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Scale-up in centrifugal partition chromatography: The "free-space between peaks" method

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ABSTRACT

Centrifugal Partition Chromatography (CPC) is a purification technique using a biphasic liquid system. As a preparative separation technique, scale-up is of primary concern. Once the separation is optimized on a lab-scale instrument, the scale-up transfer is quite straightforward simply using the instrument volume ratio as the linear transfer factor, thanks to the absence of solid support. Such linear transfer underestimates the performances of large-scale CPC rotors that are usually better than that of small rotors. It means that more material than predicted by the linear estimation could be purified. A fully practical method based on experimental observations is proposed. The first step is to determine experimentally the free space volume available between the two peaks of interest doing two analytical separations, one with the small analytical CPC instrument, giving ΔV_1, and the second with the large preparative one, giving ΔV_2. The second step is to determine on the small CPC instrument how much material can be loaded to reach the maximum mass load still giving the required purity and recovery ratio of the desired compound. Then, an accurate prediction of the maximal quantity of sample that the large-scale rotor can purify is simply obtained by multiplying the maximum mass load on the analytical CPC instrument by the free-space between peaks $\Delta V_2/\Delta V_1$ ratio. For demonstration purposes, the method is applied to the transfer of the CPC separation of a synthetic three-GUESS-compound mixture from a 35 mL-rotor to a semi-prep 239-mL rotor. The paper addresses also the operating condition optimization depending on industrial production strategy (maximal load per run or maximal productivity).

1. Introduction

Counter-current chromatography (CCC) is a liquid–liquid chromatographic technique, i.e. it requires only two immiscible liquid phases with no need for solid support [1,2]. One of the liquid phases is the stationary phase, maintained in the column by centrifugal forces. The other one is the mobile phase, pumped through the stationary phase.

Since CCC development in the mid-1960s by Yoichiro Ito [3], numerous column designs were conceived. However, only two designs were developed and commercialized: the hydrodynamic and hydrostatic systems [4]. Hydrodynamic systems are composed of Teflon tubing coiled on bobbins with two axes of rotation which generate variable centrifugal fields. On the contrary, hydrostatic systems, named Centrifugal Partition Chromatographs (CPC), are composed of steel disks stacked in a rotor spinning around a single rotation axis (constant centrifugal field). Inside each disk, interconnected cells of different shape and/or volume are engraved.

Due to the liquid stationary phase, CCC has numerous advantages compared to classic solid phase chromatography techniques, such as higher load capacity and no solute infinite retention [5]. However, despite a relatively lower solvent consumption in CCC, method development in high capacity rotors is not economically viable yet. Thus, manufacturers have recently introduced small columns for faster method development and optimization [6]. The purpose of these small volume instruments is to allow for the rapid development of the separation using minimal amounts of solvents and then to scale-up by transferring the optimized separations to higher column volumes for increased production.

Up to now, CCC scaling-up is usually performed at constant stationary phase retention ratio. Once the method developed on a small column volume is optimized, a scale-up factor is used to estimate the conditions required to work with the higher column volume. With hydrodynamic systems, due to the tube configuration of the apparatus, the scale-up factor can be calculated according to the ratio of the columns volumes or the ratio of the tube sections.

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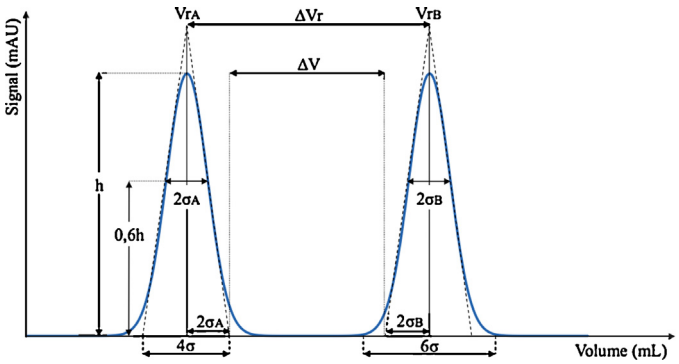

Fig. 1. Representation of the separation of two Gaussian peaks illustrating the concept of "free space between peaks", ΔV, compared to the retention difference, ΔV_r, used in the definition of the chromatographic resolution.

Luo [7] performed an analytical separation of four phenolic alkaloids using an 18-mL CCC column. He then transferred it on a 50-times larger 900-mL column. The strategy consists in increasing the flow rate and the solutes load by the same scale-up factor defined as the column volume ratio: 900/18 = 50. Results showed almost the same resolution because the stationary phase retention ratios were the same in the two CCC columns. However, this is not always the case: had the stationary phase retention been better on the preparative column, the resolution and the injected amount could have been higher. In hydrostatic systems, Sutherland [8] accomplished a scale-up example also using the ratio of the column volumes as the transfer factor. The myoglobin and lysozyme separation was optimized on a 500 mL hydrostatic column with an aqueous two-phase solvent system (ATPS). The transfer was performed on a 12.5 times larger column volume, i.e. 6.25 L working with a 12.5 times higher flow rate and injected protein amount. On this 6.25 L preparative separation, the protein resolution was greater than that observed for the 500 mL analytical separation: the sample load could have been increased significantly [8].

In these experiments, the scale-up factor based on the column volume ratio gives the flow rate and sample load to use on the larger column to have the same separation done in the same time. However, a better resolution is frequently observed with larger rotors, showing that a greater relative solute load could have been purified. Furthermore, it is often claimed that the scale-up factor must be determined for a similar stationary phase retention ratio in the small and large columns. This is not always feasible. In addition, it may be sometimes possible to work with higher stationary phase retention ratio in the larger rotors which might allow loading even more material on the preparative column.

The aim of this work is to develop a new practical scale-up methodology, in order to exactly predict the maximum loadable quantity in the large volume preparative rotor after optimization of a purification method on a small apparatus. This methodology will be developed on hydrostatic CPC columns but it should be adaptable to any device (hydrodynamic, hydrostatic) and with no parameter constraint. Several scaling-up issues will be covered: columns behavior, loading optimization, prediction of the maximal injectable load on the larger column and finally which column operating conditions should be specifically optimized to maximize productivity.

2. Experimental

2.1. Chemicals

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

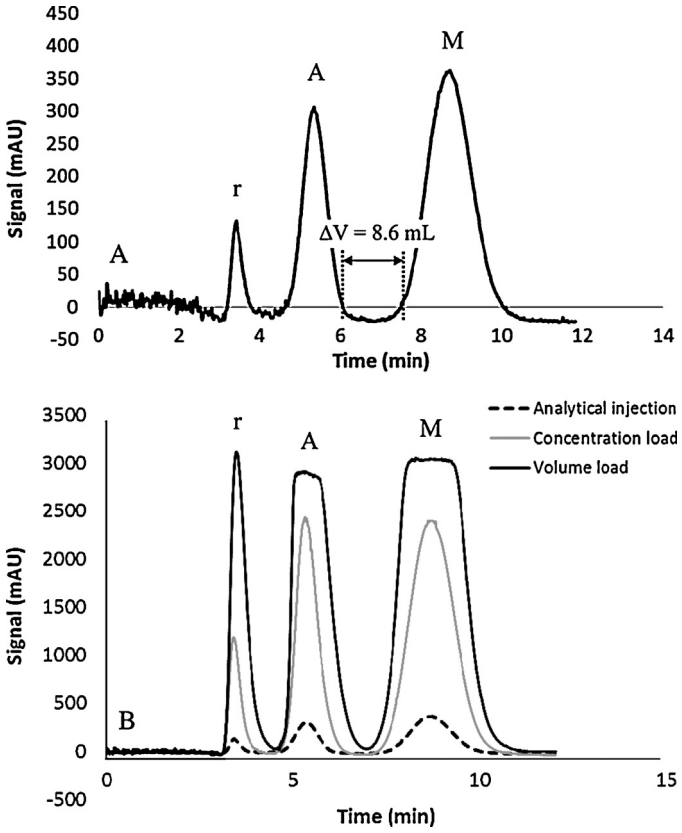

Fig. 2. (A) 35-mL rotor analytical injection of 0.36 mL (1% column volume) of 0.3 mg/mL new coccine red (r), 8.0 mg/mL aspirin (A) and 3.3 mg/mL coumarin (M) in descending mode. (B) 35-mL rotor optimization injections: analytical injection, concentration load and volume load. Solute concentrations, injected volumes and mass load are listed in Table 1. Column volume: 35 mL, descending mode, flow rate: 5 mL/min, system Arizona M, heptane/ethyl acetate/methanol/water 6/5/6/5 (v/v), 2400 rpm, stationary phase retention: 50%, 54 bar.

2.2. Instrumentation

The instrument is a hydrostatic apparatus model, FCPC-A from Kromaton Rousselet-Robatel (Annonay, France) with interchangeable columns (or rotors). The smaller analytical column has a volume of 35 mL whereas the semi-preparative rotor has a 239-mL volume. The two rotors were made of 13 stacked disks each containing 64 cells (8-shaped also called twin cells) making a total of 832 interconnected cells. Each cell of the 35-mL rotor has a volume of 31 μL with a connecting duct of 12 μL making the total cell volume 25.8 mL and the duct volume 10 mL or 28% of the rotor volume. Since during the chromatographic process the duct volume contains only mobile phase, it can be considered as "dead" volume making the theoretical maximal S_f value of the 35-mL rotor being 72%. Each cell of the 239-mL rotor has a volume of 220 μL with interconnecting duct of 67 μL making a total cell volume 183 mL and the duct volume 56 mL or 23% of the rotor volume. The larger 239-mL could theoretically retain a better S_f = 77% of stationary phase.

A refrigerated circulator F10-C Julabo (Colmar, France) was used to cool down the CPC instrument flowing chilled water in the dedicated lines. A Spot Prep II integrated system from Armen Instruments (Saint-Avé, France, a division of Gilson USA) was used. This equipment is the assembly of a quaternary pump (flow rate from 5 to 250 mL/min, maximal pressure 230 bar), an automatic