

Prediction of Formic Acid Chromatogram in Gradient Elution Chromatography

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Abstract Optimal operation in chromatography is needed to save operation time and the solvent used in multiple chromatographic runs. To this end, many simulation studies of chromatography process have been performed. The relationship between the distribution coefficient and the ionic strength is important in gradient elution ion chromatography. Experimental runs and computer simulations were carried out under linear gradient elution conditions in order to compare the experiments and the simulation. Experiments were performed with formic acid under isocratic conditions to determine the simulation equation parameters. Computer simulation was based on three equations which related distribution with ionic strength as follows; $K = \alpha I^\beta$, $K = A + BI + CI^2$ and $K = y_0 + A_1 \cdot e^{(-I/m_1)} + A_2 \cdot e^{(-I/m_2)}$. The effects of gradient slope on the chromatograms are discussed, and good agreement between the experimental and the simulated results is shown.

Keywords: gradient elution chromatography, simulation, distribution coefficient, organic acid

INTRODUCTION

Gradient elution chromatography is widely used for the separation of mixtures and plays a important role in the separation of biomolecules in both the analytical laboratory and in large scale industrial processes [1]. Gradient elution has generally not been used in ion chromatography with a conductivity detector, because many important ions can be detected isocratically. However, to elute ions with very different retention time such as mono-, di-, tri-, tetra- and even pentavalent ions in one operation run, it is necessary to vary the composition of the eluent. Gradient elution chromatography allows the detection and separation of several components in a shorter time by changing the composition of the eluent and was a development of ion chromatography [2,3]. The sample concentration in the stationary phase depends on the ionic strength, pH and solute concentration of the mobile phase during the gradient elution process in the column. Unlike the situation that excites in isocratic elution, the strength of the eluent is varied throughout the separation in gradient elution. Therefore, the ionic strength of the mobile phase becomes different with the lapse of time and affects on the distribution of solute between the mobile and stationary phases [1,4].

The recent, rapid development of the biotechnology industry demands highly selective downstream recovery and purification techniques. In addition, engineering studies of the chromatographic behavior of several

separation process steps are required to characterize the large scale behavior of a chromatographic process. Hence, the computer simulation for the chromatography process [5-8] has developed and a number of papers have been published on isocratic elution chromatography and gradient elution chromatography. Yamamoto *et al.* [3] and Billy *et al.* [5] theoretically described a linear gradient of ionic strength in protein ion chromatography. El Fallah and Guiochon [9] explained the gradient elution of lysozyme and small organic molecules and compared experiments with simulation results.

In the present study, we apply gradient elution to the separation of an organic acid in high performance ion chromatography. The linear adsorption isotherm was provided for the relationship of concentrations between the mobile and stationary phases [10,11] and a dispersive-equilibrium model was used for general mass balance to determine the band profile of an organic acid by solving the governing equations numerically. The equation proposed by Yamamoto *et al.* [3] was compared with the empirical equation based on experimental data to describe the relationship between ionic strength, I , and the distribution coefficient, K .

MATERIALS AND METHODS

Theoretical Background

Gradient Elution Chromatography Theory

The ionic strength of a mobile phase in linear gradient elution chromatography continuously increases with time as shown in Fig. 1. The input concentration is C_0 , passes through a column of length L in accordance

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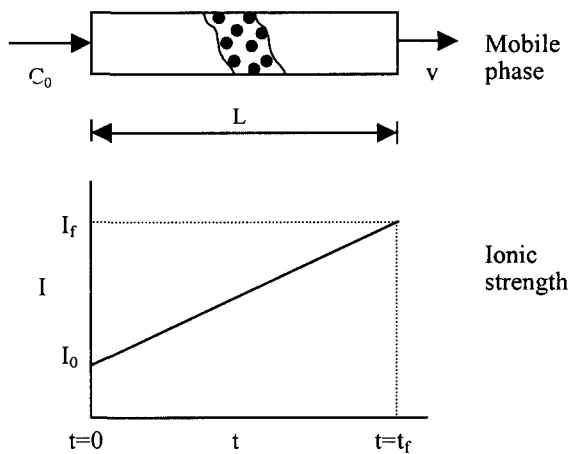


Fig. 1. Principle of the gradient elution chromatography.

with mobile phase, which flows with velocity of v .

Ionic strength with lapse of time is expressed as follows.

$$I = I_0 + (I_f - I_0) \frac{t}{t_f} \quad (1)$$

Here, I is the ionic strength at time t , and I_0 and I_f are the initial and the final ionic strengths of the mobile phase. The t_f is a total time of the chromatographic run.

Governing Equations

The equilibrium-dispersive model was used to explain the partition behavior between two phases [6,10-12]. This model has two assumptions. The first is that an instantaneous equilibrium occurs between the mobile and stationary phases and the second is that the mass transfer into the column is controlled by molecular diffusion of mobile phase passing around the packing material. The chromatography process based on the model is described by a differential mass transfer equation and is given by;

$$\frac{\partial C_i}{\partial t} + F \frac{\partial q_i}{\partial t} + u \frac{\partial C_i}{\partial z} = D_{i,a} \frac{\partial^2 C_i}{\partial z^2} \quad (2)$$

where, C_i and q_i are the equilibrium concentrations of component i in the mobile and stationary phases, respectively; z is the column length, t is the time, u is the linear velocity of the mobile phase and F is the phase ratio, $(1-\epsilon)/\epsilon$, where ϵ is the total porosity of the column. $D_{i,a}$ is the apparent axial dispersion coefficient and all the contributions to band broadening can be lumped into a single apparent dispersion coefficient.

In order to solve equation (2), we need appropriate initial and boundary conditions. The initial condition is given by

$$C_i(z,0) = 0 \quad t = 0 \quad (3)$$

Boundary conditions at inlet and outlet could be

written as, respectively

$$C_i(0,t) = C_i^0 \quad 0 < t \leq 0 \quad (4)$$

$$\frac{\partial C_i}{\partial z} = 0 \quad z = L \quad (5)$$

The solute is injected at t_p , injection start time.

Adsorption Isotherm

Adsorption isotherm can be presented as a function of solute concentration in the mobile and stationary phases, and the Langmuir isotherm is popularly used for the adsorption experimental data across a large range of concentrations [11,12].

$$q = \frac{aC}{1+bC} \quad (6)$$

where a and b are numerical coefficients related to the shape and position of the band profiles; these parameters is obtained by experiment. The Langmuir isotherm can be rewritten as a linear adsorption isotherm as follows in the low C range [12].

$$q = aC \quad (7)$$

The distribution coefficient between two phases, K , can be substituted for the parameter a in equation (7) in a low range of solute concentrations in the mobile phase.

$$q = KC \quad (8)$$

Relationship between Distribution Coefficient and Ionic Strength

When the distribution between a solute and an ion-exchanger is mathematically expressed in gradient elution chromatography, the relationship between the distribution coefficient, K , and the ionic strength of the mobile phase, I , is very important. The dependency of K on ionic strength, I was given by Yamamoto and co-workers as follows [3]:

$$K = \alpha \cdot I^{-\beta_1} \quad (9)$$

Taking the logarithms of Eq. (9) we obtain a linear expression [2,4].

$$\log K = \log \alpha - \beta_1 \log I \quad (10)$$

A plot of $\log I$ vs. $\log K$ has a straight line with a negative slope and coefficients α , β_1 are easily obtained experimentally.

To use Eq. (10), we should determine the distribution coefficient K [11,14-15]. By definition of the moments of a distribution, the n -th moment of the band profile at the exit of a chromatographic bed is given by

$$m_n = \int_0^\infty C(t) \cdot t^n dt \quad (11)$$

where $C(t)$ is a function of the solute concentration for the lapse of time in column outlet. First absolute moment, m'_1 , the mean residence time of the elution curve, is calculated according to the usual expression.

$$m'_1 = \frac{\int_0^{\infty} C(t) \cdot t dt}{\int_0^{\infty} C(t) dt} \quad (12)$$

For a linear system, the mean residence time is related to K as follows :

$$m'_1 = \frac{L}{u_0} [\varepsilon + (1 - \varepsilon)\beta(1 + K)] \quad (13)$$

where, L : column length, u_0 , ε , and β are superficial velocity ($u_0 = u\varepsilon$), void fraction of column, and particle porosity respectively.

Accordingly, a plot of m'_1 vs. L/u_0 gives a straight line through the origin and the unknown parameter K can be obtained from the slope.

Simulation

Numerical calculations based on Eqs. (1) to (13) were performed by a differential equation solver, Macsyma program (Macsyma Inc., Arlington, MA, USA) [16], in a Pentium PC. Chromatograms were simulated for various cases. Operating parameters for the simulation are summarized in Table 1.

Chromatography Experimentals

All experiments were performed with a high pressure Dionex 300 ion Chromatography System (USA). The instrument consisted of an IonPac AS12A (200 mm \times 4 mm I.D., packed particle size: 9.0 μ m), an advanced gradient pump (Dionex, USA), ASRS-I self-regenerating suppressor and a pulsed electrochemical detector (Conductivity mode, Dionex). An analogue signal of the chromatogram was transferred converted by a computer interface and saved on Dionex's PeakNet Software (V4.30).

Isocratic elution chromatography experiments were performed to determine the value of K in mobile phase of several compositions. NaOH concentrations of 2, 4, 6, 8 and 10 mM were used. The running conditions in

Table 1. Parameters varied in the simulation of Equilibrium dispersive model

Parameter	Value	Remark
C	2-8 mM	Dimensional
I	0.75-1.5 mM	Isocratic elution
	2-10 mM	Gradient elution
dI/dt	2-8 mM	Initial 2mM in linear gradient elution
V_0	0.9-1.2 mL/min	Isocratic elution

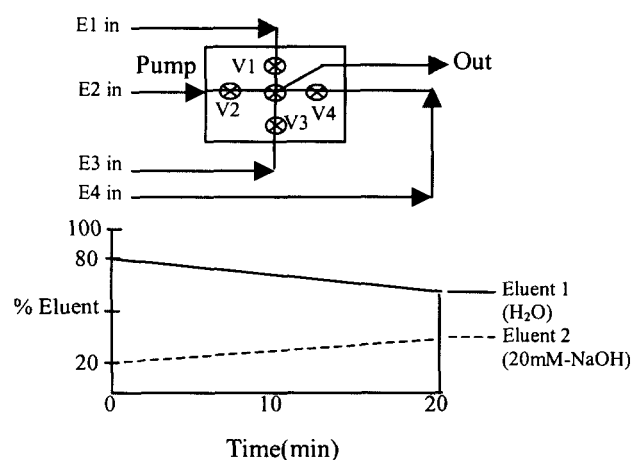


Fig. 2. Schematic diagram of pump mixing for gradient elution runs.

gradient elution mode were as follows; the initial NaOH concentration was 4 mM and the final concentrations are 6, 8 and 10 mM. The composition of the mobile phases was controlled using a gradient device as shown in Fig. 2.

Formic acid of 2 mM was used as a solute and the NaOH solution flow rate was controlled into column at 1 mL/min. The injection volume was fixed at 25 μ L.

RESULTS AND DISCUSSION

Relation between Distribution Coefficient and Ionic Strength

The parameters used to simulate the gradient elution profile were estimated from experimental data under isocratic elution conditions. The distribution coefficient, K , was obtained using the moment method with a change of ionic strength of mobile phase. A representative curve is illustrated in Fig. 3. The plot of the mean residence time m'_1 versus L/u_0 shows a straight line through the origin and the distribution coefficient K was determined from the slopes of these lines.

When the mobile phase had a low NaOH concentration (2 mM), small deviations were observed near origin, as shown in Fig. 3. This was probably caused by incomplete solvent mixing in the pump. Table 2 shows the values of distribution coefficient for variable ionic strengths in gradient elution. As a result, we can know that distribution coefficient is decreased by increasing the ionic strength.

K as calculated by the moment method can be related to a function depending on I . Fig. 4(a) was arrived at on the basis of the equation by Yamamoto and coworkers fitted with our experimental data. The parameters α , β were determined using the following linear function.

Table 2. Values of parameters obtained by the pulse response test in gradient elution

Ionic strength, I (mM)	Distribution coefficient, K	
2	19.9389	$d_p = 9 \mu\text{m}$
4	14.2062	$L = 20\text{cm}$
6	11.5605	$\epsilon = 0.756$
8	8.9805	$\beta = 0.876$
10	6.6376	$D = 0.00153$

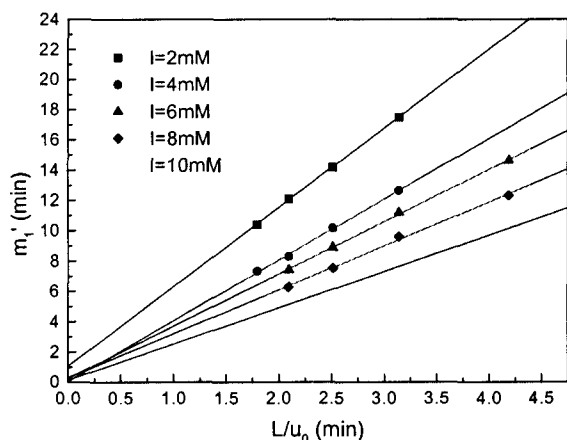


Fig. 3. Experimental relationships between m'_1 and L/u_0 for various ionic strengths.

Since the equation used by Yamamoto is valid for low eluent concentrations, a larger deviation was observed at higher eluent concentrations. Accordingly, we considered an empirical equation for the functional relation of the distribution coefficient K and the ionic strength I , as shown in Fig. 4(b). The solid line in Fig. 4(b) was obtained by calculating the following quadratic function of ionic strength.

$$K = A + BI + CI^2$$

where A , B and C are empirical parameters derived from data fitting.

The curve expressed by the dotted line in Fig. 4(b) was given by the following exponential function.

$$K = y_0 + A_1 \cdot e^{(-I/m_1)} + A_2 \cdot e^{(-I/m_2)}$$

where the parameters y_0 , A_1 , A_2 , m_1 and m_2 were determined by data fitting.

Compared with Fig. 4(a), smaller deviations between experimental data and empirical lines in Fig. 4(b) were observed. It is shown that better agreement with results can be obtained from the empirical equations with more coefficients.

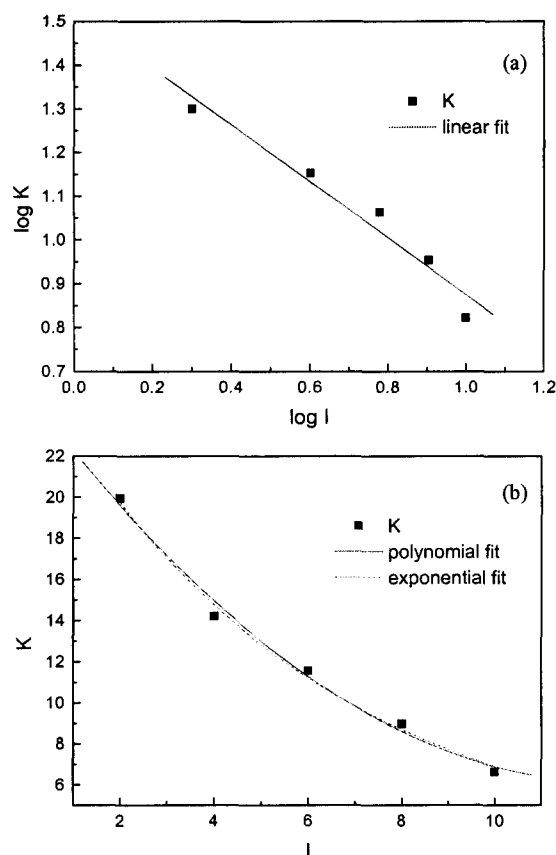


Fig. 4. Relationships between ionic strength and distribution coefficient. (a) using the Yamamoto equation, (b) using empirically derived curve fitting equations.

Comparison between Simulated Data and Experimental Results

Experimental results were compared to simulation results under conditions that initial concentrations of mobile phase fixed and final concentrations were allowed to vary. Gradient elution is accomplished by changing from the mobile phase of a weak concentration to one of a strong concentration. In ion exchange chromatography, ionic strength changes may be accomplished either by changes in the concentration gradient of the displacing ion or by a composition gradient change from a weakly retained eluent ion to a more strongly retained ion. We placed the focus on one component system and performed experiments by changing the gradient of mobile phase ionic strength such as $I = 4\text{-}6 \text{ mM}$, $4\text{-}8 \text{ mM}$ and $4\text{-}10 \text{ mM}$. The results are shown in Fig. 5.

The three equations discussed previously were applied to compare the simulation data with the experimental data. Fig. 5(a) shows comparisons based on the Yamamoto relationship. Deviations in the frontal parts of the chromatograms, result from poor matches be-

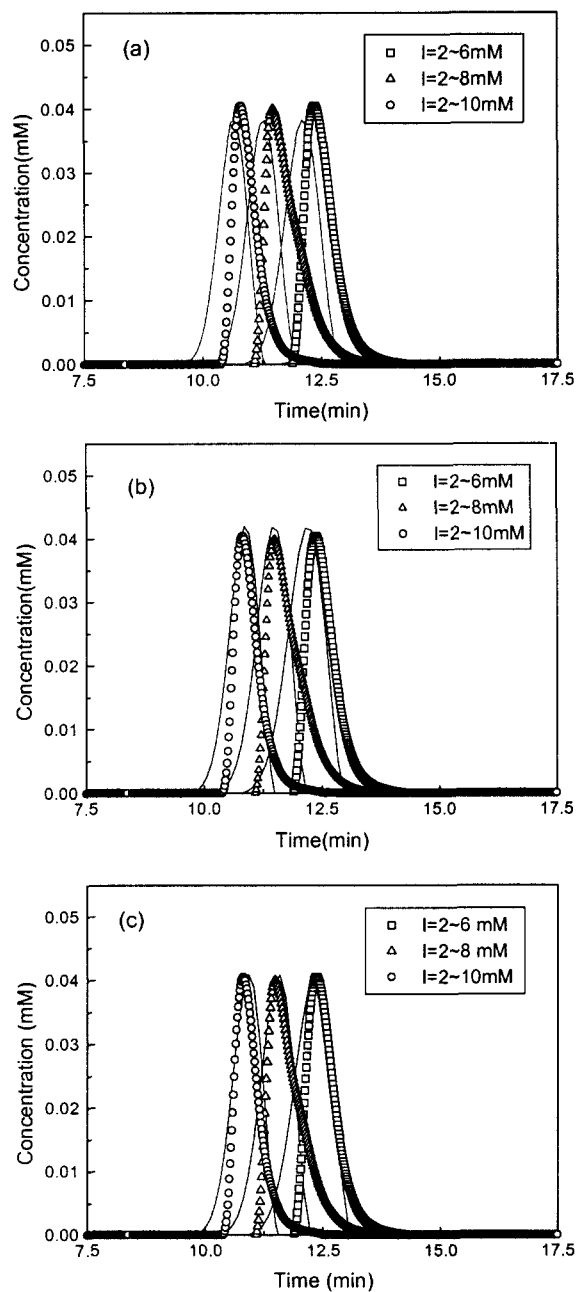


Fig. 5. Comparison of simulated and experimental results upon changing gradient slopes. (a) The result for equation used by Yamaomto *et al.* [3] (b) The result for the quadratic equation obtained by data fitting. (c) The result for the exponential equation obtained by data fitting (O, Δ , \square : experiments, - : simulations).

tween the empirical relation of Yamamoto *et al.* [3] and the experimental K values. Also, small deviation in the peak height and the retention time is found because the concentration of mobile phase is changed by passing of the time in gradient elution. Figs. 5(b) and 5(c) show

comparisons between simulation data and experimental chromatograms when the quadratic function and the exponential function are used. Both functions yield better coincidence for the low gradients ($I = 2-6$ mM). As the steepness of ionic strength increases the experimental data tend to lag behind the simulation. This could be ascribed to the fact that the basic assumption of complete mixing in the mixing device is not valid at the higher salt gradient. But, we can know that increasing slope of the gradient elution shows non-symmetry of chromatograms characterizing nonlinear system.

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NOMENCLATURE

- A, A_1, A_2, B, C : empirical parameters (dimensionless)
 a : constant in Langmuir isotherm for sample component (dimensionless)
 b : adsorption equilibrium constant for sample component (mL/ μ g)
 C_i : concentration of the solute in the mobile phase (μ g/mL)
 $D_{i,a}$: apparent dispersive coefficient (cm/min)
 F : phase ratio (dimensionless)
 F_y : flow rate of the mobile phase (mL/min)
 K : distribution coefficient (dimensionless)
 I : ionic strength (mM)
 I_0, I_f : initial and final ionic strengths of the mobile phases (mM)
 L : column length (cm)
 m'_1 : first absolute moment (min)
 m_1, m_2 : empirical constants (dimensionless)
 q_i : concentration of the solute stationary phase (μ g/mL)
 t : time (min)
 t_f : final running time of gradient elution chromatography (min)
 t_p : pulse injection time of sample (sec)
 u : interstitial velocity (cm/min)
 u_0 : superficial velocity (cm/min), $u_0 = u\varepsilon$
 y_0 : empirical constant (dimensionless)

Greek Letters

- α, β_1 : empirical parameters (dimensionless)
 β : porosity of particle
 ε : total porosity of column

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