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Fabrication of Hard Dextran DEAE: Adsorption Equilibria of BSA

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Abstract. A hard dextran-DEAE ion exchanger (Hard Dextran DEAE) was developed. It is hard and keeps good properties of dextran-DEAE for protein separation. It is not compressed in a column and can be used in much wider range of flow rate in the column than the commercial hard gel, DEAE Sepharose Fast Flow made of agarose. The saturation capacity of BSA on Hard Dextran DEAE is about 1.7 times of that on DEAE Sepharose Fast Flow at pH 6.9. Equilibrium isotherm for adsorption of BSA depends on pH considerably. When $pH \geq 5.5$, the equilibrium isotherm is correlated by the Langmuir equation. When $pH \leq 5.05$, the isotherm is correlated by the Freundlich equation. The higher the concentration of NaCl is, the smaller the amount of BSA adsorbed. When the concentration of NaCl is higher than 100 mol m^{-3} at pH 6.9 and 50 mol m^{-3} at pH 4.8, BSA was not adsorbed on the resin. This may suggest that BSA is adsorbed by electrostatic attraction. About 100 mol m^{-3} NaCl aqueous solution can be used as an eluant of proteins.

Keywords: composite resin, hard dextran, protein, BSA, equilibria

Introduction

The ion exchange chromatographic separation of proteins is important not only for analysis but also in large-scale industries, such as food industry and drug industry. The requirements to an ion exchanger for large-scale protein separation are as follows: (i) the particles are spherical and uniform; (ii) they are hard enough to prevent their compression by the pressure in column and therefore the pressure drop across the column is small; (iii) adsorption capacity is large; (iv) there is no nonspecific adsorption; (v) intraparticle diffusion is fast; (vi) desorption of protein is easy and the protein is recovered in concentrated form; (vii) harmless; and so on. We use the term "hard gel" as the resin which satisfies requirement (ii). We also use the term "soft gel" when its bed shows extremely large pressure drop even at very slow flow rate.

A number of ion-exchangers have been developed but they do not satisfy all the mentioned requirements. Dextran-DEAE and dextran-QAE ion exchangers such as DEAE Sephadex A-50 and QAE Sephadex A-50 (Pharmacia LKB Biotechnology) are very good for protein separation. They may satisfy the above requirements except (ii). Their saturation capacities may be the largest among the commercial ion exchangers. However, since those dextran ion-exchangers are soft and are compressed in column, their beds show extremely large pressure drops even at very low flow rate (Pharmacia LKB Biotechnology, 1990). Therefore, it is difficult to use them in a large-scale chromatographic separation of proteins. Ion exchangers made of agarose, such as DEAE Sepharose Fast Flow and QAE Sepharose Fast Flow (Pharmacia LKB Biotechnology), and perfusion chromatography ion exchanger, such as POROS HQ and QE (PerSeptive Biosystems) have been developed to overcome this problem. The adsorption capacity of protein in POROS is relatively smaller than that in Sepharose Fast Flow. As these ion exchangers are hard, the pressure drop across the bed is small

even at high flow rate and a high pressure pump is not necessary.

The equilibria of adsorption of BSA on the ion-exchangers, QAE Sephadex A-50 (Yoshida et al., 1993), DEAE Sephadex A-50 (Yamamoto et al., 1983; Tsou and Graham, 1985; Yoshida et al., 1994b) and DEAE Sepharose Fast Flow (Yoshida et al., 1994a) have been presented. The adsorption capacity of BSA at pH 6.9 on DEAE Sepharose Fast Flow (Yoshida et al., 1994a) is about one third of that on DEAE Sephadex A-50 (Yoshida et al., 1994b).

In the present work, we have considered the possibility of using dextran-DEAE ion exchanger to separate protein on large-scale chromatograph by making it hard and developed a new ion exchanger (hereafter called Hard Dextran DEAE). Experimental pressure drops across a column are demonstrated to prove that it can be used in wide range of flow rate. The adsorption isotherms of bovine serum albumin (BSA) are determined. The effects of the concentration of BSA, the pH of the solution and the concentration of coexisting salt, NaCl, on the equilibrium isotherm are investigated. Some of the results are compared with DEAE Sephadex A-50 (soft gel) and DEAE Sepharose Fast Flow (hard gel).

Fabrication of Hard Dextran DEAE

Figure 1 shows the conceptual diagram of Hard Dextran DEAE which we have developed in this study.

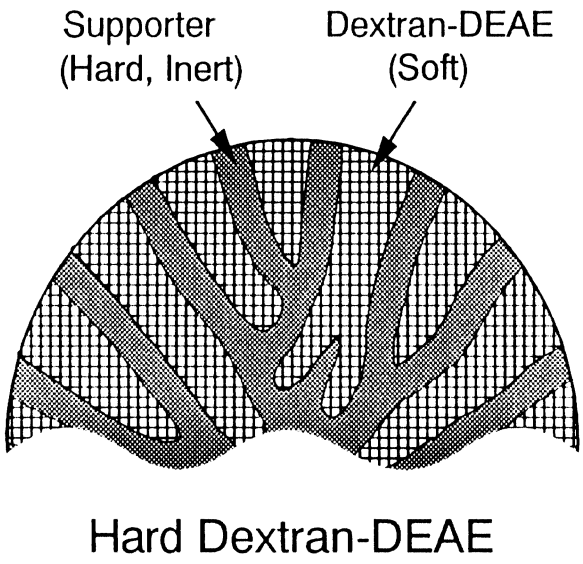

Figure 1. Conceptual diagram of Hard Dextran DEAE.

Supporter of the dextran-DEAE is SEPABEADS FP-HG05, which has been developed by Mitsubishi Chemical Co. for gel permeation chromatography. It is a synthesized hard gel made of methacrylate resin, and inert for adsorption of proteins. It is a macroreticular-type resin and the porosity of the macropore is about 0.6. We let dextran (SIGMA Chem. Co.) penetrate into the macropores of SEPABEADS FP-HG05 by contacting the gel particles and dextran solution for 12 hours at room temperature. Thereafter the particles were separated from the dextran solution. The dextran in the macropore of the gel particles was cross-linked using epichlorohydrin (Kishida Chem. Co.) in toluene at 323 K. Then the gel particles were placed in 3 kmol m^{-3} NaOH aqueous solution. 2-diethylaminoethyl chloride hydrochloride (Tokyo Kasei Co.) aqueous solution was added and stirred for 5 hours at 323 K. Diethylaminoethyl (DEAE) was introduced onto the dextran network by this reaction. The finished product, Hard Dextran DEAE, was washed by distilled water thoroughly.

Experimental

Pressure drops across a column were measured for Hard Dextran DEAE and DEAE Sepharose Fast Flow. The schematic of the apparatus is shown in Fig. 2. The inner diameter of the column was 0.8 cm. The resin particles, which had been in equilibrium with phosphate buffer solution of pH 6.9, were packed in the column. The bed was 8 cm high. The phosphate buffer solution was prepared using NaH_2PO_4 and Na_2HPO_4. The concentration of the buffer solution was 6.975 eq m^{-3}. The phosphate buffer solution was fed into the column using a HPLC pump, Shimadzu LC-8A with range of 0.1–150 cm^3 min^{-1}. The pressure drop across the bed was measured using PRESSURE TRANSMITTER, MODEL PL-611 (CHINO Co.) with range of 0–10 kg cm^{-2}.

The adsorption isotherms of BSA were determined by batch method at five different pH values: 4.8, 5.05, 5.5, 6.9, and 8.79. pH of the BSA solution was adjusted using phosphate buffer (NaH_2PO_4 and Na_2HPO_4). The concentration of the buffer in the BSA solution was 6.975 eq m^{-3}. The resin particles, which had been in equilibrium with the buffer solution of the same value of pH as the BSA solution, were contacted with the BSA solution in syringe vials. Just before using the syringe vials, they were sterilized with 75% ethanol aqueous solution and then dried. The resin

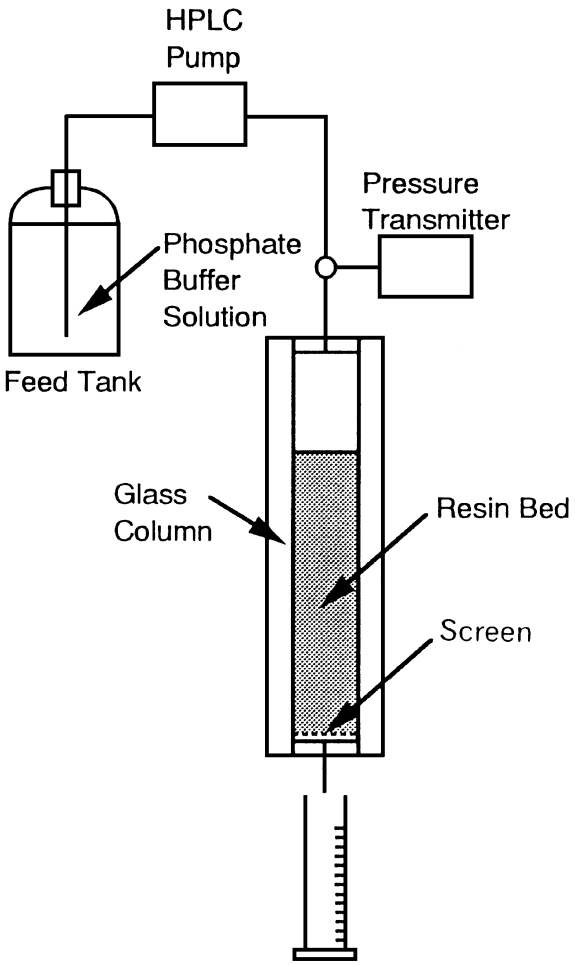

Figure 2. Equipment schematic.

particles were weighed and added into the syringe vials. The syringe vials were filled with BSA solution using Pipetman (Gilson) by which the volume of the solution was determined. There was no air in the syringe vials. Even if very small amount of air was in the syringe vials, the denaturation of BSA would of course be a serious problem. The syringe vials were rotated gently. The equilibrium was fully reached in 4 days. The solutions of BSA were analyzed by Shimadzu UV-visible recording spectro-photometer UV-260 ($\lambda = 278.0$ nm). The adsorbed phase concentration was calculated according to the equation:

$$q = \frac{V(C_0 - C)}{W} \tag{1}$$

where C_0 (kg m^{-3}) and C (kg m^{-3}) are the initial concentration and equilibrium concentration of BSA in the liquid phase, respectively, q (kg m^{-3} of wet resin) denotes the adsorbent phase concentration of BSA , and V (m^3) and W (m^3 of wet resin) are the volume of the solution and the adsorbent particles, respectively. The concentration of Na^+ was analyzed with flame analysis. The concentration of Cl^- was determined by the Mohr method. All experiments were carried out at 298 K.

Results and Discussion

Physical Properties of the Resin

Table 1 shows the physical properties of Hard Dextran DEAE and DEAE Sepharose Fast Flow. The diameters of the resin particles were measured after in equilibrium with phosphate buffer solution of pH 6.9,

Table 1. Physical properties of Hard Dextran DEAE and DEAE Sepharose Fast Flow.

	Hard Dextran DEAE	DEAE Sepharose F.F.
Particle diameter of wet resin* (μm)		
Free in buffer (pH 6.9), d_p	134	69.4
d'_p	137	79.4
Saturated by BSA (pH 6.9), $d_{p,\,BSA}$	133	72.4
Free in 100 mol m^{-3} NaCl (pH 6.9), $d_{p,\,NaCl}$	132	63.6
$d_p/d_{p,\,BSA}$	1.01	0.959
$d_{p,\,NaCl}/d_{p,\,BSA}$	0.991	0.879
$V_p/V_{p,\,BSA}$	1.03	0.881
$V_{p,\,NaCl}/V_{p,\,BSA}$	0.973	0.680
Density		
True density (kg m^{-3})	1.610	1.729
Apparent density (kg of dry particle/m^3 of wet particle)	0.469	0.130
Water content (wt%)	60.1	87.6
Concentration of DEAE fixed in the resin phase (ion exchange capacity), Q (eq m^{-3})		
Eq. (2)	267 ($K_1 = 0.259$)	446 ($K_1 = 1.30$)
Eq. (3)	254 ($K_2 = 0.139$)	448 ($K_2 = 0.335$)

*Mean value of 50 particles ($N = 50$). $d = \sum_{i=1}^{N} d_i/N$. $d' = \sum_{i=1}^{N} d_i^3/\sum_{i=1}^{N} d_i^2$.
V: volume of resin particle.

BSA solution ($C_0 = 1\,\mathrm{kg\,m^{-3}}$ and pH 6.9 adjusted using the phosphate buffer) and $100\,\mathrm{mol\,m^{-3}}$ NaCl aqueous solution (pH 6.9 adjusted using phosphate buffer) by a microscope. d and d' in Table 1 denote arithmetic mean diameter based on the length and area, respectively. Since the difference between d_p and d'_p of Hard Dextran DEAE is smaller than that of DEAE Sepharose Fast Flow, the particles of Hard Dextran DEAE is more uniform than DEAE Sepharose Fast Flow. For Hard Dextran DEAE, there is little difference between the volume of the particle, which did not adsorb BSA, in phosphate buffer solution and that saturated by BSA. There is also little difference between the volume of the particle, which did not adsorb BSA, in $100\,\mathrm{mol\,m^{-3}}$ NaCl solution and that saturated by BSA. For DEAE Sepharose Fast Flow, the volume difference is relatively large. The desorption of BSA using $100\,\mathrm{mol\,m^{-3}}$ NaCl aqueous solution reduces the volume by 32%. From these results, Hard Dextran DEAE may give an easier operation for adsorption of proteins and their desorption using NaCl aqueous solution in a column than DEAE Sepharose Fast Flow.

The densities and the water contents in Hard Dextran DEAE are similar to commercial ion exchangers for separation of metal ions. Since the porosity of DEAE Sepharose Fast Flow is greater, the apparent density is smaller and the water content is greater than Hard Dextran DEAE.

The concentration of the functional group, DEAE, fixed in the resin phase, Q ($\mathrm{eq\,m^{-3}}$ of wet resin), was determined by measuring the equilibrium isotherms for two different ion exchange systems by the batch method:

$$\mathrm{R{\cdot}Cl^- + H_2PO_4^-} \overset{K_1}{\rightleftharpoons} \mathrm{R{\cdot}H_2PO_4^- + Cl^-} \qquad (2)$$

$$\mathrm{2R{\cdot}Cl^- + HPO_4^{2-}} \overset{K_2}{\rightleftharpoons} \mathrm{R_2{\cdot}HPO_4^{2-} + 2Cl^-} \qquad (3)$$

The Cl^- form resin particles were contacted with $6.975\,\mathrm{eq\,m^{-3}}$ NaH_2PO_4 solution (Eq. (2)) and $6.975\,\mathrm{eq\,m^{-3}}$ Na_2HPO_4 solution (Eq. (3)), respectively. Applying the mass action law to Eqs. (2) and (3), Eqs. (4) and (5) are derived, respectively.

$$K_1 = \frac{q_1(C_0 - C_1)}{(Q - q_1)C_1} \qquad (4)$$

$$K_2 = \frac{q_2(C_0 - C_2)^2}{(Q - q_2)^2 C_2} \qquad (5)$$

where subscripts 1 and 2 denote $H_2PO_4^-$ and HPO_4^{2-}, respectively. q ($\mathrm{eq\,m^{-3}}$ of wet resin) and C ($\mathrm{eq\,m^{-3}}$) are the equilibrium concentration in the resin phase and liquid phase, respectively, and C_0 ($\mathrm{eq\,m^{-3}}$) is the total concentration of ionic species in the liquid phase. Equations (4) and (5) are transformed to Eqs. (6) and (7), respectively.

$$q_1 = Q - \frac{1}{K_1}\frac{(C_0 - C_1)q_1}{C_1} \qquad (6)$$

$$q_2 = Q - \frac{1}{\sqrt{K_2}}\frac{(C_0 - C_2)\sqrt{q_2}}{\sqrt{C_2}} \qquad (7)$$

The experimental values of q_1 and q_2 are plotted versus $(C_0 - C_1)q_1/C_1$ and $(C_0 - C_2)\sqrt{q_2/C_2}$ for the ion exchange systems of Eqs. (2) and (3), respectively, in Figs. 3 and 4. The data for both systems are correlated

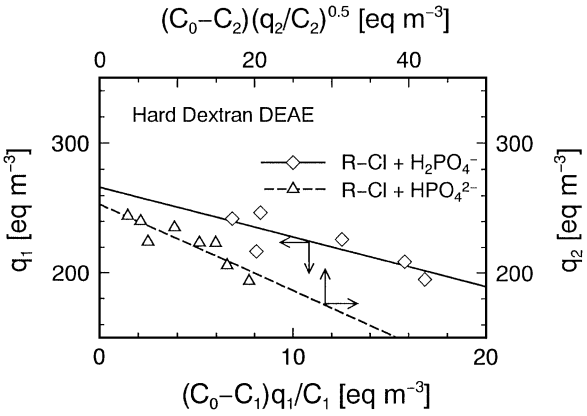

Figure 3. Ion exchange equilibria for $R\text{-}Cl^- + H_2PO_4^-$ and $R\text{-}Cl^- + HPO_4^{2-}$ system in Hard Dextran DEAE. $C_0 = 6.975\,\mathrm{eq/m^3}$. —— Eq. (6); --- Eq. (7).

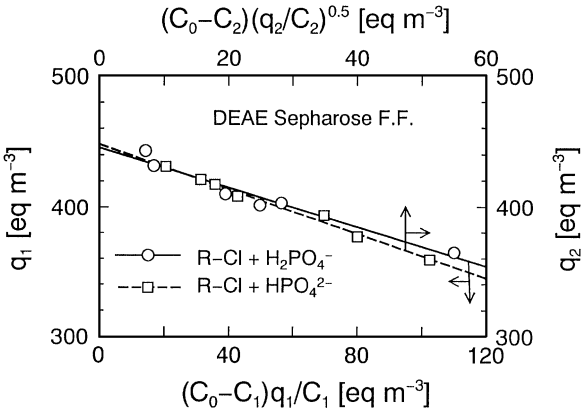

Figure 4. Ion exchange equilibria for $R\text{-}Cl^- + H_2PO_4^-$ and $R\text{-}Cl^- + HPO_4^{2-}$ system in DEAE Sepharose Fast Flow. $C_0 = 6.975\,\mathrm{eq/m^3}$. —— Eq. (6); --- Eq. (7).

by straight lines. The concentration of the functional group DEAE fixed in the resin phase (exchange capacity), Q, and the equilibrium constant, K, were determined from the intercept and the slope, respectively, and they are listed in Table 1. The value of Q obtained for the NaH_2PO_4 solution (Eq. (2)) is close to that for the Na_2HPO_4 solution (Eq. (3)). The value of Q in Hard Dextran DEAE is about 58% of that in DEAE Sepharose Fast Flow.

Pressure Drop Across Bed

Pharmacia LKB Biotechnology (1990) presented that the flow rate of water through the column in which DEAE Sephadex A-50 particles were packed (45 cm high) showed a maximum value as the pressure drop increased. The maximum superficial velocity was about 30 cm/h at about 2×10^4 Pa/m. After the maximum point, as the pressure drop increased to 5×10^4 Pa/m, the flow rate decreased to 6.6 cm/h. This was caused by compression of the soft gel particles in the column.

Figure 5 shows the experimental relation between pressure drop and *Re* number for Hard Dextran DEAE and DEAE Sepharose Fast Flow. For DEAE Sepharose Fast Flow, the pressure drop increases in proportion to *Re* in low flow rate region. However, when *Re* $(= d' u_f \rho/\mu)$ is larger than a value, the pressure drop increases rapidly, because the particles are compressed by the pressure and the void fraction of the bed decreases. This is similar to DEAE Sephadex A-50 as mentioned above, although the flow rate in the bed of DEAE Sepharose Fast Flow is much higher than that of DEAE Sephadex A-50. In this experiment, the maximum superficial velocity in the bed of DEAE Sepharose Fast Flow was about 0.66 cm/s ($Re \cong 0.5$ in Fig. 5). For Hard Dextran DEAE, the pressure drop increases in proportion to flow rate and it is much smaller than that for DEAE Sepharose Fast Flow. This is because the particles of Hard Dextran DEAE are not compressed in the bed. In Fig. 5, the maximum superficial velocity in the bed of Hard Dextran DEAE reached to about 1.3 cm/s ($Re \cong 2$). From these results, Hard Dextran DEAE may be used in wider range of flow rate than DEAE Sepharose Fast Flow.

Equilibrium Isotherm for Adsorption of BSA

First of all, the adsorption isotherm of BSA on SEPABEADS FP-HG05, the supporter of the Hard Dextran DEAE, was determined. It appeared that BSA was not adsorbed at all.

Figure 6 shows the effect of the initial concentration of BSA (C_0) on the equilibrium isotherm for Hard Dextran DEAE. pH of the solution was adjusted to 6.9 by using phosphate buffer mentioned earlier. As the equilibrium isotherm is independent of the initial concentration of BSA, it may be adsorbed on the phosphate form resin by the following Langmuir-type reaction:

$$\mathrm{R} + \mathrm{BSA} \rightleftharpoons \mathrm{R{\cdot}BSA} \tag{8}$$

The adsorption isotherms of proteins are often described by the Langmuir equation (ion exchanger: Tsou and Graham (1985), Yoshida et al. (1989, 1993, 1994a, b, c); affinity chromatography: Chase (1984), Arnold et al. (1985a, b), Arve and Liapis (1987), McCoy

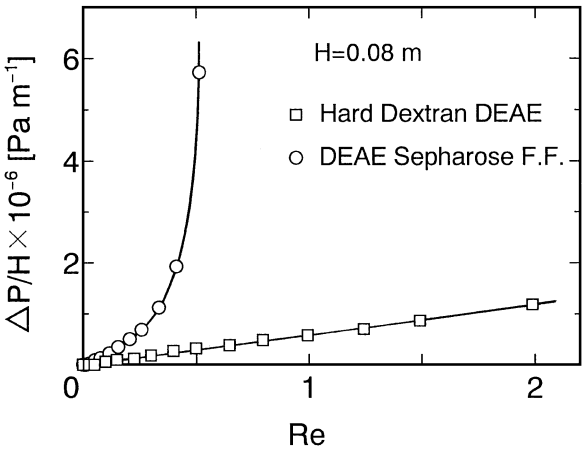

Figure 5. Relation between pressure drop and flow rate in Hard Dextran DEAE and DEAE Sepharose Fast Flow at pH 6.9.

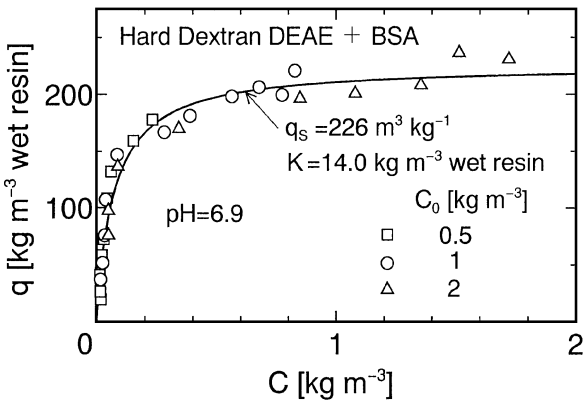

Figure 6. Effect of initial concentration of BSA on equilibrium isotherm for Hard Dextran DEAE at pH 6.9. —— Eq. (9).

and Liapis (1991)). We also applied the Langmuir equation,

$$q = \frac{K q_s C}{1 + KC} \tag{9}$$

The solid line shows the Langmuir isotherm and correlates the data well. The experimental equilibrium constant K ($m^3\,kg^{-1}$) and the saturation capacity q_s (kg of BSA m^{-3} of wet resin) are listed in Table 2.

Yoshida et al. reported that DEAE Sephadex A-50 and DEAE Sepharose Fast Flow showed a similar behavior as above (Yoshida et al., 1994a, b). Figure 7 shows the comparison between their equilibrium isotherms for $C_0 = 1\,kg\,m^{-3}$ at pH 6.9. The solid and dashed lines show the Langmuir isotherm calculated using the Langmuir constants given in Table 2. The saturation capacity of Hard Dextran DEAE is about 68% (data presented by Tsou and Graham (1985)) and 55% (data presented by Yoshida et al. (1994b)) of DEAE Sephadex A-50. Since these values are close to the porosity of the macropore (0.6) of SEPABEADS FP-HG05, which is the supporter of Hard Dextran DEAE, the dextran-DEAE formed in the macropore of SEPABEADS FP-HG05 should be similar to DEAE Sephadex A-50. Although the saturation capacity of

Table 2. Experimental Langmuir coefficients and Freundlich coefficients.

No.	pH	C_0 ($kg\,m^{-3}$)	C_{NaCl} ($mol\,m^3$)	q_s ($kg\,m^{-3}$)	K ($m^3\,kg^{-1}$)	k	n
Hard Dextran DEAE (hard gel)							
1	4.8	1	0			170	0.205
2	5.05	1	0			114	0.758
3	5.5	1	0	173	7.81		
4	6.9	0.5	0	226	14.0		
5	6.9	1	0	226	14.0		
6	6.9	2	0	226	14.0		
7	8.79	1	0	179	61.4		
8	6.9	1	20	142	7.84		
9	6.9	1	50			71.2	0.808
10	6.9	1	100	0	—		
11	4.8	1	50	0	—		
12	4.8	1	100	0	—		
DEAE Sepharose Fast Flow (hard gel)[a]							
13	4.8	1	0			86.4	0.707
14	5.05	1	0	84.1	7.34	91.7	2.47
15	6.9	1	0	131	31.7		
16	8.79	1	0	186	30.0		
DEAE Sephadex A-50 (soft gel)[b]							
17	4.8	1	0			517	0.391
18	5.05	1	0	480	6.04		
19	6.9	1	0	401	52.3		
20	8.79	1	0	360	25.0		
DEAE Sephadex A-50 (soft gel)[c]							
21	6.9	1	0	326	32.7		
22	6.5	1	170	14	2.26		

[a] Yoshida et al. (1994a).
[b] Yoshida et al. (1994b).
[c] Tsou and Graham (1985).

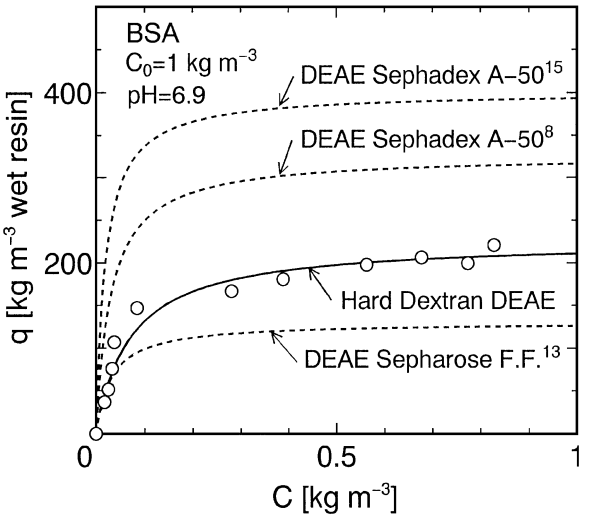

Figure 7. Comparison between equilibrium isotherms for adsorption of BSA on Hard Dextran DEAE (hard gel), DEAE Sepharose Fast Flow (hard gel), and DEAE Sephadex A-50 (soft gel) at pH 6.9. ——, --- Eq. (9).

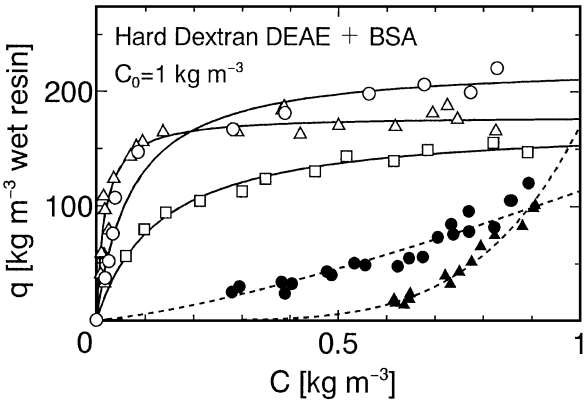

Figure 8. Effect of pH on equilibrium isotherm for adsorption of BSA on Hard Dextran DEAE. pH: ▲4.8, ●5.05, □5.5, ○6.9, △8.79; —— Eq. (9); --- Eq. (10).

BSA in Hard Dextran DEAE is smaller than DEAE Sephadex A-50 (soft gel), it is about 1.7 times of that of DEAE Sepharose Fast Flow (hard gel).

Figure 8 shows the effect of pH on the equilibrium isotherm. The equilibrium isotherm is strongly affected by pH. The isotherms are correlated reasonably well by the Langmuir equation (solid line) at pH $\geq$ 5.5, but when pH $\leq$ 5.05, the data could not be correlated by the Langmuir equation but by the Freundlich equation (broken line):

$$q = kC^{1/n} \tag{10}$$

Since the value of pI $\cong$ 4.8 (Wallevik, 1973; Evanson and Deutsch, 1978), BSA is negatively charged when pH > pI. Therefore, the results in Fig. 8 may suggest that when pH $\geq$ 5.5, BSA is adsorbed on the ion exchanger by the electrostatic attraction between positively charged fixed group of the ion exchanger and negatively charged BSA. The value of K increases with increasing pH, because the negative charges of BSA increase with increasing pH. The saturation capacity q_s is the largest at pH 6.9 as shown in Table 2. When pH $\leq$ 5.05, since the pH is close to the pI $\cong$ 4.8 of BSA, BSA molecule would be almost neutral and the selectivity for adsorption of BSA is smaller than that for pH $\geq$ 5.5. Yoshida et al. (1989, 1993, 1994a, b, c) discussed these phenomena for adsorption of BSA on QAE Sephadex A-50 in more detail.

Figure 9 shows the effect of coexisting salt, NaCl, on the equilibrium isotherm at pH 6.9. The amount of BSA adsorbed on the ion exchanger decreases with increasing the concentration of NaCl (C_{NaCl}). When $C_{\mathrm{NaCl}} = 100\,\mathrm{mol\,m^{-3}}$, BSA was not adsorbed at all on the resin. This means that 100 mol m^{-3} NaCl can be used as an eluant of BSA. In the case of DEAE Sephadex A-50, Tsou and Graham (1985) showed that BSA was still adsorbed when 170 mol m^{-3} NaCl coexisted in the BSA solution (see Table 2). We also found that BSA was not adsorbed on DEAE Sephadex A-50 when $C_{\mathrm{NaCl}} \geq 200\,\mathrm{mol\,m^{-3}}$ but was adsorbed when $C_{\mathrm{NaCl}} < 200\,\mathrm{mol\,m^{-3}}$, although the reason is not clear. Therefore, the elution of BSA from Hard Dextran DEAE may be accomplished using lower concentration of NaCl than DEAE Sephadex A-50. In addition, when $C_{\mathrm{NaCl}} \leq 20\,\mathrm{mol\,m^{-3}}$, the data are correlated

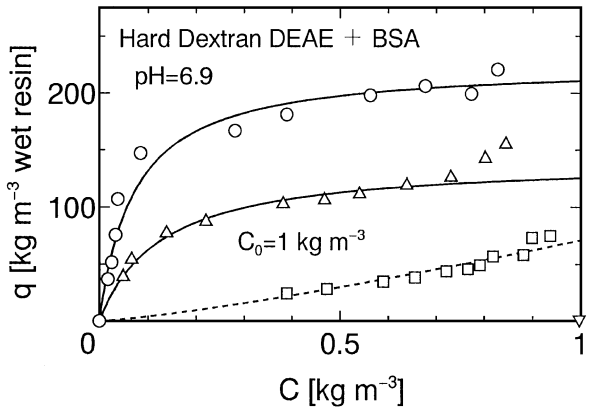

Figure 9. Effect of concentration of NaCl on equilibrium isotherm for adsorption of BSA on Hard Dextran DEAE at pH 6.9. —— Eq. (9); --- Eq. (10). C_{Nacl} (mol/m^3): ○0; △20; □50; ▽100.

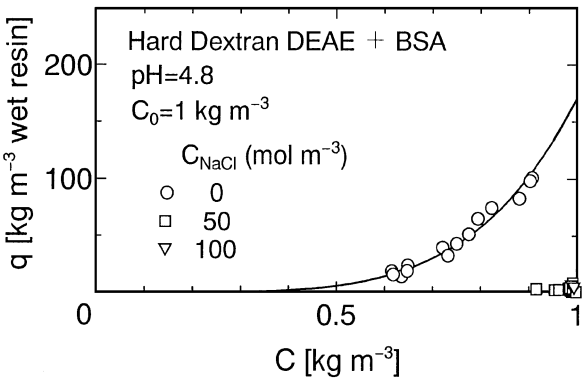

Figure 10. Effect of concentration of NaCl on equilibrium isotherm for adsorption of BSA on Hard Dextran DEAE at pH 4.8. —— Eq. (10).

well with the Langmuir equation (solid line). When $C_{NaCl} = 50\,mol\,m^{-3}$, the data could not be correlated by the Langmuir equation but agree well with the Freundlich equation (broken line). Figure 10 shows the effect of coexisting salt, NaCl, on the equilibrium isotherm at pH 4.8($\cong$ pI). When $C_{NaCl} \geq 50\,mol\,m^{-3}$, BSA was not adsorbed on Hard Dextran DEAE at all. The results in Figs. 9 and 10 suggest that BSA molecules are not adsorbed on the resin by hydrophobic interaction but by electrostatic attraction.

Conclusion

Hard Dextran DEAE, which have been developed in this study, appeared technically feasible for large-scale chromatographic separation. The following conclusions were obtained:

(i) Since Hard Dextran DEAE is very hard, it is not compressed in a column and it can be used in much wider range of flow rate in a column than the commercial hard gel, DEAE Sepharose Fast Flow made from agarose.

(ii) The saturation capacity of BSA on Hard Dextran DEAE is about 1.7 times of that on DEAE Sepharose Fast Flow at pH 6.9.

(iii) Equilibrium isotherm for adsorption of BSA depends on pH considerably. When pH $\geq$ 5.5, the equilibrium isotherm is correlated by the Langmuir equation. When pH $\leq$ 5.05, the isotherm is correlated by the Freundlich equation.

(iv) The saturation capacity of BSA is the largest at pH 6.9. Since it is about 60% of DEAE Sephadex A-50 (soft gel), the dextran-DEAE which is supported in the macropore in SEPABEADS FP-HG05 may be similar to that of DEAE Sephadex A-50.

(v) The higher the concentration of NaCl is, the smaller the amount of BSA adsorbed. When the concentration of NaCl is higher than $100\,mol\,m^{-3}$ at pH 6.9 and $50\,mol\,m^{-3}$ at pH 4.8, BSA was not adsorbed on the resin. This may suggest that BSA is adsorbed by electrostatic attraction. About $100\,mol\,m^{-3}$ NaCl aqueous solution can be used as an eluant of proteins.

Nomenclature

C	liquid phase equilibrium concentration of BSA	$kg\,m^{-3}$
C_{NaCl}	liquid phase concentration of NaCl	$mol\,m^{-3}$
C_0	liquid phase initial concentration for BSA	$kg\,m^{-3}$
	liquid phase initial concentration for NaH_2PO_4 and Na_2HPO_4	$eq\,m^{-3}$
C_1	liquid phase equilibrium concentration of $H_2PO_4^-$	$eq\,m^{-3}$
C_2	liquid phase equilibrium concentration of HPO_4^{2-}	$eq\,m^{-3}$
d	mean diameter of N particles defined by $\sum_{i=1}^{N} d_i/N$	m
d'	mean diameter of N particles defined by $\sum_{i=1}^{N} d_i^3/\sum_{i=1}^{N} d_i^2$	m
H	bed length	m
K	Langmuir equilibrium constant	$m^3\,kg^{-1}$
K_1	equilibrium constant of Eq. (2)	—
K_2	equilibrium constant of Eq. (3)	—
k	Freundlich coefficient	—
n	Freundlich coefficient	—
ΔP	pressure drop across bed	Pa
Q	exchange capacity of resin	$eq\,m^{-3}$ wet resin
q	adsorbed phase equilibrium concentration	$kg\,m^{-3}$ wet resin
q_1	adsorbed phase equilibrium concentration of $H_2PO_4^-$	$eq\,m^{-3}$ wet resin
q_2	adsorbed phase equilibrium concentration of HPO_4^{2-}	$eq\,m^{-3}$ wet resin

q_s	saturation capacity in adsorbed phase	kg m^{-3} wet resin
Re	$d' u_f \rho / \mu$	—
u_f	superficial velocity	m s^{-1}
V	volume of solution	m^3
W	volume of adsorbent	m^3

References

Arnold, F.H., H.W. Blanch, and C.R. Wilke, "Analysis of Affinity Separations I: Predicting the Performance of Affinity Adsorbers," *Chem. Eng. J.*, **30**, B9–B23 (1985a).

Arnold, F.H., H.W. Blanch, and C.R. Wilke, "Analysis of Affinity Separations II: The Characterization of Affinity Columns by Pulse Techniques," *Chem. Eng. J.*, **30**, B25–B36 (1985b).

Arve, B.H. and A.I. Liapis, "Modeling and Analysis of Elution of Biospecific Adsorption in Finite Bath," *AIChE J.*, **33**, 179–193 (1987).

Chase, H.A., "Affinity Separations Utilizing Immobilized Monoclonal Antibodies—A New Tool for the Biochemical Engineer," *Chem. Eng. Sci.*, **39**, 1099–1125 (1984).

Evanson, M.A. and H.F. Deutsch, "Influence of Fatty Acids on the Isoelectric Point Properties of Human Serum Albumin," *Clin. Chim. Acta.*, **89**, 341–354 (1978).

Ion Exchange Chromatograph: Principles and Methods, Pharmacia LKB Biotechnology, 1990.

McCoy, M.A. and A.I. Liapis, "Evaluation of Kinetic Models for Biospecific Adsorption and its Implications for Finite Bath and Column Performance," *J. Chromatography*, **548**, 25–60 (1991).

Tsou, H.S. and E.E. Graham, "Prediction of Adsorption and Desorption of Protein on Dextran Based Ion-Exchange Resin," *AIChE J.*, **31**, 1959–1966 (1985).

Wallevik, K., "Isoelectric Focusing of Bovine Serum Albumin Influence of Binding of Carrier Ampholites," *Biochim. Biophys. Acta*, **322**, 75–87 (1973).

Yamamoto, S., K. Nakanishi, R. Matsuno, and T. Kamikubo, "Ion Exchange Chromatography of Protein—Prediction of Elution Curves and Operation Conditions. II Experimental Verification," *Biotechnol. and Bioeng.*, **25**, 1373–1391 (1983).

Yoshida, H. and T. Kataoka, "Adsorption of BSA on Cross-Linked Chitosan: The Equilibrium Isotherm," *Chem. Eng. J.*, **41**, B11–B15 (1989).

Yoshida, H., H. Nishihara, and T. Kataoka, "Adsorption of BSA on QAE-Dextran: Equilibria," *Biotechnol. and Bioeng.*, **41**, 280–286 (1993).

Yoshida, H., H. Nishihara, and T. Kataoka, "Adsorption of BSA on Strongly Basic Chitosan: Equilibria," *Biotechnol. and Bioeng.*, **43**, 1087–1093 (1994a).

Yoshida, H., H. Nishihara, and T. Kataoka, "Adsorption of BSA on DEAE-Dextran: Equilibria," *Separation Sci. and Technol.*, **29**, 2227–2244 (1994b).

Yoshida, H., M. Yoshikawa, and T. Kataoka, "Parallel Transport of BSA by Surface and Pore Diffusions in Strongly Basic Chitosan," *AIChE J.*, **40**, 2034–2044 (1994c).