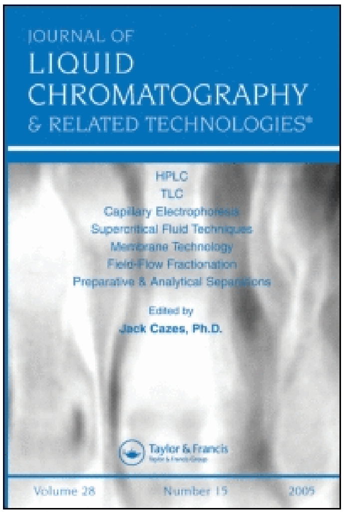

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ELUTION BEHAVIOR OF ORGANIC ACIDS IN DONNAN EXCLUSION CHROMATOGRAPHY

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ABSTRACT

The elution behavior of organic acids has been elucidated using a dextran gel-type cation-exchanger column with an eluent of 1.00 M tetramethylammonium chloride. It was found that Donnan exclusion chromatography is applicable to organic acids as well as inorganic ones. Some types of separation methods were performed by the use of the separation mode whose mechanism was confirmed by Donnan exclusion chromatography.

INTRODUCTION

Although the separation of organic acids by means of ion exclusion chromatography has been described by many investigators (1–4), only a qualitative explanation has been given to their elution behavior.

In Donnan exclusion chromatography based on electrostatic repulsion from an ion-exchanger phase, if one uses a concentrated electrolyte as an eluent, the elution behavior of a given ion is explained quantitatively by the Donnan membrane equilibrium equation. Its elution position from a column is dependent upon its charge. The treatment of this principle has been established for inorganic acids, mainly phosphorus oxoacids (5–8). In

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the present paper, the elution behavior and the separation of organic acids such as aliphatic, aromatic and aminopolycarboxylic acids are described. The investigation of the separation mechanism is emphasized here rather than the separation itself, because many separations of organic compounds have been done more efficiently by other types of chromatography.

EXPERIMENTAL

It is neccesary that an ion-exchanger contains a large volume of a non-adsorption region (distribution coefficient is 0 to 1) for Donnan exclusion chromatography. A cross-linked dextran gel-type cation exchanger, SP-Sephadex C-25, is swollen in electrolyte solution and suitable for this purpose. The column was packed with SP-Sephadex C-25 (15 mm I.D. x 92.5 cm). Tetramethylammonium chloride solution (1.00 M) was used as an eluent, because its cation does not interact appreciably with a sample anion. Then, 1 ml of the sample solution, dissolved in the eluent, was applied to the top of the column. Flow rate was maintained at 0.5 ml/min. The effluent was introduced into a flow cell of a variable-wavelength spectrophotometer (LDC Model SpectroMonitor Ⅲ) for aromatic compounds and cobalt-aminopolycarboxylate complexes, or collected by a fraction collector into fractions of 1 ml for other samples. Aromatic compounds in effluents were detected at 275 nm wavelength and cobalt-aminopolycarboxylate complexes at 515 nm wavelength. Aliphatic carboxylic acids were determined as their hydroxamic acids formed by reaction with dicyclohexylcarbodiimide (9).

The calculation of the distribution coefficient is the same as that previously described in detail (5). The distribution coefficient, K_D, is defined as

$$K_D = \frac{V_e - V_0}{V_D} \qquad (1)$$

where V_e is the elution volume, V_D the net volume of the exchanger phase except of a skeleton and V_0 the interstitial volume. In case of an equilibrium mixture with different charge states such as polybasic acids, H_nL, the distribution coefficient, D, can be written as

$$D = K_{D,L}\,\alpha_L + K_{D,HL}\,\alpha_{HL} + K_{D,H_2L}\,\alpha_{H_2L} + \cdots\cdot \qquad (2)$$

where K_{D,H_jL} is the characteristic distribution coefficient of component H_jL, and α_{H_jL} is its mole fraction.

RESULTS AND DISCUSSION

In Donnan exclusion chromatography, the principal separation factor is an electrostatic repulsion between fixed groups of an ion exchanger and the co-charged sample ions. The elution volume of a given sample ion can be elucidated from its charge. When another factor, such as adsorption or steric exclusion, acts together with ionic exclusion, the elution position should shift to a larger volume (adsorption) or to a smaller volume (steric exclusion) than that predicted from ionic exclusion only. The shift, therefore, indicates the presence of another factor. If the separation mechanism is confirmed, separation would be improved, surely, by using these factors properly. The elution behavior of organic acids on a cation-exchanger column was estimated first and represented as the distribution coefficient indicating mechanism. From these results, some types of separations were accomplished.

Distribution coefficient, K_D or D.

The distribution coefficients of organic acids, except for adsorbed compounds stated later, were obtained by eqns. (1) and (2) [TABLE 1]. Fig. 1 illustrates the plots for the distribution coefficients, K_D, of the elution standards of crotonaldehyde (neutral), phosphinate (monovalent), phosphonate (divalent) and trimetaphosphate (trivalent), and the distribution coefficients, D, of orthophosphate at different pH as an equilibrium mixture with different charge states. When the distribution coefficients of organic acids are plotted against the (mean) charge calculated from the

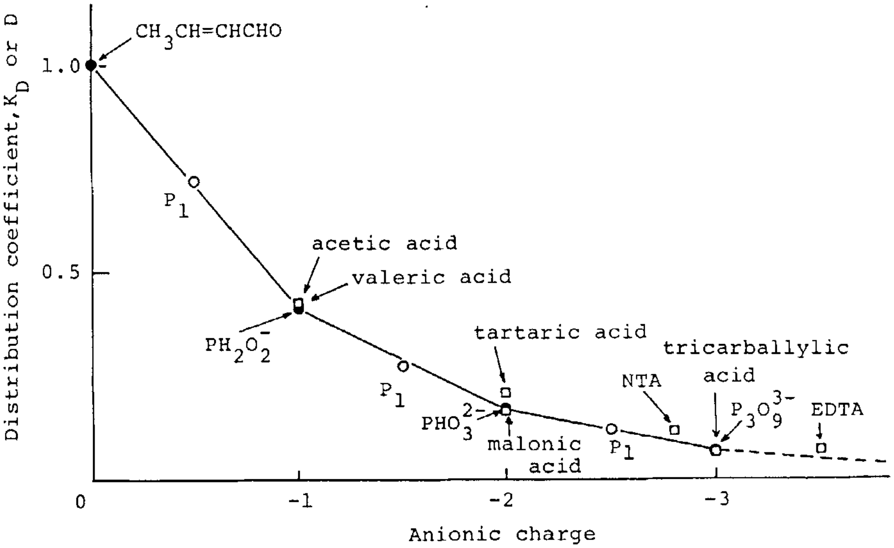

FIGURE 1

The relationship between distribution coefficient and anionic charge. ●; elution standard, o; orthophosphate (P_1) at different pH, □; organic acid. The broken line between -3 and -4 is calculated from eqns. (1) and (2).

TABLE 1

Distribution Coefficient, K_D or D.

Species	K_D or D	Species	K_D or D
Acetic acid	0.426	Oxalic acid	0.213
Valeric acid	0.426	Tricarballylic acid	0.070
Malonic acid	0.156	NTA	0.116
Tartaric acid	0.207	EDTA	0.076

dissociation constant, these values are in fair agreement with those of phosphorus oxoacids. This means that Donnan exclusion chromatography can be applied generally to organic acids as well as inorganic acids and gives a quantitative explanation for their elution behavior.

The deviation of NTA and EDTA from the line of Fig. 1 may be due to the dispersion of charge on a large molecule.

Separation of aliphatic carboxylic acids.

It has been mentioned in previous papers (5,8) that the mutual separation of mono-, di- and trivalent anions of phosphorus oxoacids is completely accomplished. To make sure that Donnan exclusion chromatography is applicable to organic compounds, some mixtures of aliphatic carboxylic acids were separated on the cation-exchanger column. Fig. 2 shows the separation of acetic, tartaric and tricarballylic acids as the completely dissociated samples at pH 10 of mono-, di- and trivalent anions, respectively. Each elution volume can be interpreted quantitatively and its distribution coefficient agrees with the corresponding standards of phosphorus oxoacids.

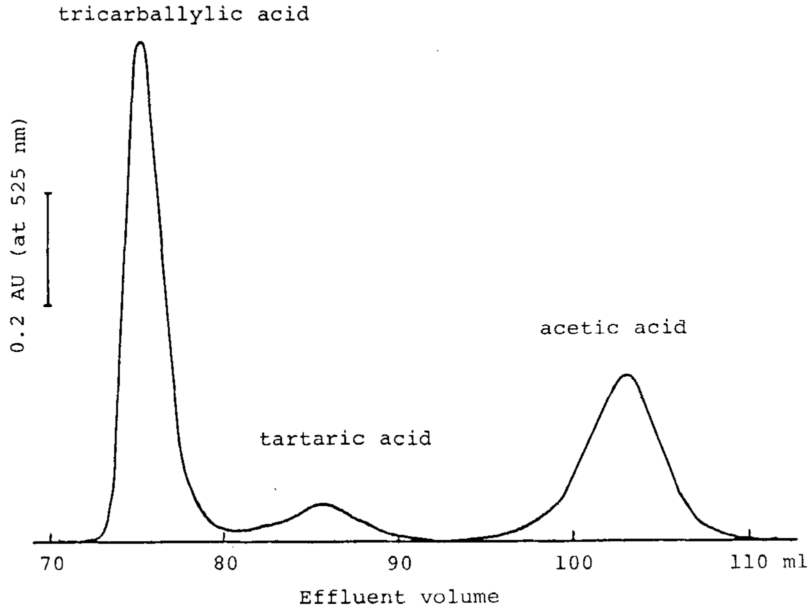

FIGURE 2

Separation of aliphatic carboxylic acids. Column; SP-Sephadex C-25 (Me_4N^+ form), 15 mm I.D. x 92.5 cm. Eluent; 1.00 M Me_4NCl (pH 10). Sample; 0.1 M $(Me_4N)_3C_6H_5O_6$, 0.1 M $Na_2C_4H_4O_6 \cdot 2H_2O$ and $NaC_2H_3O_2 \cdot 3H_2O$.

Separation of aromatic compounds.

The separation of phthalic acid, benzoic acid, salicylic acid and phenol is shown in Fig. 3. The charge states of these sample anions in the eluent at pH 10 are -2 for phthalic acid, -1 for benzoic acid and salicylic acid and *ca.* -0.5 for phenol, respectively. Although the elution volume depends upon its charge, anions with phenol group were eluted at a later position than predicted. It is possible that salicylic acid and phenol tend to be adsorbed due to the interaction between a phenol group and a dextran gel matrix. In

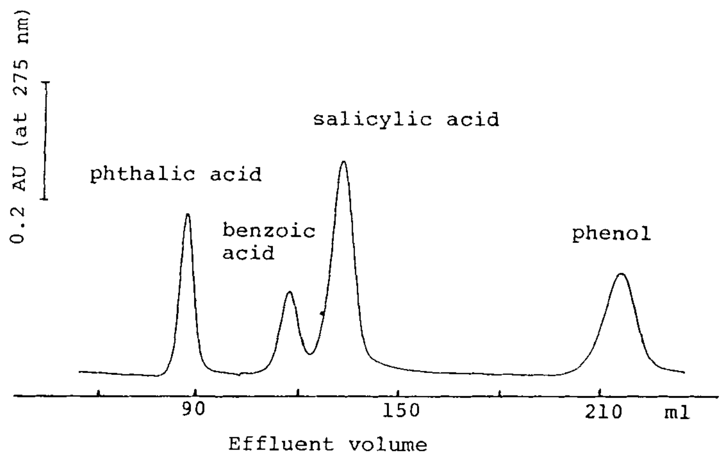

FIGURE 3

Separation of aromatic anions. Column; SP-Sephadex C-25 (Me_4N^+ form), 15 mm I.D. x 92.5 cm. Eluent; 1.00 M Me_4NCl (pH 10). Sample; each 4 x 10^{-3} M of $C_6H_4(COOK)(COOH)$, $C_6H_4(OH)COONa$, C_6H_5COONa and C_6H_5OH.

this case, the separation became more favorable by combined factors of ionic exclusion and adsorption.

Separation of highly-charged anions by formation of cobalt (II) complex.

A direct separation of highly-charged anions seems to be difficult because the difference of the ditribution coefficients decreases with the increase of each ionic charge on anions from the theoretical point of view. It may be considered that the anionic charge depression by complex formation with metal cation leads to better separation.

The elution curves for a mixture of cobalt(II) complex of IDA (iminodiacetic acid), NTA (nitrilotriacetic acid) and EDTA (ethylenediaminotetraacetic

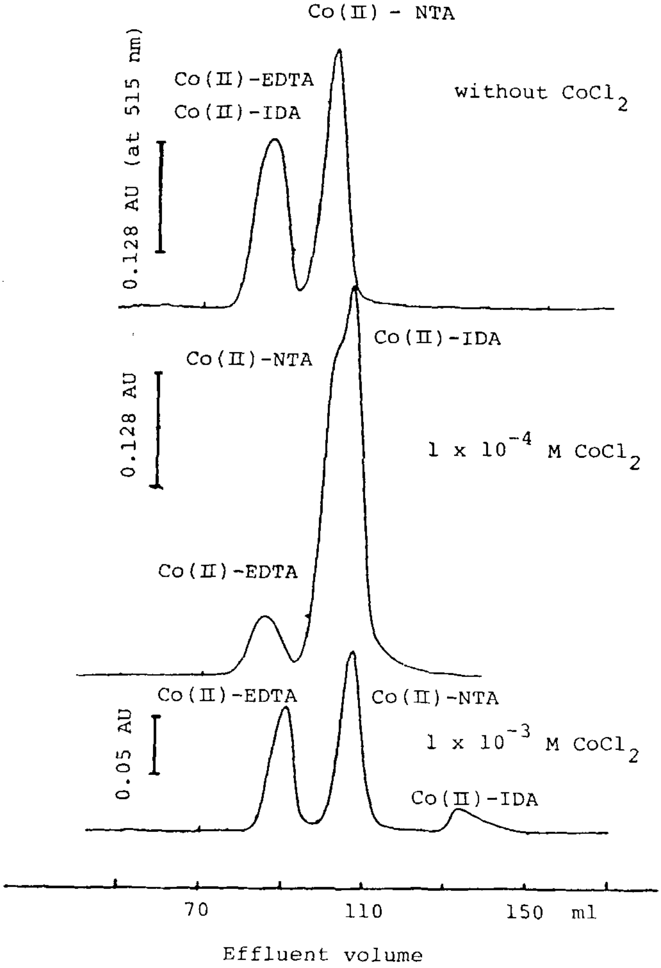

FIGURE 4

Separation of aminopolycarboxylic acid anions by Co(II) complex formation. Column; SP-Sephadex C-25, 15 mm I.D. x 92.5 cm. Eluent; 1.00 M Me_4NCl and (a) without $CoCl_2$, (b) 1 x 10^{-4} M $CoCl_2$ or (c) 1 x 10^{-3} M $CoCl_2$ (pH 6.0). Sample; each 0.1 M of Co(II)-IDA, Co(II)-NTA and Co(II)-EDTA.

acid) are shown in Fig. 4, with eluents containing cobalt(II) chloride at different concentrations (pH 6). The elution volumes of highly stable EDTA and NTA complexes are constant whether cobalt(II) ions are added to the eluent or not, whereas the elution position of the less stable IDA complex shifts gradually to a larger volume according as cobalt(II) ion concentration increases in the eluent. This indicates that cobalt(II) complexes of EDTA and NTA are completely formed but that of IDA is partially formed in the eluent at lower concentration of cobalt(II) chloride. These complexes were separated from each other with the eluent containing 1×10^{-3} M cobalt(II) chloride.

From the aspect of resolution, if adsorptive or steric exclusion effects are present, they often are present in a direction and magnitude that enhances the separation.

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