Moment Analysis Theory for Size Exclusion Capillary Electrom chromatography with Chemical Reaction of Intermolecular Interaction

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New moment equations were developed for size exclusion capillary electrom chromatography (SECEC), in which intermolecular chemical reactions simultaneously took place. They explain how the first absolute and second central moments of elution peaks are correlated with some fundamental equilibrium and kinetic parameters of mass transfer and chemical reaction in SECEC column. In order to demonstrate the effectiveness of the moment equations, they were used to predict chromatographic behavior under hypothetical SECEC conditions. It was quantitatively studied how the association and dissociation rate constants of intermolecular interaction affected the position and spreading of elution peaks. It was indicated that both the intermolecular reaction kinetics and axial dispersion of solute molecules in a capillary column had a predominant contribution to the band broadening.

Keywords Moment analysis theory, size exclusion capillary electrom chromatography, intermolecular interaction, reaction kinetics, mass transfer rate

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Introduction

It is required to study intermolecular interactions from kinetic points of view for well understanding the intrinsic characteristics and mechanisms of various functions and phenomena in many research fields. Different instrumental methods have been developed for determining rate constants, i.e., association (ka) and dissociation (kd) rate constants, of intermolecular interactions between a solute (S) and a ligand (L) to form a solute-ligand complex (X).

\[ S + L \xrightleftharpoons[k_d]{k_a} X \]  

A chromatographic approach was proposed for the kinetic study of intermolecular interaction, i.e., kinetic size exclusion chromatography (KSEC) with mass spectrometry (MS) detection (KSEC-MS).\(^1\) SEC makes it possible to separate chemical species relating to the intermolecular interaction without their immobilization. It is also possible to detect an objective component without its chemical modification (e.g., fluorescence labeling) by using MS. The KSEC-MS method was applied to the measurement of the rate constants of the interactions between carbonic anhydrase and acetazolamide\(^1\) and between dihydrofolate reductase and methotrexate.\(^2\) However, the KSEC-MS method contains a trial-and-error data analysis procedure based on curve-fitting. The values of ka and kd are determined so that the chromatogram numerically calculated by using the ka and kd values reasonably fits with that experimentally measured.

It is more preferable that the ka and kd values can be analytically and unmistakably determined from elution peak profiles. The moment analysis (MA) method based on the general rate model of chromatography is effective for the analytical determination of some fundamental parameters concerning the retention equilibrium, mass transfer rate, and reaction kinetics from the first absolute (μ1) and second central (μ′2) moments of experimental elution peaks.\(^3\) We have already developed moment equations for chromatography, in which the reaction kinetics between solute molecules and functional ligands on the stationary phase surface was represented by the Langmuir type rate equation.\(^4\) In practice, the reaction rate constants (ka and kd) were determined for the intermolecular interaction between 2-phenoxypropionic acid and β-bromo-cycloexedrin by chromatographic experiments with the MA theory.\(^5\) On the other hand, regarding separation technique, capillary electrom chromatography (CEC) has some intrinsic characteristics. Separation behavior of CEC is correlated with both the chromatographic behavior of liquid chromatography (LC) and electrophoretic behavior of capillary electrophoresis (CE) because CEC is based on the two separation techniques. It is expected that a unique separation system can be constituted by appropriately controlling some experimental conditions of CEC. Figure 1 shows a schematic illustration of size exclusion capillary electrom chromatography (SECEC), as one example. For instance, a mobile phase (running buffer) solvent containing a sufficient amount of electrically neutral ligand is filled in a capillary column, in which porous separation media are packed for SEC. The SECEC procedure is carried out by injecting a small amount of positively charged solute in an electric field by simultaneously applying a chromatographic pressure. SECEC behavior is based on the innumerable repetition of the association.

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between the solute and ligand and the dissociation of the complex. Regarding the migration of the solute and complex, in the case of SEC, larger complexes migrate faster than smaller solute molecules due to the size exclusion effect. On the other hand, in size exclusion CE, larger complexes move slower than smaller solute molecules because of the difference in their molecular size. It must be possible by appropriately adjusting experimental conditions, e.g., electric field strength and chromatographic pressure, to conduct SECEC experiments under the conditions that the migration of solute-ligand complex stops and only solute molecules migrate in the capillary column. The generation of electroosmotic flow can be suppressed by using a polymer coated capillary, as an example.

However, it is not necessarily required to actually perform SECEC experiments under the conditions that the migration of solute-ligand complex is completely stopped in the axial direction of the capillary column. In practice, SECEC data should be measured by changing the experimental conditions. Analysis of the SECEC data by a successive approximation method provides the values of $\mu_1$ and $\mu'_2$ of the elution peak, which must be measured under the objective conditions that the migration of solute-ligand complex is adequately stopped in the capillary column. Both the equilibrium and kinetic information about intermolecular interactions can be analytically obtained from the $\mu_1$ and $\mu'_2$ values of elution peaks by the MA method.10,11

In this study, as a first step, new moment equations were developed for analyzing some mass transfer phenomena and intermolecular interactions in the SECEC system, in which the association and dissociation reactions of Eq. (1) simultaneously took place. Then, the new moment equations were used to predict the chromatographic behavior of a solute under hypothetical SECEC conditions. Band broadening in chromatography is influenced by the contributions of some mass transfer resistances and intermolecular reaction kinetics. It is necessary to subtract the contributions of the mass transfer processes to band broadening for the accurate determination of the rate constants. We tried to quantitatively discuss the influence of mass transfer rate and intermolecular reaction kinetics, i.e., axial dispersion, external mass transfer, intraparticle diffusion, and reaction kinetics, on band broadening in the SECEC system.

This is a preliminary study for the determination of $k_a$ and $k_d$ of intermolecular interaction from elution peak profiles measured by SECEC. The results of this study indicate that separation behavior in SECEC systems can be theoretically analyzed on the basis of the new moment equations. It is considered possible to analytically determine the values of $k_a$ and $k_d$ for the formation and dissociation of solute-ligand complex with no immobilization and chemical modification of solute and ligand molecules. It is very important for the kinetic study of intermolecular interactions.

Theory

Moment equations had already been developed about 40 - 50 years ago for the chromatographic processes in a column packed with full-porous spherical particles.4-3,12-16 Other moment equations have also been developed for various separation media for HPLC, e.g., monoliths, core-shell particles, and so on, of which structural characteristics are quite different from those of full-porous spherical particles.6-10 It is quite important to analytically analyze elution peak profiles for deriving accurate values of $k_a$ and $k_d$. The MA method is effective for the analytical determination of $k_a$ and $k_d$. Although the values of $k_a$ and $k_d$ are also obtained by fitting the chromatogram numerically calculated with experimental elution peaks, it is unfortunate that they cannot be unambiguously determined. In addition, both mathematical knowledge and instruments for calculations are required for numerically calculating elution peak profiles. These are drawbacks of the curve-fitting method.

In this study, basic equations representing the mass balance, mass transfer rate, and reaction kinetics in the SECEC capillary column were solved in the Laplace domain. The moment equations for $\mu_1$ and $\mu'_2$ in the time domain were derived from the analytical solution of the basic equations in the Laplace domain. The derivation procedure of the moment equations is explained in more detail in Supporting Information.

Fundamental assumptions for developing moment equations

The moment equations were developed on the basis of the following set of assumptions.

1. No component in the column interacts with the stationary phase.
2. The column is axially and radially homogenous. It is controlled under isothermal conditions.
3. There is no diffusion of chemical species in the radial direction of the column.
4. All the parameters concerning the SECEC system are constant during experiments.
5. The stationary phase spherical particles consist of a solid part and internal pores. They are homogeneous. The radii of the particles and internal pores are constant. There is no size distribution.
6. Both the ligand and complex cannot penetrate into the internal pores because their molecular size is sufficiently larger than that of internal pores. The reaction of Eq. (1) takes place in the external pore space in the column. The molecular weight of the ligand and complex is sufficiently larger than that of the solute. Solute molecules diffuse in the internal pores by pore diffusion in the radial direction. The partial molar volume of the solute is constant.
(7) The mobile phase solvent flows through the external pore space. It is not adsorbed on the stationary phase. The mobile phase is incompressible. No parameter, such as the molecular diffusivity and the mobile phase viscosity, has a pressure dependence.

(8) The migration of the complex to the axial direction of the column is stopped in the SECCEC system. Neither axial diffusion nor migration of solute-ligand complex is considered. Both axial dispersion and migration of only the solute in the column are taken into account.

(9) There is an external film around stationary phase particles, in which solute molecules diffuse in the radial direction. External mass transfer rate is proportional to the difference between the solute concentration in the mobile phase (C_\text{s}) and that at the external surface of the particles (C_{\text{si}}).

(10) The influence of electric field in the internal pores of the stationary phase on band broadening is not taken into account.

**Basic equations**

The following partially differential equations, Eqs. (2) - (5), based on the general rate model of chromatography, represent the mass balance, mass transfer rate, and chemical reaction kinetics of solute molecules in the column and in the stationary phase.

\[ D_r \frac{\partial^2 C_s}{\partial z^2} - u \frac{\partial C_s}{\partial z} - \frac{A_r}{\varepsilon_s} N_0 - N_i = \frac{\partial C_s}{\partial t} \]

\[ N_0 = \k_i (C_s - C_{si,r}) = D_r \left( \frac{\partial C_s}{\partial r} \right)_r \]

\[ D_r \left( \frac{\partial^2 C_s}{\partial z^2} + \frac{2}{r} \frac{\partial C_s}{\partial r} \right) = \varepsilon_i \frac{\partial C_s}{\partial t} \]

\[ N_i = \frac{\partial C_s}{\partial t} = k_e C_s C_i - k_d C_X \]

where C_s, C_i, and C_X are respectively the concentration of solute (S), ligand (L), and solute-ligand complex (X), z the longitudinal distance along the capillary column, t the time, D_r the axial dispersion coefficient, u the average interstitial velocity of the mobile phase, A_r the ratio of the total external surface area of the stationary phase to the column volume, \varepsilon_s the column void fraction (external porosity), N_0 the mass flux of the solute molecule from the bulk mobile phase to the external surface of the stationary phase, C_s the concentration of the solute molecule within the internal pores of the stationary phase, R the radius of the stationary phase spherical particles, r the radial distance from the center of the stationary phase spherical particles, \k_i the external mass transfer coefficient, D_r the effective diffusion coefficient of the solute molecule in the stationary phase, N_i the reaction rate of Eq. (1) between S and L to form X, and \varepsilon_i the porosity of the stationary phase particles (internal porosity). The subscript R denotes the variables at r = R.

**Initial and boundary conditions**

The initial and boundary conditions are as follows.

\[ C_s (z, t = 0) = 0 \quad \text{for} \quad 0 \leq z \leq Z \]

\[ C_{si} (z, t = 0, r) = 0 \quad \text{for} \quad 0 \leq z \leq Z, 0 \leq r \leq R \]

\[ C_X (z, t = 0) = 0 \quad \text{for} \quad 0 \leq z \leq Z \]

\[ C_s (z = 0, t) = C_{\text{s0}} \quad \text{for} \quad 0 \leq t \leq \tau \]

\[ \frac{\partial C_s}{\partial r} \bigg|_{r=0} = 0 \]

where Z is the column length, C_{\text{s0}} is the solute concentration of the injection pulse, and \tau is the width of the injection pulse introduced into the capillary. A rectangular shape of the injection pulse was assumed.

**Analytical solution in the Laplace domain**

The basic equations were analytically solved in the Laplace domain. The analytical solution in the Laplace domain (\overline{C}_s) was obtained as Eq. (6). All the functions of A(p), B(p), E(p), F(p), G(p), and M(p) are included in Eq. (6).

\[ \overline{C}_s = \frac{C_{\text{s0}}}{p} \left[ 1 - \exp(-p \tau) \right] \exp[-M(p)z] \]

\[ A(p) = \frac{pk_e C_i}{p + k_d} \]

\[ B(p) = \frac{\varepsilon_i p}{D_r} \]

\[ E(p) = R \sqrt{B(p)} \]

\[ F(p) = E(p) \coth(E(p)) - 1 \]

\[ G(p) = p + A(p) + \frac{A_k}{\varepsilon_i} \left( 1 - \frac{B_i}{F(p) + B_i} \right) \]

\[ M(p) = \frac{D_r}{2D_i} \left[ 1 + \frac{4D_i G(p)}{u^2} \right] \]

where \overline{C}_s denotes the Laplace transform of C_s, p the Laplace transform variable, and B_i is the Biot number, which is defined as the ratio of k_eR to D_r.

In principle, the inverse transformation of the analytical solution in the Laplace domain, i.e., Eqs. (6) - (12), provides an analytical solution in the time domain, which represents a whole elution peak profile. However, the analytical solution in the time domain thus derived would not be useful for a practical analysis of elution peak profiles. It is predicted that the analytical solution also has a complicated formula because Eqs. (6) - (12) are complicated. On the other hand, the moment equations in the time domain can be derived from the analytical solution in the Laplace domain.

**Moment equations in the real time domain**

The moment equations for \mu_i and \mu_i' were derived as follows from the analytical solution in the Laplace domain.

\[ \frac{\mu_i - \frac{\tau}{2}}{u} = 1 + \frac{(1 - \varepsilon_i) k_i}{\varepsilon_i} + \frac{k_e C_i}{k_d} \]

\[ \frac{\mu_i' - \frac{\tau^2}{12}}{u^2} = \frac{2D_r}{u^2} \left[ 1 + \frac{(1 - \varepsilon_i) k_i}{\varepsilon_i} + \frac{k_e C_i}{k_d} \right] \]

\[ + \frac{2(1 - \varepsilon_i) k_i' (R + \frac{R^2}{15D_f}) + 2k_e C_i}{k_d^2} \]
These equations indicate how the representative characteristics of the elution peak rest on some fundamental parameters of the mass transfer processes and chemical reaction in the column. The first and second terms in the right hand side of Eq. (13) correspond to the time, in which $S$ passes through the interparticulate (external) and intraparticulate (internal) pore volume, respectively. The third term represents the influence of the reaction kinetics on $\mu_i$. The ratio of $k_e$ to $k_d$ in Eq. (13) is equal to the association equilibrium constant ($K_a$).

On the other hand, as illustrated in Fig. 1, the first, second, third, and fourth terms in the right hand side of Eq. (14) indicate the contributions to $\mu_i$ of axial dispersion, external mass transfer, intraparticle diffusion, and reaction kinetics, respectively. At first, the axial dispersion of solute molecules in the column packed with spherical particles is assumed to consist of two main mechanisms, i.e., molecular diffusion and eddy diffusion. Axial dispersion coefficient ($D_a$) was estimated by Eq. (S35) (Supporting Information). Then, the contribution of mass transfer resistance at the external liquid film surrounding stationary phase particles to band broadening is also considered in Eq. (14). The relating parameter, i.e., $k_e$, was estimated by Eq. (S36) (Supporting Information). Additionally, solute molecules migrate in the internal pores of the stationary phase particles by pore diffusion. Equation (14) also represents the contribution of intraparticle diffusion to band broadening. Equations (S37) and (S38) (Supporting Information) were used for estimating $D_p$, which corresponds to diffusive migration of solute molecules due to concentration gradient. The values of $k_e$ and $k_d$ can be determined by subtracting the contributions of the mass transfer processes described above from total band broadening.

In SEC, although $\epsilon_e$ is constant, $\epsilon_i$ depends on the molecular size of solutes. For example, small molecules can penetrate into the whole internal pore space. However, large molecules are excluded from or can partially penetrate into the internal pores. This depends on the correlation between the diameter of pores and the molecular size of the solutes. Consequently, the value of $\epsilon_i$ is different for each solute. This means that the information about the degree of penetration of solute molecules into the internal pores can be obtained from $\mu_i$ data by Eq. (13).

There is no assumption concerning the shape of elution peaks for the derivation of the moment equations. It is not a necessary condition of the moment analysis that the elution peak profile has to be represented by a Gaussian distribution curve. Additionally, as defined in Eqs. (S19) and (S20) (Supporting Information), the values of $\mu_i$ and $\mu_i'$ were calculated from the whole profile of elution peaks experimentally measured. They were not derived from specific parameters of elution peaks, e.g., position and width at half height. This means that the values of $\mu_i$ and $\mu_i'$ represent intrinsic characteristics of the whole peak shape.

**Brief Explanation of SECEC Experiments and Data Analysis**

**SECEC experiments**

Figure 1 shows a schematic illustration of one example of SECEC, in which a positively charged solute (S) interacts with an electrically neutral ligand (L) to form a complex (X). It is predicted that the elution time (i.e., $\mu_i$) of S increases with an increase in the applied chromatographic pressure ($P_{\text{in}}$). However, the elution peak of S is no longer detected when $P_{\text{in}}$ exceeds a critical level ($P_{\text{in}}^*$), at which the migration of X is just stopped in the axial direction of the capillary column, because S inversely migrates to the left direction in Fig. 1. This means that a correlation between $\mu_i$ and $P_{\text{in}}$ can be experimentally measured.

It is practically difficult to actually conduct SECEC experiments at $P_{\text{in}}$. However, analysis of the correlation between $\mu_i$ and $P_{\text{in}}$ by a successive approximation method provides the values of $\mu_i$ and $\mu_i'$ of an elution peak, which must be measured under the objective conditions that the migration of X is completely stopped in the capillary column. Both the equilibrium and kinetic information about intermolecular interaction can be analytically obtained from the $\mu_i$ and $\mu_i'$ values of elution peaks by the MA theory.\textsuperscript{10,11}

**Estimation of $P_{\text{in}}$**

The value of $P_{\text{in}}$ is calculated from the $\mu_i$ data measured by changing $P_{\text{in}}$. The average migration velocity of S ($V_{\text{S}}$) is represented as follows when both S and X migrate in the column.

$$V_{\text{S}} = \frac{\epsilon_e C_S}{\epsilon_e C_S + \epsilon_i C_X} + \frac{V_X}{V_X + \frac{\epsilon_e C_X}{\epsilon_e C_S}}$$

where $\epsilon_i = (\epsilon_e + (1 - \epsilon_e)\gamma)$ is the total porosity of the column, $\gamma$ is the ratio of $\epsilon_i$ to $\epsilon_e$, and $C_S$ and $C_X$ are the concentration of S and X, respectively. The value of $\mu_i$ is represented as follows.

$$\mu_i = \frac{Z}{V_{\text{S}}} = \frac{Z}{V_S + V_X \gamma K_a C_L} = (1 + \gamma K_a C_L)$$

The second term in the denominator represents the influence of the migration velocity of X on $\mu_i$. Equation (16) is the same as Eq. (13) when $V_X$ is equal to zero. Because $u$ is the interstitial velocity, $V_S$ is calculated as $u/\epsilon_e u$.

In this study, $P_{\text{in}}$ was estimated from the correlation between $\mu_i$ and $P_{\text{in}}$ by a successive approximation method. In Eq. (16), there are three parameters, i.e., $V_S$, $V_X$, and $K_a$, of which values are not known. However, the value of $K_a$ can be conventionally measured by other methods, e.g., affinity capillary electrophoresis.\textsuperscript{10-23} The others are the experimental parameters. At first, a $V_S$ value is assumed from the $\mu_i$ data of S at $P_{\text{in}} = 0$ Pa. The values of $V_X$ at each $P_{\text{in}}$ are calculated from those of $\mu_i$ by assuming the $V_S$ value because $K_a$ is already measured by other methods. Then, $P_{\text{in}}$ is estimated from the intercept at $V_X = 0$ m s\(^{-1}\) of the correlation between $V_{\text{S}}$ and $P_{\text{in}}$. Once $P_{\text{in}}$ is estimated, $\mu_i$ at $P_{\text{in}}$ is obtained from the correlation between $\mu_i$ and $P_{\text{in}}$. At this time, another value of $V_S$ is calculated from that of $\mu_i$ at the $P_{\text{in}}$ value by Eq. (13) because X is stopped in the column at $P_{\text{in}}$. When the two values of $V_S$ agree with each other, it must be a true value of $V_S$. If they are not in agreement with each other, the iterative calculations described above must be repeated by assuming the $V_S$ value calculated.

**Determination of $k_e$ and $k_d$**

When a true value of $P_{\text{in}}$ is estimated, both $\mu_i$ and $\mu_i'$ of the elution peak, which must be measured under the conditions that the migration of X is stopped in the axial direction of the column, are respectively estimated from the correlation between $\mu_i$ and $P_{\text{in}}$ and that between $\mu_i'$ and $P_{\text{in}}$. Then, according to Eq. (14), $k_e/k_d$ was calculated by subtracting the contribution of axial dispersion to the $\mu_i'$ value at $P_{\text{in}}$. As illustrated in Fig. 1, $\mu_i'$ is influenced by some mass transfer resistances and intermolecular reaction kinetics. However, the results of this
study indicate that the contributions of external mass transfer (k\textsubscript{t}-term) and intraparticle diffusion (D\textsubscript{r}-term) to \( \mu' \) are negligibly small. The value of \( D\textsubscript{r} \) is estimated from \( \mu' \) data of S measured in the absence of L by Eq. (14). Finally, the values of \( k_a \) and \( k_d \) are calculated from those of \( k_d/k_a \) and \( k_d/k_d' \).

**Calculation**

In order to confirm the effectiveness of the moment equations, i.e., Eqs. (13) and (14), they were applied to the analysis of chromatographic behavior of small molecules in SECEC systems, as one example. Chromatographic behavior in SECEC systems was simulated by using the moment equations under hypothetical SECEC conditions listed in Table S1 (Supporting Information). Some kinetic parameters involved in Eq. (14) were estimated by using conventional literature correlations as explained in detail in Supporting Information.

**Results and Discussion**

**First moment analysis**

Equation (13) indicates that \( \mu_1 \) is correlated with \( K_a C_L \). Figure 2 illustrates the full-logarithmic plot of the correlation between \( \mu_1 \) and \( K_a C_L \) and that between the retention factor (k) and \( K_a C_L \), which were calculated under the hypothetical SECEC conditions listed in Table S1. The linear dashed line indicates that k is equal to \( 0.50 K_a C_L (= eK_a C_L/[e, + (1 - e, C_L)]) \). The solid line illustrates the dependence of \( \mu_1 \) on \( K_a C_L \). It approaches an asymptote with a decrease in \( K_a C_L \). It corresponds to the column hold up time (\( \tau_0 \)), which is equal to \( 6.0 \times 10^2 \) s (=\( 0.30/1.0 \times 10^{-3} \))[1 +(1 - \( e, C_L /e,)] \). On the other hand, \( \mu_1 \) increases with an increase in \( K_a C_L \) in the range of high values of \( K_a C_L \). The solid line indicates that \( \mu_1 = 3.0 \times 10^3 K_a C_L \) when \( K_a C_L \) is sufficiently large. Equation (13) indicates that the solute retention depends only on the value of the product, i.e., \( K_a C_L \), not on each value of \( k_a, k_d, \) and \( C_L \).

**Second moment analysis**

As indicated in Eq. (14), \( \mu' \) is affected by the change in some parameters in Table S1. The manner in which the change of \( \mu' \) is more complicated than that of \( \mu_1 \) because plural rate processes of mass transfer and chemical reaction affect band broadening. It would be difficult without calculations to quantitatively predict how the change in the parameters influences the value of \( \mu' \). In this study, the influence of the variation in the values of four parameters, i.e., \( u, k_a, k_d, \) and \( C_L \) on \( \mu' \) was simulated while changing their values. Equation (14) indicates that \( \mu' \) consists of the contributions of four kinetic processes in the column, i.e., axial dispersion, external mass transfer, intraparticle diffusion, and intermolecular interaction.

Figure 3 illustrates the flow rate dependence of \( \mu' \) and the contributions of the four kinetic processes to \( \mu' \). As predicted from Eq. (14), the value of \( \mu' \) and the contributions of the four kinetic processes decrease with an increase in \( u \). The contribution of axial dispersion (D\textsubscript{r}-term, dotted line) indicates more significant flow rate dependence than the others. The D\textsubscript{r}-term is inversely proportional to \( u' \), although the other terms to \( u \). The flow rate dependence of the contribution of the intermolecular interaction (IMI-term, dashed line) and that of intraparticle diffusion (D\textsubscript{r}-term, double dotted-dashed line) are parallel. However, the slope of the flow rate dependence of the external mass transfer (k\textsubscript{t}-term, dotted-dashed line) is slightly different because, as indicated in Eq. (S36) (Supporting Information), \( k_t \) also depends on \( u \). In Fig. 3, the contribution of the D\textsubscript{r}-term is significant in the low flow rate range. On the other hand, the IMI-term has a predominant contribution to \( \mu' \) irrespective of the flow rate conditions. The contributions of k\textsubscript{t}-term and D\textsubscript{r}-term to \( \mu' \) are negligibly small.

Figure 4 illustrates the influence of \( k_a \) on \( \mu' \) and on the contributions of the four kinetic processes to \( \mu' \). It is reasonable that the values of the k\textsubscript{t}- and D\textsubscript{r}-terms are constant because \( k_t \) is not included in the two terms in Eq. (14). On the other hand, the contribution of the D\textsubscript{r}-term shows a curved profile. Figure 4 also indicates that the contribution of the IMI-term increases with an increase in \( k_a \). The contribution of the IMI-term to band broadening is significant in the whole range of \( k_a \) values. Although the contribution of the D\textsubscript{r}-term to \( \mu' \) is relatively large, it is about one order of magnitude smaller than that of the
IMI-term. Again, the contributions of the \( k_f \)- and \( D_e \)-term to \( \mu_2' \) can be neglected. In addition, as indicated in Eqs. (13) and (14), the calculation results obtained by changing the value of \( C_L \) are the same as those in Fig. 4, although the results are not shown.

Figure 5 illustrates the influence of \( k_d \) on \( \mu_2' \) and on the contributions of the four kinetic processes to \( \mu_2' \). Again, the contributions of the \( k_e \) and \( D_e \)-terms are constant regardless of the \( k_d \) value. Although the contribution of the \( D_e \)-term shows a curved profile, it decreases with increasing \( k_d \). The contribution of the IMI-term also decreases with an increase in \( k_d \). The contribution of the IMI-term to band broadening is significant in the whole range of \( k_d \) values. However, the \( D_e \)-term shows a comparable contribution to \( \mu_2' \) in the range of large values of \( k_d \). Again, the contributions of the \( k_e \) and \( D_e \)-term to \( \mu_2' \) are relatively small.

In Fig. 6, the values of \( k_a \) and \( k_d \) are simultaneously changed in the range from \( 10 \) to \( 1.0 \times 10^3 \) \( \text{dm}^3 \text{mol}^{-1} \text{s}^{-1} \) and from \( 0.10 \) to \( 10 \) \( \text{s}^{-1} \), respectively. However, the value of \( K_A \) is kept constant at \( K_A = 1.0 \times 10^2 \) \( \text{mol}^{-1} \text{dm}^3 \). In Fig. 6, the relative importance of the contributions of the four kinetic processes to \( \mu_2' \) is different even if \( K_A \) is kept constant at \( 1.0 \times 10^2 \) \( \text{mol}^{-1} \text{dm}^3 \). It is reasonable that the contributions of the \( D_e \)-, \( k_e \)-, and \( D_e \)-terms are constant because \( K_A \) is constant and because \( k_a \) and \( k_d \) are not included in the three terms. Although the contribution of the IMI-term decreases with increasing \( k_a \) and \( k_d \), it is significant irrespective of the \( k_a \) and \( k_d \) values under the hypothetical conditions listed in Table S1.

Figure 4 Influence of \( k_a \) on \( \mu_2' \) and the contributions of axial dispersion (\( D_L \)-term), external mass transfer (\( k_f \)-term), intraparticle diffusion (\( D_e \)-term), and intermolecular interaction (IMI-term) to \( \mu_2' \).

Figure 5 Influence of \( k_d \) on \( \mu_2' \) and the contributions of axial dispersion (\( D_L \)-term), external mass transfer (\( k_f \)-term), intraparticle diffusion (\( D_e \)-term), and intermolecular interaction (IMI-term) to \( \mu_2' \).

Figure 6 Influence of \( k_a \) and \( k_d \) on \( \mu_2' \) and the contributions of axial dispersion (\( D_L \)-term), external mass transfer (\( k_f \)-term), intraparticle diffusion (\( D_e \)-term), and intermolecular interaction (IMI-term) to \( \mu_2' \). The value of \( K_A \) is kept constant at \( 1.0 \times 10^2 \) \( \text{mol}^{-1} \text{dm}^3 \).

Conclusions

The new moment equations for \( \mu_1 \) and \( \mu_2' \), i.e., Eqs. (13) and (14), were developed for the SECEC system, in which an intermolecular reaction took place between a solute and a ligand to form a solute-ligand complex. A set of basic equations of the general rate model of SECEC representing the mass balance, mass transfer rate, and intermolecular reaction kinetics in the column were analytically solved in the Laplace domain under the conditions that the migration of the solute-ligand complex stopped in the SECEC system. The new moment equations in the real time domain were derived from the analytical solution in the Laplace domain.

In order to demonstrate the usefulness of the moment equations, they were used to predict chromatographic behavior in the hypothetical SECEC systems. The influence of some parameters relating to the intermolecular interaction on \( \mu_1 \) and \( \mu_2' \) in the SECEC systems was quantitatively evaluated. It was
indicated, as preliminary information for the determination of \( k_a \) and \( k_d \) by the MA-SECEC method, that the intermolecular reaction kinetics had a predominant contribution to peak broadening and that the axial dispersion in the column also had a relatively large contribution to \( \mu_d' \). On the contrary, the contribution of external mass transfer and intraparticle diffusion to \( \mu_d' \) was negligibly small.

Some flow analytical methods have been used for the kinetic study of intermolecular interactions. However, the MA-SECEC has two advantageous merits in comparison with the other methods. They are quite important for the study of reaction kinetics. One is that the \( k_a \) and \( k_d \) values can be determined with no immobilization and chemical modification of solute and ligand molecules. The other is that their values can be analytically determined from experimental elution peak profiles by the moment theory, not by a trial-and-error data analysis procedure based on curve-fitting.

Chromatographic methods have been used for the kinetic study of intermolecular interactions, e.g., peak profiling method,24–29 non-linear chromatography,30–37 peak decay method,38 and break through experiment.39 It is expected that the information about the intermolecular interaction between solute molecules and functional ligands can be obtained by subtracting the contribution of the mass transfer phenomena from total band broadening. However, in principle, functional ligands are fixed on the stationary phase surface in the chromatographic theories.40,42 Other drawbacks are also pointed out. For example, in the case of the peak profiling method,44 it is assumed that the contributions of some kinetic processes to band broadening were the same between retained and non-retained compounds. However, the values of some kinetic parameters relating to band broadening are different between the two compounds. This means that the assumption of the peak profiling method is not strictly appropriate. In the case of the breakthrough experiments,39 the \( k_a \) and \( k_d \) values were estimated so that the breakthrough curves calculated fit to those experimentally observed. It is concerned whether their values are unmistakably determined from experimental data or not.

In addition, the influence of equilibration rate between different forms of a solute on band broadening was studied in the field of chromatography.40–43 However, the contribution of axial dispersion to band broadening is not taken into account in the chromatographic theories.40–42

Some methods based on CE have also been developed for the kinetic study of intermolecular interactions,44 e.g., affinity capillary electrophoresis,45 exponential decay method,46 and kinetic capillary electrophoresis (KCE).47–51 In these methods, \( k_a \) and \( k_d \) are determined by matching electropherograms or exponential decay curves numerically calculated by changing the rate constants to those experimentally measured. Macroscopic approach for studying kinetics at equilibrium (MASKE) was also developed for measuring equilibrium and kinetic parameters in the state of chemical equilibrium.52,53 However, it is required to tag a detectable label to solute or ligand molecules in MESKE. It is concerned whether the chemical modification affects the analytical results of intermolecular interactions or not.

It was tried to preliminarily establish a method for extracting the information about affinity kinetics of intermolecular interactions. A unique system based on SECEC was considered for measuring experimental data. The moment equations were also developed for the analytical determination of \( k_a \) and \( k_d \) of intermolecular interactions from elution peak profiles measured by SECEC. In this study, they were used to quantitatively discuss band broadening in the SECEC column because the kinetics information about intermolecular interactions is obtained by subtracting the contribution of some mass transfer processes to total band broadening. It is expected that the \( k_a \) and \( k_d \) values must be analytically determined by the MA-SECEC method with no immobilization and chemical modification of solute and ligand molecules.

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Supporting Information

Detailed information is provided for the development of the moment equations for the analysis of elution peak profiles measured by SECEC, in which intermolecular chemical reactions simultaneously take place. Some items of information about the calculation of chromatographic behavior in SECEC systems are also explained. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

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