Combination of chelating behaviour with molecular recognition as interaction for ligand exchange chromatography

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Abstract. A novel chelating resin was synthesized, composed of chemically bonded glycinocyclodextrin (S-CD-G) to which silica gel was added as matrix and silanes with amino groups as spacer. It showed efficient complexation with transition metal cations. In this report the copper and zinc resin complexes were investigated for application as stationary phases for the separation of substituted phenol isomers. The results revealed that the copper resin complex has a high potential for this application.

Introduction

Ligand-exchange chromatography (LEC) is a well established method for separating complex-forming organic compounds. Much work has already been published on the separation of substituted phenols via LEC [1]. However, the separation of phenols on the basis of the combination of chelating behaviour and molecular recognition has so far not been dealt with. Since the glycine moiety has been used as mobile phase in ligand exchange mode for the resolution of underivatized amino acids [2–4], silica gel containing glycine as pendent group attached to the cyclodextrin (CD) cavity, i.e. glycinocyclodextrin resin, has been synthesized. Furthermore, the metal-resin complexes have also been investigated to evaluate their possible use as stationary phases for the LEC separation of phenolic compounds.

Experimental

Apparatus. The IR-spectra of the resin and its metal complexes in KBr were recorded by an infrared spectrophotometer (Perkin Elmer 983). A HPLC system (LDC/Milton Roy, USA) consisting of Constametric I and III pumps, Gradient Master (model 1601), a vari-

able-wavelength detector (LDC, SpectroMonitor D), a Rheodyne injection valve (model 7125) and an integrator (Hitachi, D-2500) were used. A Radiometer pH-meter (Copenhagen, Denmark, PHM 61) was used to measure the pH-values.

Chemicals. Most chemicals were of analytical reagent grade (E. Merck, Germany). 3-Aminopropyltriethoxysilane and phenolic compounds were supplied by Aldrich (Milwaukee, USA). Silica gel 60 G (E. Merck) was grinded to a particle size of 325–400 mesh.

Preparation of the sorbents. 3-Aminopropyltriethoxysilane (5 mL) was reacted with chloroform-washed silica gel (4 g) in toluene (100 mL) and with triethylamine (0.5 mL) as a catalyst at 75 °C for 3 h. The product(S) was washed sequentially with isopropanol, water and acetone. Tosyl chloride (4 g) in acetonitrile was dropwise added with vigorous shaking to an alkaline β -CD (4 g) solution (0.15 mol/L, 100 mL). The mixture was reacted at room temperature for 1 h at pH 12. The reaction was terminated by addition of hydrochloric acid (1 mol/L). After filtration, the product was passed through the cation-exchanger and the effluent was vacuum evaporated. The concentrated material was chiefly β -CD tosylate. 6 g of it were added to product S (3 g) in pyridine (150 mL). The mixture was reacted at 75 °C for 40 h. The resulting product was washed successively with pyridine, acetone and methanol and then dried overnight under vacuum at 50 °C. The product was S-CD. Glycylethyl ester hydrochloride salt (6 g) was added to a stirred solution of equal amounts of sodium hydroxide. The mixture was extracted with dichloromethane. S-CD (3 g) was added to the extract and reacted at 80 °C for 72 h. The product was washed with water and acetone and dried in air. The product was termed S-CD-G.

Results and discussion

Characterization of stationary phases. The tosylation step for the synthesis of the resin was followed according to Iwakura's method [5]. The binding sites of CD to the silanized silica gel might be the secondary hydroxyl

Table 1. Capacity factors of selected substituted phenol isomers on S-CD-G-Zn column with varied compositions of the mobile phase^a

Sample	Capacity factors				
	35/65 ^b	45/55 ^b	50/50 ^b	30/70 ^c	30/10/60 ^d
Phenol	0.86	0.14	0.06	0.43	0.66
2-Chlorophenol	1.55	0.14	0.06	0.95	0.93
2,4-Dichlorophenol	4.45	0.96	0.49	2.98	3.48
2-Nitrophenol	6.25	2.34	1.65	2.98	3.48
4-Nitrophenol	12.90	4.42	2.92	5.46	6.42

^a Flow rate: 0.5 mL min⁻¹^b Ratio of CH₃OH to H₂O^c Ratio of CH₃CN to H₂O^d Ratio of CH₃OH to CH₃CN to H₂O**Table 2.** Capacity factors of selected substituted phenol isomers on the S-CD-G-Cu column with varied compositions of the mobile phase^a

Sample	Capacity factors				
	40/60 ^b	50/50 ^b	63/35 ^b	30/70 ^c	30/10/60 ^d
Phenol	0.58	0.43	0.35	0.71	0.35
2-Chlorophenol	1.23	0.72	0.35	1.13	0.68
2,4-Dichlorophenol	2.78	1.86	0.88	2.71	1.97
2-Nitrophenol	4.65	3.14	2.27	3.64	3.34
4-Nitrophenol	9.25	6.92	4.29	7.43	6.92

^a Flow rate: 0.5 mL min⁻¹^b Ratio of CH₃OH to H₂O^c Ratio of CH₃CN to H₂O^d Ratio of CH₃OH to CH₃CN to H₂O

groups and glycine might be attached to the CD via the primary hydroxyl groups. The product in acid solution develops a darkish green colour when reacted with chromium trioxide. This result gives evidence to the bonding of CD in silica gel. After each step of synthesis, elemental analysis of the product was carried out in order to assess the extent of the bonding reaction. The functionality of glycine to the final product was 0.4 mmol g⁻¹. The adsorption capacities of the resin for Co(II), Cu(II), Ni(II) and Zn(II) in pH 5.2 acetate buffer were 0.15, 0.24, 0.21 and 0.29 mmol g⁻¹, respectively.

Specific evidence of the covalently bonded CD group to silica gel is provided by the infrared absorption bands of the C-H stretching, 2900 cm⁻¹, and to the glycine group by the absorption bands of C=O, 1750 cm⁻¹. In the metal-resin complexes, vibrational evidence for the coordination of the glycine groups concerning ν (C-O) and ν (N-H) is obvious. Furthermore, coordination causes these lines to move to smaller wave numbers and a new absorption line appears (1550 cm⁻¹) in addition to that at 619 cm⁻¹ for the copper-resin complex and 1440 cm⁻¹ for the cobalt-resin complex.

The pK_a-value of the resin and the stability constants of the metal-resin complexes were determined as described in the previous paper [6]. The pK_a-value of the resin ligand is 11.2. The result is rational [7], as there is an electron-withdrawing group, -C=O, besides the protonated amine. The values of the stability constants of

Fig. 1A–C. Separation of phenolic compounds. Stationary phase (10 × 4.6 mm i.d.): **A:** S-CD-G-Zn; **B** and **C:** S-CD-G-Cu. Mobile phase: **A** and **B:** 50% H₂O, 50% CH₃OH. **C:** 60% H₂O, 30% CH₃OH, 10% CH₃CN. Detection: UV (λ = 270 nm). Peaks: **A:** 1, 2 phenol + 2-chlorophenol; 3, 2,4-dichlorophenol; 4, 2-nitrophenol; 5, 4-nitrophenol; **B** and **C:** 1, phenol; 2, 2-chlorophenol; 3, 2,4-dichlorophenol; 4, 2-nitrophenol; 5, 4-nitrophenol**Table 3.** Summary of results for the determination of substituted phenols

Sample	Linear equation ^a
Phenol	$y = 0.60X + 1.22 \times 10^3$ ($r = 0.9954$)
2-Chlorophenol	$y = 0.32X + 1.98 \times 10^2$ ($r = 0.9997$)
2,4-Dichlorophenol	$y = 0.12X + 1.08 \times 10^2$ ($r = 0.9991$)
2-Nitrophenol	$y = 0.50X + 2.65 \times 10^1$ ($r = 0.9980$)
4-Nitrophenol	$y = 0.09X - 6.60 \times 10^1$ ($r = 0.9978$)

^a Dynamic range: 0–2.4 × 10⁻⁴ mol/L

copper and zinc resin complexes are 8.58 and 7.00, respectively, which are somewhat smaller than those of the monomeric copper- or zinc-glycine complexes [8]. This phenomenon might possibly be due to a steric hindrance in the resin matrix.

Analytical application. The metal (copper or zinc)-resin complexes prepared as described in the previous paper [9] were used as stationary phases for the separation of phenolic compounds. Preliminary tests revealed that the five substituted phenols examined were not separated and coeluted from the underivatized S-CD column with mobile phases of CH₃CN/H₂O (30/70), CH₃OH/H₂O (50/50) or CH₃OH/H₂O (35/65). However these compounds apparently yield a better resolution on a S-CD-G-metal complex column. Methanol and acetonitrile were used as organic modifiers in the mobile phases. The elution order of the phenols on both S-CD-G-Cu and S-CD-G-Zn columns with methanol-water is the same and the resolution was found to be better than with acetonitrile. An increased methanol content resulted in both a decreasing retention and separation efficiency (Tables 1 and 2). The combination of methanol with acetonitrile as

a modifier brings about an increase of selectivity (Fig. 1). Since the separation mode was via ligand exchange, the stability constants of the metal complexes can be considered as a measure of how stable a solute with the central metal ion is. However, the elution order of the phenolic compounds is different from the predicted order. The exceptions were also examined by Petronio et al. in the separation of phenolic compounds with the Chelex-100 iron complex as stationary phase [3]. According to Takayanagi's report, the stability of the inclusion complexes with β -CD decreases in the following order: 4-nitrophenol $\gg$ 4-chlorophenol $\geq$ 2-chlorophenol $\geq$ 2-nitrophenol [10]. The result is consistent with the prediction. Carrying out the separation in this study was possible, despite the fact that the difference in the stability of the other three compounds with β -CD is not so large [10]. As discussed above, the interaction of both ligand exchange and inclusion was assumed to improve the selectivity of the separation of phenolic compounds considerably and it is better than that of only the inclusion process or ligand exchange. The results also showed that a more efficient separation was achieved when the S-CD-G-Cu column was used instead of the S-CD-G-Zn column (Fig. 1).

Generally, methods for the determination of phenols in water are based on spectrophotometry [11]. However, this technique does not equally respond to all phenols, and the results for total phenols may be significantly erroneous. With the above LEC system, we were able to separate the phenols and reach detection limits of

0.2 nmol abs. per 20 μ L injection. The calibration graphs are summarized in Table 3.

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