Nanoadsorbents Based on NIPAM and Citric Acid: Removal Efficacy of Heavy Metal Ions in Different Media

Yifei Guo, Xuejie Zhang, Xueqing Sun, Dandan Kong, Meihua Han, and Xiangtao Wang*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 151, Malianwa North Road, Haidian District, Beijing 100193, China

ABSTRACT: Heavy metal ions in aqueous solutions are harmful to human health, but exploring and exploiting nanoadsorbents with a high adsorption capacity and low cost should be an effective method for overcoming this problem. In this study, a novel nanoadsorbent termed poly(N-isopropylacrylamide-co-citric acid) (PNCA) was designed and synthesized via free-radical polymerization. PNCA exhibits good solubility in aqueous solutions and can self-assemble into spherical nanoaggregates with a mean hydrodynamic diameter of approximately 723.1 nm. After freeze-drying, the solid powder of PNCA exhibited a loose porous structure. When PNCA is dissolved in water, the resulting copolymer solution exhibits high removal rates for Cu^{2+} and Pb^{2+} of over 80%; meanwhile, over 97% of the PNCA is precipitated with metal ions. The adsorption process of PNCA chelated with Cu^{2+} ions fit the Freundlich model. The adsorption capacity is independent of the media pH, but could be affected by the temperature. Except for herbal medicines with alkaloids as active ingredients, PNCA also presents good adsorption capacity for Cu^{2+} in herbal medicine decoctions, with a removal rate of over 80%. The cell cytotoxicity in vitro and system toxicity in vivo demonstrate the desirable biosafety of PNCA. These results suggest that PNCA with good biosafety can be utilized as a nanoadsorbent to remove the metal ions, especially Cu^{2+}, in different media.

1. INTRODUCTION

Heavy metal ion contamination represents a serious threat to human health. Such a contamination exists in many ecological systems and it is necessary to remove these harmful elements from water or other aqueous solutions prior to their use.\(^1\)\(^2\)\(^3\) For this purpose, various methods have been exploited, including chemical precipitation, ion-exchange, adsorption, ultrafiltration, and reverse-osmosis techniques.\(^4\)\(^5\)\(^6\) Among these methods, adsorption is considered to be a facile, effective, and economic method.\(^7\)\(^8\)\(^9\) Materials containing abundant functional groups can be utilized as effective adsorbents,\(^10\)\(^11\)\(^12\)\(^13\) especially carboxyl groups, which can adsorb metal ions via chelation.\(^14\)\(^15\)

Citric acid exists naturally in the human body and has been approved by the FDA as a food additive.\(^16\) Citric acid contains three carboxyl groups in each molecule, and hence, it should be applicable as an effective adsorbent to remove heavy metal ions in aqueous solutions.\(^17\)\(^18\)\(^19\) However, citric acid presents a low adsorptive efficacy based on its high water solubility. Therefore, it is generally loaded onto a substrate for use as an adsorbent.\(^20\)\(^21\)\(^22\) The adsorption efficacy of such derivatives is limited due to the low loading-efficacy of citric acid on these substrate. Hence, it is necessary to find another method for preparing adsorbents based on citric acid.

To enhance the removal efficiency, adsorbents are expected to be soluble in aqueous solutions to achieve a high specific surface area, followed by sedimentation after chelating with heavy metal ions.\(^23\) Based on the development of nanomaterials, water-soluble polymers have been explored and applied to the removal of heavy metal ions from waste water, including poly(1-vinylimidazole),\(^24\) poly(vinyl amino-N,N'-bis-propane diol),\(^25\) poly(vinyl sulfonic acid),\(^26\) polyethyleneimine,\(^27\) poly(acrylic acid),\(^28\)\(^29\) poly(vinyl alcohol),\(^30\)\(^31\) and polysaccharides.\(^32\) However, this technique carries the potential risk of secondary pollution in terms of the survival of adsorbents in water.\(^23\) To decrease residual adsorbents in aqueous solutions, various smart nanoadsorbents with stimuli-response properties have been designed.\(^33\)\(^34\) In particular, thermosensitive materials have been researched broadly based on the feasibility and practicality of their application. Poly(N-isopropyl acrylamide) (PNIPAM) is a thermosensitive nanomaterial with a low critical solution temperature of 32 °C, which is often applied as a drug/gene carrier.\(^35\)\(^36\)\(^37\) When the temperature is lower than 32 °C, PNIPAM is soluble in...
water in the form of random coils based on hydrogen bonds. As the temperature increases beyond 32 °C, hydrogen bonds are broken and PNIPAM transitions from hydrophilic to hydrophobic, causing it to precipitate from water. Based on this property, PNIPAM is often utilized as a nanoadsorbent to remove heavy metal ions and organic dyes in waste water. Additionally, it has been reported that the adsorption efficacy could be improved significantly by combining acrylic acid with PNIPAM due to the chelation efficacy between carboxyl groups and heavy metal ions. Therefore, copolymers derived from PNIPAM and carboxyl monomers can provide an excellent removal capacity.

Based on the high removal efficiency of citric acid and the thermoresponsibility of PNIPAM, in this study, a thermoresponsive copolymer named poly(NIPAM-co-citric acid) (PNCA) that combines NIPAM and a trimethyl citrate monomer (TCM) was designed and synthesized via free-radical polymerization. The adsorption capacity, residual levels, and biosafety of the proposed copolymer were studied. PNCA could chelate with Cu$^2+$ and Pb$^2+$ with a high adsorption efficacy in water. Furthermore, PNCA also presented selective adsorption for Cu$^2+$ in decoctions of Chinese herbal medicines. However, PNCA should not be applied in decoctions with alkaloids as active ingredients because the electrostatic interaction between alkaloids and PNCA is strong, which can affect the adsorption capacity and the concentration of the active ingredient.

2. RESULTS AND DISCUSSION

2.1. Synthesis of Nanoadsorbents. Based on the trimethyl citrate monomer (TCM), the proposed nanoadsorbent poly(N-isopropyl acrylamide-co-citric acid) (PNCA) was synthesized via successive free-radical polymerization and hydrolysis reactions (Scheme 1). N-Isopropyl acrylamide (NIPAM) and TCM were polymerized to prepare the copolymer poly(NIPAM-co-trimethyl citrate). Next, the copolymer was hydrolyzed using LiOH in a mixed solvent of tetrahydrofuran (THF)/H$_2$O (1:1, v/v) over 12 h. Following dialysis with distilled water and freeze-drying, PNCA was obtained as a white solid.

2.2. Characterization. The structure of PNCA was confirmed using $^1$H NMR spectroscopy; the signals at 3.6–3.7 ppm were attributed to the methenyl proton of NIPAM, the signal at 3.0 ppm was attributed to the methylene proton of citric acid, and the signals at 1.0 ppm were attributed to the methyl proton of the backbone and NIPAM (Scheme 1). The molar mass of PNCA was detected by GPC, which was 155,000 (Table 1). Due to the free-radical polymerization, the molar mass distribution of PNCA was approximately 3.2.

PNCA presented good hydrophilic properties and could be dispersed in distilled water indirectly to form a transparent solution. The particle size, size distribution, and $\zeta$-potential of the PNCA aqueous solution with a concentration of 1 mg mL$^{-1}$ were measured using dynamic light scattering (DLS). It was found that PNCA could self-assemble into nanosstructures in aqueous solutions with an average hydrodynamic diameter of approximately 723.1 nm (Figure 1a) and a $\zeta$-potential of $-25.8$ mV. Scanning electron microscopy (SEM) images showed that these aggregates were spherical nanoparticles with an average diameter of approximately 210 nm (Figure 1b). The particle size was smaller when measured by SEM than by dynamic light scattering (DLS), which was attributed to the fact that measurement of the particle size reported the actual diameter of the dried particles rather than the hydrodynamic diameter. Based on the thermosensitivity of NIPAM, the copolymer PNCA also presented thermoresponsive properties; the turbidity curve is shown in Figure 1c. PNCA exhibited typical thermoresponsive behaviors, and its low critical solution temperature (LCST) was approximately 43.2 °C at a concentration of 1 mg mL$^{-1}$.

| Table 1. Results of the Copolymer PNCA |
| sample & $M_n^{\text{D}}$ & $D_h$ (nm) & PDI$^c$ & $\zeta$ (mV)$^d$ |
|---|---|---|---|---|
| PNCA & 155,000 & 723.1 & 0.24 & $-25.8$ |

*Measured by gel permeation chromatography (GPC) using water as the eluent at 35 °C. $^a$Hydrodynamic diameter. $^b$Polydisperse index. $^c$Dispersive potential.

Reagents and conditions: (a) AIBN, DMF, 4 h, 60 °C, 92%; (b) LiOH, THF/H$_2$O, –5 to 25 °C, 14 h, 87%.
2.3. Adsorption Efficacy in Aqueous Solutions. Heavy metal ion solutions, including Cu²⁺, Pb²⁺, Cd²⁺, and Hg²⁺ separately, were mixed with citric acid solutions and PNCA solutions. No significant changes in metal ion solutions could be observed after adding citric acid solutions, implying that the chelates of citric acid and metal ions were difficult to separate from the system. In contrast, the copolymer PNCA exhibited good adsorptive properties (Figure 2a) after adding PNCA solutions; a large amount of precipitation occurred in Cu²⁺ and Pb²⁺ solutions, and a small amount of precipitation was observed in Cd²⁺ and Hg²⁺ solutions. These results suggested that PNCA exhibited different adsorption efficacies for different metal ions.

After adding 0.1 mL of PNCA solution with the concentration of 9 mg mL⁻¹ into 1 mL of heavy metal ion solutions with the concentration of 100 μg mL⁻¹, including Cu²⁺, Pb²⁺, Cd²⁺, and Hg²⁺ ions separately, precipitation occurred. After filtration, the concentrations of metal ions in the filtrates were detected by ICP-Mass, and then they were utilized to calculate the removal rates, which are presented in Figure 2b. The concentrations for Cu²⁺, Pb²⁺, Cd²⁺, and Hg²⁺ were 18.6, 8.4, 87.6, and 96.9 μg mL⁻¹, respectively, and the removal rates were 81.4, 91.6, 12.4, and 3.1% correspondingly. After filtration, the precipitates were freeze-dried and their morphologies were determined using SEM. To identify structural changes in PNCA after chelating with heavy metal ions, the PNCA solution was freeze-dried; the solid nanoadsorbent presented a loose porous sponge structure (Figure 2c, PNCA). The complex of PNCA chelated with Cu²⁺ and Pb²⁺ ions presented a tight structure, the precipitate of PNCA with Cu²⁺ ions exhibited a nanofiber structure (Figure 2c, PNCA + Cu²⁺), and the precipitate of PNCA with Pb²⁺ ions exhibited a sheet-like structure (Figure 2c, PNCA + Pb²⁺). This phenomenon revealed that chelation acted as a linker to induce dense macromolecule-chain-stacking during the adsorption process, resulting in the tight structures that were shown.

To estimate the adsorption capacity of PNCA,

\[ Q_e = \frac{C_i - C_f}{m} V \]

adsorption tests at 50 °C were conducted with heavy metal ion concentrations of 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 8.0, and 10.0 mg mL⁻¹. After heating and centrifugation, blue precipitates were collected. The filtrate was analyzed using ICP-Mass to estimate the concentration of residual Cu²⁺ ions; \( C_f \) (mmol L⁻¹) is the concentration of Cu²⁺ ions in the filtrate at equilibrium and \( Q_e \) (mmol g⁻¹) is the adsorption capacity. To determine the adsorption capacity, the adsorption isotherms at 50 °C were investigated. As shown in Figure 3a, the Cu²⁺ ion chelation efficacy of PNCA was approximately 26 mmol g⁻¹. Besides, according to the formulas of the Langmuir and Freundlich model, adsorption models were estimated and two curves were obtained (Figure 3b,c). The regression coefficient (\( R^2 \)) of the Freundlich model was 0.98, which was greater than that of the Langmuir model (\( R^2 = 0.87 \)), revealing that the adsorption model of PNCA matched the Freundlich model.

The adsorption capacity of adsorbents might be affected by the media pH; therefore, it is necessary to study the influence of the media pH on the removal rate of PNCA.\(^{47}\) PNCA and Cu²⁺ powder were dissolved in different solutions with pH values ranging from 2 to 8. The PNCA solution (0.1 mL) was added into 1 mL of Cu²⁺ ion solution, and after heating and centrifugation, the precipitates were collected. The Cu²⁺ ion concentrations in the filtrate were detected by ICP-Mass, and the adsorption capacity \( Q_e \) at different pH values was calculated (Figure 4a). As the media pH value increased from 2 to 8, the adsorption capacity was enhanced slightly, but no significant changes were shown, which indicated that the adsorption properties of PNCA would not be affected significantly by the media pH; hence, PNCA could be broadly utilized as a nanoadsorbent.

Due to the thermosensitive properties of PNCA, the influence of temperature on the adsorption properties of PNCA for Cu²⁺ ions was also studied. When a PNCA solution was added to a heavy metal ion solution at a temperature below its LCST, no changes could be observed during vortex.
However, the precipitates appeared immediately after heating the solution above the LCST, exhibiting a sharp change. The adsorption capacities of PNCA at different temperatures are shown in Figure 4b. These results indicated that PNCA did not exhibit any significant adsorptive capacity when the temperature was below 42 °C, whereas after heating above 42 °C, the adsorptive capacity enhanced significantly and was maintained at approximately 80%.

2.4. Adsorption Efficacy in a Decoction. Although Chinese herbal medicines only present a moderate therapeutic efficacy, they are broadly utilized in the clinic based on their negligible side effects. However, depending on the kind of plants, herbal medicines could hyperaccumulate heavy metals, which could pose a severe risk to human health. Therefore, it is necessary to remove heavy metal ions from herbal decoctions. Based on the good removal rate of PNCA for Cu²⁺ and Pb²⁺ in water, PNCA was applied furthermore to remove Cu²⁺ and Pb²⁺ from a ligusticum wallichii solution, which is a Chinese water. PNCA was applied to remove Cu²⁺ and Pb²⁺ in decoctions of different Chinese traditional medicine decoctions (b): 1, ligusticum wallichii; 2, scutellaria baicalensis; and 3, coptidis rhizome. These results suggested that PNCA presented a good adsorptive capacity for Cu²⁺ and Pb²⁺ in ligusticum wallichii decoctions. Meanwhile, the concentration of FA in the decoction after filtration only changed slightly to 182.2 μg mL⁻¹, representing a removal rate of 7.1%. It seemed that PNCA would not affect the therapeutic efficacy of ligusticum wallichii significantly.

To evaluate the adsorption efficacy of PNCA for Cu²⁺ ions in other Chinese herbal medicines, scutellaria baicalensis and coptidis rhizome decoctions were also tested (Figure 5b). The original concentration of Cu²⁺ ions in these decoctions was 100 μg mL⁻¹. After adding the PNCA solution, large amounts of precipitates were shown in ligusticum wallichii and scutellaria baicalensis decoctions and a small amount of precipitate was presented in coptidis rhizome decoctions. The actual concentrations of Cu²⁺ ions were 19.2, 12.2, and 86.7 μg mL⁻¹, representing removal rates of 80.8, 87.8, and 13.3%, respectively. Overall, PNCA exhibited a high removal rate for Cu²⁺ in decoctions of ligusticum wallichii and scutellaria baicalensis, but a low adsorption efficacy for decoctions of coptidis rhizome. Besides, the concentrations of active ingredients in these herbal medicine decoctions were detected by high-performance liquid chromatography, and the results showed that PNCA would not affect the therapeutic efficacy of ligusticum wallichii or scutellaria baicalensis because the losses of active ingredients in these decoctions were less than 5%. In contrast, the active ingredient of coptidis rhizome showed a significant change, with a loss of 33%. Based on these results, it seemed that PNCA could not be applied to all types of herbal medicines, which could be explained by the structure of PNCA. PNCA was formed from citric acid, indicating that it contained many carboxyl groups that could interact with compounds containing basic groups. In ligusticum wallichii and scutellaria baicalensis, the active ingredients were ferulic acid and baicalin, respectively; both of them contained carboxyl groups, revealing that the interactions between PNCA and these active ingredients were very weak. However, the active ingredient in coptidis rhizome was berberine, which was an alkaloid, representing that the electrostatic interaction between berberine and PNCA was strong, and therefore, a significant amount of berberine was adsorbed by PNCA.

2.5. Biosafety. To estimate the effects of PNCA on human health, the biosafety was assessed based on its in vitro cell cytotoxicity toward HUVEC cells and in vivo animal experiments, as the residual organic nanaosorbents in either water or the decoction might induce severe toxicities. The cytotoxicity in vitro against HUVEC cells was researched via the MTT method, and the concentrations of PNCA ranged from 1 μg mL⁻¹ to 30 mg mL⁻¹ (Figure 6a). The cell viability was over 80% when the concentration of PNCA was less than 0.5 mg mL⁻¹, revealing that PNCA did not show significant cell cytotoxicity. Although the highest concentration of PNCA was 0.9 mg mL⁻¹ in these adsorptive experiments, after chelating with Cu²⁺ and Pb²⁺ ions, nearly all of the nanaosorbent was precipitated. Based on the weight of precipitates, the residual amount of PNCA in the aqueous solution was <3%; hence, the concentration was <0.03 mg mL⁻¹, resulting in a cell viability greater than 90%. These results suggested that nanaosorbent PNCA exhibited an acceptable biosafety in vitro.

Besides, normal Balb/c mice were utilized to estimate the systemic toxicity of PNCA in vivo. The mice were randomly divided into six groups and then administered with a normal saline solution (blank control group) or with PNCA in the normal saline solution with concentrations ranging from 5 to 400 mg kg⁻¹ (test groups). The mice were monitored every day, and their body weights were recorded every 2 days. The body weights of the test groups showed no significant differences compared to that of the blank control group (Figure 6b). During the entire procedure, no weight loss, signs of distress (unresponsiveness, labored breathing, discharge), or death were observed, revealing the low systemic toxicity of PNCA.
3. CONCLUSIONS

A nanoadsorbent based on NIPAM and citric acid was designed and synthesized via free-radical polymerization with a high yield. The resulting PNCA presented good solubility in water and could self-assemble into spherical nanoparticles with a hydrodynamic diameter of approximately 723.1 nm. PNCA exhibited a good adsorption efficacy toward Cu\(^{2+}\) and Pb\(^{2+}\) ions in aqueous solutions with removal rates greater than 80%. Additionally, over 97% of the PNCA precipitated with metal ions; hence, the residual PNCA in the filtrate was <3%. PNCA presented a good adsorption efficacy of Cu\(^{2+}\) and Pb\(^{2+}\) ions not only in aqueous solutions but also in Chinese medicine decoctions, especially for Cu\(^{2+}\) ions, with a removal rate of 81%. Although PNCA presented a good adsorption efficacy in decoctions, it was not suitable for herbal medicines with alkaloids as active ingredients because the carboxyl groups in PNCA could interact with alkaloids via electrostatic interactions. PNCA revealed good biosafety, and no cell cytotoxicity in vitro or system toxicity in vivo was shown. These results suggested that PNCA with good biosafety could be utilized as a nanomaterial to remove metal ions, particularly Cu\(^{2+}\), from various media.

4. MATERIALS AND METHODS

4.1. Materials. NIPAM was purchased from Sigma-Aldrich Chemicals, Germany. Trimethyl citrate and methacryloyl chloride were obtained from TCI Shanghai. The trimethyl citrate monomer was synthesized according to previous papers.\(^{26}\) Heavy metal ions were purchased from the National Institute of Metrology, China. Ferulic acid, baicalin, and berberine hydrochloride were purchased from the National Institute of Metrology, China. Ferulic acid, baicalin, and berberine hydrochloride were purchased from the National Institute of Metrology, China. Dichloromethane (DCM) was recrystallized three times in methanol. Triethylamine (TEA) was dried over calcium hydride and distilled prior to its use. Other reagents and solvents purchased were of analytical grade and used without further purification.

4.2. Cells and Animals. The human umbilical vein endothelial (HUVEC) cell line was purchased from the National Infrastructure of Cell Line Resource (Beijing, China) and cultured in an RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 units mL\(^{-1}\) of penicillin G and streptomycin in a humidified 5% CO\(_2\) atmosphere at 37 °C.

BALB/c mice (5–6 weeks old, 20 ± 2 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). All experimental procedures were performed in accordance with the Guidelines and Policies for Ethical and Regulatory for Animal Experiments and approved by the Animal Ethics Committee of Peking Union Medical College (Beijing, China).

4.3. Syntheses. Poly(NIPAM-co-trimethyl citrate) (PNTC): the trimethyl citrate monomer (0.50 g), NIPAM (1.00 g), AIBN (1.50 mg, 0.15%, wt %), and dry DMF (1.00 mL) were added into a Schlenk tube. The solution was thoroughly deoxygenated using several freeze–pump–thaw cycles and then stirred for 4 h at 60 °C. The polymer was dissolved in DCM and purified using silica gel column chromatography with DCM as an eluent; PNTC was collected as a white powder (1.38 g, 92%). \(^1\)H NMR (CDCl\(_3\)): \(\delta = 0.8–1.2\) (br, CH\(_3\)), 1.3–1.7 (dbr, CH\(_2\)), 1.8–2.1 (br, CH), 3.1–3.3 (br, CH\(_2\)), 3.6–3.7 (br, CH\(_3\)), 3.8–3.9 (br, CH).

Poly(NIPAM-co-citric acid) (PNCA): LiOH (0.40 g) in H\(_2\)O (10 mL) was added dropwise into a solution of PNTC (0.15 mg) in THF (40 mL) at 0 °C. The mixture was stirred overnight, and then the solvent was evaporated. The crude product was purified via the dialysis method to afford PNCA as a white powder (0.13 g, 87%). \(^1\)H NMR (CDCl\(_3\)): \(\delta = 0.8–1.2\) (br, CH\(_3\)), 1.2–1.7 (dbr, CH\(_2\)), 1.8–2.0 (br, CH), 2.8–3.1 (dbr, CH\(_2\)), 3.6–3.8 (br, CH).

4.4. Characterization by NMR and GPC. NMR spectra were recorded using Bruker 300 and 600 MHz spectrometers either in DMSO-\(d_6\) or in D\(_2\)O at room temperature. Gel permeation chromatography (GPC) measurements were carried out on a Shimadzu GPC LC20 instrument with a single-column set equipped with an RID-20 refractive index detector and water (containing 0.1 N NaNO\(_3\) and 0.06% NaN\(_3\)) as the eluent at 35 °C. Calibration was performed with PEO standards.

4.5. Dynamic Light-Scattering Analysis. Particle sizes and size distributions of the PNCA aqueous solutions were detected by dynamic light scattering. These measurements were conducted by a Zetasizer Nano-ZS analyzer (Malvern Instruments, U.K.). The concentration of the PNCA solution was 1 mg mL\(^{-1}\), and all experiments were carried out in triplicate.

4.6. Scanning Electron Microscopy. An energy-dispersive spectrometer (SEM-EDS; S-4800, Hitachi) was utilized to investigate the morphologies of nanoadsorbents and precipitation. After freeze-drying, these samples were placed on a matrix and then sputter-coated with a conductive layer of gold–palladium (Au/Pd) for 1 min. A 30 mV potential was used for observation.

4.7. Adsorption Measurement. The aqueous solution (0.1 mL) of citric acid (CA, 9 mg mL\(^{-1}\)) or PNCA (9 mg mL\(^{-1}\)) was added to 1 mL of metal ion solution (100 µg mL\(^{-1}\)) containing Cu\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\). The mixture was heated and the precipitates formed. After centrifugation and filtration, the precipitates were freeze-dried and weighted, and the metal ion concentrations in the filtrates were detected by ICP-Mass.

The aqueous solution (0.1 mL) of PNCA (3 mg mL\(^{-1}\)) was added to 1 mL of Cu\(^{2+}\) ion solutions (0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 8.0, and 10.0 mg mL\(^{-1}\)). The mixtures were then heated and the precipitates formed. After centrifugation and filtration, the Cu\(^{2+}\) ion concentrations in the filtrates were detected by ICP-Mass.

The aqueous solution (0.1 mL) of PNCA (3 mg mL\(^{-1}\)) was added to 1 mL of Cu\(^{2+}\) ion solution (0.25 mg mL\(^{-1}\)) with the pH value ranging from 2 to 8. The mixtures were then heated and the precipitates formed. After centrifugation and filtration, the Cu\(^{2+}\) ion concentrations in the filtrates were detected by ICP-Mass.

The aqueous solution (0.1 mL) of PNCA (3 mg mL\(^{-1}\)) was added to 1 mL of Cu\(^{2+}\) ion solutions (0.25 mg mL\(^{-1}\)) with the temperature ranging from 38 to 48 °C. After centrifugation and filtration, the Cu\(^{2+}\) ion concentrations in the filtrates were detected by ICP-Mass.

All samples were diluted with 2–5% nitric acid and the filtrates were analyzed by an ICP spectrometer (Agilent ICP-MS 7500). The radio-frequency power was set to 15 L min\(^{-1}\), the carrier gas was argon, the plasma flow was 15 L min\(^{-1}\), the auxiliary gas flow was set to 1.5 L min\(^{-1}\), and the
nebulizer gas flow was 0.75 L min⁻¹. The axial observation detection mode with a linear calibration type was used. The viewing height and grating order were adjusted for each metal ion. Standard solutions were prepared at five different concentrations and a quality control standard was tested after every 15 samples. The wavelengths for each metal analyzed were 327.39 nm for copper, 220.35 nm for lead, and 226.50 nm for cadmium. The adsorptive capacities (Q_e) and removal rates were calculated as follows:

\[ Q_e = (C_0 - C_f) \times V/W \]

removal rate = \( (1 - C_f/C_0) \times 100\% \)

where \( C_0 \) is the initial concentration of Cu²⁺ ions, \( C_f \) is the actual concentration of Cu²⁺ ions in the filtrate at equilibrium, \( V \) is the volume of the filtrate, and \( W \) is the weight of the adsorbents.

4.8. XRD. X-ray diffraction analysis (XRD) was used to determine the compositions of the samples, using a Japan D/Max 2500PC X-ray diffractometer (Rigaku, Japan) with a 2θ range between 3 and 80° and a graphite-filtered Cu Kα radiation source (\( \lambda = 1.54 \) Å) operating at 40 kV and 100 mA (scanning rate of 8° min⁻¹). The XRD patterns were recorded at 25 °C.

4.9. Biosafety Testing. The in vitro cytotoxicity of PNCA toward a HUVEC cell line was assessed using an MTT assay. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 units mL⁻¹ of penicillin G and streptomycin at 37 °C with 5% CO₂ and seeded in 96-well plates at a density of 10⁴ cells per well. Prior to treatment with the PNCA solution, the cells were incubated for 24 h, and then the cultural medium was changed to fresh RPMI-1640. After treatment with various formulations and culturing for another 48 h, 20 μL of MTT solution (5 mg mL⁻¹ in PBS) was added to each well and incubated for 4 h. Subsequently, the medium was removed and 200 μL of DMSO was added into each well to dissolve the blue formazan crystals, and the absorbance was measured at 570 nm. The cell inhibition rate was calculated as follows: cell inhibition = \( (1 - OD_{treated}/OD_{control}) \times 100\% \), where OD_{treated} was the value after treatment with the PNCA solution and OD_{control} was the value after treatment with the culture medium.

The normal BALB/c mice were randomly divided into six groups (10 mice per group); mice were treated with normal saline (control group) or PNCA with different concentrations (test group) via gastrointestinal administration. The body weights of the mice were monitored as an index of systemic toxicity.

4.10. Statistical Analysis. All experiments were conducted in at least triplicate (at least three independent experiments). Data were presented as mean values ± SD. Comparisons between groups were performed using one-way analysis of variance (SPSS 19.0), \( P < 0.05 \) indicated statistical significance.

**ACKNOWLEDGMENTS**

This work is financially supported by CAMS Innovation Fund for Medical Sciences (CIFMS, no. 2017-12M-1-013 and no. 2016-12M-1-012).

**REFERENCES**

1. Burakov, A. E.; Galunin, E. V.; Burakova, I. V.; Kucherova, A. E.; Agarwal, S.; Tkachev, A. G.; Gupta, V. K. Adsorption of heavy metals on conventional and nanostructured materials for wastewater treatment purposes: A review. *Ecotoxicol. Environ. Saf.* 2018, 148, 702–712.

2. De Gisi, S.; Lofrano, G.; Grassi, M.; Notarnicola, M. Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review. *Sustainable Mater. Technol.* 2016, 9, 10–40.

3. Duruibe, J. O.; Ogwuegbu, M. O. C.; Egwurugwu, J. N. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.* 2007, 2, 112–118.

4. Barakat, M. A. New trends in removing heavy metals from industrial wastewater. *Arabian J. Chem.* 2011, 4, 361–377.

5. Dąbrowski, A.; Hubicki, Z.; Podkocicenly, P.; Robins, E. Selective removal of the heavy metal ions from waters and industrial wastewaters by ion-exchange method. *Chemosphere* 2004, 56, 91–106.

6. Hashim, M. A.; Mukhopadhyay, S.; Sahu, J. N.; Sengupta, B. Remediation technologies for heavy metal contaminated groundwater. *J. Environ. Manage.* 2011, 92, 2355–2388.

7. Sud, D.; Mahajan, G.; Kaur, M. P. Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions – A review. *Bioresour. Technol.* 2008, 99, 6017–6027.

8. Babel, S.; Kumiawan, T. A. Low-cost adsorbents for heavy metals uptake from contaminated water: a review. *J. Hazard. Mater.* 2003, 97, 239–243.

9. Wan Ngah, W. S.; Hanafiah, M. A. K. M. Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review. *Bioresour. Technol.* 2008, 99, 3935–3948.

10. Xin, X.; Wei, Q.; Yang, J.; Yan, L.; Feng, R.; Chen, G.; Du, B.; Li, H. Highly efficient removal of heavy metal ions by amine-functionalized mesoporous Fe₃O₄ nanoparticles. *Chem. Eng. J.* 2012, 184, 132–140.

11. Almarri, M.; Ma, X.; Song, C. Role of surface oxygen-containing functional groups in liquid-phase adsorption of nitrogen compounds on carbon-based adsorbents. *Energy Fuels* 2009, 23, 3940–3947.

12. Radovic, L. R.; Silva, I. F.; Ume, J. I.; Menéndez, J. A.; Leon, C. A. L. Y.; Scaroni, A. W. An experimental and theoretical study of the adsorption of aromatics possessing electron-withdrawing and electron-donating functional groups by chemically modified activated carbons. *Carbon* 1997, 35, 1339–1348.

13. Standek, S.; Veronovski, A.; Novak, Z.; Knez, Ž. Silica aerogels modified with mercapto functional groups used for Cu(II) and Hg(II) removal from aqueous solutions. *Desalination* 2011, 269, 223–230.

14. Wang, H.; Yu, Y.-F.; Chen, Q.-W.; Cheng, K. Carboxyl-functionalized nanoparticles with magnetic core and mesopore carbon shell as adsorbents for the removal of heavy metal ions from aqueous solution. *Dalton Trans.* 2011, 40, 559–563.

15. Anirudhan, T. S.; Deepa, J. R.; Christa, J. Nanocellulose/nanobentonite composite anchored with multi-carboxyl functional groups as an adsorbent for the effective removal of Cobalt(II) from nuclear industry wastewater samples. *J. Colloid Interface Sci.* 2016, 467, 307–320.

16. Carocho, M.; Barreiro, M. F.; Morales, P.; Ferreira, I. C. F. R. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Compr. Rev. Food Sci. Food Saf.* 2014, 13, 377–399.

17. Wuana, R. A.; Okieimen, F. E.; Imborvungu, J. A. Removal of heavy metals from a contaminated soil using organic chelating acids. *Int. J. Environ. Sci. Technol.* 2010, 7, 485–496.
(18) Bassi, R.; Prasher, S. O.; Simpson, B. K. Extraction of metals from a contaminated sandy soil using citric acid. *Environ. Prog.* 2000, 19, 275–282.

(19) Bailey, S. E.; Olin, T. J.; Bricka, R. M.; Adrian, D. D. A review of potentially low-cost sorbents for heavy metals. *Water Res.* 1999, 33, 2469–2479.

(20) Sec, N. V.; Ly, H. T. Y. Lead (II) removal from aqueous solution by chitosan flake modified with citric acid via crosslinking with glutaraldehyde. *J. Chem. Technol. Biotechnol.* 2013, 88, 1641–1649.

(21) Wartelle, L. H.; Marshall, W. E. Citric acid modified agricultural by-products as copper ion adsorbents. *Adv. Environ. Res.* 2000, 4, 1–7.

(22) Zhu, B.; Fan, T.; Zhang, D. Adsorption of copper ions from aqueous solution by citric acid modified soybean straw. *J. Hazard. Mater.* 2008, 153, 300–308.

(23) Parasuraman, D.; Serpe, M. J. Poly (N-isopropylacrylamide) microgel-based assemblies for organic dye removal from water. *ACS Appl. Mater. Interfaces* 2011, 3, 4714–4721.

(24) Takafuli, M.; Ide, S.; Ibara, H.; Xu, Z. Preparation of poly(1-vinylimidazole)-grafted magnetic nanoparticles and their application for removal of metal ions. *Chem. Mater.* 2004, 16, 1977–1983.

(25) Zerze, H.; Karagoz, B.; Ozbelge, H. O.; Bicak, N.; Aydogan, N.; Yilmaz, L. Imino-bis-propane diol functional polymer for efficient boron removal from aqueous solutions via continuous PEUF process. *Desalination* 2013, 310, 158–168.

(26) Palencia, M.; Rivas, B. L.; Pereira, E. Metal ion recovery by polymer-enhanced ultrafiltration using poly(vinyl sulfonic acid): Fouling description and membrane–metal ion interaction. *J. Membr. Sci.* 2009, 345, 191–200.

(27) Islamoglu, S.; Yilmaz, L. Effect of ionic strength on the complexation of poly(N-isopropylacrylamide) (PEI) with Cd2+ and Ni2+ in polymer enhanced ultrafiltration (PEUF). *Desalination* 2006, 200, 288–289.

(28) Canizares, P.; Pérez, A.; Camarillo, R. Recovery of heavy metals by means of ultrafiltration with water-soluble polymers: calculation of design parameters. *Desalination* 2002, 144, 279–285.

(29) Zhang, Y.-F.; Xu, Z.-L. Study on the treatment of industrial wastewater containing Pb2+ ion using a coupling process of polymer complexation-ultrafiltration. *Sep. Sci. Technol.* 2003, 38, 1585–1596.

(30) Akd, M. A.; Sarhan, A. A.; Shouier, K. R.; Atta, A. M. Application of crosslinked ionic polymer (vinyl alcohol) nanogel as adsorbents for water treatment. *J. Dispersion Sci. Technol.* 2013, 34, 1399–1408.

(31) Wang, L.-Y.; Wang, M.-J. Removal of heavy metal ions by poly(vinyl alcohol) and carboxymethyl cellulose composite hydrogels prepared by a freeze–thaw method. *ACS Sustainable Chem. Eng.* 2016, 4, 2830–2837.

(32) Crini, G. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Prog. Polym. Sci.* 2005, 30, 38–70.

(33) Özkahraman, B.; Acar, I.; Emik, S. Removal of Cu2+ and Pb2+ ions using CMC based thermoresponsive nanocomposite hydrogel. *Clean: Soil, Air, Water* 2011, 39, 658–664.

(34) Ramírez, E.; Burrilo, S. G.; Barrera-Díaz, C.; Roa, G.; Bilyeu, B. Use of pH-sensitive polymer hydrogels in lead removal from aqueous solution. *J. Hazard. Mater.* 2011, 192, 432–439.

(35) Zhang, Y.; Furyk, S.; Bergbreiter, D. E.; Cremer, P. S. Specific ion effects on the water solubility of macromolecules: PNIPAM and the hofmeister series. *J. Am. Chem. Soc.* 2005, 127, 14505–14510.

(36) Fleige, E.; Quadir, M. A.; Haag, R. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: Concepts and applications. *Adv. Drug Delivery Rev.* 2012, 64, 866–884.

(37) Mura, S.; Nicolas, J.; Couvreur, P. Stimuli-responsive nanocarriers for drug delivery. *Nat. Mater.* 2013, 12, 991.

(38) Burmistrova, A.; Richter, M.; Isele, M.; Üzüm, Ç.; Von Klitzing, R. The effect of co-monomer content on the swelling/shrinking and mechanical behaviour of individually adsorbed PNIPAM microgel particles. *Polymers* 2011, 3, 1575.