

Fundamental Properties of the Poly(hydroxycarboxylic Acid) Resin Column for High-Performance Liquid Chromatography

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The spherical copoly(2-bromomaleic anhydride/divinylbenzene) was prepared by suspension polymerization, and the spherical resin obtained (5–20 μm in diameter) was then hydrolyzed to a poly(hydroxycarboxylic acid) resin. This procedure resulted in a resin with a structure similar to the malic acid moiety in the polymer chain. We propose that it may be useful as a packing material for high-performance liquid chromatography.

Key words poly(hydroxycarboxylic acid) resin; high-performance liquid chromatography; 2-bromomaleic acid; suspension polymerization; polymer beads; divinylbenzene

Maleic anhydride (MAN) is easily copolymerized with styrene (ST), divinylbenzene (DVB), and many other monomers. The spherical MAN copolymer cross-linked with DVB has been used for selective metal sorption¹⁾ and high-performance liquid chromatography (HPLC).²⁾

In contrast to MAN, only a few studies have been carried out on the polymerization of 2-halogenated MAN derivatives, and little is known about the properties of 2-halogenated MAN copolymers.^{3,4)}

In this report the spherical copoly(2-bromomaleic anhydride(BrMAN)/DVB) was prepared by suspension polymerization, and the spherical resin obtained (5–20 μm in diameter) was then hydrolyzed to a poly(hydroxycarboxylic acid) (PC) resin. The fundamental properties of the PC resin as packing material in HPLC were studied.

Experimental

Materials DVB solution (about 55% of DVB, Wako Pure Chemical Industries, Ltd., Osaka) was washed with 2N sodium hydroxide and water to remove the polymerization inhibitor. The monomer was dried over anhydrous calcium chloride. BrMAN (Aldrich Chemical Company Inc.) was purified by distillation under reduced pressure. Hydroxyethyl cellulose (Wako Pure Chemical Industries, Ltd.) was dried *in vacuo* for 2 h at 100 °C. Benzene and 1,4-dioxane were distilled prior to use. All other chemicals were of reagent grade.

Apparatus The IR spectra were recorded on a JASCO IR Report-810 spectrophotometer using KBr disks. The bromine content in the resin was determined by the oxygen combustion method. HPLC was carried out on a PLC-5D (Tokyo Rikakikai Co., Ltd.).

Preparation of Spherical Copoly(BrMAN/DVB) Polymerization was carried out in a 1 l round-bottomed three-necked flask under a nitrogen atmosphere. A mixture of 36 g of BrMAN, 18 ml of DVB, 18 ml of benzene, 18 ml of dioxane, and 0.36 g of benzoyl peroxide was added to a suspending medium. This suspending medium contained 450 ml of glycerol, hydroxyethyl cellulose (0.9 g) and anhydrous lithium chloride (22.5 g). The monomer phase was first dispersed in the suspending medium by stirring at room temperature, the oil bath temperature was then raised gradually to 70 °C at a rate of 1 °C/2 min, and the suspension was stirred for 4 h. The polymer beads obtained were immersed in methanol, washed with methanol and benzene, and dried *in vacuo* at room temperature. Approximately 6 g of white spherical resin (copoly(BrMAN/DVB), 5–20 μm in diameter, classified by a continuous solvent elutriation method⁵⁾ with methanol) was obtained.

Preparation of PC Resin The copoly(BrMAN/DVB) was hydrolyzed in 0.1N potassium hydroxide for 5 h at 50 °C. The hydrolyzed copolymer (PC resin) was then washed with 0.1N hydrochloric acid and water, and dried *in vacuo* at room temperature. The pH titration curve of the PC resin was determined by potentiometric titration under a nitrogen atmosphere at 20 °C. The sodium salt of the resin was dispersed in distilled water with stirring and titrated with 0.05N HCl from the micrometer buret. The acid dissociation constant of the resin was

determined according to the method of Noyes.⁶⁾

Chromatography The column (25 \times 0.4 cm i.d.) was prepared by packing the PC resin into stainless steel tubing using the slurry method. The solutes were dissolved in methanol or acetonitrile at a concentration of approximately 1 mg/ml and 1–5 μl of the solution was injected with a 10 μl syringe.

Results and Discussion

Preparations of Spherical Copoly(BrMAN/DVB) and PC Resin The copoly(BrMAN/DVB) was prepared in the same manner as the copoly(MAN/DVB)²⁾ except that anhydrous lithium chloride was used in place of sodium chloride as the stronger salting-out agent for the monomer solution in the suspending medium. The bromine content in the copoly(BrMAN/DVB) and the PC resin was 0.3 and 0 meq/g, respectively. The ion-exchange capacity (H–Na) of the PC resin was 0.6 meq/g. IR spectra of the resins are shown in Fig. 1. The copoly(BrMAN/DVB) (A) shows characteristic absorption bands at 1785 and 1855 cm^{-1} for acid anhydride. The PC resin (B) shows characteristic absorption bands at 1760 cm^{-1} of carboxylic acid and around 3450 cm^{-1} due to a hydroxyl group.⁷⁾

Figure 2 shows the titration curve of PC resin. The PC resin which has malic acid moiety in the polymer chain dissociates in two steps. The titration curve is similar to

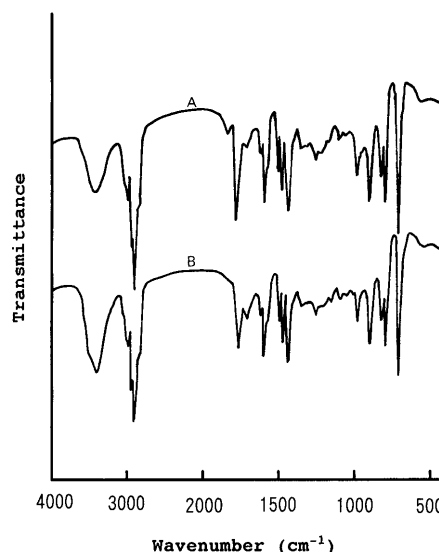


Fig. 1. IR Spectra of the Resins

A, copoly(BrMAN/DVB); B, PC resin.

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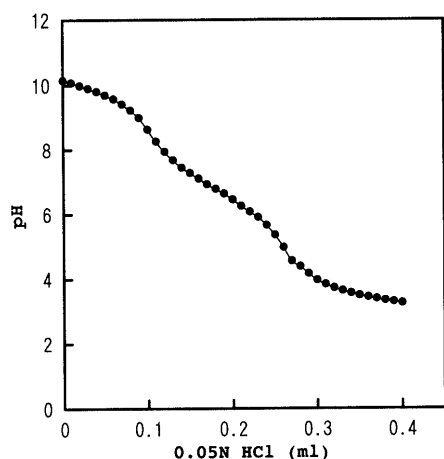


Fig. 2. A Potentiometric Titration Curve of the PC Resin
22.5 mg of PC resin (Na) was added to 10 ml of water.

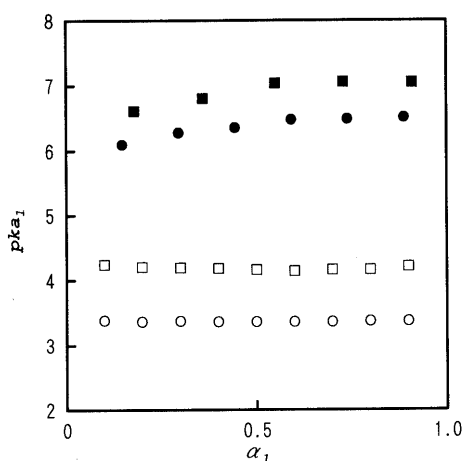


Fig. 3. Dependence of the Apparent Dissociation Constant of the Primary Carboxyl Group (pK_{a1}) on the Degree of Ionization (α_1) for PC Resin (●), CA Resin (■), L-Malic Acid (○), and Succinic Acid (□)

that of the hydrolyzed copoly(MAN/ST) having succinic acid moiety in the polymer chain.⁸⁾ Interaction between the neighboring carboxylic groups in the PC resin seems to affect their dissociation similar to the hydrolyzed copoly(MAN/ST). The relationships between the degree of dissociation (α_1) and the apparent primary dissociation constant (pK_{a1}) of the PC resin, CA resin²⁾ (carboxylic acid resin from copoly(MAN/DVB)), succinic acid, and malic acid are shown in Fig. 3. The PC resin is a stronger acid than the CA resin, and this relation is similar to the difference of acidity between malic acid and succinic acid. In a linear polyelectrolyte such as the hydrolyzed copoly(MAN/ST), the apparent pK_{a1} values are changed with the progress of the dissociation.⁸⁾ This behavior has been thought to be due to the conformational change of the polymer with the progress of dissociation in aqueous solution.⁹⁾ In the case of PC resin, however, the apparent pK_{a1} values were little changed. This may be attributed to little conformational change in the PC resin highly cross-linked by DVB.

Properties of the PC Resin Column The interaction of various solutes and the PC resin is expressed as a capacity factor (k') in Table 1. The results of CA resin and H-3010²⁾ (Hitachi gel 3010, a macroporous hydrophobic copoly(ST/DVB)) are also compared. Aromatic com-

Table 1. Capacity Factors (k') of Various Compounds on Columns Packed with PC Resin, CA Resin, and H-3010

| Sample | Capacity factor (k') | | |
|---------------------|--------------------------|------------------------|----------------------|
| | PC resin | CA resin ^{a)} | H-3010 ^{a)} |
| Benzene | 2.32 | 2.12 | 1.11 |
| Toluene | 2.55 | 2.37 | 1.45 |
| <i>m</i> -Xylene | 3.10 | 2.86 | 1.86 |
| Benzyl alcohol | 0.78 | 0.75 | 0.33 |
| Phenol | 0.68 | 0.67 | 0.35 |
| Catechol | 0.38 | 0.45 | 0.20 |
| Resorcinol | 0.29 | 0.34 | 0.15 |
| Hydroquinone | 0.27 | 0.32 | 0.13 |
| Benzoic acid | 0.89 | 0.96 | 4.11 |
| Aniline | 2.46 | 0.96 | 0.48 |
| Pyridine | 1.98 | 1.21 | 0.52 |
| 2-Aminophenol | 5.34 | 1.26 | 0.27 |
| 3-Aminophenol | 1.94 | 0.45 | 0.17 |
| 4-Aminophenol | 13.28 | 3.08 | 0.17 |
| 2-Aminobenzoic acid | 0.80 | 1.80 | 8.30 |
| 3-Aminobenzoic acid | 0.45 | 0.76 | 4.20 |
| 4-Aminobenzoic acid | 0.67 | 1.31 | 4.31 |

HPLC conditions: detection, 254 nm; mobile phase, methanol; flow rate, 1 ml/min. a) Data taken from ref. 2.

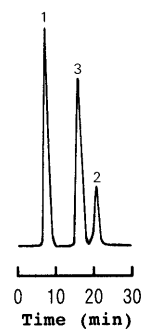


Fig. 4. Separation of Phthalic Acids on the PC Resin

1, *o*-; 2, *m*-; 3, *p*-phthalic acid. Eluent, acetonitrile-water (1:9) containing 0.05 M phosphate buffer (pH 3); flow rate, 1.0 ml/min; detection, 280 nm.

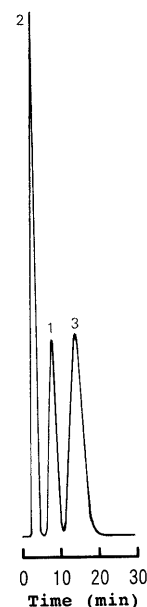


Fig. 5. Separation of Aminopyridines on the PC Resin

1, 2-; 2, 3-; 3, 4-substituted pyridine. Eluent, acetonitrile-water (6:4) containing 0.05 M phosphate buffer (pH 9); flow rate, 1.0 ml/min; detection, 240 nm.

pounds showed a similar elution behavior on the PC resin, CA resin, and H-3010 column, and this behavior seems to be due to the π - π interactions between benzene rings.^{2,10} Compounds with a carboxylic group, such as benzoic acid and aminobenzoic acids showed smaller k' values on the PC and CA resin columns than on the H-3010 column. This can be ascribed to a mutual repulsion between the carboxylic groups in the solute and the resins. In addition, the k' values on the PC resin column were smaller than those on the CA resin column. This could be attributable to the difference in the strength of acid dissociation between the resins (Fig. 3).

On the other hand, compounds with basic groups showed larger k' values on the PC and the CA resin columns than on the H-3010 column due to the acid-base interaction. The k' values on the PC resin column were larger than on the CA resin column, and again this is thought to be attributable to the difference in the strength of acid dissociation of the resins (Fig. 3).

We studied the separation of isomers of phthalic acids and aminopyridines on the PC resin column. Phthalic acids and aminopyridines were easily separated according to the difference of the acid dissociation constant of the solute, as shown in Figs. 4 and 5, respectively.

In conclusion, the PC resin, which has a structure simi-

lar to the malic acid moiety in the polymer chain, is a stronger acid than CA resin having a structure similar to the succinic acid moiety in the polymer chain. The PC resin was expected to be useful for the separation of a wide variety of solutes.

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