# Controlling Phase Transition Behavior of Thermally Responsive Metal Affinity Hydrogels: A Molecular Design Approach

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ABSTRACT: A molecular design approach is used to develop thermally responsive metal affinity hydrogels of *N*-isopropylacrylamide which can retain the nature of the overall volumetric response of the base gels and provide highly specific affinity separation. The synthesis of a novel iminodiacetic acid monomer to obtain metal affinity hydrogels with the right hydrophobic/hydrophilic balance is described and compared to a grafting technique presented in an earlier study. Copolymerization of this monomer with *N*-isopropylacrylamide and detailed characterization of both materials by various spectroscopic and elemental analysis techniques are presented. Preliminary experiments on the equilibrium swelling and critical analysis of the phase transition of the *N*-isopropylacrylamide gels incorporated with these novel metal affinity groups indicate that these gels retain the phase transition behavior of the base gels.

## Introduction

In the past decade interest in *N*-isopropylacrylamide (NIPAAm) gels has shifted from merely investigating their thermally reversible lower critical solution temperature (LCST) behavior to developing applications based on this property. These efforts have mostly involved modifying NIPAAm hydrogels through copolymerization or entrapment of functional moieties such as enzymes, drugs, and biomolecules.[1−6] Such modifications are effective in targeting specific applications but often introduce undesirable changes in the phase transition behavior of the NIPAAm gel.

Recently, we reported a functionalization technique for the incorporation of metal affinity groups into NIPAAm gel and its effect on the nature of the phase transition (PT).[7] The PT was broader and shifted to higher temperatures when copolymerized with acrylamide, which agrees with reported results.[2,8] Further, when the gel was grafted with iminodiacetic acid and chelated with $Cu^{2+}$ as a metal affinity group (Scheme 1A), the sharp PT behavior was lost. The grafting also resulted in uneven distribution of functional groups in the gel, with a dense layer of functionality on the surface and little or no functionality in the gel interior. This uneveness may have contributed to the final loss of the PT of the gel. These results suggest that an improved general approach to synthesizing thermally responsive affinity hydrogels that retain the PT behavior of the base gels needs to be developed. Copolymerization of functional comonomers into the NIPAAm backbone is another approach that has been investigated.[2] Uniform distribution of affinity groups in the gel was obtained using this approach, but undesirable changes in the PT behavior of the gel still occurred.

Hydrophobic and hydrophilic comonomers have been shown to affect the PT behavior of the NIPAAm gels inversely.[8−15] The PT becomes discontinuous and shifts to lower temperatures when NIPAAm is copolymerized with long chain hydrophobic comonomers having greater surface exposure to water. The PT shifts to higher temperatures with hydrophilic comonomers, and the continuity of the transition varies with its type and composition. This suggests that functional comonomers with the right hydrophobic/hydrophilic balance may preserve the PT behavior of the base polymer. Usually, functional molecules are developed solely for affinity functions. In order for them to have the correct hydrophobic/hydrophilic balance to blend into the backbone of the NIPAAm gels and retain their PT behavior, they must be molecularly designed to do so. To date, such a molecular design approach for functional comonomers has not been reported.

This paper presents a novel synthetic design approach to obtain metal affinity ligand comonomers which will impart the right hydrophobic/hydrophilic balance and uniform comonomer distribution in thermally responsive NIPAAm hydrogels. The general approach to the design is shown in Scheme 1B. For this work, a vinyl-terminated iminodiacetic acid (VIDA) ligand was designed, synthesized, and copolymerized into the NIPAAm polymer backbone during gel synthesis to achieve uniform distribution of the metal affinity groups within the gel. Since iminodiacetic acid is a highly hydrophilic group, a six-carbon alkyl chain was used in the design of VIDA to link the iminodiacetic acid to the vinyl end. The alkyl chain served the dual purpose of increasing the hydrophobic hydration surface area of water as well as acting as a spacer arm projecting the iminodiacetic acid groups to prevent steric hindrance with the polymer backbone. Although not shown in Scheme 1, a cross-linker is included in the polymerization step, and its composition in the gel can also affect the hydrogel phase transition. This work focuses on two areas: (i) synthesis of vinyl IDA monomer and its copolymerization with NIPAAm to form hydrogels and (ii) preliminary studies of the changes in the phase transition behavior of NIPAAm on copolymerization with this metal affinity ligand and $Cu^{2+}$ chelation. A brief description of the equilibrium swelling behavior of the copolymer hydrogels is also presented.

## Experimental Section

**Synthesis of Vinyliminodiacetic Acid Monomer (VIDA).** *Materials for Monomer Synthesis.* All reactions were carried out

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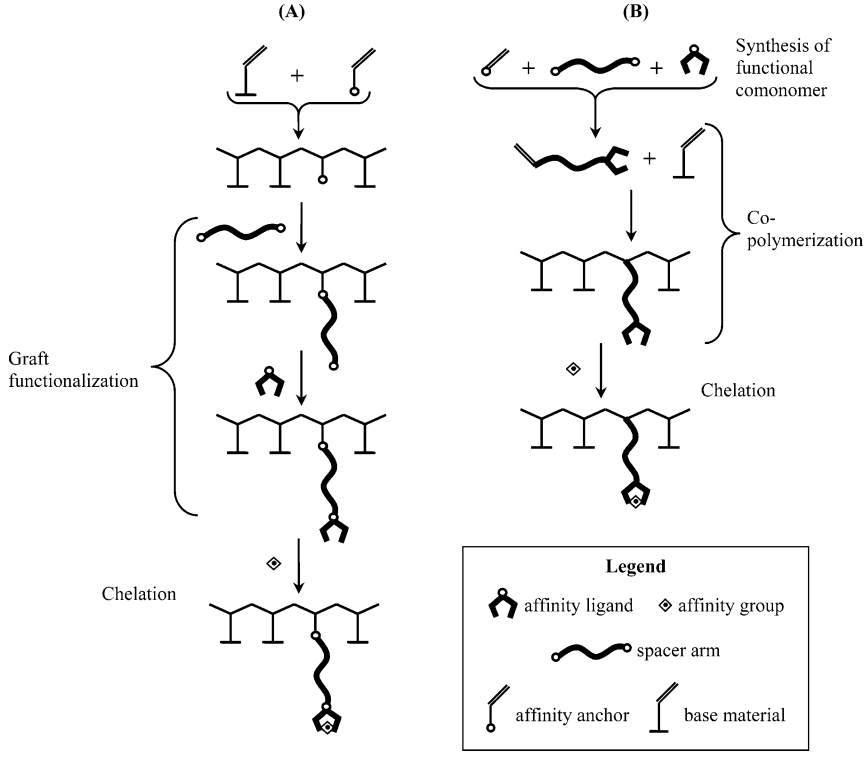
**Scheme 1. (A) Functionalization of Hydrogels with Affinity Ligands Using the Grafting Technique and (B) Functionalization of Hydrogels by Copolymerization with Ligand Synthesized by Molecular Design**




under a nitrogen atmosphere using dry solvents under anhydrous conditions unless otherwise noted. Dimethyliminodiacetate hydrochloride (97% pure) was purchased from Fluka and used directly. BOC-6-aminohexanoic acid (97% purity), *N*-ethyl-*N*-(3-(dimethylamino)propyl)carbodimide (EDC, 97% purity), anhydrous dichloromethane, 4-(dimethylamino)pyridine (DMAP, 99.9% purity), and trifluoroacetic acid (TFA, 99% purity) obtained from Sigma-Aldrich were used without further purification. Triethylamine (TEA, 99.5% pure, Sigma-Aldrich) was stored over sodium hydroxide for a couple of days before use. Acryloyl chloride (96% purity, Lancaster Synthesis) was distilled and stored at −20 °C in the dark. Anhydrous ethanol, reagent grade methanol, and acetone were obtained from Fisher Scientific.

*General Techniques.* All yields refer to chromatographically and spectroscopically ($^1$H NMR) homogeneous materials, unless otherwise noted. Thin layer chromatography (TLC) was used to monitor the course of the reaction, using 200 $\mu$m polyester baked Bakerflex silica gel plates with fluorescent $UV_{254}$ or ninhydrin indicators and UV light. NMR spectra were recorded at the University of Toledo Instrumentation Center on INOVA 600 MHz or Varian VXRS 400 MHz spectrometers in $CDCl_3$ or $D_2O$. TMS at 0 ppm and $D_2O$ at 4.67 ppm were used as internal reference for $^1$H NMR. Attached proton tests (APT) were performed to distinguish between different carbons in the $^{13}$C NMR spectra. Deuterated methanol (49 ppm) was used as an internal reference for $D_2O$ in APT. High-resolution mass spectra (HRMS) were recorded on a micromass LCT electrospray mass spectrometer, at the Mass Spectrometry and Proteomics Facility at Ohio State University. A TA Instruments Q-50 thermogravimetric analyzer was used to determine the presence of solvent of crystallization in the purified monomer.

**Dimethyl *N*-(6-(Boc-amino)hexanoyl)iminodiacetate (6).** Dimethyliminodiacetate hydrochloride (**4**) (8.43 g, 42.6 mmol, 1 equiv), BOC-6-aminopropionic acid (**5**) (9.87 g, 42.6 mmol, 1 equiv), and DMAP (0.5 g, 4.09 mmol) were suspended in anhydrous methylene chloride (100 mL) under a nitrogen atmosphere in a well-dried 250 mL flask. EDC (7.27 g, 46.86 mmol, 1.1 equiv) was then added, upon which the solution became totally clear. The reaction mixture was stirred overnight at room temperature. The methylene chloride solution was washed with water (30 mL), and the aqueous phase was extracted twice with methylene chloride. The combined methylene chloride extract was dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo to obtain the product **6** (17.37 g) as a pale yellow oil. It was used in the next step without further purification. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 4.60 (bs, 1H), 4.13 (s, 2H), 4.11 (s, 2H), 3.73 (s, 3H), 3.67 (s, 3H), 3.06−3.05 (bm, 2H), 2.27−2.24 (m, 2H), 1.62−1.58 (m, 2H), 1.45−1.413 (m, 3H), 1.384 (s, 9H), 1.32−1.27 (m, 2H).

**Dimethyl *N*-(6-(Amino)hexanoyl)iminodiacetate (7).** A 20% (v/v) solution of TFA (26 mL) was slowly added with constant stirring to a methylene chloride solution of **6** (105 mL) cooled to 0 °C. The reaction mixture was slowly brought to room temperature after 30 min and was stirred overnight at room temperature. Evaporation of solvent in vacuo gave a crude viscous brown liquid of **7**. The product was used in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.52 (bs, 2H), 4.15 (s, 2H), 4.13 (s, 2H), 3.77 (s, 3H), 3.71 (s, 3H), 3.06−3.05 (bm, 2H), 2.38−2.34 (t, 2H, $J$ = 6.4 Hz), 1.71−1.63 (bm, 4H), 1.28−1.32 (m, 2H).

**Dimethyl *N*-(6-(Acrylamido)hexanoyl)iminodiacetate (3).** To a solution of crude **7** in anhydrous methylene chloride (35 mL) cooled in an ice bath, TEA (45 mL) was added dropwise under a nitrogen atmosphere. The solution was transferred to a pressure equalizing dropping funnel and added dropwise to a solution of acryloyl chloride **8** (6.13 g, 68.11 mmol, 1.6 equiv) and 2,6-*tert*-butyl-4-methylphenol (TBMP, 500 mg) in methylene chloride (35 mL) cooled in ice for $1^1/_2$ h. The reaction mixture was allowed to slowly come to room temperature and stirred overnight. The methylene chloride solution was then washed with water (2 × 30 mL), 10% HCl solution (30 mL), and then again with water (30 mL) and 10% (w/v) sodium bicarobate solution (20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated in vacuo to obtain a yellowish-brown oil (13.0 g). Flash column chromatography on silica in EtOAc/hexanes (70:30) → EtOAc gave pure **3** as a pale yellow oil (6.15 g, 44% over three steps). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.28−6.24 (d, 1H, $J$ = 16.8), 6.13−6.06 (dd, 2H, $J_1$ = 10.4 Hz, $J_2$ = 17 Hz), 5.61−5.58 (d, 1H, $J$ = 10.4 Hz), 4.17 (s, 2H), 4.14 (s, 2H), 3.76 (s, 3H), 3.71

(s, 3H), 3.34−3.32 (q, 2H, $J$ = 6 Hz), 2.33−2.29 (t, 2H, $J$ = 7.2 Hz), 1.67−1.64 (m, 2H), 1.55−1.52 (m, 2H), 1.38−1.36 (m, 2H).

***N*-(6-(Acrylamido)hexanoyl)iminodiacetic Acid Disodium Salt (VIDA) (2).** A solution of compound **3** in water−ethanol (1:1, 20 mL) containing sodium hydroxide (0.75 g, 18.7 mmol, 1 equiv) was stirred for 3 h at room temperature. Excess ethanol (20−30 mL) was added to the reaction mixture and cooled in an ice bath with vigorous stirring. Slow addition of acetone to this solution yielded a fine bright yellow powder which was filtered and vacuum-dried to obtain whitish-yellow crystals of **2** (6.7 g, 16.6 mmol, 92.6%). $^1$H NMR (600 MHz, $D_2O$): $\delta$ 6.11−6.05 (dd, 1H, $J_1$ = 7.8, $J_2$ = 21.0), 6.0−5.96 (d, 1H, $J$ = 21.0), 5.56−5.55 (d, 1H, $J$ = 7.8), 3.80 (s, 2H), 3.73 (s, 2H), 3.08−3.06 (t, 2H, $J$ = 5.4), 2.2−2.17 (t, 2H, $J$ = 7.8), 1.45−1.36 (m, 4H), 1.22−1.16 (m, 2H).

**Synthesis of NIPAAm−VIDA Copolymer Gels.** *Materials for Gel Synthesis.* The monomer NIPAAm and cross-linker *N*,*N*-methylenebis(acrylamide) (MBAAm, ultrapure grade) were purchased from Polysciences Inc. The NIPAAm was recrystallized from hexane before use. The photoinitiator riboflavin (99% purity) and accelerator *N*,*N*,*N*,*N*-tetramethylethylenediamine (TEMED, 99.5% purity) were obtained from Sigma-Aldrich. Deionized water purified from a US Filters filtration system was used for all the experiments.

*Gel Synthesis.* While the pure NIPAAm and copolymer gels were synthesized using the same photochemical polymerization technique, the procedure for the copolymer is described in detail.[16] A monomer solution of NIPAAm (1.3 g, 11.5 mmol), MBAAm (55 mg, 0.35 mmol, 2.8 mol %), and VIDA (200 mg, 0.49 mmol, 3.9 mol %) was prepared in 10 mL of deionized (DI) water. The solution was evacuated for 3−4 min to remove any dissolved oxygen following which 50 $\mu$L of riboflavin solution (0.1% (w/v)) and 8 $\mu$L of TEMED were added. The solution was swirled gently 3−4 times and poured into a polypropylene Petri dish for polymerization under UV light (365 nm 30 W, UV products-XX-15) for 2 h at room temperature. The gel was immersed in DI water for at least 3 days with water changes at least 3 times a day to remove any unreacted monomers. In a similar manner, free-standing thin film gels were synthesized by transferring gel solution between two glass plates coated with dimethyldichlorosilane and separated by a 0.5 mm spacer using a syringe.[7] The gels were cut into round pieces of 1.6 cm diameter using a cork punch before further use.

*Metal Affinity Functionalization of Gel.* Gels were immobilized with $Cu^{2+}$ ions by incubating in excess 0.05 M copper sulfate solution[7,17] at room temperature for 24 h. They were then repeatedly washed with DI water for 3 days to remove all the unchelated $Cu^{2+}$ ions. Copper contents in the gels were measured using induction coupled plasma analysis (ICP, Thermolectron IRIS Interpid II) at the USDA Greenhouse Production Research Group at the University of Toledo. Samples for analysis were prepared by cutting at least three small pieces from different areas of a large sample using a cork punch.

## Results and Discussion

**Synthesis of Metal Affinity Comonomer (VIDA).** The synthesis of **2** was achieved in four steps, with an overall yield of ∼40%. The dimethyl ester of iminodiacetic acid (**4**) was reacted with BOC-6-aminohexanoic acid (**5**) in the presence of EDC and DMAP to give the corresponding amide **6**. EDC acts as a coupling agent to facilitate and to activate the carboxyl group toward reaction with dimethyliminodiacetate hydrochloride. It also serves to scavenge the hydrochloride salt to liberate the amine group of iminodiacetate for nucleophilic reaction. This step converts the insoluble salt to the soluble amine compound. Compound **6** was then deprotected using TFA to give the corresponding amine **7**. The reaction of this amine with acryloyl chloride **8** gave **3**. The methyl ester was finally hydrolyzed with aqueous sodium hydroxide to obtain the vinyl IDA monomer **2**.

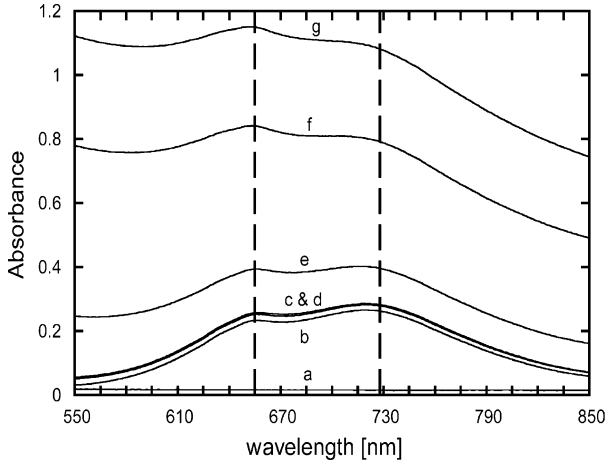
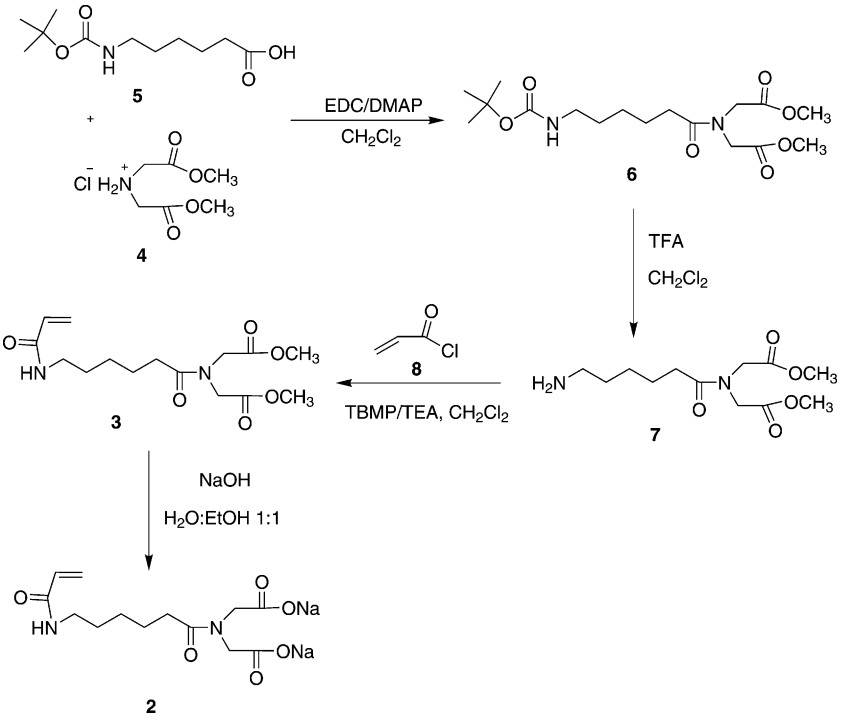



**Figure 1.** UV−vis spectra of pure VIDA, pure $NiSO_4$, and VIDA−$Ni^{2+}$ complex solutions in DI water: (a) VIDA, (b) 0.12 M $NiSO_4$. VIDA−$Ni^{2+}$ complexes were studied by mixing VIDA and $NiSO_4$ solutions in a volumetric ratio of 50:50 (v/v) and molar ratios of (c) 1:4.8, (d) 1:2.4, (e) 1:1.6, (f) 1:1.2, and (g) 1:1.

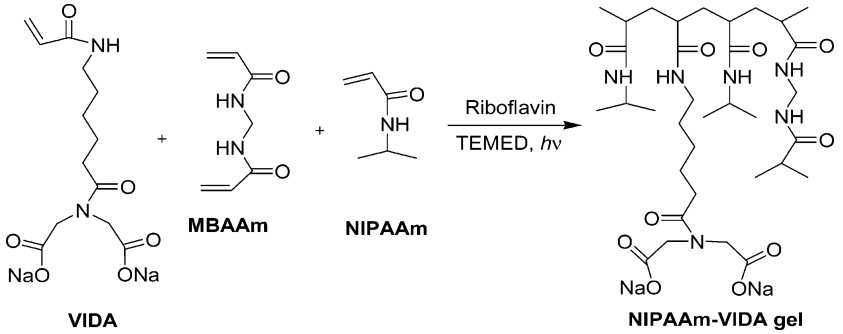
**Monomer Characterization.** *Thermogravimetric Analysis.* Thermogravimetric analysis of the monomer at 5 °C/min over 25−400 °C was used to determine content of residual solvents within the crystallized monomer. Results showed a 15.1% weight loss at ∼60 °C, which corresponded to one molecule of acetone entrapped in every molecule VIDA that crystallized. The molecular weight addition due to acetone was considered while calculating the number of moles of VIDA to be added for synthesis. The entrapped acetone molecules are not expected to affect the gels formed as they will easily separate into the aqueous phase when the VIDA crystals are dissolved in water. Further, any residual acetone in the gel will dissolve in water and would be removed during repeated washing steps.

*UV−vis Spectroscopy.* While the ability of iminodiacetic acid to strongly chelate transition metals such as $Ni^{2+}$ and $Cu^{2+}$ is well established,[18−21] a qualitative study was used to demonstrate that the newly synthesized vinyl-terminated IDA also formed complexes with these metal ions.[22] While VIDA formed complexes with both $Cu^{2+}$ and $Ni^{2+}$ ions, the discussion will focus on the VIDA−$Ni^{2+}$ complex. For this study equal volumes of 0.24 M $NiSO_4$ and VIDA were mixed in molar ratios ranging from 1:4.8 to 1:1 of VIDA:$NiSO_4$ and observed using a UV−vis spectrophotometer (Shimadzu, UV2401 PC) between 550 and 850 nm. $NiSO_4$ has distinct broad twin peaks at 655 and 720 nm while VIDA does not absorb in this region. These peaks were analyzed because they typically show a shift in absorbance to lower wavelengths on complex formation. The UV−vis spectra of the VIDA monomer, $NiSO_4$ solution, and the solution mixture at different mole ratios are shown in Figure 1. There are two features that can be clearly observed. The first of these is a change in the relative heights of the twin peaks with varying VIDA and $NiSO_4$ concentrations. Initially, the peak at 655 nm is shorter than the peak at 720 nm, but with increase in the VIDA:$NiSO_4$ ratio from 1:4.8 to 1:1, the former exceeds that at 721 nm. This suggests that the VIDA−$NiSO_4$ complex absorbs at wavelengths close to 655 nm. Since this overlaps with the broad twin peak of $NiSO_4$, it is difficult to judge the nature of the VIDA−$Ni^{2+}$peak and determine how much its absorbance shifts to lower values. The second feature in Figure 1 is the sharp jump in the absorbance values of the mixture observed from ratios of 1:1.6 to 1:1. The absorbance of the mixture at a ratio of 1:1 is 10 times that of $Ni^{2+}$ ions of similar concentration. These jumps in absorbance were accompanied by precipitation of the chelate complex. This confirmed the

**Scheme 2. Synthesis of *N*-(6-(Acrylamido)hexanoyl)iminodiacetic Acid Disodium Salt (VIDA) (2)**




**Scheme 3. Synthesis of NIPAAm−VIDA Copolymer Gel**




chelation ability of the VIDA monomer. The above results qualitatively suggest that the state of $Ni^{2+}$ ions change in the presence of VIDA and that the VIDA monomer possesses chelating ability. A detailed study of the coordination number and stability constants of VIDA−$Cu^{2+}$ or VIDA−$Ni^{2+}$ complex will be presented in a subsequent paper.

**Gel Synthesis and Functionalization.** Pure NIPAAm and copolymer hydrogels could be synthesized in a single solvent system and without the use of nitrogen environment, using the photochemical polymerization technique shown in Scheme 3. The photoinitiator riboflavin requires oxygen to form free radicals and acts as an oxygen scavenger, eliminating the need to maintain an inert atmosphere. Although small changes in oxygen content did not affect the polymerization, the presence of excess oxygen (i.e., no evacuation) in the solution led to the formation of a low molecular weight material as all of the riboflavin was consumed.

In contrast to the functional gels prepared by grafting technique, the copolymer gels formed had exceptional properties. The color of the copper chelated gels prepared using copolymerization with VIDA monomer was very uniform throughout the gel, as seen in Figure 2. The gels were transparent even after chelation, which suggested that they were highly homogeneous and had a uniform distribution of copper. This was further confirmed by analyzing the bound copper content (BCC) in different sections of the gel by ICP. The BCC of several gels synthesized and functionalized by this technique was 12.7 ± 1.0 mg/g of dry gel.

**Elemental Analysis.** *ICP Analysis.* Thoroughly dried samples were digested in nitric acid and hydrogen peroxide using standard protocol, in a microwave (1200 W, MARS-CEM Corp.). To determine whether the VIDA monomer copolymerized with NIPAAm moieties during gel formation, the $Na^+$ content of the NIPAAm−VIDA gel was measured by ICP analysis, since it is the only component which can contribute sodium atoms to the gel. The ICP results confirmed that VIDA participated in gel formation with a conversion of 51.3 ± 3.1% and 61.3 ± 4.0% in the thick and thin gels, respectively. The relatively low conversion of VIDA can be attributed to its bulkiness as compared to the NIPAAm moiety, which has a molecular weight a fourth that of VIDA. This can cause rapid polymerization of NIPAAm moieties, incorporating fewer number of VIDA groups in the backbone due to steric hindrance. This is supported by the fact that the weight percent of NIPAAm in the final gel was much higher than the expected theoretical value, as seen in Table 1. The high reaction rates can be observed from the fact that the gel point was reached in 20 min. This suggests that lowering the reaction rate could help improve conversions of VIDA. The use of different initiator systems could also improve conversion of VIDA.

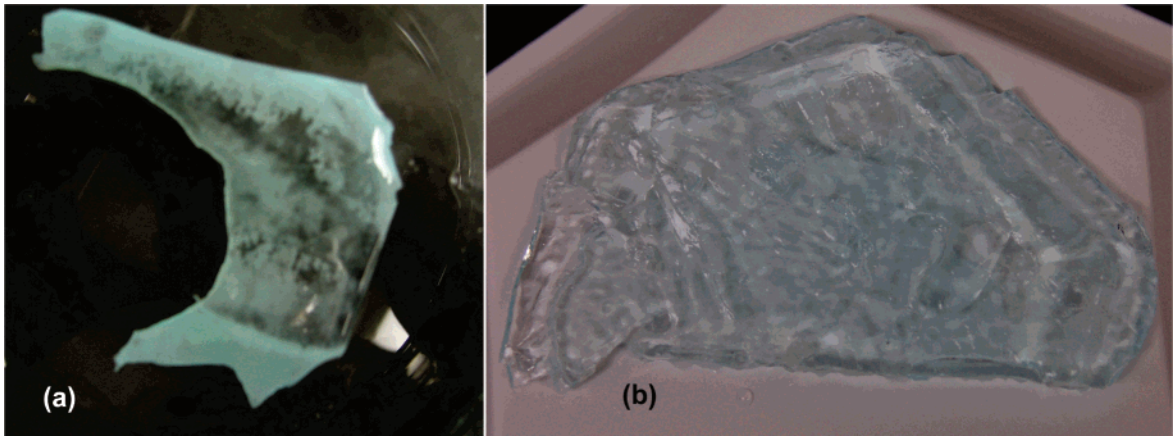



**Figure 2.** Visual comparison of the distribution of copper in affinity hydrogels: (a) gels functionalized using a grafting technique[7] showing an uneven distribution and (b) gels synthesized by in-situ polymerization in this study showing an even distribution.

**Table 1. Measured and Calculated Weight Percentages of C, H, and N and Theoretical and Calculated Composition in the Copolymer Hydrogels**

| | wt % | | | | wt % | | |
|---|---|---|---|---|---|---|---|
| | C | H | N | | NIPAAm | MBAAm | VIDA |
| measured | 61.64 ± 0.04 | 9.16 ± 0.6 | 12.14 ± 0.04 | theoretical[c] | 83.6 | 3.5 | 12.8 |
| calculated[a] | 62.03 | 9.36 | 12.29 | calculated[a] | 89.2 | 3.7 | 7.00 ± 0.4[b] |

[a] Calculated values are approximate. [b] Measured by ICP. [c] Theoretical values are the wt % of monomers used for synthesis.

ICP measurements to determine the copper chelating ability of the gel showed that 85 ± 5.0% of the VIDA in the bulk gels and 73 ± 7% in the thin films chelated copper. The higher BCC in the bulk gels suggested that there could be some entrapment of copper[7,23] in the tortuous structure of the gel. When the bulk gels were swollen and shrunk 2−3 times by decreasing and increasing the temperature, the excess copper was eluted and the BCC reduced to 73 ± 5%, which is comparable to that of the thin film gels.

*CHN Analysis.* The C, H, and N contents of thoroughly dried gels were determined using a Perkin-Elmer CHNS analyzer at the USDA Greenhouse Production Research Group at the University of Toledo. The weight percent of VIDA in the gels measured using ICP was combined with the measured C, H, and N values to estimate the overall composition of the gel. The weight percents of NIPAAm and MBAAm in the gel were varied, and the resultant C, H, and N values and H/C and N/C ratios were calculated. By plotting these calculated H/C and N/C ratios, the point at which they most closely match the measured values can be determined. Details of the calculation are given in the Supporting Information. A sensitivity analysis of the data suggests that <3% change in the C, H, and N composition caused large variations in the calculated compositions of NIPAAm and MBAAm. On the basis of the ratio of the measured and theoretical C, H, and N values, the average yield of the polymerization reaction was calculated to be ~96.0%.

*FTIR Spectroscopy.* The FTIR spectra for the fingerprint region of the NIPAAm monomer, VIDA monomer, NIPAAm−VIDA gel, and Cu−NIPAAm−VIDA gel are shown in Figure 2. The spectra of thoroughly dried samples were obtained by the KBr pellet method. Since only 1.95 mol % of VIDA is incorporated into the gel backbone, its imprints are not very clearly seen in the product. However, a few characteristic observations can be made by comparing the above graphs. The copolymer gel shows a broad peak in the range of 3650 and 3100 $cm^{-1}$ wavenumbers which is absent in the *N*-isopropylacrylamide monomer and is characteristic of the carboxylic acid groups in VIDA monomer. The peak at 1325 $cm^{-1}$ which is characteristic of carboxylic acid salts is seen in the VIDA monomer as well as the copolymer gel. Further on incorporation of $Cu^{2+}$ in the gel there is a marked difference in the nature of the carboxylic peaks in the range of 3650 and 3100 $cm^{-1}$ and in the range of 650−400 $cm^{-1}$.

**Equilibrium Swelling Studies.** The lower critical solution temperature (LCST) of 0.5 mm thick hydrogels was determined by recording the relative change in their diameter with temperature in DI water. The diameters of the gels in the temperature range of 20−80 °C was determined after equilibrating at each intermediate temperature for at least 2.5 h using a temperature-controlled water bath (a Neslab GP300 attached to a chiller Neslab-FTC 350) and a X-axis measuring microscope (Gaertner Scientific). Assuming isotropic behavior, the relative change in volume of the gel, $V/V_0$, can be calculated using the equation

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

where $V_0$ and $D_0$ are the equilibrium volume and diameter of the gel at room temperature just after synthesis and $V$ and $D$ are the final volumes and diameters of the gel at each temperature.

As seen in Figure 4a, the gels synthesized using the molecular design approach and in-situ copolymerization of metal affinity comonomer exhibited a very prominent and sharp PT. The sigmoidal nature of the equilibrium swelling curve is in sharp contrast to that of gels grafted with metal affinity groups, which exhibited a linear and gradual decrease in equilibrium swelling with temperature with no sharp PT.[7] The onset temperatures of the equilibrium swelling curves in Figure 4 were used to mark the phase transition temperature. The onset and offset temperatures were defined as the point of intersection of the tangents to the curves at the beginning and ending of the PT. As expected, the pure NIPAAm gels had a sharp and discontinuous PT at 33.5 °C.

Following copolymerization with the VIDA, the PT was retained but was shifted to 46 °C and became continuous. This 12 °C shift in the phase transition temperature clearly suggests

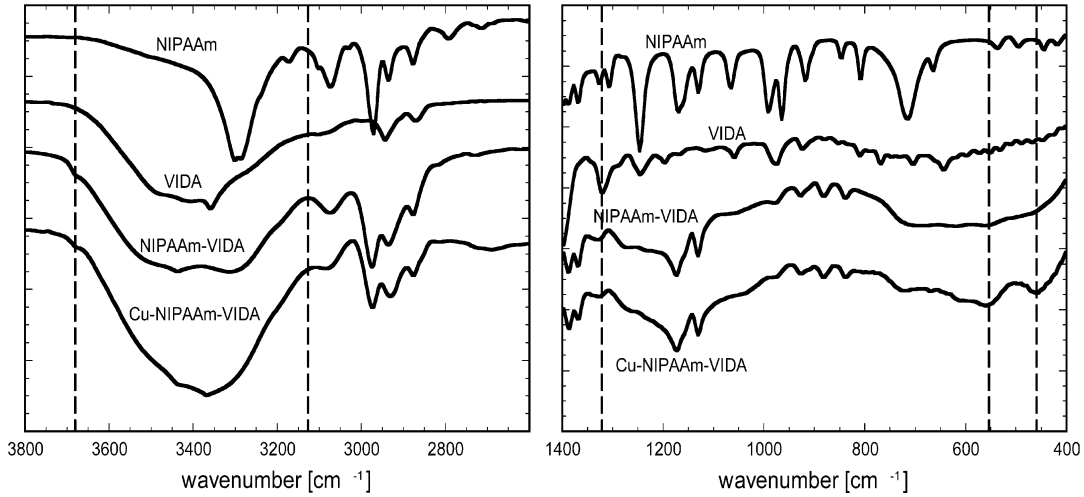



**Figure 3.** Change in the nature of the FTIR spectra of the NIPAAm and VIDA monomers on copolymerization and copper chelation.

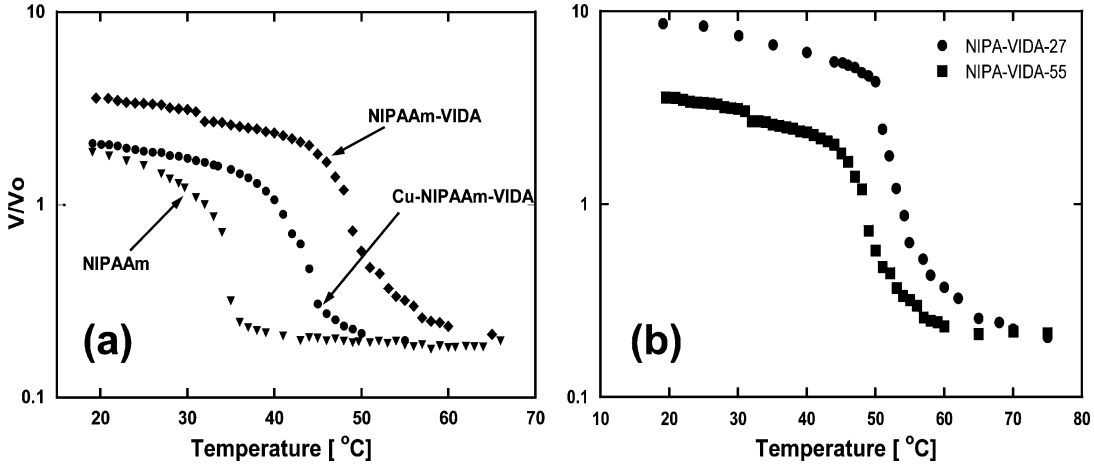



**Figure 4.** (a) Nature of the volume phase transition of NIPAAm gels containing metal affinity ligand VIDA and $Cu^{2+}$ as a metal affinity group. (b) Change in the PT temperature with change in cross-linking density of the gel. NIPAAm−VIDA-27 and NIPAAm−VIDA-55 are copolymer hydrogels with 27 and 55 mg of MBAAm cross-linker, respectively.

that the hydrophilic $(COO^-Na^+)_2$ groups in VIDA exert a resistance to collapse of the gel due to the ionization of the carboxylic acid which increases the osmotic pressure difference between the interior and exterior of the gel.[12−14] However, when sodium in these $(COO^-Na^+)_2$ groups was substituted with copper to form $(COO^-Cu^{2+-}OOC)$ complexes, the gel lost the excess osmotic pressure generated due to ionization of the carboxylic acid, and the phase transition occurred at a lower temperature of 39 °C.

Further, it can be seen from Figure 4b that the equilibrium swelling, the PT temperature, and the sharpness of the PT changed when the concentration of the MBAAm cross-linker in the gel was decreased. The PT of the gels became sharper and shifted to 50 °C on decreasing the cross-linker amount in the gel to 27 mg, as seen in Figure 4b. This effect of cross-linking density on the behavior of NIPAAm hydrogels is in accordance with general observations made by others.[24,25] The cross-linker concentration affects the hydrophobicity and chain confinement of the gel. Figure 4b suggests that increasing the cross-linking density of the metal affinity gels may reduce their PT temperature to that of the NIPAAm gels, but this may also lead to decrease in the equilibrium swelling and sharpness of the PT of the affinity gels.

There is more than one approach to estimating the effect of functionalization on the sharpness of the PT behavior. The difference $\Delta T$ between the onset and offset temperatures of the PT can be considered as a measure of sharpness. This approach suggests that the order of sharpness of the transition is NIPAAm > Cu−NIPAAm−VIDA > NIPAAm−VIDA with values of ca. 1.9, 6.9, and 10.4 °C, respectively. Hence, this type of functionalization does impact the PT behavior. However, the broadening of the PT suggested by $\Delta T$ does not consider the relative volume change of the gels, i.e., the increase in the volume change undergone by the functionalized gels as compared to NIPAAm gels. The slope of the line connecting the onset and offset temperatures does account for this and could be a more accurate measure of the sharpness of the PT of the gel. Using this approach, it is observed that there is very little broadening of the transition between the NIPAAm, NIPAAm−VIDA, and Cu−NIPAAm−VIDA gels, with sharpness values of ca. −0.41, −0.26, and −0.17, respectively. Hence, the metal affinity hydrogels developed by this technique very closely retained the nature of the PT of the pure NIPAAm gels. Further, lowering cross-linking density of gel showed an increase in the sharpness factor to ca. −0.70. The ability to make small changes in the PT temperature and sharpness factors by varying the cross-linking density suggests that it can be used as an additional parameter to control the behavior of the affinity hydrogels to achieve the desired effects.

It is also interesting to note that the equilibrium swelling of the NIPAAm gels at 20 °C increased by ∼1.75 times when copolymerized with only 1.9 mol % of VIDA. Following $Cu^{2+}$

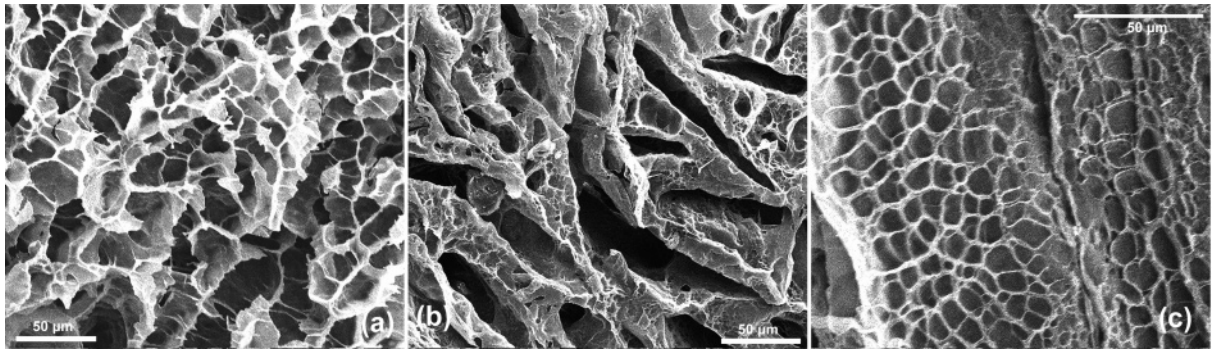



**Figure 5.** Scanning electron micrographs of the cross section of (a) NIPAAm, (b) NIPAAm−VIDA, and (c) Cu−NIPAAm−VIDA.

chelation and thorough washing with DI water the equilibrium swelling of the NIPAAm−VIDA gel decreased by up to 63 wt % at 20 °C relative to the unchleated gel. The resulting equilibrium swelling was similar to that of pure NIPAAm gels, as seen in Figure 4. Such relatively low equilibrium swelling is a characteristic of hydrophobic gels such as pure NIPAAm itself. This can be attributed to the loss of ionic nature of VIDA when it chelates copper. Thus, along with the six-carbon alkyl chain of VIDA, the copper ions play an important role in the molecular design, as a hydrophobic substituent to balance the hydrophilic components in VIDA for achieving the desired PT behavior of the gel.

**SEM Analysis.** The role of the copper ions in the success of the molecular design and to achieve metal affinity hydrogels very similar to the base NIPAAm hydrogels is further supported by observations of their morphology and three-dimensional network structures. Scanning electron micrographs in Figure 5 of freeze-dried hydrogel samples were taken with a Nova FIB microscope at the Electron Microbeam Analysis Laboratory of the University of Michigan. The SEM micrographs strongly suggest that Cu−NIPAAm−VIDA and NIPAAm hydrogels have a similar network structure, which in turn may contribute to their similar volumetric response behavior. Further, the formation of such a similar network structure in the two gels is an indication that the hydrophobic/hydrophilic balance in both the gels is also very similar and may be the reason for the similarity in the PT behavior. The SEM micrographs also show that the NIPAAm−VIDA hydrogels have very large pores, unlike the Cu−NIPAAm−VIDA and NIPAAm gels. This is due to the ionization of carboxylic acid groups which increases the osmotic pressure inside the gel and the pore sizes as discussed earlier, while copper chelation reduces the osmotic pressure and pore sizes by neutralizing the charged groups.

This study suggests that each component of an affinity group has an effect on the structure and PT behavior of the gel. These effects must be well understood and categorized to achieve a successful design scheme for the functional comonomer and the environmentally responsive gel as whole. On the basis of this understanding, it is important to balance hydrophobic regions in the affinity comonomer by incorporating hydrophilic groups in them and vice versa, which mimics the balance in the base hydrogel. Thus, the design scheme should satisfy two major conditions: (i) ensure that the hydrophobic/hydrophilic balance of the functional comonomer and the affinity gel is similar to that of the base polymer and (ii) uniform distribution of the functional comonomer throughout the gel. The cross-linker composition should also be considered when optimizing gel behavior. This engineering approach can be useful for developing environmentally responsive affinity gels with a range of ligands for various applications. Detailed characterization of PT of the copolymer gels will be presented in future articles.

## Conclusions

A novel molecular design strategy to synthesize metal affinity hydrogels which retain their thermally responsive phase transition behavior was developed. A VIDA comonomer with metal chelating ability combined with an appropriate hydrophobic/hydrophilic balance was successfully designed and synthesized. The VIDA was copolymerized with the NIPAAm to obtain hydrogels that can chelate $Ni^{2+}$ and $Cu^{2+}$. The nature of the phase transition of the gel synthesized by this technique was similar to that of NIPAAm gels. The metal affinity gel (Cu−NIPAAm−VIDA) also exhibited a phase transition temperature close to that of NIPAAm gels. By further refining the design of the comonomer, the phase transition temperature of the affinity gels can be controlled to desired values. Additionally, the cross-linking density of the gels can be used as a parameter to fine-tune the nature and temperature of the phase transition. The study demonstrates the importance of each component of the affinity ligand comonomer in the design of a thermally responsive gel. The molecular design of comonomers to suit specific application may be a route to maintaining the structural integrity and properties of the base hydrogel.

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**Supporting Information Available:** Method to determine compositions of NIPAAM, MBAAM, and VIDA in final copolymer gel. This material is available free of charge via the Internet at http://pubs.acs.org.

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