Binding of Several Heavy Metal Ions by Polyaspartyl Polymers and Their Application to Some Chinese Herbal Medicines

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Received 26 October 2006; accepted 1 May 2007
DOI 10.1002/app.26879
Published online 2 August 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Water-insoluble polyaspartyl polymers were synthesized by using water as medium instead of organic medium. Taking Ca^{2+} as a reference, the binding of several heavy-metal ions, including Pb^{2+}, Cd^{2+}, Hg^{2+}, Cr^{3+}, Cu^{2+}, and Mn^{2+}, by polyaspartyl polymers was studied. The experimental results revealed that polyaspartate is an excellent binding agent for the investigated heavy-metal ions. These cation ions were bound to polyaspartate polymer by the same mechanism as Pb^{2+}, which can be explained by ion exchange model. Since polyaspartate has a protein-resembling structure that is sensitive to trace heavy metal, it was used to remove some trace heavy-metal elements in Chinese herbal medicines. It was found that polyaspartate material was an effective agent for the removal of Pb^{2+}, Cd^{2+}, and Hg^{2+} ions from glycyrrhizin, angelica, and gynostemma pentaphyllum. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2736–2745, 2007

Key words: hydrogels; gels; hydrophilic polymers; adsorption

INTRODUCTION

Polyaspartyl polymers are typical environmentally friendly macromolecules with a biodegradable polypeptide structure. Since they have great potentials as substitutes for some harmful substances in many key fields, the development and application of polyaspartyl-based polymers have attracted a great attention worldwide.

Polyaspartate (PAA) and its derivatives have been found to possess a quite number of good performances and to exhibit prospective applications in many fields.[1–3] But it is still necessary to extend their applications, especially as water-insoluble polyaspartyl polymers. The literatures on preparation and uses of water-insoluble polyaspartyl polymers have been reported, but they were mostly centered on water-absorbing materials or drug-loading carriers.[4–8] Moreover, organic solvents, such as DMF, DMSO, and so on, were used in the preparation processes. Such a process is harmful to human being and expensive in cost, and the recovery of these solvents releases many wastewater, which brings up grave environmental concerns. It can be seen that the process is not clean and the procedure is too complicated.

This work focuses on the synthesis of PAA materials with an excellent heavy-metal-removing performance and on their application to some Chinese herbal medicines. Another objective is to explore a clean preparation process using water as the solvent medium substituting for environmentally unsafe organic solvents.

Pb^{2+} binding by polyaspartyl polymers has been investigated in our previous work.[9] On the basis of this work, we further conducted a research into the binding of other heavy-metal cations by polyaspartyl materials, including Cd^{2+}, Hg^{2+}, Cr^{3+}, Cu^{2+}, and Mn^{2+}, and utilized them to remove heavy metal in some Chinese herbal medicines.

EXPERIMENTAL

Reagents and materials

Polysuccinimide (PSI) was obtained by acid-catalyzed polycondensation of L-aspartic acid.[10] Glycyrrhizin, angelica, and gynostemma pentaphyllum were purchased from the hospital pharmacy. All other reagents were obtained from Tianjin Chemical Reagent (Tianjin, China), and were used as received without further purification.

Measurements

The free Pb^{2+} concentrations in aqueous solution were measured using potentiometer determination

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TABLE I
Instrumental Parameters and Operating Conditions for ICP-OES

Radio frequency (R.F.) power	1.2 kW
Plasma gas flow rate	15 L/min
Flow rate of Argon auxiliary	1.5 L/min
Nebuliser gas flow rate	0.9 L/min
Precision (general)	1–3%
Detection limit	0.01 mg/L
Pb element analytical line (λ)	220.3 nm
Cd element analytical line (λ)	226.5 nm
Hg element analytical line (λ)	184.9 nm
Cu element analytical line (λ)	324.7 nm
Mn element analytical line (λ)	257.1 nm
Cr element analytical line (λ)	267.7 nm
Ca element analytical line (λ)	317.9 nm

by a Model pHS-25 pH Meter (Shanghai Rex Instruments Factory, Shanghai, China) with a 0.01-mV resolution. With the potentials between lead-ion-selective electrode and saturated calomel electrode (SCE) recorded, they were determined by the aid of a calibration curve of potential versus log concentration.[11]

The total concentration of a heavy-metal ion in an aqueous solution was determined with a Vista MPX Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Varian, Palo Alto, CA). The instrumental operating conditions are shown in Table I.

Fourier transform infrared (FTIR) spectra were recorded with a Nicolet Magna-560 FTIR spectrometer (Madison, WI) in KBr pellets over a wavenumber range of 4000–400 cm^{-1} with a 4-cm^{-1} resolution.

X-ray photoelectron spectroscopy (XPS) spectra were acquired with a PerkinElmer PHI-1600 electron-spectroscopic chemical analysis (ESCA) (Wellesley, MA) equipped with a hemispherical electron energy analyzer and a Mg Kα monochromator source (light quantum energy = 1253.6 eV). The pressure in analysis chamber during data acquisition was $\sim 10^{-7}$ Pa. The analysis area was 0.8 mm^2. The survey spectra were carried out with a pass energy of 187.85 eV. The contaminative C_{1s} peak at 284.6 eV was used to calibrate the energy shift. The accuracy of the binding energy was estimated to be ±0.2 eV. All the data of spectra were treated with PHI Multipak software (version 6.0, Wellesley, MA).

The surface observation of polymer samples, both before and after adsorption of heavy metal ions by them, was run in a Philips XL-30 scanning electron microscopy (SEM) (FEI company, OR), with an INCA X-sight energy dispersive analysis of X-ray (EDAX) accessory (Oxford Instruments, UK). The polymers were mounted on a copper conductive tape and coated with Au to minimize sample charging.

Preparation of polyaspartate hydrogel using water as solvent medium

A 100 mL three-necked round-bottomed flask equipped with a thermometer, a condenser, and a magnetic stirrer was charged with 10 mL of aqueous sodium hydroxide (0.28 g, 7 mmol), 0.4 g (2.7 mmol) L-lysine, and 10 mL of absolute ethanol. The mixture was heated to about 45°C with agitation to ensure total dissolution, and then 1.0 g (0.01 mol) of PSI with an average molecular weight of 22,000 as measured by gel permeation chromatography (GPC)[10] was added into the flask under vigorous stirring. The flask contents were maintained at 45°C for about 2 h. After cooling to room temperature, pH value of the mixture was adjusted to 9 by adding 10 wt % aqueous NaOH solution. The mixture was then poured into a 100-mL beaker, and 0.4 mL of 50 wt % glutaraldehyde was added. The reaction mixture was kept at room temperature until stable gel was formed. The obtained gel was washed several times with deionized water until the pH value of washing medium was neutral, and then dried at 45°C under reduced pressure.

Preparation of aqueous solutions of glycyrrhizin, angelica, or gynostemma pentaphyllum

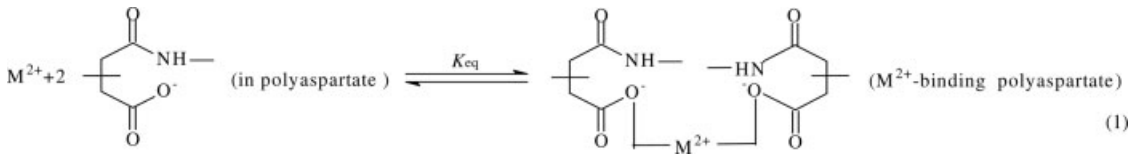
Small pieces of glycyrrhizin, angelica or gynostemma pentaphyllum (50 g) were soaked in 500 mL deionized water in a 1000 mL round-bottomed flask equipped with a reflux condenser and allowed to stand at room temperature for 0.5 h. Then, the contents of the flask were heated and maintained at reflux for 1 h. The mixture was cooled and left overnight. The upper liquid layer was carefully decanted and concentrated with a rotary evaporator to a volume.

A certain volume (e.g., 10 mL) of herbal solutions in a weighed dish was placed into an oven. The content of the dish was heated and maintained at 80°C under a reduced pressure to constant weight. The solid content can be calculated from the difference in weight of the dish before and after baking and the volume of the herbal liquor.

RESULTS AND DISCUSSION

Ion-exchange model for Pb^{2+}-binding by linear polyaspartate

Previously, Freundlich and Langmuir models have been used to explain the mechanism for Pb^{2+} binding by linear polyaspartate (PAA).[9] In this article, we alternatively describe adsorption equilibrium between Pb^{2+} and linear PAA by ion-exchange model. Supposing that adsorption equilibrium of linear PAA with Pb^{2+} solution is as follows:



(1)

Equilibrium coefficient is defined by the equation

$$K_{eq} = \frac{q/Q}{C_e(1 - q/Q)^2} \quad (2)$$

where C_e is the equilibrium concentration of Pb^{2+} in solution (mol/L), q/Q is the equilibrium uptake fraction of Pb^{2+} on 1 g or 1 mol of PAA (g/g or mol/mol), and $1 - q/Q$ is unoccupied fraction on 1 g or 1 mol of PAA (g/g or mol/mol). Equilibrium coefficients K_{eq} calculated from ion-exchange model are list in Table II.

Taking row 2 as an example to illustrate how to calculate K_{eq} shown in Table II:

$$q/Q = 0.85 \times 97 \times 2/207.2 = 0.796$$

where 97 and 207.2 are molar mass of succinyl unit and Pb^{2+}, respectively.

Substituting them into eq. (2), it gives $K_{eq} = 0.66 \times 10^5$ L/mol.

As shown in Table II, the obtained K_{eq} from eq. (2) (that is, two aspartyl unit binding one Pb^{2+} ion) are about the same order of magnitude. If the activity coefficients of Pb^{2+} ion were taken into account, the difference among equilibrium coefficients would probably be narrowed. However, in the case that 1-order or 3-order binding model was adopted (that is, one or three aspartyl unit binding one Pb^{2+}), the calculated coefficient would be different orders of magnitude.

Comparison of polyaspartate and sulfur-containing amino acids for Pb^{2+}-binding

The effects of some functional groups in side chains, such as —CONH—, —OH, and —NH_2, on the Pb^{2+}-binding have been investigated.[9] It has been confirmed that the Pb^{2+}-binding performance of PAA is the best among polyaspartamide derivatives with different side chains.

There are some sulfur-containing functional groups in natural proteins, which are generally considered as having a strong interaction with heavy metals. Table III conducts a direct comparison of PAA, cysteine (with —SH group), cystine (with —S—S-group), methionine (with CH_3S-group), and glycine (without sulfur-containing functional groups but with a carboxyl groups) in terms of Pb^{2+} binding.

Cystine fails to be compared with others by the ion-selective electrode method due to its bad solubility in water. The results in Table III reveal that cysteine exhibits a strong Pb^{2+}-binding capability than methionine, while methionine has no better performance than glycine (without S atom but with —COOH group), suggesting that not all sulfur-containing functional groups have a significant Pb^{2+}-binding capability. Among them, mercapto-group possesses a strong Pb^{2+}-binding function. When other group, such as alkyl group, substitutes the H atom in —SH group, the binding performance of the resulting substance would get worse.

It also has been observed in Table III that although PAA has no S atom, its Pb^{2+}-binding performance is

TABLE II
Equilibrium Coefficients Calculated from Ion Exchange Model (18°C)

Amount of polysuccinimide (mg)	Concentration of free Pb^{2+} ion in solution (10^5) (mol L^{-1})	Pb^{2+} uptake (g g^{-1})	q/Q	K_{eq} (10^{-5}) (L mol^{-1})
1.25	37.162	0.92	0.861	1.20
2.50	28.958	0.85	0.796	0.66
3.75	19.305	0.80	0.749	0.62
5.00	13.031	0.70	0.655	0.42
7.50	4.344	0.60	0.562	0.67
12.50	0.965	0.40	0.375	0.99
15.00	0.724	0.33	0.309	0.89
17.50	0.410	0.28	0.262	1.17
20.00	0.290	0.25	0.234	1.37

Initial c(Pb^{2+}) = 100 mg/L, total volume of $Pb(NO_3)_2$ solution = 50 mL, c($NaNO_3$) = 0.1 mol/L, pH = 6, temperature = 18°C.

TABLE III
Comparison of Polyaspartate and Sulfur-Containing Amino Acids in Terms of Pb^{2+} Binding

Absorbent	Amount (mg)	Concentration of free Pb^{2+} in solution (mg/L)	Pb^{2+} uptake (mg/g)
Polyaspartate	20	2.0	245
Cysteine	20	35	163
Cystine	20 (insoluble)	–	–
Methionine	20	80	50
Glycine	20	80	50

Initial c(Pb^{2+}) = 100 mg/L, total volume of $Pb(NO_3)_2$ solution = 50 mL, c($NaNO_3$) = 0.1 mol/L, pH = 6, temperature = 18°C.

superior to cysteine. It might be due to the following three reasons. Firstly, carboxyl group is stronger than mercapto-group in terms of Pb^{2+}-binding. Secondly, provided that two —COOH groups or two —SH groups bind one Pb^{2+} ion, the uptake capacity of PAA is greater than that of cysteine at the same dosage because molar mass of aspartyl units in PAA and cysteine is 115.1 and 121.1, respectively. In addition, PAA materials have a better chemical stability than thiol materials. Thirdly, the formation of complex between Pb^{2+} ion and PAA may strengthen the interaction between them, while cysteine is unable to form such complex. Perhaps, mercapto group has a stronger effect on lead organic compounds, which are softer than Pb^{2+} ion. Further work needs to be done to explain it.

Selectivity of Pb^{2+}-, Hg^{2+}-, and Cd^{2+}-binding on polyaspartate hydrogel

Since the pH value of solution has a substantial influence on the binding performance of PAA, it is improper to use the ion-selective electrode method to determine the binding selectivity of different ions by linear PAA. So, water-soluble PAA was converted into water-insoluble PAA hydrogel by crosslinking. In the view of PAA gel's strong hygroscopical nature, it is difficult to weigh the gel accurately. Moreover, many factors may affect the preparation process, so that it is not easy to obtain the uniform gel. Even though the gels are produced from the same batch, there may be a microstructure difference between them. To compare the binding selectivity of the chosen ions by PAA with reliability and validity, Ca^{2+} was selected to be the reference. Meanwhile, the effect of Ca^{2+} existence on adsorption of heavy-metal ions on PAA gel was also taken into account. The results are shown in Tables IV and V, respectively.

It can be seen from Table IV that PAA gel has an excellent and approximate adsorbing performances for Pb^{2+}, Hg^{2+}, and Cd^{2+}, for the molar ratios of the bound Pb^{2+}, Hg^{2+}, or Cd^{2+} to bound Ca^{2+} are approximately the same. It seems therefore that two-order ion exchange model is suitable to them all.

The results given in Table V show that although PAA gel has a strong Ca^{2+}-binding ability, the presence of a certain amount of Ca^{2+} ion has no effect on the binding of Pb^{2+}, Hg^{2+}, and Cd^{2+} by PAA, unless the gel is saturated with ions. Thus, it is of particular advantage for PAA materials to be applied to herbal medicines.

Investigation of binding action of Pb^{2+}, Hg^{2+}, and Cd^{2+} on polyaspartate hydrogel

Since lead(II), mercury(II), and cadmium(II) PAA are all soluble in water, it can be inferred that these divalent cations are bound to PAA by a chemical interaction, not by the precipitation caused by the change in pH of solution. Considering the results shown in Table IV, it can be inferred that the binding mechanisms of Hg^{2+} and Cd^{2+} by PAA are the same as that of Pb^{2+}.

The bound state of Pb associated with PAA gel has been analyzed in our previous work.[9] Similarly, the same analysis was done for Hg and Cd using IR, XPS, SEM, and EDAX. The results are shown in Figures 1–4 and Table VI.

IR spectrum of PAA hydrogel shows strong absorption at 1396.5 cm^{-1} (C=O in carboxyl group),

TABLE IV
Comparison of Adsorption of Pb^{2+}, Hg^{2+}, and Cd^{2+} on Polyaspartate Hydrogel[a]

Ions in water	pH	Initial concentration of M^{2+} in solution (mmol/L)				Final concentration of M^{2+} in solution (mmol/L)				Molar ratio of ion binding[b]
		Pb^{2+}	Cd^{2+}	Hg^{2+}	Ca^{2+}	Pb^{2+}	Cd^{2+}	Hg^{2+}	Ca^{2+}	
Pb^{2+}/Ca^{2+}	6	0.491			0.495	0.059			0.086	1.06 : 1
Cd^{2+}/Ca^{2+}	5		0.491		0.495		0.101		0.122	1.05 : 1
Hg^{2+}/Ca^{2+}	4			0.490	0.495			0.115	0.143	1.06 : 1
$Pb^{2+}/Cd^{2+}/Hg^{2+}/Ca^{2+}$	5	0.491	0.491	0.490	0.495	0.088	0.072	0.076	0.110	1.05 : 1.08 : 1.07 : 1

[a] Amount of polyaspartate hydrogel = 0.12 g, total volume of M $(NO_3)_2$ solution = 50 mL, exchange time = 1 h, temperature = 20°C, M^{2+} = Pb^{2+}, Hg^{2+}, or Cd^{2+}.
[b] Ratio of ion binding refers to the molar ratio of different ions adsorbed on the gel to Ca^{2+} ion.

TABLE V
Effect of Ca^{2+} on Adsorption of Pb^{2+}, Hg^{2+}, and Cd^{2+} by Polyaspartate Hydrogel

		Initial concentration of M^{2+} in solution (mg/L)				Final concentration of M^{2+} in solution (mg/L)				M^{2+} uptake
Ions in water	pH	Pb^{2+}	Cd^{2+}	Hg^{2+}	Ca^{2+}	Pb^{2+}	Cd^{2+}	Hg^{2+}	Ca^{2+}	(mg/g)
Pb^{2+}/Ca^{2+}	6	94			94	5.96			35.88	36.68
Pb^{2+}/Ca^{2+}	6	101.7			19.87	12.15			3.48	37.31
Cd^{2+}/Ca^{2+}	5		94		94		39.91		47.41	22.54
Cd^{2+}/Ca^{2+}	5		55.16		19.87		12.93		4.57	17.60
Hg^{2+}/Ca^{2+}	4			94	94			2.84	55.29	37.98
Hg^{2+}/Ca^{2+}	4			98.25	19.87			23.11	5.73	31.31

Amount of polyaspartate hydrogel = 0.12 g, total volume of $M(NO_3)_2$ solution = 50 mL, exchange time = 1 h, temperature = 20°C, M^{2+} = Pb^{2+}, Hg^{2+}, or Cd^{2+}.

and strong-wide absorption at 3439.0 cm^{-1} (N—H). Meanwhile IR spectra of Cd^{2+}- and Hg^{2+}-binding PAA hydrogels show strong absorption at 1397.9 and 1396.4 cm^{-1} (C=O in carboxyl group), respectively, and strong-wide absorption at 3396.1 and 3366.1 cm^{-1} (N—H), respectively, all shifting to a lower wave number region. It suggests that carboxyl groups in PAA structure are involved in binding Cd and Hg, and that N atoms in amide group probably participate in binding them.

Meanwhile, the XPS measurements for the surface of gels before and after adsorption were also carried out. The XPS spectrum of N (1s) after Cd^{2+} adsorption is similar to that after Pb^{2+} adsorption.[9] After adsorption, the N (1s) peak was separated into two sets of peaks (Gaussian curve), centered at 399.72 eV and 397.68 eV, respectively [Fig. 2(a)]. The peak at 397.68 eV may come from the N atoms in coordination state.

However, the XPS spectrum of N (1s) after Hg^{2+} adsorption is different from that after Pb^{2+} adsorption or after Cd^{2+} adsorption. Though the N (1s) peak was also separated into two sets of peaks (Gaussian curve), but centered at 399.99 eV and 401.95 eV, respectively [Fig. 3(a)], shifting to higher binding energy. This phenomenon needs further investigation.

XPS spectra of O(1s) after both Cd^{2+} and Hg^{2+} adsorption [Figs. 1(b), 2(b), and 3(b)] reveal no formation of new oxide-containing compounds. That is, there is no formation of mercury and cadmium oxide, or mercury and cadmium hydroxide. Besides, SEM photographs (Fig. 4) demonstrate that no obvious precipitates are formed on the surface of gel after adsorption. These results suggest that the binding of Cd^{2+} or Hg^{2+} on PAA is not by precipitation but by chemical interaction.

There are only a few binding energy data for Cd and Hg compounds, and the values available for different valence of Cd and Hg are too close to determine the bonding states of Cd and Hg associated with PAA.

Surface atomic ratios from XPS were given in Table VI. An atomic ratio of 2.05% Cd^{2+} and 0.92% Hg^{2+} uptake on the surface are close to the bulk composition determined by chemical analysis. This suggests that Cd^{2+} and Hg^{2+} are adsorbed uniformly in the gel rather than only on its surface.

In summary, the binding performances of Pb^{2+}, Hg^{2+}, and Cd^{2+} on PAA were very similar, and IR,

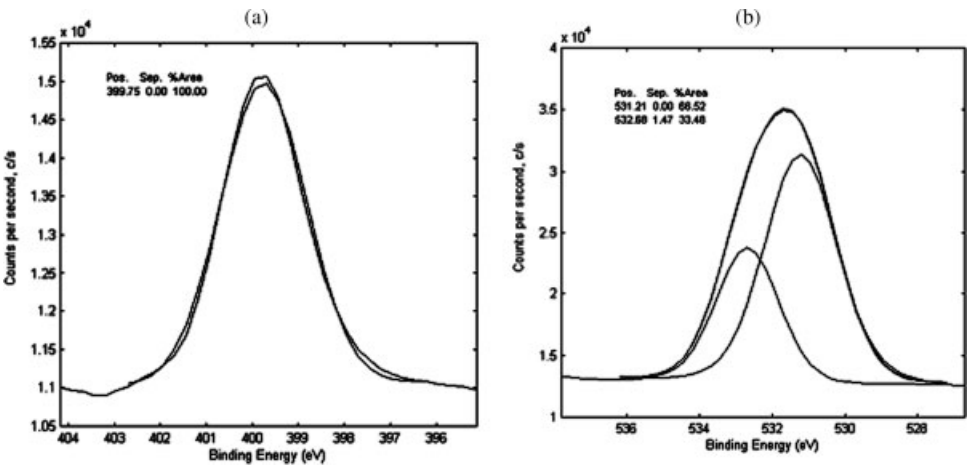


Figure 1 XPS spectra of polyaspartate hydrogel before adsorption. (a) N1s, (b) O1s.

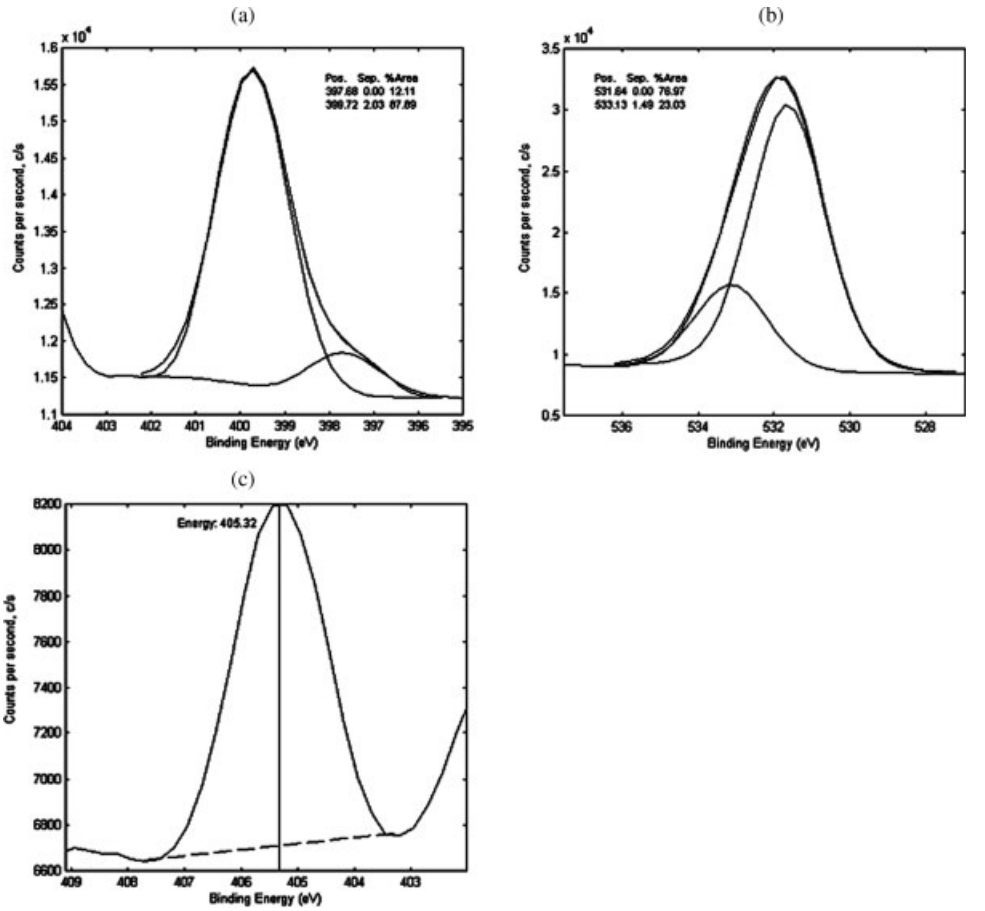


Figure 2 XPS spectra of polyaspartate hydrogel with Cd^{2+}. (a) N1s, (b) O1s, (c) $Cd3d_{5/2}$.

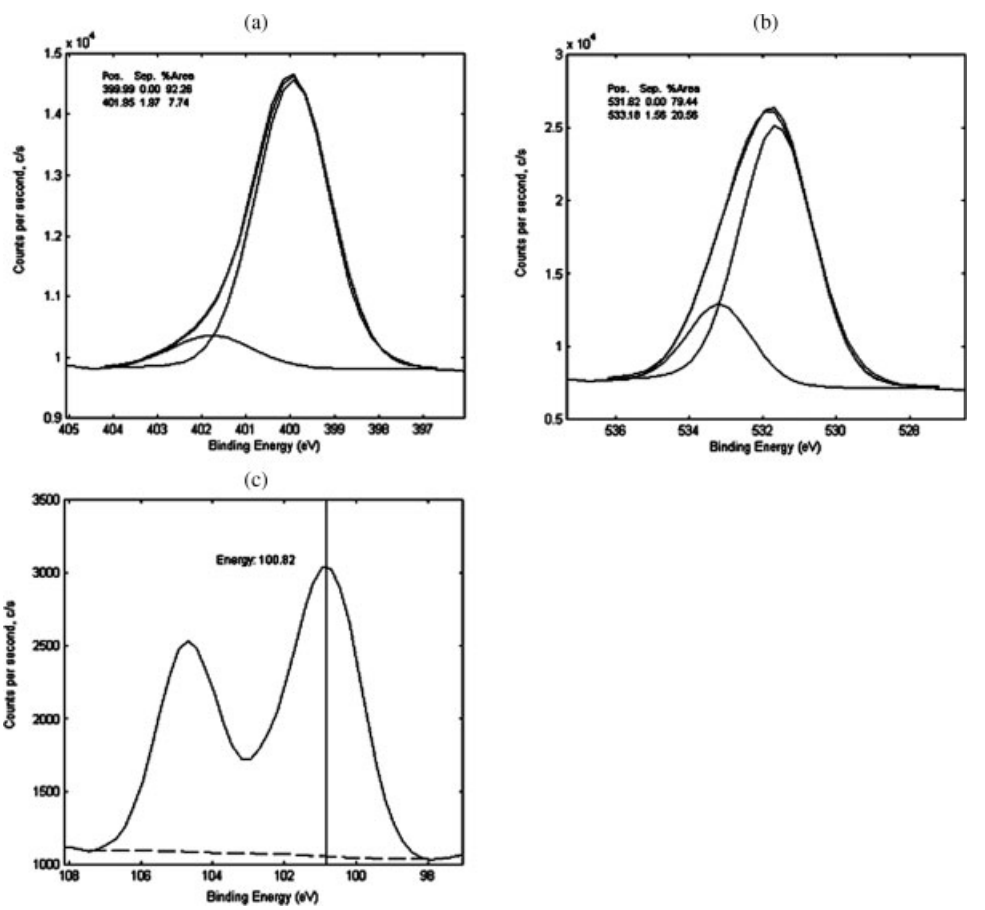


Figure 3 XPS spectra of polyaspartate hydrogel with Hg^{2+}. (a) N1s, (b) O1s, (c) $Hg4f_{7/2}$.

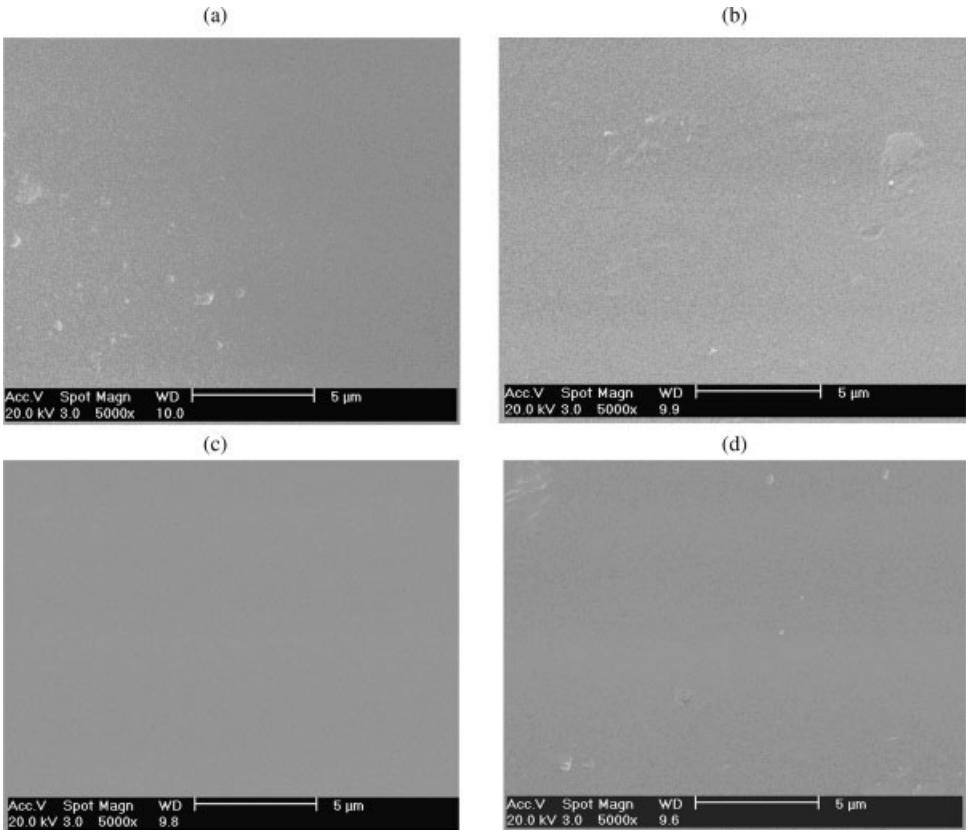


Figure 4 SEM photomicrographs after adsorption of Pb^{2+}, Cd^{2+}, and Hg^{2+} on polyaspartate hydrogel. (a) With Pb^{2+}, (b) with Cd^{2+}, (c) with Hg^{2+}, (d) before adsorption.

XPS, SEM, and EDAX analysis after adsorption were also similar. It can be concluded that the binding mechanisms of Pb^{2+}, Hg^{2+}, and Cd^{2+} are the same, which can be described with the ion-exchange model.

Heavy-metal removal from several Chinese herbal medicines, using polyaspartate hydrogel

Poisoning effects of heavy-metal elements on organisms are commonly regarded as the interactions of metals with cells and enzyme in living beings, causing the latter out of normal biological function. This indicates that some substances like protein have strong interaction with these elements. That is, protein is sensitive to heavy-metal elements. PAA possesses a protein-resembling polypeptide linkage. Compared to nature proteins, it has more groups with functions for binding heavy-metal ions in its side chains. Thus, it is conjecturable that PAA is more sensitive to trace heavy-metal elements than protein, and is more suitable to eliminate trace concentration of heavy metals. Experiments mentioned above have confirmed that Pb^{2+}, Hg^{2+}, and Cd^{2+} can be removed effectively from aqueous solutions by PAA hydrogel and that the presence of Ca^{2+} has almost no influence on the binding of these ions (ref. Tables III and IV). Therefore, an attempt to apply PAA materials for removal of heavy metal from Chinese herbal medicine was made.

TABLE VI
ESCA Parameters for Polyaspartate Hydrogel with Cd and Hg

	Hydrogel with Cd		Hydrogel with Hg	
Elements	Binding energy (eV)	Atomic ratio (%)	Binding energy (eV)	Atomic ratio (%)
O (1s)	531.64	31.21	531.62	30.91
N (1s)	398.54	9.26	398.51	12.25
Cd ($3d_{5/2}$)	405.32	2.05	–	–
Hg ($4f_{7/2}$)	–	–	100.82	0.91

The uptakes of Cd^{2+} and Hg^{2+} on the sample are about 20 mg/g dry gel and 20 mg/g dry gel, or about atomic ratio of 2.2% and 1.1%, respectively, based on polyaspartate.

TABLE VII
Content of Heavy Metals in Aqueous Solutions of Some Herbal Medicines

Solution of herbal medicine	Solid content in solution (g/L)	Initial concentration of M in solution (mg/L)		
		Pb	Cd	Hg
Glycyrrhizin	52	–	0.074	0.29
Angelica	72	–	0.011	0.13
Gynostemma pentaphyllum	62	–	0.11	0.42

M = Pb, Hg, or Cd.

Glycyrrhizin, angelica, and gynostemma pentaphyllum were chosen for their high heavy-metal element content to investigate the removal of Pb^{2+}, Hg^{2+}, and Cd^{2+} using PAA materials, and the effect of concentration or solid contents in herbal solution on the removal was also investigated. Table VII shows the original heavy-metal contents in aqueous solution of the selected herbal medicines.

The data in Table VII confirm that the selected medicines do contain a small amount of heavy metal. Since the chemical states of these elements are unknown, a certain amount of Pb^{2+}, Hg^{2+}, and Cd^{2+} were deliberately added into the herbal liquors to investigate the suitability. Table VIII summarizes the experimental results.

As shown in Table VIII, the following conclusions can be drawn:

1. PAA gel is an effective agent for the removal of Pb^{2+}, Hg^{2+}, and Cd^{2+} from selected herbal liquors. Even in the range of low heavy-metal ion concentrations, it still maintains its good performance. If a continuous operation or a multiple-stage operation is used, the removal percentage would be much higher.
2. In addition to ion concentration, the removal efficiency is influenced by the solid content of herbal solution, especially in the range of high solid contents or low ion concentrations. This can be explained as the competitive adsorption of heavy-metal ions with other components in herbal liquid, which lower the removal percentage of heavy-metal ions.
3. It should be noted that Pb and Cd are more easily removed than Hg from the chosen herbal solutions, and the residual content of Hg is close to the original content. This suggests that Hg is not in the form of Hg^{2+} ion but in others such as organic mercury compounds. Another reason may be that some components in the herb have stronger interaction with Hg^{2+}, compared with PAA gel. Further work is necessary to solve this issue.

In general, herbal liquor contains small quantities of amino acid and peptide in addition to the main components. PAA possesses a protein-resembling structure and much denser carboxyl groups in its side chains than natural protein, resulting in strong interaction with heavy-metal ions and thus more active sites for binding them, leading to lower equilibrium ion concentration. It can capture heavy metal ion from natural proteins, so that heavy metal ions in herbal medicines can be removed.

Selectivity of Cu^{2+}-, Mn^{2+}-, and Cr^{3+}-binding on polyaspartate hydrogel

Like lead(II), mercury(II), and cadmium(II) PAA, copper(II), manganese(II), and chromium(III) PAA

TABLE VIII
Results of Removal of Pb^{2+}, Hg^{2+}, and Cd^{2+} from Some Herbal Medicines Using Polyaspartate Hydrogel

Solution of herbal medicine	Solid content in solution g/L	Initial concentration of M in solution (mg/L)			Final concentration of M in solution (mg/L)			Percent of M-removing (%)		
		Pb	Cd	Hg	Pb	Cd	Hg	Pb	Cd	Hg
Glycyrrhizin	132	4.00	2.26	4.25	2.07	1.06	2.46	48.3	53.1	42.1
	104	4.00	2.24	4.17	1.67	1.00	2.14	58.2	55.4	46.5
	52	4.00	2.22	4.12	0.75	0.30	1.53	81.2	86.5	62.9
	132	1.00	0.64	1.26	0.65	0.34	0.85	35.0	46.9	32.5
	35	1.00	0.59	1.12	0.01	<0.01	0.30	99.0	99.8	73.2
Angelica	144	4.00	2.19	4.01	2.76	0.67	2.01	31.0	69.4	49.9
	72	4.00	2.17	3.95	1.83	0.57	1.66	54.2	73.7	58.0
Gynostemma pentaphyllum	248	4.00	2.59	5.16	2.03	1.04	2.65	49.2	59.8	48.6
	62	4.00	2.28	4.30	1.56	0.82	1.74	61.0	64.0	59.5
	25	0.24	0.18	0.09	0.01	<0.01	0.08	83.3	99.4	77.8

Amount of polyaspartate hydrogel = 0.2 g, total volume of solution = 50 mL, exchange time = 1 h, temperature = 22°C, M = Pb, Hg, or Cd.

TABLE IX
Comparison of Binding of Cu^{2+}, Mn^{2+}, and Cr^{3+} on Polyaspartate Hydrogel[a]

Ions in water	Initial pH	Initial concentration of cation in solution (mmol/L)				Final concentration of cation in solution (mmol/L)				Molar ratio of ion-binding[b]
		Cu^{2+}	Mn^{2+}	Cr^{3+}	Ca^{2+}	Cu^{2+}	Mn^{2+}	Cr^{3+}	Ca^{2+}	
Cu^{2+}/Ca^{2+}	4.5	0.758			0.758	0.110			0.160	1.08 : 1
Mn^{2+}/Ca^{2+}	4		0.757		0.758		0.314		0.346	1.07 : 1
Cr^{3+}/Ca^{2+}	3			0.505	0.758			0.137	0.459	1.23 : 1

[a] Amount of polyaspartate hydrogel = 0.1 g, total volume of metal nitrate solution = 50 mL, exchange time = 1 h, temperature = 20°C.
[b] Ratio of ion binding refers to the molar ratio of different ions adsorbed on the gel to Ca^{2+} ion.

are also water-soluble (unless concentrations of the ions are high enough, probably the ions can serve as the crosslinker of PAA, causing the formation of water-insoluble crosslinked PAA), indicating that the binding of these divalent cations by PAA should be attributed to the chemical interactions. Still using Ca^{2+} as the reference, binding performances of Cu^{2+}, Mn^{2+}, and Cr^{3+} by PAA were investigated, and the results are given in Table IX.

By comparison with the data in Table IV, the molar ratios of Cu^{2+}- and Mn^{2+}-binding to Ca^{2+}-binding are in good agreement with those of Pb^{2+}-, Hg^{2+}-, and Cd^{2+}-binding, suggesting that Cu^{2+} and Mn^{2+} are bound to PAA through the same mechanism as Pb^{2+}, Hg^{2+}, and Cd^{2+}, and the interactions of PAA with Cu^{2+} and Mn^{2+}can be described with the same two-order ion-exchange model. As for trivalence Cr^{3+}, the two-order ion-exchange model is not suitable. On the assumption that three carboxylic groups binds one Cr^{3+}, while two carboxylic groups binds one Ca^{2+}, if the initial molar ratio of Cr^{3+} to Ca^{2+} is 2/3, the molar ratio of bound Cr^{3+} to bound Ca^{2+} should be 2/3 (or 1/1.5). But the value from the experiment is 1.23/1, obviously higher than that calculated from the assumption. It is clear that PAA has a relatively stronger Cr^{3+}-binding ability. Further work needs to be done to explain the result.

Effect of structure of polyaspartamide on Cu^{2+}-, Mn^{2+}-, and Cr^{3+}-binding

The framework of polyaspartamides is an amide linkage as well as a polypeptide linkage resembling protein. A study on the effect of some functional groups in side chains, such as —COO^- and —OH, on Cu^{2+}-, Mn^{2+}-, and Cr^{3+}-binding was carried out.

Table X compares the effects of polyaspartyl structures in terms of Cu^{2+}-, Mn^{2+}-, and Cr^{3+}-binding.

The results show that binding performances of PAA gel for Cu^{2+}, Mn^{2+}, and Cr^{3+} are superior to poly[(2-hydroxyethyl)-DL-aspartamide] at the same dosage. This might be due to the fact that the electrostatic charge effect of —COO— in side chains is stronger than that of —OH. Additionally, the molecular mass of NaOH is less than that of ethanolamine, so it is obvious that the uptake per gram of PAA is larger than that of any other polyaspartamide materials. Poly[(2-hydroxyethyl)-DL-aspartamide] material is also a good agent for binding Mn^{2+} and Cr^{3+}, except for Cu^{2+}.

CONCLUSIONS

Water-insoluble polyaspartyl polymers were synthesized using water medium instead of poisonous and harmful organic medium. The reaction of PSI with L-lysine and hydrolysis of imide ring were carried out in one step (in one pot) by raising the reaction

TABLE X
Comparison of the Effects of Polyaspartyl Structure in Terms of Binding of Cu^{2+}, Mn^{2+}, and Cr^{3+}[a]

Polyaspartamide absorbents[b]	Ions in water	Initial pH	Initial concentration of cation in solution (mg/L)	Final concentration of cation in solution (mg/L)
Polyaspartate gel	Cu^{2+}	4.8	22.70	0.30
	Mn^{2+}	5.3	18.74	0.083
	Cr^{3+}	3.2	22.13	0.23
α,β-Poly[(2-hydroxyethyl)-DL-aspartamide] gel	Cu^{2+}	4.8	22.70	8.89
	Mn^{2+}	5.3	18.74	0.24
	Cr^{3+}	3.2	22.13	0.53

[a] Amount of polyaspartyl hydrogel = 0.2 g, total volume of metal nitrate solution = 50 mL, exchange time = 1 h, temperature = 20°C.
[b] They were obtained according to the method described in literature.[10]

temperature to accelerate the reaction of lysine with PSI, and adding ethanol to decelerate the alkaline hydrolysis rate of imide ring. There is no need to control pH, and thus simplifying the procedure.

Taking Ca^{2+} as the reference, the binding of several heavy metal ions, including Pb^{2+}, Cd^{2+}, Hg^{2+}, Cr^{3+}, Cu^{2+}, and Mn^{2+}, on polyaspartyl polymers was investigated. It is indicated that PAA is an excellent binding agent for these selected ions, and the binding of the divalent ions is through the similar mechanism for Pb^{2+}. The synthesized polyaspartyl polymers were used for the removal of trace heavy metal elements in Chinese herbal medicines. The experimental results reveal that PAA is an excellent binding agent for Pb^{2+}, Cd^{2+}, and Hg^{2+} in the Chinese herbal medicines glycyrrhizin, angelica, and gynostemma pentaphyllum.

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