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Adsorption Performance of Proteins to CM Sepharose FF and DEAE Sepharose FF Adsorbents

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Abstract−Adsorption equilibrium and kinetics were studied for the binding of proteins to CM Sepharose FF and DEAE Sepharose FF. The influence of temperature, pH, viscosity, initial concentration and the volume of adsorbents on the adsorption characteristics was investigated in detail. The results showed that the isotherms of lysozyme to CM Sepharose FF were well described by a Langmuir-type correlation. The two phase resistance model describing the dynamic adsorption process of lysozyme, papain, BSA to CM Sepharose FF was presented, and the pore diffusion coefficients were determined by using this model and the dynamic adsorption data.

Key words: Adsorption, Ion-Exchanger, Diffusion Coefficient, Lysozyme, Protein

INTRODUCTION

Ion-exchange adsorbents have found widespread use in the purification of proteins, both in the laboratory and in the production plant, since the introduction in 1956 of the first ion exchanger specifically designed for proteins [Peterson and Sobers, 1956]. This has recently been highlighted in a study by Bonnerjea [Bonnerjea et al., 1986], which showed that ion exchangers were used in 75% of all the published purification protocols that they examined. This widespread use of ion exchangers is due to their versatility, relative cheapness and their acceptance by the regulatory authorities in the production of pharmaceutical proteins [Skidmore et al., 1990; Janson, 2001; Hong and Row, 2002].

The investigation of protein adsorption at liquid-solid interfaces is of great importance because the understanding of interaction between proteins and ion exchanger will facilitate the purification of proteins and the scale-up of the process. The characteristics of the adsorption of each protein to adsorbents were determined by different types of experiment. The equilibrium capacity of Sepharose FF for each of the proteins was established by determining adsorption isotherms. However, the adsorption of protein by an ion exchanger is not an instantaneous event and mass transfer effects must also be considered. The dynamic approach to equilibrium was therefore examined by studying the rate of uptake of protein in a shaking vessel.

Adsorption of protein to a porous ion-exchange entails the following macroscopic steps [Johnston and Hearn, 1990]: 1) protein movement from the bulk, mobile phase to the adsorbent surface layer (film diffusion); 2) protein transfer across a stagnant film layer surrounding the adsorbent particles; 3) protein diffusion into the pores of the particles (pore diffusion); and 4) adsorption of protein to the solid phase. Each of these steps contributes to the overall adsorption, but the slowest and most significant step will be the rate-controlling mechanism. External, bulk diffusion is often considered infinitely fast, resistance to film mass transfer can be minimal provided mixing is adequate, whilst pore diffusion can actually hinder adsorption, particularly if the protein size is large and the pore openings small.

The main aims of the work reported here are to study the static and dynamic adsorption characteristics of lysozyme to CM Sepharose fast flow. The same procedure will be further extended to examine the adsorption performance of papain, BSA and cellulase to adsorbents to provide an effective and general evaluation method.

MATERIAL AND METHODS

1. Material

CM Sepharose Fast Flow, DEAE Sepharose Fast Flow were all purchased from Amersham Pharmacia Biotech. Lysozyme, papain and bovine serum albumin (BSA) from Sangon Bioengineeing Co. in Shanghai, cellulase from our Institute of Bioengineering. All other chemicals used were of analytical grade and obtained from commercial sources.

2. Apparatus

The UV-1 ultraviolet spectrophotometer and REC101 chart recorder were the products of Amersham Pharmacia Biotech. Peristaltic pump from LG-PUMP Co. in Baoding, Hebei Province.

3. Buffer

Tris-HCl buffer (0.05 mol/L, pH8.0) was obtained by mixing 50 mL 0.1 mol/L Tris(hydroxymethyl)aminomethane solution with 29.2 mL 0.1 mol/L HCl, diluted into 100 mL distilled water.

4. Assay

The concentrations of lysozyme of the pure proteins were determined by measuring the optical density of the solution at 280 nm. The assays were calibrated by measuring the optical density of protein solutions of known concentration.

EXPERIMENTAL

1. Equilibrium Measurements

1 mL of 1 : 1 suspension CM Sepharose FF in buffer was added to each of a series of flasks containing known concentrations of lysozyme in Tris-HCl buffer, pH 8.0. The flasks were incubated for 18 h in a shaking water bath to allow equilibrium to be established.

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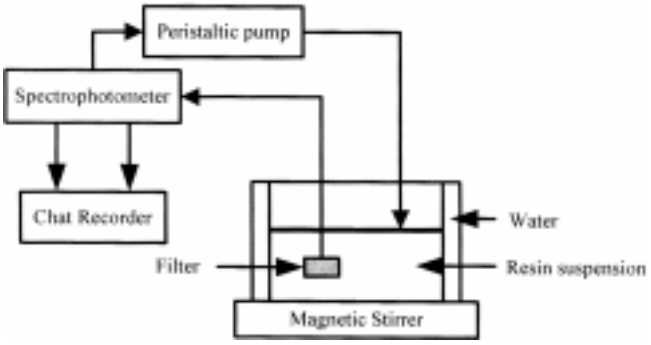

Fig. 1. Apparatus for determining the dynamic adsorption performance.

Samples were removed and centrifuged and the supernatants were assayed to determine the equilibrium concentration of protein in the soluble phase by UV spectrophotometry. The amount of protein adsorbed to the CM Sepharose FF was then calculated by mass balance. The effect of temperature variation on the equilibrium adsorption capacity was considered in this measurement.

2. Kinetic Measurements

The apparatus for determining the dynamic adsorption performance is described in Fig. 1. At a certain temperature, the UV monitor was equilibrated with buffer prior to the experiment, ascertaining the baseline of the chart recorder. Then a 100 mL known concentration of protein solution was recycled by a peristaltic pump; the liquid stream was continuously returned to the reactor so that the overall volume of the system remained constant. After a steady state, a defined amount of 1 : 1 suspension adsorbent in buffer was added into the system, and soluble phase was continuously removed from the batch reactor by passage through a filter and pumped through a continuous flow UV monitor to measure protein concentration. The output of the spectrophotometer was connected to a chart recorder so that a permanent record could be kept of the time course of reduction of protein concentration in the soluble phase. The protein concentrations were normalized by dividing the concentration C, at time t, by the protein concentration at time zero, C_0. Finally, C/C_0 was plotted against t.

The influence of pH, viscosity, initial concentration and amount of adsorbents on the kinetics was studied in this experiment.

THEORY

1. An Equilibrium Model

For adsorption behavior study on proteins to ion exchanger, the following approach was developed by Chase [Chase, 1984]

$$q^* = \frac{q_m C^*}{K_d + C^*} \tag{1}$$

where $K_d=k_2/k_1$ is the dissociation constant of the protein-ion exchanger complex, k_1 and k_2 are the adsorption and desorption rate constants, respectively. q is the adsorbed protein concentration, q_m the maximum protein capacity of the ion exchanger, C the soluble protein concentration, the superscript* denotes values when equilibrium has been established between solid and liquid phases. Eq. (1) is a form of the Langmuir adsorption isotherm, from which the dissociation constant K_d and the maximum protein capacity of the ion exchanger q_m can be calculated by least squares linear regression analysis. We have previously used this to calculate the adsorption capacity of BSA to DEAE Sepharose FF with an accurate correlation [Hu et al., 2001]. But it should be kept in mind that Eq. (1) represents a very simplistic picture of a protein binding to adsorbents, and it was correlated only by solute and type of adsorbents rather than amount and size of adsorbents.

2. Two-Phase Resistance Model

Tsou [Tsou and Graham, 1985], Graham [Graham and Fook, 1982] have developed a solution to the mass balance equation from the two-phase diffusion model, which assumes the overall resistance to diffusion may be approximated by two effective films, one in the bulk liquid phase surrounding the resin and the other in the resin itself. While it is true that this theory is only a very rough approximation to the actual diffusional process which must involve much more complex diffusional models, it is an extremely convenient approximation which, due to its relative simplicity, is often used in describing rates of adsorption or ion exchange in columns. In particle diffusion controlling, the resulting equation based upon above assumptions is

$$\ln[1-F(t)] = \frac{-3\bar{k}\theta}{r_0} = \frac{-\pi^2\bar{D}}{r_0^2}\theta \tag{2}$$

where $\bar{k} = \frac{\pi^2\bar{D}}{3r_0}$ represents resin side mass transfer coefficient, r_0 radius of resin particle, θ total elasped time, $\bar{D}$ the diffusion coefficient of protein in resin. The definition of F(t) is

$$F(t) = \frac{q_0 - q}{q_0 - q_\infty} \tag{3}$$

where q_0, q and q_∞ represents the adsorption capacity at t=0, t=θ and t=∞ respectively. The deduction of Eq. (2) was explained in detail in references [Tsou and Graham, 1985; Graham and Fook, 1982]. The modelling of adsorption capacity of proteins on dextran and cellulose based ion exchange resin by this assumption demonstrated a good correlation.

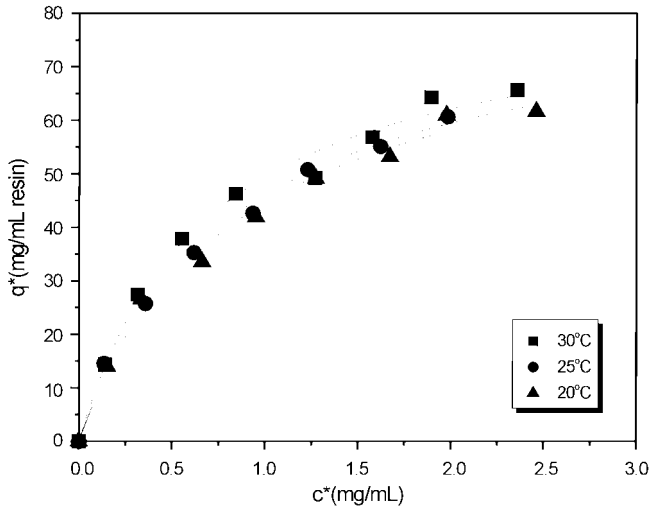

Fig. 2. Adsorption isotherm for the binding of lysozyme to CM Sepharose FF at different temperature.

Table 1. Values of q_m and K_d for the static adsorption of lysozyme to CM Sepharose FF at different temperature (pH=8.0)

Temperature (°C)	q_m (mg/mL)	K_d
20	83.31	0.869
25	83.52	0.832
30	83.87	0.691

RESULTS AND DISCUSSION

1. Adsorption Isotherms

The isotherms for the adsorption of lysozyme to CM Sepharose FF at 20 °C, 25 °C and 30 °C are shown in Fig. 2, suggesting that the experimental data approximately conform to Langmuir behaviour. This adsorption behaviour allows the estimation of the equilibrium parameters q_m and K_d, as tabulated in Table 1. That q_m maximum lysozyme capacity of CM-Sepharose FF changed slightly while K_d decreased with the elevation of the temperature indicated the equilibrium adsorption of lysozyme is temperature favorable, the same phenomenon as that found by others [Kim et al., 1998; Hu et al., 2001].

2. Kinetic Adsorption of Lysozyme

The kinetic adsorption of lysozyme to CM Sepharose FF was investigated according to the experimental operation described in this paper. Effects of pH, viscosity, initial concentration and volume of adsorbents on adsorption rate studies were presented here.

2-1. Effect of pH

Fig. 3 shows the adsorption profile of lysozyme to CM Sepharose FF at decreasing pH of the mobile phase. We can see that with the decrease of pH, more lysozyme would be adsorbed onto the ion exchanger after the equilibrium was approached. As lysozyme has an isoelectric point (pI) of pH 11.0: the lower pH of mobile phase, the higher the equilibrium adsorption capacity. But it is worth commenting on the selectivity of pH because extremely low pH of mobile phase often leads to the loss of lysozyme activity. The pore diffusion coefficients correlated by two phase resistance model are tabulated in Table 2. The effective diffusion coefficients $\overline{D}$ increase with the decrease of pH, well according with our experimental data.

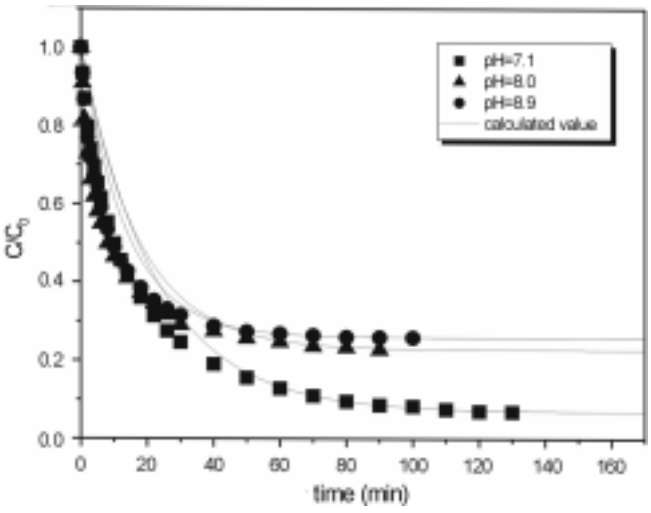

Fig. 3. Adsorption profile of lysozyme to CM Sepharose FF by two phase resistance model at different pH.

Table 2. Diffusion coefficients of lysozyme in CM Sepharose FF particle calculated by two phases resistance model at different pH

pH	q_m (mg/mL)	$\overline{D}$ (cm^2/s)
7.1	93.10	1.40×10^{-9}
8.0	77.24	1.13×10^{-9}
8.9	73.20	0.87×10^{-9}

2-2. Effect of Solution Viscosity

The adsorption rate was greatly influenced by the solution viscosity as depicted in Fig. 4 and Fig. 5. From them we know that with the increase of solution viscosity, the time required to achieve an equilibrium will be prolonged while the maximum adsorption capacity remains constant irrespective of it. This can be elucidated by the molecular movement mechanism. The increase of solution viscosity would cause slower molecular movement of lysozyme in solution and lower mass transfer rate in the liquid film and pore. Again,

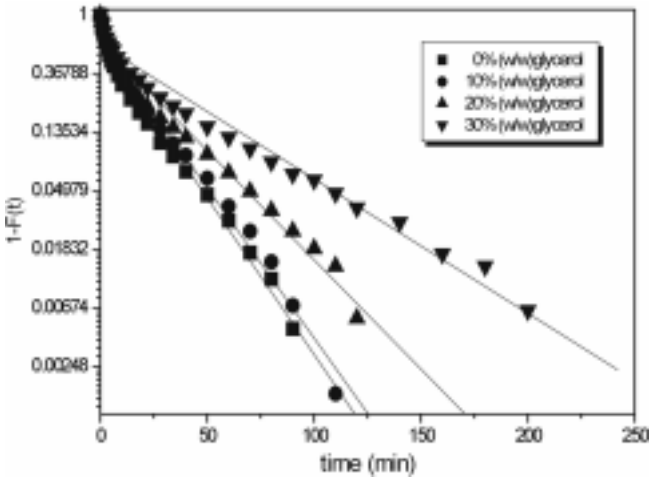

Fig. 4. Plot of ln[1−F(t)] vs. time for adsorption of lysozyme at different viscosities of solution.

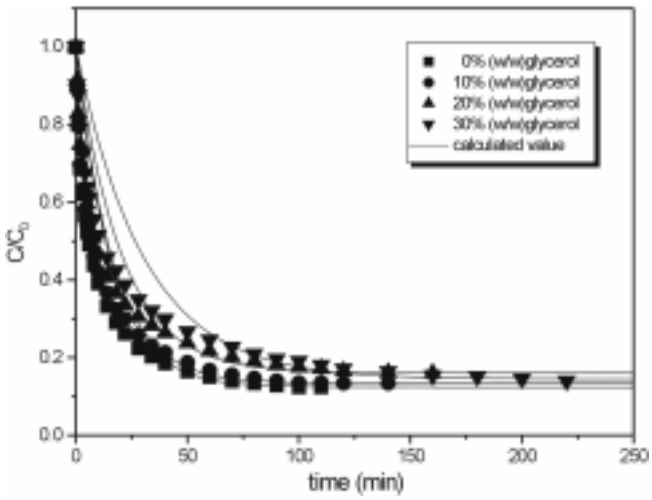

Fig. 5. Adsorption profile of lysozyme to CM Sepharose by two phase resistance model at different viscosities of solution.

Table 3. Diffusion coefficients of lysozyme in CM Sepharose FF particle calculated by two phase resistance model at different viscosities of solution

Glycerol (%)	Viscosity (cP)	Diffusion coefficient $\overline{D}$ (cm^2/s)
0	0.96	1.13×10^{-9}
10	1.52	1.00×10^{-9}
20	2.41	0.73×10^{-9}
30	3.86	0.45×10^{-9}

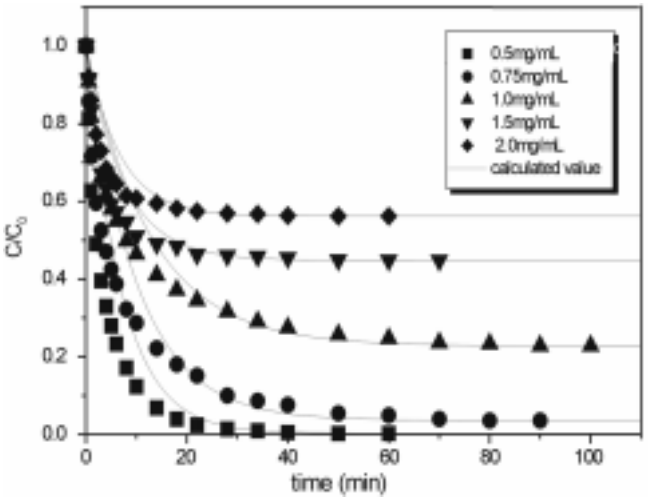

Fig. 6. Adsorption profile of lysozyme to CM Sepharose by two phase resistance model at different initial concentrations.

Table 4. Diffusion coefficients of lysozyme in CM Sepharose FF particle calculated by two phase resistance model at different initial concentration

Initial concentration (mg/mL)	q_m (mg/mL)	$\overline{D}$ (cm^2/s)
0.5	49.88	2.79×10^{-9}
0.75	72.34	1.36×10^{-9}
1.0	77.24	1.13×10^{-9}
1.5	82.23	2.2×10^{-9}
2.0	87.65	2.55×10^{-9}

the pore diffusion coefficients correlated by two phase resistance model is illustrated in Table 3. The effective diffusion coefficients $\overline{D}$ decrease with the increase of solution viscosity, as explained above.

2-3. Effect of Initial Concentration

The adsorption profile of lysozyme to CM Sepharose FF at different initial concentrations is given in Fig. 6. The equilibrium adsorption capacity of ion exchanger went up with the increasing of initial concentration of the solution, as shown in Table 4. The most striking feature of these results is that the initial effective diffusion coefficient $\overline{D}$ correlated by two phase resistance model went down followed by a second rise with the increase of initial lysozyme concnetration. This demonstrated that the mass transfer resistance in the resin contributed differently according to the initial concentration of solution.

2-4. Effect of Volume of Adsorbent

From Fig. 7, it can be seen that the adsorption experiment would

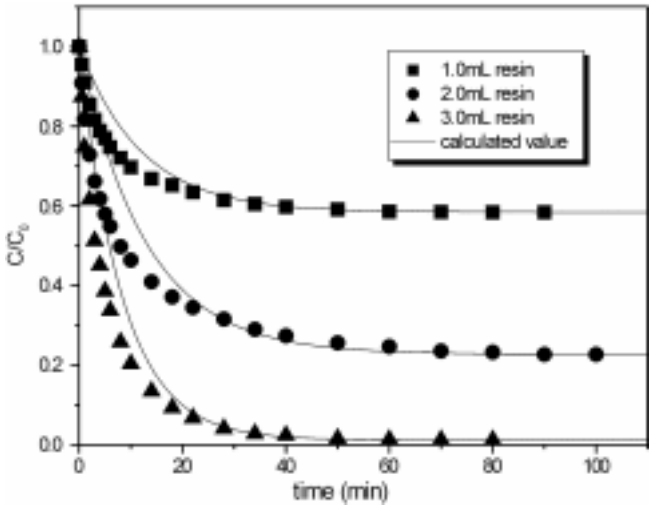

Fig. 7. Adsorption profile of lysozyme to CM Sepharose by two phase resistance model at different adsorbent volumes.

Table 5. Diffusion coefficients of lysozyme in CM Sepharose FF particle calculated by two phase resistance model at different adsorbent volumes

Adsorbent (1 : 1, mL)	q_m (mg/mL)	$\overline{D}$ (cm^2/s)
1.0	83.15	1.63×10^{-9}
2.0	77.24	1.13×10^{-9}
3.0	65.86	2.36×10^{-9}

be carried out thoroughly as well as a low soluble protein equilibrium concentration with the adding of adsorbent. When 3 mL CM Sepharose FF was applied to 1.0 mg/mL lysozyme solution, nearly all the lysozyme in the solution is bound to the resin. But it should be kept in mind that the equilibrium adsorption capacity per unit weight resin will drop with the increase of volume of adsorbents, as shown in Table 5.

3. Kinetic Adsorption of Papain and BSA

3-1. Effect of Initial Concentration

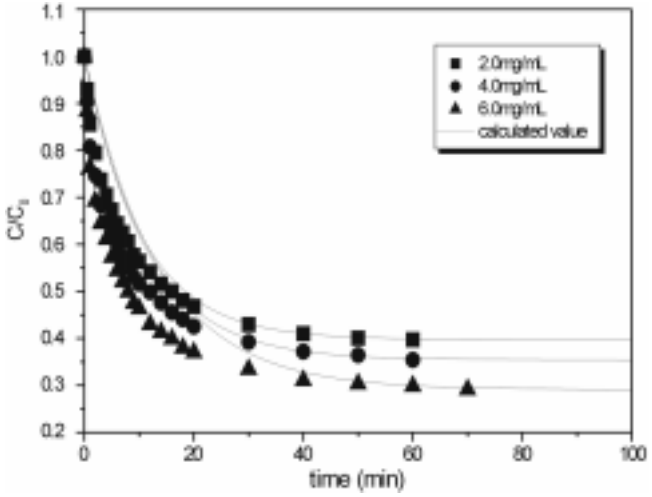

Fig. 8. Adsorption profile of papain to CM Sepharose by two phase resistance model at different initial concentrations.

An adsorption profile of papain and BSA to CM Sepharose FF at different initial concentrations was determined in this work, and the same two phase resistance model was applied to the above systems too. From Fig. 8, we found that this adsorption can be well described by this model. But we have to pay attention to a slight deviation between the correlation and experimental data at the initial stage; that is accounted for by the film diffusion control dominating at the initial rate period and the intraparticle diffusion control at the final rate period. The same phenomena occurred in the adsorption of lysozyme.

3-2. Effect of Volume of Adsorbent

The adsorption performance of papain and BSA to CM Sepharose FF is further measured at different volume of adsorbents. There is a good agreement between experimental data and correlation by two phase resistance model as presented in Fig. 9, suggesting that an effective and general method could be established to evaluate the binding behaviour of proteins in a resin.

4. Kinetic Adsorption of Cellulase

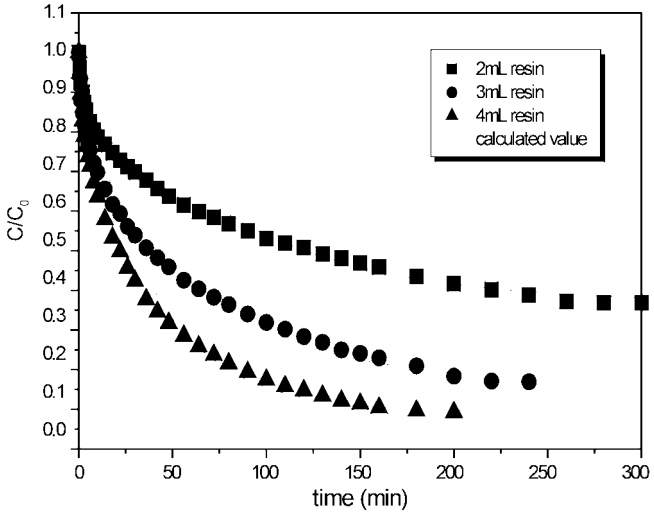

Fig. 9. Adsorption profile of BSA to CM Sepharose by two phase resistance model at different resin volumes.

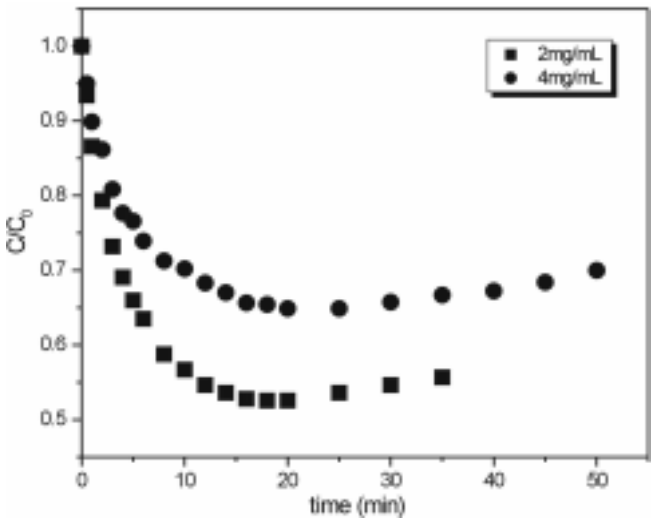

Fig. 10. Adsorption profile of cellulase to DEAE Sepharose FF.

The kinetic adsorption of cellulase to DEAE Sepharose FF was carried out in this work; the results are shown in Fig. 10. At the final adsorption period, an unusual appearance was observed: there is somewhat of an increase of soluble protein concentration again after the fall. This is attributed by the dissociation of protein-ion exchanger complex, implying that another resin has to be found to apply in the cellulase purification.

CONCLUSION

Some meaningful results were obtained from the equilibrium and kinetic measurements of proteins to CM and DEAE Sepharose FF adsorbents.

1. The Langmuir isotherm has been successfully used to describe the adsorption of lysozyme to the cation exchanger CM Sepharose FF in single component experiments, and the equilibrium adsorption of lysozyme is temperature favorable.

2. Rate studies were carried out to investigate the adsorption performance of lysozyme, papain and BSA to CM Sepharose FF. pH, viscosity, initial concentration and volume of adsorbents exert an influence upon the adsorption characteristics of above three proteins in the same mode.

3. The two phase resistance model was employed to correlate the dynamic adsorption experiments and the effective pore diffusion coefficients were obtained. The apparent success of the theoretical model in accounting for the experimental observations has enabled prediction of the influence of many of the operation parameters on the performance of an ion exchange separation.

4. The kinetic adsorption of cellulase to DEAE Sepharose FF is quite different from that of lysozyme, papain and BSA to CM Sepharose FF. This unfavorable operation should be avoided and be examined in depth.

ACKNOWLEDGEMENT

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NOMENCLATURE

C : soluble protein concentration [g/mL]
C_0 : initial, or inlet liquid phase protein concentration [g/mL]
$\overline{D}$: effective pore diffusivity [cm^2/s]
$\overline{k}$: resin side mass transfer coefficient [cm/s]
k_1 : adsorption rate constant [$cm^3/(g \cdot s)$]
k_{-1} : desorption rate constant [s^{-1}]
K_d : dissociation constant for the protein-ion-exchanger complex [g/mL]
q : concentration of protein adsorbed to the ion exchanger [g/cm^3]
q_m : maximum protein capacity of the ion exchanger [g/cm^3]
q_0 : protein capacity of the ion exchanger at t=0 [g/cm^3]
q_∞ : protein capacity of the ion exchanger at t=∞ [g/cm^3]
r_0 : effective resin particle radius [cm]

Greek Letter

θ : time [s]

Superscript

* : value when system is at equilibrium

REFERENCES

Bonnerjea, J., Oh, S., Hoare, M. and Dunnill, P., "Protein Purification: The Right Step at the Right Time," *Bio/Technology*, **4**, 954 (1986).

Chase, H. A., "Prediction of the Performance of Preparative Affinity Chromatography," *J. Chromatogr.*, **297**, 179 (1984).

Graham, E. E. and Fook, C. H., "Rate of Protein Absorption and Desorption on Cellulosic Ion Exchangers," *AIChE J*., **28**(2), 245 (1982).

Hong, S. B. and Row, K. H., "Protein Separation by High-Performance Membrane Chromatography," *Korean J. Chem. Eng*., 1**9**, 314 (2002).

Hu, H. B., Yao, S. J. and Zhu, Z. Q., "Study on Adsorption Performance of BSA in Streamline DEAE and DEAE Sepharose FF Adsorbents," *Chem. Eng. (China)*, **29**(1), 37 (2001).

Hu, H. B., Yao, S. J., Zhu, Z. Q. and Hur, B. K., "Comparison of the Adsorption Characteristics of Expanded Bed Adsorbent with Conventional Chromatographic Adsorbent," *Korean J. Chem. Eng*., **18**, 357 (2001).

Janson, J. C., "Optimization of Large-Scale Chromatography of Proteins," *Korean J. Chem. Eng*., **18**, 149 (2001).

Johnston, A. and Hearn, M. T. W., "High-Performance Liquid Chromatography of Amino Acids, Peptides and Proteins," *J. Chromatogr.*, **512**, 101 (1990).

Kim, D. W., Jang, Y. H. and Jeong, Y. K., "Adsorption Kinetics and Behavior of Two Cellobiohydrolases from *Trichoderma Reesei* on Microcrystalline Cellulase," *Biotechnol. Appl. Biochem.*, **27**, 97 (1998).

Peterson, E. A. and Sobers, H. A., "Chromatography of Proteins. I. Cellulose Ion-Exchange Adsorbents," *J. Am. Chem. Soc.*, **78**, 751 (1956).

Skidmore, G. L., Horstmann, B. J. and Chase, H. A., "Modelling Single-Component Protein Adsorption to the Cation Exchange S Sepharose FF," *J. Chromatogr*, **498**, 113 (1990).

Tsou, H. S. and Graham, E. E., "Prediction of Adsorption and Desorption of Protein on Dextran Based Ion-Exchange Resin," *AIChE J*., **31**(12), 1959 (1985).