Kinetic Study of the Intermolecular Interaction between 2-Phenoxypropionic Acid and β-Bromo-cyclodextrin Affixed on the Stationary Phase by Liquid Chromatography

Kanji MIYABE*† and Satoshi KAMIYA**

*Department of Chemistry, Faculty of Science, Rikkyo University, 3-34-1 Nishi-Ikebukuro, Toshima, Tokyo 171-8501, Japan
**Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan

The intermolecular interaction between 2-phenoxypropionic acid and β-bromo-cyclodextrin affixed on the stationary phase surface in a chiral HPLC system was studied by the moment analysis method. At first, pulse response and peak parking experiments were conducted to measure some parameters concerning the column geometry, adsorption equilibrium, and mass-transfer kinetics. Then, the first absolute moment (μ₁) and second central moment (μ₂) of the elution peaks were analyzed by the moment equations, which were developed by assuming that the reaction kinetics between the solute molecules and the functional ligands can be represented by the Langmuir-type rate equation. Finally, the flow-rate dependence of HETP calculated from μ₁ and μ₂ was analyzed by using the values of the parameters to determine the association and dissociation rate constants of the intermolecular interaction. It was demonstrated that the combination of the chromatographic experiments and moment analysis is one of the effective strategies for the kinetic study of intermolecular interactions.

Keywords Intermolecular interaction, moment analysis, reaction kinetics, association rate constant, dissociation rate constant, Langmuir-type rate equation, equilibrium constant, chiral HPLC system

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Introduction

Intermolecular interaction is one of the important subjects that must be studied for well understanding the intrinsic characteristics and mechanism of various functions and phenomena. Different types of methods have been developed for analyzing the intermolecular interactions between solute and ligand molecules. Information about the equilibrium of intermolecular interactions, e.g., association constant (K₁) and dissociation constant (K₂), can be obtained by these methods. In addition, some of them, e.g., surface plasmon resonance and fluorescence cross-correlation spectroscopy, have been used for determining the kinetic parameters of intermolecular interactions, i.e., association rate constant (k₁) and dissociation rate constant (k₂).

Chromatography has also been used for the kinetic study of intermolecular interactions. Band spreading in a column is correlated with the mass-transfer rate and the reaction kinetics of intermolecular interactions between solute molecules and functional ligands on the stationary phase surface. It is expected that information about the intermolecular interaction can be obtained by subtracting the contribution of the mass-transfer phenomena from the total band broadening. Kinetic information about intermolecular interactions has been derived from chromatographic data measured in different experimental systems on the basis of some analytical procedures, e.g., peak-profiling method, non-linear chromatography, peak-decay method, and a break-through experiment. The peak-profiling method is based on the comparison of the elution peaks of the retained and non-retained compounds. However, there are some points that affect the validity of the peak-profiling method based on the plate height equation proposed by Horvath and Lin. At first, in the plate-height equation, the contribution of surface diffusion to intraparticle mass transfer is not taken into account. The formulae of the moment equations for the first absolute moment (μ₁) and the second central moment (μ₂) were not reported, either. Only a plate-height equation was proposed. It is not mathematically clarified how the mass-transfer and reaction kinetic processes contribute to μ₂.

Second, k₂ was calculated on the assumption that the contributions of all kinetic processes other than stationary phase mass transfer to band broadening were the same between the 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. For example, the pore diffusivity is different between the retained and non-retained compounds because the values of the molecular diffusion coefficient, itself, are not the same between them. Similar to pore diffusion, the difference in the contribution of the external mass transfer to band broadening

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† To whom correspondence should be addressed.
E-mail: kmiyabe@rikkyo.ac.jp
must be taken into account between the two compounds. Irrespective of the retention behavior of the compounds, the external mass-transfer resistance affects the band broadening. In addition, the magnitude of the contributions of both the external mass transfer and pore diffusion depends on the retention factor of the compounds. This means that their contributions to band broadening are different between the retained and non-retained compounds. In conclusion, the contributions of the external mass transfer and pore diffusion cannot be cancelled between the two compounds by a simple subtraction of \( H \). Therefore, an accurate value of \( k_d \) is not determined by the peak profiling method.

In non-linear chromatography,\(^7\) an equation representing the correlation between the ratio of the concentration of the solute in the mobile phase to that injected into the column and the ratio of time to the hold-up time is derived by solving the basic equations concerning the mass balance of a solute compound and the Langmuir-type reaction kinetics at some boundary conditions. On the other hand, in the peak-decay method,\(^1\) it is assumed that only two reversible rate processes, \( i.e. \), a diffusion mass transfer and an adsorption-desorption step, take place in the column. However, there are some drawbacks in these methods. The contribution of several mass-transfer processes in the column to band broadening, such as eddy diffusion, external mass transfer, and pore and surface diffusion in the stationary phase, is not considered. On the other hand, regarding the peak-decay method,\(^2\) the elution profile experimentally measured is analyzed on the basis of an extreme assumption that only a single dissociation will take place for each solute-ligand complex if re-adsorption and diffusion back into the stagnant mobile phase are prevented.

In breakthrough experiments,\(^6\) the values of \( k_i \) and \( k_d \) were estimated so that the breakthrough curves calculated on the basis of the general rate model of chromatography fit to those experimentally observed. However, the contribution of surface diffusion to the intraparticle mass transfer was not considered, although surface diffusion had a significantly predominant role for the molecular migration in the stationary phase.\(^1\)

The moment analysis of elution peak profiles measured by pulse-response experiments is one of methods used for the kinetic study of intermolecular interactions. In this study, kinetic information about intermolecular interactions was derived on the basis of moment theory by subtracting the contribution of the mass-transfer resistance to the band broadening in chromatography. In a previous paper,\(^1\) new moment equations for \( \mu_i \) and \( \mu'_i \) were developed by assuming that the reaction kinetics between solute molecules and functional ligands could be represented by the Langmuir-type rate equation. They are essential for determining the values of \( k_i \) and \( k_d \) from the elution peak profiles. In this study, new moment equations were used for analyzing the chromatographic behavior in a chiral HPLC system. It was tried to demonstrate that the two rate constants (\( k_i \) and \( k_d \)) can be quantitatively determined by the moment analysis method.

**Theory**

New moment equations were developed to study the equilibrium and kinetic characteristics of intermolecular interactions by chromatography.\(^20\) The basic equations concerning the mass balance, mass transfer, and reaction kinetics in the column were solved in the Laplace domain. The reaction kinetics between the solute molecules and functional ligands was expressed by the Langmuir-type rate equation.

\[
\frac{d\eta}{dt} = k_i C_i (\eta - \eta) - k_d \eta
\]

The moment equations in the time domain were derived from the analytical solution of the basic equations in the Laplace domain. The following equation represents how \( \mu_i \) is correlated with some parameters.

\[
\mu_i = \frac{\int_0^\infty \left[ e^{-t/\mu} \left( 1 - e^{-t/\mu} \right) \right] dt}{\int_0^\infty e^{-t/\mu} dt} = \frac{L}{u_0} \left[ \alpha_i + (1 - \alpha_i) \left( \beta_i + (1 - \beta_i) \frac{k_i}{k_d} \eta \right) \right]
\]

where \( u_0 \) is the superficial velocity of the mobile phase, which is defined as the ratio of the volumetric flow rate of the mobile phase to the cross-sectional area of the empty column. Equation (2) indicates that \( K_i \), which is equal to \( k_i/k_d \), is obtained from \( \mu_i \).

On the other hand, \( \mu'_i \) consists of the contributions (\( \delta \)) of some rate processes in the column, \( i.e. \), \( \delta_a, \delta_i, \delta_e, \) and \( \delta_k \).\(^21,22\)

\[
\mu'_i = \frac{\int_0^\infty \left[ e^{-t/\mu} \left( 1 - e^{-t/\mu} \right) \right]^2 dt}{\int_0^\infty e^{-t/\mu} dt} = \frac{2L}{u_0} \left( \delta_a + \delta_i + \delta_e + \delta_k \right)
\]

\[
\delta_a = \left( \alpha_i + (1 - \alpha_i) \left( \beta_i + (1 - \beta_i) \frac{k_i}{k_d} \eta \right) \right)
\]

\[
\delta_i = (1 - \alpha_i) \left( \frac{k_i}{3k_i} \right) \left( \beta_i + (1 - \beta_i) \frac{k_i}{k_d} \eta \right)^2
\]

\[
\delta_e = (1 - \alpha_i) \left( \frac{k_i}{15D_i} \right) \left( \beta_i + (1 - \beta_i) \frac{k_i}{k_d} \eta \right)^2
\]

\[
\delta_k = (1 - \alpha_i) \left( \frac{k_i}{k_d} \right) \frac{k_i}{k_d} \eta
\]

These equations indicate how the important characteristics of the elution peak rest on several fundamental parameters of the retention equilibrium, mass transfer rate, and reaction kinetics in the column.

The flow-rate dependence of the height equivalent to a theoretical plate (HETP), calculated from \( \mu_i \) and \( \mu'_i \), is analyzed by the method of moments. The combination of Eqs. (2) - (8) provides the following equation.

\[
\frac{H_{total}}{H_{total}} = \frac{\mu'_i}{H_{total}} \frac{L}{u_0} = \frac{2L}{u_0} \left( \delta_a + \frac{2u_0}{\delta_i} \delta_i + \frac{2u_0}{\delta_e} \delta_e + \frac{2u_0}{\delta_k} \delta_k \right)
\]

\[
= H_a + H_i + H_e + H_k
\]

The experimental value of HETP is expressed as \( H_{total} \), because it is the sum of the contributions of the four kinetic processes in the column. Equation (9) indicates that the information about the contribution of \( H_a \) to \( H_{total} \) is obtained by subtracting the values of \( H_a, H_i, \) and \( H_k \) from \( H_{total} \). This means that the value of \( k_i/k_d \) is calculated from \( \delta_a \). Finally, the individual values of \( k_i \) and \( k_d \) can be determined because the values of \( k_i/k_d \) and \( k_i/k_d \) are calculated form \( \mu_i \) and \( \mu'_i \), respectively. It is explained in Supporting Information how the contributions of \( H_a, H_i, \) and \( H_k \) are subtracted from \( H_{total} \).
Experimental

Apparatus

Pulse-response (PR) and peak-parking (PP) experiments were carried out using an HPLC system, which consisted of a high-pressure pump (PU-980) (JASCO, Japan), a 7725i valve injector (20 μL) (Rheodyne, USA), and a UV-VIS spectrophotometric detector (UV-2075) (JASCO, Japan). A thermostated water bath (NCB-1200) (EYELA, Japan) was used for maintaining the column temperature. BOWIN software (JASCO, Japan) was used for acquiring and analyzing the experimental data.

Columns and reagents

Two columns were used in this study. One was a chiral separation column for optical isomers (CHIRAL CD BR) (YMC, Japan) (4.0 × 23 mm). It was packed with separation media prepared by a modification of the surface of full-porous silica gel particles (particle diameter (d_p) = 5 μm) with β-bromo-cyclodextrin (CD). It was kindly gifted by the manufacturer. A second column (Presto FT-Silica) (Imtakt, Japan) (4.6 × 30 mm) was packed with non-porous silica gel particles (d_p = 2 μm). This column was gently gifted from Imtakt. It was used for PP experiments using benzene and one of the polystyrene standards, of which the molecular weight was ca. 3.0 × 10^4, as the probe compounds.

A mixture of acetonitrile (HPLC grade) and an aqueous solution of sodium chloride (0.2 mol dm^{-3}) and acetic acid (1.1%) (10/90, v/v) was used as the mobile phase solvent. Water was prepared by distilling ion-exchanged water. Two optical isomers, i.e., R-(-)-2-phenoxypionic acid (R-PPA) and S-(+)-2-phenoxypionic acid (S-PPA), were used as sample compounds. They were of reagent grade and used without further purification. Their solutions were separately prepared by dissolving each compound into the mobile phase solvent at a concentration of 1 mg mL^{-1}.

Another column (Presto FT-Silica) (Imtakt, Japan) (4.6 × 30 mm) was packed with non-porous silica gel particles (d_p = 2 μm). This column was gently gifted from Imtakt. It was used for PP experiments under non-retained conditions to determine the molecular diffusivity (D_m) of R-PPA and S-PPA in the mobile phase solvent. Additionally, this column was also used for determining D_m of benzene and the polystyrene standard, of which the molecular weight was ca. 3.0 × 10^4, as the probe compounds.

Procedures

Both PR and PP experiments were conducted under SEC and chiral separation conditions for deriving information about some related parameters. The experimental conditions are explained in Supporting Information. It was confirmed that, throughout all of the experiments, the relative error of the mobile phase flow velocity was less than 1%. The column temperature was kept at 298 ± 0.1 K or 303 ± 0.1 K.

Results and Discussion

Measurement of porosities of the CHIRAL CD BR column

Information about the porosities (ε_c, ε_e, and total porosity (ε_t)) of the chiral separation column was determined by inverse size exclusion chromatography (ISEC), in which a series of polystyrene standards of different molecular weights (M_w = 5.8 × 10^6 to 3.3 × 10^6) and benzene were used as probe compounds. Tetrahydrofuran (THF) was used as the mobile phase solvent. The concentration of the probe compounds in THF was 1 mg mL^{-1}. In order to derive information about the mass transfer in the stationary phase, this column was also used for PP experiments using benzene and one of the polystyrene standards, of which the molecular weight was ca. 3.0 × 10^4, as the probe compounds.

A mixture of acetonitrile (HPLC grade) and an aqueous solution of sodium chloride (0.2 mol dm^{-3}) and acetic acid (1.1%) (10/90, v/v) was used as the mobile phase solvent. Water was prepared by distilling ion-exchanged water. Two optical isomers, i.e., R-(-)-2-phenoxypionic acid (R-PPA) and S-(+)-2-phenoxypionic acid (S-PPA), were used as sample compounds. They were of reagent grade and used without further purification. Their solutions were separately prepared by dissolving each compound into the mobile phase solvent at a concentration of 1 mg mL^{-1}.

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where \( C \) is the concentration, \( q \) the amount adsorbed, \( q_{\text{st}} \) the saturated amount adsorbed, and \( K_L \) the Langmuir coefficient.

Two linear correlations were observed between the reciprocal of \( q \) and that of \( C \) for the two solute compounds (refer to Fig. S1 in Supporting Information). The values of \( q_{\text{st}} \) were obtained from the intercept of the linear lines as 11.1 and 9.8 mg mL\(^{-1}\) for R-PPA and S-PPA, respectively.

**Measurement of \( D_m \) of R-PPA and S-PPA**

The molecular diffusion coefficient (\( D_m \)) was experimentally measured by PP experiments in order to obtain \( D_m \) as accurately as possible.

At first, PP experiments were carried out using benzene as the probe compound in an LC system consisting of the non-porous silica gel column and methanol to measure the obstructive factor (\( \gamma_m \)) in Eq. (S10) (Supporting Information). A linear correlation was observed between variance (\( \sigma_{\text{ax,t}}^2 \)) and peak parking time (\( t_p \)) (not shown). The value of \( \gamma_m \) was calculated to be 0.78 from the slope of the linear line. The value of \( D_m \) of benzene in methanol at 303 K had separately been measured as \( 2.2 \times 10^{-5} \) cm\(^2\) s\(^{-1}\).28,29

Then, PP experiments were carried out at 298 K using the non-porous silica gel column and the mobile phase solvent.

**Measurement of \( D_m \) of benzene and the polystyrene standard**

The values of \( D_m \) of benzene and the polystyrene standard (\( M_w = \text{ca.} 3.0 \times 10^4 \)) in THF are necessary to derive the obstructive factors (\( \gamma_m, \gamma_e, \) and \( \gamma_i \) in Eq. (S10)) of the CHIRAL CD BR column. Figure 3 illustrates two linear lines for the two probe compounds at 303 K. The value of \( D_m \) of benzene in THF was calculated from the slope of the linear line as \( 2.1 \times 10^{-5} \) cm\(^2\) s\(^{-1}\), because \( \gamma_m \) of the non-porous silica gel column was already measured as being 0.78, as explained above. The \( D_m \) value is also estimated as \( 2.4 \times 10^{-5} \) cm\(^2\) s\(^{-1}\) by the Wilke-Chang equation. The two \( D_m \) values are comparable to each other.

Similarly, \( D_m \) of the polystyrene standard was calculated as \( 1.3 \times 10^{-6} \) cm\(^2\) s\(^{-1}\) from the slope of the straight line in Fig. 3. It is also estimated as \( 1.0 \times 10^{-6} \) cm\(^2\) s\(^{-1}\) by the following literature correlation.33

\[
D_m = 3.45 \times 10^{-4} M_w^{0.564}
\]

The two \( D_m \) values are of the same order of magnitude.

**Measurement of \( D_p \) of R-PPA and S-PPA**

PP experiments of benzene and the polystyrene standard were conducted by using THF as the mobile phase solvent to estimate the pore diffusivity (\( D_p \)) in the CHIRAL CD BR column. Because benzene molecules can penetrate into all of the pore spaces in the column, band broadening of benzene provides information about the mass transfer in both the interparticulate and intraparticulate pore spaces. On the other hand, information about the mass transfer in the interparticulate pore space is
obtained from band spreading of the polystyrene standard. Roughly speaking, information about the mass transfer in the intraparticulate pore space is obtained by subtracting the band spreading of the polystyrene standard from that of benzene. Figure 4 illustrates linear correlations between the increment of $\sigma_{ax,t^2}$ and $t_p$ for benzene and the polystyrene standard. The PP experiments were carried out at 303 K because $M_w$ of the polystyrene standard is relatively large. The maximum value of $t_p$ for the polystyrene standard is longer than that for benzene because of its large molecular weight. The slope of the linear line for the polystyrene standard is obviously smaller than that for benzene.

According to Eq. (S10), $\gamma_m$ of the CHIRAL CD BR column was calculated as 0.67 from the slope of the straight line for benzene in Fig. 4, because benzene molecules can penetrate into all of the pore spaces in the column. On the other hand, $\gamma_e$ of the column was calculated as 0.66 from the slope of the straight line for the polystyrene standard in Fig. 4, because it can penetrate into only interparticulate pore space. In this case, the second term in the right-hand side of Eq. (S10) is neglected because the polystyrene standard cannot penetrate into intraparticulate pore space because of its large molecular size. On the basis of the $\gamma_m$ and $\gamma_e$ values, $\gamma_i$ was calculated as 0.69 according to Eq. (S11) (Supporting Information). At this time, the value of $K_h/k_t^2$ was calculated as 0.37 by Eq. (S13) (Supporting Information). The value of $k_t^2$ was calculated as 2.1 because that of $K_h$ was estimated to be 0.79 by Eq. (S4) (Supporting Information). The value of $k_t^2$ is comparable with the observation that $k_t^2$ of porous silica gel ranging from 2 to 6.22 The value of $D_p$ was calculated by Eq. (S3) (Supporting Information) as $1.1 \times 10^{-6}$ cm$^2$ s$^{-1}$ for R-PPA and S-PPA in the chiral separation media at 298 K because those of $\varepsilon_i$ and $D_m$ were obtained as 0.64 and $4.6 \times 10^{-5}$ cm$^2$ s$^{-1}$, respectively. In this study, the geometrical values of the packing materials, i.e., $\varepsilon_i$, $K_h$, and $k_t$, were measured by the peak-parking experiments using THF as the solvent. However, the value of $D_p$ was calculated from that of $D_m$ experimentally measured in the mobile phase solvent.

Measurement of $D_s$ of R-PPA and S-PPA

The surface diffusion coefficient ($D_s$) was measured by PP experiments in the chiral HPLC system using the CHIRAL CD BR column and the mobile phase solvent. Figure 5 illustrates the linear correlations between $\sigma_{ax,t^2}$ and $t_p$. At first, $D_{ax}$ was calculated by Eq. (S14) (Supporting Information) from $D_{ax}$, which was obtained from the slope of the linear lines in Fig. 5. Then, $D_s$ was calculated from $D_{ax}$ by Eq. (S17) (Supporting Information). The values of $D_s$ at 298 K were calculated as $1.3 \times 10^{-6}$ and $1.2 \times 10^{-6}$ cm$^2$ s$^{-1}$ for R-PPA and S-PPA, respectively.

Measurement of $K_h$

In this study, $\mu_1$ data were measured with changing $u_0$. Two
linear correlations were observed for R-PPA and S-PPA between \((\mu_l - \mu_0)(1 - \varepsilon_c)\) and \(L/u_0\) (not shown). Both of them almost passed through the origin. From Eq. (2), \(t_0\) is represented as follows.

\[
t_0 = \frac{L}{u_0} \left[ \varepsilon_c + (1 - \varepsilon_c) \varepsilon_d \right]
\]

(13)

Because \(K_s\) was calculated from plural data of \(\mu_l\), it was measured more accurately as 93 and 1.1 \(\times 10^3 \text{ M}^{-1}\) for R-PPA and S-PPA, respectively, from the slope of the two linear lines.

**Measurement of \(k_a\) and \(k_d\)**

According to Eq. (S6) (Supporting Information), \((H_{\text{total}} - H_l - H_d)\) is plotted against \(u_0\) in Fig. 6. The value of \(H_l\) was calculated by Eqs. (6) and (9) from \(k_i\), which was estimated by Eq. (S1) (Supporting Information). On the other hand, the value of \(H_d\) was calculated by Eqs. (7) and (9) from \(D_d\), which was estimated by Eq. (S2) (Supporting Information) because \(D_d\) and \(D_i\) were experimentally measured previously. Although there is some scatter, two linear correlations were observed in Fig. 6 between \((H_{\text{total}} - H_l - H_d)\) and \(u_0\).

The value of \((H_{\text{total}} - H_l - H_d)\) is ca. 0.012 cm at the lowest flow rate, i.e., \(u_0 = \text{ca.} 0.04 \text{ cm s}^{-1}\). On the other hand, the value of the first term in the right-hand side of Eq. (S6), i.e., \(2\gamma_d/\mu_0 u_0\), is calculated as 7.5 \(\times 10^3 \text{ cm}^{-1}\) when \(\gamma_d\) is assumed as 0.7.  According to Eq. (S6) \((H_{\text{total}} - H_l - H_d)\) is negligibly small under the flow-rate conditions that \(u_0\) is larger than 0.04 cm s\(^{-1}\) and that a linear correlation is observed between \((H_{\text{total}} - H_l - H_d)\) and \(u_0\).

The value of \(k_d/k_a^2\) was obtained as 3.1 \(\text{ M}^{-1} \text{ s}\) for R-PPA from the slope of the linear solid line in the high flow rate range (\(u_0 > 0.04 \text{ cm s}^{-1}\)). On the other hand, the ratio of \(k_d/k_a = (K_s)\) was calculated for R-PPA as 93 \(\text{ M}^{-1}\) from the slope of the linear line between \((\mu_l - \mu_0)(1 - \varepsilon_c)\) and \(L/u_0\). The values of \(k_d\) and \(k_a\) were, respectively, obtained for R-PPA as 2.7 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and 30 s\(^{-1}\) from those of \(k_d/k_a\) and \(k_d/k_a^2\). In the same manner, \(k_d\) and \(k_a\) for S-PPA were obtained as 3.6 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and 33 s\(^{-1}\), respectively.

The moment theory suggests that there is a linear correlation between \((H_{\text{total}} - H_l - H_d)\) and \(u_0\) under sufficiently high flow-rate conditions, i.e., \(u_0 > 0.04 \text{ cm s}^{-1}\) because the contribution of the first term in the right-hand side of Eq. (S6) can be neglected. On the basis of the considerations, the solid lines in Fig. 6 were drawn, and their slope was analyzed to determine the values of \(k_a\) and \(k_d\). However, because there is some scatter in Fig. 6, the dotted lines were also drawn for plots between \((H_{\text{total}} - H_l - H_d)\) and \(u_0\) at \(u_0 > 0.1 \text{ cm s}^{-1}\). In the same manner described above, \(k_a\) and \(k_d\) for R-PPA were obtained as 2.1 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and 22 s\(^{-1}\), respectively. For S-PPA, \(k_a\) and \(k_d\) were obtained as 2.6 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and 24 s\(^{-1}\), respectively.

**Conclusions**

It was demonstrated that the combination of the chromatographic (PR and PP) experiments and the moment analysis method can provide quantitative information about the equilibrium and kinetics of intermolecular interactions. As a concrete example, the values of \(K_s\), \(k_a\), and \(k_d\) concerning the intermolecular interaction between 2-phenoxypionic acid and \(\beta\)-bromocyclodextrin were determined by analyzing the chromatographic behavior in the chiral HPLC system. It seems that \(k_a\) and \(k_d\) of R-PPA were in the range from 2.1 \(\times 10^3\) to 2.7 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and from 22 to 30 s\(^{-1}\), respectively. Similarly, \(k_a\) and \(k_d\) of S-PPA were in the range from 2.6 \(\times 10^3\) to 3.6 \(\times 10^3 \text{ M}^{-1} \text{ s}^{-1}\) and from 24 to 33 s\(^{-1}\), respectively. It is expected that the chromatographic method with the moment analysis is effective for a kinetic study of intermolecular interactions. However, it is still required to acquire more experimental data. In addition, it is also required to develop a method for the kinetic study of intermolecular interactions under the conditions that both the solute and ligand molecules are free in solution.

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