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Separation and Concentration of Adenosine Triphosphate and Adenosine Monophosphate by Using Two Chromatographic Columns

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Abstract

A new technique is proposed for simultaneous separation and concentration. Adenosine triphosphate (ATP) and adenosine monophosphate (AMP) as model solutes were separated and concentrated by using two fixed-bed columns. Appropriate adsorbents were selected from measurements of adsorption isotherms. Intraparticle effective diffusivities were determined from batchwise adsorption. Experimental breakthrough curves were in good agreements with calculated ones for columnwise adsorption. ATP and AMP could be concentrated about 18 times of initial concentrations for columnwise elution.

INTRODUCTION

Bioproducts usually involve multisolutes in dilute solutions. In this work, a new technique is proposed by which each component can be separated and concentrated by using chromatographic columns. The number of columns corresponds to the number of species to be separated. Two kinds of nucleotide monomers, adenosine triphosphate (ATP) and adenosine monophoshate (AMP), are used to test this technique.

ATP plays an important role in many enzymatic pathways such as the synthesis of polysaccharides, lipids, polypeptides, and nucleic acids (*1*). ATP and AMP, however, exist together in a mixture because the reaction of ATP to AMP is reversible in such enzymatic pathways.

In this paper, simultaneous separation and concentration of ATP and AMP are investigated by our proposed technique.

PRINCIPLE

Figure 1 shows the principles of separation and concentration of two solutes by using two columns.

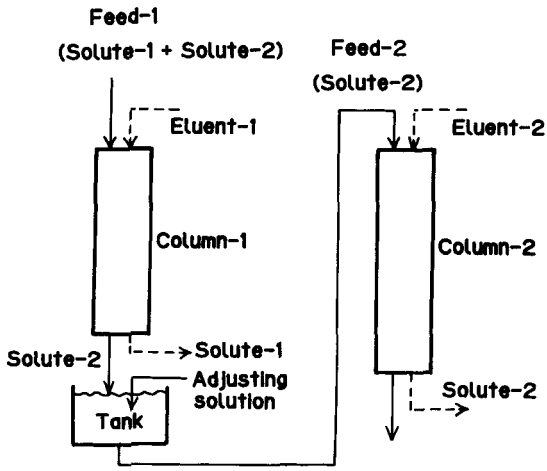

FIG. 1. Principle of separation and concentration by using two columns.

Feed-1 involves both Solute-1 and Solute-2 in a dilute solution. Solute-1 can be selectively adsorbed in Column-1 while Solute-2 flows without adsorption. Following Column-1, the solution is stored in a tank.

At the breakthrough point of Solute-1, the flow is switched from Feed-1 to Eluent-1. Solute-1 can be desorbed by Eluent-1 and concentrated at the exit of Column-1.

An adjusting solution is added in the tank to improve the adsorption capacity of Solute-2 in Column-2, if necessary. Feed-2, which involves Solute-2, is prepared and flowed through Column-2. Solute-2 can be adsorbed in Column-2 until the breakthrough point is reached. Concentrated Solute-2 can be obtained by using Eluent-2.

More than two kinds of solutes can be separated and concentrated if the same procedure is continued by using the corresponding number of columns packed with suitable adsorbents.

EXPERIMENTAL

Materials

Adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) were purchased from Oriental Yeast Co., Japan.

The adsorbent to adsorb ATP in the first column was a weakly basic anion-exchange resin, FD-DA12 (manufactured by Mitsubishi Kasei Co.,

Japan), a hydrophilic polyvinyl, hard porous polymer with a diethyl amino function. The sizes of 125 particles were measured by microphotography, and the average diameter of the resin was 0.0644 mm.

The adsorbent to adsorb AMP in the second column was a strong basic anion-exchange resin, Dowex 1-X2, in the Cl form (purchased from Dow Chemical Co.). The average diameter was determined to be 0.172 mm from microphotography measurements.

The buffer solutions were composed of 0.05 *M* Trisbase and 0.05 *M* (or 0.2 *M*) NaCl. The pH was adjusted by adding HCl solution in the buffer. Trisbase was purchased from Sigma Chemical Co., USA.

Apparatus and Operation Conditions

Column-1 (25.2 cm high, 1 cm i.d.) was a glass tube packed with FP-DA 12. The second column was a glass tube (7.5 cm high, 0.6 cm i.d.) packed with Dowex 1-X2 in the Cl form.

Feed-1 contained both ATP and AMP in Buffer-A1 (0.05 *M* Tris-base + 0.05 *M* NaCl, pH 7). ATP was selectively adsorbed in Column-1. The solution exiting Column-1 was collected until the breakthrough point of ATP was reached. Then the pH of this solution was adjusted up to 10 by adding the concentrated NaOH solution in order to satisfy the adsorption condition in Column-2. However, the concentration of NaCl increases to about two times that of the original solution, and then the adsorption capacity of AMP decreases in Column-2. For this reason the same volume of 0.05 *M* Trisbase solution was added. Finally, Feed-2 contained only AMP in Buffer-A2 (0.05 *M* Trisbase + 0.05 *M* NaCl, pH 10) and the feed concentration of AMP was decreased to half that of Feed-1.

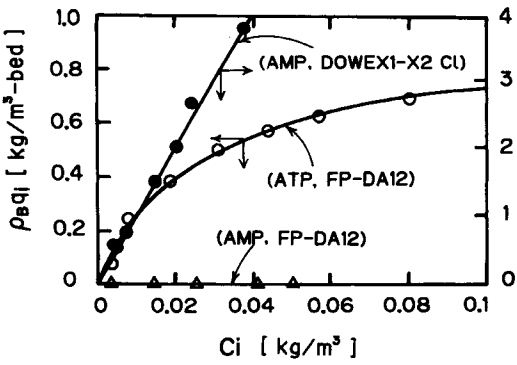

FIG. 2. Adsorption isotherms of ATP and AMP.

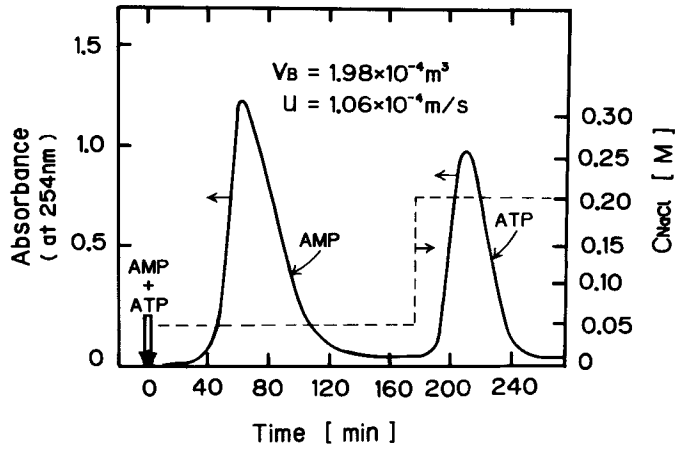

FIG. 3. Complete separation of ATP and AMP in pulse response.

Buffer-E (0.05 *M* Trisbase + 0.2 *M* NaCl, pH 7) was used for both Eluent-1 and Eluent-2 to desorb ATP and AMP in Column-1 and Column-2, respectively.

All experiments were performed at 298 K. The absorbance of ATP and AMP in the buffer solutions at 254 nm was measured with a spectrophotometer (Shimazu UV-150).

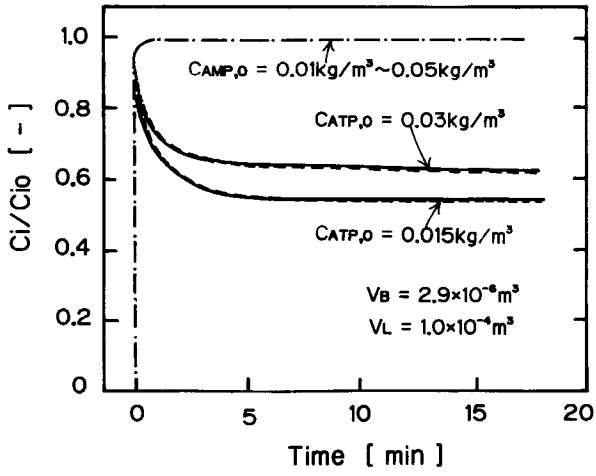

FIG. 4. Experimental and calculated results for batchwise adsorption of ATP and AMP by FP-DA 12.

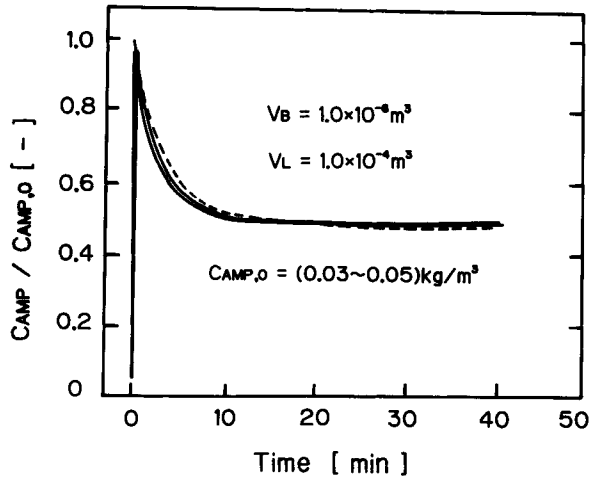

FIG. 5. Experimental and calculated results for batchwise adsorption of AMP by Dowex 1-X2 in the Cl form.

RESULTS AND DISCUSSIONS

Adsorption Isotherm

The equilibrium adsorption isotherms of ATP and AMP were measured. Test tubes containing FP-DA 12 and Dowex 1-X2 in the Cl form were immersed in the ATP and AMP solutions and shaken in a thermostat at 298 K for 2 days. The adsorption capacities were calculated from the

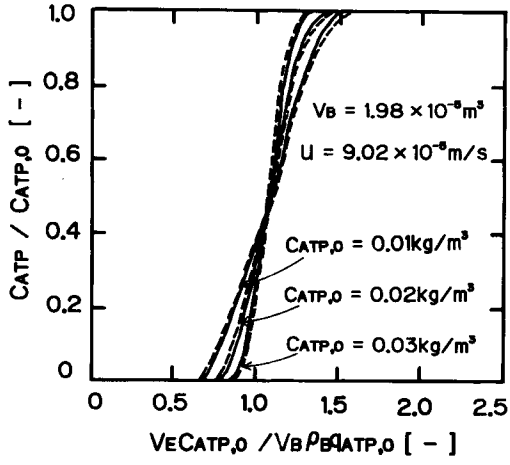

FIG. 6. Effect of feed concentration on breakthrough curves of ATP in Column-1.

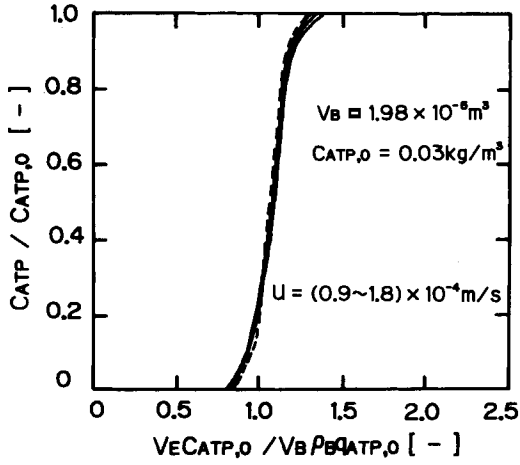

FIG. 7. Effect of liquid velocity on breakthrough curves of ATP in Column-1.

differences between the initial and final concentrations of ATP and AMP in the solutions.

The results are shown in Fig. 2. The adsorption isotherm of ATP on FP-DA 12 in Buffer-A1 was found to have a good fit to a Langmuir-type expression as given by the equation

$$\rho_B q_{ATP}/0.95 = 35 \times C_{ATP}/(1 + 35 \times C_{ATP}) \quad (1)$$

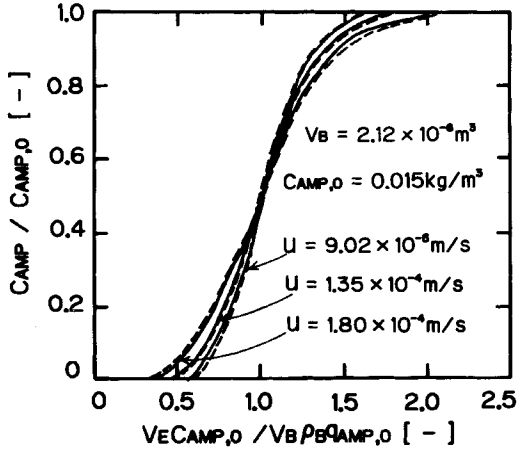

FIG. 8. Effect of feed concentration on breakthrough curves of AMP in Column-2.

However, AMP was not adsorbed in the same conditions. Therefore, ATP can be selectively adsorbed on FD-DA 12. This selective adsorption may be explained by the stronger affinity of ATP with three phosphoric acid radicals to the anion-exchange resin than AMP with one phosphoric acid radical in the neutral buffer solution (pH 7).

In order to find a suitable adsorbent for the adsorption of AMP, many kinds of ion-exchange resins were investigated under various conditions. Finally, Dowex 1-X2 in the Cl form was chosen because of its excellent adsorption ability in Buffer-A2 (pH 10) and easy regeneration in Buffer-E (0.2 *M* NaCl).

The adsorption isotherm of AMP on Dowex 1-X2 in the Cl form in Buffer-A2 was obtained by a linear equation:

$$\rho_B q_{AMP} = 101 \times C_{AMP} \tag{2}$$

As the concentration of NaCl in the buffer was increased from 0.05 to 0.2 *M*, the adsorption capacities of both AMP and ATP decreased and became almost zero in Buffer-E. An increase of ionic strength in the buffer solutions causes a decrease of affinity between adsorbate and adsorbent.

Chromatograms of ATP and AMP on a FP-DA 12 Column

The rapid partial separation of ATP and AMP was tested in Column-1 packed with FD-DA 12 by utilizing a stepwise increase of NaCl, as shown in Fig. 3.

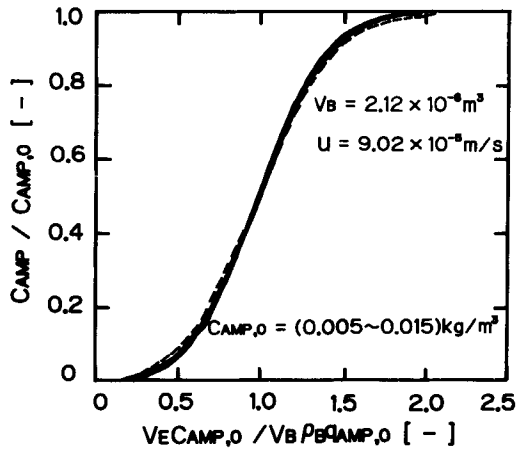

FIG. 9. Effect of liquid velocity in breakthrough curves of AMP in Column-2.

When a mixture of ATP and AMP was pulse injected, the sharp peak of AMP immediately appeared because AMP was not adsorbed by the FP-DA 12 resin. ATP was tightly adsorbed in the same conditions.

When the NaCl concentration in the buffer was increased from 0.05 to 0.2 *M*, the ATP peak appeared. Therefore, a mixture of AMP and ATP can be separated by using FP-DA 12 resin. However, ADP cannot be separated from ATP because the ADP peak overlaps that of ATP.

Batchwise Adsorption

Batchwise adsorption experiments were carried out in a slurry bubble column with air to determine the intraparticle effective diffusivity.

The transient concentration profiles of ATP in Buffer-A1 for batchwise adsorption on FP-DA 12 are shown as solid lines of Fig. 4 for two initial concentrations. The broken lines in Fig. 4 indicate the calculated curves obtained from the model of intraparticle mass transfer rate-control (*2*). The intraparticle effective diffusivity was calculated to be 2.2×10^{-11} m^2/s through comparison of the experimental data with the calculated curves.

Since AMP was not adsorbed on FP-DA 12 in Buffer-A1, its transient concentrations are always equal to the initial ones, as shown in Fig. 4.

Figure 5 shows the transient concentration profiles of AMP in Buffer-A2 on Dowex 1-X2 in the Cl form for three initial concentrations. Since the adsorption isotherm is linear, as shown in Fig. 2, the normalized profiles, C/C_0, are independent of the initial concentrations. From a comparison of the experimental data (solid lines) with the calculated curve

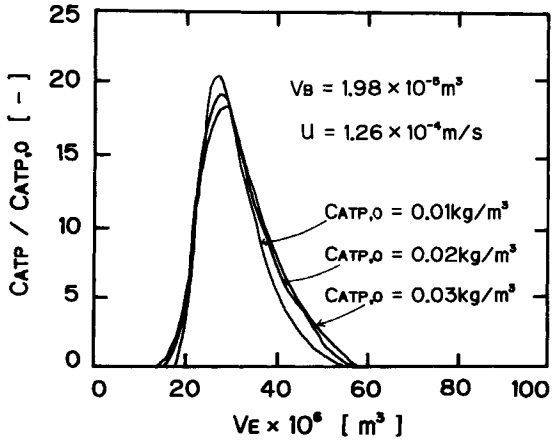

FIG. 10. Elution curves of ATP in Column-1.

(broken line), the intraparticle effective diffusivity was determined as 1.2×10^{-10} m^2/s.

Columnwise Adsorption

Based on preliminary experiments on the adsorption isotherm and batchwise adsorption, ATP and AMP were chosen as Solute-1 and Solute-2 in Fig. 1, respectively.

Figures 6 and 7 show the breakthrough curves of ATP in Column-1. The solid lines show the experimental results and the broken lines show the calculated results obtained by assuming an approximate linear driving force (*2*).

As the feed concentration of ATP increases, the slopes of the breakthrough curves become steeper, as shown in Fig. 6. The breakthrough curves in Fig. 7 are independent of liquid velocities because the resistance of liquid–solid mass transfer is not significant.

Figures 8 and 9 show the breakthrough curves of AMP in Column-2. The feed concentrations of AMP are independent of the breakthrough curves, as shown in Fig. 8, due to the linear isotherm in Fig. 2. Liquid velocities, however, affect the breakthrough curves (see Fig. 9).

Columnwise Elution

Buffer-E was used for the elution steps for both Column-1 and Column-2. Figure 10 shows the elution curves of ATP after columnwise adsorption in Fig. 6. When the eluent was loaded to Column-1, ATP was

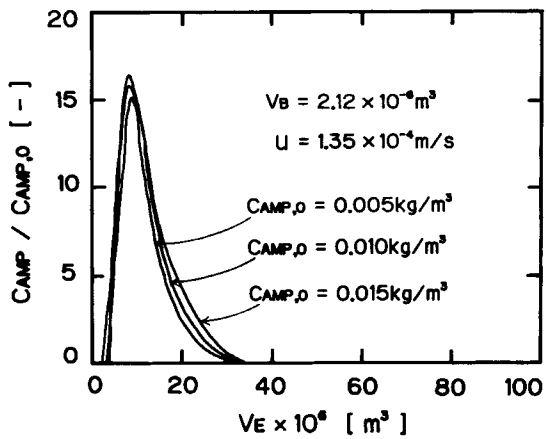

FIG. 11. Elution curves of AMP in Column-2.

easily desorbed. The maximum concentration at the exit was about 18 times larger than the initial concentration.

Figure 11 shows the elution curves of AMP after columnwise adsorption in Fig. 8. AMP was concentrated by about 16 times the initial concentration.

The adsorption and elution procedure in the two columns could be repeated more than 10 times without any change of resins.

CONCLUSION

ATP and AMP have been separated from each other and concentrated by the proposed technique. The experimental results agree with the theoretical calculation. Any multisolutes may be separated and concentrated by choosing the appropriate adsorbents and operating conditions.

SYMBOLS

$C_{AMP,0}$	feed concentration of AMP (kg/m^3)
$C_{ATP,0}$	feed concentration of ATP (kg/m^3)
C_i	concentration of i-component (ATP or AMP) (kg/m^3)
D_e	intraparticle effective diffusivity (m^2/s)
q_i	adsorbed amount (kg/kg)
u	superficial velocity (m/s)
V_B	volume of adsorbent (m^3)
V_E	volume of effluent (m3)
V_L	volume of liquid (m^3)
ρ_B	density of fixed bed (kg/m^3)

REFERENCES

1. O. Miyawaki, K. Nakamura, and T. Yano, *J. Chem. Eng. Jpn., 15,* 142 (1982).
2. B. L. Yang, M. Goto, and S. Goto, *Colloids Surf., 37,* 369 (1989).

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