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Adsorption Isotherms of Amino Acids and Kinetic Analysis of Ion-Exchange Chromatographs by the Moment Method

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Abstract

Adsorption isotherms of three amino acids (aspartic acid, glutamic acid, and glycine) were measured in a single solute or multisolutes by the batchwise method. Kinetic parameters were determined in a column packed with ion-exchange resin by the moment method. The equilibrium constants obtained by both methods were in good agreement. Experimental elution curves agreed well with calculated one.

INTRODUCTION

Both anion- and cation-exchange resins have been widely used for preparative chromatographic separations of amino acids which are amphoteric molecules.

Gosling et al. (*1*) used an anion-exchange resin and two components of amino acids (aspartic acid and tryptophan), and emphasized the role of adsorption isotherms in ion-exchange chromatography. Saunders et al. (*2*) used a cation-exchange resin and two components of amino acids (phenylalanine and tyrosine), and measured the equilibrium uptake. Also, they showed that intraparticle transport was dominated by the slow diffusion of amino acid cations through the resin.

Usual ion-exchange chromatographs (including those in the two papers cited above) consist of several different operations such as adsorption, washing, elution, and regeneration. However, if a strong acid cation-exchange resin in the sodium form is used, only elution is required because amino acids can be separated by different interactions between the resin and amino acids. This operation is similar to a conventional chromatograph.

In this paper a strong acid cation-exchange resin of the gel type, Dowex 50W-X8 in the sodium form, is used to separate three components of amino acids (aspartic acid, glutamic acid, and glycine). Adsorption isotherms were measured and then the kinetic parameters were determined by the moment method. In previous papers (*3*, *4*) this method was successfully applied to ion retardation and ion exchange processes. This work was mainly performed to obtain the values of kinetic parameters required in a companion paper (*5*).

EXPERIMENTAL

Chromatographic peaks for pulse injection of an aqueous solution containing three kinds of amino acids, that is, aspartic acid (Asp), glutamic acid (Glu), and glycine (Gly), were measured in a packed bed at 303 K. The column used in this work consisted of a glass tube of 6 mm i.d. and 43 cm height. Sodium citrate buffer solution was used as the eluent. The pH of the buffer solution was adjusted at pH 3.4. The resin used was a strong acid cation-exchange resin, Dowex 50W-X8. It was sieved at the portion of 0.14 mm average diameter. The resin was conditioned in the usual manner and finally changed to the sodium form. The density of the swollen resin was 1210 kg/m^3. Concentrations of amino acids were measured by UV absorbance at 570 nm by the ninhydrin method.

To obtain adsorption isotherms, test tubes which contained swollen resins (about 1 to 2 g) and amino acid solution (pH was adjusted at 3.4 by sodium citrate buffer) were immersed in the thermostat of 303 K for about 60 h. The equilibrium uptake was calculated from the difference between the initial and final concentrations of amino acid in the solution.

To investigate the effect of the presence of other amino acids on the adsorption isotherm, uptakes for binary mixtures as well as a single component of amino acid were measured.

RESULTS

Adsorption Isotherms

Adsorption isotherms of three kinds of amino acids in the sodium citrate buffer solution of pH 3.4 on the ion-exchange resin at 303 K are shown in Fig. 1 for both a single component and binary mixtures. Since the data were on a straight line through the origin, the adsorption isotherms might be linear. Then the following equation holds:

$$\overline{C} = KC \tag{1}$$

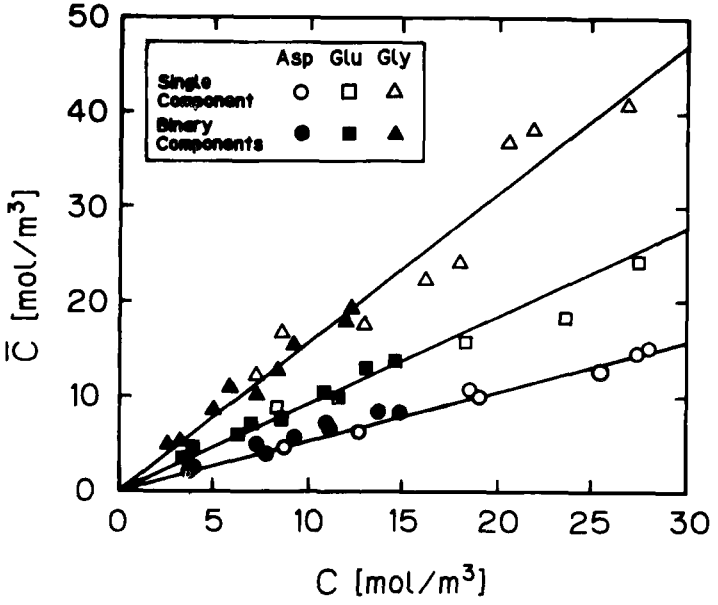

FIG. 1. Adsorption isotherm for three kinds of amino acids in solution of a single or binary mixtures components at 303 K and pH 3.4.

The adsorption equilibrium constant, K, could be determined from the slope.

Since the isotherms of binary mixtures are the same as those of a single component, there are little interactions between the amino acids at equilibrium. For an anion-exchange resin in the hydroxide form (*1*), the aspartic acid isotherm was independent of tryptophan but the equilibrium uptake of tryptophan decreased as the aspartic acid concentration increased. This may be due to hydrophobic interactions, since tryptophan is one of the more hydrophobic amino acids.

First Moment Analysis

Since the isotherm is linear, the moment method can be employed to analyze the chromatogram obtained experimentally by the same procedure as in previous papers (*3*, *4*).

The first absolute moment can be expressed as

$$\mu_1' = \frac{z\varepsilon_B}{u}\left(1 + \frac{1 - \varepsilon_B}{\varepsilon_B} K\right) + \frac{t_0}{2} \qquad (2)$$

where the value of μ_1' can be obtained from the experimental chromatograph as

$$\mu_1' = \int_0^\infty tC(t)dt / \int_0^\infty C(t)dt \tag{3}$$

Figure 2 shows plots of $\mu_1' - t_0/2$ versus $1/u$ for amino acids. The volume of pulse injection was in the range of 0.1 to 0.3 $\times$ 10^{-6} m^3. The concentration of each amino acid in the injection was fixed at 20 mol/m^3. Since the value of t_0 is about 30 s, it is negligible. The void fraction of the bed and the adsorption equilibrium consant for amino acids were determined from Eq. (2). To obtain the value of the void fraction of the bed, an aqueous solution of sodium chloride was used. Since sodium chloride is excluded by the Donnan effect from the resin of the sodium form, the value of K is almost zero. Equation (2) may be simplified as

$$(\mu_1')_{NaCl} - t_0/2 = z\varepsilon_B/u \tag{4}$$

The void fraction of the bed, ε_B, was determined to be 0.394 from sodium chloride data. The data of amino acids in Fig. 2 fell on straight lines through

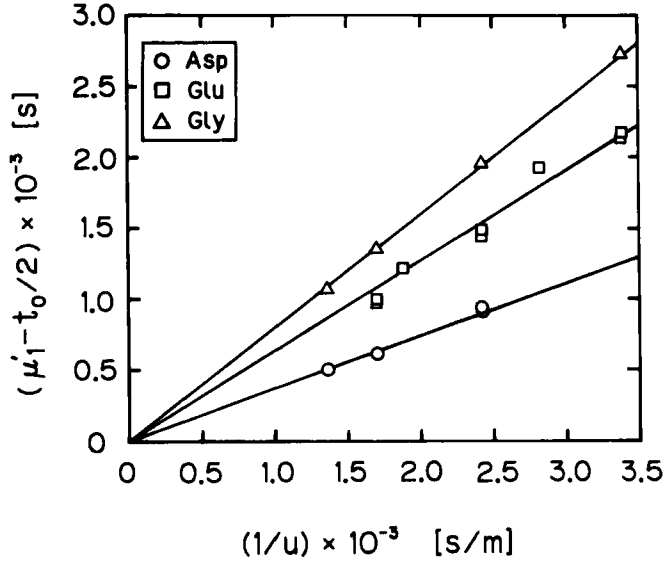

FIG. 2. First absolute moment plots.

the origin. The slope of the line in Fig. 2 gave the adsorption equilibrium constant, K, for amino acids as summarized in Table 1. These value are almost equal to those from the batchwise method of adsorption isotherm measurements.

Second Moment Analysis

The second moment, expressed in Eq. (2) of the previous paper (*4*), consists of separate and additive terms of kinetic constants based on axial dispersion, intraparticle diffusion, and liquid film mass transfer.

The linear regression was compared with the nonlinear optimization method for the analysis of moment (*4*). In this work, the linear regression will be used because the difference between the two methods may not be significant.

Figure 3 shows the plots of $(\mu_2 - t_0^2/12)/2z\varepsilon_B$ versus $1/u$. It is evident that there exist linear relations. For the estimation of liquid film mass transfer coefficient, molecular diffusivities were calculated from the Wilke-Chang equation (*6*). The estimated values of D_m are presented in Table 1. The values of k_f at the flow rate $u = 1.0 \times 10^{-4}$ m/s were calculated from the Wakao's equation (*7*) and are shown in Table 1. The effect of flow rate on the value of k_f was less than 20% in experimental conditions.

The slope and intercept of the lines in Fig. 3 were calculated by the least-square method. The values of Pe in Table 1 are expressed as

$$\mathrm{Pe} = 2uR/\varepsilon_B D_L \tag{5}$$

The intraparticle diffusivities were calculated from the intercept by using k_f at the flow rate $u = 1.0 \times 10^{-4}$ m/s. Saunder et al. (*2*) measured both

TABLE 1

The Value of Parameters Determined at 303 K

	Aspartic acid	Glutamic acid	Glycine
	Batchwise Method		
K (−)	0.525	1.02	1.57
	Moment Method		
K (−)	0.541	1.14	1.60
$D_m \times 10^{10}$ (m²/s)	8.4	7.8	10.7
$k_f \times 10^5$ (m/s)	2.07	1.94	2.54
$\bar{D} \times 10^{11}$ (m²/s)	4.93	1.48	3.95
Pe (−)	0.0293	0.0686	0.181

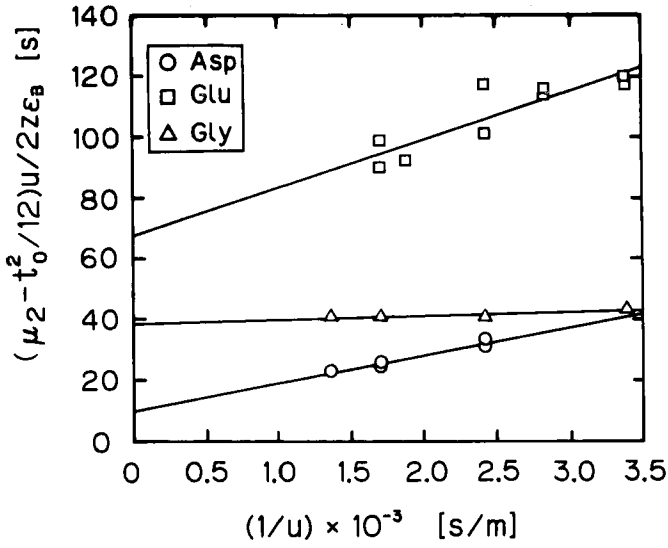

FIG. 3. Second central moment plots.

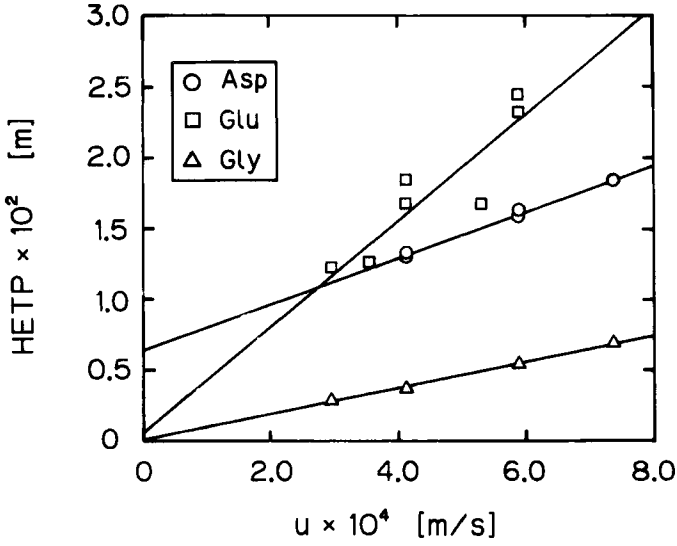

FIG. 4. Dependence of HETP on flow rate.

macropore and microparticle effective diffusivities for phenylalanine and tyrosine by using a cation-exchange resin of the macroporous type. For phenylalanine, the value of macropore effective diffusivity was 2.5×10^{-10} m^2/s and that of microparticle effective diffusivity was 1.4×10^{-12} m^2/s. For tyrosine they were 2.3×10^{-10} and 1.8×10^{-12} m^2/s, respectively. In this work the resin is of the gel type and intraparticle effective diffusivities, $\overline{D}$, were in the middle of macropore and microparticle effective diffusivities of Saunder et al. (*2*).

DISCUSSION

The column efficiency may be evaluated by the height equivalent to theoretical plate (HETP) in plate theory and expressed as

$$\text{HETP} = \mu_2 z/(\mu_1')^2 \tag{6}$$

The values of HETP were calculated from the experimental data. Figure 4 shows the experimentally obtained HETP together with HETP calculated

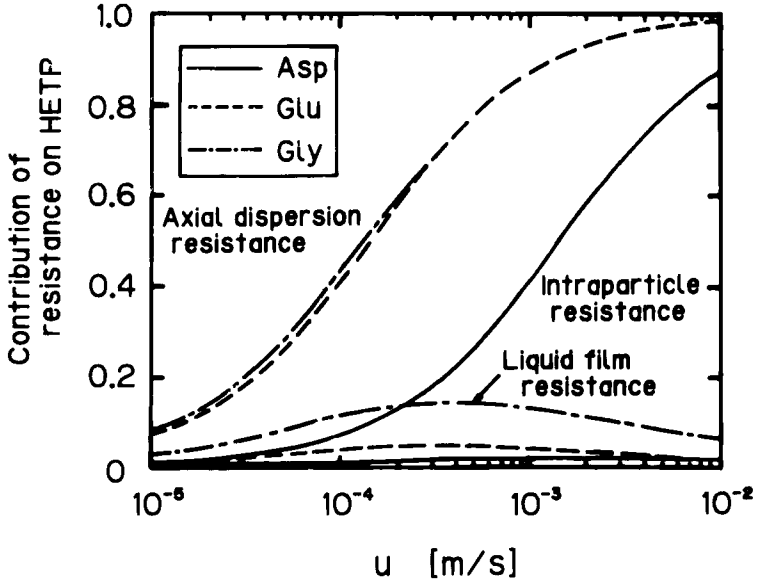

FIG. 5. Effect of flow rate on relative contribution of each resistance to HETP for amino acids.

by Eq. (6) in a previous paper (*4*) by using the kinetic parameters determined in this work. The values of HETP increased with an increase in the flow rate for all amino acids.

To evaluate the relative importance of the three mass transfer resistances, the contribution of each resistance to HETP was calculated. Figure 5 shows the effect of the flow rate on the relative importance of the respective resistances for the amino acids. The resistance of liquid film mass transfer is not significant. Although the resistance of liquid film mass transfer of glycine is the largest of the three amino acids, the maximum is about 15%.

The contribution of axial dispersion decreased as the liquid velocity increased. The resistance of intraparticle diffusion as aspartic acid was less than that of the other amino acids. For aspartic acid, the resistance of axial dispersion was most important. For glutamic acid and glycine, the resistances of intraparticle diffusion and axial dispersion were comparative in their experimental flow rates.

Typical elution curves for the three amino acids are shown in Fig. 6. The solid line indicates one of the experimental results and the broken line indicates the theoretical curve, which was calculated by using the analytical

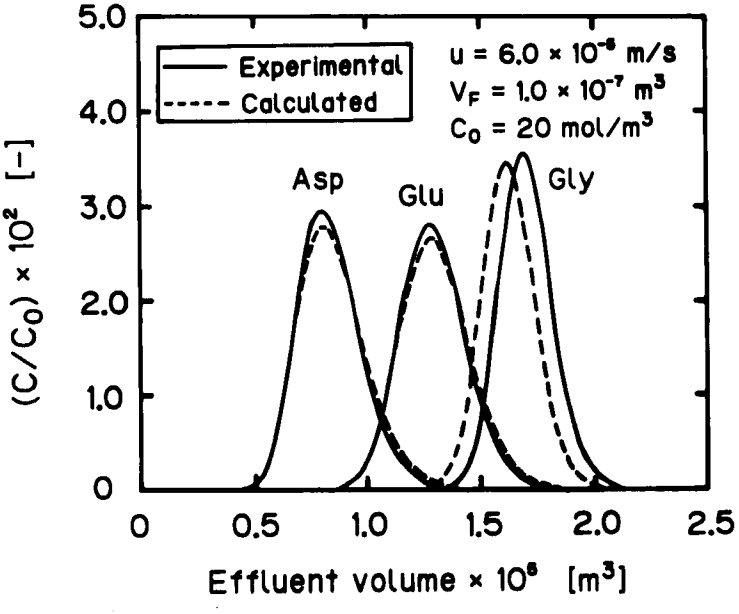

FIG. 6. Comparison of elution curves for amino acids.

equation solved by Rasmuson et al. (*8*) with the kinetic parameters determined by the moment method. The experimental curve is in good agreement with the calculated one, although the calculated curve for glycine slightly precedes the experimental one. The order of elution of the amino acids may be followed by the magnitude of adsorption of the equilibrium constant, K.

CONCLUSION

Adsorption isotherms of three amino acids (aspartic acid, glutamic acid, and glycine) on Dowex 50W-X8 in the sodium form were linear in a buffer solution of pH 3.4. Therefore, moment analysis of the chromatograph elution curves could be applied. The experimental elution curves of the three amino acids agreed well with their theoretical curves when using parameters determined from experimental data.

SYMBOLS

C	concentration of amino acid in the liquid (mol/m^3)
$\overline{C}$	concentration of amino acid in the resin phase based on the volume of swollen resin (mol/m^3)
$\overline{D}$	intraparticle diffusivity (m^2/s)
D_L	axial dispersion coefficient (m^2/s)
D_m	molecular diffusivity (m^2/s)
K	adsorption equilibrium constant (−)
k_f	liquid film mass transfer coefficient (m/s)
R	radius of the spherical particle (m)
t	time (s)
t_0	injection time (s)
u	superficial velocity (m/s)
V_F	volume of pulse injection (m^3)
z	length of the packed bed (m)

Greek

ε_B	interparticle void fraction in the bed (−)
μ_1'	first absolute moment of the chromatographic curve (s)
μ_2	second central moment of the chromatographic curve (s^2)

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