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Effects of process parameters on the efficiency of chromatographic separations using a cuboid packed-bed device

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ABSTRACT

In recent papers we have proposed cuboid packed-bed devices as viable alternatives to preparative columns with low bed-height to diameter ratios that are typically used for biopharmaceutical purification processes. In this case study, we systematically examined operating parameters such as mobile phase flow rate, sample injection volume and feed protein concentration on the characteristics of flow-through and bound-and-eluted protein peaks. The current study was carried out using strong anion exchange media packed in a cuboid packed-bed device and its equivalent column, i.e. with identical volume and bed-height. Our experimental results showed that the cuboid packed-bed device outperformed its equivalent column at all conditions examined. However, the improvement in performance in flow-through experiments was more significant at lower flow rates, and when smaller sample volumes (i.e. less than 20% of bed volume) were injected. Also, the performance of the cuboid packed-bed was significantly better when a concentrated protein sample was injected using a small volume loop. In bind-and-elute experiments, the flow rate had a very significant impact on the performance of the cuboid packed-bed device, with better results being obtained at the lower flow rates examined. By choosing appropriate experimental conditions, significantly sharper peaks and thereby efficient separations could be achieved using the cuboid packed-bed device than with its equivalent column.

1. Introduction

Preparative column chromatography is widely used for separation and purification of high-value products such as biopharmaceuticals [1]. For instance, monoclonal antibody (mAb) purification is carried out over a wide range of scales, utilizing columns that are a few milliliters to several thousand of liters bed-volume [2,3]. Scale-up of preparative columns begin with studies on small columns, based on which appropriate scale-up parameters and strategies are decided. Generally, a scaling rule of constant (L/d_p), which means a fixed ratio of column length (L) to particle size (d_p) is widely used for a whole range of liquid chromatography applications [4]. Hence, if the same media is to be used, the bed-height is maintained constant during scale-up. This means that during the scale-up of preparative columns, the diameter is the only parameter that is increased, the implication being that larger columns have very low bed-height to diameter ratios when compared to smaller columns. Therefore, larger columns are not able to operate at the same efficiency as smaller ones, i.e. lower numbers of theoretical plates are obtained as the column diameter is increased [5–7]. The main reasons for this are poor distribution of fluid in the column header, flow maldistribution within the column itself, variability in axial shear stresses, dispersion in peripherals and radial temperature gradient [8–10].

Much of the effort for solving the above problems are focused around improved header design and development [11,12], monitoring and improvement of packing methods [13,14], and alleviation of radial temperature gradient [15]. Alternative methods such as the use of parallel flow [16], curtain flow [17] and radial flow [18] columns have also been reported. From our group, we have proposed a radically different solution, i.e. the use of a cuboid or box-shaped packed-bed device (see Fig. 1) [19–21], whose design is inspired by that of a laterally-fed membrane chromatography device [22–27], another genre of separation devices developed in our lab. As shown in Fig. 1, a cuboid device which houses within itself a box-shaped packed bed has a feed inlet on the upper left side and a feed outlet on the lower right hand side. This configuration ensures a high degree of uniformity in flow, and a very small variability in solute residence time along the length of the device [20]. Using such novel cuboid packed-bed devices, significantly higher efficiencies in different types of separations were obtained when compared with equivalent conventional columns [19–21]. The cuboid devices with their efficient separation features have great potential for application in different types of process-scale

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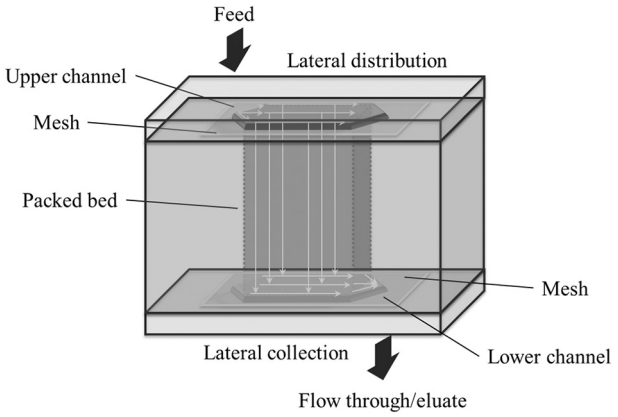

Fig. 1. Schematic diagram of cuboid packed-bed device.

chromatography, including biopharmaceutical purification.

In our previous studies, superior performance of the cuboid packed-bed devices has been demonstrated using different types of cation exchange and anion exchange media [19–21]. The cuboid packed-bed devices outperformed their equivalent commercial resin columns in terms of efficiency metrics such as peak width at half height, number of theoretical plates, tailing factor and asymmetry factor [19–21]. In the current study, we systematically examined effects of operating parameters such as mobile phase flow rate, sample injection volume and feed protein concentration on the attributes of flow-through and eluted protein peaks. Such studies are useful for determining operating ranges for high-efficiency protein separations using the cuboid packed-bed device. Head-to-head studies were carried out using anion exchange media (Capto Q) packed in a cuboid packed-bed device and its equivalent commercial column, i.e. with identical volume and bed-height. In the flow-through studies, lysozyme was used as the unbound tracer protein, while in the bind-and-elute experiments, bovine serum albumin served as the model protein. The results obtained are discussed.

2. Material and methods

Lysozyme (isoelectric point 11, L6876), bovine serum albumin (BSA, isoelectric point 4.8, A7906), and other chemicals such as Trizma base (T1503), Trizma hydrochloride (T3253), sodium hydroxide (795429), and hydrochloric acid (258148)), were purchased from Sigma-Aldrich (St. Louis, MO, USA). HiTrap Capto Q strong anion exchange column (bed volume: 5 mL, 16 mm diameter × 25 mm bed height, 11-0013-03) and strong anion exchange Capto Q media (17-5316-03) were purchased from GE Healthcare Biosciences, QC, Canada. Sodium chloride (SOD002.205) was purchased from Bioshop (Burlington, ON, Canada). All the buffers and the solutions were prepared using water obtained from a SIMPLICITY 185 water purification unit Millipore (Molsheim, France). Prior to use, the buffers and solutions were filtered and degassed.

The cuboid packed-bed device used in this study had dimension of 20 mm (length) × 10 mm (width) × 25 mm (height), and therefore the same cross-sectional area and bed-height as its equivalent commercial HiTrap Capto Q column. A schematic diagram of the cuboid packed-bed device is shown in Fig. 1. It consisted of centrally located box-shaped space for housing the resin, fed and drained respectively by two lateral channels containing in retaining plates of either side. The media was retained within the cuboid housing space using suitable nylon mesh [19–21]. The device was packed as described earlier [19–21]. Chromatography experiments using the cuboid packed-bed device and its equivalent column were carried out using an AKTA prime liquid chromatography system (GE Healthcare Biosciences, QC, Canada). High salt concentration containing buffer, 50 mM Tris-HCl, 1 M NaCl, pH 8.0 was used for regular cleaning between the runs, while 0.5 M NaOH solution was used after several runs for a more thorough cleaning.

3. Results and discussion

Table 1 summarizes the peak widths at half height of the lysozyme flow through peaks obtained with the same feed solution using different loops, with the cuboid packed-bed device and its equivalent column. These experiments were carried out at two different flow rates (i.e. 5 and 1 mL/min). The peaks obtained with the cuboid packed-bed device were consistently narrower than those obtained with the column for all the loops and flow rates examined, especially when the loop volume was 1 mL or less. Fig. S1 (Supplementary information) and Fig. 2 show representative lysozyme flow-through peaks obtained by injecting of 2 mL and 0.1 mL samples respectively. When the 2 mL loop was used, plateaued peaks were observed with the cuboid packed-bed device at both flow rates examined. With the column, some front and back end peak dispersion was observed and the peaks did not level off in a similar fashion. However, the difference in peak width at half height was not that significantly different with the two devices. When a smaller loop (i.e. 0.1 mL) was used, significant differences in peak attributes were observed. The peak obtained with the cuboid packed-bed device was not only sharper and higher, but also significantly more symmetric. The differences in peak attributes were even greater at the lower flow rates examined (i.e. 1 mL/min). From the results shown in Table 1, Fig. S1 (Supplementary information) and Fig. 2, it may be summarized that while the performance of the cuboid packed-bed device was consistently better than that of its equivalent column, the difference was more significant when a smaller loop (i.e. less than or equal to 20% of the bed volume) was used. This is consistent with our earlier study [21], where the difference in peak attributes with a cuboid packed-bed device and its equivalent column was found to be only marginally different when a big loop i.e. 5 mL was used for sample injection. However, with loops smaller than 0.5 mL, significantly superior peak attributes were obtained with the cuboid packed-bed device. Also, the difference was greater at lower flow rates (i.e., 1 mL/min). This is consistent with results reported in [21] where it was shown that the differences in the number of theoretical plates per meter ($\overline{N}$) between a cuboid packed-bed device and its equivalent column increased with decrease in flow rate. To further examine other factors affecting performance of the

Table 1
Peak widths at half height for flow-through peaks obtained with the cuboid packed-bed device and its equivalent column using different loops (bed volume: 5 mL, media: Capto Q anion exchange, running buffer: 50 mM Tris-HCl, pH 8.0, protein sample: 1 mg/mL lysozyme).

	Flow rate (mL/min)		Loop (mL)				
			0.1	0.5[a]	1	2	5[a]
Peak width at half height	5	Column	0.59	0.98	1.26	2.29	4.44
		Cuboid	0.42	0.62	0.96	2.23	4.4
	1	Column	0.75	1.1	1.37	2.29	4.61
		Cuboid	0.35	0.6	0.95	2.19	4.57

[a] Data from [21].