AFFINITY PURIFICATION BY TAPERED BED

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Key Words: Adsorption, Affinity, Elution, Pressure Drop, Tapered Bed, Fixed Bed, Fluidized Bed

Introduction

Affinity chromatography plays a significant role in the separation and purification of biologically active macromolecules. It is generally operated in a fixed-bed mode by using a cylinder column. However, there are some operating problems in this method; (1) the bed easily clogs when crude extracts or high by viscous materials are treated; (2) adsorbent beads with mechanically soft properties are destroyed at the high flow rate; and (3) pressure drops may be high.

A fluidized bed can be used for affinity purification²⁾. However, due to well-mixing, the adsorption efficiency is low. To solve these problems, we have proposed a method that combines batchwise adsorption with columnwise elution^{4, 5)}, although the operation is discontinuous.

In this work, a tapered bed with continuous upflow is used rather than a straight cylindrical fixed bed to improve the performance of affinity chromatography. The superficial liquid velocity decreases with vessel height, and a longer retention time of separation component as well as higher operation efficiency can be obtained than in a fluidized bed. This kind of apparatus has been successfully used in continuous culture³⁾ and crystal growth processes¹⁾.

To demonstrate the efficiency of this operation, human serum albumin was purified by Blue-Sepharose CL-6B. The results for adsorption, elution and pressure drop are compared with those with the fixed-bed method.

1. Experimental Apparatus and Method

A schematic diagram of the experimental apparatus is shown in **Fig. 1**. The inside diameter of the inlet tapered bed was 0.88 cm and the tapered angle was 5.4°. The adsorbent Blue-Sepharose CL-6B 2 g (made by Pharmacia Co.Ltd, the average diameter 84 μm) was used. The initial height of bed was 7 cm. As the liquid

passed through, the bed was expanded up to 9.3 cm. The pressure drop was measured by a manometer. The absorbance of albumin in the buffer solution was measured at 280 nm by a spectrophotometer. All the apparatus was kept at 293 K. A straight column of 1 cm ID was used for comparison with the tapered bed. The height of the bed packed with 2 g of the adsorbent in the straight column was 9.2 cm, which was nearly equal to that of the tapered bed at the expanded state. The feed was downwardly introduced into the straight column to confirm the state of fixed bed. The voidage was kept at 0.271 independently of liquid flow rate. The buffer solution was composed of 50 mol/m³ Tris-HCl plus 50 mol/m³ NaCl, pH 8.0. The eluent was 200 mol/m³ sodium thiocymate plus 50 mol/m³ Tris-HCl, pH 8.0.

2. Results and Discussion

2.1 Adsorption isotherm and intraparticle effective diffusivity

A series of batchwise adsorption experiments were carried out to obtain the adsorption isotherm and the intraparticle effective diffusivity at 293K. The results show that the isotherm equation corresponds to the Langmuir type:

$$q = 0.075 \times 4.3 C / (1 + 4.3 C) \quad (1)$$

Intraparticle effective diffusivity was evaluated to be $1.05 \times 10^{-11} \text{ m}^2/\text{s}$ by the curve-fitting method.

2.2 Breakthrough curves

Figure 2 shows the breakthrough curves of albumin of different adsorption methods under the same operation conditions. The calculated results are shown by solid lines. In the calculation of the fixed-bed, the pore diffusion-controlling and linear driving forces were assumed. Similar assumptions were used for calculation of the tapered bed. However, the liquid velocity was treated as a function of bed height. Although the voidage might be longitudinally varied, the average voidage was used to simplify the calculation³⁾. The value was deter-

* Received July 19, 1993. Correspondence concerning this article should be addressed to S. Goto.

Fig. 1 Schematic diagram of apparatus

Fig. 2 Comparison of breakthrough curves

mined as 0.412 from the ratio of the adsorbent volume to the total expanded volume of tapered-bed. Good agreement between calculation and experiment was obtained. By using the well-mixed model, adsorption in the fluidized bed was also calculated for comparison with the other two operations. As expected, the breakthrough curve was slightly more gentle for the tapered-bed than that for the fixed-bed because of larger bed voidage. On the other hand, it is rather steeper than that for the fluidized bed.

2.3 Elution curves

Since the adsorbed albumin is easily eluted from Blue-Sepharose CL-6B, the elution operation was finished in a shorter time. Figure 3 shows the elution curves after adsorption. There are no differences in the elution curves between the tapered-bed and the fixed-bed. The maximum elution ratio C/C_0 is about 20 times. The mass balance was almost satisfied for the affinity system in both operations.

2.4 Pressure drop

The pressure drop was also compared between the tapered-bed and the fixed-bed. To make a standard for

Fig. 3 Comparison of elution curves

Fig. 4 Comparison of pressure drops

comparison: (1) the resistance of the apparatus was eliminated by subtracting the pressure drop of the empty column, and (2) the same quantity of adsorbent (0.67 g) was used in both cases. Figure 4 shows plots of the pressure drop vs. flow rate. Because the voidage of the tapered-bed increases with increasing flow rate, the tapered-bed has a much lower pressure drop than the fixed-bed.

Conclusions

The tapered bed was evaluated for affinity chromatography. This operation indicates the following advantages: a lower pressure drop than that of the cylindrical fixed-bed without bed clogging, longer contact time between adsorbent and adsorbates, and higher purification efficiency than that of the fluidized-bed.

Acknowledgment

The authors wish to thank Mr. H. Ito for his assistance in experiments and Mr. T. Imura for his kind help in making the apparatus.

Nomenclature

C	= concentration of solute	[kg/m³]
C_0	= feed concentration of solute	[kg/m³]
P	= pressure drop in bed	[Pa]
q	= adsorbed amount	[kg/kg]
V_E	= volume of liquid	[m³]

v = flow rate in adsorption or elution [m³/s]
 W = weight of adsorbent [kg]

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