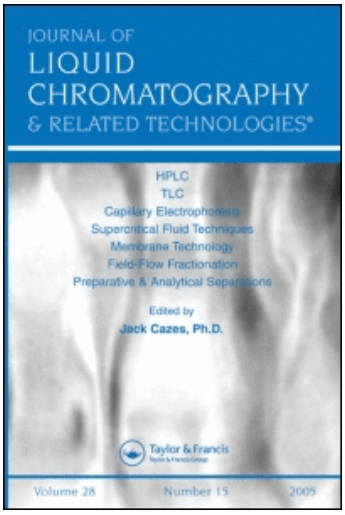

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Partitron 25, a Multi-Purpose Industrial Centrifugal Partition Chromatograph: Rotor Design and Preliminary Results on Efficiency and Stationary Phase Retention

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Partitron 25, a Multi-Purpose Industrial Centrifugal Partition Chromatograph: Rotor Design and Preliminary Results on Efficiency and Stationary Phase Retention

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Abstract: A new preparative chromatograph with a capacity of 25 liters has been designed to meet the most rigorous industrial criteria. All parts contacting liquid phases are made of titanium. The rotor, cast in one piece, can be operated up to 150 bars (2,000 psi). Preliminary results on retention of the liquid stationary phases from non-aqueous and aqueous–aqueous biphasic solvent systems are presented and commented. A special emphasis is made on the theoretical plate number generated by each individual partition cell. The results highlight the benefit of a new concept of rotor featuring a better mass transfer than that observed in the stacked disk rotors. Finally, the use of neoteric solvents (ionic liquids, supercritical carbon dioxide) is mentioned.

Keywords: Centrifugal partition chromatography CPC, Counter-current chromatography CCC, Industrial chromatography, Proteins, ATPS

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INTRODUCTION

Since its introduction by Dr. Yoichiro Ito in 1966, Counter-Current Chromatography (CCC)[1] has become very popular among natural products chemists,[2,3] pharmacognosists, and also chemists dealing with extraction-separation of metallic cations.[4]

The success of industrial HPLC is mainly linked to new needs originating from the development of more structurally complicated active molecules associated with more stringent FDA recommendations, making chromatography essential. These needs continue to increase, making CCC more and more attractive not only for its separation performances, but also for reduced purification costs and environmental linked problems.

Unanimously recognized as a preparative technique, it is astonishing to note that CCC just started, very recently, to be scaled up to the industrial production level with the help of the pharmaceutical industry.[5] The British team at Brunel (London, UK) developed preparative devices based on the Ito hydrodynamic two-axis CCC design.[5] We choose to focus on the hydrostatic single-axis CCC design.

In order to develop a production scale machine we had to consider the critical parameters required in industry. First of all, the machine has to be reliable to allow semi-continuous operation by a succession of identical purification cycles over at least 100 hours (one week). Secondly, the productivity of the processes has to be at an industrial level: long elution times at low flow-rates, often practiced in laboratory, are no longer acceptable. Consequently, high flow-rates are absolutely necessary. These considerations reduced the choice to hydrostatic mono-axis [Centrifugal Partition Chromatography (CPC)] and hydrodynamic bi-axis [Counter-Current Chromatography (CCC)] instruments. We focused on the former.

This preliminary communication presents the original solutions, which have been successfully developed for the design of an industrial CPC counter-current chromatograph. Chromatographic performances, evaluated using a non-aqueous biphasic solvent system and an aqueous two phase system (ATPS) using the current CCC methods of evaluation, have validated the new concepts. These preliminary results make Partitron 25 the prototype of a new class of very powerful instruments and a serious alternate to production HPLC.

EXPERIMENTAL

Reagents

Acetonitrile, heptanes (mixture of isomers), both of prep HPLC grade, were from Carlo Erba. Naphthalene, and myoglobin were from Sigma-Aldrich.

Polyethylene glycol, PEG 1000, and di-potassium hydrogen phosphate were from Acros Organics. Water was deionized.

Apparatus

The Partitron 25 CPC (Figure 1) of dimensions: 1.50 (L) × 1.00 (D) × 1.85 (H) m; weight: 1,200 kg (see description of the rotor in Results and discussion) was connected through 2 rotating seals (tungsten carbide, 150 bar; 1,500 rpm) from Deublin (Vaukegan, IL, USA) to: A Varian PrepStar pump fitted with 800 mL/min pump heads for mobile and stationary phase delivery; an Isco 500D syringe pump for sample injection; a Knauer K-2501 UV detector, and a Kipp and Zonen recorder. The experiments have been conducted at room temperature ($25^\circ \pm 1^\circ$C). The temperature was just measured, not controlled.

Figure 1. The Partitron 25 hydrostatic preparative chromatograph, 1.5 m (L) × 1 m (D) × 1.85 m (H), weight: 1,200 kg (control panel not shown).

Solvent Systems

The biphasic solvent system heptanes-acetonitrile : 1 : 1 (v/v) was prepared by mixing equal volumes of solvents, shaking, and settling. The system PEG 1,000 K_2HPO_4 : 12.5% : 12.5% (w/w) was prepared by dissolving 6.25 kg of each compound in 37.5 liters water to obtain 50 kg of the aqueous two phase liquid system (ATPS).

RESULTS AND DISCUSSION

Partitron 25 Rotor Design

With over 10 years of R & D experience with a Sanki Engineering Limited (now EverSeiko Corp.) laboratory scale 245 mL model LLB-M and a pilot scale 5.4 L model LLI-7 chromatographs, we concluded that an industrial production scale model should have at least a capacity of 25 liters and feature high pressure limits. Indeed, high flow-rates are required to meet the modern productivity needs. High flow rates mean a high centrifugal field to avoid bleeding of the stationary phase, that is to say high rotational speeds and, consequently, high back-pressures.

The pressure limit of about 60 bars of the two Sanki models was imposed, not only by the rotating seals, but also by the stacked disk assembly, which needs Teflon® sheet gaskets between the individual disks. The assembly is held in place by bolting, and a limited pressure is applied to the mechanical assembly by bolts to prevent excessive creep of the Teflon®. Larger disks would exacerbate the problem. Therefore, a more robust rotor was designed in order to meet the high pressure and industrial production requirements. This purpose was achieved by drilling cylindrical partition cells radially through the wall of a cylindrical one-piece metallic cylinder. Titanium was chosen for its lightness, chemical inertness, and biocompatibility. The cells are arranged side by side in the cylinder body and connected in series to each other by ducts also radially drilled through the cylinder wall. The cells are distributed along a helical spiral around the axis of the rotor cylinder. Connection of cells with ducts are made by removable closings or plugs in which a communication channel has been hollowed out to connect a cell to its associated duct. These closings are in contact with a fluoro-elastomer seal and held directly or indirectly by a screwing element. Unscrewing of an outer closing allows direct access to an individual cell without having to dismantle the whole rotor (Figure 2).

Cells and ducts are linked axially, under rotation of the rotor the incoming mobile phase flow is diverted by the Coriolis force on the cell wall, lowering the efficiency of mass transfer.[6,7] This phenomenon has been avoided by filling the cylindrical cells with tampons made from knitted stainless steel or titanium wire (Multiknit®, Tissmetal, Reims, France, Figure 3). Multiknit®

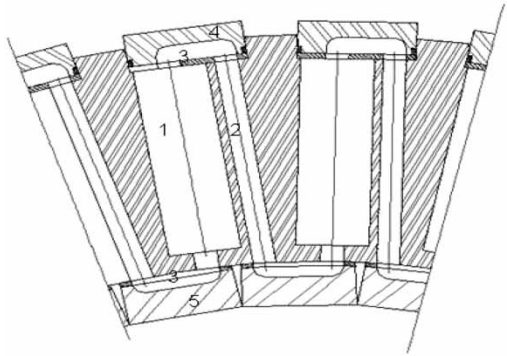

Figure 2. Cross section of the rotor wall. 1-cell (7 × 2.2 cm L × ϕ; ∼27 mL), 2-duct (7.8 × 0.7 cm L × ϕ; ∼4 mL), 3-communication channel (∼1.6 mL), 4 & 5 bolted closings.

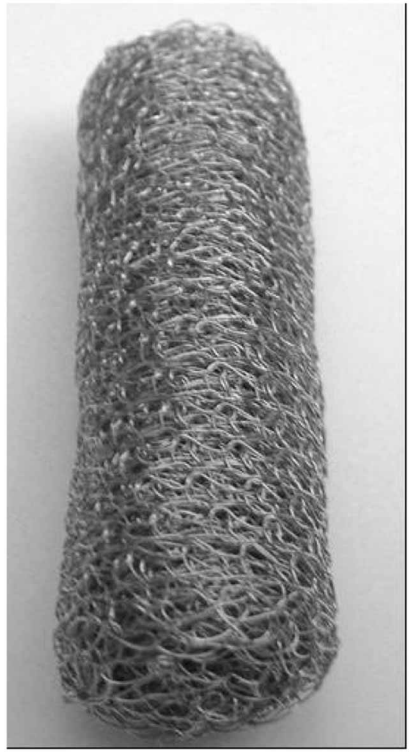

Figure 3. Multiknit® tampon 70 × 22 mm. Titanium wire 200 μm, 10 m, 0.3 mL.

tampons are used in column distillation for their efficiency and for a negligible volume occupancy and back-pressure. The Partitron 25 has been designed to enable good manufacturing practice (GMP) compliance, and is currently validated in commercial pharmaceutical processes (Figures 4–6). More information on the complete cleaning of all cells between cycles will be the subject of coming publications.

Specifications

Rotor: 60 × 73 cm (Ø × H), 766 cells of size 70 × 22 mm (L × Ø), ducts: Ø 7 mm, duct volume: 17.2%, nominal total volume: 25.022 mL, operating volume with tampons: 22,400 mL, relative centrifugal force at 1,500 rpm: ~600 × g, power: 7.5 HP (5.7 kW), 380 V AC, 15 A, three electrical phases, process parts: titanium, Teflon®, weight: 1,200 kg (2,645 lbs).

Chromatographic Performances

Using Heptanes-Acetonitrile System for a Low Molecular Weight Solute

Before constructing the 25 L titanium rotor, a reduced 5 L aluminium rotor with identical characteristics, except a reduced cell number: 146

Figure 4. Detail from outer rotor surface. The couple of cavities above cell and duct are for bolts.

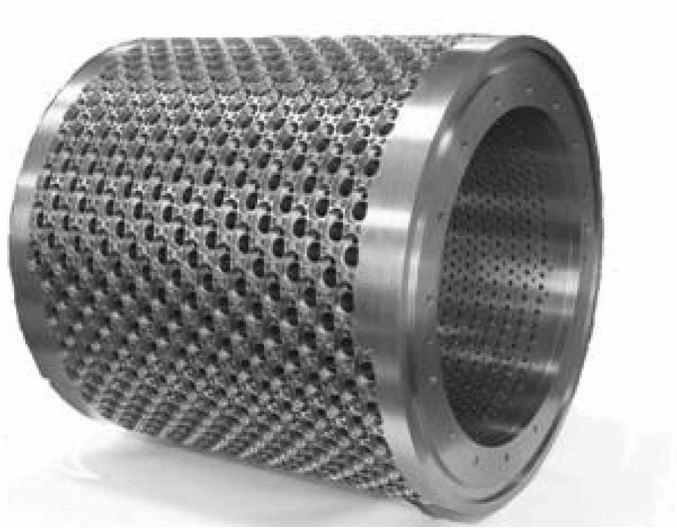

Figure 5. Rotor without closings (almost 500 kg of pure titanium, 60 × 73 cm (Ø × H)).

instead of 766 was built. Using this smaller rotor with the system heptanes-acetonitrile:1:1(v,v) in descending mode, naphthalene was used as a test solute to evaluate the apparatus performances (Table 1).

At 1,300 rpm with an acetonitrile mobile phase at a 200 mL/min flow rate, the retention of stationary phase was about 84% (4.2 L of heptane phase retained) and the back-pressure was 43 bars. The theoretical plate number for the naphthalene peak was found higher than the cell number: 177 plates versus 146 cells. This means that one physical cell is able to generate 1.2 theoretical plates (0.8 plate without Multiknit). This was never seen before with hydrostatic CCC devices that always needed several channels, locules, or tubes to generate one theoretical plate. For example, under the same conditions, the 5 L LLI-7 chromatograph from Sanki generates only 400 plates for 1040 cells. It is almost three cells for one plate, or 0.38 plate per cell. The tampon and cell design are responsible for the very good liquid phase mixing and consecutive increase of the solute mass transfer between phases inside each cell.

Scaling up to the 25 L rotor, at 900 rpm with an acetonitrile mobile phase at 500 mL/min flow rate, the back-pressure was 93 bars and the naphthalene plate number only 610, somewhat less than the cell number (766 cells). Since less heptane phase was retained (only about 19 L or Sf = 75%) due to the high 0.5 L/min flow rate, this 1.2 cell needed to generate one theoretical plate may be considered as good compared to classical machines (3 to 5 cells per plate). At 1,300 rpm the back-pressure crossed the 120 bar pressure limit of the Varian Prep star pump equipped with the 800 mL/min heads and it was not

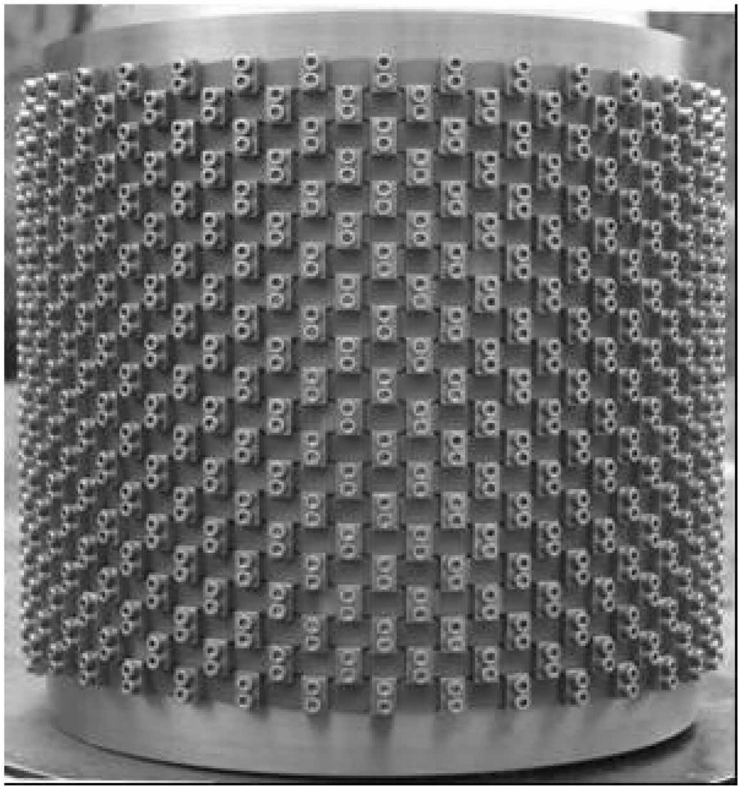

Figure 6. Rotor with closings (60 × 73 cm (ØxH), 766 cells).

possible to work or to measure the expected 900 Plates. These preliminary results confirm the interest of a high centrifugal field for optimizing the retention and the dramatic effect of the Multiknit® lining on efficiency.

Using an Aqueous—Aqueous Polymer Phase System for a Protein

The system PEG 1,000—dibasic potassium phosphate, 12.5%:12.5% (w/w) has been extensively studied for protein separation on the Sanki LLB-M

Table 1. Theoretical plate number N for naphtalene on the 146 cell, 5 liter aluminium rotor of the Partitron 25

Flow-rate (mL/min)	200	200	400
Speed (rpm)	900	1,300	1,300
Theoretical plates	137	175	177

CPC and on the cross-axis coil planet centrifuge by K. Shinomiya et al.[7,8] This system is selected to check its phase retention in the new device.

The rotor was filled with the upper PEG-rich phase and the lower phosphate-rich phase was used as the mobile phase pumped at 200 mL/min in the descending mode under a 700 rpm rotation. The equilibrium was reached after expulsion of 8.3 L of PEG-rich stationary phase corresponding to a Sf retention ratio of 62.8% and a pressure of 70 bars. Next, the speed was increased to 800 rpm and the flow-rate to 300 mL/min, giving a new equilibrium at 84 bars with the same Sf = 74% (no PEG phase bleeding). After injection of 250 mL of a 2 g/L myoglobin solution (500 mg injected in a 1:1 mixture of both ATPS phases), the myoglobin peak eluted with a 14 L retention volume (45 min retention time) with about 580 theoretical plates. The retention of stationary phase, Sf = 74%, is in accordance or better than the published[8] 55 to 65% values obtained with the same ATPS on a Sanki LLB-M CPC. However, the efficiency is 5 times higher, undoubtedly as a consequence of the presence of the Multiknit® lining working as a mixer-disperser of the mobile phase in the stationary phase.[9] From this, though unique, experiment it can temporarily be concluded that the new cell design makes the Partitron 25 CCC hydrostatic instrument especially suitable for protein purification. As more experimental data will be available, these preliminary conclusions will be firmly established.

CONCLUSION

The Partitron 25 apparatus is able to retain high volumes of stationary phase including the difficult-to-retain aqueous two phase systems. It makes it an apparatus of exceptional value for the large scale purification of proteins. Of particular interest are the monoclonal antibodies directed toward tumors, which are currently worldwide under development. The absence of dead zones and the ease of sanitization make it particularly suitable for the isolation of antibodies from plants (farming) or cell cultures. From a speculative point of view, the high operating pressure could allow for the use of supercritical carbon dioxide, which is known as an efficient diluent for ionic liquids, making the combination of these neoteric solvents attractive for the near future of "green chromatography". In these conditions, the new Partitron 25 could well be used as a reactor-separator.

The actual 150 bar pressure limit will be increased in the forthcoming series of machines to 200 bars by preparing larger rotor diameters up to 100–110 cm and, subsequently, reducing the maximum rotational speed from 1,500 to 500 rpm. These changes will permit use of rotary seals working up to 200 bars with L/min flow rates. This extension of the working pressure will correlatively allow a greater number of separation cells, i.e., 1,000 to 1,500 in order to pass the 2,000 theoretical plate count in

efficiency. The apparatus volume could also be extended as high as 30 to 50 liters.

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