



Mass transfer mechanism in liquid chromatography columns packed with shell particles: Would there be an optimum shell structure?

Fabrice Gritti, Georges Guiochon*

Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA

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ABSTRACT

The mass transfer mechanisms in columns packed with old (55 μm Zipax and 5 μm Poroshell) and recently commercialized shell particles (2.7 μm Halo-C₁₈ and Kinetex-C₁₈) were investigated from a *physico-chemical* point of view. Combining a model of diffusion in heterogeneous packed beds (effective medium theory) with values of the heights equivalent to a theoretical plate (HETPs derived from the first and second central moments of the elution profiles) and of the peak variances provided by the peak parking method, we demonstrate that columns packed with current shell particles perform better than those packed with fully porous particles in resolving low molecular weight compounds because the eddy diffusion term of the van Deemter equation of the former is markedly smaller. The calculation of eddy diffusion in column beds suggests that the smaller *A* terms are due to smaller trans-column velocity bias in columns packed with shell particles. We also show that the mass transfer of large molecules (e.g., proteins) is faster when the internal volume accessible to the analyte increases. Therefore, it is suggested that shell particles made of concentric layers with average pore sizes increasing with increasing diameter would provide columns with higher efficiency.

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1. Introduction

Horváth et al. [1] conceived pellicular particles as packing materials for liquid chromatography in the late 1960s. They prepared 50 μm glass silica beads coated with a thin film of ion exchange resin and used columns packed with them to separate nucleotides, expecting two advantages: (1) a high loading capacity due to the large saturation capacity of the resin and (2) a low solid–liquid mass transfer resistance, due to the thin stationary phase layer [2,3]. In spite of the impressive separations reported by Horváth et al., this type of stationary phase was not adopted by the community because ion-exchange is a retention mechanism specific to ions, which did not interest much early chromatographers who preferred to develop liquid–liquid (LLC) rather than liquid–solid (LSC) chromatography, due to the poor results generally obtained in gas–solid chromatography. Huber [4,5], Halasz et al. [6], and Karger [7] pioneered LLC for a few years. This was why, 50 and later 35 μm shell particles were made, with a solid core of glass beads similar to the beads used by Horváth et al., surrounded by a

ca. 1 μm thin layer of fine silica particles. This layer was impregnated with a liquid serving as the stationary phase. Several brands of these shell particles were commercialized in the early 1970s, including the 37–50 μm Corasil I and II (Waters Associates, 1970), the 50 μm Zipax (Dupont de Nemours, 1972), and the 50 μm Pellicosil (Macherey-Nagel, 1975) [8–10]. The volume fraction of the particle occupied by the porous shell was between 5 and 10% and the minimum reduced HETPs of the columns packed with them was between 2.0 and 2.5. However, it rapidly proved difficult to find two liquids practically insoluble in each other and between which the sample components would equilibrate with constants different from either zero or infinity. Furthermore, it was realized that LLC columns were unstable, rapidly losing stationary phase and providing irreproducible analyses. The use of liquid stationary phases was abandoned. Manufacturers of superficially porous packing materials tried to convert them to shell particles for LSC. However, the porous layers that had been designed to hold pools of the stationary liquid phase had a relatively small surface area, hence provided insufficient retention and a low loading capacity, becoming overloaded with very small size samples. In the same time, finer and finer fully porous particles were produced, permitting the production of more efficient columns and nullifying the potential advantages of the existing shell particles.

A second generation of shell particles appeared in 1992, with the 5 μm Poroshell (Agilent). It met only with limited success, in spite of allowing excellent separations [11]. Real success came

* Corresponding author at: Department of Chemistry, University of Tennessee, 552 Buehler Hall, Knoxville, TN 37996-1600, USA.

Tel.: +1 865 974 0733; fax: +1 865 974 2667.

E-mail addresses: guiochon@utk.edu, guiochon@ion.chem.utk.edu (G. Guiochon).

in 2006, with the introduction of the 2.7 μm Halo shell particles of Advanced Material Technologies [12,13]. The drawback of the low loading capacity of shell particles was eliminated by building a 0.5 μm thick porous shell around a 1.7 μm solid silica core. Therefore, the porous volume occupies about 75% of the particle volume. The most striking result was the achievement of 4.6 mm I.D. columns with a minimum reduced plate height of 1.5 for small molecules. This new packing material was designed for the separation of small molecular weight compounds [14]. Three years later, Phenomenex offered the 2.6 then the 1.7 μm Kinetex particles which exhibit exceptional performance, with a small $h_{\text{min}} = 1.2$ [15,16], a small C term [14,17], and a very flat HETP curve for both low and medium-size molecules. The C term results from the combination of two independent, additive mass transfer resistances. The first resistance accounts for the kinetics of diffusion of the sample molecules from the moving eluent (inter-particle volume) to the internal eluent (intra-particle volume), across the stagnant film of eluent surrounding the particles. The second mass transfer resistance term is due to the diffusivity of the sample across the particles, including the contributions of mesopore and surface diffusion. Early this year, Agilent came up with the new 2.7 μm Poroshell 120 while Advanced Material Technologies launched a second brand, the 2.7 μm Halo-ES-peptide shell particles [18] providing exceptional performance for peptides and small proteins. Packed in 4.6 mm I.D. tubes, all these particles give columns exhibiting plate heights equivalent to those achieved with the latest state-of-the-art sub-2 μm particles, with H between 3 and 4 μm .

We report here on the results of physico-chemical investigations of the reasons why 4.6 mm I.D. columns packed with modern shell particles perform so much better than fully porous particles. The analysis of the longitudinal diffusion coefficient (B coefficient) of the HETP equation was performed by applying the peak parking method to columns packed with fully and superficially porous particles [19–21]. The trans-particle mass transfer resistance coefficient (C_p coefficient) was determined by analyzing the results of peak parking measurements with a combination of several models of diffusion in heterogeneous packed beds and the general expression of C_p for mass transfer in porous media [2,3]. The external film mass transfer coefficient, C_f , was derived from the Wilson & Geankoplis correlation for small molecules [22]. This correlation was recently validated for the transfer of small molecules through porous particles [23]. The eddy diffusion term, A , was derived by subtraction of the $\frac{B}{v}$, $C_p v$, and $C_f v$ terms from the experimental reduced HETPs. Finally, we discuss the possibilities of improving the performance of these shell particles toward the separation of large molecules.

2. Theory

The overall reduced HETP of a chromatographic column can be accounted for as the sum of five main independent contributions that can be measured separately [21]: (1) longitudinal diffusion (the B term); (2) eddy diffusion (the A term); (3) the external film mass transfer resistance (the C_f term); (4) the trans-particle mass transfer resistance (the C_p term); and (5) an additional contribution due to the heat friction of the eluent percolating across the bed, the h_{Heat} term [24–27].

$$h = \frac{B}{v} + A(v) + C_f v + C_p v + h_{\text{Heat}} \quad (1)$$

The term h_{Heat} is negligible at small flow rates, for weakly adsorbed compounds and eluents having high thermal conductivities or under adiabatic conditions [28].

The reduced interstitial velocity v and the reduced HETP are defined as

$$v = \frac{ud_p}{D_m} \quad h = \frac{H}{d_p} \quad (2)$$

where H is the column HETP, d_p the average particle size of the packing material, D_m is the bulk molecular diffusivity, and u the interstitial linear velocity given by:

$$u = \frac{F_v}{\epsilon_e \pi R_c^2} \quad (3)$$

where F_v is the volume flow rate of the mobile phase, ϵ_e is the interstitial porosity, and R_c the inner radius of the column tube. The determination of the diffusion coefficients was explained elsewhere [21].

3. Experimental

3.1. Chemicals

The mobile phases were either mixtures of water and acetonitrile or pure tetrahydrofuran. Dichloromethane ($\rho_{\text{CH}_2\text{Cl}_2} = 1.323 \text{ g/cm}^3$) was used to measure the column hold-up volumes by pycnometry in combination with tetrahydrofuran ($\rho_{\text{THF}} = 0.883 \text{ g/cm}^3$). These four solvents were HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). The mobile phase was filtered before use on a surfactant-free cellulose acetate filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Insulin was a generous gift from Eli Lilly (Indianapolis, IN, USA). Trifluoroacetic acid (TFA), thiourea, and naphtho[2,3-*a*]pyrene were also purchased from Fisher Scientific. The sample test mixture containing uracil, acetophenone, toluene, and naphthalene in pure acetonitrile was generously offered by Phenomenex (Torrance, CA, USA).

3.2. Columns

The Kinetex 2.6 μm C_{18} 100 Å and Luna 3.0 μm $C_{18}(2)$ 100 Å columns (100 mm \times 4.6 mm) were offered by the manufacturer (Phenomenex, Torrance, CA, USA). The 2.7 μm Halo- C_{18} 90 Å and Halo-ES-peptide- C_{18} 160 Å columns (150 mm \times 4.6 mm) were generous gifts from the column manufacturer (Advanced Material Technologies, Wilmington, DE, USA). The 1.7 μm BEH- C_{18} 135 Å and 300 Å (100 mm \times 3.0 mm, 150 mm \times 4.6 mm, and 50 mm \times 2.1 mm) and 3.0 μm Atlantis-d C_{18} (150 mm \times 4.6 mm) columns were also provided by the manufacturer (Waters, Mildford, MA, USA).

It is worth noting that, out of a lot of 133 columns packed with the same batch of Kinetex particles, following the same packing procedure, the efficiency of the best column deviated by less than 10% from the mean efficiency. The average particle size of Kinetex particles (2.5 μm) was measured by the Coulter counter technique after calibration of this instrument with the scanning electron microscopy (SEM) data obtained for the same lot of particles.

3.3. Measurement of the HETP data

3.3.1. Low molecular weight compounds: uracil, acetophenone, toluene, and naphthalene

The mobile phase was a mixture of water and acetonitrile (20/80, v/v). The sample volume injected was 1 μL . Although it is theoretically possible that a mismatch between the composition of the eluent (80% ACN) and that of the sample solution (100% ACN) affects somewhat the evaluation of the mass transfer kinetics parameters, in practice it would be difficult to detect the influence of the injection of a 1 μL sample on the efficiency of a 100 mm \times 4.6 mm

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