

Enhancing Detection Sensitivity of Responsive Microgel-Based Cu(II)
Chemosensors via Thermo-Induced Volume Phase Transitions

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We report on the fabrication of responsive microgel-based Cu²⁺ chemosensors possessing tunable detection sensitivity via thermo-induced microgel collapse/swelling. A novel phenanthroline-containing fluorescent monomer capable of Cu²⁺-binding and fluorescence sensing, PhenUMA (**4**), was synthesized at first by reacting 5-amino-1,10-phenanthroline (**2**) with 2-(3-isocyanato-propionyloxy)ethyl methacrylate (**3**). Near-monodisperse Cu²⁺-sensing microgels were synthesized via emulsion polymerization of *N*-isopropylacrylamide (NIPAM) in the presence of *N,N'*-methylenebis(acrylamide) (BIS), an anionic surfactant, and PhenUMA (**4**) at around neutral pH and 70 °C. At 20 °C, PhenUMA-labeled microgels in their swollen state can selectively bind Cu²⁺ over other metal ions (Al³⁺, Mg²⁺, Zn²⁺, Fe³⁺, Mn²⁺, Ni²⁺, Ag⁺, Cd²⁺, Hg²⁺, and Pb²⁺), leading to prominent quenching of fluorescence emission intensity. At a microgel concentration of 0.25 g/L, the Cu²⁺ detection limit can be down to ~125 nM. When heated above 32 °C, fluorescence intensity of PhenUMA-labeled microgels in the absence of Cu²⁺ exhibits an approximately 33% increase due to their volume phase transition, which is reasonable considering that fluorescent PhenUMA moieties are now located in a nonpolar environment. Furthermore, Cu²⁺ detection sensitivity of PhenUMA-labeled microgels can be dramatically enhanced via thermo-induced microgel collapse at elevated temperatures. At a microgel concentration of 0.083 g/L, detection limits of Cu²⁺ ions can be drastically improved from ~28 nM at 20 °C to ~8 nM at 40 °C. A plausible mechanism for the thermo-induced enhancement of Cu²⁺ detection sensitivity has been proposed. To the best of our knowledge, this proof-of-concept work represents the first example of responsive microgel-based metal ion chemosensor with thermo-tunable detection sensitivity, which simultaneously combining advantageous properties of small molecule sensing moieties and stimuli-responsive soft matter entities.

Introduction

Being capable of efficiently catalyzing atom transfer radical polymerization (ATRP),^{1,2} Huisgen azide–alkyne 1,3-dipolar cycloaddition,^{3–6} and the recently discovered meta-position arylation reaction of certain benzene derivatives,⁷ copper ions have exhibited tremendous importance in synthetic organic and polymer chemistry in the past decade. However, residual copper contamination has always been an issue because copper species is highly toxic to organisms, such as certain

algae, fungi, and bacteria,^{8–10} and might even cause liver damage in children.^{11,12} Thus, highly sensitive and selective detection and imaging of Cu²⁺ ions in tissues and organisms is quite crucial. Previously, a variety of fluorescent and colorimetric Cu²⁺ chemosensors based on small molecules,^{13–23}

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conjugated polymers,²⁴ nanomaterials,^{10,25–33} and biomolecules³⁴ have been developed.

Small molecule-based chemosensors for cations typically consist of an ensemble of covalently linked ion-binding or ion-reactive receptor and fluorophore reporter units.³⁵ As a unique example, 1,10-phenanthroline (Phen) exhibits intense fluorescence emission and also contains two amine moieties, which can serve as binding sites for H⁺/metal cations and concomitantly produce significant changes in emission properties.³⁶ Thus, Phen itself can act as a stand-alone sensing ensemble due to self-contained binding and fluorescence readout structural motifs. In the past few years, a variety of Phen-based small molecule chemosensors have been developed for the detection of pH,³⁷ metal cations (Zn²⁺, Mg²⁺, Cu²⁺, Co²⁺, etc.),^{38–45} oxygen and temperature,^{46,47} and even organic molecules (urea, benzene, etc.).^{48–50}

Recently, the concept of self-organization has also been introduced to the field of ion-selective chemosensors to achieve functional cooperativity and adaptability, mainly using surfactant micelles and functionalized latex or silica

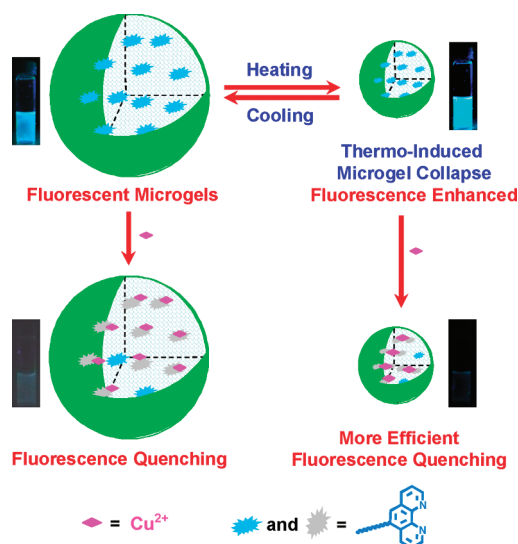
nanoparticles as the sensing matrix.^{51–54} Unlike most chromogenic and fluorogenic chemosensors based on molecular-level host–guest recognition or analyte-specific reaction, self-organized chemosensors do not require the direct covalent linkage between receptor and fluorophore units.⁵⁴ Thus, their fabrication allows for the choice from a rich library of receptors and fluorescent dyes without extra synthetic works, provided that the receptor capable of selectively recognizing the substrate is available. In one notable example,⁴⁴ Jung and co-workers reported the fabrication of organic/inorganic hybrid fluorescent chemosensor for Cu²⁺ ions via covalent immobilization of Phen derivatives to silica nanotubes and nanoparticles, as well as mesoporous silica. A Cu²⁺ detection limit of 30 nM was achieved in the acetonitrile–water mixture (2:8 v/v) for functionalized silica nanotubes, though the prerequisite of using an organic cosolvent rendered this approach less favorable if it will be applied to biological systems.

A crucial point in designing sophisticated self-organized chemosensors is the proper placement of receptor and fluorescence report moieties, which are typically not chemically connected to each other, to achieve the most efficient fluorescence turn-on or turn-off detections.^{35,54} Though Phen-based sensors do not seem to have this problem due to their ability of combined ion-recognition and fluorescence readout, maximizing the average coordination numbers of the metal cation–Phen complex, which might range from 1:1 to 1:3, will surely enhance the detection sensitivity.^{44,55,56} Theoretically, this can be achieved by optimizing the spatial arrangement of Phen moieties within self-organized chemosensors by embedding them into responsive soft matters.

Our group has a long-standing interest in stimuli-responsive polymers and microgels.⁵⁷ Recently, we attempted to further exploit their practical applications in the field of chemosensors. It is worthy of noting that poly (*N*-isopropylacrylamide) (PNIPAM) labeled with polarity-sensitive dyes has been employed as digital thermometers in previous literature reports.^{58–64} However,

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Scheme 1. Strategies Employed for Enhancing the Detection Sensitivity of Microgel-Based Cu^{2+} Chemosensors via Thermo-Induced Microgel Collapse



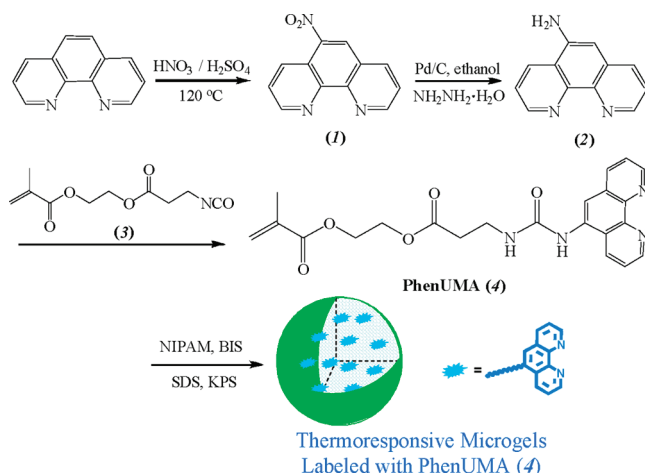
uncontrolled aggregation of PNIPAM chains and even macroscopic phase separation above the lower critical solution temperature (LCST, $\sim 32^\circ\text{C}$) dramatically limits the range of detection temperatures.⁶⁵

In this work, we report on the fabrication of responsive PNIPAM microgel-based Cu^{2+} chemosensors possessing tunable detection sensitivity via thermo-induced microgel collapse/swelling (Scheme 1). Near-monodisperse thermoresponsive microgels were synthesized via emulsion polymerization of *N*-isopropylacrylamide (NIPAM) and a novel Phen-based Cu^{2+} -sensing monomer, PhenUMA (4), in the presence of *N,N'*-methylenebis(acrylamide) (BIS) and an anionic surfactant (Scheme 2). The detection sensitivity and selectivity of as-synthesized microgels to Cu^{2+} ions at varying temperatures and microgel/metal cation concentrations have been characterized in detail. We have found that PhenUMA-labeled microgels in their swollen state can selectively bind Cu^{2+} over other metal ions, leading to prominent quenching of fluorescence emission intensity. Moreover, Cu^{2+} detection sensitivity of PhenUMA-labeled microgels can be dramatically enhanced via thermo-induced microgel collapse at elevated temperatures. At a microgel concentration of 0.083 g/L, detection limits of Cu^{2+} ions can be drastically improved from ~ 28 nM at 20°C to ~ 8 nM at 40°C . To the best of our knowledge, this proof-of-concept work represents a novel example of metal ion-sensing responsive microgels possessing thermo-enhanced detection sensitivity.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAM) (97%, Tokyo Kasei Kogyo Co.) was purified by recrystallization from a mixture of benzene and *n*-hexane (1/3, v/v). 2-Hydroxyethyl methacrylate (HEMA) (98%, Acros) was passed through a basic alumina column, vacuum distilled over calcium hydride (CaH_2),

Scheme 2. Synthetic Schemes Employed for the Synthesis of PhenUMA-Labeled Thermoresponsive Microgels for Selective Cu^{2+} Sensing with Tunable Detection Sensitivity



and stored at -20°C prior to use. *N,N'*-Methylenebis(acrylamide) (BIS) and potassium persulfate (KPS) were recrystallized from methanol and ethanol, respectively, and then stored at -20°C prior to use. Sodium azide (NaN_3) was purchased from Aldrich and used as received. Nitrate salts (Mg^{2+} , Pb^{2+} , Cd^{2+} , Al^{3+} , Ag^+ , Zn^{2+} , Mn^{2+} , Ni^{2+} , Fe^{3+} , Hg^{2+} , and Cu^{2+}) were purchased from Sinopharm Chemical Reagent Co., Ltd., and used for all sensing experiments. *N,N*-Dimethylformamide (DMF) was dried over calcium sulfate and distilled at reduced pressure. Triethylamine (TEA), dichloromethane (DCM), and chloroform were dried by refluxing over CaH_2 and distilled prior to use. Tetrahydrofuran (THF) and toluene were dried by refluxing over sodium and distilled prior to use. 1,10-Phenanthroline (99% purity), sodium dodecyl sulfate (SDS), Pd/C (10%), and all other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., and used as received. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of $18.4\text{ M}\Omega\text{ cm}$. 2-(3-Isocyanatopropoxy)ethyl methacrylate (3) was synthesized according to literature procedures.⁶⁶

Sample Synthesis. Synthetic approaches employed for the preparation of Phen-based monomer, PhenUMA (4), and PhenUMA-labeled Cu^{2+} -sensing microgels are shown in Scheme 2.

5-Nitro-1,10-Phenanthroline (1). 5-Nitro-1,10-phenanthroline was synthesized according to slightly modified literature procedures.⁶⁷ Into a 500 mL round-bottom flask equipped with a condenser and a magnetic stirring bar were charged 1,10-phenanthroline (30.0 g, 0.17 mol) and concentrated H_2SO_4 (160 mL), followed by the addition of concentrated HNO_3 (80 mL). After thermostating at 120°C in an oil bath and stirring for 3 h, the reaction mixture was cooled to 0°C and then gradually diluted with ice water while maintaining the temperature at less than 5°C . After neutralization with NaOH, the formed slightly yellow precipitates were collected by filtration and thoroughly washed with ice-cold water. The residues were further purified by recrystallization from 95% ethanol. After drying in a vacuum oven overnight at 50°C , 1 was obtained as a slightly yellow solid (16.87 g, 45% yield). ^1H NMR (CDCl_3 , δ , ppm, TMS, Figure 1a): 9.34–9.32 (2H, 2-CH and 9-CH), 9.05

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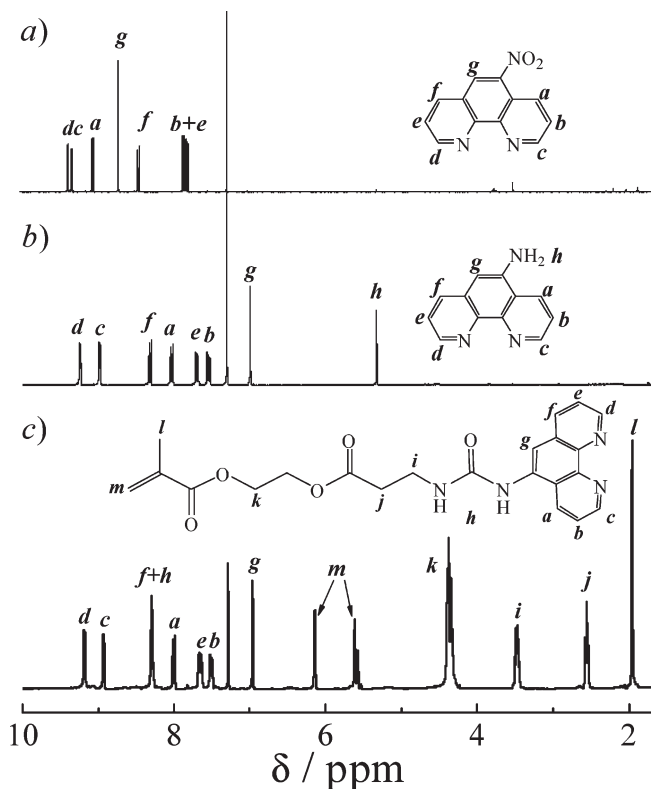


Figure 1. ^1H NMR spectra recorded in CDCl_3 for (a) 5-nitro-1,10-phenanthroline (**1**), (b) 5-amino-1,10-phenanthroline (**2**), and (c) 2-(3-(1,10-phenanthrolin-6-yl)ureido)propanoyloxyethyl methacrylate (PhenUMA, **4**).

(1H, 4-CH), 8.70 (1H, 6-CH), 8.44 (1H, 7-CH), and 7.81 (2H, 3-CH and 8-CH).

5-Amino-1,10-Phenanthroline (2). Into a three-neck round-bottom flask equipped with an air-leak and a magnetic stirring bar, 5-nitro-1,10-phenanthroline (**1**, 4.5 g, 19.98 mmol), Pd/C (10%) (0.8 g), and ethanol (100 mL) were charged. The suspension was heated to 70 °C and then degassed by bubbling with N_2 for 30 min. Hydrazine hydrate (3.0 mL, 61.5 mmol) was added, and the reaction mixture was refluxed for 5 h. After filtration at ~60–70 °C, the filtrate was evaporated to dryness on a rotary evaporator, yielding a brightly yellow powder. The crude compound was further purified by recrystallization from ethanol. After drying in a vacuum oven overnight at room temperature, **2** was obtained as a yellow crystal (3.0 g, yield: 76.9%). ^1H NMR (CDCl_3 , δ , ppm, TMS, Figure 1b): 9.25–8.99 (2H, 2-CH and 9-CH), 8.32 (1H, 7-CH), 8.03, (1H, 4-CH), 7.70–7.54 (2H, 3-CH and 8-CH), 7.0 (1H, 4-CH), and 5.33 (2H, $-\text{NH}_2$).

2-(3-(1,10-Phenanthrolin-6-yl)ureido)propanoyloxyethyl Methacrylate (4). Into a three-neck round-bottom flask equipped with a dropping funnel and a magnetic stirring bar, freshly prepared 2-(3-isocyanatopropanoyloxy)ethyl methacrylate (**3**) (0.46 g, 2.0 mmol) and anhydrous toluene (19 mL) were charged. 5-Amino-1,10-phenanthroline (**2**, 0.21 g, 1.08 mmol) in dry CHCl_3 (30 mL) was added dropwise over 1 h. The reaction mixture was stirred at ambient temperature for 3 days, followed by washing with saturated NaHCO_3 solution (3×15 mL) and deionized water (3×15 mL). After drying over anhydrous MgSO_4 and filtration, all the solvents were removed under reduced pressure. The residues were further purified by neutral alumina column chromatography using methanol/ethyl acetate (5:95 v/v) as the eluent. After drying in a vacuum oven overnight at room temperature, **4** was obtained as an orange solid (0.20 g,

yield: 44%). ^1H NMR (CDCl_3 , δ , ppm, TMS, Figure 1c): 9.19 (1H, 9-CH in Phen), 8.93 (1H, 2-CH in Phen), 8.30 (3H, 7-CH in Phen and $-\text{NHCONH}-$), 8.01 (1H, 4-CH in Phen), 7.66–7.51 (2H, 3-CH and 8-CH in Phen), 6.97 (1H, 6-CH in Phen), 6.14–5.62 (2H, $\text{CH}_2=\text{C}(\text{CH}_3)-$), 4.36 (4H, $-\text{OCH}_2\text{CH}_2\text{O}-$), 3.46 (2H, $-\text{OCOCH}_2\text{CH}_2\text{NHCONH}-$), 2.56 (2H, $-\text{OCOCH}_2\text{CH}_2\text{NHCONH}-$), and 1.96 (3H, $-\text{CH}_3$).

Synthesis of Cu^{2+} -Sensing Thermoresponsive Microgels. Into a three-neck round-bottom flask equipped with a mechanical Teflon stirrer, a thermometer, and a nitrogen-bubbling tube, NIPAM (0.49 g, 4.33 mmol), BIS (10 mg, 64.86 μmol), **4** (8.6 mg), SDS (0.02 g), and deionized water (50 mL) were charged. After degassing by bubbling with N_2 for 30 min and thermostating at 70 °C under mechanical stirring (800 rpm), KPS (25 mg, 92.5 μmol) was introduced to initiate the emulsion polymerization. The dispersion was left stirring at 70 °C for 7 h. After cooling to room temperature, the obtained microgel dispersion was further purified by dialysis (cellulose membrane, molecular weight cutoff: 14 kDa) against deionized water for 4 days, and fresh media was replaced every 4 h.

Characterization. **Nuclear Magnetic Resonance (NMR) Spectroscopy.** All ^1H NMR spectra were recorded on a Bruker AV300 NMR spectrometer (resonance frequency of 300 MHz for ^1H) operated in the Fourier transform mode. CDCl_3 was used as the solvent. All UV–vis spectra were acquired on a Unico UV/vis 2802PCS spectrophotometer. SEM images were taken on a JSM-6700F scanning electron microscope working at an acceleration voltage of 5 keV.

Laser Light Scattering (LLS). Dynamic and static laser light scattering (LLS) measurements were conducted on a commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-tau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He–Ne laser ($\lambda_0 = 632$ nm) as the light source. Scattered light was collected at a fixed angle of 90° for a duration of ~10 min. Distribution averages and particle size distributions were computed using cumulants analysis and CONTIN routines. All data were averaged over three measurements. All samples were filtered through 0.45 μm Millipore Acrodisc-12 filters to remove dust.

Atomic Force Microscope (AFM). AFM measurements were performed on a Digital Instrument Multimode Nanoscope IIID operating in the tapping mode under ambient conditions. A silicon cantilever (RFESP) with resonance frequency of ~80 kHz and spring constant of ~3 N/m was used. The set-point amplitude ratio was maintained at 0.7 to minimize sample deformation induced by the tip. The sample was prepared by dip-coating 0.01 g/L microgel dispersion onto freshly cleaved mica surface, followed by natural drying.

Fluorescence Measurements. Fluorescence spectra were recorded using a RF-5301/PC (Shimadzu) spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The slit widths were set at 5 nm for excitation and 5 nm for emission.

Results and Discussion

Synthesis of PhenUMA-Labeled Microgels. As shown in Scheme 2, PhenUMA-labeled thermoresponsive PNIPAM microgels were synthesized via emulsion polymerization of *N*-isopropylacrylamide (NIPAM) and a novel Phen-based Cu^{2+} -sensing monomer, PhenUMA (**4**), in the presence of BIS and SDS. PhenUMA (**4**) was synthesized by reacting 5-amino-1,10-phenanthroline (**2**) with

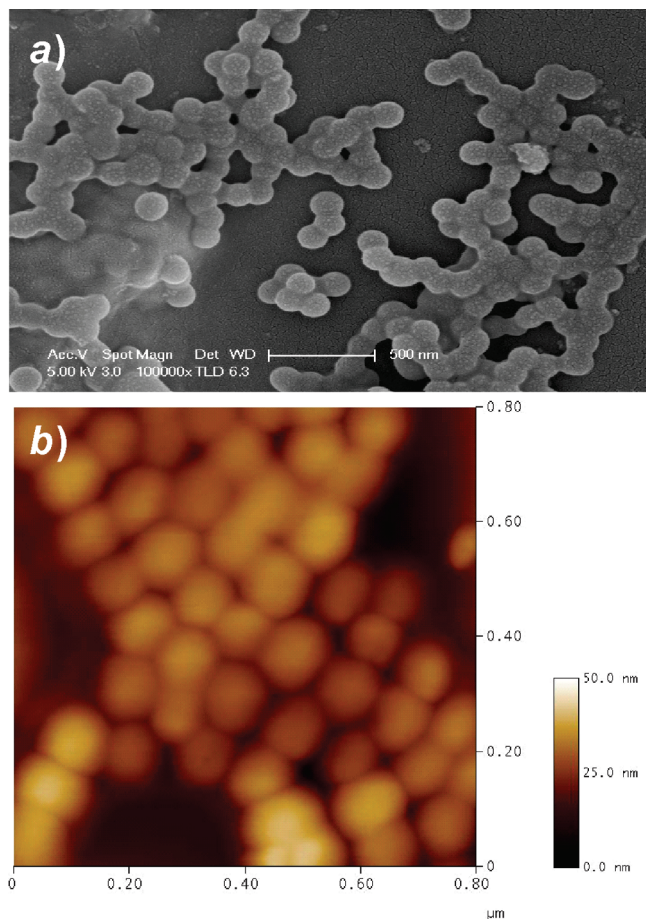


Figure 2. (a) SEM and (b) AFM height images obtained for PhenUMA-labeled thermoresponsive PNIPAM microgels.

an excess of freshly prepared 2-(3-isocyanato-propionyloxy)ethyl methacrylate (**3**). The ^1H NMR spectrum of **4** is shown in Figure 1, together with the peak assignments. Relative integral ratios between characteristic resonance signals (peaks *c*, *d*, and *m*) are determined to be 1:1:2, and this confirms the chemical structure of **4**.

PhenUMA-labeled thermoresponsive PNIPAM microgels with a designed cross-linking density of 2.0 wt % were characterized by LLS, SEM, and AFM analysis (Figures 2 and 3). Aqueous dispersion of as-synthesized microgels at 20 °C exhibit an intensity-average hydrodynamic radius, $\langle R_h \rangle$, of 100 nm and a polydispersity index, μ_2/Γ^2 , of 0.071, as revealed by dynamic LLS (Figure 2). Moreover, PhenUMA-labeled microgels possess an apparent molar mass, $M_{w,\text{app}}$, of 1.58×10^8 g/mol at 20 °C. Upon heating to 40 °C, which is above the volume phase transition (VPT) temperature of conventional PNIPAM microgels, initially swollen microgels collapse and dynamic LLS revealed an $\langle R_h \rangle$ of 42 nm and a polydispersity of 0.028. Thus, heating the microgel dispersion from 20 to 40 °C led to ~ 13.5 times of volume shrinkage. If we assume that PhenUMA moieties possess a uniform distribution within microgels, we can calculate that the average distance between neighboring PhenUMA residues decreased from ~ 8.7 to 3.7 nm upon heating from 20 to 40 °C. It is quite expected that thermo-induced microgel collapse will enhance the formation of

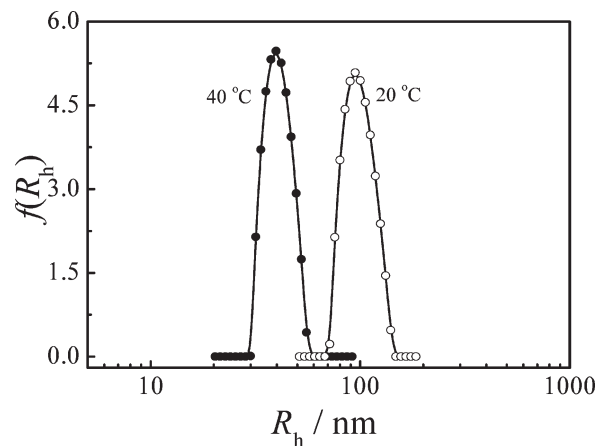


Figure 3. Hydrodynamic radius distributions, $f(R_h)$, recorded for the aqueous dispersion (0.02 g/L) of PhenUMA-labeled thermoresponsive PNIPAM microgels at 20 and 40 °C, respectively.

Cu^{2+} -PhenUMA complex with higher coordination numbers due to much closer spatial location of PhenUMA residues.

SEM and AFM images obtained by drying the aqueous dispersion of PhenUMA-labeled microgels at 20 °C revealed the presence of fairly monodisperse and spherical nanoparticles with sizes of ~ 124 and 110 nm, respectively, which is in reasonable agreement with that of collapsed microgels determined by dynamic LLS. It is well-known that SEM and AFM determine nanoparticle dimensions in the dry state, whereas dynamic LLS report the intensity-average dimensions in solution.

PhenUMA-Labeled Microgel Chemoensors for Cu^{2+} Ions. In this section, the Cu^{2+} -sensing capability of PhenUMA-labeled microgels in their swollen state at 20 °C was discussed in detail. Typical absorbance and fluorescence emission spectra obtained for the aqueous dispersion (pH 7.0 phosphate buffer) of PhenUMA-labeled microgels (0.25 g/L, $[\text{PhenUMA}] = 1.0 \times 10^{-5}$ M) upon gradual addition of Cu^{2+} ions are shown in Figures 4 and 5.

As shown in Figure 4, the UV-vis absorbance intensity of the microgel dispersions considerably increases upon gradual addition of Cu^{2+} ions (0–0.6 equiv relative to that of PhenUMA residues) and the absorbance intensity at 202 nm exhibits approximately 1.5-fold enhancement (Figure 4, inset). Concomitantly, we can discern a red shift of the maximum absorbance peak from 202 to 207 nm in the same range of $[\text{Cu}^{2+}]$. The increase of absorbance intensity and the red shift can be clearly ascribed to the formation of the Cu^{2+} -PhenUMA complex.

PhenUMA-labeled microgels (0.25 g/L, $[\text{PhenUMA}] = 1.0 \times 10^{-5}$ M) in phosphate buffer exhibit relatively intense fluorescence emission at 452 nm when excited at 280 nm, which is in agreement with that reported by Jung and co-workers.⁴⁴ This indicates that the incorporation of Phen moieties into thermoresponsive PNIPAM microgels does not alter their photophysical properties. As shown in Figure 5, the fluorescence emission intensity substantially decreases upon addition of Cu^{2+} ions and stabilizes above 0.5 equiv of Cu^{2+} ions (relative to PhenUMA residues),

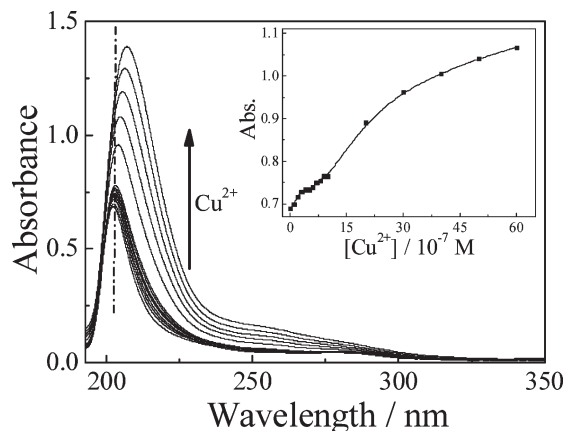


Figure 4. UV-vis absorption spectra obtained for the aqueous dispersion (pH 7.0 phosphate buffer; 20 °C) of PhenUMA-labeled thermoresponsive PNIPAM microgels (0.25 g/L, $[\text{PhenUMA}] = 1.0 \times 10^{-5} \text{ M}$) upon gradual addition of 0–0.6 equiv of Cu^{2+} ions relative to that of PhenUMA residues. The inset shows the change in absorbance intensity at 202 nm as a function of $[\text{Cu}^{2+}]$.

exhibiting approximately 80% cumulative fluorescence quenching (Figure 5b). Concomitantly, a slight blue shift of the maximum emission peak from 452 to 443 nm in the same range of $[\text{Cu}^{2+}]$ can be observed. The fluorescence quenching by Cu^{2+} ions can also be easily visualized by the naked eye under UV 365 nm irradiation (Scheme 1). If we define the detection limit as the Cu^{2+} concentration at which a 10% fluorescence quenching can be measured by employing 0.25 g/L aqueous dispersion of the PhenUMA-labeled microgels, the Cu^{2+} detection limit can be determined to be $1.25 \times 10^{-7} \text{ M}$.

The selectivity of PhenUMA-labeled thermoresponsive microgels for Cu^{2+} ions was then investigated (Figures 6 and 7). Among a series of cations including Mg^{2+} , Pb^{2+} , Cd^{2+} , Al^{3+} , Ag^{+} , Zn^{2+} , Mn^{2+} , Ni^{2+} , Fe^{3+} , Hg^{2+} , and Cu^{2+} (1.0 equiv), only Cu^{2+} exhibits the most prominent fluorescence quenching. Other metal ions exhibit relatively weak fluorescence quenching though Pb^{2+} and Mg^{2+} ions tend to slightly enhance the fluorescence intensity ($\sim 5\text{--}6\%$ increase). Interestingly, the addition of 1.0 equiv Zn^{2+} ions induce the shift of maximum emission peak from 452 to 460 nm, which might be attributed to the unique atomic structure of Zn^{2+} , and it does not exhibit fluorescence quenching to PhenUMA residues.^{68–70}

Most importantly, fluorescence spectra recorded in the presence of Cu^{2+} (1.0 equiv) and 1.0 equiv of competing metal ions (Mg^{2+} , Pb^{2+} , Cd^{2+} , Al^{3+} , Ag^{+} , Zn^{2+} , Mn^{2+} , Ni^{2+} , Fe^{3+} , and Hg^{2+}) reveal that other metal ions do not interfere in the detection of Cu^{2+} ions (Figures 6b and 7). The coexistence of equimolar amount of Fe^{3+} and Cu^{2+} ions in the PhenUMA-labeled microgel dispersion results in a weak emission peak at 365 nm, whereas the fluorescence intensity at 452 nm decreases to the same level when Cu^{2+} ions alone are added. We are currently unclear

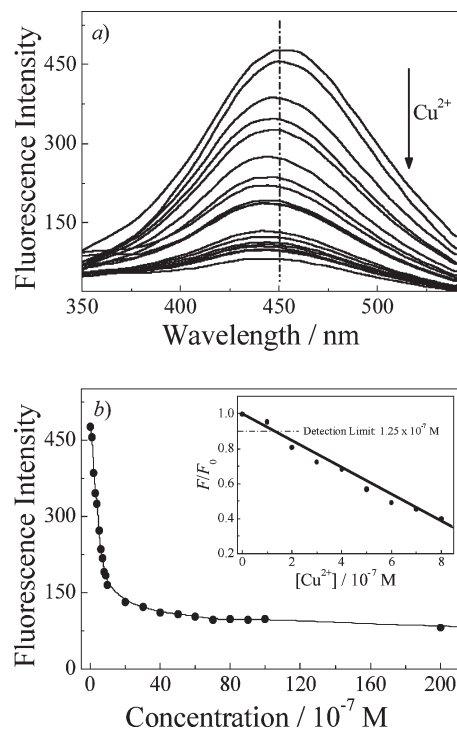


Figure 5. (a) Fluorescence emission spectra and (b) change in fluorescence intensity ($\lambda_{\text{ex}} = 280 \text{ nm}$, $\lambda_{\text{em}} = 452 \text{ nm}$; slit widths: Ex. 5 nm, Em. 5 nm) recorded for the aqueous dispersion (pH 7.0, phosphate buffer; 20 °C) of PhenUMA-labeled thermoresponsive PNIPAM microgels (0.25 g/L, $[\text{PhenUMA}] = 1.0 \times 10^{-5} \text{ M}$) upon gradual addition of 0–2.0 equiv of Cu^{2+} ions relative to that of PhenUMA residues. The inset in (b) shows the change in fluorescence intensity ($\lambda_{\text{em}} = 452 \text{ nm}$) upon gradual addition of Cu^{2+} ions (0–0.08 equiv) and the determination of detection limit.

about the detailed mechanism to explain the appearance of the 365 nm emission peak.

In our initial trials to fabricate Phen-labeled microgel-based chemosensors, we synthesized *N*-(1,10-phenanthroline-6-yl)acrylamide (PhenAM) monomer and copolymerized it into PNIPAM microgels. Unfortunately, we found that PhenAM-labeled microgels exhibit poor selectivity to Cu^{2+} ions. It seems that the urea functionality within PhenUMA is essential for Cu^{2+} selectivity, and this strongly suggests that the urea structural motif might also participate in the coordination with Cu^{2+} ions.

In the absence of Cu^{2+} ions, the fluorescence intensity of PhenUMA-labeled microgels exhibits $\sim 33\%$ increase upon heating from 20 to 40 °C (Figure 8), which can also be checked by the naked eye under UV 365 nm irradiation (Scheme 1). To verify that the fluorescence enhancement is because fluorescent PhenUMA residues are located in a hydrophobic environment within collapsed microgels,^{58,59,62,71,72} we also measured the temperature-dependent fluorescence emissions of PhenUMA monomer (Figure 8b), which actually exhibit a slight and monotonic decrease of fluorescence intensity upon heating. Thermo-induced collapse of PhenUMA-labeled microgels is expected to provide extra advantages in terms of

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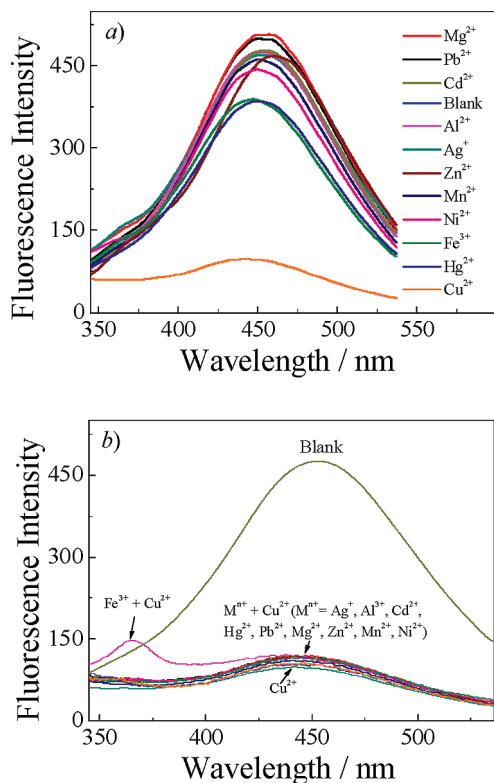


Figure 6. Fluorescence emission spectra ($\lambda_{\text{ex}} = 280$ nm, $\lambda_{\text{em}} = 452$ nm; slit widths: Ex. 5 nm, Em. 5 nm) recorded for the aqueous dispersion (pH 7.0, phosphate buffer; 20 °C) of PhenUMA-labeled thermoresponsive PNIPAM microgels (0.25 g/L, [PhenUMA] = 1.0×10^{-5} M) upon addition of (a) 1.0 equiv of Mg^{2+} , Pb^{2+} , Cd^{2+} , Al^{3+} , Ag^+ , Zn^{2+} , Mn^{2+} , Ni^{2+} , Fe^{3+} , Hg^{2+} , and Cu^{2+} ions, respectively, and (b) followed by addition of 1.0 equiv of Cu^{2+} ions.

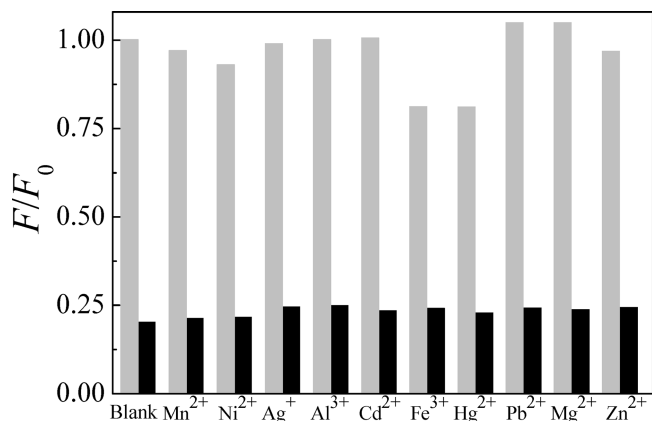


Figure 7. Selectivity of PhenUMA-labeled PNIPAM microgel chemosensors (0.25 g/L, [PhenUMA] = 1.0×10^{-5} M) for Cu^{2+} ions in the presence of other metal ions (pH 7.0, phosphate buffer; 20 °C). The fluorescence response ($\lambda_{\text{ex}} = 280$ nm, $\lambda_{\text{em}} = 452$ nm; slit widths: Ex. 5 nm, Em. 5 nm) was normalized with respect to the initial fluorescence intensity of the blank microgel sample. F and F_0 represent the fluorescence intensity of microgel chemosensors in the presence and absence of metal ions, respectively. Grey bars: F/F_0 ratios upon addition of 1.0 equiv of different metal ions: Mn^{2+} , Ni^{2+} , Ag^+ , Al^{3+} , Cd^{2+} , Fe^{3+} , Hg^{2+} , Pb^{2+} , Mg^{2+} , and Zn^{2+} , respectively. Black bars: F/F_0 ratios upon addition of 1.0 equiv of different metal ions followed by the addition of 1.0 equiv of Cu^{2+} , respectively.

Cu^{2+} sensing. At elevated temperatures, the collapse of microgels will lead to the decrease of relative distances between neighboring PhenUMA residues. This will result in more efficient capture of Cu^{2+} ions via the cooperative

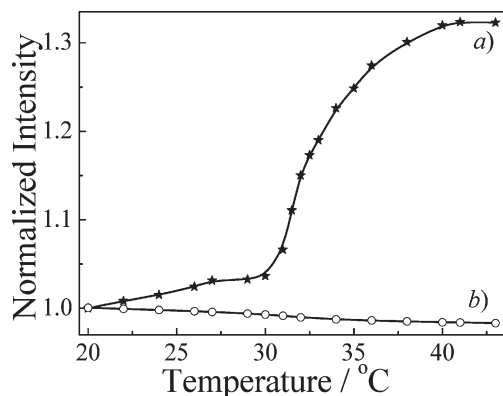


Figure 8. Change in relative fluorescence intensity ($\lambda_{\text{ex}} = 280$ nm, $\lambda_{\text{em}} = 452$ nm; slit widths: Ex. 5 nm, Em. 5 nm) recorded in the temperature range of 20–43 °C for (a) the aqueous dispersion (pH 7.0, phosphate buffer) of PhenUMA-labeled PNIPAM microgel chemosensors (0.25 g/L, [PhenUMA] = 1.0×10^{-5} M) and (b) the aqueous solution of PhenUMA monomer at a concentration of 1.0×10^{-5} M.

complexation, and concomitantly more prominent fluorescence quenching. Moreover, thermo-enhanced fluorescence emission of PhenUMA-labeled microgels can partially amplify signal changes via more efficient fluorescence quenching by Cu^{2+} ions at elevated temperatures.

On the basis of LLS data (Figure 2), we get to know that $\langle R_h \rangle$ of PhenUMA-labeled microgels exhibits a decrease from 100 to 42 nm in the temperature range of 20–40 °C. Thus, thermo-induced microgel VPT can lead to the decrease of relative distance between neighboring PhenUMA residues from 8.7 nm at 20 °C to 3.7 nm at 40 °C. In an effort to investigate the effect of detection sensitivity temperatures on Cu^{2+} -sensing, we measured Cu^{2+} detection limits of PhenUMA-labeled microgels at 20 and 40 °C, respectively, and the results are shown in Figure 9. The Cu^{2+} detection limits of PhenUMA-labeled thermoresponsive microgels at a concentration of 0.083 g/L ([PhenUMA] = 3.0×10^{-6} M) considerably improve from ~ 28 nM at 20 °C to ~ 8 nM at 40 °C. A simple calculation can roughly estimate that, at these detection limits, one Cu^{2+} ion can quench the fluorescence of ~ 10.7 PhenUMA residues at 20 °C, whereas at 40 °C, one Cu^{2+} ion can quench ~ 37.5 fluorescent moieties.²⁵ Thus, the collapse of PhenUMA-labeled PNIPAM microgels at elevated temperatures has indeed been confirmed to considerably enhance the Cu^{2+} detection sensitivity due to the cooperative and collective effects resulting from increased local PhenUMA concentrations.

Fluorescence Quenching Mechanism. As to the quenching mechanism of Cu^{2+} ions to phenanthroline moieties, we tentatively propose as follows. Upon Cu^{2+} ion addition, the formation of the Cu^{2+} –PhenUMA complex leads to the fluorescence quenching of PhenUMA residues, possibly via the electron transfer mechanism. This type of quenching mechanism has been proposed by Jung et al.⁴⁴ and Prasad et al.⁷³ for Cu^{2+} –Phen systems. However, considering that electron transfer must occur within a short distance (< 2 nm) between quenchers and

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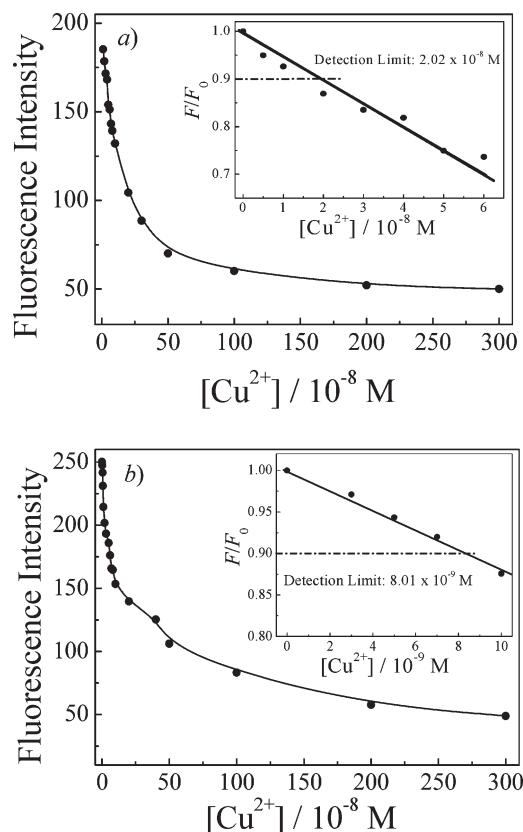


Figure 9. Change in fluorescence emission intensity ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 452$ nm; slit widths: Ex. 5 nm, Em. 5 nm) recorded at (a) 20 °C and (b) 40 °C for the aqueous dispersion (pH 7.0, phosphate buffer) of PhenUMA-labeled thermoresponsive PNIPAM microgels (0.083 g/L, [PhenUMA] = 3.0×10^{-6} M) upon gradual addition of 0–1.0 equiv of Cu^{2+} ions relative to that of PhenUMA residues. The insets in (a) and (b) show the determination of detection limits at 20 and 40 °C, respectively.

fluorophores, this mechanism alone cannot explain the fact that one single Cu^{2+} ion is able to quench approximately 10.7 and 37.5 PhenUMA residues at 20 and 40 °C, respectively.⁷⁴ On the basis of our previous calculation of the relative distance between PhenUMA residues in swollen and collapsed microgel states, Cu^{2+} ions are

being able to quench PhenUMA fluorophores located at > 2 nm distances. Recently, Williams and co-workers reported that the Cu^{2+} –bipyridine complex can efficiently quench the fluorescence of $Ru(bpy)_3^{2+}$ via combined electron transfer and energy transfer mechanisms.⁷⁵ We tentatively propose here that the energy transfer mechanism might also play an important role for the observed fluorescence quenching.

Conclusion

In summary, near-monodisperse Cu^{2+} -sensing microgels were synthesized via emulsion polymerization of NIPAM in the presence of BIS, an anionic surfactant, and PhenUMA monomer. The microgel-based chemosensors present a high selectivity and sensitivity for Cu^{2+} over other metal ions including Al^{3+} , Mg^{2+} , Zn^{2+} , Fe^{3+} , Mn^{2+} , Ni^{2+} , Ag^+ , Cd^{2+} , Hg^{2+} , and Pb^{2+} . The fluorescence intensity of PhenUMA microgels can be efficiently quenched by Cu^{2+} ions, possibly via combined electron transfer and energy transfer mechanism. We further found that the quenching efficiency of Cu^{2+} ions to PhenUMA residues can be considerably improved via thermo-induced microgel collapse, and the detection limits were prominently enhanced from ~ 28 nM at 20 °C to ~ 8 nM at 40 °C. A plausible mechanism was tentatively proposed. The employed new strategy to improve detection limits by combining the concept of self-organized chemosensors with stimuli-responsive soft matter entities might be expanded to other systems. Further works toward the fabrication of microgel-based ratiometric FRET sensors are currently underway.

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