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Kinetic and equilibrium study of the separation of propranolol enantiomers by high performance liquid chromatography on a chiral adsorbent

G. Duan *, C.B. Ching, S. Swarup

Department of Chemical Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Singapore

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Abstract

The kinetic and equilibrium constants for the chromatographic enantioseparation of racemic propranolol, using cellulose tri(3,5-dimethyl-phenylcarbamate) as the chiral stationary phase and hexane and isopropanol as the mobile phase, were evaluated by moment analysis on the basis of equilibrium-dispersive and linear driving force models. The overall mass transfer coefficients were evaluated to be 0.56 s^{-1} and 1.08 s^{-1} for the *S*-(−) and *R*-(+) enantiomers of propranolol. Other system properties, required as input parameters for the modelling and simulation of the process under consideration, including the voidage of the column and the axial dispersion coefficient, were also determined. The parameters obtained were used to simulate the two single enantiomer band profiles at different flow rates. The simulated results were found to match the experimental results quite well. These parameters are very important for the design of preparative chromatography.
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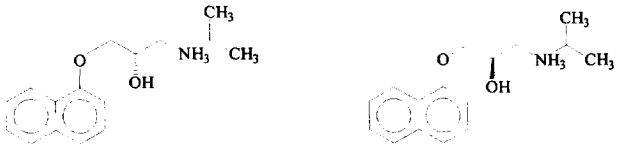
Keywords: Kinetic; Equilibrium; Propranolol enantiomers; High performance liquid chromatography

1. Introduction

Owing to the strict regulation by governments and pressure from the public and academic organizations, there is a need to develop optically pure drugs [1,2]. In addition, most companies prefer to manufacture a single isomer in order to avoid exhaustive bioevaluation of the wrong enantiomer. Several methods are available for the preparation of optically pure compounds (for a review, see Ref. [3]). Recently, with the advent of some powerful chiral stationary phases, high performance liquid chromatography (HPLC) has received increasing attention as a large-scale preparative method [1,4–7]. The advantages of this method are its high selectivity, simple product recovery, ease of further purification and very short product recovery time. For the optimization of such separation, certain kinetic and equilibrium parameters are needed.

Propranolol is a commonly used drug (in its racemic form) for hypertension and angina; the structures of the two enantiomers are shown in Fig. 1. *S*-(−)-Propranolol can be used for cardiovascular problems and hypertension, whereas the *R*-(+) enantiomer is a contraceptive [8]. For a safer and more effective drug, it is better to separate the enantiomers before use. Enantioseparation of racemic propranolol using a chiral adsorbent, cellulose tri(3,5-dimethyl-phenylcarbamate) (Chiralcel OD, structure shown in Fig. 2), has been demonstrated using an analytical column [9,10]. Recently,



(R)-Propranolol (S)-Propranolol

Fig. 1. Structural formulae of the two enantiomers of propranolol.

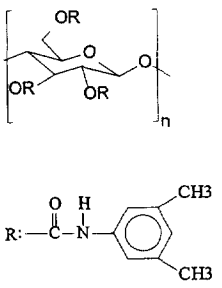


Fig. 2. Structure of Chiralcel OD.

* Corresponding author.

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Fornstedt et al. [11] studied the adsorption behaviour and mass transfer kinetics of the chiral separation of *rac*-propranolol hydrochloride using immobilized protein cellobiohydrolase I on silica as the reversed phase in an analytical column. The adsorption isotherms were studied carefully, and the separation process was modelled with a solid film linear driving force model. Different mass transfer coefficients were assumed for the simulation.

In this study, a preparative column of Chiralcel OD was employed for the separation of propranolol (free base), using hexane and isopropanol as the mobile phase, instead of the tertiary mixture demonstrated in the previous study [9–11]. The characterization of the preparative chiral column and the kinetics of mass transfer for preparative use are presented. The overall mass transfer coefficient, together with other parameters, such as the axial dispersion coefficient, bed voidage and equilibrium constants, are determined experimentally by a chromatographic method: moment analysis. In addition, a simulation of the band profiles, using the parameters obtained from the experiment, not the assumed mass transfer coefficient as in the previous study [11], is demonstrated.

2. Theoretical section

2.1. The rate model of chromatography

The mass balance equation for each component using the equilibrium-dispersive model in the mobile phase can be written as [12]

$$u\frac{\partial c}{\partial z}+\frac{\partial c}{\partial t}+F\frac{\partial \bar{q}}{\partial t}=D_L\frac{\partial^2 c}{\partial z^2} \tag{1}$$

where D_L is the axial dispersion coefficient, which is the sum of the molecular and eddy diffusion coefficients, F is the phase ratio and equals $(1-\varepsilon)/\varepsilon$, where ε is the bed voidage, $\bar{q}$ and c are the average values of the stationary and mobile phase concentrations over the entire particle, respectively, z and t are the space and time coordinates and u is the mobile phase velocity. The assumptions made in the derivation of the above equation include homogeneous packing, a constant volumetric flow rate, a constant dispersion coefficient, an isothermal bed and no radial gradients. In the linear rate model

$$\frac{\partial \bar{q}}{\partial t}=k_f(q^*-\bar{q}) \tag{2}$$

$$F\frac{\partial \bar{q}}{\partial t}=\left[\frac{1-\varepsilon}{\varepsilon}\right]k_f(q^*-\bar{q}) \tag{3}$$

where k_f is the mass transfer coefficient. This rate expression will be used in this study as it is difficult to obtain accurate estimates of the various mass transfer resistances from the presently available experiments. If the kinetics of adsorption–desorption are infinitely fast, then

$$q^*(z,t)=Kc(z,t) \tag{4}$$

Furthermore, the parameters estimated will be used in a numerical solution of the above equations under linear isotherms.

2.2. Moment analysis

The hydrodynamics of a chromatographic column can be described through the analysis of the residence–time distribution of the eluent, which is classically derived from the response curve to a Dirac injection. This response curve of the column to a Dirac injection contains information associated with the properties of the column. These properties, which include the equilibrium and mass transfer parameters, can be extracted by several methods: solution in the time domain, solution in the Laplace domain, solution of the Fourier domain and the method of moments [13]. The merits and limitations of each method have been discussed by Ramachandran and Smith [14]. Parameter estimation by the method of moments has been covered in detail previously [15–17].

By definition of the moments of a distribution, the nth moment of the band profile at the exit of a chromatography bed of length $z=L$ is

$$M_n=\int_0^\infty c(t,z=L)t^n\mathrm{d}t \tag{5}$$

The n absolute or normalized moment is

$$\mu_n=\frac{M_n}{M_0}=\frac{\int_0^\infty c(t,L)t^n\mathrm{d}t}{\int_0^\infty c(t,L)\mathrm{d}t} \tag{6}$$

and the nth central moment is

$$\overline{\mu_n}=\frac{\int_0^\infty c(t,L)(t-\mu_1)^n\mathrm{d}t}{\int_0^\infty c(t,L)\mathrm{d}t} \tag{7}$$

Assuming, as kinetic model, the solid film linear driving force model (Eq. (2)), and that the kinetics of adsorption–desorption are infinitely fast

$$q^*=Kc \tag{8}$$

Thus, the first and second moments can be expressed as [15]

$$\mu_1=\frac{L}{u}\left[1+\left(\frac{1-\varepsilon}{\varepsilon}\right)K\right] \tag{9}$$

$$\overline{\mu_2}=\frac{2L}{u}\left\{\frac{D_L}{u^2}\left[1+\left(\frac{1-\varepsilon}{\varepsilon}\right)K\right]^2+\left(\frac{1-\varepsilon}{\varepsilon}\right)\frac{K}{k_f}\right\} \tag{10}$$

From the moment analysis of the solution of the general rate model in the Laplace domain, we can obtain the expression for the height equivalent to a theoretical plate (HETP) [15], which equals

$$\mathrm{HETP}=\frac{L}{N}=\frac{\overline{\mu_2}}{\mu_1^2}L \tag{11}$$

Eqs. (9) and (10) can be combined with Eq. (11)

$$\mathrm{HETP}=\frac{2D_L}{u}+2u\left(\frac{1-\varepsilon}{\varepsilon}\right)\frac{K}{k_f}\left[1+\left(\frac{1-\varepsilon}{\varepsilon}\right)K\right]^{-2} \tag{12}$$

The differences between the various expressions reported in the literature for the dependence of the column HETP on the experimental parameters result from the use of different expressions for the axial dispersion D_L and mass transfer coefficient k_f.

2.3. Axial dispersion in packed beds

The sources of band broadening of kinetic origin include molecular diffusion, eddy diffusion, the mass transfer resistances and the finite rate of the kinetics of adsorption–desorption. Thus, it is important to study diffusion in porous media. Ruthven [15] pointed out that the rates of adsorption and desorption in porous adsorbents are usually controlled by the rate of diffusion within the pore network, more than by the kinetics of adsorption–desorption. This is especially true in chromatography, where the adsorbents are prepared carefully so that they exhibit a moderately strong energy of physisorption and no chemisorption. In a packed bed, it is impossible to move very far along a straight line without hitting the surface of a particle. The channels follow tortuous paths around the particles. When modelling chromatography, the phenomena that contribute to axial mixing are included in a single axial dispersion coefficient. The two main mechanisms contributing to axial dispersion are molecular diffusion and eddy diffusion. In a first approximation, their contributions are additive, and the axial dispersion coefficient D_L is given by

$$D_L=\gamma_1 D_m+\gamma_2 d_P u \tag{13}$$

where d_P is the particle diameter, D_m is the molecular diffusivity, and γ_1 and γ_2 are geometrical constants, whose values are usually around 0.7 and 0.5, respectively [12]. The second approximation was suggested by Chung and Wen [18]. It can be expressed as follows

$$\mathrm{Pe}=\frac{L}{\varepsilon_b d_P}\left[0.2+0.011\ \mathrm{Re}^{0.48}\right] \tag{14}$$

where $\mathrm{Re}=(\gamma\varepsilon_b d_P u)/\eta$ is the Reynolds number, $\mathrm{Pe}=uL/D_L$ is the column Peclet number, L is the column length, d_P is the particle size, ε_b is the interparticle void fraction, γ is the density of the mobile phase, and η is its viscosity. In this work, Eq. (13) was used to evaluate the axial coefficients of the propranolol enantiomers on two chiral columns because of its simplicity.

3. Materials and instrumentation

3.1. Materials and equipment

Propranolol hydrochloride and its pure enantiomers were purchased from Sigma. The hydrochlorides were converted to propranolol free base by precipitation using 1.0 M aqueous sodium hydroxide solution, and extracted with chloroform three times. The organic phase chloroform was dried with anhydrous sodium sulphate before evaporation to dryness. Tri-*tert*-butylbenzene (TTBB) was purchased from Aldrich (USA). The experiments were carried out using a single column liquid chromatographic system. A Chiralcel OD column (1×25 cm, Daicel Chemical Industries) was used, the chiral stationary phase being cellulose tri(3,5-dimethylphenylcarbamate). The HPLC system consisted of a Waters 486 tunable UV-absorbance detector (MA, USA) set at 254 nm, a 2510 HPLC pump (Varian), equipped with a Reodyne (CA, USA) model 9125 sample injector, and an HP-3390A reporting integrator (Hewlett Packard, USA). The temperature was controlled at 25°C by a B. Braun water bath with Frigomix U and Thermomix BU (Germany). A computer with an AD/DA card was also used for data storage and for calculating the values of the first and second moments by integration. The experimental set-up is shown in Fig. 3.

3.2. Experimental procedures

TTBB and the two enantiomers of propranolol were dissolved separately in the mobile phase, which was first degassed in a Branson model 1200 ultrasonic bath. After a

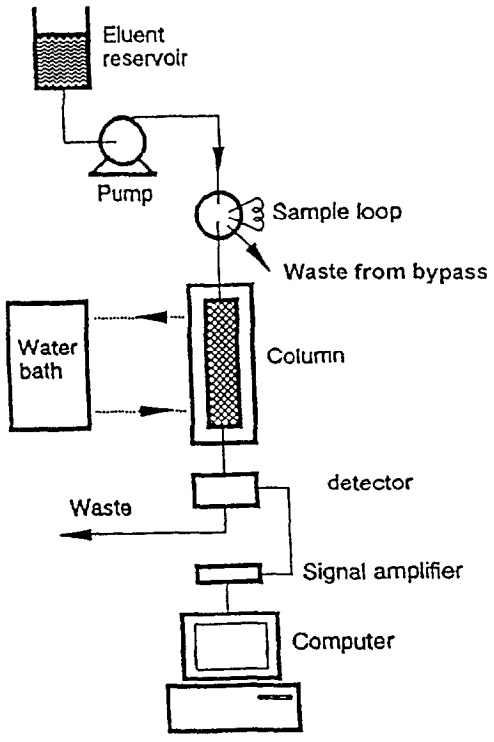

Fig. 3. Diagram of the experimental set-up.

period of time for stabilization of the system, a small pulse of sample (20 μl) was injected, and the concentration response at the outlet of the column was monitored continuously by the UV detector. For a given chromatographic system (sample–mobile phase–column), several response peaks were measured at different flow rates of the mobile phase. These response peaks gave comprehensive data on the determination of the adsorption equilibrium and kinetic coefficients.

4. Results and discussion

4.1. Determination of the bed voidage

The usual definition of the zero retention time in chromatography is

$$t_{0R} = \frac{\varepsilon_T L}{u} \quad (15)$$

where u and L are the superficial velocity and the length of the chromatographic column, and ε_T is the total porosity of the column. In order to evaluate the voidage of the column, the following correlation was used in this work, which was suggested by Ruthven [15] and Suzuki [16]

$$\varepsilon_T = 0.45 + 0.55\varepsilon \quad (16)$$

TTBB has been widely used for the determination of the column dead-time for cellulose and amylose derivative chiral stationary columns [19]. Although the sorption of solutes for the cellulose and amylose derivatives is strongly supported by the phenyl group, the latter is shielded by the *tert*-butyl groups in TTBB. On the other hand, the molecular size of TTBB is relatively small. Therefore, this compound is not retained on Chiralcel OD, and was chosen to determine the value of ε_T using Eq. (16).

By plotting the first moment of TTBB on the chiral column against the inverse superficial velocity, as shown in Fig. 4,

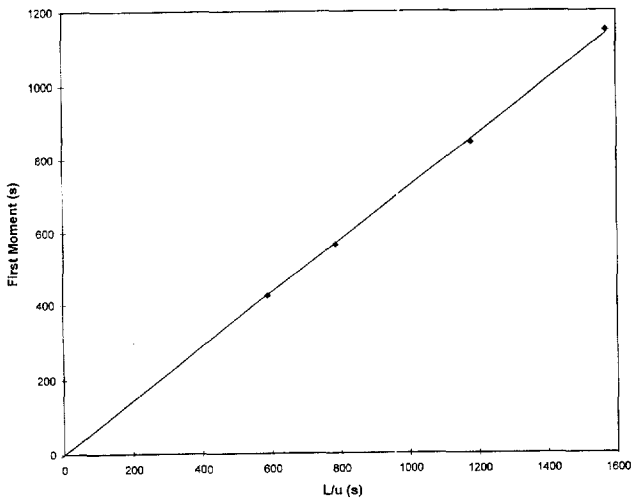

Fig. 4. Plot of the first moment of TTBB versus L/u of the column. L is the column length and u is the superficial velocity.

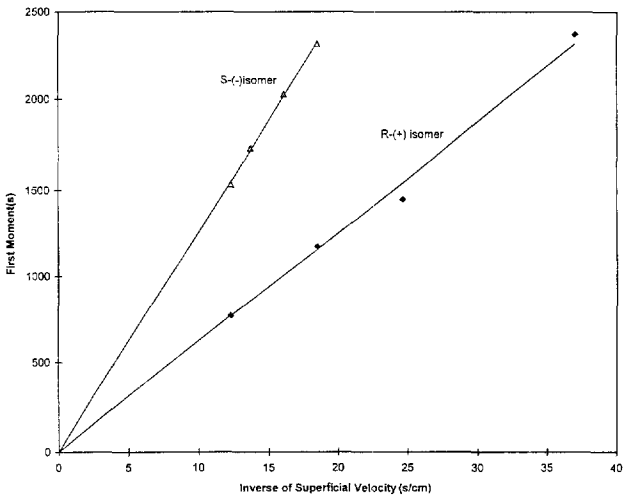

Fig. 5. Plot of the first moments of the two enantiomers of propranolol versus the inverse superficial velocity on the column.

ε_T was found to be 0.72; the voidage ε of Chiralcel OD for this system is 0.49.

4.2. Determination of equilibrium data

As mentioned above, we assume that the kinetics of adsorption–desorption are infinitely fast, i.e., the isotherm is linear when Eqs. (9) and (10) are used to evaluate the first and second moments. Therefore, all pulse experiments need to be carried out under linear conditions. Hence, linear equilibrium isotherms can be assumed under these conditions. The results obtained on the chiral columns demonstrate that this assumption is correct, because the first moment data at different initial sample concentrations for the propranolol enantiomers and TTBB are essentially the same, despite a tenfold variation in the amount of sample injected.

The first moment data for the two propranolol enantiomers obtained on the Chiralcel OD column were plotted against the inverse superficial velocity. The results are shown in Fig. 5. According to Eq. (9), the equilibrium constants were determined from the slopes of the lines in Fig. 5. The equilibrium constants were found to be 6.71 and 2.89 for *S*-(−)- and *R*-(+)-propranolol, respectively.

The equilibrium results under linear conditions show that the K values for the two enantiomers are greater than unity. This means that there are strong interactions between both enantiomers and the chiral column. Moreover, the chiral column has a greater affinity for *S*-(−)-propranolol than for *R*-(+)-propranolol. This may be due to their different steric interactions with the chiral stationary phase.

4.3. Determination of the axial dispersion coefficient and mass transfer coefficient of the chiral column

When a fluid flows through a packed bed, there is a tendency for axial mixing to occur. In the model used here, the effects of all mechanisms which contribute to axial mixing are included together in a single effective axial dispersion

coefficient D_L (Eq. (13)). In a liquid system, the molecular diffusivities of the liquids are too small to contribute significantly to axial dispersion, even at low Reynolds numbers [15]. Therefore, the molecular diffusivities in the systems studied can be neglected. According to Eq. (13), we have

$$D_L = \gamma_2 d_P u = \lambda u \quad (17)$$

The axial mixing in the chiral column is determined by the flow pattern rather than by molecular diffusion. This contribution to HETP (Eq. (12)) should therefore be approximately the same for the two propranolol enantiomers and TTBB. Combining Eqs. (12) and (17), we obtain

$$\mathrm{HETP} = 2\lambda + 2u\left(\frac{1-\varepsilon}{\varepsilon}\right)\frac{K}{k_f}\left[1+\left(\frac{1-\varepsilon}{\varepsilon}\right)K\right]^{-2} \quad (18)$$

In Fig. 6, HETP data for TTBB are plotted against the interstitial velocity. The results show that the axial dispersion of TTBB is dominant on the OD column, although there are slight changes in HETP with different flow rates. D_L can be determined from HETP corresponding to $v = 0$, which gives

$$\lambda = \frac{2D_L}{v} = 0.02 \text{ cm} \quad (19)$$

The small difference in D_L determined from Fig. 6, may be caused mainly by experimental error. In addition, as axial mixing in a liquid system is determined by the flow pattern in the bed rather than by molecular diffusion, this contribution to HETP should be approximately the same for all sorbates. This implies that all solutes should give the same HETP, at zero velocity, so that the D_L value is equal to $0.01v$ cm for all components in the modelling process in future studies.

The plots of HETP against the interstitial velocity for the two propranolol enantiomers in Fig. 7 show that HETP increases approximately linearly with velocity. The overall mass transfer coefficients k_f can be determined using Eq. (18): 0.56 s^{-1} and 1.08 s^{-1} for the *S*-(−) and *R*-(+) enantiomers of propranolol, respectively. Normally, the chiral columns, which are packed with chemically modified silica particles, are developed to permit the achievement of a high resolution and fast separation of the chiral compounds. Therefore, mass transfer is fast in these columns. In the solid film linear driving force model the overall mass transfer coefficient, k_f includes contributions from the resistance to mass transfer due to diffusion across the boundary layer between the flowing mobile phase around the packing materials and the stagnant mobile phase inside these particles [20]. The results show that the mass transfer on the chiral column is fast according to the magnitudes of the k_f values.

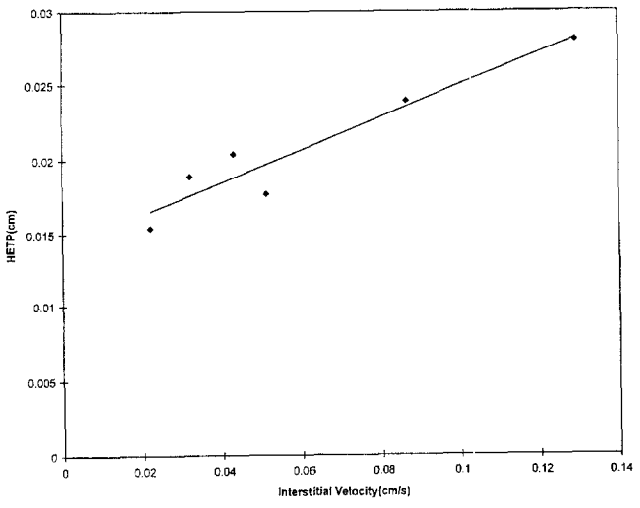

Fig. 6. Plot of HETP of TTBB versus the interstitial velocity on the column.

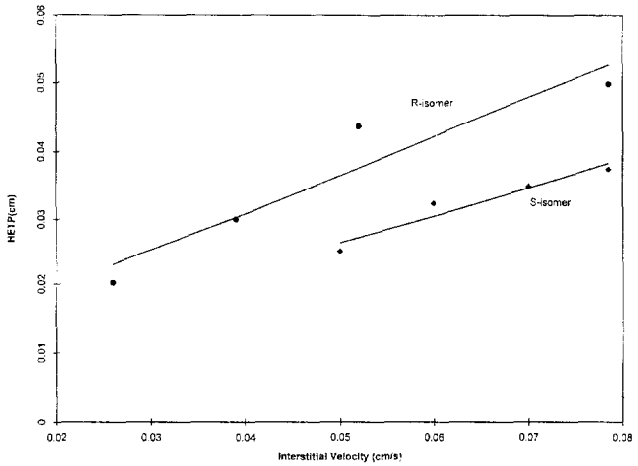

Fig. 7. Plot of HETP of the two enantiomers of propranolol versus the interstitial velocity on the column.

Any small error in the experiments will lead to an uncertainty in the calculated kinetic parameters due to the principal disadvantage of the moments method. In general, the precise nature of the dispersive effects (external film resistance, intraparticle mass transfer resistance) has only a very modest influence on the transient response curves of a reasonably long chromatographic column [21]. For an approximate calculation, as in the modelling of the chiral column–propranolol enantiomer system, a lumped mass transfer resistance parameter can therefore be used quite effectively.

4.4. Simulation of the elution profile with determined parameters

The mass transfer coefficient k_f, bed voidage ε, axial dispersion coefficient D_L and equilibrium constants obtained from this study were used to simulate the elution profiles of the single isomers to test the effectiveness of these values. The corresponding initial and boundary conditions for Eqs. (1) and (2) are given by

$$c(z, t=0) = q(z, t=0) = 0 \quad (20)$$

$$D_L\frac{\partial c}{\partial z}(z=0, t) = -v[c(z=0^-, t) - c(z=0^+, t)] \quad (21)$$

$$\frac{\partial c}{\partial z}(z=L, t) = 0 \quad (22)$$

The feed to a column can be represented as a square pulse as

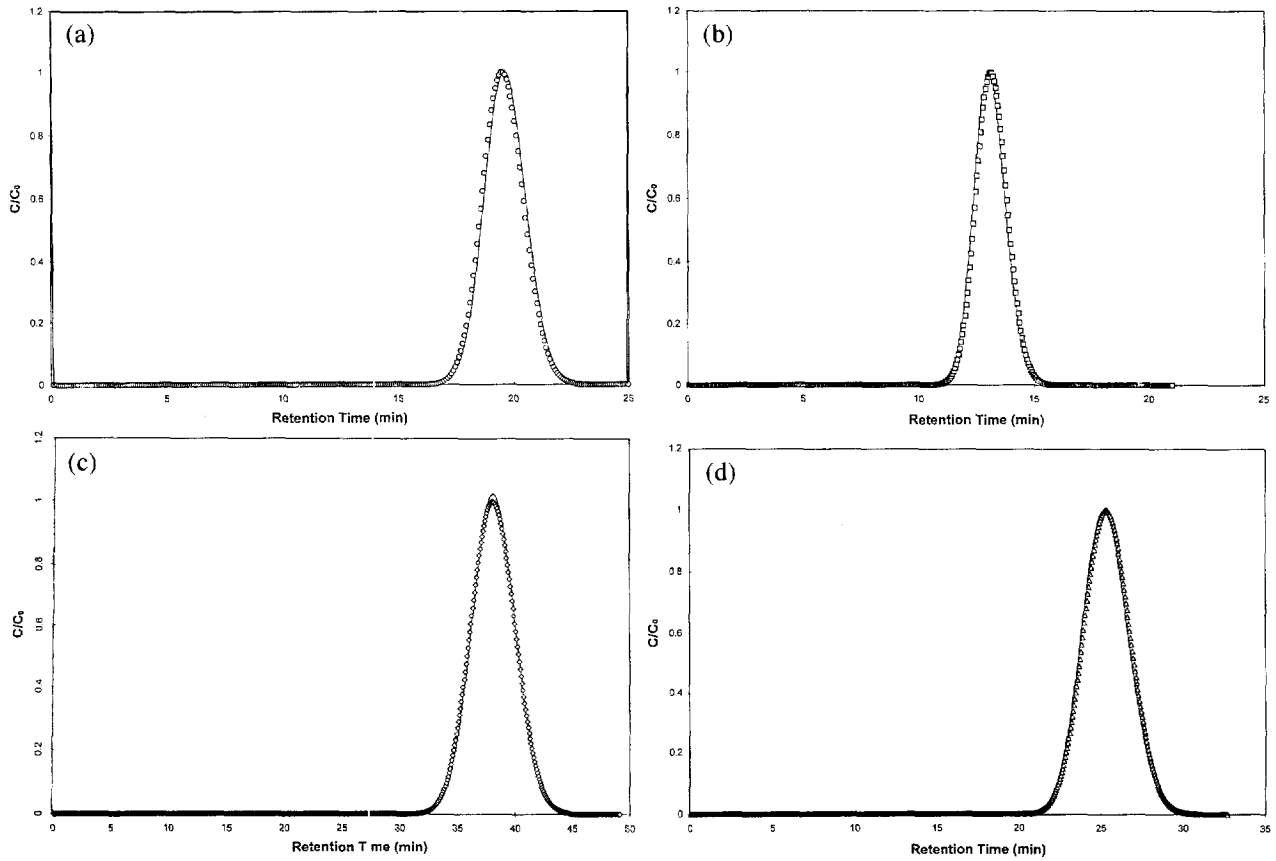

Fig. 8. Comparison of the simulated and experimental band profiles of the two isomers at different flow rates: *R* isomer at flow rate of 2 ml min^{-1} (a) and 3 ml min^{-1} (b); *S* isomer at flow rate of 2 ml min^{-1} (c) and 3 ml min^{-1} (d).

$$c(z=0^-, t) = c_F \quad 0 \le t \le t_F$$
$$c(z=0^-, t) = 0 \quad \text{otherwise} \tag{23}$$

The isotherm used here is linear

$$q^* = Kc \tag{24}$$

The solution of different types of differential equation in chromatography is described in Ref. [11]. In this study, Eqs. (1) and (2) were solved by the technique of finite elements with PDECOL [21]. The parameters used were obtained from the experimental results. The simulated results are compared with the experimental results for the two enantiomers at different flow rates in Fig. 8. It was found that the experimental and simulated results match quite well.

5. Conclusions

In this study, the overall mass transfer coefficient, axial dispersion coefficient, equilibrium constants of the two isomers and bed voidage of the Chiralcel OD column for the enantioseparation of racemic propranolol were determined by moment analysis. The chiral stationary phase exhibits a greater affinity for the *S* enantiomer of propranolol than for the *R* enantiomer. The equilibrium constants are 6.71 and 2.89 for the *S* and *R* enantiomers, respectively. The magnitude of the overall mass transfer coefficient shows that the mass transfer on the chiral column is fast. The axial dispersion coefficient was also estimated, and is approximately equal to 0.01ν for both enantiomers. These parameters were utilized to simulate the band profiles of the single isomers. The simulated results fit the experimental observations very well. After the determination of the adsorption isotherm, these parameters will be used in the optimization of simulated moving bed chromatography.

6. Nomenclature

c	Concentration (mmol)
c_f	Feed concentration (mmol ml^{-1})
c_o	Concentration of outlet (mmol ml^{-1})
D_L	Axial dispersion coefficient (min^{-1})
D_m	Solute molecular diffusivity (cm^2 min^{-1})
F	Phase ratio, equal to $(1-\varepsilon)/\varepsilon$
HETP	Height equivalent to a theoretical plate (cm)
k_f	Overall mass transfer coefficient (min^{-1})
K	Equilibrium distribution constant
L	Length of the chromatographic column (cm)
t	Time (s)
t_0	Mean retention time for an unretained compound (s)
t_{0R}	Mean retention time for an unretained compound (chromatographer's definition) (s)

t_R Mean retention time (s)
u Superficial velocity (cm s^{-1})
v Interstitial fluid velocity (cm s^{-1})

Greek symbols

ε External porosity or bed voidage
ε_i Internal porosity
ε_T Total porosity
η Liquid viscosity
λ Flow geometry-dependent constant
μ_1 First moment (s)
μ_2 Second moment (s^2)

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