Journal of Chromatography A, 1549 (2018) 25–30

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Feasibility study for high-resolution multi-component separation of protein mixture using a cation-exchange cuboid packed-bed device

Guoqiang Chen, Alisha Gerrior, Raja Ghosh*

Department of Chemical Engineering, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4L7, Canada

ARTICLE INFO

Article history:
Received 22 December 2017
Received in revised form 19 February 2018
Accepted 14 March 2018
Available online 15 March 2018

Keywords:
Cuboid packed-bed
Chromatography
Multi-component separation of proteins
Efficiency
Resolution

ABSTRACT

In recent papers we have discussed the optimization of design and operating conditions for cuboid packed-bed device for chromatographic separations. The efficiency metrics used in these studies included the number of theoretical plates per unit bed height as well as attributes of flow-through and eluted peaks. These studies were carried out using equivalent columns as benchmarks. The cuboid packed-bed devices consistently outperformed the columns in terms of the above metrics. The current study examines how well, or indeed if at all these superior efficiency metrics translate to superiority in multi-component protein separation. Cation exchange resin was examined in the current study using appropriate multi-component model protein system which was chosen with close isoelectric points to make the separation challenging. Effects of operating and experimental parameters such as flow rate, loop size and linear gradient length on separation performance were systematically investigated. Separation metrics examined included peak width, tailing factor, asymmetry factor and resolution of separated protein peaks. The results obtained showed that the cation exchange cuboid packed-bed device significantly outperformed its equivalent commercial column (e.g., the number of theoretical plates per unit bed height was 8636/m for the cuboid packed-bed device as opposed to 1480/m for the column at a flow rate of 0.5 mL/min). The difference in efficiency was particularly high at lower flow rate and when shorter gradients were employed. The results suggest that the cuboid packed-bed devices could potentially have promising application in preparative separations such as biopharmaceutical purifications.

1. Introduction

Packed-columns represent the gold-standard in chromatographic separation. However, they have many limitations such as high capital cost, low-throughput and poor scalability. To overcome some of these limitations, non-chromatographic methods such as membrane chromatography, ultrafiltration, precipitation, flocculation and combination of multiple non-chromatographic steps have proposed [1–5]. Although some of these methods are more scalable or give faster separation, most of these cannot match column chromatography in terms of selectivity or resolution in separation. Consequently, columns continue to rule the roost in both analytical and preparative separations.

The field of fast and high-resolution analytical separations has seen several advances such as the development of very fine resin particles such as the sub-2 μm porous silica particles [6], and the sub-3 μm core-shell porous particles with solid core diameter of 1.7 μm [7], monolith columns [8], supercritical fluid columns [9], high-temperature liquid chromatography (HTLC) [10], and instrumentation compatible with these new techniques [11]. On the other hand, developments in the field of preparative chromatography have been rather sluggish. Preparative chromatographic processes are typically carried out with columns having low bed height to diameter ratios. Radial flow maldistribution, mainly within the top and bottom headers, and the inlet/outlet frit, which is negligible in the analytical columns becomes very significant at the preparative scale. Also, dispersion in peripherals and uneven packing can result in non-uniform flow distribution. Also, these columns have to be packed with larger particles to minimize the pressure drop. On account of these factors [12–16], large preparative columns have to be operated at extremely low efficiency. In addition, radial temperature gradients that typically exist in these large columns further reduce column efficiency [17]. Some of the new technologies described in the earlier part of this paragraph in the context of analytical separations, e.g., monolith [18] and supercritical fluid chromatography [19], have been tried with very limited success at the preparative scale. Efforts at improving performance of process columns are focused around addressing the heterogeneities that

* Corresponding author.
E-mail address: rghosh@mcmaster.ca (R. Ghosh).

https://doi.org/10.1016/j.chroma.2018.03.029
0021-9673/

typically drag down performance efficiency. This includes header re-design [13,20,21], adjustment of temperature gradient [22], and monitoring column packing [23,24].

Fig. 1A shows the flow pattern within a typical preparative column. The solute residence time along the central flow path is much shorter than those closer to the periphery [25]. Such variability in flow path length and consequent variability in solute residence time results in, as shown in Fig. 1A, broad and poorly resolved eluted peaks [25]. In a recent paper, we had proposed a solution to this problem i.e. flow maldistribution in packed-beds, through the use of a box-shaped or cuboid packed-bed device [25], whose design was inspired by lateral-fed membrane chromatography (LFMC) devices [26–29]. Fig. 1B shows the flow pattern within such a cuboid packed-bed device, based on which, the variability in flow path length and residence time distribution could be significantly reduced [25,30]. The lateral flow distribution in the upper channel and the corresponding flow collection in the lower channel ensure almost equal residence time within the packed-bed from the inlet to the outlet, irrespective of flow path [30]. Consequently, eluted peaks would be sharper than those obtained from an equivalent (i.e. same resin, bed-height and volume) column, and resolution of multiple sequentially eluted components from the chromatography device could be significantly improved (as shown in Fig. 1B). In a recent study [31], we have further improved the performance of a cuboid packed-bed device optimizing the device aspect ratio. In that study [31], which was carried out using anion exchange resin, the numbers of theoretical plates per meter within the cuboid packed-bed device could be boosted up to ~10,000/m, while the corresponding number for its equivalent column was ~2000/m at the same experimental conditions. The voidage in the Capto Q resin containing cuboid packed-bed device and its equivalent HiTrap column were similar (1.84 mL and 1.56 mL respectively). Quite clearly, cuboid packed-bed devices seem quite promising for application in process scale high-resolution, multi-component separations.

This paper presents a feasibility study for high-resolution, multi-component protein separation with a cuboid packed-bed device containing strong cation exchange resin. The ternary protein mixture used consisted of proteins ribonuclease A, cytochrome C and lysozyme, which have close isoelectric points, the goal being to make the separation challenging. Experiments were also carried out using an equivalent commercial column, i.e. packed with same resin and having same bed-volume and bed-height. Effects of process and experimental parameters such as flow rate, loop size, and linear gradient length were studied. Separation performance metrics such as attributes of protein peaks and their resolution were systematically examined.

2. Material and methods

Ribonuclease A (R6513), cytochrome C (C7752), lysozyme (L6876), hydrochloric acid (258148), sodium phosphate monobasic (S0751), sodium phosphate dibasic (S0876), and sodium hydroxide (795429) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The properties of the model proteins used in the study are shown in Table S1 of Supplementary material. Sodium chloride (SOD002.205) was purchased from Bioshop (Burlington, ON, Canada). All the buffers and solutions were prepared using water obtained from a SIMPLICITY 185 water purification unit (Millipore, Molsheim, France). The buffers and solutions were filtered and degassed prior to use. Capto S media (17-5441-03), and 5 mL HiTrap Capto S column (17-5441-23) were purchased from GE Healthcare Biosciences, QC, Canada. The characteristic properties of Capto S media are also listed in Table S2 of Supplementary material. The HiTrap Capto S column had 16 mm diameter and 25 mm bed height.

Table 1
Comparison of characteristics of salt peaks obtained with the Capto S resin containing cuboid packed-bed device and its equivalent column at different flow rates (bed volume: 5 mL, mobile phase: 0.4 M NaCl, tracer: 0.8 M NaCl, loop: 0.1 mL).

	Capto S		
	Flow rate (mL/min)	Column	Cuboid
$w_{0.5}$	0.5	1.74	0.81
	1	1.56	0.84
	2	1.62	0.94
	5	1.74	1.2
	10	1.9	1.5
$\tilde{N}s$ (/m)	0.5	1480	8636
	1	1829	8272
	2	1773	6737
	5	1675	4287
	10	1527	2929
N_{cuboid}/N_{column}	0.5	5.84	
	1	4.52	
	2	3.8	
	5	2.56	
	10	1.92	
TF	0.5	1.81	1.01
	1	1.82	1.00
	2	1.58	1.03
	5	1.30	1.01
	10	1.25	1.02
AF	0.5	2.66	1.03
	1	2.67	1.02
	2	2.13	1.07
	5	1.63	1.06
	10	1.50	1.06

The 5 mL cuboid packed-bed device used in this study had a length of 20 mm, a width of 10 mm and a height of 25 mm. The 2:1 aspect ratio (length to width) had been determined to be optimum for this scale (5 mL bed volume) in an earlier study [31]. The detailed design of the cuboid packed bed device and its components have been described elsewhere [25,30,31]. The chromatography devices, i.e. HiTrap columns and the cuboid packed-bed device were connected to an AKTA prime liquid chromatography system using appropriate PEEK tubing and connectors. The same tubing and connectors were used for the column and cuboid experiments to keep the extra-device volume constant. During the chromatography experiments, pressure, conductivity, and UV absorbance at 280 nm were monitored. Theoretical plate measurements and salt peak attribute characterization experiments were carried out using 0.4 M NaCl solution as mobile phase and 0.8 M NaCl solution as conductivity tracer. In these experiments, the packed-bed was equilibrated with the mobile phase until the baseline was stable, followed by injection of 0.1 mL tracer (2% of packed-bed) at different flow rates. 20 mM phosphate buffer (pH 7) and 20 mM phosphate buffer with 1 M NaCl (pH 7) were used as binding and eluting buffer respectively for the Capto S resin based protein separation experiments. The protein mixture in these experiments consisted of 0.5 mg/mL ribonuclease A + 0.5 mg/mL cytochrome C + 0.5 mg/mL lysozyme prepared in binding buffer. All experiments using the cuboid packed-bed device and its equivalent column were performed in duplicate.

3. Results and discussion

Fig. 2 shows representative salt peaks obtained with the Capto S resin containing cuboid packed-bed device and its equivalent column at flow rates of 5 mL/min and 0.5 mL/min. Clearly, the peaks obtained with the cuboid packed-bed device were sharper and more symmetric. Table 1 summarizes the characteristics of all the salt peaks obtained with the Capto S resin containing cuboid packed-bed device and its equivalent HiTrap Capto column. The parameters measured include peak width at half height ($w_{0.5}$), the