Journal of Chromatography A, 1376 (2015) 112–125

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

The relative importance of the adsorption and partitioning mechanisms in hydrophilic interaction liquid chromatography

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ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form
28 November 2014
Accepted 29 November 2014
Available online 9 December 2014

Keywords:
HILIC
Retention mechanism
Adsorption–partitioning mechanism
Intra-particle diffusivity
Torquato's model
Landauer's model

ABSTRACT

We propose an original model of effective diffusion along packed beds of mesoporous particles for HILIC developed by combining Torquatos model for heterogeneous beds (external eluent + particles), Landauers model for porous particles (solid skeleton + internal eluent), and the time-averaged model for the internal eluent (bulk phase + diffuse water (W) layer + rigid W layer). The new model allows to determine the analyte concentration in rigid and diffuse W layer from the experimentally determined retention factor and intra-particle diffusivity and thus to distinguish the retentive contributions from adsorption and partitioning. We apply the model to investigate the separation of toluene (TO, as a non-retained compound), nortriptyline (NT), cytosine (CYT), and niacin (NA) on an organic ethyl/inorganic silica hybrid adsorbent. Elution conditions are varied through the choice of a third solvent (W, ethanol, tetrahydrofuran (THF), acetonitrile (ACN), or *n*-hexane) in a mobile phase (MP) of ACN/aqueous acetate buffer (pH 5)/third solvent (90/5/5, v/v/v). Whereas NA and CYT retention factors increase monotonously from W to *n*-hexane as third solvent, NT retention reaches its maximum with polar aprotic third solvents. The involved equilibrium constants for adsorption and partitioning, however, do not follow the same trends as the overall retention factors. NT retention is dominated by partitioning and NA retention by adsorption, while CYT retention is controlled by adsorption rather than partitioning. Our results reveal that the relative importance of adsorption and partitioning mechanisms depends in a complex way from analyte properties and experimental parameters and cannot be predicted generally.

1. Introduction

Reversed phase (RPLC) [1–5] and hydrophilic interaction (HILIC) [6–13] are the most widely applied retention modes of liquid chromatography used in the pharmaceutical, the biological, and the food industry fields [14]. Very polar and/or ionizable compounds are best analyzed by HILIC, which uses stationary phases with polar functional groups and mixtures of an aqueous buffer and organic solvent (usually ACN) as the MPs. The adsorbent is never completely inert toward the components of the MP. In terms of the excess adsorption of one eluent component with respect to the other (excess adsorption is well documented in RPLC [15–18]), the polar surface adsorbs W preferentially from the ACN-rich MP [19], so that the mesoporous eluent is richer in W at the surface than in the bulk region farther away from the surface.

As a result, a complex interfacial region forms whose properties differ from those of the bulk eluent. This was confirmed by molecular dynamics (MD) simulations that investigated the adsorption of W/ACN mixtures to hydrophilic silica surfaces [20–25]. According to these simulations, a W-rich layer with a rigid and diffuse part forms as the interface between the solid silica surface and the liquid MP. The properties of the rigid W layer (ca. 4 Å wide) are governed by the surface whereas those of the diffuse W layer (ca. 11 Å wide) reflect the influence of surface and MP. Therefore, the retention of analytes depends on their distribution between the internal bulk phase and the diffuse W layer and on their distribution between the diffuse and the rigid W layer. The composition, the structure, and the mobility of solvent molecules gradually transition across the thickness of the W-rich layer. The very low, almost frozen mobility in the rigid W layer increases to the values of the diffuse W layer and of the bulk. This microscopic description is consistent with the partial exclusion observed for the hydrophobic compound TO from the internal pore volume [26]. The HILIC adsorption system should be regarded as a three-phase system, as showed for RPLC [27–29]. In RPLC, the three phases are the silica surface modified

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http://dx.doi.org/10.1016/j.chroma.2014.11.087

Nomenclature

Roman letters

a	coefficient defined in Eq. (7)
a_1	coefficient defined in Eq. (27)
b_1	coefficient defined in Eq. (28)
c_1	coefficient defined in Eq. (29)
B	reduced longitudinal diffusion coefficient with reference to the interstitial linear velocity
C_i	ith sample concentration data (kg/m^3)
c_a	sample concentration in the rigid W layer (kg/m^3)
c_b	sample concentration in the bulk phase (kg/m^3)
c_d	sample concentration in the diffuse W layer (kg/m^3)
$c_{mesopore}$	average sample concentration in the mesopore volume (kg/m^3)
c_p	average sample concentration in the particle volume (kg/m^3)
$c_{skeleton}$	sample concentration in the solid skeleton (kg/m^3)
D_{bed}	effective diffusion coefficient across the packed bed immersed in the mobile phase with respect to the concentration gradient in the bulk eluent (m^2/s)
D_p	effective diffusion coefficient across the porous particles with respect to the concentration gradient in the mesoporous eluent (m^2/s)
D_{part}	effective diffusion coefficient across the porous particles with respect to the concentration gradient in the bulk eluent (m^2/s)
$D_{p,eff}$	effective diffusion coefficient across the porous particles with respect to the concentration gradient in the porous particle (m^2/s)
$D_{skeleton}$	sample diffusion across the skeleton volume (m^2/s)
$D_{mesopore}$	effective sample diffusivity across the mesopore volume (m^2/s)
D_m	diffusion coefficient of the sample in the bulk phase (m^2/s)
f_a	sample molar fraction in the rigid W layer
f_b	sample molar fraction in the bulk phase
f_d	sample molar fraction in the diffuse W layer
i	counting number of data points
$\vec{j}_p$	total flux density across the porous particle (mol/m^2/s)
$\vec{j}_{skeleton}$	flux density across the skeleton volume (mol/m^2/s)
k'	retention factor
k_1	zone retention factor
K_a	equilibrium constant for the sample adsorption–desorption between the rigid W phase and the diffuse W-rich phase
K_d	equilibrium constant for the sample partitioning between the diffuse W-rich phase and the bulk eluent
N	number of data point
L	column length (m)
t_p	parking time (s)
t_{ex}	extra-column time (s)
t_R	retention time (s)
t_{TO}	retention time of toluene (s)
u_R	migration linear velocity (m/s)

Greek letters

β	parameter in Torquato's model of effective diffusion in packed beds defined by Eq. (18)
β_1	root of a second order equation given by Eq. (26)
δ	ratio of the average diffusion coefficient in the diffuse W layer to the bulk diffusion coefficient
ϵ_e	external column porosity
ϵ_p	particle porosity
γ_e	external obstruction factor caused by randomly packed non-porous particles to the diffusion in the external bulk mobile phase
γ_p	internal obstruction factor to the diffusion across the porous particles
μ_1	first moment (s)
μ'_2	second central moment (s^2)
σ_t^2	second central moment measured from the half-height peak width (s^2)
Ω	ratio of the effective diffusivity of the sample in the porous particle (the concentration gradient is taken in the bulk eluent) to its bulk diffusion coefficient
Ω_p	ratio of the effective diffusivity of the sample in the porous particle (the concentration gradient is taken in the mesoporous eluent) to its bulk diffusion coefficient
ξ_2	adjustable parameter in Torquato's model of effective diffusion Eq. (16)

with the hydrophobic alkyl chains, an organic-solvent rich layer accumulated on top of the alkyl chains, and the aqueousorganic bulk eluent. In HILIC, the three phases are the silica surface with the adsorbed rigid W layer, the diffuse W layer, and the organic-solvent rich aqueousorganic bulk eluent.

A puzzling and unsolved enigma in HILIC is the nature of the relative contributions of adsorption (analyte adsorption onto the surface of the rigid W layer from the diffuse W layer) and partitioning (analyte accumulation in the diffuse W layer from the bulk phase) to the overall retention factor. Small molecules may behave differently in the rigid W layer and the diffuse W layer. Their adsorption may be due to weak electrostatic interactions through hydrogen bonding and dipole–dipole interactions as well as strong ionic interactions when analytes and stationary phase surface bear permanent charges. Partitioning involves the diffuse W layer while adsorption requires analytes to penetrate into the rigid W layer. Because the retention factor reflects the sum of both types of retentive contributions, measurements of the retention factor do not allow one to distinguish between partitioning- and adsorption-driven retention. Typically, experiments addressing the retention mechanism in HILIC are based solely on measurements of the overall retention factor [30–35]. The relative contributions from adsorption and partitioning to the overall retention factor remain unknown. This shows that the determination of HILIC properties other than the retention factor is needed to distinguish between the amounts of analyte adsorbed in the rigid W layer and dissolved in the diffuse W layer. The goal of this work is to differentiate between the contributions of adsorption and partitioning to the retention of small, polar, and ionizable compounds in HILIC.

Our method consists in measuring both the retention factors (from the elution time) and the intra-particle diffusivity (using the peak parking method [36–38]) of analytes in order to accurately estimate their fractions present at the adsorbent surface (due to adsorption) and in the diffuse W layer (due to partitioning). Analytes interacting with the rigid W layer at the adsorbent silica surface contribute differently to the average diffusion coefficient across the HILIC particles than analytes interacting with the diffuse W layer. The local values of the diffusion coefficients of analytes in the solid silica skeleton, in the rigid W layer, in the diffuse W layer, and in the internal bulk eluent are different and they can be assumed on a reasonable basis according to the results given by MD simulations [24]. The calculation requires a new model for effective