

Note

Computer-assisted prediction of retention times for inorganic polyphosphates in gradient ion-exchange chromatography

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Retention prediction methods^{1–4} have been successfully applied to the optimization of the gradient elution conditions for liquid chromatography. A computer-assisted retention prediction system³ had the advantage of a substantial saving in the time taken for the optimization compared with the conventional (trial-and-error) method.

Various kinds of theories have been proposed for retention prediction in gradient elution chromatography^{5–8}. These theories were usually based on the relationship between the capacity factors, k' , of the solutes and the eluent concentrations, C , under isocratic elution conditions. Therefore the capacity factors were measured under isocratic conditions of various eluent concentrations and then the relationship between k' and C ($k' = aC^{-b}$ in ion-exchange mode) applied to the prediction of solute retention time in gradient elution.

For example, the retention times of polyphosphates (P_n) with polymerization number, n , less than 10 were successfully predicted². On the other hand, no successful attempts have been reported for gradient ion-exchange chromatography of higher polyphosphates ($n \geq 10$), mainly because of the difficulty in purifying the individual polyphosphates to be used for the measurement of capacity factors under isocratic conditions.

The purpose of this work was to develop a theory useful for the prediction of retention times of higher polyphosphates ($n \geq 10$). The $\log k'$ values of oligophosphates were measured under isocratic conditions and found to be linearly related to their n values. The retention times for higher polyphosphates could be predicted by extrapolation of this linear relationship. The predicted retention times were compared with the observed ones under various gradient elution conditions.

EXPERIMENTAL

Guaranteed reagents from Katayama (Osaka, Japan) were used without further purification. Sodium phosphate glass (a mixture of $\text{Na}_{n+2}\text{P}_n\text{O}_{3n+1}$; P_n^-) with average polymerization number, \bar{n} , of 10 was prepared by thermal dehydration of orthophosphate.

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

High-performance liquid chromatography (HPLC) and flow injection analysis (FIA)^{4,9} were used for the separation and the detection of polyphosphates. The mixture of polyphosphates was separated on a column (250 × 4.0 mm I.D.) packed with an anion exchanger (TSK gel SAX, 10 μm). The column temperature was maintained within 0.1°C.

Retention times and band widths were calculated with a personal computer PC-9801 (NEC, Tokyo). The program was written in BASIC. The full listings are available on request to the authors.

RESULTS AND DISCUSSION

Theory for the prediction of retention times

The retention times, t_R (min), were predicted by use of the theory proposed by Jandera and Churáček⁷ for gradient ion-exchange chromatography. This theory was chosen because of its applicability to a great variety of gradient profiles. The solute retention time, t_R , was calculated as

$$t_R = (1/u)\{(1/B')[(xb + 1)B' at_0u + C_i^{(xb + 1)/x}]^{1/(xb + 1)} - C_i^{1/x}/B'\} + t_0 \quad (1)$$

where u is the flow-rate (ml/min), a and b are constants for the individual solute obtained from plots of $\log k'$ vs. $\log C$ under isocratic conditions⁹ and t_0 is the column dead time (min); C_i , B' and x are adjustable parameters for the gradient profile.

The gradient profile can be expressed as a function of the eluent concentration and the time

$$\begin{aligned} C &= (C_i^{1/x} + Bt)^x \\ B &= (C_f^{1/x} - C_i^{1/x})/t_f \end{aligned} \quad (2)$$

where C is the eluent concentration at time t , C_i is the initial eluent concentration at the beginning of the gradient elution ($t = 0$) and C_f is the final one at the end of the gradient elution ($t = t_f$); $B' = B/u$. The parameter x characterizes the shape of the gradient profile: at $x = 1$, the change in eluent concentration is linear, while the concentration gradient is convex for $x < 1$ and concave for $x > 1$.

Constants a and b determine the retention times for individual solutes under given gradient elution conditions (x , C_i , B' and u) according to eqn. 1. They can be obtained from the relationship between the eluent concentration and the capacity factor under isocratic elution conditions:

$$k' = aC^{-b} \quad (3)$$

Prior to the calculation of retention times in gradient elution from eqn. 1, it is necessary that the capacity factors be measured at various eluent concentrations under isocratic conditions and constants a and b calculated from eqn. 3.

Estimation of capacity factors for higher polyphosphates ($10 \leq n \leq 35$)

Previously⁹ we measured the capacity factors of inorganic oligophosphates

($1 \leq n \leq 9$) at various eluent concentrations under isocratic conditions and determined constants a and b from eqn. 3. It is difficult to determine such constants for higher polyphosphates ($n \geq 10$)^{2,9}. Therefore the retention times of these polyphosphates in gradient elution chromatography have never been predicted.

The R_M values of polyphosphates are known to be related to the n values in thin-layer chromatography¹⁰, and R_M corresponds to $\log k'$. The $\log k'$ values of polyphosphates ($n < 10$) were found to be linearly related to their n values in isocratic ion-exchange chromatography (each correlation coefficient = 0.999) at various eluent concentrations (Fig. 1) and at various column temperatures (Fig. 2). The relationship can be expressed as

$$\log k' = c(n - 2) + d \quad (4)$$

where $(n - 2)$ is the number of central phosphorus atoms of the polyphosphate. Iida and Yamabe¹⁰ reported that the constants c and d corresponded to the free energy necessary for transportation of the solute from the mobile phase to the stationary phase.

The capacity factors at various eluent concentrations could be estimated by the extrapolation of these relationships (Fig. 1) at a given temperature. Therefore

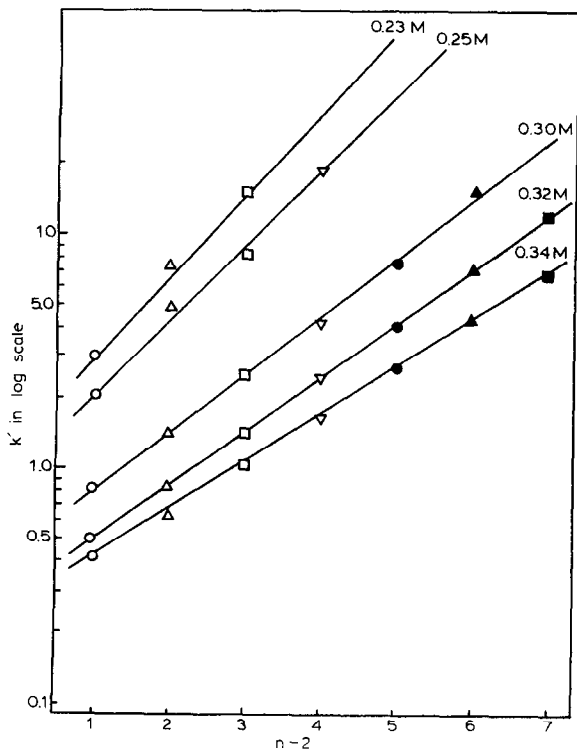


Fig. 1. Plots of $\log k'$ vs. the number of central phosphorus atoms of polyphosphates, $n - 2$, at various eluent concentrations (molar concentrations of potassium chloride are shown). Column temperature: 25°C. \circ — \circ , Tri-; \triangle — \triangle , tetra-; \square — \square , penta-; ∇ — ∇ , hexa-; \bullet — \bullet , hepta-; \blacktriangle — \blacktriangle , octa- and \blacksquare — \blacksquare , nonaphosphate.

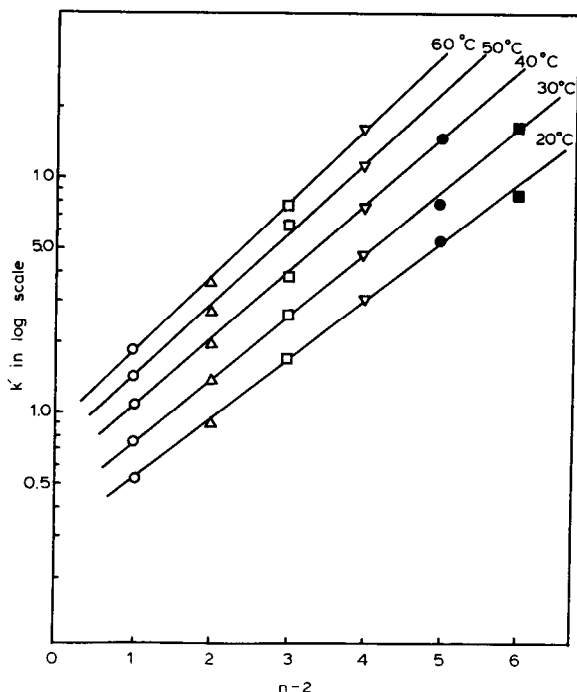


Fig. 2. Plots of $\log k'$ vs. the number of central phosphorus atoms of polyphosphates, $n - 2$, at various column temperatures. Eluent: 0.30 *M* potassium chloride and 0.1% Na_4EDTA .

constants a and b for higher polyphosphates ($10 \leq n \leq 35$) were calculated from eqn. 3 at various column temperatures. Table I lists the estimated values at 25 and 60°C. The constants a and b for oligophosphates ($7 \leq n \leq 9$) at 60°C were also estimated by this method and are listed in Table I. The capacity factors for such oligophosphates could not be measured at 60°C under isocratic conditions⁹.

Prediction of retention times of polyphosphates by a computer-assisted system

The retention times for polyphosphates were predicted from eqn. 1 by use of the estimated constants a and b (Table I). We first examined how well the present approach predicts retention times. Tables II–IV compare observed and calculated values of retention times of polyphosphates. The same gradient profile ($x = 0.3$, $C_i = 0.2 \text{ M}$, $C_f = 0.5 \text{ M}$ and $t_f = 180 \text{ min}$) was employed for Tables II and III, but the column temperatures were different (25°C in Table II, 60°C in Table III). On the other hand, in Tables III and IV the same column temperature (60°C) was used, while the gradient profile in Table III was different from that in Table IV ($x = 0.3$, $C_i = 0.2 \text{ M}$, $C_f = 0.6 \text{ M}$, $t_f = 480 \text{ min}$). The 75 observed t_R values were predicted with an average error of 4.6%. This suggests that the relationship between $\log k'$ and n can be successfully applied to the estimation of capacity factors for higher polyphosphates ($10 \leq n \leq 35$) under any gradient elution conditions and at any column temperatures.

TABLE I

ESTIMATED $\log a$ AND b VALUES AT COLUMN TEMPERATURES OF 25 AND 60°C

<i>n</i>	25°C		60°C	
	<i>b</i>	$\log a$	<i>b</i>	$\log a$
7	—	—	9.17	-3.29
8	—	—	10.1	-3.47
9	—	—	11.1	-3.65
10	11.4	-4.34	12.1	-3.84
11	12.3	-4.55	13.0	-4.02
12	13.2	-4.77	14.0	-4.20
13	14.1	-4.98	15.0	-4.39
14	15.0	-5.20	15.9	-4.57
15	15.9	-5.42	16.9	-4.76
16	16.8	-5.63	17.9	-4.94
17	17.7	-5.85	18.8	-5.12
18	18.6	-6.06	19.8	-5.31
19	19.5	-6.28	20.8	-5.49
20	20.4	-6.50	21.7	-5.67
21	21.3	-6.71	22.7	-5.86
22	22.2	-6.93	23.7	-6.04
23	23.1	-7.14	24.6	-6.22
24	24.0	-7.36	25.6	-6.41
25	24.9	-7.57	26.5	-6.59
26	25.8	-7.79	27.5	-6.78
27	26.7	-8.01	28.5	-6.96
28	27.6	-8.22	29.4	-7.14
29	28.4	-8.44	30.4	-7.33
30	29.3	-8.65	31.4	-7.51
31	30.2	-8.87	32.3	-7.69
32	31.1	-9.09	33.3	-7.88
33	32.0	-9.30	34.3	-8.06
34	32.9	-9.52	35.2	-8.24
35	33.8	-9.73	36.2	-8.43

TABLE II

OBSERVED AND CALCULATED RETENTION TIMES (min)

 $x = 0.3$; $C_i = 0.2 M$; $C_f = 0.5 M$; $t_f = 180$ min; $T = 25^\circ\text{C}$.

<i>n</i>	Obs.	Calc.	Error (%)	<i>n</i>	Obs.	Calc.	Error (%)
1	6.37	6.13	-3.8	11	61.9	65.9	+6.5
2	10.5	9.81	-6.6	12	68.6	72.5	+5.7
3	13.5	13.4	-0.74	13	74.6	78.9	+5.8
4	17.7	18.5	+4.5	14	80.3	85.0	+5.9
5	23.2	24.7	+6.5	15	85.8	91.0	+6.1
6	29.4	31.5	+7.1	16	91.9	96.7	+5.2
7	35.9	37.8	+5.3	17	95.9	102	+6.4
8	42.5	45.5	+7.0	18	101	108	+6.3
9	49.5	51.9	+4.8	19	106	113	+6.9
10	55.9	59.2	+5.9	20	110	118	+7.3

TABLE III

OBSERVED AND CALCULATED RETENTION TIMES (min)

 $x = 0.3$; $C_i = 0.2 M$; $C_f = 0.5 M$; $t_f = 180$ min; $R = 60^\circ\text{C}$.

<i>n</i>	<i>Obs.</i>	<i>Calc.</i>	<i>Error (%)</i>	<i>n</i>	<i>Obs.</i>	<i>Calc.</i>	<i>Error (%)</i>
1	6.94	7.46	+ 7.5	11	100	100	0
2	12.6	14.0	+10.1	12	110	110	0
3	17.3	19.7	+13.9	13	120	120	0
4	24.9	27.3	+ 9.6	14	129	130	+0.78
5	34.9	37.1	+ 6.3	15	139	139	0
6	45.6	47.9	+ 5.0	16	147	147	0
7	56.9	58.2	+ 2.3	17	155	156	+0.65
8	68.1	68.9	+ 1.2	18	162	164	+1.2
9	79.1	79.6	+ 0.63	19	169	171	+1.2
10	89.5	90.1	+ 0.67	20	177	179	+1.1

TABLE IV

OBSERVED AND CALCULATED RETENTION TIMES (min)

 $x = 0.3$; $C_i = 0.2 M$; $C_f = 0.6 M$; $t_f = 480$ min; $T = 60^\circ\text{C}$.

<i>n</i>	<i>Obs.</i>	<i>Calc.</i>	<i>Error (%)</i>	<i>n</i>	<i>Obs.</i>	<i>Calc.</i>	<i>Error (%)</i>
1	7.64	7.55	-1.2	19	240	228	-5.0
2	16.3	15.1	-7.4	20	250	239	-4.4
3	20.3	22.1	+8.9	21	260	249	-4.2
4	30.2	31.6	+4.6	22	270	258	-4.4
5	43.5	44.4	+2.1	23	280	267	-4.6
6	58.7	58.8	+0.17	24	289	276	-4.5
7	74.6	72.5	-2.8	25	298	285	-4.4
8	90.8	86.9	-4.3	26	307	293	-4.6
9	107	101	-5.6	27	314	301	-4.1
10	123	116	-5.7	28	322	308	-4.3
11	138	130	-5.8	29	330	315	-4.5
12	154	144	-6.5	30	337	322	-4.5
13	168	157	-6.5	31	345	329	-4.6
14	181	170	-6.1	32	351	335	-4.6
15	194	183	-5.7	33	358	342	-4.5
16	206	195	-5.3	34	364	348	-4.4
17	217	206	-5.1	35	370	353	-4.6
18	229	218	-4.8				

The band widths of polyphosphates could also be calculated from the relationship between retention times and band widths, as discussed previously⁴.

To demonstrate the accuracy of the retention prediction system, Fig. 3 shows predicted (upper) and observed (lower) chromatograms for the gradient elution conditions in Table III. In the upper chromatogram, the peaks were given Gaussian profiles by use of the predicted retention times (Table III) and band widths and the area of each peak was assumed to be the same. The characteristics of each chromatogram are almost identical with respect to the retention times, band widths and resolution.

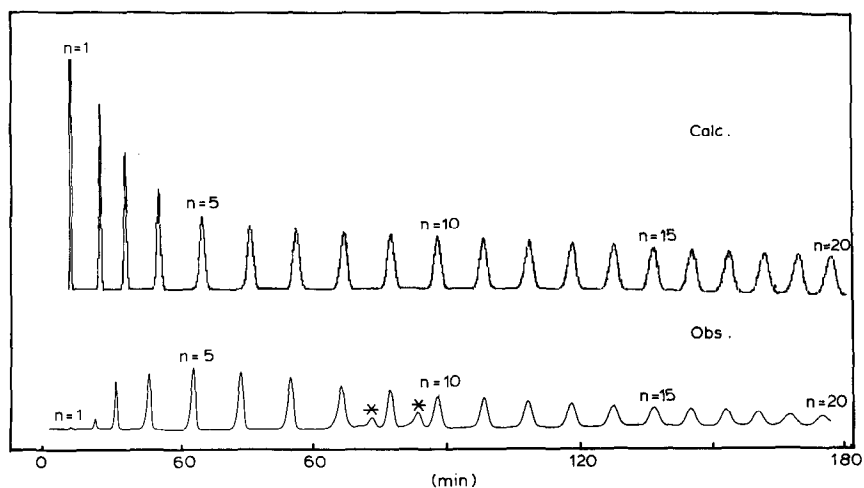


Fig. 3. Observed and calculated chromatograms for $P_n(\bar{n} = 10)$. Gradient elution conditions as in Table III. Peaks indicated with asterisks are due to cyclic phosphates. The n values represent the polymerization number of the polyphosphates.

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