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REVIEW

COMPUTER-ASSISTED RETENTION PREDICTION FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE ION-EXCHANGE MODE

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CONTENTS

1. INTRODUCTION

High-performance liquid chromatography (HPLC) in the ion-exchange mode (HPIEC) has been widely used for the separation of ionic species since the early days of HPLC. Nowadays, HPIEC is a routine established method utilized mainly in two research areas, biochemical and inorganic analysis. For example, HPIEC methods for the separation and analysis of protein mixtures are assuming major importance in the biochemical area[1,2]. HPIEC also provides an excellent means of rapidly and efficiently purifying synthetic oligonucleotides from crude reaction mixtures[3–5]. In the analysis

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of inorganic ions, ion chromatography[6–8] has been one of the fastest growing areas of HPLC in recent years.

Optimization of the separation is an especially important aspect of any routine HPIEC method[9–11]. Although most routine HPIEC methods are still being developed by trial-and-error methods, because a large number of parameters, *e.g.*, mobile phase composition, gradients, column temperature, column conditions and flow-rate, that can be varied to find the desired separation, must be taken into consideration.

This situation is rapidly changing with the increasing availability of powerful personal computers (PC) for HPLC method development[11–15]. A computer-assisted retention prediction system which can predict retention times has been developed that enables chromatographers easily to find optimum elution conditions. Recently a more efficient system, the so-called HPLC computer simulation system, which can simulate visually HPLC separations, was developed by combining a computer-assisted retention prediction system with computer graphics.

This review deals with advances in the computer-assisted retention prediction and computer simulation of HPIEC. Fundamental theories and applications of a computer-assisted retention prediction system with isocratic and gradient elution will be described separately. Applications are focused on the HPIEC separations of biopolymers and inorganic ions.

2. OVERVIEW OF COMPUTER-ASSISTED RETENTION PREDICTION

2.1. Introduction

Many papers have reported computer-assisted retention prediction but few have concentrated on its practical aspects. This section gives a practical overview of computer-assisted retention prediction, including its history, general procedures and software and hardware requirements.

2.2. Historical aspects

The theoretical basis for retention prediction was first given by Martin and Synge[16] when they introduced the plate theory in 1941. The plate theory was subsequently extended to the prediction of retention times under isocratic[17,18] and gradient elution conditions[19–28]. These theories successfully predicted retention times in isocratic and gradient ion-exchange chromatography. Computer-assisted retention prediction based on these theories has not been widespread among chromatographers, however, because all theories were limited in general applicability and in the 1950s and 1960s computers were rarely used in the laboratory.

To overcome the drawbacks of these theories two groups derived general theories for retention prediction: (1) Jandera and Churáček's approach[29–32] and (2) Snyder and co-workers' approach[33–40], the so-called linear solvent strength (LSS) theory. These theories have been successfully applied to a computer-assisted retention prediction system because of the general applicability and relative simplicity of the resulting equations.

In the 1980s, chromatographers used computers more frequently, and especially the advent of personal computers has enabled retention times in HPLC to be predicted

more easily. A combination of the general theories for retention prediction and the powerful ability of personal computers with respect to calculation and graphical presentation led to a new method for predicting HPLC separations, the so-called computer simulation of HPLC[14,41–53]. A computer simulation system predicts retention times and band widths, and then presents a simulated chromatogram visually on the monitor screen and/or printer.

2.3. Procedure for computer-assisted retention prediction

In this section, the specific steps that should be taken in computer-assisted retention prediction are summarized. A flow scheme is given in Fig. 1 to illustrate the steps in retention prediction, and these can be explained as follows.

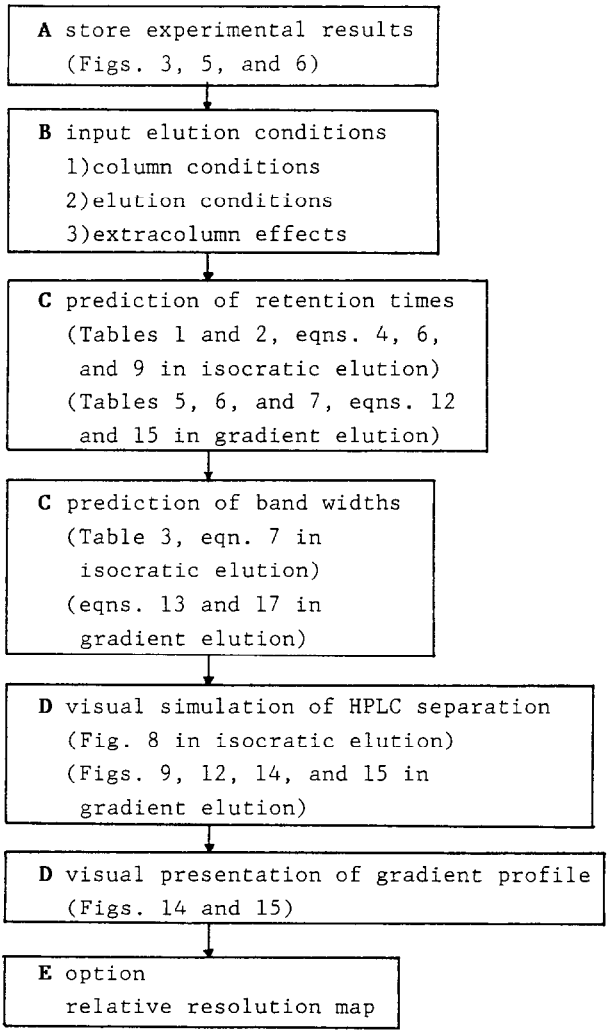


Fig. 1. Flow scheme of the computer-assisted retention prediction system.

(A) Store experimental results on a floppy disk

Although the most powerful retention prediction is an absolute one, which predicts an HPLC separation before the actual HPLC run, such predictions are rarely available. Almost all computer-assisted retention prediction systems operate in an extrapolative mode. After the minimum number (two or three) of actual HPLC runs under specific elution conditions, it is possible to predict HPLC separations with further changes in conditions. Experimental results, therefore, should be obtained prior to prediction and then one must input them into the system or store them on a floppy disk or a hard disk for later re-use. Details of the method for obtaining experimental results are described in section 3.2.

(B) Input elution conditions

To predict chromatograms, the following parameters are input into the prediction system: (1) column conditions; (2) elution conditions; and (3) extra-column effects.

The column conditions, which include column diameter, column length, particle size and mobile phase flow-rate, affect the resolution, analysis speed, column pressure, peak height and solvent consumption per run. The theoretical plate number and column dead time can be predicted from the column conditions[54,55], otherwise one must measure the plate number and column dead time for the specific analytical column and input them into the prediction system.

Elution conditions in isocratic elution include salt concentration, pH of the mobile phase and column temperature. Elution conditions in gradient elution are the gradient profile (the salt concentration of the mobile phase at the beginning and the end of the gradient), gradient shape (linear, convex, concave, multi-step and combinations of different gradient shapes), the steepness of the gradient and the gradient time. Extra-column effects include the extra-column residence time and band broadening.

(C) Prediction of retention times and band widths

After inputting of several of the parameters listed above, the retention prediction system predicts retention times and band widths. Details of these steps are described in Sections 3 (isocratic elution) and 4 (gradient elution).

(D) Visual presentation of chromatogram and gradient profile

Simulated chromatograms are displayed on the monitor screen using predicted retention times and band widths. Simulated chromatograms are given ideal Gaussian profiles with arbitrary units of area for each peak. The gradient profile is also presented visually on the monitor screen.

In these cases, the personal computer exhibits its powerful ability for graphical presentation, *i.e.*, visual simulation. Such computer graphics help the chromatographer to understand how the chromatographic separation changes on changing various elution conditions.

(E) Other option

To help in optimizing procedure and method development, other optional functions are available in the retention prediction system, such as the presentation of a relative resolution map.

2.4. Computer software and hardware requirements

Almost all the software developed for retention prediction, except in Japan, run on IBM PC, PC-XT, PC-AT and compatible computers equipped with 640K memory, two floppy disk drives, a monitor with graphics capability and a dot-matrix printer for output. A math coprocessor and a hard disk drive are optional. The operation of the program requires access to proprietary IBM files which must be copied from the operating system diskette (DOS 2.0 or later, MS-DOS 3.0 or later) before the system will function.

The retention prediction systems developed by Japanese researchers run on NEC PC-9801 and compatible computers equipped with 640K memory, two floppy disk drives, a monitor with graphics capability and a dot-matrix printer for output. A math coprocessor and a hard disk drive are optional. The system is supplied with MS-DOS 2.0 or later. The NEC (Nippon Electric, Tokyo, Japan) personal computer, which is more popular than the IBM-PC in Japan, is partly compatible with the IBM-PC but has no compatibility in its graphical presentation.

Programs are written in BASIC or FORTRAN. Most software is commercially available or registered as public domain software. For example, DryLab™ software is available from LC Resources (Lafayette, CA, U.S.A.).

3. ISOCRATIC ELUTION HPLC

3.1. Introduction

An isocratic elution HPIEC method is useful in the analysis of sample mixtures containing several ionic species. For example, several inorganic ions and some small biomolecules such as amino acids and nucleotides can be separated isocratically[1,6,7]. Complex mixtures with a wide retention range, however, cannot be eluted in one run by isocratic separation. Gradient elution is adequate for separating such mixtures.

There are several advantages of isocratic over gradient elution HPLC, *e.g.*, relatively simple equipment is required, relatively few parameters need to be optimized to find the desired separation, without re-equilibration of the column after every HPLC run, and low baseline drift. In addition, the theory of retention prediction in isocratic elution can be expressed as a simple function, so it has been studied for a long time. In this section the theory and applications of retention prediction for an isocratic elution HPIEC method are described.

3.2. Theoretical aspects

3.2.1. Prediction of retention times

In ion-exchange chromatography, the distribution of a solute ion I^i and an eluent ion El^e is described by the selectivity coefficient K for the following ion-exchange equilibrium[29–32,56–58]:

$$eI^i + iEl^e_{(s)} \rightleftharpoons eI^i_{(s)} + iEl^e \tag{1}$$

$$K = \frac{[I]^e[El]^i_{(s)}}{[I]^e_{(s)}[El]^i} \tag{2}$$

where the subscript (s) represents ionic species distributed in the stationary phase, the square brackets refer to the concentrations of the solute or eluent ions and superscripts i and e are the absolute values of the solute and eluent ionic charges.

The relationship between the capacity factor (k') of I and [El] can be derived as

$$k' = \frac{t_i - t_0}{t_0} = \frac{V_s}{V_m} \cdot D = \varphi' K^{1/i} Q^{e/i} [El]^{-(e/i)} \quad (3)$$

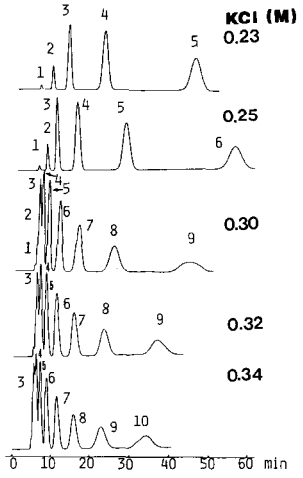
where V_m is the volume of the mobile phase, V_s is the volume of the stationary phase, t_i is the retention time in isocratic elution, t_0 is the column dead time, D is the distribution ratio for solute, φ' is a constant for a given column (phase ratio, V_s/V_m) and Q is the total exchange capacity of the ion exchanger for ion El. The relationship can be simplified if K and Q are assumed to be constant:

$$k' = a[El]^{-b} \quad (4)$$

where $b = e/i$ and $a = \varphi' K^{1/i} Q^{e/i}$.

This assumption has been demonstrated to be valid experimentally (Fig. 2) by several investigators[23,24,29,31,32,44–46], where linear relationships were observed for plots of log k' against log [El], as shown in Fig. 3 and expressed as follows:

$$\log k' = -b \log [El] + \log a \quad (5)$$



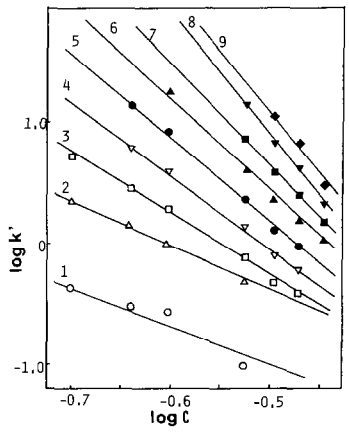


Fig. 2. Effect of changing eluent concentration on the elution profiles of inorganic polyphosphates at pH 10 and a column temperature of 25°C. Peak numbers indicate polymerization numbers: 1 = ortho-; 2 = di-(pyro-); 3 = tri-; 4 = tetra-; 5 = penta-; 6 = hexa-; 7 = hepta-; 8 = octa-; 9 = nona-; 10 = decaphosphate. Column, 250 × 4.0 mm I.D. packed with porous strong anion exchanger (TSKgel SAX, 10 μm, 3.7 mequiv./g). Flow-rate, 1.0 ml/min. Eluent, appropriate concentrations of KCl + 0.1% (w/v) EDTA sodium salt. (From ref. 46, with permission.)

Fig. 3. Plot of log k' *vs.* log C (= log [El]) using retention data of inorganic polyphosphates shown in Fig. 2. Numbering as in Fig. 2. (From ref. 46, with permission.)

These plots were obtained by measurement of capacity factors in isocratic elution with changing eluent concentrations as shown in Fig. 2.

The simple expressions in eqns. 4 and 5 were found to describe the retention accurately even in the presence of complex-forming equilibria and in the absence of complexes[29,31,32].

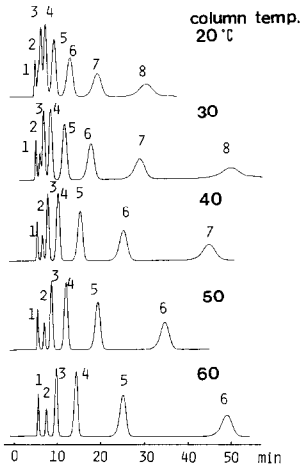
Eqns. 3 and 4 predict the retention times with changing eluent concentrations at given pH and column temperature. In addition, to predict how the retention times change with column temperature (Fig. 4), the following fundamental equation for chromatographic retention must take into consideration:

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \beta \quad (6)$$

where ΔH^0 and ΔS^0 are the enthalpy and entropy changes, respectively, associated with the solute retention process and the parameter β is assumed to be constant for a given ion exchanger at a given eluent concentration. These parameters were calculated from the plots (Fig. 5) of $\ln k'$ against $1/T$, from the measurement of chromatograms using isocratic elution with varying column temperature (Fig. 4).

Figs. 4 and 5 show that the enthalpy changes for inorganic polyphosphates are positive. The result is contrary to the effect of temperature on the ion-exchange chromatographic behaviour of organic compounds[59,60], but is consistent with that of inorganic ions[61]. The positive enthalpy changes for inorganic polyphosphates may be ascribed to the less hydrophobic interaction of inorganic polyphosphate anions with the ion exchanger compared with that of organic compounds.

In contrast, the effect of pH on the retention is too complicated to describe by a simple expression as shown in Fig. 6, because the way in which retention times change



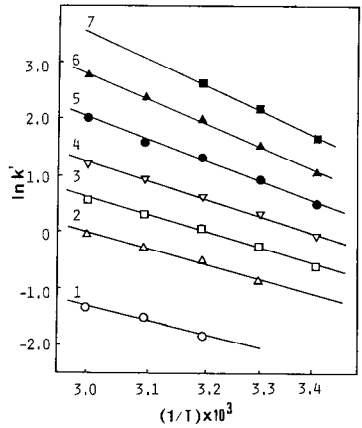


Fig. 4. Effect of column temperature on the elution profile of inorganic polyphosphates at pH 10. Eluent, 0.30 *M* KCl + 0.1% (w/v) EDTA sodium salt. Column, flow-rate and numbering as in Fig. 2. (From ref. 46, with permission.)

Fig. 5. Plots of $\ln k'$ *vs.* $10^3/T$ using retention data of inorganic polyphosphates shown in Fig. 4. Numbering as in Fig. 2, eluent as in Fig. 4. (From ref. 46, with permission.)

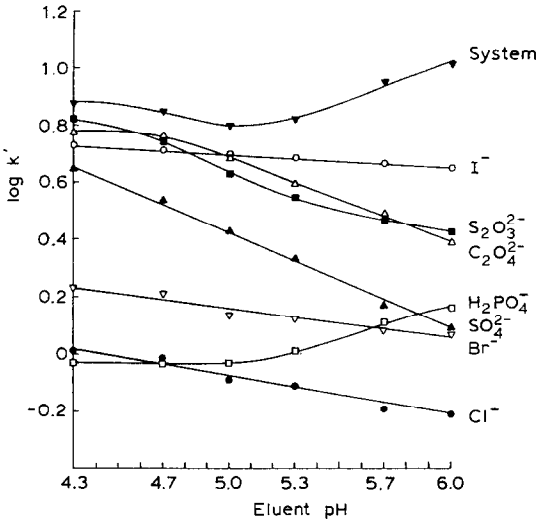


Fig. 6. Retention data for inorganic anions obtained with 5.0 m*M* phthalate eluents in the pH range 4.3–6.0. (From ref. 64, with permission.)

with pH of the mobile phase has not yet been elucidated. Hence, the empirical equation $k' = f(\text{pH})$ has been proposed by several workers.

Prior to the retention prediction, it is necessary for b, a, ΔH^0, ΔS^0 and the empirical function $k' = f(\text{pH})$ to be obtained experimentally. Constants and function are saved to a floppy disk as described in Section 2.3(A) and then it is possible to predict retention times.

3.2.2. Prediction of band width

The band width, w_i, in isocratic elution can be related to the number of theoretical plates, N, as follows:

$$w_i = 4t_i/N^{1/2} \tag{7}$$

N is approximately constant for a given set of elution conditions (column conditions, mobile phase and temperature). Changes in band width with varying column conditions can be calculated easily by eqn. 7, because N can be accurately predicted under various column conditions, such as length, diameter, particle size, and mobile phase flow-rate[54,55]. On the other hand the theoretical basis of the effect of temperature on N is obscure. At present, changes in band widths with varying column temperature should be calculated by the empirical equation[46].

Eqn. 7 predicts band widths under various elution and column conditions when N has been previously determined.

3.3. Retention prediction in HPLC separations of inorganic ion and biopolymers

3.3.1. Inorganic ions

Several investigators[62–66] have developed computer-assisted retention prediction systems in ion chromatography. Table 1 shows an example of retention prediction

TABLE I

COMPARISON OF PREDICTED AND OBSERVED RETENTION TIMES IN ION CHROMATOGRAPHY OF INORGANIC ANIONS UNDER ISOCRATIC ELUTION[62]

Anion separation column: Vydac Model 3021C4.6. Column temperature: 22.5 ± 2.0°C.

Eluent	*Species*	*Retention time (min)*	
		Predicted	*Observed*
$3.5 \cdot 10^{-3}$ *M* KHP[a] (pH 5.85)	Cl^-	1.78	1.74
	Br^-	2.16	2.16
	NO_3^-	2.45	2.45
	SO_4^{2-}	2.83	2.84
	$S_2O_3^{2-}$	3.70	3.73
$1.0 \cdot 10^{-3}$ *M* KHP (pH 4.00)	Cl^-	2.65	2.65
	NO_3^-	3.74	3.58
	Br^-	3.34	3.23
	SO_4^{2-}	13.56	14.00
$1.0 \cdot 10^{-3}$ *M* KHP (pH 4.20)	Cl^-	3.63	3.63
	NO_3^-	6.50	6.55
	SO_4^{2-}	18.40	18.40
$1.0 \cdot 10^{-3}$ *M* KHP (pH 5.20)	Cl^-	2.00	2.00
	NO_3^-	2.72	2.63
	SO_4^{2-}	5.30	5.15

[a] KHP = potassium hydrogen phthalate.

for some inorganic anions with varying eluent concentration and pH[62]. Agreement of the predicted retention times with observed values is satisfactory within a relative standard deviation (R.S.D.) of 3%, which is equivalent to the experimental precision. Senyavin *et al.*[66] also proposed a method for retention prediction of inorganic ions in ion chromatography and the predicted retention times agreed well with the observed values. Jenke and Pagenkopf[62] and Haddad and Cowie[64] demonstrated the use of a retention prediction system in selecting the correct eluent concentration and pH.

Baba and co-workers[41,44,46] investigated computer-assisted retention prediction in anion-exchange HPLC for inorganic condensed phosphates such as orthophosphate, diphosphate (pyrophosphate) and triphosphate. The retention prediction system can predict retention times and band widths with varying eluent concentration and column temperature, and then present the simulated chromatograms visually. The system, therefore, should be defined as a computer simulation system rather than a computer-assisted retention prediction system. The results are summarized in Tables 2 and 3. Observed capacity factors and band widths are predicted with average errors of 5% for retention times and 10% for band widths at each constant eluent concentration while column temperature is changed. Some predicted values of band widths deviate from the observed values with large errors of more than 10%, because the band widths are calculated based on the assumption that N is constant for different solutes.

Baba *et al.*[46] applied a computer simulation method in optimizing the elution conditions to maximize sample resolution and minimize the analysis time. Many more simulated chromatograms were obtained over a wide range of ionic strength and column temperature, and a resolution map (Fig. 7) was drawn for a specific peak pair,

TABLE 2

OBSERVED AND CALCULATED VALUES OF CAPACITY FACTOR IN ANION-EXCHANGE HPLC OF INORGANIC POLYPHOSPHATES UNDER ISOCRATIC ELUTION AT VARIOUS COLUMN TEMPERATURES[44]

Anion separation column: 250 × 4.0 mm I.D. packed with TSK gel SAX. Eluent: 0.25 *M* KCl + EDTA sodium salt.

Solute[a]	*Temperature (°C)*									
	20		*30*		*40*		*50*		*60*	
	Obs.	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*
1	0.185	0.170	0.256	0.220	0.300	0.290	0.337	0.370	0.474	0.460
2	0.841	0.790	1.03	1.09	1.35	1.33	1.63	1.69	2.12	2.12
3	1.54	1.38	2.09	1.88	2.69	2.51	3.52	3.30	4.49	4.26
4	2.93	2.73	4.14	3.88	5.67	5.39	7.55	7.34	10.2	9.81
5	6.11	5.56	9.11	8.34	13.2	12.2	18.6	17.4		
6	13.0	11.2	20.6	17.8						

[a] Numbering as in Fig. 2.

TABLE 3

OBSERVED AND CALCULATED VALUES OF BAND WIDTH ($\sigma_t = w_i/4$) IN ANION-EXCHANGE HPLC OF INORGANIC POLYPHOSPHATES UNDER ISOCRATIC ELUTION AT VARIOUS COLUMN TEMPERATURES[44]

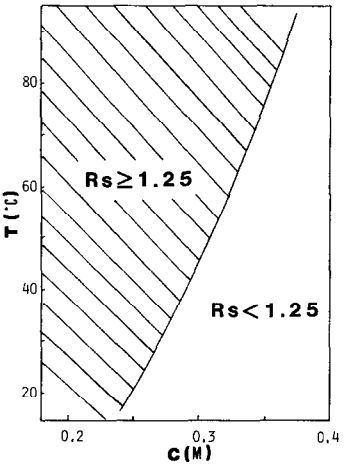
Column and eluent as in Table 2.

Solute[a]	*Temperature (°C)*									
	20		*30*		*40*		*50*		*60*	
	Obs.	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*
1	9.00	12.2	9.00	10.7	9.00	10.0	7.80	9.68	7.80	9.52
2	12.6	16.9	12.6	15.4	12.0	15.2	12.6	15.4	13.2	16.1
3	19.8	21.7	19.2	20.9	20.4	21.6	21.0	23.2	24.0	25.6
4	31.2	33.1	33.0	34.1	36.0	38.0	37.8	43.7	42.6	51.3
5	49.2	57.6	54.0	64.5	64.2	77.4	73.6	95.4		
6	90.0	106	102	129						

[a] Numbering as in Fig. 2.

which was always the poorest resolved, of di- and triphosphates. They concluded that the optimum conditions lay in the region close to the boundary of the shaded area in Fig. 7. Fig. 8 represents an example of simulated and observed chromatograms obtained under the optimum conditions. All peaks are resolved and analysed in less than 30 min.

Rojas *et al.*[65] described the computer simulation of ion-exchange separation of hydrochloric acid and malic acid. Several simulations were performed by changing the pH and the concentration of the eluent. Computer simulation was demonstrated as a powerful tool for optimizing elution conditions.



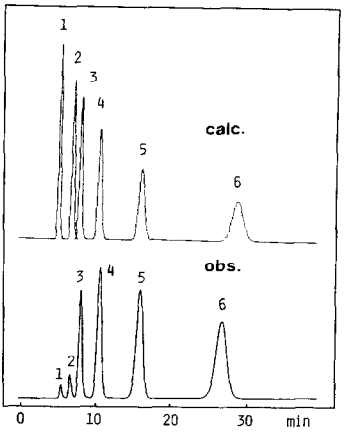


Fig. 7. Optimum isocratic elution conditions in anion-exchange separation of inorganic polyphosphates as a function of column temperature and eluent concentration at pH 10. *Rs* is the resolution between di- and triphosphate, which is the poorest resolved pair. (From ref. 46, with permission.)

Fig. 8. Observed (bottom) and simulated (top) chromatograms for inorganic polyphosphates under optimum isocratic elution condition. Eluent, 0.35 *M* KCl + 0.1% (w/v) EDTA sodium salt. Column temperature, 80°C. Other conditions as in Fig. 2. (From ref. 44, with permission.)

3.3.2. Biopolymers

In protein analysis by ion-exchange HPLC, eqns. 4 and 5, which are valid for small molecules, cannot be applied to retention prediction because the three-dimensional structure of proteins makes their electrostatic interactions with ion exchangers complex. Regnier and co-workers[67–69] proposed a stoichiometric displacement model to express the retention of proteins on ion-exchange supports. The model was based on the equilibrium

$$P \cdot CI + ZDI_b \rightleftharpoons P_b + ZcDI_0 + ZdCI \tag{8}$$

where P · CI refers to protein (P) in solution with an accompanying counter ion (CI), P_b is protein bound on the stationary phase, DI_b and DI_0 refer to displacing ions associated with the ion-exchange surface and in the mobile phase, respectively, Z is the number of charges that are associated with adsorption–desorption process and constants c and d are needed to adjust for valency.

Regnier and co-workers[67–69] derived an expression that relates the capacity factor to the concentration of displacing agent in the mobile phase and the number of charged groups involved in the adsorption–desorption process:

$$k' = K_Z/[(DI_0)(CI)]^Z \tag{9}$$

where K_Z is a constant.

The stoichiometric displacement model has predicted accurately the retention of proteins such as β-lactoglobulin A, conalbumin and ovalbumin[67,68]. The model has

TABLE 4
COMPUTER-ASSISTED RETENTION PREDICTION SYSTEM IN ISOCRATIC ELUTION

Method[a]	*Sample*[b]	*Variables*	*Ref.*
CARP	RE	[El]	17
CARP	IP	[El], pH	18
CARP	P	[El]	68
CARP	II	[El]	63
CARP-CS	IP	[El], Temp.	41,44
CARP-CS	II,OI	[El], pH	65
CARP	NA	[El]	69
CARP	II	[El]	66

[a] CARP = Computer-assisted retention prediction system; CARP-CS = computer-assisted retention prediction system having the availability of computer simulation.

[b] II = Inorganic ions; IP = inorganic polyphosphates; NA = nucleic acids; OI = organic ions; P = proteins; RE = rare earth metal ions.

also been demonstrated to be applicable to the prediction of the retention of nucleic acids, which have complex three-dimensional structures[69].

Table 4 gives examples of computer-assisted retention predictions in isocratic elution HPIEC separations.

4. GRADIENT ELUTION HPLC

4.1. Introduction

Gradient elution is a powerful technique for the separation of complex mixtures of biopolymers and inorganic ions. For example, more than 35 species of inorganic polyphosphates could be easily resolved by HPLC with gradient elution, as shown in Fig. 9. On the other hand, only six species could be separated by HPLC with isocratic elution, as shown in Fig. 8.

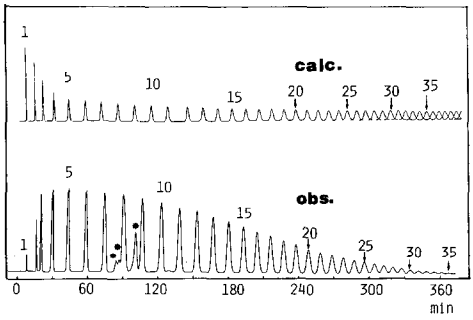


Fig. 9. Gradient HPLC separation of inorganic polyphosphates under the optimum conditions. Bottom and top chromatograms are observed and simulated, respectively. Column, 250 × 4.0 mm I.D. packed with porous strong anion exchanger (TSKgel SAX, 10 μm, 3.7 mequiv./g). Flow-rate, 1.0 ml/min. Eluent: (A) 0.2 M KCl + 0.1% (w/v) EDTA sodium salt (pH 10), (B) 0.6 M KCl + 0.1% (w/v) EDTA sodium salt (pH 10). Gradient profile expressed as eqn. 11 using the following parameters; $x = 0.3$, $C_i = 0.2$ M, $C_f = 0.6$ M, $t_f = 480$ min and $B = 3.70 \cdot 10^{-4}$. Column temperature, 60°C. (From ref. 47, with permission.)

In developing the HPLC method, more parameters to be optimized must be taken into account in gradient elution than in isocratic elution. Several workers have proposed theories for retention prediction in gradient elution since the mid-1950s when Drake[19] and Freiling[20,21] derived the fundamental equation for the prediction of the peak position in gradient elution.

The theories proposed in the 1950s and 1960s, however, were limited in their applications, because early gradient LC hardware was simple, relying on a linear or simple curved gradient. On the other hand, modern gradient hardware is typically equipped with an electronic programmable device and is capable of accurately generating either linear, convex, concave or multi-step gradients.

In the past 10 years, general theories have been proposed by two research groups, the theories of which are excellent from the viewpoints of applicability and generality. Emphasis will therefore be placed on these theories: Jandera and Churáček's approach[29–32] and Snyder and co-workers' LSS theory[33–37] and their applications.

4.2. Theoretical aspects

4.2.1. Theory derived in the early days

The theory based on the plate theory was developed for predicting retention times in gradient elution chromatography. Drake[19] and Freiling[20,21] proposed the fundamental concept for the prediction of retention in gradient elution chromatography as follows:

$$\int_0^{t_0} \mathrm{d}t_0 = \int_0^{t_g - t_0} \frac{1}{k'} \mathrm{d}t \tag{10}$$

After Drake and Freiling had derived the fundamental equation, several workers modified their equation to make it applicable in predicting retention in gradient ion-exchange chromatography. Schwab *et al.*[22], Ohashi and co-workers[23–25] and Molnár *et al.*[26] presented equations for prediction in anion-exchange chromatography using exponential gradients of salt concentration. The equation was extended by Massart and Bossaert[27] so as to be applicable to more general gradient functions such as linear, concave, convex and exponential gradients.

Inczédy[28] published an equation for prediction in the cation-exchange chromatography under a linear concentration gradient. Details of these theories will not be given here and readers are referred to the original papers and a comprehensive book[32] on the theory of gradient elution.

4.2.2. General theories

4.2.2.1. Jandera and Churáček's approach. These workers introduced a new function for gradient profiles[30–32]. The equation is applicable to a wide variety of gradient shapes, linear, concave and convex. Fig. 10 shows typical gradient profiles obtained from the equation

$$C = (C_i^{1/x} + Bt)^x$$

$$B = B'u = (C_f^{1/x} - C_i^{1/x})/t_f \tag{11}$$

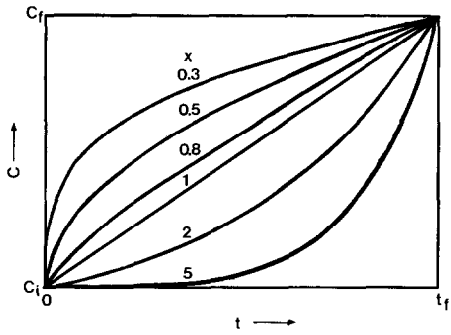


Fig. 10. Gradient profiles expressed as eqn. 11 by changing x values. (From ref. 80, with permission.)

where C is the eluent concentration at time t, C_i is the initial elution concentration at the beginning of the gradient elution ($t=0$), C_f is the final concentration at the end of the gradient elution ($t=t_f$), t_f is the gradient time, B represents the steepness of the gradient profile and x characterizes the shape of the gradient profile (linear gradient shape at $x=1$, convex gradient shape at $x<1$ and concave gradient shape at $x>1$).

Combining eqns. 4, 10, and 11 gave the equation for the prediction of retention times in gradient elution[30–32]:

$$t_g = (1/u)\{(1/B')[(xb+1)B'at_0u + C_i^{(xb+1)}]^{1/(xb+1)} - C_i^{1/x}/B'\} + t_0 \qquad (12)$$

Prior to the prediction of retention times in gradient elution using eqn. 12, it is necessary to obtain constants a and b, *i.e.*, the capacity factors must be measured at various eluent concentrations as shown in Fig. 2 (at least three sets of experiments) under isocratic conditions as demonstrated in Section 3.2.1. The constants are entered into the program or saved to a floppy disk as described in section 2.3(A).

Eqn. 12 was further extended[30–32] so as to be applicable to the prediction of retention with gradient elution which is combined using subsequent steps with different forms of the gradient profile and gradient elution with a mobile phase of constant composition in the first step followed by gradient elution in the second step.

Band widths, w_g, with gradient elution can be predicted from the equation[30–32]

$$w_g = (4t_0/N^{1/2})\{1+a[C_i^{1/x}+B(t_g-t_0-t_D)]^{-xb}\} \qquad (13)$$

where N is the plate number and t_D is the system dwell time between the outlet of the gradient-generating device and the column inlet.

4.2.2.2. Snyder and co-workers' approach (LSS theory). Snyder and co-workers introduced the concept of a "linear solvent strength" (LSS) system[33–37] for retention prediction in gradient elution. In LSS gradient systems, the k' value of a solute at the column inlet (k_i) varies with time t as

$$\log k_i = \log k_0 - b'(t/t_0) \qquad (14)$$

where k_0 is the value of k_i at the start of the gradient, and b' is a constant for a given solute.

In LSS systems, the retention time, t_g, in gradient elution can be predicted by the equation

$$t_g = (t_0/b') \log (2.3k_0b') + t_0 + t_D \quad (15)$$

In reversed-phase chromatography with linear gradient elution, the LSS-type solvent programme in eqn. 14 is applicable and parameter b' should remain constant as follows:

$$b' = V_m \Delta\varphi S/t_f u \quad (16)$$

where V_m is column dead-volume ($V_m = t_0 u$), $\Delta\varphi$ is the total change in volume fraction φ of the organic solvent during the gradient, S is equal to $-\mathrm{d}(\log k')/\mathrm{d}\varphi$, t_f is the gradient time and t_D is the system dwell time.

Ion-exchange separations are not of the LSS type, because k_i varies with time in a non-linear fashion in linear gradient elution. The LSS theory can nevertheless be adapted to deal with this situation. It is assumed that the gradient is approximately LSS over the time during which the solute band is migrating through the column, so that eqn. 15 is obeyed over this interval.

Prior to the prediction of retention from eqn. 15, two actual gradient runs are needed over the same composition range with two different times. The retention times are entered into the program as described in section 2.3(A).

Eqn. 17 can predict band widths, $\sigma_t (= w_g/4)$, in ion-exchange separations with an LSS gradient system.

$$\sigma_t = J(t_0 N^{-1/2}) G[1 + (1/2.3b')] \quad (17)$$

where J is an empirical parameter that recognizes "anomalous band broadening" with very steep gradients, N is the column plate number and G is a gradient compression factor.

Snyder and co-workers[14,48–50] developed PC-based simulation/optimization software, DryLab G (LC Resources), based on the LSS theory.

4.3. Retention prediction in HPLC separations of inorganic ions and biopolymers

4.3.1. Inorganic ions

Several investigators have studied retention prediction in exponential gradient elution for ion-exchange separations of phosphorus oxo acids[22–25], simple inorganic ions[22] and rare earth metal ions[26]. Massart and Bossaert[27] and Inczédy[28] also described retention prediction for the ion-exchange chromatography of metal complexes using linear gradients. The predictions achieved in those studies had errors of 5–10%.

Baba and co-workers[42–44,47] developed a computer-assisted retention prediction system that is an alternative to computer simulation. The system, which is based on Jandera and Churáček's approach, predicted the retention times of inorganic polyphosphates[42–44] in anion-exchange HPLC with binary-convex gradient shape. Jandera and Churáček's theory was modified by extrapolation of the linear

TABLE 5

OBSERVED AND CALCULATED RETENTION TIMES (min) IN ANION-EXCHANGE HPLC OF INORGANIC POLYPHOSPHATES (n=POLYMERIZATION NUMBER) UNDER GRADIENT ELUTION[43]

Gradient elution conditions, eluents and column as in Fig. 9.

n	*Obs.*	*Calc.*	*Error (%)*	*n*	*Obs.*	*Calc.*	*Error (%)*
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				

relationship between log k' and polymerization number of polyphosphates so as to be applicable in predicting the retention of polymers that could not be predicted from the original theory. The simulated chromatogram can be displayed on the monitor screen[47] as shown in Fig. 9.

In this study[43,47], retention times for more than 35 kinds of inorganic polyphosphates could be predicted within less than 5% error under various gradient elution conditions as listed in Table 5. Simulated and observed chromatograms are shown in Fig. 9 to demonstrate its accuracy.

Gradient elution has been demonstrated to be a powerful technique for the separation of polyvalent ion mixtures and combined mixtures of monovalent and polyvalent ions in ion chromatography[70]. LSS theory has also been investigated in gradient ion chromatography and is applicable to the prediction of retention times[70]. The complete separation of 36 ions was demonstrated with optimum gradients obtained by computer simulation as shown in Fig. 11.

A method for numerically solving a mathematical model of gradient elution ion-exchange chromatography of multi-component mixtures has been developed[71]. The method was demonstrated to predict retention times accurately (within 3% error) for some organic acids with linear gradients and to be applicable to elucidating the effect of the gradient on the retention times and the resolution.

4.3.2. Biopolymers

Snyder and co-workers[14,40,48–53] described the computer simulation of HPLC DryLab G based on the LSS theory. Calculated retention and band width data were

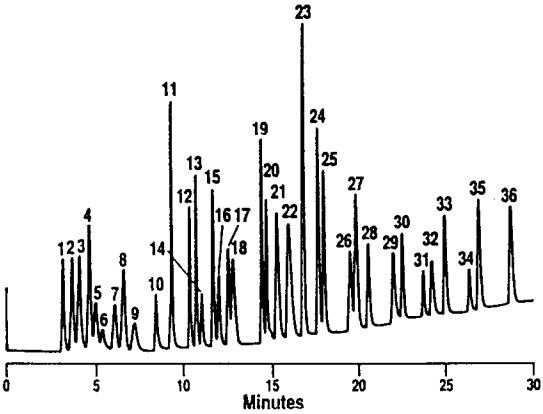


Fig. 11. Gradient elution of inorganic and organic anions. All anions are 10 ppm unless indicated otherwise. Peaks: 1 = fluoride (1.5 ppm); 2 = α-hydroxybutyrate; 3 = acetate; 4 = glycolate; 5 = butyrate; 6 = gluconate; 7 = α-hydroxyvalerate; 8 = formate (5 ppm); 9 = valerate; 10 = pyruvate; 11 = monochloroacetate; 12 = bromate; 13 = chloride (3 ppm); 14 = galacturonate; 15 = nitrite (5 ppm); 16 = glucuronate; 17 = dichloroacetate; 18 = trifluoroacetate; 19 = phosphite; 20 = selenite; 21 = bromide; 22 = nitrate; 23 = sulphate; 24 = oxalate; 25 = selenate; 26 = α-ketoglutarate; 27 = fumarate; 28 = phthalate; 29 = oxaloacetate; 30 = phosphate; 31 = arsenate; 32 = chromate; 33 = citrate; 34 = isocitrate; 35 = *cis*-aconitate; 36 = *trans*-aconitate. The following eluents were used. Eluent: (A) 0.75 m*M* NaOH; (B) 100 m*M* NaOH. Gradient programme: 0–5 min, 100% A; 5–15 min, from 100% A to 70% A; 15–30 min, from 70% A to 14% A. Flow-rate, 1.0 ml/min. Column, Dionex HPIC-AS5A. (From ref. 70, with permission.)

compared with the observed values and the validity of eqns. 15 and 17 in the analysis of protein samples such as ribonuclease and lysozyme was demonstrated. Experimental retention and band width data for the gradient elution of proteins shows good agreement with the LSS model. The system was applied to HPLC method development for the separation of proteins by gradient ion-exchange chromatography.

Hodges *et al.*[72] developed a computer simulation of separations of peptides and proteins by HPLC in the ion-exchange mode and also in size-exclusion and reversed-phase modes. The computer program, called Pro Digest-LC, accurately predicts the retention behaviour of peptides of known composition and simulates the chromatogram.

Baba *et al.*[45] extended the computer-assisted retention prediction system described above to be applicable in simulating anion-exchange separations of oligonucleotides. The results are summarized in Tables 6 and 7. HPLC separations of oligonucleotides were predicted and simulated visually as shown in Fig. 12 within an error of 8% under binary-linear (Table 6) and binary-convex (Table 7) gradient shapes.

Parente and Wetlaufer[73] proposed a theory for the retention prediction of some proteins. The theory was based on the stoichiometric displacement model[67–69] which was mentioned in section 3.3.2. They also developed a computer-assisted retention prediction system, which predicted retention times of α-chymotrypsinogen A, trypsin inhibitor, cytochrome *c* and lysozyme within *ca.* ±1%.

The theory proposed by Parente and Wetlaufer is similar to Jandera and Churáček's approach[30–32], which has been applied to the computer-assisted retention

TABLE 6

OBSERVED AND CALCULATED RETENTION TIMES (min) IN ANION EXCHANGE HPLC OF OLIGOADENYLATES, A_n, UNDER LINEAR GRADIENT ELUTION[45]

Column and eluents as in Fig. 12. Gradient profile is expressed as eqn. 11 using the following parameters: $x=1$, $C_i=0.01$ M, $C_f=0.3$ M, $t_f=128$ min; column temperature, 40°C.

Solute	*Obs.*	*Calc.*	*Error(%)*	*Solute*	*Obs.*	*Calc.*	*Error(%)*
A_1	4.72	4.05	−14	A_{14}	76.3	79.9	4.7
A_2	11.6	11.6	0	A_{15}	79.4	82.3	3.7
A_3	19.8	20.3	2.5	A_{16}	82.2	84.4	2.7
A_4	28.2	30.9	9.6	A_{17}	84.9	86.4	1.8
A_5	35.5	40.2	13	A_{18}	87.5	88.1	0.69
A_6	42.1	45.9	9.0	A_{19}	89.8	89.7	−0.11
A_7	47.9	52.3	9.2	A_{20}	92.0	91.2	−0.87
A_8	53.3	57.9	8.6	A_{21}	94.1	92.6	−1.6
A_9	57.2	62.8	9.8	A_{22}	96.1	93.8	−2.3
A_{10}	61.7	67.1	8.8	A_{23}	97.4	94.9	−2.6
A_{11}	65.8	70.9	7.8	A_{24}	99.2	96.0	−3.2
A_{12}	69.5	74.3	6.9	A_{25}	101	97.0	−4.0
A_{13}	73.0	77.2	5.8				

TABLE 7

OBSERVED AND CALCULATED RETENTION TIMES (min) IN ANION-EXCHANGE HPLC OF OLIGOADENYLATES, A_n, UNDER CONVEX GRADIENT ELUTION[45]

Gradient elution conditions, eluents and column as in Fig. 12.

Solute	*Obs.*	*Calc.*	*Error(%)*	*Solute*	*Obs.*	*Calc.*	*Error(%)*
A_1	5.04	3.74	−26	A_{19}	122	116	−4.9
A_2	11.7	8.70	−26	A_{20}	127	120	−5.5
A_3	17.8	14.8	−17	A_{21}	132	123	−6.8
A_4	23.7	23.7	0	A_{22}	136	126	−7.4
A_5	30.7	33.3	8.5	A_{23}	140	130	−7.1
A_6	38.0	39.8	4.7	A_{24}	145	132	−9.0
A_7	45.0	48.1	6.9	A_{25}	149	135	−9.4
A_8	52.0	56.0	7.7	A_{26}	153	137	−10
A_9	58.8	63.8	8.0	A_{27}	158	140	−11
A_{10}	65.1	70.6	8.4	A_{28}	162	142	−12
A_{11}	72.0	77.2	7.2	A_{29}	165	144	−13
A_{12}	78.9	83.4	5.7	A_{30}	169	146	−14
A_{13}	86.0	89.2	3.7	A_{31}	172	147	−15
A_{14}	92.9	94.6	1.8	A_{32}	175	149	−15
A_{15}	99.6	99.6	0	A_{33}	179	151	−16
A_{16}	106	104	−1.9	A_{34}	182	152	−16
A_{17}	112	109	−2.7	A_{35}	185	154	−17
A_{18}	117	113	−3.4				

prediction system for inorganic polyphosphates[42–44] and oligonucleotides[45] by Baba and co-workers. The results by Parente and Wetlaufer represented more accurate prediction of retention times than those by Baba and co-workers, because the dwell time was taken into consideration in the work by Parente and Wetlaufer but not in the work by Baba and co-workers. However, the theory proposed by Parente and

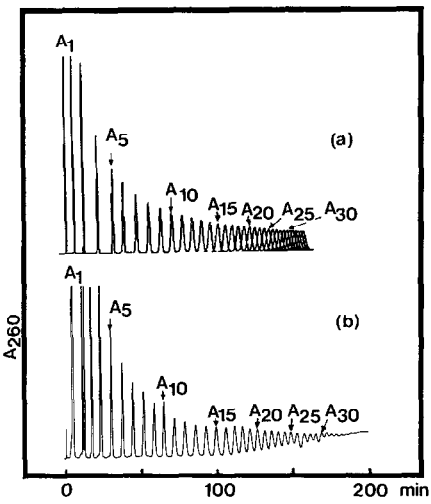


Fig. 12. (a) Simulated and (b) observed chromatograms for a polyadenylate partial hydrolysate, A_n. Gradient profile expressed as eqn. 11 using the following parameters: $x = 0.58$, $t_f = 240$ min, $C_i = 0.01$ *M* and $C_f = 0.3$ *M*. Column: Shim-pack WAX-1 (weak anion exchanger, 50 mm × 4.0 mm I.D.). Flow-rate, 1.0 ml/min. Buffer: (A) 0.01 *M* phosphate (pH 6.8) containing 20% acetonitrile; (B) 0.3 *M* phosphate (pH 6.8) containing 20% acetonitrile. Column temperature, 40°C. (From ref. 45, with permission.)

TABLE 8

COMPUTER-ASSISTED RETENTION PREDICTION SYSTEM IN GRADIENT ELUTION

Method[a]	*Sample*[b]	*Gradient shape*[c]	*Ref.*
CARP	II	EXP	22
CARP	ILP	EXP	23
CARP	IP	EXP	24, 25
CARP	RE	EXP	26
CARP	RE	LIN	27
CARP	MI	LIN	28
CARP	TH	LIN, CONC, CONV	30
CARP-CS	OI	LIN	71
CARP	P	LIN	74, 75
CARP-CS	IP	CONV	42–44
CARP	P	LIN(LSS)	40
CARP	P	LIN	73
CARP	II, OI	LIN(LSS)	70
CARP-CS	P	LIN	72
CARP-CS	ON	LIN, CONV	45

[a] See Table 4.

[b] II = Inorganic ions; ILP = inorganic lower oxo acids of phosphorus; IP = inorganic polyphosphates; MI = metal ions; OI = organic ions; ON = oligonucleotides; P = proteins; RE = rare earth metal ions; TH = theoretical treatment.

[c] CONC = Concave; CONV = convex; EXP = exponential; LIN = linear.

Wetlaufer is applied only to a linear gradient shape, whereas Jandera and Churáček's approach is applicable to a wide variety of gradient shapes.

Yamamoto and co-workers[74–76] investigated retention prediction in gradient elution for the ion-exchange chromatography of proteins. The simulated chromatograms were found to agree with the observed chromatograms with stepwise and linear gradient elution.

Table 8 gives examples of the computer-assisted retention prediction of HPIEC separations in gradient elution.

5. OPTIMIZATION AND HPLC METHOD DEVELOPMENT BY COMPUTER-ASSISTED RETENTION PREDICTION

To complete this review, the important area of the application of computer-assisted retention prediction systems, optimization and HPLC method development[77–80], will be briefly discussed. Optimization of isocratic elution conditions has been described in section 3.3. Emphasis will therefore be placed on the optimization of gradient elution conditions.

Computer-assisted retention prediction systems or HPLC computer simulation have been successfully applied to the optimization of gradient profiles (see refs. 11, 14, 40, 46–53, 77–80) and isocratic elution conditions[46,62,64]. The computer simulation

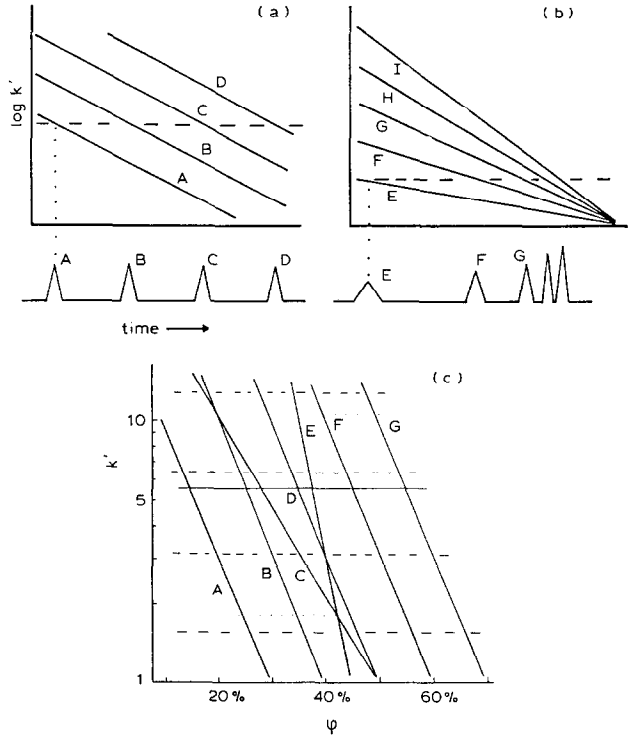


Fig. 13. Illustration of the relationships between mobile phase composition and capacity factor for three types of samples: (a) case I; (b) case II; (c) case III. (From ref. 52, with permission.)

system with its capability of graphical presentation of simulated chromatogram has especially been demonstrated to be an efficient approach for HPLC method development, because visual simulation assists chromatographers in the selection of optimum elution conditions.

Snyder and co-workers[48–53] have extensively studied the overall strategy for the development of a gradient elution method using computer simulation as the main tool. Possible sources of errors in computer simulation of gradient elution have been investigated[51]. Snyder and co-workers[52,53] classified samples to be separated in gradient elution into three groups according to their separation characteristics. Fig. 13 shows the relationships between solvent strength and capacity factors for cases I, II and III. HPLC separations for all cases were illustrated with changing gradient elution profiles using computer simulation to find the desired separation, *e.g.*, computer simulations for case III samples as shown in Fig. 14. The optimization strategy for each case was well documented[52,53].

In specific separation problems, computer simulation has also been recognized as a powerful tool for optimization. Examples are listed in Table 9. In isocratic and gradient elution chromatography of oligonucleotides[80] and inorganic polyphosphates[46,47], computer simulation has been successfully applied to the optimization of eluent conditions to maximize sample resolution and to minimize analysis time. For example, computer simulations have been performed in gradient HPLC separations for oligonucleotides[80], which corresponded to case II samples according to Snyder and co-workers' classification, by changing the gradient shapes as shown in Fig. 15.

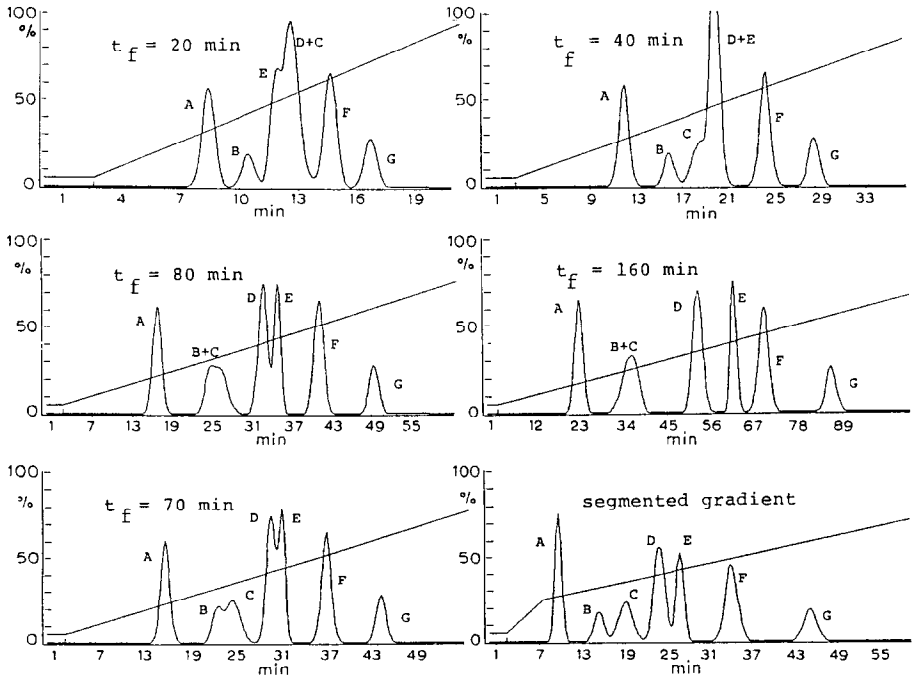


Fig. 14. Illustration of a case III sample. Gradient runs with t_f varying (5–100% gradients). DryLab G simulations. (From ref. 52, with permission.)

TABLE 9

DEVELOPMENT OF AN OPTIMIZED HPLC-IE SEPARATION BY A COMPUTER-ASSISTED RETENTION PREDICTION SYSTEM

Method[a]	*Type*[b]	*Sample*[c]	*Parameters to be optmized*[d]	*Ref.*
CARP	G	TH	LIN, CONC, CONV	77
CARP	I	II	[El], pH	62
CARP	I	II	[El], pH	64
CARP-CS	I	IP	[El], Temp	46
CARP-CS	G	IP	CONV	47
CARP	G	P	LIN (LSS)	40
CARP-CS	G	TH	LIN, CONV (LSS)	48–53
CARP-CS	G	HER	MULTI (LSS)	79
CARP-CS	G	ON	LIN, CONV	80

[a] See Table 4.

[b] G = Gradient; I = isocratic.

[c] HER = Herbicides; II = inorganic ions; IP = inorganic polyphosphates; ON = oligonucleotides; P = proteins; TH = theoretical treatment.

[d] CONC = Concave; CONV = convex; EXP = exponential; LIN = linear; MULTI = multi-step gradient shapes.

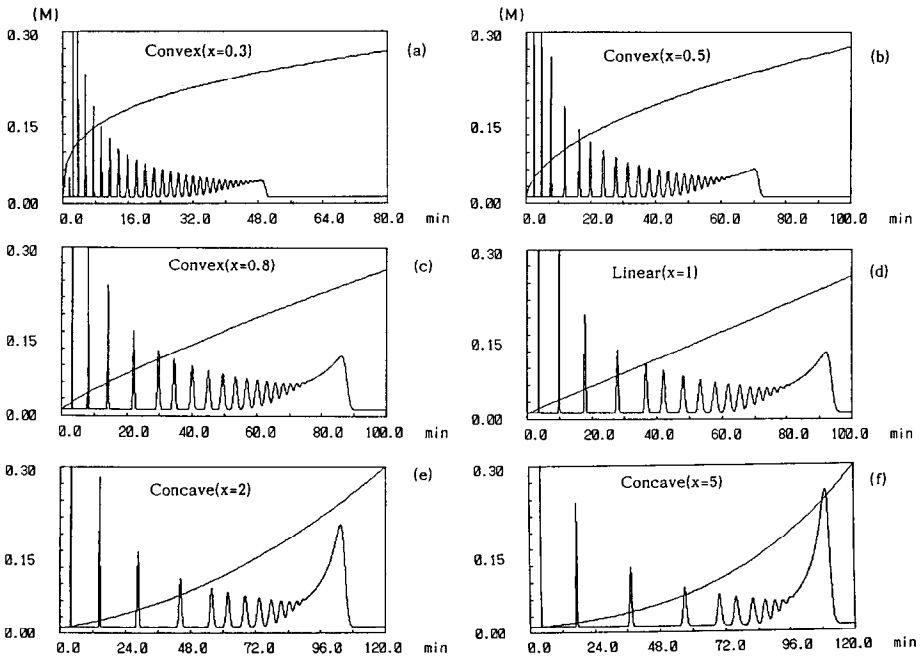


Fig. 15. Effect of gradient shape on the anion-exchange separation of oligonucleotides; computer simulation using the computer program developed by Baba and co-workers[42,43,45]. Conditions: anion-exchange column (50 × 4.0 mm I.D.); flow-rate, 1.0 ml/min. Gradient with buffers A ($C_i = 0.01$ *M* phosphate buffer) and B ($C_f = 0.3$ *M* phosphate buffer) and gradient time $t_f = 120$ min. (From ref. 80, with permission.)

A convex gradient shape gave a better resolution than linear and concave shapes in oligonucleotide analysis, especially for the later eluted samples. A systematic approach to obtaining an adequate separation by computer simulation has provided substantial savings in the time required for optimization over the non-systematic method (trial-and-error method).

6. CONCLUSIONS

In the few several years, significant advances have been made in computer-assisted retention prediction and HPLC computer simulation systems for the HPIEC method. Fundamental theories for retention prediction have been established for both isocratic and gradient elution. The theory is almost adequate to predict band widths in isocratic elution, whereas the theory of gradient elution is insufficient for the accurate prediction of band widths. The reason is that eqn. 13 neglected the band compression due to the change in k' during the elution of the sample band in gradient elutions and all effects involving the empirical parameters in eqns. 13 and 17 have been confirmed for separations of small molecules but not for large-molecule separations, which have many complexities. Further research in ion-exchange chromatography may provide a sufficient understanding of the relationship between band widths and gradient elution conditions. A computer-assisted retention prediction system has accurately predicted the retention in HPIEC separations of inorganic ions and biopolymers in both isocratic and gradient elution. Recently, the combination of computer-assisted retention prediction with computer graphics has provided a new method, called computer simulation, for the visual simulation of HPIEC separations. Computer-assisted retention prediction and HPLC computer simulation systems, especially the latter, greatly simplify the task of optimizing isocratic and gradient elution conditions for the HPIEC separations of complex mixture of ionic samples.

7. SYMBOLS

B, B'	gradient steepness parameters in eqn. 11
C	eluent concentration at time t in gradient elution
C_i, C_f	initial and final eluent concentrations in gradient elution (eqn. 11)
CI	counter ion (eqn. 8)
D	distribution ratio
DI_b, DI_0	displacing ions associated with the ion-exchange surface and in the mobile phase (eqn. 8)
El, $El_{(s)}$	an eluent ion in the mobile and the stationary phases (eqn. 1)
G	gradient compression factor[36]
ΔH^0	enthalpy change associated with the solute retention process
I, $I_{(s)}$	a solute ion in the mobile and the stationary phases (eqn. 1)
J	anomalous band broadening factor for gradient elution (eqn. 17)
K	ion-exchange selectivity coefficient
K_Z	constant in eqn. 9
N	column plate number
P, P_b	protein in solution and bound on the stationary phase
Q	total exchange capacity of the ion exchanger

S	change in k' with the moble phase composition in isocratic reversed-phase separation; $S=\mathrm{d}(\log k')/\mathrm{d}\varphi$ (eqn. 16)
ΔS^0	entropy change associated with the solute retention process
V_m	volume of the mobile phase
V_s	volume of the stationary phase
Z	number of charges associated with adsorption–desorption process (eqn. 8)
a	constant in eqn. 4
b	exponential constant in eqn. 4
b'	gradient steepness parameter (eqn. 14)
c, d	constants that are needed to adjust for valency in eqn. 8
i, e	absolute values of ionic charges (eqn. 1)
k'	solute capacity factor
k_i	k' at the column inlet during gradient elution (eqn. 14)
k_0	value of k' at the beginning of gradient elution (eqn. 14)
t	time (min)
t_D	dwell time of gradient equipment (min) between the outlet of the gradient-generating device and column inlet
t_f	gradient time (min)
t_i, t_g	retention times (min) in isocratic and gradient elutions
t_0	column dead time (min)
u	flow-rate (ml/min)
w_i, w_g	band widths in isocratic and gradient elutions
x	parameter characterizing gradient shape in eqn. 11
β	parameter in eqn. 6
σ_t	band width (1 S.D.) in gradient elution (s)
φ'	phase ratio, V_s/V_m
φ	volume fraction of organic solvent in a reversed-phase eluent
$\Delta\varphi$	change in φ from beginning to end of a reversed-phase gradient (eqn. 16)

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9. SUMMARY

The present state of studies on computer-assisted retention prediction for high-performance liquid chromatography (HPLC) in the ion-exchange mode is surveyed. Practical aspects of the retention prediction system are summarized. Attention is paid to fundamental theory for the prediction of retention times and band widths in both isocratic and gradient elutions. The article also deals with the applicability of the theory to the retention prediction and computer simulation system for HPLC separations of inorganic ions and biopolymers. Optimization and HPLC method development using the retention prediction system are briefly described.

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