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Complete Separation of Albumin and Hemoglobin by Metal Chelate Affinity Chromatography

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Abstract

Hemoglobin was adsorbed on Zn^{2+} chelating Sepharose 6B but albumin was not adsorbed. Then albumin and hemoglobin could be separated completely by metal chelate affinity chromatography. In a conventional columnwise adsorption step, unusual breakthrough curves were observed because some parts of metal ions could be released from the adsorbent. A combined process with batchwise adsorption and columnwise elution was used to avoid this problem and to shorten the operating time.

INTRODUCTION

Metal chelate affinity chromatography was originally proposed by Porath et al. (*1*) in 1975 and has been developed as a useful method for protein purification. It is based on the interaction between a metal ion and histidine or cysteine exposed on the surface of protein. Metal chelate affinity chromatography is usually operated in a column with four steps, such as adsorption, washing, elution, and washing. When proteins in a feed solution are very dilute, columnwise adsorption requires the passage of a large quantity of liquid through the column. High flow rates result in poor adsorption efficiency. The mechanically soft properties of the chelate affinity adsorbent may also preclude high flow rates due to consolidation. Under these circumstances, columnwise adsorption requires a very long operating time. Also, since metal ions have a higher affinity for proteins to be separated than for the matrix in some cases, they may be released from the matrix and flow together with the solution during the adsorption step. Although the release of metal ions may be prevented by tightening the interaction during a coupled operation (for example, by changing the pH of a metal ion solution), proteins cannot be eluted in a elution step.

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Like other adsorption operations, metal chelate affinity adsorption can be done in a stirred slurry tank. In this case, since all of the adsorbent particles can be contacted by proteins in the solution at the same time, the adsorption rate will be large. The operating time for metal chelate affinity separation can therefore be reduced by adopting a batchwise adsorption step.

On the other hand, the elution step is generally carried out easily by changing either the pH or the concentration of the buffer salt. It requires a much shorter time because only a small quantity of eluent is used. Therefore, the combination of batchwise adsorption and columnwise elution should produce efficient and rapid purification. The combined process has been used for the purification of albumin (*2*) and trypsin (*3*) in our previous work.

The purpose of the present work is to extend the idea of the combined process to binary systems for the separation and purification from a mixture solution. Albumin and hemoglobin were chosen.

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EXPERIMENTAL

Materials

Albumin bovine was purchased from Sigmal and hemoglobin bovine was purchased from Wako. The adsorbent was prepared by charging with metal ions by elution with an excess solution of $ZnCl_2$ having a concentration of 3 mg/mL and with the pH adjusted to 6.1 to Chelating Sepharose 6B (from Pharmacia). The average diameter of the adsorbent was 0.103 mm. The buffer solution was composed of 0.8 *M* NaCl plus 25 m*M* $Na_2HPO_4 \cdot 12H_2O$, pH 8.0 (denoted as buffer A). The eluent was of 0.8 *M* NaCl plus 0.1 *M* $Na_2HPO_4 \cdot 12H_2O$, pH 6.5 (denoted as buffer E). After each experiment, the Zn^{2+} was stripped with 0.05 *M* EDTA, pH 7.0, and the charging operation was repeated.

Apparatus

All experiments were performed at 298 K. Figure 1 shows the experimental apparatus constructed to carry out the combined operation. It consisted of a stirred slurry tank for adsorption and a fixed-bed column for elution. The volume of the stirred slurry tank was 0.25×10^{-3} m^3. The inside diameter of the fixed-bed column was 6.0×10^{-3} m.

The combined process is carried out as follows. The adsorbent is first transferred to the lower column to form a fixed bed. After the adsorbent is completely washed with water and then buffer A, it is moved to the upper stirred slurry tank with buffer A for the adsorption operation. For a comparison of batchwise and columnwise in the adsorption step, columnwise adsorption was carried out by using the lower column.

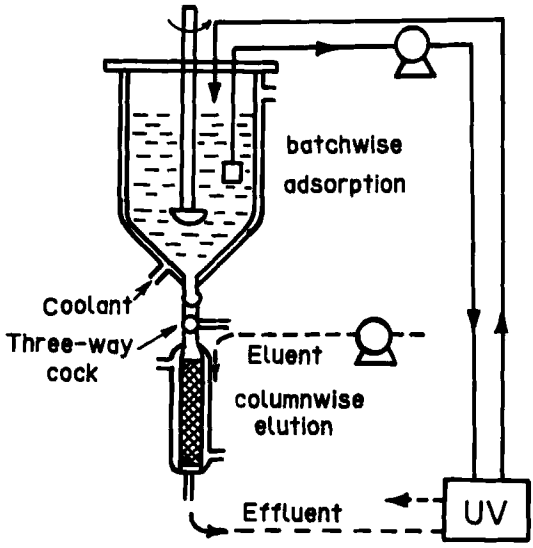

FIG. 1. Experimental apparatus for the combined process.

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RESULTS AND DISCUSSION

Adsorption Isotherm

A series of batchwise adsorptions were carried out to obtain equilibrium capacities at 298 K. Different initial concentrations of albumin or hemoglobin in the solution of buffer A were prepared. The final concentrations after adsorption were used as the equilibrium values. The capacities were calculated from the difference between the initial and final concentrations of albumin or hemoglobin in the solution.

The results are shown in Fig. 2. Albumin was not adsorbed, and the adsorption isotherm is expressed as

$$\rho_B q_A = 0 \tag{1}$$

Hemoglobin could effectively be adsorbed, and the isotherm is expressed by the following Langmuir-type equation:

$$\rho_B q_H = 47.7 \times 14C_H/(1 + 14C_H) \tag{2}$$

The maximum adsorption was $\rho_B q_m = 47.7$ kg/m^3.

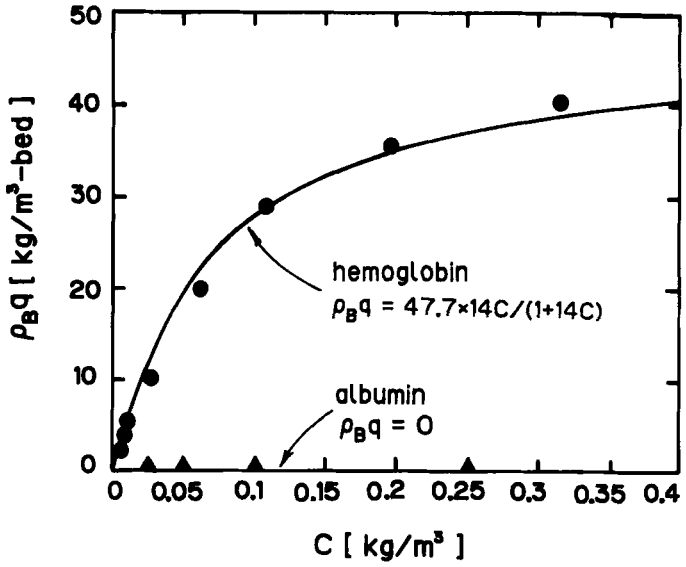

FIG. 2. Adsorption isotherm.

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Pulse Response

The complete separation of albumin and hemoglobin was first tested in a lower column by the pulse method. As shown in Fig. 3, when the binary mixture of proteins in buffer A was introduced in a column packed with the Zn^{2+} load chelating gel, the albumin peak was detected because albumin was not adsorbed. However, the hemoglobin was not detected due to complete adsorption. When the solution was changed from buffer A to buffer E, the hemoglobin peak appeared. Albumin and hemoglobin could be separated completely by using buffers A and E.

Batchwise Adsorption

The transient profile of the bulk liquid concentration in buffer A for batchwise adsorption is shown in Figs. 4 and 5. The value of bed volume V_B in these figures was determined from the volume of the fixed bed before the adsorbent was moved to the upper stirred tank. The effect of initial concentrations of hemoglobin is shown in Fig. 4. As the initial concentrations decreased from 0.2 to 0.05 kg/m^3, the transient profiles become steeper.

Since albumin was not adsorbed, the transient concentrations were always equal to the initial ones.

The effect of stirring speed on the adsorption of hemoglobin is shown in Fig. 5. An increase in the stirring speed in a given range did not affect

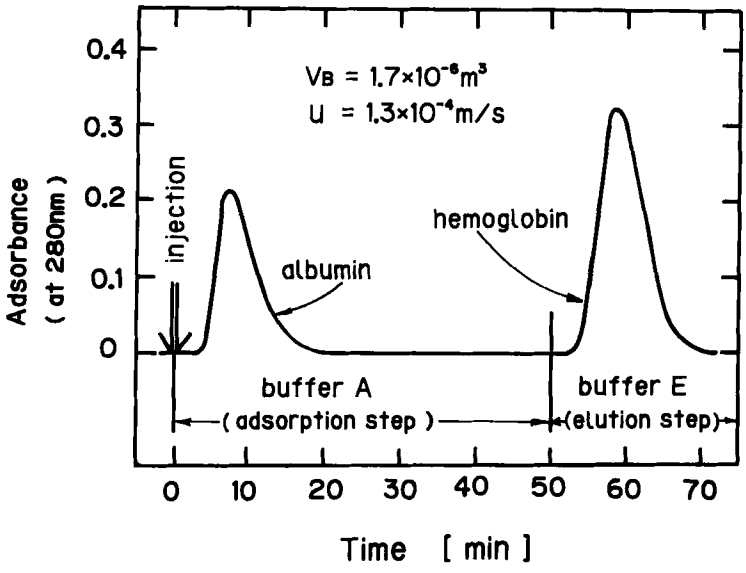

FIG. 3. Complete separation of albumin and hemoglobin in pulse response.

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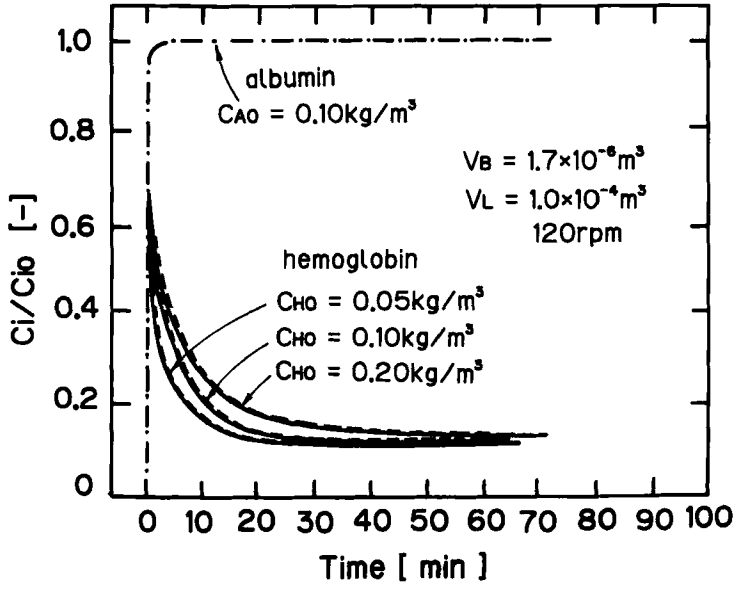

FIG. 4. Experimental and calculated results for batchwise adsorption.

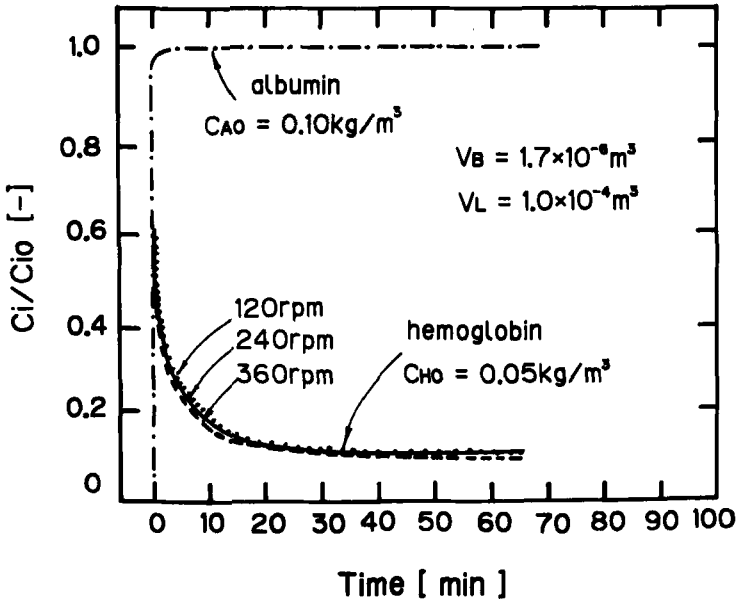

FIG. 5. Effect of stirring speed for batchwise adsorption.

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the transient profiles for the adsorption operation. This demonstrates that the adsorption process of hemoglobin in chelate gel is not affected by film mass transfer but is largely governed by intraparticle diffusion. In order to protect the mechanically soft chelate gel, higher stirring speeds are not recommended. The following experiments were fixed at 120 rpm.

The broken lines in Fig. 4 indicate the calculated curves which were obtained from mass balance equations for the particles (*2*). The intraparticle effective diffusivity was evaluated to be 1.17×10^{-11} m²/s as a result of comparison of the experimental data with the calculated curves.

Columnwise Adsorption

Figures 6 and 7 show the breakthrough curves obtained from the columnwise adsorption in buffer A. The peaks in the breakthrough curves are about two times the initial concentration. This unusual behavior may be explained by the release of metal ions from the adsorbent. When metal ions are released from the chelating gel, hemoglobin coupled with metal ions may also be detached from the adsorbent. Hemoglobin is concentrated during the adsorption step. The phenomenon of metal ion release from the gel was observed for peptides bound to Ni^{2+} chelating gel by Hochuli et al. (*4*).

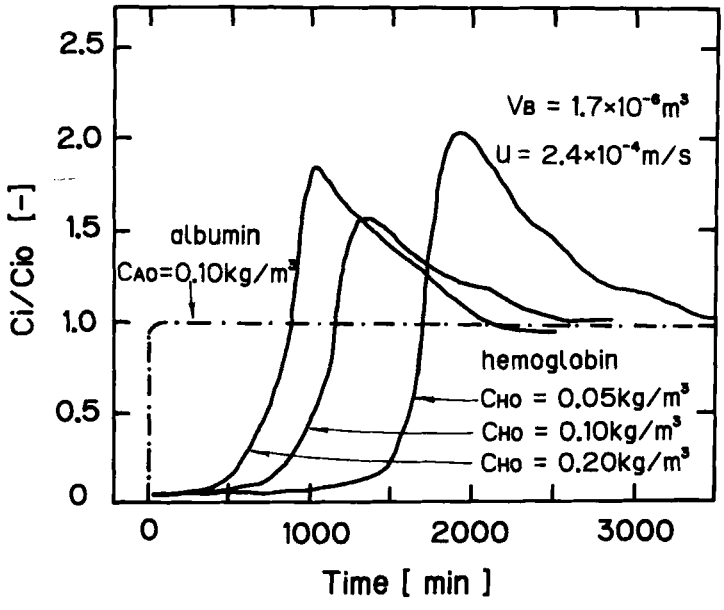

FIG. 6. Effect of feed concentration in breakthrough curves for columnwise adsorption.

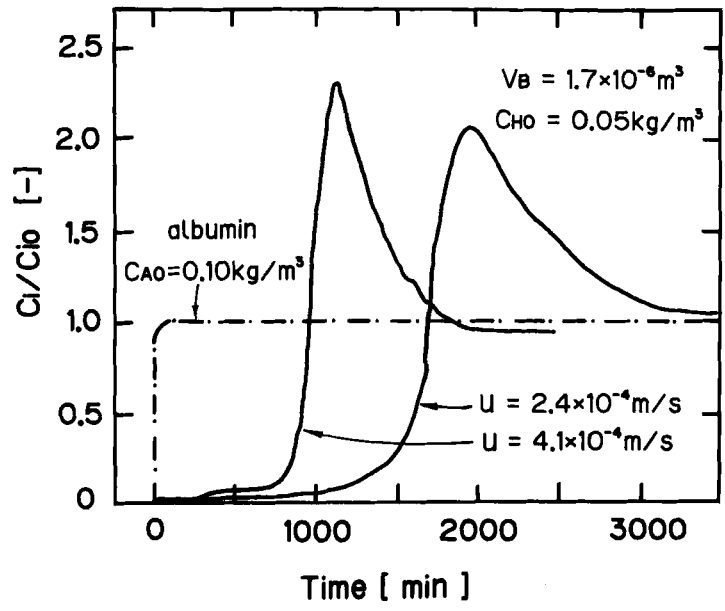

FIG. 7. Effect of liquid velocity in breakthrough curves for columnwise adsorption.

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Since the Zn^{2+} chelating gel has a high adsorption capacity for hemoglobin, a long time was required to reach the breaking point.

As the feed concentration of hemoglobin is increased, the time to the break point becomes shorter, as shown in Fig. 6. The exit concentration of albumin is always equal to the feed concentration because there is no adsorption of albumin.

The results of the investigation of different liquid velocities are shown in Fig. 7. As the liquid velocity becomes higher, the time to the break point becomes shorter. In order to shorten the operation time, the liquid velocity was increased further. However, a liquid velocity of 4.1×10^{-4} m/s was the upper limit due to the very high pressure drop.

Columnwise Elution

Buffer E was used for the elution step. Figure 8 shows the elution curves after both the batchwise adsorption in Fig. 4 and the columnwise adsorption in Fig. 6 with the feed concentration of 0.05 kg/m^3. The time required for elution was short. The elution was completed in about 25 min for columnwise adsorption and 30 min for batchwise adsorption when the liquid velocity was 3.5×10^{-4} m/s. No traces of albumin appear in either curve, which means that the albumin and hemoglobin were completely separated by the Zn^{2+} chelating gel. It should be noted that the elution quantity after

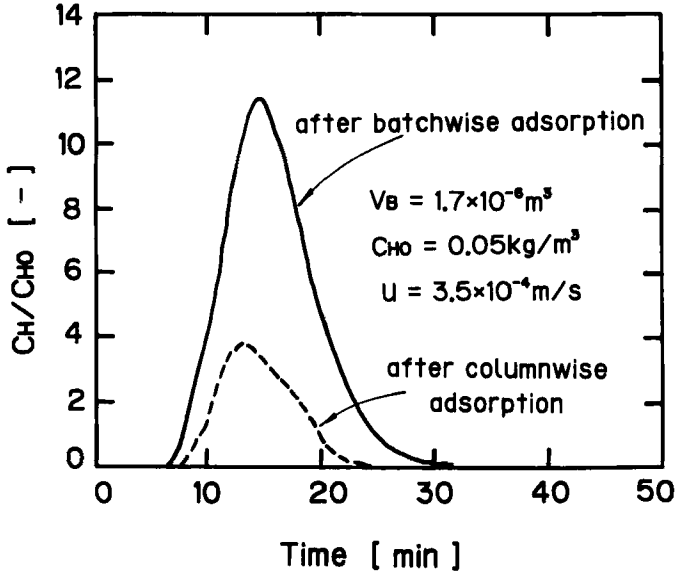

FIG. 8. Elution curves after batchwise and columnwise adsorptions.

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the columnwise adsorption (broken line) is much less than the elution quantity after the batchwise adsorption (solid line).

The maximum elution ratio after columnwise adsorption was about 4, while that after batchwise adsorption was about 12. Some parts of the hemoglobin adsorbed were already eluted in the adsorption step of the columnwise operation, as shown in Fig. 6. The total quantity of hemoglobin eluted during adsorption and the elution step is approximately equal to the quantity adsorbed. This kind of phenomenon was not found in batchwise adsorption. This may be explained by the presence of metal ions in the liquid phase during the adsorption step.

CONCLUSION

The Zn^{2+} chelate affinity adsorbent has high selectivity. The use of a combined batchwise adsorption and columnwise elution process leads to efficient and complete separation of albumin and hemoglobin in a short time. This method can be used for other separation processes.

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SYMBOLS

C_i	concentration of solute i (kg/m^3)
C_{i0}	feed concentration of solute i (kg/m^3)
$\rho_B q$	adsorbed amount (kg/m^3)
t	time (s)
u	superficial velocity (m/s)
V_B	volume of adsorbent (m^3)
V_L	volume of liquid (m^3)

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