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# High-resolution protein separation using a laterally-fed membrane chromatography device

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ABSTRACT

Radial-flow membrane chromatography devices which are used for flow-through separation are generally unsuitable for bind-and-elute chromatography, particularly where multiple components need to be separated. We discuss a laterally-fed membrane chromatography device, suitable for high-resolution, multi-component protein separation in the bind-and-elute mode. In the current study, a stack of cation exchange membranes was housed within the membrane device and its performance was compared with an equivalent commercial radial-flow device having the same membrane bed volume and bed height. Tracer experiments were carried out using sodium chloride solution to compare their residence time distributions. The laterally-fed device showed superior flow distribution characteristics, which could be attributed to a lower variability in solute-flow path-lengths, and a smaller dead volume. Single protein bind-and-elute experiments carried out using lysozyme showed that the peaks obtained with the laterally-fed device were significantly sharper and more symmetrical. Excellent separation of three model proteins ovalbumin, conalbumin and lysozyme demonstrated that the laterally-fed membrane chromatography device was indeed suitable for carrying out high-resolution multi-component protein purification. These proteins could be fractionated in about 10 membrane bed volumes using the laterally-fed device as opposed to 25 bed volumes with the radial-flow device. The design of the laterally-fed device is simple and its flat shape gives it significantly lower footprint and offers additional advantages such as stackability and ease of multiplexing.



## 1. Introduction

Downstream processing capacity in the biotechnology industry has struggled to keep up with the remarkable improvements in both bioreactor titer and volume. Chromatography, which is the linchpin in most purification processes, has become the major bottleneck in bio-manufacturing [1]. Economy of scale does not exist for large columns due to the high resin cost. Also, the resolution decreases with increase in scale. Membrane chromatography has long been suggested as a fast and cost-effective alternative to resin-based column chromatography [2–7]. The predominantly convection-based transport of target bio-molecules to and from their binding sites on a membrane, as opposed to the largely diffusion-limited mass transport of these molecules within the resin bed makes membrane chromatography significantly faster [2–7]. Speed of separation translates to both higher productivity and lower product degradation. Also, in membrane chromatography, the efficiency of binding of even large solutes such as monoclonal antibodies is relatively independent of the superficial velocity. This offers significant flexibility in process design. Other advantages include lower buffer usage and pressure drops, and the absence of problems such as channeling and fracturing of resin beds. Moreover, the disposable nature of membrane devices eliminates the need for cleaning and validation steps, and thereby contributes toward practicality and ease of use [8,9].

Devices for membrane chromatography are commonly available in the stacked-disk and radial-flow formats [10,11]. The use of hollow-fiber membrane devices has also been reported but they are not very commonly used, and are not suitable for pulse chromatography [12,13]. Stacked-disk membrane devices are most commonly used for laboratory-scale separations and process development. However, it is impractical to use these devices in large-scale processes as when their diameter is increased, the radial to axial dimension aspect ratio, and thereby the variability of flow path lengths become large enough to severely affect separation performance. Stacked-disc devices which incorporate efficient flow-distribution features have been shown to be suitable for high-resolution protein fractionation [14]. However, even with these improved devices, the separation efficiency decreases when the diameter exceeds 50–60 mm. Radial-flow devices which are

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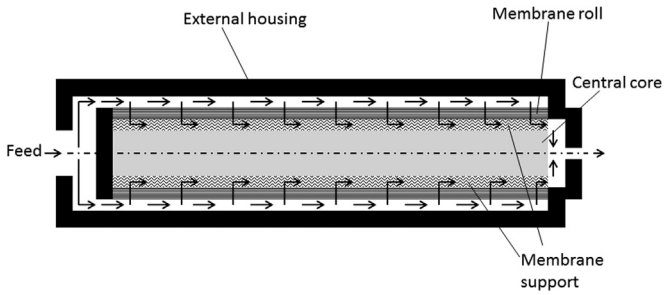


**Fig. 1.** Radial-flow membrane chromatography device.

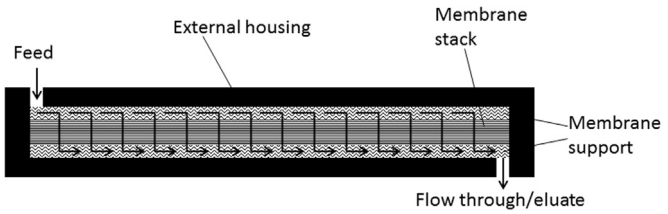


**Fig. 2.** Laterally-fed membrane chromatography device.

made by wrapping long pieces of flat-sheet membranes over central collection channels are commonly used in large-scale applications [15–17]. These devices offer large cross-sectional area to bed volume ratios, can be operated at low back pressures, and are scalable. They perform quite well in flow-through separations where the target bio-molecules are typically collected as unbound material going through the device, while impurities that are present in relatively small amounts are adsorbed. Major applications of radial-flow devices include polishing of monoclonal antibodies (mAbs), i.e. removal of impurities such as host cell proteins (HCPs), endotoxins, and viruses [18–22].

While membrane chromatography is widely used for polishing applications in the biotech industry, there has been very limited use of this technique for large-scale purification in the bind-and-elute mode, particularly where multiple components need to be separated [23]. As shown in Fig. 1, a radial-flow device has a complicated design and thereby a complicated flow-path. The liquid is first distributed in a radially outward direction to the annular space surrounding the membrane roll. It is then forced into the membrane in a radially inward direction and collected on the outer surface of the central core which supports the membrane roll. As demonstrated in this paper, such complexity creates large variability in flow path lengths within the device, resulting in dispersion effects such as peak broadening and loss of peak resolution. It is also difficult to balance the pressure drop in the axial direction on both sides of the membrane. The feed space is typically an open channel while the permeate collection space consists of either a porous or grooved structure. Pressure balancing is critically important for efficient membrane utilization as is discussed at the end of the paper. The effective membrane area in a membrane roll changes along the bed height, i.e. decreases in a radially inward direction. This results in an increase in superficial velocity in a radially inward direction, which could adversely affect chromatographic separation. Radial-flow devices have large dead volumes, both on the feed and the permeate sides, which could aggravate the above dispersion effects. Also, the large supporting central core does not play any active role in the separation process, and therefore device volume utilization is poor. While none of the above factors are perhaps critically limiting in separations carried out in the flow-through mode, these could adversely affect high-resolution, multi-component separations carried out in the bind-and-elute mode. Elution chromatography is very subtle process where desorption of bound species from an adsorbent bed is dependent on the uniformity of species binding and sensitive to the manner in which the eluent composition changes within the bed.

We feel that availability of membrane devices which address the above issues would contribute to the wider acceptance and use of membrane chromatography in the biotechnology industry. In our recent studies, we have compared the performance of such a laterally-fed device with a stacked-disc membrane device [24,25]. The laterally-fed device outperformed the stack-disc device in terms of all the attributes examined, i.e. flow distribution, membrane binding capacity utilization, and peak resolution. In the current paper, we discuss how a laterally-fed membrane chromatography device could be used to carry out high-resolution, multiple-component protein separation in the bind-and-elute mode. The device (see Fig. 2) is designed to house a stack of rectangular flat sheet adsorptive membranes. Liquid is laterally distributed over the feed-side of the device and thereby enters the membrane stack at different locations along its length, eventually emerging at corresponding locations on the permeate side, where it flows laterally to the outlet of the device. This configuration makes it possible to balance the pressure-drop on the feed side with that on the permeate side, thereby ensuring uniformity of flow along the length of the membrane stack. Also, unlike in a radial-flow device, where the superficial velocity within the bed increases in a radially inward direction, the flow of liquid is anticipated to be more uniform in the laterally-fed membrane chromatography device. As idealized in Fig. 2, the flow path lengths are similar throughout the device. This is expected to improve both, the efficiency of membrane utilization, and the resolution of eluted peaks in chromatographic separation.

In this paper, we discuss the design, fabrication and use of a laterally-fed membrane chromatography device, within which a stack of rectangular flat sheet cation exchange membranes, having a bed volume of 7 mL was housed. The performance of this device was compared with that of an equivalent commercial radial-flow device having the same bed volume and bed height. Tracer experiments were carried out using sodium chloride in both pulse- and step-input modes to investigate their residence time distributions. Single protein, bind-and-elute experiments were then carried using lysozyme and peaks thus obtained were compared. Protein separation experiments were then carried out in the pulse-mode using ovalbumin as the model unbound protein, and conalbumin and lysozyme as model bound proteins, to demonstrate the suitability of the laterally-fed device for carrying out high-resolution, multi-component, protein purification. The results obtained are discussed.

## 2. Materials and methods

Lysozyme (L6876), conalbumin (C7786), ovalbumin (A5503), sodium phosphate monobasic (S0751), sodium phosphate dibasic (S0876), citric acid (C0759), sodium citrate dihydrate (S4641), sodium chloride (S7653), hydrochloric acid (258148), ammonium persulfate (A3678), 30% acrylamide solution (A3699), glycerol (G2025), bromophenol blue (B0126), Brilliant Blue R concentrate (B8647), glycine (G8898), sodium dodecyl sulfate (L3771), Trizma-hydrochloride (T3253), Trizma base (T1503), N,N,N′,N′-tetramethyl ethylenediamine (T9281), and DL-dithiothreitol (43817) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (1000-1) and methanol (6700-1) were purchased from Caledon Laboratories LTD. (Georgetown, ON, Canada). Ultra-4 centrifugal filters (3 kDa MWCO, UFC800324) were purchased from EMD Millipore Co. (Billerica, MA, USA). RTV 108 silicon-based adhesive was purchased from MOMENTIVE (Columbus, OH, USA). Sartobind S cation-exchange membrane sheets (94IEXS42-001) and Sartobind SingleSep Mini (92IEXS42D4-SS-A, 7 mL bed volume) were