Temperature dependence of the anion-exchange chromatographic behaviour of cyclic phosphate oligomers and their anion-exchange separation

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ABSTRACT

The temperature dependence of the chromatographic behaviour of a series of cyclic condensed phosphates with degree of polymerization from 3 to 8 on an anion-exchange column was investigated. The efficiency of Jandera's equation in predicting the elution behaviour was confirmed and the elution conditions for separating cyclic phosphates were established with the aid of computer simulations. An increase in column temperature resulted in a significant increase in the number of theoretical plates. Conditions for separating the six phosphates were not found at 30°C; however, the computer simulations suggested that suitable conditions for their separation existed at 70°C and this was confirmed experimentally. The enthalpy changes of the ion-exchange reaction of all phosphates were determined from the temperature dependences of their retention times and varied in proportion to their anionic charges. The ion-exchange reaction of these cyclic phosphates was governed by the dehydration of the anions and partial dissociation of ion pairs between cyclic phosphate anions and K^+ ions in the eluent.

INTRODUCTION

Cyclic phosphates, belonging to inorganic condensed phosphates and represented as $M^I_n P_n O_{3n}$ (M = metal), from the viewpoint of electrolyte solution chemistry are very interesting materials, having high negative charges on their relatively compact molecules [1]. They also can be used for other purposes, such as complexing agents with various cations or food additives, in place of higher membered linear polyphosphates. Of these cyclic phosphates, cyclotri- and cyclotetraphosphate have long been known and can be easily prepared. The existence of

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cyclic phosphates above the tetramer was confirmed in 1956 [2]. Since that time, the chemistry of cyclic phosphates has developed considerably. We have studied various chemical properties of these oligomers with degree of polymerization less than eight [3–5].

Recently, HPLC has been successfully applied to studies of the hydrolysis of cyclic oligophosphates [6–8]. Further, we have reported on the rapid optimization of the separation conditions for four inorganic cyclic phosphates under gradient elution conditions in anion-exchange chromatography [9].

In this work, the separation of six cyclic phosphate anions from each other and the chromatographic behaviour of these anions were investigated. Some of the condensed phosphates, including cyclotri- and cyclotetraphosphate have been used to

derive the equations describing their elution behaviour and to test the plate theory [10–12]. Since these condensed phosphate oligomers are useful polyprotic acids in which each of the PO_4 tetrahedra units is linked to each of its neighbours by an oxygen atom, their degree of protonation can be changed with the pH of the eluents except for cyclic phosphates. Despite extensive studies of their chromatographic behaviour, few theoretical approaches to their selectivities in ion exchangers have been reported.

It has been said that the enthalpy changes in ion-exchange reactions are usually small and temperature does not have a great effect on the ion-exchange equilibria [13]. However, the importance of column temperature was pointed out in the ion-exchange chromatographic separation of linear polyphosphate anions having highly negative charges [14,15]. In this work, the effect of temperature on the chromatographic behaviour of cyclic phosphate anions was studied in detail. The retention time of each of the cyclic phosphate increases with increase in temperature. The theoretical plate number also increases with increase in temperature. We found that the four cyclic phosphate mixtures mentioned above can be separated at 70°C faster and with a better resolution owing to the increase in the number of theoretical plates than at 30°C.

Further, the separation of six cyclic oligomers from the trimer to octamer has been achieved, where the pentamer and heptamer were isolated by the column chromatography of the fractionated mixture of Graham's salt [3].

From the temperature dependence of the retention times of six cyclic phosphate anions, the enthalpy changes of the anion-exchange reaction were determined. The enthalpy changes were positive, and hence the anion-exchange reactions were endothermic. Large differences in the enthalpy changes per unit anionic charge on the cyclic phosphate anions were not observed. The enthalpy changes can be regarded as corresponding to desolvation energies and the dissociation energies of K^+ which form ion pairs with cyclic phosphate anions when the anions enter into the exchanger phase. These steps are important in the anion-exchange reaction of these cyclic phosphate anions. The effect of temperature should be considered for the ion-exchange reactions of highly charged ions such as condensed phosphate ions.

EXPERIMENTAL

Chemicals

Sodium salts of cyclotri- (P_{3m}), $Na_3P_3O_9\cdot6H_2O$, and cyclotetra phosphate (P_{4m}), $Na_4P_4O_{12}\cdot4H_2O$, were prepared by the usual methods [16]. Sodium cyclohexa- (P_{6m}) and cyclooctaphosphate (P_{8m}) were prepared by the methods of Griffith and Buxton [17] and Schülke [18], respectively. Cyclopenta- (P_{5m}) and cycloheptaphosphate (P_{7m}) were isolated from the fractionated solution of Graham's salt by the use of a QAE-Sephadex A-25 column [3]. All other chemicals were of analytical-reagent grade.

Chromatographic system

The anion exchanger TSK Gel SAX, 10 μm, was packed into a 500 mm × 4 mm I.D. column. A Hitachi L-6200 liquid chromatograph was used and a Hitachi 655A-13 reaction pump was connected to mix the Mo(V)–Mo(VI) reagent with the column effluents for the spectrophotometric determination of the phosphates with a Hitachi L-4200 UV–Vis detector. The flow system was similar to that described previously [14]. The separation column was surrounded by a jacket, the temperature of which was kept constant within ± 0.1°C by circulating water.

Elution procedure

The sample solution (0.5 ml) was injected into a separation column and chromatographed at a flow-rate of 1.0 ml/min. The concentrations of the samples were $(1.5–4.5)\cdot10^{-5}$ M. The eluent consisted of appropriate concentrations of potassium chloride and 0.1% (w/v) Na_2EDTA buffered at pH 10.2. The column interstitial volume and the extra-column volume were 2.88 and 2.04 ml, respectively.

Computer simulations

The computer simulation procedure and the notations used in the equations have been described previously [9,19].

RESULTS AND DISCUSSION

In a previous paper [9], we predicted the elution behaviour of four cyclic phosphates, cyclotri-, -tetra-, -hexa- and -octaphosphate, which can be relatively easily prepared, at 30°C. The four were sep-

arated from each other as predicted by the computer simulations. However, the retention times for the phosphates did not differ much and the separation of the six cyclic phosphate anions known so far (the above four plus cyclopenta- and -heptaphosphate) seemed to be difficult at that temperature. Therefore, the elution behaviour of these cyclic phosphates at higher temperatures was investigated. It was found that the retention volume of the cyclic phosphate anions increased with increase in the column temperature. The temperature dependence of the retention volume of cyclooctaphosphate is shown in Fig. 1. The number of plates in the column used (N) was calculated from the analysis of the
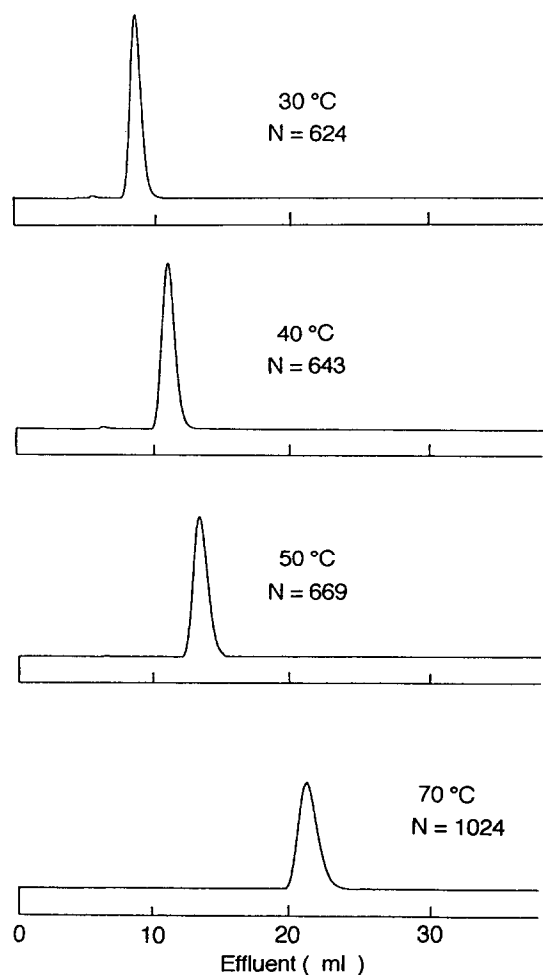
elution curves with the usual plate theory. The retention volume increased with increase in elution temperature and the number of plates also increased considerably. The separation of four cyclic phosphates at 70°C was examined for comparison with the data at 30°C. To find the optimum conditions for separating the four cyclic phosphates by computer simulation, the dependence of the capacity factor on the eluent concentration at 70°C was determined. As described previously [9], a plot of the logarithm of the capacity factor (k') against the logarithm of the concentration of eluting ion (c') was found to be linear:

$$k' = ac'^{-b} \tag{1}$$

The plot of ln k' against ln c' at 70°C is shown in Fig. 2. The values of a and b obtained are given in Table I. The slope, b, is referred to as the ionic charge of cyclic phosphate anion in the exchanger phase [3]. Substituting the above a and b values in Jandera's equation [9,19,20] and the computer simulations yielded the best conditions for the rapid and complete separation of the four cyclic phos-
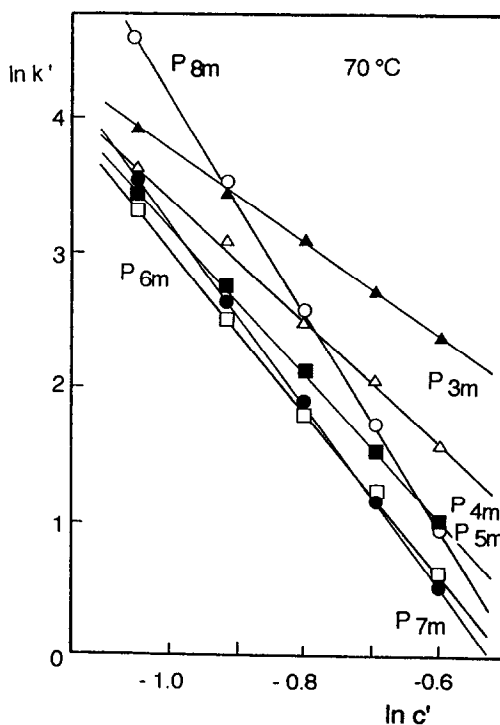

Fig. 1. Chromatograms of cyclooctaphosphate with 0.4 M KCl as eluent at various temperatures.

Fig. 2. Plots of ln k' vs. ln c' at a column temperature of 70°C.

TABLE I

CONSTANTS a AND b IN EQN. 1 AT A COLUMN TEMPERATURE OF 70°C

Solute	a	b
$P_3O_9^{3-}$	1.56	−3.29
$P_4O_{12}^{4-}$	0.351	−4.48
$P_5O_{15}^{5-}$	0.115	−5.36
$P_6O_{18}^{6-}$	0.0631	−5.78
$P_7O_{21}^{7-}$	0.0342	−6.58
$P_8O_{24}^{8-}$	0.0241	−7.86

TABLE II

OBSERVED AND PREDICTED RETENTION VOLUMES FOR FOUR CYCLIC PHOSPHATES UNDER GRADIENT ELUTION CONDITIONS AS GIVEN IN FIG. 3 AT 70°C

Phosphate	Retention volume (ml)		Difference (%)
	Observed	Predicted	
P_{3m}	36.7	39.8	+8.3
P_{4m}	22.3	23.6	+5.8
P_{6m}	13.6	13.5	−0.7
P_{8m}	16.7	17.0	+1.6

phates with gradient conditions as $C_i = 0.5$, $C_f = 0.6$, $t_f = 40$ min, $x = 5$ [9].

Fig. 3 shows the observed and predicted chromatograms under the optimum gradient conditions. The four cyclic phosphates are completely separated within 40 min, which is half of the separation time at 30°C. The retention times observed are in fair agreement with the predicted values, as shown in Table II. The increase in the column temperature from 30 to 70°C resulted in an increase in the plate number and hence the column separation efficiency.

Next, the separation of the six cyclic phosphates from cyclotri- to cyclooctaphosphate was examined. As the preparation of cyclopenta- and cycloh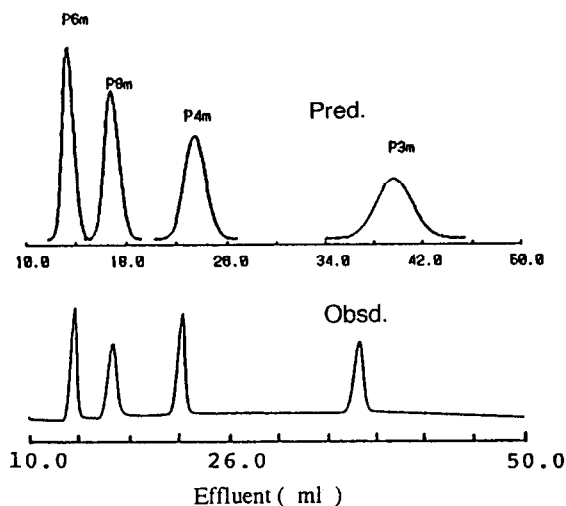eptaphosphate is difficult by the usual method, they were isolated by column chromatography as described previously [3]. Computer simulations at lower column temperatures did not give satisfactory results. However, a good separation at 70°C was. predicted with gradient conditions of $C_i = 0.4$, $C_f = 0.5$, $t_f = 350$ min, $x = 1$ [9]. The chromatogram predicted by the computer simulation is shown in Fig. 4. The chromatogram observed is also shown in Fig. 4, and the separation of the six cyclic phosphates was satisfactory as predicted. The predicted and observed retention volumes of each phosphate are given in Table III, showing good agreement.

It can be concluded that by increasing the column temperature to 70°C and by considering the gradient profile of the eluent, the six cyclic phosphates can be separated from each other. As shown in the chromatograms finally obtained (Fig. 4), the cyclic

Fig. 3. Predicted and observed chromatograms for a mixture of four cyclic phosphates with gradient elution condtions of $C_i = 0.5$, $C_f = 0.6$, $t_f = 40$ min, $x = 5$.
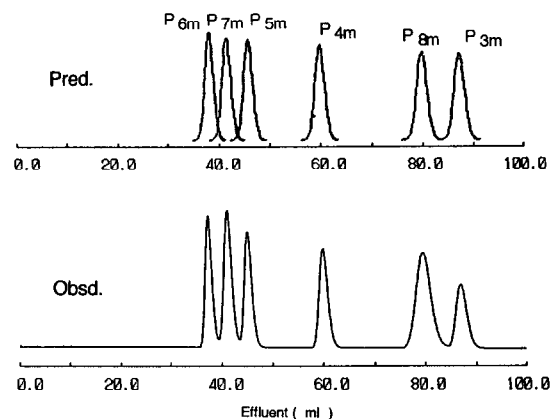

Fig. 4. Predicted and observed chromatograms for a mixture of six cyclic phosphates with gradient elution conditions of $C_i = 0.4$, $C_f = 0.5$, $t_f = 350$ min, $x = 1$.

TABLE III

OBSERVED AND PREDICTED RETENTION VOLUMES FOR SIX CYCLIC PHOSPHATES UNDER THE GRADIENT ELUTIONS CONDITION GIVEN IN FIG. 5 AT 70°C

Phosphate	Retention volume (ml)		Difference (%)
	Observed	Predicted	
P_{3m}	88.1	88.6	+ 0.5
P_{4m}	61.1	60.7	− 0.8
P_{5m}	46.6	46.4	− 0.4
P_{6m}	38.9	38.7	− 0.6
P_{7m}	42.7	42.2	− 1.2
P_{8m}	80.6	82.4	+ 2.2

Fig. 5. Temperature dependence of the logarithm of the capacity factor with 0.5 M KCl as an eluent.

phosphates were not eluted in order of increasing charge. The cyclic phosphates from the trimer to hexamer are eluted in order of decreasing charge, contrary to the elution order of linear phosphate oligomers [19]. However, the heptamer and octamer are eluted in order of increasing charge. The elution order depends completely on the relationship between the capacity factors and the eluent concentration. From the usual law of mass action for ion exchange, a linear relationship can be derived for the plots of ln k' vs. ln [Cl⁻] and the values of the slopes of the straight lines correspond to the charge of each cyclic phosphate anion (eqn. 1). Finally, the ln k' vs. ln c' relationships in Fig. 2 are governed by the free energy changes of ion-exchange reactions of the cyclic phosphate anions, but at the present stage it is not possible to determine or predict true free energy changes of such high-valent anions.

The mechanism of the ion-exchange reaction of these cyclic phosphate anions was considered in some detail from the temperature dependence of the capacity factors. When the concentration of the eluting reagent, KCl, was kept constant at 0.5 M, the results for the variation of ln k' for each phosphate with the reciprocal of absolute temperature of the column are as shown in Fig. 5. Good linearity was obtained. As mentioned previously, the capacity factor can be related to column temperature by eqn. 2, as the ionic strength of the system was kept constant:

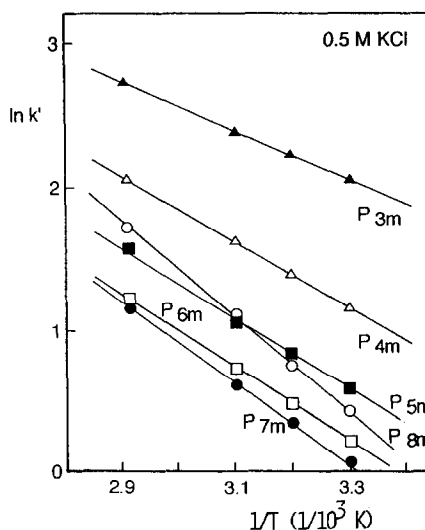$$\ln k' = -\Delta H/RT + \Delta S/R + \text{constant} \qquad (2)$$

In this study, only the capacity factors were determined as a function of the concentration of KCl and temperature. Although selectivity coefficients were not obtained, the enthalpy changes for the anion-exchange reactions were calculated.

The enthalpy changes for the ion-exchange reaction were calculated from the slopes of the linear ln k' versus $1/T$ plots. We measured the temperature dependence of the capacity factors of the cyclic
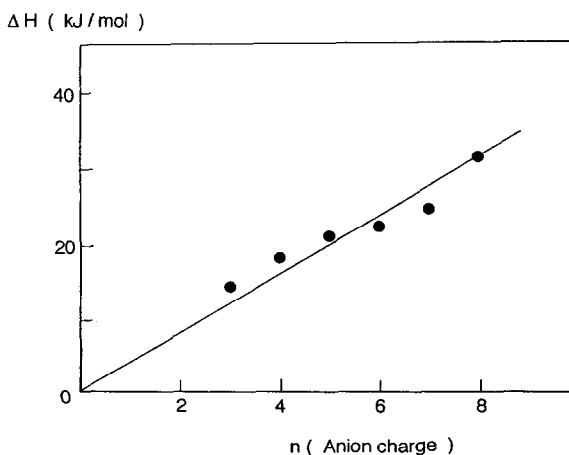

Fig. 6. ΔH vs. charge of cyclic phosphate anions in 0.5 M KCl.

phosphates at constant KCl concentrations from 0.3 to 0.5 M. The enthalpy changes thus obtained for cyclotetraphosphate were 21.2, 20.5, 21.9 and 19.8 kJ/mol at KCl concentrations of 0.3, 0.4, 0.45 and 0.5 M, respectively. For the remaining solutes, the ΔH values did not change so much with KCl concentration, but for the six cyclic phosphates they strongly depended on the charge of the cyclic phosphate anions. The positive values of ΔH indicated that the ion-exchange reaction is endothermic and as an example ΔH values in 0.5 M KCl solution are shown in Fig. 6 as a function of the anionic charges. The experimental data yielded an approximately linear plot passing through the origin. Thus, the ΔH values per unit anionic charge were almost same for each cyclic phosphate anion.

No theory has been proposed to predict the selectivity order for a series of cyclic phosphate oligomers on anion exchangers because of the difficulty involved in the determination of thermodynamic selectivity coefficients. The anionic selectivity has been discussed for some oxygen-containing anions by Chu et al. [21]. They attributed the stronger absorption of the anions to their weaker hydration. In our case, the enthalpy changes seemed to involve energy necessary for the partial dissociation of the ion pair between the phosphate anion and K^+ ion in the solution phase and also the dehydration energy of the phosphate anion entering into the ion-exchanger phase, and the dehydration step of the phosphate anion on adsorption might be one of the important factors that control the ion-exchange reaction of these cyclic phosphate anions.

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