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The Ion-exchange Kinetics of SAM^+/H^+ System with JK110 Resin

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Abstract−S-adenosyl-L-methionine (SAM) is an important small molecule with many medical interests. The ion-exchange kinetics of SAM^+/H^+ system with JK110 resin has been studied in this work. The results showed that intra-particle diffusion was the rate limiting step in the ion-exchange process. A suitable model of particle diffusion has been proposed to describe the effects of pH, temperature and SAM concentration in the solution on the ion-exchange rate. By simulating experimental data with model equation, some parameters such as diffusion constant B, internal diffusion coefficient D_i and activation energy E_a are obtained.

Key words: S-adenosyl-L-methionine Separation, JK110 Resin, Exchange Velocity, Particle Diffusion Model

INTRODUCTION

S-Adenosyl-L-methionine (SAM), which is found in all living organisms [Alessandra et al., 2002], is one of the most important small molecules with biological activity. As shown in Fig. 1, the molecular structure of SAM consists of an active methyl moiety and an energy-rich sulfur atom. The significance of SAM results from the fact that SAM is the principal biological methyl group donor and the precursor of aminopropyl groups in polyamine biosynthesis [Giulidori et al., 1984; Alessandra et al., 2000]. Moreover, SAM is the unique natural sulfonium compound in the metabolism of sulfur amino acids. It is also a precursor of glutathione (GSH) through its conversion to cysteine via the trans-sulfuration pathway [Lu, 2000]. Given the importance of SAM in cell function, it is not surprising that this molecule is being administered as an important therapeutics for the treatment of various clinical disorders, such as liver diseases [Mato et al., 1997; Lieber, 1999], osteoarthritis [Vettere et al., 1987] and depressive syndromes [Anderer et al., 2002]. In addition, SAM has potential importance as a cancer chemopreventive agent [Pascale et al., 1992] and has therapeutic effectiveness on hyperlipemia, arteriosclerosis, Parkinsons disease, and senile dementia [Ftiedel et al., 1989].

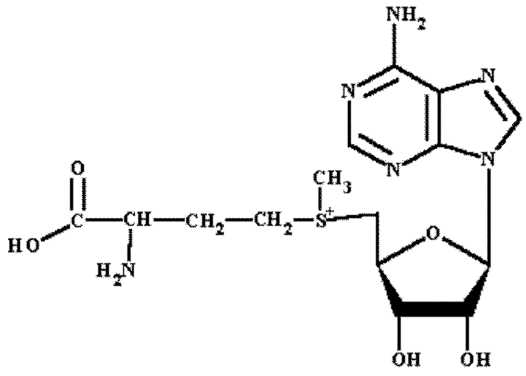

Fig. 1. Chemical structure of the SAM molecule.

SAM is an intracellular product of yeast cells when L-methionine is added as precursor. The traditional separation procedure of SAM includes: extraction of SAM from yeast cells, precipitation of SAM by picrolonic acid and formation of stable salt [Fiecchi, 1977]. The process involves many steps and the overall recovery is relatively low. Also, the procedure consumes large amount of several kinds of organic solvent, which will make the process more complex and lead to possible environmental pollution.

In our previous work [Hui, 2002], SAM separation by ion-exchange method was developed instead of picrolonic acid precipitation. It was found that JK110 resin was the most suitable one for the recovery of SAM from the yeast extract because of its high ion-exchange capacity and rapid adsorption rate.

In this work, the effects of various operation conditions, such as pH, temperature and SAM concentration, on the rate of ion exchange will be examined, and the rate limiting step of the ion-exchange process will be analyzed.

EXPERIMENTS

1. Materials

JK110 resin, which is a kind of weak acid cationic exchange resin, used in this work is provided by Shanghai Huazhen Resin Corporation (China). And the basic physicochemical properties of JK110 resin are listed in Table 1. Before application, the resin is pretreated to H^+ type. All the chemicals are in AR grade or better. The yeast extract containing SAM is prepared from *Saccharomyces cerevisiae* in this laboratory.

2. Methods

2-1. Preparation of the Extract Containing SAM

SAM is prepared by the cultivation of *Saccharomyces cerevisiae* in the existence of L-methionine. Through fed-batch fermentation,

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Table 1. Basic physicochemical properties of JK110 resin

Average particle diameter (mm)	Matrix	Functional group	Exchange capacity (mmol/g)	Regeneration
0.64	Acrylic acid	Carboxyl	12.5	1 mol/*l* NaOH 1 mol/*l* HCl

yeast cells were harvested and treated with 0.12 vol. of ethyl acetate; and then 0.50 vol. of 0.18 mol/l sulfuric acid were added under vigorous stirring for 2 hours. The cell debris was removed by centrifugation in a Sorvall centrifuge (Du Pont Company, U.S.A.) at 5,000 rpm and 4 °C for 20 minutes. The clear supernatant containing SAM, namely extract, was obtained for ion-exchange experiments.

2-2. Measurement of Ion-exchange Rate of SAM

The ion-exchange rates of SAM on JK110 resin were measured in a beaker on a thermostatic magnetic stirrer (Model 78HW-1, Hang-Zhou Instrument and Meter Plant, China) at various initial pH (adjusted by 0.05 mol/*l* phosphate buffer). In the beaker, 50 ml of solution with known initial SAM concentration was contained, and then 1.0 gram of wet resin was added to start the ion exchange process. Samples of solution were taken and the SAM concentration was measured by HPLC in certain time interval. The amount of ion-exchange of SAM (q_t) and the fractional attainment of equilibrium (F) are calculated with following equations, respectively.

$$q_t = \frac{(c_0 - c_t)\times v}{w} \tag{1}$$

$$F = \frac{(c_0 - c_t)\times v}{wq_\infty} = \frac{q_t}{q_\infty} \tag{2}$$

2-3. Analytical Methods [Hoffman, 1986; Wei et al., 2001]

An Agilent 1100 series HPLC system equipped with a Hypersil BDS C_{18} reversed-phase analytical column (250×4.6 mm I. D., 5 μm particle) was used to analyze the SAM concentration The mobile phase consists of 2 mmol/*l* hetanesulfonic acid sodium salt, 40 mmol/*l* $NH_4H_2PO_4$ and 18% (v/v) methanol, and the flow rate is 1.0 ml/min. The wavelength of UV detector is set at 254 nm. Before analysis, the samples should be diluted properly.

RESULTS AND DISCUSSIONS

1. Dissociation Equilibrium of SAM in Aqueous Solution

According to the molecular structure of SAM as shown in Fig. 1, in each SAM molecule, there are four groups which can be dissociated: one amino group in methionine residue, another amino group in purine moiety, one carboxyl group and one sulfonium group. The pK values are 11.5, 7.8, 1.8 and 3.4, respectively [Farooqui et al., 1983]. The multilevel dissociation equilibrium occurs in the aqueous solution as follows:

$$SAM^{3+} \overset{pK_1'}{\rightleftharpoons} SAM^{2+} \overset{pK_2'}{\rightleftharpoons} SAM^{1+} \overset{pK_3'}{\rightleftharpoons} SAM^{0} \overset{pK_4'}{\rightleftharpoons} SAM^{1-}$$

The calculated distribution curves of four kinds of ionic forms as well as the molecular SAM, according to an equation derived by Handerson-Hasselbalch, are shown in Fig. 2. It is obvious that the ionized forms of SAM will change from SAM^{3+}, SAM^{2+}, SAM^{+}, SAM^{0} to SAM^{-} with the increase of pH value.

The SAM is more stable at acidic condition according to the literatures [Alessandra et al., 2002; Hoffman, 1986; Lu, 2000]. For H^+-type weak acidic cationic ion-exchange resin, the ion exchange can be performed at low pH value, where SAM will be positively charged and stable.

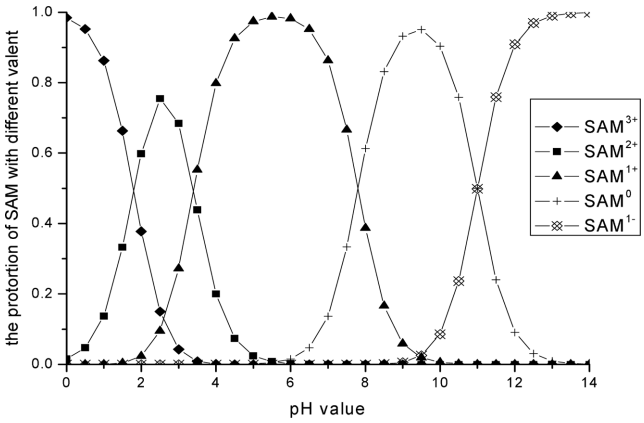

Fig. 2. Dissociation curves of SAM in aqueous solution at different pH value.

2. Determination of Rate Limiting Step

The overall rate of the ion-exchange is depended on both mass transfer resistance and mass-action mechanism. Generally, in the ion-exchange process, the ion-exchange reaction is very fast and the diffusion is the major factor affecting the overall ion-exchange rate. The mass transfer resistance for ion-exchange process consists of both liquid film diffusion and intra-particle diffusion [Selvaraj et al., 2004]. It is preferred to know which step is the rate-limiting step for the purposes of process enhancement and design.

In the case of liquid-film diffusion control, the diffusion rate equation can be developed based on the Fick's Law and mass balance as follows [Boyd et al., 1947; Yue and Wang, 2000].

$$\ln(1-F) = -k_1 t \tag{3}$$

where, k_l is the mass transfer rate constant of liquid-film diffusion, $k_1 = 3D_l/r_0\delta\alpha$. It is obvious that a linear relationship should be reliable between ln(1−F) and t in liquid-film diffusion and the slope is k_1.

For intra-particle diffusion control, according to the Fick's Law of spherical particles, the uptake curve in ion-exchange process can be described as:

$$F = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp(-n^2 Bt) \tag{4}$$

where B is the internal diffusion constant, $B = D_i\pi^2/r_0^2$. For each experimental value of F, a value of Bt is obtained from Eq. (4). When the ion-exchange rate complies with particle diffusion, Bt should follow a linear relationship with t, and the slope is B.

A typical uptake curve of SAM on JK110 resin is shown in Fig. 3. According to Eqs. (3) and (4), the relationships of ln(1−F) vs. t as well as Bt vs. t are plotted in Fig. 4, respectively. It is obvious

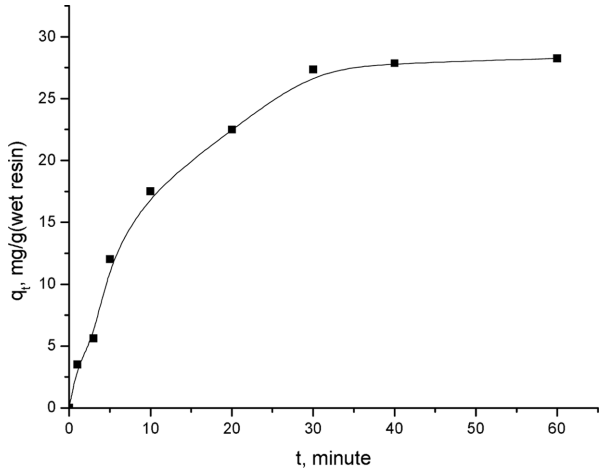

Fig. 3. Ion-exchange uptake curve of SAM on JK110 resin (c_0=4.0 g/*l*, T=298 K, pH 7.0).

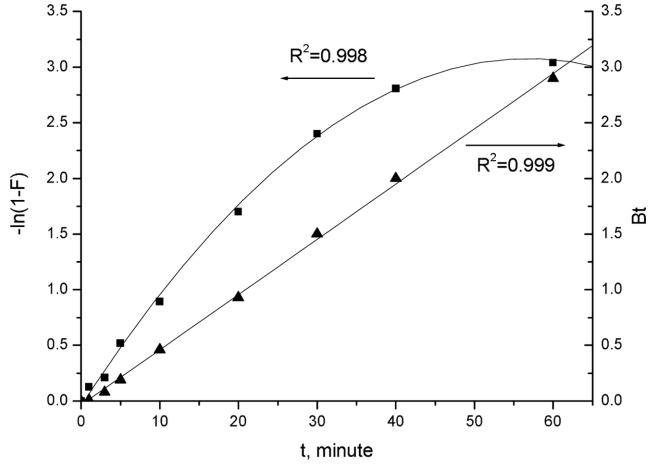

Fig. 4. Estimation of interaction mechanism between SAM^+ and H^+ (R^2: correlation coefficient).

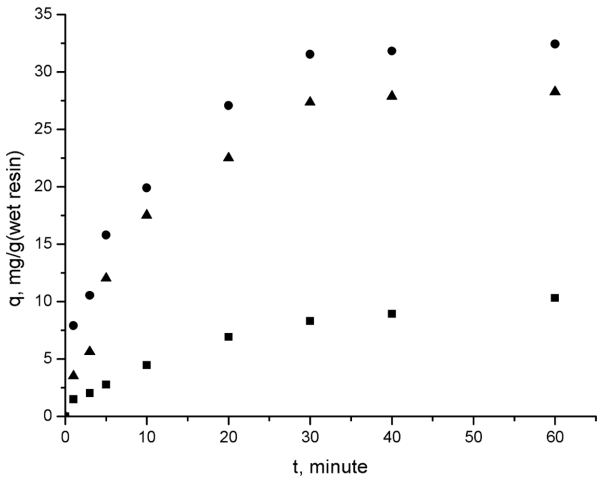

Fig. 5. Uptake curves of SAM on JK110 at different pH values (T= 298 K, c_0=4.0 g/*l*). pH values: ■ 3.0, ● 5.0, ▲ 7.0.

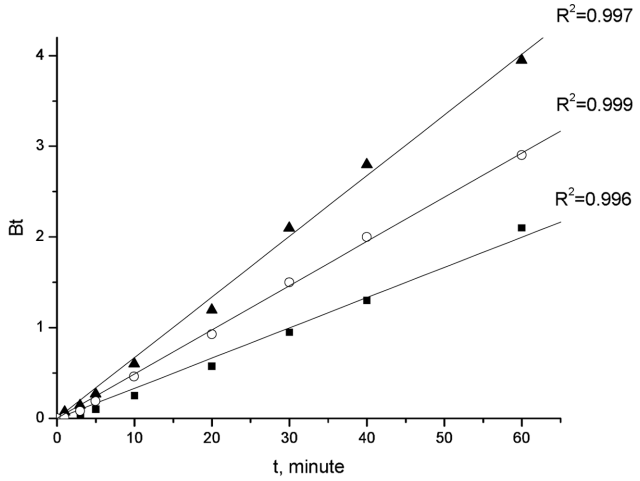

Fig. 6. Effect of pH on exchange velocity. ■ ○ ▲ experimental data; — model prediction, pH values: ■ 3.0, ▲ 5.0, ○ 7.0.

that ln(1−F) vs. t does not exhibit a linear relationship; therefore, film diffusion is not the limiting step in the overall ion-exchange rate. A straight line was observed between Bt and t, which explained that the intra-particle diffusion is the limiting step and determines the overall ion-exchange rate.

3. Effect of pH Values on Ion-exchange Rate

As shown in Fig. 2, the dissociation of SAM is very complex and depends greatly on the pH value. The ionic form of SAM at different pH value is a major factor affecting the ability of ion exchange. Thus, the pH value is an important factor affecting the dissociation of ion-exchange resin as well as the rate of ion exchange. The effects of pH value on the uptake curves of SAM on JK110 resin are shown in Fig. 5. The pH values were set at pH 3.0, 5.0 and 7.0, respectively.

The uptake curves were able to be correlated by Eq. (4), and the results are shown in Fig. 6. Table 1 lists the simulated B values and intra-particle diffusivities. It is clear that the ion-exchange rate is controlled by intra-particle diffusion regardless of what the pH value is.

It was found that when the pH value was raised from pH 3.0 to pH 5.0, the ion-exchange amount at equilibrium increased from 10.32 mg/g to 32.41 mg/g. Also from Table 2, the intraparticle diffusivity increased from 8.38×10^{-6} to 1.64×10^{-5} cm^2/s. Table 3 lists the cal-

Table 2. The values of B and D_i at different pH values

pH	3.0	5.0	7.0
B, s^{-1}	5.55×10^{-4}	1.09×10^{-3}	8.12×10^{-4}
D_i, cm^2/s	8.38×10^{-6}	1.64×10^{-5}	1.23×10^{-5}

Table 3. The calculated proportion of SAM with different valent in the solution

pH value	SAM^{3+} %	SAM^{2+} %	SAM^{1+} %	SAM^0 %	SAM^{1-} %
3.0	4.3	68.4	27.3	-	-
5.0	-*	2.4	97.4	-	-
7.0	-	-	86.3	13.7	-

*indicates the fraction lower than 0.5%.

culated fractions of various SAM dissociation patterns at different pH value. At pH 3.0, the fractions of SAM^{3+}, SAM^{2+} and SAM^{1+} are 4.3%, 68.4% and 27.3%, respectively, whereas <0.5%, 2.4% and 97.6% at pH 5.0. The ion exchange of each SAM^{3+} or SAM^{2+} will occupy three or two active sites in JK110 resin, therefore resulting in low ion-exchange capacity. Also, the low pH value is not preferred for the dissociation of weak acidic ion-exchange resin JK110; thus, it is unfavorable for ion exchange of SAM. In the meanwhile, the ionic radii of SAM^{3+} and SAM^{2+} are larger than that of SAM^{+}, which will cause a decrease in the intra-particle diffusivity.

Further raising the pH value to 7.0 will cause the decrease in the ion-exchange capability as show in Fig. 5. From Fig. 2, it is clear that 13.7% of neutral form of SAM^{0} appears except SAM^{+}, which does not have the ability of ion exchange. The decreases in the ion-exchange capability as well as the diffusivity reflect the effect of SAM^{0} indeed.

4. Effect of SAM Concentration on Uptake Curves

The effect of the initial SAM concentration on the uptake curves was evaluated at 25 °C and pH 5,0, and the results are shown in Fig. 7. The simulated curves and diffusivities according to Eq. (4) are shown in Fig. 8 and Table 4. It is obvious that the ion-exchange capacity is higher at higher initial SAM concentration. The controlling step affecting ion-exchange rate is still the intra-particle diffusion. The apparent diffusivity is enhanced with the increase of initial SAM concentration.

Table 4. The values of B and D_i at different initial concentration of SAM

c_0, g/*l*	2.0	4.0	10.0
B, s^{-1}	5.59×10^{-4}	1.09×10^{-3}	1.32×10^{-3}
D_i, cm^2/s	8.47×10^{-6}	1.64×10^{-5}	1.99×10^{-5}

5. Effect of Temperature on Uptake Curves

The effects of temperature on the uptake curves were investigated at pH 5.0, and the results are illustrated in Fig. 9. The plots of Bt

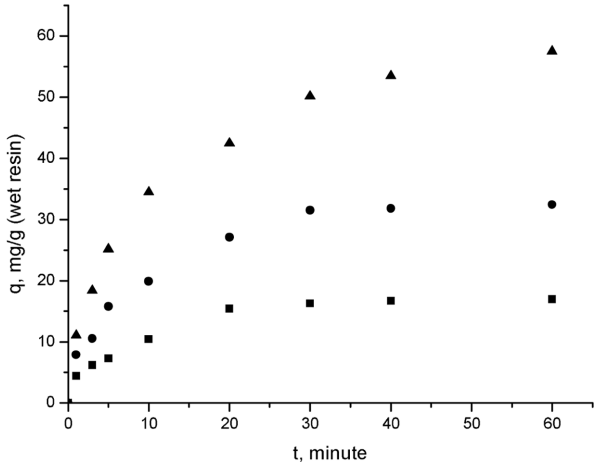

Fig. 7. Uptake curves of SAM on JK110 at different concentration of SAM. T=298 K, pH 5.0; c_0: ■ 2 g/*l*, ● 4 g/*l*, ▲ 10 g/*l*.

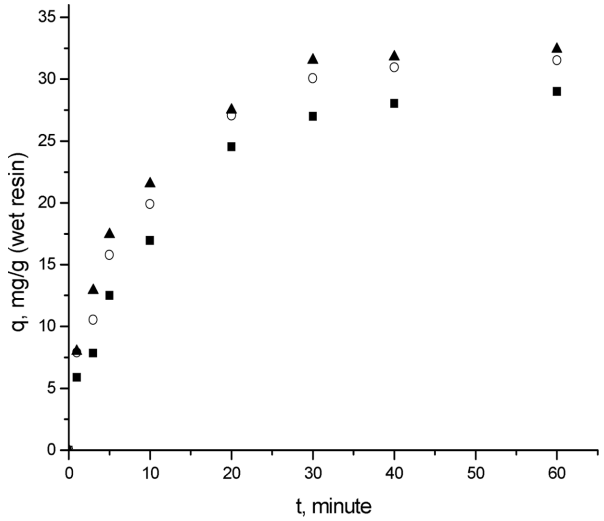

Fig. 9. Uptake curves of SAM on JK110 at different temperature. c_0=4.0 g/*l*, pH 5.0; T, K: ■ 288, ○ 298, ▲ 308.

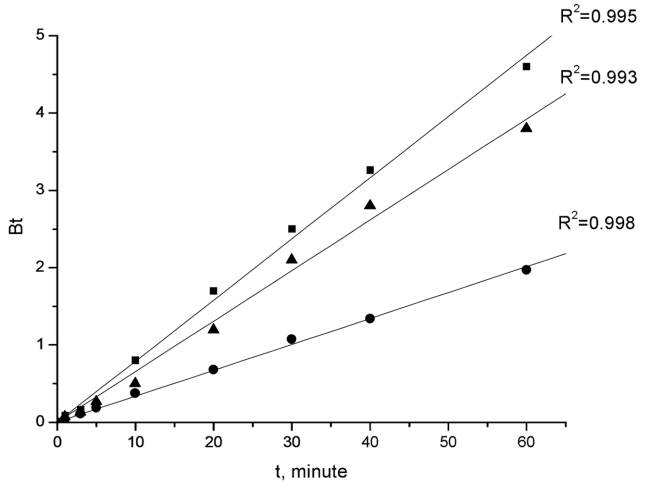

Fig. 8. Effect of concentration on diffusion coefficient. ■ ● ▲ experimental data, — model prediction; c_0: ● 2 g/*l*, ▲ 4 g/*l*, ■ 10 g/*l*.

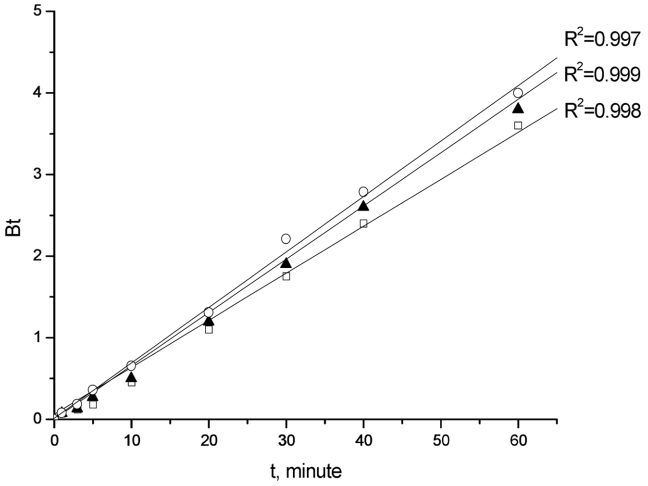

Fig. 10. Effect of temperature on exchange velocity. □ ○ ▲ experimental data, — model prediction; T, K: □ 288, ▲ 298, ○ 308.

Table 5. Correlated values of B and D_i at different temperature

T, K	288	298	308
B, s^{-1}	9.66×10^{-4}	1.09×10^{-3}	1.14×10^{-3}
D_i, cm^2/s	1.54×10^{-5}	1.64×10^{-5}	1.72×10^{-5}

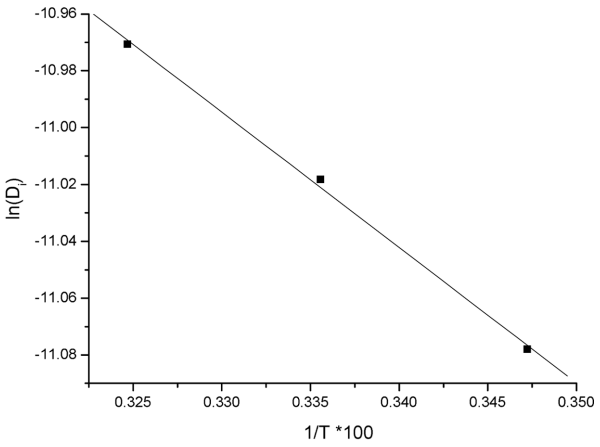

Fig. 11. The effect of temperature on intra-particle diffusivity.

vs. t and the correlated parameters B, D_i are shown in Fig. 10 and Table 5, respectively. When the temperature was increased from 288 K to 308 K, the ion-exchange rate was speeded up, but the increase was quite small. The results indicated that the ion-exchange between SAM^+ and H^+ was an endothermic process. While, the weak dependency of the rate on temperature also showed that the rate-limiting step of the ion-exchange process was not the chemical reaction but the mass transfer.

Diffusion coefficient D_i and temperature T comply with Arrhenius' formula:

$$D_i=D_0 \exp(-E_a/RT) \quad (5)$$

Fig. 10 shows the relationship between log D_i and 1/T. And the activation energy of this process calculated from Arrhenius' formula was quite low (3.96 KJ/mol), which further indicated that the limiting step was mass transfer.

CONCLUSIONS

1. The rate-controlling step of ion exchange between JK110 week acidic resin and SAM is the intra-particle diffusion.

2. The dissociation pattern of SAM at different pH values makes a significant effect on the ion-exchange rate. At pH 5.0, the ion-exchange rate as well as the ion-exchange capacity is the highest.

3. The exchange rate rises with the increase of SAM concentration in the solution and temperature. The activation energy of ion-exchange process calculated from Arrhenius' formula is 3.96 KJ/mol.

ACKNOWLEDGMENT

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NOMENCLATURE

B : internal diffusion constant [s^{-1}]
c_0 : initial concentration of SAM [g/*l*]
c_t : the concentration of SAM in the solution at t time [g/*l*]
D_0 : the activation energy when $E_a=0$
D_i : internal diffusion coefficient [cm^2/s]
D_l : diffusion coefficient in the liquid film [cm^2/s]
E_a : apparent activation energy [kJ/mol]
F : fractional attainment of equilibrium
k_1 : constant of liquid-film diffusion
q_t : ion-exchange capacity of SAM on JK110 resin at t time [mg/g (wet resin)]
q_∞ : equilibrium exchange capacity of SAM [mg/g (wet resin)]
r_0 : radius of resin particle [cm]
v : volume of the extract [ml]
w : weight of wet resin [g]
α : distribution coefficient
δ : thickness of liquid film

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