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Short communication

Relationship between the flow-rate of the mobile phase and retention of the stationary phase in counter-current chromatography

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Abstract

The retention percentages of the stationary phase (S_F) at various flow-rates (F_C) of 15 two-phase solvent systems were determined in three types of counter-current chromatographic apparatus equipped with small-, middle- and large-bore coiled columns. The regression analysis of S_F showed a linear relationship between the square root of the flow-rate and the retention percentage of the stationary phase, i.e. $S_F = A - B\sqrt{F_C}$ where A indicates the difference in the solvent composition of the solvent system and B indicates the difference in the volume ratio of the solvents for the same composition of the solvent system. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Counter-current chromatography; Flow-rate; Retention of stationary phase

1. Introduction

Counter-current chromatography (CCC) has made great progress in the past 30 years [1–4]. Nevertheless, the relationship between the parameters that influence the chromatography behavior has not been thoroughly studied. The retention percentage of the stationary phase (S_F), the percentage of the stationary phase volume retained in the column relative to the total column capacity, is one of the most important parameters in CCC, and it is used for the derivation of the column efficiency, peak resolution and solute retention [2].

Though the S_F value changes with various param-

eters such as rotation speed, flow-rate of the mobile phase, sample volume and its concentration, the flow-rate is a unique parameter that influences the chromatographic behavior after all other conditions are set. Therefore, reliable prediction of retention levels of the stationary phase at a given flow-rate of the mobile phase will greatly contribute to the application of CCC.

2. Experimental

2.1. Apparatus

Two different rotary devices were employed in the present studies: The type J multilayer coil planet

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centrifuge with a 10 cm revolution radius which produces a synchronous planetary motion of the column holder. It was fabricated at the Beijing Institute of New Technology Application, Beijing, China. The second apparatus is a large rotary device which rotates a large diameter coil holder around its own horizontal axis in the unit gravitational field. This apparatus was constructed at the Zhejiang University, Hangzhou, China. The coiled column was coaxially mounted on the holder of each apparatus. The dimensions of the columns used with these instruments are described below.

2.2. Procedure

The percentage retention of the stationary phase (S_F) at a given flow-rate of the mobile phase was determined by the following procedure: The column was first entirely filled with the stationary phase. Then the mobile phase was pumped into the inlet of the column at the desired flow-rate while the apparatus was rotated at the desired speed. After the mobile phase front emerged and the two phases had established the hydrodynamic equilibrium throughout the column, the volume of the stationary phase eluted from the column was measured.

The S_F values of 12 two-phase solvent systems (Table 2), including ethyl acetate–water, hexane–ethyl acetate–water (from 1:9:10 to 8:2:10), hexane–ethyl acetate–methanol–water (from 1:9:1:9 to 8:2:8:2) and hexane–water were determined using the multilayer coil planet centrifuge. The coiled column was prepared from 130×1.6 mm I.D. PTFE (polytetrafluoroethylene) tubing by winding it coaxially onto the column holder hub of 8 cm diameter forming 14 coiled layers with a total capacity of 230 ml. The β value ranged from 0.4 to 0.78 ($\beta=r/R$ where r is the distance from the holder axis to the coil, and R is the distance between the holder axis and the central axis of the centrifuge).

The S_F values of two solvent systems including chloroform–methanol–acetic acid–water (5:3:1:4) and hexane–ethanol–water (6:5:3) were determined in the second apparatus equipped with a rotating holder coaxially around its horizontal axis (Table 2). The apparatus is equipped with a 4-layer column that was prepared by winding 134 m×2.6 mm I.D. PTFE tubing coaxially onto a 15 cm diameter hub making

70 loops in each layer. The total column capacity is 710 ml.

The above apparatus was also used to determine the S_F values of a chloroform–water system using a 6-layer coiled column that was prepared from 97 m×5.5 mm I.D. PTFE tubing by winding it coaxially onto a 15 cm diameter holder hub making 26 loops per layer. The total column capacity was 2300 ml.

3. Results and discussion

The S_F values of 15 solvent systems in three types of columns at various flow-rates were listed in Tables 1–3. With a regression analysis of $S_F = A - B\sqrt{F_C}$, we found all the correlation coefficients were less than -0.992 . This indicates that there is a linear relationship between the square root of the flow-rate and the retention percentage of the stationary phase. Table 4 showed the linear quotations and correlation coefficients. In most cases the A value substantially exceeds 100, suggesting that the linearity of the curve may be limited within some range of S_F , probably between 80% and 20%. Nevertheless, these results indicate that, once the S_F values at two different flow-rates are determined, the S_F at any flow-rate can be predicted for most solvent systems in a broad range which is very useful in actual separations. A comparison between the A and B values of hexane–ethyl acetate–water and ethyl acetate–water and hexane–water shows that both the A and the B values of hexane–ethyl acetate–water are close to those of hexane–water, but are far from those of ethyl acetate–water. In the hexane–ethyl acetate–methanol–water system at different volume ratios, the A values were little different, while the B values decrease in a gradient fashion as the relative volume of hexane to ethyl acetate increases. Although much more study will be required to confirm the statement, it seems likely that the B value corresponds to the volume ratio of the solvents in a given two-phase solvent system, whereas the A value represents the solvent composition in the two-phase solvent system. The regression analysis applied to the data from aqueous two-phase solvent systems using an eccentric multilayer coil planet centrifuge reported by Xia et al. [5] also showed a linear

Table 1
Determination of S_F in the apparatus equipped with a 1.6 mm I.D. tube column at various flow-rates

Solvent system	Mobile phase	Rotary speed of apparatus (rpm)	Flow-rate of mobile phase (ml/min)	Retention rate of stationary phase (%)
Ethyl acetate–water (10:10)	Lower	750	0.8	80.0
			1.6	62.7
			1.8	51.2
			2.4	39.7
			3.0	31.5
			3.6	22.6
Hexane–ethyl acetate–water (1:9:10)	Lower	750	1.0	75.4
			1.7	67.2
			2.6	58.3
			3.4	53.8
			4.8	42.5
			6.3	31.6
Hexane–ethyl acetate–water (2:8:10)	Lower	750	1.0	79.7
			2.0	68.7
			3.2	59.5
			4.1	53.8
			6.4	43.9
			10.0	31.6
Hexane–ethyl acetate–water (5:5:10)	Lower	750	1.0	86.5
			2.1	76.7
			3.3	71.2
			4.2	66.0
			5.1	61.2
			10.0	31.6
Hexane–ethyl acetate–water (8:2:10)	Lower	750	1.0	89.6
			2.1	79.8
			3.1	75.9
			4.1	70.3
			5.8	63.3
			10.0	31.6
Hexane–water (10:10)	Lower	750	1.4	82.0
			2.5	73.8
			3.5	65.3
			4.3	62.0
			5.2	58.5
			10.0	31.6
Hexane–ethyl acetate–methanol–water (1:9:1:9)	Lower	750	1.0	77.1
			1.9	67.7
			3.0	58.0
			4.1	50.78
			5.3	44.0
			10.0	31.6
Hexane–ethyl acetate–methanol–water (2:8:2:8)	Lower	750	1.1	72.0
			2.2	60.7
			3.2	52.7
			4.0	47.5
			4.8	43.4
			10.0	31.6
Hexane–ethyl acetate–methanol–water (4:6:4:6)	Lower	750	1.0	79.1
			2.0	66.8
			3.0	59.9
			4.1	53.4
			5.0	48.8
			10.0	31.6

Table 1. Continued

Solvent system	Mobile phase	Rotary speed of apparatus (rpm)	Flow-rate of mobile phase (ml/min)	Retention rate of stationary phase (%)
Hexane–ethyl acetate–methanol–water (5:5:5:5)	Lower	750	1.0	81.1
			2.0	71.3
			3.0	63.9
			4.0	59.2
			5.2	54.9
Hexane–ethyl acetate–methanol–water (6:4:6:4)	Lower	750	1.5	81.1
			2.3	76.7
			4.7	66.6
			5.6	61.7
			7.5	56.0
Hexane–ethyl acetate–methanol–water (8:2:8:2)	Lower	750	1.1	86.4
			2.1	81.7
			3.7	77.5
			5.0	74.1
			7.0	70.0

Table 2

Determination of S_F in apparatus equipped with 2.6 mm I.D. tube column at various flow-rates

Solvent system	Mobile phase	Rotary speed of apparatus (rpm)	Flow-rate of mobile phase (ml/min)	Retention rate of stationary phase (%)
Chloroform–methanol–water–acetic acid (5:3:4:1)	Upper	90	0.5	93.8
			1.5	88.8
			3.0	82.7
			6.0	70.1
			9.0	64.9
Hexane–ethanol–water (6:5:3)	Lower	46	1.0	61.7
			3.0	59.3
			6.0	53.1
			10.0	48.1
			15.0	44.4

Table 3

Determination of S_F in apparatus equipped with the 2.6 mm I.D. tube column at various flow-rates

Solvent system	Mobile phase	Rotary speed of apparatus (rpm)	Flow-rate of mobile phase (ml/min)	Retention rate of stationary phase (%)
Chloroform–water (1:1)	Lower	900	3.0	65.0
			3.5	64.0
			4.2	63.5
			8.0	57.5
			16.5	48.4
			24.0	42.0
			35.0	35.1
			44.0	30.0

Table 4
Regression analysis between $\sqrt{F_C}$ and S_F of 15 solvent systems

Solvent system	Equation of linear regression	Correlation coefficient
Ethyl acetate–water (10:10)	$S_F = 132.59 - 58.51\sqrt{F_C}$	-0.99293
Hexane–ethyl acetate–water (1:9:10)	$S_F = 104.62 - 28.58\sqrt{F_C}$	-0.99768
Hexane–ethyl acetate–water	$S_F = 102.23 - 23.51\sqrt{F_C}$	-0.99765
Hexane–ethyl acetate–water (5:5:10)	$S_F = 105.91 - 19.59\sqrt{F_C}$	-0.99769
Hexane–ethyl acetate–water (8:2:10)	$S_F = 107.51 - 18.36\sqrt{F_C}$	-0.99507
Hexane–water (10:10)	$S_F = 107.97 - 22.05\sqrt{F_C}$	-0.99976
Hexane–ethyl acetate–methanol–water (1:9:1:9)	$S_F = 102.87 - 25.73\sqrt{F_C}$	-0.99673
Hexane–ethyl acetate–methanol–water (2:8:2:8)	$S_F = 98.18 - 25.20\sqrt{F_C}$	-0.99942
Hexane–ethyl acetate–methanol–water (4:6:4:6)	$S_F = 102.27 - 24.19\sqrt{F_C}$	-0.99712
Hexane–ethyl acetate–methanol–water (5:5:5:5)	$S_F = 100.90 - 20.66\sqrt{F_C}$	-0.99565
Hexane–ethyl acetate–methanol–water (6:6:6:4)	$S_F = 102.88 - 17.13\sqrt{F_C}$	-0.99487
Hexane–ethyl acetate–methanol–water (8:2:8:2)	$S_F = 96.81 - 10.14\sqrt{F_C}$	-0.99918
Chloroform–water (1:1)	$S_F = 77.75 - 7.22\sqrt{F_C}$	-0.99977
Chloroform–methanol–water–acetic acid (5:3:4:1)	$S_F = 104.20 - 13.25\sqrt{F_C}$	-0.99419
Hexane–ethanol–water (6:5:3)	$S_F = 68.91 - 6.38\sqrt{F_C}$	-0.99235

relationship of $S_F = 50.95 - 17.10\sqrt{F_C}$ ($r = -0.9946$). This indicates that the S_F at a certain flow-rate for an aqueous two-phase solvent system can also be estimated from the data obtained from two experiments at different flow-rates of the mobile phase.

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References

- [1] Y. Ito, in: N.B. Mandava, Y. Ito (Eds.), Counter-current Chromatography: Theory and Practice, Marcel Dekker, New York, 1986, pp. 79–442.
- [2] W.D. Conway, Counter-current Chromatography: Apparatus, Theory and Application, VCH, New York, 1990.
- [3] W.D. Conway, J.R. Petroski (Eds.), Modern Counter-current Chromatography, ACS Symp. Series No. 593, American Chemical Society, Washington, DC, 1995.
- [4] Y. Ito, W.D. Conway (Eds.), High-speed Counter-current Chromatography, Wiley-Interscience, New York, 1996.
- [5] L. Xia, J.-T. Hsu, J. Liq. Chromatogr. 15 (1992) 2801–2817.