The pH Dependence of Anion Exchange Chromatographic Separation of Tri- and Tetraphosphate Anions

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The anion-exchange distribution ratios of tri-, tetra-, and pentaphosphate were determined in a Dowex 1-X4-potassium chloride system at different pH by the batch method. The difference between the mean charges of the phosphates in resin phase and solution was explained in terms of a higher pH of resin phase than that of the solution. pH dependence of the separation factors for tri- and tetraphosphate and for tetra- and pentaphosphate was estimated from the batch equilibrium data, and the relative elution positions of these phosphates were confirmed by the practical chromatographic runs.

We employed gradient-elution chromatography with anion-exchange resins for the separation of a series of linear oligophosphates.[1,2] In the course of the experiments[1,2] we found that when ammonium acetate solutions with pH 7 or potassium chloride solutions with pH 8 are used as an eluent, a mixture of linear oligophosphates can be almost completely separated from one another, but the difference between the elution positions of tri- and tetraphosphate is remarkably smaller than that between the elution positions of other adjacent pairs. However, when the mixture is eluted with potassium chloride solutions with pH 9.3, separation of tri- and tetraphosphate does not occur. The same pH dependence of the elution positions of these two phosphates can be seen in the elution diagrams for a series of linear oligophosphates by Matsuhashi.[3]

The present investigation was undertaken to clarify the pH dependence of the anion-exchange chromatographic separation of tri- and tetraphosphate. The mean charges of these phosphates in a resin phase and in a solution at equilibrium are discussed.

Experimental

Batch Experiment. *Resin*: The anion-exchange resin Dowex 1-X4 (100—200 mesh) was conditioned to chloride form and dried at 60°C in a vacuum desiccator for 5 hr.

Preparation of Sample Solutions: Commercial pentasodium triphosphate hexahydrate was purified by recrystallization with ethyl alcohol, and dissolved in potassium chloride solutions of various concentrations and pH values. Chloride concentrations; 0.150, 0.200, 0.250, 0.300, and 0.350 M. pH 5.2; 0.001 M potassium hydrogen phthalate-dilute sodium hydroxide buffer. pH 7.1; 0.003 M sodium maleate-dilute acetic acid buffer. pH 9.5; 0.010 M ammonium chloride-dilute aqueous ammonia buffer. Solutions of tetra- and pentaphosphate were prepared from glassy sodium polyphosphate with an average degree of polymerization of 4.5 by means of the gradient-elution chromatographic method. Fractions of the effluent-containing tetra- or pentaphosphate were collected, and after addition of 1 g of dry Dowex 1-X4 the volume of this mixture was increased by a factor of more than 100 times with water so that the greater part of the phosphate anions was adsorbed in the resin phase. The resin was then separated from the solution and immersed in a concentrated potassium chloride solution for 1 hr. The phosphate anions adsorbed in the resin phase were thus recovered into the solution. From this solution, tetra- or pentaphosphate solutions of the different chloride concentrations and pH values were prepared.

Determination of Distribution Ratios: A weighed amount of dry Dowex 1-X4 (about 0.5 g) was put into a stoppered Erlenmeyer flask which contains 10 m*l* of a potassium chloride solution with a definite chloride concentration and pH. This was kept standing overnight, and after 5 m*l* of a linear oligophosphate solution of the corresponding chloride concentration and pH value was added, the mixture was shaken for 2 hr at 20°C.

The phosphorus content in the solution was determined colorimetrically with the molybdenum(V)-molybdenum(VI) reagent.[4] The distribution ratio D is defined by the equation

$$D = \frac{\text{millimoles of phosphorus per m}l\text{ resin bed}}{\text{millimoles of phosphorus per m}l\text{ solution}} \qquad (1)$$

Gradient-Elution Chromatography. *Glassy Sodium Polyphosphate*: Glassy sodium polyphosphate with an average degree of polymerization of 4.5 was prepared by quenching the melt of a mixture of sodium dihydrogen orthophosphate dihydrate and disodium hydrogen orthophosphate dodecahydrate with a molar ratio of 5 to 4 after complete dehydration and condensation.

Chromatographic Run: An aliquot of a solution containing 1 mg of sodium dihydrogen orthophosphate dihydrate and 50 mg of the glassy sodium polyphosphate was loaded on a column of Dowex 1-X4 (100—200 mesh, ϕ 1.24×66.5 cm) and eluted with potassium chloride solution by means of gradient-elution technique. The initial chloride concentration of the eluent in a mixing bottle and the chloride concentration of the eluent in a reservoir were 0.209 and 0.468 M, respectively. The pH of the eluents was adjusted to 4.5 with 0.001 M potassium hydrogen phthalate and dilute sodium hydroxide solution or to 3.0 with 0.001 M potassium hydrogen phthalate and dilute hydrochloric acid. The volume of the eluent in the mixing bottle was kept to 1000 m*l*. Chromatographic runs were carried out with the flow rate of 36—45 m*l*/hr at 20°C. Effluents were collected into 9 m*l* fractions with an automatic fraction collector.

Determination of Phosphorus and Chloride Concentrations: Molybdenum(V)-molybdenum(VI) reagent was used for the determination of phosphorus contents. Determination of chloride

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[1] T. Nakamura, K. Ujimoto, N. Yoza, and S. Ohashi, *J. Inorg. Nucl. Chem.*, **32**, 3191 (1970).

[2] S. Ohashi, N. Tsuji, Y. Ueno, M. Takeshita, and M. Muto, *J. Chromatogr.*, **50**, 349 (1970).

[3] M. Matsuhashi, *J. Biochem.*, **44**, 65 (1957).

[4] F. Lucena-Conde and L. Prat, *Anal. Chim. Acta*, **16**, 473 (1957).

concentrations was carried out by Mohr's method for the fractions containing no phosphate anions.

Results and Discussion

Koguchi *et al.*[5] reported that the mean charge of the phosphate species in a resin phase $\bar{i}_R$ is given by the following equation for the system of an anion-exchange resin and an ammonium acetate solution.

$$\partial \log D/\partial \log(\mathrm{AcO^-}) = \bar{i}_R \qquad (2)$$

This treatment is applicable to the system where potassium chloride is used in place of ammonium acetate as follows. The linear phosphate anion with a degree of polymerization of n, $\mathrm{H}_{n+2-j}\mathrm{P}_n\mathrm{O}_{3n+1}{}^{j-}$, is abbreviated to $\mathrm{H}_{a-j}\,\mathrm{P}^{j-}$ or P^{j-}. The distribution ratio D and the dissociation constants in a resin phase and in a solution $\bar{K}_j$ and K_j are defined by the following equations for a given linear phosphate.

$$D = \sum[\mathrm{P}^{j-}]/\sum(\mathrm{P}^{j-}) = \{[\mathrm{P}^0]+[\mathrm{P}^-]+\cdots+[\mathrm{P}^{j-}]+\cdots +[\mathrm{P}^{a-}]\}/\{(\mathrm{P}^0)+(\mathrm{P}^-)+\cdots+(\mathrm{P}^{j-})+\cdots+(\mathrm{P}^{a-})\} \qquad (3)$$

$$\bar{K}_j = [\mathrm{H}^+]^j[\mathrm{P}^{j-}]/[\mathrm{H}_a\mathrm{P}] \qquad (4)$$

$$K_j = (\mathrm{H}^+)^j(\mathrm{P}^{j-})/(\mathrm{H}_a\mathrm{P}) \qquad (5)$$

where the concentrations of ions in the resin phase and in the solution are distinguished by [ ] and ( ), respectively. Donnan's equilibrium gives the following equation for the pair of hydrogen and chloride ions.

$$[\mathrm{H}^+]\cdot[\mathrm{Cl}^-] = (\mathrm{H}^+)\cdot(\mathrm{Cl}^-)\cdot\gamma_{(\mathrm{H}^+)}\cdot\gamma_{(\mathrm{Cl}^-)}/\gamma_{[\mathrm{H}^+]}\cdot\gamma_{[\mathrm{Cl}^-]}$$

where γ's represent activity coefficients. The distribution coefficient of a non-ionized phosphate species between the resin phase and the solution are considered to be constant,

$$[\mathrm{P}^0]/(\mathrm{P}^0) = \gamma_{(\mathrm{P}^0)}/\gamma_{[\mathrm{P}^0]} = \text{constant} \qquad (7)$$

From Eqs. (3), (4), and (5), we get

$$D = \{[\mathrm{P}^0]/(\mathrm{P}^0)\}\cdot\{1+\cdots+K_j/[\mathrm{H}^+]^j+\cdots+K_a/[\mathrm{H}^+]^a\}/\{1+\cdots+K_j/(\mathrm{H}^+)^j+\cdots+K_a(\mathrm{H}^+)^a\} \qquad (8)$$

If a pH of the solution is kept constant,

$$\{[\mathrm{P}^0]/(\mathrm{P}^0)\}/\{1+\cdots+K_j/(\mathrm{H}^+)^j+\cdots+K_a/(\mathrm{H}^+)^a\} = K\,(\text{const.}) \qquad (9)$$

From Eqs. (8) and (9), we get

$$\log D = \log K + \log(1+\cdots+\bar{K}_j/[\mathrm{H}^+]^j+\cdots+\bar{K}_a/[\mathrm{H}^+]^a) \qquad (10)$$

In order to differentiate Eq. (8) with respect to log(Cl⁻), the following relation is used.

$$\{\partial \log D/\partial \log(\mathrm{Cl}^-)\}_{(\mathrm{H}^+)=\mathrm{const.}} = \left\{\frac{\partial \log D}{\partial[\mathrm{H}^+]}\cdot\frac{\partial[\mathrm{H}^+]}{\partial(\mathrm{Cl}^-)}\cdot\frac{\partial(\mathrm{Cl}^-)}{\partial \log(\mathrm{Cl}^-)}\right\}_{(\mathrm{H}^+)=\mathrm{const.}} \qquad (11)$$

The components of the right-hand side of Eq. (11) are expressed as follows.

$$\partial[\mathrm{H}^+]/\partial(\mathrm{Cl}^-) = \{(\mathrm{H}^+)/[\mathrm{Cl}^-]\}\cdot\gamma_{(\mathrm{H}^+)}\gamma_{(\mathrm{Cl}^-)}/\gamma_{[\mathrm{H}^+]}\gamma_{[\mathrm{Cl}^-]} \qquad (12)$$

$$\partial \log D/\partial[\mathrm{H}^+] = \{-\bar{K}_1/[\mathrm{H}^+]^2-\cdots-j\bar{K}_j/[\mathrm{H}^+]^{j+1}-\cdots -a\bar{K}_a/[\mathrm{H}^+]^{a+1}\}/\{1+\bar{K}_1/[\mathrm{H}^+]+\cdots+\bar{K}_j/[\mathrm{H}^+]^j+\cdots +\bar{K}_a/[\mathrm{H}^+]^a\} \qquad (13)$$

$$\partial(\mathrm{Cl}^-)/\partial \log(\mathrm{Cl}^-) = (\mathrm{Cl}^-) \qquad (14)$$

Thus, Eq. (11) can be written as

$$\{\partial \log D/\partial \log(\mathrm{Cl}^-)\}_{(\mathrm{H}^+)=\mathrm{const.}} = \{[\mathrm{P}^-]+\cdots +j[\mathrm{P}^{j-}]+\cdots+a[\mathrm{P}^{a-}]\}/\{[\mathrm{P}^0]+[\mathrm{P}^-]+\cdots+[\mathrm{P}^{j-}]+\cdots +[\mathrm{P}^{a-}]\} = \sum j[\mathrm{P}^{j-}]/\sum[\mathrm{P}^{j-}] = \bar{i}_R \qquad (15)$$

The distribution coefficient of a particular ionic species P^{j-}, f_j, is derived from Donnan's equilibrium for the pair of this ionic species and chloride ion:

$$f_j = [\mathrm{P}^{j-}]/(\mathrm{P}^{j-}) = \{[\mathrm{Cl}^-]/(\mathrm{Cl}^-)\}^j\cdot\gamma_{(\mathrm{P}^{j-})}\gamma^j_{[\mathrm{Cl}^-]}/\gamma_{[\mathrm{P}^{j-}]}\gamma^j_{(\mathrm{Cl}^-)} \qquad (16)$$

Eq. (15) can then be transformed into the following equation.

$$\bar{i}_R = \{f_1(\mathrm{P}^-)+\cdots+jf_j(\mathrm{P}^{j-})+\cdots+af_a(\mathrm{P}^{a-})\}/\{f_0(\mathrm{P}^0) +f_1(\mathrm{P}^-)+\cdots+f_j(\mathrm{P}^{j-})+\cdots+f_a(\mathrm{P}^{a-})\} = \sum jf_j(\mathrm{P}^{j-})/\sum f_j(\mathrm{P}^{j-}) \qquad (17)$$

On the other hand, the mean charge of the phosphate species in the solution, $\bar{i}$, is given by

$$\bar{i} = \sum j(\mathrm{P}^{j-})/\sum(\mathrm{P}^{j-}) \qquad (18)$$

The concentration of chloride ion in the resin phase is usually much greater than that in the solution, thus Eq. (16) gives

$$f_j \gg 1 \qquad (19)$$

From Eqs. (17), (18), and (19), the following relation can be obtained.

$$\bar{i}_R \geqq \bar{i} \qquad (20)$$

This relation can be understood from the fact that a weak acid such as phosphoric acids is much dissociated at a higher pH and that the hydrogen ion concentration in an anion-exchange resin phase is in general higher than that in a solution.

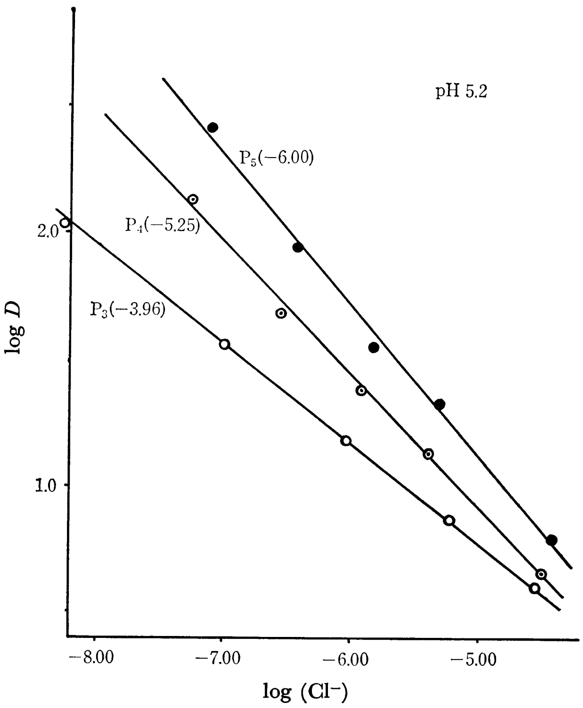

Fig. 1. log D *vs.* log(Cl⁻) for tri-(P_3), tetra-(P_4) and penta-(P_5) phosphate at pH 5.2. The slope for each straight line is given in parentheses.

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[5] K. Koguchi, H. Waki, and S. Ohashi, *J. Chromatogr.*, **25**, 398 (1966).

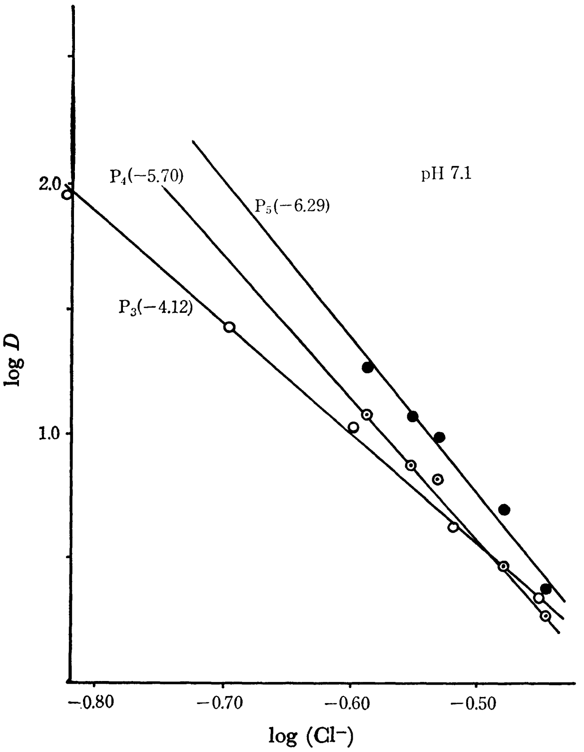

Fig. 2. log D *vs.* log(Cl$^-$) for tri-, tetra-, and pentaphosphate at pH 7.1.

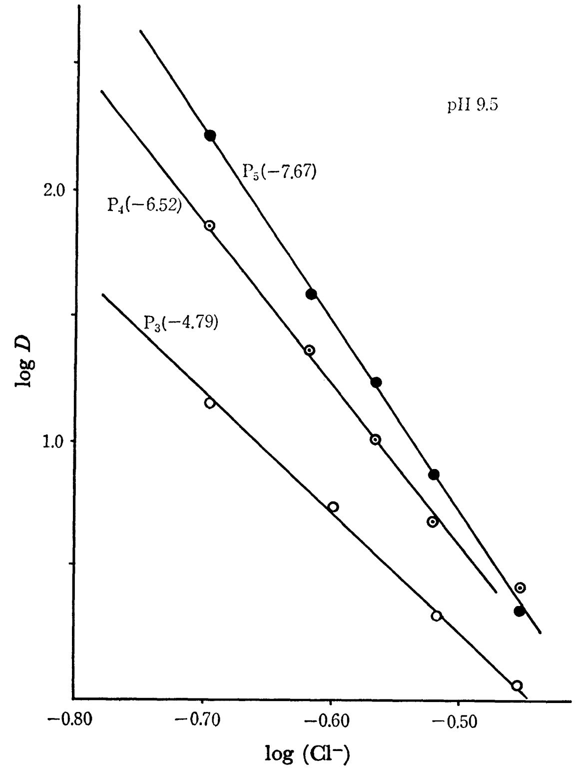

Fig. 3. log D *vs.* log (Cl$^-$) for tri-, tetra-, and pentaphosphate at pH 9.5.

The relationship between the distribution ratio and the chloride ion concentration in the solution is given in Figs. 1, 2, and 3. From Eq. (15), the slope of each straight line gives the mean charge of each phosphate species in the resin phase at a given pH of the solution. In Fig. 4, the resulting mean charges are compared with the calculated mean charges in the solution for tri- and tetraphosphate. For these calculations the dissociation constants obtained by Irani and Callis[6] were employed. The relationship of Eq. (20) is confirmed by Fig. 4. Since in the higher pH region the fully dissociated phosphate species predominate in both phases, the decrease of the difference between $\bar{i}_R$ and $\bar{i}$ is expected from Eqs. (17) and (18). The difference still remaining will be attributed to the change of the activity coefficients.

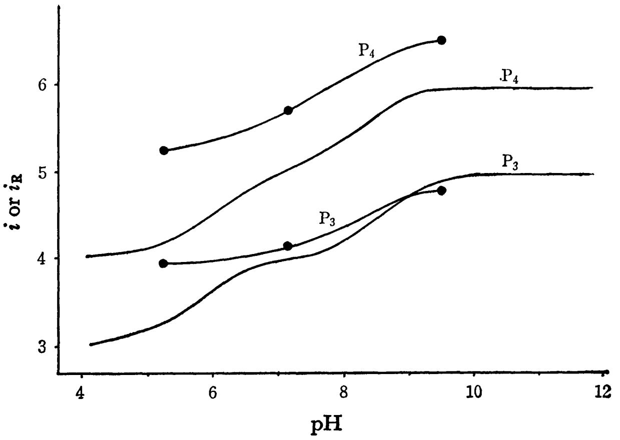

Fig. 4. Relationship between $\bar{i}$ or $\bar{i}_R$ and the pH values in solution.
——: calculated $\bar{i}$ values, ●—●: observed $\bar{i}_R$ values.

The chromatographic separation of linear oligophosphates will be discussed in terms of a separation factor α_a^b which is defined by

$$\alpha_a^b = D_b/D_a \qquad (21)$$

where D_a and D_b represent the distribution ratios of phosphate species a and b, respectively. From the definition, α_3^4 and α_4^5 were calculated from the batch distribution ratios for the pairs of tri- and tetraphosphate

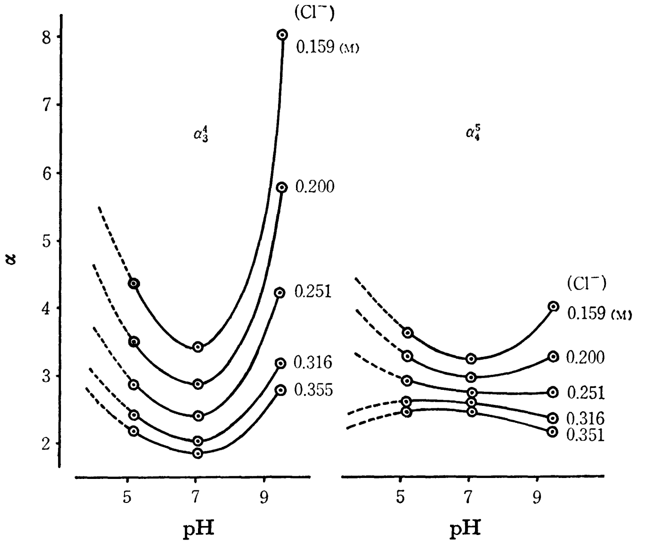

Fig. 5. The pH dependence of $\alpha_3{}^4$ and $\alpha_4{}^5$ at various chloride concentrations.

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[6] R. R. Irani and C. F. Callis, *J. Phys. Chem.*, **65**, 934 (1961).

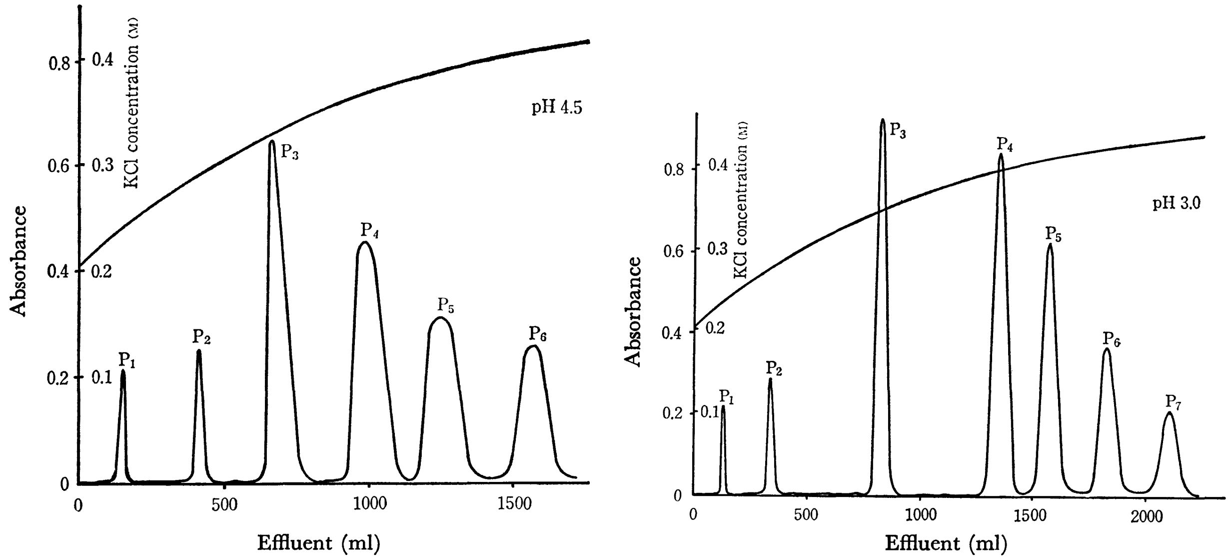

Fig. 6. Elution curves of linear oligophosphates at pH 4.5 and 3.0.

and of tetra- and pentaphosphate. The pH dependence of these separation factors is shown in Fig. 5. All the curves for α_3^4 give the minium values near pH 7 for all chloride concentrations. On the other hand, the curves for α_4^5 exhibit a tendency similar to those for α_3^4 at low chloride concentrations, but those at the high chloride concentrations seem to have maximum values. This suggests that the separation of tri- and tetraphosphate will be improved if the pH of eluent is kept apart from pH 7, but the separation of tetra- and pentaphosphate will not appreciably improved by changing the pH of eluent in the high chloride concentration region where these two phosphates are practically eluted out. This was confirmed by the chromatographic runs at different pH values of 4.5 and 3.0, which are shown in Fig. 6. The two elution diagrams indicate that the separability of tri- and tetraphosphate is improved by decreasing a pH, but not of tetra- and pentaphosphate.