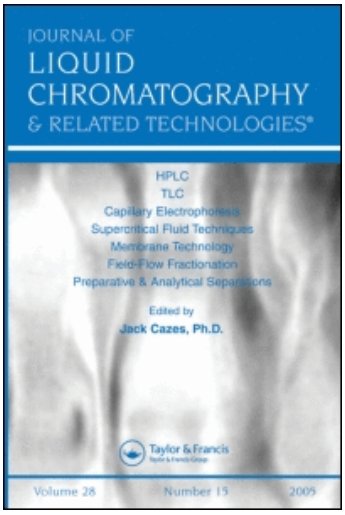

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Large Convoluted Tubing for Scale-Up of Slow Rotary Countercurrent Chromatograph

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Large Convoluted Tubing for Scale-Up of Slow Rotary Countercurrent Chromatograph

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ABSTRACT

The chromatographic performance of 1.5 cm I.D. convoluted teflon tubing was studied at low rotary speeds ranging from 34 to 105 rpm. Results were as follows: (i) many solvent systems showed excellent retention of the stationary phase even at a high flow rate of the mobile phase; (ii) a good mixing of the two phases gave efficient solute partitioning resulting in high peak resolution; (iii) increasing the sample size caused only minor peak broadening; and (iv) increase in peak width and resolution using

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longer columns is predictable. Our data show that it is feasible to scale up a slow rotary countercurrent chromatograph with 1.5 cm I.D. convoluted tubing for industrial use.

Key Words: Countercurrent chromatography; Slow rotary countercurrent chromatography; Scale up.

INTRODUCTION

Utilizing the slow rotary mode of column for countercurrent chromatography, was first described by Ito and Bhatnagar.[1,2] In this system, the best result was attained by rotating the coil slowly around its horizontal axis at a critical speed that yields high retention of the stationary phase. In our previous studies,[3] the performance of various coiled columns was evaluated in terms of stationary phase retention and partition efficiency at low speeds of rotation. The results showed that for slow rotary countercurrent chromatography the convoluted tubing performed better. An apparatus equipped with a 10-L capacity column made of 8.5 mm I.D. convoluted tubing was prepared based on the studies on various parameters such as tubing size, column capacity, and rotary speed. Using this apparatus, 150 g of crude tea extracts were successfully separated, yielding 40 g of biologically active epigallocatechin gallate (EGCG) in high purity at a recovery rate of 82.6%. In the present paper, we examine the chromatographic parameters using a convoluted Teflon tubing with an average I.D. of 1.5 cm for developing a large chromatographic column.

EXPERIMENTAL

Apparatus

Figure 1 shows our trial apparatus equipped with a tubing hub of 15 cm outer diameter. Two pieces of 1.5 cm average I.D. PTFE tubing with 7.3 m length (1280 mL) and 8.4 m length (1470 mL), respectively, were used for the preparation of a coiled column. A Knauer HPLC pump 64 with preparative pump head (Knauer, Berlin, Germany) was used for the delivery of the mobile phase. Fractions were collected with a Pharmacia LKB collector (Uppsala, Sweden).

Reagents

All the organic solvents were analytical grade (Riedel-de Haen, Germany). 11-hydroxylprogesterone (HPT) and progesterone (PT) were

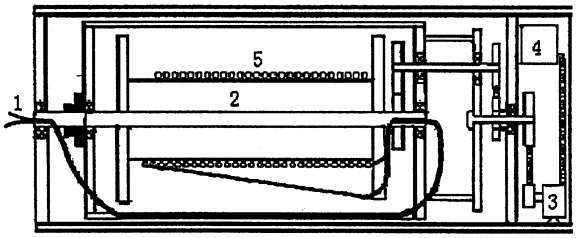

Figure 1. The scheme of the trial apparatus of slow rotary countercurrent chromatograph. 1: inlet and outlet; 2: column hub; 3: motor; 4: speed controller; 5: tubing of helical column.

purchased from Sigma (USA). Pure standards of EGCG and epicatechin gallate (ECG) were prepared at the Institute of Food Chemistry, Technical University of Braunschweig, Germany.

Measurement of Stationary Phase Retention

For each solvent system, the retention of the stationary phase was determined at two or three different flow rates of the mobile phase, at a given rotational speed as follows: The column was first completely filled with the stationary phase and the mobile phase was then introduced into the column from head to tail at a flow rate of 5 or 10 mL/min. Here, head means the left end when the tubing was deasil wound onto the hub and the column also deasil rotated. The effluent was collected into a graduated cylinder to measure the volume of the stationary phase displaced from the column. Immediately after hydrodynamic equilibrium was established in the column (as indicated by no carryover of stationary phase), the volume of stationary phase displacement was noted. Then, the flow rate of the mobile phase was increased to the second level (in some cases even to a third level) and the volume of the resulting stationary phase displacement was determined again. From the volume of the stationary phase eluted from the column and the total column capacity, the percent retention of stationary phase was determined for each flow rate of the mobile phase.

Determination of Chromatographic Parameters

Effects of flow rate, injection volume, and sample concentration, as well as column length on retention volume and peak width were studied. In each chromatographic procedure, the column was first completely filled with the

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designed stationary phase and mobile phase of the selected immiscible solvent system, and rotated at a given speed. Then, the sample was injected through a sample loop and the mobile phase was eluted through the column. The effluent was collected into test tubes, and each fraction was analyzed by either UV or HPLC, in order to construct the chromatogram.

UV and HPLC Analysis

The obtained chromatographic fractions were analyzed by UV spectrometers at a given wavelength. The fractions containing two components were analyzed by HPLC with a reversed phase C18 HPLC column where 60% and 30% of acetonitrile in water were used as the mobile phase to elute the mixture of HPT and PT and the mixture of ECG and EGCG, respectively.

Determination of Partition Coefficient

A saturated solution of a certain analyte was prepared by using the layer of a biphasic solvent system in which the analyte was better soluble. The saturated solution was then diluted one time with the same phase. Two milliliter of this diluted solution and 2.0 mL of the second layer were thoroughly mixed in a test tube. After the two phases were separated, the solute concentration in each layer was determined by UV spectroscopy. The partition coefficients were calculated on the basis of the measured absorbance ratios.

Calculation of Partition Coefficient and Resolution from Chromatograms

The partition coefficient (K) component is calculated by applying the following formula:

$$K = 1 + \frac{(V_R - V_C)}{(S_F V_C)}$$

Here, V_C and S_F are the column capacity and percent retention of stationary phase in the column, respectively. V_R is the elution volume of a component at its maximum concentration from the chromatogram. K is the partition coefficient expressed as concentration in stationary phase/concentration in mobile phase.

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Resolution between two components is calculated with the formula:

$$R_S = \frac{2(V_{R2} - V_{R1})}{(W_2 + W_1)}$$

Here, V_{R1} and V_{R2} are the elution volume of the two components at their maximum, and W_1 and W_2 are the peak widths of the two components.

RESULTS AND DISCUSSION

Retention of Stationary Phase

Table 1 lists the retention of stationary phase in six solvent systems at various rotary speeds and flow rates, respectively. Five systems [except the mixture of *n*-hexane/ethyl acetate/1-butanol/water/acetic acid (0.5/1/2/6/0.1)], produced over 50% of retention of stationary phase at a flow rate of 40 mL/min, which is likely to be used for an industrial-scale CCC. The results also indicate that in the case of the 1.5 cm I.D. convoluted tubing, large differences in density between the two phases yield higher retention of stationary phase. The data of the *t*-butyl methyl ether/water system shows that the retention is better when the organic upper phase is acting as stationary phase. It is also apparent that the longer the tubing the better the retention of stationary phase.

Partition Coefficient

Table 2 lists the partition coefficients of HTP, PT, and EGCG determined from CCC chromatograms and test tube measurements. The results show that the partition coefficients obtained from these two methods are very close to each other. It further indicates that no significant amount of carryover of stationary phase took place after the solvent front emerged.

Broadening of Peak Due to Increased Flow Rate

Figure 2 shows the chromatogram obtained for EGCG after partitioning through the 1280 mL column, using a binary solvent system composed of *t*-butyl methyl ether/water. It is apparent that peak broadening is caused by a higher flow rate. The broadening amounted to 22% when the flow rate increases from 20 mL/min to 40 mL/min.

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Table 1. Retention of stationary phase for six solvent systems at various rotary speeds and flow rates.

Solvent system	Column capacity (mL)	Stationary phase	Rotary speed (rpm)	Flow-rate (mL/min)	Retention of stationary phase (in %)
t-Butyl methyl ether/water	2,750	Lower phase	100	10	66
			100	23	53
		Upper phase	105	10	70
			105	40	66
t-Butyl methyl ether/water	1,280	Upper phase	105	10	63
			105	40	58
n-Hexane/ethyl acetate/ 1-butanol/water/acetic acid (0.5/1/2/6/0.1)	2,750	Upper phase	34	5	82
			34	10	49
			34	40	23
n-Hexane/methanol/water (6 : 5 : 5)	1,280	Upper phase	55	10	71
			55	40	53
Chloroform/water	1,280	Lower phase	100	10	78
			100	40	69
n-Hexane/1-butanol/water (1/1/2)	1,280	Upper phase	80	10	68
			80	40	53

Table 2. Comparison of two different methods for determination of partition coefficients (CCC chromatogram vs. test tube experiment).

Flow rate (mL/min)	Column capacity (mL)	Sample amount	Partition coefficient from CCC chromatogram		Partition coefficient using the test tube experiment	
			I	II	I	II
23	2,750	80 mg HPT (I) + 250 mg PT (II)	0.076	1.42	0.03	1.67
45	2,750	30 mg HPT (I) + 90 mg PT (II)	0.076	1.42	0.03	1.67
40	2,750	500 mg EGCG (I)	1.44		1.37	
20	1,280	500 mg EGCG (I)	1.34		1.37	
40	1,280	500 mg EGCG (I)	1.34		1.37	
40	1,280	1,000 mg EGCG (I)	1.33		1.37	
40	1,280	2,000 mg EGCG (I)	1.31		1.37	

Note: HPT, 11-hydroxylprogesterone; PT, progesterone; EGCG, epigallocatechin gallate.

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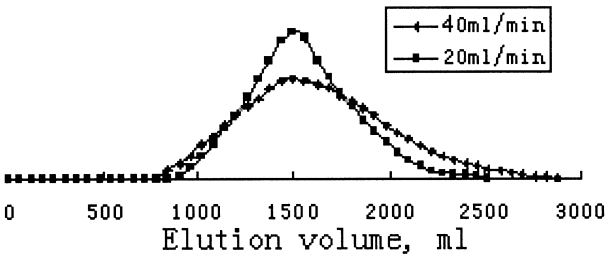

Figure 2. Chromatograms obtained for EGCG using different flow rates. Volume of stationary phase: 640 mL; mobile phase: lower phase; Sample solution: 500 mg EGCG in 50 mL lower phase; Column capacity: 1280 mL; Solvent system: *t*-butyl methyl ether/water; Rotary speed: 105 rpm; UV absorbance determination: 280 nm.

Peak Broadening Due to Increased Tube Length

Figure 3 shows three chromatograms obtained for the partitioning of EGCG using columns of different lengths, i.e., 7.3 m, 8.4 m, and 15.7 m of 1.5 cm I.D. convoluted tubing. The peak widths, excluding the tailing ends, are 1740 mL, 1890 mL, and 2600 mL, respectively, while the absolute peak widths based on the UV spectrophotometric data are 2040 mL, 2220 mL, and 2760 mL. Approximately, there is a relationship of $W = L^{0.5}$ between the peak width W (excluding the tailing end) and column length L (Fig. 4), which is consistent with normal liquid chromatography.

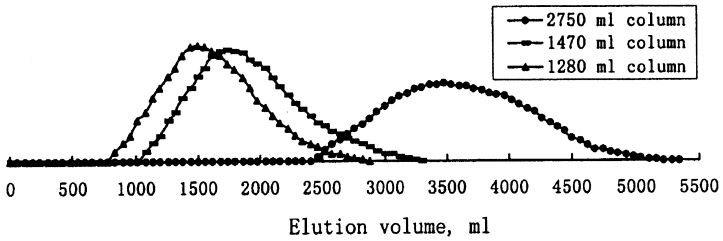

Figure 3. Chromatograms of EGCG obtained on 1.5 cm I.D. convoluted columns of different length. Mobile phase: lower phase.

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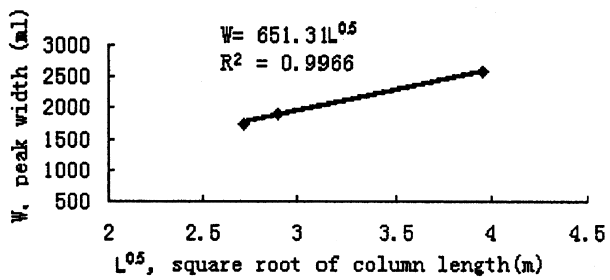

Figure 4. Plotting of peak width excluding the tailing end (W) vs. square root of the column length ($L^{0.5}$).

Peak Broadening Due to Increased Sample Size

Figure 5 shows peak broadening when the sample size is increased. The absolute peak width of 0.5 g EGCG in 50 mL lower phase, 1 g EGCG in 100 mL lower phase, and 2 g EGCG in 200 mL lower phase were 1740, 1870, and 2100 mL, respectively. The percent increase of peak width with double increase of sample is 7.5% and 12.3%, respectively. This demonstrates that the peak broadening caused by an increased sample volume is not significant.

Figure 6 shows the chromatograms produced from different sample concentration in the same injection volume. For higher sample concentration, the peak broadening became significant and, in addition, the high sample concentration causes a severe loss of stationary phase. These results indicate that the best way to increase the sample size is to load a large volume at a low concentration.

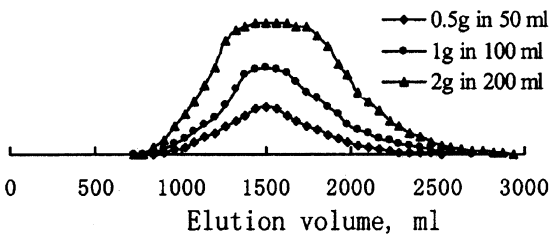

Figure 5. Peak broadening due to increasing sample volumes. Volume of stationary phase: 640 mL; Sample solution: EGCG in lower phase; Column capacity: 1280 mL; Solvent system: *t*-butyl methyl ether/water; mobile phase: lower phase; Flow rate: 40 mL/min; Rotary speed: 105 rpm; UV absorbance determination: 280 nm.

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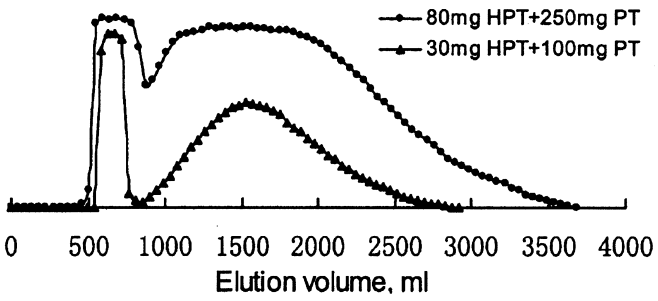

Figure 6. Peak broadening caused by increased sample concentration in the same injection volume. Volume of stationary phase: 1320 mL; Sample solution: the given sample weight in 25 mL upper and 25 mL lower phase; Column capacity: 2750 mL; Solvent system: *n*-hexane/methanol/water (6 : 5 : 5); mobile phase: lower phase; Flow rate: 43 mL/min; Rotary speed: 55 rpm; UV absorbance determination: 254 nm.

Peak Resolution

In order to study the effect of column length on peak resolution, a mixture of EGCG and ECG was separated using a two-phase solvent system composed of *t*-butyl methyl ether/methanol/water (10 : 1 : 10) in which the two compounds gave partition coefficients of 0.63 and 3.68, respectively, when the upper phase is used as the stationary phase. The resulting separations using 7.3 m and 15.7 m-columns are shown in Figs. 7 and 8, respectively. The resolution in the case of the 7.3 m column is 0.89, while the 15.7 m column

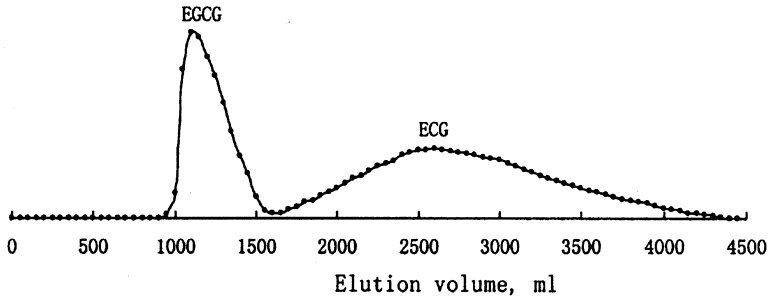

Figure 7. Chromatograms of the separation of the mixture of EGCG and ECG using the 7.3 m convoluted column. Sample solution: 250 mg EGCG and 250 mg ECG in 50 mL of lower phase; Column capacity: 1280 mL; Solvent system: *t*-butyl methyl ether/methanol/water (10 : 1 : 10); mobile phase: lower phase; Flow rate: 20 mL/min; Rotary speed: 100 rpm; UV absorbance determination: 280 nm.

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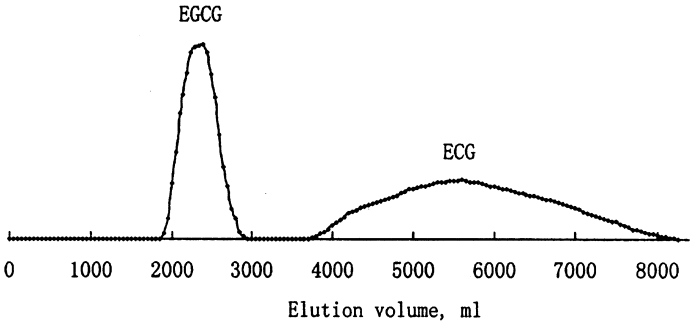

Figure 8. Chromatogram of the separation of a mixture of EGCG and ECG using 15.7 m convoluted column. Sample solution: 250 mg EGCG and 250 mg ECG in 50 mL of lower phase; Column capacity: 2750 mL; Solvent system: *t*-butyl methyl ether/methanol/water (10 : 1 : 10); mobile phase: lower phase; Flow rate: 20 mL/min; Rotary speed: 100 rpm; UV absorbance determination: 280 nm.

gives a resolution of 1.27. It seems that the resolution is directly proportional to the square root of the column length, as in high-speed countercurrent chromatography (HSCCC).[4]

In summary, it can be concluded that 1.5 cm I.D. convoluted teflon tubing can be used to scale up slow rotary countercurrent chromatograph for industrial-scale separations because of the following reasons: (i) It provides excellent retention of the stationary phase for many solvent systems, even at a high flow rate of the mobile phase. (ii) It produces a good mixing of the two phases, thus enabling efficient partitioning. (iii) Increase in sample size only yields relatively small peak broadening. (iv) Increase in peak width and resolution using longer columns is predictable.

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