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Adsorption and desorption behaviors of S-adenosyl-L-methionine in a fixed-bed ion-exchange column

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Abstract−S-adenosyl-L-methionine (SAM) is one of most versatile molecules in nature and has wide medical applications. The ion-exchange separation process of SAM of the extract of yeast cells has many advantages over selective precipitation by picrolonic acid. Experiments of the dynamic column process of SAM on JK110 resin were carried out in a fixed-bed ion-exchange column. The effects of different operation parameters on the adsorption and desorption behaviors of SAM were investigated. The results show that the ion-exchange adsorption of SAM is successfully implemented at 2BV/h, 10 g/L, pH 5.0; the adsorbed SAM in the ion-exchanged bed is efficiently desorbed by 0.2 N H_2SO_4 solution at the flow rate of 2BV/h. According to material balance, the recovery yield of SAM for this ion-exchange process is 90.1%. Finally, this ion-exchange process was successfully scaled up to separate SAM at high yield and purity.

Key words: S-Adenosyl-L-Methionine, JK110 Resin, Fixed-Bed Ion-Exchange, Separation

INTRODUCTION

S-adenosyl-L-methionine (SAM), called "active methionine," represents one of the most versatile molecules in nature [Ftiedel et al., 1989]. The molecular structure of SAM consists of an activated methyl moiety and an energy-rich sulfur atom, which are the elements of carrying out biochemical functions and pharmacological effects [Gaetano et al., 1989]. The significance of SAM results from the fact that SAM is the principal biological methyl donor, and the precursor of aminopropyl groups in polyamine biosynthesis. Moreover, SAM is a 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. The well-established biochemical roles and pharmacological effects of the sulfonium compound have attracted great interest in its therapeutic applications. In the clinical field, SAM, as a therapeutic drug, is administered in human therapy of liver diseases, osteoarthritis and depressive syndromes [Shelly, 2000; Charles, 1999; Vettere et al., 1987]. In addition, SAM has potential importance as cancer chemopreventive agent [Pascale et al., 1992].

Because of its increasing market capability, many efforts were paid to how to produce this magic molecular efficiently, including screening high-producing yeasts or constructing recombinant yeasts, high-density cultivation of *Saccharomyces cerevisiae*, biotransformation from L-methionine using waste yeasts, etc [Lin et al., 2004; Liu et al., 2004]. However, much less work was reported about how to separate SAM from different SAM-containing sources. 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 amounts of several kinds of organic solvent, which will make the process more complex and lead to possible environmental pollution. In our previous work, one efficient ion-exchange separation process of SAM was proposed, and the ion-exchange equilibrium and exchange velocity of SAM on weak-acid cation-exchange resin JK110 were studied [Liu, 2002; Chen et al., 2005]. In the present work, further efforts were made to examine the effects of different operation conditions on the separation of SAM in a fixed-bed ion-exchange column. As a result, the ion-exchange dynamic properties of SAM with JK110 resin were determined, and the processes of the adsorption and desorption of SAM were optimized successfully.

EXPERIMENTAL

1. Materials

JK110 resin used in this work, which was provided by Shang-Hai HuaZhen Resin Corporation (Shanghai, China), is a kind of weak-acid cation-exchange resin. The basic physicochemical properties of JK110 resin are listed in Table 1. According to the procedure provided by the manufacturer, the resins are pretreated to hydrogen form before use. All the chemicals are in AR grade or better. The SAM extract for this research is prepared from *Saccharomyces cerevisiae* grown in our laboratory.

2. Methods

2-1. Preparation of the Extract Containing SAM

After fed-batch cultivation, yeast cells are harvested and extracted with 12% ethyl acetate. The detailed procedure for the SAM extract preparation was the same as that reported before [Chen et al., 2005].

2-2. Experimental System and Procedure of Dynamic Ion-exchange Column

The dynamic column experiments were performed with Gradi-

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Table 1. Basic physicochemical properties of JK110 resin

Average particle diameter (mm)	Matrix	Functional group	Exchange capacity (mmol/g)	Regeneration
0.60	Acrylic acid	carboxyl	12.0	1 mol/L NaOH 1 mol/L HCl

Table 2. Comparison of dynamic breakthrough capacities at a variety of experimental requirements

	U (BV/h)[1]			C (g/l)[2]			pH[3]		
	1.0	2.0	4.0	2.01	3.86	9.05	3.0	5.0	7.0
Q (mg/g wet resin)	57.90	50.18	15.44	40.2	50.18	54.3	10.29	50.18	27.02

[1]Experimental requirements: c_0=3.86 g/L, pH=5.0
[2]Experimental requirements: u=2 BV/h, pH=5.0
[3]Experimental requirements: c_0=3.86 g/ L, u=2 BV/h

Frac System (Amersham Pharmacia Biotech, Sweden), which consists of RediFrac (fraction collector), Optical Unit UV-1, Control Unit UV-2, Peristaltic Pump P-1 and Recorder 111. All the experiments were carried out by using a glass column (Model XK16, 16×200 mm, Amersham Pharmacia Biotech), packed with 15 ml of JK110 resin. The SAM solution of concentration c_0 was pumped into a chromatographic column with a jacket via peristaltic pump at a fixed flow rate (u). The concentration c_t of SAM at the exit of the column was analyzed at time interval until complete breakthrough of SAM. The amount of ion exchange of SAM (q_t) was calculated with the following equation:

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

The saturated ion-exchange column was washed thoroughly with deionized water, and then was eluted by a certain concentration of H_2SO_4 solution for SAM recovery and column regeneration. The elution efficiency (η) was calculated with the following equation:

$$\eta = \frac{c_1 \times v_1}{q_t w} \quad (2)$$

The eluent was concentrated and then crystallized with methanol. All the experiments were performed at constant temperature (T= 25 °C).

2-3. Analytical Method

An Agilent 1100 series HPLC system equipped with chemical station was used, which consisted of Quartpump, ALS injector, VWD detector, and Degasser. Separation was carried out with a Hypersil BDS C_{18} reversed-phase analytical column (250*4.6 mm I.D., 5 μm particle) (Elite Scientific Instrument Corporation, Dalian, China). To protect the analytical column, a Hypersil guard pre-column was fitted. The mobile phase consisted of 2 mmol/*l* hetanesulfonic acid sodium salt, 40 mmol/*l* $NH_4H_2PO_4$ and 18% (v/v) methanol, which was pumped at 1.0 ml/min. The wavelength of UV detector was 254 nm. Before analyzing, the sample should be diluted properly.

RESULTS AND DISCUSSION

1. Adsorption of SAM on Dynamic Ion-Exchange Column

The effects of feed flow rate (u), inlet concentration (c_0) and pH of the feed solution on the breakthrough curves were investigated systematically. Above all, we defined that the point at which the outlet concentration of SAM becomes 10 percent of inlet concentration was the breakthrough point. Based on this set point, the breakthrough volume and breakthrough capacity under different operation parameters could be estimated. Table 2 lists the dynamic breakthrough capacities (Q) at a variety of experimental requirements.

1-1. Effects of Feed Flow Rate on the Breakthrough Curve

The effects of flow rate on the breakthrough curves were investigated at 1 BV/h, 2 BV/h and 4 BV/h. As shown in Fig. 1, with the increase in the flow rate, the breakthrough time advanced. The breakthrough capacities at 1.0, 2.0, 4.0 BV/h were 57.90, 40.18 and 15.44 mg per gram of wet resin, respectively (Table 2). This might be explained by insufficient contact time between SAM and resin particles when the flow rate increased. However, at an early stage of the column process, the exchange capacity almost increased linearly and had the same trend for the different flow rates, which indicated that flow rate has little effect on the exchange rate at early stage. After about 10 hr, the exchange capacity would approach to a fixed value, which meant that the flow rate affected saturated capacity significantly. The saturated capacities at 1.0, 2.0, 4.0 BV/h were 76.22, 68.07 and 49.84 mg per gram of wet resin, respectively. Considering the stability of SAM [Morana et al., 2000, 2002] and production efficiency, in order to avoid SAM degradation a short operation time was more favorable, although the breakthrough curve at 2 BV/h is not as sharp as that at 1 BV/h. So the flow rate of 2 BV/h was chosen for the adsorption process of SAM.

1-2. Effect of Inlet Concentration on the Breakthrough Curves

Breakthrough curves at various inlet concentrations at u=2 BV/h were examined in this fixed-bed column (Fig. 2). When the inlet concentration increased, the shape of the curves became steep and ion-exchange rate increased as well, while the breakthrough time was brought forward. The higher the inlet concentration of SAM, the higher the SAM concentration gradient between the feed liquid and the resin, which was one of the reasons for the increase of exchange rate as shown in Fig. 2(B).

1-3. Effect of pH on the Breakthrough Curve

SAM was a weak electrolyte with multiple ionizable groups, and its *pK'* values were 1.8, 3.4, 7.8 and 11.5, respectively [Farooqui, 1983]. A multilevel dissociation equilibrium occurs in the aqueous solution. The ionic forms of SAM in the solution are determined by the solution pH, and the proportion of SAM with different valences

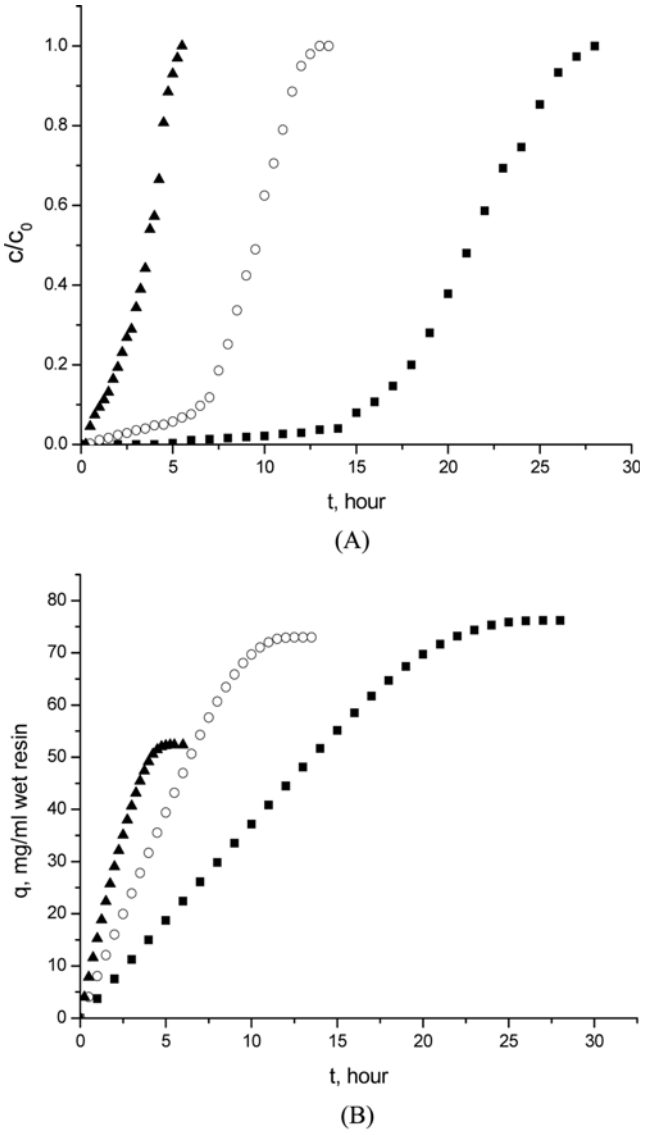

Fig. 1. Effects of flow rate on the breakthrough curves (A) and the exchange rate (B). c_0=3.86 g/L, pH=5.0; u: ■ 1 BV/h; ○ 2 BV/h; ▲ 4 BV/h.

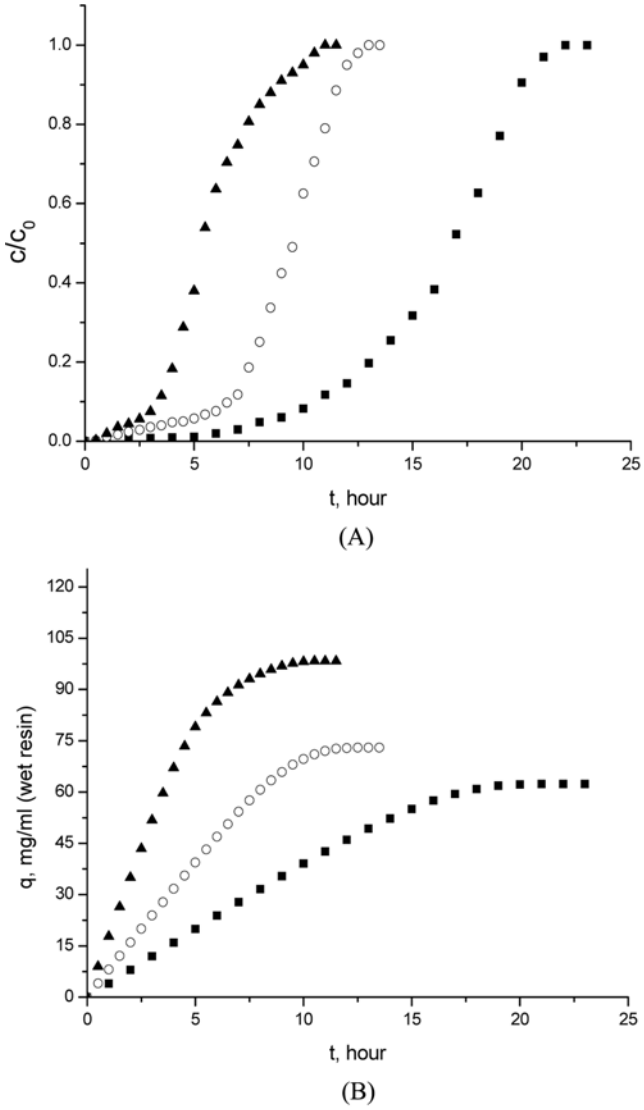

Fig. 2. Effects of inlet concentration on the breakthrough curves (A) and the exchange rate (B). u=2 BV/h, pH=5.0; c_0: ▲ 8.98 g/L; ○ 4.06 g/L; ■ 2.01 g/L.

in the solution at various pH values can be calculated theoretically. Dissociation curves of SAM in aqueous solution at different pH values were figured out in our previous work [Chen et al., 2005]. According to previous results on the ion-exchange equilibrium and interaction kinetics of SAM^+/H^+ system, pH 5.0 should be favorable to the SAM separation. Our experimental results supported it as in Fig. 3. With the pH values changing from 5.0 to 3.0, the breakthrough volume and the exchange rate in column process declines, indicating that pH 3.0 was unsuitable for the separation of SAM. The reason could be that the employed resin hardly dissociated at pH 3.0, and more resins were needed to exchange one mole of SAM when lower pH SAM extract was subjected. It was also shown that the breakthrough time of SAM would advance when the pH value varied from 5.0 to 7.0. According to the dissociation curves, the ionic form of SAM would shift from SAM^+ to SAM^0 when the pH of feed liquid was adjusted to higher than 5.0 by NaOH solution. Moreover, the existing Na^+ ions in the feed liquid would compete with SAM^+ for the exchange with H^+ ions on the resin. The best adsorption behavior was obtained when the pH of SAM extract was adjusted to 5.0. The saturated exchange capacity at pH 5.0 was around two times as that of pH 3.0 and about 1.5 times as that of pH 7.0. As a conclusion, pH vale of feed solution was very important and needed to be adjusted to 5.0 for the good performance of SAM adsorption in this fixed-bed ion exchange operation.

2. Determination of Desorption Requirements

After SAM was adsorbed by JK110 resin, H_2SO_4 solution was selected as the eluent to perform the desorption of SAM from the saturated resin phase. In the literature [Fiecchi, 1977; Gennari and Fedrico, 1991], it was reported that SO_4^{2-} ion was more favorable for the stability of SAM than Cl^-, I^-, etc. The effects of the concentration of H_2SO_4 solution and the eluting flow rate on the elution curves were investigated. As shown in Fig. 4(A), the concentration of H_2SO_4 solution affects greatly the elution curves. With the H_2SO_4

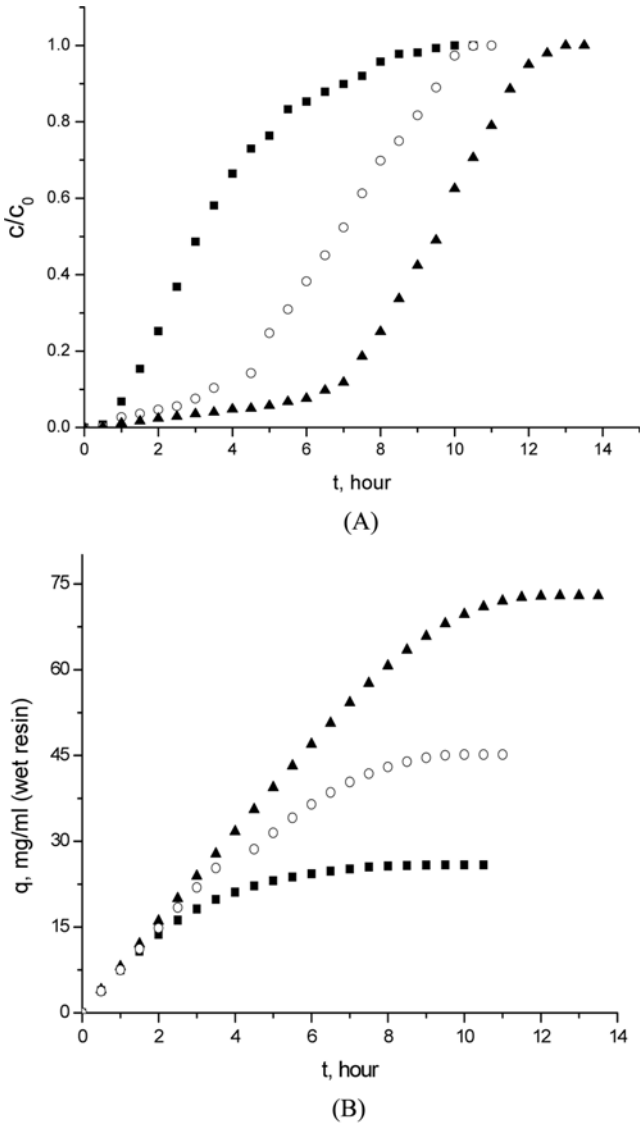

Fig. 3. Effect of pH on the breakthrough curves (A) and the exchange rate (B). c_0=3.86 g/L, u=2 BV/h; pH: ■ 3.0; ▲ 5.0; ○ 7.0.

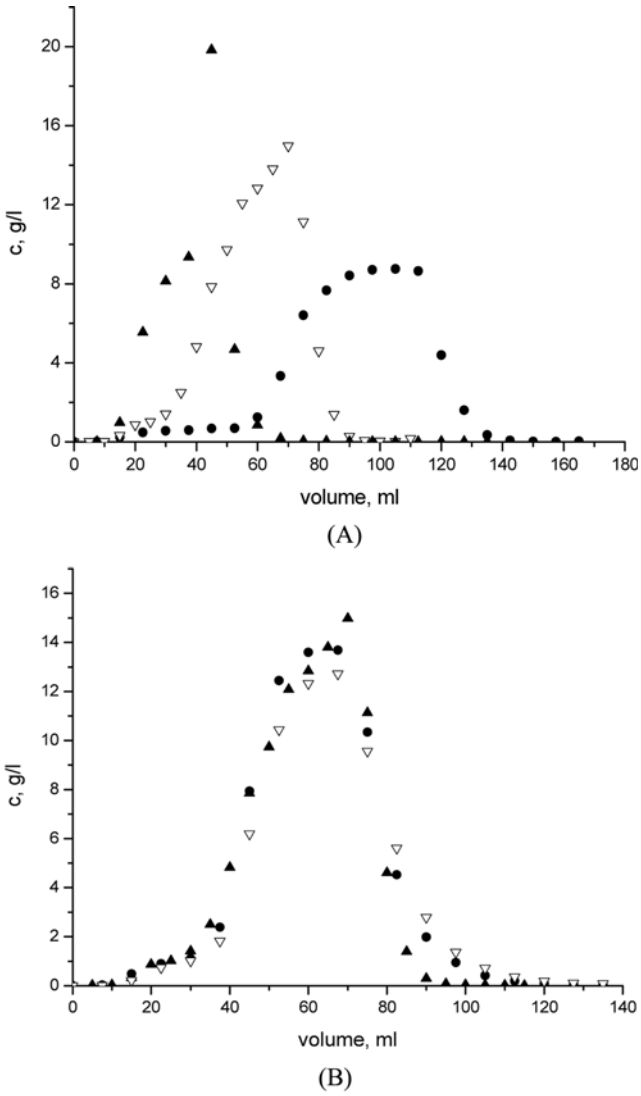

Fig. 4. Effect of H_2SO_4 concentration (A) and flow rate (B) on elution curves. (A): elution rate=1 BV/h, H_2SO_4 concentration: ● 0.1 N; ▽ 0.2 N; ▲ 0.4 N; (B): 0.2 N H_2SO_4 solution, elution rate: ▲ 1 BV/h; ● 2 BV/h; ▽ 3 BV/h.

concentration increasing, the eluting peak focuses and the amount of eluent used sharply decreases (Table 3). Contrary to this, the flow rate had little effect on the elution results as shown in Fig. 4(B); considering the recovery ratio and production efficiency, the elution process of SAM was carried out by 0.2 N H_2SO_4 solution at a flow rate of 2 BV/h.

In addition, this process of fixed-bed ion exchange was successfully scaled up at a large column (L=70 cm, D=5.5 cm). The adsorption of SAM was carried out when 10 g/l SAM solution (pH 5.0) was fed at a flow rate of 2 BV/h. Then desorption was performed with 0.2 N H_2SO_4 solution at a flow rate of 2 BV/h. According to a material balance, the total yield of SAM purified by the whole ion-exchange process was 90.1%. After desorption, the eluate was concentrated and then crystallized with methanol. The white powders obtained were analyzed by HPLC and the purity was 95.1%.

CONCLUSIONS

Table 3. Effects of H_2SO_4 concentration on the eluent volume and elution efficiency

H_2SO_4 concentration	Elution volume (mL)	Elution efficiency (%)
0.1 N	120	86.9
0.2 N	75	91.9
0.4 N	60	68.9

The dynamic column process of SAM separation was carried out at various experimental requirements. The effects of flow rate, inlet concentration and pH of the feed liquid on the breakthrough curves were investigated. The pH vale of the feed liquid would affect the adsorption of SAM in JK110 resins greatly. The optimal bioprocess for the ion-exchange separation of SAM was achieved when 4.06 g/l SAM extract (pH 5.0) was fed with a rate of 2 BV/h. The suitable elution requirements were further examined by investigating

the effects of the concentration of H_2SO_4 and elution rate. Under optimal conditions, the recovery yield of SAM for this ion-exchange separation attained 90.1% even when this ion-exchange process was scaled up in large scale.

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NOMENCLATURE

c_0 : initial concentration of SAM [g/*l*]
c_t : the concentration of SAM in the solution at t time [g/*l*]
c_1 : the concentration of SAM in the eluent [g/*l*]
q_t : ion-exchange capacity of SAM on JK110 resin at t time [mg/g (wet resin)]
v : volume of the extract [ml]
v_1 : volume of the eluent [ml]
w : weight of wet resin [g]
η : elution efficiency [%]

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