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Retention Properties of Poly(carboxylic acid) Resin Column for High-Performance Liquid Chromatography

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Maleic anhydride was copolymerized with divinylbenzene by suspension polymerization, and the spherical beads obtained (10—30 μm in diameter) were then hydrolyzed to a carboxylic acid (CA) resin. This CA resin had a low exchange capacity (0.40 meq/g), and was proposed to be useful as a packing material for high-performance liquid chromatography. The retention behavior of various compounds on a column packed with the CA resin was studied using methanol or a mixture of acetonitrile and buffer solution as the mobile phase. The acid–base and hydrophobic interactions, which occur because of the ionogenic and hydrophobic moieties of the CA resin, work effectively for the separation of the solutes. The CA resin column, with the appropriate selection of a mobile phase, is suggested to be useful for the separation of various kinds of solutes such as neutral, acidic and basic compounds.

Keywords poly(carboxylic acid)resin; high-performance liquid chromatography; maleic anhydride; suspension polymerization; polymer bead; divinylbenzene

Maleic anhydride (MA) is easily copolymerized with many monomers, the anhydride moieties introduced in the copolymers are expected to have high reactivity with many compounds, and the various derivatives of the copolymer are expected to have a wide range of applications. Copolymers of maleic anhydride and other monomers have been prepared as fine powders and have been successfully used for the immobilization of enzymes(1) and drug carriers.(2) Cross-linked spherical beads of maleic anhydride copolymer have been prepared by suspension polymerization and used for the immobilization of urease(3) and selective metal sorption.(4)

Herein, we report the applicability of spherical poly(carboxylic acid) resin (CA resin) with particles 10—30 μm in diameter as packing material in high-performance liquid chromatography (HPLC). Both the ionogenic and hydrophobic moieties of the CA resin are expected to work effectively for the separation of many solutes. The retention behaviors of hydrocarbons, carboxylic acids, phenols, and substituted aromatic compounds were studied.

Experimental

Chemicals Divinylbenzene (DVB) solution (nominally containing about 55% of DVB, Wako Pure Chemical Industries, Ltd., Osaka) was washed with 2 N sodium hydroxide and water to remove the polymerization inhibitor. The monomer was dried over anhydrous calcium chloride. The MA, benzoyl peroxide, sodium chloride and glycerol used were of a reagent grade (Wako Pure Chemical Industries, Ltd., Osaka). Hydroxyethyl cellulose (reagent grade, Tokyo Kasei Kogyo Co., Ltd., Tokyo) was dried *in vacuo* for 2 h at 100 °C. Benzene and dioxane were distilled before use. Hitachi gel 3010 (H-3010), a macroporous hydrophobic styrene–DVB copolymer (20 μm particle size), was obtained from Hitachi.

Apparatus Infrared (IR) spectra were taken on a JASCO IR-810 spectrometer in a KBr disk. A JASCO 880-PU Intelligent Pump was used together with a JASCO UV-970 Intelligent UV/VIS Detector.

Preparation of Spherical MA–DVB Resin Polymerization was carried out in a round-bottomed, three-necked flask under a nitrogen atmosphere. A mixture of 40.5 g of MA, 36 ml of DVB, 36 ml of benzene, 36 ml of dioxane, and 0.72 g of benzoyl peroxide was added to a suspending medium of 750 ml of glycerol containing hydroxyethyl cellulose (1.5 g) and sodium chloride (37.5 g). The organic phase was first dispersed in the suspending solution at 35 °C, then the bath temperature was gradually raised to 70 °C, at a rate of 1 °C/2 min, and stirred for 6 h. The resulting beads were immersed in water, washed with water, methanol, and benzene and dried *in vacuo* at 60 °C. About 20 g of white spherical beads (10—30 μm in diameter, classified by a continuous solvent elutriation method(5) with methanol) were obtained.

Preparation of CA Resin MA–DVB resin (7 g) was refluxed with 50 ml of 2 N sodium hydroxide and then washed with 2 N hydrochloric acid and water and dried *in vacuo* at 60 °C.

Chromatography The columns (25 × 0.4 cm i.d.) were made of stainless-steel tubing packed with a methanol slurry of CA resin and H-3010. The solutes were dissolved in methanol or acetonitrile at a concentration of approximately 1 mg/ml and 2—5 μl of the solution was injected with a 10 μl syringe. Unless otherwise stated, methanol or acetonitrile–water (1 : 1) containing 0.05 M phosphate buffer was used as the eluent at a flow rate of 0.8, or 1 ml/min. The dead volume of the column was determined by using sodium nitrate, which might not be retained.

Results and Discussion

Preparation of MA–DVB and CA Resins MA–DVB resin was prepared by using a glycerol solution as the suspending medium for the monomer phase. Continuous glycerol phase to dispersed monomer phase, at a ratio of about 5 : 1, was used for suspension polymerization to

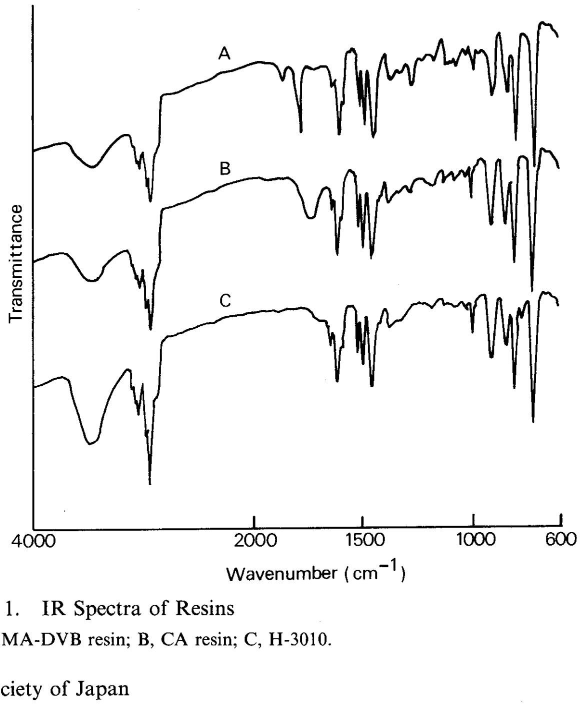

Fig. 1. IR Spectra of Resins

A, MA-DVB resin; B, CA resin; C, H-3010.

Table I. Height Equivalent to a Theoretical Plate (HETP) of the Columns

Sample	HETP (mm)	
	CA resin	H-3010
Benzene	0.11	0.04
Phenol	0.07	0.03
Benzoic acid	0.81	3.43
Aniline	0.19	0.10

HPLC conditions: detection, 254 nm; mobile phase, methanol; flow rate, 1 ml/min.

Table II. Capacity Factors (k') of Various Compounds on Columns Packed with CA Resin and H-3010

Sample	Capacity factor (k')	
	CA resin	H-3010
Benzene	2.12	1.11
Toluene	2.37	1.45
m-Xylene	2.86	1.86
Naphthalene	5.56	3.76
Benzyl alcohol	0.75	0.33
Phenol	0.67	0.35
Catechol	0.45	0.20
Resorcinol	0.34	0.15
Hydroquinone	0.32	0.13
Benzoic acid	0.96	4.11
Aniline	0.96	0.48
Pyridine	1.21	0.52
Quinoline	2.58	1.45
o-Phenylenediamine	1.46	0.32
m-Phenylenediamine	1.39	0.24
p-Phenylenediamine	14.56	0.27
3-Aminopyridine	4.74	0.26
o-Aminophenol	1.26	0.27
m-Aminophenol	0.45	0.17
p-Aminophenol	3.08	0.17
o-Aminobenzoic acid	1.80	8.30
m-Aminobenzoic acid	0.76	4.20
p-Aminobenzoic acid	1.31	4.31

HPLC conditions: detection, 254 nm; mobile phase, methanol; flow rate, 1 ml/min.

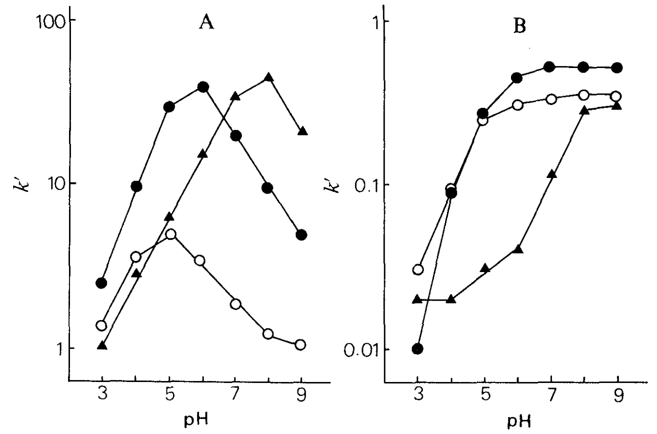

Fig. 2. Effect of Eluent pH on the Capacity Factor, k', of Aminopyridines

A, CA resin; B, H-3010. ●, 2-; ○, 3-: ▲, 4-substituted pyridine. Eluent, acetonitrile–water (1 : 1) containing 0.05 M phosphate buffer; flow rate, 0.8 ml/min.

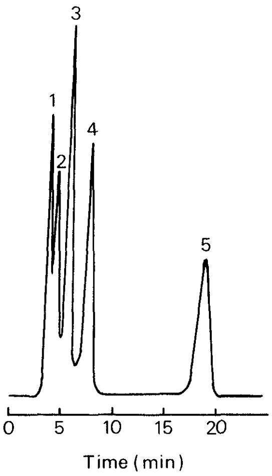

Fig. 3. Separation of Polyhydric Phenols on the CA Resin

1, pyrogallol; 2, resorcinol; 3, hydroquinone; 4, catechol; 5, phenol. Eluent, acetonitrile–water (3 : 7) containing 0.05 M phosphate buffer (pH 4.0); flow rate, 0.8 ml/min.

obtain a CA resin with a relatively low exchange capacity. Figure 1 shows the IR spectra of the MA–DVB resin, the CA resin, and H-3010. The MA–DVB resin shows characteristic absorption bands at 1860 and 1780 cm^{-1} for acid anhydride. The exchange capacity (H–Na) of the CA resin was 0.40 meq/g, and the IR spectrum shows characteristic absorption at 1750 cm^{-1} due to a carboxylic acid.

Evaluation of CA Resin Column in Methanol The efficiency of the column packed with CA resin was determined with some organic compounds by comparison with that of H-3010 (Table I). The interaction of various solutes and the polymers was expressed as a capacity factor (k') (Table II). Dieter and Walton reported that π–π interactions between aromatic compounds and the benzene rings of the polystyrene chains contributed to the retention.[6] Since the CA polymer cross-linked with DVB and H-3010 have benzene rings in their polymer chain, the π–π interaction would affect the separation of aromatic compounds. Compounds having a carboxylic group, benzoic acid and aminobenzoic acids showed smaller k' values on the CA resin column than on the H-3010 column. This may be attributed to a mutual repulsion between the carboxylic groups in solute and CA resin.

When a compound has a basic group, the acid–base interaction would mainly take part in the retention of the solute on the CA resin column. The k' values of the solutes shown in Table II generally increase with an increase in pK_a value of the solute. This is in reverse relation to the result obtained using a vinylpyridine polymer column with acidic solutes.[7] On the other hand, H-3010 did not show either relation.

Effect of Eluent Composition In ion-exchange chromatography, the retention of solutes is affected by the nature of the solutes and the pH of the mobile phase. The apparent pK_a values of ionogenic groups in cation exchange resins are approximately 2, 5—6, 10 for sulfonic, carboxylic, and phenolic resins, respectively.[8] The apparent pK_a values of the CA resin in water, determined from the titration curves,[9] was about 6. Figure 2 shows the relationships between the eluent pH and the k' values for 2-, 3-, and 4-aminopyridine (pK_a[10]: 6.86, 5.98, and 9.11, respectively) on H-3010 and the CA resin column. The k' values for aminopyridines obtained on the H-3010 column, which has no ion-exchanging group, are small, and the values

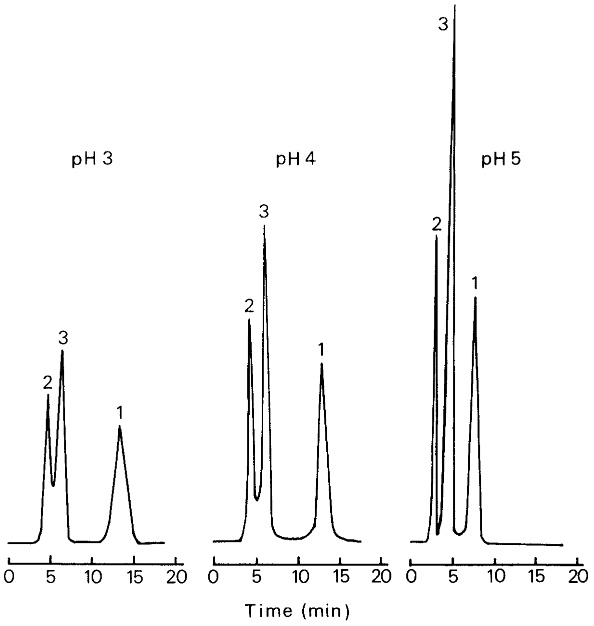

Fig. 4. Separation of Aminobenzoic Acids on the CA Resin

1, 2-; 2, 3-; 3, 4-substituted benzoic acid. Eluent, acetonitrile–water (3:7) containing 0.05 M phosphate buffer; flow rate, 0.8 ml/min.

generally increase as the eluent pH increases. These behaviors are similar to the results observed for aniline on the H-3010 column.[11] Since the degree of dissociation of the aminopyridines decreases with increasing pH, the hydrophobic interaction of the solutes with H-3010 contributes to retention in the higher pH region. These behaviors indicate that the retention of aminopyridines on the H-3010 column is mainly due to a hydrophobic interaction. The k' values for aminopyridines are higher on the CA resin column than on the H-3010 column, and a maximum k' value was observed in the pH range studied. The order of the position of the maximum k' value corresponded to the pK_a value of the aminopyridine; that is, aminopyridine with a higher pK_a value was situated in a higher eluent pH region. The appearance of a maximum retention peak showed that the retention of the aminopyridines is affected by the degree of dissociation of both the CA resin and the aminopyridines in the eluent; thus, the pH which shows maximum retention is dependent on the degree of dissociation of the CA resin and the solutes. Considering the characteristics of the CA resin in HPLC, the selection of eluent pH is more important than the kind of solvent used as the eluent.

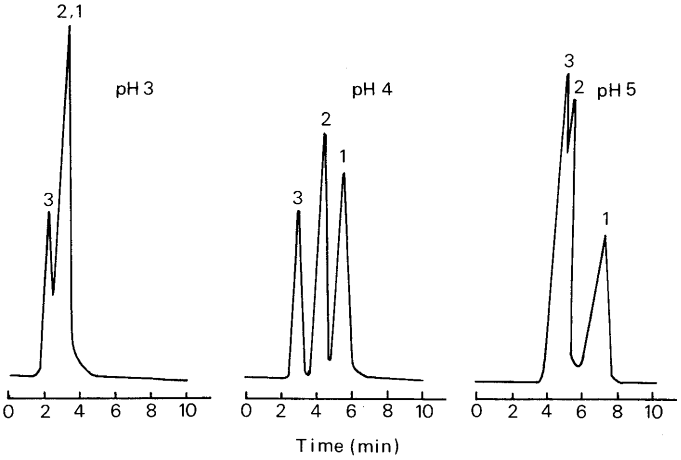

Fig. 5. Separation of Aminophenols on the CA Resin

1, 2-; 2, 3-; 3, 4-substituted phenols. Other conditions as in Fig. 4.

The separation of phenols and structural isomers of aminobenzoic acids and aminophenols on the CA column was studied. As shown in Fig. 3, phenols were easily separated. Figures 4 and 5 show the elution patterns for aminobenzoic acids and aminophenols, respectively. The mutual separation of isomers of aminobenzoic acids and aminophenols are strongly affected by the eluent pH.

In conclusion, the CA resin described here, with appropriate selection of mobile phase composition, is promising material for the separation of a wide variety of solutes.

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