Polymer 49 (2008) 3744–3750

Contents lists available at ScienceDirect

Polymer

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

Equilibrium swelling behavior of thermally responsive metal affinity hydrogels, Part II: Solution effects

Ganesh Iyer [a], L.M. Viranga Tillekeratne [b], Maria R. Coleman [a], Arunan Nadarajah [a,*]

[a] Department of Chemical and Environmental Engineering, University of Toledo, Toledo, OH 43606, USA
[b] Department of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH 43606, USA

ARTICLE INFO

Article history:
Received 11 April 2008
Received in revised form 23 June 2008
Accepted 23 June 2008
Available online 27 June 2008

Keywords:
Hydrogels
Thermal responsive
Metal affinity

ABSTRACT

In a previous study the effect of compositional variations on the equilibrium swelling of co-polymer hydrogels designed and synthesized from *N*-isopropylacrylamide (NIPAAm) and vinyl iminodiacetic acid (VIDA) monomers was investigated [Iyer G, Tillekeratne LMV, Coleman MR, Nadarajah A. Polymer, in this issue, doi:10.1016/j.polymer.2008.06.037]. The gels have both thermally responsive swelling and metal affinity properties and the effect of solution conditions on the equilibrium swelling of copper chelated and unchelated gels is studied here. In contrast to their sharp phase transition behavior in DI water, buffer solutions unexpectedly caused swelling of both gels to be the same and lose the sharp phase transition. Imidazole solutions had the expected phase transition behavior with increasing swelling and loss of phase transition of the unchelated gels which were partially reversed by copper chelation. Other small non-binding molecules, such as phenol, had minimal effects on the swelling behavior. Chicken egg white lysozyme solutions caused both gels to have reduced but equal equilibrium swelling and lose their sharp phase transition. These solution effects are explained in terms of salting in/salting out phenomena, the polarization of amide groups in VIDA, the solution pH and protein adsorption on hydrogel surfaces.

1. Introduction

In a previous study we described our efforts to develop *N*–isopropylacrylamide (NIPAAm)-based hydrogels incorporating the metal affinity ligand vinyl iminodiacetic acid (VIDA) groups employing a molecular design approach [1,2] The temperature induced phase transition behavior of these hydrogels was shown to be quite sensitive to the composition of NIPAAm, VIDA and crosslinker. Some combinations of these groups produced sharp phase transition behaviors similar to pure NIPAAm gels, while others resulted in more linear changes in swelling with temperature and the lack of a complete collapse even at high temperatures. These effects allowed us to propose a *phase transition phase diagram* for these gels [2].

The phase transition behavior of NIPAAm-based hydrogels is also quite sensitive to the solution conditions, such as solute concentrations and pH [3–6]. Pure NIPAAm hydrogels in fact have a salt concentration induced phase transition behavior in addition to a temperature induced one [7]. Such effects are of importance for the affinity hydrogels being developed here as pH and salt and/or buffer concentration are employed in metal affinity binding for the binding and release of target molecules. The concentration of the target molecules themselves can have an effect. Given the complexity of the composition effects on the phase transition behavior of these gels, a similar complexity is likely in the effects of solution conditions.

As discussed in the previous study the compositional effects on the phase transition behavior is largely driven by the balance between the hydrophobic and hydrophilic groups in the VIDA incorporated co-polymer hydrogel [2]. Temperature induced swelling and collapse of the gel are driven by pentagonal clathrate water structures around hydrophobic groups [7–15]. Hydrophilic groups instead contribute to swelling by binding to water molecules through hydrogen bonds which are less sensitive to temperature changes. When considering solution effects on these hydrogels other factors have to be considered as well. In ionic gels the addition of salt diminishes the osmotic pressure inside the gel and reduces its swelling [16]. The molecular processes giving rise to the osmotic pressure are also responsible for a more subtle effect known as the "salting out" phenomenon. This is the process by which the salt ions bind water molecules reducing the amount of free water available to form hydrogen-bonded networks [7,17]. As the salt concentration is increased this causes gel collapse to occur at lower temperatures for NIPAAm-based hydrogels. At low salt concentrations yet another effect is the "salting in" phenomenon where salt ions provide electrostatic shielding through counter-ions for charged groups [18,19]. This allows other nearby groups to become ionized increasing the swelling of the gel as a result.

* Corresponding author. Tel.: +1 419 530 8030; fax: +1 419 530 8076.
E-mail address: nadarajah@utoledo.edu (A. Nadarajah).

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doi:10.1016/j.polymer.2008.06.036

Table 1
Compositions, phase transition temperatures and sharpness factors of NIPAAm, NIPAAm–VIDA and Cu-NIPAAm–VIDA gels studied

Gel type	Composition (mg)	Solution	LCST (°C)	Sharpness factor
NIPA	CI-55	0.05 M buffer	31.5	−0.45
NIPA–VIDA	VIDA-200, CI-55	Phenol solution	40.5	−0.16
	VIDA-200, CI-55	1 M solution	NTO	–
	VIDA-200, CI-55	0.05 M buffer	NTO	–
	VIDA-200, CI-55	0.01 M buffer	NTO	–
	VIDA-200, CI-55	CEWL	NTO	–
Cu-NIPA–VIDA	VIDA-200, CI-55	Phenol solution	37.5	−0.21
	VIDA-200, CI-55	1 M solution	53.2	−0.23
	VIDA-200, CI-55	Phenol solution†	39.2	−0.17
	VIDA-200, CI-55	1 M solution†	41.1	−0.17
	VIDA-200, CI-55	0.05 M buffer	NTO	–
	VIDA-200, CI-55	0.01 M buffer	NTO	–
	VIDA-200, CI-55	CEWL	NTO	–

The VIDA and crosslinker amounts in these gels are listed while the remainder is NIPAAm for a total gel weight of 1500 mg. Here NTO means no sharp phase transition was observed, CI is the crosslinker and IM is imidazole. Solutions marked with † had the swelling of gels first measured in a salt solution and then again in DI water.

In this work we study the effect of buffer concentration, pH and target molecule on the temperature induced swelling and phase transition behavior of the metal affinity gels developed here. There have been very few studies of the effect of non-binding small molecule on the temperature induced phase transition behavior of affinity hydrogels [20,21], and none on the effect of binding small molecules and the effect of the size of the binding molecule. These studies will be done here for the VIDA co-polymer hydrogel. Based on these studies we will examine the effect of these conditions on the phase transition phase diagram proposed earlier for these gels. Such an understanding of these effects is needed to further improve the molecular design approach, so as to ensure that the gels retain their phase transition behavior under various environmental conditions.

2. Experimental

The syntheses of NIPAAm, NIPAAm–VIDA and copper chelated NIPAAm–VIDA (Cu-NIPAAm–VIDA) were described in detail in the first part of this study [2]. In brief, gels of 0.5 mm thickness were prepared by free radical polymerization of NIPAAm with co-monomer *N*-(6-(acrylamido)hexanoyl)-iminodiacetic acid sodium salt (VIDA) [1] and crosslinker *N*,*N*-methylenebisacrylamide (MBAAm) using photoinitiator riboflavin, in the proportions shown in Table 1. The gels synthesized were cut into discs of 1.6 cm diameter, washed thoroughly and chelated with copper using 0.05 M copper sulfate solution to obtain Cu-NIPAAm–VIDA gels. The gels were then thoroughly washed with DI water for 3–4 days and equilibrated overnight in DI water and the solution in which their equilibrium swelling behavior was to be studied.

Solutions for equilibrium swelling studies were prepared in DI water using sodium phosphate buffer (mono-basic and dibasic), imidazole (IM), phenol and chicken egg white lysozyme (CEWL), all purchased from Sigma–Aldrich. Deionized (DI) water purified from a US Filters filtration system was used for all the experiments. The change in equilibrium swelling diameter of the hydrogel samples in the above solutions between 20 °C and 80 °C was determined after equilibrating them at each intermediate temperature for 2.5 h, and is described in detail in the first part of this study [1]. Assuming isotropic behavior, the relative change in volume, V/V_0 can be calculated using the following equation:

$$V/V_0 = \left(\frac{D}{D_0}\right)^3 \quad (1)$$

where, V_0 and D_0 are the volume and diameter of the gel just after synthesis and V and D are the equilibrium final volume and diameter of the gel. The points of intersection of the tangents to the phase transition (PT) curve at the beginning and end of the phase transition were used to designate the onset and offset temperatures of the phase transition. The onset temperature was defined as the lower critical solution temperature (LCST) and the slope of the line connecting the onset and offset temperatures was defined as the sharpness of the phase transition of the gel. The NIPAAm–VIDA co-polymer gels were used as controls for the studies of solution effects on the phase transition behavior of the metal affinity gels. The various gels used for these studies, their compositions and solution conditions are listed in Table 1.

3. Results and discussion

3.1. Effect of buffer concentration

The equilibrium swelling of the gels as a function of temperature was studied in a pH 7 sodium phosphate buffer, which is a commonly used medium for chromatographic separations and biological applications. The salt effects of the buffer on pure NIPAAm and on unchelated and copper chelated VIDA–NIPAAm copolymer gels are shown in Fig. 1(a). The first observation is that the pure NIPAAm hydrogels are largely unaffected by the buffer retaining the phase transition behavior. There is a small decrease in

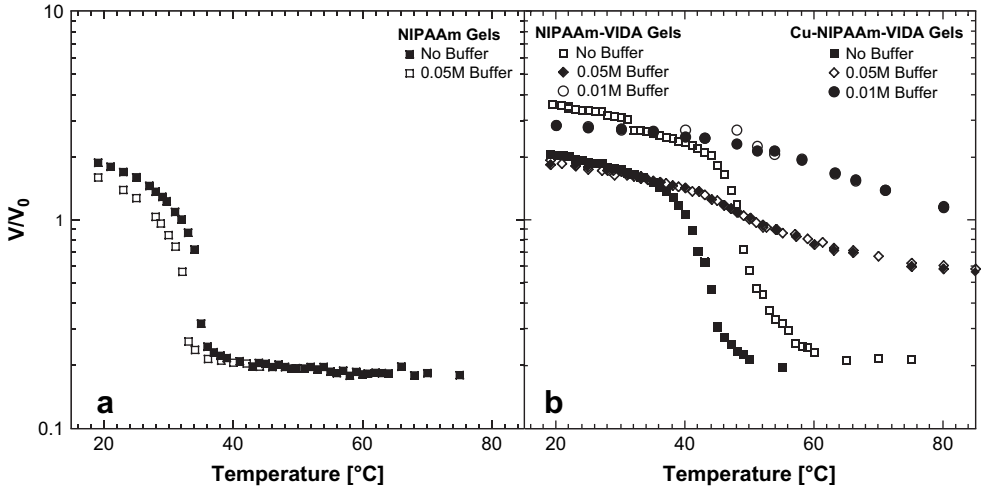

Fig. 1. The equilibrium swelling of gels in sodium phosphate buffer of pH 7 at various temperatures: (a) NIPAAm and (b) NIPAAm–VIDA and Cu-NIPAAm–VIDA gels.

the equilibrium swelling and the LCST from buffer addition. This effect on NIPAAm gels is well studied and the osmotic pressure/salting out phenomenon is the accepted explanation [5,22,23]. The buffer ions scavenge the free water to form bound water layers around themselves and this reduces the free water separating the clathrates around the hydrophobic groups in NIPAAm gels diminishing their swelling. Additionally, these hydrophobic groups can now be brought into closer proximity at lower temperatures, reducing the LCST.

Unlike the small changes seen in the swelling of pure NIPAAm hydrogels, the buffer concentration has a dramatic effect on the swelling and phase transition behavior of the VIDA co-polymer gels. First, both the NIPAAm–VIDA and the Cu-NIPAAm–VIDA hydrogels lost their phase transition behavior, as seen in Fig. 1(b). Both gels exhibited equilibrium swelling at all temperatures studied and did not attain their fully collapsed state even at temperatures over 80 °C. Second, the extent of the equilibrium swelling of the gels decreased with increase in the ionic strength of the buffer used. Third, and most significant, the extent of swelling behavior of both the copper chelated and unchelated co-polymer gels became the same over the studied temperature range.

The second of these effects can be readily understood. The higher buffer concentration reduces the osmotic pressure inside the gels by better balancing the ionic strength inside and outside the gels. As mentioned before, the osmotic pressure effect is related to the salting out effect of the buffer ions which will deplete the available free water through bound water shells. These effects will contribute to the shrinking of the gels with higher buffer concentrations. It should be noted that at 20 °C the buffer concentration caused the swelling of the unchelated NIPAAm–VIDA gel to be less than that in DI water, but not so for the copper chelated gel.

The other two effects are somewhat surprising and not as easily understood. If we consider only the NIPAAm–VIDA gel, then it is clear that the presence of the buffer salts causes the gel to remain swollen at high temperatures. This is contrary to most observations where the effect of salts, particularly sodium salts, is to suppress the swelling of NIPAAm-based hydrogels [4,6,7]. One possible reason for this is that when the hydrogels first come into contact with the salt solution, the pores in the outer layer of the gel become closed due to the elimination of free water [24]. This would cause an impermeable outer skin to form in the gel, thereby isolating the gel interior and preventing its collapse at higher temperatures.

In order to test this hypothesis, samples of both NIPAAm–VIDA and Cu-NIPAAm–VIDA gels were first swollen by equilibrating them in DI water at 20 °C and then collapsed at 70 °C. The collapsed gels were then transferred to a 0.05 M phosphate buffer solution equilibrated at 70 °C. Finally the gels were equilibrated at 20 °C in the same buffer solution. The results of these experiments are shown in Fig. 2. If an outer skin was the reason, the gels were swollen in buffer solutions at high temperatures, then transferring an already collapsed gel to a buffer solution at high temperature should prevent it from becoming swollen. Fig. 2 shows that the gel does swell from a collapsed state at this condition which means that an impermeable outer layer is not the explanation for this behavior.

In our view the only other explanation for this behavior involves the salting in of the hydrogel, in particular the carboxylic acid groups on it. For the unchelated NIPAAm–VIDA hydrogel, the close proximity of the carboxylic acids in the IDA group means that at most only one of these will be ionized in DI water. However, the presence of salt can cause these groups to ionize completely due to the electrostatic shielding provided by the Na^+ ions. Unlike the pentagonal clathrate water structures around hydrophobic groups which begin to collapse as water is heated above 30 °C, bound water around ions or ionic groups can remain even at high temperatures. These bound waters greatly limit the ability of neighboring hydrophobic groups to combine and initiate the phase transition. Thus, the addition of buffer salts to the solution has an effect similar to the addition of very high amounts of VIDA to the hydrogel by elimination of its phase transition behavior altogether, as discussed in our previous study [2].

The behavior of Cu-NIPAAm–VIDA gels is more puzzling. The NIPAAm–VIDA and the Cu-NIPAAm–VIDA gels are quite different because the former has ionic groups while the latter has no ionic groups due to Cu^{2+} chelation [1]. The loss of ionic nature and increase in hydrophobicity in Cu-NIPAAm–VIDA gels suggest that they should behave similar to pure NIPAAm gels as was observed in DI water. However, the close similarity of the swelling behavior of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels as shown by Fig. 1, suggests that at least in the presence of salts their swelling occurs by similar mechanisms. This means that the salting in mechanism described above for NIPAAm–VIDA gels should also work for Cu-NIPAAm–VIDA gels.

One possible explanation for the behavior of copper chelated gels comes from an earlier study which indicated that for gels of similar composition only ~73% of the functionalized iminodiacetic acid (IDA) groups in an NIPAAm-based co-polymer hydrogel were chelated by copper [1]. This suggests that the remainder was

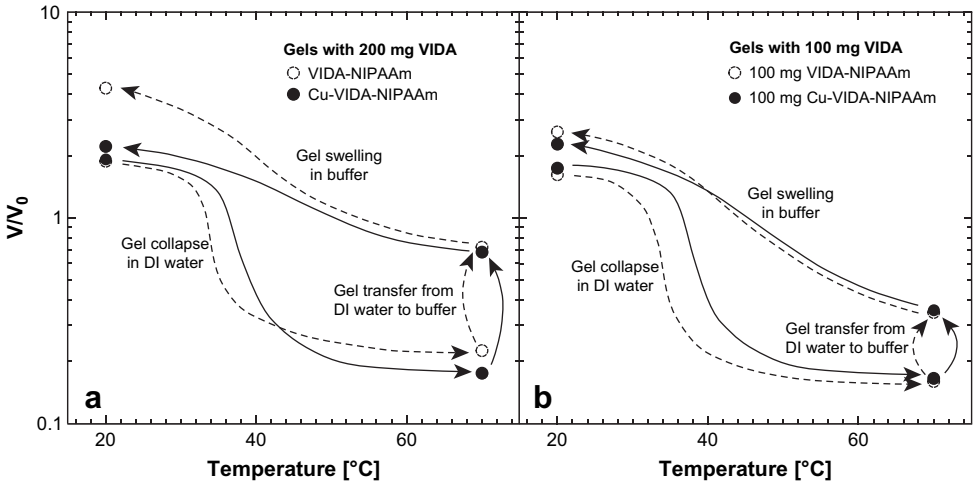

Fig. 2. The swelling of co-polymer hydrogels when they are cycled through DI water and buffer solutions and different temperatures: (a) Cu-NIPAAm–VIDA and NIPAAm–VIDA gels with 200 mg VIDA and 55 mg crosslinker and (b) Cu-NIPAAm–VIDA and NIPAAm–VIDA gels with 100 mg VIDA and 55 mg crosslinker. The solid lines show the changes in swelling for the copper chelated gels and the dashed lines for the unchelated ones.

unavailable for copper chelation, possibly because of their inaccessibility due to the tortuous nature of the gel network. It is likely that a similar fraction of the IDA groups in the NIPAAm–VIDA gel in this study is chelated by copper. This would not only partly explain why the phase transition behavior of these gels in DI water does not revert to that of pure NIPAAm gels upon copper chelation observed in our previous study [2] but may also partly explain why their behavior in salt solutions mimic that of the unchelated gels. By completely ionizing the unchelated ~27% of carboxylic acids in these gels through the salting in mechanism, the salt solution prevents the collapse of the gel at high temperatures.

While unchelated groups may provide part of the explanation, their small fraction is unlikely to completely explain why the swellings of both chelated and unchelated gels are identical in buffer solutions. For this to happen most of the VIDA groups should no longer be chelated by copper. In other words the presence of buffer ions must be causing tridentate bonds of the chelated copper to break down.

To understand this lack of copper chelation it is necessary to consider the structure of VIDA. VIDA was synthesized from an aminohexanoic acid, with the nitrogen being attached to a carbonyl group, rather than the customary alkyl group, resulting in an amide linkage in the final structure. The electron withdrawing nature of the carbonyl in the amide linkage will produce a resonance structure with positively charged nitrogen, as shown in Fig. 3 [25,26]. This nitrogen can no longer be involved in copper chelation as it cannot share its lone pair of electrons with the Cu^{2+} to form a coordinate covalent bond. The results from our earlier study suggest that this resonance structure is not the dominant one in DI water as the copper chelated gels have swelling behavior that is quite different from the unchelated ones as expected. However, in the presence of buffer ions Fig. 1 suggests that this resonance structure may be the dominant one resulting in copper chelated gels behaving the same as unchelated ones. Other studies have also shown that amide groups in NIPAAm hydrogels become similarly destabilized in the presence of salt solutions [22].

To verify the above hypothesis the effect of buffer solutions on the bound copper content (BCC) of the hydrogels was measured. Thick and thin samples of the Cu-NIPAAm–VIDA hydrogels were subjected to washing cycles with 0.05 M sodium phosphate buffer and DI water and the BCC of the gels was measured using induction coupled plasma (ICP) analysis before and after each washing step and the results are shown in Table 2. ICP analyses of thoroughly dried samples were performed in a Thermolectron IRIS Interpid II ICP analyzer, after digestion in nitric acid and hydrogen peroxide using a standard protocol. As expected the copper chelation is unaffected by DI water. Washing with buffer did result in the leaching of Cu^{2+} ions from the gels. In a single washing step the thin (~0.5 mm) gel had greater leaching than the thick (5–7 mm) one which suggests that there were some diffusional limitations for the leaching process. However, after three washing steps the extent of leaching was the same for the thick and thin gels suggesting that equilibrium had been reached.

The results shown in Table 2 suggest that at best no more than ~20% of the chelated Cu is leached by the buffer. While some of the remaining Cu^{2+} ions in the gel may exist in a loosely bound state,

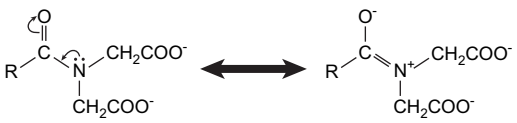

Fig. 3. Resonance structures of VIDA. The lone pair of electrons on the nitrogen is delocalized by resonance, forming a partial double bond with the carbonyl carbon. This results in a structure with positive charge on the nitrogen atom and a negative one on the oxygen.

Table 2
Bound copper content of gels and percentage of Cu^{2+} leached before and after washing with DI water and buffer

Washing solution	Number of washings	BCC (mg/g dry gel)		Cu^{2+} leached	
		Thick	Thin	Thick (%)	Thin (%)
DI water	3	13.55 ± 0.01	13.77 ± 0.66	0	0
Buffer	1	12.09 ± 0.52	11.69 ± 1.49	10	15
	3	10.91 ± 0.68	11.28 ± 0.29	19	18

most are likely to remain in a chelated state. This means that the polarization of the amide group in VIDA in the presence of buffer solutions does occur and its effects on the swelling of the gel are significant. However, this affects less than a quarter of the VIDA groups and so cannot completely account for the observed swelling behavior of the Cu-NIPAAm–VIDA hydrogels in buffer.

While the two mechanisms considered above provide only partial explanations for the buffer effects on Cu-NIPAAm–VIDA hydrogels, they may provide a complete one when considered together. In other words the buffer may cause the ~27% of unchelated VIDA groups to completely ionize and may cause a similar or greater fraction of the chelated ones to do the same. The combined effect of these ionizations will result in the Cu-NIPAAm–VIDA hydrogels having the same swelling behavior as the NIPAAm–VIDA hydrogels as shown in Fig. 1.

The above results suggest that the *phase transition phase diagram* proposed in the previous study [2] will have to be modified to include the effect of buffer or salt solutions on the affinity hydrogels. This is shown in Fig. 4 indicating the large effect of buffer concentration on the phase transition. While this large effect is somewhat unexpected, under normal circumstances this could be reversed through copper chelation as accomplished for these gels in DI water. Unfortunately, the effect of the salt solutions on the polarization of VIDA prevents this here, greatly limiting the region where sharp phase transition behaviors can be expected.

3.2. Effect of small molecule binding

To determine the effect of binding and non-binding molecules on the temperature dependent equilibrium swelling behavior of

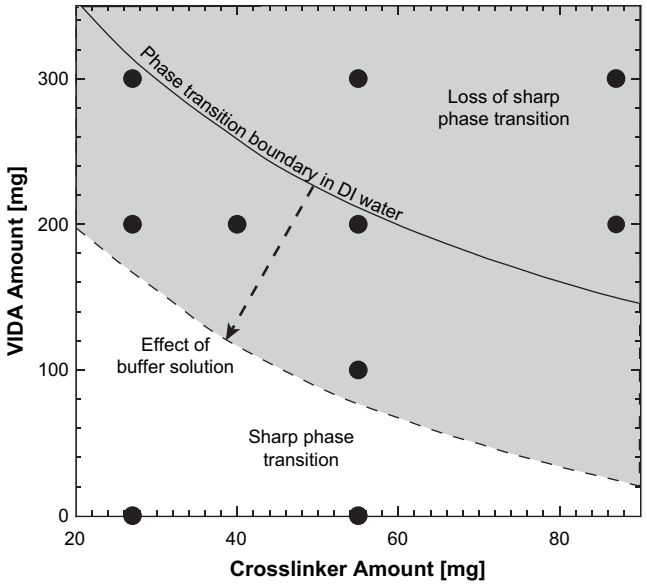

Fig. 4. Phase transition phase diagram for chelated and unchelated NIPAAm–VIDA gels in DI water and buffer solutions, showing the regions with and without sharp phase transition behavior. For all the gels considered here the amounts of VIDA and crosslinker are shown, with the remainder being NIPAAm for a total gel weight of 1500 mg.

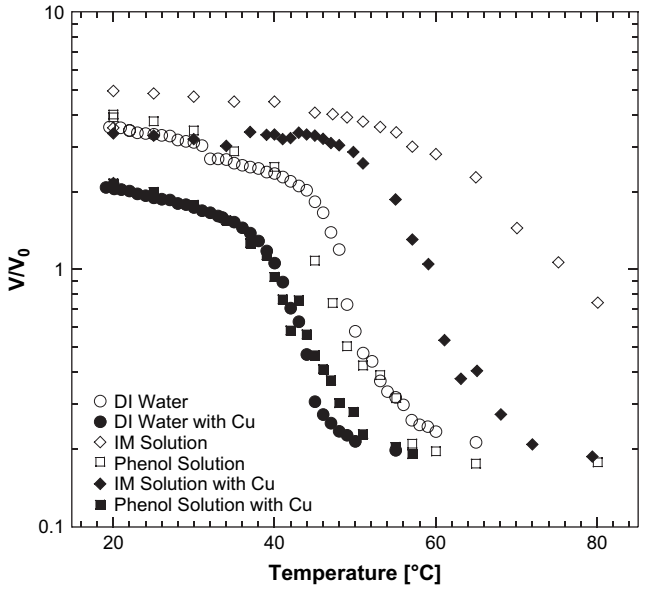

Fig. 5. Effect of DI water, 8.8×10^{-3} mmol/ml imidazole (IM) solution and 8.5×10^{-3} mmol/ml phenol solution on the equilibrium swelling of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels at different temperatures.

the NIPAAm–VIDA and Cu-NIPAAm–VIDA gels, their swelling was tested separately in equimolar solutions of imidazole and phenol. Of these two molecules, only imidazole can bind to Cu^{2+} in the Cu-NIPAAm–VIDA gel through a coordinate covalent bond and serves as a model-binding molecule. Neither imidazole nor phenol is expected to have any specific interactions with the unchelated NIPAAm–VIDA gel. These molecules also serve as low molecular weight analogs of proteins and other macromolecules.

As expected, the phase transition of both the gels remained unaffected by the presence of phenol in solution, but this was not the case for imidazole. The imidazole solution increases the swelling and the LCST for both hydrogels, as seen in Fig. 5 and Table 1. While Cu-NIPAAm–VIDA gels reached their fully collapsed state, the complete transition of the NIPAAm–VIDA gels could not be measured over the studied temperature range. There are three possible explanations for this behavior. First, imidazole ionizes in DI water to produce a positively charged imidazolium ion and an OH^- ion, increasing the pH of the solution. This pH change could be causing the observed behavior. Second, imidazole could be affecting the swelling through the same salting in mechanism as sodium phosphate solutions. Third, imidazole binds to the chelated Cu^{2+} ions in Cu-NIPAAm–VIDA gels, and at least for these gels this binding could be affecting their swelling behavior.

The second salting in explanation relies on the fact that imidazole, with a p*K* of 7, will be in a mostly ionized state in solution and will act as a $<10^{-2}$ M salt solution. The effect of the imidazole solution fits in with salt effects for the NIPAAm–VIDA hydrogel, but its effect on the swelling is even higher than for DI water at 20 °C. At this low concentration of the imidazole solution the osmotic/salting out effects are negligible, but it can contribute a little to the salting in effect on carboxylic acid groups described earlier. As a result the swelling of the gel is slightly higher than that for DI water or 0.01 M buffer at low temperatures. Similarly, the limited salting in effect is insufficient to completely eliminate the phase transition behavior as was the case with the buffer solutions of much higher concentration. However, the effect is sufficient to ensure that only a partial collapse of the NIPAAm–VIDA gel occurs even at 80 °C. These observations with imidazole solutions serve to confirm the salting in explanation of the effect of buffer solutions on swelling discussed earlier. They also suggest that the strong effect of salt solutions on the phase transition phase diagram shown in Fig. 5 is valid for higher concentrations, such as the 0.01 M and 0.05 M buffer solutions used earlier, but not for the $<10^{-2}$ M imidazole solution.

Considering the effect of imidazole binding to copper chelated gels, it should be noted that the imidazole solution did not cause the chelated affinity gels to have the same swelling behavior as the unchelated ones, as was the case with sodium phosphate buffer solutions. In fact Fig. 5 shows that the Cu-NIPAAm–VIDA gels retain the sharp phase transition even in imidazole solutions, although they do have a hydrophilic group-induced "tail" between 60 °C and 80 °C. This suggests that the copper ions remain chelated in the presence of imidazole solutions.

Imidazole molecules form a coordinate covalent bond with Cu^{2+} ions, which is quite stable unless imidazole is protonated. It is likely that this binding largely stabilizes the chelated copper in the affinity gels, despite the presence of excess imidazolium ions in solution. However, the imidazolium ions do cause some of the amide groups in VIDA to become more polarized and lose their copper chelation. This results in increased swelling and higher LCST than for these affinity gels in DI water and the formation of the hydrophilic group-induced tail. This suggests that the target molecules can stabilize copper chelation in affinity gels and partially counteract the effect of salt solutions which cause the loss of sharp phase transition as seen in Fig. 4.

3.3. Effect of pH

As mentioned earlier, the solution pH may be affected by the addition of phenol and imidazole to water in an unbuffered solution when examining the effect of small molecules on the swelling of co-polymer hydrogels. Buffered solutions were not used due their own effects on the phase transition which will preclude efforts to determine the effect of individual components on the phase transition behavior of the gels. Measurements showed that the pH of phenol and imidazole solutions varied between 6.9–7.7 and 8.9–9.6, respectively, which is likely to be significant enough to effect the swelling [3,27].

To determine whether solution pH was responsible for the changes in the phase transition behavior, the equilibrium swelling of the gels was studied as a function of pH in buffers of 0.01 M ionic strength at 20 °C and the results are shown in Fig. 6. Due to the buffer effect, the equilibrium swelling of both gels is almost equal and its value remain almost flat and unchanged over most of the pH range of 4–12. A small peak in the swelling was observed around pH 9 which is the pH range of imidazole solutions. A similar swelling peak at this pH for pure NIPAAm gels has been observed, but the reason for this is unclear [3]. This peak could partly explain the increased swelling of the affinity gels in imidazole solutions at 20 °C. However, it is unlikely to completely explain the large effects of imidazole solutions on the swelling of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels, particularly the loss of the sharp phase transition.

3.4. Effect of protein binding

Since the use of environmentally responsive hydrogels for protein separations has been of major research interest, the effect of macromolecules on the phase transition of the gels was studied using chicken egg white lysozyme (CEWL), which has a molecular weight of ~14,400 g/mol. The equilibrium swelling of the gels in CEWL was studied up to 60 °C with short equilibration times to ensure that the protein did not denature. The results are shown in Fig. 7. The equilibrium swellings of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels in CEWL solution were found to be equal at all temperatures and the nature of their swelling curves changed from

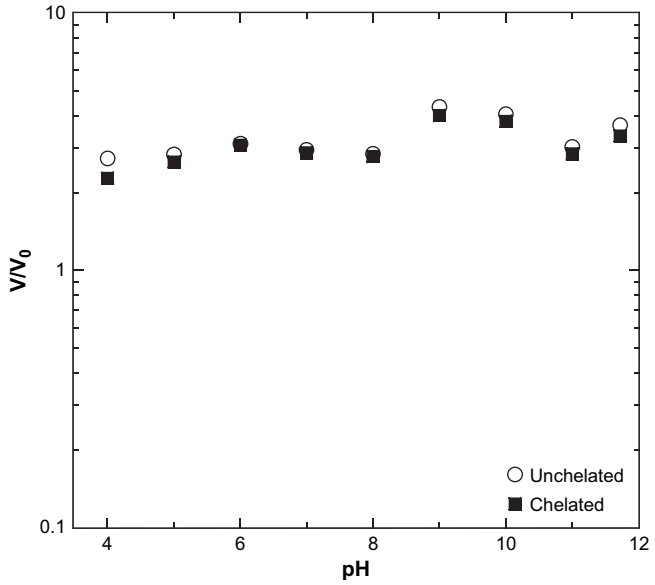

Fig 6. Effect of pH on the equilibrium swelling of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels.

sigmoidal to linear. Thus, the temperature dependent swelling of the gel is affected differently by large and small molecules and by binding and non-binding molecules.

While environmentally responsive affinity hydrogels are being studied as potential media for protein separations, Fig. 7 suggests that these materials loose their equilibrium swelling in the presence of even small proteins such as CEWL. One possible explanation is based on the fact that at neutral pH CEWL has a high overall positive charge. This suggests that they will bind with carboxylic ions from multiple VIDA groups in the unchelated NIPAAm–VIDA gels. Such binding could cause the gel to shrink as the VIDA groups wrap around the protein. However, given the relatively low fraction of VIDA groups in the gel it is not clear if this effect is enough to explain the observed gel shrinkage in the presence of CEWL solutions. For the chelated Cu-NIPAAm–VIDA gels the observed swelling behavior could be due to protein binding through the single histidine group in CEWL to the chelated copper. The swelling

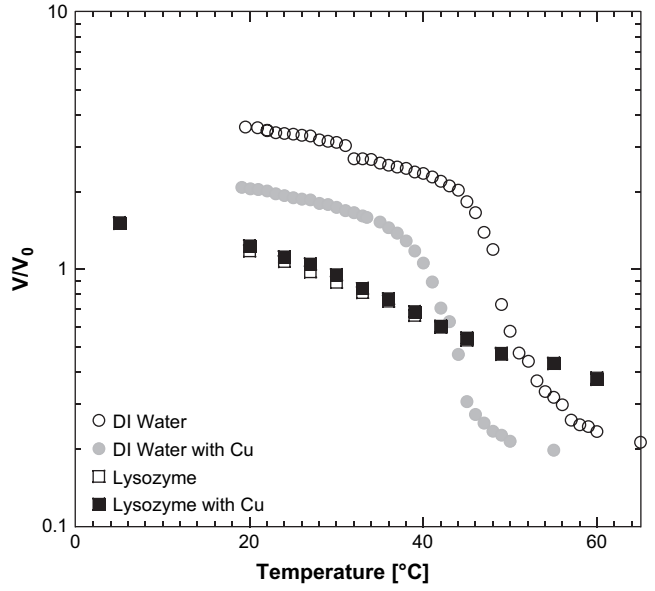

Fig. 7. Effect of chicken egg white lysozyme binding on the equilibrium swelling of NIPAAm–VIDA and Cu-NIPAAm–VIDA gels.

results with the imidazole solution suggest that the histidine group in CEWL should stabilize the chelated copper in Cu-NIPAAm–VIDA and bind to it. However, since there is only one histidine group in CEWL this mechanism does not allow for multiple bonds to explain the gel shrinkage.

The most likely explanation for the observed swelling behavior is the surface adsorption of proteins on the hydrogel surface. This is particularly true if the hydrogel has small pores that proteins cannot penetrate. The affinity hydrogels used here have pores larger than those of small proteins [1], but protein adsorption will still be significant given the hydrophobic nature of the NIPAAm gels. This means that protein adsorption can cause significant pore blockage preventing the diffusion of lysozyme molecules inside the gels. Consequently, an osmotic pressure gradient across the gels will develop as a result of the lysozyme concentration gradient, and this in turn will lead to the observed gel shrinkage in CEWL solutions. This shrinkage will occur in both chelated and unchelated gels. Such protein adsorption and associated gel shrinkage have been observed in NIPAAm gels before [28,29].

While this study was restricted to the effect of various molecules on the swelling behavior of the VIDA co-polymer gels, the above results suggest that in order to bind proteins to the affinity groups the gels may have to be designed more carefully. The pore sizes of the gels may have to be matched with those of the target protein, gels restricted from shrinkage, and gel surfaces engineered to minimize protein adsorption [29]. These efforts will have to be coupled with those to minimize the polarization of the amide groups in VIDA due to the presence of salt ions.

4. Conclusions

The swelling of chelated and unchelated NIPAAm–VIDA gels behave in some unexpected ways in the presence of solutions with different solutes. Sodium phosphate buffer solutions cause both gels to have the same swelling behavior with loss of sharp phase transition and lack of complete gel collapse even at 80 °C. The change in the phase transition behavior is most likely due to the salting in effect on the carboxylic acid groups in VIDA. The same swelling of the two gels is likely due to a combination of (a) the polarization of amide groups in the presence of the buffer ions causing the loss of chelated copper in Cu-NIPAAm–VIDA gels and (b) the salting in effect on the unchelated VIDA groups.

Phenol solutions had no effect on both types of gels. However, imidazole solutions cause the unchelated gel to swell more and lose its sharp phase transition behavior, but copper chelation partly reverses this effect suggesting that, unlike the buffer solution, imidazole stabilizes copper chelation in the gel. Changes in the solution pH also had a small contribution to the swelling of the gels. Chicken egg white lysozyme solutions caused both gels to shrink the same extent, with loss of sharp phase transition behavior. It is likely that the shrinking is caused by the surface adsorption of the protein on the gel resulting in pore blockage and an osmotic pressure gradient which reduces swelling. Use of these gels for protein and small molecule separations may require further modification of the gels to minimize the effect of polarization of amide groups due to the presence of salt ions and to reduce protein adsorption on gel surfaces.

Acknowledgements

This research was supported by a research grant from the USDA and by the Department of Chemical and Environmental Engineering, University of Toledo. Douglas Sturtz and Jonathan Frantz of the USDA assisted with the induction coupled plasma analysis of the gels samples.

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