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Determination of chromatographic separation parameters of tryptophan enantiomers on a Chirosil-SCA chiral stationary phase by using the inverse method based on the initial guesses estimated from elution by characteristic point method

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ABSTRACT

An effective chiral stationary phase (CSP) for enantioseparation of amino acids was established previously by bonding (18-crown-6)-2, 3, 11, 12-tetracarboxylic acid to silica gel. This CSP has recently been commercialized under the name of Chirosil-SCA. As a first step for developing a Chirosil-SCA simulated moving bed chromatographic process for separation of tryptophan enantiomers, the adsorption isotherm and mass-transfer parameters of each tryptophan enantiomer on the Chirosil-SCA CSP were determined in this study while using only water as a mobile phase. For this task, inverse method (IM) was applied on the basis of the initial guesses estimated from elution by characteristic point (ECP) method, which was found to be more advantageous in the aspects of both accuracy and computational efficiency than the case of utilizing individually only IM or ECP method. The results revealed that the adsorption behavior of each tryptophan enantiomer on the Chirosil-SCA could be well described by the Langmuir–Freundlich isotherm. The model predictions based on the determined parameter values were in close agreement with the experimental chromatograms from a series of single-component or mixture pulse tests that were performed under various feed concentrations and flow rates. It was also found that the Langmuir–Freundlich isotherm parameters of each enantiomer were largely affected by temperature. Such a marked dependence of the parameters on temperature was investigated quantitatively. The results of such an investigation indicated that as the temperature decreases, the adsorption affinities of both enantiomers become higher and the heterogeneity of the Chirosil-SCA becomes more pronounced.

1. Introduction

More than 18% of the top 500 drugs worldwide are known to be in the category of racemic compounds [1,2]. The necessity for fractionating the racemic drugs into two pure enantiomers has been raised over several decades, which has aroused a lot of interests in the development of efficient enantioseparation processes. One of the widely accepted processes is a chromatographic separation process based on a properly synthesized adsorbent, which has been referred to as chiral stationary phase (CSP) in the literature [1–5].

Various types of CSPs have been developed and applied to chromatographic enantioseparation processes so far. Among them, it is quite worth noting a recently developed CSP that was prepared by bonding (18-crown-6)-2, 3, 11, 12-tetracarboxylic acid to silica gel [6–9]. This CSP was reported to be highly effective in chromatographic enantioseparation of amino acids. Due to such a separating power verified, the CSP was commercialized under the names of Chirosil-RCA and Chirosil-SCA, which were based on (+)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid and (−)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid respectively.

Until now, most of previous researches on the aforementioned Chirosil CSP have been focused on the investigation of retention factors, separation factors, and resolution factors of several chiral compounds including amino acid enantiomers [6–12]. All of these previous studies were performed under analytical conditions and under the mobile phase containing an organic solvent and/or an acidic modifier such as acetic acid, sulfuric acid, and perchloric acid. No previous studies have hitherto attempted to extend the application scope of the Chirosil CSP to preparative or large-scale enantioseparation processes under a more environmentally benign and more cost-effective mobile phase solution.

The goal of this study is to complete the first task for the aforementioned preparative application of the Chirosil CSP under the condition of using only water as a mobile phase. The corresponding task is to acquire the chromatographic separation parameters

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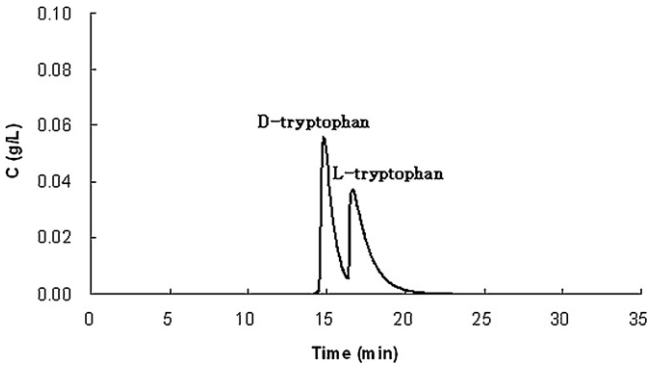

Fig. 1. Result of the preliminary pulse test with a mixture of tryptophan enantiomers at 20 °C. Experimental conditions (solid phase: Chirosil-SCA, mobile phase: water, column length: 25 cm, column diameter: 1 cm, flow rate: 2 mL/min, injection volume: 1 mL, feed concentration: 0.1 g/L for each enantiomer).

(or intrinsic parameters) of amino acid enantiomers under overloaded conditions while employing Chirosil-SCA and water as solid and mobile phases respectively. The intrinsic parameters to be obtained under such conditions include adsorption isotherm and mass-transfer parameters. For the task of this study, the mixture of D- and L-tryptophan was chosen as a target separation system because the tryptophan enantiomers were found to have an explicit difference in retention time in the preliminary pulse test of this study (Fig. 1), where only water was used as a mobile phase.

In regard to the determination of adsorption isotherm parameters and/or mass-transfer parameters, there are several methods available in the literature [13–16]. Among them, the inverse method (IM) and the elution by characteristic point (ECP) method, which will be briefly explained in the following theory section, were employed in the present work. It should be mentioned that the usage of IM and ECP method in this study is different from that in the literature. The two methods were utilized individually without any inter-dependence on each other in the literature [15–18]. By contrast, in this study, IM was applied on the basis of the initial guesses estimated from ECP method for a given parameter-determination task. The results of this study revealed that such an ECP-assisted IM application were markedly effective in determining adsorption isotherm and mass-transfer parameters with high accuracy and high computational efficiency, particularly for the case where the isotherm equation was highly nonlinear and complex.

The intrinsic parameters of tryptophan enantiomers that are reported in this first part of a series of papers will be applied to our subsequent work, which is to develop an optimal SMB process for separation of D- and L-tryptophan while using only water as a mobile phase. The results about such an enantioseparation SMB process will be provided in the next papers of the series.

2. Theory

2.1. Column model

One of the important tools for optimal design of a continuous chromatographic separation process is the column model, i.e., the mathematical model that can allow description or prediction of migration behavior of each species through a chromatographic column packed with adsorbent. The detailed column model consists of the following material balance equations [19,20]:

$$\frac{\partial C_i}{\partial t} + \frac{(1-\varepsilon)}{\varepsilon}\frac{\partial q_i}{\partial t} - E_{b,i}\frac{\partial^2 C_i}{\partial z^2} + u_0\frac{\partial C_i}{\partial z} = 0 \quad (1a)$$

$$\frac{\partial q_i}{\partial t} = k_{m,i}a_p(q_i^* - q_i) \quad (1b)$$

where the subscript i indicates the different components; C and q are the component concentrations in liquid and solid phases respectively; ε is the total porosity; E_b is the axial dispersion coefficient from the Chung and Wen correlation [21]; q^* is the solid-phase concentration in equilibrium with the liquid-phase concentration (C); u_0 is the liquid-phase interstitial velocity; k_m is the mass-transfer coefficient; $a_p = 3/R_p$ for spherical particles; and R_p is the radius of solid particle.

In the above model equations, the equilibrium relationship between q_i^* and C_i is commonly expressed by the following adsorption isotherms for a racemic mixture:

$$q_i^* = f(C_1, C_2), \quad i = 1 \text{ or } 2 \quad (1c)$$

where components 1 and 2 stand for D-tryptophan and L-tryptophan respectively in the separation system of our interest, and the functionality, f, depends on the type of adsorption isotherm.

2.2. Inverse method (IM)

An essential prerequisite for applying the column model equations (Eq. (1)) to process design is the determination of the adsorption isotherm parameters and the mass-transfer parameter for each component. Inverse method (IM) has been recognized as one of the well-established methods for determining such intrinsic parameters [16,17]. This method begins with acquisition of experimental chromatogram data under overloaded conditions. The acquired chromatogram data are compared with the calculated band profiles, which are based on the column model (Eq. (1)) with the assumed values of the unknown intrinsic-parameters. The band-profile calculations are then continued until the calculated band profiles (or model predictions) match the experimental chromatogram the most closely. During these calculation processes, the values of the intrinsic parameters for each component are tuned such that the discrepancies between the experimental chromatogram and the model predictions are minimized. Usually, an efficient optimization routine is applied to such a minimization task.

IM, however, could have some difficulties in its application process, if the adsorption isotherm would be a highly complex and nonlinear type of equation. The reason is that in such case, the selection of an appropriate initial guess for each isotherm parameter becomes difficult or sometimes impossible, which in turn causes the necessity for widening the searching region for each parameter. Since IM always requires a huge number of numerical computations (or simulations), the aforementioned problems concerning initial guesses and searching region can sometimes hinder the convergence of solutions for each parameter. Furthermore, this situation can be made worse, if several types of adsorption isotherms are to be tested in the stage of the IM application as in this study.

2.3. Elution by characteristic point (ECP) method

Another attractive method for determination of intrinsic parameters is the ECP (elution by characteristic point) method [15,17,18]. Since this method is based on the ideal model, it allows the determination of only adsorption isotherm parameters. As far as such a task is concerned, the ECP method must be a highly simple and fast one because the acquisition of only one overloaded elution profile per each component is quite enough [17,18]. Furthermore, no numerical simulations are needed in the calculation procedure. Only some algebraic calculations are sufficient for determination of adsorption isotherm parameters.

The fundamental equation of the ECP method can be derived by setting $E_{b,i}$ and $k_{m,i}$ to zero and infinity respectively in the column model (Eq. (1)). The resultant equation is reduced to that of the

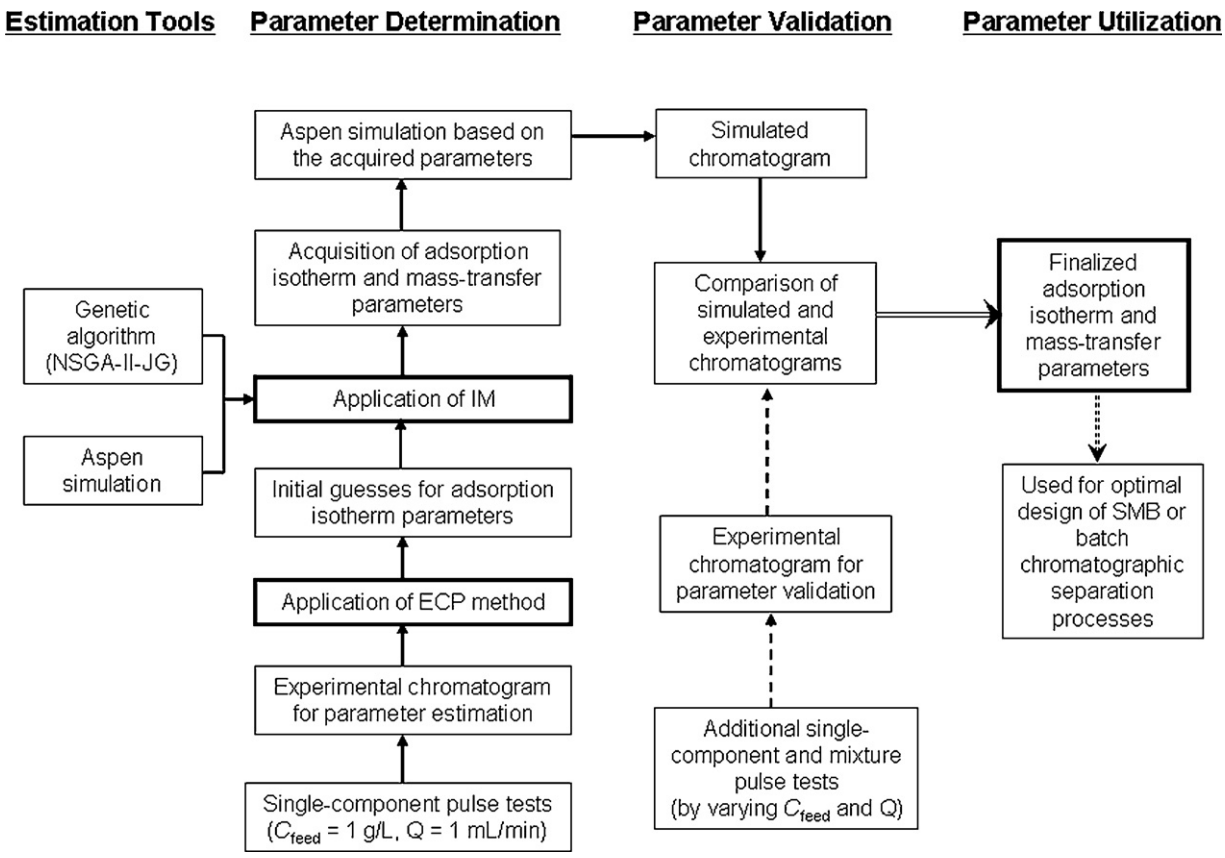

Fig. 2. Systematic strategy for determining, validating, and utilizing the adsorption isotherm and mass-transfer parameters of tryptophan enantiomers on the Chirosil-SCA CSP under the condition of using only water as a mobile phase.

ideal model, which can be transformed again into the following form [18]:

$$t_{R,i}(C_i) = t_p + t_0 \left(1 + F \left. \frac{dq_i^*}{dC_i} \right|_{C_i} \right) \quad (2)$$

where $t_{R,i}(C_i)$ is the retention time corresponding to C_i in a dispersed wave in the overloaded elution profile; t_p is the width of the injected pulse in time unit; t_0 is the hold-up time ($t_0 = L/u_0$); F is the phase ratio ($F = (1-\varepsilon)/\varepsilon$); and $(dq_i^*/dC_i)|_{C_i}$ is the slope of the isotherm at C_i.

For a given isotherm equation, the slope of the isotherm can be estimated from two different sources. One is from the above ECP equation (Eq. (2)) based on the experimentally measured elution profile under overloaded conditions, and the other from the isotherm equation itself. By minimizing the deviations between the slopes resulting from these two sources, one can obtain the isotherm parameters.

Although the ECP method allows a quick estimation of isotherm parameters as explained above, the isotherm parameters from the ECP method cannot be utilized directly for process design because their estimation is based on the assumption of the absence of mass-transfer effects, which is quite unrealistic. In addition, the mass-transfer parameter, which is as important as the isotherm parameters, cannot be obtained from the ECP method.

2.4. Use of ECP-assisted IM application

Considering the advantages and disadvantages of IM and ECP method, the individual use of one seems to be either inefficient or inappropriate, particularly for the case where the isotherm equation is highly nonlinear and complex as in the case of our study. Thus, the strategy of using IM with a partial assistance from ECP method was employed in this study. The detailed scheme of this strategy is delineated in Fig. 2, where the overall research work is divided into three tasks such as determination, validation, and utilization of intrinsic parameters. Among the three tasks, the first two will be addressed in this paper while the last task will be treated in the subsequent publication.

As shown in Fig. 2, the core of the proposed strategy is that the ECP method is applied first to estimation of the parameter values of the isotherm equation considered. The estimated parameter values from the ECP method serve as initial guesses in the following stage of the IM application, where the best-fit isotherm and mass-transfer parameters are determined with the help of an efficient optimization tool coupled with Aspen simulator. In addition, the best-fit isotherm model was determined based on the results from the IM application.

The validity of the determined parameter values is checked before their application to process design. For this, a series of overloaded pulse-injection experiments are carried out while varying the operating conditions differently from those in the IM application. The resultant experimental profiles are then compared with the model-prediction results based on the parameter values determined above. If the two results are in reasonable agreement, the parameter values will be finalized as those for process design.

3. Experimental

3.1. Materials and equipments

Both D-tryptophan and L-tryptophan were purchased from Sigma–Aldrich Co. (St. Louis, MO). The water utilized in this study belonged to the level of distilled deionized water (DDW), which

was obtained from a Milli-Q system by Millipore (Bedford, MA). The adsorbent used was the Chirosil-SCA CSP ((−)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid bonded to silica gel), which was manufactured by RS Tech Co. (Daejeon, South Korea). The adsorbent particle size is 10 μm. The column containing the adsorbent has a length of 25 cm and a diameter of 1 cm, and it was pre-packed by the manufacturer (RS Tech Co. South Korea). The total porosity of the pre-packed column was 0.7, which was determined from the retention time of DDW pulse under the condition of using 30% ethanol in DDW as a mobile phase.

All the experiments of this study were conducted with the Waters HPLC system (Milford, MA), which consisted of two HPLC pumps (Waters 515), a PDA detector (Waters 996), and an injector (Rheodyne 9725i). The experimental data from the HPLC system were collected and analyzed with the help of Waters Millennium software operating in the Windows environment.

3.2. Procedures

For pulse tests, a sampling loop was connected to the injector in the aforementioned HPLC system. In the load position, the loop was filled with a feed solution. The eluent flow rate was controlled by the Millennium software. Then the injection valve was switched to the inject position in order to start the injection. Data recording was started simultaneously. The effluent concentration was monitored by the PDA detector. The column temperature was maintained constant using a Mistral Column Thermostat 880 (Plainsboro, NJ). The extra-column dead volume was 0.123 mL, which was measured by a pulse test without the column.

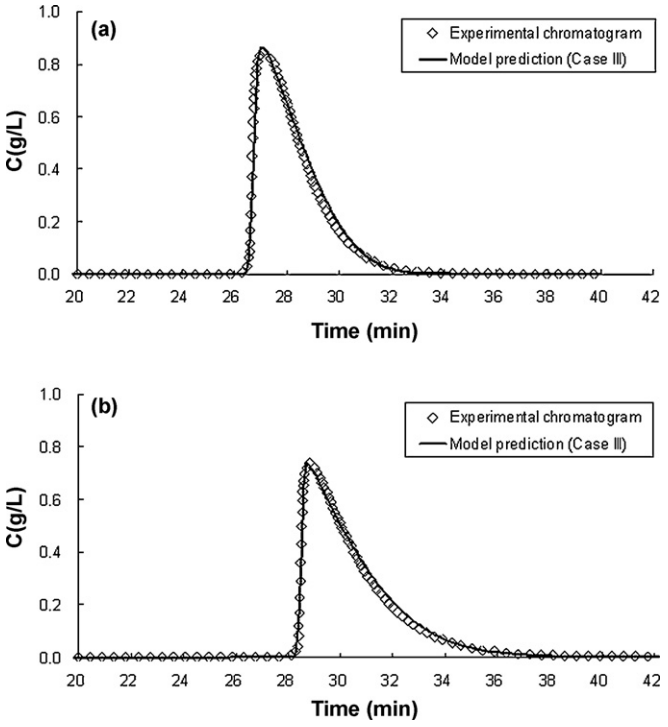

Fig. 3. Comparison of the experimental chromatogram and the model-prediction result from case III (Langmuir–Freundlich isotherm) at 20 °C. (a) D-Tryptophan, (b) L-tryptophan. Experimental conditions (feed concentration: 1 g/L for each enantiomer, injection volume: 2 mL, flow rate: 1 mL/min).

4. Results and discussion

4.1. Parameter determination by use of ECP-assisted IM application

4.1.1. 1st step: application of ECP method to estimating the initial guesses for IM

To obtain the experimental chromatograms necessary for the ECP-method application, two single-component pulse tests were carried out for D-tryptophan and L-tryptophan respectively at 20 °C. The results are presented in Fig. 3. It is clear from this figure that the overloaded condition, which is the essential requirement for justifying the use of the ECP method, was satisfied.

Based on the experimental profiles in Fig. 3, the values of dq^*/dC were estimated using the ECP method. This task was carried out by measuring the retention times at several discrete concentration points along the dispersed wave in Fig. 3 and plugging the measured retention times into the ECP-related equation (Eq. (2)), which was then solved for dq^*/dC. The resultant values of dq^*/dC, namely, the values of dq^*/dC originating from the experimental profile and the ECP method were plotted as a function of C in Fig. 4 for each enantiomer. Such data points plotted in this figure will be called "*ECP data*" hereafter.

Note in Fig. 4 that the ECP data points, which signify the isotherm slopes, decrease with increasing C. In addition, Fig. 3 shows that the experimental chromatogram of each enantiomer has a sharp front and a dispersed rear. This trend is one of the typical phenomena occurring in nonlinear adsorption systems with convex upward isotherm curves, which can be represented by one of the following isotherm equations: Langmuir, bi-Langmuir, or Langmuir–Freundlich isotherm equation. Prior to the determination of the isotherm type and parameters for tryptophan enantiomers, a brief introduction regarding each isotherm equation is given below.

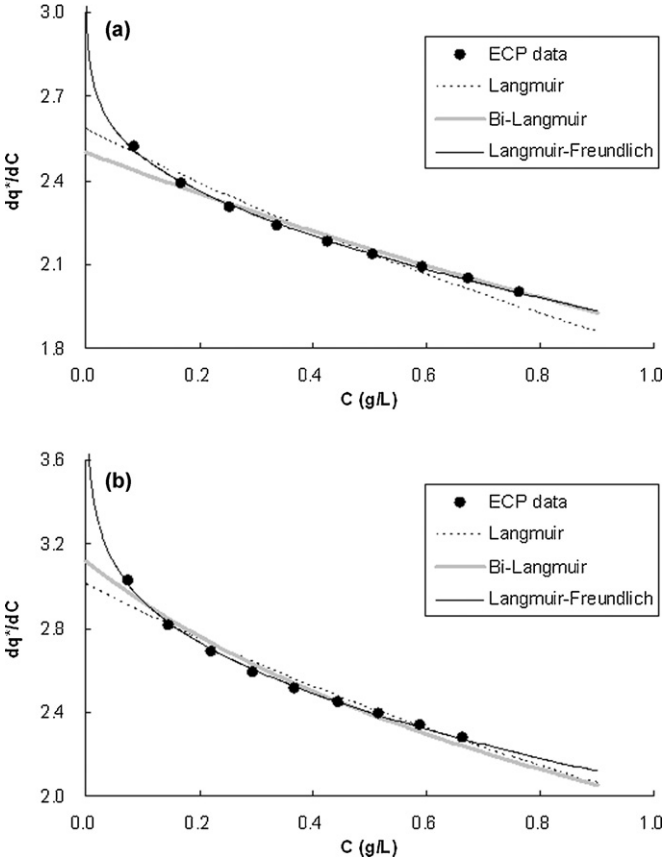

Fig. 4. Comparison of the ECP data and the calculated results from the three isotherm model equations. (a) D-Tryptophan, (b) L-tryptophan.

Table 1
Adsorption isotherm parameters resulting from the ECP-method application (1st step of the proposed strategy) at the temperature of 20 °C.

Langmuir model (20 °C)		Bi-Langmuir model (20 °C)		Langmuir–Freundlich model (20 °C)	
q_s (g/L)	13.0113	q_{ns} (g/L)	15.5290	q_s (g/L)	22.8197
b_1 (L/g)	0.1985	b_{ns} (L/g)	0.1555	b_1 ((L/g)$^{\beta_1}$)	0.1056
b_2 (L/g)	0.2313	q_s (g/L)	0.7026	β_1	0.9585
		$b_{s,1}$ (L/g)	0.1225	b_2 ((L/g)$^{\beta_2}$)	0.1216
		$b_{s,2}$ (L/g)	1.0054	β_2	0.9344

First, the single-component Langmuir isotherm equation for each enantiomer is as follows [18]:

$$q_1^* = \frac{q_s b_1 C_1}{1 + b_1 C_1}, \qquad q_2^* = \frac{q_s b_2 C_2}{1 + b_2 C_2} \tag{3}$$

where the subscripts 1 and 2 indicate D-tryptophan and L-tryptophan respectively. In the above Langmuir equation, there are three isotherm parameters to be determined, which include q_s, b_1, and b_2. Secondly, the single-component bi-Langmuir isotherm equation for each enantiomer [18] is:

$$q_1^* = \frac{q_{ns} b_{ns} C_1}{1 + b_{ns} C_1} + \frac{q_s b_{s,1} C_1}{1 + b_{s,1} C_1}, \qquad q_2^* = \frac{q_{ns} b_{ns} C_2}{1 + b_{ns} C_2} + \frac{q_s b_{s,2} C_2}{1 + b_{s,2} C_2} \tag{4}$$

where a total of five isotherm parameters including q_{ns}, b_{ns}, q_s, $b_{s,1}$, and $b_{s,2}$ should be determined. Thirdly, the single-component Langmuir–Freundlich isotherm equation for each enantiomer [22,23] is:

$$q_1^* = \frac{q_s b_1 C_1^{\beta_1}}{1 + b_1 C_1^{\beta_1}}, \qquad q_2^* = \frac{q_s b_2 C_2^{\beta_2}}{1 + b_2 C_2^{\beta_2}} \tag{5}$$

where a total of five isotherm parameters including q_s, b_1, β_1, b_2, and β_2 should be determined.

The aforementioned isotherm parameters were obtained by fitting the first derivative of each isotherm equation to the ECP data in Fig. 4. Here, it should be mentioned that the ECP data of D-tryptophan (1) and L-tryptophan (2) were fitted simultaneously to the first derivatives of their corresponding isotherm equations. This is because several parameters (q_s in Eq. (3), q_s, q_{ns}, and b_{ns} in Eq. (4), and q_s in Eq. (5)) are common to both the isotherm equations of D-tryptophan and L-tryptophan.

The results from the above task are presented in Table 1 and Fig. 4. It is easily seen that the best-fit of the ECP data was attained by the Langmuir–Freundlich isotherm equation. On the other hand, the Langmuir isotherm equation appears to be the most unsuitable for describing the ECP data. However, these results do not necessarily mean that the Langmuir–Freundlich isotherm is the most adequate for the system of interest, because the isotherm parameters from the above ECP-method application (Table 1) are based on the ideal model (i.e., highly simplified model). The best-fit isotherm model will thus be determined in the following IM application.

4.1.2. 2nd step: application of IM on the basis of the estimates from ECP method

The isotherm parameters estimated above from the ECP method (Table 1) were employed in this section as the initial guesses for the IM application. To determine the most adequate isotherm model among the three isotherm candidates considered, a total of three IM applications were conducted for cases I, II, and III, which were based on the Langmuir, the bi-Langmuir, and the Langmuir–Freundlich isotherms respectively.

For each case, the isotherm and mass-transfer parameters of both tryptophan enantiomers were determined simultaneously by minimizing the squared deviations between the experimental concentrations and the model-predicted concentrations along the elution profiles of both enantiomers, which was assisted by a highly robust optimization tool based on non-dominated sorting genetic algorithm with jumping genes (NSGA-II-JG) [24,25]. The corresponding computer-program codes for the NSGA-II-JG were prepared using Visual Basic Application (VBA) in Excel software. Since the NSGA-II-JG optimization requires a huge number of detailed rate-model simulations, the VBA codes were prepared to include the function of calling Aspen Chromatography simulator as well as of implementing the NSGA-II-JG algorithm. Further details on this optimization algorithm are listed in Supplementary data file # 1.

Table 2 lists the resulting parameter values from the above optimizations for the three cases. Based on these parameter values, the model-predicted profiles for each case were generated and compared with the experimental profiles. The comparison results for case III are presented in Fig. 3 and the others for cases I and II are available in Supplementary data file # 2. It was found that the predicted profiles from the case III based on the Langmuir–Freundlich isotherm agree the most closely with the experimental profiles for both tryptophan enantiomers (Fig. 3). The validity of this observation can also be confirmed statistically by comparing the standard deviation (σ) and the F-ratio [16] for the three cases in the following manner.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{\mathrm{sim}} - C_i^{\mathrm{exp}})}{n - p}} \tag{6a}$$

$$F\text{-ratio between cases I and III} = \frac{\sigma_{\mathrm{I}}^2}{\sigma_{\mathrm{III}}^2} \tag{6b}$$

Table 2
Adsorption isotherm and mass-transfer parameters resulting from the IM application (2nd step of the proposed strategy) at the temperature of 20 °C.

Case I (Langmuir, 20 °C)		Case II (bi-Langmuir, 20 °C)		Case III (Langmuir–Freundlich, 20 °C)	
q_s (g/L)	16.8175	q_{ns} (g/L)	15.8399	q_s (g/L)	24.2000
b_1 (L/g)	0.1491	b_{ns} (L/g)	0.1345	b_1 ((L/g)$^{\beta_1}$)	0.0994
b_2 (L/g)	0.1729	q_s (g/L)	1.2358	β_1	0.9670
$k_{m,1}$ (cm/min)	0.8561	$b_{s,1}$ (L/g)	0.3214	b_2 ((L/g)$^{\beta_2}$)	0.1140
$k_{m,2}$ (cm/min)	0.0361	$b_{s,2}$ (L/g)	0.8716	β_2	0.9350
		$k_{m,1}$ (cm/min)	0.4867	$k_{m,1}$ (cm/min)	0.0667
		$k_{m,2}$ (cm/min)	0.5590	$k_{m,2}$ (cm/min)	0.0682

$$F\text{-ratio between cases II and III} = \frac{\sigma_{II}^2}{\sigma_{III}^2} \quad (6c)$$

where n is the number of data points in the chromatograms and p is the number of parameters.

The results from the aforementioned statistical analyses are presented in Table 3. One can see that the σ value of the case III based on the Langmuir–Freundlich isotherm is much smaller than those of the other cases based on the Langmuir and the bi-Langmuir isotherms. This in turn leads cases I and II to have more than twice the F-ratio value compared to cases III, which implies that the fitting performance of the case III based on the Langmuir–Freundlich isotherm is more than two times better than those of the other cases. For this reason, the Langmuir–Freundlich isotherm was selected as the best-fit isotherm model for the system of interest.

4.2. Validation of the parameters determined from the IM application

To investigate the validity of the case III parameters determined above (Table 2), a series of pulse tests were conducted under different operating conditions from those in the previous section. First, three pulse tests were performed for each enantiomer while varying the feed concentration. The resulting experimental profiles were then compared with the model-predicted profiles that were obtained based on the case III parameters in Table 2. It was confirmed that the model-predicted profiles were in close agreement with the experimental profiles for both enantiomers (the corresponding figures were included in Supplementary data file # 3).

Secondly, a single pulse test was carried out for each enantiomer while varying both the feed concentration and the flow rate. The results of these pulse tests were also found to be in good agreement with the model-predicted results (Fig. 5).

Finally, three pulse tests were conducted, in which the mixture of D-tryptophan and L-tryptophan was loaded at various feed concentrations and flow rates. The resultant experimental profiles are presented in Fig. 6. The model-predicted profiles corresponding to such experimental conditions were also obtained on the basis of the case IIII parameters in Table 2. Here, it should be mentioned that a binary-component Langmuir–Freundlich isotherm equation was used in the model predictions, which is due to the fact that the adsorption behavior of one component is always affected by the presence of the other in nonlinear adsorption systems [18]. The binary-component Langmuir–Freundlich isotherm equation is given by:

$$q_1^* = \frac{q_s b_1 C_1^{\beta_1}}{1 + b_1 C_1^{\beta_1} + b_2 C_2^{\beta_2}}, \qquad q_2^* = \frac{q_s b_2 C_2^{\beta_2}}{1 + b_1 C_1^{\beta_1} + b_2 C_2^{\beta_2}} \quad (7)$$

where all the relevant parameter values in the above equations come from those of the single-component Langmuir–Freundlich isotherm equations that are available in Table 2.

The model-predicted profiles based on the aforementioned binary-component isotherm equation were compared with the experimental profiles from the pulse tests based on the loading of enantiomer mixture. As shown in Fig. 6, the former profiles are in satisfactory agreement with the latter profiles.

Table 3
Statistical analyses for determining the best-fit isotherm model from the results of the IM application at the temperature of 20 °C.

	σ[a]	F-ratio[b]
Case I (Langmuir)	0.0246	2.79
Case II (bi-Langmuir)	0.0219	2.20
Case III (Langmuir–Freundlich)	0.0148	1.00

[a] The number of data points in the chromatograms was $n = 441$ and the number of parameters was $p = 5$ for case I and $p = 7$ for cases II and III.
[b] Case III was chosen as a reference when calculating the F-ratio for each case.

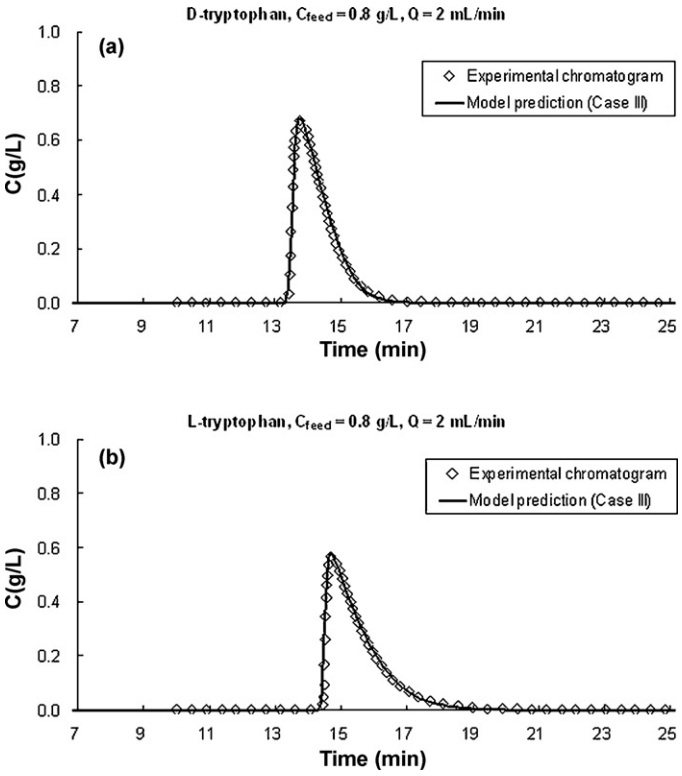

Fig. 5. Parameter validation by varying both the feed concentration (C_{feed}) and the flow rate (Q) in the single-component pulse tests at 20 °C. All the other experimental conditions than specified in each figure are the same as in Fig. 3.

Therefore, the Langmuir–Freundlich isotherm and mass-transfer parameters reported in Table 2 are sufficiently applicable to optimal design of the SMB process that employs Chirosil-SCA and water as solid and liquid phases for the separation of tryptophan enantiomers.

4.3. Effect of temperature on the Langmuir–Freundlich isotherm parameters

In the previous sections, the Langmuir–Freundlich isotherm parameters were obtained at only one temperature, 20 °C. To investigate the effect of temperature on the Langmuir–Freundlich isotherm parameters, additional pulse tests were carried out in this section while varying the temperature. The results from these pulse tests, including the results from the previous pulse tests at 20 °C, are compared in Fig. 7. Note that the retention time of each enantiomer is largely affected by temperature, indicating that the adsorption affinity of each enantiomer to the Chirosil-SCA CSP is strongly dependent on temperature. Obviously, such a marked dependence of the adsorption affinity on temperature ensures that the Chirosil-SCA CSP deserves to be a potential adsorbent of a temperature-gradient SMB process.

One of the prerequisites for the aforementioned application to a temperature-gradient SMB is to express the isotherm parameters as a continuous function of temperature. As a first step for this task, the Langmuir–Freundlich isotherm parameters at 5 °C, 10 °C, and 15 °C were determined discretely on the bases of the additional pulse test results in Fig. 7 and the proposed strategy (i.e., use of ECP-assisted IM application). In these determination tasks, only the four isotherm parameters such as b_1, b_2, β_1, and β_2 were allowed to depend on temperature. But the remaining isotherm parameter q_s, which is virtually representative of saturation capacity, was made

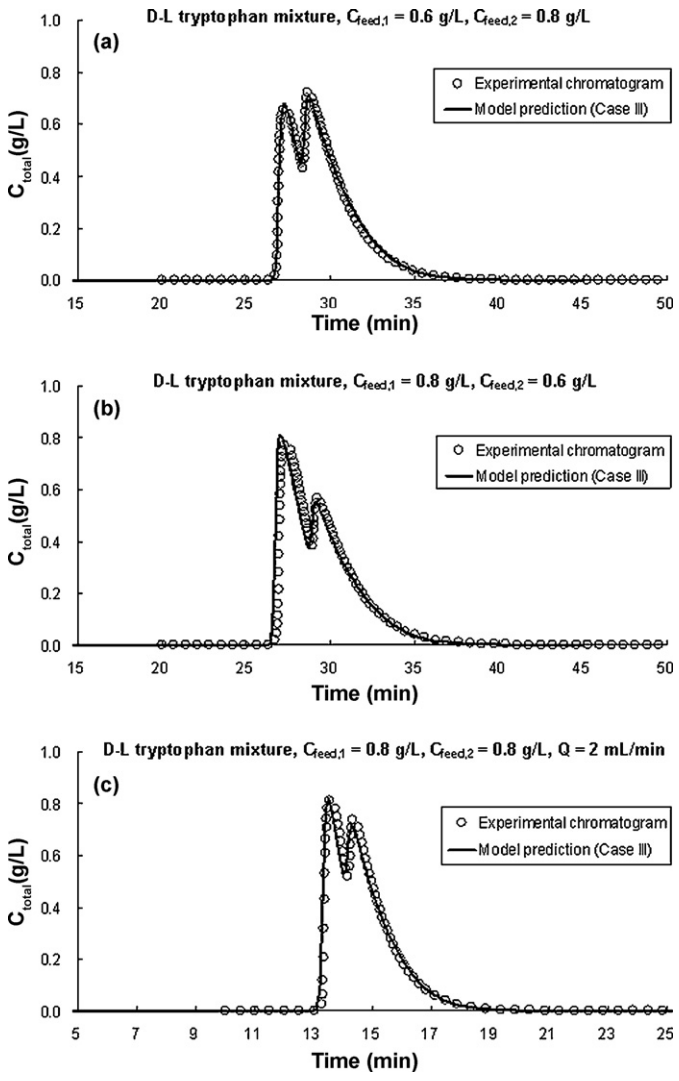

Fig. 6. Parameter validation by varying the feed concentration (C_{feed}) and/or the flow rate (Q) in the mixture pulse tests at 20 °C. All the other experimental conditions than specified in each figure are the same as in Fig. 3.

to be independent of temperature, which is a matter of common practice in the area of a temperature-gradient process development [26,27]. Thus, the values of q_s and mass-transfer parameters were kept the same as those at 20 °C during the parameter determination tasks of this section.

The resulting parameter values from the aforementioned tasks are presented in Table 4. It was confirmed that the model-prediction results based on these parameter values agreed well with all the experimental chromatograms acquired at the temperatures of 5 °C, 10 °C, and 15 °C (Fig. 7).

Table 4
Langmuir–Freundlich isotherm and mass-transfer parameters obtained from the proposed strategy (i.e., simultaneous use of IM and ECP method) at the temperatures of 5 °C, 10 °C, and 15 °C.

	T = 5 °C	T = 10 °C	T = 15 °C
q_s (g/L)	24.2000		
b_1 ((L/g)$^{\beta_1}$)	0.1270	0.1175	0.1075
β_1	0.9530	0.9573	0.9619
b_2 ((L/g)$^{\beta_2}$)	0.1476	0.1362	0.1241
β_2	0.9050	0.9149	0.9257
$k_{m,1}$ (cm/min)		0.0667	
$k_{m,2}$ (cm/min)		0.0682	

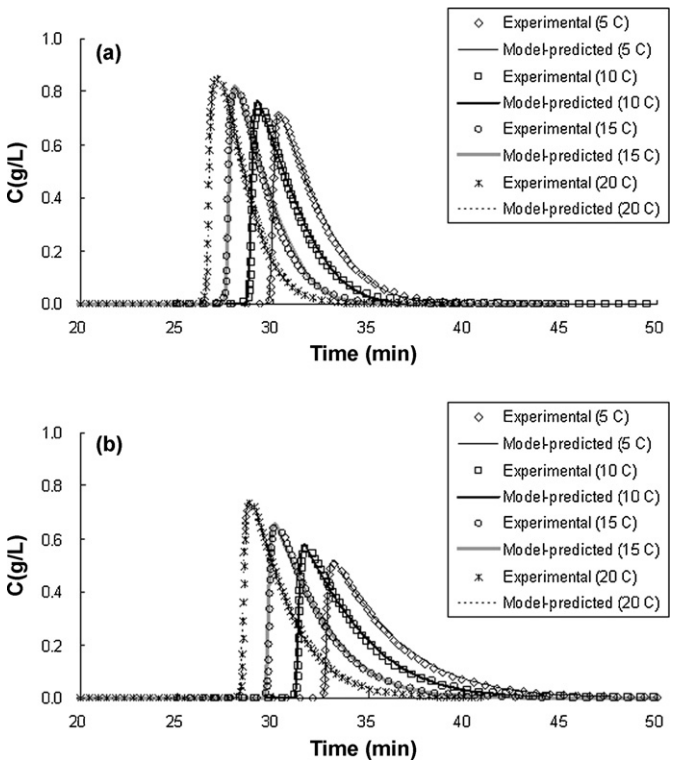

Fig. 7. Effect of temperature on the experimental and the model-predicted chromatograms resulting from the single-component pulse tests. (a) D-Tryptophan, (b) L-tryptophan. The Langmuir–Freundlich isotherm and mass-transfer parameters used in the model prediction were obtained at each temperature from the proposed strategy (i.e., use of ECP-assisted IM application). Experimental conditions (feed concentration: 1 g/L for each enantiomer, injection volume: 2 mL, flow rate: 1 mL/min).

One of the noteworthy observations in Table 4 is that the b_1 and b_2 values increase with decreasing temperature. In light of the physical meanings of the Langmuir–Freundlich isotherm parameters [23], such trend implies that the adsorption affinities of both enantiomers increase with decreasing temperature. The relative magnitude between b_1 and b_2 values also reveals that the adsorption affinity of L-tryptophan is larger than that of D-tryptophan at all the temperatures investigated. Another interesting observation is that the β_1 and β_2 values decrease with decreasing temperature. This phenomenon indicates that the heterogeneity of the adsorbent under consideration becomes more pronounced as the temperature decreases.

For the aforementioned isotherm parameters (Table 4), it will be a worthwhile task to establish a proper equation that can allow the accurate description of their temperature dependences and can thus be applied to the design of a temperature-gradient process. To facilitate this task, we employed the following form of mathematical expressions that relate b_i and β_i to temperature [28]:

$$b_i = A_i \exp\left(\frac{B_i}{T}\right) \tag{8a}$$

$$\beta_i = C_i - \frac{D_i}{T} \tag{8b}$$

where the subscript i stands for component 1 or 2; A_i, B_i, C_i, and D_i are the fitting parameters; and T is the temperature in K. The values of A_i and B_i in Eq. (8a) were determined by the linear regression of $\{\ln b_i\}$ versus $\{1/T\}$. In addition, the values of C_i and D_i in Eq. (8b) were determined by the linear regression of $\{\beta_i\}$ versus $\{1/T\}$. The resulting values of A_i, B_i, C_i, and D_i are listed in Table 5. These values and the above equations will be highly useful in developing

Table 5
The fitting parameters of Eqs. (8a) and (8b) that can allow the prediction of the Langmuir–Freundlich isotherm parameters (b_i and β_i) as a function of temperature*.

	D-Tryptophan (1)	L-Tryptophan (2)
A_i	1.0166×10^{-3}	9.1499×10^{-4}
B_i	1343.6	1414.9
C_i	1.2257	1.4959
D_i	75.935	164.390

* The correlation coefficients (R^2) of the four linear-regression tasks for determining the above parameters (A_1, B_1), (A_2, B_2), (C_1, D_1), and (C_2, D_2) were 0.9990, 0.9987, 0.9971, and 0.9994 respectively. (More detailed information was included in Supplementary data file # 4.)

a temperature-gradient SMB process for separation of tryptophan enantiomers.

5. Conclusions

The intrinsic parameters of D-tryptophan and L-tryptophan were determined for the chromatographic system where the Chirosil-SCA CSP and water were employed as solid and liquid phases respectively. For such a task, two overloaded pulse tests were carried out and the results were analyzed by both ECP and inverse method (IM).

First, the ECP method was applied to estimate the parameters of the three isotherm models considered. These parameters from the ECP method served as initial guesses in the following IM application, where the finalized isotherm and mass-transfer parameters were obtained and the best-fit isotherm model was selected. Such pattern of ECP and IM applications in series was highly effective in determining the intrinsic parameters with high accuracy and high computational efficiency.

The results from these applications showed that the adsorption behavior of both tryptophan enantiomers followed the Langmuir–Freundlich isotherm. The model predictions based on the finalized parameter values were in close agreement with the experimental chromatograms from a series of single-component or mixture pulse tests that were performed under various feed concentrations and flow rates. In addition, the temperature-dependency of the Langmuir–Freundlich isotherm parameters of each enantiomer was examined quantitatively. It was found that as the temperature decreases, the adsorption affinities of both enantiomers become higher and the heterogeneity of the Chirosil-SCA becomes more pronounced. The results of this study will contribute to development of an optimal Chirosil-SCA SMB process for enantioseparation of tryptophan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.12.102.

References

[1] I.H. Kim, News Inform. Chem. Eng. 21 (2003) 175.
[2] J.S. Park, W.S. Kim, J.M. Kim, I.H. Kim, J. Chem. Eng. Jpn. 41 (2008) 624.
[3] Y. Xie, B. Hritzko, C.Y. Chin, N.-H.L. Wang, Ind. Eng. Chem. Res. 42 (2003) 4055.
[4] T.H. Yoon, B.H. Chung, I.H. Kim, Biotechnol. Bioprocess Eng. 9 (2004) 285.
[5] K.B. Lee, C.Y. Chin, Y. Xie, G.B. Cox, N.-H.L. Wang, Ind. Eng. Chem. Res. 44 (2005) 3249.
[6] M.H. Hyun, J.S. Jin, W. Lee, J. Chromatogr. A 822 (1998) 155.
[7] M.H. Hyun, J.S. Jin, W. Lee, Bull. Korean Chem. Soc. 19 (1998) 819.
[8] M.H. Hyun, H.J. Min, Y.J. Cho, Bull. Korean Chem. Soc. 24 (2003) 911.
[9] M.H. Hyun, Y.J. Cho, J.S. Jin, J. Sep. Sci. 25 (2002) 648.
[10] M.H. Hyun, J.S. Jin, H.J. Koo, W. Lee, J. Chromatogr. A 837 (1999) 75.
[11] M.H. Hyun, S.C. Han, B.H. Lipshutz, Y.-J. Shun, C.J. Welch, J. Chromatogr. A 959 (2002) 75.
[12] H.J. Choi, Y.J. Park, M.H. Hyun, J. Chromatogr. A 1164 (2007) 235.
[13] Y.J. Cho, H.J. Choi, M.H. Hyun, J. Chromatogr. A 1191 (2008) 193.
[14] A. Lee, H.J. Choi, M.H. Hyun, Chirality 22 (2010) 693.
[15] H. Guan, B.J. Stanley, G. Guiochon, J. Chromatogr. A 659 (1994) 27.
[16] A. Felinger, D. Zhou, G. Guiochon, J. Chromatogr. A 1005 (2003) 35.
[17] A. Seidel-Morgenstern, J. Chromatogr. A 10375 (2004) 255.
[18] K. Petrusevska, M.A. Kuznetsov, K. Gedicke, V. Meshko, S.M. Staroverov, A. Seidel-Morgenstern, J. Sep. Sci. 29 (2006) 1447.
[19] J.S. Hur, P.C. Wankat, Ind. Eng. Chem. Res. 44 (2005) 1906.
[20] J.S. Hur, P.C. Wankat, Ind. Eng. Chem. Res. 45 (2006) 8713.
[21] S.F. Chung, C.Y. Wen, AIChE J. 14 (1968) 857.
[22] W. Fritz, E.V. Schluender, Chem. Eng. Sci. 29 (1974) 1279.
[23] R.J. Umpleby, S.C. Baxter, Y. Chen, R.N. Shah, K.D. Shimizu, Anal. Chem. 73 (2001) 4584.
[24] R.B. Kasat, S.K. Gupta, Comput. Chem. Eng. 27 (2003) 1785.
[25] K.B. Lee, R.B. Kasat, G.B. Cox, N.-H.L. Wang, AIChE J. 54 (2008) 2852.
[26] J.K. Kim, N. Abunasser, P.C. Wankat, Adsorption 11 (2005) 579.
[27] W. Jin, P.C. Wankat, Ind. Eng. Chem. Res. 46 (2007) 7208.
[28] K.-H. Moon, B.-K. Na, H.-K. Song, S.-S. Suh, J. Korean Inst. Chem. Eng. 33 (1995) 621.