Parameter Estimation of Perillyl Alcohol in RP-HPLC by Moment Analysis

Kyung Ho Row*, Chong Ho Lee, and Ji Hoon Kang

Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, Incheon 402-751, Korea

Abstract Parameter estimations were made for the reversed-phase adsorption of perillyl alcohol (POH), a potent anti-cancer agent, on octadecylsilyl-silica gel (ODS). The average particle diameter of ODS was about 15 μm, and the particles were packed in the column (3.9 $\times$ 300 mm). The mobile phase used was a mixture of acetonitrile and water, in which the acetonitrile ranged between 50 and 70 (v/v %). The first absolute moment and the second central moment were determined from the chromatographic elution curves by moment analysis. Experiments were carried out using POH solutions within the linear adsorption range. The fluid-to-particle mass transfer coefficient was estimated using the Wilson-Geankoplis equation. The axial dispersion coefficient and the intraparticle diffusivity were determined from the slope and intercept of a plot of H vs $1/u_0$, respectively. The contributions of each mass-transfer step were axial dispersion, fluid-to-particle mass transfer, and intraparticle diffusion.

Keywords : moment analysis, parameter estimation, perillyl alcohol

INTRODUCTION

The study of the mass transfer rate is important in understanding and designing large-scale processes such as chromatography. The diffusion resistance in adsorbent particles seems to have a significant effect on the overall adsorption rate, and consequently on the efficiency of a chromatographic separation. Intraparticle diffusion has been studied in various porous adsorbents by several methods such as batch adsorption [1,2], chromatographic techniques [3-6], and steady-state experiments [7,8].

In order to simulate chromatographic separation processes in a column and to make a rational design of chromatographic systems for the separation of mixtures, information on the adsorption rate of each component is essential. All mass-transfer steps in a column contribute to peak broadening and column efficiency in chromatographic separations. Axial dispersion and fluid-to-particle mass-transfer phenomena have been studied in detail in the field of chemical engineering.

However, comparatively little work has been carried out on the diffusion phenomena in reversed-phase packing materials, in contrast to the extensive studies concerning retention behaviors. Detailed analyses of adsorption and mass-transfer phenomena on the surface of packing materials are essential to construct an accurate simulation models of chromatographic separations. It has already been clarified that intraparticle diffusion

* **Corresponding author**
Tel: +82-32-860-7470   Fax: +82-32-872-0959
e-mail: rowkho@inha.ac.kr

contributes to overall mass-transfer resistance in ODS particles [9-11]. Accordingly, kinetic studies on the surface diffusion phenomena in reversed-phase packing materials will surely provide significant information about the separation mechanisms of reversed-phase chromatography.

The parameters estimated in the current study were : the intraparticle diffusion coefficient, axial dispersion coefficient, adsorption equilibrium constant, molecular diffusivity, and the fluid-to-particle mass transfer coefficient. This study of these parameters provided significant information about the separation mechanisms of reversed-phase chromatography. An accurate estimation of these parameters wis also required to predict chromatographic profiles.

THEORY

The chromatographic peaks leaving from the column were analyzed using the method of moments [9,12,13]. The adsorption equilibrium constant was determined from the first moment analysis, and the second moment analysis provided information on all the mass-transfer steps involved in the adsorption processes. The intraparticle diffusion coefficient was determined by subtracting the effect of the axial dispersion and fluid-to-particle mass transfer. The first absolute moment and the second central moment of a chromatographic peak were expressed as follows :

$$\mu_1 = \frac{\int C e(t) t \, dt}{\int C e(t) \, dt} = \frac{z}{u_0} \delta_0 \tag{1}$$

Biotechnol. Bioprocess Eng. 2002, Vol. 7, No. 1

17

$$\mu_2' = \frac{\int Ce(t)(t-\mu_1)2dt}{\int Ce(t)dt} = \frac{2z}{u_0}(\delta_{ax} + \delta_f + \delta_d) \tag{2}$$

where

$$\delta_0 = \varepsilon + (1-\varepsilon)(\varepsilon_P - \rho_P K) \tag{3}$$

$$\delta_{ax} = \frac{Ez}{u_0^2}\delta_0^2 \tag{4}$$

$$\delta_f = (1-\varepsilon)(R/3k_f)(\varepsilon_p + \rho_p K)^2 \tag{5}$$

$$\delta_d = (1-\varepsilon)(R^2/15De)(\varepsilon_p + \rho_p K)^2 \tag{6}$$

The first moment was analyzed by Eq. (7) derived from Eq. (1) in the following way.

$$(\mu_1 - t_0)/(1-\varepsilon) = (z/u_0)\rho_p K \tag{7}$$

where

$$t_0 = (z/t_0)[\varepsilon + (1-\varepsilon)\varepsilon_p] \tag{8}$$

According to Eq. (7), a linear relationship should be observed between $(\mu_1 - t_0)/(1 - \varepsilon)$ and (z/u_0). The adsorption equilibrium constant, K, was calculated from the slope of the plot. To analyze the second moment, the parameter H was calculated as follows:

$$H = (\mu_2'/\mu_1^2)(z/2u_0) = (Ez/u_0^2) + H_0 \tag{9}$$

$$H_0 = \delta_d/\delta_0^2 \tag{10}$$

The value of δ_f was calculated from Eq. (5), and its contribution to the second moment was corrected. The fluid-to particle mass-transfer coefficient, k_f, was estimated using the equation of Wilson-Geankoplis for liquid-phase adsorption.

$$Sh = (1.09/\varepsilon)Sc^{1/3}Re_p^{1/3} \tag{11}$$

where

$$Sh = \frac{2k_fRe_p}{D_m} \tag{12}$$

$$Sc = \frac{\eta}{\rho_p D_m} \tag{13}$$

$$Re_p = \frac{2u_0 r_p \rho_p}{\eta} \tag{14}$$

The molecular diffusivity, D_m, of an adsorbate in the mobile phase was estimated by the Wilke-Chang equation.

$$D_m = \frac{7.4 \times 10^{-8}(\alpha_2 M_2)^{1/2}T}{\eta_2 V_1^{0.6}} \tag{15}$$

The contribution of the adsorption rate at an adsorption site to the second moment was assumed to be negligible. The plot of H vs $1/u_0$ provided the axial dispersion coefficient, Ez, and the intraparticle diffusivity, D_e,

from the slope and intercept, respectively.

MATERIALS AND METHODS

A high performance liquid chromatographic equipment (Waters 600S) was employed. A small volume of the sample solutions was introduced into the fluid flow using a sample injector. The stainless steel column (3.9 × 300 mm) was packed with 15 μm C_{18} packing (Lichrosphere, Merck). The chromatographic peak of POH in the effluents was monitored using an ultraviolet detector at 205 nm. The pulse response experiments were performed under various chromatographic conditions. The concentration of perillyl alcohol (POH) was varied within a range of 0.1 to 10 mg/mL and the injection volume was 3 μL. The flow rate of the mobile phase was varied within a range of 0.6 mL/min to 1.4 mL/min. Acetonitrile/water mixtures ranging in the composition of acetonitrile from 50 to 70 (v/v %) were used as the mobile phase.

RESULTS AND DISCUSSION

Parameter estimations were made for the reversed-phase adsorption of perillyl alcohol (POH), a potent anti-cancer agent, on octadecylsilyl-silica gel (ODS). The average particle diameter of ODS was about 15 μm and the particles were packed in the column (3.9 × 300 mm). The first absolute moment and second central moment were determined from chromatographic elution curves by moment analysis.

Fig. 1 shows the plot of $(\mu_1 - t_0) / (1 - \varepsilon)$ vs. z/u_0 for POH at various concentrations. The POH concentration was were 0.1, 1, and 10 mg/mL, and the flow rates of the mobile phase were 0.6, 0.8, 1.0, 1.2, and 1.4 mL/min. Acetonitrile/water (70/30, v/v %) was used as the mobile phase. The adsorption equilibrium constant (K) was calculated from the slopes of the linear plots at the various concentrations of POH. Based on the current experimental range of POH concentration, the adsorption equilibrium remained was almost constant. This means that the experiments were carried out with POH solutions within the linear adsorption range. The adsorption equilibrium constant was 1.678 with only a slight deviation of 0.05. Therefore, the range of POH concentration used did not affect the retention factor.

In Fig. 2, the mobile phase of acetonitrile/water mixtures ranged in its composition of acetonitrile from 50 to 70 (v/v %). Straight lines of different slopes through the origin were observed. The slope increased with an increase in the water content of the mobile phase. The adsorption of POH on ODS increased with decreasing acetonitrile content. As the acetonitrile content increased in the mobile phase, the retention factor decreased.

The second moment analysis provided information on all the mass-transfer steps involved in the adsorption processes. The axial dispersion coefficient consisted of
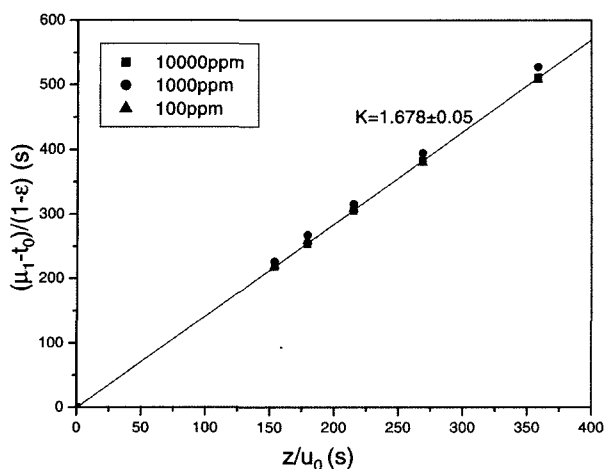
18

Biotechnol. Bioprocess Eng. 2002, Vol. 7, No. 1

Fig. 1. First moment plots of POH at various concentrations (ACN/Water = 70/30 v/v %).
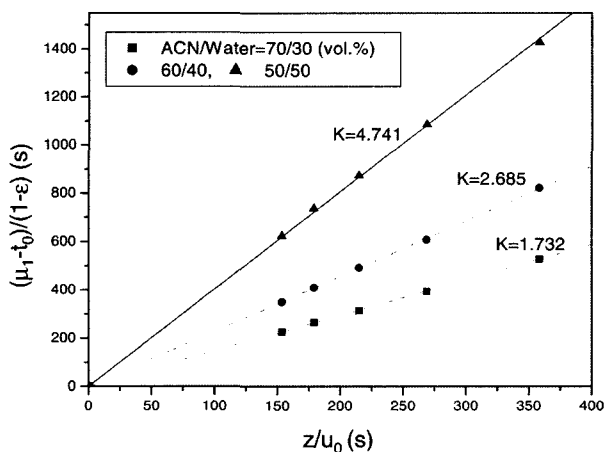

Fig. 2. First moment plots of POH with various mobile phases (1,000 ppm of POH).

two terms: the first was the molecular diffusion, and the second was the fluid dispersion [14,15].

$$Ez = \gamma_1 D_m + \gamma_2 (2R_p) u_0 \tag{16}$$

where γ_1 and γ_2 are constants that normally have values of about 0.7 and 0.5, respectively. The molecular diffusion ($\gamma_1 D_m$) of a liquid is negligible compared to eddy diffusion or fluid dispersion, even at low Reynolds numbers [14]. In the following Eq. (17), the eddy diffusivity is the dominant term in liquid chromatography, especially when the flow velocity is not low, thus $Ez \propto u_0$. The importance of this relationship has been acknowledged by certain researchers [16]. A simpler, but perhaps rougher estimation, can be obtained using

$$Ez = \gamma_2 (2R_p) u_0 \tag{17}$$

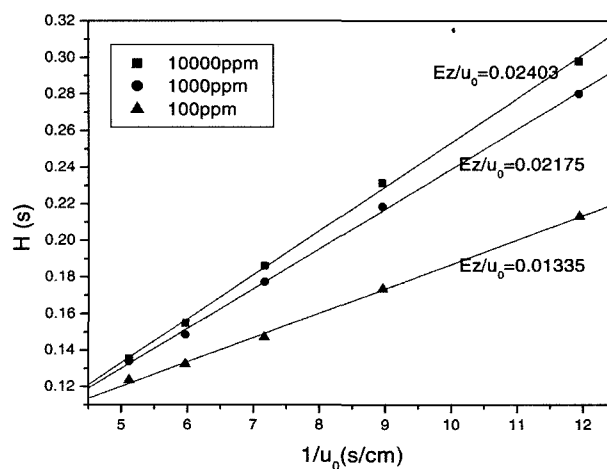Thus, $\quad Pe = \dfrac{u_0 L}{Ez} = \dfrac{L}{2\gamma_2 R_p} \tag{18}$

Fig. 3. Second moment plots of POH at various cocentrations (ACN/Water=70/30 v/v %).

In the case of particle, the axial dispersion coefficient can be expressed as :

$$Ez = u_0 d_p / Pe \tag{19}$$

Under such a condition, a linear relation between H and $1/u_0$ is derived from Eq. (9). In Fig. 3, H is plotted against $1/u_0$ for each concentration of POH. Similarly, Fig. 4 shows the plot of H and $1/u_0$ at various mobile phase compositions. The values of Ez/u_0 and the pore diffusivity can be determined from the slope and intercept of the plot, respectively. The value of Ez/u_0 increased with increased POH concentration and content of acetonitrile in the mobile phase composition. The Peclet number decreased with an increase in the Ez/u_0 value. This number reflects the ratio of the convection rate to the dispersion rate. An increase in the Pe value, while other parameters are fixed, sharpens the concentration profiles in the effluent [15]. The mass transfer effects tend to diffuse the concentration profiles. The parameter values estimated in this work are listed in Tables 1 and 2.

CONCLUSION

The parameters of POH were studied by moment analysis, and the effect of the POH concentration and mobile phase composition on there parameters was also studied. The first and second moments of the POH chromatographic data were analyzed. The molecular diffusivity, D_m, was estimated using the Wilke-Chang equation and the external film mass transfer coefficient, k_f, was estimated by the Wilson-Geankoplis equation. The adsorption equilibrium constant was found to be 1.732, 2.68, and 4.74, when the mobile phase composition was 70/30, 60/40, and 50/50 (ACN/Water, v/v %), respectively. The effect of increasing the POH concentration and the content of acetonitrile in the mobile
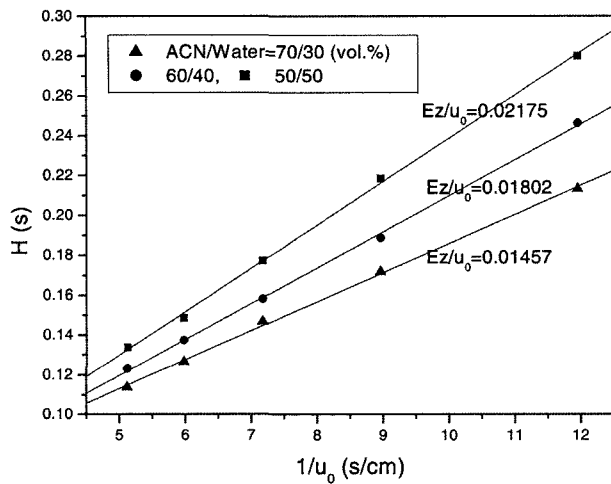
Fig. 4. Second moment plots of POH with various mobile phases (1,000 ppm of POH).

Table 1. Parameters estimated by moment analysis

Parameter \ % ACN	70	60	50
K [-]	1.732	2.685	4.741
$Ez_{0.6}$ [cm^2/s]	1.82×10^{-3}	1.51×10^{-3}	1.22×10^{-3}
$Ez_{0.8}$ [cm^2/s]	2.43×10^{-3}	2.01×10^{-3}	1.63×10^{-3}
$Ez_{1.0}$ [cm^2/s]	3.03×10^{-3}	2.51×10^{-3}	2.03×10^{-3}
$Ez_{1.2}$ [cm^2/s]	3.64×10^{-3}	3.02×10^{-3}	2.44×10^{-3}
$Ez_{1.4}$ [cm^2/s]	4.25×10^{-3}	3.52×10^{-3}	2.85×10^{-3}
D_e [cm^2/s]	8.23×10^{-9}	2.50×10^{-9}	1.41×10^{-9}

* Subscript describes flow rate of mobile phase [mL/min].

Table 2. Parameters estimated by correlation equations

Parameter \ % ACN	70	60	50
D_m [cm^2/s]	9.70×10^{-6}	7.81×10^{-6}	6.98×10^{-6}
$k_{f0.6}$ [cm/s]	7.75×10^{-2}	6.70×10^{-2}	6.22×10^{-2}
$k_{f0.8}$ [cm/s]	8.53×10^{-3}	7.38×10^{-3}	6.85×10^{-3}
$k_{f1.0}$ [cm/s]	9.19×10^{-3}	7.95×10^{-3}	7.38×10^{-3}
$k_{f1.2}$ [cm/s]	9.76×10^{-3}	8.45×10^{-3}	7.84×10^{-3}
$k_{f1.4}$ [cm/s]	10.28×10^{-3}	8.89×10^{-3}	8.25×10^{-3}

* Subscript describes flow rate of mobile phase [mL/min].

phase composition resulted in an increase in Ez/u_0 and a decrease in the Peclet number.

NOMENCLATURE

a_2	: association coefficient of solvent
C_e	: concentration in effluent [g/cm^3]
D_e	: intraparticle diffusion coefficient [cm^2/s]
D_m	: molecular diffusivity [cm^2/s]
d_p	: particle diameter [cm]
Ez	: axial dispersion coefficient [cm^2/s]
K	: adsorption equilibrium constant
k_f	: fluid-to-particle mass transfer coefficient [cm/s]
Re_p	: particle Reynolds number
R_p	: mean pore radius [cm]
r_p	: particle radius [cm]
Sc	: Schmidt number
Sh	: Sherwood number
t_0	: retention time of unretained component [min]
u_0	: superficial velocity [cm/s]

Greek Symbols

ε	: void fraction of a chromatographic column
ε_p	: porosity
η	: viscosity [Pa·s]
μ_1	: first absolute moment
μ_2	: second central moment
ρ_p	: particle density [g/cm^3]

REFERENCES

[1] Suzuki, M. and K. Kawazoe (1975) Effective surface diffusion coefficients of volatile organics on activated carbon during adsorption from aqueous solution. *J. Chem. Eng. Jpn.* 8: 379.

[2] Suzuki, M., T. Kawai, and K. Kawazoe (1976) Adsorption of poly(oxyethylene) of various molecular weights from aqueous solutions on activated carbon. *J. Chem. Eng. Jpn.* 9: 203-208.

[3] Schneider, P. and J. M. Smith (1968) Chromatographic study of surface diffusion. *AIChE J.* 14: 886-895.

[4] Kawazoe, K., M. Suzuki, and K. Chihara (1974) Chromatographic study of diffusion in molecular-sieving carbon. *J. Chem. Eng. Jpn.* 7: 151-157.

[5] Chihara, K., M. Suzuki, and K. Kawazoe (1978) Adsorption rate on molecular sieving carbon by chromatography. *AIChE J.* 24: 237.

[6] Awum, F., S. Narayan, and D. Ruthven (1988) Measurment of intracrystalline diffusivities in nax zeolite by liquid chromatography. *Ind. Eng. Chem. Res.* 27: 1510-1515.

[7] Suzuki, M. and T. Fujii (1982) Concetration dependence of surface diffusion coefficient of propionic acid in activated carbon particles. *AIChE J.* 28: 380-385.

[8] Takeuchi, Y., E. Furuya, and H. Ikeda (1984) On the concentration dependency of intraparticle effective diffusivity for tetrahydrofuran-activated carbon system. *J. Chem. Eng. Jpn.* 17: 304-309.

[9] Miyabe, K. and M. Suzuki (1992) Chromatography of liquid-phase adsorption on octadecylsily-silica gel. *AIChE J.* 38: 901.

[10] Miyabe, K. and M. Suzuki (1993) Adsorption characteristics of octadecylsilyl-silica gel in gaseous system. *AIChE J.* 39: 1791-1798.

[11] Suzuki, M. (1973) Notes on determining the moments of the impulse response from the basic transformed equations. *J. Chem. Eng. Jpn.* 6: 540.

[12] Miyabe, K. and M. Suzuki (1994) Mass-transfer phenomena on the surface of adsorbent in reversed-phase chromatography. *Ind. Eng. Chem. Res.* 33: 1792-1802.

[13] Miyabe, K. and M. Suzuki (1995) Chromatographic study on liquid-phase adsorption on octadecylsilyl-silica gel. *AIChE J.* 41: 548-558.

[14] Ruthven, D. M. (1984) *Principles of Adsorption and Adsorption Processes*, Wiley, New York, USA.

[15] Jonsson, J. A. (1987) *Chromatographic Theory and Basic Principles*, Marcel Dekker, New York, USA.

[16] Weber, S. G. and P. W. Carr (1989) *High Performance Liquid Chromatography*, P. R. Brown and T. A. Hartwick, (eds.), Wiley, New York, USA.