Influence of the column radial heterogeneity on the determination of single-component isotherms by the elution by characteristic point method

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Abstract

The influence of the column radial heterogeneity on the determination of equilibrium isotherm data by the elution by characteristic point (ECP) method is studied using nondimensional numerical calculations and taking into account typical radial distributions of the mobile phase flow velocity and the column efficiency across a column. Overloaded elution peaks were calculated with the equilibrium-dispersive model as a function of four dimensionless parameters, the number of theoretical plates at the center of the column, the Langmuir equilibrium constant, the retention and the loading factors. The influence of the mass transfer resistances and the radial heterogeneity of the column on the ECP data was analyzed by comparing the true isotherm and the one estimated from the diffuse profile of overloaded peaks. The results provide information on the accuracy of the ECP method. The error made increases with increasing degree of radial heterogeneity. This error can be corrected by using the results of the nondimensional numerical calculations, allowing a further extension of the applicability of the ECP method. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

A wide variety of information on the thermodynamics and the mass transfer kinetics of phase equilibrium can be derived easily from experimental data measured on phase systems packed inside chromatographic columns [1]. Classical methods can be used to analyze the characteristics of the curves recorded at the column outlet as a response to pulse or step perturbations of any physico-chemical parameter, usually a concentration, change made at the column inlet. Appropriate theories and/or models are used in this analysis. So far, these methods are based on the implicit assumption that the packing properties of the column, for instance, the structure of the packed bed, its density, and its void fraction, are radially homogeneous.

Unfortunately, it is now well known that packed columns are rarely homogeneous enough to warrant this assumption [1,2]. Heterogeneous radial distributions of numerous physical parameters, e.g., the mobile phase flow velocity, the axial dispersion of the band along the column, the solute concentration, were demonstrated for all HPLC columns studied

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[3–16]. Experimental results arise either from investigations of the behavior of the whole bed with PFG-NMR [3–6] or from local detection carried out with optical fiber [7–9] or electrochemical detection [10–14]. In linear chromatography, the radial heterogeneity of both the mobile phase flow velocity and the column axial dispersion cause asymmetrical peaks, tailing or fronting, depending on the experimental conditions [17–19]. The asymmetry of the elution peaks is a source of more serious troubles in physico-chemical studies because most theories and equations are based on the implicit assumption of the radial homogeneity of the eluent composition and a Gaussian distribution of the concentration along the column axis. Therefore, it is necessary to clarify the influence of the column radial heterogeneity on the chromatographic determination of important physico-chemical properties.

Phase equilibrium correlations or isotherms are the most essential characteristic of an adsorption system. Isotherms are usually measured by batch and/or column methods. In chromatography, column methods are conventionally used rather than batch ones for the sake of convenience. Experimental data are acquired with conventional chromatographs, without special modifications. The following methods are most frequently used, frontal analysis (FA), the pulse, perturbation or elution on a plateau method (PT), frontal analysis by characteristic point (FACP), elution by characteristic point (ECP), and the retention time method [1,20,21]. Although the radial distributions of the flow velocity and the column efficiency cause some changes in the retention time, these perturbations are small for most columns used in practice [17–19]. Therefore, the FA and the pulse methods are not much affected by column radial heterogeneity. Unfortunately, these methods require the successive acquisition of numerous breakthrough curves or pulse response peaks in a wide range of solute concentration. The accuracy of the objective isotherm depends on their number because each of them gives only one point of the isotherm. The iterative operation is tedious and requires the waste of large amounts of sample solutions.

These disadvantages of FA and pulse methods are overcome by the FACP, ECP, and retention time methods. In FACP and ECP, each point on the diffuse boundary of the elution profile of a large sample band corresponds to one point of the objective isotherm [1,21]. Numerous points of this isotherm can be derived from the profile of one overloaded band. In the retention time method [1,20,22], the two parameters of the Langmuir isotherm for a single component are determined from the retention time of only two chromatographic peaks, an elution peak at infinite dilution and an overloaded band. The use of one of these three methods allows considerable reduction of the amount of sample wasted.

However, the profiles of the bands used in FACP and ECP are influenced by the kinetics of mass transfer between the phases in the column [1,20,23–25]. In these methods, local equilibrium is assumed everywhere in the column during the measurement process. The retention time method is also based on the assumptions that stationary and mobile phases are in equilibrium and that there is no axial dispersion in the column. However, in this method, the retention times of two peaks only are measured. Compared with the peak width, the peak asymmetric factor, or the diffuse part of the profile, the retention time of an elution peak is less affected by the column radial heterogeneity [17–19]. So, it is expected that the retention time method is less sensitive to radial heterogeneity of the column. Radial heterogeneity affects more strongly the results of FACP and ECP. Guan et al. [23] made a detailed study of the errors of accuracy and precision of ECP due to nonideal effects and indicated that the use of high efficiency columns having more than 2000, and preferably close to 5000, theoretical plates is required.

Previously, we studied the influence of the mass transfer resistances in a radially homogeneous column on the accuracy of the ECP method [26]. Single-component isotherms were estimated by ECP from the diffuse profile of the overloaded elution peaks numerically calculated using the equilibrium-dispersive model. The comparison of these calculated isotherms with the original ones provided detailed information on the error of ECP arising from the finite mass transfer kinetics in the column. We also proposed a correction procedure for the error made on equilibrium isotherms derived by ECP and demonstrated its usefulness by applying the method to a concrete case of the determination of equilibrium isotherm in an enantiomeric separation system [26].

Radial heterogeneity of conventional HPLC col-

umns is generally observed [2]. It is either impossible or extremely difficult to prepare a really homogeneous column. In previous papers [17–19], we reported that the radial distributions of the mobile phase flow velocity and the column efficiency cause a distortion of the elution peaks and a reduction of the apparent number of theoretical plates of a column. Data obtained by FACP and ECP are most probably affected by a radial heterogeneity of the column. The aim of this study was to assess the applicability of the ECP method for the determination of single-component isotherms in spite of the radial heterogeneity of the column. This work was performed using nondimensional numerical calculations of the influence of the radial heterogeneity of the flow velocity and of the axial dispersion along the column on band profiles.

2. Theory

We consider here the profiles of the overloaded elution peaks and the desorption curves obtained under nonlinear isotherm conditions and used in ECP and FACP. In order to investigate the influence of the column radial heterogeneity on the accuracy of the determination of equilibrium isotherms, numerical calculations of elution peak profiles were made on the basis of the following assumptions:

1. the equilibrium isotherm is represented by the simple Langmuir equation;
2. the column is hypothetically divided into 50 coaxial, annular columns of constant thickness, equal to $1/50$ radius of the column;
3. each of these annular columns is considered as homogeneous;
4. the distributions of the mobile phase flow velocity and the column efficiency in the radial direction of the column are represented by parabolic functions;
5. there is no radial dispersion of the studied compound in the column;
6. the overall profile of the overloaded elution peaks and the desorption curves given by an heterogeneous column will be obtained as the summation of the corresponding profiles obtained for all the annular columns, each profile being weighed in proportion to the cross-sectional area of the column.

The overloaded elution peaks and desorption curves leaving from each annular column were calculated numerically using the equilibrium-dispersive model. The mass balance equation of the equilibrium model is written:

$$u \frac{\partial C}{\partial z} + F \frac{\partial q}{\partial t} + \frac{\partial C}{\partial t} = 0 \tag{1}$$

where C and q are the mobile and stationary phase concentrations, respectively, t is the time, z the length along the column, F the phase ratio, and u the mobile phase velocity. The calculation scheme of numerical solutions of this equation can be adjusted easily to include the proper amount of numerical dispersion and obtain the profiles predicted by the equilibrium-dispersive model [1] (see calculation method later in this section). Then, the equation is rewritten in nondimensional form as follows [26]:

$$\frac{\partial C_d}{\partial Z_d} + \frac{\partial Q_d}{\partial T_d} + \frac{1}{F\beta} \frac{\partial C_d}{\partial T_d} = 0 \tag{2}$$

The dimensionless parameters used in this equation are defined as follows:

$$C_d = \frac{C}{C_0}, Q_d = \frac{q}{q_0}, Z_d = \frac{zu}{D_a}, T_d = \left(t - \frac{L}{u}\right)\frac{u^2}{D_a F\beta},$$

$$F = \frac{1-\varepsilon}{\varepsilon}, \beta = \frac{q_0}{C_0}, r_L = \frac{1}{1+KC_0}$$

$$T_{d,0} = \frac{Pe_L}{r_L k'}, T_{d,p} = \frac{Pe_L L_f}{1-r_L}, Pe_L = \frac{Lu}{D_a} = 2N,$$

$$L_f = \frac{n}{SL(1-\varepsilon)q_s}, F\beta = r_L k' \tag{3}$$

where C_d and C are the dimensionless and the actual concentration of the solute, respectively, Q_d and q the dimensionless and the actual amount adsorbed, respectively, C_0 the solute concentration of the feed solution, q_0 the amount adsorbed at equilibrium with C_0, Z_d and z the dimensionless and the actual longitudinal distance along the column, respectively, u the linear velocity of the mobile phase, D_a the apparent axial dispersion coefficient, T_d and t the dimensionless and the actual time, respectively, L the column length, F the phase ratio, ϵ the total porosity of the column, β the ratio of q_0 to C_0, r_L the dimensionless Langmuir equilibrium constant, K the coefficient of the Langmuir equation related to the adsorption energy, $T_{d,0}$ and $T_{d,p}$ the dimensionless hold-up time and the dimensionless time width of the

injected pulse (a rectangular injection profile is assumed), respectively, Pe_L the column Peclet number, k' the retention factor, L_f the loading factor, N the number of theoretical plates, n the amount of the solute injected, S the cross-sectional area of the column, and q_s the saturation capacity of the column, arising from the Langmuir isotherm used to account for the phase equilibrium.

The nondimensional Langmuir equation was used to represent the equilibrium correlation between the mobile and the stationary phase:

$$Q_d = \frac{C_d}{r_L + (1 - r_L)C_d} \tag{4}$$

The initial and boundary conditions are as follows:

$$T_d = -(T_{d,0} + T_{d,p}), \quad Z_d > 0; \quad C_d = 0, Q_d = 0 \tag{5a}$$

$$-(T_{d,0} + T_{d,p}) < T_d < -T_{d,0}, \quad Z_d = 0; \quad C_d = 1 \tag{5b}$$

$$T_d > -T_{d,0}, \quad Z_d = 0; \quad C_d = 0 \tag{5c}$$

with the parameters defined above, under Eq. (3). The numerical calculations were made by using the forward-backward finite difference scheme with the following nondimensional increments of the longitudinal distance of the column and the time [1]:

$$\partial Z_d = 2 \tag{6a}$$

$$\partial T_d = \frac{4(1 + k')}{r_L k'} \tag{6b}$$

The calculation of numerical solutions of the equilibrium-dispersive model are carried out by replacing the second-order term (axial dispersion term) in the mass balance of this model by an equivalent numerical dispersion [1]. This is achieved by selecting the space and time increments as indicated in Eqs. (6a) and (6b) above.

In the next step, the influence of the radial distributions of both the mobile phase flow velocity and the column efficiency on the peak profile must be considered. According to previous experimental studies [7–9,12,13], the radial distributions of the flow velocity and of the column efficiency are represented by the following parabolic functions:

$$u_r = a_u \left(\frac{r}{R}\right)^2 + b_u \tag{7}$$

$$N_r = a_N \left(\frac{r}{R}\right)^2 + b_N \tag{8}$$

where r is the radial distance from the center of the column, R the column radius, u_r and N_r the linear flow velocity of the mobile phase and the number of theoretical plates at a radial position r, respectively, and a_u, b_u, a_N, and b_N are numerical parameters. In previous studies [7–14], it was experimentally demonstrated that the relative difference of the flow velocity at the center (u_c) and near the wall of the column (u_w) was usually a few percent in both tailing and fronting peaks. On the other hand, the HETP was shown to be several times larger near the wall than at the center of the column in either tailing or fronting peaks. The presence of a correlation between the radial distributions of the flow velocity and the column efficiency was indicated by a comparison of the experimental data with the results of numerical calculations [18,19]. Previous studies [7–14] found the ratio u_w/u_c to be between 0.93 and 1.075. However, the average mobile phase flow velocity (u_{av}) was adjusted to be constant and equal to unity (i.e., constant flow-rate). The value of u_{av} is given by:

$$u_{av} = \int\limits_0^R 2\pi r \frac{u_r}{\pi R^2} \, dr \tag{9}$$

Combination of Eqs. (7) and (9) gives $u_{av} = (a_u/2) + b_u$. From Eq. (7), $u_c = b_u$, and $u_w = a_u + b_u$. The values of a_u and b_u were chosen so that u_{av} be equal to unity. For example, in the case when $u_w/u_c = 0.93$, $a_u = -0.0726$ and $b_u = 1.0363$. Similarly, the ratio N_w/N_c was varied from 0.2 to 1.0 because the HETP was shown to be several times larger near the wall than at the center of the column [7–14]. When $N_w/N_c = 0.20$ and $N_c = 1000$, the coefficients of Eq. (8) were taken as $a_N = -800$ and $b_N = 1000$.

An objective isotherm was estimated by analyzing the diffuse profile of the overall elution peaks by the ECP method. The accuracy of the ECP method was evaluated by comparing the coefficients of the isotherms obtained and those of the original (true) Langmuir isotherms. In the nondimensional basic equations, there are four parameters, N_c, r_L, k', and

L_f, which all affect the accuracy and the concentration range of the objective isotherm determinable by the ECP method. In this study, the influence of the column radial heterogeneity, i.e., u_w/u_c and N_w/N_c, on the error made in the determination of the objective isotherm by the ECP method was calculated, taking into account the effect of variations of these four parameters. The influence of u_w/u_c and N_w/N_c on the concentration range of the objective isotherm determinable by the ECP method was also investigated.

3. Results and discussion

We first studied the influence of the radial distributions of the mobile phase flow velocity and the column efficiency on the accuracy of single-component isotherms determined by ECP in the two cases of $u_w/u_c < 1.0$ and $u_w/u_c > 1.0$. The typical correlation between u_w/u_c and N_w/N_c was estimated from the experimental data. Then, the influence of the column radial heterogeneity on the error made on the equilibrium isotherm under various chromato-

graphic conditions was estimated from the results of numerical calculations made for different values of the four parameters. Finally, the correlation between the maximum concentration of the solute for which the equilibrium isotherm can be determined by ECP ($C_{d,max}$) and the extent of column radial heterogeneity was clarified.

3.1. Correlation between u_w/u_c and N_w/N_c

Fig. 1 (see also Table 1) shows the correlation between u_w/u_c and N_w/N_c. In linear chromatography, tailing or fronting peaks are observed for u_w/u_c smaller or larger than unity, respectively [17–19].

The solid lines in Fig. 1 represent the limited ranges of u_w/u_c and N_w/N_c, within which experimental data are available in reversed-phase liquid chromatography (RPLC) to determine such plots, which is done by comparing the recorded elution peaks with the results of numerical calculations. In previous papers [17–19], we reported that the radial distribution of the mobile phase flow velocity across
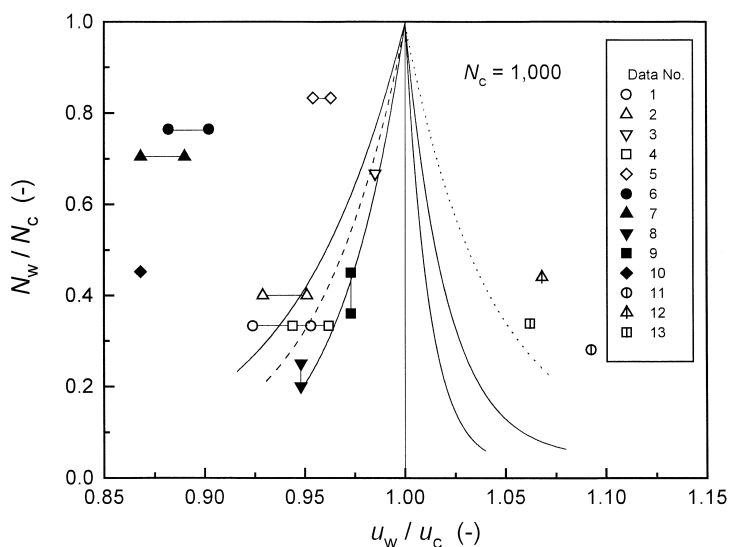

Fig. 1. Correlation of the radial distributions of the mobile phase flow velocity and the column efficiency. The column efficiency in the center region is 1000 theoretical plates. Data numbers refer to Table 1. The solid lines represent the limited region, in which some experimental data were practically observed in RPLC. The dashed line is the average correlation of the solid lines. The dotted line corresponds to the reciprocal of the plots on the dashed line. Note that the symbol (–) used in all figures means that the parameter is dimensionless.

Table 1
Experimental data on the radial heterogeneity of the mobile phase flow velocity and the local column efficiency

Column			Efficiency			Radial heterogeneity data			No.	Ref. no.
Packing materials	d_p[a] (μm)	Size (mm)	h_c[b]	h_w[c]	N_c[d]	N_w/N_c[e]	$u_w/u_c(N_c)$[f]	$u_w/u_c(1000)$[g]		
Glass beads	40	4.6×200	5.5	16.5	900	0.33	0.92–0.95	0.92–0.95	1	14
Porous silica	10	4.6×200	10	25	2000	0.4	0.95–0.965	0.93–0.95	2	14
Zorbax C_{18}	10	4.6×100	10	15	1500	0.67	0.988	0.985	3	7
Zorbax C_{18}	10	7.8×300	6	18	5000	0.33	0.975–0.983	0.94–0.96	4	9
Zorbax C_8	5	9.4×250	5.4	6.5	9300	0.83	0.985–0.988	0.95–0.96	5	9
Zorbax C_{18}	10	50×150	3.9	5.1	3900	0.77	0.94–0.95	0.88–0.90	6	8
Zorbax C_{18}	10	50×150	3.1	4.4	4800	0.71	0.94–0.95	0.87–0.89	7	8
RP18	3	3.2×100	5	20–25	6700	0.20–0.25	0.98	0.95	8	12
RP18	3	3.2×100	4.5	10–12	7400	0.36–0.45	0.99	0.97	9	12
RP18	3	3.2×40	1.9	4.2	7000	0.45	0.95	0.87	10	13
Glass beads	64	11.7×775	1.7	6.1	7100	0.28	1.03	1.09	11	10
Glass beads	76	17.3×600	1.6	3.5	5100	0.44	1.03	1.07	12	11
Glass beads	76	15.8×600	1.7	4.9	4800	0.34	1.03	1.06	13	11

[a] Particle diameter.
[b] Reduced HETP at the center of the column.
[c] Reduced HETP near the wall of the column.
[d] Number of theoretical plates at the center of the column.
[e] Ratio of the column efficiency near the wall to that at the center of the column.
[f] Ratio of the flow velocity near the wall to that at the center of the column under the conditions that the column efficiency at the center of the column is equal to N_c.
[g] Conversion ratio of the flow velocity near the wall to that at the center of the column under the conditions that the column efficiency at the center of the column is equal to 1000.

columns having different efficiencies could be correlated by the following equations:

$$\frac{1-\left(\frac{u_w}{u_c}\right)_i}{1-\left(\frac{u_w}{u_c}\right)_j}=\sqrt{\frac{N_j}{N_i}} \quad \left(\frac{u_w}{u_c}<1.0\right) \tag{10}$$

$$\frac{\left(\frac{u_w}{u_c}\right)_i-1}{\left(\frac{u_w}{u_c}\right)_j-1}=\sqrt{\frac{N_j}{N_i}} \quad \left(\frac{u_w}{u_c}>1.0\right) \tag{11}$$

where the subscripts i and j correspond to two columns having different efficiencies.

The symbols in Fig. 1 represent experimental results previously published [7–14]. Since these data were not obtained for $N_c = 1000$, a conversion of the observed values of u_w/u_c at the original N_c in each experiment to this reference plate number was made, using Eqs. (10) and (11). Most of the experimental data regarding u_w/u_c and N_w/N_c that are available are in the range $u_w/u_c < 1.0$ (tailing peaks) [7–9,12–

14]. Many are found in the region between the two solid lines. The symbols corresponding to radial distributions of the flow velocity and the column efficiency of columns providing tailing peaks (for example, slurry packed columns) are located in the region between the two solid lines. The dashed line represents the average correlation between u_w/u_c and N_w/N_c.

In contrast, few values of u_w/u_c and N_w/N_c were measured for $u_w/u_c > 1.0$ (fronting peaks) [10,11]. There is a discrepancy between the experimental data of u_w/u_c and N_w/N_c [10,11] and the solid lines estimated separately [19]. Sufficiently reliable results have not yet been obtained for the radial heterogeneity of columns providing fronting peaks (for example, dry packed columns). More experimental data should be acquired to elucidate this issue. In this work, we study the influence of the column radial heterogeneity on the accuracy of the ECP method for the determination of single-component isotherm on the basis of the correlations expressed by the dashed and dotted lines in Fig. 1. The dotted line in Fig. 1

was calculated from the dashed line by taking the reciprocal of u_w/u_c in the range smaller than unity. Although the dotted line is not included in the range between the two solid lines corresponding to $u_w/u_c > 1.0$, the dotted line was used for the numerical calculations made in this study, in order to compare the situations between the columns providing tailing and fronting peaks.

3.2. Influence of the column radial heterogeneity on the determination of single-component isotherms

Fig. 2 represents a series of three overloaded elution peaks at $L_f = 0.2$. The peak profiles are influenced by the distributions of the mobile phase flow velocity and of the column efficiency in the radial direction of the column. Tailing and fronting peaks would be observed in linear chromatography under the conditions of cases (2) and (3). However, as seen in Fig. 2, only tailing peaks are observed under Langmuir isotherm conditions, with $r_L = 0.5$ (see Eq. 4), irrespective of the type of radial distribution of the flow velocity. The figure shows that the influence of the nonlinear behavior of the isotherm at $r_L = 0.5$ on the peak distortion is larger than that of the column radial heterogeneity in both cases (2) and (3). Fig. 2 also shows that the profile of the

diffuse rear boundary of the overloaded elution peaks are in good agreement with the decay profile of the desorption curves, irrespective of the degree of column radial heterogeneity. This suggests that the isotherms estimated from the rear of all the elution peaks can be nearly overlaid, although the maximum concentration ($C_{d,max}$) determinable by ECP is affected by the peak distortion caused by the column radial heterogeneity. It is smaller than that achieved with the FACP method, i.e., $C_{d,max} = 1.0$.

The equilibrium isotherms estimated by ECP from the overloaded elution peaks are compared with the original (true) Langmuir isotherm at $r_L = 0.5$ in Fig. 3. Although the discrepancy between the isotherms estimated and the original Langmuir one is small, it increases with increasing degree of column radial heterogeneity. The discrepancy observed for a radially homogeneous column represents the influence of the finite mass transfer kinetics at $N = 1000$ on the determination of the equilibrium isotherm by ECP. The error made in the determination of the single-component isotherm by ECP is plotted versus C_d in Figs. 4 ($u_w/u_c < 1.0$) and 5 ($u_w/u_c > 1.0$). The ordinate is the ratio of the amounts adsorbed at a given concentration C_d estimated by ECP ($Q_{d,ECP}$) and at equilibrium ($Q_{d,True}$). $Q_{d,ECP}$ is an overestimate of $Q_{d,True}$ in Figs. 4 and 5 if the column is radially
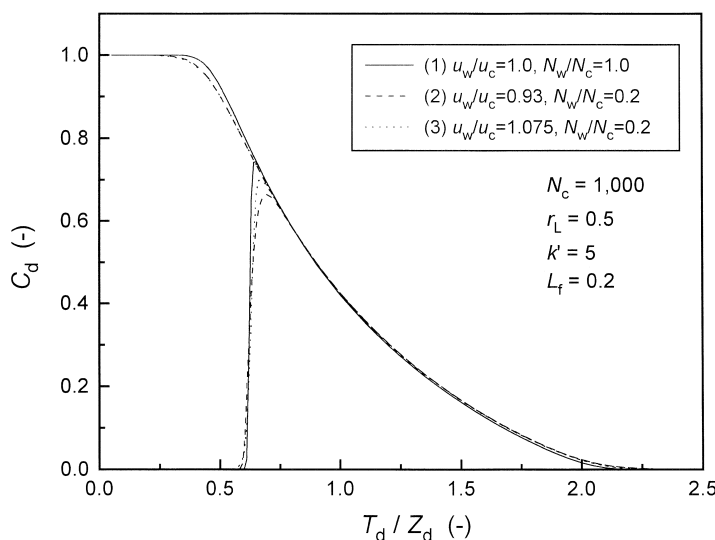

Fig. 2. Peak and desorption curve profiles calculated for three combinations of radial distributions of the mobile phase flow velocity and the column efficiency.
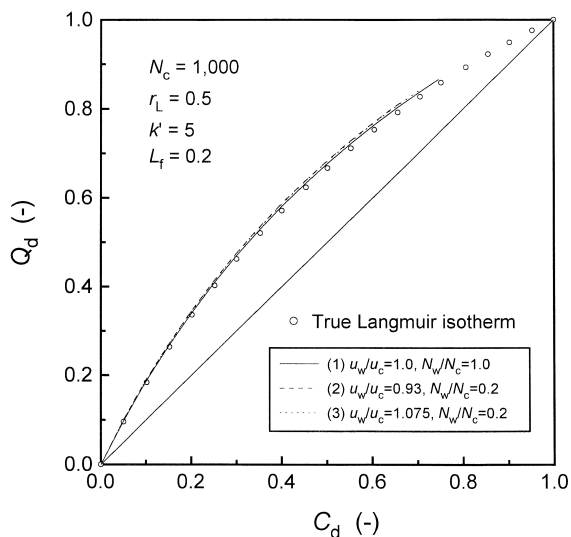
Fig. 3. Comparison of the isotherms estimated from the over-loaded peaks in Fig. 2 by the ECP method with the original (true) Langmuir isotherm.

heterogeneous. The ratio $Q_{d,ECP}/Q_{d,True}$ decreases with increasing C_d. It should reach unity at $C_d = 1.0$ [26]. The error on the single-component isotherm made by ECP increases with increasing extent of the radial heterogeneity of the column, irrespective of

whether the maximum flow velocity is at the center or near the wall of the column. The correlation between $Q_{d,ECP}/Q_{d,True}$ and C_d at $u_w/u_c = 1.0$ and $N_w/N_c = 1.0$ arises from the finite mass transfer kinetics in the column. The difference between the other correlations and that at $u_w/u_c = 1.0$ and $N_w/N_c = 1.0$ represents the influence of the column radial heterogeneity on the determination of single-component isotherms by ECP. The measurement error increases with increasing degree of column radial heterogeneity. The variation in $Q_{d,ECP}/Q_{d,True}$ is far more important at low than high concentrations. Comparing Figs. 4 and 5 suggests that the error in the estimation of single-component isotherms by ECP is larger for $u_w/u_c < 1.0$ than for of $u_w/u_c > 1.0$. The tailing behavior of elution peaks due to the nonlinearity of the Langmuir isotherm seems to be somewhat compensated by the fronting effect resulting from the column radial heterogeneity when $u_w/u_c > 1.0$.

3.3. Influence of the column radial heterogeneity at different N values

When an isotherm is determined by ECP, a high efficiency column must be used because ECP is
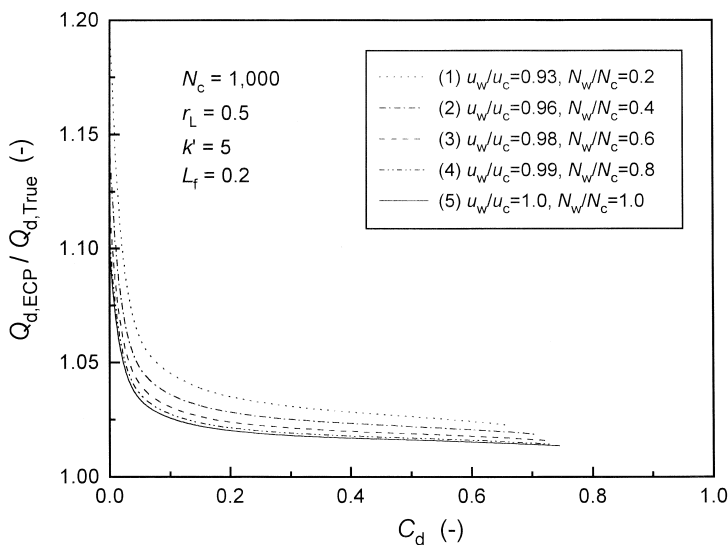

Fig. 4. Accuracy of the ECP method as a function of C_d at five combinations of radial distributions of the mobile phase flow velocity and the column efficiency, which are represented by the dashed line in Fig. 1. Tailing peaks would be observed in linear chromatography under the conditions of the column radial heterogeneity.
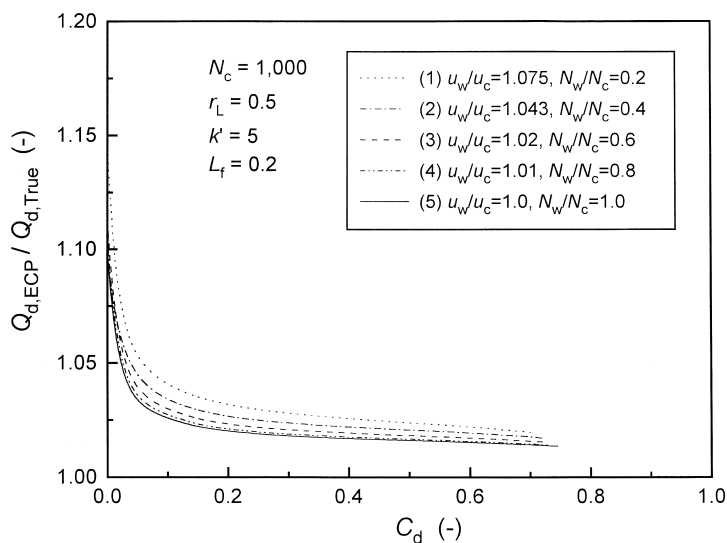
Fig. 5. Accuracy of the ECP method as a function of C_d at five combinations of radial distributions of the mobile phase flow velocity and the column efficiency, which are represented by the dotted line in Fig. 1. Fronting peaks would be observed in linear chromatography under the conditions of the column radial heterogeneity.

based on the assumption that mass transfers in the column proceed so fast that measurements are made at equilibrium. In a previous paper [23], it was shown that the column efficiency should exceed 5000 theoretical plates for the error made to become negligible, and that this error becomes important for

efficiencies below 2000 theoretical plates. Fig. 6 shows the influence of the radial distributions of the mobile phase flow velocity and the column efficiency on the isotherms obtained by ECP at two different N_c values, 1000 and 2000 theoretical plates. When N_c = 1000—2000, the values of $Q_{d,ECP}$ are larger than
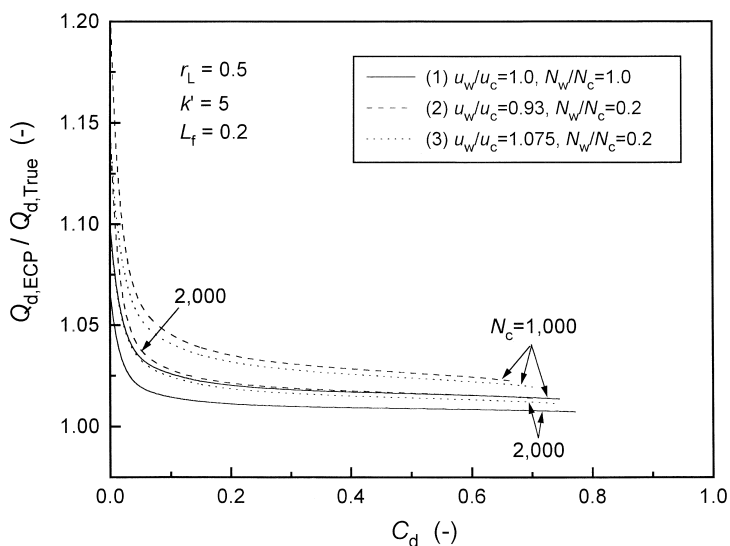
Fig. 6. Accuracy of the ECP method as a function of C_d at three combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The column efficiency in the center region is assumed to be 1000 or 2000 theoretical plates.

those of $Q_{d,True}$ by only a few percent, even in the middle of the range of C_d, in spite of the relatively low values of N_c. The influence of the radial distribution of the mobile phase flow velocity on the systematic error of measurement of single-component isotherms by ECP is larger when $u_w/u_c < 1.0$ than when $u_w/u_c > 1.0$, irrespective of N_c.

It is well known that peak distortion is accompanied by an increase in the peak variance. In previous papers [17–19], we studied the quantitative influence of peak tailing and fronting on the second central moment of asymmetric peaks. Fig. 7 shows the ratio of the apparent column efficiency (N_M) calculated from the first two moments of the peak profile to N_c at different N_w/N_c values. The dot-dashed lines in Fig. 7 give the ratio N_M/N_c for various combinations of N_w/N_c and u_w/u_c. As in Fig. 1, the solid lines represent the boundaries of the range of u_w/u_c and N_w/N_c, within which experimental data were acquired in RPLC. The dashed and dotted lines are the same as in Fig. 1. The dot-dashed lines in Fig. 7 are symmetrical, the same values of N_M/N_c are obtained for a given value of u_{max}/u_{min}, whether the maximum flow velocity (u_{max}) is in the center or at the wall of the column. This suggests

that the ratio N_M/N_c is influenced only by the ratio u_{max}/u_{min}, not by the values of u_{max} and u_{min}. Fig. 7 shows that the apparent efficiency of a column for which $u_w/u_c = 0.93$, $N_w/N_c = 0.2$, and $N_c = 1000$ is about 400. The same value of N_M is also predicted at $u_w/u_c = 1.075$, $N_w/N_c = 0.2$, and $N_c = 1000$. The apparent efficiency of this radially heterogeneous column is 40% of that of the radially homogeneous column prepared with the same packing material.

Fig. 8 shows three peak profiles calculated for (1) $u_w/u_c = 1.0$, $N_w/N_c = 1.0$, and $N_c = 400$, (2) $u_w/u_c = 0.93$, $N_w/N_c = 0.2$, and $N_c = 1000$, and (3) $u_w/u_c = 1.075$, $N_w/N_c = 0.2$, and $N_c = 1000$. The profiles of these overloaded elution peaks are nearly overlaid, even though the calculation parameters are so different. Fig. 9 compares the isotherms estimated from the rear boundaries of these peaks by ECP with the original (true) Langmuir isotherm. Nearly the same isotherms are obtained. The correlations between $Q_{d,ECP}/Q_{d,True}$ and C_d are shown in Fig. 10. Even if a homogeneous column is used, there is a measurement error of about 2% in this case (4), with $u_w/u_c = 1.0$, $N_w/N_c = 1.0$, and $N_c = 1000$ [23]. The error in case (1) is twice that in case (4), the difference arising from the lower efficiency. The column radial
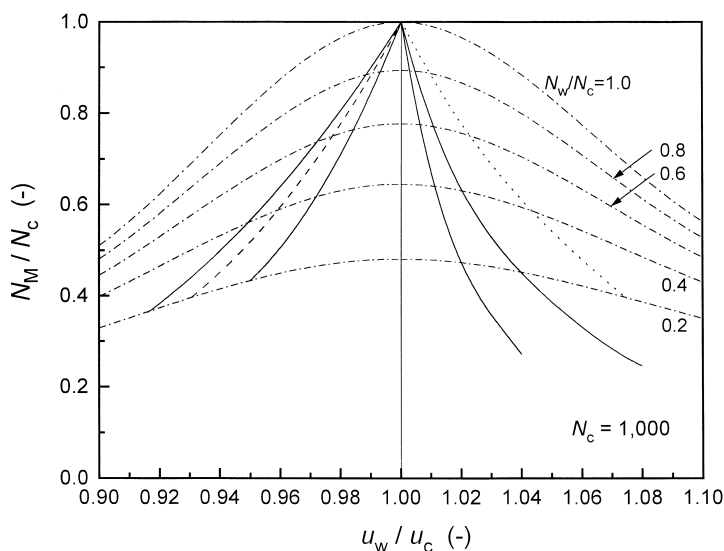

Fig. 7. Correlation of the radial distribution of the mobile phase flow velocity and the ratio of the apparent column efficiency calculated from the first and second moments of elution peak to that in the center region of a column. The solid, dashed, and dotted lines, respectively, correspond to those in Fig. 1.
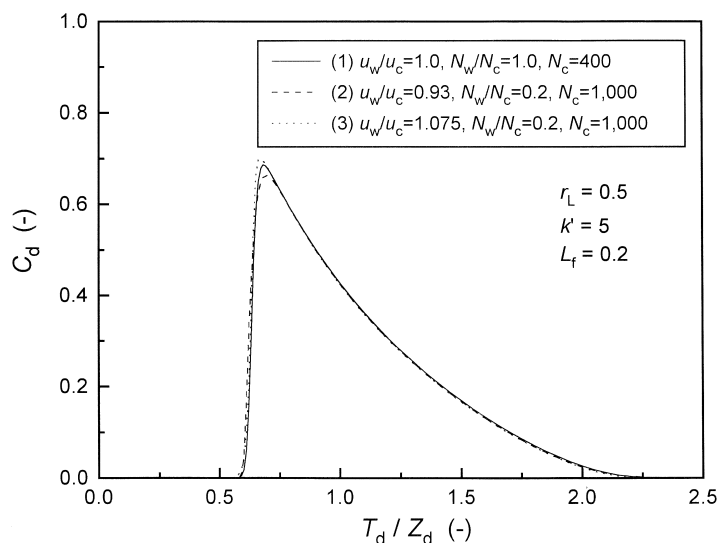
Fig. 8. Peak profiles calculated for three combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The column efficiency in the center region is assumed to be 400 or 1000 theoretical plates.
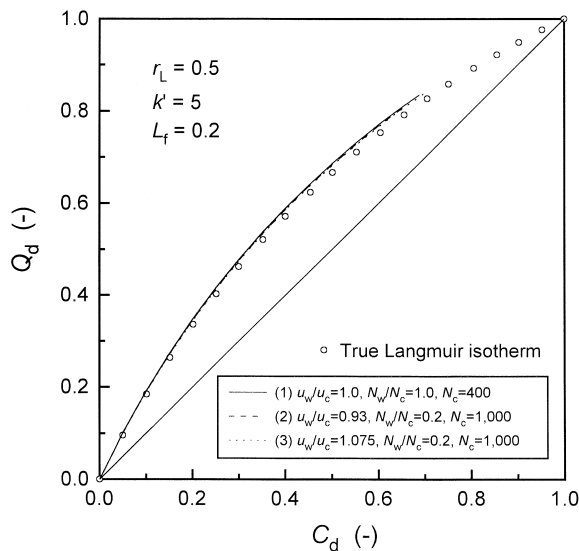

Fig. 9. Comparison of the isotherms estimated from the over-loaded peaks in Fig. 8 by the ECP method with the original (true) Langmuir isotherm.

heterogeneity increases the measurement error caused by ECP. The error in case (3) is again slightly smaller than that in case (2).

Table 2 illustrates the influence of the mass transfer kinetics and the column radial heterogeneity on the accuracy of the isotherm determination. Using a Langmuir isotherm ($q = aC/[1 + bC]$), band profiles were generated for columns having efficiencies of 500, 1000, and 2000 theoretical plates and different degrees of radial heterogeneity characterized by their values of u_w/u_c (between 0.93 and 1.075) and N_w/N_c (between 0.2 and 1.0). The rear boundaries of these peaks were used to determine an estimate of the best value of the second coefficient (b) of the Langmuir isotherm. As previously shown [17–19], the retention time, hence the first coefficient of the isotherm, can be estimated with little systematic error from a chromatogram run under linear conditions. The ratio of the estimate (b) to the true value (b_o) is derived from Eqs. (3) and (4) to be $b/b_o = r_{L,o}(1 - r_L)/[r_L(1 - r_{L,o})]$. A simple estimate of the relative error made is thus $(1 - 2r_L)/r_L$. The data in Table 2 show that the increase in the systematic error is insignificant for columns exhibiting a low degree of radial heterogeneity, but becomes significant for values of u_w/u_c larger than 1.01 or smaller than 0.99. Note the significant error already introduced by the mass transfer kinetics (at $u_w/u_c = 1.0$), in agreement with previous results [23].
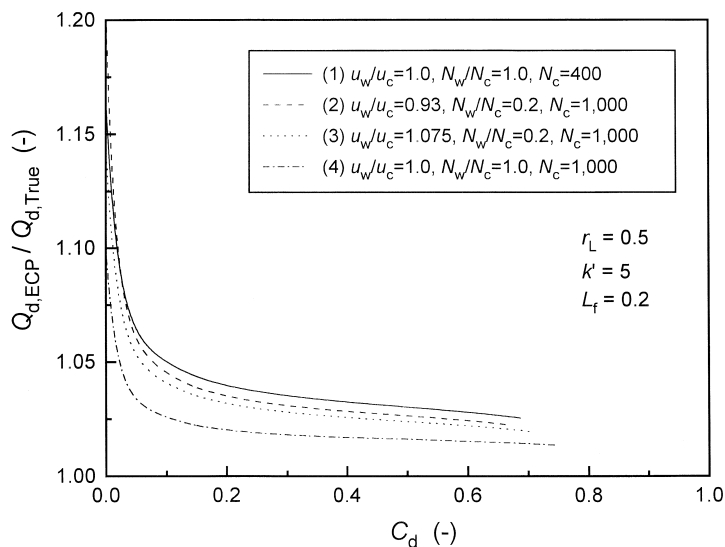
Fig. 10. Accuracy of the ECP method as a function of C_d at four combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The column efficiency in the center region is assumed to be 400 or 1000 theoretical plates.

When the equilibrium isotherm of an expensive compound is required, the smallest possible amount should be used. Then, a narrow bore column is preferable, to prevent wasting and to achieve a high value of L_f. Shortening the column is not an option as it brings about a reduction of its efficiency and an

Table 2
Errors made on the estimates of the second Langmuir coefficient

N_c	u_w/u_c	N_w/N_c	r_L	Relative error of	
				r_L (%)	K (%)
1000	0.93	0.2	0.467	6.6	14.1
1000	0.96	0.4	0.472	5.6	12.0
1000	0.98	0.6	0.476	4.9	10.2
1000	0.99	0.8	0.478	4.4	9.2
1000	1.0	1.0	0.479	4.2	8.7
1000	1.01	0.8	0.478	4.3	9.0
1000	1.02	0.6	0.476	4.7	9.9
1000	1.043	0.4	0.473	5.3	11.3
1000	1.075	0.2	0.469	6.2	13.1
2000	0.93	0.2	0.479	4.3	8.9
2000	1.0	1.0	0.488	2.4	4.9
2000	1.075	0.2	0.482	3.7	7.7
500	0.93	0.2	0.448	10.4	23.2
500	1.0	1.0	0.467	6.5	13.9
500	1.075	0.2	0.451	9.7	21.6

increase in the error of measurement. Jacobson et al. [24] claimed that no correction could be made to account for band spreading due to the mass transfer resistances in the column in either FACP or ECP and that this is the major drawback of these methods. However, the results in Figs. 4–6 and 10 provide valuable information on the influence of the mass transfer resistance and the radial heterogeneity of the column on the error made on equilibrium isotherms measured by ECP. This error can be corrected by using the results provided by the nondimensional calculations reported in this study. It is reasonable to expect that a correction strategy based on these calculations could extend the usefulness of the ECP method.

3.4. Influence of the column radial heterogeneity at different L_f values

Fig. 11 illustrates the influence of L_f on the correlation between $Q_{d,ECP}/Q_{d,True}$ and C_d. In each case (1) to (3), the correlations corresponding to different L_f values can be overlaid. The arrows in Fig. 11 indicate $C_{d,max}$ corresponding to each value of L_f. The whole arc $0 < C_d < 1$ of the objective
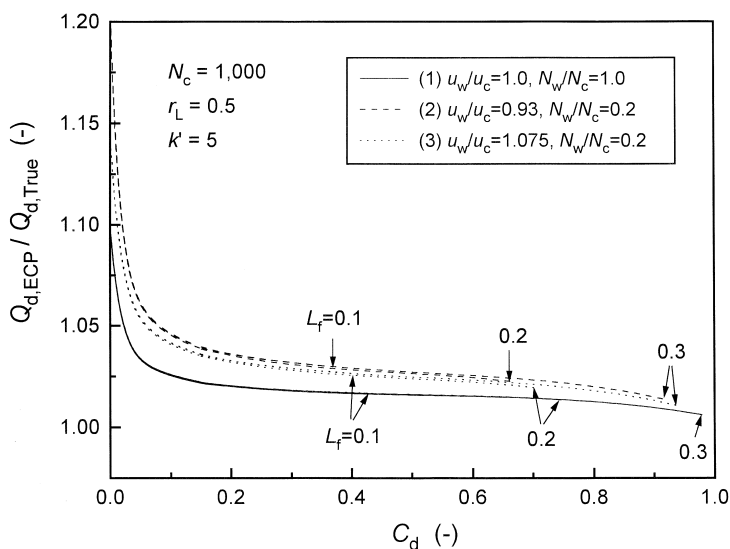
Fig. 11. Accuracy of the ECP method as a function of C_d at three combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The loading factor is taken as 0.1, 0.2, and 0.3. The arrows indicate $C_{d,max}$ at each value of L_f.

isotherm cannot be estimated at $L_f < 0.3$ under the conditions of Fig. 11. In conclusion, L_f influences little the accuracy of single-component isotherms determined by ECP. Only the concentration range within which the objective isotherm is derived changes with L_f.

3.5. Influence of the column radial heterogeneity at different r_L values

Similar to Fig. 11, Fig. 12 shows the influence of the column radial heterogeneity on the accuracy of single-component isotherms measured by ECP at different r_L values. In all three cases, the error at low concentrations is smaller for small than for large values of r_L because the lower r_L, the stronger the influence of isotherm nonlinear behavior on the peak profile. As shown in the figure, if L_f is kept constant at 0.2, the objective isotherm can be determined accurately in a narrow concentration range if r_L is small. The degree of tailing of the overloaded elution peaks increases decreasing r_L. The broadening and tailing of the elution peaks is accompanied with a reduction of its apical height, i.e., $C_{d,max}$. The error on the objective isotherm is smaller in case (3) than in case (2), irrespective of the value of r_L. Although

the difference between the curves in Fig. 12 is important in the low concentration range, it decreases with increasing C_d.

3.6. Influence of the column radial heterogeneity at different k' values

The error on single-component isotherms measured by ECP at different k' values is plotted in Fig. 13 as a function of C_d. The discrepancy between the ECP-measured isotherms and the true Langmuir one decreases with increasing k' in all three cases in Fig. 13. Nearly the same correlations are observed between $Q_{d,ECP}/Q_{d,True}$ and C_d for k' larger than about 5. This suggests that, like observed in Figs. 4–6 and 10–12, the objective isotherm can be determined more accurately in case (3) than in case (2).

3.7. Influence of the column radial heterogeneity on $C_{d,max}$

Fig. 14 illustrates the correlation between $C_{d,max}$ and L_f for $N_c = 1000$ and $k' = 5$ and the influence of the column radial heterogeneity on this correlation. The value of $C_{d,max}$ depends on the experimental conditions. Among them, r_L has a strong influence
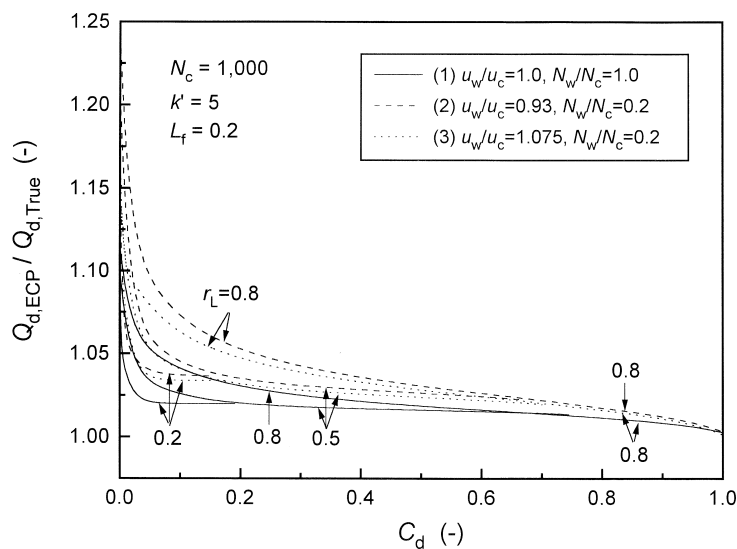
Fig. 12. Accuracy of the ECP method as a function of C_d at three combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The dimensionless Langmuir equilibrium constant is taken as 0.2, 0.5, and 0.8.

on $C_{d,max}$, as shown in Fig. 14. $C_{d,max}$ increases faster with increasing L_f at high than at low values of r_L. When $r_L = 0.8$, $C_{d,max}$ is almost equal to unity at $L_f = 0.1$, even though the column radial heterogeneity is assumed to be relatively large in this study. $C_{d,max}$ increases more slowly with increasing L_f at $r_L = 0.2$ than at $r_L = 0.8$. The values of $C_{d,max}$ at $r_L = 0.2$ are close to 0.5 at $L_f = 0.5$, which corresponds to ex-
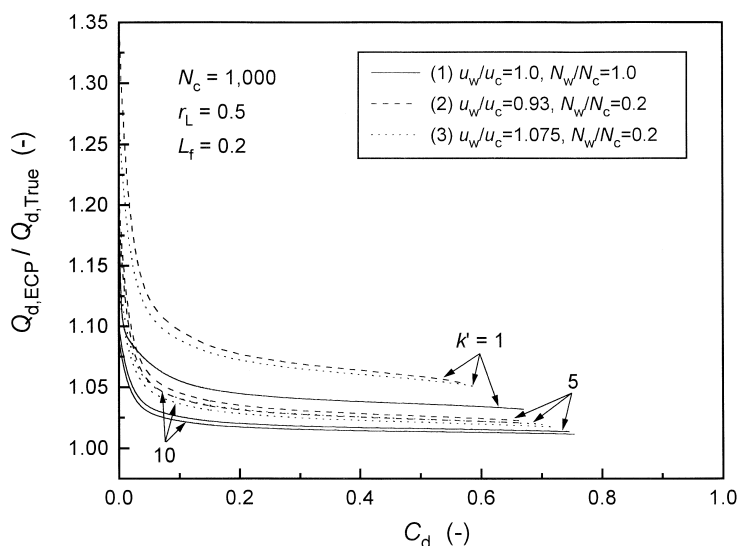

Fig. 13. Accuracy of the ECP method as a function of C_d at three combinations of radial distributions of the mobile phase flow velocity and the column efficiency. The retention factor is taken as 1, 5, and 10.
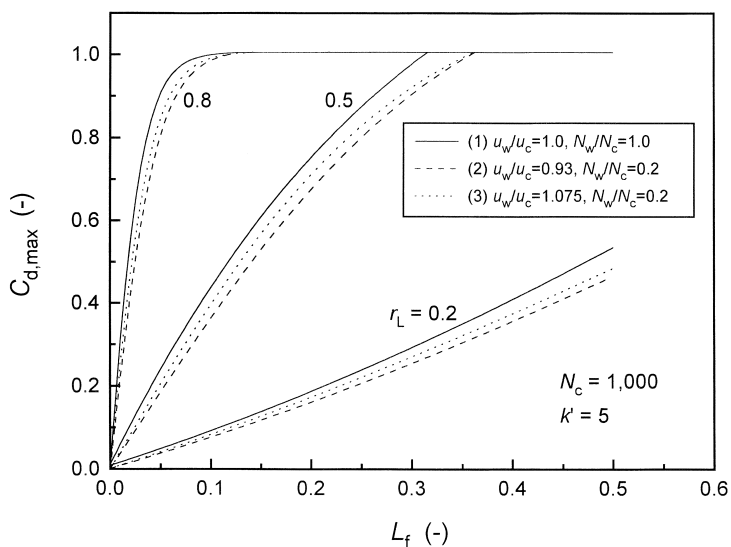
Fig. 14. Correlation of $C_{d,max}$ with L_f at three combinations of radial distributions of the mobile phase flow velocity and the column efficiency.

tremely overloaded conditions in preparative chromatography.

As shown in Figs. 6 and 10, a variation of N_c does not affect significantly the apical height of overloaded elution peaks ($C_{d,max}$). When there is no radial distribution of the mobile phase flow velocity and of the column efficiency, the difference in peak height between $N_c = 1000$ and 2000 is about 0.05, the same as between $N_c = 1000$ and 400.

Almost the same observation is made when the apparent value of N decreases due to an increasing column heterogeneity. The influence of a change in k' on $C_{d,max}$ is also small, as shown in Fig. 13. Nearly the same value of $C_{d,max}$ is observed, irrespective of k' for k' larger than about 5. The influence of the flow velocity and column efficiency radial distributions on $C_{d,max}$ are not large either. In Fig. 12, the value of $C_{d,max}$ at constant L_f is larger in case (3) than in case (2), irrespective of the value of r_L. However, the difference between these two values of $C_{d,max}$ is small. Thus, it seems that the results in Fig. 14 can be used as a general reference for the prediction of $C_{d,max}$ in the ECP method, under ordinary chromatographic conditions. Finally, the necessary amount of sample to be injected in order to be able to measure the isotherm within the

required concentration range can be predicted depending on the experimental conditions. This is useful to prevent unnecessary waste of chemical compounds and time.

3.8. Correction procedure

With a high efficiency column (e.g., $N > 2000$), the objective isotherm can be determined by ECP with a reasonable accuracy. However, even in this case, the radial heterogeneity of the column has a detrimental influence on the accuracy of the isotherm. If a column with a moderate or low efficiency (1000 plates or fewer) must be used, a correction is required to reduce the errors introduced by ECP due to the finite mass transfer kinetics and the column radial heterogeneity. First, the influence of the column radial heterogeneity on the isotherm must be eliminated, using the results of the numerical calculation made taking the radial distributions of the mobile phase flow velocity and the local column efficiency into account. These distributions can be estimated by analyzing the profile of elution peaks recorded under linear conditions, using the ratio of peak widths at different peak heights and the peak asymmetry factor [18,19].

Then, the influence of the mass transfer resistance on the error made by ECP should be corrected. First, an apparent isotherm is estimated, following the conventional procedure, from the rear diffuse profile of an overloaded elution peak. The two parameters, r_L and L_f, are calculated from this apparent isotherm. The other two parameters, N_c and k', are derived from a chromatogram acquired under linear conditions. The correction of the apparent isotherm is obtained as the result of a numerical calculation using the four parameters, r_L, L_f, N_c, and k', and taking into account the radial distributions of the mobile phase flow velocity and the local column efficiency. As shown in Figs. 3 and 9, the isotherm thus obtained differs usually little from the objective isotherm. However, the error in the estimation of the objective isotherm in this manner increases with decreasing column efficiency. A more detailed study would be necessary to derive the correlation between the correction error and the column efficiency.

4. Conclusion

The numerical study of the influence of the column radial heterogeneity on the accuracy of single-component isotherms determined by ECP shows the importance of the radial distributions of the mobile phase flow velocity and of the column efficiency in these measurements. This influence combines with the better known effect of the mass transfer kinetics. The error made increases with increasing degree of column radial heterogeneity, whether $u_w/u_c < 1.0$ or $u_w/u_c > 1.0$. It is larger for $u_w/u_c < 1.0$ than for $u_w/u_c > 1.0$, irrespective of the values of the four parameters that completely characterize the experimental conditions (efficiency along the column axis, retention factor at infinite dilution, curvature of the isotherm, and loading factor). In linear chromatography, elution peaks show fronting and tailing profiles for $u_w/u_c > 1.0$ and $u_w/u_c < 1.0$, respectively. For a Langmuir isotherm, overloaded peaks exhibit tailing even in a homogeneous column. If $u_w/u_c > 1.0$, the fronting effect due to the column radial heterogeneity and the tailing effect due to the nonlinear behavior of the isotherm compensate to a degree. If $u_w/u_c < 1.0$, the two effects add up, resulting in a larger error.

The correlation between $Q_{d,ECP}/Q_{d,True}$ and C_d gives valuable information on the influence of the mass transfer resistances and the radial heterogeneity of the column on the error made on the equilibrium isotherm determined by ECP. Numerical calculations using the nondimensional equations of nonlinear chromatography provide the value of $Q_{d,ECP}/Q_{d,True}$ as a function of C_d under given experimental conditions. Knowing this correlation allows the derivation of proper corrections for the influence of the mass transfer resistances and the column radial heterogeneity, hence a more accurate determination of the objective isotherm by ECP when using relatively low efficiency columns. The numerical calculations allows also the prediction of the concentration range of the arc of isotherm which can be derived from an ECP experiment. The results of the nondimensional calculations permit a preliminary optimization of the experimental conditions. The results of this work extend the validity of the ECP method for the accurate determination of equilibrium isotherms.

5. Nomenclature

a_N	coefficient in Eq. 8
a_u	coefficient in Eq. 7
b_N	coefficient in Eq. 8
b_u	coefficient in Eq. 7
C	actual concentration
C_d	dimensionless concentration
C_0	actual concentration of a feed solution
D_a	apparent axial dispersion coefficient
F	phase ratio ($=(1-e)/e$)
k'	retention factor
K	Langmuir parameter
L	column length
L_f	loading factor
n	amount of a solute injected
N	number of theoretical plates
N_M	number of theoretical plates calculated from first and second moments
Pe_L	column Peclet number
q	actual amount adsorbed
q_s	saturation capacity
q_0	actual amount adsorbed in equilibrium with C_0

Q_d	dimensionless amount adsorbed
$Q_{d,ECP}$	dimensionless amount adsorbed estimated by the ECP method
$Q_{d,True}$	dimensionless amount adsorbed in equilibrium with C_d
r	Radial distance from the center of a column
r_L	dimensionless Langmuir equilibrium constant
R	column radius
S	column cross-sectional area
t	actual time
T_d	dimensionless time
$T_{d,p}$	dimensionless time width of an injection pulse
$T_{d,0}$	dimensionless hold-up time
u	linear velocity of a mobile phase
z	actual longitudinal distance
Z_d	dimensionless longitudinal distance

Greeks

β	ratio of q_0 to C_0
ϵ	total porosity of a column

Subscripts

av	average
c	at the center of a column
i	ith column
j	jth column
max	maximum
min	minimum
r	at a radial position r
w	Near the wall of a column

Acknowledgements

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