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Assessment of intra-particle diffusion in hydrophilic interaction liquid chromatography and reversed-phase liquid chromatography under conditions of identical packing structure

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ABSTRACT

A recently developed stripping protocol to completely remove the stationary phase of reversed-phase liquid chromatography (RPLC) columns and turn them into hydrophilic interaction liquid chromatography (HILIC) columns with identical packing characteristics is used to study the underlying mechanisms of intra-particle diffusion in RPLC and HILIC. The protocol is applied to a column with a large geometrical volume (250 × 4.6 mm, 5 μm) to avoid extra-column effects and for compounds with a broad range in retention factors (k" from ~0.6 to 8). Three types of behavior for the intra-particle diffusion (D_{part}/D_m) in RPLC versus HILIC can be distinguished: for nearly unretained compounds (k" < 0.6), intra-particle diffusion in HILIC is larger than in RPLC; for compounds with intermediate retention behavior (k" ~ 0.9–1.2), intra-particle diffusion in HILIC and RPLC are similar; and for well retained compounds (k" > 1.8), intra-particle diffusion in RPLC is larger than in HILIC. To explain these observations, diffusion in the stationary phase ($\gamma_s D_s$) and in the stagnant mobile phase in the mesopore zone ($\gamma_{mp} D_m$) are deduced from experimentally determined values of the intra-particle diffusion, using models derived from the Effective Medium Theory. It is demonstrated that the larger intra-particle diffusion obtained for slightly retained compounds under HILIC conditions is caused by the higher mesopore diffusion in HILIC (γ_{mp} = 0.474 for HILIC versus 0.435 for RPLC), while the larger intra-particle diffusion obtained for strongly retained compounds under RPLC conditions can be related to the much higher stationary phase diffusion in RPLC ($\gamma_s D_s/D_m$ = 0.200 for RPLC versus 0.113 for HILIC).

1. Introduction

Liquid chromatography is nowadays a well-established separation tool in various fields of analysis. Many research efforts are currently devoted to the progression of this technique, focusing on fundamental studies of separation theory [1–3], the study and development of faster, and more efficient columns [4–8], and the creation of suitable chromatographic systems to operate these high efficiency columns [9,10]. In the last decade, column and instrument manufacturers have commercialized shorter and narrower columns packed with finer particles, and ultra-high pressure liquid chromatographic equipment (UHPLC) that can reach pressures up to 1500 bar. By far the most popular column format in (U)HPLC is the packed bed of porous silica particles. The silanol groups at the surface of these porous particles can be bonded to different ligands to obtain various surface chemistries. The large specific surface area inherent to porous particles, in combination with dedicated surface chemistries, results in a number of important interaction sites for compounds of interest. To access these interaction sites, analyte molecules must, however, diffuse across the intra-particle zone. The extent to which this intra-particle diffusion occurs can be expected to have a large impact on the band broadening and hence efficiency obtained in these porous particles. A detailed understanding of intra-particle mass transfer in (U)HPLC is therefore essential to improve the performance of current chromatographic columns.

Intra-particle diffusion combines the parallel contributions of diffusion in the (obstructed) stationary phase ($\gamma_s D_s$) and diffusion in the stagnant mobile phase in the mesopores ($\gamma_{mp} D_m$), in short referred to as mesopore diffusion in the rest of the paper. Stationary phase diffusion is either obtained by molecules adsorbed on or dissolved in the stationary phase. The driving force for station-

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ary phase diffusion is the concentration gradient of the molecules in this adsorbed/dissolved state. Mesopore diffusion on the other hand is obtained when molecules diffuse through the stagnant mobile phase in the particle pores with their concentration gradient as the main driving force. In this case, there is no interaction with the stationary phase [11]. To explain the observed intra-particle diffusion (D_{part}) in a certain packing material, the values of its contributing mechanisms should be known accurately.

Two approaches can be used to deduce γ_{mp} and $\gamma_s D_s/D_m$ from experimentally measured values of D_{part}/D_m, based on the following equation derived from the Effective Medium Theory (EMT):

$$\frac{D_{part}}{D_m} = \frac{\varepsilon_e \gamma_{mp} + (1-\varepsilon_e) K_{A,part} \gamma_s D_s/D_m}{\varepsilon_e + (1-\varepsilon_e) K_{A,part}} \quad (1)$$

with ε_e the external porosity and $K_{A,part}$ the solid phase-based equilibrium constant. An existing geometrical model can be used to estimate the value of γ_{mp}, after which the value of $\gamma_s D_s/D_m$ can be derived directly from Eq. (1). Alternatively, Eq. (1) can be fitted to experimentally obtained values of D_{part}/D_m for random values of γ_{mp} and $\gamma_s D_s/D_m$ until a good agreement between estimated and experimentally obtained D_{part}/D_m value is obtained [12]. The shortcoming of the former approach is that various expressions exist to calculate γ_{mp}, each with their own range of applicability and degree of accuracy [13–15]. Most existing correlations to predict the value of γ_{mp} are moreover based on a number of assumptions (pores are treated as cylindrical tubes, analytes are considered to be hard spheres). The latter approach on the other hand can easily be performed by a two-parameter fitting of Eq. (1) to experimental D_{part}/D_m data using the solver function of a spreadsheet calculator such as MS Excel or any other software performing nonlinear regression.

Hydrophilic interaction liquid chromatography (HILIC) has steadily been gaining interest in the past few years in many applications dealing with polar and ionizable compounds [16,17]. Only little work on the fundamental study of intra-particle diffusion in HILIC has been done so far, hence little data is available on the importance of intra-particle diffusion in HILIC versus RPLC [12,18,19]. Whereas in RPLC, stationary phase diffusion is facilitated by the low-viscosity layer of acetonitrile that is present in the stationary phase, in HILIC, stationary phase diffusion will take place in a high viscosity water layer [20]. Since solute diffusion is inversely proportional to viscosity, stationary phase diffusion in HILIC is expected to occur much less compared to RPLC [21,22]. Packing differences between HILIC and RPLC columns additionally make it difficult to accurately assess differences in intra-particle mass transfer between HILIC and RPLC.

Recently, we have developed a new strategy that allows to compare mass transfer phenomena in HILIC and RPLC without having to account for differences in packing structure [18]. In this procedure, a full kinetic performance characterization of C_{18}-coated columns is first performed under RPLC conditions. The C_{18} coating of the column is subsequently completely stripped away by acid-catalyzed hydrolysis to obtain a bare silica column. This column is then characterized under HILIC conditions. It has been demonstrated that the column structure remains unaffected by the stripping procedure, allowing to compare fundamental mass transfer properties of exactly the same column under HILIC and RPLC conditions. The proof-of-principle of this protocol has been presented for two 50×4.6 mm HPLC columns evaluated for compounds with zone retention factors ranging between k" = 5.5 and 16.5. Under these circumstances, it was demonstrated that differences in intra-particle diffusion also affect eddy dispersion, a contribution that is normally ascribed to phenomena occurring outside the particles [18].

In this paper, the developed stripping model will be applied to study the underlying mechanisms of intra-particle mass transfer in HILIC and RPLC columns with an identical packing structure and to quantify the extent of each contribution (mesopore diffusion and stationary phase diffusion) to intra-particle diffusion. For this purpose, a broader range of zone retention factors will be studied (k" from ~0.6 to 8), while a column with a large geometrical volume (250×4.6 mm, 5 μm) will be used to minimize extra-column effects.

2. Experimental

2.1. Chemicals and columns

Ammonium acetate, adenine, thymidine, trifluoroacetic acid (TFA), toluene, thiourea, fructose and $NaNO_3$ were obtained from Sigma-Aldrich (Steinheim, Germany); guanine, thymine, cytosine, uracil and adenosine from Janssen chimica (Geel, Belgium). Milli-Q water was prepared in the lab using a Milli-Q gradient water purification system from Millipore (Bedford, MA, USA). HPLC grade acetonitrile (ACN) was purchased from Fisher Chemicals (Erembodegem, Belgium). HPLC grade tetrahydrofuran was from VWR (Leuven, Belgium). Glacial acetic acid and benzene were from Merck (Darmstadt, Germany) and acenaphthene from Merck (Hohenbrunn, Germany). Polystyrene standards (MW = 500, 2000, 3000, 10.000, 20.000, 30.000, 70.000, 150.000, 300.000, 700.000, 1.000.000 and 2.000.000) used for inverse size-exclusion chromatography (ISEC) experiments were purchased from Sigma–Aldrich (Bornem, Belgium). A Zorbax Eclipse Plus C_{18} (4.6×250 mm, $d_p = 5$ μm) column was obtained from Agilent Technologies (Diegem, Belgium).

2.2. Apparatus

All band-broadening and column-pressure measurements were performed on an Agilent 1290 UHPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, autosampler and diode array detector with a flow cell of 1 μL. The overall system volume was experimentally determined from the elution time of an unretained marker by replacing the column with a zero-dead volume connector. It was determined to be on average 10 μL (for flow rates ranging between 0.02 and 1.6 mL/min). Chemstation software (Agilent Technologies) was used to control the UHPLC system and for data acquisition and process.

Column stripping and peak-parking experiments were performed on a high-performance liquid chromatography system from Dionex Softron GmbH (Thermo Scientific, Germering, Germany) equipped with a high pressure pump, autosampler and UV/VIS variable wavelength detector with a flow cell of 11 μL. The overall system volume was experimentally determined to be 20 μL. Data acquisition and instrument control were performed by Chromeleon software (Thermo Scientific). Absorbances were measured at 254 nm and the data collection rate was maintained at 40 Hz for all these experiments. Viper tubing with an I.D. of 75 μm and lengths of 550 mm and 250 mm (Thermo Scientific) were used between the injector and the inlet of the column, and the outlet of the column and the detector, respectively.

Molecular diffusion coefficient (D_m) measurements and ISEC experiments were carried out on a Perkin Elmer 275 UHPLC system (Perkin Elmer, Massachusetts, USA) equipped with a binary high pressure pump, an autosampler, and a variable wavelength detector with a flow cell of 2.6 μL. The detection wavelength was set at 254 nm with a sampling rate of 50 Hz. A stainless steel viper (125 μm I.D.) was used between the injector and the inlet of the column. Between the outlet of the column and the detector, PEEK tubing with an internal diameter of 125 μm was used. The overall system volume was determined to be 13 μL. Chromera software

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