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Mathematical model of the dynamics of countercurrent chromatography*

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Abstract: A mathematical model of the dynamic behavior of countercurrent chromatography was proposed, and the model parameters, including the partition coefficient, the axial dispersion coefficient, the intraparticle diffusion coefficient and the external mass ransfer coefficient were calculated by the method of chromatogram moment analysis. Comparison of the experimental chromatograms of caffeine and theophylline determined in this work with the simulated curves computed by the proposed model showed fairly good agreement. Further, the difference between the average identified the partition coefficients by chromatogram moment analysis and the experimental values was small also, and the relationship between the external mass transfer rate and the linear velocity was similar to that obtained with solid-liquid chromatography.

Key words: Countercurrent chromatography, Model simulation, Caffeine, Theophylline
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INTRODUCTION

High speed countercurrent chromatography (HSCCC) uses the special centrifugal force resulting from the planetary motion of a coiled column (Mandava et al., 1988). Countercurrent chromatography's use of many solvent systems yielded sufficient retention of the stationary phase. Rare earth metals, natural botanic compounds, proteins, etc. had been separated successfully (Pukhovskaya et al., 1993; Abe et al., 1994; Lee, 1994). Although countercurrent chromatography has been widely applied as an analysis tool and a preparative method, the theoretical counterpart, particularly the dynamic behavior of the chromatographic process in the column, is not well studied yet. The main purpose here is to do some exploratory study on this aspect. A preliminary mathematical model was proposed to describe the dynamics of countercurrent chromatography. Then the model was tested by experimental chromatograms of caffeine and theophylline determined in this work.

EXPERIMENTAL DETAILS

HSCCC apparatus and materials

The HSCCC apparatus used in the present work was constructed in this laboratory. A pair of column holders were mounted symmetrically on the rotary frame at a distance of 10 cm from the central axis of revolution. The multilayer coiled column was prepared by winding a 90 m × 2 mm I.D. polyethylene tube onto the holder hub. The capacity was 270 ml. The revolution speed could be regulated up to 1500 r/min.

Schematic drawing of the experimental set-up is shown in Fig.1. More information on the HSCCC apparatus can be found in Yoichiro Ito (Mandava et al., 1988). Caffeine and theophylline were purchased from SIGMA. Chloroform (analytical grade) was purchased from Zhejiang Chemical Reagent Factory, Hangzhou, China. Water was dem-

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ineralized in the laboratory.

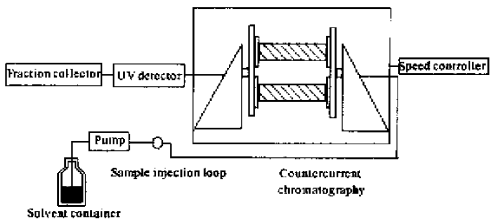

Fig. 1 Schematic drawing of the experimental set-up

Operations

1. Solvent system and sample preparation

The solvent system prepared by mixing equal volume of chloroform and water at room temperature was shaken vigorously, then left overnight to reach equilibrium. The two phases were separated shortly before use.

Sample solutions were prepared by dissolving caffeine and theophylline in a 5 mg/ml mixture of equal volume of chloroform and water.

2. Operation of the HSCCC apparatus

The column was first filled with the upper phase composed mainly of water. The lower phase composed mainly of chloroform was then pumped into the head end of the column as mobile phase while the apparatus was running at a certain revolution speed. After hydrodynamic equilibrium was reached as indicated by a clear mobile phase at the tail end, the sample solution (0.7 ml) was injected through the sample valve. The effluent from the tail end of the column was monitored with a UV detector at 254 nm and collected by a fraction collector. At the end of the experiment, all the liquid in the column was purged out by nitrogen and collected. The volumes of the two phases were measured, and the volume of the stationary phase relative to the total column capacity was calculated.

3. Partition coefficients determination

Caffeine and theophylline were added into an equilibrated two-phase solvent system (equal volume of each phase); was then stirred vigorously; and then left overnight to reach equilibrium again. The phases were separated and analyzed by UV spectrophotometer at 254 nm to determine each phrase's concentration. Then the partition coefficient was calculated.

FUNDAMENTAL THEORY

Differential equations of the model

The theory of conventional liquid-solid chromatography had been extensively studied by many researchers. Countercurrent chromatography is essentially a liquid-liquid chromatography process. In other words, countercurrent chromatography differs mainly from conventional chromatography in that the stationary phase is liquid instead of solid. An attempt was made here to use the model of liquid-solid chromatography to describe the dynamics of countercurrent chromatography. The following assumptions were made to establish the model.

1. The partition coefficients of caffeine and theophylline are constant in the range of the concentrations studied.

2. Constant temperature.

3. The hydrodynamic state is steady and uniform throughout the column.

4. The effect of the injected sample on the retention of the stationary phase is negligible.

5. Although there should be high polydispersity of the droplets in HSCCC, as approximation, spherical droplets with average diameter based on the mass transfer area could be used instead of polydispersed droplets distribution.

6. The mass transfer in the droplet obeys Fick's law and the rate of external mass transfer on the surface of the droplet is defined by the linear driving force equation.

Based on the above assumptions the mathematical model can be written as follows:

The equation of mass conservation of mobile phase:

$$E_a\left(\frac{\partial^2 C_m}{\partial Z^2}\right)_t - V\left(\frac{\partial C_m}{\partial Z}\right)_t - \left(\frac{\partial C_m}{\partial t}\right)_z - \frac{N_t a}{\alpha} = 0 \quad (1)$$

C_m, concentration of solute in mobile phase (μg/ml);

Z, axial dimension of the column (m);

t, time(min);

E_a, axial diffusion of solute (cm^2/sec);

V, velocity of continuous phase (m/min);
a, area of mass transfer in unit volume(m^2/m^3);

$$\alpha = \frac{1 - S_f}{S_f};$$

S_f, retention of stationary phase (%);

That of the stationary phase:

$$\left(\frac{\partial^2 C_p}{\partial r^2}\right)_t + \frac{2}{r}\left(\frac{\partial C_p}{\partial r}\right)_t - \frac{1}{D_c}\left(\frac{\partial C_p}{\partial t}\right)_r = 0 \quad (2)$$

C_p, concentration of solute in stationary phase ($\mu g/ml$);
r, radium dimension of droplet (m);
D_c, intraparticle diffusion of solute (cm^2/sec);

The equation of phase equilibrium:

$$C_{p,R} = K_d C_{m,R} \quad (3)$$

R, radium of droplet (m);
K_d, equilibrium constant;

Mass transfer on the surface of the stationary phase:

$$N_t = k_f(C_m - C_{p.R}/K_d) \quad (4)$$

k_f, mass transfer coefficient (m/min);

Boundary conditions on the surface and at the center of the droplet:

$$D_c\left(\frac{\partial C_p}{\partial r}\right)_{r=R} = k_f\left(C_m - \frac{C_{p.R}}{K_d}\right)$$
$$\left(\frac{\partial C_p}{\partial r}\right)_{r=0} = 0 \quad (5)$$

Boundary condition of pulse injection at $Z = 0$ of concentration C_0:

$$C_m = C_0(U(t) - U(t-\tau)), \text{at } Z = 0 \quad (6)$$

C_0, concentration of solute in sample (mg/ml);
$U(t)$, unit pulse function;
τ, time of injection(min);

Initial conditions:

$$C_m = 0, \text{at } Z \geqslant 0,\ t = 0$$
$$C_p = 0, \text{at } r \geqslant 0,\ t = 0 \quad (7)$$

Laplace transformation of Eqs.(1) and (2) yields the resulting ordinary differential equation which can be solved by using the transformed boundary conditions. Since it is very difficult to derive the inverse transformation, only two extreme cases, namely the case of internal mass transfer control and the case of external mass transfer control, were studied separately.

In the case of internal diffusion control (Model 1) in which the Biot number is much larger than 1 ($B_i = k_f R/D_c$) and the axial dispersion is negligible, the solution (Houghton, 1964; Van Deemter et al., 1956) is:

$$\frac{C_m}{C_0} = 0.5\left[\operatorname{erf}\left(\frac{Y\theta - X}{2\sqrt{X/5}}\right) - \operatorname{erf}\left(\frac{Y(\theta-\tau) - X}{2\sqrt{X/5}}\right)\right]$$

where:
$$Y = 3D_c/R^2 \quad (8)$$
$$X = Y\frac{K_d Z}{\alpha V}$$
$$\theta = t - Z/V$$

In the case of external mass transfer control (Model 2) in which Bi is much less than 1 and the axial dispersion is negligible, the solution (Van Deemter et al., 1956; Lapidus et al., 1952) is:

$$\frac{C_m}{C_0} = X_0\left(\frac{N_p}{2\pi M\theta^3}\right)^{1/2}\exp\left[-\frac{N_p M}{2\theta}\left(\theta - \frac{1}{M}\right)^2\right]$$

where:
$$X_0 = \frac{\tau}{Z/V}$$
$$\theta = \frac{t}{Z/V}$$
$$M = \frac{1}{1 + K_d/\alpha} \quad (9)$$
$$N_p = \left[\frac{2(E_a/V)}{Z} + \frac{2M(1-M)}{(Z/V)K_f}\right]^{-1}$$
$$K_f = \frac{3k_f}{RK_d}$$

Identification of the model parameters

Generally, the partition coefficient (K_d), the axial dispersion coefficient (E_a), the intraparticle diffusion coefficient (D_c) and the mass transfer coefficient (k_f) are supposed to affect the effluent concentration profile and the resolution. Analysis of the relationships between the four parameters and the operation conditions are required for the prediction and optimization of an HSCCC separation.

The method of chromatogram moment analysis was used to obtain the model parame-

ters, whose relationship with the chromatogram moments are (Kucera, 1965; Schneider et al., 1968a; 1968b):

$$\mu_1' = \frac{Z}{V}(1+\delta_0) + \frac{\tau}{2} \quad (10a)$$

$$\mu_2 = \frac{2Z}{V}\left[\delta_1 + E_a\frac{(1+\delta_0)^2}{V^2}\right] + \frac{\tau^2}{12} \quad (10b)$$

$$\mu_3 = \frac{3Z}{V}\left[\delta_2 + \frac{4E_a\delta_1(1+\delta_0)}{V^2} + \frac{4E_a^2(1+\delta_0)^3}{V^4}\right] \quad (10c)$$

The nth absolute moment,

$\mu_n' = m_n/m_0$ where $m_n = \int_0^\infty t^n C_m(z,t)\mathrm{d}t$ $(n = 0, 1, 2, \cdots)$;

The nth central moment,

$$\mu_n = \frac{\int_0^\infty (t-\mu_1)^n C_m(z,t)\mathrm{d}t}{m_0};$$

where

$$\delta_0 = K_d\alpha$$

$$\delta_1 = \alpha\frac{R^2}{15}\left(\frac{K_d}{D_c} + \frac{5K_d^2}{Rk_f}\right)$$

$$\delta_2 = \alpha\frac{K_dR^4}{D_c^2}\left(-\frac{4}{315} + \frac{4}{45}x + \frac{2}{9}x^2\right)$$

$$x = \frac{D_cK_d}{Rk_f}$$

K_d is irrelevant to D_c, E_a, k_f, and R, and can be directly identified from the first-order moment. In the case of HSCCC, the axial dispersion terms with Ea can be ignored because the ratio of column length to drop diameter (L/d_p, where L is the length of column, d_p is the diameter of droplet) is very large (Carberry, 1958; Carberry, 1976), in our case, larger than 44570 (L/d_t, where d_t is the diameter of the column). Since R could not be determined, D_c and R were combined into a parameter D_c/R^2, and k_f and R were combined into another parameter k_f/R, which were calculated from the second-order and third-order moments.

RESULTS AND DISCUSSION

A series of experiments were performed. Among them, four were run at the same revolution speed of the column ω(308 r/min), but with different flowrates of the mobile phase (F); four were run at the same F(10 ml/min), but with different ω. The experimental and computed results are listed in Table 1.

Table 1 The model parameters computed from experimental chromatograms

F (ml/min)	ω (r/min)	S_f (%)	$V\times10^{-2}$ (m/sec)	Caffeine	Theophylline			
				K_d	K_d	D_c/R^2	k_f/R	$B_i = \frac{k_f/R}{D_c/R^2}$
4.0	308	73.21	7.922	0.04831	3.546	0.2072	22.90	110.5
6.4	308	62.50	9.055	0.05243	3.652	0.2565	21.31	83.06
8.4	308	48.21	8.605	0.05060	3.681	0.2908	24.18	83.13
10.0	308	19.64	6.602	0.05101	3.514	0.2933	15.96	54.41
10.0	339	53.57	11.427	0.04612	3.744	0.3692	29.81	80.71
10.0	533	73.57	20.067	0.04524	3.719	0.3322	53.42	160.8
10.0	292	7.143	5.713	0.04895	3.560	0.3509	13.99	39.87

Comparison between model simulation and experimental chromatogram

Biot number is defined as:

$$B_i = \frac{\text{the internal mass transfer resistance}}{\text{the external mass transfer resistance}} = \frac{k_f/R}{D_c/R^2} = \frac{k_fR}{D_c}$$

The Biot number magnitude determines which of the two mass transfer resistances has more effect on the effluent concentration profile and the resolution. Biot number in our ex-

periments were calculated and listed in Table 1. Under the conditions studied in the work, Biot number was very large (from 40 to 110), so the external mass transfer resistance was small compared to the internal mass transfer resistance and can be ignored. Therefore the internal mass transfer control model (Model 1) was used to simulate the effluent concentration profiles. By using the parameters obtained by moment analysis, the chromatogram of theophylline was simulated by Eq.(8). Two examples of simulated curves together with experimental curves are shown in Figs.2 and 3. The results showed that the model of internal diffusion control fits the experimental results quite well.

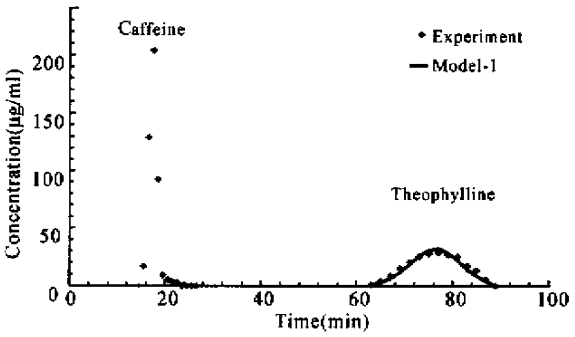

Fig.2 The chromatographic curve of caffeine and theophylline

Revolution speed ω = 308 r/min; Flow rate F = 8.4 ml/min; Pulse size 0.7 ml × 5.0 mg/ml

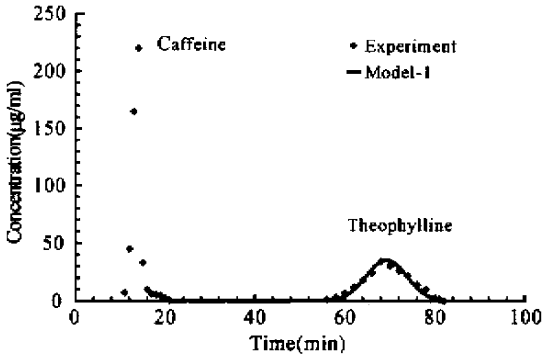

Fig.3 The chromatographic curve of caffeine and theophylline

Revolution speed ω = 339 r/min; Flow rate F = 10 ml/min; Pulse size 0.7 ml × 5.0 mg/ml

For caffeine, the retention volume was so small that caffeine' peak broadening was mainly due to off-column mixing but not mass transfer resistence. It is not meaningful to deduce D_c/R^2 and k_f/R and to fit internal or external mass transfer control model to the experimental chromatogram.

Partition coefficients determined by HSCCC vs. partition coefficients determined by shake flask

The variation of the partition coefficients (K_d) identified by the model for different conditions is quite small, within 14.69% of the average for caffeine and 6.33% of the average for theophylline. Further, the difference between the average identified value and the experimental value was small also; for caffeine, it was 0.0490 compared to 0.0507, and for theophylline, it was 3.628 compared to 3.524.

The internal mass transfer rate

The internal mass transfer rate D_c/R^2 calculated from moments are listed in Table 1. Although the internal diffusion coefficient D_c, unlike the molecular counterpart, is of a complex physical nature in aspects such as the internal circulation of the droplets, the internal diffusion rate D_c/R^2 might not vary with the flowrate of mobile phase F and revolution speed ω. In our case, the D_c/R^2 value varied from 0.2072 to 0.2933 while F changed from 4 to 10 ml/min at constant ω, and from 0.2933 to 0.3692 while ω changed from 292 to 533 r/min at constant F. It turned out that the D_c/R^2 value did not vary with F or ω; the variation was in the range of the error of determination.

The external mass transfer rate as a function of the linear velocity

The results showed that the external mass transfer rate k_f/R was nearly proportional to the linear velocity V of the mobile phase defined by $V = 4F/\pi d_t^2(1 - S_f)$ (Fig.4) under the conditions studied in this work. The equation obtained by correlation was similar to that obtained with solid-liquid chromatography:

$$k_f/R = 4.5161\ V - 0.9218$$

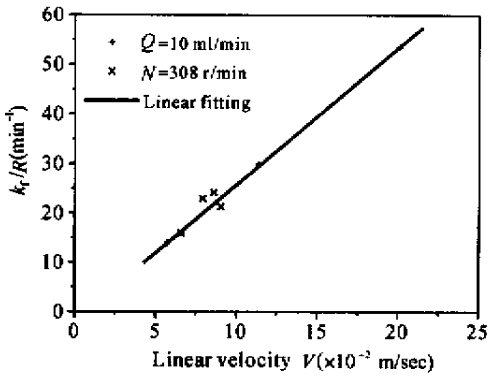

Fig.4 The relationship between the external mass transfer rate and linear velocity for theophylline

CONCLUSIONS

A preliminary model for countercurrent chromatography was established, and a set of parameters were identified by moment analysis. It seemed that the model could describe the system studied quite well, the parameters identified by moment analysis were reasonable, compared to the liquid-solid chromatography using a solid fixed phase.

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