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DONNAN EXCLUSION CHROMATOGRAPHY:
THE ELUTION BEHAVIOR OF PHOSPHORUS
OXOPOLYANIONS OF LARGE SIZE IN DEC

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ABSTRACT

The elution behavior of ions of large size in Donnan exclusion chromatography has been elucidated by an assorted ionic and steric exclusion. An effective model for the swollen ion-exchanger phase such as a cross-linked dextran cation-exchanger and a structural size parameter for various oxoanions of phosphorus have been proposed. These studies may help the prediction for elution positions of ions excluded from an ion-exchanger column.

INTRODUCTION

Donnan exclusion chromatography (DEC) is a new type of separation method utilizing an electrostatic repulsion between sample ions and fixed ionic groups of an ion-exchanger. Accordingly, cation-exchangers should be used for the mutual separation of anions and anion-exchangers for cations(1-7). In the previous paper(1), a theoretical ground was given on the basis of the Donnan distribution equilibrium of sample ions between solution

and ion-exchanger phase; the logarithm of the distribution coefficient is found to be proportional to the practical ionic charge on sample ions. Further, it was pointed out that species of large size, such as phosphorus oxoanion polymers, undergo both ionic and steric exclusion effects and are eluted at a volume position before that which would be predicted purely from the ionic exclusion effect.

For a steric exclusion effect with ion-exchangers, Samuelson(8,9) performed many experiments with various organic substances. He generally correlated the elution behavior of oligomers such as sugar derivatives with their partial molar volumes, using the Gibbs-Donnan relationship. However, his treatment could not easily be applied to our case, where the sample components are highly charged and hydrated, and their conformations in solution or their effective sizes are not very clear.

It is very important to elucidate the elution behavior in terms of basic properties of sample ions, such as the molecular structure under an appropriate model on the interior structure of the ion-exchanger. For this purpose we employed oxopolyanions of phosphorus, which have a variety of sizes and structures.

EXPERIMENTAL

Chemicals Orthophosphate; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ or Na_2HPO_4 , diphosphate(pyrophosphate); $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$, phosphonate (phosphite); $\text{Na}_2\text{PHO}_3 \cdot 5\text{H}_2\text{O}$ and phosphinate(hypophosphite); $\text{NaPH}_2\text{O}_2 \cdot \text{H}_2\text{O}$ were of commercially available reagent grade. Triphosphate; $\text{Na}_5\text{P}_3\text{O}_{10} \cdot 6\text{H}_2\text{O}$ was prepared by recrystallization from commercial anhydrous triphosphate. Other linear polyphosphates, salts of lower oxoacid of phosphorus and metaphosphates were prepared in our

laboratory. Crotonaldehyde; *trans*- $\text{CH}_3\text{CH}=\text{CHCHO}$ was of reagent grade.

Eluent The eluent was 1.00 M tetramethylammonium chloride solution prepared with a small amount of hydrochloric acid or tetramethylammonium hydroxide to desired pH. The solution of tetramethylammonium chloride was standardized titrimetrically with silver nitrate solution.

Elution procedure A pyrex tubing column of 15 mm I.D. x 95.8 cm was packed with SP-Sephadex C-25 (40–120 μm particle size, tetramethylammonium form). After the column was conditioned with the eluent, one ml of a sample solution, adjusted into the same pH as the eluent, was added onto the top of the column, then the eluent was passed through the column. The effluent was collected by a fraction collector (each one ml volume), and analyzed as described below.

Determination of sample ions Phosphorus for various oxoanions were determined colorimetrically with a molybdenum(V)-molybdenum(VI) reagent by a standard method(11). For lower oxoacid of phosphorus, sodium hydrogensulfite solution was added as oxidizing agent. Crotonaldehyde was determined UV-spectrophotometrically at wavelength 224 nm.

RESULTS AND DISCUSSION

It was found that the Donnan exclusion distribution coefficient of a certain ion $\text{X}^{\text{X}-}$ eluted from a $\text{B}^{\text{+}}$ -cation-exchanger column is expressed by the following equation (1);

$$K_D = R^{\text{X}} \quad (1)$$

$$R = \frac{[B^+] \cdot Y_X^{1/x}}{[B^+]_r \cdot \bar{Y}_X^{1/x}} \cdot \frac{Y_B}{\bar{Y}_B} \quad (2)$$

where y and \bar{y} are activity coefficients of subscript species for solution and exchanger phase. Here R may be considered to be approximately constant under a given eluting condition. K_D is related to its elution position by an ordinary chromatographic relationship,

$$V_e = V_0 + K_D \cdot V_D \quad (3)$$

where V_e is the elution volume, V_0 void volume and V_D the net internal volume of the exchanger phase. It has been experimentally supported by a linear relationship between $\log K_D$ and an anionic charge that R is constant if the size of sample anion is not very large. However, this does not fit ions which are of large size, exceeding a certain limit. In Fig. 1-(A), there was a considerable difference between elution volumes for octametaphosphate $P_8O_{24}^{8-}$ and long-chained linear polyphosphate P_nO_{3n+1} ($\bar{n}=55$) ions, although these ions should be eluted at nearly the same volume position ($K_D \approx 0$). Conventional theories could not explain such distribution of highly-charged ions which should be completely excluded by the ionic exclusion effect. On the other hand, as the ionic exclusion effect can be evaluated by Donnan exclusion chromatography, the steric effect can be discriminated from the ionic exclusion effect. Therefore, another model for the interior of an ion-exchanger of the present type must be proposed for quantitative explanation and a structural parameter for the steric effect is introduced.

The ion-exchanger interior can be divided, for convenience, into two phases where the fixed ionic groups are present in a concentrated state (Donnan exchanger phase) and in a diluted state (pseudo exchanger phase),

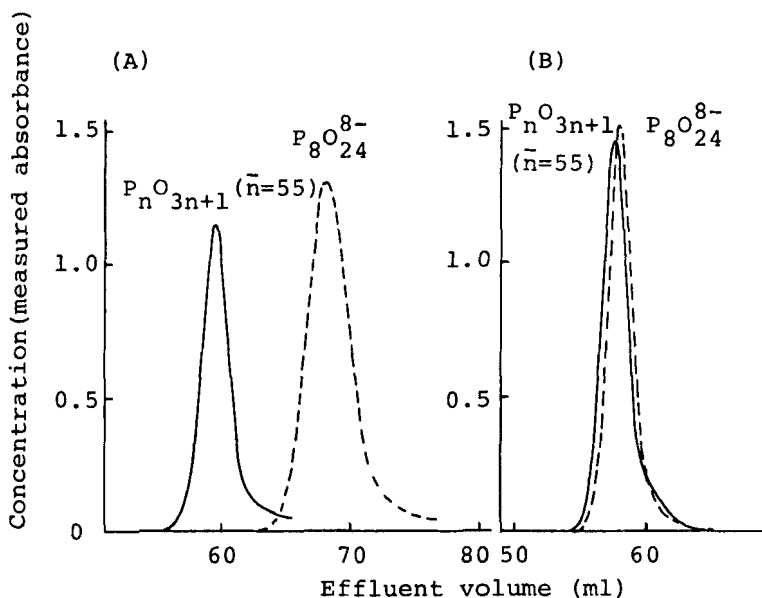


FIGURE 1

Elution behavior of Species sterically excluded.

Column; SP-Sephadex C-25, $(\text{CH}_3)_4^+$ -form, (A) 1.5 cm I.D. x 95.8 cm and (B) 1.5 cm I.D. x 95.5 cm. Eluent; (A) 1.00 M and (B) 0.10 M $(\text{CH}_3)_4\text{NCl}$ (pH 9.8). Flow rate; 0.75 ml/min. Sample amount; 0.7×10^{-6} mol $\text{Na}_8\text{P}_8\text{O}_{24} \cdot 8\text{H}_2\text{O}$, 3.0×10^{-6} mol (as P) $\text{Na}_n\text{P}_n\text{O}_{3n+1}$ ($\bar{n} = 55$).

as shown in Fig. 2. The phase of concentrated fixed ionic groups may maintain its ion-exchanger characteristics such as adsorption or ionic exclusion even after a considerable invasion of outside electrolytes takes place. On the other hand, the phase of dilute fixed ionic groups may be no longer considered as a real ion-exchanger phase, when the exchanger is immersed in a concentrated electrolyte solution, because the internal solution of this phase becomes nearly the same solution

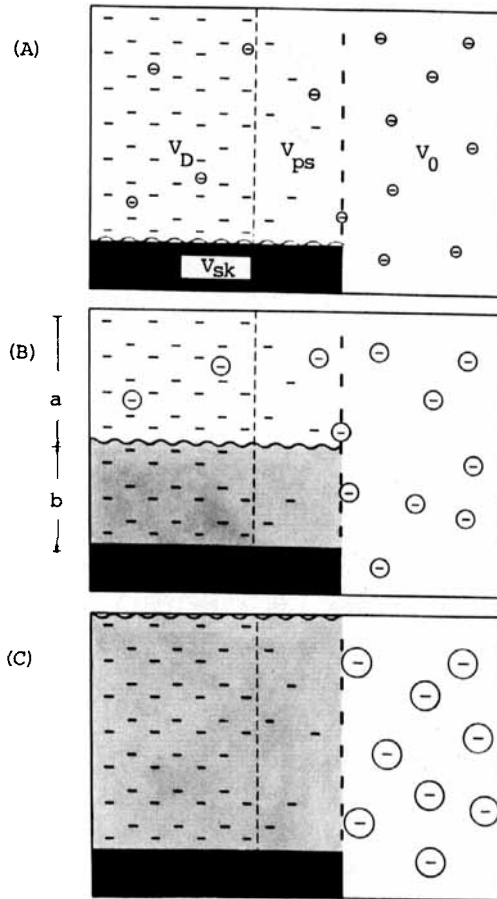


FIGURE 2

A proposed structure model for a swollen dextran cation-exchanger. (A) Anions of small size which cannot be excluded by steric exclusion effect. (B) Anions partially excluded by steric exclusion effect. (C) Anions of very large completely excluded by steric effect. V_0 ; external interstitial phase volume, V_{ps} ; pseudoe exchanger phase volume, V_D ; Donnan exchanger phase volume, V_{sk} ; skeleton volume, $-$; fixed anion of cation-exchanger, \ominus ; sample anion, α ; ion-penetratable volume fraction in ion-exchanger ($\alpha = a/a+b$).

as the external solution by remarkable electrolyte invasion. Even highly-charged anions could not be excluded from this pseudo exchanger phase as long as they are of small size while anions of very large size are completely excluded from both phases of cation-exchanger. However, the volume fraction of such pseudo exchanger phase varies with the electrolyte concentration of external solution(Fig. 1-(B)). When the exchanger is merely immersed in water, the difference between this phase and the external water becomes significant, and therefore, all movable anions should be excluded completely by the ionic exclusion effect irrespective of molecular size; this was confirmed by the fact that all anions are eluted together at the same effluent volume from a cation-exchanger column. Of course, it is not argued here that the distinct boundary between the inside phases is actually present. The concentration of fixed ionic groups may vary somewhat more continuously over the whole exchanger phase. Therefore, our treatment is just an approximation for theoretical convenience.

Under these assumptions, the elution volume V_e for large anions which undergo both ionic and steric exclusions, can be given by the following equation

$$V_e = V_0 + \alpha (V_{ps} + K_D \cdot V_D) \quad (4)$$

instead of Eqn (3). Here V_0 is the volume of the external interstitial solution, V_{ps} that of pseudo exchanger phase and V_D that of the Donnan exchanger phase. The α is the volume fraction of the ion-penetratable part in an ion-exchanger($a/a+b$ in Fig, 2-(B)), which depends upon the ion structure and becomes unity in case of small ions (corresponding to the distribution coefficient K_d in gel-permeation chromatography). The K_D in Eqn (4) is not an apparent distribution ratio, but an authentic distribution coefficient between the Donnan exchanger

phase and the solution phase. Among these quantities, V_0 can be determined from the elution volume for extremely large ions such as long-chained polyphosphate ions ($\bar{n}=55$), and evaluated to be 59.5 ml for a column of 169.3 ml. On the other hand, V_{ps} can not directly be measured since the small ions of extremely high charge are not available. This was determined by a trial and error treatment with the consideration that the assumption of an appropriate V_{ps} value should bring about the linear relationship between $\log K_D$ and ionic charge(1). The $\log K_D$ -ionic charge plot with small ions at $V_{ps} = 18.2$ ml for the same column gave a straight line, as shown in Fig. 3.

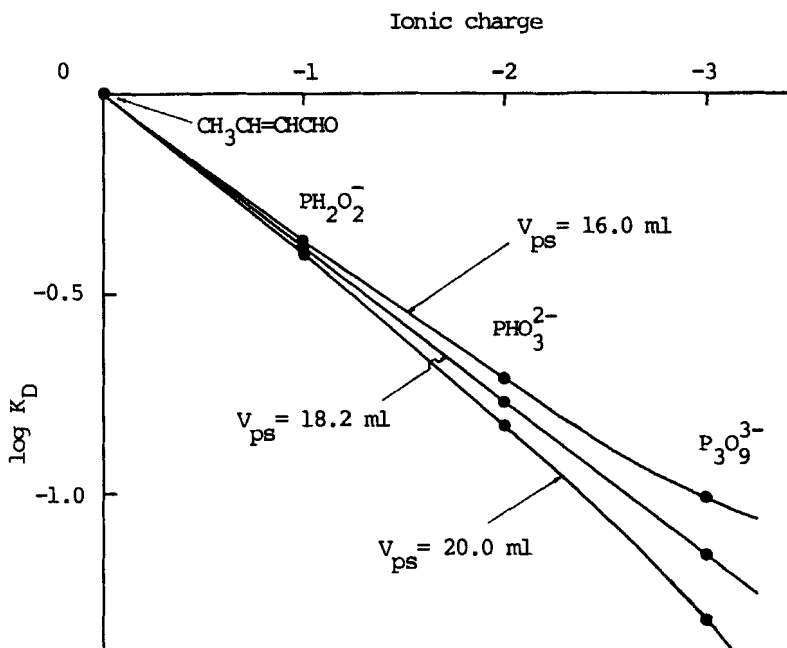


FIGURE 3
Estimation of V_{ps} .

Next, V_D was estimated to be 74.8 ml by subtracting $V_0 + V_{ps}$ from the elution volume of neutral species of 152.5 ml (crotonaldehyde; $K_D = 1$, $\alpha = 1$). The skeleton volume of an ion-exchanger can also be calculated from these data and total column volume. The volume allocation of the cation-exchanger, SP-Sephadex C-25 equilibrated with 1.00 M tetramethylammonium chloride solution is given in TABLE 1. Although these relative volumes are, of course, dependent upon types of ion-exchangers and compositions of external solutions, the understanding of the phase composition of a swollen ion-exchanger may serve to interpret the elution phenomena of various ions from such an ion-exchanger.

The α value indicates to what extent, in terms of volume fraction, the exchanger permits the penetration of a particular ion and directly depends upon the dimension of the ion. To know the dependence of α on the ion size, we measured α on series of linear polyphosphates, cyclic metaphosphates and lower oxoanions of phosphorus. The K_D of a highly charged anion was estimated from the theoretical straight line in Fig. 3, since it can not be determined experimentally. Then K_D and V_e values of individual sample anions were

TABLE 1
Composition of SP-Sephadex C-25 Column Equilibrated
with 1.00 M $(\text{CH}_3)_4\text{NCl}$ Solution

Volume allocation(%)	
V_0	35.1
V_{ps}	10.8
V_D	44.2
V_{sk}	9.9

TABLE 2
Structural Size Parameters for Oxoanions of Phosphorus

Number	Species	z	i_e	p	α
Linear phosphorus oxoanions					
1	PH_2O_2^-	3	1	4	1.00
2	PHO_3^{2-}	3	2	5	1.00
3	H_2PO_4^-	3	1	4	1.00
4	$\text{H}_2\text{P}_2\text{O}_7^{2-}$	5	2	7	0.95
5	$\text{HP}_2\text{O}_7^{3-}$	5	3	8	0.93
6	$\text{P}_2\text{O}_7^{4-}$	5	4	9	0.86
7	$\text{P}_2\text{H}_2\text{O}_5^{2-}$	5	2	7	1.00
8	$\text{P}_3\text{O}_{10}^{5-}$	5	4	9	0.83
9	$\text{P}_3\text{O}_9^{5-}$	6	4	10	0.68
10	$\text{P}_4\text{O}_{13}^{6-}$	7	4	11	0.65
Cyclic phosphorus oxoanions					
11	$\text{P}_3\text{O}_9^{3-}$	5	2	7	1.00
12	$\text{P}_4\text{O}_{12}^{4-}$	7	2	9	0.86
13	$\text{P}_6\text{O}_{18}^{6-}$	9	2	11	0.61
14	$\text{P}_8\text{O}_{24}^{8-}$	11	2	13	0.47
15	$\text{P}_6\text{O}_{12}^{6-}$	6	2	8	0.86

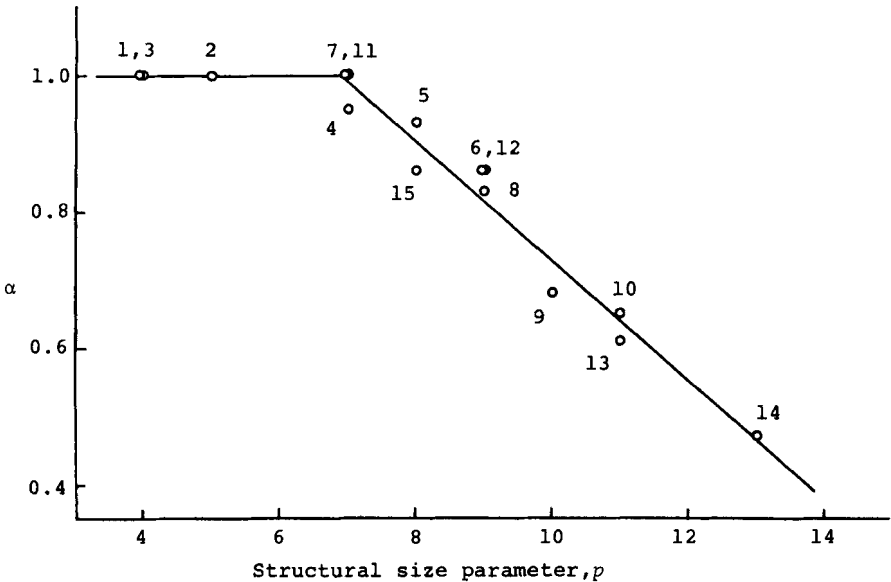


FIGURE 4

Relationship between α and the structural size parameter. Numbers in the figure are the same as in TABLE 2.

substituted in Eqn (4) to calculate α . The α values obtained are tabulated in TABLE 2; this order of α is essentially the same as that of the distribution coefficient obtained by gel chromatography in which influences of eluting agents are taken into account(10).

In order to correlate α with the ion structure, a structural size parameter p was introduced. The parameter was, in principle, taken as the molecular length of hydrated phosphorus oxoanion. Since the conformation of various phosphorus polymers are, in general, not exactly known, we first assumed the net molecular chain length l as the total number of P and O atoms along the molecular chain as a first approximation; for example, $l = 5$ and 7 for trimeta- and tetrametaphosphate ions,

respectively. For triphosphate and longer linear phosphates, the l value was assumed to have the same value as that in the corresponding metaphosphate of the same phosphorus numbers, for instance, l for triphosphate is not seven but five, because a linear chained anion is considered to take a spiral conformation. Furthermore, we added to l the total anionic charge of the two end groups i_e to make a contribution by bound water molecules which affect the actual length of species. The p thus obtained should reflect, at least qualitatively, the hydrated length of an polymer anion in the simplest way. These parameters are given in TABLE 2 together with α 's; the correlation between α and p is shown in Fig. 4. For large phosphorus oxoanions which undergo a steric exclusion from an ion-exchanger, a fairly good relationship can be seen between α and the structural size parameter. This relationship may serve in elucidating the exclusion or elution behavior of a large ionic species and, in some cases, in deducing the molecular conformation in solution state.

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