Simplistic Determination of Operation Parameters for Simulated Moving Bed (SMB) Chromatography for the Separation of Ketoprofen Enantiomer

Tae Hyun Park, Tae Ho Yoon, and In Ho Kim*

Department of Chemical Engineering, Chungnam National University, Daejeon 305-764, Korea

Abstract Since it is troublesome to estimate adequate flow rates in four sections of SMB chromatography, a systematic determination of the flow rates has been suggested by using ketoprofen as a model chiral enantiomer. S-ketoprofen, less retained species, was separated from raffinate stream and the variation in its purity was dependent on the changes of the flow rate of section 4 (Q_4), the raffinate flow rate (Q_{raf}), and the feed flow rate (Q_{feed}) under a fixed switching time t^*. When one parameter was changed at the given experimental condition, purities of product were changed and these phenomena has be well explained by the triangle theory.

Keywords: SMB chromatography, ketoprofen, chiral separation

INTRODUCTION

Ketoprofen is chiral in nature and used as an anti-inflammatory drug for relieving rheumatoid pain, and composed of two enantiomers that can be separated by commercially available chiral columns. For the preparative chiral separation of ketoprofen, SMB chromatography is used to obtain the S-ketoprofen which has pharmaceutical activity from the ketoprofen racemate. SMB is composed of four separation sections where flow rates change due to inflows and outflows. The operation of SMB is rather complex, and determination of flow rates in all four sections of SMB is quite difficult. There are some theories for estimation of operating conditions of SMB chromatography. Among these methods, we use triangle method as suggested by Morbidelli *et al.* to estimate operating conditions of SMB [1]. The triangle method is based on the calculation of dimensionless flow rates, m values which are the ratios of flow rate between mobile phase and stationary phase in four sections of True Moving Bed (TMB) as well as the retention times of R- and S-ketoprofen. Henry's constants of R- and S-ketoprofen are derived from retention times, and then the 'm' values (m_2 and m_3) of second and third sections of SMB are limited by the Henry's constants for getting pure R- and S-ketoprofen [2]. A triangle can be generated in the m_2 and m_3 diagram by horizontal, vertical and diagonal lines. Morbidelli *et al.* suggested that the points inside the triangle region are the candidates for getting both the pure raffinate (S-ketoprofen) and the extract (R- ketoprofen). Many criteria other than the triangle theory were suggested for determining the appropriate

flow rates in SMB [3,4]. For example, commercial ASPEN engineering suite simulator is a convenient software for determining SMB operation conditions from several batch experimental data and adsorption isotherms.

The present study was carried out to show the effect of flow rate variation in SMB on the purities of raffinate or extract stream by a simple method based on the triangle theory. It starts with changing flow rates of section 4 (Q_4) by fixing feed and raffinate flow rate, and switching time. And then, the flow rate of raffinate, one of the products streams, was varied to understand its effect on the purities of raffinate and extract streams. After that, the feed flow rate was also changed to know the variation effect of feed flow on the purities of products (raffinate and extract). These procedures will be a useful tool to represent the effects of flow rate changes in SMB.

MATERIALS AND METHODS

Chemicals

Ketoprofen racemate, acetone, methanol and acetic acid were purchased from Sigma Co. (USA), and HPLC-grade hexane and *tert*-butylmethylether (TBME) from J. T. Baker (USA). In order to make a calibration curve for the concentration determination of ketoprofen, pure ketoprofen enantiomer (99%, Sigma Cat. No. K2135) was used. Stationary phases utilized in batch and SMB experiments were Kromasil TBB (O,O'-bis(4-*tert*-butyl-benzoyl)-N,N'-diallyl-L-tartar diamid bonded on spherical silica, particle size=10 μm, pore size=100 Å), and packed into empty stainless steel columns (10 mm ID × 100 mm L) by slurry packing with a packing device (Model 1666 Slurry Packer, Alltech, USA).

*Corresponding author
Tel: +82-42-821-5685   Fax: +82-42-822-8995
e-mail: ihkim@cnu.ac.kr

Table 1. Validation of column identity by measuring zero retention time, voidage, and Henry's constant of S- and R-ketoprofen

Column number	t_0 (min)	ε	H (S-ketoprofen)	H (R-ketoprofen)
1	0.89	0.535	11.26	13.47
2	0.897	0.540	11.16	13.36
3	0.927	0.558	11.42	13.69
4	0.923	0.556	11.50	13.81
5	0.920	0.554	11.49	13.80
6	0.887	0.534	11.12	13.73
Average	0.907	0.55	11.32	13.64

Batch Chromatography

Batch experiments were performed on a HPLC pump (M-930, Younglin, Korea) and detector (Model M720, Younglin, Korea) with DataApex data acquisition system at 254 nm. Mobile phase was the mixture of hexane and TBME with acetic acid as a pH adjuster (85/15/0.1% v/v), and flow rate was 4.7 mL/min with 100 μL sample loading amount. Column identity experiments were performed by measuring retention times of R- and S-ketoprofens as well as voidage volume for each column [2]. Henry's constants for the columns were calculated by the following equations.

$$t_r = t_0\left(1 + \frac{(1 - \varepsilon)}{\varepsilon}H\right)$$

$$\varepsilon = t_0\,Q/V$$
$$q = HC$$

where, t_r: retention time, t_0: zero retention time, ε: voidage, Q: volumetric flow rate of mobile phase, V: Column volume, q: solute concentration in stationary phase, C: solute concentration in mobile phase and H: Henry's constant.

SMB Operation

SMB chromatography system used in this experiment was explained in the previous report [2]. Variation of parameters was achieved by changing the flow rate in section 4 (Q_4), raffinate flow rate (Q_{raf}), feed flow rate (Q_{feed}), and feed concentration. Flow rates for SMB operation are related as follows;

$$Q_1 = Q_4 + Q_{elu}. \quad Q_2 = Q_1 - Q_{ext}$$
$$Q_3 = Q_2 + Q_{feed}, \quad Q_4 = Q_3 - Q_{raf}$$
$$Q_{feed} + Q_{elu} = Q_{ext} + Q_{raf}$$

Dimensionless flow rate in the j section, 'm_j', is related with switch time 't^*' by the following equation.

$$m_j = (Q_j\,t^* - V\varepsilon) / (V(1 - \varepsilon))$$

where, V and ε are the column volume and the column

voidage, respectively.

The analytical column for purity determination of extract and raffinate streams was purchased from Eka Nobel (0.46 × 25 cm, 5 μm, Kromasil TBB, Sweden). Analytical conditions were 1 mL/min of eluent flow rate and 50 μL loading at the same mobile phase condition mentioned above.

RESULTS AND DISCUSSION

S-ketoprofen is eluted earlier than R-ketoprofen enantiomer, and the retention times of raffinate (S-form) and extract (R-form) are measured to calculate Henry's constants of six columns used in SMB (Table 1). The relative differences between the average value and the measured Henry's constants of the columns are within 5%, therefore the packed SMB columns could be accepted for installation into SMB. This permission criterion was suggested by Kaspereit et al. [5].

The flow rate in section 4 (Q_4), recycle flow rates, changes starting from an arbitrary condition (m_2 and m_3 are 12.2 and 12.8), which is one point in m_2-m_3 diagram. From this point all flow rates can be calculated using equations listed above, and a detail calculation step is explained in [2]. In this case, the first SMB experiment was started from 4.5 mL/min of flow rate in section 4, and continued with other flow rates of Q_4. The sum of input flow rates of Q_{feed} and Q_{elu} should be equal to the sum of raffinate and extract flow rates. Therefore, the change of Q_4 with a fixed set of Q_{feed}, Q_{raf}, and t^* implies that Q_1, Q_2, and Q_3 are automatically varied. As the Q_4 decreases, purities of extract and raffinate decline (Fig. 1). This means that the internal concentration profile through connected columns is shifting to the opposite direction of mobile phase flow. Therefore, proper Q_4 could be selected as 4.4 mL/min in the region between 4.0 and 4.5 mL/min.

With the flow rate Q_4 of 4.4 mL/min in Fig. 1, we made a series of SMB experiments by varying the raffinate flow rate Q_{raf}. There is also a maximum value of raffinate purity as shown in Fig. 2. The points in Fig. 2 correspond to the points in m_2-m_3 diagram of Fig. 3, and the purity variation in Fig. 2 can be explained by observing the point positions in Fig. 3. According to Juza et al. [1],
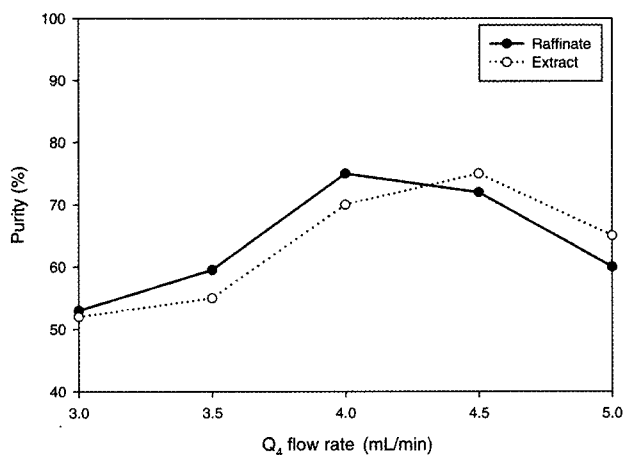
Fig. 1. Purity variation of extract and raffinate streams with changes in Q_4 flow rate (Q_{feed}=0.2 mL/min, Q_{Raf}=0.5 mL/min, t^*=9.9 min, and feed concentration=0.5 mg/mL).
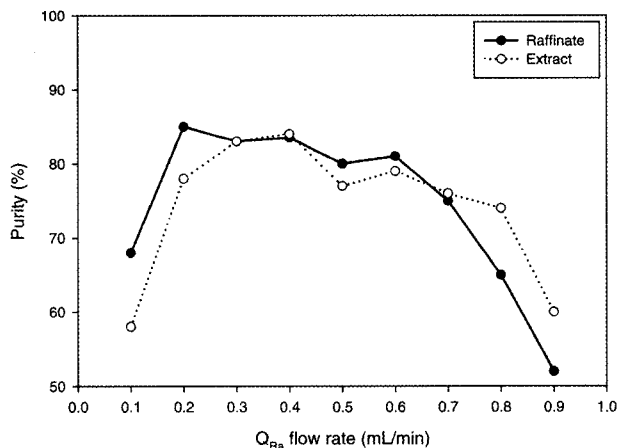

Fig. 2. Purity change of extract and raffinate streams with changes in raffinate flow rate (Q_{feed}=0.2 mL/min, Q_4=4.4 mL/min, t^*=9.9 min, and feed concentration=0.5 mg/mL).

Fig. 3. Operation points movement with changes in raffinate flow rate in the m_2-m_3 plane.

Fig. 4. Purity change of raffinate and extract streams with changes in feed flow rate (Q_4=4.4 mL/min, Q_{Raf}=0.2 mL/min, t^*=9.9 min, and feed concentration=0.5 mg/mL).

there is a criterion on the purity of extract and raffinate, related with the position in the triangle diagram. In the right upper side, there is no pure raffinate but pure extract, and opposite phenomena can be occurred in the left lower side. When the Q_{raf} is low, experimental condition located in the left lower side in Fig. 3. The purity of raffinate is higher than that of extract in this case. Subsequently, the flow rate of raffinate being increased, experimental conditions are shifted to the right diagonal direction in Fig. 3. In these cases, purities of raffinate and extract increase together up to 80% as shown in Fig. 2. When the Q_{raf} exceeds 0.7 mL/min, the purity of raffinate decreases more rapidly than that of extract. Therefore, the necessary condition to get high purities of raffinate and extract is the location of operation points inside the triangle of m_2-m_3 plane [7].

The purity curves of raffinate and extract decline with the increase feed flow rate from 0.1 to 0.5 mL/min (Fig.
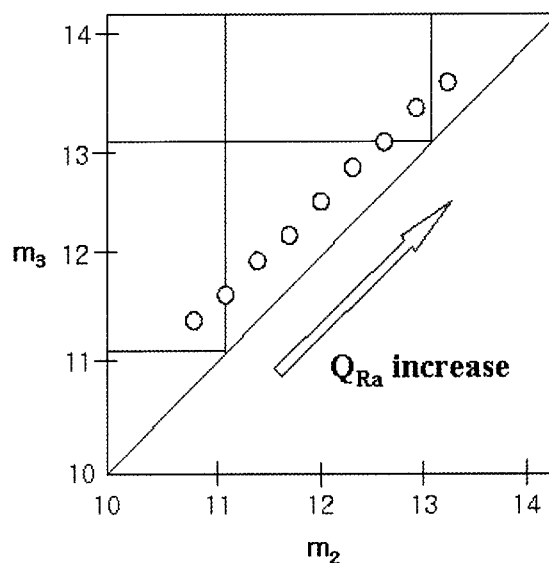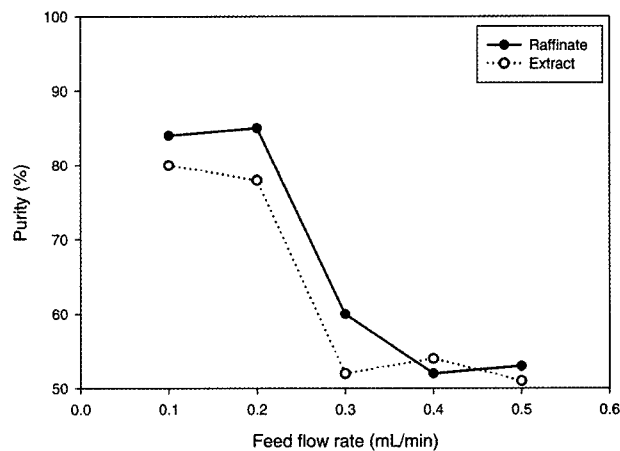
4). In this case, the other flow rates were set as Q_4=4.4 mL/min, Q_{raf}=0.2 mL/min with t^*=9.9 min. And the corresponding operation points of Q_{feed}=0.1, 0.2, 0.3, 0.4 and 0.5 mL/min plotted in Fig. 4 are represented as five points in Fig. 5. When the flow rate feed exceeds 0.3 mL/min, the purities of both extract and raffinate decrease steeply. The two points plotted on the right side in Fig. 5 are located in the proper region to yield high purity. Since the left three points in the m_2-m_3 diagram are in the inadequate region, the purity of raffinate becomes lower.

Feed concentration is an important factor in SMB process development since higher feed amount under allowable solubility in eluant promises higher productivity. But, solubility of chiral compound is so low generally that the search of good eluant is one of important procedure in
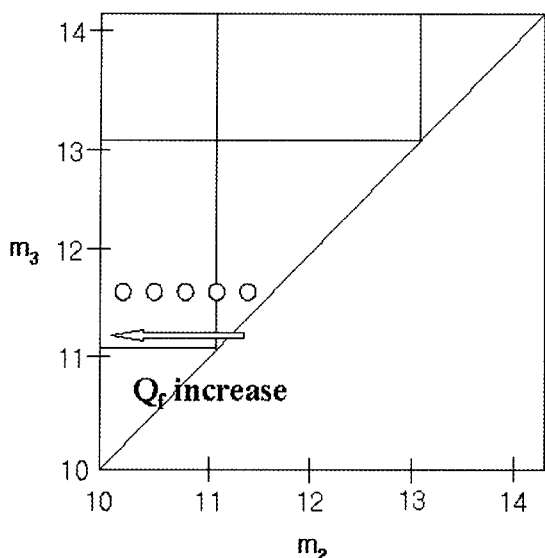
Fig. 5. Operation points movement with changes in feed flow rate in the m_2-m_3 plane.
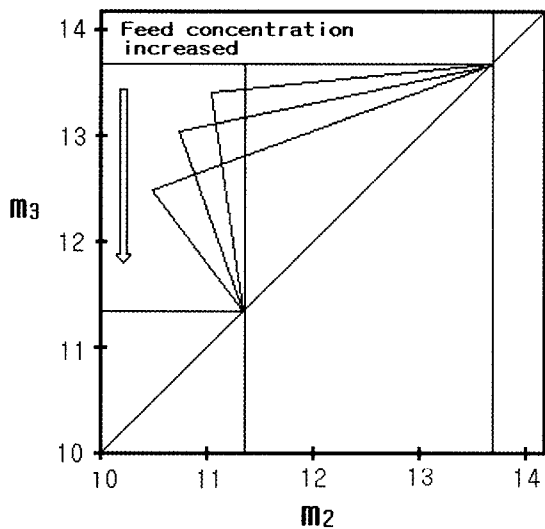

Fig. 6. The shape of triangle diagram with the increase in feed concentration.

SMB researches as well as the selection of chiral stationary phase suitable to the eluant [8]. Moreover, adsorption isotherm equation should be modified in case of nonlinear system due to a high concentration of feed. A distorted shape of triangle (Fig. 6) comes from the high feed concentration. The distorted triangle has a narrow internal area so that high purity region is not easily accessible [9]. The purity of raffinate decreases with the increase of feed concentration as expected (Fig. 7). As the feed concentration becomes over 1 g/L, the adsorption isotherm seems to be in the range of nonlinear region and the triangle region has a narrow separation region as shown in Fig. 6.
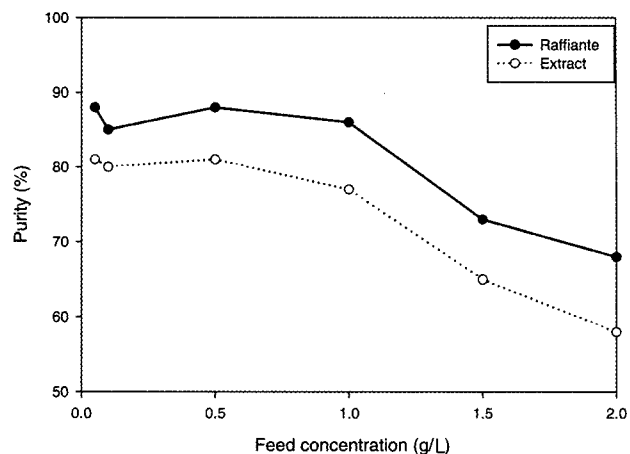

Fig. 7. Purity change of raffinate and extract streams with variation in feed concentration (Q_4=4.4 mL/min, Q_{Ra}=0.2 mL/min, Q_{feed}=0.2 mL/min, t^*=9.9 min).

CONCLUSION

SMB experiments for separation of S-ketoprofen enantiomer from ketoprofen racemate were performed systematically to determine the adequate flow rates of SMB. Operation parameters of the flow rate of section 4 (Q_4), the raffinate flow rate (Q_{raf}), and the feed flow rate (Q_{feed}) under a fixed switching time t^* (=9.9 min) were changed within the range of 3~5 mL/min, 0.1~0.9 mL/min, and 0.1~0.5 mL/min, respectively. In these cases, purity variations were observed from 53% to 78% for the Q_4 change and from 52% to 87% for the Q_{raf} and Q_{feed} changes. The purity variations can be explained by the location of operation points in the m_2-m_3 plane. Because the maximum purity attained as 87% is still low, we need further to perform optimization researches by theoretical and experimental works.

REFERENCES

[1] Juza, M., M. Mazzotti, and M. Morbidelli (2000) Simulated moving-bed chromatography and its application to chirotechnology. *TIBTECH* 18: 108-118.

[2] Yoon, T. H., B. H. Chung, and I. H. Kim (2004) A novel design of simulated moving bed chromatography for separation of ketoprofen enantiomer. *Biotechnol. Bioprocess Eng.* 9: 285-291.

[3] Xie, Y., Y.-M. Koo, and N.-H. L. Wang (2001) Preparative chromatographic separation: Simulated moving bed and modified chromatography methods. *Biotechnol. Bioprocess Eng.* 6: 363-375.

[4] Yang, Y. J., C. H. Lee, and Y.-M. Koo (2004) Separation of amino acids by simulated moving bed using competitive

Langmuir isotherm. *Biotechnol. Bioprocess Eng.* 9: 331-338.

[5] Kaspereit, M., P. Jandera, M. Skavrada, and A. Seidel-Morgenstern (2002) Impact of adsorption isotherm parameters on the performance of enantioseparation using simulated moving bed chromatography. *J. Chromatogr. A* 944: 249-262.

[6] Mihlbachler, K., J. Fricke, T. Yun, A. Seidel-Morgenstern, H. Schmidt-Traub, and G. Guiochon (2001) Effect of the homogeneity of the column set on the performance of a simulated moving bed unit. I. Theory. *J. Chromatogr. A* 908: 49-70.

[7] Leao, C. P. and A. E. Rodrigues (2004) Transient and steady-state models for simulated moving bed processes: Numerical solutions. *Comp. Chem. Eng.* 28: 1725-1741.

[8] Antos, D. and A. Seidel-Morgenstein (2002) Two-step solvent gradients in simulated moving bed chromatography: Numerical study for linear equilibria. *J. Chromatogr. A* 944: 77-91.

[9] Houwing, J., H. A. H. Billiet, and L. A. M. van der Wielen (2002) Optimization of azeotropic protein separations in gradient and isocratic ion-exchange simulated moving bed chromatography. *J. Chromatogr. A* 944: 189-201.