Fresenius J Anal Chem (1991) 339:877–881

Fresenius' Journal of Analytical Chemistry

Synthesis of chelating resins and its application in ligand exchange chromatography

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Summary. Three kinds of new chelating resin containing β-hydroxydithiocinnamic acid, ethyl β-hydroxydithiocinnamate and N-(hydroxymethyl)-thioamide functional groups were synthesized. Cu(II), Cd(II) and Hg(II) were used as complex forming metal ions. By varying the combination of metal ion and chelating resin, a great variety of separation systems could be realized. For the on-line trace enrichment of 2-mercaptobenzimidazole, the copper-loaded N-(hydroxymethyl)-thioamide concentration column was found most suitable. UV detection at 300 nm was used. The detection limit was 0.2 ng/g. A 10 ml sample was found to be sufficient to attain the described sensitivity, and linear relationship was obtained in the range of 0.75 ng/g to 12 ng/g. The best separation of the thiol compound mixture thioglycolic acid, methionine, cysteine and 2-mercaptoethanol was obtained with copper-loaded ethyl β-hydroxydithiocinnamate resin; UV absorbance detection was at 215 nm.

Introduction

Ligand exchange chromatography (LEC) was first suggested by Helfferich in 1961 as a novel separation technique [1]. LEC differs from ion exchange, adsorption and other types of chromatography in its basic process of interaction between the sorbate and stationary phases. It is a process in which interaction between the stationary phase and the molecules to be separated occurs during the formation of coordination bonds inside the coordination sphere of the complex-forming ion [2]. Hence, the ability of compounds to form complexes with metals is an essential condition for the separation of compounds by LEC. The major requirement for complex-forming ions in LEC is the formation of kinetically labile sorption complexes. Theoretically, any sorbent that can be used for the selective sorption of transition metals is suitable for LEC. However, chelating resins were proved to be better than others. Unfortunately, among the numerous chelating sorbents synthesized so far, only resins with iminodiacetate ligands and resins with α-amino acid groups have been widely used in LEC. In the present research, some chelating resins were synthesized. These were complexed with metal ions and used as the stationary phase for ligand exchange chromatography for the selective on-line trace enrichment of 2-mercaptobenzimidazole and for the separation of some sulfur-containing compounds.

Experimental

Apparatus

A LCD/Milton Roy (Riviera Beach, FL, USA) LC system consisting of Constametric I and III pumps, model 1601 Gradient Master was used in combination with a Spectro Monitor D UV-VIS detector, a Rheodyne (Berkeley, CA, USA) model 7125 injection valve and model 7000 switching valve. A schematic diagram of the main components is shown in Fig. 1.

Breakthrough curves and chromatograms were recorded on a CI-10B integrator (LDC/Milton Roy) or Yokogawa two-pen recorder and processed manually.

Chemicals

Most chemicals were of analytical reagent grade and were purchased from E. Merck, Darmstadt, Germany. 2-Mercaptobenzimidazole was supplied by Aldrich Chemical Co., USA. The thiol compounds cysteine, 2-mercaptoethanol, methionine and thioglycolic acid were supplied by Sigma, USA.

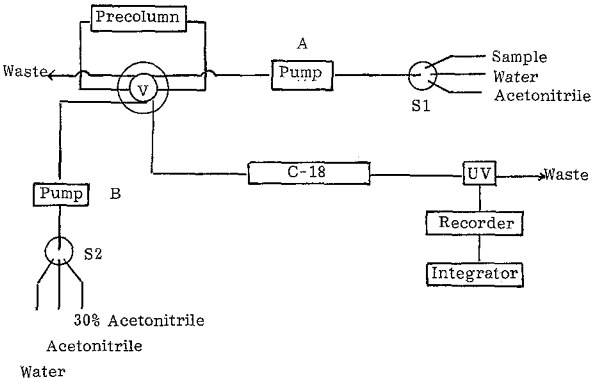

Fig. 1. Schematic diagram of the on-line trace enrichment of 2-mercaptobenzimidazole. S1, S2: low-pressure solvent selection switching valve. V: high-pressure switching valve. Precolumn: 20 × 2 mm I. D., Cu-HMTA resin. Analytical column: 250 × 4.6 mm I. D. packed with 10 μm LiChrosorb RP-18

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Preparation of the sorbents

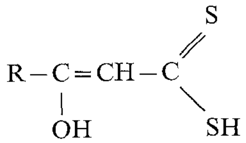
Three kinds of chelating resin containing β-hydroxydithiocinnamic acid (β-HDCA), ethyl β-hydroxydithiocinnamate (β-HDCAE) and N-(hydroxymethyl)thioamide (HMTA) were synthesized by the following procedures.

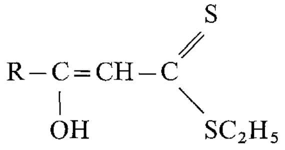
I. Synthesis of the chelating resin containing β-hydroxydithiocinnamic acid [3]. The polystyrene-divinylbenzene, XAD-4 (Rohm and Haas, Philadelphia, PA, USA) macroreticular resin was used as starting material. To 10 g of XAD-4 resin, 90 g of anhydrous aluminium trichloride and 50 ml of petroleum ether (100° – 140° C) were added. 50 ml of 1:1 petroleum ether/acetic anhydride were added over a period of 30 min through an addition funnel, and the resulting mixture was refluxed at 83° C for 5 h. This product was cooled to room temperature and hydrolyzed in 12 mol/l hydrochloric acid and ice. It was then collected by suction filtration and washed sequentially with 15 mol/l ammonium hydroxide, 12 mol/l hydrochloric acid, water and acetone. These procedures were repeated to obtain a higher yield. The product was then slurried in 50 ml of petroleum ether to which 20 g of potassium tert.-butoxide and 40 ml of carbon disulfide were added. The mixture was allowed to react at 43° C for 5 h. The final product was collected by suction filtration and washed sequentially with 2.5 mol/l sulfuric acid, water and acetone.

II. Synthesis of the chelating resin containing ethyl β-hydroxydithiocinnamate [4]. Forty ml of tetrabutylammonium hydroxide was mixed with 50 ml of dioxane. Then 2 ml of ethyl iodide was added. After stirring for 5 min, 10 g of β-hydroxydithiocinnamic acid resin, prepared by the above procedures, was added. The mixture was reacted at 45° C for 5 h. The product was collected by filtration under suction and washed sequentially with water, then acetone.

III. Synthesis of the chelating resin containing N-(hydroxymethyl)-thioamide [5]. A macroporous cross-linked copolymer was prepared by the reaction of acrylonitrile and divinylbenzene by the procedure given in ref. [6]. A 1 l, round-bottomed flask equipped with a mechanical stirrer, reflux condenser, thermometer and an inlet tube for nitrogen gas was charged with 450 ml of dioxane; then 25 ml of conc. sulfuric acid and 20 g of thioacetamide were added. After stirring under nitrogen for 10 min, 30 g of the above product (acrylonitrile-divinylbenzene copolymer) was added. The mixture was reacted at 90° C for 48 h. The product was collected by filtration under suction and washed sequentially with water, methanol and acetone. It was then mixed with 220 ml formaldehyde. Then 6.5 ml of 2 mol/l sodium hydroxide were added dropwise, and the mixture was left to react at 45° C for 24 h. The product was collected by suction filtration and washed sequentially with methanol, acetone and water. The result was immersed in 500 ml of 0.1 mol/l sodium chloride for 24 h, and was finally washed free of chloride with water.

The structure of these sorbents is the following:

I.


II.


III. $R^1-CSNHCH_2OH$

Where: R: Styrene-divinylbenzene copolymer
R^1: Acrylonitrile-divinylbenzene copolymer

Preparation of the metal-loaded sorbents. Two g of the synthesized chelating resin was suspended in 85 ml of 0.1 mol/l Cu(II), Cd(II) or Hg(II) solution, respectively, and 15 ml 1 mol/l, pH 5 acetic acid-sodium acetate buffer; each mixture was mechanically shaken for 4 h. The metal-loaded phases were collected on glass filters and washed with 100 ml of LC grade water and dried for 2 h at 100° C.

The capacity of the metal-loaded sorbents was calculated by determining the amount of metal ion remaining in solutions by the procedures in complexometric titration with EDTA [7].

Procedures

Trace enrichment was carried out on 20 × 2 mm I. D. home made precolumns which were hand-packed with the metal-loaded stationary phase with a microspatula, using a thick slurry of the packing material in methanol. The stainless steel analytical column was a 250 × 4.6 mm I. D. packed with the synthesized 100 – 200 mesh β-hydroxydithiocinnamate or N-(hydroxymethyl)thioamide resin or 10 µm LiChrosorb RP-18 (E. Merck, Darmstadt, Germany).

Stock solutions of the model compounds were prepared by weighing, and dissolving in methanol. The solutions were diluted with methanol-water mixtures to obtain standard solutions at the ng/g level. Solutions of 2-mercaptobenzimidazole were freshly prepared each day, degassed in an ultrasonic bath, purged with nitrogen and stored in the dark.

Breakthrough studies were carried out in triplicate using 60 ng/g standard solutions of the model compound 2-mercaptobenzimidazole at a flow rate of 0.5 ml min^{-1}.

Chromatograms were generally run at ambient temperature with a flow rate of 1 ml min^{-1}. All mobile phases were degassed ultrasonically before use. Columns were considered to be equilibrated when a stable baseline was obtained, and when two successive injections of sample yielded the same retention time.

Results and discussion

Characterization

The structures of the synthesized resin were characterized after each step of the synthesis by infrared spectra. The composition of the synthesized chelating resin was also confirmed by elementary analysis. The sulfur content corresponded to 0.26 mmol of β-hydroxydithiocinnamic acid, 0.18 mmol of ethyl β-hydroxydithiocinnamate and 0.43 mmol of N-(hydroxymethyl)thioamide groups per gram of the resin. Some physical and chemical properties of the synthesized resin, such as the water regain, sulfur content, dis-

Table 1. Physical and chemical properties of various resins

	β-HDCA	β-HDCAE	HMTA
Particle size (mesh)	100–200	100–200	100–200
Water regain (g/g)	0.77	–	0.57
Sulfur content (mmol/g)	0.26	0.18	0.43
pK_a	6.54	–	6.56
Cd(II) Capacity (mmol/g)	0.02	0.01	0.20
Cu(II) Capacity (mmol/g)	0.05	0.03	0.30
Hg(II) Capacity (mmol/g)	0.59	0.45	1.10
$\log K_f$ [Cd(II)]	1.10	–	3.7
$\log K_f$ [Cu(II)]	2.44	–	3.9

sociation constant, metal capacity and formation constant of the synthesized resins have been determined, as noted in previous papers [3–5, 8]. The results are summarized in Table 1. They show that the N-(hydroxymethyl)-thioamide resin provided, in nearly all cases, the highest capacity. All of these resins were used as metal-loaded sorbents in ligand exchange chromatography for the on-line trace enrichment or separation of some sulfur-containing compounds.

The model compound of the trace enrichment experiment was 2-mercaptobenzimidazole. This imidazole is a corrosion inhibitor for several metals and an antioxidant in tyres and rubber products. However, it may be a carcinogen, and a method is urgently needed for its selective determination in industrial waste water and surface water. Brinkman et al. [9, 10] used precolumns packed with Hg(II)-loaded phases for the selective, on-line trace enrichment of 2-mercaptobenzimidazole. The metal-loaded sorbents they used were the commercially available resins Spheron thiol and Spheron oxine. Excellent selectivity was demonstrated at low ng/g levels. However, Brinkman et al. showed that there were still various experimental problems in retention, elution and regeneration of the metal-loaded precolumns. In this investigation, we used N-(hydroxymethyl)thioamide resin as metal-loaded sorbent for the trace enrichment experiment. The metal ions chosen were cadmium, copper and mercury.

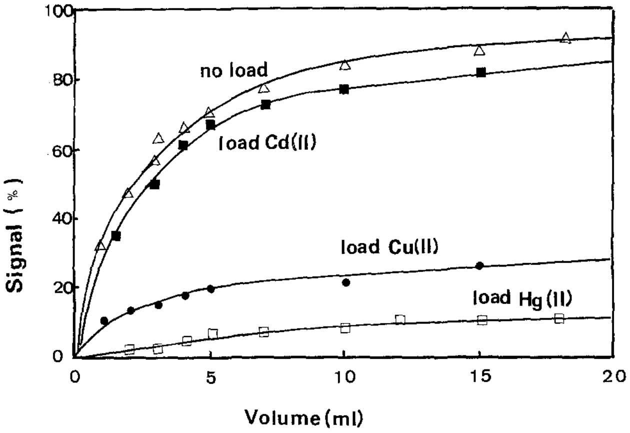


Fig. 2. Breakthrough curves of 2-mercaptobenzimidazole on metal-loaded HMTA resin. Concentration: 4×10^{-7} mol/l; flow rate: 0.5 ml min^{-1}

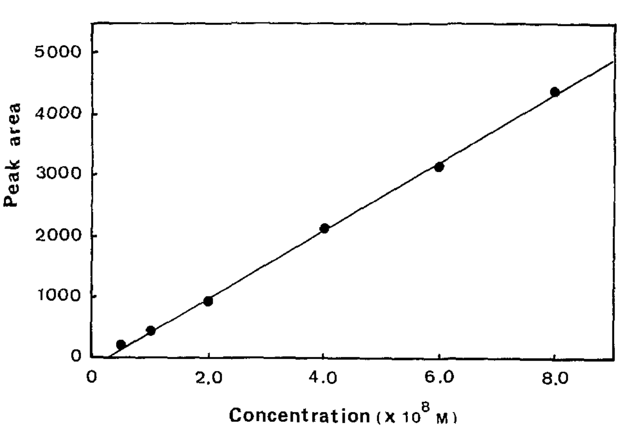


Fig. 3. Determination of 2-mercaptobenzimidazole by on-line concentration. Precolumn: Cu-HMTA; analytical column: C-18; flow rate: 1 ml min^{-1}; mobile phase: $CH_3CN:H_2O$ = 3:7

Table 2. Analytical data for the selective on-line trace enrichement of 2-mercaptobenzimidazole

Sample volume	10 ml
Concentration rate	0.5 ml min^{-1}
Capacity factor	0.47
Average recovery (n = 5)	95% (8 ng/g)
Linearity (n = 5)	0.75–12 ppb (r = 0.9992)
Detection limit	S/N = 3, 0.2 ng/g

From the studies for 2-mercaptobenzimidazole, it was found that the breakthrough capacity was dependent on several factors such as the dimension of the column, the flow conditions, the size of the resin, and the capacity of the resin etc. The breakthrough curves are shown in Fig. 2 at a flow rate of 0.5 ml min^{-1}. The order of the breakthrough capacity was HMTA-Hg > HMTA-Cu > HMTA-Cd > HMTA, where the unmodified HMTA column showed poor breakthrough capacity and the HMTA-Hg column gave satisfactory results, compared with any other HMTA-metal column. Therefore, the HMTA-Hg phase would be the best precolumn for on-line concentration of 2-mercaptobenzimidazole. However, it was found that the 1:1 acetonitrile-water mixture could not completely elute the concentrated sample from the column; a stronger eluent, such as cysteine or any other thiol-containing ligand must be used. Although the concentration factor would be higher in this case, the precolumn could not be used continuously. For this investigation, the HMTA-Cu phase was chosen as the precolumn for the on-line concentration of 10 ml of 2-mercaptobenzimidazole sample solution and LiChrosorb C-18 as the analytical column. The mobile phase was water-acetonitrile (7:3). The linearity of this method was between 0.75–12 ng/g as shown in Fig. 3, and the detection limit of S/N = 3 was 0.2 ng/g as shown in Table 2. The concentration factor was 100, in comparison with that without precolumn of which the detection limit was 20 ng/g (Fig. 4). The mercury loaded β-HDCA and β-HDCAE resins were also tested as precolumns for the concentration of 2-mercaptobenzimidazole. However, the results showed that these columns could only be used as 2-mercaptobenzimidazole remover and not properly as precolumn for it, since the proper eluent could not be easily obtained for the high capacity of these precolumns.

Table 3. Distribution coefficients for thiols on various copper(II)-loaded resins[a]

Resin	Solvent	Thiol compound				
		Thioglycolic acid	Cysteine	2-Mercapto-ethanol	Methionine	2-Mercapto benzimidazole
β-HDCA	0.1 mol/l NaCl	793			903	
	30% CH_3OH	1386	<1	838		2071
	0.02 mol/l KH_2PO_4	892				
	0.02 mol/l KH_2PO_4 – 0.02 mol/l Citric acid	<1	<1	<1		
	0.01 mol/l KH_2PO_4 – CH_3CN	637		574	601	
β-HDCAE	0.1 mol/l NaCl		22	10	7	
	30% CH_3OH	12	16	22		
	0.02 mol/l KH_2PO_4	6	8	25	19	
	0.02 mol/l KH_2PO_4 – 0.02 mol/l Citric Acid	<1	6	21		
	0.02 mol/l KH_2PO_4 – CH_3CN	3	5	24	6	
HMTA	0.1 mol/l NaCl		572	603	642	
	30% CH_3OH	574	445	542		1978
	0.02 mol/l KH_2PO_4	638		589		

[a] Amount of resin: 0.3 g; volume of solution: 25 ml

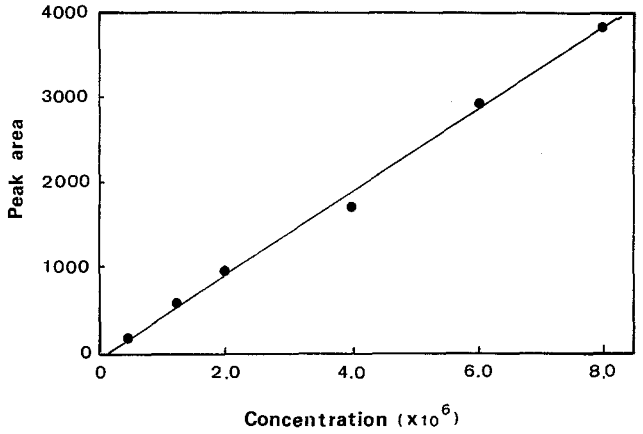


Fig. 4. Determination of 2-mercaptobenzimidazole by direct injection. Analytical column: C-18; sample volume: 20 μl; flow rate: 1 ml min^{-1}; mobile phase: $CH_3CN:H_2O = 3:7$

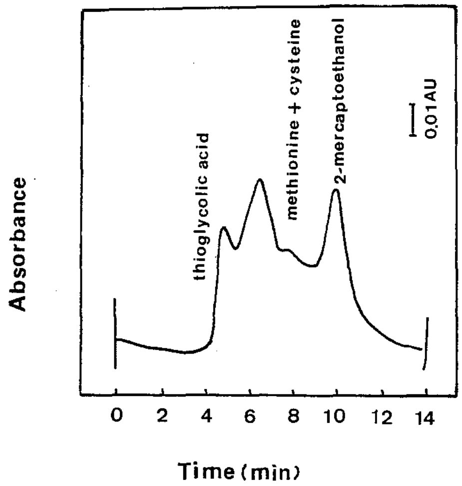


Fig. 5. Separation of thiol mixtures. Stationary phase: Cu-β-HDCAE. Sample: 0.2 μg 2-mercaptoethanol, thioglycolic acid, cysteine and methionine, respectively. Mobile phase: 0.02 mol/l KH_2PO_4-CH_3CN (7:3) (v/v). Flow rate: 0.5 ml min^{-1}. Detection: 0.2 AUFS, 215 nm

In the separation of some thiol compounds, β-HDCA, β-HDCAE and HMTA resins were used as copper-loaded sorbents. The thiol compounds investigated were thioglycolic acid, cysteine, 2-mercaptoethanol, methionine and 2-mercaptobenzimidazole. The distribution coefficients for these thiols on various copper-loaded resins in various media are shown in Table 3. The results revealed that all the distribution coefficients for these thiols on copper-loaded β-HDCAE are very small as compared with those on copper-loaded β-HDCA and copper-loaded HMTA phases. This phenomenon may be due to the lowest metal capacity of the β-HDCAE resin among them as shown in Table 1. Both the distribution coefficients for 2-mercaptobenzimidazole on copper-loaded β-HDCA and on copper-loaded HMTA phase are large enough and are more than 10^3. The results indicated that these two stationary phases could be used satisfactorily for the concentration and determination of 2-mercaptobenzimidazole. The results coincide with those of the previous trace enrichment experiment. They also showed that methanol-water mixture, sodium chloride, potassium dihydrogen phosphate or its citric mixture were not suitable for the separation of these thiols. However, the potassium dihydrogen phosphate-acetonitrile mixture was apparently quite suitable for separating these thiols at the copper-loaded β-HDCAE stationary phase. Various ratios of acetonitrile to 0.02 mol/l potassium dihydrogen phosphate were tested. The separation of thiol mixtures with 7:3 and 4:1 0.02 mol/l KH_2PO_4-CH_3CN mixture are shown in Figs. 5 and 6. The ratio of one to four was found to be most appropriate. However, cysteine and methionine could not be separated

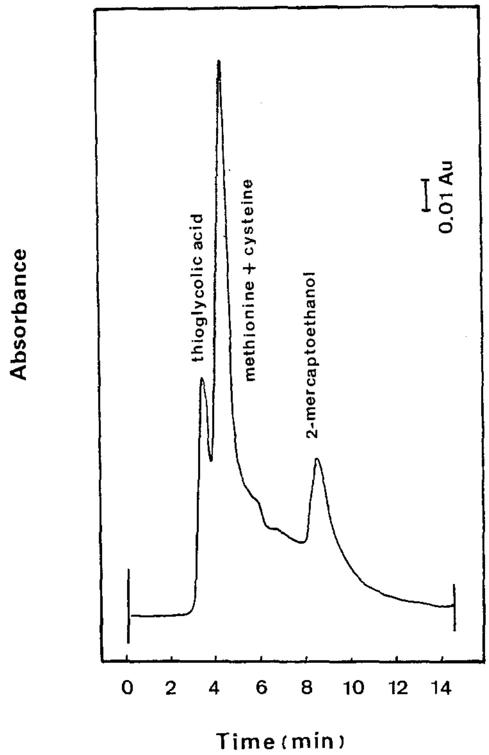


Fig. 6. Separation of thiol mixtures. Stationary phase: Cu-β-HDCAE. Sample: 0.2 µg 2-mercaptoethanol, thioglycolic acid, cysteine and methionine, respectively. Mobile phase: 0.02 mol/l KH_2PO_4-CH_3CN (4:1) (v/v). Flow rate: 0.5 ml min^{-1}. Detection: 0.2 AUFS, 215 nm

by this system; they could however be well separated by the copper-loaded β-HDCAE phase using merely 0.02 mol/l KH_2PO_4 as mobile phase as shown in Fig. 7.

Conclusion

The results showed that copper loaded β-HDCAE resin was the best stationary phase for the separation of thiol compounds. Since the β-HDCAE sorbent showed stronger hydrophobic properties than the other two sorbents, β-HDCA and HMTA, the result is rational. For the trace enrichment of 2-mercaptobenzimidazole, the copper loaded HMTA resin showed superior properties among the tested systems. Though the mercury loaded HMTA, β-HDCA and β-HDCAE resins also showed high breakthrough capacity, a simple eluent system could not easily be obtained. However, they could be used as sulfide compound removers, both in the gaseous or liquid phases of industrial waste.

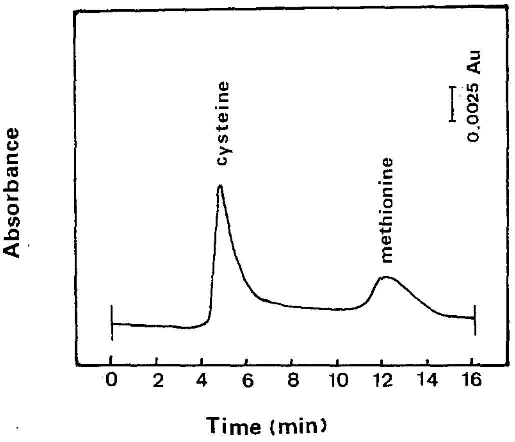


Fig. 7. Separation of thiol mixtures. Stationary phase: Cu-β-HDCAE. Sample: 0.2 µg cysteine and methionine, respectively. Mobile phase: 0.02 mol/l KH_2PO_4. Flow rate: 0.4 ml min^{-1}. Detection: 0.05 AUFS, 215 nm

Acknowledgements. The financial support for this study by the National Science Council of the Republic of China is gratefully acknowledged.

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Received September 15, 1990