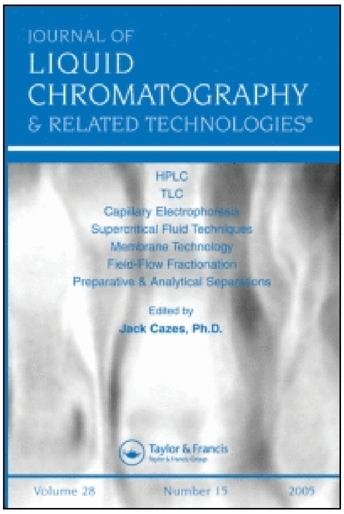

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Gel Chromatographic Behavior of Inorganic Linear and Cyclic Phosphates: Influences of Eluting Agents

Tohru Miyajima[a]; Yukiharu Yoshikawa[a]; Norimasa Yoza[a]; Shigeru Ohashi[a]
[a] Department of Chemistry, Faculty of Science, Kyushu University 33 Hakozaki, Higashiku, Fukuoka, JAPAN

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GEL CHROMATOGRAPHIC BEHAVIOR OF INORGANIC LINEAR AND CYCLIC PHOSPHATES

INFLUENCES OF ELUTING AGENTS

Tohru Miyajima, Yukiharu Yoshikawa, Norimasa Yoza
and Shigeru Ohashi
Department of Chemistry, Faculty of Science, Kyushu University 33
Hakozaki, Higashiku, Fukuoka, 812 JAPAN

ABSTRACT

Inorganic phosphates were chromatographed on a Sephadex G-25 column using lithium chloride, sodium chloride, potassium chloride, rubidium chloride, cesium chloride and tetramethylammonium chloride as an eluting agent at pH 10. When tetramethylammonium chloride was used the distribution coefficient of a linear phosphate was apparently smaller than that of the cyclic phosphate which has the same number of phosphorus atoms in its molecule. It was concluded that gel chromatographic behavior of phosphate species is greatly influenced by the nature of counter ions in an eluent.

INTRODUCTION

As well as in organic polymer chemistry, gel chromatography has a great potentiality in characterizing inorganic polymers such as polyphosphates(1-6) and polysilicates(7). This technique has been used not only for separations of these polymers but also estimation of effective radii of these anions(2). It has been pointed out that the plots of K_{av} values of linear phosphates versus logarithms of the degree of polymerization give a straight line(2,3). In all these cases electrolyte solution of an appropriate concentration has been used as an eluent in order to prevent the Donnan effect(8). Sodium or potassium salt has usually been used for this purpose. The linear and cyclic phosphates which have the same number of phosphorus atoms in a molecule showed almost the same distribution coefficient in these electrolyte solutions(3).

It has recently been recognized that complex formation plays an important role on the gel chromatographic behavior of ionic solutes(9-13). Since sodium or potassium ions bind some oxoanions of phosphorus to form ion-pair, the resulting elution behavior of these anions is expected to be greatly dependent on the nature of the eluting agent.

In this study, the elution behavior of orthophosphate(P_1), diphosphate(P_2), triphosphate(P_3), tetraphosphate(P_4), trimetaphosphate(P_{3m}) and tetrametaphosphate(P_{4m}) were examined using 0.1 M chloride salts of lithium, sodium, potassium, rubidium, cesium and tetramethylammonium ions, because it was expected that the affinity of these cations to phosphate anions is different from each other. When tetramethylammonium chloride was used, a linear phosphate was eluted faster than the cyclic phosphate which has the same number of phosphorus atoms in its molecule. This was attributed to their molecular shapes in tetramethylammonium chloride solution.

EXPERIMENTAL

Materials

Analytical grade disodium monohydrogen orthophosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and tetrasodium diphosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) were used without further purification. Sodium triphosphate hexahydrate ($Na_5P_3O_{10} \cdot 6H_2O$) was recrystallized from ethanol-water mixed solvent. Hexaammonium tetraphosphate hexahydrate ($(NH_4)_6P_4O_{13} \cdot 6H_2O$) (14), trisodium trimetaphosphate trihydrate ($Na_3P_3O_9 \cdot 3H_2O$) (15) and tetrasodium tetrametaphosphate tetrahydrate ($Na_4P_4O_{12} \cdot 4H_2O$) (15) were prepared according to the literaures. Other reagents were analytical grade.

Procedure for elution

Sephadex G-25 columns (15x900 mm or 15x450 mm) were used. 0.1 M electrolyte solutions whose pH were adjusted to 10 by use of NH_3-NH_4Cl buffer were used as eluents. The phosphate anions except for orthophosphate were considered to be almost completely dissociated at this pH. Orthophosphate anion was expected to be monoprotonated, i.e., HPO_4^{2-}, at pH 10. The pH value of an eluent was not raised more than 10 because it has been reported that at pH higher than 10 the gel phase bears negative charges which leads to abnormal behavior of ionic species(11). 0.5 ml portions of sample solutions which contained each phosphate as well as an electrolyte and buffer agents of the same concentration as the eluent were applied to the column. Elution flow rate was 1.7 ml/min. Total phosphate concentration of an effluent was monitored with AutoAnalyzer II (Technicon). The details of the applicability of AutoAnalyzer II as a post column detector of a liquid chromatography was described previously(16). The residence time in the detector was measured to be ca. 18 min. In order to determine the elution volumes of crotonaldehyde ($CH_3CH = CHCHO$), a UV detector (224 nm) was used.

The elution volume of the eluting agents were determined using a conductometric detector. 0.5 ml of water was applied to the column which was equilibrated with 0.1 M background electrolyte and then eluted with the solution. The elution profile gave one negative peak. Since the cations and anions of background electrolyte should migrate with the same velocity through the column in order to satisfy the principle of electrical neutrality, the resulting negative peak shows the elution volume of the eluting agent.

Calculation of a distribution coefficient, K_{av}

A K_{av} value was calculated by the equation

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$

where V_t is the total column volume, V_e is the elution volume of the sample and V_0 is the void volume which is determined with sodium phosphate glass (a mixture of long-chain polyphosphate whose average degree of polymerization is estimated to be 100) or with Blue Dextran 2000 (Pharmacia).

RESULTS AND DISCUSSION

Elution behavior of eluting agents

It has been reported that the elution volumes of anionic species are dependent on the penetrability of the counter cations in the eluent into gel pores(17). If the sizes of counter cations are larger than that of orthophosphate anion, the penetration of the anion may be restricted.

As can be seen from Table 1, the K_{av} values of the eluting agents are always greater than those of orthophosphates, which excludes the idea that the penetration of orthophosphate anion into gel pores was prevented by the cations of supporting electrolytes.

Effect of salt concentration of an eluent

It has been stressed that electrolyte solution of an appropriate concentration should be used as an eluent in order to prevent the Donnan effect(8). The effect of the concentration of the electrolyte on the elution behavior of phosphate anions has been examined. The K_{av} values of orthophosphate (HPO_4^{2-}), trimetaphosphate and tetrametaphosphate obtained by elution with tetramethylammonium chloride as an eluting agent did not change when the electrolyte concentration was between 0.02 and 0.2 M (Fig. 1). It can be concluded that the Donnan effect can be sufficiently suppressed by use of a 0.02 M electrolyte solution as an eluent. However, when sodium chloride was used as an eluting agent, K_{av} values increased gradually with an increase in the concentration. No constancy of the K_{av} values may mislead the conclusion that the Donnan effect is not sufficiently suppressed even in such a high electrolyte concentration as 0.5 M (Fig. 2). The increase in K_{av} values in the sodium chloride system should be explained by an alteration of the solute species due to ion-pair formation between sodium ion and phosphate anions.

Higher concentration of tetramethylammonium chloride solution than 0.2 M leads to a great increase in the K_{av} values (Fig. 1). This phenomenon may be attributed to the solvophobic effect.

TABLE 1

K_{av} Values of Inorganic Phosphates,
Crotonaldehyde and Eluting Agents

	LiCl	NaCl	KCl	RbCl	CsCl	$(CH_3)_4NCl$
P_1	0.54	0.58	0.65	0.60	0.60	0.45
P_2	0.41	0.41	0.50	0.52	0.48	0.20
P_3	0.30	0.31	0.40	0.39	0.39	0.14
P_{3m}	0.30	0.33	0.39	0.40	0.37	0.23
P_4	0.25	0.26	0.33	0.34	0.34	0.10
P_{4m}	0.25	0.27	0.33	0.32	0.32	0.15
Croton-aldehyde	0.71	0.71	0.71	0.72	0.73	0.73
H_2O	0.68	0.70	0.74	0.73	0.74	0.64

Column size was 15x900 mm. Phosphate concentrations of each sample were kept to be ca. 10^{-3} M as P. Concentrations of crotonaldehyde were ca. 5 ppm. Concentrations of eluting agents were 0.1 M.

Effect of cations in the eluent

The K_{av} values of inorganic phosphates obtained in various eluents are summarized in Table 1. When a 0.1 M tetramethylammonium chloride solution was used as an eluent, a linear triphosphate, P_3, was eluted faster than a cyclic trimetaphosphate, P_{3m}, with the same number of phosphorus atoms in a molecule. Similar elution behavior was also observed for a pair of tetrameric phosphates, P_4 and P_{4m}. On the other hand, no marked difference was observed between the K_{av} values of linear and cyclic phosphates with the same number of phosphorus atoms in a molecule, when 0.1 M alkali metal chloride solution was used as an eluent.

It is believed that the tetramethylammonium cation has little tendency toward complex formation with phosphate anions. The surface charge of linear phosphate is not likely to be shielded by complexation with tetramethylammonium cations. Due to the mutual repulsion among charged phosphate groups, linear phosphate anions tend to form somewhat extended structures. Hence the

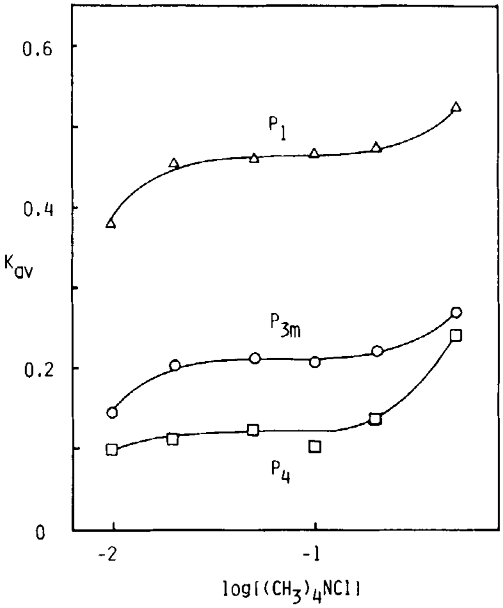

FIGURE 1.

Effect of tetramethylammonium chloride concentration on the K_{av} values of inorganic phosphates.

Column size was 15x450 mm.

effective sizes of linear phosphate become greater than those of the corresponding cyclic phosphates. Alkali metal cations have greater associating ability to phosphate anions than tetramethylammonium ion. They are considered to bind preferentially end phosphate groups of linear phosphates. Owing to the shielding of end phosphate groups the mutual repulsion of the end phosphate groups are deduced, which leads to somewhat coiled structures. The effective size of a linear phosphate may be similar to that of the corresponding cyclic phosphate in 0.1 M alkali metal chloride solution.

It is clear that distribution coefficients for a phosphate obtained in various alkali metal chloride solutions are greatly different from each other. This behavior is contrast to the elution behavior of neutral solute (crotonaldehyde) whose K_{av} values are almost unchanged. Though a satisfactorical explanation can not be made at the present stage, it should be pointed out that the greater

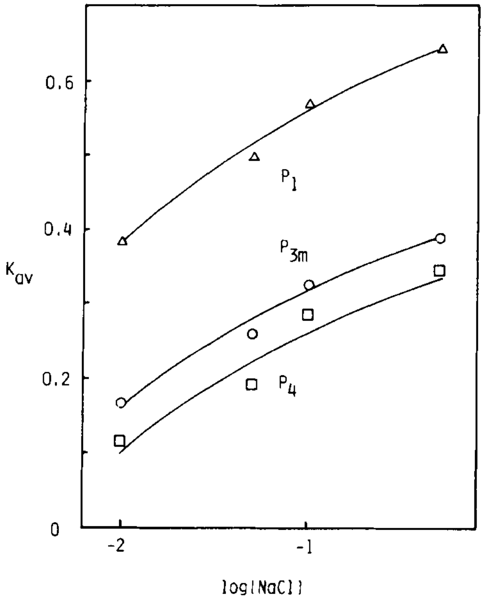

FIGURE 2.

Effect of sodium chloride concentration on the K_{av} values of inorganic phosphates.

Column size was 15x450 mm.

the K_{av} value of eluting agent is, the greater the K_{av} value of phosphate anion is.

In conclusion, gel chromatographic behavior of inorganic phosphate anions is greatly dependent on the nature of the cations of background electrolytes. Complexation with these cations should be taken into account. Since the situation is quite different from that of the neutral solutes such as dextrans(18), estimation of sizes of the ionic solutes by gel chromatography seems to need further precise study.

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REFERNCES

1. Ohashi, S., Yoza, N. and Ueno, Y., J. Chromatogr., 24, 300(1966).
2. Ueno, Y., Yoza, N. and Ohashi, S., J. Chromatogr., 52, 469, 481(1970).
3. Felter, S., Dirheimer, G. and Ebel, J. P., J. Chromatogr., 35 207(1968).
4. Yoza, N., Tokuda, M., Inoue, T. and Ohashi, S., J. Inorg. Nucl. Lett., 9, 385(1973).
5. Yoza, N. Ishibashi, K. and Ohashi, S., J. Chromatogr., 134, 497(1977).
6. Yoza, N., Yoshidome, H. and Ohashi, S., J. Chromatogr., 150, 393(1978).
7. Shimada, K. and Tarutani, T., J. Chromatogr., 168, 401(1979).
8. Neddermeyer, P. A. and Rogers, L. B., Anal. Chem., 40, 755 (1968).
9. Miyajima, T., Yoza, N. and Ohashi, S., Anal. Lett., 10, 709 (1977).
10. Deguchi, T., J. Chromatogr., 120, 159(1976).
11. Ujimoto, K., Yoshimura, T., Ando, I. and Kurihara, H., J. Chromatogr., 174, 123(1979).
12. Sinibaldi, M. and Corradini, O., J. Chromatogr., 148, 553(1978).
13. Miyajima, T., Ibaraki, M., Yoza, N. and Ohashi, S., J. Liq. Chromatogr., 4, 259(1981).
14. Griffith, E. J., J. Inorg. Nucl. Chem., 26, 1381(1964).
15. Brauer, G., "Handbuch der Präparativen Anorganischen Chemie" Ferdinand Enke Verlag, Stuttgart (1960) p.495.
16. Hirai, Y., Yoza, N. and Ohashi, S., J. Liq. Chromatogr., 2, 677(1979).
17. Yoza, N., Ogata, T., Ueno, Y. and Ohashi, S., J. Chromatogr., 61, 295(1971).
18. Granath, K. A. and Kvist, B. E., J. Chromatogr., 28, 69(1967).