EFFECT OF COLUMN TEMPERATURE ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC BEHAVIOUR OF INORGANIC POLY-PHOSPHATES

II*. GRADIENT ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

The column temperature as well as a chloride concentration gradient were found to be effective for the optimization of the analysis of inorganic polyphosphates in gradient ion-exchange chromatography. The effects of column temperature on both retention time and band width are discussed. A computer-assisted retention prediction system was successfully applied to the optimization of the gradient elution conditions. More than 35 kinds of polyphosphates could be separated completely under the optimum conditions.

INTRODUCTION

Inorganic polyphosphate (P_n), where n is the degree of polymerization or polymerization number, can usually be prepared by the thermal dehydration of monomeric orthophosphate (P_1)[1,2]. In most cases, mixed polyphosphates ($P_{\bar{n}}$), where $\bar{n}$ is the average polymerization number, are obtained. In order to characterize the distribution of P_n in such dehydration products, high-performance liquid chromatography (HPLC) has widely been employed[3–6]. For the separation of oligophosphates with n less than 6, isocratic ion-exchange chromatography has been shown to be satisfactory[3]. On the other hand, for polyphosphates with greater values of n gradient techniques have been recommended[4–6].

This paper deals mainly with the optimization of gradient ion-exchange chromatography for the separation of polyphosphates, and the problems of how to minimize the analysis time and to maximize the resolution. In the solution of these problems, two factors, the pH and chloride concentration of the eluent, have been shown to be important[4]. On the other hand, variation of the temperature has scarcely been employed for improving the separation of polyphosphates. The present series of stud-

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* For Part I, see ref. 3.

ies was initiated to improve the separation by controlling the column temperature. In the first paper[3] we demonstrated the marked effect of column temperature on the isocratic ion-exchange chromatography of oligophosphates. Similar experiments have now been made for polyphosphates.

In isocratic elution, the increase in temperature resulted in an increase in retention time and an improvement in the resolution. The combined effects of the column temperature and the chloride concentration gradient were found to enable the separation of polyphosphates. Optimum conditions for the separations of polyphosphates with $n = 1$–10, 1–20 and 1–35 are discussed.

EXPERIMENTAL

Chemicals and reagents

Unless otherwise stated, guaranteed reagents from Katayama (Osaka, Japan) were used without further purification. Sodium phosphate glass (a mixture of $Na_{n+2}P_nO_{3n+1}$; $P_{\bar{n}}$) with average polymerization number, $\bar{n}$, of 10 was prepared by thermal dehydration of orthophosphate[2]. The phosphate glass was dissolved in distilled water.

A molydenum (V)–molybdenum (VI) reagent for the analysis of polyphosphates was prepared by the method described previously[3].

The eluents for the chromatographic separation of polyphosphates comprised appropriate concentrations of potassium chloride and 0.1% (w/v) Na_4EDTA (pH 10).

Apparatus

An Hitachi liquid chromatograph 635 with a solvent programmer was used for the separation of polyphosphates. The sample solution (100 μl) was injected into a water-jacketed column (250 × 4.0 mm I.D.) packed with polystyrene-based anion exchanger (TSK gel SAX, 10 μm; Toyo Soda) and chromatographed at a flow-rate of 1.0 ml/min. The column temperature was kept constant within 0.1°C. The effluent was introduced into an FIA (flow injection analysis) system to permit the hydrolysis of polyphosphates to orthophosphate and the subsequent colour development. The absorbance of the resulting heteropoly blue complex was measured at 830 nm with a spectrophotometer. Details of the HPLC–FIA system were described previously[3,7].

Calculation

All predicted retention times and band widths were calculated with a personal computer PC-9801 (NEC, Tokyo). Details of the program written in BASIC were described elsewhere[8]. The full listings can be obtained on request to the authors.

RESULTS AND DISCUSSION

Optimum gradient profile and eluent pH

The gradient elution technique is widely accepted as a powerful tool for the analysis of complex sample mixtures[9] and has been applied to the separation of inorganic polyphosphates[4–6]. In addition to the gradient shapes, the eluent pH has been carefully selected for the optimization of the elution conditions[4]. One successful separation was carried out at pH 10 (ref. 3).

There are three kinds of gradient shapes, convex, concave and linear. The first shape has been shown to be effective especially for the separation of inorganic polyphosphates ($n \geqslant 10$)[4,8]. Therefore a gradient elution system with a convex shape at pH 10 was designed in this study for the rapid analysis of polyphosphates.

The eluent concentration–time function proposed by Jandera and Churacek[10] was used in this work.

$$C = (C_i^{1/0.3} + Bt)^{0.3} \tag{1}$$

where C is the molar eluent concentration, t the time in min and C_i is the initial concentration of the eluent. The parameter B can be expressed as follows

$$B = (C_f^{1/0.3} - C_i^{1/0.3})/t_f \tag{2}$$

where C_f is the final eluent concentration and t_f is the time (min) spent required to attain this concentration. A typical gradient profile is shown in Fig. 1 with the parameters $C_i = 0.2\ M$, $C_f = 0.5\ M$ and $t_f = 180$ min.

Effect of temperature on retention and resolution

In the first paper[3] of this series, the column temperature was found to play a key rôle in isocratic ion-exchange chromatography. In the present gradient ion-exchange chromatography, HPLC profiles of polyphosphates at 5, 25 and 60°C were recorded (Fig. 2) at a fixed pH and gradient profile (Fig. 1). The peaks with asterisks refer to cyclic phosphates. The retention time for each solute increased with increasing temperature. Higher temperatures were found to result in improved resolution. These effects were similar to those observed under isocratic elution conditions.

Fig. 2 indicates that the retention time for decaphosphate varied by 4–5 min for a 5°C in temperature. This variation corresponded to a 10% error in the retention time at 25°C. The variation in retention time could be controlled within 1 min when the column temperature was kept constant (± 1°C). A similar effect of temperature on the retention time was also observed for the other polyphosphates. For the identification of a solute on the basis of its retention, without the elution of a reference sample, such temperature effects should be taken into consideration[11].
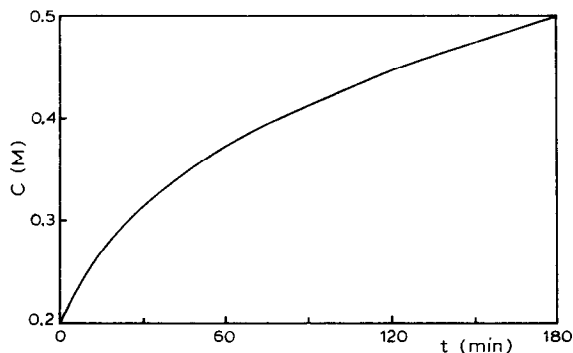

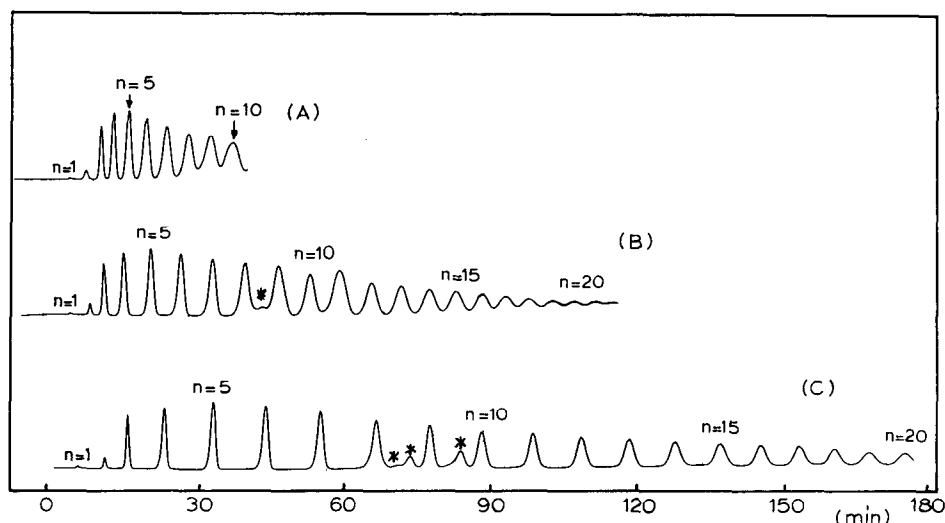Fig. 1. Typical gradient profile. The values of the parameters are listed in Table I (No. 1).

Fig. 2. Effects of column temperature on the gradient elution profiles of polyphosphates at pH 10 and 5°C (A); 25°C (B) and 60°C (C). Sample: $P_{\bar{n}}$ ($\bar{n}$ = 10). Column: 250 × 4.0 mm I.D., porous strong anion exchanger (TSK gel SAX, 10 μm, 3.7 mequiv./g). Flow-rate: 1.0 ml/min. Peaks with asterisks correspond to cyclic phosphates. Gradient profile as in Fig. 1.

The resolution, R_s, is related to the separation factor, α, the plate number, N, and the capacity factor, k'[12]:

$$R_s = (1/4)\,(\alpha - 1)\,N^{1-2}\,[k'/(1 + k')] \tag{3}$$

Fig. 3 illustrates the relationship between the band widths and the retention times observed in Figs. 2 and 4. The band width of any peak decreased with increasing
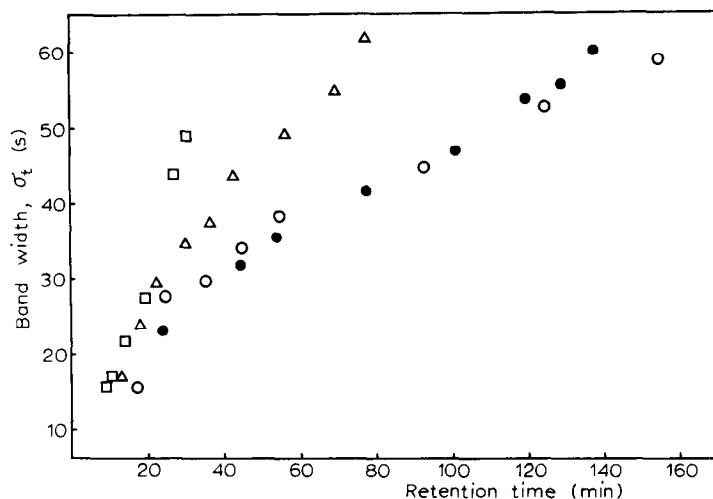

Fig. 3. Relationship between the band width (standard deviation) and retention time at various temperatures: □, 5°C; △, 25°C; ●, 60°C; ○, 70°C.

temperature. In other words, the value of N increased with increasing temperature. The separation factor, α, also increased with increasing temperature. For example, the separation factor between tetraphosphate and pentaphosphate increased from 1.3 at 5°C to 1.5 at 60°C.

Optimization of the gradient elution conditions

Elution profiles were predicted by using a computer-assisted retention prediction system[8,13] based on the theory[10] developed for application to higher polyphosphates ($n \geqslant 10$)[13]. The upper and the lower elution profiles in Figs. 4 and 5 represent the calculated and observed chromatograms, respectively. Calculated peaks were presented as Gaussian peaks by use of predicted retention times and band widths obtained from the relationship between retention times and band widths in Fig. 3. The areas of all calculated peaks were assumed to be the same. The calculated chromatograms were in good agreement with the observed ones. This prediction system was found to be applicable to the optimization of the gradient elution conditions for the analysis of inorganic polyphosphates.

In order to minimize the analysis times for various polyphosphates and at the same time achieve complete resolution ($R_s \geqslant 1.25$), the combined effects of a concentration gradient and the column temperature at pH 10 were utilized. The optimum conditions were classified into three categories (Table I) according to the ranges of polymerization numbers: $n = 1$–10 (Fig. 2B); $n = 1$–20 (Fig. 4); $n = 1$–35 (Fig. 5).

Fig. 2B shows the recommended procedure for the separation of polyphosphates having $n = 1$–10. The analysis time was within 60 min even at room temper-
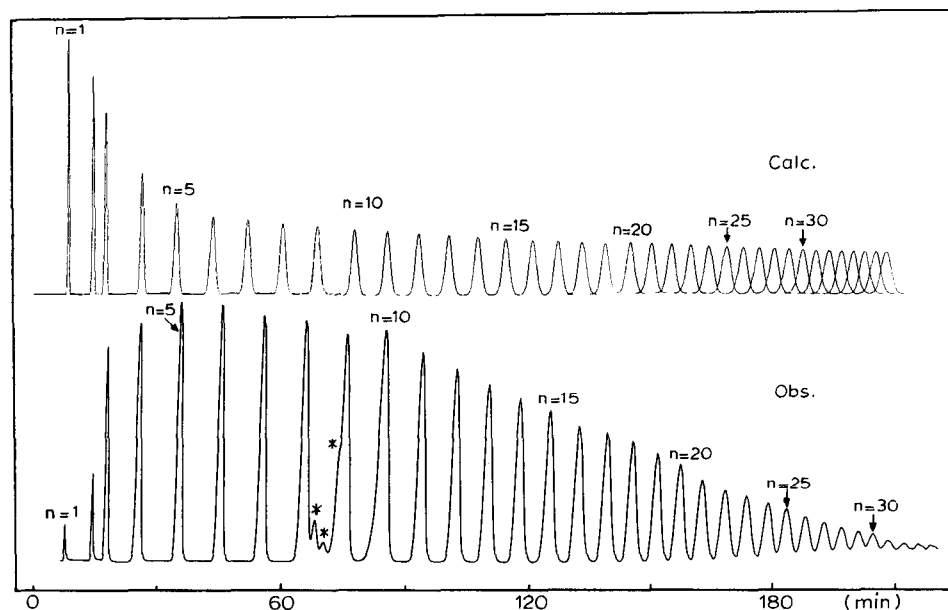

Fig. 4. Elution profiles obtained under the optimum conditions for the separation of polyphosphates ($P_{\bar{n}}$; $\bar{n} = 10$). The column temperature and the parameters for the gradient profile are listed in Table I (No. 2). Other conditions as in Fig. 2. The upper and lower profiles represent calculated and observed chromatograms, respectively.
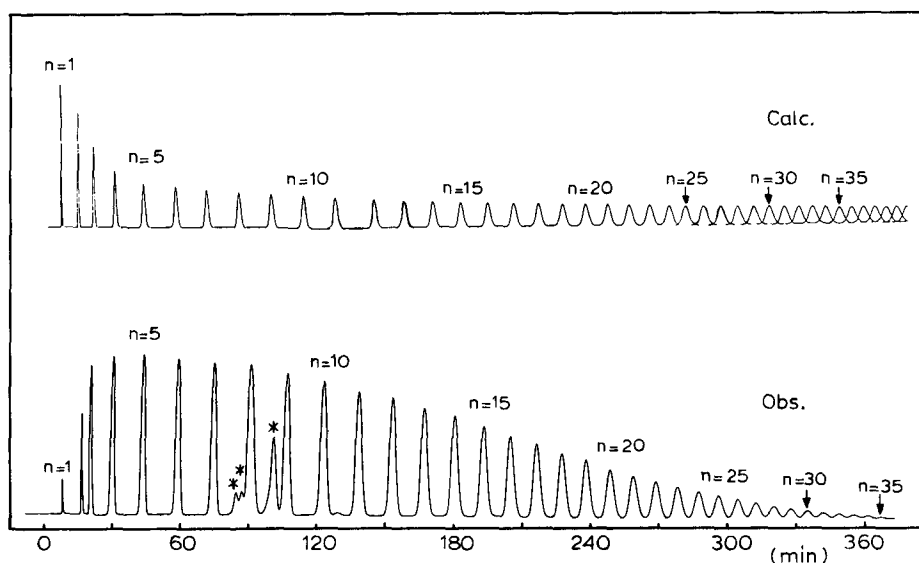
Fig. 5. Elution profiles obtained under the optimum conditions for the separation of polyphosphates ($P_{\bar{n}}$; $\bar{n} = 10$). The column temperature and the parameters for the gradient profile are listed in Table I (No. 3). Other conditions as in Fig. 2.

ature. This gradient system was also able to separate polyphosphates having $n = 1$–20 at 60°C, but the analysis time was unsuitable as shown in Fig. 2C.

Fig. 4 shows the elution profile for a mixture of polyphosphates ($\bar{n} = 10$) obtained by the gradient system of 0.2–0.6 M potassium chloride at 70°C. It is surprising that the last peak corresponds to polyphosphate with $n = 34$. At least three peaks (with asterisks) due to cyclic polyphosphates appeared between the peaks of linear polyphosphates ($n = 8$–9)[4]. The peak height of orthophosphate ($n = 1$) in Figs. 4 and 5 increased compared with that in Fig. 2 since polyphosphates were hydrolyzed during storage of the sample solution. The polyphosphates ($n = 1$–20) could be separated completely within 2.6 h. Therefore, this gradient elution system is recommended especially for the analysis of samples containing polyphosphates having ($10 \leqslant n \leqslant 20$). A further increase in the column temperature from 70 to 80°C did not result in complete separation of polyphosphates ($n > 20$) when the other parameters (No. 2 in Table I) were kept constant.

TABLE I

OPTIMUM CONDITIONS FOR THE SEPARATION OF INORGANIC POLYPHOSPHATES

No.	Parameters for gradient profile in eqns. 1 and 2				Column temperature (°C)
	C_i (M)	C_f (M)	t_f (min)	$B \times 10^4$	
1	0.2	0.5	180	9.86	25
2	0.2	0.6	240	7.40	70
3	0.2	0.6	480	3.70	60

The elution system used at 70°C in Fig. 4 was not suitable for the complete separation ($R_s \geqslant 1.25$) of polyphosphates having $n > 20$, though individual peaks were well defined. These compounds could be separated completely by increasing the value of t_f at 60°C as shown in Fig. 5. Gradient elution at temperatures lower than 60°C was also attempted, but failed to give complete separation even upon increasing t_f, because of the increase in band widths and the decrease in separation factors. On the other hand, the analysis time at temperatures higher than 60°C was longer. The parameters in Table (No. 3) were thus concluded to be optimal for the gradient elution of polyphosphates ($n = 1$–35) from the viewpoint of maximizing the resolution and minimizing the analysis time.

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