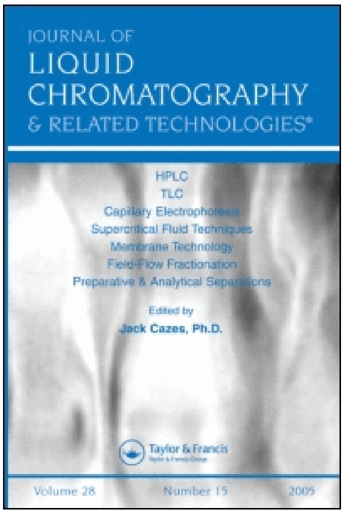

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DONNAN EXCLUSION CHROMATOGRAPHY WITH NONIONIC ADSORPTION

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ABSTRACT

The Donnan exclusion chromatography associated with nonionic adsorption has been studied on nucleotides (AMP, ADP and ATP) and aromatic acids (phthalic acid, benzoic acid, salicylic acid and phenol) using crosslinked dextran cation-exchangers, SP-Sephadex and CM-Sephadex. It has been clarified experimentally based on a theoretical prediction that the combination of ionic exclusion and adsorption effects can make the separation in the Donnan exclusion range more effective in some cases.

INTRODUCTION

The phenomena of ionic exclusion from ion-exchangers have quantitatively been elucidated by Donnan exclusion distribution and extended to a mutual separation of differently charged ions

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(not the counter ions but the co-ions for an ion-exchanger) under the term " Donnan exclusion chromatography " (1,2), such as phosphorus oxoanions (1), organic ions (3) and metal ions (1). Also the property that the distribution coefficient or the elution position of a typical ion depends only on its ionic charge (the average charge in the case of mixture of two states in equilibrium) has been utilized to the charge determination or the evaluation of stability constants for labile complexes (4). It has also be suggested that other factors such as molecular size (5) and nonionic adsorption (3) can sometimes make the separation by Donnan exclusion chromatography more effective. In this paper, details of the latter effect will be demonstrated with nucleotides and aromatic acid anions using swollen crosslinked-dextran cation exchangers.

EXPERIMENTAL

Chemicals

The sodium salts of *cyclo*-triphosphate $Na_3P_3O_9 \cdot 6H_2O$ and linear polyphosphate($\bar{n}$=100) were prepared in our laboratory. Adenosine monophosphate $AMP \cdot 2Na \cdot H_2O$, adenosine diphosphate $ADP \cdot 2Na \cdot 2H_2O$, adenosine triphosphate $ATP \cdot 2Na \cdot 3H_2O$, phthalate $C_6H_4(COOK)(COOH)$, benzoate C_6H_5COONa, salicylate $C_6H_4(OH)(COONa)$, phenol C_6H_5OH, phosphite $Na_2PHO_3 \cdot 5H_2O$, hypophosphate $NaPH_2O_2 \cdot H_2O$ and crotonaldehyde *trans*-$CH_3CH{=}CHCHO$ were of commercially available reagent grade. All other chemicals were also of reagent grade.

Cation-exchange columns

Both a cross-linked dextran gel type strong acid cation-exchanger, SP-Sephadex C-25(Na^+ form), and the same gel type weak acid cation-exchanger, CM-Sephadex C-25(Na^+ form), were converted into the tetramethylammonium ion form before chromatographic runs. These ion-exchangers were packed into a column to make a bed dimension 1.0 cm I.D. X 66.5 cm, 1.0 cm I.D. X 92.0 cm or 1.5 cm I.D. X 96.0 cm in water.

Eluents

The tetramethylammonium chloride eluents of appropriate concentrations were prepared at a desired pH adjusted with a small amount of hydrochloric acid or tetramethylammonium hydroxide. The mixed solution of 0.40 M tetramethylammonium chloride and 0.10 M ammonium chloride-ammonia buffer was also used as an eluent.

Elution procedure

After the column was pretreated with the eluent, 0.1 ml (or 1.0 ml for 1.5 cm I.D. column) of sample solution dissolved in the same eluent was added to the top of the column. Then elution was carried out at room temparature (20 ± 2 °C) and flow rate (50 ml/h). The effluent was collected by fractions of 0.5 ml (or 1.0 ml for 1.5 cm I.D. column) or introduced directly to a flow cell of a variable wavelength spectrophotometer (LDC Model SpectroMonitor III) for the components with light-absorptivity.

Analyses of sample components

Inorganic phosphorus oxoanions were determined spectrophotometrically with a molybdenum (V) - molybdenum (VI) reagent. Lower phosphorus oxoanions were determined by the same reagent after oxidation with hydrogen sulfite. Crotonaldehyde, nucleotides and aromatic acids were directly determined at 224 nm, 260 nm and 275 nm, respectively, utilizing their own UV light absorption.

Determination of distribution coefficient

The distribution coefficient, K_D, of a given sample component was estimated from the relation $V_e = V_0 + K_D V_D$, where V_e is the peak elution volume, V_0 the void volume and V_D the net volume of the exchanger phase, which were determined according to the literture (1).

RESULTS AND DISCUSSION

The Donnan exclusion distribution coefficient of an anion X^{x-} eluted by ionic exclusion from a B^+-form cation exchange column is expressed by the following equation.

$$K_D = \frac{[\ \overline{X^{x-}}\ ]}{[\ X^{x-}\ ]} = R^x \tag{1}$$

where

$$R = \frac{[\ B^+\ ]}{[\ \overline{B^+}\ ]} \cdot \frac{y_x^{1/x}\ y_B}{\overline{y_x^{1/x}}\ \overline{y_B}} \tag{2}$$

Here blacket and y are molar concentration and activity coefficient, respectively, and bar refers to the ion-exchanger phase. When the activity coefficient quotient R is constant, K_D may depend only on the ionic charge of the sample component, as far as a steric exclusion or a nonionic adsorption to the ion-exchanger matrix dose not take place. The reliability of this assumption was shown experimentally by a linear relationship between log K_D and the ionic charge with various ions and SP-Sephadex previously (1). This was also confirmed at another ionic strength (I = 0.5) and at another ion-exchanger, CM-Sephadex. (Fig. 1)

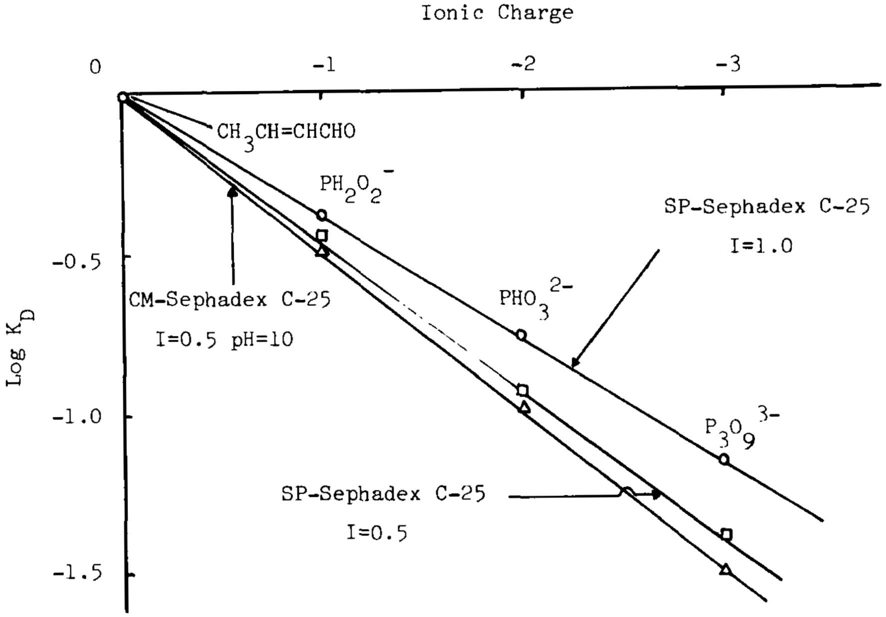

FIGURE 1

The relationship between distribution coefficient K_D and ionic charge

The situation may become a little more complicated when non-ionic adsorption of the sample components onto an ion-exchanger is concerned, as encountered for aromatic compounds. In this case we should distinguish two states for the sample ion present in an ion-exchanger phase. The concentration ratio of these two states should be kept constant by the adsorption constant.

$$K^* = \frac{[\overline{X^{*x-}}]}{[\overline{X^{x-}}]} \qquad (3)$$

Thus the distribution coefficient can be written as

$$K_D = \frac{[\overline{X^{x-}}] + [\overline{X^{*x-}}]}{[X^{x-}]} = R^x (1 + K^*) \qquad (4)$$

Next we consider the mutual separation of differently charged components X_1^{x-} and X_2^{x-}. If the separation factor is defined as the ratio of the two distribution coefficients, the separation factor in the Donnan exclusion chromatography where an nonionic adsorption is involved can be expressed by

$$S_2^1 = K_{D,1} / K_{D,2} = R^{x_1 - x_2} \cdot \frac{1 + K^{*1}}{1 + K^{*2}} \qquad (5)$$

which is easily derived from eqn (3) and (4). When $x_1 > x_2$ and $K^{*1} > K^{*2}$, that is, an ion of the lower charge has the stronger adsorptivity, both $R^{x_1 - x_2}$ and $1 + K^{*1} / 1 + K^{*2}$ become larger than unity since R is less than unity. Therefore, eqn (5) indicates that the adsorption can assist the Donnan exclusion chromatography and also that the Donnan exclusion principle can make the adsorption chromatography more effective in some cases. In the present work this was confirmed in the separation of nucleotides or some aromatic acids by comparing the neutral gel and the ion-exchanger from the same crosslinked dextran matrix.

Fig. 2 shows the elution curves of ATP, ADP and AMP from two columns of the same dimension with Sephadex G-25 and SP-Sephadex C-25, and with the same eluent. At pH 3.0 where the separation was carried out, the charge of ATP, ADP and AMP are considered to be approximately -2, -1 and 0, respectively (6). The separation with the neutral gel Sephadex G-25 is undoubtedly caused by the difference of their adsorptivities. It can be seen from the Fig. 2 that the Donnan exclusion chromatography with SP-Sephadex renders a better separation for any pair of the sample components than with Sephadex G-25.

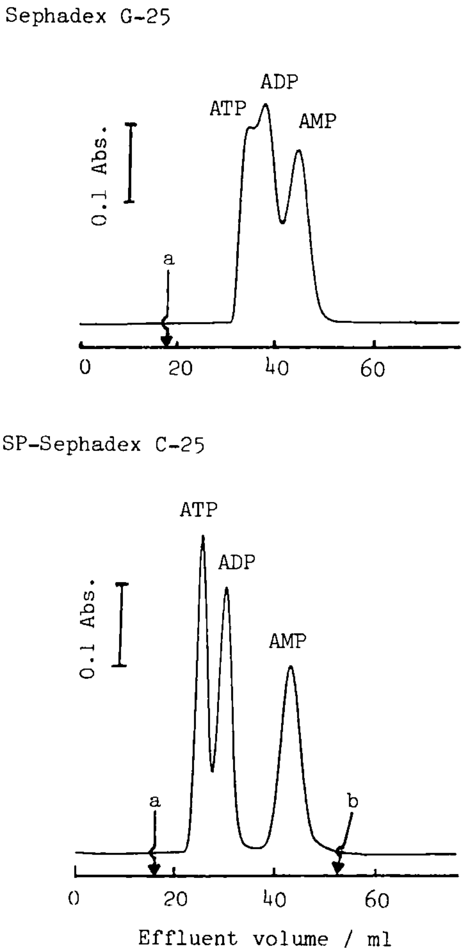

FIGURE 2

Separation of nucleotides Column; 1.0 cm I.D. X 66.5 cm Eluent; 0.5 M Me_4NCl (pH 3.0) Sample; each 7.5×10^{-7} mol of ATP, ADP and AMP a; interstitial volume b; one column volume

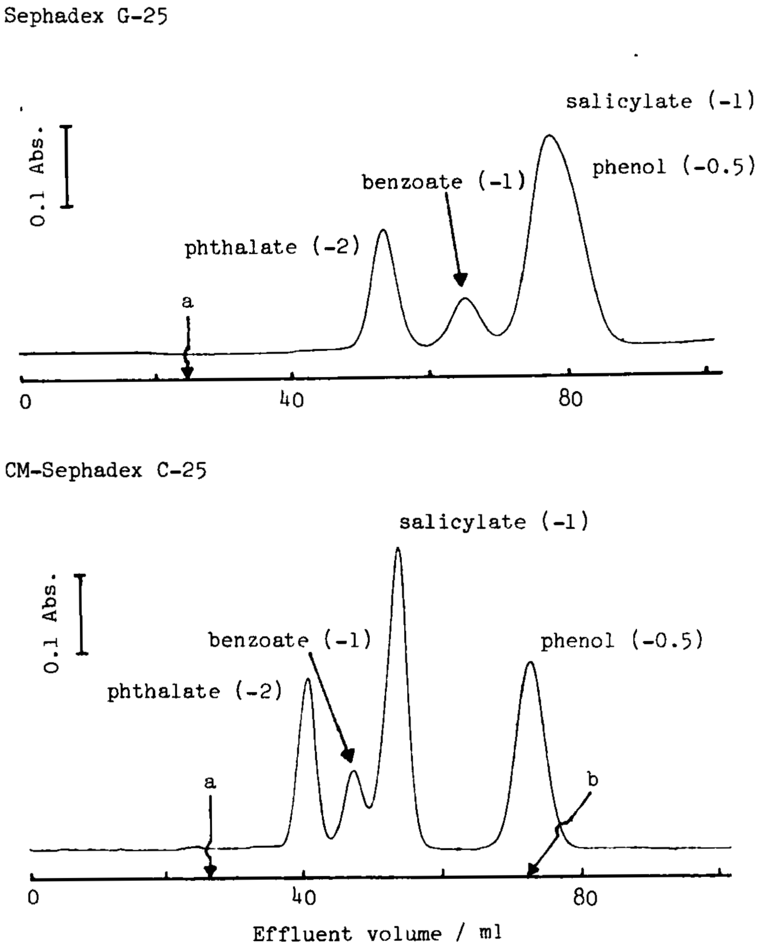

FIGURE 3

Separation of aromatic anions. Column; 1.0 cm I.D. X 92.0 cm Eluent; 0.4 M Me_4NCl + 0.1 M NH_4Cl-NH_3 buffer(pH 10) Sample; each 8 X 10^{-7} mol of $C_6H_4(COOK)(COOH)$, C_6H_5COONa, $C_6H_4(OH)(COONa)$, C_6H_5OH a; interstitial volume b; one column volume

A complete separation may be accomplished by the use of a larger column, if necessary.

Another instance of the comparison is demonstrated with phthalate, benzoate, salicylate and phenol using Sephadex G-25 and CM-Sephadex C-25 columns of the same dimension in Fig. 3. The charges of these components are considered to be approximately -2, -1, -1 and -0.5 at pH 10, respectively (7). The fact that the elution volumes for some components exceed one column volume indicates the presence of nonionic adsorption to the gel matrix. Again better separation factors could be observed on each pair of components for the ion-exchanger than for the other. The phenomena that ion-exclusion and adsorption chromatography assist to each other may be utilized in many other practical separations.

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