



Mass transfer mechanism in chiral reversed phase liquid chromatography



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ABSTRACT

The mechanism of mass transfer in chiral chromatography was investigated using an experimental protocol already applied in RPLC and HILIC chromatography. The different contributions to the reduced height equivalent to a theoretical plate (HETP) include the longitudinal diffusion HETP term, the solid–liquid mass transfer resistance HETP term, the short-range eddy dispersion HETP term, and the long-range eddy dispersion HETP term. Their accurate measurement permits the determination of the adsorption rate constant k_{ads} of *trans*-stilbene enantiomers on a column packed with Lux 5 μm Cellulose-1 particles. The experimental results demonstrate that the number of adsorption–desorption steps per unit time of chiral compounds on polysaccharide-based chiral stationary phases is four orders of magnitude smaller than that of achiral compounds.

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1. Introduction

Polysaccharide-based chiral stationary phases (CSP) are the class of phases that is most widely used for the analysis of biochemicals and pharmaceutical compounds by HPLC [1–4]. Besides their broad range of applications in NPLC, these phases can be used in RPLC, with aqueous mixtures of either methanol or acetonitrile as mobile phases [5,6]. Their numerous applications to the separation of racemic mixtures were reviewed by Tachibana [7]. The most successful polysaccharide-based CSPs are derived from cellulose tris(3,5-dimethyl-phenylcarbamate), which has a wide chiral recognition ability, a good chemical stability, a good loadability, and is highly repeatable [8].

In addition to their selectivity, the efficiency of columns packed with these polysaccharide-based CSPs plays a major role in the successful separation of important pairs of enantiomers in chiral RP-HPLC for which the selectivity factor is insufficient [6]. The efficiencies reported for polysaccharide-based CSPs [9] are generally smaller than those measured for either RPLC silica- C_{18} [10,11] or HILIC [12–15]. This fact holds true for low molecular weight compounds eluted with similar mobile phases which have the similar viscosity and diffusion coefficients. In both the RPLC and HILIC retention modes, the mass transfer resistance around and above

the optimum velocity is essentially controlled by the eddy diffusion term that originates from velocity biases taking place over long-range distances, like the column diameter [11]. In practice, the HETP of columns packed with sub-3 μm particles remains nearly constant at high mobile phase velocities for small molecules [12], so the rate constants of adsorption on all sites in both RPLC and HILIC are assumed to be very large. In contrast, the apparent C term of the van Deemter plots measured in chiral chromatography is relatively large compared to those measured in RPLC or HILIC. The question remains of whether such a steep C -behavior is due to a slow diffusivity of analytes across the CSP particles or to a small number of adsorption–desorption events (e.g., to a small value of the adsorption rate constant) on both non-selective and selective sites [16]. Recent calculation of the macroscale transport and the HETP in the bulk region of column beds demonstrated the crucial role of the microscopic characteristics of the adsorption–desorption kinetics on chromatographic performance [17]. For a retention factor of 1.0 and a reduced velocity of 60, an increase of the reduced plate height by 1.0 unit is expected if the average residence time in the stationary phase increases from 0.02 to 200 μs (e.g., with a decreasing frequency of adsorption–desorption steps from about $5 \times 10^7 \text{ s}^{-1}$ to only $5 \times 10^3 \text{ s}^{-1}$) [17].

The first goal of this work was to understand the mechanism of mass transfer in chiral RP-HPLC, using an experimental protocol reported earlier [18]. A method based on extrapolation was previously used [19]. A cellulose-based CSP, a mobile phase made of methanol and water, a neutral racemic compound, and a reference achiral compound were selected for the purpose of

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List of symbols*Roman letters*

$A(v)$	reduced eddy diffusion plate height
B	reduced longitudinal diffusion coefficient with reference to the interstitial linear velocity
C_i	sample concentration at the discrete elution time t_i (mol/m ³)
C_p	sample concentration in the internal mesoporous volume (mol/m ³)
c_s	sample concentration in the stationary phase (mol/m ³)
C_a	reduced solid–liquid mass transfer resistance coefficient due to a slow adsorption–desorption process
C_p	reduced solid–liquid mass transfer resistance coefficient due to the finite rate of diffusion across the particle
D	dimensionless constant defined in Eq. (14)
Dn	Dean number
D_p	effective sample diffusivity across the particle (m ² /s)
D_{eff}	effective diffusion coefficient of the packed bed (m ² /s)
d_p	average particle diameter (m)
D_m	bulk molecular diffusion coefficient (m ² /s)
$F(\lambda_m)$	Hindrance diffusion factor
F_v	flow rate (m ³ /s)
$F_{v,PP}$	flow rate applied during the peak parking method (m ³ /s)
h	reduced plate height
h_{TC}	reduced long-range eddy dispersion plate height
$h_{TC,comp}$	compensation reduced long-range eddy dispersion plate height from the Luna to the Lux column
H	plate height (m)
j	molecular flux through the particle (mol/m ² /s)
k'	retention factor
k_1	zone retention factor
k_p	particle retention factor
k_{ads}	adsorption rate constant (s ⁻¹)
K_c	Kozeny–Carman constant
k_0	specific permeability (m ²)
K_a	equilibrium Henry's constant for the sample distribution between the solid phase in the particle and the liquid eluent phase
N	column efficiency
L	column length (m)
P_{tot}	total pressure drop (Pa)
P_{ex}	system pressure drop in absence of the chromatographic column (Pa)
r_c	column inner radius (m)
R_{coil}	radius of the coiled capillary tube (m)
r_{tube}	capillary tube inner radius (m)
Sc	Schmidt number
t_1	first discretized elution time (s)
t_N	last discretized elution time (s)
t_{left}	elution time (adsorption) at 10% of the peak height (s)
t_{right}	elution time (desorption) at 10% of the peak height (s)
t_i	discretized elution time (s)
t_p	parking time (s)
t_R	retention time at the peak apex (s)
Δt_p	increment of the peak parking time (s)

u	interstitial linear velocity (m/s)
u_{center}	linear velocity in the center of the capillary tube (m/s)
u_s	superficial linear velocity (m/s)
V_0	column hold-up volume (m ³)

Greek letters

β	parameter in Torquato's model of effective diffusion in packed beds defined by Eq. (7)
β_1	positive root of the quadratic Eq. (26)
β_2	negative root of the quadratic Eq. (26)
$\Delta\sigma_{pp}^2$	increment of the peak variance measure in the peak parking method (s ²)
ϵ_e	external column porosity
ϵ_p	particle porosity
ϵ_t	total column porosity
γ_p	intra-particle obstruction factor
γ_e	obstruction factor caused by randomly packed non-porous particles to the diffusion in the external bulk mobile phase
η	eluent viscosity (Pa s)
μ_1	first moment (s)
$\mu_{1,PP}$	first moment recorded during the PP experiments for a zero parking time (s)
$\mu_{1,PP,ex}$	extra-column first moment in the PP conditions (s)
μ'_2	second central moment (s ²)
v	reduced interstitial linear velocity
Ω	ratio of the effective diffusivity of the sample in the porous region of the particle to its bulk diffusion coefficient
ω_1	diffusion eddy dispersion coefficient related to trans-channel velocity bias
λ_1	flow eddy dispersion coefficient related to trans-channel velocity bias
ω_2	diffusion eddy dispersion coefficient related to short-range inter-channel velocity bias in columns packed with non-porous particles
λ_2	flow eddy dispersion coefficient related to short-range inter-channel trans-column velocity bias in columns packed with non-porous particles
ρ	eluent density (kg/m ³)
$\sigma_{v,ex}^2$	extra-column volume variance (m ⁶)
$\sigma_{v,tube}^2$	total volume variance (m ⁶)
ξ_2	adjustable parameter in Torquato's model of effective diffusion Eq. (6)

applying the method. The experimental protocol used had already been tested and validated in both RPLC [10] and HILIC [14,15]. The total reduced HETP h was measured from the moments of peaks recorded in a wide range of flow rates [20,21]. The diffusion coefficients D_m of the analytes in the bulk phase were measured by the capillary method [22,23]. The reduced longitudinal diffusion coefficient B was directly measured by the peak parking method [24–26]. The mass transfer resistance coefficient C_p due to a finite diffusion rate across spherical and fully porous particles was obtained by combining the results of the peak parking method and a validated model of effective diffusion along packed beds [27–29]. The short-range eddy dispersion HETP term was obtained from statistical analysis of packed beds [30]. The long-range eddy dispersion HETP was measured under RPLC conditions for a column of the same dimension and packed with particles of the same size as the chiral column used in this work [31]. The second goal of this work was to use the results of this experimental deconvolution

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