Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Ching, C. B. , Hidajat, K. and Uddin, M. S.(1989) 'Evaluation of Equilibrium and Kinetic Parameters of Smaller Molecular Size Amino Acids on KX Zeolite Crystals via Liquid Chromatographic Techniques', Separation Science and Technology, 24: 7, 581 — 597
To link to this Article: DOI: 10.1080/01496398908049793
URL: http://dx.doi.org/10.1080/01496398908049793

SEPARATION SCIENCE AND TECHNOLOGY, 24(7 & 8), pp. 581-597, 1989

Evaluation of Equilibrium and Kinetic Parameters of Smaller Molecular Size Amino Acids on KX Zeolite Crystals via Liquid Chromatographic Techniques

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Abstract

Liquid chromatographic techniques have been employed to evaluate the sorption and diffusion characteristics of smaller molecular size amino acids on large zeolite KX-type crystals. The three amino acids investigated in the present study were cysteine, threonine, and serine. All three amino acids adsorbed quite rapidly but there are significant differences in both the equilibrium constants and intracrystalline diffusivities. Combined with the kinetic and equilibrium data of the other three amino acids reported previously, it can be concluded that the larger molecules generally show a lower equilibrium constant. However, no obvious trend can be observed for intracrystalline diffusivity data.

INTRODUCTION

The accurate determination of the number and kind of amino acids present in a sample has long been appreciated to be one of the most important types of analysis in the methodological catalog of biological research.

The analysis is usually accomplished by separating amino acid mixtures using classical ion-exchange chromatography followed by column derivatization with ninhydrin or a fluorogenic reagent. This technique resolves most amino acids with good detection limits, especially if either fluorogene or *o*-phthaldialdehyde is used as the postcolumn derivatizing reagent. In order to improve the efficiency and

to decrease the analysis time for the resolution of amino acid mixtures, several precolumn techniques have been developed which utilize reverse-phase HPLC. These techniques offer greater efficiency, ease of use, and higher flow rates than the conventional ion-exchange techniques. However, the procedure generally requires specialized equipment and long analysis time is required.

In the present work the feasibility of applying the liquid chromatography technique using a column packed with large unaggregated zeolite crystals as a means of separating the smaller common amino acids was investigated. As this method does not require any pre- or post-column derivatization techniques, it should become a rapid technique in the amino acids analysis and separation.

This study is an extension of our work (*1*) on evaluation of the kinetics and equilibria of sorption of four representative amino acids (glycine, lysine, alanine, and phenylalanine) on type X zeolites (K^+ form). Considering the molecular size of amino acids against pore size of the crystal, it suggests that any such applications will be limited only to the lower molecular weight of species. Hence, the present study was performed using cysteine, threonine, and serine as the sorbates.

EXPERIMENTAL

The experimental system is shown schematically in Fig. 1. A Pharmacia liquid-chromatograph column (1.6 cm i.d.) was packed with unaggregated zeolite crystals to a bed height of 12 cm. The eluent (water) was degassed and filtered through a 2-μm filter. Injection was performed with a Rheodyne Type 7125 sampling valve fitted with a 200 μL loop. Detection was by a refractive index detector (Hewlett Packard model 1037a). Signal from the RI detector was handled by a Hewlett Packard model 3497A data acquisition/control unit which performed the logging of the chromatographic response data and computation of equilibrium and kinetic data.

The inlet and outlet lines of the column were made as short as possible in order to reduce the effects of dispersion outside the packed bed and between the injection valve and refractive index detector. The hold-up of these lines was found to be 0.36 cm^3.

The zeolite framework consists of an assemblage of SiO_4 and AlO_4 tetrahedra, joined together in various regular arrangements through shared oxygen atoms, to form an open crystal lattice containing pores of molecular dimensions into which guest molecules can penetrate.

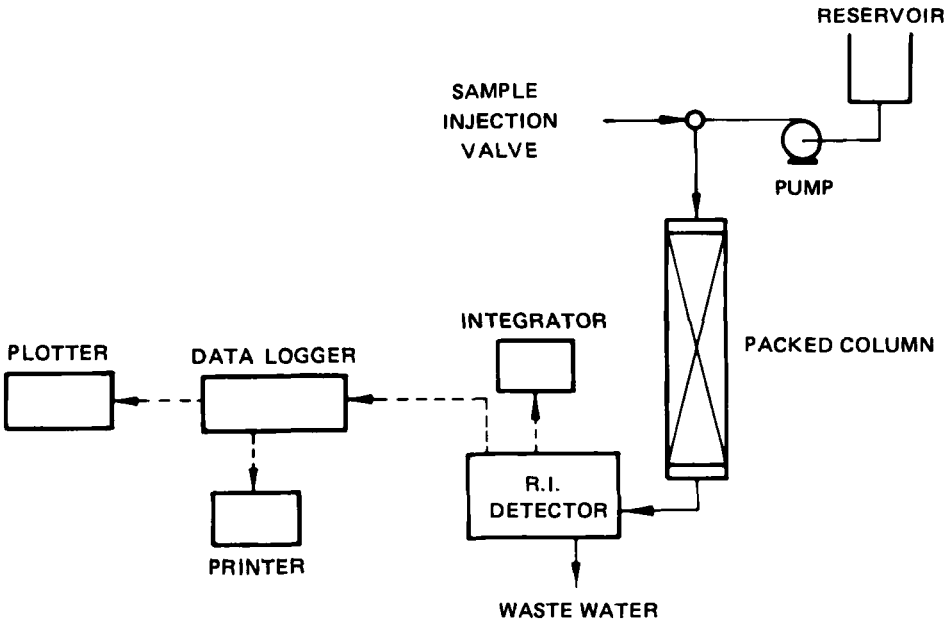

FIG. 1. Schematic diagram of the experimental set-up.

In most of the previous chromatographic studies of zeolites, pelletized adsorbent particles have been used. The commerically available zeolite crystals are generally too small (<5 μm) to be packed directly into the chromatographic column without avoiding the generation of a high pressure drop across the column. Furthermore, small crystals are often undesirable for diffusion studies because the overall diffusion process is much too rapid. The use of pelletized adsorbent complicates the analysis and interpretation of kinetic data since an additional resistance to mass transfer caused by diffusion through the macropores of the particle must be accounted for.

In this study, large crystals (≈ 53 μm) were used. The use of large crystals maximized the intracrystalline contribution to mass transfer resistance, thereby improving the accuracy of the diffusivity measurements.

THEORETICAL

The chromatographic response curves were analyzed by the method of moments. For a column packed with unaggregated crystals, the first and

second moments of the pulse response are related to the adsorption equilibrium constant (K) and the intracrystalline diffusional time constant (D_c/r^2) by (*2*, *8*)

$$\mu \equiv \frac{\int_0^\infty ctdt}{\int_0^\infty cdt} = \frac{L}{\varepsilon v}[\varepsilon + (1-\varepsilon)K] \tag{1}$$

$$\frac{\sigma^2}{\mu^2} L \equiv \text{HETP} = \frac{2D_L}{v} + \frac{2\varepsilon v}{(1-\varepsilon)}\left[\frac{r}{3k_f} + \frac{r^2}{15D_cK}\right]\left[1 + \frac{\varepsilon}{(1-\varepsilon)K}\right]^{-2} \tag{2}$$

where

$$\sigma^2 \equiv \int_0^\infty c(t-\mu)^2 dt \Big/ \int_0^\infty cdt$$

In a liquid-phase system the Peclet number ($2Lv/D_L$) has been shown (*9*, *10*) to be almost constant and independent of fluid velocity especially in the low Reynold number regime. A plot of HETP vs fluid velocity should therefore be linear with the slope directly related to the mass transfer resistance and the intercept giving the axial dispersion contribution $2D_L/v$.

The magnitude of the external mass transfer resistance may be estimated from Wakao's correlation (*3*). Since the particle size is small, the Reynolds number is always low over the entire range of velocity used in these experiments. In this regime Sh $\approx$ 2.0 of $k_f \approx D_m/r$. Clearly, if $KD_c \ll D_m$ and $r/3k_f \ll r^2/15KD_c$, the external resistance to mass transfer will be negligible in comparison with the intracrystalline diffusional resistance.

Since water is adsorbed more strongly than other components and since the intracrystalline diffusivity will be lower than the free liquid diffusivity, this condition should always be fulfilled in an aqueous system ($K < 1$, $D_c < D_m$). Equation (2) therefore simplifies to

$$\frac{\text{HETP}}{2} = \frac{D_L}{v} + \left(\frac{\varepsilon v}{1-\varepsilon}\right)\left(\frac{r^2}{15KD_c}\right)\left(1 + \frac{\varepsilon}{(1-\varepsilon)K}\right)^{-2} \tag{3}$$

and the intracrystalline diffusional time constant can be found unambiguously from the slope of a plot of HETP vs fluid velocity.

In the calculation of the moments of the experimental response curves, small corrections were applied to allow for the hold-up and dispersion in the detector (volume $\approx$ 2.3 cm^3). These corrections were determined directly from pulse response measurements with the column removed from the system.

RESULTS AND DISCUSSION

The sorbates used in this study are at very low concentrations (<1 wt%). They are assumed to be further diluted in the column since the sample size used is very small compared to column volume. Therefore system linearity with diffusivity independent of concentration can be assumed at this low concentration range.

Determination of Voidage and Axial Dispersion

Figure 2 shows plots of the first moment of the response over bed height vs reciprocal superficial fluid velocity for blue dextran, starch, and D_2O pulses.

Since starch and blue dextran are large carbohydrate molecules which cannot penetrate the zeolite micropores, there can be no significant diffusion or adsorption ($K = 0$). According to Eq. (1), the plot should be linear with a slope which gives a value of the voidage equal to 0.571.

In contrast, D_2O penetrates the micropores fully. It was verified experimentally in our previous work (*1, 6*) that D_2O and H_2O had the same K at ambient temperature by measuring their vapor-phase isotherms. Therefore, K should be equal to the total fractional micropore volume of the crystal. According to Breck (*7*), this value was found to be approximately 0.51. The slope of the D_2O plot should therefore be equal to $[\varepsilon + 0.51(1 - \varepsilon)]$. The voidage derived from this slope was found to be 0.572, in excellent agreement with the value derived from blue dextran and starch data.

Studies of axial dispersion of liquids in packed bed showed that (D_L/v) is essentially independent of fluid velocity at low Reynolds number. Thus, under conditions of axial dispersion control, one would expect to find a constant HETP. Since D_2O penetrates rapidly with no significant mass transfer resistance, the HETP values should represent the axial dispersion contribution and should be independent of both the velocity and temperature. From Fig. 3, HETP was found to be independent of

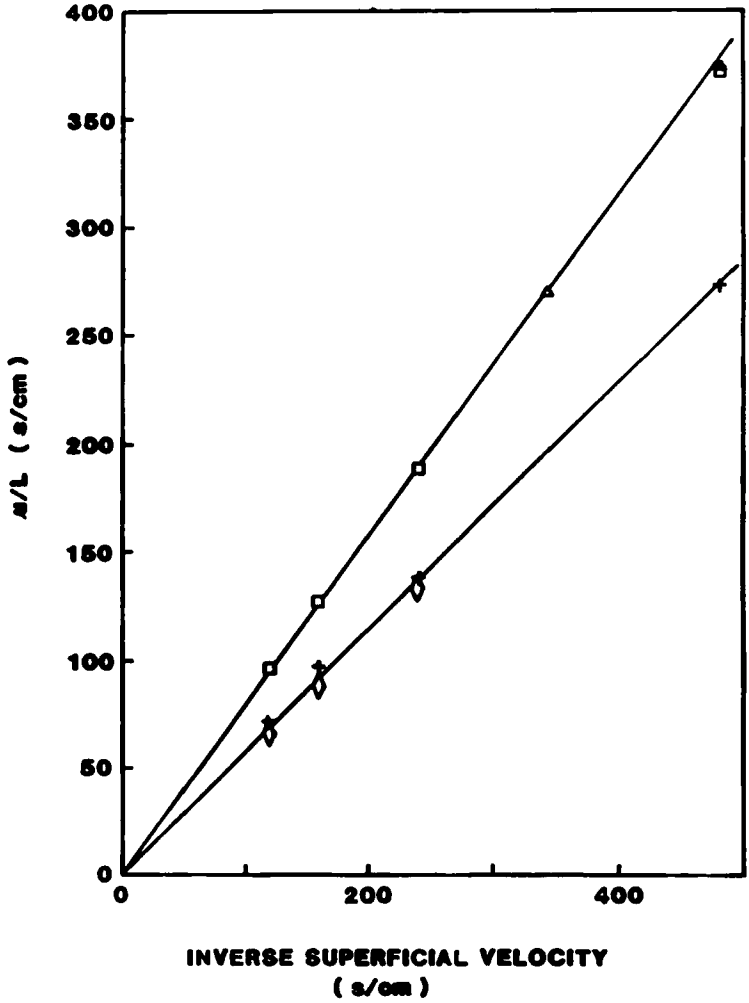

FIG. 2. Graph of μ/L against inverse of superficial velocity for D_2O, blue dextran, and starch: (+) blue dextran, (◇) starch, (□) D_2O sample size = 100 μL, (△) D_2O sample size = 200 μL.

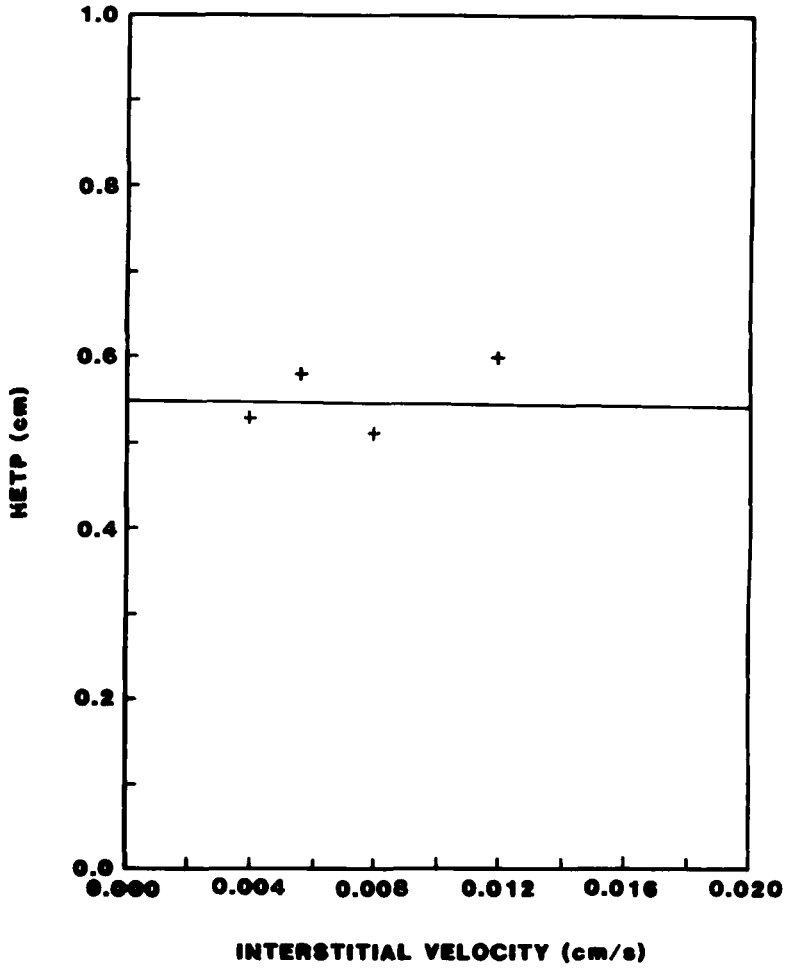

FIG. 3. Graph of HETP against V for D_2O.

velocity and equal to 0.45 cm. The contribution was therefore taken to be the axial dispersion term in Eq. (3).

Since axial mixing in a liquid system is determined by the flow pattern in the bed rather than by the molecular diffusion, this contribution to the HETP should be approximately the same for all sorbates.

Equilibrium Data

Plots of μ/L against reciprocal superficial velocity for the three amino acids, namely cysteine, threonine, and serine, are shown in Figs. 4 and 5. Equilibrium adsorption constants were derived from the gradients of these plots according to Eq. (1).

It can be seen that serine, being the smallest molecule among the three amino acids studied, has the highest K values. The K value decreases with

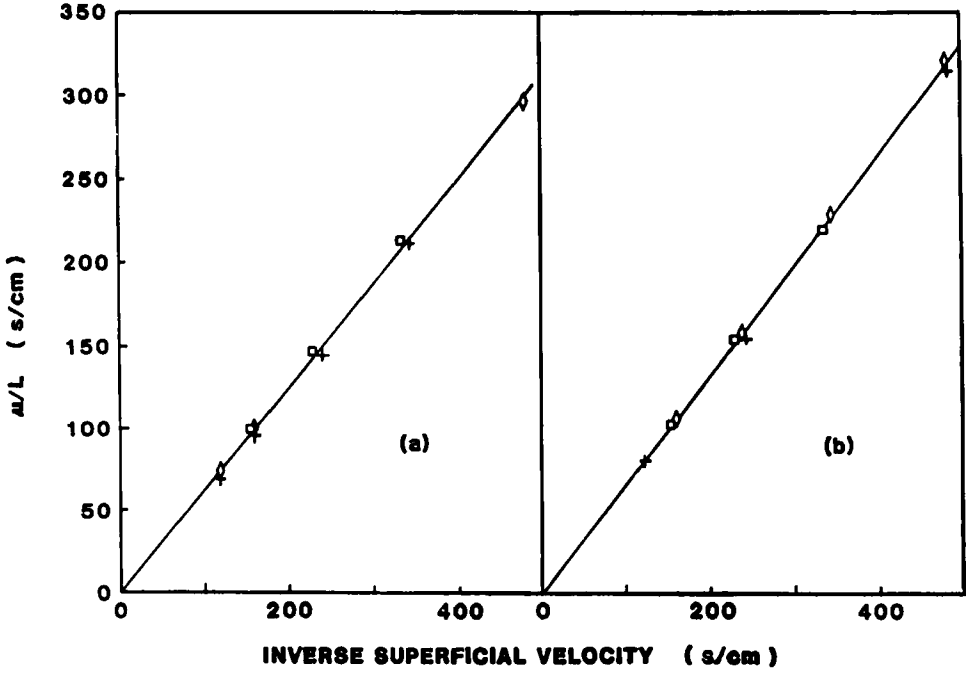

FIG. 4. Graph of μ/L against inverse of superficial velocity. (a) Cysteine and (b) threonine: (□) 5°C, (+) 30°C, (◇) 50°C.

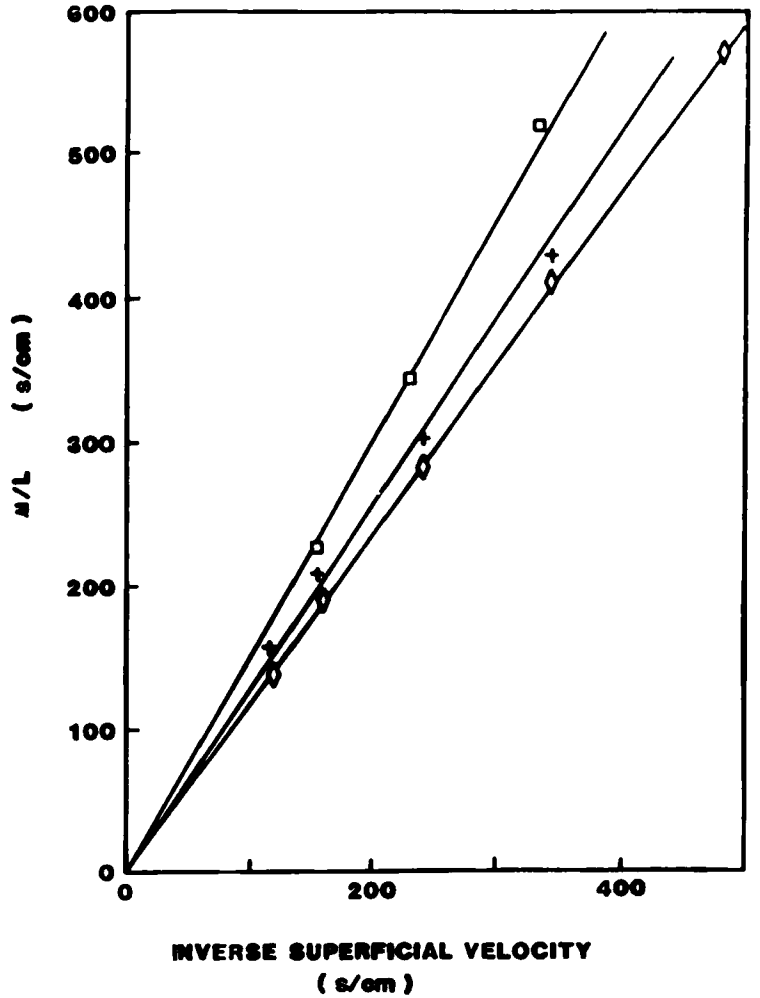

FIG. 5. Graph of μ/L against inverse of superficial velocity for serine: (□) 5°C, (+) 30°C, (◇) 50°C.

increasing temperature, showing an exothermic heat of exchange adsorption ($\Delta H = -1.79$ kcal/mol). In contrast, cysteine and threonine, being larger molecules, are less strongly adsorbed. There is no significant temperature dependence of the equilibrium constant for these two amino acids.

The K values for these three amino acids were compared to values for the other three amino acids reported by Ching and Ruthven (*1*). A summary of all these data is presented in Table 1, and the Arrhenius plot showing the temperature dependence of the equilibrium constant is shown in Fig. 5.

It is observed that serine has a much higher K value when compared to alanine and glycine, both of which possess smaller molecular structures. Threonine, another hydroxyl group containing amino acids, also has a higher K value when compared to alanine which has a smaller molecular structure. It is thus suspected that hydroxyl groups containing amino acids generally have higher K values as compared to other amino acids having approximately the same size.

TABLE 1
Equilibrium Constants and Intracrystalline Diffusivities

Sorbate	T (°C)	K	$-\Delta H$ (kcal/mol)	$D_c/r^2 \times 10^4$ (s^{-1})	$D_c \times 10^9$ (cm^2/s)	E (kcal/mol)
Cysteine	5	0.113		1.28	0.897	
	30	0.113	0	1.59	1.11	1.56
	50	0.113		1.90	1.33	
Serine	5	2.228		1.42	1.00	
	30	1.525	1.79	3.24	2.43	5.43
	50	1.428		5.55	3.90	
Threonine	5	0.203		1.90	1.33	
	30	0.203	0	2.85	2.00	3.23
	50	0.203		4.44	3.12	
Glycine[a]	0	0.82		13.4	8.40	
	25	0.72	1.10	30.4	19.0	6.60
	42	0.64		75.0	47.0	
Lysine[a]	0	0.043		2.20	1.40	
	25	0.043	0	2.82	1.80	2.30
	44	0.043		3.93	2.50	
Alanine[a]	0	0.16		6.79	4.20	
	25	0.16	0	11.5	7.20	4.60
	43	0.16		22.5	14.0	

[a]Data reported by Ching and Ruthven (*1*).

TABLE 2
Size of Amino Acid Molecule Estimated Based on Bond Length and Bond Angle (5)

	Length (Å)	Width (Å)
Alanine	5.5	3.3
Arginine	11.0	4.7
Arparagine	7.5	3.5
Aspartic acid	6.5	3.5
Cysteine	6.4	3.7
Glycine	3.9	3.5
Glutamic acid	8.0	3.5
Glutamine	9.0	3.5
Histidine	8.5	6.1
Isoleucine	8.5	5.0
Leucine	8.5	5.0
Lysine	11.3	3.5
Methionine	10.3	3.5
Phenylalanine	9.7	4.7
Proline	6.2	5.1
Serine	6.1	3.5
Threonine	6.1	5.0
Tryptophan	10.9	5.6
Tyrosine	10.4	4.7
Valine	7.0	5.0

Excluding the hydroxyl-containing group, the K values for other amino acids generally follow the sequence of our estimated molecular size.

At this point it has to be pointed out that the estimated size of the molecules presented in Table 2 is based on the assumption that the molecules are linearly stretched. The degree of coiling is not considered in this estimation. Thus the estimation can only provide a general idea of the molecular size but little information on the diffusion characteristics. However, comparison of size among the molecules from the same class is still reliable.

Kinetic Data

Plots of HETP (after subtracting the axial dispersion term) against interstitial liquid velocity for the three amino acids are shown in Figs. 6 and 7. By substracting the HETP contributed by the axial dispersion

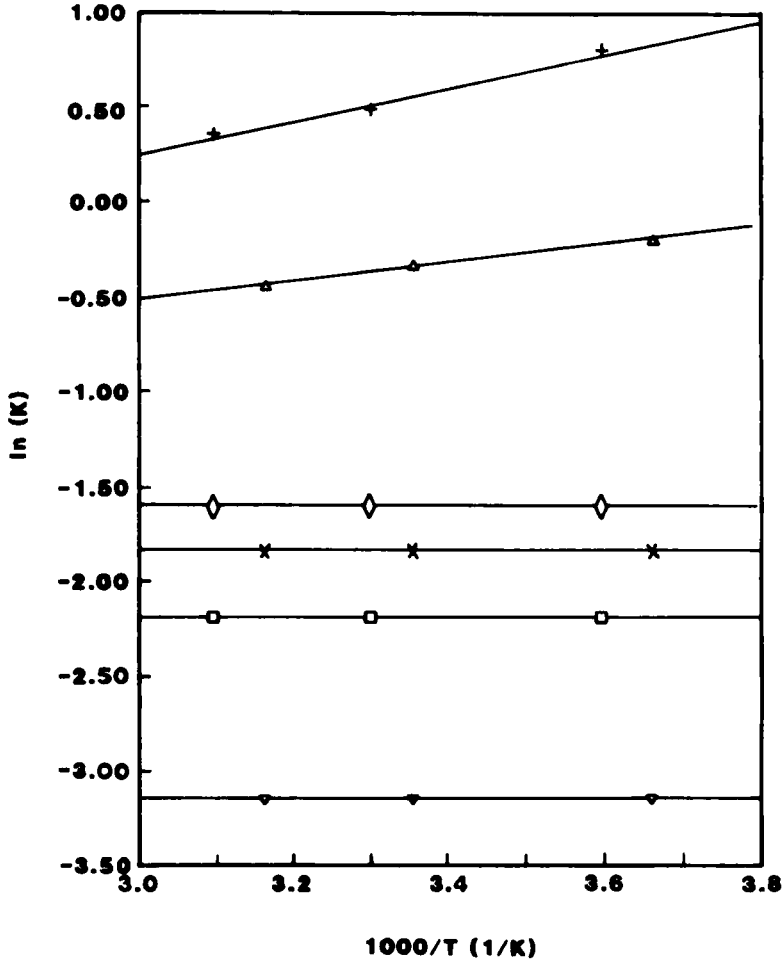

FIG. 6. Temperature dependence of equilibrium constants: (□) cysteine, (+) serine, (◇) threonine, (△) glucine, (×) analine, (▽) lysine.

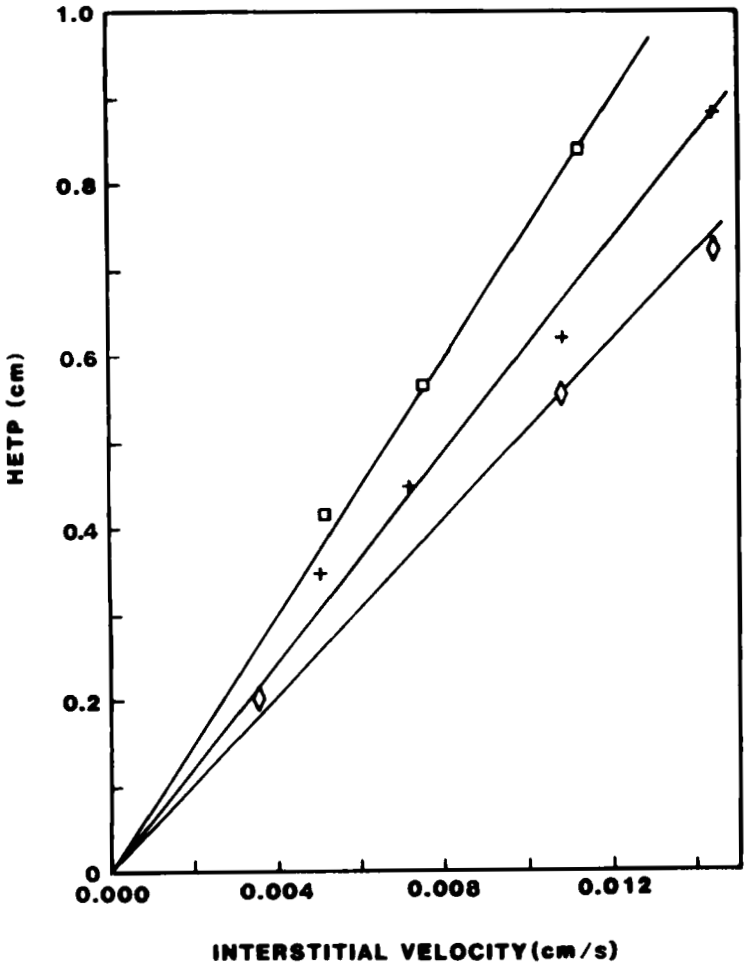

FIG. 7. Graph of HETP against interstitial velocity for cysteine: (□) 5°C, (+) 30°C, (◊) 50°C.

term, such plots should be linear and intercept at the origin. Despite some scatter, the data still conform to this trend.

As unaggregated zeolite crystals were used, macropore resistance was eliminated. By assuming negligible film resistance, diffusional time constants (D_c/r_c^2) and hence intracrystalline diffusivities (D_c) were derived from the gradients of these plots. The diffusivities are in the range of 10^{-9} to 10^{-10} cm^2/s and seem qualitatively reasonable for hindered diffusion processes. From these D_c values, it can be shown that $KD_c \ll D_m$, where D_m is in the order of 10^{-6} cm^2/s estimated using the Wilke and Chang equation (*9*). Hence the assumption of negligible film resistance is valid.

It is noticed that the HETP increases with decreasing temperature, suggesting higher mass transfer resistance at lower temperature. These values, together with those reported previously by Ching and Ruthven (*1*), are tabulated in Table 1. The Arrhenius plot showing the temperature dependence of the diffusivities is shown in Fig. 8.

From the results it is observed that the sequence of diffusivities and activation energies does not seem to follow the sequence of molecular size. Lysine, threonine, and serine, which have significant different molecular sizes, are expected to have different D_c values. But the Arrhenius plot for the three amino acids were found to criss-cross each other. Cysteine, which is estimated to possess a smaller molecular structure than lysine, has smaller values of D_c and E. It can be due to the coiling effect of some of the amino acids.

CONCLUSION

The application of the liquid chromatographic technique using a column packed with large synthetic zeolite crystals as a means of studying sorption equilibrium and intracrystalline diffusion rates of smaller amino acids has been demonstrated. The three amino acids (serine, threonine, and cysteine) are all adsorbed quite rapidly, but there are significant differences in both the equilibrium constants and intracrystalline diffusivities. Combined with the kinetic and equilibrium data of the other three amino acids (glycine, lysine, and analine) reported previously (*1*), it can be concluded that the larger molecules generally show a lower equilibrium constant, but no obvious trend can be observed for intracrystalline diffusivity data. However, it can be deduced that separation based on the combined effects of equilibrium and kinetics should be feasible for these amino acids.

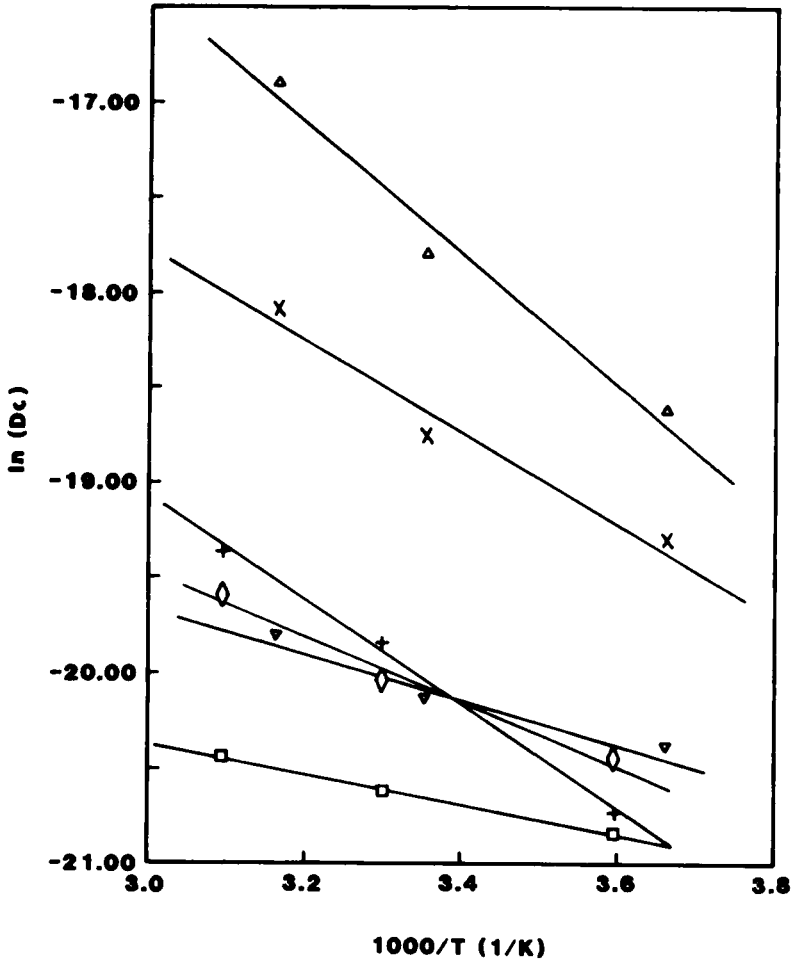

FIG. 8. Temperature dependence of intracrystalline diffusivities: (□) cysteine, (+) serine, (◇) threonine, (△) glycine, (×) analine, (▽) lysine.

From the size of the molecules estimated based on the bond length and bond angle, it was found that larger amino acids such a tryptophan, tyrosine, and phenylalanine have sizes approaching the size of the pore of the zeolite crystals (7.6 Å × 12.6 Å). These big molecules may impose some limitations on the possibility of complete separation of the common amino acids. Nevertheless, separation using a column packed with large zeolite crystals still seems to be quite promising, and a study of the sorption and diffusion of other amino acids is currently being pursued.

NOTATIONS

c	sorbate concentration in fluid phase (mol/cm^3)
c_0	fluid concentration at inlet of packed column (mol/cm^3)
c_0'	initial ($t < 0$) steady-state value of c (mol/cm^3)
D_c	intracrystalline diffusivity (defined relative to adsorbed phase concentration gradient as driving force (cm^2/s)
D_m	molecular diffusivity in fluid phase (cm^2/s)
D_p	pore diffusivity (cm^2/s)
D_L	axial dispersion coefficient (cm^2/s)
E	diffusional activation energy (cal/mol)
HETP	height equivalent to a theoretical plate (cm)
k_f	external film mass transfer coefficient (cm/s)
K	dimensionless adsorption equilibrium constant [(mol/cm^3 crystal)/(mol/cm^3 solution)]
L	bed height (cm)
Pe	Peclet axial dispersion number for column ($= vL/D_L$)
q	sorbate concentration in crystal (mol/cm^3)
$\bar{q}$	value of q averaged over crystal and pellet (mol/cm^3)
r	radial coordinate for microparticle or crystal (cm)
r_c	crystal radius (cm)
R	radial coordinate for macroparticle or pellet (cm)
R_p	pellet radius (cm)
Re	Reynolds number ($= 2\rho v \varepsilon R_p/\mu$)
Sc	Schmidt number ($= \mu/\rho D_m$)
Sh	Sherwood number ($= 2R_p k_f/D_m$)
t	time (s)
$\bar{t}$	mean time (s)
v	interstitial liquid velocity (cm/s)
z	distance measured from column inlet (cm)

u mean of response curve (s)
σ^2 variance of response curve (s^2)
γ separation factor ($= K_A/K_B$ for linear system)
ε voidage of adsorbent bed
ε_p porosity of adsorbent particle
ΔH enthalpy change for sorption of 1 mol of amino acid with displacement of the equivalent quantity of water (cal/mol)

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Received by editor May 16, 1988
Revised August 31, 1988