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Methodology for optimally sized centrifugal partition chromatography columns☆

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ABSTRACT

Centrifugal Partition Chromatography (CPC) is a separation process based on the partitioning of solutes between two partially miscible liquid phases. There is no solid support for the stationary phase. The centrifugal acceleration is responsible for both stationary phase retention and mobile phase dispersion. CPC is thus a process based on liquid–liquid mass transfer. The separation efficiency is mainly influenced by the hydrodynamics of the phases in each cell of the column. Thanks to a visualization system, called "Visual CPC", it was observed that the mobile phase can flow through the stationary phase as a sheet, or a spray. Hydrodynamics, which directly governs the instrument efficiency, is directly affected during scale changes, and non-linear phenomena prevent the successful achievement of mastered geometrical scale changes. In this work, a methodology for CPC column sizing is proposed, based on the characterization of the efficiency of advanced cell shapes, taking into account the hydrodynamics. Knowledge about relationship between stationary phase volume, cell efficiency and separation resolution in CPC allowed calculating the optimum cell number for laboratory and industrial scale CPC application. The methodology is highlighted with results on five different geometries from 25 to 5000 mL, for two applications: the separation of alkylbenzene by partitioning with heptane/methanol/water biphasic system; and the separation of peptides by partitioning with *n*-butanol/acetic acid/water (4/1/5) biphasic system. With this approach, it is possible to predict the optimal CPC column length leading to highest productivity.

1. Introduction

Centrifugal Partition Chromatography (CPC) is a separation process, developed by Murayama et al. [1] and based on the partitioning of solutes between two partially miscible liquid phases. A CPC column consists of a series of cells linked by ducts in cascade and arranged in a centrifuge (one axis, two rotary seals). The centrifugal acceleration (50–800 times the gravity) is responsible for both stationary phase retention and mobile phase dispersion. There is no solid support for the stationary phase. As a consequence, CPC can support larger mass overload than silica-based chromatography, making it especially suitable in the preparative scale.

As highlighted by Sutherland et al. [2], counter current separations technologies (including CCC and CPC apparatuses) present some advantages compared to conventional separations techniques. The first advantage of counter current technologies is that they provide high productivities (1 kg/day at a laboratory scale). When the chromatographic system is well optimized (*i.e.* both the stationary and the mobile phase as well as the development mode), the sample loading can be easily increased.

An other benefit noted by the authors is the robustness of the separations, due to the reproducibility of the column "packing", the stationary phase being a liquid. Moreover, these solid support free technologies did not require expensive packing materials and are more tolerant of particulate matter.

Finally they are also environmentally friendly processes thanks to their low solvent consummation (in comparison of conventional processes) and their capacity to be easily cleaned due to the liquid nature of the stationary phase (no adsorption phenomenon).

At first, the technology was mainly studied, described and developed at the laboratory scale [3]. Main developments of the

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separation process were on the solvent choice for biphasic system selection [4,5], on new separation methods (pH zone refining [6]; or ion exchange which are displacement chromatography modes [7]) or new application fields [8–10]. In the two past decades, technological improvements lead to new CPC apparatuses. Columns were built on the same principle; novelty was in the cell design. In fact, studies on flow pattern [11–14] put in evidence that the column efficiency is mainly controlled by the interfacial mass transfer kinetics and the stationary phase retention, both directly correlated to the two liquid phases hydrodynamics. Effects of the Coriolis acceleration, the entrance velocity and the phase properties on the observed flow patterns were demonstrated. The increase of the energy input (*via* larger rotational speed or flow rate) introduces instabilities in the flow patterns, going from single droplets to oscillating sheets and then sprays. New CPC cells were designed to promote the more dispersed flow patterns (spray conditions), good stationary phase retention and sufficient contact time in each cell [15–17].

Until a recent period, CPC users communicated on the fact that as CPC operates without overload, the scale change can be predicted by simple proportionality rule: one can inject twice more product, in a column which volume is two times larger, with a double flow rate. As an example, Sutherland et al. [18] presented a geometrical scale-up from a CPC apparatus of 0.5 L to a second one of 6.5 L using the ratio between the two column volumes as the single invariant number. The authors remarked that in scaling-up the method worked well and that the analytes separation was better at larger scale.

In another work, Hamzaoui et al. [19] allowed bringing out that the scale change was not precisely a linear phenomenon. The authors compared a Centrifugal Partition Extractor (CPE) column to a CPC one. The two apparatuses have close volumes (200 mL for the CPC and 303 mL for the CPE) but the CPE column has 231 cells while the CPC one has 1320 cells. The obtained separation was identical with an increased productivity by 6.7 while increasing the column volume by 1.5.

To realize a predictive scale change, the convenient invariant number(s), which take into account the relevant parameters, need to be determined. We propose here to introduce a correlation between dimensionless numbers that include: the two-phases properties, the operating conditions and the CPC cells size (on the Vashy–Buckingham theorem basis [20]). This approach was previously proposed by Zhao et al. [21] to control scale changes in CCC using the Sherwood number (Sh), the Schmidt number (Sc) and the rotational Reynolds number (Re). The correlation for CPC will be fitted to a large panel of experimental separations, giving a correlation for mass transfer efficiency prediction, whatever the scale or the conditions are. Validation of the method will be proposed for two applications and its use for CPC column engineering detailed.

2. Materials and methods

2.1. Visualization experimental device

The Visual CPC is a prototype designed to visualize and take pictures of the flow pattern within the cells of a CPC column (Fig. 1 on the top). In the Visual-CPC rotor, disks engraved with different cell's design and/or size can be inserted. The disk is clamped between a glass ring of optical quality (EDIVER, Rebais, France) and a steel plate. The rotor can be placed in vertical or horizontal position [22]. For the hydrodynamics study, commercial disks from Kromaton were used (Table 1). The cell designs are twin cells (Fig. 1, below).

Two pumps were used according to the column scale: an APTrix 1000 pump (Armen Instrument, Vannes, France) for flow rates from

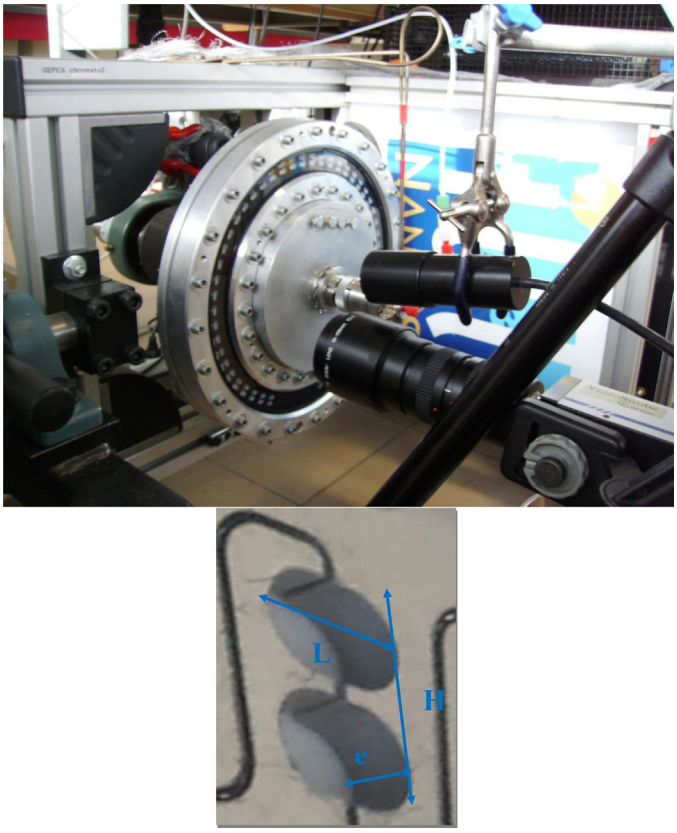

Fig. 1. Close view of the Visual CPC device used for flow pattern visualization (on the left) and description of a CPC twin cell (on the right) where *H* is the height, *L* the width and *e* the thickness of the cell.

30 to 1000 mL/min and a Spectra P4000 (ThermoQuest, San Jose, USA) for flow rates from 0.1 to 30 mL/min.

Two 6-port switching valves in peek material (UpChurch, Oan Harbor, USA) were used for the injections of the compounds and the selection of either ascending (upper phase mobile) or descending (lower phase mobile) mode. The CPC rotor is connected to the chromatographic system with 1/8″ PTFE tubing (UpChurch, Oan Harbor, USA) and two rotary seals (Tecmeca, Epernay, France). The rotational speed of the CPC rotor is comprised between 300 and 3000 rpm.

Solutes detection was achieved by a Spectra 100 UV detector (ThermoQuest, USA) linked to aSA32 data acquisition box (AOIP, Evry, France) recording 7–50 points per second.

2.2. Video instrumentation

A slightly modified Video Strobe system was used (Sysmat Industrie, St Thibault des Vignes, France), consisting of a TMC-9700 progressive scan charge-coupled device (CCD) color camera (Pulnix, Sunnyvale, CA, USA) with an asynchronous shutter, two Phylec stroboscopic units (Sysmat) and a VLS7T optical speed sensor (Compact, Bolton, UK) which triggered both stroboscopes and camera. The camera was equipped with an 18–108 mm F 2.5 TV zoom lens.

A Xenon lamp emitting a 2 μs flash was used. This short time flash allows "freezing" the acquired image, even for high rotational speed.

2.3. Centrifugal Partition Chromatography columns

The CPC columns were manufactured by Rousselet–Robatel–Kromaton (Annonay, France). Twin cells at different scales were used for columns between 38 mL (FCPC25)