ELSEVIER

Journal of Hazardous Materials B83 (2001) 219–236

Journal of Hazardous Materials

www.elsevier.nl/locate/jhazmat

Comparison and evaluation of the synthetic biopolymer poly-L-aspartic acid and the synthetic "plastic" polymer poly-acrylic acid for use in metal ion-exchange systems

Thomasin C. Miller, James A. Holcombe*

Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712, USA

Received 31 October 2000; received in revised form 30 January 2001; accepted 30 January 2001

Abstract

Poly-L-aspartic acid (PLAsp), a biopolymer, and a similar synthetic polymer, poly-acrylic acid (PAA), each consisting of ~50 repeating Asp and acrylic acid monomers, respectively, were immobilized onto controlled pore glass (CPG) and evaluated for use as metal ion-exchange materials. Both polymers achieve metal complexation primarily through their repeating carboxylate side groups resulting in a similar binding trend for the metals tested (Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}), with metal binding capacities ranging from <0.1 to 12 μmol metal/g column and <0.1 to 32 μmol metal/g column for PLAsp and PAA respectively. Cu^{2+} and Pb^{2+} exhibited strong binding to both materials, while the other metals demonstrated only weak or minimal binding. Both columns allowed for quantitative release of bound metals through acid stripping and experienced increased overall metal binding with increasing pH. Both systems also maintained similar structural and chemical stability when continuously exposed to neutral buffered, highly acidic, oxidizing, large molecule rich, and elevated temperature environments. The main differences between the two systems are the material cost and system biodegradability.

Keywords: Poly-L-aspartic acid; Poly-acrylic acid; Controlled pore glass

1. Introduction

The future of metal waste cleanup depends to a great extent on the development of novel ion-exchange systems for the selective removal of metals from natural and industrial wastewaters. Environmental contamination by metals has increased, partly because they

* Corresponding author. Tel.: +1-512-471-5140; fax: +1-512-471-0985.
E-mail address: holcombe@mail.utexas.edu (J.A. Holcombe).

0304-3894/01/$ – see front matter
PII: S0304-3894(01)00184-4

represent a recirculating problem, unlike many organic contaminants that can be metabolized or decomposed. "Degradation" is not an option for metal removal, and only isolation and recovery can address this issue. Since permissible limits for many metals are at the ppm to sub-ppb levels, common methods of bulk removal (e.g. precipitation, filtration) often fail to bring discharge streams and waste sites into compliance. As a consequence, successful, efficient metal ion-exchangers are needed for preconcentration and remediation efforts to achieve these low level metal standards.

The ideal system should demonstrate a large capacity and selectivity for target metals of interest. To minimize column length and throughput, the chelator should demonstrate strong metal binding with fast binding/release kinetics as well as allow for on-demand metal release requiring minimal elution volume. The stability and resiliency of the column with exposure to harsh chemical and physical environments is equally important as the column's metal extraction properties. For example, natural and industrial waste streams often have other noxious chemicals present, such as acids, bases, oxidizers, large "sticky" molecules, such as bioadsorbants, or elevated temperatures that may alter a columns ability to efficiently extract metals. Ideally, the metal exchange system should remain chemically and structurally stable when exposed to these types of conditions with minimal loss of metal binding capacity.

A means of metal binding with the potential for selectivity and column structural stability in different chemical environments is the novel approach of using short chain polymers immobilized on the surface of mechanically stable structural supports [1–10]. Attachment to the support is made by a chemical bond between one end of the polymer and the substrate surface, thereby yielding a chelator possessing 3 d.f. for metal binding by the "tail", which provides unique metal chelation capabilities. Two types of bonded-phase materials that are currently being investigated for their metal binding properties in our laboratory are simple biopolymers and synthetic "plastic" polymers.

Both types of materials have very similar qualities. Both biopolymers and synthetic polymers can be synthesized or purchased as short linear chains (50–100 units) and prepared with a variety of different metal binding functionalities. Selectivity for target metals can be achieved through functional groups contained in the linear polymer chain, and the presence of multiple functionalities along the polymer allow for polydentate complexation. Both the biopolymer peptide backbone and the synthetic polymer's carbon backbone are flexible and can facilitate weak interchain cross-linking, permitting the chain to "wrap" around target metals, finally reaching a free energy minimum that results in its optimal tertiary conformation for binding a particular metal.

The purpose of this study was to characterize, compare and evaluate a biopolymer and structurally similar "plastic" polymer bonded-phase ion-exchange system for use in metal preconcentration and remediation. The two systems were constructed using a biopolymer, poly-L-aspartic acid (PLAsp), and poly-acrylic acid (PAA) immobilized on controlled pore glass (CPG). CPG is a well-characterized, porous bonded-phase support material that has a high surface area (94 m^2/g) for ligand immobilization and retains its structural stability under a variety of harsh chemical environments. PLAsp was chosen for this study due to its success as a bonded-phase metal chelator in preliminary studies [4]. PAA was chosen as the complementary "plastic" polymer due to its similar chemical composition to PLAsp. The structures of both polymers are shown in Fig. 1.

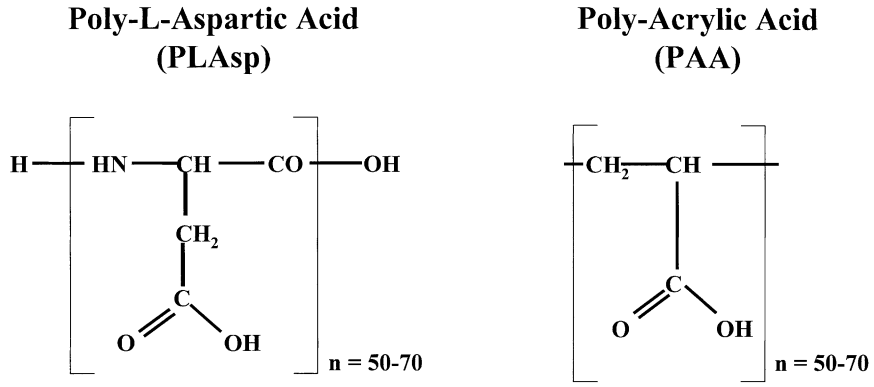

Fig. 1. Structures of poly-L-aspartic acid (PLAsp) and poly-acrylic acid (PAA), respectively.

Table 1
Parameters used in evaluating lifetime of immobilized chelator and support

Test condition	Reagent	Comment
Control (pH 7.0)	pH 7.0 ammonium acetate buffer solution	Normal operating condition for metal binding studies
Oxidizing acid	1.0 M HNO_3	Harsh, oxidizing acid used for metal recovery in binding experiments
Oxidizing agent	5% H_2O_2	Moderate oxidizing agent to augment the nitric acid study
Bioadsorbant	0.5% albumin	Experiment performed to evaluate the impact of large molecules that may adsorb to the chelator surface on chelator activity
Temperature	pH 7.0 ammonium acetate buffer solution at 60°C	Experiment accentuates any instability in the immobilization and emphasizes reversibility of the conformational structure of the chelator

Specifically, metal binding characteristics, such as capacity, binding strength, selectivity, and reversibility were evaluated for both systems. The pH dependence on metal binding was also investigated for both systems. Additionally, the chemical and mechanical stability of both materials were evaluated by monitoring the metal binding characteristics upon constant exposure to a variety of solution conditions (listed in Table 1) that may be common in natural or industrial waste streams. These findings were then compared to carboxylate resins employed in current ion-exchange technologies and used to evaluate the potential success of both systems as environmental metal extraction agents.

2. Experimental

2.1. Instrumentation

A flow injection manifold, described previously [4], was used to provide flow of acid, buffer and sample to the column. All connections were made with 0.76 mm i.d. PTFE tubing.

A Kel-F tee was placed between the column and the nebulizer to provide air compensation and minimize noise.

A Perkin-Elmer model 4000 atomic absorption spectrometer with an air/acetylene flame was used for determination of the metals. Hollow cathode lamps were operated at the currents recommended by their manufacturers. Wavelengths for Ca, Cd, Co, Cu, Mg, Mn, Na, Ni, and Pb were 422.7, 228.8, 240.7, 324.8, 285.2, 279.5, 589.0, 232.0, and 217.0 nm, respectively, and were used in conjunction with a monochrometer bandpass of 0.7 nm for Ca, Cd, Cu, Mg, Na, and Pb and 0.2 nm for Co, Mn, and Ni.

2.2. Reagents

All reagents used were reagent grade unless noted. Deionized distilled water was used to prepare solutions, and all glassware was soaked overnight in 4 M HNO_3 prior to use. Poly-L-aspartic acid (Sigma) (DP(vis) 50, MW (vis) 11,100) and poly-acrylic acid (Aldrich) (50 wt.%, MW $\sim$ 5000 kDa) were used as received. Controlled pore glass (PG 240-120; Sigma) had an average pore diameter of 226 Å and a mesh size of 80–120. Other reagents included hydrochloric acid, acetic acid, ammonium hydroxide, 3-mercaptopropyltrimethoxysilane (95%) (Aldrich); 3-aminopropyltriethoxysilane (98%), glutaraldehyde (25%), trypsin, albumin, *N*-ethylmaleimide (Sigma); sodium hydroxide, ferric chloride, hydroxylamine hydrochloride (Fisher Scientific); potassium hydroxide (Baker); ammonium acetate, hydrogen peroxide (30%), methanol, ethanol, and disodium hydrogen phosphate (EM Science). Stock solutions of Ca^{2+} (Fisher), Cd^{2+} (Fisher) and Pb^{2+} (SPC Science) atomic absorption standards were used to prepare the 10 ppm loading solutions for the metal binding experiments. For Co^{2+} (Baker), Mn^{2+} (Matheson, Colleman & Bell), Na^{+} (Baker), and Ni^{2+} (Baker), the loading solutions were prepared from standardized solutions of the reagent grade nitrate salt. Cu^{2+} loading solutions were prepared from the appropriate amount of Cu metal in concentrated HNO_3.

2.3. Procedures

2.3.1. Immobilization of PLAsp onto CPG

The immobilization of PLAsp onto CPG was described previously by Gutierrez [4]. The process was slightly modified for this study in that both the glutaraldehyde and PLAsp attachment steps were performed at pH $\sim$ 7–8.

2.3.2. Immobilization of PAA onto CPG

The immobilization of PAA onto CPG was modeled after procedures previously described [11–14]. The PAA polymer acquired from Aldrich was analyzed and found to possess a terminal double bond, a fundamental feature that makes it possible for the following chemical immobilization to be performed.

Approximately 1.0 g of CPG was boiled in 100 ml of 5% v/v HNO_3 for 30 min. Then the glass was collected by filtration and rinsed thoroughly with deionized water. The acid activated glass was then dried in an oven at 95°C for 3 h.

A solution prepared by dissolving 2.5 ml of 95% 3-mercaptopropyltrimethoxysilane (3-MPS) in 25 ml of methanol was purged under $N_{2(g)}$ for 5 min. The dried, acid-activated

glass was then added to the reaction system with stirring (facilitated by $N_{2(g)}$ bubbler). The reaction system was heated to 80°C for 12 h with stirring. Then the reaction mixture was cooled and the silanized glass was collected by filtration. The glass was then rinsed thoroughly with methanol and dried in an oven overnight at 80°C.

To facilitate the Michael reaction between the unsaturated end of the PAA polymer and the thiol on the immobilized 3-MPS, the carboxylate groups on the polymer were esterified to form poly-acrylic methyl ester. PAA 5 ml, methanol 200 ml, H_2SO_4 2 ml and the silanized CPG were placed in a three-neck round bottomed flask equipped with a reflux condenser, $CaCl_2$ drying tube, and a stir bar. The reaction was heated to reflux for 6 h under $N_{2(g)}$ and then cooled. The reaction mixture was then allowed to stir at room temperature under $N_{2(g)}$ overnight.

After reacting overnight, some of the glass was isolated from the bottom of the flask using the suction of a Pasteur filter pipette. This glass was subjected to the hydroxamate test (using MeOH, EtOH, KOH, hydroxylamine hydrochloride, and $FeCl_3$ reagents) to confirm the presence of ester (glass turns pinkish-red) [15]. After a positive test, the glass was then collected as described previously and dried under $N_{2(g)}$ overnight.

The methyl-esterified PAA-CPG was then acid hydrolyzed by exposure to 50 ml of 3 M HCl for 1 h with stirring to return the polymer to its native structure. The glass was then collected and rinsed with deionized water.

During PAA immobilization, it is unlikely that all of the 3-MPS thiol groups were reacted with PAA polymer. To attempt to block any remaining thiol groups, the PAA-CPG was then exposed for 1 h to a solution of *N*-ethylmaleimide (NEM) prepared by dissolving 0.11 g NEM in 50 ml of 0.01 M NaH_2PO_4 buffered at pH 6.5. The glass was then collected and washed as described above and allowed to dry under $N_{2(g)}$ overnight.

2.3.3. Binding of metals to PLAsp and PAA

The flow injection analysis (FIA) system described earlier was employed in all experiments [4]. All pumps and tubing were warmed up for 15 min prior to use. Before binding experiments were performed, the column was conditioned by passing 0.1 M HNO_3 through the column for 2 min followed by ammonium acetate buffer (pH 7.0) for 30 s at a flow rate of 1 ml/min. After initial conditioning, complete breakthrough curves were obtained for several metals (Ca, Cd, Co, Cu, Mg, Mn, Ma, Ni, and Pb) by monitoring the flame atomic absorption (FAA) signal as a 10 ppm solution of the target metal at pH 7.0 was passed through the microcolumn at a flow rate of 1.0 ml/min. After ~20 min (or when a nearly constant, high absorbance suggested that the effluent concentration had reached the influent concentration), the sample flow was stopped as well as FAA monitoring of the signal. Buffer was then passed through the lines for 15 s to remove metal-containing solution from the column dead volume and line tubing. HNO_3 (0.1 M) was switched in-line to strip the metals into a 25 ml volumetric flask for subsequent analysis. Previous studies have shown that by stripping the column with acid, quantitative recoveries of the metals are obtained [4]. While past studies have shown that only a few 100 μl were needed to strip metals from the columns, 25 ml was employed to ensure complete removal and to have sufficient sample to conduct the needed analysis of the strip. Acidified metal standards were used to construct a calibration curve for the strip solutions to determine the relative binding capacities of these metals. These same acidified standards, which are not retained by the column, were pumped

through the system after stripping and passed to the FAA. The signals from these standards were used to construct a calibration curve in order to evaluate the breakthrough curve data. All metal binding experiments were performed in triplicate to ensure reproducibility for the PLAsp and the PAA columns.

2.3.4. Binding of Cu^{2+} to PLAsp and PAA with varying pH

The PLAsp-CPG and PAA-CPG column capacities were determined for pH values ranging from 4.0 to 8.0 using a 10.0 ppm Cu^{2+} influent solution at pH 7.0 pumped through the column at a flow rate of 1.0 ml/min. Breakthrough curves and strips were collected and evaluated using the metal binding procedure described in the previous section. All metal binding experiments were performed in duplicate to ensure reproducibility for the PLAsp and the PAA columns.

2.3.5. Stability studies on PLAsp-CPG and PAA-CPG

Using the metal binding and stripping procedure described previously, the breakthrough curve shapes (i.e. binding strengths) and capacities of the PLAsp-CPG and PAA-CPG columns were used as indications of degradation when subjected to the physical and chemical conditions listed in Table 1. The columns were continuously exposed to the chemical or thermal environment and tested for degradation at regular intervals. The columns were subjected to a total of five intervals of exposure to the respective chemical or physical environment with fresh reagents prepared before each new exposure period. At the end of each exposure period, breakthrough data was collected using a 10.0 ppm Cu^{2+} influent solution at pH 7.0 pumped through the column at a flow rate of 1.0 ml/min. Similarly, acidified Cu^{2+} standards were used to construct breakthrough and strip calibration curves for subsequent analysis. All metal binding experiments were performed in duplicate to ensure reproducibility for the PLAsp and the PAA columns.

3. Results and discussion

3.1. Verification of PLAsp and PAA immobilization onto CPG

Unblocked silanol groups contained on the surface of modified CPG have the ability to act as metal binders independent of the immobilized chelators bonded to the surface [4,16,17]. Similarly, any groups introduced to the CPG surface during chelator attachment, such as aminopropyl or glutaraldehyde groups during the PLAsp immobilization or thiol groups from the PAA binding procedure, could also act as potential metal binders if not terminated with PLAsp or PAA. It is desirable that the chelators of interest (PLAsp or PAA) be the only metal binding functionalities in the bonded-phase ion-exchange system.

To determine if PLAsp and PAA were immobilized onto the surface of CPG as the main component of metal binding, the affinity of the glass for various target metals was tested at each stage of the immobilization procedure for PLAsp and PAA. Gutierrez et al. reported the results for the different stages of immobilization of PLAsp onto CPG with the conclusion that PLAsp was the main component of metal binding in that system [4]. Using a similar approach, overall breakthrough capacities for Cd^{2+}, Cu^{2+}, and Ni^{2+} were determined for

Table 2
Binding capacity of CPG system during different stages of PAA immobilization[a]

Metal Ion	Capacity (μmol/g)			
	Acid-activated CPG	MPS silanized CPG	PAA-CPG	Thiol blocking with NEM
Cd^{2+}	0.41	83[b]	48	16
Cu^{2+}	7.6	4.9	36	32
Ni^{2+}	0.54	2.9	15	15

[a] Flow rate 1.0 ml/min; pH 7.0; metal loading concentration 10 ppm.
[b] Flow rate 1.0 ml/min; pH 7.0; metal loading concentration 40 ppm.

the four main stages of PAA immobilization (acid activation, silanization, PAA linkage, and thiol blockage), and the results are shown in Table 2. The major trend that is illustrated by the data is that the metal binding capacity increased dramatically for Cd^{2+} as the glass was silanized with a terminal thiol group. A 40 ppm Cd^{2+} loading solution was used for capacity determination at this stage due to the high concentration of thiol groups immobilized on the surface. Cd^{2+} is a soft metal acid and prefers to bind to soft donor ligands like thiols to harder oxygen containing silanol groups [18]. There was a much less dramatic increase for Ni^{2+}, even resulting in a decrease in capacity for Cu^{2+}, both metals preferring to bind to hard and moderately hard donor ligands [18]. The capacities of Cu^{2+} and Ni^{2+} both increased after linkage of PAA to the silanized CPG surface, while the capacity of Cd^{2+} decreased. This is consistent with the metal binding behavior predicted by the deactivation of many reactive thiols in the system through binding of the carboxylate rich PAA. To insure that all of the thiols were blocked on the surface and could not contribute to Cd^{2+}, Cu^{2+}, or Ni^{2+} binding (however, minimal), the exchange system was exposed to an excess of *N*-ethylmaleimide (NEM), a common thiol-protecting agent. This led to a significant decrease in Cd^{2+} binding in the system with a minimal decrease in Cu^{2+} and Ni^{2+} capacities. Therefore, the remaining metal binding capacity should, in all likelihood, be due to the presence of PAA carboxylate ligands.

3.2. Metal binding characteristics of PLAsp and PAA

Both PLAsp and PAA are chelators that contain carboxylate groups as their main metal binding functionalities. Due to the comparable chemical nature of both of the polymers, it is predicted that PLAsp and PAA would have similar metal binding characteristics. On both columns, breakthrough curves were analyzed by monitoring the metal analyte concentration in the column effluent phase as a function of influent volume. Fig. 2 shows a typical Cu^{2+} breakthrough curve for both PLAsp and PAA. On both curves, the baseline region represents the strong metal/chelator binding sites ($\log K_{\text{effective}} > 10$), while the sloped region signifies weaker metal binding sites. The Cu^{2+} and Pb^{2+} breakthrough curves were characterized by significant strong and weak metal binding site regions for both columns. The remaining metals were characterized by only weak site binding and exhibited no measurable strong sites. The only metal for which there was minimal binding of any type was Na^{+}. The total amount of each metal bound on each of the columns was determined by integration of the area above the obtained breakthrough curves for each metal. Table 3 summarizes

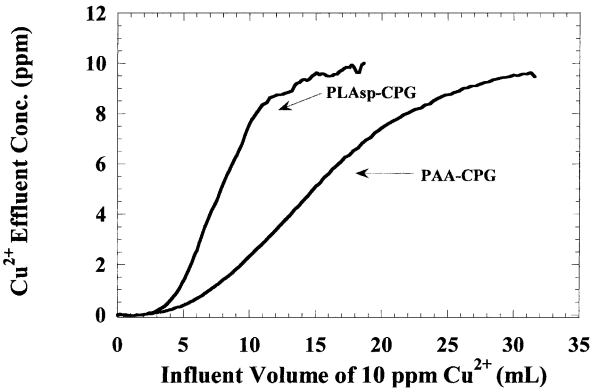

Fig. 2. Breakthrough curves of Cu^{2+} on PLAsp-CPG and PAA-CPG loaded at pH 7.0 at a flow rate of 1.00 ml/min.

the metal binding results for both polymers, and it is easily seen that indeed the metal binding trends are similar in both cases: for PLAsp: $Cu^{2+} \gg Pb^{2+} > Cd^{2+} > Ni^{2+} > Co^{2+} \approx Mn^{2+} > Ca^{2+} > Mg^{2+} \sim Na^{+}$; for PAA: $Cu^{2+} \gg Pb^{2+} > Cd^{2+} \approx Mn^{2+} \sim Ni^{2+} > Co^{2+} \sim Ca^{2+} \sim Mg^{2+} \gg Na^{+}$. This trend is in general agreement with that reported for metal binding to carboxylate ligands [19]. It is also apparent from the table that the overall metal binding capacity is approximately three times higher for PAA-CPG than PLAsp-CPG. This is most likely attributed to variability in the effectiveness of the immobilization of the chelators onto the glass substrates. In previous studies, the overall immobilization efficiency with silane reagents has been known to range between 1 and 10% coverage of chelator [3,4,9]. Thus, for the purposes of this study, it is more important to evaluate and compare the metal binding trends between the two chelators than to directly compare their overall capacities.

Table 3
Effective breakthrough capacity and strip recovery of selected metals on the PLAsp-CPG and PAA-CPG columns[a]

Metal Ion	Breakthrough capacity (μmol/g) on PLAsp-CPG[b]	Recovery (%)[b]	Breakthrough capacity (μmol/g) on PAA-CPG	Recovery (%)[c]
Cu^{2+}	12 ± 1	100 ± 12	32 ± 1	110 ± 17
Pb^{2+}	4.6 ± 0.1	98 ± 3	20 ± 2	105 ± 18
Cd^{2+}	3.7 ± 0.5	84 ± 12	16 ± 1	106 ± 15
Ni^{2+}	3.1 ± 0.2	94 ± 7	15 ± 1	93 ± 9
Co^{2+}	1.6 ± 0.1	100 ± 9	11 ± 1	91 ± 12
Mn^{2+}	1.5 ± 0.2	93 ± 14	16 ± 2	98 ± 19
Ca^{2+}	1.0 ± 0.1	120 ± 16	11 ± 2	85 ± 18
Mg^{2+}	<0.1	–	9.1 ± 0.6	100 ± 16
Na^{+}	<0.1	–	< 0.1	–

[a] Flow rate 1.0 ml/min; pH 7.0; $n = 3$.
[b] Data initially reported by Gutierrez et al. [4].
[c] Stripping performed with 25 ml of 0.1 M HNO_3 at 1 ml/min.

Table 4
Type and capacity of common cross-linked carboxylate resin-based ion-exchangers [21]

Resin name and designation	Capacity (meq/g)
Amberlite IRC 50	10
Duolite CS 101	10.2
Ionenaustauscher IV	10
Kastel C 100	7
Kationit KB$	8.55
Permutit C	11
Wolfatit CP 300	4.5
Zeokarb 226	9.0

As discussed previously, in addition to strong, selective metal binding, the ideal ion-exchange system should also allow for efficient release of the target metals. The breakthrough capacity was directly compared to the concentration of the isolated strip solution to provide the percent metal recovery as the fully metal loaded columns were stripped with 25 ml of 0.1 M HNO_3 (Table 3). The data shown here demonstrates that recoveries in excess of 80% were obtained for all metals and quantitative recovery was seen for most. Efficient recovery is likely due to an H^+ displacement combined with a conformational change disrupting the polymers optimal tertiary structure responsible for metal binding as the pH is lowered [9,10,20].

Some of the most common cross-linked carboxylate resin-based ion-exchangers are listed in Table 4, along with their capacities listed in milli-equivalents per gram (meq/g). One major difference between the resin-based chelators listed in Table 4 and the bonded-phase chelators characterized in this study is the metal binding capacity of the chelators. The carboxylate resins in Table 4 are able to bind metals in the meq/g range while the immobilized PLAsp and PAA chelators have capacities that are two to three orders of magnitude lower. This is not unexpected for a bonded-phase chelator where a significant mass of the material is associated with the support material. However, structural stability (e.g. negligible shrinking or swelling with pH changes) and wide flexibility in chelator selection is gained. As a consequence, bonded-phase chelators are potentially useful for waste solution polishing with the potential for selectivity, albeit with possible loss in utility for application requiring bulk metal removal.

As noted above, an advantage of the bonded-phase chelators over the cross-linked resin-based chelators is the lower swelling effects of the former. When resin-based exchangers come into contact with water or other solvents the outermost functional groups are solvated and the randomly arranged polymer chains unfold to accommodate the larger solvated ions. This leads to an increased ion concentration inside the ion-exchanger causing an increase in osmotic pressure, which causes the resin to swell. For example, when the common carboxylate resin ion-exchanger Amberlite IRC-50 is exposed to water, the resin will swell anywhere from 48–202% [21]. These swelling effects can impact flow characteristics leading to blockage or flow channeling within the column that can adversely affect the metal binding properties of the resin. Due to the more rigid support structure of CPG, the PLAsp and PAA bonded-phase chelators do not exhibit any significant swelling characteristics in

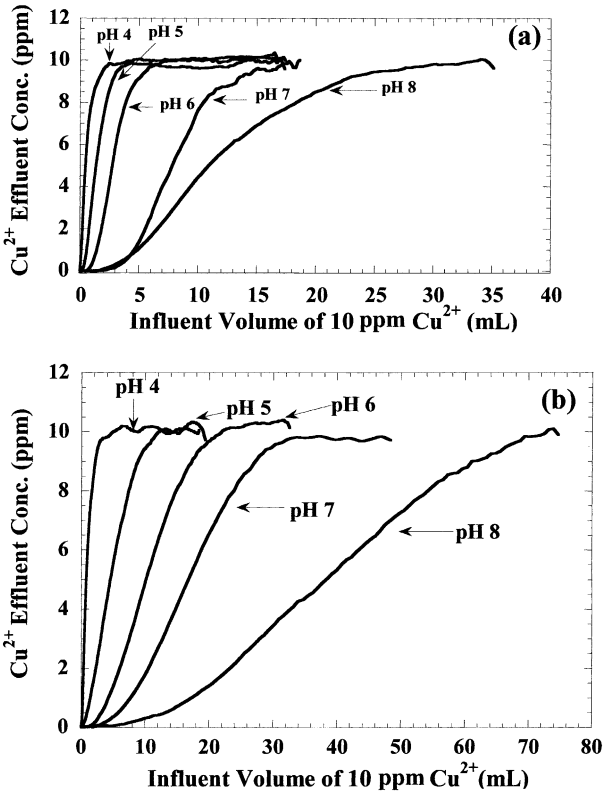

Fig. 3. Breakthrough curves of 10 ppm Cu^{2+} (pH 4.0–8.0) loaded at 1.00 ml/min for (a) PLAsp-CPG and (b) PAA-CPG. Data collection was stopped after a nearly constant, high absorbance suggested that the effluent concentration had reached the influent concentration or after a total of 70 min.

a variety of aqueous environments although the silica substrate of CPG does degrade in alkaline solutions, viz. pH≲9.

3.3. Binding of Cu^{2+} to PLAsp and PAA with varying pH

The PLAsp-CPG and PAA-CPG column capacities were determined for pH values ranging from 4.0 to 8.0, using a 10.0 ppm Cu^{2+} influent solution pumped through the column at a flow rate of 1.0 ml/min. The breakthrough curves for the PLAsp and PAA columns are presented in Fig. 3. All of the breakthrough curves have an early-time baseline region, indicating quantitative binding of the influent Cu^{2+} that is attributed to strong metal binding sites. Also present are the sloped weaker binding site regions. For both columns, the weak site capacity increased with increasing pH while the strong site capacity only increased up to pH 7.0. Above this pH, only an increase in the capacity of the weak site region is observed. The total binding capacities (strong plus weak sites) and strip recoveries for each pH are tabulated in Table 5 for both columns. In accordance with the breakthrough curves, the

Table 5
Cu^{2+} binding capacities and strip recoveries for PLAsp-CPG and PAA-CPG with pH (10 ppm Cu^{2+} influent, 1.0 ml/min flow, $n = 3$)

pH	PLAsp-CPG		PAA-CPG	
	Cu^{2+} bound (μmol/g of CPG)	Cu^{2+} Recovered (%)	Cu^{2+} bound (μmol/g of CPG)	Cu^{2+} Recovered (%)
4	1.3 ± 0.1	85 ± 10	2.0 ± 0.3	90 ± 19
5	3.7 ± 0.6	85 ± 14	13 ± 1	85 ± 7
6	5.9 ± 0.1	95 ± 2	24 ± 3	104 ± 13
7	13 ± 1	100 ± 11	32 ± 5	109 ± 17
8	28 ± 3	108 ± 12	79 ± 1	113 ± 2

overall binding capacity of each of the columns increased with increasing pH. Table 5 also shows that the total Cu^{2+} recovered in the acid effluent was also in good agreement with the total Cu^{2+} binding capacity from the breakthrough curves at each pH. To directly compare the total Cu^{2+} binding capacity trends with pH for PLAsp and PAA, the Cu^{2+} capacities were plotted in Fig. 4, as a function of pH after being normalized to the capacity at pH 8.0. The nearly identical behavior suggests that the conformations and modes of Cu^{2+} binding to the two polymers may be very similar at the different pH values. It further suggests that the peptide bond in PLAsp exhibits minimal influence on both binding and pK_a of the chain.

Francois et al. [22] have extensively studied the binding of Cu^{2+} to PAA in homogeneous solution at varying pH. They describe PAA as a polymer whose charge density can be varied by changing the pH, and at partial neutralization, the charges do not have fixed positions along the chain, but organize themselves in order to favor the formation of complexes with cationic species. PAA exhibits a conformational transition when its degree of neutralization increases. At low pH, PAA exhibits a compact conformation with dimensions intermedi-

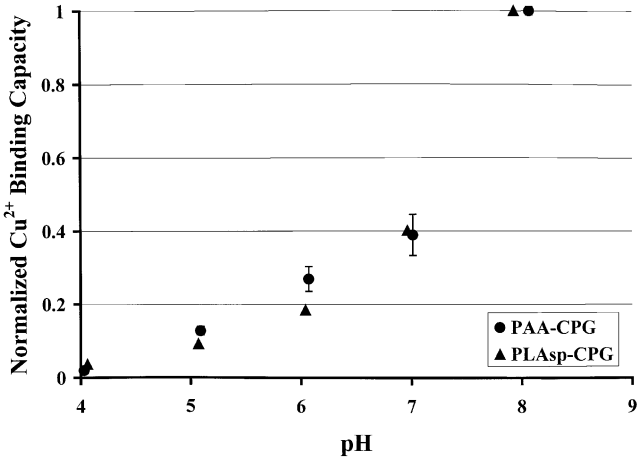

Fig. 4. pH dependence of Cu^{2+} binding capacity for PLAsp-CPG and PAA-CPG. Data for both columns were normalized to the respective capacities at pH 8.0.

ate between a "globule without solvent" and a statistical coil. This conformation favors the formation of a "binuclear" complex, where two Cu^{2+} are bound by four carboxylate ligands. At high pH, the conformation changes to that of an expanded, random coil due to the presence of electrostatic repulsions between the ionized carboxylate groups. Due to chain expansion, the "binuclear" complex dissociates into a "mononuclear" complex where one Cu^{2+} binds for every two carboxylates. These complexes are both found as intra and intermolecular structures.

Similar conformational changes have been reported for PLAsp in homogeneous solution [23]. The PLAsp prefers a compact helical geometry at low pH while converting to an extended random coil above pH $\sim$ 7.0. The conformational change is induced because the helix, which is normally stabilized by hydrogen bonds between the amide and carboxyl groups of the biopolymer backbone, becomes destabilized by the strong electrostatic repulsion between charged carboxylate side chains.

It is likely that the immobilized polymers exhibit similar conformational properties with varying pH to those in homogeneous solution. The curves in Fig. 4 show a similar proportional, exponential increase in relative Cu^{2+} binding with increasing pH for both systems. At low pH (i.e. pH $\leq$ 4), both systems exhibit minimal Cu^{2+} binding. The conformational structures of the polymers may be so compact that metal can only weakly bind to the few carboxylate groups located on the outside of the immobilized helices or tightly coiled structures. As the pH is increased, more carboxylate groups become deprotonated, causing the polymeric structures to expand. Both intra and intermolecular strong and weak sites become increasingly available for binding as the chains are extended. It is likely that the strong binding sites are formed from one or more chains "wrapping" around the Cu^{2+} during chelation, possibly forming similar structures to the binuclear complex described by Francois. The weak sites may be formed from less condensed structures, such as the mononuclear structures. As the chains expand, it seems that the chains have only a finite ability to form condensed structures around the metal.

New atomic force microprobe evidence presented by Miller et al. [20] suggests that definite changes in conformation, similar to those described above, do take place as the immobilized polymers are exposed to different pHs. Polymers exposed to an acidic environment are present as tightly coiled structures on the glass substrate surface, with minimal metal binding. By changing the binding environment to pH 7.0, the polymer conformation changes to a much more extended structure due to chain deprotonation and increased ion-dipole interactions that produce elongation of the chain. This results in greater access to exposed metal binding sites along the chain, which permits greater interactions with metals in solution.

3.4. Stability studies on PLAsp-CPG and PAA-CPG

To test the durability of the PLAsp-CPG and PAA-CPG columns under conditions that might reasonably be present in natural and industrial metal clean-up sites, each type of column was exposed independently to the environmental conditions listed in Table 1, respectively, for $\sim$70–80 h. Each of the columns was subjected to a total of five intervals of exposure to each test condition. The column capacities at each of these intervals were measured in duplicate and plotted as a function of exposure time in Figs. 5–9. The trendlines pictured in these figures are featured to help display the overall effect of the test condition on

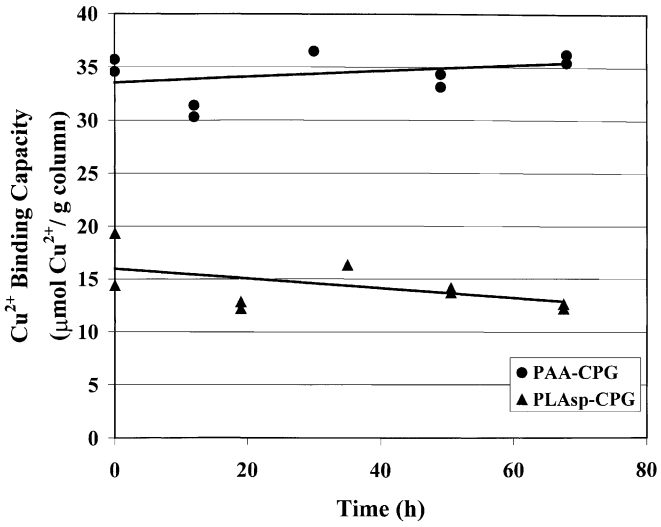

Fig. 5. Cu^{2+} binding capacity as a function of exposure time to 0.05 M ammonium acetate at pH 7.0 for PLAsp-CPG and PAA-CPG.

the column metal binding behavior (i.e. metal binding increase, decrease, no change, etc.). They are not meant to suggest that the functions displayed are necessarily linear in nature.

Fig. 5 shows the effect of constant exposure of 0.05 M ammonium acetate (pH 7.0) on the Cu^{2+} binding characteristics of both systems. This buffer is used in the normal oper-

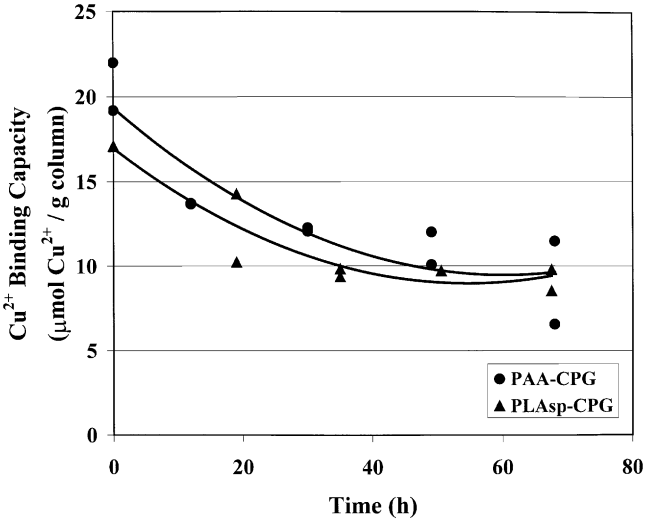

Fig. 6. Cu^{2+} binding capacity as a function of exposure time to 1 M HNO_3 for PLAsp-CPG and PAA-CPG.

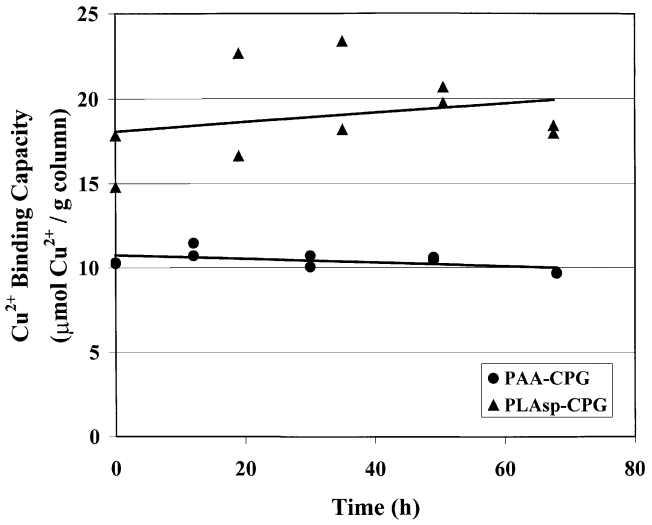

Fig. 7. Cu^{2+} binding capacity as a function of exposure time to 5% H_2O_2 at pH 7.0 for PLAsp-CPG and PAA-CPG.

ating conditions for metal binding experiments and represents the "ideal" metal extraction environment due to its buffer action near the pH of neutral water. In this case, it was used as a control to determine any baseline degradation instabilities that may be taking place. Fig. 5 clearly shows that the Cu^{2+} binding capacity remained relatively stable over ~80 h of constant cycling, with no visible column degradation.

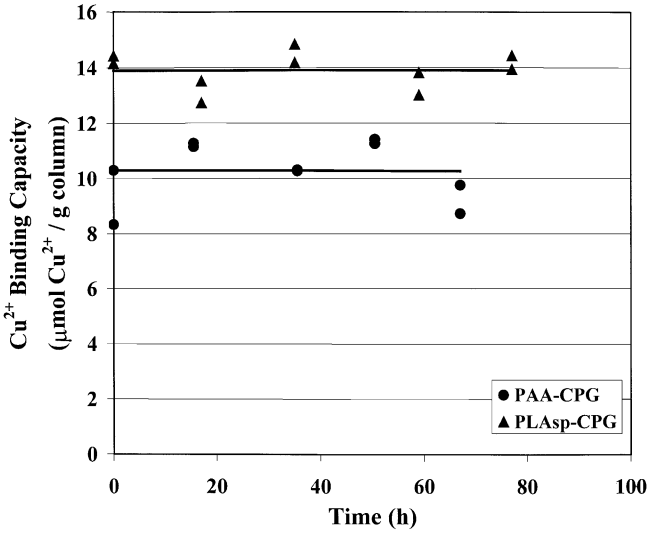

Fig. 8. Cu^{2+} binding capacity as a function of exposure time to 0.05 M ammonium acetate at pH 7.0 elevated to a temperature of 60°C for PLAsp-CPG and PAA-CPG.

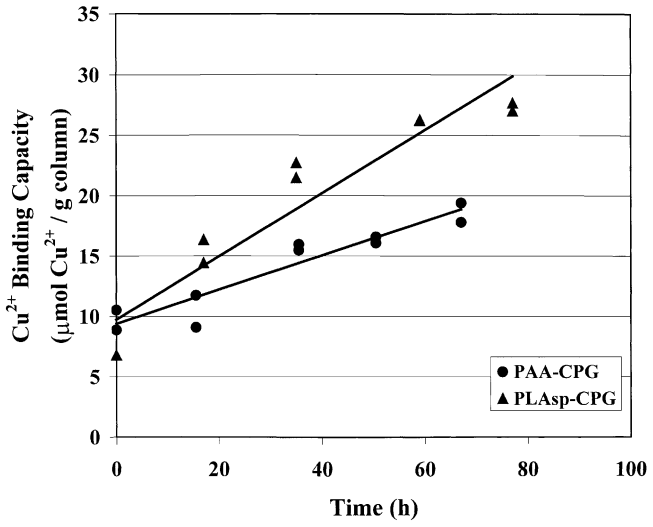

Fig. 9. Cu^{2+} binding capacity as a function of exposure time to 0.5% albumin for PLAsp-CPG and PAA-CPG.

The oxidizing acid, HNO_3 is one of the potential "stripping" agents that can be used for column metal recovery. Even though it has been used in this study to aid in the efficient recovery of metals from the PLAsp-CPG and PAA-CPG systems, 1 M HNO_3 creates a unique low pH, oxidizing, possibly hydrolyzing environment that may degrade the polymer systems over time. Fig. 6 indeed shows that both the PLAsp and PAA systems did slowly loose metal binding capacity upon constant exposure to 1 M HNO_3. According to Schnabel, it is common for polyamides (such as poly-amino acids) to undergo hydrolysis at their respective amide linkages at pH $\leq$ 7. However, he also states that polyhydrocarbons are quite stable under such conditions [24]. These statements seem to contradict the data shown in Fig. 6 which suggests that the PLAsp and PAA columns were loosing metal binding capacity at approximately the same rate even though PAA lacks the amide linkage. Schnabel also states that siloxane linkages are also susceptible to hydrolysis, which is the likely [24] explanation of the loss in metal binding capacity.

Exposing the polymers systems to 5% H_2O_2, a mild oxidizing environment, augments the study exposing both systems to an oxidizing acid. It can be seen from Fig. 7, that there is no loss in Cu^{2+} binding capacity upon constant exposure of the anchored polymers to 5% H_2O_2, suggesting that neither the polymers nor the linkages to CPG are affected by mild oxidizing agents at neutral pH. This suggests that the acidic, oxidizing character of HNO_3 interferes with the stability of the siloxane anchoring of the ligands to the glass substrate.

According to Schnabel, organic molecules are stable only below a certain limiting temperature (typically 100–200°C) due to the limitation of the strength of the covalent linkages within the polymer [24]. Typical modes of degradation include rupture of main and side chain chemical bonds as well as cyclization, elimination and cross-linking reactions in linear polymers. These effects in synthetic polymers, such as polymethylmethacrylate, have been shown, to a great extent, to be reversible, allowing for reconstitution of the original

polymeric material. However, in the case of natural high molecular weight biopolymers, such as proteins, heat induced variations of intra and intermolecular interactions prove to be mostly irreversible causing denaturation of predetermined secondary and tertiary structures needed for proper biological functioning.

To study the effects of temperature on the PLAsp and PAA systems, the immobilized polymers were continuously exposed to a 0.05 M ammonium acetate solution (pH 7.0) elevated to a temperature of 60°C. This temperature was chosen due to the documented denaturation of natural proteins, such as lysozyme and collagen at this temperature [24]. Fig. 8 illustrates the effects of exposure of the polymer/CPG exchangers to this environment. From the account given above concerning the stability of natural biopolymers in a thermal environment, it might be surmised that the PLAsp system should experience some degradation. However, Fig. 8 clearly demonstrates that minimal degradation of both polymer systems occurs at this temperature. This behavior may be due to the small size of the polymers. According to [25,26], long chain polymers make it possible for reactions to occur, such as the unzipping of monomers from a reactive chain end, that are impossible in small molecules. For example, products of decomposition of one monomer unit in a long chain polymer may activate the rapid unit-to-unit decomposition of adjacent units, a process that becomes increasingly more difficult as the polymer length is shortened. The contradictory behavior of the PLAsp biopolymer may also be explained by the fact it is not a "naturally made" peptide. A major advantage of synthetic biopolymers over natural systems is that while natural proteins must sustain their tertiary structure to maintain their metal binding activity, synthetic peptides do not have a (preformed) tertiary structural dependence. Rather the flexibility of the biopolymers backbone allows it to "wrap" around a metal as it binds, finally reaching a free energy minimum that results in its optimal tertiary conformation for binding that particular metal, even at temperatures where a natural proteins intra and intermolecular interactions would normally be disrupted [24].

Large molecules, typically biological molecules, such as proteins, present in industrial and natural waste streams have many polar and non-polar groups that would allow for the adsorbance of these types of molecules to the surface of the ion-exchange column (through dipole, van der Waals, and hydrogen bonding interactions) and interfere with chelator metal binding activity. To evaluate the impact of the presence of large molecules on chelator activity, albumin, a typical large biological protein (MW $\sim$ 69,000 kDa), was continuously cycled through the PLAsp and PAA columns at a 0.5% concentration in water at pH 7.0. According to Fig. 9, the albumin did "stick" to both exchange columns, causing an increase in Cu^{2+} binding capacity after each $\sim$18 h of cycling. It is possible that the albumin both adsorbed to the ionic polymeric material in each column as well as became caught inside the pores of the CPG. The increase in Cu^{2+} binding is attributed to the amount of albumin that became stuck on the columns. Rinsing the columns with copious amount of deionized H_2O as well as 1 M HNO_3 did nothing to restore the columns to their original Cu^{2+} binding capacities. However, by cycling the enzyme trypsin through the two columns (prepared from a procedure described by Eggerer [27]), the adsorbed albumin was sufficiently digested to restore the columns to their original capacities. Therefore, even though the metal binding properties of both the biopolymer and "plastic" polymer columns were affected by the presence of large, sticky molecules, the columns were easily restored to their original binding properties through the use of an enzymatic protease.

4. Conclusion

The results of this study showed that the biopolymer system, PLAsp-CPG, and the synthetic "plastic" polymer system, PAA-CPG, had very similar metal binding characteristics. They both demonstrated similar binding selectivity for the metals tested. Both materials exhibited similar strong and weak metal binding characteristics as well as allowed for quantitative, on-demand release of the bound metals through acid stripping. PLAsp-CPG and PAA-CPG both demonstrated increased overall metal binding as well as proportional changes in strong and weak metal binding sites with increasing pH. This suggests that both systems undergo similar conformational transitions and form related binding complexes as their carboxylate chains become increasingly electrostatically charged. Interestingly, both systems exhibited similar stabilities when exposed to a variety of environmental conditions. Both PLAsp and PAA showed minimal loss in metal binding capacity upon exposure to 0.05 M ammonium acetate buffer, 5% H_2O_2, and elevated temperature (60°C). Both demonstrated slight losses in capacity when exposed to 1 M HNO_3, probably as a result of chelator loss through siloxane bond degradation. Also, upon exposure to the large molecule albumin, the column capacities increased due to adsorbance of albumin to the columns' surfaces. However, the columns were easily restored to their original binding capacities through the use of the protease trypsin, indicating that they are resistant to enzymatic degradation as well, provided that the enzyme does not readily attack the backbone linkage.

These results indicate that both polymers would be good candidates for bonded-phase ion-exchange agents in varied chemical and thermal environments. These species could also be used to preconcentrate metals from sodium-rich matrices, since they exhibit little propensity for such alkali metals. The main advantage of using PAA over PLAsp is the fact that it is less expensive in the current market. A distinct advantage of PLAsp over PAA is the fact that it is composed of a peptide backbone rather than a repeating carbon unit chain. For example, both columns remain very structurally stable in mild chemical environments, indicating they will retain a long lifetime of use. However, once it is decided that a column is no longer to be used, methods of degradation must be employed that will not harm the environment. Biopolymers containing peptide bonds may be much more easily biodegraded using selected proteases or bacteria that specifically attack and break up the peptide chain, such as pepsin and chymotrypsin [28]. "Plastic" polymers, having a hydrocarbon backbone, must be depolymerized by harsher means [24]. Therefore, the original starting materials of the PLAsp column can be much more easily isolated than the PAA column.

In conclusion, both polymeric systems are equally suitable agents for trace metal preconcentration and remediation from natural and industrial waste streams. However, the system cost and biodegradability are conflicting factors that must be weighed in choosing which is most suitable for a particular application.

Acknowledgements

This work was supported by Grants from the Gulf Coast Hazardous Substance Research Center, The Texas Hazardous Substance Center and the Robert A. Welch Foundation.

References

[1] D. Bhattacharyya, J.A. Hestekin, P. Brushaber, L. Cullen, L.G. Bachas, S.K. Sikdar, J. Membr. Sci. 141 (1998) 121–135.
[2] S.M.C. Ritchie, L.G. Bachas, T. Olin, S.K. Sikdar, D. Bhattacharyya, Langmuir 15 (1999) 6346–6357.
[3] T.C. Miller, J.A. Holcombe, Anal. Chem. 71 (1999) 2667–2671.
[4] E. Gutierrez, T. Miller, J.R. Gonzalez-Redondo, J. Holcombe, Environ. Sci. Technol. 33 (1999) 1664–1670.
[5] M. Howard, H. Jurbergs, J.A. Holcombe, Anal. Chem. 70 (1998) 1604–1609.
[6] M.E. Howard, H.A. Jurbergs, J.A. Holcombe, J. Anal. At. Spectrom. 14 (1999) 1209–1214.
[7] M.E. Howard, J.A. Holcombe, in: A. Iskander (Ed.), Environmental Restoration of Metal Contaminated Soil, CRC Press, New York, 2000.
[8] M.E. Howard, Ph.D. Dissertation, University of Texas, Austin, TX, 1999.
[9] H.A. Jurbergs, J.A. Holcombe, Anal. Chem. 69 (1997) 1893–1898.
[10] H. Jurbergs, Dissertation, University of Texas, Austin, TX, 1997.
[11] C. Hirayama, H. Ihara, T. Muaki, Macromolecules 25 (1992) 6375–6376.
[12] T. Fukomoto, H. Ihara, S. Sakaki, H. Shosenji, C. Hirayama, J. Chromatogr. A 672 (1994) 237–241.
[13] H. Ihara, T. Fukumoto, C. Hirayama, Anal. Sci. 9 (1993) 711–713.
[14] J. March, Advanced Organic Chemistry, 3rd Edition, Wiley, New York, 1985.
[15] D.W. Mayo, R.M. Pike, P.K. Trumper, Microscale Organic Laboratory with Multistep and Multiscale Synthesis, 3rd Edition, Wiley, New York, 1994.
[16] A. Legrand, The Surface Properties of Silicas, Wiley, Chichester, 1998.
[17] D.E. Leyden, W. Collins, Silylated Surfaces, Gordon and Breach, New York, 1980.
[18] D.F. Shriver, P. Atkins, C.H. Langford, Inorganic Chemistry, 2nd Edition, Freeman, New York, 1994.
[19] M. Gulumian, R.D. Hancock, H.B. Rollin, in: G. Burton (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, Vol. 1, Marcel Dekker, New York, 1995, p. 108.
[20] T.C. Miller, E.-S. Kwak, D.A. Vanden Bout, J.A. Holcombe, A new "look" at metal complexation by immobilized biopolymers using liquid cell tapping mode AFM, in: Proceedings of the 27th Annual Conference of the Federation of Analytical Chemistry and Spectroscopy Studies, Nashville, TN, 2000.
[21] J. Inczedy, Analytical Applications of Ion Exchangers, Pergamon Press, Oxford, 1966.
[22] J. Francois, C. Heitz, M.M. Mestdagh, Polymer. 38 (1997) 5321–5332.
[23] A.G. Walton, J. Blackwell, S.H. Carr, Biopolymers, Academic Press, New York, 1973.
[24] W. Schnabel, Polymer Degradation Principles and Practical Applications, Macmillan, New York, 1981.
[25] N.S. Allen, M. Edge, Fundamentals of Polymer Degradation and Stabilization, Elsevier, London, 1992.
[26] N. Grassie, G. Scott, Polymer Degradation and Stabilization, Cambridge University Press, Cambridge, 1985.
[27] J. Eggerer, Eur. J. Biochem. 143 (1984) 205–212.
[28] L. Stryer, Biochemistry, 3rd Edition, Freeman, New York, 1988.