



Effect of operating parameters on a centrifugal partition chromatography separation



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ABSTRACT

Centrifugal partition chromatography (CPC) is the branch of countercurrent chromatography (CCC) that works with single axis hydrostatic columns with rotary seals. The hydrodynamic of the liquid stationary phase-liquid mobile phase equilibrium in the CPC chambers has been studied theoretically and with specially designed CPC columns. In this work, we selected a simple analytical separation (no loading study) of three test solutes, coccine red, coumarin and carvone, with a commonly used heptane/ethyl acetate/methanol/water 1:1:1 v/v biphasic liquid system and two different rotors: a commercially available 30-mL CPC instrument and a 80-mL prototype rotor designed for productivity. We fully studied this separation in many possible practical operating conditions of the two rotors, aiming at a generic column characterization. The rotor rotation was varied between 1000 and 2800 rpm, the aqueous mobile phase flow rate was varied between 1 and 22 mL/min with the 30-mL rotor and 10 and 55 mL/min with the 80-mL rotor, the upper limits being mechanical constraints and some liquid stationary phase remaining in the rotor. The variations of S_f , the volume ratio of stationary phase in the rotor, were studied versus mobile phase flow rate and rotor rotation speed. A maximum mobile phase linear velocity was found to depend on the centrifugal field for the 30-mL rotor. This maximum velocity was not observed with the 80-mL rotor. Studying the changes in coumarin and carvone peak efficiencies, it is established that the number of cells required to make one theoretical plate, i.e. one chromatographic exchange, is minimized at maximal rotation speed and, to a lesser extent, at high mobile phase flow rate (or linear velocity). Considering the throughput, there is evidence of an optimal flow rate depending on the rotor rotation that is not necessarily the highest possible.

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1. Introduction

Countercurrent chromatography (CCC) is a separation technique that uses two liquid phases without solid support. Two main advantages compensate for the need of a centrifugal field to hold the liquid stationary phase steady while the liquid mobile phase percolates through it [1–3]. The first advantage is the high load possible in the volume of the liquid stationary phase compared to the overload problems commonly encountered with saturated surfaces of solid stationary phases. The second advantage is that CCC offers a huge selectivity panel since chemists can finely tune their solvent system to the sample to be purified. Solvent selection in CCC is crucial since it is selecting at the same time the stationary phase, which would be the column in other chromatographic techniques, and the mobile phase. Any composition change in one liquid phase may induce a change in the other liquid phase. To help in the del-

icate and time-consuming step of liquid system selection in CCC, databases now gather the literature worldwide experience [4,5].

Two types of CCC columns were made commercially available: i) the hydrodynamic CCC columns with rotating coils of simple tubing and ii) the hydrostatic CCC columns called Centrifugal Partition Chromatographs (CPC) with disks of interconnected cells. A great deal of efforts have been carried out since the past 10 years by suppliers to provide robust and efficient technologies, for both hydrodynamic CCC and hydrostatic CPC instruments. However, it is still not rare to encounter users complaining about long runs (hours) and broad peaks when working with CCC columns. This common observation comes from the fact that, while spending days to work on selectivity finely tuning the liquid system for the purification, the instrument operating parameters are overlooked and not optimized, leading to a false image of CCC and discouraging beginners. In 2005, Ito provided general rules for hydrodynamic CCC instruments [6]. In this work, we would like to study how the CPC operating parameters are related to throughput with a simple analytical separation. The numerous problems associated with large mass and/or volume injections were not examined.

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In CPC, the two main concerns are i) stationary phase retention, that influences retention volumes and hence resolution as well as time- and solvent-consumption, and ii) band broadening, related to peak sharpness, peak overlaps, resolution and final purity of the collected fractions. A special parameter, S_f , has to be introduced in CCC which describes variable stationary phase volumes [1–3]. S_f is defined as the ratio of V_S , the volume of stationary phase over V_C , the column volume. Two groups have extensively worked on S_f and band broadening in CPC. The group of Marchal from St Nazaire (France) developed an impressive work on mass transfer and flow regimes based on visualization using a specially designed CPC instrument with a transparent disk. They modeled mass transfer and efficiencies and proposed improvements of cell design [7–9]. Introducing the concept of height of a transfer unit (HTU), they established that increasing both centrifugal field and flow rate improved mixing, interfacial area and hence mass transfer. Schembecker at Dortmund (Germany) also used flow visualization to study flow patterns in a transparent disk CPC working with various solvent systems and comparing different cell designs. This group pointed out the impact of phase viscosity on stationary phase retention [10–12]. While suggesting improvements on cell design and a preferential selection of solvent systems with low interfacial tension, their advice on operating parameters is limited to the use of maximal rotation speed.

While these two groups provided a work of tremendous quality in understanding the effect of operating parameters on hydrodynamics, their main purpose remained CPC cell engineering and their tools, such as flow visualization instruments and mathematical models, seem only accessible to experts.

Our purpose is to confirm the general trends that were previously exposed and practically observed in CPC practice. The systematic study is based on a simple separation of standards working with two different commercial CPC instruments at low concentration. The influence of the mobile phase flow rate and rotor rotation speed (centrifugal field) on stationary phase retention, band broadening, resolution and throughput will be experimentally studied with these two different rotors and the same test solute sample (low concentration) and liquid system.

2. Experimental section

2.1. Apparatus

The frame instrument is a hydrostatic apparatus model, FCPC-A from Kromaton Rousselet-Robatel (Annonay, France) including safety casing, motor with its electronic regulation and a fan with a liquid cooling circulation. Its central shaft can receive interchangeable columns (or rotors). Two 32-cm diameter rotors were used. The first one is a commercially available rotor. It had a measured exact volume of 33.25 mL with 832 twin-cells with a number-eight shape at an average distance of 10 cm from the central axis of rotation. The Kromaton Company proposed to test a prototype rotor of larger volume designed for preparative purification at high flow rates. The prototype rotor has an exact volume measured as 83.4 mL with 406 twin-cells also at an average distance of 10 cm of the central axis. The exact shape of the cell is proprietary. The rotor could fit into the FCPC-A frame. All known characteristics of the two rotors are listed in Table 1. For convenience, the analytical and preparative rotors will be referred as the 30-mL and 80-mL rotors, respectively.

A refrigerated circulator F10-C Julabo (Colmar, France) was used to cool down the CPC instrument by flowing chilled water in the dedicated lines of the FCPC-A frame. A Puriflash integrated system from Interchim (Montluçon, France) was used for solvent delivery, injection and detection. This equipment is the assembly of a quaternary pump (flow rate from 1 to 60 mL/min, maximal pres-

sure 200 bar), an automatic loop injection valve fitted with a 10 mL sample loop, a UV/VIS dual wavelength spectrophotometer set at 254 nm and 280 nm and a fraction collector. An integrated computer with touch-screen allows for full apparatus control and data acquisition.

The volume of connecting tubing or extra-rotor volume has been measured to be 4.9 mL from injection to detection points.

2.2. Phase system and test solutes

All reagents were of analytical grade. Methanol, heptane and ethyl acetate as well as the three model solutes new coccine red, coumarin and carvone were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

The selected solvent system on all experiments was the heptane/ethyl acetate/methanol/water 1:1:1:1 (v/v) mixture also referred as Arizona N or HEMWat zero system [13]. After full equilibration, one liter of this solvent system splits at room temperature in two phases: 412 mL of the upper phase with heptane, ethyl acetate, methanol and water composition 62.5/34.4/2.6/0.5% v/v, density 0.752 g/mL, viscosity 0.40 cP or mPa.s, and 588 mL of lower phase with 0.1/18.3/39.9/41.7%v/v, density 0.898 g/mL, viscosity 1.45 cP or mPa.s. The phase density difference is 0.146 g/mL and the interfacial tension is 2.5 mN/m [11,13]. With the relatively polar test solutes selected, this solvent system was used in the reversed phase mode i.e. the mobile phase was the aqueous polar lower phase flown in the descending mode. The liquid stationary phase was the less polar organic upper phase.

The test solutes selected to carry on the study were picked up from the solute list proposed by Friesen and Pauli [14,15]. The selection was based on UV absorptivity and a wide polarity range implying a large range of partition coefficients in very different liquid systems. New coccine red is a charged compound that did not partition in the selected solvent system being exclusively located in the aqueous lower phase coloring it red. It was therefore used as a non-retained marker for mobile phase volume determination. Coumarin and carvone were selected with partition coefficient of 1.3 ± 0.3 and 7.5 ± 0.5 , respectively, in the 1:1:1:1 Arizona N or HEMWat 0 system. Coumarin spends as much time in the stationary organic phase as in the aqueous mobile phase of this system. Carvone favors the less polar stationary phase of the selected liquid system: it is a compound that exhibits a high retention factor in the selected solvent system. Resolution and efficiency will be studied with coumarin and carvone even at very low stationary phase retention volume ratio.

2.3. Experimental procedure

The rotor to be used, either the 30-mL standard rotor or the 80-mL prototype rotor, was installed inside the FCPC-A frame, connected to the upper and lower rotary seals and rinsed first with the lower phase and next with the upper phase of the HEMWat 0 (or AZ N) solvent system. The cooling unit was set to circulate water at 15 °C to remove calories out of the FCPC-A chamber whose temperature would otherwise rise due to heat generated by rotary seal rotation. With the rotor spinning at 2500 rpm, the FCPC-A chamber temperature was monitored stable at 21 °C.

For a given experiment, the rotor spinning at 600 rpm is entirely filled with the upper stationary phase at 5 mL/min (30-mL rotor) or 15 mL/min (80-mL rotor) in the descending mode. Then the rotation is set up at the speed needed for the experiment. Table 2 indicates the relationship between centrifugal fields and rotation speeds for the two rotors with cells at an average 10 cm distance from the central axis. After the working rotational speed is stabilized (less than 2 min), the lower aqueous mobile phase is pumped through the stationary phase in the descending mode. The driving pres-

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