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Chromatographic Separation of Bupivacaine Enantiomers by HPLC: Parameters Estimation of Equilibrium and Mass Transfer Under Linear Conditions

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Abstract. Bupivacaine is an amide type local anesthetic widely used in surgery and obstetrics because of its sustained peripheral and central nerve blockade. *R*-(+)-bupivacaine is more toxic to the central nervous and the cardiovascular systems than *S*-(−)-bupivacaine. To obtain *S*-(−)-bupivacaine with high degree of purity using a continuous simulated moving bed (SMB) unity, equilibrium and mass transfer parameters under dilute conditions were obtained by pulse experiments using 0,0′-bis[4-*terc*-butyl-benzoyl]-*N*,*N*′-diallyl-*L*-tartar diamide immobilized in silica (Kromasil® CHI-TBB). The linear equilibrium constants were found to be 2.12 and 2.91 for *R*-(+)- and *S*-(−)-bupivacaine, respectively. Axial dispersion coefficients were found to be practically the same for both enantiomers. A fast kinetic of mass transfer was observed. The internal resistance to the mass transfer controls all the mass transfer process in this chiral column and the pore diffusion coefficients were of the order 10^{-7} cm^2/s. The equilibrium and mass transfer parameters will be employed in future simulation and design of operating conditions of SMB unity.

Keywords: Chiral separations, preparative liquid chromatography, mass transfer, equilibrium, bupivacaine

1. Introduction

The necessity to generate individual enantiomers for testing has become a growing priority in pharmaceutical research and development. This necessity is directly related to the increased knowledge of the effect differing enantiomers have in biological systems (Miller et al., 1999). It is well known that some optical isomers may exhibit completely different, and even opposing, pharmacological effects. As a consequence, new chiral separation methods are being proposed nowadays to produce single enantiomeric forms of chiral drugs (Azevedo et al., 1999). The use of chromatographic technique to obtain significant quantities of enantiomerically pure drug intermediates is well established and a simulated moving bed (SMB) chromatography in recent years has become a routine technique for the separation of enantiomers (Wang and Ching, 2004; Santos et al., 2004; Yu and Ching, 2003; Xie et al., 2003; Pais et al., 2000).

The behavior of a chromatographic system is governed by three basic phenomena (Schulte et al., 1997): (i) the adsorption thermodynamics, described by equilibrium isotherms which give the composition in the stationary phase versus the composition in the mobile phase when equilibrium is reached, at given temperature; (ii) the column hydrodynamics, i.e., the properties of the flow through the porous medium; (iii) the mass transfer kinetics. These parameters are very important in the modeling and simulation of chromatographic processes as well as for design of operating conditions of an SMB unit.

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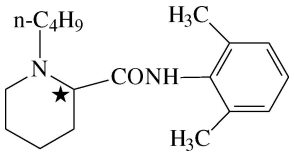

Figure 1. Chemical structure of bupivacaine. The symbol (⋆) indicates the chiral center.

In a chromatographic packed bed column, the position of elution peak in chromatography depend essentially on the thermodynamics of phase equilibrium, especially at high concentrations, in which range the asymmetry of the elution profile is strongly and foremostly influenced by the curvature of the nonlinear equilibrium isotherm (Miyabe and Guiochon, 2000). At the low concentrations (dilute conditions) usually used in analytical chromatography, the isotherms are linear. In large-scale applications of chromatography more concentrated solutions are usually separated and non-linear solutions are usually required. However, the linear chromatographic solutions are very useful for the insight they give on the importance of mass transfer and dispersion (Wankat, 1994). Under dilute conditions a small pulse is dispersed by mass-transfer effects as it migrates through a column. Axial dispersion and the mass-transfer processes provide the sources of band broadening in linear chromatography (Miyabe and Guiochon, 1999).

Bupivacaine, (±)-1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide (Fig. 1), an amide type local anesthetic widely used effects in surgery and obstetrics for several decades due to its sustained peripheral and central nerve blockade, is synthesized and used as the racemate (Gu et al., 1998; Fawcett et al., 2002; Tanaka et al., 2003). Bupivacaine enantiomers differ pharmacologically. For example, *R*-(+)-bupivacaine is more toxic to the central nervous and the cardiovascular systems than *S*-(−)-bupivacaine. Bupivacaine enantiomers also differ pharmacokinetically. For example, it has been found in sheep and humans that the clearance of *R*-(+)-bupivacaine is 20–40% greater than *S*-(−)-bupivacaine and it has been found that the tissue-blood distribution coefficient for many tissues is greater for *R*-(+)-bupivacaine that for *S*-(−)-bupivacaine. Hence it is clear that an in depth understanding of the pharmacology of bupivacaine is not possible without a thorough study of its component enantiomers (Gu et al., 1998).

In this work the chromatographic separation of bupivacaine enantiomers Kromasil® CHI-TBB as a chiral column was investigated. Small pulse experiments with diluted solutions were accomplished to obtain total porosity, linear equilibrium constants (Henry constants), axial dispersion coefficients and mass transfer parameters. These parameters will be employed to design the operating conditions in a continuous chromatographic SMB unit.

2. Theory

2.1. Total Porosity and Linear Equilibrium Constant

Total porosity (ε_T) of packed bed columns includes all the volumes available to the fluid phases, both inside and outside the particles constituting the packing material. In principle, this can be estimated from the retention time of an inert tracer (t_0) as follows (Böcker et al., 2002):

$$\varepsilon_T = \frac{t_0 Q}{V_c} \tag{1}$$

where Q is the volumetric flow-rate of mobile phase and V_c is the total column volume. Bed porosity (ε) and particle porosity (ε_p) are related to ε_T by the following equation:

$$\varepsilon_T = \varepsilon + (1 - \varepsilon)\varepsilon_p \tag{2}$$

According to Eq. (2), if the internal particle porosity is known, the bed porosity can be calculated from the total porosity of the column (Wang and Ching, 2002).

In linear chromatography, solute concentrations are low, within the Henry's law domain of the adsorption isotherms. The solid-liquid curve can be represented by a straight line and the Henry constant (H) represents the slope of the adsorption isotherm of a pure component at infinite dilution. Under dilute conditions competition is absent and chromatograms using the racemic mixture provide the same information as those using the pure enantiomers (Francotte et al., 1998; Migliorini et al., 2002; Böcker et al., 2002). H can be determined from the retention times (t_R) of a small racemic chromatographic pulse as follows:

$$H = \left(\frac{t_R - t_0}{t_0}\right)\frac{\varepsilon_T}{1 - \varepsilon_T} \tag{3}$$

2.2. Plate Equation for Linear Chromatography

For a system with linear adsorption isotherms, mass transfer parameters and axial dispersion coefficients

of a chromatographic column can be evaluated by a height equivalent to a theoretical plate (HETP), which is defined according to the van Deemter equation as follows (Ruthven, 1984):

$$\text{HETP} = \frac{L}{N} = 2\frac{D_L}{u} + 2u\left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{1}{Kk_m} \times \left(1+\left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{1}{K}\right)^{-2} \quad (4)$$

where

$$N = 5.545\left(\frac{t_R}{w_{1/2}}\right)^2 \quad (5)$$

and

$$K = \varepsilon_p + (1-\varepsilon_p)H \quad (6)$$

In the above equations, L is the column length, N the theoretical plate number, $w_{1/2}$ the peak width at half height, u the superficial velocity of mobile phase, D_L the axial dispersion coefficient, k_m the overall mass transfer coefficient and K is the equilibrium constant.

2.3. Mass Transfer Parameters

The overall mass transfer resistance ($1/k_m$) is composed of two separate mass transfer mechanisms—the external and the internal mass transfer resistances to mass transfer (Ma and Wang, 1997):

$$\frac{1}{k_m} = \frac{d_p}{6k_f} + \frac{d_p^2}{60\varepsilon_p D_p} \quad (7)$$

where d_p is the particle diameter, D_p the pore diffusion coefficient and k_f the external mass transfer coefficient. k_f can be obtained by the Wilson and Geankoplis correlation, which is valid for liquid systems in which $0.0015 < \text{Re} < 55$ (Wilson and Geankoplis, 1966):

$$\text{Sh} \equiv \frac{d_p k_f}{D_m} = \frac{1.09}{\varepsilon}(\text{Sc})^{1/3}(\text{Re})^{1/3} \quad (8)$$

where Sh, Sc and Re are dimensional numbers of Sherwood, Schmidt and Reynolds and D_m is the molecular diffusion coefficient. D_m can be estimated by the Wilke-Chang equation (Guiochon et al., 1994):

$$D_m = 7.4\times10^{-8}\frac{(\phi M)^{1/2}T}{\eta V_b^{0.6}} \quad (9)$$

where ϕ denotes the association coefficient (it takes into account the molecular interactions of solute-solvent due to hydrogen binding), M is the molecular mass, η the solution viscosity, T the absolute temperature, and V_b is the molar volume at normal boiling point.

2.4. Axial Dispersion in Packed Bed

When a fluid flows through a packed bed, there is a tendency for axial mixing to occur contributing to the band broadening and reducing the efficiency separation. All of the phenomena contributed to axial mixing, except that of mass transfer resistance, are lumped into an axial dispersion coefficient. It is usually assumed that axial dispersion is the combined result of two different mechanisms, molecular diffusion and eddy diffusion. In a packed bed, it is impossible for the mobile phase to move along a straight line without colliding with the particles. As a first approximation, molecular diffusion and eddy diffusion are additive, and the axial dispersion coefficient (D_L), is given by:

$$D_L = \gamma_1 D_m + \gamma_2 d_p u \quad (10)$$

where γ_1 e γ_2 are geometrical constants (Ruthven, 1984; Guiochon et al., 1994). For enantioseparation of chiral substances whose physical properties (including diffusion coefficients D_m) are identical, their axial dispersion coefficients are equal (Wang and Ching, 2002).

D_L can also be expressed as $D_L = \tau D_m + \lambda u$ for convenience, where τ is the tortuosity factor for a packed bed column and λ the flow-geometric dependent constant. As the molecular diffusivity of liquid is too small to contribute significantly to axial dispersion even at low Reynolds numbers, Eq. (10) can be simplified as (Wang and Ching, 2002):

$$D_L = \lambda u \quad (11)$$

3. Experimental Section

3.1. Materials and Equipments

Racemic mixture of bupivacaine (free base) was furnished by Cristália Pharmaceutical Company (Itapira–SP, Brazil). 1,3,5-tri-*tert*-butylbenzene (TTBB) was purchased from Aldrich (USA). The mobile phase used in this work was hexane/2-propanol/acetic

acid/triethylamine (98/2/0.3/0.05 v/v) was purchased from J. T. Baker (USA).

The preparative chiral column used in experiments was Kromasil® CHI-TBB (25 × 1 cm I.D.) furnished by Eka-Nobel (Sweden). The Kromasil® CHI-TBB column was packed with 16 μm of particle diameter and 100 Å of internal pore diameter of Kromasil silica which is covalent bonded with 0,0′-bis[4-terc-butyl-benzoyl]-*N*,*N*′-diallyl-*L*-tartar diamide. The experiments were carried out using a single chromatographic column in a HPLC system consisting of a dual pump Waters 1525 equipped with UV detector Waters 2487 and digital data acquisition system. The signal was monitored by the UV detector with a wavelength of 270 nm.

3.2. *Experimental Procedures*

The inert tracer TTBB and racemic mixture of bupivacaine were dissolved separately in the mobile phase. These solutions were previously filtered (0.45 μm milipore filter) and degasified in a Cole Parmer 8892 ultrasonic bath. Initially, the hold-up volume of TTBB was measured and corrected for the dead volume contribution of the liquid chromatography, by replacing the column with a zero-volume connector. The experiments were carried out at four different mobile phase flow rates (0.017–0.067 cm^3/s) and 25°C (±1°C). For this aim, small pulses (20 μL) of dilute solutions of TTBB and racemic mixture were injected into the column after a time interval necessary for the stabilization of the system. The response peaks gave the necessary information on the determination of the adsorption equilibrium and mass transfer coefficients.

4. Results and Discussion

A small pulse of a dilute solution of TTBB and racemic mixture of bupivacaine was introduced in the column and the elution profiles were recorded. Figure 2 shows the elution profiles of TTBB and racemic bupivacaine at the flow rate 0.05 cm^3/s. We can observe a good separation performance of bupivacaine enantiomers on Kromasil® CHI-TBB. The separation occurs for base line. The retention times and width at half height of each peak provided important information for the calculation of the equilibrium constants (Henry constants) and column efficiency in terms of theoretical plates.

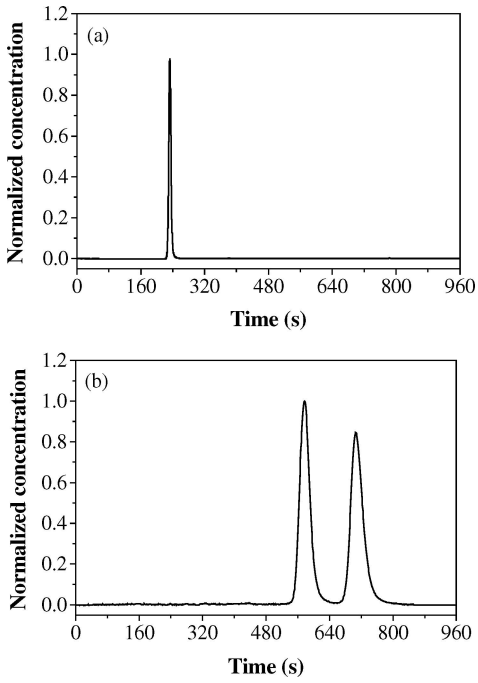

Figure 2. Elution profile of (a) TTBB and (b) racemic mixture of bupivacaine. The less-retained enantiomer is *R*-(+)-bupivacaine and the more-retained enantiomer is *S*-(−)-bupivacaine. Experimental conditions: flow-rate: 3.0 mL/min; injection volume: 20 μL; detector response: $\lambda = 270$ nm; temperature: 25°C.

4.1. *Determination of Total Porosity and Henry Coefficients*

In practice, the major problem in chromatography is to identify such an inter tracer, i.e., a chemical species, which does not adsorb on the stationary phase, since even a small adsorptivity would lead to significant errors in the determination of the porosity (Böcker et al., 2002). TTBB (Fig. 3) is a molecule with low molecular mass (264.44 g/gmol) and is considered non-retained in most chiral stationary phases (CSPs). Thus, TTBB

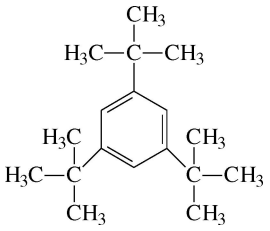

Figure 3. Chemical structure of TTBB.

has been widely used for the determination of column dead time (t_0) for various CSPs (Silva Jr et al., 2005; Santos et al., 2004, Xie et al., 2003, Wang and Ching, 2002; Cavazzini et al., 2001; Miyabe and Guiochon, 1999; Duan et al., 1998). As reported by Wang and Ching (2002), the exclusion mechanisms probably do not occur due to the relatively small molecular size of TTBB. As a result of this small size TTBB enters the pore system, but does not adsorb on the surface of the CSP.

In this work, the ε_T of the column was determined from the response to a pulse injection of TTBB according to the Eq. (2). The ε_T was found to be 0.58. The following correlation suggested by Ruthven (1984) was used for estimation of bed porosity:

$$\varepsilon_T = 0.45 + 0.55\varepsilon \tag{12}$$

The ε was found to be 0.24; the ε_p was found to be 0.45. This correlation has been used for estimation of the bed porosity of CSPs (Silva Jr. et al., 2005; Wang and Ching, 2002; Duan et al., 1998). Recently, Silva Jr et al. (2005) measured ε_T and ε of a polysaccharide based CSP by pulse experiments with TTBB and blue dextran, respectively. The experimental result of ε was compared with the values obtained by the Eq. (12) and the match was found to be very close. The utilization of Eq. (12) is considered satisfactory due to the difficulty of finding a high molecular weight inert polymer dissolved in a convenient mobile phase appropriate for bed porosity determination experiments.

The H was calculated from the retention times of a small pulse of racemic bupivacaine and TTBB according to Eq. (3). The values were found to be 2.12 and 2.91 to *R*-(+)- and *S*-(−)-bupivacaine enantiomers, respectively. The results obtained under dilute conditions show that the H values are greater than unity. From these results we can concluded that there exist a strong interactions between both enantiomers and the chiral column. The chiral column has a greater affinity for *S*-(−)-bupivacaine than for *R*-(+)-bupivacaine. This behaviour can be attributed to different interactions that occur between the bupivacaine enantiomers and the stationary phase. The separation factor (or selectivity) α was found to be 1.37. The retention and selectivity are mainly caused by multiple hydrogen bonds, although steric and π-π interactions are also considered to be present.

4.2. *Determination of Axial Dispersion and Mass Transfer Coefficients*

In order to obtain the HETP expression we combined Eqs. (4), (7) and (11) and rearranging,

$$\mathrm{HETP} = 2\lambda + 2u\left(\frac{\varepsilon}{1-\varepsilon}\right)\left(\frac{d_p}{6k_f} + \frac{d_p^2}{60\varepsilon_p D_p}\right) \times \left(1 + \left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{1}{K}\right)^{-2} \tag{13}$$

The external mass transfer coefficient, which is flow-rate dependent, was estimated from Eq. (8). So, isolating the term regarding to the external resistance of mass transfer of right hand side in Eq. (13):

$$\mathrm{HETP_{mod}} = 2\lambda + 2u\left(\frac{\varepsilon}{1-\varepsilon}\right)\left(\frac{d_p^2}{60\varepsilon_p D_p}\right) \times \left(1 + \left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{1}{K}\right)^{-2} \tag{14}$$

where

$$\mathrm{HETP_{mod}} = \mathrm{HETP} - 2u\left(\frac{\varepsilon}{1-\varepsilon}\right)\left(\frac{d_p}{6k_f}\right) \times \left(1 + \left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{1}{K}\right)^{-2} \tag{15}$$

Figure 4 shows the van Deemter plot for bupivacaine enantiomers. It was possible to observe a linear dependence between $\mathrm{HETP_{mod}}$ versus u for both enantiomers. In the measured range of flow-rate (0.017–0.067 mL/min), the experimental data failed to show a minimum in the van Deemter plot. This result indicates that the effects of axial dispersion and mass transfer resistance control the efficiency of the column. D_p and D_L were obtained from the slope and intercept of straight lines in the van Deemter plot and their values are reported in Table 1. The result shows that the D_L was found to be approximately the same value for both enantiomers. The small difference among the results can be attributed of experimental errors. As discussed previously in Section 2.2, the overall mass transfer resistance is composed of external and internal resistances. Since the particle Reynolds number in this study is small, reaching values from 0.099 to 0.398, Eq. (8) was used to estimate k_f. According to the magnitude of k_f values, in which it was found ranging from 0.042 to 0.066 cm/s, we concluded that the internal resistance

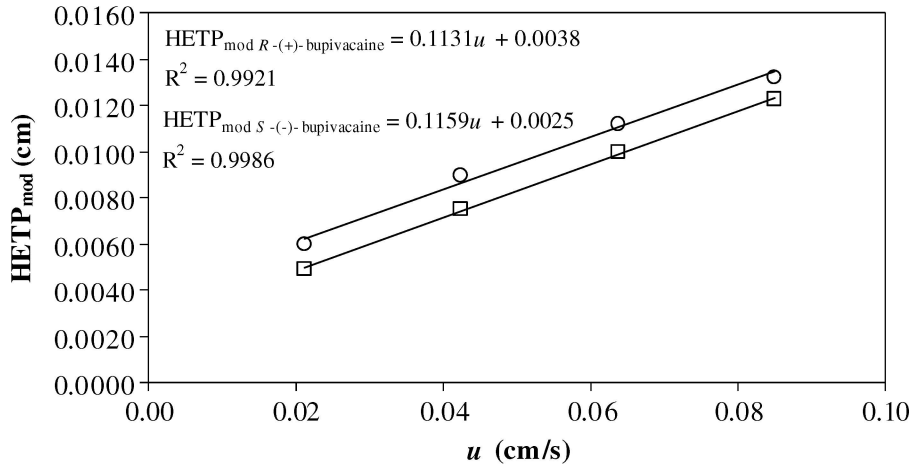

Figure 4. van Deemter plot of (O) *S*-(−)-bupivacaine and (□) *R*-(+)-bupivacaine.

to the mass transfer controls the mass transfer process in this chiral column because $d_p/6k_f \ll d_p^2/60\varepsilon_p D_p$. Therefore, D_p controls all mass transfer process in this chiral column.

As reported by Wang and Ching (2002), enantiomers have identical physical and chemical properties in an achiral environment. Therefore, the contributions of k_f and D_p to the overall mass transfer coefficient k_m should be equal for both enatiomers. So, the difference in the k_m could be due to the different kinetics of adsorption and desorption processes. The chiral stationary phase provides an excellent efficiency, probably due to a more favorable mass transfer kinetic. In fact, the slopes of both lines in the Fig. 4 are quite low, which means that k_m of each enantiomer inside the particles of CSP has high order of magnitude. According to the magnitude of the k_m values showed in Table 1 we can conclude that the Kromasil® CHI-TBB presents a fast mass transfer. Duan et al. (1998) reported that chiral stationary phases packed with chemically modified silica particles present high resolution and fast separation of the chiral compounds.

Table 1. Axial dispersion coefficients and mass transfer parameters.

	R-(+)-bupivacaine	*S*-(−)-bupivacaine
D_L ($cm^2 \cdot s^{-1}$)	$0.00190u$	$0.00125u$
k_f ($cm \cdot s^{-1}$)	Wilson and Geankoplis equation (1966)	
D_p ($cm^2 \cdot s^{-1}$)	2.29×10^{-7}	1.89×10^{-7}
k_m (s^{-1})	2.42	2.00

5. Conclusions

In this study, the Henry constants, axial dispersion and mass transfer parameters for the separation of bupivacaine enantiomers in Kromasil® CHI-TBB column were determined by pulse experiments. The chiral column exhibits good separation performance for bupivacaine enantiomers with a grater affinity to the *R*-(+)-bupivacaine than to the *S*-(−)-bupivacaine. A fast mass transfer was observed in the column and the axial dispersion coefficients were found to be practically identical for both enantiomers. The presented results demonstrate potential for the use of this chiral stationary phase in a continuous chromatographic SMB unity. The operating conditions of a SMB unity will be designed using the parameters estimated in this work under linear conditions of the adsorption isotherms.

Nomenclature

d_p	Particle diameter (cm)
D_L	Axial dispersion coefficient ($cm^2\ s^{-1}$)
D_m	Molecular diffusion coefficient ($cm^2\ s^{-1}$)
D_p	Pore diffusion coefficient ($cm^2\ s^{-1}$)
H	Henry constant
HETP	Height equivalent to a theoretical plate (cm)
k_m	Overall mass transfer coefficient (s^{-1})
k_f	Film mass transfer coefficient ($cm\ s^{-1}$)
K	Equilibrium constant in Eq. (4)
L	Column length (cm)
N	Theoretical plate number
T	Temperature (°C)

t_R Solute retention time (s)
t_0 Retention time of non-retained compound (s)
u Superficial velocity of mobile phase (cm s^{-1})
$w_{1/2}$ Peak width at half height (s)

Greek symbols

α Selectivity
ε Bed porosity
ε_p Particle porosity
ε_T Total column porosity
τ Tortuosity factor

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