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DONNAN EXCLUSION CHROMATOGRAPHY

I. THEORY AND APPLICATION TO THE SEPARATION OF PHOSPHORUS OXOANIONS OR METAL CATIONS

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SUMMARY

Donnan exclusion chromatography is a special type of ion-exchange separation method based on the electrostatic repulsion between the sample ions and fixed ionic groups of an exchanger. The elution behaviour in this chromatography is well explained by the Donnan equilibrium relation of ions between two phases. Practical separations for various oxoanions of phosphorus and for metal cations are reported. Possibilities of this chromatography in other applications are also discussed, as well as theoretical considerations.

INTRODUCTION

Donnan exclusion chromatography (DEC) is a new type of separation method based on the principle by which anions are separated using a cation exchanger or cations using an anion exchanger. In both cases ions of higher charge are eluted earlier according to charge exclusion by the fixed ionic groups of the ion exchanger. A simple separation of undissociated weak acid from the dissociated anions has previously been carried out by Wheaton and Baumann¹, and by Crone². Also, a few examples of anion-exclusion liquid chromatography with cation exchangers have been reported^{3–5}, though they have not given any explanation on quantitative grounds. We have studied the behaviour of phosphorus oxoanions and carboxylic acids, introducing the theory of DEC. The separation of metals in their cationic state was also examined with an anion-exchanger column.

EXPERIMENTAL

Chemicals

Orthophosphate (P_1^V), diphosphate (P_2^V), hypophosphite and phosphite samples were commercially available $NaH_2PO_4 \cdot 2H_2O$ or Na_2HPO_4 , $Na_4P_2O_7 \cdot 10H_2O$, $NaPH_2O_2 \cdot H_2O$ and $Na_2PHO_3 \cdot 5H_2O$ of reagent grade. Triphosphate (P_3^V , $Na_5P_3O_{10} \cdot 6H_2O$) samples were purified from the commercial reagent. Trimeta-, tetrameta- and

octametaphosphate, $\text{NaP}_3\text{O}_9 \cdot 3\text{H}_2\text{O}$, $\text{Na}_4\text{P}_4\text{O}_{12} \cdot 4\text{H}_2\text{O}$ and $\text{Na}_8\text{P}_8\text{O}_{24} \cdot 8\text{H}_2\text{O}$ were prepared in our laboratory. Sodium and magnesium chlorides, and silver, potassium and cobalt nitrates were all of reagent grade.

Cation-exchange columns

SP-Sephadex C-25 (Na^+) was converted into the tetramethylammonium ion form and made to a bed of 46×1.5 cm I.D. (column A) or 96×1.5 cm I.D. (column B). Column A was used in the determination of distribution coefficients of various ions. Column B was used for relatively difficult separations.

Eluents for anion separation

Tetramethylammonium chloride (1.00 M), or 0.95 M tetramethylammonium chloride plus 0.05 M tetramethylammonium acetate was employed at the required pH. The pH of the eluent was adjusted by the addition of a small amount of tetramethylammonium hydroxide and acetic acid.

Elution procedure for anions

The column was preconditioned with the eluent, then 1 ml of a sample solution containing a small amount of the ions to be studied and the same concentration of the eluting agent was added to the top of the column. The eluent was passed through the column at room temperature and at a definite flow-rate. The effluent was collected into a fraction collector (each 1-ml volume) and analyzed as described below.

Determination for anions

Phosphorus in various oxoanions was determined colorimetrically with a molybdenum(V)–molybdenum(VI) reagent by the usual method. For lower oxoanions of phosphorus sodium bisulfite was also used. Ammonia was determined by direct pH measurement of the effluent. In the case of weak acid anions, the concentrations were evaluated from the pH change of the effluent after addition of a certain amount of hydrochloric acid with aid of a calibration curve. Crotonaldehyde was determined by UV spectrophotometry at 224 nm. Sodium and magnesium were determined with an atomic absorption spectrophotometer.

Elution for cations on anion exchanger

QAE-Sephadex A-25 (Cl^-) was converted into the nitrate ion form and made to a bed of 39×1.0 cm I.D. After the column was conditioned with 0.3 M sodium nitrate, 0.5 ml of sample solution containing small amounts of silver, potassium and cobalt nitrates was added to the top of the column, then the eluent, 0.3 M sodium nitrate, was passed through. Silver ions in the effluent were analyzed titrimetrically with potassium thiocyanate, potassium ions potentiometrically with an ion-selective electrode and cobalt ions spectrophotometrically after the addition of concentrated hydrochloric acid.

The distribution coefficient, K_D , of a given sample ion was chromatographically determined from the relation $V_e = V_0 + K_D V_D$, where V_e is the elution volume, V_0 the void volume and V_D the net internal volume of the exchanger phase. V_e was determined from the effluent volume at the maximum concentration of the eluted component. V_0 was assumed from the elution volume of ions of very high charge.

V_D was calculated from the elution volume for neutral species such as ammonia molecules and the V_0 value. Both V_0 and V_D were *ca.* 43% of a column bed volume for column A at 1.00 M tetramethylammonium chloride. The other 14% corresponds to the skeleton volume of exchanger material.

RESULTS AND DISCUSSION

In order to make an effective separation by DEC highly swollen ion exchangers must be used, because a sufficient volume of effluent in the non-sorption region is required for good resolution. From a comparison of ordinary Dowex 50W-X1 and cross-linked dextran ion exchanger SP-Sephadex C-25, it was concluded that the latter was much better for the resolution of phosphite and hypophosphite.

In the system of a B^+ -form cation exchanger and a bulk electrolyte B^+A^- solution, the Donnan equilibrium of a given anion X^{x-} of small size (no steric exclusion) can be expressed by

$$[X^{x-}]_R [B^+]_R^x \bar{y}_X \bar{y}_B^x = [X^{x-}] [B^+]^x y_X y_B^x \quad (1)$$

where y is an activity coefficient and the bar or subscript R refers to the exchanger phase.

The distribution coefficient, K_D , is defined and represented as

$$K_D \equiv \frac{[X^{x-}]_R}{[X^{x-}]} = \left(G \cdot \frac{[B^+]}{[B^+]_R} \right)^x \quad (2)$$

where

$$G \equiv \frac{y_X^{1/x} y_B}{\bar{y}_X^{1/x} \bar{y}_B}$$

When G is constant irrespective of the kind of ion, the term $G \cdot [B^+]/[B^+]_R$ remains constant and we use a factor R for this term, which is dependent only on the concentration of the background electrolyte (eluent). Then we have

$$K_D = R^x \quad (3)$$

It can be deduced from eqn. 3 that K_D is determined only by the ionic charge under a given eluent and decreases with the increase of anionic charge since R is usually less than unity. For a given ion, K_D increases with the eluent concentration through faster increase of $[B^+]$ than that of $[B^+]_R$.

The logarithm of K_D determined chromatographically is plotted against the anionic charge in Fig. 1. It can be seen that this plot gives a good linearity when a value of 35.0 ml for V_0 in the 81.6-ml bed (column A) is assumed, which shows constancy of R . An important result is that the ionic size has no effect on the elution position as long as the ion is not very large, as indicated by the same K_D values of acetate and valerate ions. Consequently this principle may be applied to a group separation of different charges in some cases. When the sample anions are the equilibrium mixture of different charge states, the anions are eluted in one peak at a posi-

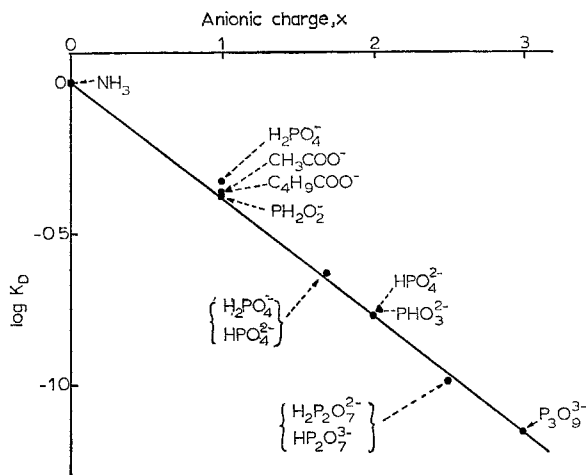


Fig. 1. The relationship between charge and distribution coefficient, K_D . Column: SP-Sephadex C-25 $(\text{CH}_3)_4\text{N}^+$ -form, 46.0×1.5 cm I.D. Eluent: $0.95 \text{ M } (\text{CH}_3)_4\text{NCl} + 0.05 \text{ M } (\text{CH}_3)_4\text{NOAc}$.

tion corresponding to their average charge. This behaviour may also be applicable to the charge determination of unknown species.

Assuming the constancy of R in eqn. 3, it is easy to calculate the maximum separability for the combination of different charges. Separability, S , between anions of adjacent charge number x and $x + 1$ may be written as the difference of corresponding distribution coefficients, considering that the elution position does not affect the peak width very much in the exclusion region.

$$S \equiv K_{D(x)} - K_{D(x+1)} = R^x(1 - R) \quad (4)$$

S is differentiated with R and this is set zero to get the maximum separability. When $R_{\text{max}} = x/x + 1$, the separability gives a maximum value.

$$S_{\text{max}} = \frac{x^x}{(x + 1)^{x+1}} \quad (5)$$

The higher the charges, the more difficult the separation becomes. Higher concentration of eluent may be preferable for ions of higher charge. Separation of divalent and trivalent ions seems to be a practical limit ($S_{\text{max}} = 0.15$) for easy operation.

The separation of monovalent and divalent anions was very easy and each component was quantitatively recovered in a small volume of effluent (Fig. 2).

The direct separation of highly charged anions such as tri-, tetra- and penta-valent ions was rather difficult, but a parallel depression of charge by protonation or ion-pair formation with cations may improve the separation (Fig. 3). A small amount of EDTA was added to the eluent to make a masking iron impurity.

A successive separation of five phosphorus oxoacids of different anionic charges was achieved at a good resolution (Fig. 4). Here large octameta- and tetrametaphosphate ions may be considered to be eluted out by both charge and steric exclusion.

DEC combined with ion exchange, *i.e.* the simultaneous separation of anionic, neutral and cationic species over a non-sorption and a sorption region, was carried out for -2 to $+2$ charges (Fig. 5). All species were separated from each other with a single cation-exchange column.

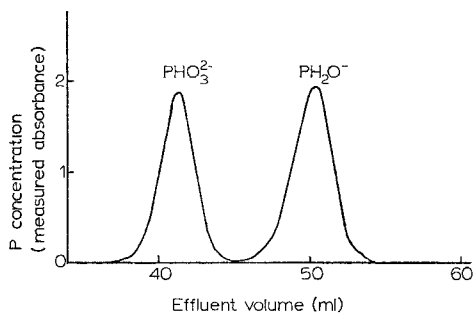


Fig. 2. Separation of phosphite and hypophosphite ions by DEC. Column: SP-Sephadex C-25 $(\text{CH}_3)_4\text{N}^+$ -form, 46.0×1.5 cm I.D. Eluent: $0.95 \text{ M } (\text{CH}_3)_4\text{NCl} + 0.05 \text{ M } (\text{CH}_3)_4\text{NOAc}$ (pH 9.8). Flow-rate: 0.6 ml/min . Sample: $\text{PHO}_3^{2-} 2.5 \cdot 10^{-3} \text{ mmol}$, $\text{PH}_2\text{O}_2^- 2.7 \cdot 10^{-3} \text{ mmol}$.

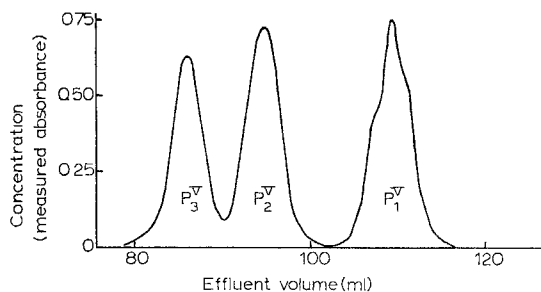


Fig. 3. Separation of linear phosphate anions by DEC at depressed charge. Column: SP-Sephadex C-25 K^+ -form, 96.0×1.5 cm I.D. Eluent: 0.5 M KCl , $0.1\% \text{ EDTA}$ (pH 4.7). Sample: $\text{P}_1^{\text{V}} 4 \cdot 10^{-3} \text{ mmol}$, $\text{P}_2^{\text{V}} 2 \cdot 10^{-3} \text{ mmol}$, $\text{P}_3^{\text{V}} 1.3 \cdot 10^{-3} \text{ mmol}$.

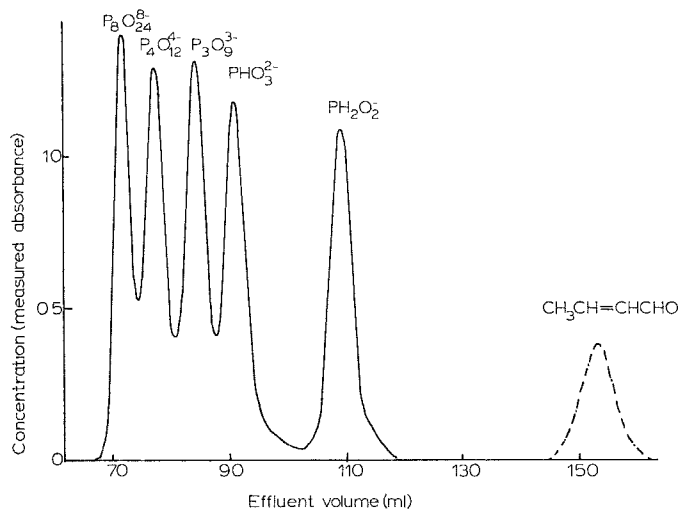


Fig. 4. Separation of phosphorus oxoanions by DEC. Column: SP-Sephadex C-25 $(\text{CH}_3)_4\text{N}^+$ -form, 95.8×1.5 cm I.D. Eluent: $1.00 \text{ M } (\text{CH}_3)_4\text{NCl}$ (pH 9.8). Flow-rate: 0.75 ml/min . Sample: each 10^{-3} – 10^{-2} mmol .

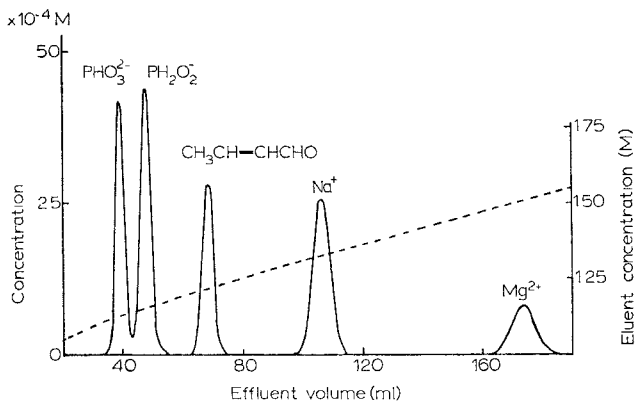


Fig. 5. Donnan exclusion chromatography combined with ion-exchange chromatography. Column: SP-Sephadex C-25 $(\text{CH}_3)_4\text{N}^+$ -form, 46.0×1.5 cm I.D. Eluent: $(\text{CH}_3)_4\text{NCl}$ (pH 9.8) gradient elution. Flow-rate: 0.6 ml/min. Sample: each 10^{-3} mmol.

DEC is applicable to cations as well as to anions. The separation of metals in their cationic states was carried out with an anion-exchange column (Fig. 6). Each component was collected in only a few millilitres of effluent. The equilibrium mixture of silver was eluted at a position between the monovalent and neutral positions, indicating that the mean charge is *ca.* 0.5 for nitrate complex formation.

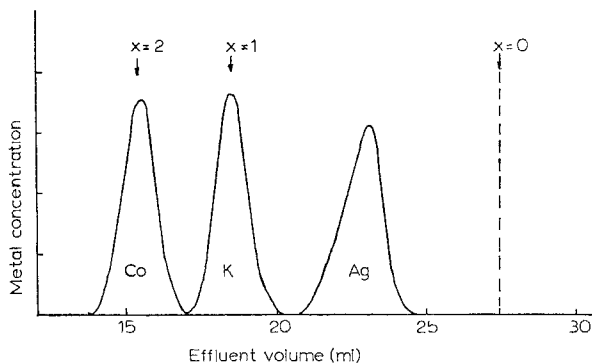


Fig. 6. Donnan exclusion chromatography for cations. Column: QAE-Sephadex A-25 NO_3^- form, 39.0×1.0 cm I.D. Eluent: 0.3 M NaNO_3 . Flow-rate: 0.4 ml/min. Sample: each 0.025 mmol.

The principal advantage of DEC is that the time required to separate ions is shorter and the elution volume is smaller than with an ordinary ion-exchange separation, because these ions are eluted within one column volume.

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