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Copper(II)–iminodiacetic acid chelating resin as a stationary phase in the immobilized metal ion affinity chromatography of some aromatic amines

YU LIU and SHILIN YU*

Department of Applied Chemistry, Beijing Institute of Chemical Technology, 15 Beisanhuan East Road, Changyang District, Beijing 100029 (China)

ABSTRACT

Styrene–divinylbenzene copolymer 60–80 mesh was used as the matrix and ground to a reasonable particle size in a colloid mill. Small and uniform particles (5–15 μm) can be obtained in a laboratory-made continuous acetone elutriation device. After elutriation the iminodiacetic acid (IDA) chelating resin was synthesized, and the infrared spectrum of the product indicated that the synthetic reaction is effective. The Cu(II)-modified IDA chelating resin was used as a stationary phase in high-performance immobilized metal ion affinity chromatography, packed into a column by the high-pressure slurry-packing technique. Methanol, to which ammonia was added as a competitive ligand, was used as the mobile phase for the separation of aromatic amines. The retention value of aromatic amines depends mainly on their basicity and molecular size. The results showed that some aromatic amines can be separated with the Cu(II)–IDA column.

INTRODUCTION

Ligand-exchange chromatography or metal chelate affinity chromatography, which was introduced by Helfferich¹ and Porath and co-workers^{2,3}, was more recently renamed immobilized metal ion affinity chromatography (IMAC)^{4,5}. It has been widely applied to the analysis of biomolecules such as amino acids, proteins and peptides^{6–10}, and also molecules of small size¹¹.

IMAC is a versatile separation method based on interfacial interactions between sample molecules in solution and metal ions fixed to a chelating ligand which is covalently coupled to a solid support. The simplest chelating ligand is iminodiacetic acid (IDA), bonded to an insoluble matrix of biological (agarose gel)⁴, inorganic (silica gel)^{6,7} or synthetic organic polymers (TSK gel G5000PW)^{8–10}, and relatively stable coordinate compounds with heavy metal ions are formed.

A buffer solution or salt solution is often used as the mobile phase in IMAC. In some instances, modifiers such as chelators, detergents or organic solvents may be added to the buffer to improve the chromatographic selectivity.

Shimomura *et al.*¹² used 200–400 mesh Chelex 100–Ni(II) as the stationary phase for the separation of aliphatic amines using classical column chromatography. In our work, an attempt was made to prepare IDA chelating resin on a styrene–divinylbenzene (S–DVB) copolymer matrix of small particle size (5–15 μm) using a new method. The Cu(II)-modified IDA chelating resin was used as the stationary phase in high-performance IMAC for the separation of aromatic amines.

EXPERIMENTAL

Apparatus

The grinding and elutriation of S–DVB copolymer were carried out in a Model JTM-50 colloid mill and a laboratory-made continuous elutriation device, respectively. The particle size was measured in a Model S-250 III scanning electron microscope. The identification of IDA resin was made by IR spectroscopy (Nicolet Model 60SX-B) and elemental analysis (Carlo Erba Model 1106 analyser). The adsorption properties of IDA resin for metal ions were determined by atomic absorption spectrometry (Model WFD-Y spectrometer). A slurry-packing apparatus (CHEMCO Model 124A) was employed for column packing. The chromatographic separations were carried out on a liquid chromatograph (Shimadzu Model LC-1) equipped with a reciprocating single-piston pump (Model LC-6A) and a UV detector (Model SPD-1, 254 nm).

Grinding and elutriation of S–DVB copolymer

The 60–80-mesh GDX-101 S–DVB copolymer was immersed in aqueous acetone for 30 min, then ground for 5 min twice in a colloid mill (160 V, a.c.). Finally, a suspension was obtained.

According to Stokes' law, particles of different size can be ranged by varying the velocity of a fluid^{13,14}. Using a laboratory-made continuous acetone elutriation device (Fig. 1), narrow-sized fractions of S–DVB copolymer were obtained. As the flow-rate

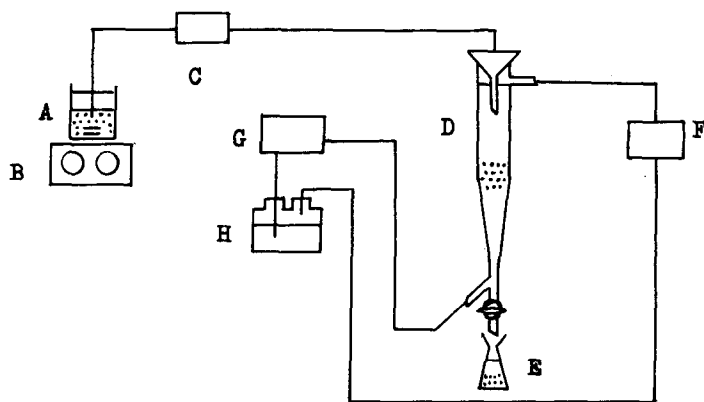


Fig. 1. Apparatus for continuous elutriation. A = Feed supply; B = magnetic stirrer; C = slurry pump; D = elutriation column; E = bottom product receiver; F = filter (overhead product receiver); G = peristaltic pump; H = acetone reservoir.

of acetone was 0.5 ml/min the small and uniform particles (5–15 μm) can be screened out.

Both the grinding and screening steps were examined by scanning electron microscopy.

Synthetic procedures for IDA-S-DVB chelating resin

The 5–15- μm GDX-101 was chloromethylated with chloromethyl methyl ether in the presence of anhydrous zinc(II) chloride. After washing and drying under vacuum, the chloromethylated copolymer (S-DVB- CH_2Cl) was immersed in dimethylformamide (DMF) for 4 h at room temperature, diethanolamine was added with gentle stirring at 110°C on oil-bath and reflux distillation was performed for 7 h. The gel [S-DVB- $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$] was filtered on a Buchner funnel and washed with 5% dilute hydrochloric acid, water and 95% ethanol, sucked dry and then returned to the reaction flask. At 50°C, 33% nitric acid containing a small amount of iron(III) chloride was added to the gel in the reaction flask, with slow stirring, followed by reflux distillation for 4 h at 95°C. The product gel (IDA-S-DVB chelating resin) was cooled, washed thoroughly on a Buchner funnel with water until the washings were neutral, then washed again with 95% ethanol and dried at room temperature.

Preparation of Cu(II)-IDA column

The IDA chelating resin in a methanol-butanol-carbon tetrachloride (5:10:25) slurry was packed into a 250 mm \times 2 mm I.D. stainless-steel column by the high-pressure slurry packing technique.

First the column was washed with methanol, then copper(II) ions were loaded on the column by pumping 0.01 M copper nitrate solution at 0.5 ml/min. The copper nitrate solution was equilibrated with the chelating resin for 4 h. In order to ensure no leakage of metal ion in the experimental work, the column was washed with methanol to removing excess copper(II).

RESULTS AND DISCUSSION

The infrared spectrum of the product [S-DVB- $\text{CH}_2\text{N}(\text{CH}_2\text{COOH})_2$, IDA] (Fig. 2) shows the strongest hydroxyl stretching absorption peak at 3435 cm^{-1} , a strong carbonyl stretching absorption peak at 1700 cm^{-1} , a stronger C-N stretching band around 1170 cm^{-1} and a weak C-O stretching band around 1420 cm^{-1} . Therefore, it was concluded that the synthetic reaction is effective.

The elemental composition of IDA resin is C 59.55, H 7.89, N 2.69 and O 29.87%. The functional group (IDA) content calculated from elemental analysis is 2.11 mmol/g (S-DVB).

The IDA chelating resin can absorb many divalent metal ions. The absorption capacity (at pH 5) for Cu^{2+} , Ni^{2+} , Co^{2+} and Zn^{2+} is 2.00, 1.18, 0.75 and 0.61 mmol/g, respectively. The rate of chelation between IDA resin and metal ions is so rapid that chemical equilibrium, under operating conditions, can almost be attained in 5 min.

The distribution coefficient of Cu^{2+} on the IDA resin increases with increasing pH, but at higher pH (>6), the distribution coefficient decreases rapidly with increasing pH owing to the formation of an amino complex. The ionic strength of the solution does not affect the distribution coefficient because the IDA resin possesses

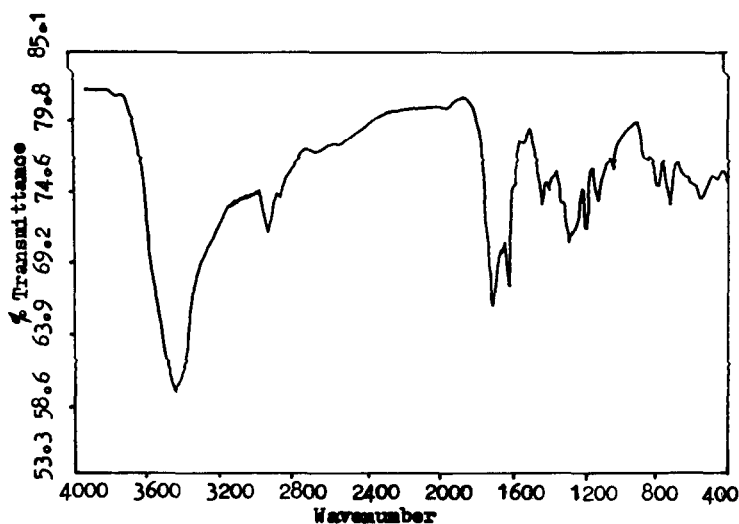


Fig. 2. Infrared spectrum of IDA chelating resin.

a strong chelating tendency towards heavy metal ions. The strong binding of copper ion to the IDA chelating groups minimizes the bleeding of copper ion (about 10^{-8} – 10^{-7} *M*) from the column, and the Cu(II)–IDA column is stable.

The S–DVB matrix cannot be operated under high pressure (> 200 kg/cm²) as the apparent column efficiency decreases. Some aromatic amines appear to have a very high affinity to copper(II) ion, and are difficult to elute by using methanol as the mobile phase. We therefore added ammonia to the mobile phase as a competitive ligand, and examined the effect of the ammonia ligand concentration on the capacity factor of sample ($k'\alpha[\text{NH}_3]^{-1}$, Table I).

The retention values of aromatic amine depend mainly on their basicity¹⁵. As the affinity action of amines for copper(II) ion increases with increasing basicity of the

TABLE I
CAPACITY FACTORS (k') OF AROMATIC AMINES ON THE CU(II)–IDA COLUMN

Compound	Mobile phase	
	Methanol	Methanol + 0.3 mM NH ₃
Aniline	3.98	2.10
N-Methylaniline	1.84	1.28
N,N-Dimethylaniline	0.62	0.30
2,4-Dimethylaniline	4.24	2.51
<i>o</i> -Nitroaniline	4.15	2.44
<i>m</i> -Nitroaniline	5.67	4.23
<i>p</i> -Nitroaniline	5.77	4.36
Pyridine	13.50	6.64
8-Hydroxyquinoline	6.31	4.98

amines, the retention value increases. On the other hand, steric hindrance will also affect the affinity action, and the retention value depends on the above two factors.

CONCLUSION

The results indicate that the Cu(II)-IDA chelating resin column has advantageous properties for use in IMAC, and may be applied to the analysis of aromatic amines. If the high-pressure resistance of the S-DVB matrix could be improved by the synthesis of highly cross-linked S-DVB copolymer, an effective column for use under high pressures could be developed. Such a column should be useful for the separation and analysis of biomacromolecular samples.

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