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

This new chromatograph with a capacity of 25 liters has been designed to meet the most rigorous industrial criteria. All wetted parts are made of titanium and 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 N 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.

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].

Unanimously recognized as a preparative technique, it is astonishing to note that CCC has not, up to now, been scaled up to the industrial production level in spite of several assets of value for the pharmaceutical industry.

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.

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 direct the choice between mono-axis [Centrifugal Partition Chromatography (CPC)] and bi-axis [Counter-Current Chromatography (CCC)] machines undoubtedly towards the former.

We present here 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 performant machines and a serious alternate to production HPLC.

EXPERIMENTAL

Reagents

Acetonitrile, heptanes (mixture of isomers), both of prep HPLC grade, were from Carlo Erba

Naphtalene , and Myoglobin were from Sigma-Aldrich. Polyethylene glycol PEG 1000 and di-Potassium hydrogen phosphate were from Acros Organics. Water was deionized.

Apparatuses

The Partitron 25 CPC (fig. 1) of dimension: 1.50 (L) x 1.00 (D) x 1.85 (H) m; weight: 1,200 kg (see description of the rotor in section Results and discussion) was connected through 2 rotating seals (tungsten carbide, 150 bars; 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 (26° ± 1°C).

Solvent systems

Biphasic solvent system heptane - acetonitrile: 1: 1 (vol. / vol.) 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 solute in 37.5 liters water.

RESULTS AND DISCUSSION

Partitron 25 rotor design[7]

With an over 10 years R & D experience with Sanki Engineering Limited (now EverSeiko Corp.) laboratory scale 245 ml model LLB-M and pilot scale 5.4 liters model LLI - 7, we concluded that an

industrial production scale model would have at least a capacity of 25 liters and feature a higher pressure limit.

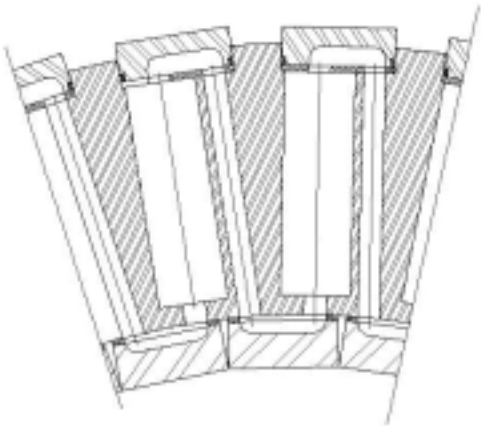
Actually, high flow-rates enforced by productivity need high centrifugal fields to avoid bleeding of the stationary phase, that is to say high rotational speeds and consequently high back-pressures.

The pressure limit of about 60 bars of the above models is imposed, not only by the rotating seals, but by the stacked disk assembly which needs Teflon® sheet gaskets between the individual disks. The assembly is held in place by bolting and a limiting pressure is applicable to the mechanical assembly by bolts to prevent excessive creep of the Teflon®.

Larger disks would exacerbate the problem.

Therefore, we decided to overcome this disadvantage by designing a robust rotor in order to push back well this pressure limitation beyond the hundred bars and which can be used in industrial production.

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 body and connected in series to each other by ducts too radially drilled through the wall.

The cells are distributed along a helical spiral around the axis of the rotor.

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 an associated duct.

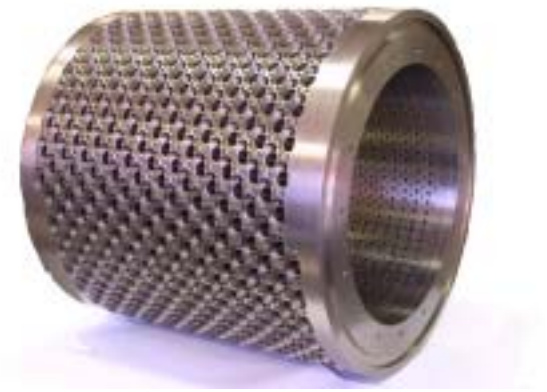
These closings are in contact with a fluoro-elastomer seal and held directly or indirectly by a screwing element.

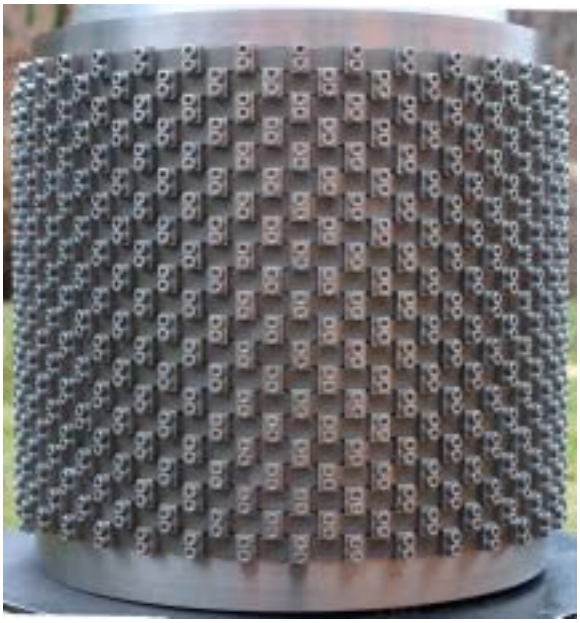
Unscrewing of an outer closing allows direct access to an individual cell without having to dismantle the whole rotor.

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 [5, 6]. This phenomenon has been avoided by filling the cylindrical cells with tampons made from knitted stainless steel or titanium wire (Multiknit®, TISSMETAL, Reims, France).

Multiknit® tampons are used in column distillation for their efficiency and for a negligible back-pressure.

Partitron 25 has been designed to enable cGMP compliance and is currently validated in commercial pharmaceutical processes.





Specifications

Rotor: 60 x 73 cm (∅ x H) 466 kg

Cells (766): 70 x 22 mm (Lx∅)

Ducts: ∅ 7 mm

Duct volume: 17.2%

Nominal volume: 25,022 mL

Operating volume with tampons: 22,400 mL

Relative centrifugal force at 1,500 rpm: ~ 600 x g

Power: 7.5 HP, 380 V AC, 15 A, 3∅

Process Parts: titanium, Teflon®

Weight: 1,200 kg

Chromatographic performances

Using heptane - acetonitrile system for a low molecular weight solute

Before constructing the 25 liter titanium rotor we made a 5 liter aluminium rotor with identical characteristics except a reduced cell number: 146 instead of 766.

Using this rotor with the system heptane - acetonitrile: 1: 1(v,v) in descending mode, we measured for naphthalene injections:

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

The retention of stationary phase was 84% at 900 rpm, and the back-pressure at 1,300 rpm and 200 ml/min was 43 bars.

The theoretical plate number is higher than the cell number: 177 versus 146 and one physical cell generate 1.2 theoretical plates. Removal of the Multiknit tampons lower this value to 0.8 theoretical plate.

Under the same conditions, the 5 liters LLI-7 from Sanki generates only 400 plates for 1040 cells, or 0.38 plate per cell.

Scaling up to the 25 liter rotor could be done at 900 rpm: at 500 ml/min the back-pressure was 93 bars and the plate number 610, somewhat less than the expected 715, probably because of the lowered retention of 75% following the increase in flow-rate from 200 to 500 ml/min.

At 1,300 rpm the back-pressure crossed the pressure limit of the Varian Prep star pump equipped with the 800 ml/min heads (probably in the range 120 - 130 bars) and we couldn't 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

We chose the system PEG 1,000 - dibasic potassium phosphate: 12,5%: 12,5% (w/w) which has been extensively studied for protein separation on the Sanki LLB-M CPC and on the cross-axis coil planet centrifuge by K. Shinomiya *et al.*[6,7]

The rotor was filled with the upper PEG phase and the lower phosphate phase was pumped at 200 ml/min under rotation at 700 rpm. The equilibrium was reached after expulsion of 9.0 liters of stationary phase corresponding to retention of 60% and a pressure of 70 bars.

The speed was increased to 800 rpm and the flow-rate to 300 ml/min giving a new equilibrium at 84 bars with no bleeding.

After injection of 500 mg myoglobin in solution in 250 ml of a 1: 1 mixture of both phases, we measured 579 theoretical plates for the peak eluting after 45 minutes.

The retention of stationary phase is in accordance with the published[6] 55.0/65.5% for the same ATPS on a Sanki LLB-M CPC, but the efficiency is 5 times higher, undoubtedly as a consequence of the presence of the Multiknit® lining working as a disperser of the mobile phase.

From this, though unique, experiment we can nevertheless conclude that the new cell design makes Partitron 25 especially suitable for protein purification.

CONCLUSION

Partitron 25 has high retention values for the stationary phase including aqueous two phase systems making it of exceptional value for the purification of proteins. Of particular interest are the monoclonal antibodies directed toward tumors and 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.

The high operating pressure allows 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" and for the use of Partitron 25 as a reactor-separator.

The actual pressure limit of 150 bars will be brought in the forthcoming series of machines to 200 bars by increasing the rotor diameter from 60 to 100 -110 cm and subsequently reducing the maximum rotational speed from 1,500 to 500 rpm permitting thus the use of rotary seals working up to 200 bars.

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 reach 2,000 theoretical plates and capacities in the range of 30 to 50 liters.

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