Development of Environmentally Responsive Hydrogels with Metal Affinity Behavior

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Received 6 October 2006; accepted 27 November 2006
DOI 10.1002/app.26294
Published online 12 April 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This work describes initial efforts to incorporate affinity ligands within an environmentally responsive hydrogel. Metal affinity ligands were chosen as model affinity groups and thermally responsive *N*-isopropyl acrylamide/acrylamide copolymers were used as the base hydrogels. The $-NH_2$ group of the acrylamide serves as a reactive group for functionalization with metal affinity ligands. The gels were synthesized by free radical polymerization and Cu^{2+} was bound to the gel via 1,4-butanediol diglycidyl ether (BDE) as a linker and iminodiacetic acid (IDA) as a chelating ligand. The base acrylamide gels were also functionalized with metal affinity ligands to allow for comparison with thermally responsive affinity gels. The results show the effectiveness of this technique for both these types of gels, and an improved method to immobilize metal affinity groups on to thermally sensitive *N*-isopropyl acrylamide gels was also developed. It was seen that the yields for the reaction with BDE decreased with increased reaction time in both kinds of gels, whereas reaction with IDA showed a decrease in yields with increase in temperature for *N*-isoporpyl acrylamide gels and increase in yields for acrylamide gels. Further techniques were developed to overcome diffusional resistances and stresses in the thermally responsive *N*-isopropyl acrylamide gels so as to improve the distribution of Cu^{2+} ions. J Appl Polym Sci 105: 1210–1220, 2007

Key words: hydrogels; separation techniques; functionalization of polymers; phase behavior

INTRODUCTION

A variety of environmentally nonresponsive gels including agarose, cellulose, chitosan, and acrylamide with or without affinity have been studied as separations media for purification of organic/bio molecules by chromatography.[1–7] In contrast, hydrogels with environmentally responsive phase transition behavior (PTB) have found abundant interest for applications such as drug delivery/controlled release[8,9] and biocatalysis,[10,11] but not much interest has been shown in applying environmentally responsive gels towards purely selective separations or separation and detection strategies. Researchers such as Okano and coworkers have used the thermal responsiveness of *N*-isopropylacryamide gels towards the hydrophobic/hydrophilic separation of moieties in liquid chromatography.[12,13] Others, such as Mattiasson and coworkers, have used soluble *N*-isopropylacryamide gels with affinity groups to bind to a targeted biomolecule in a mixture and precipitate them out of solution along with the bound molecule by triggering their phase transition (affinity precipitation).[14] Other than these few studies, gels exhibiting PTB have not been employed for immobilized selective affinity separations.

Chromatographic media are required to withstand high packing volumes and pressure heads. As a result, composite and highly crosslinked gels are the most common media used today.[15,16] PTB gels cannot be crosslinked in this manner, as they tend to loose the desired phase transition behavior. These drawbacks have prevented their use for large scale separations media.

The advent of the ''Lab on a Chip'' concept[17,18] opens numerous potential applications that could take advantage of the PTB of environmentally responsive gels for *small scale separations media*. For example, there is a need to develop high sensitivity media for separating and concentrating analytes for sensors. Hydrogels that exhibit PTB would be an attractive material for such applications. While simple thermally responsive hydrogels could be used to concentrate analytes, the process would be nonspecific and would not allow for isolation of individual species. Combining highly specific affinity ligands within the size and shape selectivity of environmentally responsive hydrogels could overcome this disadvantage.

Our approach is to synthesize thermally responsive affinity hydrogels which incorporate immobilized metal affinity (IMA) ligands within NIPAAm gels, as a model system. Although any affinity group should be effective, metal affinity ligands were chosen as they are a widely used and well understood system. In this

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Contract grant sponsor: Interdisciplinary Research Initiation Awards Program, University of Toledo.

method, multidentate chelators which can complex metals are immobilized on supports.[5] Histidine tagged proteins bind to these chelated metals (transition metals such as Cu^{2+}, Ni^{2+}, and Zn^{2+}), allowing for their easy recovery from a protein mixture. Thus, incorporating metal affinity ligands into thermally responsive NIPAAm gels would provide a mechanism to selectively bind and concentrate histidine tagged moieties.

NIPAAm has a prominent phase transition at 32–35°C.[19,20] Copolymerizing NIPAAm with other monomers tends to shift the temperature and change the shape of the phase transition.[21–23] Thus, covalently binding affinity groups to the hydrogel backbone can effect the PTB. Copolymer gels of thermally responsive *N*-isopropylacrylamide-acrylamide (poly(NIPAAm-*co*-AAm)) and homopolymer gels of nonresponsive acrylamide (PAAm) were synthesized and functionalized with metal affinity ligands. Small concentrations of AAm were used in copolymer synthesis to provide reactive groups within the gel that could be used as sites for reacting with metal affinity ligands. The metal affinity ligand, iminodiacetic acid, was bound to the AAm pendant groups using an epoxide spacer arm and chelated with Cu^{2+} ions.

Specifically this article focuses on methods used to form thermally responsive gels with metal affinity groups. A comparative study of the effect of functionalization chemistries on both acrylamide and copolymers of *N*-isopropylacrylamide and methods to improve them will be presented. A systematic comparison of the impact of several parameters on the functionalization of environmentally responsive gels will be discussed. A brief discussion of the effect of metal affinity groups on the phase transition behavior of these gels will also be included.

EXPERIMENTAL

Materials

N-isopropyl acrylamide (*N*-IPAAm), *N*,*N*-methylenebisacrylamide (MBAAm) (ultra pure grade), and acrylamide (AAm) (ultra pure, electrophoresis grade) were obtained from Polysciences. *N*-IPAAm was recrystallized from hexane before use, but the rest were used without further purification. The photo-initiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPA, 99% purity), the linker 1,4-butanediol diglycidyl ether (95% purity), the chelating ligand disodium salt of iminodiacetic acid (IDA), and disodium salt of ethyl enediaminetetraacetic acid (EDTA, electrophoresis grade) were obtained from Sigma–Aldrich. Absolute alcohol was purchased from Fisher Scientific. Deionized water purified from a US Filters filtration system was used for all the experiments.

METHODS

Spectroscopic techniques

The chemical structure of copolymer gels was characterized using Fourier transform infrared (FTIR) spectroscopy (Digilab FTS 4000). Horizontal attenuated total reflectance (HATR) spectroscopy technique on the internal work bench of the FTIR was used to obtain sharp spectra. For this, thin films were directly cast on the germanium HATR crystal, dried under vacuum, and scanned at least 1024 times. Induction coupled plasma (ICP) analyses was done using a PerkinElmer Plasma II Emission spectrophotometer to determine the copper content of metal chelated polymer gels and to monitor the extent of functionalization with IDA and BDE.

Free standing hydrogel synthesis

UV-polymerization exhibited several advantages over thermal redox systems for this work, including short reaction times and better reproducibility in preparing hydrogels of various shapes. Syntheses of poly *N*-isopropyl acrylamide (PNIPAAm), polyacrylamide (PAAm), or copolymer gels (Poly(*N*-IPAAm-AAm)) were carried out by free radical polymerization using a photoinitiator (DMPA). The typical procedure for the synthesis of PNIPAAm gels is as follows. A monomer solution of 2 g (17.6 mmols) of *N*-IPAAm and 0.1 g (0.649 mmols) of crosslinker MBAAm was prepared in 5 mL of deionized (DI) water. Dry nitrogen gas was bubbled through the solution for 15 min at room temperature to remove any dissolved oxygen. A solution of 30 mg (0.117 mmols) of DMPA was prepared in 5 mL of ethyl alcohol and added to the monomer solution. The mixture was stirred and cast in polypropylene petri dishes under a UV-light of 365 nm (30 W, UV products XX-15). The gel formed at room temperature within 10 min, but the reaction was continued for 30 min to ensure completion. Following the reaction, gels were immersed in DI water for a week, with frequent water changes to remove any unreacted monomers. A method similar to the above was used to produce PAAm and poly(NIPAAm-*co*-AAm) copolymer gels of 7.72–23.8 wt % AAm. The yields of gelation were quantified using a simple gravimetric method. The yield is defined as the ratio of the dry weight of a thoroughly washed sample to the total grams of monomer used to make the sample. The samples were dried in a vacuum oven at 60°C and then weighed to obtain the dry weight. The average yields were as follows: PAAm, 90%; PNIPAAm, 95%; poly(NIPAAm-*co*-AAm), 98.6%.

Preparation of supported gel films

Supported gels were cast in a mold consisting of two glass or tinoxide coated glass substrates separated by

a spacer of 0.5 mm, 0.75 mm, or 1 mm thickness. One of the glass plates was coated with 3-methacryloxypropyltrimethoxysilane (bind silane) and the other with dimethyldichlorosilane (repel silane) to obtain a mechanically stable thin gel bound to one of the glass plates. A gel solution of the same composition as that used for free standing gels was transferred to the space between the plates by capillary action using a pipette. The mold was then UV irradiated and the supported hydrogel films were thoroughly washed before further use.

Gel functionalization

Both copolymer and PAAm hydrogels were functionalized with metal affinity ligands using well established chemistries.[24,25] Functionalization was carried out by cutting pieces $\sim$ 1.7 mm in diameter with a cork punch from different regions of a large hydrogel of 6–7 mm thickness to ensure consistency of the chemistry. A three step process consisting of successive reaction with BDE, IDA, and $CuSO_4$ was used to produce metal affinity hydrogels. For the BDE reaction step, the hydrogels were incubated at room temperature for 12 h in 60 mL of 0.3*M* NaOH solution containing 10 mL BDE and 60 mg $NaBH_4$. The modified hydrogels (PNIPAAm-AAm-BDE and PAAm-BDE) were then soaked in DI water for 30 min to remove residual reactants. For the second step, the hydrogels were immersed in 70 mL of 1*M* $NaHCO_3$ solution containing 5 g of IDA at 5°C for 24 h. The dependence of the extent of functionalization on the IDA reaction temperature from 5 to 60°C was examined. The 5°C reaction gave highest degree of functionalization with IDA for copolymer gels and was used for all subsequent studies. These gels were designated as PNIPAAm-AAm-IDA or PAAm-IDA.

To prepare metal chelated hydrogels, the PNIPAAm-AAm-IDA or PAAm-IDA hydrogels were incubated for 24 h in excess 0.05*M* $CuSO_4$ solution, washed multiple times, and stored in DI water. The IDA terminated gels can be regenerated by soaking the metal chelated gels in a solution of EDTA to strip off bound copper ions. The copper content of the gels was determined by incubating the gels in $CuSO_4$, washing with water to remove unbound copper, followed by a wash in 0.05*M* EDTA to remove the bound copper. The bound copper contents were determined by ICP using the wash solutions and reported on a normalized mass or area basis.

Equilibrium swelling studies

The lower critical solution temperature (LCST) of all hydrogels was determined by recording the relative mass ratios of the gels in water. The weights of the hydrogels at various temperatures were determined using a temperature controlled water bath (a Neslab GP300 attached to a chiller Neslab FTC 350) and a microbalance (Denver Instruments Model 2200). The gels were stabilized for 30 min at each temperature prior to data collection, to ensure that it was at steady state. The percent relative mass ratio (RM) was used to quantify the swelling of hydrogels as a function of temperature in the range of 5–70°C, PAAm concentration and functionalization chemistry. RM is defined as follows:

$$\mathrm{RM} = \left(\frac{W_w}{W_{\max}}\right) \times 100 \quad (1)$$

where W_w is the wet weight of the hydrogel at each temperature and $W_{\max}$ is the maximum swelling weight of the hydrogel measured at 5°C. The equilibrium swelling ratios (ESC) were also calculated for the above, but are not reported in this article.

RESULTS AND DISCUSSION

FTIR spectroscopy

The characteristic peaks for the AAm and NIPAAm gels were compared with the copolymer gels using HATR spectroscopy. The FTIR spectra for the fingerprint region of the pure polymer gels and of the copolymer gels with 13.6 wt % AAm monomer are shown in Figure 1. Although the percentage of acrylamide in the copolymer gels was very low and both acrylamide and *N*-isopropyl acrylamide are similar molecules, HATR spectroscopy produced excellent results in distinguishing their spectra. The characteristic single peak of *N*-isopropyl acrylamide (—NH, 3300 cm^{-1}) overlaps with the characteristic double peak from acrylamide (—NH_2, 3335–3190 cm^{-1}) which appears as a shoulder at a wavenumber of around 3190 cm^{-1} in the copolymer gel. As the acrylamide is 13.6% (wt %) of the copolymer gel, the intensity of this peak is rather diminished, but the intensity increased with increased AAm concentrations (not shown). Also the isopropyl group of NIPAAm was prominently observed in the copolymer at wavenumbers of 1366–1385 cm^{-1} and 2970 cm^{-1}. These results indicate that both monomers were successfully incorporated into the backbone of the gel network. The amide bond in the copolymer is observed at an intermediate wavenumber of 1650 cm^{-1} between those of NIPAAm and AAm.

Functionalization and chelation studies

The protocol used was originally employed to functionalize supports with —OH groups only.[24,25] This was employed in this study assuming that under the same reaction conditions the —NH_2 groups in the gels

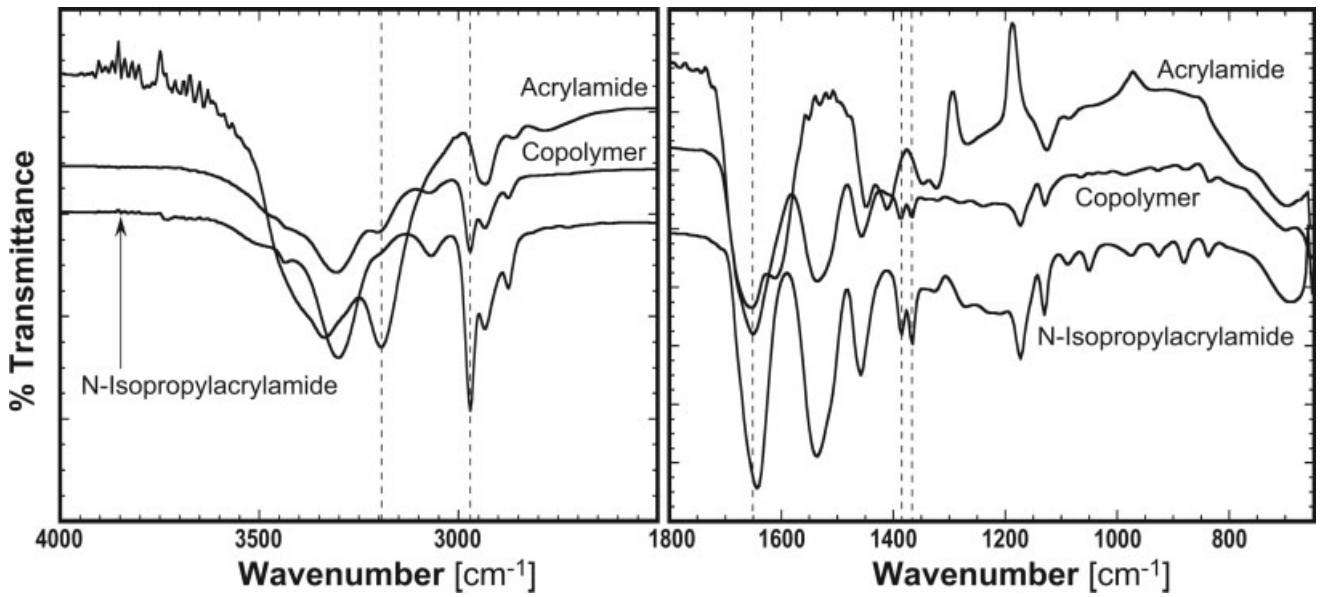


Figure 1 Comparison of FTIR spectra of AAM, NIPAAm, and copolymer gels. In addition to the NIPA peak at 3300 cm^{-1}, the copolymer gel shows a shoulder at 3190 cm^{-1} consistent with that of acrylamide. Peaks at 2970 and 1386–1385 cm^{-1} are consistent with NIPAAm in the gel. The amide peak at 1650 cm^{-1} is intermediate between that of AAm and NIPAAm.

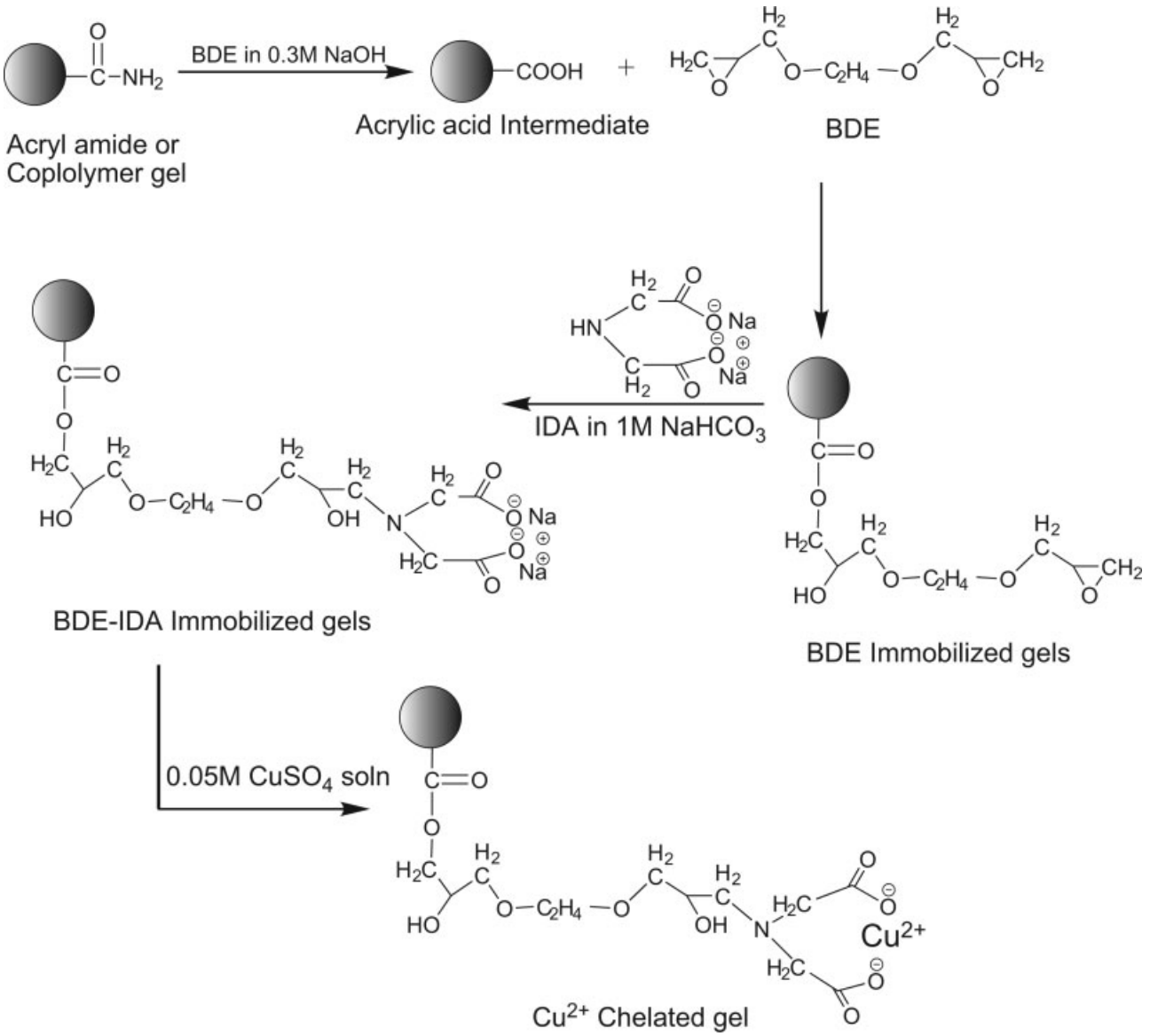
would have similar reactivity for BDE as the —OH groups. The gels successfully chelated copper, suggesting that the amide groups reacted with BDE and IDA under the prescribed reaction conditions. Although the protocol was effective for the hydrogels in this study, the reaction mechanism for the amide and hydroxyl functional groups with BDE could be different as shown in Scheme 1. There is considerable evidence in the literature showing that acrylamide can easily undergo conversion to acrylic acid through reaction with a base. For example, this principle has been used to develop partially ionized acrylamide gels by adjusting the basicity of the solution.[26,27] The $—NH_2$ of acrylamide is not a strong nucleofile, whereas the carboxylate anion of acrylic acid can react more readily with the epoxide. This suggests that the amide is first converted into an intermediate carboxylate anion, i.e., acrylic acid anion by NaOH, which can react with the epoxide to form an ester linkage to give BDE immobilized gels.

The chelating capacities of the various types of gels were estimated by measuring the bound copper ion contents (BCC). This reported capacity is based on an assumption of one to one binding between the IDA ligands and the Cu^{2+} ions. For analytical purposes, the compositions of the gels were fixed at the following weight ratios: for acrylamide gels AAm : MBAAm ratio of 18 : 1 and for copolymer gels NIPAAm : AAm : MBAAm ratio of 18 : 3 : 1. The average values of the BCC for functionalized and unfunctionalized gels are given in Table I. The values confirmed that both virgin PAAm and PNIPAAm-AAm copolymer hydrogels were successfully functionalized with BDE and IDA. Although there have been reports regarding chelation in gels with $—NH_2$ groups,[28] the ICP results indicate that the unfunctionalized hydrogels had negligibly low BCC and that the copper in the gels was only due to functionalization with metal affinity ligands. As shown in Table I, the large copper content of c-PAAm relative to c-PNIPAAm gels indicates that the acrylamide groups are necessary for functionalization of copolymers. The BCC will be used in the following sections as a measure of the effect of process parameters on the extent of functionalization with metal affinity ligands.

Effect of BDE reaction conditions

Initially the functionalized gels exhibited poor reproducibility of BCC. In particular there appeared to be considerable variation in BCC with wash time following the BDE reaction. Therefore, a study of the impact of washing time was performed to select optimum conditions. For all cases, the IDA step was carried out for 24 h at 5°C. For both PAAm and poly(NIPAAm-AAm) gels, an increase in washing time following the BDE reaction step led to a decrease in the BCC of the gels as shown in Figure 2.

To ascertain whether BDE was reacting with the polymer backbone or merely diffusing in and out of the gel, the BCC of the gels reacted with BDE for different time periods was measured. For all these cases the reaction was carried out at room temperature and a wash time of 30 min was used. As shown in Figure 3, the functionalization first increased with time, reached a maximum at about 12 h, and then decreased



Scheme 1 Functionalization carried out by reacting the amine functional groups in the gel with the epoxide BDE. The unreacted epoxy end of the BDE was further reacted with IDA, which in turn chelated the Cu^{2+} from $CuSO_4$.

steeply. This suggests that while the BDE reacts with the acrylamide groups on the gel backbone, the bound groups degrade with time.

This loss of functionality/reactivity of BDE during the reaction or washing step can be explained by two possible mechanisms. The first mechanism relies on the fact that BDE is bound to the acrylamide in the gels by an ester linkage. Ester linkages can easily undergo hydrolysis when subjected to a strong basic medium. Hence, although initially BDE reacts with the gel backbone through an ester linkage, a prolonged exposure to the basic medium could cleave this bond. This is suggested by a decrease in the copper content of the gels for BDE reaction times of greater than 12 h. A similar phenomenon could explain the loss of BDE groups during extended washing

TABLE I
BCC Values of Functionalized and Base Hydrogels

Sample	c-PAAm	c-PNIPAAm	c-copolymer	u-PAAm	u-PNIPAAm	u-copolymer
BCC (mg/g wet gel)	47.9	0.00	4.17	0.00	0.00	0.00

c-, functionalized gels; u-, unfunctionalized gels.

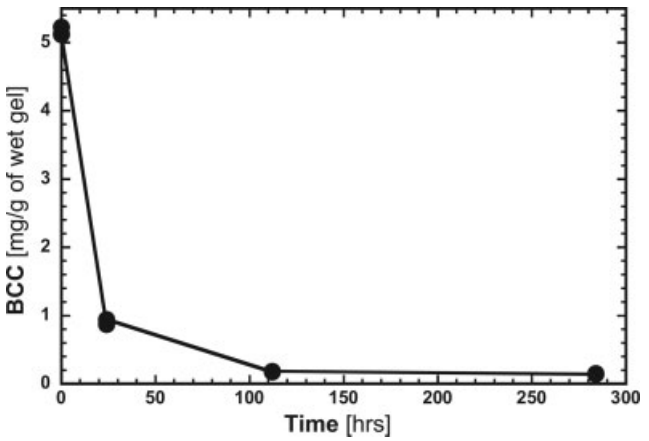


Figure 2 Change in BCC of copolymer hydrogels with an increase in washing time after the BDE functionalization step.

cycles. In addition, the gels can shrink in response to the ionic strength of the surrounding solution.[29–31] Such shrinkage was observed during BDE functionalization in 0.3*M* NaOH solution. The gels being washed are initially saturated with sodium hydroxide and are in a shrunken state. As may be expected, the rate of diffusion through gels in the shrunken state is very slow, causing localized increase in pH within the gel. This slow desorption rate would give ample reaction time for the residual NaOH in the gel to cleave the BDE from the gel backbone and compound the problem. This can be verified from the fact that the maximum loss of Cu is just within the first 24 h, during which time the gel is recovering from its collapsed state.

The second mechanism is based on the fact that epoxide functional groups undergo a nucleophilic ring opening to form corresponding alcohols in basic media.[32] This reaction is time and temperature dependent. Hence, a prolonged exposure (greater than

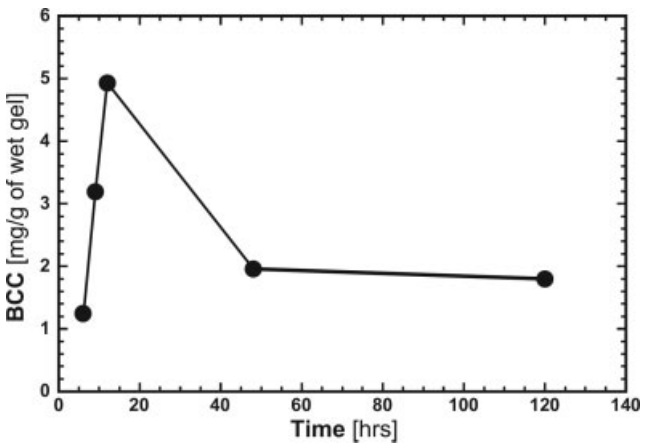


Figure 3 BCC of copolymer hydrogels as a function of BDE reaction time at 5°C.

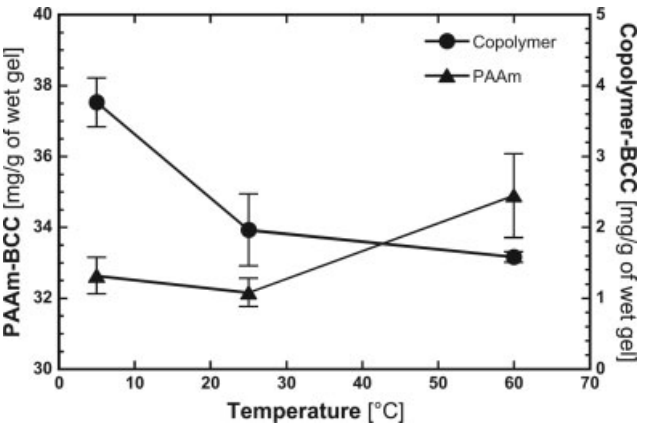


Figure 4 Impact of IDA reaction temperature on the BCC of PAAm and poly(NIPAAm-AAm) gels.

12 h) of the immobilized BDE groups to excess sodium hydroxide solution may cause the opening of the epoxide groups making them unreactive with IDA. Long washing times can denature the BDE in the gel in a similar manner, due to the extremely slow diffusion rate of basic medium out of the gel. It is likely that both mechanisms are contributing to the loss of BDE functionality. Following these studies, the BDE reaction and washing times were standardized to 12 h and 30 min, respectively, before functionalization with IDA.

Effect of temperature on IDA reaction

Traditionally, IDA functionalization has been performed by heating BDE activated supports in an IDA solution at 60°C.[24] Since the copolymer gels exhibit a lower critical solution temperature at about 32°C, a high reaction temperature of 60°C would result in a significant collapse of the gel matrix. To investigate the impact of temperature on the reactivity of IDA with bound BDE groups, PAAm-BDE and NIPAAm-AAm-BDE gels were reacted with IDA at temperatures ranging from 5 to 60°C. There was a small increase in the BCC with increasing reaction temperature for the PAAm gels as shown in Figure 4. This was expected since the rate of reaction would increase with temperature. In contrast, there was considerable decrease in BCC for the copolymer gels with increasing temperature (Fig. 4). These results suggest that there are two rate limiting steps governing IDA functionalization: the diffusion of reactant into the gel and the reaction rate. Either of these steps can be rate limiting, depending on the temperature and degree of swelling of the hydrogel. For the PAAm gel, which exhibits no thermally responsive swelling/collapse, an increase in temperature resulted in an increase in the rate of reaction with an overall increase in the BCC. However, the NIPAAm component of the copolymer exhibits a lower critical solution tempera-

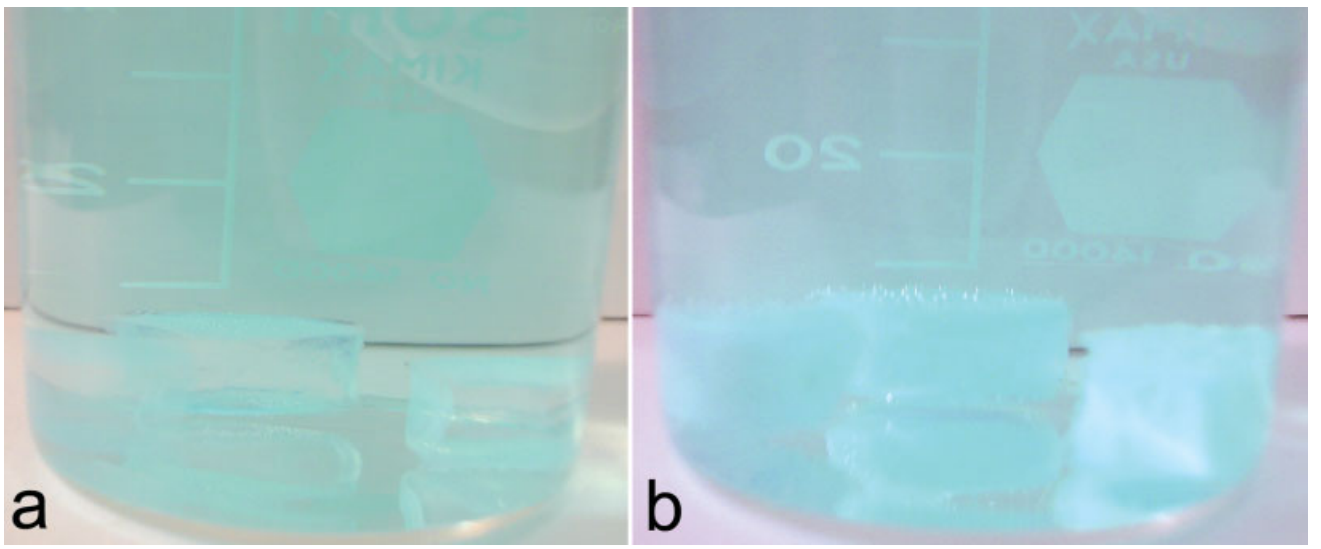


Figure 5 Copper adsorption on copolymer hydrogels; (a) functionalized copolymer hydrogel just immersed in 0.05*M* $CuSO_4$ solution and (b) functionalized copolymer hydrogel after 12 h in $CuSO_4$ solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ture (LCST) behavior[19,20] at temperatures greater than 32–35°C.

This shrinkage of the copolymer would result in high diffusional resistance for IDA moieties into the gel resulting in reduction of IDA concentration within the gel and corresponding decrease in overall reaction rates. In addition to the temperature effects, the high salt concentration of 1*M* $NaHCO_3$ and IDA in the surrounding solution can cause extreme osmotic pressure differences between the inside and the outside of the gel, causing the gel to collapse. This would result in even greater diffusional resistance in thermally responsive NIPAAm relative to the PAAm based gels. Therefore, IDA functionalization was carried out at 5°C for 24 h to ensure that diffusional resistance is minimized and sufficient reaction time is allotted.

Based on studies of the impact of reaction conditions on the functionalization of hydrogels, the following procedure was used for all further studies: (i) BDE reaction time was limited to 12 h, (ii) the washing time was 30 min to remove as much unreacted BDE as possible, and (iii) the IDA functionalization was carried out at 5°C for 24 h for both copolymer and AAM gels for comparison. To confirm the effectiveness of the low temperature functionalization procedure and the washing and storing steps, functionalized AAm and NIPAAm-AAm gels were tested with bound copper for leaching in DI water for up to 20 days. Samples of these gels were tested for their BCC by ICP at regular intervals and no evidence of leaching was found over time.

Physical properties of hydrogels

The impact of functionalization and copper chelation on the physical properties and appearance of the hydrogel was investigated. The transparent-colorless PNIPAAm-AAm-IDA hydrogel in Figure 5(a), turned bluish-green and opaque [Fig. 5(b)] on copper chelation. The gels had similar equilibrium swelling as virgin copolymer gels and did not show any significant changes in rigidity due to the presence of bound copper. However, PAAm-IDA hydrogels loaded with copper exhibited a change in color from white to blue and were significantly more rigid as seen in Figure 6(a). This transformation can be partially attributed to the larger concentration of copper ions in these gels when compared with the copolymer gels. The gels regained their original physical characteristics when copper was stripped using EDTA as shown in Figure 6(b).

A critical issue in developing environmentally responsive affinity hydrogels by this technique is the homogenous functionalization of the hydrogel. When

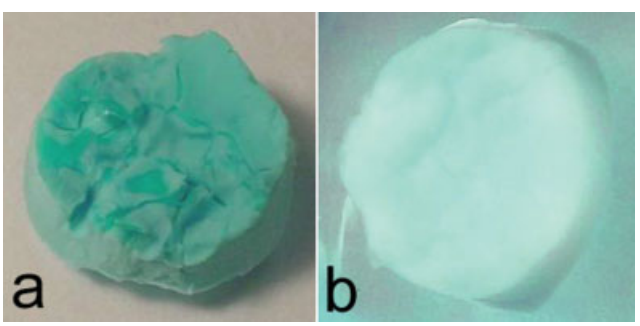


Figure 6 Photographs showing the stripping of copper from PAAm hydrogels; (a) color and texture of PAAm hydrogels loaded with copper; (b) PAAm gels turns back to its original color (white) following Cu^{2+} stripping using EDTA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

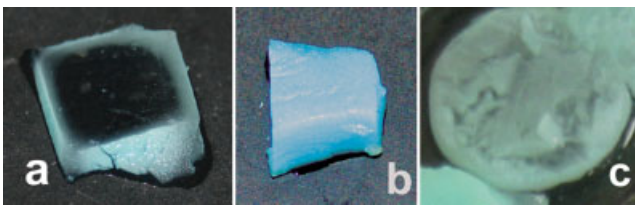


Figure 7 Photographs of chelated copper in gels; (a) cross-sectional view of copper chelated PAAm-AAm copolymer gel appears transparent inside and blue on the outside; (b) cross-sectional view of copper chelated PAAm gel appears uniformly blue inside and outside; and (c) cross section of the PAAm-AAm copolymer gel which turned blue outside and inside when placed in $CuSO_4$ solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

small pieces of both *N*-IPAAm-AAm and PAAM gels were functionalized, they exhibited uniform distribution of copper on the surface as seen in Figures 5 and 6. However, a cross-section of the copolymer gel showed highly nonuniform distribution of copper throughout, with the gel being almost transparent at the center, as seen in Figure 7(a), while the PAAm gels showed very uniform distribution [Fig. 7(b)]. To determine whether this problem was due to lower functionalization of the interior of the gels, a sliced gel was incubated in Cu^{2+} solution. As shown in Figure 7(c), these gel slices chelated copper and exhibited a uniform distribution of copper throughout the sliced surface of the gel. The BCC of the gels with sliced surfaces were measured using ICP and were found to be comparable to gels with an unsliced surface. This experiment suggests that, although BDE and IDA can easily permeate into the gel, the copper ions cannot.

Further chelation studies on larger copolymer gel pieces showed nonuniform distribution of copper even along the surface of the gel. Although typical functionalization studies were done with small samples punched from larger hydrogels, two large copolymer gels were functionalized to evaluate surface variation in properties. The two gels had a thickness of ~ 7 mm and ~ 3 mm. While the 3 mm thick gel showed a BCC in the range of 3.0–5.6 mg/g of wet gel, with a couple of concentration spikes of about 9–13 mg/g of wet gel, the 7-mm thick gel showed a larger variations in BCC ranging from 9.4 to 0.24 mg/g of wet gel.

This poor distribution of bound copper in functionalized environmentally responsive copolymer gels can be attributed to several factors. The first of these is simple pore blockage by colloidal clusters of copper, which may cause diffusional resistances as well as charged barriers for further permeation of copper ions into the gel. This may explain the nonuniform distribution of copper ions which were eliminated when the interior of the gel was exposed to $CuSO_4$. The above experiment also showed that the interior of the gel was functionalized by BDE and IDA and that diffusional resistance was the main cause for the lack of bound copper in the interior of the gel.

Another reason for pore blockage either locally or along the surface of the gel could be the loss of ionic nature of the gel on substitution by copper, i.e., the $(COO^-)_2$ sites become the nonionic $COO^-Cu^{2+}COO^-$. This can lead to a rapid collapse of the gel matrix near the surface, resulting in a diffusion-resistant pathway for the $CuSO_4$ ions into the interior of the gel. Finally, thermally responsive gels may be locally affected by the heat of reactions generated during the various functionalization and chelation procedures, which may cause local differences in pore distribution, entrapping unreacted groups or allowing better functionalization in particular areas. The problem of entrapment of unreacted groups is further aggravated by the smaller washing times. This phenomenon is suggested by the fact that high values of BCC were observed at certain points in the gel. As hydrogels have a highly flexible network and are three-dimensionally interlinked, these local changes can cause large stresses along the entire gel area, affecting pore distribution, permeation, and copper distribution. In smaller gels, these effects are only visible in one dimension, while in larger gels a two dimensional variation can be readily observed. The above observations suggested that reduction in gel thicknesses and restriction of three-dimensional mobility of the gel matrix could produce more uniform copper distribution in thermally responsive affinity gels produced using post gel functionalization chemistries.

Thin film gels

To address the issues discussed earlier, very thin immobilized copolymer gels were synthesized on supports and then functionalized. As thin gels exhibit very low mechanical strength, supporting the gels makes characterization much easier. Additionally, this constrained the mobility of the gel to a single dimen-

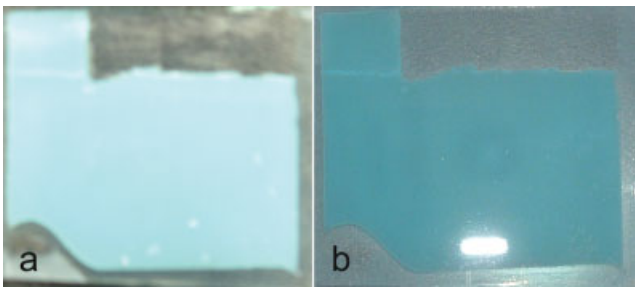


Figure 8 Photographs showing the different colors of copper chelated gels; (a) gels washed only with water are opaque and deep bluish green, while (b) gels further washed with salt and buffer turn transparent sky blue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Change in Average BCC with Thickness of Gel Before and After Salt Wash

Sample thickness (mm)	BCC without salt wash (mg Cu^{2+}/cm^2)	BCC after salt wash (mg Cu^{2+}/cm^2)
0.75	0.42 ± 0.09	0.25 ± 0.04
0.5	0.16 ± 0.05	0.17 ± 0.01

sion, which satisfied one of the suggested criteria for uniform copper distribution. Supported gels were prepared using well established techniques, similar to electrophoresis technology.[2,33] They were cast either on glass or tin oxide coated glass surfaces precoated with bind silane-3-methacryloxypropyltrimethoxysilane.

The supported gels were functionalized using the same protocol as the bulk gels. As shown in Figure 8(a), the copper chelated supported gels have a very uniform distribution of copper along the surface. Additionally a cross-section of the gel did not exhibit any nonuniformity in color through the thickness. As the gels were very thin with a defined thickness and area, the copper distribution in the gel will be reported in terms of measurable unit surface area. The BCC from different sections of the same gel was found to be uniform and reproducible.

The deep bluish green color of the gel also suggests that some physisorbed or entrapped Cu^{2+} ions may remain in the gel following water washes. Similar observations were made for bulk gels. Copper leaching studies were done under UV at 280 nm in a 0.05*M* sodium phosphate buffer containing 1*M* NaCl to test this hypothesis. A steady increase in the signal was observed with time. The gels were washed with this buffer followed by a DI water wash to determine the relative concentration of bound and unbound copper within the gels. The copper content remaining in gels following the wash was determined by placing gels in an EDTA solution to strip bound copper and by monitoring the copper concentration of the solution using ICP.

The copper concentrations in the gels, following a salt solution/water wash, are shown in Table II. Salt subdues ionic interactions in the gel, which should release copper ions into solution in a process known as salting in. The total copper content of a 0.75 mm gel decreased almost by half after the salt wash. This process was accompanied by a very prominent change in color of the gel from an opaque deep bluish green to a nearly transparent sky blue as observed in Figure 8(b). This phenomenon may be due to interactions between the excess copper ions trapped by residual BDE-IDA acid groups in the gel due to decreased washing times or interactions between the unreacted $—NH_2$ groups and neighboring bound/unbound IDA ligands leading to the formation of complex colloidal clusters within the gel matrix.

For a better understanding of diffusional resistance, gels with varying thickness and crosslinking densities were investigated. Gels of 0.5 mm and 0.75 mm were prepared, functionalized and chelated. As expected, the 0.75 mm samples had larger amounts of copper than 0.5 mm samples on a per area basis before salt wash. After the salt wash, the copper content of the 0.5 mm thick gels did not change while that of the 0.75 mm thick gels was reduced by half. However, the final copper content of the 0.75 mm gels was proportional to its thickness as can be seen in Table II. This change in copper content suggests that salt carries away the unbound colloidal clusters of copper. These results are consistent with the explanation that there may be pore blockage by copper clusters causing resistance to diffusion, which may result in poorer distribution of copper in the gel above a critical thickness of $\sim$ 0.5 mm. This means a uniform distribution of copper ions at depths greater than 0.5 mm will be difficult to achieve. Also, the uniform copper distribution along the surface of the gel suggests that the pore distribution is uniform in supported gels unlike free standing gels. Thus, immobilization could potentially be used as a technique to control the pore distribution of these gels in the dimension of interest.

Studies on the effects of varying the crosslinking density were done on 0.5 mm thick gels following washes with salt buffer to ensure that there was no unbound copper. There was an increase in copper content with decrease in crosslinking density as shown in Table III. This suggests that the increase in pore sizes with decrease in crosslinking densities allows BDE, IDA, and copper easier access within the gel matrix. This is consistent with expected results for hydrogels with decreased crosslinking density.

Equilibrium swelling studies

Although this work primarily focuses on developing consistent chemistries for gel formation and functionalization, a short analysis of swelling of free standing hydrogels following functionalization will be presented.

TABLE III
Relationship between Average BCC of the Gels and their Crosslinking Densities

Amount of crosslinker (mg)	BCC (mg Cu^{2+}/cm^2)
25	0.85 ± 0.062
47	0.50 ± 0.056
100	0.33 ± 0.027

BCC were measured after salt wash on 0.5 mm thick gels.

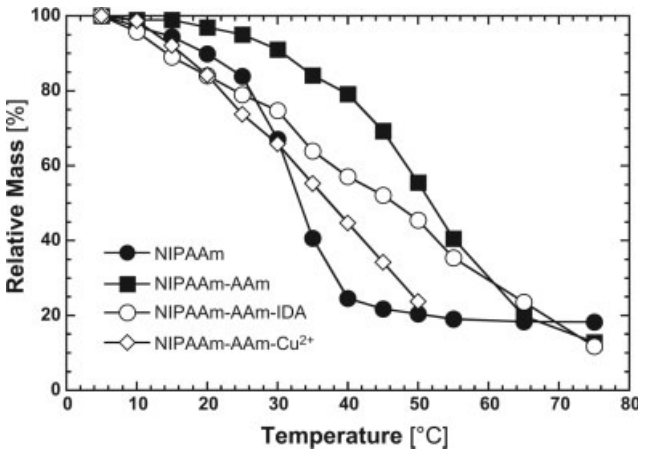


Figure 9 Change in the equilibrium swelling of *N*-isopropylacrylamide gels with copolymerization, funtionalization, and copper chelation; (a) pure NIPAAm gel, (b) NIPAAm-AAm copolymer gel, (c) NIPAAm-AAm-BDE-IDA functionalized gel, and (d) NIPAAm-AAm-BDE-IDA-Cu^{2+} chelated gel.

A more detailed study will be provided in subsequent papers. The relative mass as a function of temperature for the base copolymer and functionalized gel samples is shown in Figure 9. As can be seen from Figure 9(a), virgin NIPAAm gels show a sharp phase transition between 30 and 35°C. The transition appears a little broader and more continuous because the samples used were much thicker, had a higher crosslinking density (10 : 1, NIPAAm/MBAAm) and were synthesized by a different method[34] than those usually reported in the literature, to ensure mechanical stability during functionalization and characterization. This sluggish response of the gel could be directly attributed to the combined effect of increase in dimensions,[35,36] difference in shape,[36] and increase in crosslinking density.[20,31]

The incorporation of acrylamide resulted in a broadening and shifting of the phase transition temperature to higher values as shown in Figure 9(b). This indicates the addition of the acrylamide $—NH_2$ groups increases the hydrophilicity of the gel. This observation is consistent with the greater affinity for water of amine groups relative to the hydrophobic *N*-isopropyl groups. On functionalizing with BDE and IDA, the gels still loose water continuously with increasing temperature, but not as sharply as the base copolymer gel. This behavior is not well understood, but may be due to the stresses developed in the gel during functionalization, nonuniform distribution of affinity groups, and the presence of entrapped unreacted ligands and spacers in the gel matrix. As was discussed earlier, the IDA-Cu^{2+} complex forms a dense surface layer on the gels, rendering the interior and the exterior of the gel heterogeneous. In contrast to the definition of hydrogels as semisolids, the dense surface layer appears to give the gel more solid-like properties. Also, the loss of ionizable groups following copper chelation reduces the water content of the gel which can be observed from its low equilibrium swelling. These two factors may disrupt the delicate balance between the hydrophilic and hydrophobic groups in the gel responsible for a sharp phase transition and make it a linear one instead.

CONCLUSIONS

Both thermally sensitive *N*-isopropylacrylamide-acrylamide copolymers and nonresponsive acrylamide hydrogels were synthesized easily using a photopolymerization technique. Metal chelating ligands were incorporated in the gel by functionalizing the acrylamide linkage using a traditional chromatographic grafting technique. Although the technique can be applied directly to the acrylamide hydrogels, a number of modifications were required for their use in environmentally sensitive gels. The copper diffusion and distribution problems in thermally responsive gels led to the development of thin film copolymer gels which showed a number of advantages over the bulk gels and also aided in understanding phenomena related to gel functionalization. Thus, for the first time it was shown that immobilized thermally responsive hydrogels with uniformly distributed metal affinity groups can be developed using conventional metal functionalization chemistries.

Equilibrium swelling studies suggest that the transition behavior of gels is severely affected by the incorporation and distribution of affinity groups and the presence of entrapped unreacted functional groups. Although the gels did not show the desired sharp transition behavior exhibited by the virgin hydrogels, they were environmentally responsive and changed their state in response to temperature. The study suggests that the effect of incorporation of affinity groups on the phase transition and the effect of phase transition on the distribution of affinity groups is significant and must be considered when designing environmentally responsive affinity gels. Immobilized thin film gels may be effective in isolating and overcoming these problems which makes them excellent candidates for small scale affinity separations.

We thank Dr. Viranga Tillekeratne of the University of Toledo, Department of Medicinal and Biological Chemistry for sharing his insights into the mechanism of various reactions. We also thank Dr. Srikanth Varanasi of Pilkington North America for donating tin oxide coated glass plates. Dr. Panne Burckel of the University of Toledo Instrumentation Center assisted us with the induction coupled plasma analyses.

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