



Revisiting resolution in hydrodynamic countercurrent chromatography: Tubing bore effect[☆]



A. Berthod^{*}, K. Faure

Institut des Sciences Analytiques, Université de Lyon, CNRS, 5 rue de la Doua, 69100 Villeurbanne, France

ARTICLE INFO

Article history:

Received 7 January 2015
Received in revised form 17 February 2015
Accepted 17 February 2015
Available online 24 February 2015

Keywords:

Resolution
Hydrodynamic countercurrent chromatography
Coiled bobbin
Tubing bore
Efficiency
Equipment design
Theoretical study

ABSTRACT

A major challenge in countercurrent chromatography (CCC), the technique that works with a support-free biphasic liquid system, is to retain the liquid stationary phase inside the CCC column (S_f parameter). Two solutions are commercially available: the hydrostatic CCC columns, also called centrifugal partition chromatographs (CPC), with disks of interconnected channels and rotary seals, and the hydrodynamic CCC columns with bobbins of coiled open tube and no rotary seals. It was demonstrated that the amount of liquid stationary phase retained by a coiled tube was higher with larger bore tubing than with small bore tubes. At constant column volume, small bore tubing will be longer producing more efficiency than larger bore tube that will better retain the liquid stationary phase. Since the resolution equation in CCC is depending on both column efficiency and stationary phase retention ratio, the influence of the tubing bore should be studied. This theoretical work showed that there is an optimum tubing bore size depending on solute partition coefficient and mobile phase flow rate. The interesting result of the theoretical study is that larger tubing bores allow for dramatically reduced experiment durations for all solutes: in reversed phase CCC (polar mobile phase), hydrophobic solutes are usually highly retained. These apolar solutes can be separated by the same coil at high flow rates and reduced S_f with similar retention times as polar solutes separated at smaller flow rates and much higher S_f .

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Countercurrent chromatography is a liquid–liquid chromatographic separation technique that works with a support-free biphasic liquid system [1,2]. The mobile phase is one phase of the biphasic liquid system. The liquid stationary phase is the other phase that is maintained in the CCC “column” by centrifugal fields. Two types of CCC devices are commercially available: the hydrostatic CCC columns with disks of interconnected channels and rotary seals [3], and the hydrodynamic CCC columns with bobbins of coiled open tube and no rotary seals [4]. In a recent work, two hydrodynamic CCC columns of similar volumes were compared [5]. The first one was an 18 mL column made with a narrow-bore 0.8 mm Teflon tubing and the second one was a 19 mL column with a coiled 1.6 mm Teflon tubing. Even though the narrow-bore column presented a much better efficiency with thin peaks, the larger bore column will work much faster still giving acceptable resolution factors between broader peaks because the liquid

stationary phase is better retained in bobbins containing larger bore tubing.

Since the parameters acting on the resolution factor are known and the relationship between liquid stationary phase retention by a coiled bobbin, flow rate and tubing bore was established [5], it is possible to estimate theoretically the tubing bore that would give an optimal chromatographic resolution with a constant volume bobbin. This study investigates several different cases: different tubing bores, solutes of very different polarity, different flow rates and different volumes of stationary phase (S_f parameter), but working with hypothetical coils of 500 mL all having a radius large enough (15 cm or more) so that it is possible to build them mechanically without generating a tubing curvature that would change the nature of the fluid flow. Also all 500 mL coils work in the same centrifugal field separating solutes having the same selectivity ratio. The focus is put on the chromatographic resolution obtained.

2. Theory of countercurrent chromatography

2.1. Resolution factor

The resolution factor, R_s , is defined as the ratio of the volume retention difference between two peaks 1 and 2, divided by the

[☆] Presented at the 8th International Conference on Countercurrent Chromatography - CCC 2014, 23 - 25 July 2014, Uxbridge, United Kingdom.

^{*} Corresponding author. Tel.: +33 437 423 553.

E-mail address: berthod@univ-lyon1.fr (A. Berthod).

average peak width, W , at base:

$$R_s = \frac{V_{R_2} - V_{R_1}}{(W_2 + W_1)/2} \quad (1)$$

The solute retention volume in CCC is related to its liquid–liquid partition coefficient K (ratio of the solute concentration in the stationary phase to that in the mobile phase at equilibrium) and to V_M and V_S , respectively the mobile and stationary phase volume contained in the column:

$$V_R = V_M + KV_S \quad (2)$$

The stationary phase retention ratio, Sf , defined as:

$$Sf = \frac{V_S}{V_C} \quad (3)$$

in which V_C is the column volume equal to the sum $V_M + V_S$. The column efficiency, N , is expressed in theoretical plate number [1–4],

$$N = 16 \left(\frac{V_R}{W} \right)^2, \quad (4)$$

The CCC resolution equation can be derived using Eqs. (1)–(4) for Compounds 1 and 2, and assuming a constant efficiency or taking the average efficiency $N = (N_1 + N_2)/2$, as:

$$R_s = Sf \frac{\sqrt{N}}{4} \frac{(K_2 - K_1)}{(1 - Sf(1 - (K_1 + K_2)/2))} \quad (5)$$

Column efficiency, N , and the solute retention parameter (K , partition coefficient) are parameters acting on the resolution factor in all chromatographic techniques. The unique point of CCC is that the R_s chromatographic resolution factor is dependent on Sf , the amount of liquid stationary phase retained by the column. Also, the column efficiency, N , is not a constant for all solutes: it very often observed that N decreases as the solute resides a longer time in the stationary phase [5].

CCC is the only chromatographic separation technique in which the stationary phase volume contained in the column is an adjustable parameter. A CCC apparatus may produce completely different chromatograms when the same sample is separated by the same column using the same biphasic liquid system, i.e. the same mobile and same liquid stationary phase. Chromatograms showing that the resolution in CCC depends on the amount of liquid stationary phase retained by the column, Sf , were published [4].

Fig. 1 plots the variation of the resolution factor, R_s , versus the amount of liquid stationary phase, Sf , retained in a CCC column. Fig. 1 is prepared by calculating the R_s factors for pairs of solutes having the same K_2/K_1 partition coefficient ratio, i.e. the same chromatographic selectivity ($\alpha = 1.5$) and taking into account the decrease in peak efficiency, N , associated with higher solute retention (higher K) in liquid–liquid partitioning. It must be pointed out that the selectivity factor, α , is the important parameter in studying resolution even if α does not directly appear in Eq. (5). Working with a constant selectivity factor does increase the difference $K_2 - K_1$ in the numerator of Eq. (5). It seems to increase directly the resolution factor R_s . So the question is: Is it better to work with couples of solutes having a constant $K_2 - K_1$ difference or with a constant selectivity factor α ? Intuitively it seems easier to separate two solutes with $K_1 = 0.1$ and $K_2 = 0.6$ than two solutes with the same ΔK difference but larger K_s such as 30.0 and 30.5. The first couple of solutes has a selectivity factor of $\alpha = 6$, and only $\alpha = 1.016$ for the second couple. Eq. (5) could be expressed including the selectivity factor α : it is a more complicated expression that still includes K_s in both the numerator and denominator. Hence, as K increases, the fraction tends toward a constant value and the resolution factor R_s tends toward the value: $((\sqrt{N}/2) \times [(\alpha - 1)/(\alpha + 1)])$. For example, with $\alpha = 1.5$ and $N = 250$ plates, the constant is 1.581 as observed for the $K_1 = 20$ line in Fig. 1. With this assumption of constant selectivity

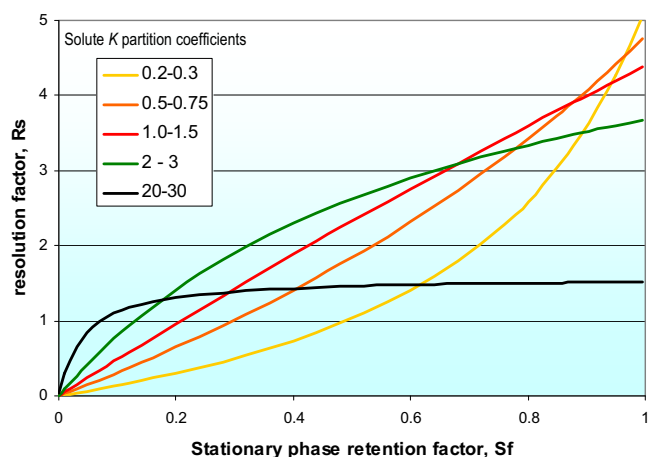


Fig. 1. Variation of the chromatographic resolution factor, R_s , as a function of the amount of liquid stationary phase retained in a given CCC column (Eq. (5)). The efficiencies used for the calculation were $N = 2700$ plates for $K = 0.2$, $N = 2300$ plates for $K = 0.5$, $N = 1900$ plates for $K = 1.0$, $N = 1350$ plates for $K = 2$, and $N = 250$ plates for $K = 20$ from experimental observations with GUESS solutes and Arizona L (heptane/ethyl acetate/methanol/water 2:3:2:3, v/v) liquid system [5].

factor $\alpha = 1.5$, a convex shape of the R_s vs. Sf curves is obtained for rapidly eluting solutes being preferentially located in the mobile phase ($K < 1$). A concave shape is seen for highly retained solutes ($K > 1$) preferring the liquid stationary phase and a straight line represents solutes partitioning equally between mobile and stationary liquid phases ($K \approx 1$). The curved shape is produced by the denominator of Eq. (5) whose value decreases for $K < 1$, increases for $K > 1$ and is constant for $K = 1$ solute, when the Sf parameter increases (Eq. (5)).

Fig. 1 shows that the maximum amount of stationary phase retained by the CCC column is desirable to have a high resolution power for the rapidly eluting solutes. For intermediately eluting solutes, the linear relationship between R_s and Sf also favors a maximum Sf ratio. Highly retained solutes may be preferably separated with lower Sf ratios because this does not significantly reduce the resolution capability of the CCC column but the lower volume of stationary phase, V_S , will greatly reduce the elution time (Eq. (2)). This theoretical study fully agrees with the Ito “golden rules”. Rule 2 recommends working with solutes having K values between 0.5 and 2. Rule 5 suggests a selectivity equal or higher than 1.5 for the resolution of two compounds [6]. Also Fig. 1 is valid for any CCC columns, hydrodynamic as well as hydrostatic types.

2.2. Liquid stationary phase retention ratio

The support-free liquid stationary phase is the heart of the CCC technique. Common sense and Eq. (5) show that some liquid stationary phase must be retained by the CCC column so that it is able to separate compounds. For hydrodynamic CCC columns only, the Sf liquid stationary phase retention ratio was found to be related to the mobile phase flow rate, F , by [7,8]:

$$Sf = A - B\sqrt{F} \quad (6)$$

A and B are constants depending on the experimental conditions. Since A corresponds to the proportion of stationary phase in the CCC column when the mobile phase is not flowing, i.e. $F = 0$ mL/min, it should be equal to 1% or 100%. Wood et al. studied the A term showing that it was containing the extra-coil volume. Hence when Sf is measured directly, the A constant is always slightly lower than 1% or 100% [9].

The B constant of Eq. (6) corresponds to the gradient slope between the mobile phase flow rate and the proportion of retained

Download English Version:

<https://daneshyari.com/en/article/1199565>

Download Persian Version:

<https://daneshyari.com/article/1199565>

[Daneshyari.com](https://daneshyari.com)