Chemical Engineering Science 102 (2013) 99–105

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Comparison of fully-porous beads and cored beads in size exclusion chromatography for protein purification

Jian Luo [a], Weiqing Zhou [a], Zhiguo Su [a], Guanghui Ma [a], Tingyue Gu [b,*]

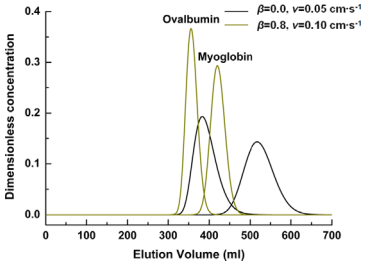
[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[b] Department of Chemical and Biomolecular Engineering, Ohio University, Athens, OH 45701, USA

HIGHLIGHTS

- Solves a general model for size exclusion chromatography using cored beads.
- Demonstrates that cored beads are surprisingly superior to fully-porous beads.
- Describes methods for mass-transfer parameter estimation.

GRAPHICAL ABSTRACT

Comparison of cored beads (β=0.8) and fully-porous beads using the same column dimensions (5 × 60 cm) and feed volume (12.7 ml) when the flow rate for cored beads was doubled.



ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form
5 July 2013
Accepted 27 July 2013
Available online 6 August 2013

Keywords:
Size exclusion chromatography
Cored beads
Modeling
Protein purification
Mass transfer

ABSTRACT

Size-exclusion chromatography (SEC) relies exclusively on intraparticle diffusion to separate solutes of different molecular sizes and shapes. Thus, its feed volume can only be a small fraction of the column volume. Much larger columns are required for SEC than other forms of liquid chromatography. Becasue of this, SEC often employs less expensive soft gels in large-scale applications to reduce costs. Excessive bed compression forces engineers to use pancake-shaped columns instead of more desirable slim columns during scale-up. Cored beads have impenetrable rigid cores that result in lower pressure drops and better pressure resistance. They also provide sharper peaks due to shortened radial distance for diffusion. Using a new general rate model for SEC with cored beads, this work demonstrated that cored beads performed better than fully-porous beads for myoglobin and ovalbumin separation through computer simulation. This theoretical work could encourge the research and product development of cored beads for large-scale SEC that has not been reported.

1. Introduction

Among various forms of liquid chromatography (LC), size exclusion chromatography (SEC), also known as gel filtration chromatography, stands out as often having very large columns in industrial separations (Bérot et al., 2005; Hofmann, 2003; Stickel and Fotopoulos, 2001). In gradient-elution chromatography involving adsorption, ion-exchange or affinity binding, the feed loading-capacity can be many times the column volume (Gu et al., 1990; Yamamoto et al., 1987; Zhou et al., 2005). However, SEC lacks a binding mechanism. Its separation ability is entirely based on diffusion in the particle macropores. Molecules with different sizes and shapes migrate at different speeds with the larger molecules coming out the column faster because they penetrate no or fewer particle macropores in the stationary phase. The bands of the solutes in the feed invariably become more diffused as they travel inside the column. The bands have to be separated within a time frame between the retention time for a non-penetrating large

* Corresponding author. Tel.: +1 740 593 1499; fax: +1 740 593 0873.
E-mail address: gu@ohio.edu (T. Gu).

0009-2509/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ces.2013.07.044

solute (shortest retention time) and the retention time for a very small molecule such as a salt and a solvent (longest retention time). This is why the feed to a typical SEC column is only a few percentages of its column volume in desalting or solvent exchange operations (Terry et al., 2004) and a only fraction of a percent for the purification of proteins (Bérot et al., 2005). Soft gels are often used in preparative- and large-scale SEC due to their low costs (Afeyan et al., 1991). One disadvantage is that scale-up of SEC using soft gels is often forced to increase column diameter, rather than column length because of pressure drop limitation. A large pressure drop would compress a gel too much. This distorts particle marcopores undesirably and makes mobile phase flow more difficult.

Despite its shortcoming of a low feed volume to column volume ratio, SEC is widely used in large-scale bioseparations (Wheelwright, 1994) because of its irreplaceable separation mechanism. For example, a total bed volume of 707 L containing Sephacryl S200 gel filtration medium was used in the manufacturing of human albumin from donated human plasma (Adcock et al., 1998). Because of bed compression, three pancake-shaped sub-columns, each with a diameter of 100 cm and a height of 30 cm, were linked in series instead of using a single column with a height of 90 cm to achieve the 707 L total bed volume.

Cored beads (Wang et al., 2007) are also known as pellicular (Greibrokk, 2004; Zhou et al., 2004), superficially porous (Kirkland et al., 2000) and fused-core beads (Schuster et al., 2010). They have a rigid nonporous solid core that makes them hydrodynamically superior to fully-porous beads because cored beads result in column pressure drops several times lower than fully-porous beads (Wang et al., 2007). This means SEC columns packed with cored beads can be scaled up in the axial direction to a much greater extent than columns packed with fully-porous beads, leading to better column performances compared with pancake columns.

Cored beads have recently generated considerable interests for liquid chromatography separations because of their unusually high column efficiencies and low column pressure drops (Ning et al., 1998; Schuster et al., 2010; Zhou et al., 2004). They have been used for separations of proteins (Zhou et al., 2007), peptides (Kiss et al., 2010), nucleotides (Kirkland et al., 2000) and other compounds (Ning et al., 1998) in adsorption, ion-exchange, and reversed-phase chromatography. Yang and Hu (1996) derived the theoretical expressions for elution and frontal linear chromatography for ion-exchange resins that were cored beads. Zhou et al. (2004) obtained intraparticle diffusion coefficients for cored beads used for ion-exchange. Wang et al. (2007) studied the pressure-flow correlation with the ion-exchange resin of cored Q beads. They found that the cored beads provided significantly enhanced rigidity and permeability compared with fully-porous homogeneous agarose beads. The maximal flow velocities were more than 2-fold higher for cored beads. The superficial ("empty-tower") mobile phase velocity at 30 psi for a 20 cm-long bed was 1120 cm/h for cored beads, much higher than the 324 cm/h for homogeneous agarose beads. Based on the Kozeny–Carman equation, column pressure drop is directly proportional to the superficial velocity and the column length (Davies and Dollimore, 1980; Tamon et al., 1981). Thus, a column with cored beads can achieve higher resolution by increasing the column length to a greater extent compared with fully-porous beads (Miyabe, 2008). This means it is possible to employ an SEC column with cored beads having a column length 100% or even longer than an SEC column with fully-porous beads while still achieving a lower or comparable pressure drop. On the other hand, with the same column dimensions, cored beads would allow a much higher flow rate at a comparable or even lower pressure drop compared with fully-porous beads.

Gu et al. (2011) compared the gradient-elution performances of cored beads and fully-porous beads theoretically through computer simulation using a general rate model for gradient LC with a Langmuir isotherm. Their results showed that cored beads performed better, because cored beads had the advantages of shorter separation times and sharper peaks.

So far, SEC with cored beads has only been reported for analytical applications. Kiss et al. (2010) compared the performances of 2.7 μm fused-core silica beads with different pore sizes in analytical high-performance SEC for the separation of peptides and small proteins. To the best of our knowledge, there have no reported theoretical or experimental work on using cored beads for preparative- and large-scale SEC in the open literature. There is a major concern that with reduced net gel volume due to the existence of a core, cored beads may suffer from feed loading-capacity losses. Perhaps due to this uncertainty, SEC media makers have yet to make such preparative LC media. The performance of cored beads in SEC warrants a serious theoretical investigation. For the first time, this work presented theoretical evidence demonstrating that cored beads could offer improved preparative-scale performance over fully-porous beads in SEC for protein purification due to improved mass transfer.

2. Theory

2.1. Mathematical model

Eqs. (1) and (2) can be obtained from mass balances of an SEC column's bulk-fluid phase and the stationary phase, respectively,

$$-D_b\frac{\partial^2 C_b}{\partial Z^2}+v\frac{\partial C_b}{\partial Z}+\frac{\partial C_b}{\partial t}+\frac{3k(1-\varepsilon_b)}{\varepsilon_b R_p}(C_b-C_{p,R=Rp})=0 \quad (1)$$

$$\frac{\partial C_p}{\partial t}-D_p\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_p}{\partial R}\right)=0 \quad (2)$$

It is assumed that the isothermal column is packed with cored beads with a uniform particle radius (R_p). Its inner core is inert and impenetrable (non-porous) with a radius of R_{core} (Fig. 1). Intraparticle diffusion occurs only in the porous shell of the cored beads. For simplicity, it is also assumed that there are no interactions between solutes in SEC. In reality, solutes may interfere with each other because diffusion coefficients can be concentration dependent. Such a dependency is not considered in the coefficients. Another possible interaction is that large solutes may block smaller solutes from accessing vacant smaller pores.

Eq. (2) requires $R_{core}\le R\le R_p$. The Partial Differential Equation (PDE) system above can be nondimensionalized using the following dimensionless groups to yield the following equations.

$$c_b=C_b/C_0,\ c_p=C_p/C_0,\ r=R/R_p, z=Z/L,\ Pe_L=vL/D_b,$$
$$Bi=kR_p/(\varepsilon_p^a D_p),\ \eta=\varepsilon_p^a D_p L/(R_p^2 v), \xi=3Bi\eta(1-\varepsilon_b)/\varepsilon_b,\ \tau=vt/L$$
$$-\frac{1}{Pe_L}\frac{\partial^2 c_b}{\partial z^2}+\frac{\partial c_b}{\partial z}+\frac{\partial c_b}{\partial \tau}+\xi(c_b-c_{p,r=1})=0 \quad (3)$$

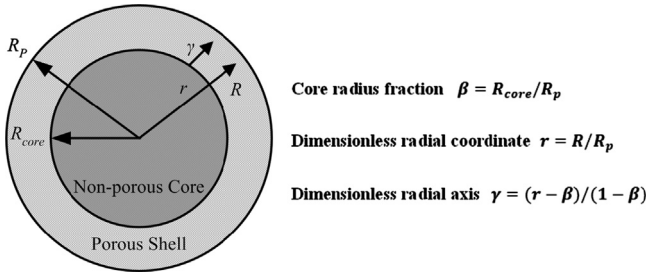


Fig. 1. Schematic of a spherical cored bead with an inert non-porous core and a porous shell (gel layer).