Elution peak positions of linear phosphates in gradient elution chromatography with an anion-exchange resin

Gradient elution chromatography has been used with good results for the separation of a series of similar ionic species such as linear phosphates[1-4] with an ion exchanger. An apparatus suitable for gradient elution has a constant-volume mixing bottle which delivers an eluent whose concentration follows the equation

$$C = C_r - (C_r - C_0) \cdot \exp\left(- V/V_m\right) \tag{1}$$

where C denotes the concentration of the eluent when V ml of the eluent has flowed from the mixing bottle, C_0 the initial concentration of the eluent in the mixing bottle, C_r the concentration of the eluent in a reservoir, and V_m the constant volume of the eluent in the mixing bottle.

In previous papers[5,6] some equations have been proposed for calculating where an elution peak will appear in such a gradient elution. The purpose of this paper is to compare the elution peak positions of linear phosphates obtained experimentally with those predicted from the equations.

The abbreviated notations, P_1, P_2, P_3, etc. are used in this paper for ortho-, di-, triphosphate, etc.

Experimental

Resin. An anion-exchange resin, Dowex 1-X4 (100–200 mesh), in the chloride form, was dried at 60° in a vacuum desiccator for 20 h. The conversion factor between the resin bed volume and resin gravity was found to be 3.3 ml/g.

Reagents. The phosphates employed in this investigation were as follows: potassium dihydrogen orthophosphate, KH_2PO_4, tetrasodium diphosphate decahydrate, $Na_4P_2O_7 \cdot 10H_2O$, pentasodium triphosphate hexahydrate, $Na_5P_3O_{10} \cdot 6H_2O$ and glassy sodium polyphosphate with an average degree of polymerization, $\bar{p}$ of 10. Potassium chloride solutions adjusted to pH 9.3 with an ammonia–ammonium chloride buffer were used as eluents. The concentration of ammonium ion in the eluents was 0.01 M.

Gradient elution. The apparatus shown in Fig. 1 was devised for the separation of linear phosphates. Reservoir 2 was inserted between reservoir 1 and the mixing bottle in order to keep the liquid level constant. A small hole, G in Fig. 1, plays the role of regulator for the liquid level. For the purpose of reference a typical chromatogram for a series of linear phosphates is illustrated in Fig. 2.

Although a borate buffer was used for the elution shown in Fig. 2, an ammonia–ammonium chloride buffer was employed in the present case in order to simplify the system of ionic species in an eluent.

C_0 and C_r were chosen as 0.200 and 0.500 M, respectively. For these values of C_0 and C_r, the parameter α is 0.600.

$$\alpha = (C_r - C_0)/C_r \tag{2}$$

As was mentioned in the previous paper[6], when a value of α is simple such as 0.500, 0.600 and 0.700, the calculation of the elution peak positions can be easily
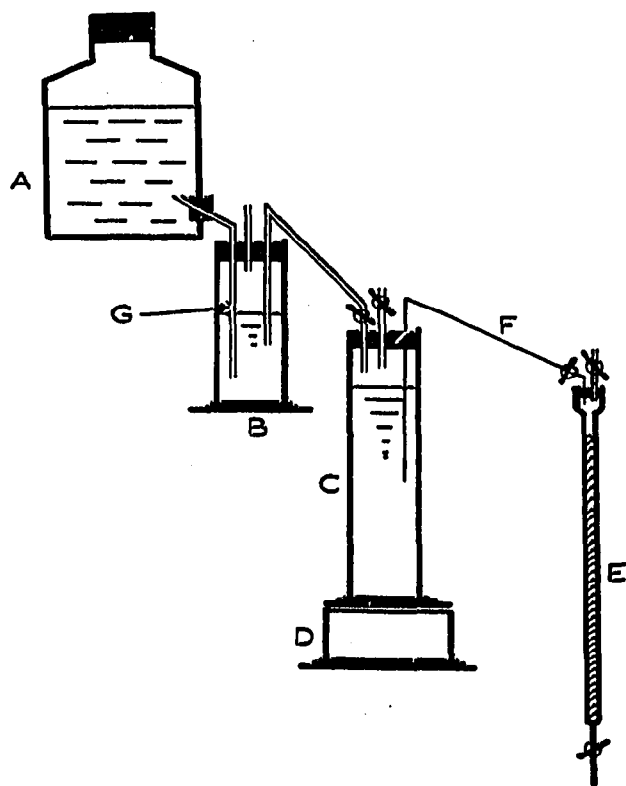
Fig. 1. Apparatus for gradient elution. A = reservoir 1, 2000-ml bottle; B = reservoir 2, 250-ml measuring cylinder; C = mixing bottle, 1000-ml measuring cylinder; D = magnetic stirrer; E = column; F = Teflon tube, I.D. 1 mm; G = a small hole.
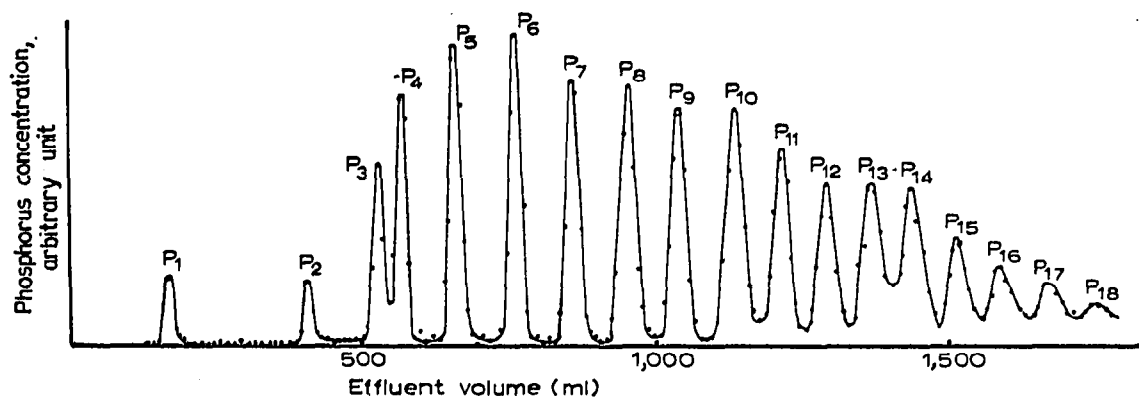

Fig. 2. Elution curve of linear phosphates. Sample: a mixture of orthophosphate, diphosphate and glassy sodium polyphosphate with a $\bar{p}$ of 10. Total amount of the phosphates loaded: 18 mg P. Resin: Dowex 1-X4 (100–200 mesh), Cl$^-$ form. Bed size: 1.2 × 69 cm. Eluent: KCl solution, pH 8.0, buffered with borate. $C_0 = 0.2\ M$; $C_r = 0.45\ M$; $V_m = 1000$ ml. Flow rate: 0.3 ml/min. Temperature: room temp.

carried out. V_m was kept constant at 1,000 ml. The bed volume of the resin, S, was 87.8 ml, and the interstitial volume of the resin bed was 41.4 ml. A phosphate mixture was eluted into 8.7 ml fractions at a flow rate of 0.56 ml/min at 21 ± 1°. Phosphate concentrations in the fractions were determined colorimetrically. Chloride concentrations in the effluent were determined titrimetrically.

Determination of distribution ratios. Potassium dihydrogen orthophosphate, tetrasodium diphosphate decahydrate and pentasodium triphosphate hexahydrate were used as the linear phosphates with degrees of polymerization, p of 1, 2 and 3. Linear phosphates with p 4 to 9 were obtained from a glassy sodium polyphosphate with a $\bar{p}$ of 10 by means of the gradient elution chromatography described above.

Dry resin (0.5–1.0 g) and 15 or 20 ml of a 0.1–0.5 M potassium chloride solution adjusted to pH 9.3 with an ammonia–ammonium chloride buffer were allowed to stand in a stoppered erlenmeyer flask overnight. 5 ml of phosphate solution (0.1–0.2 mg as P) was then added to the flask, the mixture was shaken mechanically for about 1 h at 21 $\pm$ 1°. The phosphorus content in the solution or in the resin was determined colorimetrically. The distribution ratio, D, is calculated as follows

$$D = \frac{\text{mmoles of phosphorus per ml resin phase}}{\text{mmoles of phosphorus per ml solution}} \tag{3}$$

Results and discussion

A mixture of potassium dihydrogen orthophosphate, tetrasodium diphosphate and glassy sodium polyphosphate with $\bar{p}$ of 10 was loaded onto a resin bed and separated by the method described above. The elution curve thus obtained is shown in Fig. 3. It is evident that the linear phosphates with p 4 to 9 were almost completely separated from each other. These isolated linear phosphates were immediately employed for the measurement of their distribution ratios.
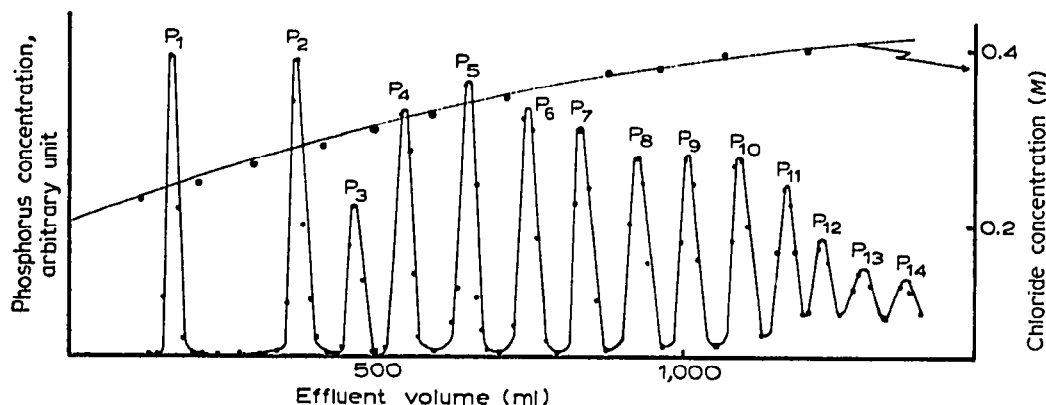

Fig. 3. Elution curve of linear phosphates. Sample: a mixture of orthophosphate, diphosphate and glassy sodium polyphosphate with a $\bar{p}$ of 10. Total amount of the phosphates loaded: 6 mg P. Resin: Dowex 1-X4 (100–200 mesh), Cl⁻ form. Bed size: 1.3 × 66.2 cm. Eluent: KCl solution, pH 9.3 buffered with NH_3 and NH_4Cl. $C_0 = 0.200$ M; $C_r = 0.500$ M; $V_m = 1000$ ml. Flow rate: 0.56 ml/min. Temperature: 21 $\pm$ 1°. Concentration of chloride ion: observed ●, calculated —.

In the separate chromatographic run, it was demonstrated that the elution peaks of tetrameta- and trimetaphosphate appear at 1122 and 1383 ml of the effluent, respectively. Since a glassy sodium polyphosphate contains, in general, small amounts of trimeta- and tetrametaphosphate, the fractions containing the linear phosphates with p 10 to 14 may be contaminated with tri- and tetrametaphosphate.

It was verified experimentally that there is a linear relationship between log D and log C for each member of the linear phosphates with p 1 to 9.

$$\log D \propto - n \log C \tag{4}$$

As examples, the distribution ratios of the linear phosphates with p of 2, 5 and 9 at various concentrations of chloride ion are shown in Fig. 4. n in eqn. 4 can be obtained as the slope from such a diagram.
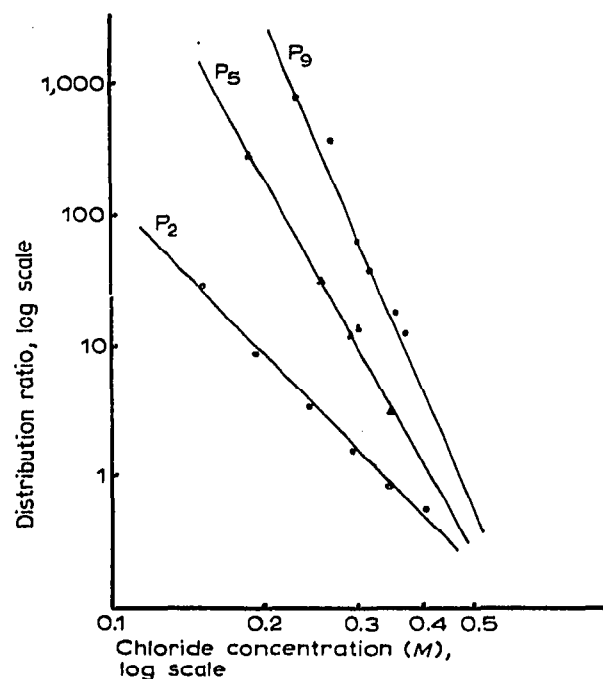

Fig. 4. Distribution ratios of P_2, P_5 and P_9 at various concentrations of chloride ion.

According to the method described in the previous paper[6], the elution peak position, $V_{max.}$, can be calculated from the following equation

$$y = x - f_n (\alpha) + f_n (\alpha e^{-x})$$ (5)

where $y = D_r S/V_m$ and $x = V_{max.}/V_m \cdot D_r$ is the distribution ratio at C_r and S is the volume of the resin bed. The relationships between x and y when $\alpha = 0.6$ and $n = 0$–6

TABLE I

CALCULATED AND OBSERVED VALUES OF V_{max}

Phosphate species	n	D_r	y	$V_{max.}$		
				Calculated (ml)	Observed (ml)	Error (%)
P_1	2.8	0.092	0.008	151	165	+9.3
P_2	4.1	0.22	0.019	371	374	+0.8
P_3	5.2	0.21	0.018	481	470	—2.3
P_4	5.9	0.19	0.017	566	548	—3.2
P_5	6.9	0.22	0.019	711	653	—8.2
P_6	7.5	0.22	0.019	776	748	—3.6
P_7	8.5	0.25	0.022	914	844	—7.9
P_8	9.5	0.24	0.021	986	931	—5.6
P_9	10.2	0.26	0.023	1078	1018	—5.6

are given graphically in the previous paper[6]. When n is larger than 6 the relationship between x and y can be calculated in a similar way.

Since the values of α, n, D_r, S and V_m are known, one can obtain a value of V_{max}. The values of V_{max}. should be corrected by adding the interstitial volume of the resin bed. The results obtained are summarized in Table I. The observed values are in rough accordance with the calculated values. It can be said that elution peak positions of linear phosphates in gradient elution chromatography with an anion-exchange resin are predicted by the method previously described.

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