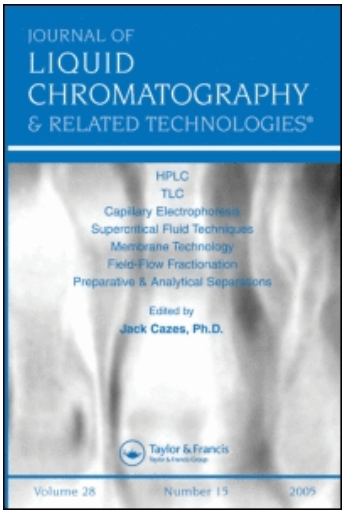

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VAN DEEMTER PLOT IN HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY WITH A FIXED VOLUME OF STATIONARY PHASE

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VAN DEEMTER PLOT IN HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY WITH A FIXED VOLUME OF STATIONARY PHASE

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ABSTRACT

An adaptability of countercurrent chromatographic data to Van Deemter equation ($H = A + B/u + Cu$) is examined under a fixed volume of the stationary phase. Three test samples, epigallochatechin gallate (EGCG), gallocatechin-gallate (GCG), and epicatechin gallate (ECG) were separated using a two-phase solvent system of n-hexane/ethyl acetate/water (3.5:1:10, v/v/v) at various linear flow rates (u).

Our mathematical treatment produced a set of formulae, $H_{EGCG} = 6.5 + 1/u + 6u$; $H_{GCG} = 6.5 + 1.3/u + 7u$ and $H_{ECG} = 6.5 + 2.5/u + 9u$, which showed excellent fit to the experimental data

for each component. Constants B and C show high correlation with the partition coefficient values of three components.

Based on the high correlation between log u and log H when $u > 1$, the plate height (H) for three components may be expressed by $H = Du^k$, where D is the plate height at $u = 1$ cm/s and k, the constant for each component.

INTRODUCTION

In liquid chromatography, the flow rate of the mobile phase is an important factor that determines the partition efficiency of solutes under a given set of other conditions. The relationship between the partition efficiency and flow rate may be expressed by Van Deemter Equation,[1-4]

$$H = A + B/u + Cu \quad (1)$$

where H (height equivalent per theoretical plate) is given as partition efficiency in terms of theoretical plate number divided by the length of the separation column; u, the flow rate of the mobile phase; and A, B, and C are constants.

When H is plotted against u in the coordinate, it forms a characteristic U-shape curve where the flow rate that gives the highest partition efficiency is at the bottom of the curve. A lower flow rate will result in a loss of efficiency due to the longitudinal diffusion caused by increased elution time (due to increased B/u), whereas a higher flow rate will cause loss of efficiency by insufficient time for solute partitioning between the two phases (due to increased Cu).

In the past, this Van Deemter plot was applied to countercurrent chromatography (CCC) that yielded an inverted U-shape.[4,5] This result is unexpected because CCC is a chromatographic technique based on the common principle to all other liquid partition chromatographic methods. However, the unique feature of CCC is a lack of solid supports; and the method uses a liquid stationary phase that is retained in the column by the aid of gravity or a centrifugal force. Consequently, the amount of the stationary phase retained in the column varies according to experimental conditions such as the applied force field and flow rate of the mobile phase.

Since the retention of the stationary phase decreases with increased flow-rate of the mobile phase and this, in turn, causes an increase in theoretical plate number, the anomalous shape of Van Deemter plot of CCC data may be attributed to the loss of the stationary phase with an increased flow rate of the mobile phase.

In this paper, we examined the adaptability of Van Deemter plot to high-speed CCC under a fixed volume of the stationary phase in the column.

EXPERIMENTAL

Apparatus

The experiments were performed using a coil plant HSCCC centrifuge that was designed and fabricated at the Beijing Institute of New Technology Application, Beijing, China. It was equipped with a two-layer coil separation column prepared by winding a 10 m long, 2.6 mm ID PTFE (polytetrafluoro-ethylene) tubing coaxially onto the column holder hub of 8 cm diameter (β = 0.5). The total column capacity measured 54 mL.

The HSCCC centrifuge was rotated at 900 rpm with an 8 cm revolution radius. The system was equipped with two HPLC pumps (Waters 510, Waters, MO, USA), a 4.5 mL-capacity sample injection valve, an UV-detector (UV-752, Shanghai Analytical Instrument Factory, Shanghai, China) and a recorder (S-1000, Shanghai Analytical Instrument Co.).

Reagents

Methanol and n-hexane were of an analytical grade and purchased from Shanghai Chemical Factory, Shanghai, China. High purity (over 99% by HPLC) standards samples, epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and epicatechin gallate (ECG), were prepared from tea extracts in our laboratory.

Computation of the Plate Height

The evaluation of n (theoretical plate) from an experimental chromatography peak was made by numerical integration methods. If the peak was of Gaussian distribution, n was calculated according to the following equation:

$$n = 16(V_r/W_b)^2 \quad (2)$$

or

$$H = L/16(V_r/W_b)^2 \quad (3)$$

where V_r is retention volume and W_b the peak width at the base.

In the present paper, Eq.3 is used for computing H.

HSCCC Procedure

The experiments were performed with a solvent system composed of n-hexane/ethyl acetate/water (3.5:1:10, v/v/v). The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

In each experiment, the coil was first entirely filled with the lower aqueous phase, and 22 mL of the upper organic phase was introduced into the column as a stationary phase by displacing the excess amount of aqueous phase from the outlet of the column. Then, the aqueous mobile phase was pumped into the inlet of the column at a given flow-rate, while the column was rotated at 900 rpm. The sample was injected after the two phases had established hydrodynamic equilibrium in the column.

The effluent from the outlet of the column was continuously monitored with a UV-detector to record the chromatogram.

RESULTS AND DISCUSSION

The H values of EGCG, GCG, and ECG obtained at different flow rates were listed in Table 1. The order of these values, $H_{EGCG} < H_{GCG} < H_{ECG}$, correlated with that of their partition coefficients, i.e., $K_{EGCG} < K_{GCG} < K_{ECG}$ which are 1.08, 2.33, and 6.00, respectively. The minimum H values were observed at a linear velocity of about 0.63 cm/s for all three components.

Table 1

The Plate Heights of EGCG, GCG and ECG at Different Flow Rates

Flow Rate (mL/min)	u (cm/s)	H of EGCG (cm/plate)	H of GCG (cm/plate)	H of ECG (cm/plate)
16	5.02	36.70	41.5	51.67
12	3.78	28.42	33.5	38.98
8	2.51	22.72	25.3	32.40
4	1.26	15.47	17.7	21.99
3.2	1.01	12.62	15.9	19.50
2	0.63	11.01	14.3	18.22
1	0.32	12.08	15.2	20.47
0.5	0.16	12.35	15.7	22.73

With a mathematical treatment of the data in Table 1, we obtained the dispersion equations of EGCG, GCG, and ECG into the Van Deemter forms as follows:

$$H_{EGCG} = 6.5 + 1/u + 6u \quad (4)$$

$$H_{GCG} = 6.5 + 1.3/u + 7u \quad (5)$$

$$H_{ECG} = 6.5 + 2.5/u + 9u \quad (6)$$

Here, u is an average linear velocity of the mobile phase through the column space occupied by the mobile phase (2.6 mm I.D. tube). However, u can be expressed simply as a volume velocity in mL/min. In this case, the above eqs., 4-6, should be modified by proportionally changing the values for constants B and C accordingly.

Figure 1 is a plot of H versus μ from the experimental data and Eqs. 4-6. It shows an excellent fit between the experimental data (dots) and theoretical curves derived from the above three equations. This indicates that the solute partition behavior in HSCCC, under a fixed volume of the stationary phase, is similar to that in gas-solid and liquid-solid chromatography.[6-8]

Different from the original equation, B (the constant in the second term) is mainly the function of the longitudinal band spreading due to violent mixing of the two phases in the column, rather than due to diffusion, whereas, the mass transfer resistance of solutes is mainly responsible for the broadening at a high flow rate (Cu).

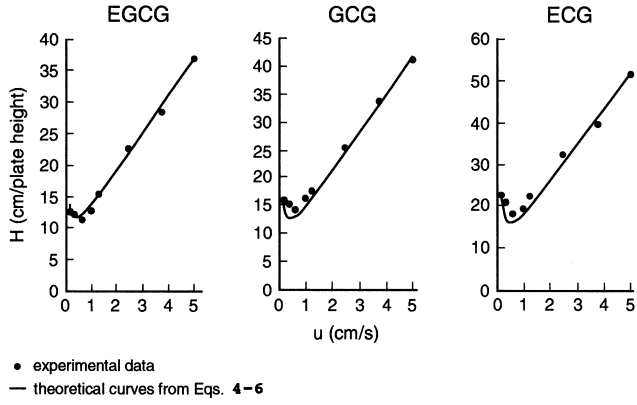

Figure 1. Plots of H versus u from the experimental data and the equations.

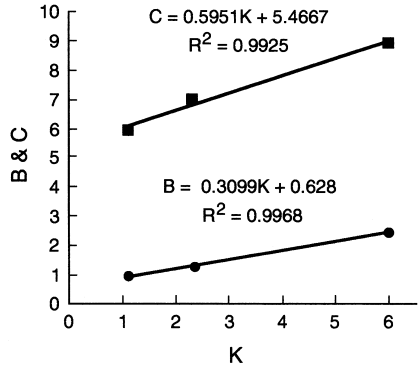

Figure 2. The correlation between the values of two constants (B & C) and K (solute partition coefficient).

There is a clear linear correlation between the value of B or C and the partition coefficient of each component (Figure 2). It implies that the longitudinal band spreading and mass transfer resistance of solutes are functions of both solute partition coefficient and the linear velocity of mobile phase.

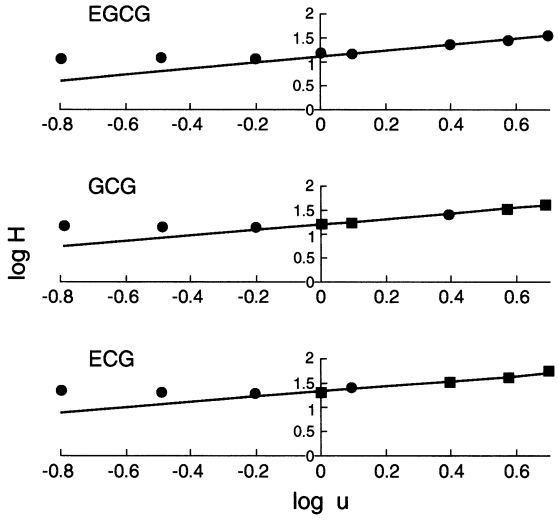

Figure 3. Plots of log H versus log u.

Figure 3 shows the plot of log H versus log u, indicating a linear correlation between these two parameters when u is 1 cm/s or greater. Therefore, H values for these three components may be expressed by the following formula provided $u \geq 1$ cm/s:

$$H = Du^k \quad (7)$$

where D is the plate height at u = 1 cm/s, and k is a constant for each component, i.e., 0.6299 for EGCG, 0.5905 for GCG, and 0.5386 for ECG.

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