce the degree of swelling and induce rapid collapse at a "critical" concentration (c_{crit}) that is characteristic of the guest molecule (Fig.1, Table 1). The data in Table 1 also show that these critical concentrations are related neither to the pK_a nor to the solubility of these compounds. Comparing the phenols having OH groups in meta positions (phenol, 1,2-dihydroxybenzene and 1,3,5-

trihydroxybenzene) a systematic shift can be observed in c_{crit} with the increasing number of hydroxyl groups: more OH groups results in lower critical concentration (Fig.1a). In the case of ortho phenols (1,2-dihydroxybenzene and 1,2,3-trihydroxybenzene) the systematic shift exists but in a slightly different way: with increasing number of OH groups the critical concentration increases (Fig.1b). In other words, ortho positions increase, and meta positions decrease the critical concentration relative to phenol at 20 °C (Table 1), and the shift in the critical concentration is the greatest for trihydroxy-benzenes. On comparing phenols with two OH groups, the sequence is meta < para < ortho. The shape of the swelling curves is also affected by the dissolved small molecule. Multiple OH substituted phenols show a stronger influence already below the critical concentration and at the same time their transition range is wider. Generally speaking, if the effect of the concentration below VPT is more pronounced then the phase transition appears to be wider and less steep (Table 1). Introducing an aminoethyl group into 1,2-dihydroxybenzene, however, changes the interactions dramatically: contrary to phenols, no VPT occurs even in very concentrated (c_0 = 1 M) solutions of dopamine (not plotted here).

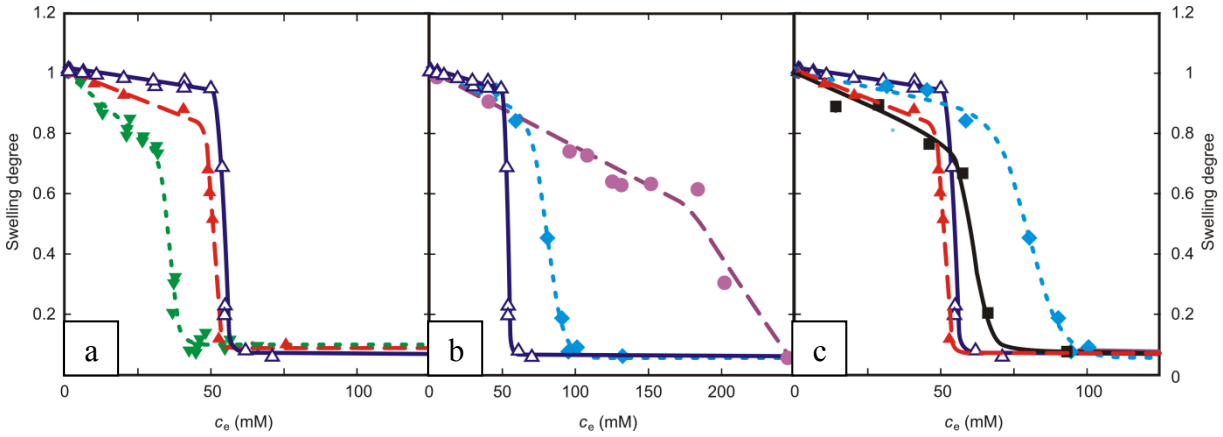

Fig.1 Swelling degree of PNIPA hydrogel in different phenolic molecule solutions at 20 °C as the function of equilibrium concentration in the free liquid phase. (a) Meta substituted phenol solutions: Δ phenol, ▲1,3-dihydroxybenzene (resorcinol), ▼ 1,3,5-trihydroxybenzene (phloroglucinol) (b) Ortho substituted phenol solutions: Δ phenol, ◆ 1,2-dihydroxybenzene (catechol), ● 1,2,3-trihydroxybenzene (pyrogallol) (c) Δ phenol, ◆ 1,2-dihydroxybenzene (catechol), ▲1,3-dihydroxybenzene (resorcinol), ■1,4-dihydroxybenzene (hydroquinone).

Table 1. c_{crit} and ΔH values of the phase transition, and slopes[a] of the swelling isotherms of PNIPA hydrogel in aqueous phenol solutions

	Phenol	Meta		Ortho		Para
		1,3-dihydroxy-benzene (resorcinol)	1,3,5-trihydroxy-benzene (phloroglucinol)	1,2-dihydroxy-benzene (catechol)	1,2,3-trihydroxy-benzene (pyrogallol)	1,4-dihydroxy-benzene (hydroquinone)
pK_a (25 °C)[b]	9.99[c]	9.32; 11.1[c]	8.45[c]	9.34; 12.6[c]	9.01[d]	9.85; 11.4[c]
Solubility in water, 20 °C (M)[e]	0.892[f]	9.082[g]	0.079[h]	4.096[i]	4.956[j]	0.636[k]
c_{crit} (mM)	53	50	36	81	194	62
ΔH (J/g) (c = 25 mM)	71±6	68±6	64±6	67±6	67±6	59±5
Initial slope (1/mM)	$-1.4\cdot10^{-3}$	$-4.7\cdot10^{-3}$	$-6.0\cdot10^{-3}$	$-2.7\cdot10^{-3}$	$-2.8\cdot10^{-3}$	$-5.5\cdot10^{-3}$
Slope in transition range (1/mM)	$-161*10^{-3}$	$-111*10^{-3}$	$-87*10^{-3}$	$-35*10^{-3}$	$-7.3*10^{-3}$	$-47*10^{-3}$

[a] the error of the slopes is 8-20 %; [b] pK_a values for dopamine are 9.0, 10.6 and 12.1 [23]; [c] [24]; [d] [25]. [e] Solubility of dopamine hydrochloride is up to 0.527 M [26]; [f] [27], [g][28], [h][29], [i][30], [j][31], [k][32].

3.2 Differential scanning microcalorimetry (DSC)

The phase transition of PNIPA swollen in the aqueous aromatic solutions was also measured by high sensitivity differential scanning microcalorimetry. All the phenols lower the phase transition temperature T_{VPT} proportionally to their concentration (Fig.2). The slope of the T_{VPT} - concentration plot rises, while the enthalpy corresponding to the phase transition ΔH in 25 mM solutions decreases slightly but systematically with increasing number in the group of meta-substituted phenols (Fig.2, Table 1). These observations, however, are not valid for ortho and para substitutions (Table 1). Dopamine increases the phase transition temperature, but the impact is much more limited. As neither the solubility nor the acid/base properties provide an explanation for the opposite effect of phenols and their aminoethyl derivative, an understanding of the intramolecular interactions is required.

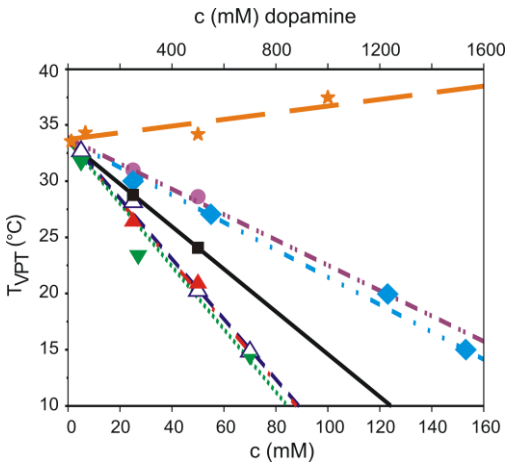

Fig.2 T_{VPT} of PNIPA in aqueous solutions from DSC measurements. For dopamine the upper concentration scale applies. Δ phenol, ◆ 1,2-dihydroxybenzene (catechol), ▲1,3-dihydroxybenzene (resorcinol), ■ 1,4-dihydroxybenzene (hydroquinone), ● 1,2,3-trihydroxybenzene (pyrogallol), ▼ 1,3,5-trihydroxybenzene (phloroglucinol), ★4-(2-aminoethyl)benzene-1,2-diol (dopamine).

For comparative investigations we selected one of the T_{VPT} decreasing molecules, phenol, and the T_{VPT} increasing dopamine. The effect of the aromatic concentration was studied by DSC on gel samples fully swollen at the initial temperature (20 °C) of the measurement. Raising the phenol concentration spectacularly lowers the temperature of the transition and broadens the response curve. The process becomes slightly but systematically more endothermic (Fig.3a, Table 2). With dopamine only a moderate upward shift in the position of the endothermic signal is observed. The broadening of the phase transition peak becomes obvious only from 500 mM dopamine concentration on (Fig. 3b). Although at 20 °C no macroscopic deswelling occurred, the enthalpy of the transition drops sharply in the 1 M dopamine solution in the scanning experiment (Fig. 3b). The broadening of the phase transition with both phenol and dopamine may indicate slower relaxation of the PNIPA chains during the VPT as the concentration increases and/or reduced heat conductivity as water is expelled.

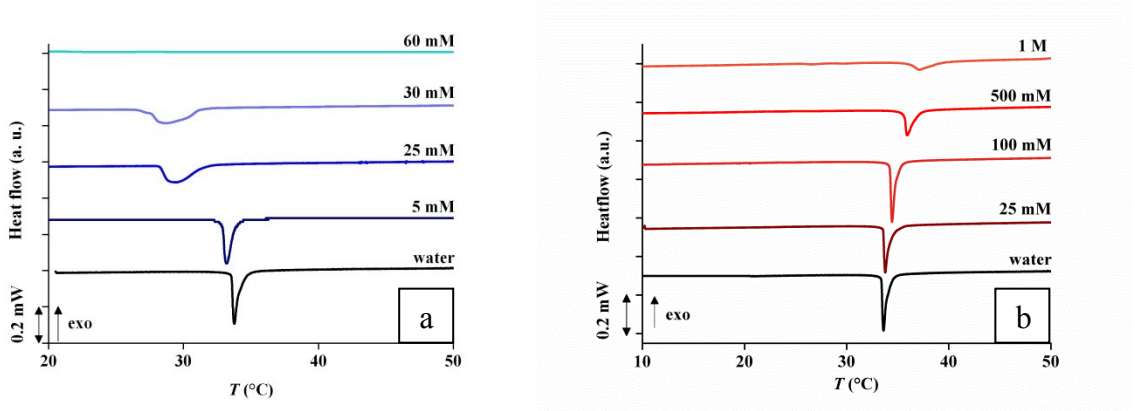

Fig.3 DSC response of powdered PNIPA gel in (a) phenol and (b) dopamine solutions with different concentrations. Successive curves are shifted vertically for clarity.

Table 2. ΔH values of the phase transition of PNIPA hydrogel swollen in aqueous phenol and dopamine solutions

Phenol		Dopamine	
c (mM)	ΔH (J/g)	c (mM)	ΔH (J/g)
0	57±5	0	57±5
5	60±5	25	55±5
25	71±6	100	60±6
30	70±6	500	50±5
60	72±6	1000	33±3

Guest molecules and ions affect the macroscopic properties of PNIPA by specific interactions with the polymer chains, or by changing the quality of the solvent. When aromatic molecules associate with the polar groups of the gel, they replace matter in the hydration layer, and disrupt the cooperative water ordering around the chain. As a result, the chain becomes more hydrophobic and the LCST shifts to lower temperatures. Phenols can associate with PNIPA by hydrogen bonding between the hydroxyl group(s) and the amide group. Thus, the entropy of the system increases and the reduction in the number of the hydrogen bonds leads to a complex that segregates from water. The effect of phenols on the swelling and thermal behaviour of PNIPA is complex, because both the number and the steric position of the hydroxyl groups influence the hydrogen bridging affinity and thus the critical concentration as well as the temperature and enthalpy of the phase transition (Figs. 2, 3b, Table 2). Although phenol has a significant effect on the swelling and thermal properties of PNIPA in aqueous media, this effect in the dry state is limited, indicating that the interaction between phenol and PNIPA can be mediated by the presence of water molecules [33]. Dopamine has virtually no influence on the swelling properties up to 1 M concentration and only a slight effect on the

thermal behavior of the gel, shifting T_{VPT} to higher temperatures, which suggests a physically different effect from phenol and an improvement in solvent quality.

3.3 ^{1}H proton solid-state NMR measurements

To understand the effects of the two aromatic guest molecules, two kinds of ^{1}H techniques providing different information were performed. The gels were studied above LCST conditions, where the otherwise freely moving organic chains form thick hydrophobic walls that still confine the swelling liquid [34]. The single pulse spectra (Fig.4) supply information about the more mobile species, the signals of the less mobile ones being broadened by dipolar interactions. The CRAMPS technique suppresses the broadening effect of the dipole-dipole interactions and the signals of the less mobile components are better resolved (Fig.5). In exchange for the improvement in resolution, the signals of the mobile components are distorted. Both single pulse spectra (Figs 4a and 4b) contain sharp signals. These signals (4.5 – 5 ppm) in the spectrum of the phenol swollen gel belong to the cavities containing liquid water [15]. No sharp signal corresponding to phenol can be observed, which indicates that these voids are free from solute molecules. The spectrum of the gel swollen in dopamine solution contains only one water signal, but sharp resonances of dopamine are also present, implying that the cavities are filled with dopamine solution. This solution is extremely concentrated: the ratio of the integral values indicates that the concentration is 8 M. In the CRAMPS spectra (Fig.5) the sharp signals are distorted, but the signals of the less mobile components are clearly distinguishable. These signals correspond to the precipitated hydrophobic polymer and small molecules with low mobility. Previous two dimensional solid state NMR measurements [14-15] proved that the phenol molecules are connected by strong H-bonds to the acrylamide side groups. By contrast, interactions between dopamine and the gel molecules could not be detected [16]. The dopamine signals present in the CRAMPS spectra showed that a substantial part of the dopamine molecules have only very low mobility, indicating oligomerization or even the polymerization of dopamine. Lee et al. [35] have suggested that the presence of catechol and amine groups could be the driving force of such polymerization. The detailed mechanism, however, is not known.

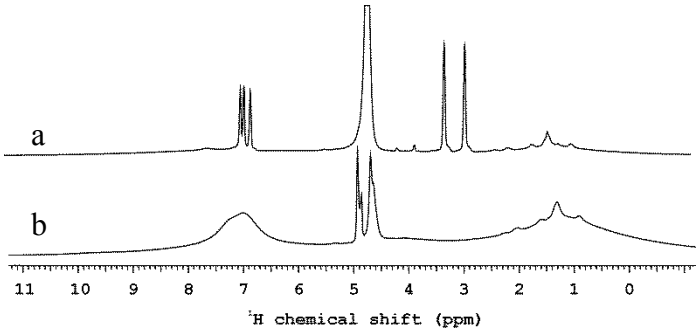

Fig.4 Single pulse solid state ^{1}H NMR spectra of PNIPA above the LCST at spinning speed of 10 kHz; (a) dopamine containing gel (b) phenol containing gel.

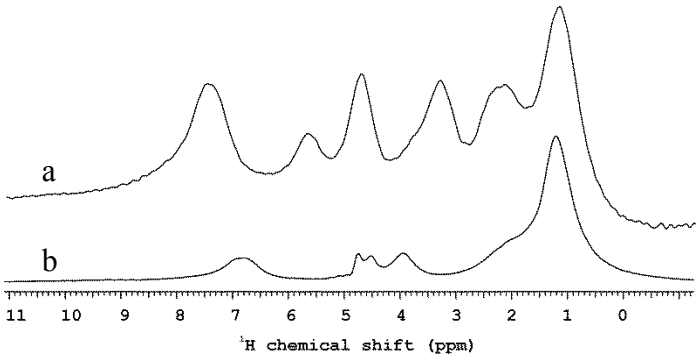

Fig.5 CRAMPS solid state ^{1}H NMR spectra of PNIPA above the LCST with spinning speed of 10 kHz: (a) dopamine containing gel (b) phenol containing gel.

3.4 X-ray powder diffraction (XRD)

Direct interactions between the solute molecules and the polymer chains are expected to influence the drying process of the guest molecule - gel systems. The interactions revealed above can influence the crystallinity of the guest molecules inside the gel after drying. The drying of gel disks swollen in the two aqueous solutions of the two selected model drugs – T_{VPT}-decreasing phenol and T_{VPT}-increasing dopamine – were measured by XRD. The XRD signals of phenol and dopamine recrystallized from their 500 mM aqueous solutions are compared in Fig. 5a and c. The signals obtained from PNIPA swollen in pure water showed that the gel remains amorphous. The lack of crystallinity in the XR diffractograms of the phenol-loaded gel (Fig.5b) is the sign that crystallization of phenol confined in the gel is inhibited. Phenol has a strong effect on the swelling behaviour of PNIPA in aqueous solutions, but its influence on the thermal decomposition of the gel in dry conditions is moderate and mediated by water [33]. This implies that the interaction between phenol and water plays a major role in the phenol - PNIPA system. This behaviour contrasts with that

observed during the drying of the dopamine-loaded gel, which is characterised by slow crystallisation of the dopamine (Fig.5d). A strong dopamine-dopamine interaction will inhibit the dopamine - PNIPA interaction, and thus foster crystallization.

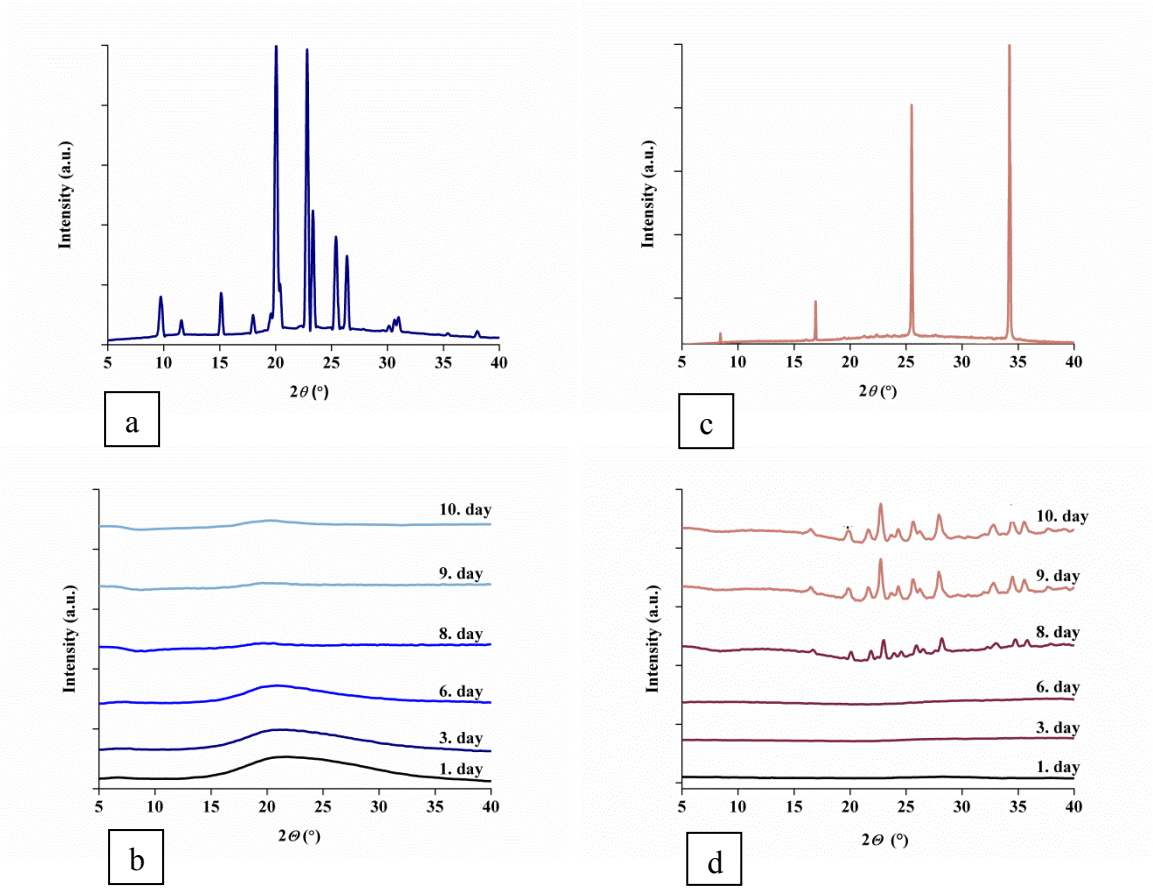

Fig.5 XRD responses of the guest molecules and the guest – PNIPA systems during the drying process: (a) phenol, (b) phenol loaded PNIPA gel, (c) dopamine hydrochloride oriented by the glass sample holder during drying, (d) dopamine loaded PNIPA gel: all the peaks can be identifyed in the reference patterns of polycrystalline dopamine hydrochloride (CSD [36], Mercury [37], DOPAMN01 [38], DOPAMN02 [39], PDF-ICDD [40], PDF-00-27-1942 [41],PDF-00-35-1914 [42], PDF-45-1642 [43]).
Successive curves are shifted vertically for clarity.

Conclusions

The influence of guest molecules and ions on the phase transition of PNIPA gels strongly depends on their chemistry. It was found that all the phenols have a major impact on the phase transition of PNIPA gels. In aqueous solution they reduce the temperature of the transition proportionally to their concentration and affect the rate of heat release. The extent depends

both on the number and on the position of the OH groups. When the OH groups are in meta position, increasing their number reduces the concentration required to initiate a phase transition already at 20 °C. Increasing the number of the OH groups in adjacent position, on the contrary, shifts this concentration to higher values. According to the NMR results, as the phase transition conditions are approached the phenol molecules interact practically exclusively with the porous polymer walls, leaving behind free water molecules in the confined voids. This interaction hinders the crystallisation during the drying of the gel, giving rise to amorphous phenol. Dopamine has only a limited influence on the phase transition temperature, even slightly increasing it at 1M. The results of various methods prove that a preferential strong dopamine – dopamine interaction prevents the interaction with the polymer even in confined conditions. The dopamine crystals that gradually develop during the drying process indicate that the lack of polymer – dopamine interaction and the strong dopamine – dopamine interaction fosters crystallization.

From these observations two conclusions can be drawn. Firstly, embedding drugs in polymer matrices for use as controlled delivery vehicles can influence the crystallinity of the stored molecules. As morphology is one of the crucial factors during delivery, the rate of the release may be severely influenced. Secondly, by corollary, strong drug – polymer interactions also reduce the amount of drug released.

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