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Using a box instead of a column for process chromatography

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ABSTRACT

Columns with relatively short bed-height to diameter ratios are frequently used for process-scale chromatography applications such as biopharmaceutical purification. Non-uniform flow distribution within such columns could result in broad and poorly resolved eluted peaks, which could in turn affect purity, recovery and productivity of the process. Different strategies centered on improved column header design have been proposed for addressing this problem. This paper describes a radically different approach, i.e. the use of a chromatography box (or chromato-box) instead of a column, for addressing the challenges posed by flow mal-distribution in process-scale, packed-bed chromatography devices. The design of the chromatography box devices used in this study is based on a laterally-fed membrane chromatography (or LFMC) device, that has been described and discussed in several recent papers. The performances of two chromatography box devices were compared with their equivalent columns in terms of sharpness and asymmetry of flow-through and eluted peaks, number of theoretical plates per metre, and peak resolution in binary and ternary protein separations. In each of the above comparisons, the chromatography box devices performed better than their equivalent columns, clearly indicating their potential as an alternative in process-scale chromatography applications.

1. Introduction

Columns are so ubiquitous in chromatography that the use of the word "chromatography" without a qualifier such as thin layer- or membrane- implies by default, column chromatography. Large-scale or process chromatography is almost entirely carried out using columns [1,2]. While membranes [3,4], monoliths [5,6] and other alternatives [7,8] are gradually finding acceptance in niche applications in biopharmaceutical purification, columns continue to hold sway. All initial work on preparative chromatography including those carried out by Mikhail Tsvett, the inventor of chromatography, was based on the use of columns i.e. a device of circular cross-section [9]. A column is easy to pack with stationary phase, and the flow of mobile phase within it is axis-symmetric. Samples can therefore be conveniently distributed in a symmetric fashion over its entire cross-section, and it is easy to visualize the segregation of separated bands of solutes as they move towards the outlet. Also, a circular cross-section gives the maximum bed-volume per unit conduit perimeter and this is a vital factor when designing any large packed-bed separation or reaction device. Perhaps a more banal reason for the widespread use of columns is the availability of glass- and plastic-ware of circular cross-section, such as burettes, pipettes, syringes and tubes, all of which could easily be fashioned into columns.

Process chromatography is widely used for the purification of biopharmaceuticals such as monoclonal antibodies, interferons, growth factors and vaccines [10–12]. One of the major attributes of columns used in such applications is their small bed-height to diameter ratios [13]. Chromatographic separation processes are commonly scaled-up by increasing the column diameter while maintaining the bed height constant [14]. Several factors make is necessary to do so. Firstly, the pressure drop increases with bed-height and beyond a certain point becomes a limiting factor [15]. Also, columns with small bed-heights and larger cross-sectional areas can be operated at significantly higher flow rates than tall columns of similar bed-volumes, and are therefore more productive. Chromatographic resins used for bioseparation tend to be "soft" and more compressible compared to those used in other applications [16]. In a tall column, the sheer weight of resin could result in severe compaction in material closer to the bottom of the bed, leading to inconsistencies in separation.

The use of columns with small bed-height to diameter ratios gives rise to some major engineering challenges such as fluid scaling and achieving uniformity in resin packing [17]. Achieving uniform flow distribution within such columns is particularly difficult [17–25]. During sample injection, non-uniform distribution due to inadequate fluid scaling could result in distortion of the

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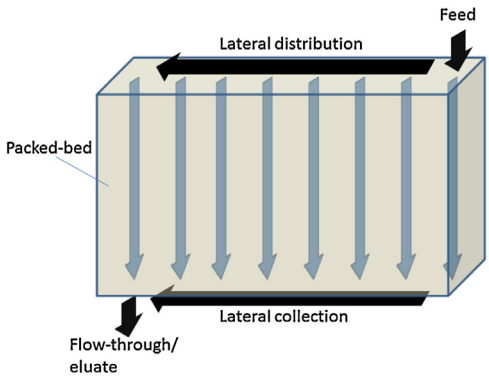

Fig. 1. Chromato-box device with idealized flow paths shown.

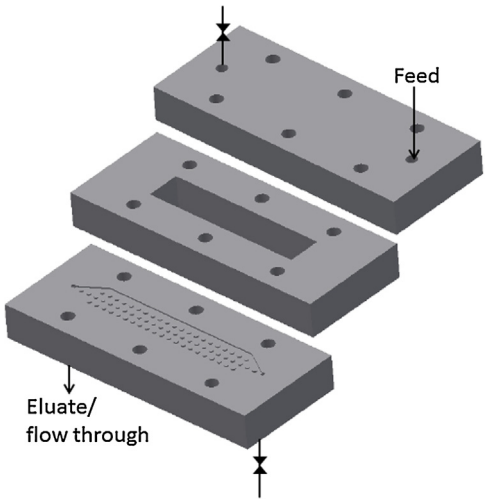

Fig. 2. Diagram of a chromato-box device.

sample front [17]. This has been demonstrated using experimental techniques such as magnetic resonance imaging [17,21–23]. Similarly, during elution, the eluent front could get distorted and interfere with the separation [17]. Moreover, the viscosity difference between the eluting buffer and protein bands could exacerbate these distortion effects [17]. Overall, these factors lead to broad and poorly resolved eluted peaks, which ultimately affect purity, recovery and productivity of a separation process. Peak broadening also results in the dilution of the material eluted from a column, which in turn could necessitate further downstream concentration steps. It has been widely reported that the efficiency of chromatographic separation decreases if column diameter is increased [26–32]. Gerontas et al. [32] have reported that in experiments carried out using Capto S cation exchange media, when the column diameter was increased from 70 mm to 600 mm while maintaining the bed height fixed (~20 cm), the number of theoretical plates per metre decreased from 5000 to 3704. Columns are provided with headers to distribute the fluid entering the packed-bed, and collecting that exiting it. Column mal-distribution challenges have been primarily addressed through improvements in column header design. Such efforts include the use of tapered, parabolic- and hyperbolic-shaped headers and collimators, incorporation of radial channels and fractal surface distributors, the use of manifolds, and the inclusion of anti-jetting features [17,25,33–39]. Alternative approaches include the development of radial-flow columns [40,41] for process-scale chromatography, and segmented parallel flow columns [42], and curtain flow columns [43] for analytical chromatography.

This paper discusses a radically different approach for addressing mal-distribution in process-scale chromatography. Laterally-fed membrane chromatography (or LFMC) devices designed specifically for high-resolution multi-component protein separations have been described and discussed in several recent papers [44–46]. In these devices, a lateral flow channel is first used to distribute the influent liquid over the feed-side of a rectangular membrane stack while permeate is collected on the other side using a similar lateral flow channel. This flow arrangement makes it easy to balance the pressure-drop across the membrane bed, and thereby ensure uniformity of flux (or superficial velocity). This design also ensures uniformity of solute flow path lengths throughout the membrane bed, and thereby reduces residence time distribution [46]. The current paper examines whether the design features and flow arrangements of an LFMC device could be adopted and adapted for packed-bed chromatography. The device thus obtained could be best described as a chromatography box (or chromato-box) and is picturized in Fig. 1, along with the idealized flow paths through such a device. Experiments were carried out using two chromatobox devices, one containing 9 mL of Capto S strong cation exchange media, and the other, 50 mL of Capto Q strong anion exchange media. The results obtained with these chromato-box devices were compared with those obtained using and their equivalent (or control) columns (i.e. having identical bed-height and volume, and thereby same cross-sectional area). The shapes of flow-through and eluted protein peaks, and the number of theoretical plates obtained are compared. Separation of different binary and ternary model protein mixtures was carried out and the resolutions of the peaks thus obtained are also compared.

2. Materials and methods

Strong cation exchange Capto S (product number 17-5441-01), and strong anion exchange Capto Q (product number 17-5316-02) chromatographic media were purchased from GE Healthcare Biosciences, QC, Canada. Proteins lysozyme (pI = 11.0, catalog number L6876), bovine serum albumin (pI = 4.8, catalog number A7906), myoglobin (from horse heart, pI = 6.8 and 7.2, catalog number M1882), ribonuclease A (from bovine pancreas, pI = 9.6, catalog number R6513) and conalbumin (pI = 6.0-6.6, catalog number C0755), and chemicals used to prepare buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). All buffers and the solutions were prepared using water obtained from a SIMPLICITY 185 water purification unit Millipore (Molsheim, France). Buffers and solutions used in chromatography experiments were micro-filtered and degassed prior to use.

The chromato-box devices used in this study and their equivalent (or control) columns were designed and fabricated in-house. These were made of white Delrin acetal resin (product number 8573K123, McMaster-Carr, USA). The design of the chromato-box devices (Fig. 2 shows the device with 9 mL bed volume) was based on that of an LFMC device [44–46] and each consisted of three pieces: a rectangular frame with a rectangular slot to house the packed-bed, and plates on both sides, each with a recessed lateral channel provided with small pillars for flow distribution (or collection). The equivalent column (Fig. 3 shows the 9 mL column) consisted of a circular frame provided with a circular slot to house the packed-bed, with headers on both side. The column headers were provided with pillars similar to those in the lateral channels of the chromato-box, to facilitate flow distribution and collection. The packed-bed dimensions within the 9 mL chromato-box device were 58.9 mm (length) × 12 mm (width) × 12.7 mm (height), and those for the 50 mL bed volume device were 132 mm (length) × 10 mm (width) × 38 mm (height). The 9 mL column had a 30 mm diameter and a bed-height of 12.7 mm, while the 50 mL column had a 40.7 mm packed-bed diameter and 38 mm bed-height. The bed-