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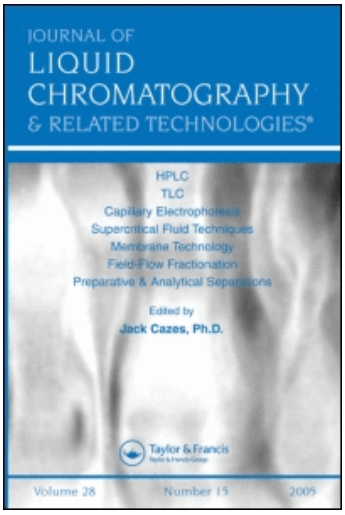

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PREPARATION AND CHROMATOGRAPHIC BEHAVIOR OF A BIFUNCTIONAL CONTINUOUS ROD FOR WEAK CATION EXCHANGE AND IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY

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ABSTRACT

A procedure for the preparation of a bifunctional continuous rod for weak ion exchange chromatography (WCX) and immobilized metal affinity chromatography (IMAC) is presented. Without chelated metal, from the retention behavior of proteins, the rod, which carries iminoacetic acid and iminodiacetic acid (IDA) groups was found to be a relatively strong cation exchanger and exhibits an excellent separation of proteins at the flow rates of both 1.0 mL/min and 8.0 mL/min, respectively. When Cu(II), Zn(II), or Ni(II) was chelated, the rod displayed the property of IMAC and the selectivity for protein separation to be different from that obtained from the naked rod. The effects of pH and

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temperature on protein retention were investigated on both the naked and metal chelated rods. An abnormal increase in retention times of proteins was found under the condition of very high pH and very low pH on the metal chelated rods.

INTRODUCTION

Immobilized metal affinity chromatography (IMAC) and weak cation exchange chromatography (WCX) have been widely employed in the protein separation. With the both, the tertiary or quaternary structure of protein molecules generally maintained, so is their bioactivity maintained. Depending on elution conditions, selectivity can be derived from the multiplicity or local environment of metal-coordinating residues of protein. With regard to the adsorbent, iminodiacetic acid (IDA) has been the most frequent chelating ligand in IMAC, and in the carboxylic group in WCX. IDA-bonded adsorbent loaded with Cu(II), Zn(II), or Ni(II) ion can be employed as the metal chelate adsorbent for protein separation by IMAC. Without a metal ion, IDA-bonded adsorbent is expected to be a relatively strong cation exchanger, but it has only limited efficiency, so it is frequently used as an IMAC adsorbent. A typical weak cation exchanger with fixed carboxylic groups cannot be attached by a metal ion and, thus, it is only used as an ion exchange adsorbent. IDA-bonded adsorbent and carboxylic group-bonded adsorbent are, therefore, usually used as a single mechanism, respectively. A mixed-mode interaction mechanism in the protein separation was reported.(1,2) Based on this idea, a mixed-mode adsorbent used in both hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC) was synthesized.(3)

The inherent problem of all of particulate media is that the chromatographic column could not be completely occupied. Generally, interparticulate porosity contributes to peak broadening, so column efficiency decreases. Introduction of media with a higher degree of continuity results in the decrease or even elimination of the column void volume.

There are many kinds of continuous media,(4-10) and one of them is a continuous rod.(9,10) Swollen polyacrylamide gel was compressed in the shape of a column and formed a continuous rod, and can be used successfully for a style of high performance liquid chromatography (HPLC) to separate proteins and peptides.(9,11-13) Fréchet et al.(10,14-18) reported a HPLC separation media with no discontinuity, consisting of a continuous “molded” rod of rigid, highly porous polymer. This rod, being essentially the equivalent to a very large single cylindrical particle, is prepared in a single step by a free-radical polymerization, directly inside an empty stainless steel tube acting as a mold. This approach has been used for the preparation of poly (glycidyl methacrylate - co - ehthylene dimethacrylate),(14,15) poly (styrene -co-divinylbenzene), (16,17) poly (acrylamide-co-

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butyl methacrylate-co-N, N'-methylenebisacryl-amide),(18) and molecularly imprinted polymer.(19) These molded rods were proven to be very efficient for protein separation by affinity chromatography,(14) weak anion exchange chromatography,(15) reversed phase liquid chromatography,(16) HIC,(18) and WCX,(20) and for the separation of small and midsize molecules,(17) isomers, and enantiomers.(19)

In this paper, we report the preparation of a bifunctional continuous rod based on macroporous poly (glycidyl methacrylate-co-ethylene dimethacrylate) for IMAC and WCX, the chromatographic property of the rod prepared, and its use for the fast protein separation.

EXPERIMENTAL

Chemicals

Glycidyl methacrylate (GMA) (99%, Institute of Chemical Reagent in Tianjing, China) was distilled under vacuum. Ethylene dimethacrylate (EDMA) (Aldrich Co. in Milwaukee, WI, USA) was extracted three times with 10% aqueous solution of sodium hydroxide and distilled water, respectively, and then dried by nonhydrate magnesium sulfate. Azobisisobutyronitrile (AIBN) was obtained from Xi'an Chemical Company in China. Lysozyme (chicken egg white, Lys), ribonuclease A (bovine pancreatic, RNase-A), myoglobin (horse skeletal muscle, Myo), α-chymotrypsinogen A (bovine pancreatic, α-Chy-A), and cytochrome C (horse heart, Cyt-C) were obtained from Sigma (Saint Louis, MO, USA). Other reagents were A.R.grade.

Preparation of the Continuous Rod

The procedure for the preparation of the continuous rod is as follows:(15) The continuous rod was prepared by an in-situ polymerization in a stainless-steel tube of 50×8 mm I.D. The 40:60 (v/v) mixture of monomers (GMA and EDMA, 75:25 vol%) and porogenic diluents (cyclohexanol and dodecanol, 90:10 vol%), in which AIBN (1%, w/v, with respect to monomers) was dissolved, and was purged with nitrogen for 15 min. The stainless-steel tube was filled with the above mixture and then sealed at both ends and was immersed into a water bath. The temperature rose to 55°C in 1 hour. The polymerization proceeded at 55°C for 12 hours. Then the tube was removed from the bath and the column was attached to the HPLC system.

Tetrahydrofuran (THF) (100 mL) was pumped through the column at a flow rate of 1.0 mL/min to remove the alcohols and other soluble compounds in

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the polymer rod under 45°C. Ethylenediamine solution was then pumped circularly through the column for 24 hours at 80°C. After that, the rod was washed in the order of dioxane/ 20% NaOH (3:2, v/v), dioxane/H_2O (3:2, v/v), dioxane, and alcohol (non-aqueous) solutions.

Finally, 100 mL of 14% chloroacetic acid solution (pH 11.0) was allowed to flow through the column circularly for 24 hours at 70°C. A bifunctional rod was prepared in such a way that the rod was chelated with different metal ions to obtain a required metal chelate column, and a weak cation exchange column without metal ion attached.

The procedure for the preparation of the metal chelated rod is as follows: the rod was washed first with 0.03 mol/L ethylene diamine tetraacetic acid (EDTA) + 0.5 mol/L NaCl, pH6, in order to remove metal ions or contaminants, then water, then 0.015 mol/L metal ion dissolved in 0.05 mol/L acetate buffer, pH5, and, finally, with 0.05 mol/L acetate buffer, pH5, in order to elute those metal ions which are adsorbed due to ionic interactions. The rod was equilibrated with 20-25 column volumes of 0.02 mol/L phosphate buffer.

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Characterization of Pore Properties

After all of the chromatographic experiments were finished, the rod was washed with 0.05 mol/L EDTA solution, 1.0 mol/L NaCl solution, water, and methanol at a flow rate of 1.0 mL/min for 1h with each solution; the bottom column fitting was removed, and the polymer rod was pushed out of the tube using the pressure provided by the mobile phase. The rod was then cut into small pieces and dried at 70°C. The weight of the dried rod was 1.1 g. The pore size distribution in the dry state was determined by mercury porosimeter from Porous Materials, Inc., Ithaca, NY, USA.

Determination of the Capacity of Carboxylic and Loading Capacity for Metal Ion

The titration of carboxylic in the rod is as follows: small pieces of the rod (10.0 mg) were titrated in 10 mL 1M NaCl. Enough 0.1 M HCl was initially added to bring the pH to 2. Then 50 μL portions of 0.1 M NaOH were added and the pH recorded until it reached 12. pH was monitored using a coming pH meter, Model pHS-25 and Orion combination pH electrode (Shanghai Leici Instrument Co., China).

The loading capacity of the synthesized IMAC rod for metal ions, Cu (II), Ni (II), or Zn (II) was determined in the following way: metal ion solution was pumped through the rod until saturation. The rod was then washed with 100 mL

of 0.1 mol/L acetate buffer (pH 4.5) containing 0.5 mol/L sodium chloride and 50mL of water. Metal ion was then eluted with 100 mL of 0.05 mol/L EDTA solution. The eluate was determined by a M6 atomic absorption spectrometer (TJI Co., USA).

Chromatograph

All the chromatographic experiments were carried out with a Shimadzu LC-6A HPLC system (Shimadzu Co., Japan) consisting of two HPLC pumps, SPD-6AV UV-VIS spectrophotometric detector, SCL-6A system controller, and C-R3A chromatopac. The detector was set at 280 nm.

RESULTS AND DISCUSSION

Preparation of the Continuous Rod and Its Pore Properties

Because of the similarity of both cyclohexanol and dodecanol with the monomers in properties, the mixture of two solvents was chosen as porogen diluents for the preparation of poly (glycidyl methacrylate-co-ethylene dimethacrylate) rod in this study. As is well known, the porous structure of the polymer can be easily controlled by adjusting the proportion of the total porogen in solution and by the relative ratio of cyclohexanol to dodecanol, respectively. In order to increase the content of the epoxide groups in the polymer that are necessary for the subsequent reaction, a higher percentage of GMA must be used. The ratios of monomer to porogen (40/60, v/v) and GMA to EDMA (75/25, v/v) were selected in this research. Such proportions not only provide a porous polymer with a pore size and mechanic intensity that is very suitable for the HPLC separation of proteins, but also offer the polymer with a quite high content of epoxide groups.

Figure 1 shows the pore size distribution profile of the rod obtained by mercury intrusion porosimetry. The pore range from 0.40 μm to 2.0 μm covers about 80% of the total pore volume. This result indicates that the continuous rod polymer has very large pores. The total pore volume of the porous polymer is 1.23 mL/g, and translates into a porosity of about 60% that correlates well with the volume of the porogenic diluents used during the preparation. These pores form the channels through which the mobile phase flows, and make this “mold” material permeable for the mobile phase, even at very high flow rates. Figure 2 shows the effect of the flow rate on the backpressure of the rod column. The almost linear dependence of the backpressure of the column on the flow rate in the whole range from 0 to 8.0 mL/min documents the pressure stability of the column and the lack of rod deformation at high flow rates. It should be pointed

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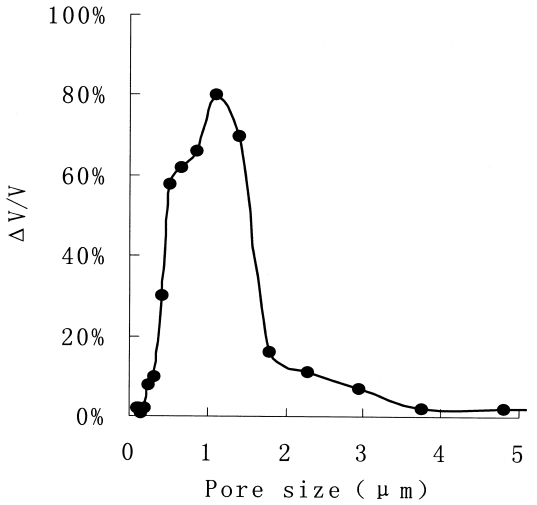

Figure 1. The profile of the pore size distribution of the continuous rod.

out, that even at a flow rate of 8.0 mL/min, the backpressure does not exceed 7.75 Mpa, being much lower than that for the same size column packed with 10 μm monodisperse beads. As expected, the rod could be used for the rapid separation of biopolymers.

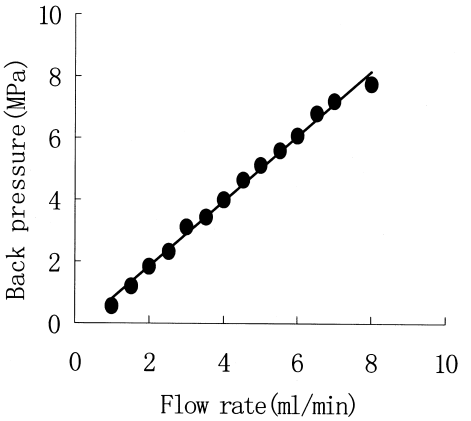

Figure 2. Effect of flow rate on backpressure in the continuous rod column. Mobile phase: 0.02 mol/L sodium phosphate buffer (pH 7.0).

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Chemical Modification of the Continuous Rod and the Adsorption of Metal Ion

Many reactions can be used for the chemical modification of the poly (glycidyl methacrylate-co- ethylene dimethacrylate) due to the presence of the epoxide groups. Because the reaction must be carried out within the column, the reactions that proceed under a warm reaction condition, and without forming insoluble product, are only suitable. An amination procedure, followed by reaction with chloroacetic acid solution was selected. In the first step, two products can be obtained, one is -$NHCH_2CH_2NH$- because of the "crosslinking" reaction within the polymer, and the other is –$NHCH_2CH_2NH_2$. Then, these two groups react with chloroacetic acid, and more than four kinds of ligands would be obtained. However, all ligands obtained in the second step reaction could be divided into two categories, -$NHCH_2CH_2COOH$ and IDA, according to their interaction property with metal ion. In other words, the rod was modified into a carboxylic/IDA-bonded adsorbent.

The rod column with such a chemical structure can be employed for both WCX and IMAC. Elemental analysis revealed the nitrogen content of the rod to be 4.87%. Carboxylic capacity of the rod is determined to be 0.351 mmol/g. The loading capacity of the rod for Zn(II), Ni(II), and Cu(II) is 202.0 μmol/g, 89.5 μmol/g, and 134.0 μmol/g, respectively. They are several times higher than that for the IDA-based silica adsorbent in the literature.(21) Therefore, IDA groups may play a dominant role for attaching those metal ions. The large difference in the loading capacity between the three metal ions employed could be explained as that the complicated chemical structure on the surface of the medium synthesized can make the medium have selective adsorption to Zn(II). As a result, a large increase in the loading capacity of the rod for Zn(II) was obtained.

A typical modified procedure by reacting with IDA and the polymer, was adopted for the preparation of the metal chelate supports.(22) But the separation efficiency for proteins on this kind of metal chelate adsorbent is not very good in most cases, due to lack of a molecular arm to connect IDA ligands with the support. However, as for the route in this study, ethylenediamine is able to play two roles as both the activating group and the molecular arm. Thus, a good separation efficiency may be obtained in the protein separation by IMAC.

Separation Ability for Proteins on the Bifunctional Continuous Rod

The separation ability and retention behaviors of proteins on the naked and chelate rods were investigated using gradient elution with increasing sodium chloride concentration in 25 mmol/L phosphate buffers at pH 7.0.

Without metal ion, the carboxylic/IDA-bonded rod is expected to be a relatively strong cation exchanger, because the pKa value of one of the carboxylic groups in the state of covalently bound IDA is 2.65.(23) As expected, the pH range to be used for protein separation may be broader, from 4.0 to 12.0 for the rod, while the typical weak ion exchanger is from 6.0 to 12, because of the usual carboxylic functions of pKa being 4.0-5.0. As stated above, as loading metal ion, the carboxylic/IDA-bonded rod is an actual IMAC column. The protein mixture, Cyt-C (pI 10.6), α-Chy-A (pI 9.5), Lys (pI 11.0), Myo (pI 7.0), and RNase-A (pI 8.2) was separated by the naked and the chelated rods with Zn(II), Ni(II), and Cu(II) at pH 7.0. The four chromatograms are illustrated in Figure 3.

The naked rod could be employed to separate, completely, five proteins. For the metal chelated rods, the similar efficiency and same elution order were found from Zn(II)-IDA and Ni(II)-IDA rods. However, with the chelated Cu(II) rod under the same chromatographic condition, the separation efficiency was found to be worse than the other two metal chelated rods mentioned above, and the elution order of proteins was also changed. This indicates different metal ions have various effects on the protein separation. This fact elucidates that a protein mixture could be separated and purified with different selectivity by selecting the rod with bifunction of weak cation exchange or metal ion affinity mechanism. The lower separation efficiency of Cu(II) chelated rod may be explained as the relatively slow kinetics of the interaction between the protein and the bound metal chelate groups of the stationary phase because of the strong metal chelate interaction.(24)

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Behavior of Proteins on the Metal Chelated Rod

The retention times of four proteins by linear gradient elution on the naked and the metal chelated rods are listed in Table 1. Compared to the naked and other two chelated rods, the four proteins were strongly retained on the chelated Cu(II) rod and had a longer retention time. Chelated Zn(II) and naked rods have a very closed retention behavior. These results are consistent with the results reported by Rassi.(24) Quite unexpectedly, the retention times of the four proteins are slightly shorter on chelated Ni(II) than on chelated Zn(II) rod.

The retention times depend, of course, not only on the strength of protein-metal interaction, but also on the amount of metal loading on the column. From the literature,(22) the binding constants of IDA in solution and in immobilized form on the stationary phase are closed to each other, and the binding strength follows the order of Cu(II) > Ni(II) >Zn(II). The short retention times of proteins on the chelated Ni(II) rod could be, thus, attributed to a much lower degree of chelation by Ni(II) than by Zn(II).

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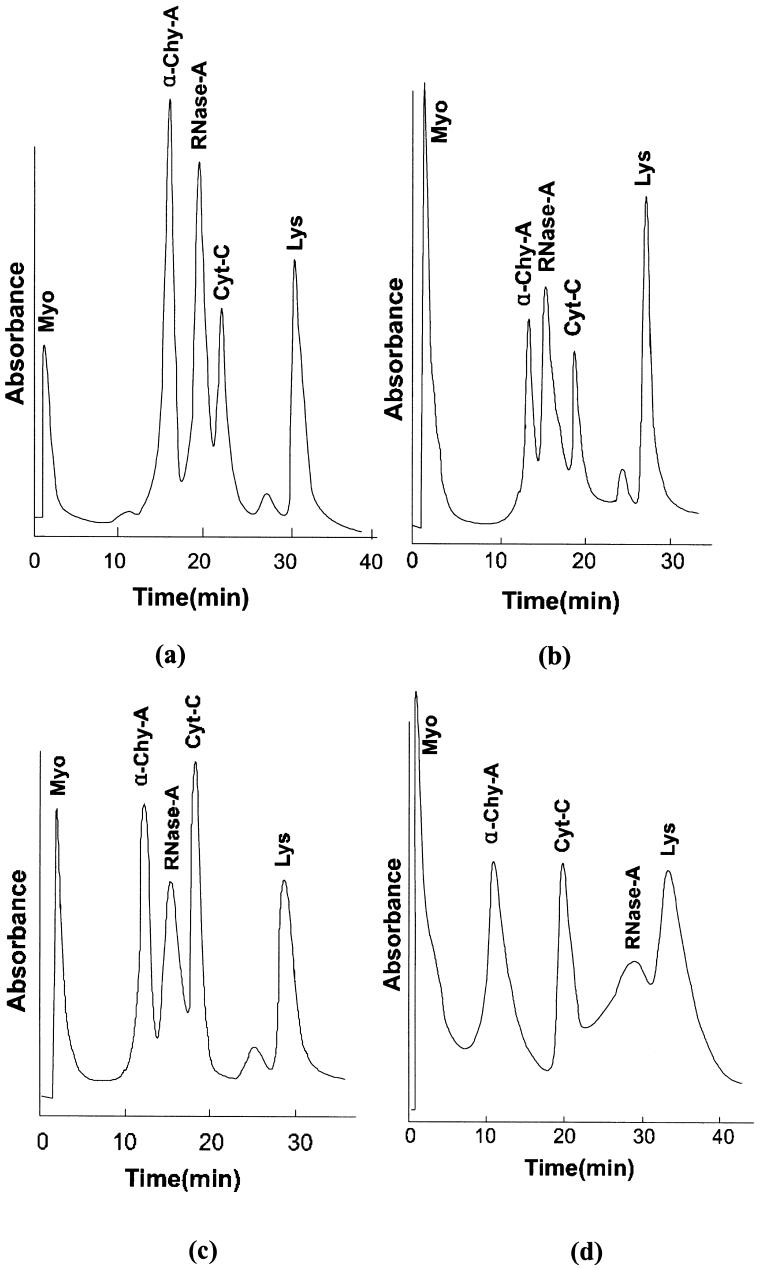

Figure 3. Chromatogram of five proteins on the naked (a), and chelated Zn(II) (b), Ni(II) (c), and Cu(II) (d) rod. Condition: mobile phase gradient from 0 to 1.0 mol/L sodium chloride buffer (pH 7.0) in 30 min; flow rate, 1 mL/min; UV detection at 280 nm.

Table 1. Retention Times of Five Proteins on the Naked and Chelated Rods[a]

	Lys	Cyt-C	RNase-A	α-Chy-A
Naked column	12.80	10.09	10.39	8.60
Chelated Zn(II)	12.84	10.33	8.96	8.06
Chelated Ni(II)	12.08	9.45	8.24	7.89
Chelated Cu(II)	17.63	11.47	17.64	8.20

[a]Conditions: mobile phase gradient from 0 to 1.0 mol/L sodium chloride buffer in 20 min; flow rate, 1 ml/min.

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pH Effect on Protein Retention

As shown in Figure 4, the pH value of the mobile phase has an important influence on the protein retention in WCX and IMAC. The retention times of proteins on the naked, and chelated Cu(II), Ni(II), and Zn(II) rods reduce gradually with increasing in the pH range from 4.0 to 9.0, this fact is consistent with the results observed by the literature.(21) However, when pH<4.0 and pH>9.0, the phenomenon is clearly different. When the pH values of the mobile phase are in the range from 3.0 to 4.0 and from 10.0 to 11.0, these proteins did not retain on the naked column, whereas, they retained on the chelated rod and the retention times had an abnormal increase.

In order to determine whether metal ions are chelated on the column at the extreme condition employed, we took Ni(II) as an example to determine the residue of metal ions on the column. It was found to be 33.6 μmol/g after the experiments on the retention of proteins at pH 3.0 and 11.0 of the mobile phase had been completed, respectively. Though Ni(II) ions lose seriously from the rod in comparison with the original amount of the chelated Ni(II), this value was found to be still relatively large with respect to that of silica-based IMAC packings for Ni(II).(21) So the rod at this extreme condition might still act as an IMAC column. The reasons for the abnormal increase in the retention times in the pH range from 4.0 to 3.0 and from 9.0 to 11.0 remained for further study.

Effect of Temperature on Protein Retention

The interactions between protein and the chelate adsorbent include hydrophobic, chelate, and static electric interactions. For the naked rod, only two of them, hydrophobic and static electric interactions exist. The change in the retention times of proteins with different temperatures in LC, except reversed phase liquid chromatography, is usually employed as one of the criteria to judge whether the retention mechanism of a column is hydrophobic or not. A series of

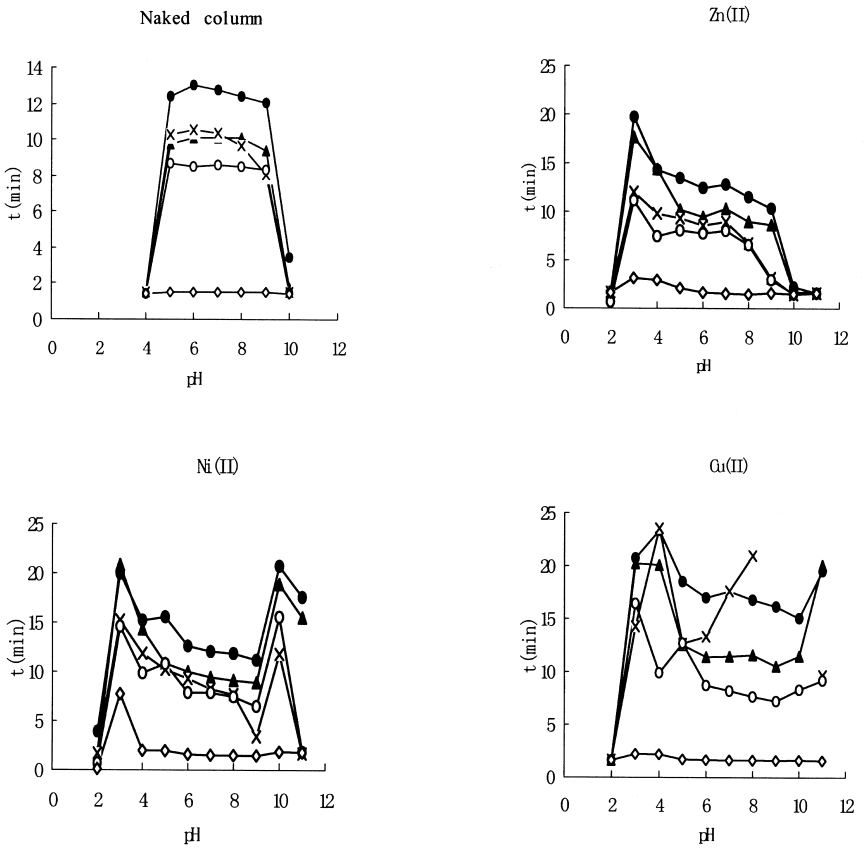

Figure 4. Effect of pH values on the retention of five proteins by the naked and chelate rods. Condition: mobile phase gradient from 0 to 1.0 mol/L sodium chloride buffer (pH 7.0) in 25 min. Other conditions are the same as that indicated in Figure 3. Protein: (●) Lys, (▲) Cyt-C, (×) RNase-A, (○) α-Chy-A, (◊) Myo.

chromatograms of three proteins, RNase-A, Cyt-C, and Lys, were obtained from the naked and chelated rods under column temperatures of 0°C, 10°C, 25°C, 35°C, and 45°C. It was found from Figure 5, that the retention times of the three proteins did not change in the temperature range from 0°C to 45°C on the naked rod, whereas, on the chelated Ni(II) rod the retention time did not change from 0°C to 35°C, but the retention time of Lys decreases to about 10 min at 45°C. Beside chelated Ni(II) rod, the similar phenomena were also found from the chelated Zn(II) and Cu(II) rods. This fact indicates the hydrophobicity of the rod

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is very weak, and the static electric interaction on the naked rod and chelate interaction on the chelated rod dominate the retention behavior of proteins, respectively. Otherwise, the retention times of the three proteins would increase with rising temperature.

However, as shown in Figure 5, the peak area of each protein at 45°C is much smaller than that at 5°C on all rods, even though the sample size of those proteins was the same, but was not found to change in the temperature range of from 0°C to 35°C. This phenomenon could be explained as that, if the molecular conformations of the three proteins had been changed at 45°C, it might be partially denatured.(25) As a result, the peak areas of these natural proteins decreased.

Rapid Separation of Biopolymer

Compared to the usual column packed with porous particles, the rod with macroporous structures could provide more efficient separations with respect to dynamic adsorptive capacity and required pressure drop.(26) Recently, a mono-

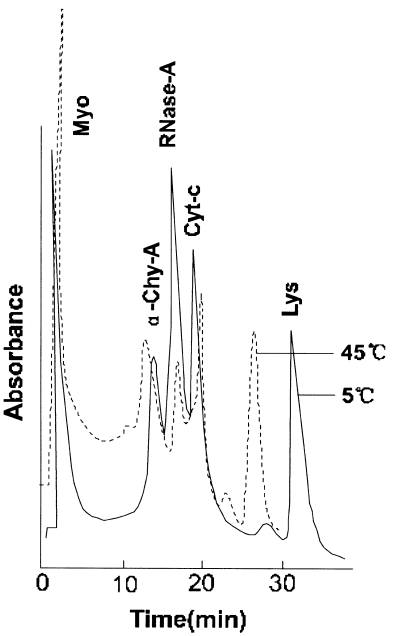

Figure 5. Chromatogram of five proteins on the chelated Ni(II) rod at 5°C and 45°C. Other conditions are the same as that indicated in Figure 3.

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lithic poly (styrene-co-divinylbenzene) rod was reported to separate proteins and peptides within 15 sec.(27) On the naked rod synthesized in this research, five proteins could be separated completely by ion exchange mechanism at the flow rate of 8.0 mL/min within 3.5 min, as shown in Figure 6a. The column efficiency in this circumstance was almost the same as that at the flow rate of 1.0 mL/min. This is because mass transfer could be accelerated by convection instead of the slow diffusion procedure.(28,29) However, when loading metal ion, the column efficiency became obviously worse at the flow rate of 8.0 mL/min, compared with the flow rate of 1.0 mL/min.

Figure 6b is the chromatogram of rapid separation of five proteins by the chelated Ni(II) rod. Five proteins could be separated within four minutes, but not completely, and the column efficiency reduces very seriously. Beside chelated Ni(II) rod, the Zn(II), and Cu(II) chelated rods also had similar phenomena. This is probably due to the relatively slow kinetics of the interactions between the pro-

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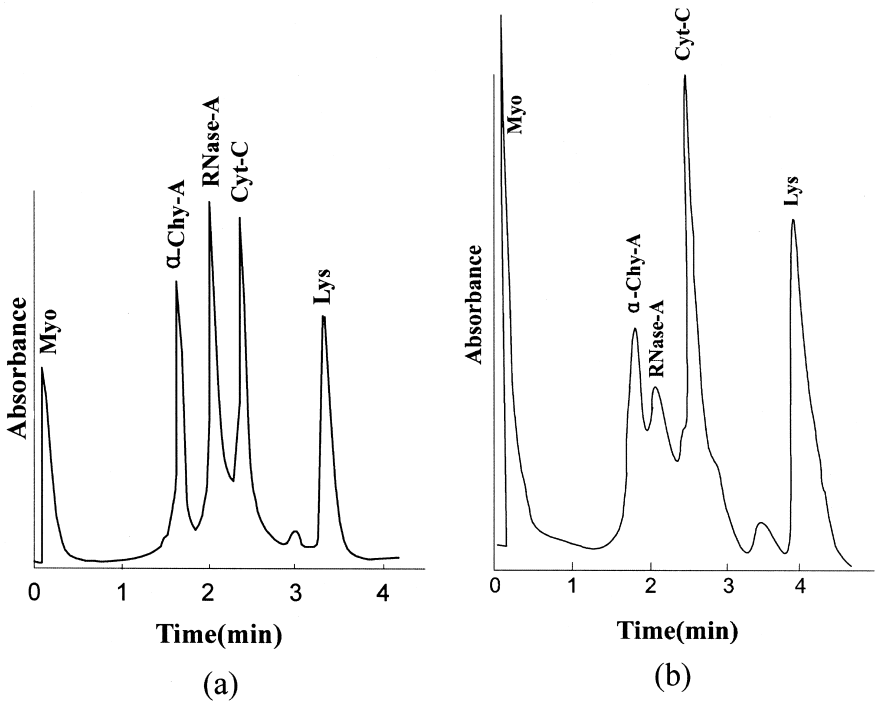

Figure 6. Chromatogram of rapid separation of five proteins on the naked (a) and the chelated Ni(II) (b) rod. Condition: mobile phase gradient from 0 to 1.0 mol/L sodium chloride buffer in 4 min; flow rate, 8.0 mL/min. Other conditions are the same as that indicated in Figure 3.

Table 2. Recovery of Proteins from Chelated Ni(II) Rod at the Flow Rate of 8.0 ml/min

Protein	Recovery (%)
Lys	88
Cyt-C	93
RNase-A	95
α-Chy-A	100

tein and the bound metal ions of the stationary phase. When the flow rate rises, the column efficiency decreases.

The rod column prepared has been proven to have good batch-to-batch reproducibility, as well as, long-term stability for the separation of proteins.(30) Table 2 lists the recoveries of proteins at the flow rate of 8.0 mL/min on chelated Ni(II) rod. All proteins tested, gained recoveries greater than 85%. The relative standard deviations of recoveries of four proteins in three parallel tests are all less than 10%. This result shows that protein can obtain high recovery on the rod.

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CONCLUSIONS

The procedure of the preparation of a bifunctional rod for weak ion exchange chromatography and immobilized metal affinity chromatography was demonstrated. Without chelated metal, the rod appears to be a relatively strong cation exchanger and exhibits excellent separation ability for proteins. In immobilized metal affinity chromatography (IMAC) with the rod, chelated by Cu(II), Zn(II), or Ni(II), all metal chelated rods separated the proteins under this investigation with different selectivity at the flow rates of 1.0 mL/min and 8.0mL/min. The use of a bifunctional rod in different ways may afford a convenient way to expand the scope of its application and offer a degree of versatility not available with other stationary phases. In addition, the both rods can be employed for the rapid separation of biopolymers.

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REFERENCES

1. Melander, W.R.; Corraini, D.; Horvath, C. J. Chromatogr. **1984**, *317*, 67-85.
2. Wei, Y.M.; Luo, Q.-Z.; Liu, T.; Geng, X.-D. Chin. J. Chem. **2000**, *18*, 60-65.
3. Kennedy, L.A.; Kopaciewicz, W.; Regnier, F.E.; J.Chromatogr. **1986**, *359*, 73-84.
4. Tennikova, T.B.; Svec, F. J.Chromatogr. **1993**, *646*, 279-288.
5. Gerstner, J.A.; Hamilton, R.; Kramer, S.M. J. Chromatogr. **1992**, *596*, 173-180.
6. Kennedy, J.F.; Paterson, M. Polymer Int. **1993**, *32*, 71-81.
7. Yang, Y.; Velayudhan, A.; Ladish, C.M.; Ladisch, M.R. J. Chromatogr. **1992**, *598*, 169-180.
8. Pretorius, V.; Davidtz, T.C.; Desty, D.H. J. High Resolut. Chromatogr. Commun. **1979**, *2*, 583-589.
9. Hjerten, S. Nature **1992**, *356*, 810-811.
10. Svec, F.; Fréchet, J.M.J. Science **1996**, *273*, 205-211.
11. Hjerten, S.; Liao, J.L.; Zhang, R. J.Chromatogr. **1989**, *473*, 273-275.
12. Liao, J.-L.; Zhang, R.; Hjerten, S. J. Chromatogr. **1991**, *586*, 21-26.
13. Liao, J.-L.; Chang, N.; Ericson, C.; Hjerten, S. Anal. Chem. **1996**, *68*, 3468-3472.
14. Petro, M.; Svec, F.; Fréchet, J.M.J. Biotechnol. Bioeng. **1996**, *49*, 355-363.
15. Svec, F.; Fréchet, J.M.J. J. Chromatogr. A **1995**, *702*, 89-95.
16. Wang, Q.C.; Svec, F.; Fréchet, J.M.J. Anal. Chem. **1993**, *65*, 2243-2248.
17. Wang, Q.C.; Svec, F.; Fréchet, J.M.J. J. Chromatogr. **1994**, *669*, 230-235.
18. Xie, S.; Svec, F.; Fréchet, J.M.J. J. Chromatogr. **1997**,*775*, 65-72.
19. Matsui, J.; Kato, T.; Takeuchi, T.; Suzuki, M.;Yokoyama, K.; Tamiya, E.; Karube, I. Anal. Chem. **1993**, *65*, 2223-2224.
20. Luo, Q.-Z.; Wei, Y.-M.; Liu, T.; Geng, X.-D. Chinese Chemical Letters **1999**, *10*, 215-218.
21. Li, R.; Chen, G.-L.; Lei, J.-D.; Li, B.-Z; Li, H.-R. Chinese J. Chromatogr. **1998**, *27*, 297-300.
22. Unger, K.K. *Packings and Stationary Phase in Chromatographic Techniques*; Marcel Dekker, Inc.: New York, 1990,
23. Horvath, Zs.; Nagydiosi, Gy. J. Inorg. Nucl. Chem. **1968**, *30*, 1899-1906.
24. Rassi, Z.E.; Horvath, Cs. J. Chromatogr. **1986**, *359*, 241-253.
25. Shi, Y.-L.; Geng, X.-D. Chinese J. Anal. Chem. **1994**, *22*, 453-456.
26. Liapis, A.I.; Meyers, J.J.; Crosser, O.K. J. Chromatogr. A **1999**, *865*, 13-25.
27. Xie, S.; Allington, R.W.; Svec, F.; Fréchet, J.M.J. J. Chromatogr. A **1999**, *865*, 169-174.
28. Afeyan, N.B.; Gordon, N.F. Mazsaroff, I.; Varady, L.; Fulton, S.P.; Yang, Y.B.; Regnier, F.E. J. Chromatogr. **1990**, *519*, 1-29.

29. Afeyan, N.B.; Fulton, S.P.; Regnier, F.E. J. Chromatogr. **1991**, *544*, 267-279.
30. Wei, Y.-M.; Huang, X.-D.; Chen, Q.; Geng, X.-D. Chinese J. Anal. Chem. **2000**, *28*, 1194-1198.

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