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Mass transfer kinetic mechanism in monolithic columns and application to the characterization of new research monolithic samples with different average pore sizes

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ABSTRACT

A general reduced HETP (height equivalent to a theoretical plate) equation is proposed that accounts for the mass transfer of a wide range of molecular weight compounds in monolithic columns. The detailed derivatization of each one of the individual and independent mass transfer contributions (longitudinal diffusion, eddy dispersion, film mass transfer resistance, and trans-skeleton mass transfer resistance) is discussed. The reduced HETPs of a series of small molecules (phenol, toluene, acenaphthene, and amylbenzene) and of a larger molecule, insulin, were measured on three research grade monolithic columns (M150, M225, M350) having different average pore size ($\simeq$ 150, 225, and 350 Å, respectively) but the same dimension (100 mm $\times$ 4.6 mm). The first and second central moments of 2 μL samples were measured and corrected for the extra-column contributions. The h data were fitted to the new HETP equation in order to identify which contribution controls the band broadening in monolithic columns. The contribution of the B-term was found to be negligible compared to that of the A-term, even at very low reduced velocities ($\nu < 1$). At moderate velocities ($1 < \nu < 3$), the contribution of the A-term decreases with increasing mesopore size and molecular diffusivity of the compound studied due to faster mass transfer across the column. Experimental chromatograms exhibited variable degrees of systematic peak fronting, depending on the column studied. The heterogeneity of the distribution of eluent velocities from the column center to its wall (average 5%) is the source of this peak fronting. At high reduced velocities ($\nu > 5$), the C-term of the monolithic columns is controlled by film mass transfer resistance between the eluent circulating in the large throughpores and the eluent stagnant inside the thin porous skeleton. The experimental Sherwood number measured on the monolith columns increases from 0.05 to 0.22 while the adsorption energy increases by nearly 6 kJ/mol. Stronger adsorption leads to an increase in the value of the estimated film mass transfer coefficient when a first order film mass transfer rate is assumed ($j \propto k_f \Delta C$). The average pore size and the trans-skeleton mass transfer have no ($< 0.5\%$, small molecules) or little ($< 10\%$, insulin) effect on the overall C-term.

1. Introduction

The development of continuous, porous silica rods in the late 1980s and during the 1990s [1–6] provided chromatographic columns that are potentially faster and more efficient than standard packed columns. The structure of silica monolithic rods are characterized by their domain size, which is the sum of the average through pore diameter and the average diameter of the porous silica skeleton. The macroscopic self-similarity of their structure is often characterized by the size ratio of their domain and of the skeleton ($\simeq$2.5). The silica monolithic columns initially commercialized by Merck (Darmstadt, Germany) was a 100 mm $\times$ 4.6 mm cylindrical rod sealed with a poly(ether ether ketone) (PEEK) tube. Its through pore and skeleton size were 2 μm and 1.3 μm, respectively. Monolithic silica rods were also prepared inside glass capillaries of inner diameter smaller than 500 μm [7]. For wider cores, breakage occurs between the monolith and the tube wall, due to shrinkage during the sol–gel preparation process. Such columns showed high sample capacity per unit adsorbent volume [8], a permeability comparable to that of 11 μm packed columns [9], and an efficiency equivalent to that of 3.5 μm packed columns [10]. As a result, these columns provided a much lower separation impedance than columns packed with either 5 or 3.5 μm particles [11]. When 5 μm particles were the standard packing material, monolithic columns appeared as a major breakthrough development of new supports. A recent review covering nearly a decade of research development of monolithic columns reports on the preparation, the characterization, and the chromatographic properties of monolithic columns [12]. It concluded that the

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large hopes initially generated by these columns at the beginning of their development eventually lead to disappointment when it was realized that structural features inherent to their fabrication process limit the efficiency of these new columns [13]. Significant progress never materialized.

The first structural feature that limited the success of silica monolithic over packed columns technology is due to the large size distribution, the random spatial distribution, and the variable geometry of the throughpores (the name of the macropores in the bimodal pore structure of monoliths), the equivalent of the interstitial channels in packed beds. Calculations made on the flow distribution in ordered and disordered two-dimensional throughpores confirmed this observation [14]. Scanning electron micrographs (SEM) of monolithic columns reveal the random spatial distribution of macropores [11,15], which causes a high degree of band spreading along the columns. Compared to the more densely and regularly packed columns, monoliths exhibit an eddy dispersion term (or A-term after the classical van Deemter definition) that presents a serious limiting factor to the preparation of more efficient silica monolithic structures. In theory, the A-term can be treated according to the Giddings coupling theory of eddy dispersion as the combination of a transchannel, a short-(one domain size) and/or a long-range (a few domain sizes) interchannel velocity biases. The equivalent for the interstitial channel in packed beds is the throughpore in monolithic columns.

A second limiting structural feature is the actual size of the throughpores. Due to the recent trend toward the use of finer particles (the average particle size decreased from 5 to 3.5, then to sub-2 μm in about a decade) modern columns can now operate with much smaller throughpore size while keeping a very similar geometry. Comparable decrease in the domain size of silica monolithic columns has not yet been achieved.

The last structural feature of silica monolithic columns which limit their efficiency is the macroscopic radial heterogeneity of the cylindrical rods, which is due to the shrinkage of silica occurring during the sol–gel preparation process. The main challenge now encountered in the preparation of very efficient silica monolithic columns is to reduce the radial gradient of mobile phase linear velocities from the center to the rod wall. Although this radial heterogeneity is nearly impossible to assess from SEM pictures, it can be measured locally using chromatographic techniques. Micro-electrodes placed directly at different radial positions of the outlet cross-section area of the silica monolithic rod permit the direct measurement of the linear velocity of the mobile phase. The velocity was found to be 4% higher at the wall than at the center of a 100 mm × 10.0 mm monolithic column [16]. A minor difference in the average external porosity near the wall, hence a larger local bed permeability, could easily explain this experimental observation. Interestingly, the reversed trend was observed with packed columns, suggesting that their center is less densely packed than their wall [17]. The local HETP of the wide monolithic rod increases nearly twice from its center to its wall, which significantly diminishes the average cross-section HETP measured with the usual detectors located downstream the column and analyzing the bulk mobile phase. This radial heterogeneity of the monolith can be considered as a transcolumn velocity bias contributing to the A-term.

Therefore a key to the improvement of the structure of silica monolithic silica column may lay in the production of a monolith having a more ordered macropore distribution of the three-dimensional space, i.e., a more ordered spatial organization of the quasi-cylindrical skeleton elements that are randomly connected to each other during the sol–gel formation and the drying process. Smaller domain size were also considered but the improvement was not comparable to what is achieved by a decrease in packed particle size because the domain size distribution of monoliths seems to widen with decreasing average domain size. For instance, Tanaka and co-workers [18] showed that the minimum of the HETP curve of amylbenzene increased from 7 to 9 μm when the domain size was decreased from 3.8 to 2.9 μm, respectively. The minimum HETP was not significantly decrease when the domain and skeleton sizes were reduced to 2.3 and 1 μm, respectively. It was still larger than 5 μm. For the sake of comparison, the minimum HETP of columns packed with 1.7 μm particles, with a corresponding domain size of 2.1 μm is typically 2.8 μm [19].

In this work, we investigate the adsorption and mass transfer of a series of small neutral compounds (phenol, toluene, acenaphthene, amylbenzene) and a small protein (insulin) on three research prototype silica monolithic columns having different average pore sizes (150, 225, and 350 Å). The size of the analyte molecule compared to the average pore size can affect retention and column efficiency, due to the simultaneous effects of pore exclusion, pore diffusion hindrance, and surface diffusion [20,21]. The experimental data were compared and fitted to a general HETP equation model derived for monolithic columns. The mass transfer resistance was taken as the sum of four independent contributions, longitudinal diffusion, eddy dispersion, film mass transfer, skeleton mass transfer. The peak parking method [22,20,23] was used to measure the effective sample diffusivities through the whole chromatographic bed (longitudinal and radial diffusion coefficients) and through the mesoporous cylindrical skeleton (trans-skeleton mass transfer resistance). The eddy dispersion term of the monolithic column was modeled based on the known structure of monolithic silica rods [12], as was done by Giddings for packed beds [24], and completed for transcolumn effects based on data available in the literature [16,17]. The film mass transfer resistance was derived from the value of the film mass transfer coefficient, k_f, given by the penetration theory [25] and assuming a first-order rate film mass transfer model between solution and the solid cylinders [26]. The impact of the average mesopore size of the rod silica monolith on the retention of the different samples and on the different kinetic contributions will be discussed.

2. Theory

2.1. HETP equation for a monolithic column

Consider a monolithic column at constant temperature T. The general HETP equation corrected for the extra-column contributions is the sum of four main independent mass transfer terms [24], accounting for the longitudinal diffusion of the analyte during its migration along the column ($h_{Long.}$), the eddy dispersion of the analyte in the throughpores, due to a differential migration velocity across and along the column (h_{Eddy}), the resistance to mass transfer by diffusion through the porous skeleton ($h_{Skel.}$), and the film mass transfer resistance between the mobile phase in the throughpores and the stagnant eluent inside the mesopores of the cylindrical silica skeleton (h_{Film}):

$$h = h_{Long.} + h_{Eddy} + h_{Skel.} + h_{Film} \quad (1)$$

These four HETP terms are derived in terms relevant to the structure of monolithic columns. By definition, all the reduced HETP terms refer to the average skeleton diameter d_{skel}.

(1) The analyte band spreads axially along the column, due to the relaxation of the axial concentration gradient. At a zero linear velocity, the band spreads as the result of the combination of diffusion taking place in the throughpore volume (the volume fraction is equal to the external porosity ϵ_e, bulk molecular diffusion coefficient D_m) and in the porous skeleton volume (volume fraction $1 - \epsilon_e$, effective skeleton diffusivity D_{Skel}). Assuming as a first approximation a parallel contribution of these two diffusion pro-