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# STUDIES IN LIGAND-EXCHANGE CHROMATOGRAPHY

## PART IV. SEPARATION OF HYDROXYBENZOIC AND HYDROXY-NAPHTHOIC ACID ISOMERS

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## SUMMARY

The separation of isomers of hydroxybenzoic and hydroxynaphthoic acids by ligand-exchange chromatography using the metallic form of a cation-exchange resin as the stationary phase and dilute aqueous ammonia or water as the mobile phase was studied. The distrubution coefficients and the separation factors of hydroxybenzoic acid isomers were larger on the Fe(III) form than on the Ti(IV) form of the resin, but a faster rate of adsorption was observed on the Ti(IV) form rather than on the Fe(III) form of the resin.

The separation of hydroxybenzoic acid isomers was best achieved by eluting a mixture of the *o*- and *p*-isomers or of the *m*- and *p*-isomers with water through a column of 50 cm in length and 9 mm I.D., packed with the Fe(III) form of the resin which had been equilibrated at pH 4.7 with aqueous ammonia.

The controlled surface resin, having sulphonic acid groups only on the surface of the resin beads, was also tested as a stationary phase for the resolution of hydroxynaphthoic acid isomers.

## INTRODUCTION

Successful separations in ligand-exchange chromatography require not only a large difference in the adsorptivities of the ligands to be separated, but also a fast rate of equilibration. The difference in the adsorptivities of the ligands is greatly influenced by the type of metallic ion present in the resin and the type and concentration of the eluent, while the rate of equilibration is related to the rates of diffusion of the sample ligands and of the ligand-exchange reaction. If the sample ligands are not bulky and the film and particle diffusion rates are both fast, the ligand exchange will become the rate-determining step in equilibration, and the sorption equilibrium will be influenced only by the type of metallic form of the resin, *i.e.*, by the number of $d$ electrons in the metallic ion, except in instance of square-planar tetra-coordination, such as in copper(II) complexes.

For hexa-coordinated complexes of transition metal ions, it can be predicted

that the ligand-exchange reaction of $d^0$, $d^1$, $d^2$ and $d^{10}$ metal complexes would always be fast, whether the metal forms a high-spin or a low-spin complex or whether the reaction proceeds by an $S_N1$ or an $S_N2$ mechanism. In fact, zinc(II), cadmium(II), etc., whose $d$ orbitals are filled with electrons, have been used satisfactorily as the central ion in ligand-exchange chromatography[1,2], but titanium(IV), titanium(III), yttrium(III), etc., which have three or more empty $d$ orbitals, have rarely been used.

Controlled surface cation-exchange resins, *i.e.*, resins that have sulphonic acid groups only on the surface, are also considered to be useful for obtaining the adsorption equilibrium rapidly, because the steps of solute particle diffusion and of complex formation at inner sites in the beads can both be neglected.

The purpose of the present work was to compare the effectiveness of the Fe(III) ($d^5$) and Ti(IV) ($d^0$) forms of resins in terms of the distribution coefficients, separation factors and rate of equilibration of hydroxybenzoic acid isomers; the former ionic form of the resin would be expected to form stable (not necessarily inert) complexes with hydroxybenzoic acids and the latter to form labile (not necessarily unstable) complexes. Some experiments were also carried out in order to establish the usefulness of the controlled surface cation-exchange resin as a stationary phase in the ligand-exchange separation of hydroxynaphthoic acid isomers.

Numerous chromatographic methods for the separation of hydroxybenzoic acid isomers have been reported, including thin-layer[3-7], column-partition[8], ion-exchange[9], column-adsorption[10], salting-out[11] and gas chromatography[3,12,13]. In many instances, however, attempts to separate the *m*- from the *p*-isomer resulted in failure or in only partial success. On the other hand, only a few papers have dealt with the separation of hydroxynaphthoic acids[14,15].

EXPERIMENTAL

*Materials*

Each isomer of hydroxybenzoic and hydroxynaphthoic acids, purchased from Tokyo Kasei Kogyo Co. Ltd., was purified by recrystallization from water until the melting-point agreed with the literature value. Standard solutions were prepared by dissolving the compounds in 30% v/v ethanol so as to give concentrations of $1 \cdot 10^{-3}$ $M$ (138.1 $\mu$g/ml), and were stored in the dark in amber bottles. If necessary, they were diluted to $5 \cdot 10^{-4}$ $M$ (69.1 $\mu$g/ml) with the same solvent.

The ion-exchange resins used were Amberlite CG-120 (sulphonated polystyrene type; Rohm&Haas Co.), 200–400 mesh, and a special grade of Diaion SK 1, 200–400 mesh, supplied by Mitsubishi Chemical Industries Ltd. The latter resin is also of the sulphonated polystyrene type, but the functional groups are situated only on the surface of the resin beads. This special resin had a capacity of 0.37 mequiv./g (0.25 mequiv./ml) and a moisture content of 8.8% (w/w). After conditioning in the usual way, the resins were converted into the Fe(III) or Ti(IV) form in a wide preparatory column by treating them with iron(III) chloride or titanium sulphate solution. They were washed thoroughly with water and then air-dried by spreading them on a filter-paper, for use in the batch method.

The eluent used was either aqueous ammonia or distilled water.

*Equipment*

The column was of 9 mm I.D. and length 50 cm and was packed with the

metallic form of the resins, which had been pre-equilibrated with an eluent by a procedure described earlier[16].

Chromatography was performed using a liquid chromatograph (Model JLC-3; JEOL Co. Ltd.) with a variable-speed pulseless pump (Model JLC-P2). The sample solutions were introduced by a valve through a 1.0-ml PTFE sample loop. From the column effluents, 5-ml portions were collected successively with a fraction collector (Model SF-160K; Toyo Kagaku Co. Ltd.).

Detection was carried out with a UV spectrophotometer (Model QV-50; Shimazdu Seisakusyo Co. Ltd.) using a quartz cell of 1.00-cm path length. The absorbances of hydroxybenzoic and hydroxynaphthoic acid isomers were measured at the following wavelengths: o-hydroxybenzoic acid, 230.0 nm; m-hydroxybenzoic acid, 228.0 nm; p-hydroxybenzoic acid, 247.0 nm; 1-hydroxy-2-naphthoic acid, 250.0 nm; 2-hydroxy-1-naphthoic acid, 226.0 nm; and 3-hydroxy-2-naphthoic acid, 234.0 nm.

For the measurement of the concentration of ammonia, a pH meter (Model HM-5A; Toa Denpa Co. Ltd.) was used.

## RESULTS AND DISCUSSION

### Distribution coefficients of hydroxybenzoic acids

In order to establish the most suitable chromatographic conditions, the distribution coefficients ($K_d$) of hydroxybenzoic acids between the metallic form of the resin and aqueous ammonia or water were first measured by the batch method as described in a previous paper[16].

In Fig. 1 and Table I, the $K_d$ values are shown as a function of the pH of the solutions that had been equilibrated with the resin. Owing to the hydration of the metal aquo-complex in the resin phase, the pH of the equilibrated solution was below 5.0 when very dilute ammonia was used as the solvent.

It is obvious from Fig. 1 and Table I that the $K_d$ values decreased with increase in ammonia concentration and that the adsorptivity of the isomers was greater on
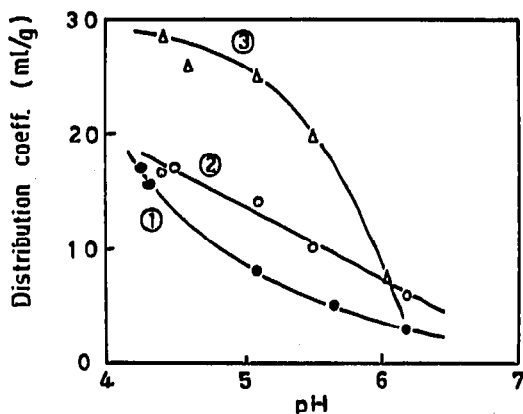


Fig. 1. Effect of pH on the distribution coefficients of hydroxybenzoic acids. (1) o-Hydroxybenzoic acid; (2) m-hydroxybenzoic acid; (3) p-hydroxybenzoic acid. Resin, Amberlite CG-120, Fe(III) form.

TABLE I

$K_d$ VALUES OF HYDROXYBENZOIC ACIDS ON THE Ti(IV) FORM OF AMBERLITE CG-120 AT VARIOUS pH VALUES

| Substance | pH | | | | |
|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 |
| o-Hydroxybenzoic acid | 5.3 | 4.6 | 3.5 | 2.4 | 1.3 |
| m-Hydroxybenzoic acid | 8.4 | 6.7 | 4.9 | 2.9 | 0.6 |
| p-Hydroxybenzoic acid | 11.0 | 8.9 | 6.6 | 4.2 | 1.9 |

the Fe(III) form than on the Ti(IV) form of the resin, suggesting that the Fe(III) complexes of hydroxybenzoic acids are more stable than the Ti(IV) complexes. In both instances, the p-isomer was adsorbed more strongly, and the o-isomer less strongly, than the m-isomer. The separation factors between the p- and o-isomers and between the p- and m-isomers on the Fe(III) form of the resin were about 3.3 and 1.7, respectively, in solutions of pH 4.7–5.3. This result suggests that separations might be possible under such conditions. On the other hand, the separation factors between the p- and m-isomers on the Ti(IV) form of the resin were not large enough to permit their quantitative separation.

In ligand-exchange adsorption, the differences in adsorptivity between isomeric ligands can be correlated with the basicity of the ligands, i.e., the larger the pK value of the ligand, the more strongly it is adsorbed. If a molecule of hydroxybenzoic acid acts as a monodentate and coordinates with the metallic ion through its carboxylic oxygen atom in weakly acidic or neutral solutions, the order of the adsorptivities obtained in this experiment is in agreement with this generalization, as the order of the pK values of the hydroxybenzoic acids is p-isomer ($pK_1 = 4.127$) > m-isomer ($pK_1 = 4.060$) > o-isomer ($pK_1 = 2.754$). It should be noted, however, that the difference in $K_d$ values between the o- and m-isomers is much smaller than that expected from their pK values. This result is probably due to the fact that only the o-isomer behaves as a bidentate ligand and forms a chelate with the metallic ion in the resin phases.

*Rate of adsorption*

The rate of adsorption of hydroxybenzoic acid isomers on the Fe(III) and Ti(IV) forms of the resins, measured in water at room temperature (23°), are shown in Figs. 2 and 3, respectively.

The amount of the acids adsorbed on the Fe(III) form of the resin still tends to increase even after 20 min or longer, but that on the Ti(IV) form of the resin becomes constant in less than 20 min. Hence, the rate of adsorption of the acids on the resin in the Ti(IV) form is much faster than on that in the Fe(III) form.

*Separation of hydroxybenzoic acid isomers*

Elution curves obtained from a column 50 cm long and 9 mm I.D., packed with the Ti(IV) form of the resin that had been equilibrated with aqueous ammonia at pH 4.5, are shown in Fig. 4. The separation of the o- from the p-isomer was nearly
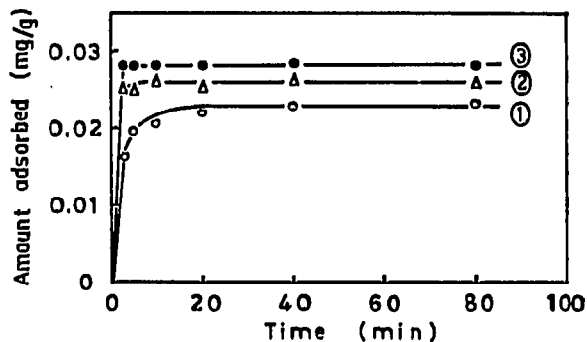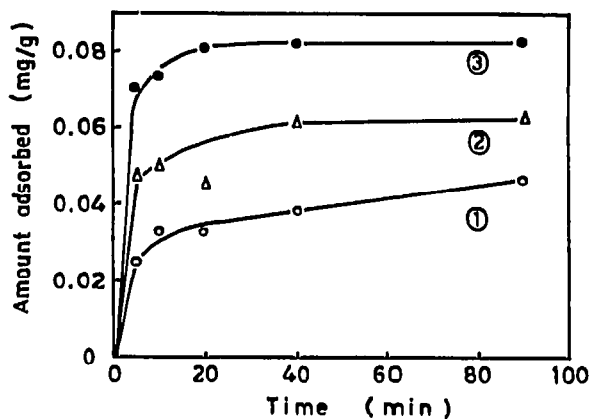
Fig. 2. Rates of absorption. (1) *o*-Hydroxybenzoic acid; (2) *m*-hydroxybenzoic acid; (3) *p*-hydroxybenzoic acid. Resin, Amberlite CG-120, Fe(III) form; solvent, water.

Fig. 3. Rates of adsorption. (1) *o*-Hydroxybenzoic acid; (2) *m*-hydroxybenzoic acid; (3) *p*-hydroxybenzoic acid. Resin, Amberlite CG-120, Ti(IV) form; solvent, water.
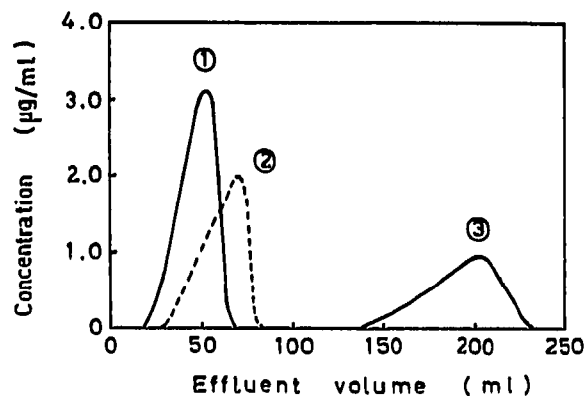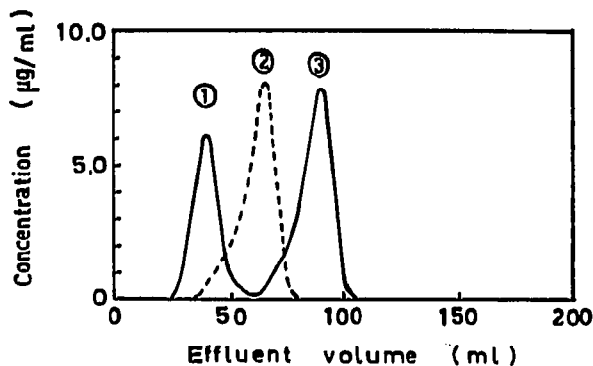


Fig. 4. Elution curve. (1) *o*-Hydroxybenzoic acid; (2) *m*-hydroxybenzoic acid; (3) *p*-hydroxybenzoic acid. Column, Amberlite CG-120, Ti(IV) form (equilibrated with aq. NH₃ at pH 4.5), 9 mm × 50 cm; eluent, water at a flow-rate of 0.21 ml/min.

Fig. 5. Elution curve. (1) *o*-Hydroxybenzoic acid; (2) *m*-hydroxybenzoic acid; (3) *p*-hydroxybenzoic acid. Column, Amberlite CG-120, Fe(III) form (equilibrated with aq. NH₃ at pH 4.7), 9 mm × 50 cm; eluent, water at a flow-rate of 0.21 ml/min.

complete, but neither separation of the *o*- from the *m*-isomer nor that of the *m*- from the *p*-isomer could be achieved. When a column of the same dimensions, packed with the Fe(III) form of the resin, was used, however, the *p*-isomer was separated completely from both the *o*- and the *m*-isomer, as shown in Fig. 5, although with some broadening of the peaks. The recoveries of the isomers were satisfactory in both instances as shown in Table II.

*Separation of hydroxynaphthoic acid isomers*

In order to establish the adsorption equilibrium rapidly, chromatography

TABLE II

RECOVERIES OF HYDROXYBENZOIC ACID ISOMERS

| Form of resin in column | Experiment No. | Amount loaded (μg) | | | Amount found (μg) | | | Recovery (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | o- | m- | p- | o- | m- | p- | o- | m- | p- |
| Fe(III) | 1 | 69.1 | | 69.1 | 68.3 | | 67.1 | 98.8 | | 97.1 |
| | 2 | 69.1 | | 69.1 | 67.7 | | 67.5 | 98.0 | | 97.7 |
| | 3 | | 69.1 | 69.1 | | 66.8 | 66.9 | | 96.7 | 96.8 |
| | 4 | | 69.1 | 69.1 | | 67.2 | 67.5 | | 97.3 | 97.7 |
| Ti(IV) | 5 | 69.1 | | 138.1 | 69.8 | | 136.6 | 101.0 | | 98.9 |
| | 6 | 69.1 | | 138.1 | 69.3 | | 137.1 | 100.3 | | 99.3 |

was performed using the special grade of Diaion SK 1 mentioned above.

In a previous paper[16], it was reported that hydroxynaphthoic acids could not be retained on a column of Amberlite CG-120 in the Fe(III) form when aqueous ammonia of pH 9.5 was used as the eluent. In the present work, therefore, the elution was first tried through a column packed with the resin in the Ti(IV) form, using water as the eluent. However, even under these conditions, the separation of the isomers could not be achieved successfully because of the similarity of their adsorptivities. Accordingly, the elution was tried again using a column 50 cm long and 9 mm I.D. packed with the Fe(III) form of the resin that had been equilibrated with aqueous ammonia at pH 5.5, with water as the eluent instead of aqueous ammonia.

Typical elution curves are shown in Figs. 6 and 7.

The order of adsorptivity was 1-hydroxy-2-naphthoic acid > 2-hydroxy-1-naphthoic acid ≈ 3-hydroxy-2-naphthoic acid. The separation of a small amount of 2-hydroxy-1-naphthoic acid or 3-hydroxy-2-naphthoic acid from a large amount of 1-hydroxy-2-naphthoic acid was achieved satisfactorily.
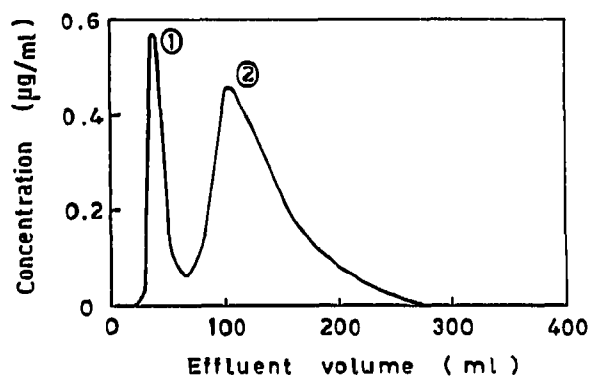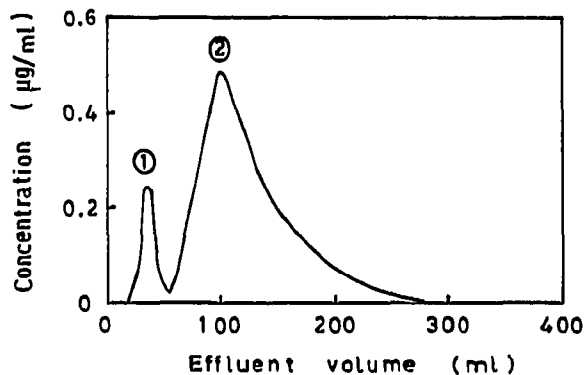


Fig. 6. Elution curve. (1) 2-Hydroxy-1-naphthoic acid (10 μg); (2) 1-hydroxy-2-naphthoic acid (50 μg). Column, Diaion SK 1 (special grade), Fe(III) form (equilibrated with aq. NH₃ at pH 5.5), 9 mm × 50 cm; eluent, water at a flow-rate of 0.16 ml/min.

Fig. 7. Elution curve. (1) 3-Hydroxy-2-naphthoic acid (4 μg); (2) 1-hydroxy-2-naphthoic acid (50 μg). Column, Diaion SK 1 (special grade), Fe(III) form (equilibrated with aq. NH₃ at pH 5.5), 9 mm × 50 cm; eluent, water at a flow-rate of 0.16 ml/min.

With respect to the adsorptivity of the isomer, it can be considered that the order of the adsorptivity would become 2-hydroxy-1-naphthoic acid > 1-hydroxy-2-naphthoic acid > 3-hydroxy-2-naphthoic acid, in agreement with the basicity[17] effect and the resonance effect[18], whether these isomers behave as monodentate or bidentate ligands in the resin phase. However, except for the fact that 3-hydroxy-2-naphthoic acid was adsorbed to a lesser extent, the experimental results were not necessarily the same as this generalization, for unknown reasons. At present, it is difficult to draw any conclusions about the influence of the surface-controlled resin on the ligand-exchange adsorption equilibrium. Detailed studies on the problems will be reported in the future.

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