Application of binary packing for chromatographic separation

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Abstract

Separation of dextran and polyethylene glycol of different molecular mass was performed using a binary packed column of glass beads (size ratio ~ 10) and a binary packed column formed by kieselguhr-G (for thin layer chromatography, Merck) and glass beads as the large size particulate fraction (size ratio ~ 30). In addition, data on the separation of micro-spheres, bacillus and yeast cells using monosized and binary glass beads columns are presented. Obtained results show the advantages of using binary packed columns formed by fine and coarse particles instead of a monosize packing of fine particles. The importance of pore channels tortuosity effect on the separation of shaped microparticles using a binary packing is demonstrated.

1 Introduction

Liquid chromatography is widely used for the separation of biological macromolecules and microparticles. The information about the role of beds pore topology on shaped macromolecules and micro-objects transport is limited and an actual subject of investigation. Fine hydrodynamic effects are expected to play a significant role in the development of shape based separation methods: Peyrin et al. (2000b); Peyrin et al. (2001); Perrin et al. (2002); André and Guillaume (2004); Hirabayashi and Kasai (1996); Hirabayashi and Kasai (2000).

Conventionally, a chromatographic packing with a narrow particle size distribution is recommended as a narrower inter-particle pore size distribution is obtained. Nevertheless, composite particles packings with a significant difference in the size of the fractions may be considered as binary mixture, virtually (Carta and Bauer, 1990).

Some of the currently used chromatographic columns with binary composite packing are formed by non-porous beads covered by a micro-spheres layer, where the ratio between beads and micro-spheres diameters varies in a wide range of values (up to 60) making difficult its production and application Honda et al. (1992).

In comparison with currently used composite chromatographic columns, it is more reasonable to use a column packing formed by binary mixtures of particles with the appropriate size ratio. The optimal binary mixture composition is in the range of minimum mixture porosity when large particles (diameter D) form the skeleton of the packing and small size particles d are distributed within the skeleton void. For the binary mixture of spherical particles, the minimum porosity corresponds to a volume fraction of large particles in the mixture x_D = 0.65 – 0.7, Dias et al. (2004) and Mota et al. (2004). Besides increasing the packing rigidity, the binary bed puts a limit on the radial dispersion and gives the possibility to control the separation process by playing with the particle size ratio D/d.

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The porosity of a binary packing in the region of x_D = 0.65 – 0.7 is smaller than the porosity of a monosize packing and the difference increases as the particle ratio D/d increases. This difference may be as high as 200%, depending on D/d. Therefore, binary packing is characterised by a larger capacity factor $k' = t_R/t_0 - 1 = V_R/V_0 - 1$, where t_R and V_R are the retention time and retention volume of the solute, respectively; t_0 and V_0 are the retention time and retention volume, respectively, of an unretarded solute. The capacity factor k' is a measure of solute retention expressed in units of void volume and an increase in k' will correspond to a better separation of solutes. If V_t is the total packing volume, the ratio V_t/V_0 is equal to the inverse of column porosity $V_t/V_0 = 1/\varepsilon$ and, assuming $V_R = V_t$, the capacity factor is described by $k'_t = (1-\varepsilon)/\varepsilon$. For example, at $V_R = V_t$ for a monosize glass beads porosity of 0.36 and for a binary mixture with D/d ~ 10 and ε = 0.22 the corresponding capacity factors are, respectively, 1.78 and 3.54; the transition from kieselguhr packing (ε = 0.8) to a mixture of glass beads and kieselguhr (ε = 0.4) results in a 6 fold increase in k', from 0.25 to 1.5. Moreover, due to the use of the binary packing, the overall tortuosity of the packing increases 1.3 – 1.4 times, Mota et al. (2000) and Mota et al. (2001), and this increase in tortuosity is similar to a 20 – 30% decrease of the packing length.

In this work, two types of binary mixture columns were tested: a mixture formed by glass beads and kieselguhr-G and a binary mixture of glass beads with different size ratios; the following macromolecules - dextran, polyethylene glycol (PEG) and Red Starch were used. Separation of micro-spheres, bacillus and yeast cells on the binary glass beads columns was also performed.

2 Materials

The mixed bed of kieselguhr and glass beads consists of a mixture of kieselguhr-G, (Merck, for thin layer chromatography) with mean particle size d = 12.33 microns and glass beads with average size D = 375 microns (size range 250 – 500 micron). The glass beads volume fraction in the mixture x_D = 0.85 is close to the minimum porosity ε = 0.4 corresponding to V_t/V_0 = 2.5. The pure kieselguhr-G packing has the following parameters: porosity ε = 0.73 and ratio V_t/V_0 = 1.225 – 1.25.

For the binary mixtures of glass beads having a large size particle fractions in the mixture x_D = 0.7, the porosity of the packings with particle size ratio D/d = 10 and 13.3 was 0.213 – 0.22, respectively. Corresponding V_t/V_0 were 4.5 and 4.69.The porosity of the monosize glass bead column is 0.362 that corresponds to $V_t/V_0 = 1/\varepsilon$ = 2.76.

Aqueous solutions of dextran, polyethylene glycol (PEG-35, molecular weight 35,000) and Red Starch (*Megazyme*, UK, molecular weight ~ 30 000 kDa) were applied to the columns.

The used micro-particles, in aqueous suspension, were: yeast cells, diameter 5 microns, micro-spheres, diameter 1 micron and rod-like bacilli *Lactobacillus bulgaricus* with 6 microns average length and diameter ~ 0.5 micron. Yeast diameter is close to the bacillus length and micro-sphere diameter is in the scale of the bacillus diameter. All particle types have similar density ~ 1.05 – 1.1 g/cm^3.

3 Results and conclusion

Data on the solute concentration on the outlet of binary packing columns formed by glass beads and kieselguhr and a mixture of glass beads (D = 2000 micron and d = 150 micron) is shown in Figs. 1 and 2. Curves represent the fitting distribution function.

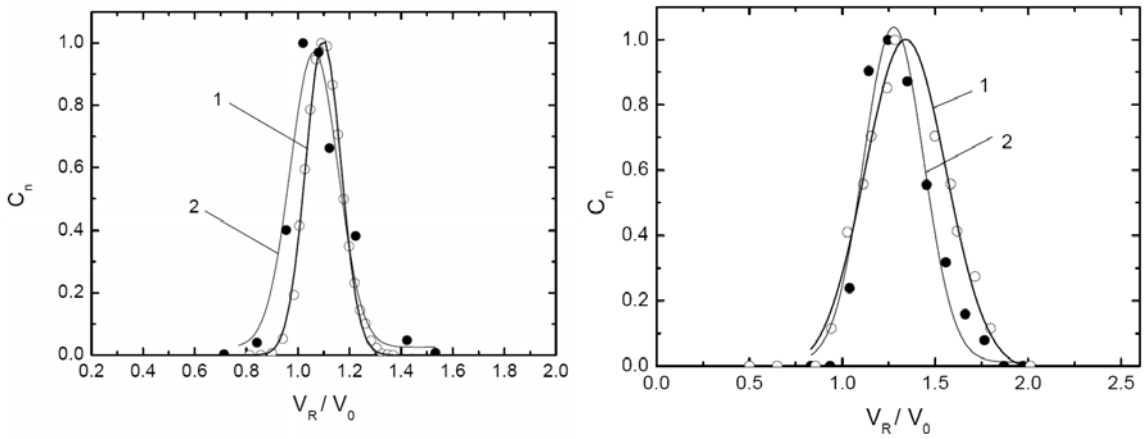

Figure 1. Normalised concentration profiles C_n vs. V_R/V_0 for (a) dextran blue and (b) PEG-35 at the outlet of binary columns: 1 – glass beads mixture and 2 – glass beads and kieselguhr. Sample solute concentration C_s = 10 g/L and volume 2 mL; flow velocity 1 mL/min.

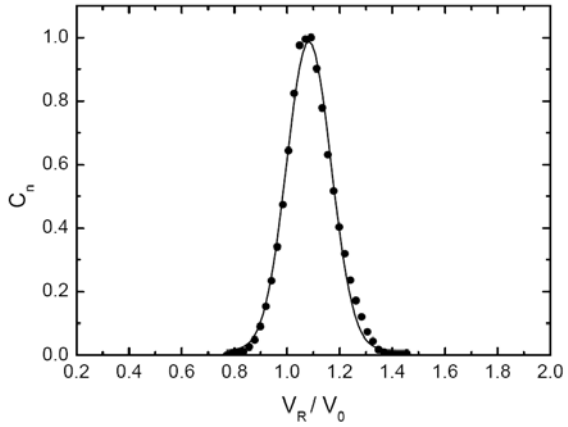

Figure 2. Red Starch concentration profile C_n vs. V_R/V_0 on binary columns of glass beads. Sample concentration C_s = 10 g/L and volume 2 mL, flow velocity 2 mL/min. Large size particle fractions in the mixture x_D = 0.7. Porosity of the packing 0.213, V_t/V_0 = 4.69.

The peak shape and position obtained on columns with a binary packing are similar to monosize packing and fitted by a Gaussian distribution function. The deviation observed in peak positions for columns 1 and 2 in Fig. 1 is due to a hydrodynamic chromatographic effect (HDC), which becomes more significant with the increase of the solute size to the pore size ratio when moving from the mixture of glass beads to the mixture of kieselguhr and glass beads.

Kieselguhr has a tendency to compress under he applied pressure (compressibility effect), being a similar behaviour observed in the present experiments for pure kieselguhr-G packing. In contrary, when a binary column (glass beads and kieselguhr) is used this effect does not occur and, simultaneously, a porosity around 0.4, close to the porosity of uniform spheres packing, is obtained.. We can speculate that the proposed binary packing, when the small particles are distributed within the large particles skeleton, is able to significantly increase the working amplitude a range of stationary phases from compressible materials (gels, cellulose, etc.).

Another positive effect that can be observed is the reduction of radial dispersion in large diameter industrial columns as consequence of the constrains imposed by the large particles.

The use of a binary packing would be interesting, also, for slalom chromatography based on the "snaking" effect, where the pathway tortuosity factor plays a significant role on the effectiveness of the separation, Peyrin et al. (2000a).

In Fig. 3, the dependence of the solute peak position on the solute molecular weight (MW) is shown, being demonstrated that an increased resolution is obtained for the binary packing; it is also observed that, for the binary packing, all peaks appear within the bed volume V_t/V_0 = 2.5 against V_t/V_0 = 1.25 for the kieselguhr column. The results also point out to the importance of molecule shape as opposite

dependences on the solute MW are observed for PEG vs. dextran.

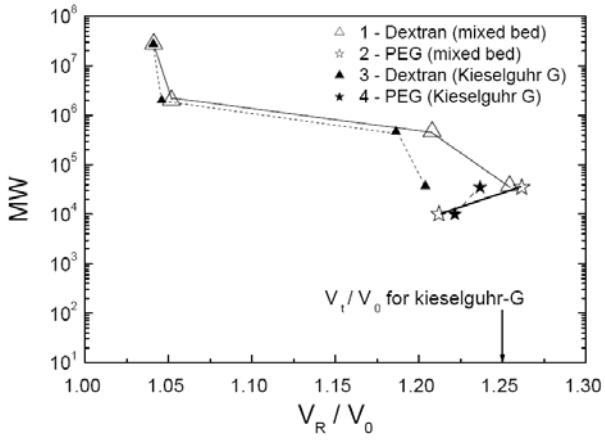

Figure 3

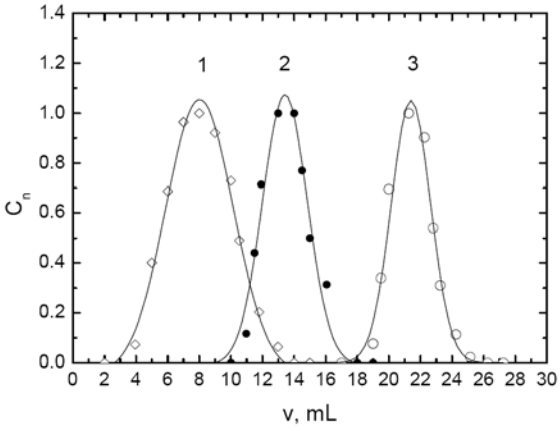

Figure 4

Figure 3. Normalised peak position V_R/V_0 of individual solutes vs. molecular weight (MW) for dextran and polyethylene glycol (PEG): 1, 2 – mixed bed column, V_t/V_0 = 2.5 (D/d ~ 30), and 3, 4 – pure kieselguhr-G column, V_t/V_0 = 1.25.

Figure 4. Normalised micro-particles concentration (C_n) breakthrough curves vs. elution volume v, V_t/V_0 = 4.4 (D/d = 10) for:1 – 1 micron micro-spheres; 2 – *S. cerevisiae*, spheroids with 5 microns diameter; 3 – *L. bulgaricus*, rods of size ~ 6×0.55 microns. Curves represent the Gaussian distribution fit.

Results on micro-particle separations obtained in a binary glass beads column packing are shown in Fig. 4, clearly highlighting the effects of the particle shape on the separation.

The analysis of the experimental data leads to the conclusion that the tortuosity pathway factor plays a significant role on the effectiveness of the separation of rod-like objects, in our case PEG and bacillus: Peyrin et al. (2000b); Peyrin et al. (2001); Perrin et al. (2002); André and Guillaume (2004); Hirabayashi and Kasai (1996); Hirabayashi and Kasai (2000).

The tortuous pathway, especially in the form of bends, may create a disorder momentum for the rod-like object. This fact will reduce the rod-like micro-particle flow velocity as compared with the flow of spherical particles of similar weight, resulting in a retardation effect. The constrain acts as a disordering factor of the particle axial orientation relative to the flow streamlines and rod-like particles will expose a higher cross-section to the flow, increasing the friction force that results in the retardation phenomenon.

The retardation effect is well seen in the micro-particle separation, Fig. 5. Theoretical estimations made taking in account the tortuosity and bend effect confirm a retardation effect for the rods. Two models were used for the analysis of the experimental results: pure exclusion effect and hydrodynamic separation model (HDC).

The exclusion model for particles was formulated in the general form as $R = A/(1-\lambda)^z$, where $R = t_m/t_0$ is a retention value, t_m and t_0 are the migration time of the micro-particle and of an infinite small sized marker, respectively, A is a coefficient proportional to the tortuosity, $\lambda = d_e/d_{por}$ is the aspect ratio (d_e is the microparticle effective diameter and d_{por} is the diameter of the capillary or pore) and parameter z = 1, 2 or 3 depends mainly on the pore shape. For instance, in a parallel-plate channel $R \sim 1/(1-\lambda)$, for a cylindrical pore $R \sim 1/(1-\lambda)^2$, and for a 3-D

pore $R \sim 1/(1-\lambda)^3$.

The HDC model is described by $R = B/(1+2\lambda-2.8\cdot\lambda^2)$, where B = 1.0. It must be pointed out that most of the HDC theories have been derived from the flow of the dispersed phase in open capillaries where the tortuosity has no influence.

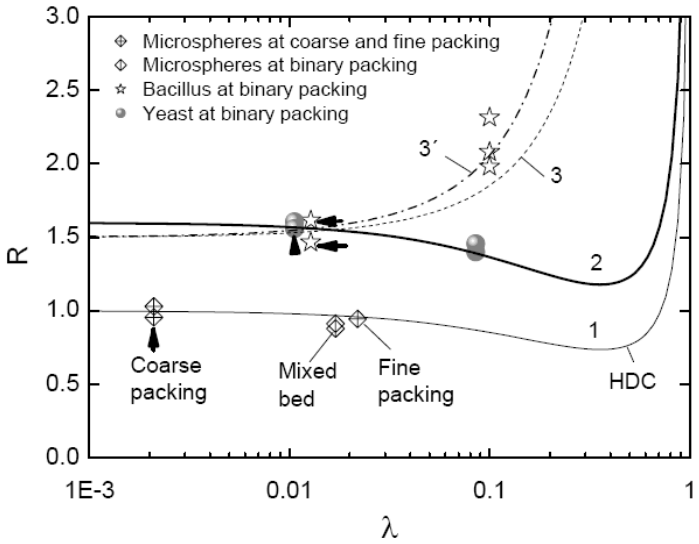

Figure 5. Results obtained on the separation of particles on different packings. Curves: 1 – $R = 1/(1+2\lambda-2.8\lambda^2)$; 2 – $R = 1.6/(1+2\lambda-2.8\lambda^2)$; 3 – $R = 1.5/(1-\lambda)^2$; and 3´ – $R = 1.5/(1-\lambda)^3$. In the fine particles packing, all cells (bacillus and yeast) were captured within the packing. Points marked by thick arrows belong to coarse particle packing.

Data presented in Fig. 5, shows, for spheres, that the classic HDC model (B = 1.0) fits well to the data whenever the ratio of particle size to the bend scale is high (~ 1/100, as is the case of micro-spheres). However, if this ratio increases and becomes ~ 1/20, the HDC model needs to be corrected due to the effect of channel wall curvature on the exclusion effect. This assumption leads to a modified HDC equation of the form $R = B/(1+2\lambda-2.8\lambda^2)$, where $B \geq 1$. The effect of the pore topology plays important role in the separation of shaped particles when the aspect ratio approaches λ = 0.1 being observed that the bacillus separation occurs by an exclusion mechanism. For binary packing, the rod-like particles behave differently from the spherical particles of the same scale of bacillus length and diameter. The explanation is the interference of rod-like particles with the pore topology. Further investigation is needed to clarify the particle – pore topology mechanism of the interference hereby identified.

As can be seen for the binary packing, the rod-like particles behave differently from the spherical particles of the same scale of bacillus length and diameter. Theoretically, a significant retardation of bacilli can be the consequence of the attachment (adhesion) – detachment effect in the porous media. However, the low ionic strength used and the absence of a tail in the bacillus breakthrough curve allows the exclusion of the mentioned reasons. The other plausible explanation is the interference of the rod-like particles with the pore topology. Moreover, comparing curves 3 and 3´ it is possible to assume that rods, due to their similarity to a one-dimensional object (λ is defined as the ratio of length to the pore size), recognise the pore channel as a three-dimensional space rather than a two-dimensional one.

It is possible to assume that an increase in the overall tortuosity as a result of the increase in the number f bends and an increase in the flow velocity may have a decisive impact in the flow of shaped particles through a porous media. Anyway, by reducing the bend scale in relation to the particle size, the opposite effect may occur as bends will be looked at as channel surface roughness.

Presented results are preliminary and a more detailed investigation is needed to confirm the suggested importance of tortuosity in the efficiency of separation processes. Clarification of the particle – pore topology interference mechanism is required.

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