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Mass-transfer properties of insulin on core–shell and fully porous stationary phases

Nándor Lambert[a], Ibolya Kiss[b], Attila Felinger[a,b,*]

[a] MTA–PTE Molecular Interactions in Separation Science Research Group, Ifjúság útja 6, H-7624 Pécs, Hungary
[b] Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Ifjúságútja 6, H-7624 Pécs, Hungary

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ABSTRACT

The mass-transfer properties of three superficially-porous packing materials, with 2.6 and 3.6 μm particle and 1.9, 2.6, and 3.2 μm inner core diameter, respectively, were investigated and compared with those of fully porous packings with similar particle properties. Several sources of band spreading in the chromatographic bed have been identified and studied according to the general rate model of chromatography. Besides the axial dispersion in the stream of the mobile phase, and the external mass transfer resistance, the intraparticle diffusion was studied in depth. The first absolute and the second central moments of the peaks of human insulin, over a wide range of mobile phase velocities were measured and used for the calculation of the mass-transfer coefficients. The experimental data were also analyzed using the stochastic or molecular dynamic model of Giddings and Eyring. The dissimilarities of the mass-transfer observed in the different columns were identified and evaluated.

1. Introduction

During the recent years, a number of HPLC columns packed with superficially porous particles of different particle and core radii and pore size distributions have been introduced. The most frequent particle diameter of such packing materials is between 2.5 and 5 μm; the currently available smallest core–shell particles have 1.3 μm diameter. The performance of the superficially porous particles is significantly higher than the conventional packings. The efficiency of the columns packed with 2.6 μm core–shell particles is nearly the same as that of columns packed with sub-2-μm fully porous particles, but they can be operated at lower pressures, consequently lesser efficiency loss due to thermal effects is observed [1,2].

The physical reasons of the increased efficiency lie in the decrease of the mass-transfer contributions to the sample band spreading during the chromatographic separation processes. The description of the mass transfer processes in columns packed with core–shell particles has received great attention [3–9].

Mass transfer in a chromatographic column has several contributions, such as the axial dispersion in the stream of the mobile phase, the external mass-transfer resistance at the particle boundary, the intraparticle diffusion or the adsorption–desorption process on the surface of the stationary phase [10]. Several models are utilized to describe the chromatographic process. One of the most detailed model is the general rate model of chromatography (GR model), which considers a number of possible sources of mass-transfer resistances. The model assumes that the mobile phase percolates through the interstitial space between the stationary phase particles. Diffusion drives the molecules from the flowing mobile phase to the pores, where the mobile phase is stagnant and adsorption and desorption take place within the pores at the surface of the stationary phase. The mass-transfer of the solute through the internal network of the mesopores inside the particles is derived from two parallel diffusion mechanisms. One through the mobile phase that fills the pores, and the other along the stationary phase surface. Kaczmarski and Guiochon applied the general rate model to superficially porous particles, and studied the influence of an inner solid core on the column efficiency [3].

Besides the unusually low value of the eddy dispersion and thus the reduced longitudinal diffusion caused by the presence of the solid core, the decrease of the intraparticle diffusion is particularly important in core–shell packed columns. These advantages are more expressive during the analysis of large molecules, such as proteins and other biomolecules, due their lower diffusion coefficients [11].

An alternative to the traditional macroscopic models that usually formulate a mass balance equation, e.g. the general rate

* Corresponding author at: Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary. Tel.: +36 72 501500x24582; fax: +36 72 501518.
E-mail address: felinger@ttk.pte.hu (A. Felinger).

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model, is the microscopic approach by the description of the chromatographic process with the movement of the chromatographic bands at molecular level. The molecular dynamic model of Giddings and Eyring describes the chromatographic process with the random migration of the molecules along the column and with randomly occurring adsorption–desorption events [12]. This stochastic model provides time constants that represent the residence time of a molecule in the stationary phase or in the mobile phase, when it is adsorbed or is in liquid state.

Besides the well known sub-3-μm partially porous particles such as Halo or Kinetex, novel core–shell packing materials, such as Aeris peptide and Aeris Widepore dedicated for the separation of macromolecules, are available on the market. The differences of the structure of these column packing material originate from the different core-to-particle ratios and average pore diameters. The aim of this study was to compare the mass-transfer coefficients of the core–shell particles with that of fully porous packing materials with help of a macromolecule (human insulin). Two different methods were used to calculate the coefficients: the general rate model and the stochastic model of chromatography.

2. Theory

2.1. The general rate model

The general rate model of chromatography assumes that diffusion drives the sample molecules from the stream of the mobile phase into the particles and inside the pores of the stationary phase particles. The mobile phase is stagnant in the pores of the particles, and the adsorption–desorption process takes place between the stagnant mobile phase and the surface of the pores [10].

The moments of a chromatographic peak, calculated from the general rate model allow the derivation of a detailed plate height equation [4,7].

The retention time, or first moment (μ_1) of a chromatographic peak is obtained as

$$\mu_1 = \frac{\int C(t)t dt}{\int C(t)dt} = \frac{L}{u_h}(1+k_1) \tag{1}$$

with

$$k_1 = F[\varepsilon_p + K(1-\varepsilon_p)] \tag{2}$$

and

$$F = \frac{1-\varepsilon_e}{\varepsilon_e} \tag{3}$$

where $C(t)$ is the chromatographic band profile, L the length of the column, u_h the interstitial velocity of the mobile phase, ε_e and ε_p the external and internal porosity respectively, and K the equilibrium constant of the adsorption or partition, and F is the column phase ratio.

The second central moment calculated via the general rate model is expressed as follows:

$$\mu_2' = \frac{\int C(t)(t-\mu_1)^2 dt}{\int C(t)dt} = \frac{2L}{u_h}\left[\frac{D_L}{u_h^2}(1+k_1)^2 + \frac{Fa^2}{u_h}\left(\frac{r_p}{3k_{ext}} + \frac{r_p^2}{15D_e}\right)\right] \tag{4}$$

where D_L is the axial dispersion coefficient, k_{ext} the external mass-transfer coefficient, D_e the intraparticle diffusion coefficient, and r_p the average particle radius. The moments calculated from the general rate model allow the derivation of the following plate height equation:

$$H = L\frac{\mu_2'}{\mu_1^2} = \frac{2D_L}{u_h} + \frac{2u_h}{F}\left(\frac{k_1}{k_1+1}\right)^2\left(\frac{r_p}{3k_{ext}} + \frac{r_p^2}{15D_e}\right) \tag{5}$$

In case of core–shell particles, the plate height equation (Eq. (5)) must be corrected, because of the altered geometry of diffusion paths inside the particles. Due to the presence of the solid core, the plate height equation will take the following form [3]:

$$H = L\frac{\mu_2'}{\mu_1^2} = \frac{2D_L}{u_h} + \frac{2u_h}{F}\left(\frac{k_1}{k_1+1}\right)^2\left(\frac{r_p}{3k_{ext}} + \frac{r_p^2}{15D_e}R\right) \tag{6}$$

where R is expressed as follows:

$$R = \frac{r_p^4 + 2r_p^3 r_i + 3r_p^2 r_i^2 - r_p r_i^3 - 5r_i^4}{(r_p^2 + r_p r_i + r_i^2)^2}, \tag{7}$$

where r_i and r_p are the internal, and the external radii of the porous shell of the superficially particles. For a fully porous particle $R = 1$.

The axial dispersion coefficient, D_L, must be known to characterize the band broadening in the interstitial volume of the column. Gunn's correlation [13] is widely used to estimate the axial dispersion coefficient. In this study a simplified form of the Gunn correlation was used, written with convenient chromatographic terms resulting in the following equation [4,7]:

$$\frac{2D_L}{u_h d_p} = \frac{2\gamma}{\nu} + \frac{2\lambda\omega\nu/F}{2\lambda + \omega\nu/F} \tag{8}$$

where $\gamma = 0.714$, $\lambda = 2.586$, $\omega = 0.0712$, ν is the reduced interstitial velocity and F is the phase ratio (Eq. (3)). The numerical values of the parameters were determined by the fitting of Eq. (8) to the Gunn correlation over a wide range of reduced interstitial velocities [4]. For the systems investigated in this study, the axial dispersion calculated by Eq. (8) resulted in values rather similar to those that can be obtained by the protocol suggested by Gritti and Guiochon [14].

The external mass transfer coefficients were calculated by using the Wilson–Geankoplis equation [15]:

$$k_{ext} = \frac{1.09}{\varepsilon_e}u_h^{1/3}\left(\frac{D_m}{d_p}\right)^{2/3} \tag{9}$$

where D_m is the molecular diffusion coefficient which can be estimated for macromolecules with the correlation derived by Young et al [16]:

$$D_m = 8.31 \times 10^{-8}\frac{T}{\eta M_w^{1/3}} \tag{10}$$

where D_m is given in cm^2/s, the viscosity of the mobile phase (η) is given in cP, M_w is the molecular weight of the analyte, and T is the absolute temperature.

2.2. Stochastic model

The molecular dynamic model introduced by Giddings, and Eyring [12] describes the movement of chromatographic bands with the random migration of molecules along the column with randomly occurring adsorption–desorption events. When we apply the stochastic theory to reversed phase separations, the model will characterize the mass-transfer process – including the external mass-transfer resistance, the diffusion in the pore of the particle and the adsorption kinetics itself – rather than the adsorption process itself.

According to the stochastic model, the chromatogram is determined as the probability density function of the residence times of the individual molecules in the column. The random number of