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Impact of the nature and composition of the mobile phase on the mass transfer mechanism in chiral reversed phase liquid chromatography. Application to the minimization of the solvent cost in chiral separations

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ABSTRACT

The mechanism of mass transfer was studied on a cellulose-based chiral stationary phase (CSP, Lux Cellulose-1) using aqueous mixtures of acetonitrile (50/50–90/10, v/v) or methanol (90/10 and 100/0, v/v) as the mobile phase. An experimental protocol validated in RPLC and HILIC chromatography and recently extended to chiral RPLC was applied. The five mass-transfer contributions (longitudinal diffusion, short-range and long-range eddy dispersion, solid–liquid mass transfer resistances due to finite intra-particle diffusivity and slow adsorption–desorption) to the reduced height equivalent to a theoretical plate (HETP) were measured. The experimental results show that the adsorption rate constants k_{ads} of *trans*-stilbene enantiomers onto the CSP are three times larger with acetonitrile than with methanol as the organic modifier. This is correlated to the decrease of enantioselectivity from 1.4 (in methanol) to only 1.1 (in acetonitrile). The amount of solvent needed to achieve a separation factor of exactly 2.0 was determined. This showed that analysis cost could be reduced seven times by selecting pure methanol as the eluent for a 5 cm long column rather than an acetonitrile–water mixture for a longer (20–45 cm) column.

1. Introduction

Chiral stationary phases (CSP) based on polysaccharide derivatives are a routinely used class of CSP for the separation of racemic mixtures by HPLC [1–3]. These phases are particularly advantageous in the RPLC mode when used with aqueous mixtures of either methanol or acetonitrile as the mobile phase [4,5]. This avoids using highly toxic, flammable, and expensive solvents in NPLC such as *n*-hexane or *n*-heptane. Their numerous applications to the separation of racemic mixtures by RPLC were recently reviewed by Tachibana and Ohnishi [6]. One of the most popular polysaccharide-based CSP is 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 [7].

While the thermodynamics of chiral recognition has been thoroughly investigated in the past [8], reports on the mass transfer kinetics (band broadening or peak width) on CSPs are scarce [9,10]. The efficiency of columns packed with cellulose-based CSPs plays a major role in the successful separation of important pairs of enantiomers for which the selectivity factor on other CSPs is insufficient [5]. Most often, analysts rely on trial and error screening of mobile phases to select the best experimental conditions. It was impossible to find a single review published on this issue, for the lack of consistent experimental results. The main reason is that five sources of band broadening affect simultaneously the overall column efficiency, the longitudinal diffusion, short-range eddy dispersion, long-range eddy dispersion, and solid–liquid mass transfer resistances due to a slow diffusion rate across the particles and to a slow adsorption–desorption process [11]. Because classical rate models are lumped models, they do not separate the contributions of these sources and do not permit the extraction of physically sound and reliable kinetic information from experimental data [12].

Efficiencies reported for polysaccharide-based CSPs used at high reduced velocities are generally smaller than those measured for either RPLC silica-C_{18} [11,13] or HILIC [14–17] stationary phases. A comparison of plots of the reduced HETP *vs.* the reduced velocity showed that, for methanol–water eluents, the B/ν branches of these CSPs and of HILIC phases were comparable but that the slopes of the $C\nu$ branches was steeper for the former [18]. Extension from RPLC and HILIC to chiral RPLC of an experimental protocol

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[19,20] suggested that it was possible to measure separately the five different band broadening contributions to the total band width of the *trans*-stilbene enantiomers on a 5 μm Lux Cellulose-1 CSP [18]. Even though long-range eddy diffusion controls mass transfer resistances around and above the optimum velocity [13], it was shown that the slow adsorption–desorption process could account for about 25% of the total plate height at a reduced velocity of about 35. Adsorption–desorption mass transfer kinetics is due to a small number of adsorption–desorption events and a large average residence time [21] on both non-selective and selective adsorption sites [22]. According to experimental data, the rate of adsorption of *trans*-stilbene onto the Lux Cellulose-1 CSP was found to be between 600 and 1200 s^{-1}. The limited accuracy of the extended experimental protocol prevents from measuring k_{ads} values larger than 10^4 s^{-1} unless the retention factor is impractically huge (>30). In routine HPLC, when $k_{ads} > 5 \times 10^4$ s^{-1}, its effect on the overall column efficiency is negligible.

The goal of this work was 3-fold: first, to measure the effects of the nature of the organic modifier used (methanol *vs.* acetonitrile) and of the mobile phase composition (water concentration from 50/50 to 90/10, v/v) on the adsorption–desorption kinetic process in chiral RPLC. This study uses an experimental protocol recently reported [18] that was designed to measure accurately the five sources of band broadening in chromatographic columns. The second goal was to provide experimental speed-resolution properties as plots of the analysis time per resolution factor unit *vs.* the resolution factor in logarithm scales for different mobile phases used with a Lux Cellulose-1 CSP. The last goal was to see whether analysts can select the mobile phase composition providing a satisfactory resolution (R_s = 2.0) and a minimum solvent cost per injection at a limited maximum back pressure (150 bar).

2. Theory

2.1. Definitions

For all necessary definitions in this section, the reader is referred to those listed in Ref. [18].

2.2. Reduced HETP equation

The overall reduced plate height h is the sum of the longitudinal diffusion term B/ν, the total eddy diffusion term $A(\nu)$, the trans-particle mass transfer resistance term due to the finite diffusivity of the analyte through the particles $C_p\nu$ and its (slow) adsorption–desorption kinetics $C_a\nu$. It is written:

$$h = \frac{B}{\nu} + A(\nu) + C_p\nu + C_a\nu \quad (1)$$

The final mathematical expressions of these four HETP terms were given previously [18]. They are summarized in the next subsections.

2.2.1. The longitudinal diffusion term

The effective diffusion coefficient in random packed beds is provided by the Torquato model written as [23–25]:

$$D_{eff} = \frac{1}{\epsilon_e(1+k_1)}\left[\frac{1+2(1-\epsilon_e)\beta - 2\epsilon_e\xi_2\beta^2}{1-(1-\epsilon_e)\beta - 2\epsilon_e\xi_2\beta^2}\right] D_m \quad (2)$$

with

$$\beta = \frac{\Omega - 1}{\Omega + 2} \quad (3)$$

where ξ_2 = 0.59 and γ_e = 0.627. The reduced B coefficient is written [24]:

$$B = 2(1+k_1)\frac{D_{eff}}{D_m} \quad (4)$$

2.2.2. Eddy dispersion HETP

The term $A(\nu)$ is the overall reduced eddy dispersion term; its expression is based on the one derived in the coupling theory of eddy dispersion by Giddings [26]:

$$A(\nu) = \frac{1}{(1/2\lambda_1) + (1/\omega_1\nu)} + \frac{1}{(1/2\lambda_2) + (B/2\gamma_e\omega_2\nu)} + h_{TC}(\nu) \quad (5)$$

where λ_1 = 0.45, ω_1 = 0.0041, λ_2 = 0.23, and ω_2 = 0.12 [27]. The method used to assess the long-range eddy dispersion HETP $h_{TC}(\nu)$ was presented in [18].

2.2.3. The solid–liquid mass transfer resistance term

The general expression of the solid–liquid mass transfer resistance coefficient due to the finite sample diffusivity across the particles (C_p) is given by [26,28]:

$$C_p = \frac{1}{30}\frac{\epsilon_e}{1-\epsilon_e}\left(\frac{k_1}{1+k_1}\right)^2\frac{1}{\Omega} \quad (6)$$

2.2.4. The adsorption–desorption mass transfer resistance term

The general expression of the HETP associated with a slow adsorption–desorption kinetics ($C_a\nu$) is given by the Laplace transform [28,29]. The mass transfer resistance coefficient C_a is written:

$$C_a = 2\frac{\epsilon_e}{1-\epsilon_e}\frac{1}{1-\epsilon_p}\left(\frac{k_1}{1+k_1}\right)^2\left(\frac{k_p}{1+k_p}\right)^2\frac{1}{D} \quad (7)$$

where D is a dimensionless constant

$$D = \frac{k_{ads}d_p^2}{D_m} \quad (8)$$

where k_{ads} is the adsorption rate constant (unit s^{-1}) and k_p is given by

$$k_p = \frac{1-\epsilon_p}{\epsilon_p}K_a \quad (9)$$

where K_a is the Henry's constant.

3. Experimental

3.1. Chemicals

The mobile phases were aqueous mixtures of acetonitrile (50/50, 60/40, 70/30, 80/20, and 90/10, v/v) or methanol (90/10 and 100/0, v/v). Acetonitrile was filtered before use on a surfactant-free cellulose acetate filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Tri-*tert*-butylbenzene (TTBB) and the racemic mixture of *trans*-stilbene were also purchased from Fisher Scientific, with a minimum purity of 99%.

3.2. Apparatus

All measurements were performed with a 1290 Infinity HPLC system (Agilent Technologies, Waldbrön, Germany) liquid chromatograph. The system includes a 1290 Infinity Binary Pump with Solvent Selection Valves and a programmable auto-sampler. The injection volume is drawn into one end of the 20 μL injection loop. The instrument includes a two-compartment oven and a multi-diode array UV–vis detection system. The system is controlled by the Chemstation software. The sample trajectory in the equipment involves the successive passage of its band through the series of:

- A 20 μL injection loop attached to the injection needle. The design of the First In – Last Out (FILO) injection system is such that the