

# Determination of Retention Factors of Aromatic Compounds by Gradient-Elution Reverse-Phase High Performance Liquid Chromatography

Ju Weon Lee and Kyung Ho Row<sup>†</sup>

Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University,  
253 Yonghyun-Dong, Nam-Ku, Incheon 402-751, South Korea  
(Received 4 March 2002 • accepted 3 May 2002)

**Abstract**—The retention mechanism of solutes under gradient conditions has been studied. Separation of a mixture of seven aromatic compounds in the two binary mobile phase, water/methanol and water/acetonitrile, was considered as an example. Retention factors were experimentally correlated by mobile phase composition. In this work, gradient-deviation time was newly introduced to compensate for ideal steep band along a column and experimentally determined by a linear equation form. An analytical expression in terms of the calculated retention factor and peak width was presented to predict the elution profile under gradient conditions. The calculated elution profile considered by the gradient-deviation time was closer to experimental data, and this mathematical model showed the feasibility of a predictive tool under gradient conditions.

Key words: Retention Factor, Aromatic Compound, Gradient Elution, Mathematical Model, RP-HPLC

## INTRODUCTION

The important parameter for quantification in HPLC is retention factor ( $k$ ) [Sofer and Hagel, 1997; Row and Lee, 1999]. Retention volume of a sample compound ( $V_R$ ) can be expressed in terms of the elution volume of a nonretained material ( $V_0$ ).  $k$  is given as the ratio of ( $V_R - V_0$ ) to  $V_0$ . The retention factor is proportional to the free energy change associated with the chromatographic distribution process, and is also related to the partition coefficient. Thus solute retention is affected by the thermodynamics of distribution between the two phases.

In isocratic elution, the mobile phase composition is unchanged during the separation. The various components of sample have wide range of  $k$  values. However, the disadvantages of isocratic mode are poor resolution of early-eluting bands, broadening of late-eluting bands to the point of difficult detection, tailing peaks, and unnecessarily long separation time. It is often overcome by changing the strength of the solvent during the operation. Gradient elution is usually performed by changing the mobile phase compositions [Row, 1989]. The changes in the solvent strength can be made stepwise or continuously. Gradient elution offers several advantages: total analysis time can be significantly reduced, overall resolution of a mixture is increased, peak shape is improved (less tailing) and effective sensitivity is increased since there is little variation in peak shape. More importantly, it provides the maximum resolution per unit time. Optimization of gradient elution is very important for analytical HPLC and scale-up column chromatography. The theory of gradient elution processes contains two general problems. The first one is connected with a total theory of solute migration under stepwise gradient conditions. Under the assumption that the relationships between the capacity factor and composition of the mobile phase are known, this problem was considered in [Lee et al., 1998].

To calculate the retention of solutes in the gradient program having five steps, Markowski and Golkiewicz obtained the analytical expressions. The second one is to predict the value of retention factor for any compositions of the multicomponent mobile phase by empirically determined equations [Row and Lee, 2000]. More often the correlations are based on a linear dependence of  $\log k$  via content of one or more components in mobile phase for binary and ternary mixtures [Lee et al., 1996]. One study [Row and Lee, 1999] demonstrated that linear models were apparently not applicable for ternary and quaternary mixtures.

In this proposed procedure, the analytical migration velocity was proposed based on retention factor which changed with time under a gradient condition to predict the Gaussian elution profile. In addition, the gradient-deviation time was introduced to the mathematical model to consider the actual phenomena deviating from the ideal step function along a column. The experimental study was performed with a mixture of seven aromatic compounds. We used binary mixture systems (water/methanol and water/acetonitrile). The purpose of this work was to optimize the separation conditions of aromatic compounds under gradient mode of reversed-phase HPLC with the modified mathematical model using a polynomial regression between the retention factor and the binary mobile phase composition and the gradient-deviation time.

## THEORY

### 1. Retention Factor

In linear chromatography, the adsorption isotherm is linear and is written as:

$$C = K \cdot q \quad (1)$$

$$\frac{dC}{dq} = K \quad (2)$$

where  $C$  is the concentration of solute in the mobile phase,  $q$  is the

<sup>†</sup>To whom correspondence should be addressed.

E-mail: rowkho@inha.ac.kr

concentration of solute in the stationary phase, and  $K$  is the equilibrium constant. The equilibrium constant is defined as the ratio of the concentration of solute in the stationary phase to the concentration of solute in the mobile phase. A fundamental chromatographic parameter is the retention factor. The retention factor,  $k$ , is defined as [Said, 1981]:

$$k = \frac{\text{amount of solute in the stationary phase}}{\text{amount of solute in the mobile phase}} \quad (3)$$

The relationship of the equilibrium constant and the retention factor is given by:

$$k = F \cdot K \quad (4)$$

$$F = \frac{V_s}{V_M} \quad (5)$$

where  $F$  is the phase ratio,  $V_s$  is the volume of the stationary phase, and  $V_M$  is the volume of the mobile phase. The relationship of the retention factor and the mobile phase composition is expressed as [Lee et al., 1996]:

$$k = k_0 e^{-S \cdot F_M} \quad (6)$$

where  $k_0$  and  $S$  are the empirical coefficients and  $F_M$  is the volume fraction or percentage of organic modifier in the mobile phase. The retention time is calculated by the following equation:

$$t_R = t_0(1+k) \quad (7)$$

where  $t_R$  is retention time and  $t_0$  is dead time.

## 2. Gradient Elution

If we assume that axial dispersion does not affect the retention time, a mixing effect between different mobile phases does not exist, and the adsorption isotherm is linear, we then obtain the following equation for the band profile of a single component:

$$\frac{\partial C}{\partial t} + F \frac{\partial q}{\partial t} + u \frac{\partial C}{\partial z} = 0 \quad (8)$$

where  $u$  is the linear velocity of the mobile phase. The migration of an injected band and the progressive change of its profile can be conveniently studied by using the theory of characteristics [Guiochon et al., 1994]. Eq. (8) can be rewritten as:

$$\frac{\partial C}{\partial t} + \left( \frac{u}{1 + F \frac{dq}{dC}} \right) \frac{\partial C}{\partial z} = 0 \quad (9)$$

Eq. (9) shows that the retention factor is associated the solute migration velocity,  $u_s$ , given by

$$u_s = \frac{u}{1 + F \frac{dq}{dC}} \quad (10)$$

In linear chromatography, the velocity  $u_s$  depends only on mobile phase composition (see Eqs. (1)-(2), (4), and (6)). If the mobile phase composition is constant, the velocity  $u_s$  is constant. Combining Eqs. (1)-(2), (4), (6), and (10) gives:

$$u_s = \frac{u}{1 + k(t)} \quad (11)$$

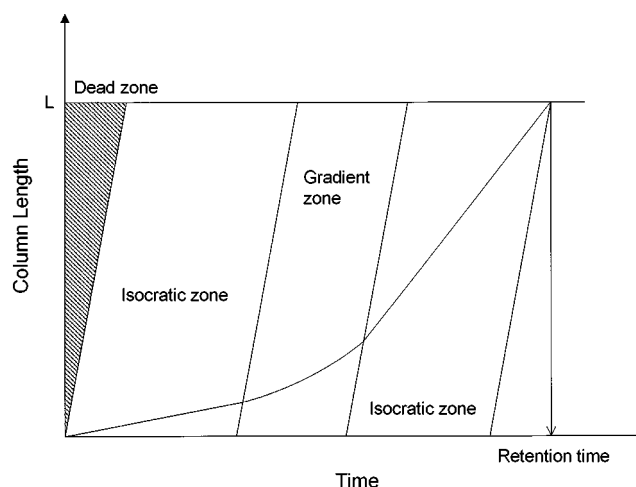


Fig. 1. Schematic drawing of solute migration trajectory under the gradient elution.

In the gradient mode, the mobile phase composition changes with time, so the retention factor is a function of time.

Fig. 1 shows the solute migration in the column. In the gradient mode, the mobile phase composition of the column is different at any time. It is difficult to calculate the solute migration trajectory; therefore, we propose the reduced time expressed as:

$$\tau = t - t_0 \frac{y_s}{L} \quad (12)$$

where  $\tau$  is the reduced time and  $L$  is the column length,  $z$  is the axial distance along chromatographic column, and instead of  $z$ ,  $y_s$  is adopted as a dependent variable to calculate the migration velocity. At the column outlet,  $y_s = L$ , the adjusted retention time is  $t_R - t_0$ . Eq. (11) is rewritten as:

$$\frac{dy_s}{d\tau} = u_s = \frac{u}{k(\tau)} \quad (13)$$

Analytical solution of Eq. (13) is given as:

$$y_s = \int_{\tau_0}^{\tau} \frac{u}{k(\omega)} d\omega + y_0 \quad (14)$$

where,  $\tau_0$  and  $y_0$  are based on the starting points of solute at the subsequent gradient times. At  $y_s = L$ ,  $\tau = \tau_R$  where it is the same as the adjusted retention time.

In isocratic mode, the retention factor does not change. We assume that the number of theoretical plates of each solute is constant in an identical column irrespective of mobile phase compositions and the bandwidth is gradually built up linearly along a column. The bandwidth at the column outlet is equal to the peak width. The peak width is expressed in terms of theoretical plate number as follows:

$$w = 4 \frac{t_R}{\sqrt{N}} \quad (15)$$

where  $w$  is the peak width and  $N$  is the theoretical plate number. The slope of a front bandwidth ( $u_f$ ) is  $L/(\tau_R - w/2)$  and that of a back of bandwidth ( $u_b$ ) is  $L/(\tau_R + w/2)$ . The migration velocities of the front and back of bandwidth,  $u_f$  and  $u_b$ , respectively, are expressed as:

$$\frac{dy_f}{d\tau} = u_f = \frac{\sqrt{Nu}}{(\sqrt{N}-2)k(\tau)-2} \quad (16-1)$$

$$\frac{dy_b}{d\tau} = u_b = \frac{\sqrt{Nu}}{(\sqrt{N}+2)k(\tau)+2} \quad (16-2)$$

where  $y_f$  and  $y_b$  are the front and back of the bandwidth migration distance. Analytical solution of Eqs. (16-1) and (16-2) is given by;

$$y_f = \int_{\tau_{f,0}}^{\tau} \frac{\sqrt{Nu}}{(\sqrt{N}-2)k(\omega)-2} d\omega + y_{f,0} \quad (17-1)$$

$$y_b = \int_{\tau_{b,0}}^{\tau} \frac{\sqrt{Nu}}{(\sqrt{N}+2)k(\omega)+2} d\omega + y_{b,0} \quad (17-2)$$

where  $\tau_{f,0}$  and  $y_{f,0}$  are based on the starting point of the bandwidth front and  $\tau_{b,0}$  and  $y_{b,0}$  on the starting points of the bandwidth back. At  $y_f = y_b = L$ , the reduced times become  $\tau_{r,f}$  and  $\tau_{r,b}$ , and the peak width is equal to  $(\tau_{r,b} - \tau_{r,f})$ . These calculated retention times (Eq. (14)) and peak width (Eq. (17)) are used to estimate the peak profile. The peak profile is calculated by Gaussian profile [Guiochon et al., 1994]:

$$C_{eff} = 2 \frac{A}{w\sqrt{\pi/2}} \exp\left(-\frac{1}{2} \frac{(t-t_R)^2}{w^2}\right) \quad (18)$$

where  $A$  is peak area and  $C_{eff}$  is the concentration of solute at the column outlet.

When the mobile phases are changed by stepwise gradients generated by a pump system with a mixer, S-shaped curves can be usually observed. To correct these deviations from the step function, the following modified equations are used with experimental breakthrough curve [Kaltinbrunner and Jungbauer, 1997]:

$$I = I_{Max} \exp(-\exp(-k_s(t-t_c))) \quad (19-1)$$

$$\frac{dI}{dt} = I_{Max} k_s \exp(-\exp(-k_s(t-t_c)) - k_s(t-t_c)) \quad (19-2)$$

where  $I_{Max}$ ,  $k_s$ , and  $t_c$  are empirical coefficients. The step-input injection shows the S-shaped response profile after it comes out of a column. So we assume that the step-input is actually injected as a linear gradient. The simplified form of S-shaped curve is represented by a linear equation,  $I_M$ :

$$I_M = \frac{I_{Max} k_s}{e} (t - t_c) + \frac{I_{Max}}{e} \left( t_c - \frac{1}{k_s} \leq t \leq t_c + \frac{e-1}{k_s} \right) \quad (20)$$

The system dwell time is expressed as:

$$t_{dwell} = t_c - \frac{1}{k_s} - t_0 - t_G \quad (21)$$

where  $t_{dwell}$  is the system dwell time and  $t_G$  is the gradient time. The system dwell time is spent mainly at a mixer of an HPLC system. We further consider a factor to affect the migration velocity of solute during gradient elution. The gradient-deviation time,  $t_{GD}$ , is included to compensate for the deviation from an ideal step function profile. The time is obtained by following relation (ref. to Eq. (20)):

$$t_{GD} = \left( t_c + \frac{e-1}{k_s} \right) - \left( t_c - \frac{1}{k_s} \right) = \frac{e}{k_s} \quad (22)$$

So  $t_{GD}$  is easily obtained by experimentally determined  $k_s$ . Finally,  $t_{GD}$  is considered both in Eqs. (14) and (17). The more precise elution

profiles can be predicted by Eq. (18).

## EXPERIMENTAL

### 1. Reagents

Seven aromatic compounds were used as solutes: benzene (BZ) (Oriental Chemical Industry, Incheon, Korea), chlorobenzene (CB) (Samchun Chemical, Kyungki-Do, Korea), toluene (TO) (Oriental Chemical Industry, Incheon, Korea), styrene (ST) (Kanto Chemical, Tokyo, Japan), *o*-dichlorobenzene (*o*-DCB) (Duksan Pharmaceutical, Kyungki-Do, Korea), *p*-dichlorobenzene (*p*-DCB) (Samchun Chemical, Kyungki-Do, Korea), and *m*-xylene (*m*-XY) (Junsei Chemical, Tokyo, Japan). The concentration of solutes dissolved in methanol was fixed as 20 µg/ml. Solvent as the mobile phase was water-filtered by a Milipore ultra pure water system (Milipore, Bedford, MA, USA). Methanol (Duksan Pure Chemical, Kyungki-Do, Korea) and acetonitrile (Duksan Pure Chemical, Kyungki-Do, Korea) were added in the mobile phase.

### 2. HPLC

The HPLC system was composed of a 515 pump (Waters, Milford, MA, USA), 600S controller (Waters, Milford, MA, USA), 486 UV detector (Waters, Milford, MA, USA), and Rheodyne injector (20 µl sample loop, Rheodyne, Cotati, CA, USA). The data acquisition system was CHROMATE Ver. 3.0 (Interface Engineering, Seoul, Korea). OptimaPak C18 (250×4.6 mm, RS Tech., Taejon, Korea) column was used. Injection volume was 1 µl through-

Table 1. Experimental parameters

Dead time, $t_0$	2.65 min
Dwell time, $t_{dwell}$	3.20 min
Gradient deviation, $t_{GD}$	1.52 min
Linear velocity, $u$	9.43 cm/min
$I_{Max}$	0.05
$k_s$	1.78
$t_c$	7.41 min

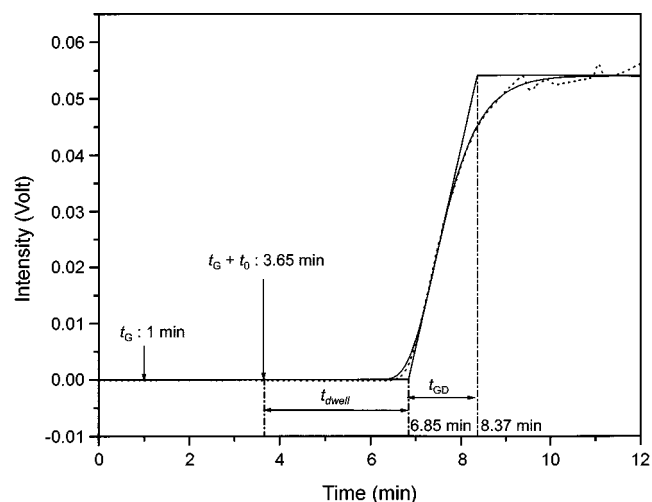


Fig. 2. Stepwise gradient breakthrough curve (dotted line: experimental data, solid curve line: curve fitting, solid straight line: imaginary linear gradient line, before  $t_G$ : water/methanol = 40/60 vol%, after  $t_G$ : water/methanol = 25/75 vol%).

out the experiments. The flow rate of mobile phase was 1 ml/min and monitored at the fixed wavelength of 254 nm.

### 3. Procedure

To measure the column dead volume, the pure water was injected in a pure methanol mobile phase. The retention time of the negative peak of water was designated as the dead time, which was equal to 2.65 min in this work as listed in Table 1. The system dwell time,  $t_{dwell}$  and gradient-deviation time,  $t_{GD}$  were measured from a breakthrough curve in the stepwise-gradient mobile phase system in Fig. 2. When the mobile phase was changed as a step function,  $t_{dwell}$  could be calculated from the differences between 6.85 and 3.65 min, which was 3.20 min. The value of  $t_{GD}$  was also obtained by the duration of the lowest and the highest response of the imagi-

nary linear equation and it was 1.52 min.

The mobile phases were two binary systems, water/methanol and water/acetonitrile. In the isocratic mode, the compositions of methanol were varied as 65, 70, 75, 80, 85, and 90 (vol%), while those of acetonitrile were as 55, 60, 65, 70, 75, and 80 (vol%). The experimental data were used to determine the coefficients of the retention model (Eq. (6)) by a simple linear regression analysis. Four gradient conditions of each binary mobile phase were experimentally performed.

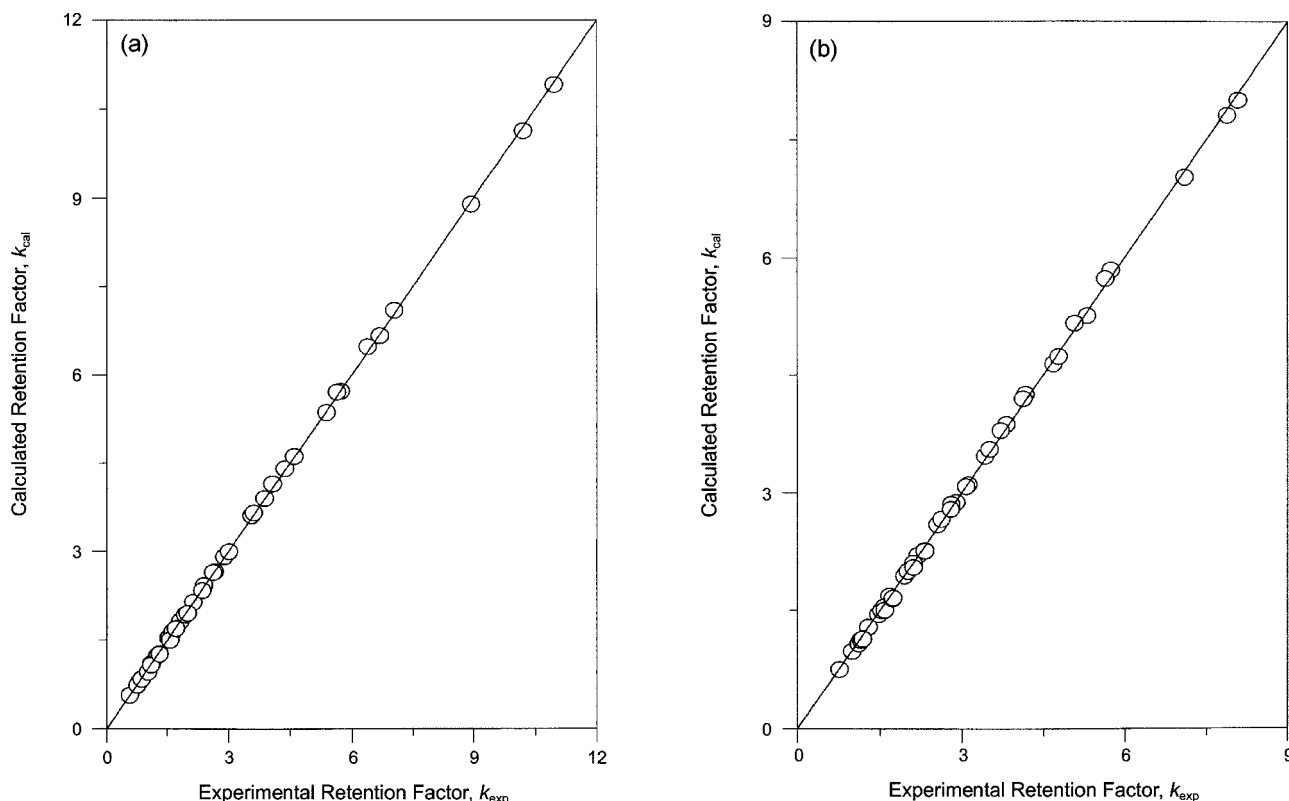
## RESULT AND DISCUSSION

Most HPLC systems are composed of a dual-pump with a mixer.

**Table 2. Empirical coefficients of retention and model of Eq. (6)**

Solute*	Methanol			Acetonitrile		
	$k_0$	S	$r^2$	$k_0$	S	$r^2$
BZ	227.675	0.06668	0.99999	54.685	0.05353	0.99967
CB	930.442	0.07933	0.99984	114.793	0.05831	0.99916
TO	820.839	0.07641	0.99993	112.672	0.05760	0.99920
ST	1447.168	0.08278	0.99986	151.474	0.06110	0.99907
<i>o</i> -DCB	2891.881	0.08898	0.99968	208.027	0.06160	0.99875
<i>p</i> -DCB	3411.381	0.08951	0.99899	259.113	0.06321	0.99885
<i>m</i> -XY	2766.938	0.08518	0.99920	236.732	0.06202	0.99891
Average			0.99964			0.99909

\*Refer to abbreviation.



**Fig. 3. Comparison of the calculated and experimental retention factors under isocratic elution (a: methanol, b: acetonitrile, straight line:  $y=x$ ).**

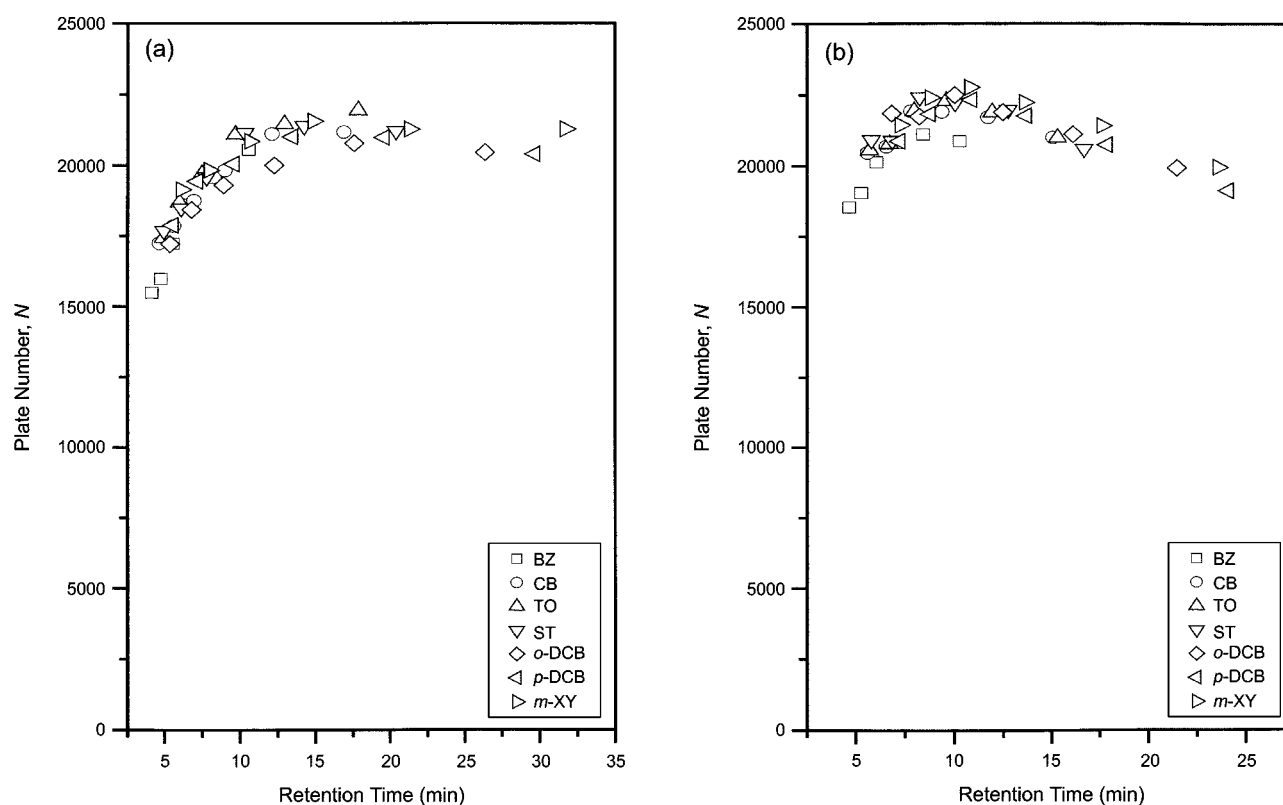
Pure solvents from their reservoirs were mixed, and passed into an inlet of the column. For a stepwise input, it is apparent to describe the response function from a mixing device as an S-curved function, which was correlated as Eq. (19). Previous researchers have neglected this deviation from the ideal stepwise form. They only considered the delay, expressed by the dwell volume. In practice, the linear and step gradient might be modified to an asymmetrical S-shaped curve [Kaltinbrunner and Jungbauer, 1997]. But this S-shaped curve function is very complicated to express a simple equation. Therefore, the S-shaped function is simplified to a linear function of Eq. (20) (Fig. 2 shows the S-shaped curve and its simplified linear line). The deviation from linearity is equal to the gradient-deviation time (Eq. (22)), and the dwell time (Eq. (21)) is determined as the inset of elution profile after the summation of gradient time and dead time. Table 1 shows the parameters of Eq. (20) including the dwell time and the gradient-deviation time. The gradient effect is delayed for the dwell time, and the deviation by gradient condition is added to the range that the mobile phase is varied.

Table 2 shows the regression results of retention model of Eq. (6). The averages of regression coefficients,  $r^2$ , of each organic modifier had sufficient precision, very close to 1.0. Fig. 3 shows that the calculated and experimental retention factors were in fairly good agreement. However, the deviation of the calculated and experimental retention factors of more-retained solutes (i.e. *o*-DCB, *p*-DCB, and *m*-XY) was larger than less-retained solutes. These results enabled the prediction of difficult gradient elution. It is essential to find the proper empirical equation of the retention factor in isocratic condition, because it is more difficult to predict the elution profile in gradient condition. Fig. 4 shows the theoretical plate num-

**Table 3. Averages of theoretical plate numbers (N) of solutes**

Solute	Average of theoretical plate numbers, N	
	Methanol	Acetonitrile
BZ	17901	20071
CB	19321	21274
TO	20046	21393
ST	19917	21500
<i>o</i> -DCB	19360	21494
<i>p</i> -DCB	19956	21101
<i>m</i> -XY	20653	21693

ber, N, with the retention times for seven aromatic components. Below ca. 7 of the retention time, the theoretical plate numbers were increased with retention times, especially with the addition of methanol. However, it was centered around 20,000. These appearances are attributed to the distorted peak shape of the less-retained solute from the Gaussian distribution. Moreover, the peak tailing caused the asymmetry of a peak, so average values of the theoretical plate number of components were designated irrespective of mobile phase compositions. Table 3 shows the arithmetic average values of N which were utilized to calculate the gradient elution profiles [Lee et al., 1998]. To prove that the consideration of gradient-deviation time helps to predict more precisely the description of real gradient mode, the error percentages of retention times and comparison of calculated and experimental profiles are presented in Fig. 5. This figure clearly shows that the inclusion of gradient-deviation time contributes to the more precise prediction under gradient condition.



**Fig. 4. Variation of theoretical plate numbers with retention times (a: methanol, b: acetonitrile).**

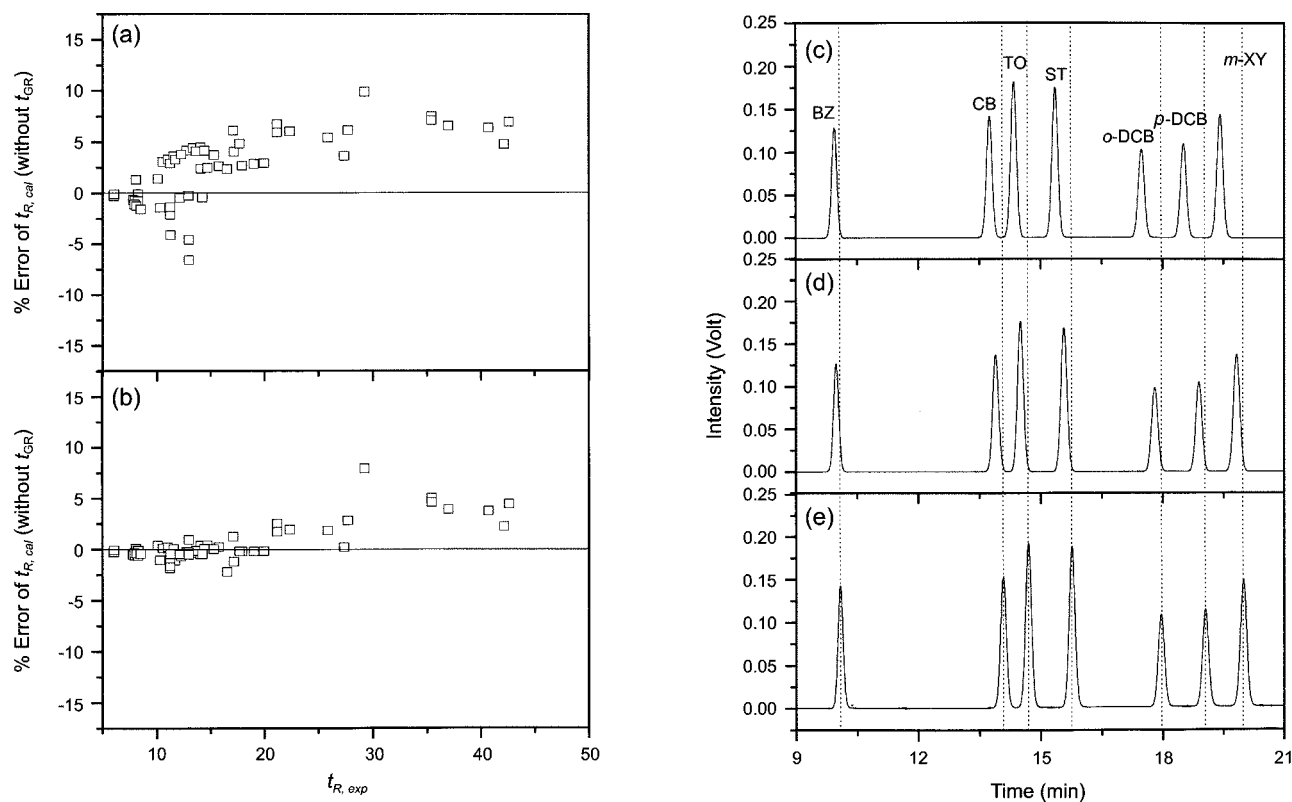


Fig. 5. Comparison of elution profiles with/without gradient-deviation times (a: error percentage of calculated retention time without gradient-deviation time, b: error percentage of calculated retention time with gradient-deviation time, c: calculated elution curve without gradient-deviation time, d: calculated elution curve with gradient-deviation time, e: experimental data, gradient condition of c, d, and e: Gradient #1).

Table 4. Experimental gradient conditions

	Time (min)	Water (vol%)	Methanol (vol%)	Curve
Gradient #1	Initial	35	65	Linear
	20	15	85	
Gradient #2	Initial	30	70	Linear
	10	10	90	
Gradient #3	Initial	30	70	Stepwise
	10	10	90	
Gradient #4	Initial	30	70	Stepwise
	4	20	80	
Gradient #5	Initial	30	70	Linear
	30	50	50	
Gradient #6	Initial	30	70	Linear
	10	50	50	
Gradient #7	Initial	70	30	Linear
	10	40	60	
Gradient #8	Initial	80	20	Linear
	30	40	60	

When methanol was used as organic modifier, better resolution and shorter separation time were observed in gradient #2 (see Table 4 and Fig. 6). With acetonitrile as an organic modifier, the gradient condition of #5 showed the better result as shown in Fig. 7. With the acetonitrile modifier, some components of CB, TO, *p*-DCB,

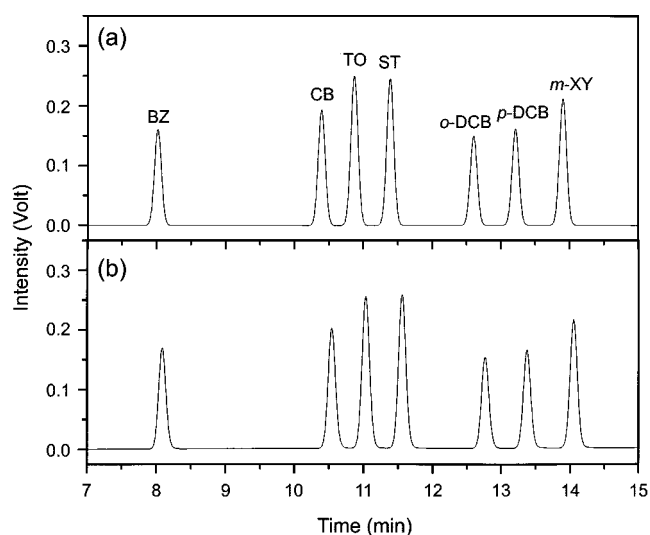


Fig. 6. Comparison of calculated and experimental elution profiles (a: calculated elution curve, b: experimental elution curve, Gradient #2).

and *m*-XY could not be separated. The calculated elution profiles (Figs. 6-a and 7-a) have relatively good agreement with the experimental chromatograms (Figs. 6-b and 7-b). Table 5 shows the error percentage of calculated retention time. The error percentages in gradient conditions #1-6 were lower than #2. However, those in

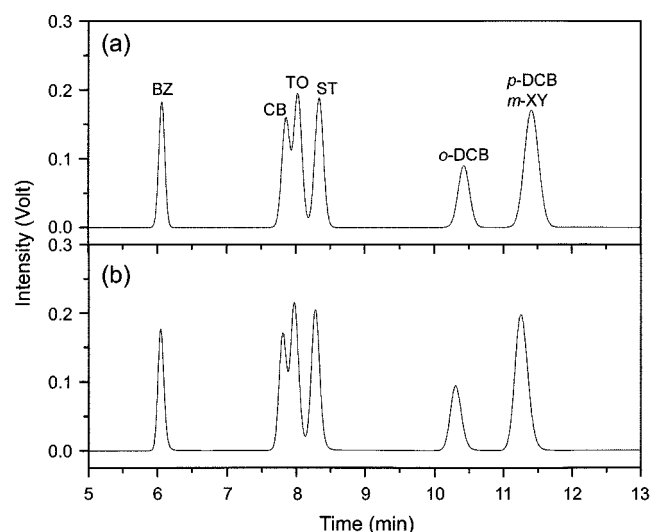


Fig. 7. Comparison of calculated and experimental elution profiles (a: calculated elution curve, b: experimental elution curve, Gradient #5).

the gradient conditions #7-8 were mostly higher than #2. The contents of acetonitrile in #7-8 were 30 and 20 (vol%), respectively, and these are experimentally out of the ranges experimented. The extrapolated values by the retention model of Eq. (6) are not exactly coincident to the real values in the gradient condition (see Fig. 8(a) and (b)). For a certain solute, the  $k_0$  values of organic modifiers of methanol and acetonitrile should be identical, but they were quite different as listed in Table 2 [Lee et al., 1996].

## CONCLUSION

A considerable increase in the application of gradient elution in more complex analytical problems has been observed. To reveal the chromatographic behavior of a solute under gradient conditions, more complicated mathematical models are required as the retention mechanism is changed with mobile phase composition. The gradient-deviation time was considered for the variation of mobile phase composition along a column. Under the binary mobile phases of water/methanol and water/acetonitrile in step/linear gradient conditions, the agreement between the resulting calculated elution pro-

Table 5. Percentage errors of calculated retention times

Material	Err. %							
	Gradient #1	Gradient #2	Gradient #3	Gradient #4	Gradient #5	Gradient #6	Gradient #7	Gradient #8
BZ	0.995	0.838	0.123	0.425	0.311	0.121	1.726	8.207
CB	1.298	1.399	0.501	1.364	0.635	0.628	2.933	5.258
TO	1.315	1.498	0.291	0.661	0.708	0.709	2.136	4.860
ST	1.235	1.449	0.488	0.219	0.736	0.800	2.337	4.152
o-DCB	0.867	1.291	1.803	0.303	1.238	1.142	2.171	3.989
p-DCB	0.871	1.193	0.717	0.494	1.849	1.414	1.777	3.549
m-XY	0.851	1.051	0.283	0.440	1.097	0.106	1.899	3.576

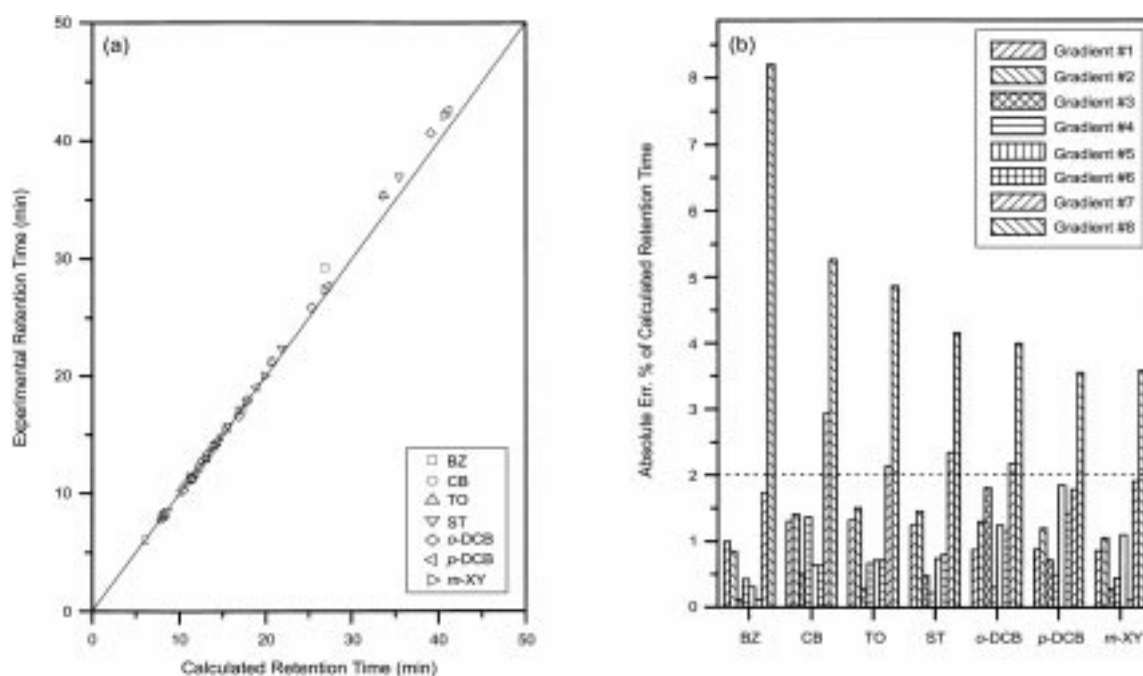


Fig. 8. Comparison of calculated and experimental retention times under the gradient elutions (a: comparison of calculated and experimental retention times, b: error percentages of calculated retention times).

file and experimental data of seven aromatic compounds was fairly good.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of the Center for Advanced Bioseparation Technology, and this work was performed in the High-Purity Separation Laboratory, Inha University.

### NOMENCLATURE

A	: peak area
C	: concentration of solute in the mobile phase [ $\mu\text{g/ml}$ ]
$C_{\text{eff}}$	: concentration of solute at the column outlet [ $\mu\text{g/ml}$ ]
F	: phase ratio
I	: response of breakthrough curve
$I_{\text{Max}}$	: empirical coefficient of Eq. (19)
K	: equilibrium adsorption constant
k	: retention factor
$k_0$	: empirical coefficient of Eq. (6)
$k_s$	: empirical coefficient of Eq. (19)
L	: column length [cm]
N	: theoretical plate number
q	: concentration of solute in the stationary phase [l]
S	: empirical coefficient of Eq. (6)
$t_0$	: dead time of the column [min]
$t_c$	: empirical coefficient of Eq. (19)
$t_{\text{dwell}}$	: system dwell time [min]
$t_{\text{GD}}$	: gradient deviation time [min]
$t_R$	: retention time of solute [min]
u	: linear velocity of mobile phase [cm/min]
$u_f, u_b$	: front and back of bandwidth migration velocity [cm/min]
$u_s$	: solute migration velocity [cm/min]
$V_M$	: volume of the mobile phase in the column [ml]
$V_S$	: volume of the stationary phase in the column [ml]
w	: peak width [min]

$y_f, y_b$	: front and back of bandwidth migration function [cm]
$y_s$	: solute migration function [cm]
$\tau$	: reduced time [min]
$\tau_R$	: reduced retention time [min]

### REFERENCES

- Guiochon, G., Golshan-Shirazi, S. and Katti, A. M., "Fundamentals of Preparative and Nonlinear Chromatography," Academic Press, London (1994).
- Kaltinbrunner, O. and Jungbauer, A., "Simple Model for Blending Aqueous Salt Buffers Application to Preparative Chromatography," *J. of Chromatogr. A*, **769**, 37 (1997).
- Lee, C. H., Lee, J. W. and Row, K. H., "Optimum Solvent Selectivity and Gradient Mode for Deoxyribonucleosides in Reversed-Phase High-Performance Liquid Chromatography," *J. of Chromatogr. A*, **828**, 337 (1998).
- Lee, Y. W., So, M. S., Lee, J. W., Chung, S. T. and Row, K. H., "Retention Models of Capacity Factor with Different Compositions of Organic Modifier in RP-HPLC," *Korean J. Chem. Eng.*, **13**, 578 (1996).
- Markowski, W. and Golkiewicz, W., "Optimization of Stepwise Gradient Elution in Columns Chromatography," *Chromatographia*, **25**, 339 (1988).
- Row, K. H., "Separation of Oligonucleotides by Reversed-Phase High Performance Liquid Chromatography," *Korean J. Chem. Eng.*, **6**, 347 (1989).
- Row, K. H. and Lee, J. W., "Gradient Separation of Soybean Phospholipids with Retention Factors of a New Polynomial Correlation," *Korean J. Chem. Eng.*, **16**, 170 (1999).
- Row, K. H. and Lee, J. W., "Determination of Retention Factors of Phospholipids in Gradient HPLC," *Chromatographia*, **51**, 61 (2000).
- Said, A. S., "Theory and Mathematics of Chromatography," Dr. Alfred Huthig Verlag GmbH, Heidelberg (1981).
- Sofer, G. and Hagel, L., "Handbook of Process Chromatography A Guide to Optimization, Scale-up and Validation," Academic Press, London (1997).