

Chromatographic Separation of Bupivacaine Racemate by Mathematical Model with Competitive Langmuir Isotherm

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Abstract—HPLC (High Performance Liquid Chromatography) was utilized for the chiral separation of racemic bupivacaine, and mathematical modeling with competitive Langmuir isotherm was performed to determine the optimum feed condition. For each racemic compound, the isotherm parameters a , b and mass transfer coefficients k were obtained by parameter estimation and maximum likelihood method. The agreement of elution profiles between the experimental data and the calculated values was fairly good. In order to find the optimum separation condition, simulations were carried out to determine the feed conditions such as concentration and injection volume. To preparatively separate racemic bupivacaine, the desirable injection volumes were 0.05 ml at 2.0 mg/ml of the concentration of racemic mixture or 0.01 ml at 20 mg/ml.

Key words: Bupivacaine, Chiral Separation, Mathematical Model, HPLC, Optimum Condition

INTRODUCTION

In a chromatographic column, materials injected are separated based on their differences of the retention with stationary phase. Amount of sample treated ranges from small scale as in an analytical instrument to large scale including absorbent towers [Bailly and Tonderur, 1982]. Compared to gas chromatography which has limitations of volatility, molecular weight, and capacity of feed material, liquid chromatography has definite advantages, so it has been mainly used in the separation of pharmaceutical chemicals as well as fine chemicals.

Enantiomer has the same physical properties with the exception of optical properties, and it has different activity in the human body. For example, one enantiomer pair may be medically active in the human body, while another may not be or may possibly have serious side effects [Medvedovici et al., 1997]. For this reason, interest in the preparation of high-purity enantiomer pair has increased. Because of difficult synthesis of high-purity enantiomer pair, the method of separation with LC has been widely used.

The chromatography models include the simple model of linear adsorption, isothermal and local equilibrium to a rather complicated equation of axial dispersion, resistance of fluid and stationary phase, non-isothermal, and non-linear adsorption [Row, 1999]. The modeling of chromatography is varied. Plate theory and rate theory have been mainly used. Generally, the solution of plate theory is more easily calculated than rate theory, but rate theory is mainly used. The solution of rate theory is solved by numerical methods. Among the large number of numerical methods devoted to the solution of PDE or PDAE systems, which are by nature very difficult to solve, a well established classification would be the following ones: the use of method of lines (MOL) [Carver, 1981; Schiesser, 1977, 1991],

finite difference methods [Anderson and al., 1992], weighted residuals methods [Finlayson, 1971; Villadsen and Michelsen, 1978], finite element methods [Strang and Fox, 1973; Carey and Finlayson, 1975], finite volume methods [Pantakar, 1980], adaptive grid methods [Verwer et al., 1989; Arney and Flaherty, 1990], and moving grid methods [Pilipis, 1990; Miller and Miller, 1981].

The sample was bupivacaine(1-Buthyl-N(2,6-dimethylphenyl)-2-piperidinecarboxamide) for local anesthetic and sold as racemate. The medical activity resides in the S-bupivacaine, while the R-bupivacaine causes a side effect as poison and reducing medical activity [Yang et al., 2003]. The aim of this study was to find the optimum feed condition for enantiomer separation by mathematical modeling of HPLC with competitive isotherm.

THEORY

1. Mathematical Model

To establish a mathematical model of the chromatographic column, the following assumptions are considered:

- (1) process variables are a function of time and the column length (no radial variation occurs in the column).
- (2) axial dispersion flow for the liquid phase
- (3) bed void fraction, radius and porosity of the particles are constant along the column.
- (4) constant flow rates in the column
- (5) negligible pressure drop in the column
- (6) the intraparticle mass transfer is described by a linear driving force (LDF).
- (7) isothermal condition (negligible thermal effects)

The differential material balances for the component i in the mobile phase along a chromatographic column could be expressed as follows:

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$$\frac{\partial C(i,z)}{\partial t} = -u \frac{\partial C(i,z)}{\partial z} + D_L \frac{\partial^2 C(i,z)}{\partial z^2} - \frac{(1-\varepsilon)}{\varepsilon} \frac{6}{R_p} \frac{\partial q(i,z)}{\partial t} \quad \forall z \in (0, L), i=1,2 \quad (1)$$

The dependent variables q and C were the sample concentrations in stationary and mobile phases, respectively. In Eq. (1), the accumulation term, convection, axial dispersion, and mass in stationary phase were considered. By a lumped kinetic model, the accumulation rate of a component in the stationary phase was expressed by mass-transfer driving force. Here a linear driving force (LDF) model was used.

$$\frac{\partial q(i,z)}{\partial t} = k(i) \frac{6}{R_p} (q^*(i,z) - q(i,z)) \quad \forall z \in [0, L], i=1,2 \quad (2)$$

Finally, the equilibrium adsorption was assumed as the following competitive Langmuir isotherm.

$$q^*(i,z) = \frac{a(i)C(i,z)}{1 + \sum_{j=1}^2 b(j)C(j,z)} \quad \forall z \in [0, L], i=1,2 \quad (3)$$

For Eqs. (1)–(3), the boundary and initial conditions were given as

$$\text{BCs} \quad @z=0 \quad C(i,0) = C_{in}(i) \quad i=1,2 \quad (4)$$

$$@z=L \quad \frac{\partial C(i,L)}{\partial z} = 0 \quad i=1,2 \quad (5)$$

$$\text{ICs} \quad @t=0 \quad C(i,z) = 0 \quad \forall z \in (0, L), i=1,2 \quad (6)$$

$$@t=0 \quad q(i,z) = 0 \quad \forall z \in (0, L), i=1,2 \quad (7)$$

2. Method of Solution

As seen in the previous governing equations, the mathematical description of a chromatographic column is described by a set of partial differential and non-linear algebraic equations (PDAEs). A solution of the PDAE systems is generally a complicated problem and no universal method effectively deals with all types of such problems.

In this work, the method of lines (MOL) was employed to convert partial differential equations (PDEs) to ordinary differential equations (ODEs) with respect to time. The advantage of this procedure is that sophisticated computer programs, which permit fast and accurate integration of large sets of ODEs over time, can be employed. In particular, integration codes based on backward differentiation formula can solve stiff as well as non-stiff systems of equations. They utilize sophisticated algorithms for automatic step-size adjustment and integration order selection to maintain a user-specified error tolerance. The family of the method of lines comprises collectively a number of finite difference, finite element and weighted residual methods in which piecewise local or global approximation functions in the space dimensions are used to convert evolutionary PDE problems into initial value ODE problems. One promising numerical method for this purpose is an orthogonal collocation method in which orthogonal polynomials and their roots are used to discretize the continuous domain and approximate spatial derivatives. This method is well known for its efficiency and accurateness in the field of reaction engineering. However, when the solution has steep gradients, it is more beneficial to use it in conjunction with a finite element approach. This gives rise to the orthogonal collocation method on finite elements, and this method is employed to deal

with the PDAE system described in the preceding chapter [Oh, 1995].

In this work, the MOL was employed to convert PDEs to ODEs with respect to time.

The method of lines consists of two steps, respectively:

Step 1: The continuous spatial domains are discretized into finite grid of points ("nodes"), thus reducing PDAEs into DAEs.

Step 2: The DAEs are integrated over time by employing appropriate integration software.

A number of sophisticated integration codes are already available for step 2 above. This work uses the DASOLV code [Jarvis and Pantelides, 1992]. This is based on backward differentiation formulae, and automatically adjusts the time step size as well as the integration order to maintain the error of integration within a user-specified tolerance.

There are several different spatial discretization techniques that can be used in the context of the MOL family. The finite difference techniques, which were employed in this work, replace the continuous problem domain by a grid such that the dependent variables from the continuous domain exist only at a finite number of discrete points. In other words, the continuous problem domain is discretized on a finite set of grid points. The derivation of finite difference is achieved by Taylor-series expansion.

Eqs. (1)–(7) were solved by using gPROMS v2.2 [gPROMS, 2003], a software package for modeling, simulation and optimization of processes with both discrete and continuous as well as lumped and distributed characteristics. This package can handle systems of algebraic equations, partial differential equations, integral equations or mixed systems of these types of equations [Barton and Pantelides, 1994; Oh, 1995]. They were reduced into a set of ordinary DAEs in time by discretization of the axial domain. A first order method in backward finite difference method (BFD) with 1,200 grid points was found to be satisfactory for solution of the model equations. All parameter estimations and simulations were performed on a Pentium IV (2.66 GHz processor) with 1 GB RAM.

EXPERIMENTAL

1. Chemical

The standard chemical of racemic bupivacaine (1-Buthyl-N(2,6-dimethylphenyl)-2-piperidinecarboxamide) hydrochloride used in this experiment was purchased from Sigma Co. (refer to Fig. 1). The extra-pure grade solvents of IPA (iso-propanol) and n-hexane, acetic acid used in the mobile phase were purchased from J. T. Baker (Phillipsburg NJ, USA), and triethylamine was purchased from Sigma Co. Sodium hydrogen carbonate and DCM (dichloromethane) was used to remove hydrochloride in standard chemical, and they were purchased from Kanto Chemical (Japan) and J. T. Baker, respec-

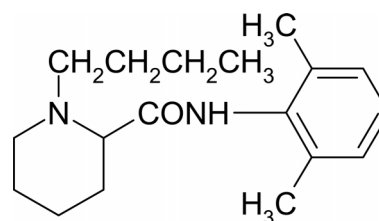


Fig. 1. Chemical structure of bupivacaine.

tively.

2. Instrument

HPLC was equipped with an M 930 HPLC pump (Younglin, Korea) and a M 720 (Younglin, Korea) detector of UV-visible tunable wavelength absorbance, and an injector (0.1 ml sample loop) of Rheodyne. The data acquisition system was AutoChro-WIN (ver. 2.0, Younglin, Korea) installed in a PC. The chromatographic column (1×10 cm, 10 mm) packed with Kromasil® CHI-TBB (Eka Chemical, Sweden). The chiral monomer was 0,0'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamideis, and polymerized. The chiral network polymer was covalently bonded to sphere silica. For the chiral network polymer, an enantiomer pair has different selectivity in a column.

3. Experiment

The extra-purity racemic bupivacaine was extracted from standard chemical of racemic bupivacaine hydrochloride. 2 ml of saturated solution of sodium hydrogen carbonate and DCM was added into 3 mg of sample. The saturated solution of sodium hydrogen carbonate used 100 ml of extra-pure water per 7 g of sodium carbonate. Sample was dissolved into DCM, and saturated solution of sodium carbonate into DCM solution. It was stirred for one minute to increase mass transfer between phases. Layer separation was observed. The bottom side of DCM phase was isolated with separatory funnel and then it was vaporized by an electric heater. The concentration of bupivacaine dissolved in *n*-hexane was 1 to 20 mg/ml and the injection volume was changed from 0.001 to 0.05 ml. The flow rate of mobile phase was fixed at 2 ml/min. The mobile phase composition was *n*-hexane/IPA/acetic acid/triethylamine, 99/1/0.1/0.05 (vol%). The UV wavelength was fixed at 260 nm. The experiment was performed at room temperature. The constants and operating conditions in this work are summarized in Table 1.

RESULTS AND DISCUSSION

1. Calculation Procedures

In the mathematical equations, Eqs. (1)-(7), mass transfer coefficient, *k* and the parameters of competitive Langmuir isotherm, *a* and *b* were normally estimated from correlation or experimental data. The model parameters required were estimated by gEST with the gPROMS software package [Process Systems Enterprise Ltd., 2003]. gEST perform parameter estimation for complex models uses both dynamic and steady-state experimental data. The parameter esti-

mation was performed to determine the unknown parameters, *k*(*i*), *a*(*i*) and *b*(*i*), in order to maximize the probability that the mathematical model would predict the values obtained from the experiments. Assuming independent, normally distributed measurement errors with zero means and standard deviations, σ_{ijk} , this maximum likelihood goal can be established through the following objective function:

$$\Phi = \frac{N}{2} \ln(2\pi) + \frac{1}{2} \min_{\theta} \left\{ \sum_{i=1}^{NE} \sum_{j=1}^{NV} \sum_{k=1}^{NM_i} \left[\ln(\sigma_{ijk}^2) + \frac{(\bar{z}_{ijk} - z_{ijk})^2}{\sigma_{ijk}^2} \right] \right\} \quad (8)$$

The maximum likelihood objective function, Eq. (8), gives flexibility for several types of variation models to be specified. These take the general form:

$$\sigma^2 = \sigma^2(\beta, z, \bar{z}) \quad (9)$$

In a heteroscedastic variance model, the measurement error depends on the measured values but is proportional to $\bar{z}^{y^2}$ or z^{y^2} . The mathematical description of a heteroscedastic variance model is as follows:

$$\sigma^2 = w^2(\bar{z} + \varepsilon)^y \text{ or } \sigma^2 = w^2(z + \varepsilon)^y \quad (10)$$

The total computational CPU time was 55086.9 sec, and the number of optimization iterations was 45. The number of line-search steps was 88. The objective function value (Φ) was -1.88057×10^3 . Finally, Table 2 lists the estimated parameters obtained for Eq. (2), *k*(*i*) and Eq. (3), *a*(*i*) and *b*(*i*) of racemic bupivacaine.

2. Estimation of Parameters in the Mathematical Model

A chromatographic column has a long cylindrical shape, and especially in a small scale, the band-broadening often may be neglected. The axial dispersion coefficient was calculated by Eq. (11) which assumed to vary along the length of column [Slater, 1991]. The calculated value of the axial dispersion coefficient was $9.051 \times 10^{-7} \text{ m}^2/\text{min}$ for racemic bupivacaine. It was used throughout the simulation work. With the several orders of magnitudes of axial dispersion coefficient, the elution profiles were almost unchanged (Fig. 2).

$$\frac{uR_p}{\varepsilon_i D_L} = \frac{0.2}{\varepsilon_i} + \frac{0.011}{\varepsilon_i} \left(\frac{Re}{\varepsilon_i} \right)^{0.48} \quad (11)$$

The sample components are normally separated by several factors, but the different adsorption characteristics predominantly contribute to the resolution of mixture. The relationship is expressed in terms of an adsorption isotherm. The Langmuir isotherm is frequently assumed as a equation in terms of the concentrations of stationary phase and mobile phase. From an elution curve in the experimen-

Table 1. Constants and operating conditions in this work

Constants	
Column length (m)	0.1
Column diameter (m)	0.01
Column volume (m ³)	7.854×10^{-6}
Bed porosity (-)	0.7
Particle diameter (m)	10×10^{-6}
Operating conditions	
Fluid flow rate (m ³ /min)	2×10^{-6}
Interstitial fluid velocity (m/min)	3.638×10^{-2}
Injection volume (ml)	0.02
Feed concentration (mg/ml)	20

Table 2. Estimated equilibrium and kinetic parameters in the mathematical model

Parameter (1: R-form, 2: S-form)	Estimate
a(1) (-)	5.331
a(2) (-)	6.982
b(1) (ml/mg)	0.372
b(2) (ml/mg)	0.636
k(1) (1/min)	0.000457
k(2) (1/min)	0.004034

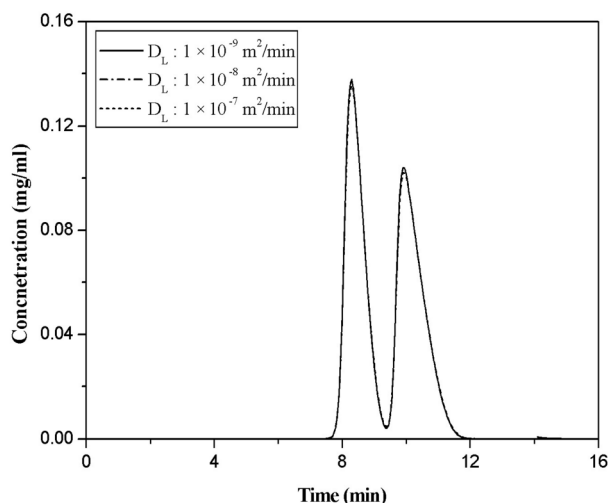


Fig. 2. Sensitivity analysis of axial dispersion coefficients.

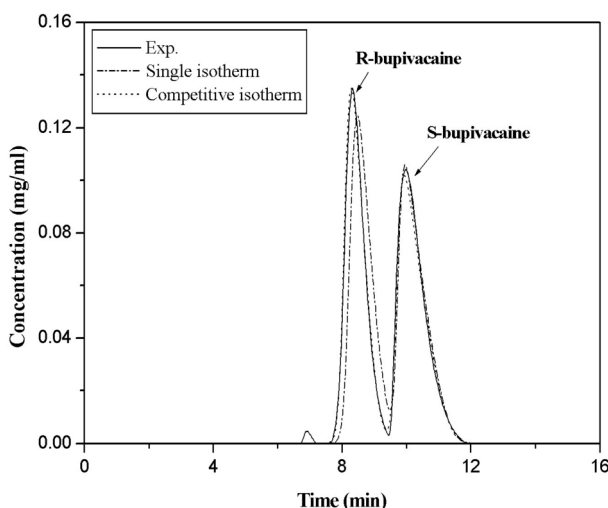


Fig. 3. Comparison of bupivacaine concentration profiles by experiment and calculated values.

tal condition, 0.02 ml of injection volume and 20 mg/ml of sample concentration, the parameters a and b in Eq. (3) were estimated by PIM (pulsed input method) [Choi, 2000] and the resulting values are listed in Table 2.

Fig. 3 shows the two calculated values with the parameters from Table 2 and experimental bupivacaine elution profiles. The single isotherm in Fig. 3 means that no competitive term was included. When a competitive isotherm was used, better agreement between experimental data and calculated values was observed in the first peak of R-bupivacaine. In a large amount of injection, the first peak will be influenced by the second peak, so the retention may be altered. For the racemic mixture of bupivacaine, the competitive Langmuir isotherm was well fitted.

3. Optimized Feed Condition

The principle of chiral separation is based on the differences in binding force between enantiomer pair and chiral selector bonded to CSP (Chiral Stationary Phase) in a chromatographic column. Generally, the binding force is a hydrogen bond and π - π bond between chiral selector and enantiomer pair. Binding force between them is

more determined by a hydrogen bond stronger than a π - π bond. The side of the binding site in the chiral selector has a structure that causes steric hindrance, so it leads to differences in affinity between the enantiomer pair. To reduce the retention time, various types of alcohol were added into the mobile phase to adjust its polarity, then it increased the desorption rate of enantiomer from the CSP.

With an increased amount of a more affinity component in the stationary phase, the adsorption amount increased in the stationary phase. And a desorption rate of less affinity component increased. For this reason, the rate of increasing peak height of the less affinity component was big, but the rate of increasing peak width was small. In this case, the enantiomer pair has competitive adsorption in the stationary phase. When injection amount in the column was increased, the peak widths of the enantiomer pair were increased, and overlapped. Then, the optimum determination of feed condition was important in a preparative work.

The purpose of mathematical modeling is to find the optimized separation condition by comparing elution profiles with a few experimental runs. The optimized condition is referred to as retention time (or factor), resolution or purity of a target material [Lee, 2002]. Resolution is defined by the difference in retention times divided by average peak width. Therefore, it decreased with larger peak width. In the preparative mode, the resolution does not have to be high, and the amount of purified material is determined by feed condition, concentration and injection volume. The effect of injection volume on resolution with sample concentration was investigated and shown on semi-log plot in Fig. 4. The resolution of R,S-bupivacaine decreased with increasing injection volume. The amount of sample injected in the unit of mass is calculated by multiplying the injection volume by sample concentration. Also, the area of a peak designates the mass of sample, so when the injection volume or sample concentration is larger, the resolution should be deteriorated by overlapped part between the two components [Row, 1999]. For example, if the resolution is set above 1.2, where baseline separation is occurring, the desirable feed condition is easily recommended in Fig. 4. For a real case of racemic mixture (1 to 1 mg/ml) as shown in Fig. 5, peak width was also considered with injection volume.

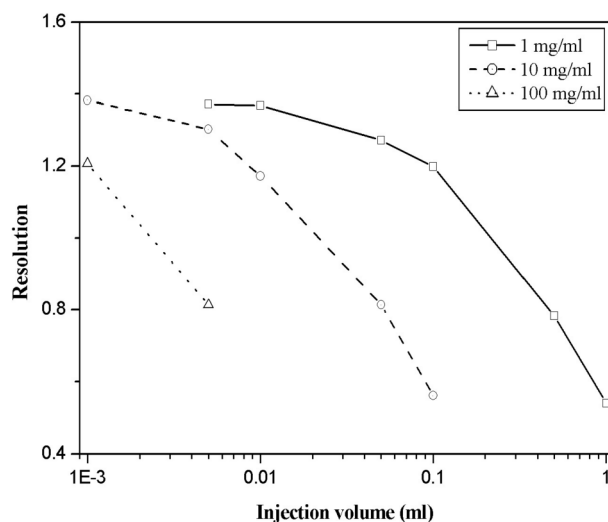


Fig. 4. Effect of injection volumes on resolution with sample concentration.

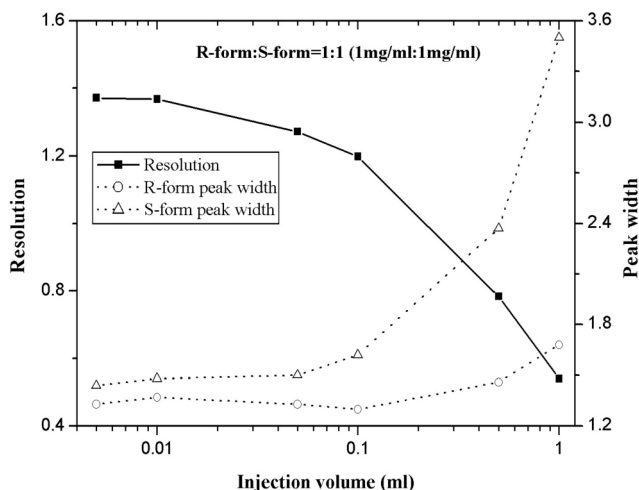


Fig. 5. Effect of injection volumes on resolution and peak width.

The increasing rate of peak width of S-form was steeper than that of R-form, because the second peak of S-form bupivacaine was interfering competitively with R-form bupivacaine on limited adsorption sites in CSP.

In an analytical scale, a tiny amount of sample was injected, and resolution may be enough to explain the degree of separation. However, in a larger amount of injection, purity and yield are often calculated in a preparative mode. For the binary mixture (A plus B), the purity of component A was calculated as the ratio of the peak area A of the total area for a specific time of collection, while the yield of component A was done by the ratio of purified fraction to the total amount of component A. When the value of resolution was above 1.2, the components were completely separated. The purity was obtained at 100% of yield. Similarly, the yield was found at 100% of purity. Figs. 6 and 7 show the purity and yield of R,S-bupivacaine racemic mixture in terms of injection volume, respectively. As the same in the resolution, they decreased with increasing injection volume since racemic mixtures were more overlapped.

To verify the feasibility of the usefulness of the mathematical

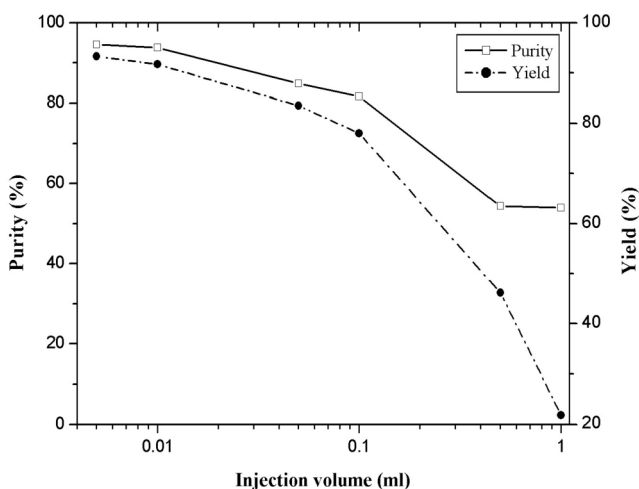


Fig. 6. Effect of injection volumes on purity and yield of R-bupivacaine.

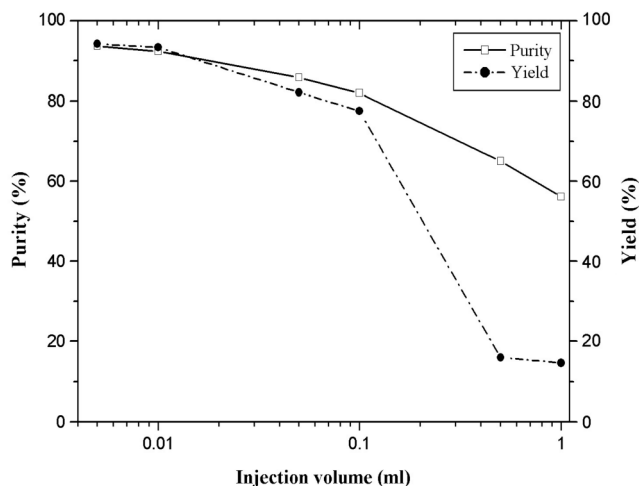


Fig. 7. Effect of injection volumes on purity and yield of S-bupivacaine.

model developed, calculations were made at the optimized condition as in above 1.2 of the resolution in Fig. 4, and compared with the experimental data. As shown in Figs. 8 and 9, the experiment data coincided with the calculated values. The deviation of retention time of the experiment data from the calculated value was less than 0.05 min at 2 mg/ml and 0.21 at 20 mg/ml racemic mixture.

CONCLUSION

To do the chiral separation of racemic bupivacaine, mathematical equations were established. In the governing equations, the parameters of the competitive Langmuir isotherm and mass transfer coefficients were estimated from the experimental data and correlation, respectively. From the simulation results, the optimized separation conditions were suggested, and the agreement between experimental data and calculated values was considerably good. This predictive mathematical model was successfully achieved, and might be utilized in preparative scale to collect the target material in a pure form.

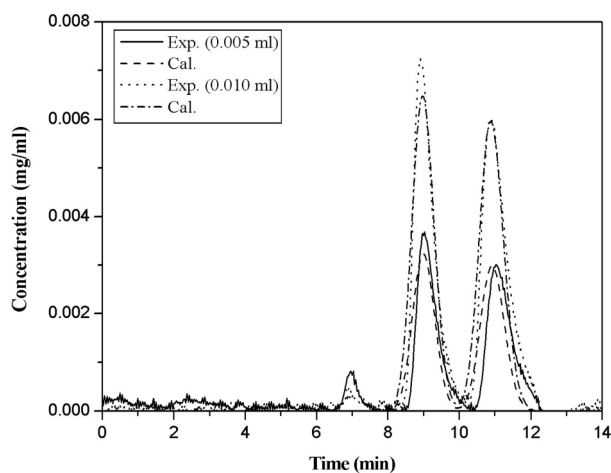


Fig. 8. Bupivacaine concentration profile (Feed concentration=1 mg/ml, injection volume=0.005, 0.01 ml).

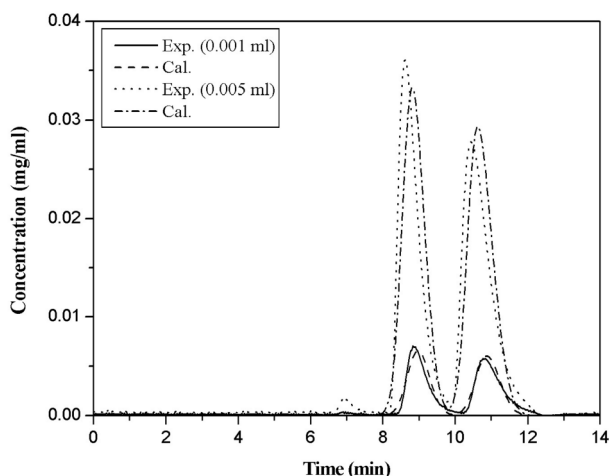


Fig. 9. Bupivacaine concentration profile (Feed concentration=10 mg/ml, injection volume=0.001, 0.005 ml).

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NOMENCLATURE

- a : parameter of competitive Langmuir isotherm [-]
 b : parameter of competitive Langmuir isotherm [ml/mg]
 C : concentration in mobile phase [mg/ml]
 C_{in} : injection concentration in mobile phase [mg/ml]
 D_L : axial dispersion coefficient [m²/min]
 i : component index ($i=1, 2$)
 j : component index ($j=1, 2$)
 k : mass transfer coefficient [1/min]
 L : column length [m]
 N : total number of measurements taken during all the experiments
 NE : number of experiments performed
 NM_{ij} : number of measurements of the j^{th} variable in the i^{th} experiment
 NV_i : number of variables measured in the i^{th} experiment
 Re : Reynolds number [-]
 R_p : radius of particle [m]
 t : time variable [min]
 q : average adsorbed phase concentration [mg/ml]
 q^* : adsorbed phase concentration in equilibrium with C [mg/ml]
 u : interstitial velocity [m/min]
 z : axial coordinate [m]
 z_{ijk} : k^{th} (model-)predicted value of variable j in experiment i
 $\bar{z}_{ijk}$: k^{th} measured value of variable j in experiment i

Greek Letters

- β : set of parameters in the general statistical variance model
 ε : bed porosity (Eq. (1)), absolute tolerance (Eq. (10))

- Φ : maximum likelihood estimation objective function
 γ : static variance model parameter
 θ : set of model parameters to be estimated
 σ_{ijk}^2 : variance of the k^{th} measurement of variable j in experiment i
 ω : static variance model parameter

REFERENCES

- Anderson, D. A., Tannehill, J. C. and Pletcher, R. H., "Computational Fluid Mechanics and Heat Transfer," McGraw-Hill, New York (1992).
 Arney, D. C. and Flaherty, J. E., "An Adaptive Mesh-moving and Local Refinement Method for Time-Dependent Partial Differential Equations," *ACM Trans. Math. Soft.*, **16**(1), 48 (1990).
 Bailly, M. and Tondeur, D., "Recycle Optimization in Non-linear Productive Chromatography. I. Mixing Recycle with Fresh Feed," *Chem. Eng. Sci.*, **37**, 1199 (1982).
 Barton, P. I. and Pantelides, C. C., "Modeling of Combined Discrete/Continuous Processes," *AIChE J.*, **40**, 966 (1994).
 Carey, F. and Finlayson, B. A., "Orthogonal Collocation on Finite Elements," *Chem. Eng. Sci.*, **30**, 587 (1975).
 Carver, M. B., "Method of Lines Solution of Differential Equations Fundamental Principles and Recent Extensions," in Mah, R. S. H. and Seider, W. D., editors, *Foundations of Computer-Aided Process Design*, Engineering Foundation, New York (1981).
 Choi, Y. S., Lee, J. W., Koo, Y. M., Choi, D. K. and Row, K. H., "Single and Competitive Isotherms of Phenol and o-Cresol by Pulsed-Input Method," *Korean J. Chem. Eng.*, **17**(6), 625 (2000).
 Finlayson, B. A., "Packed Bed Reactor Analysis by Orthogonal Collocation," *Chem. Eng. Sci.*, **26**, 1081 (1971).
 "gPROMS v2.2 User Guide," Process Systems Enterprise Ltd., London (2003).
 Jarvis, R. B. and Pantelides, C. C., "DASOLV - A Differential-algebraic Equation Solver," Technical report, Centre for Process Systems Engineering, Imperial College, London (1992).
 Lee, J. W. and Row, K. H., "Determination of Retention Factors of Aromatic Compounds by Gradient-Elution Reverse-Phase High Performance Liquid Chromatography," *Korean J. Chem. Eng.*, **19**(6), 978 (2002).
 Medvedovici, A., Sandra, P., Toribio, L. and David, F., "Chiral Packed Column Subcritical Fluid Chromatography on Polysaccharide and Macrocyclic Antibiotic Chiral Stationary Phases," *J. Chromatogr. A*, **785**, 159 (1997).
 Miller, K. and Miller, R. N., "Moving Finite Elements," *SIAM J. Numer. Anal.*, **18**, 1019 (1981).
 Oh, M., "Modelling and Simulation of Combined Lumped and Distributed Processes," Ph.D. Thesis, University of London, London (1995).
 Pantakar, V., "Numerical Heat Transfer and Fluid Flow," McGraw-Hill, New York (1980).
 Pilipis, G., "Higher Order Moving Finite Element Methods for Systems Described by Partial Differential-algebraic Equations," PhD Thesis, University of London, London (1990).
 Row, K. H., "Principles and Applications of Liquid Chromatography," Inha Press, Incheon (1999).
 Schiesser, W. E., "An Introduction to the Numerical Method of Lines Integration of Partial Differential Equations (Differential Systems

- Simulator, Version 2),” Lehigh Universities and Naval Air Development Center (1977).
- Schiesser, W. E., “The Numerical Method of Lines,” Academic Press, New York (1991).
- Slater, M. J., “The Principles of Ion Exchange Technology,” Butterworth Heinemann, Oxford (1991).
- Strang, G. and Fox, G. J., “An Analysis of the Finite Element Method,” Prentice-Hall, New Jersey (1973).
- Verwer, G., Blom, J. G., Furzeland, R. M. and Zegeling, P. A., “A Moving Grid Method for One-Dimensional PDEs Based on the Method of Lines,” in Flaherty, J. E., Paslow, P. J., Shephard, M. S. and Vasilakis, J. D, editors, Adaptive Methods for Partial Differential Equations, Clarendon Press, Oxford (1989).
- Villadsen, J. and Michelsen, M. L., “Solution of Differential Equation Models by Polynomial Approximation,” Prentice Hall, Englewood Cliffs, NJ (1978).
- Yang, H. Y., Shin, M. H., Chang, H. K., Jang, M. H., Lee, T. H., Kim, Y. J., Chung, J. H. and Kim, C. J., “Bupivacaine and Ropivacaine Suppress Glycine-and Glutamate-Induced Ion Currents in Acutely Dissociated Rat Hippocampal Neurons,” *Neurosci. Lett.*, **344**, 33 (2003).