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Mass transport of small retained molecules in polymer-based monolithic columns

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ABSTRACT

The mass transport properties of a non-retained (thiourea) and three retained low molecular weight compounds (acetophenone, valerophenone, and octanophenone) along a 4.6 mm × 45 mm PROSWIFT™ RP-1S monolithic column made of rigid cross-linked poly(styrene-divinylbenzene) copolymer was investigated in depth. Accurate protocols (peak parking experiments, measurement of the first and second central moments of peak profiles by numerical integration) combined with the use of validated models of effective diffusion along monolithic structures were applied for the determination of the longitudinal diffusion, the eddy dispersion, and the skeleton–eluent mass transfer resistances due to the finite analyte diffusivity across the polymer skeleton and to the slow absorption kinetics into the polymer volume. Experimental results show by increasing order of importance evidence that the resolution performance of this short and wide polymer-based monolithic HPLC column is limited by the slow analyte diffusivity across the polymer skeleton (smaller than one tenth of the bulk diffusion coefficient for $k' > 1$), its large eddy dispersion HETP ($H_{eddy} \simeq 100\,\mu m$), and the slow rate of absorption ($\simeq$10 Hz only) in the polymer volume for retained analytes. The column performance could be improved by preparing a more homogeneous material with a rigid internal mesoporous structure. This would provide a column bed having a larger specific surface area, allowing faster analyte diffusion across the mesoporous skeleton, a smaller eddy dispersion HETP, and a faster absorption kinetics in the polymeric monolith than those observed for the currently available materials.

1. Introduction

Analytical HPLC columns based on monolithic, polymeric stationary phases were first prepared in the late 1980s and early 1990s [1–4]. Their recent developments were thoroughly documented [5] and the numerous chemistry routes available for their preparation were reviewed [6]. They include free radical processes (initiated thermally, by photolysis or by radiations), polymerized emulsions, cryogels, living polymerizations (using nitroxide, organotellurium, atom transfer or ring-opening metathesis polymerizations), and polycondensations. First attempts at establishing relationships between the preparation, the morphology, the porous properties and the chromatographic properties of polymer-based monolithic materials were recently documented [7,8]. Yet, research to optimize monolithic beds suitable for chromatographic columns, filtration systems, or capture/release devices remains an open field. Little is known about the flow profiles and the detailed mass transfer mechanism in these media [9]. It seems that the minimum plate heights of commonly available poly(styrene-co-divinylbenzene) monolithic columns often exceeds 20 μm and that the Cu HETP term (the polymer–eluent mass transfer resistance) in their van Deemter equation is excessively large for small retained analytes [10,7]. In comparison, the first and second generations of silica-based monolithic columns have similar or even larger domain sizes than those of polymer-based monoliths but provide minimum plate heights of only 4–10 μm while their Cu HETP terms are relatively low [11–16]. This difference is puzzling and suggests that investigations of the mass transfer mechanism of small and large molecules across polymer-based monoliths are needed, to provide a better understanding of the actual mass transfer mechanism in polymer-based monolithic columns. Nevertheless, it is important to remember that polymer monoliths perform better than silica monoliths for the separation of large molecules because proteins are essentially excluded from the polymer volume and their mass transfer is not limited by their slow diffusion across

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the stationary phase as it is along the mesopores of silica monoliths.

Image reconstruction of a hyper-cross-linked poly(styrene-divinylbenzene) monolith confined in a 100 μm i.d. fused-silica capillary tube was carried out by serial block-face scanning electron microscopy [9]. This work provided quantitative information on the solvated-state morphology of this polymer monolith, identified domains of two different sizes in the bulk structure, and revealed a degree of heterogeneity in the radial macroporosity profile of the column. These short- and long-range structural heterogeneities could explain the rather large values of the minimum HETP observed for small, non-retained compounds in polymer-based monolithic columns [7]. In contrast, the reasons for the excessively large peak widths of small retained molecules was not elucidated. It is unclear whether it is due to a very slow diffusivity of these analytes across the solvated, permeated polymer skeleton or to a slow absorption kinetics into the polymer globules that agglomerate to form the monolithic bed. Therefore, a detailed, meticulous, quantitative investigation is needed to accurately measure the contributions of longitudinal diffusion, eddy dispersion, and solid–liquid mass transfer resistance in polymer-based monolithic columns. Accurate procedures were previously designed to answer this challenge [17–19]. They were applied to determine mass transfer mechanisms in RPLC [20,19], HILIC [21,22], and chiral RPLC retention modes [23,24].

The goal of this work is a quantitative determination of the mass transfer mechanism of low molecular weight analytes across a rigid cross-linked poly(styrene-divinylbenzene) copolymer monolith. The polymer is equilibrated with an eluent made of acetonitrile and water. The porosity of the monolith was derived from the retention time profile of a non-retained marker (thiourea) over the whole concentration range of acetonitrile in water (0–100%). The permeation of the hydrophobic cross-linked polymer by the organic modifier (acetonitrile) was derived from the excess absorption isotherm of acetonitrile from water into the polymer skeleton measured by the minor disturbance method (MDM). Peak parking (PP) experiments were used to estimate the longitudinal diffusion of the analytes. They were combined with three different models of effective diffusivity across monolithic structures in order to estimate the skeleton–liquid mass transfer resistance due to the finite diffusivity of analytes across the polymer skeleton. These models are the Torquato model for interconnected cylinders [25], the Landauer model for random inclusions in a continuous medium [26–28], and the conventional time-averaged diffusion model [29]. The eddy dispersion HETP term was derived from the dispersion data of non-retained analytes after correction for the obstruction factor due to the polymer monolith structure. The possible slow absorption kinetics of analytes into the monolith was measured by subtracting the longitudinal diffusion, the eddy dispersion, and the skeleton–liquid mass transfer resistance HETP terms from the total HETP. The results suggest possible sources of band broadening that limit the resolution of small retained analytes on polymeric monolithic columns and new approaches to prepare a new generation of polymer monoliths providing fast and efficient resolution of small molecules.

2. Theory

Three different types of column beds are known; they are made of packed particles, of randomly aligned fibers, and of monoliths. Particle beds have been used forever in chromatography and their properties are well known [30]. Columns made with fibers have been investigated several times, with little success due to their poor efficiency caused by their radial heterogeneity [31–33]. Monolithic columns have been more successful. They are made of either silica or cross-linked polymers [5]. The structure of the space around and inside these three types of packing materials have important differences that strongly affect the column performance.

Most particles used to pack chromatographic columns are compact, ovoid objects, which have dimensions nearly equal along the three directions of their main inertia axes. The presence of the column wall prevents their beds from being fully homogeneous by causing a slight radial distribution of the bed density. The diffusion coefficients of analytes are nearly the same in all directions and the mobile phase velocities exhibit only a slight local decrease from the column center to its wall. Their average values over distances of the order of several average particle diameters are nearly constant along the column. In contrast, columns made of quasi-parallel fibers are strongly heterogeneous. Occasionally, a fiber is not parallel to its neighbors but runs across a number of them, causing serious local perturbations of the distributions of mobile phase velocities and diffusion coefficients, hence drastically affecting the migration of analyte bands [31] and the column performance. The properties of monoliths are intermediate. They are made of elongated masses of porous material, similar to needles for silica monoliths, intermediate between bundles and heaps of wires for polymeric monoliths. The properties of these mesoporous silica needles are very similar to those of silica particles. Because the needles and wires are thin and the relative amount of the space available to the mobile phase stream between them is high, the radial mass transfer of analytes is fast and the column efficiency comparable to that of columns packed with particles. While mass transfer across silica needles is very similar to that across silica particles, however, mass transfer across bundles of polymeric chains depends very much on the flexibility of the polymer molecules that controls diffusion across the stationary phase.

2.1. Definitions

Four different porosities can be distinguished in a polymer monolithic. By decreasing order of their average size, the total porosity includes the volumes of the inter-skeleton (average skeleton size, 1.1 μm) macropores (>50 nm), of the inter-globular (the average size of agglomerated PS-DVB globules was adjusted to less than 500 nm, according to the manufacturer) macropores (>50 nm), the intra-skeleton mesopores (2–50 nm), and the intra-globular micropores (<2 nm).

In this work, the porosity ϵ of the polymer monolith, a crosslinked poly(styrene-divinylbenzene) co-polymer, in contact with the mobile phase is defined as the sum of the macroporous and the mesoporous volumes in the flow-through channels between aggregates of polymer globules that forms the skeleton and in the stagnant inter-globular eluent, respectively. The microporous volume made of stagnant liquid pools inside the polymer globules is considered as part of the stationary phase. The average size of the polymer skeleton is $d_{skel} \simeq 2$ μm. The analyte diffusion coefficient in the bulk mobile phase is D_m; its diffusivity through the permeable globules is $D_g = \Omega D_m$, with Ω being the dimensionless ratio of the analyte diffusivity through the poly(styrene-divinylbenzene) globules to its bulk diffusion coefficient. The reference concentration gradient taken for the definition of the diffusion coefficient D_g is the bulk concentration [28]. The effective diffusion coefficient along the heterogeneous polymer-based monolithic column (polymer skeleton and external eluent) is D_{eff}.

The retention factor k' of an analyte is [34,19]:

$$k' = \frac{1-\epsilon}{\epsilon} K_a \tag{1}$$

where $K_a = (c_s/c_m)$ is the equilibrium ratio of the analyte concentrations in the polymer volume and in the bulk mobile phase volume.