Salt-Promoted Adsorption of an Antibody onto Hydrophobic Charge-Induction Adsorbents

Hai-Feng Xia,[†,§] Dong-Qiang Lin,[*,‡] Zhen-Ming Chen,[†] and Shan-Jing Yao[*,†]

Department of Chemical and Biological Engineering and State Key Laboratory of Chemical Engineering, Zhejiang University, Hangzhou 310027, China

Hydrophobic charge-induction chromatography (HCIC) is a novel technology for antibody separation. In this paper, the immunoglobulin of egg yolk (IgY) was chosen as a model antibody to investigate the effects of salt on HCIC. The adsorption behavior of antibody IgY on several HCIC adsorbents as a function of salt concentration was studied using adsorption isotherms and adsorption kinetics. The hydrodynamic diameters and ζ potentials of IgY at various salt concentrations were also determined. It was found that the saturated adsorption capacities increased linearly with increasing salt concentration because of the improvement of hydrophobic interactions between IgY and the HCIC ligands. The pore diffusion model was used to evaluate the dynamic adsorption process. The total effective diffusivity (D'_e) showed a maximum value at an ammonium sulfate concentration of 0.2 M. The results indicate salt-promoted adsorption under the appropriate concentration due to a reduction of protein size and the enhancement of hydrophobic interactions between IgY and the HCIC ligand. Therefore, the addition of a proper amount of salt is beneficial for antibody adsorption in the HCIC process.

Introduction

Antibodies can be important therapeutic drugs or in vitro diagnostics, and purification methods for antibodies have been widely studied, especially affinity-based chromatography.[1−3] The purification platform with Protein A affinity adsorbents has a dominating application in research areas and industrial productions.[4,5] The specificity of Protein A for the Fc fragment of the antibody facilitates the isolation of antibodies, but this technique is quite costly and is associated with practical complications.[6] This situation has prompted a search for new alternatives to Protein A chromatography. In 1998, Burton and Harding[7] introduced a new technique for the selective capture of antibodies under physiological conditions, called hydrophobic charge-induction chromatography (HCIC). Chemical pseudo-biospecific ligands for HCIC are pH-dependent, commonly combining thiophilic, hydrophobic, and electrostatic interactions.[7] 4-Mercaptoethylpyridine (MEP), a typical HCIC ligand, contains a pyridine ring with a pK_a of 4.8 and a sulfur atom in the hydrophobic chain. The target protein can be adsorbed on uncharged ligands at neutral pH by thiophilic and hydrophobic forces and eluted by the electrostatic repulsion between the protein and the charged ligands when the pH is lower than the pK_a of MEP and the isoelectric point (pI) of the protein.[8] Boschetti[9] has reported that the dynamic binding capacities of MEP HyperCel (a commercial adsorbent from Pall Biosepara) for murine IgG1 can reach (25 to 35) mg·mL^{-1}. HCIC is a cost-effective alternative to protein A affinity chromatography, and the efficiency has been proved through several applications.[10,11]

* Corresponding authors. S.-J.Y.: Fax: +86-571-87951982. E-mail: yaosj@zju.edu.cn. D.-Q.L.: Fax: +86-571-87951015. E-mail: lindq@zju.edu.cn.
† Department of Chemical and Biological Engineering.
‡ State Key Laboratory of Chemical Engineering.
§ Current address: Key Laboratory of Industrial Biotechnology of the Ministry of Education and Research Center of Industrial Microbiology, School of Biotechnology, Jiangnan University, Wuxi 214122, China.

The binding of target proteins to HCIC adsorbents is complicated because of the dual-mode hydrophobic and ionizable ligands, whose behavior is determined by both hydrophobic and electrostatic interactions. Normally, pH and salt addition are two important factors to interpret the binding behavior of proteins toward HCIC adsorbents. The pH-dependent adsorption mechanism was studied in our previous work.[12] The pH controls the ionization states of the ligand and protein together. The uncharged high-density heterocyclic ligand at neutral pH is of great benefit to the protein binding as a result of the strong hydrophobic interactions, while the positive charge on the ligand at acidic pH induces desorption through repulsive electrostatic interactions between the ligand and the protein. The salt concentration in the solution also influences the adsorption and desorption of a protein on HCIC adsorbents through the adjustment of hydrophobic and electrostatic interactions. Basically, a high salt concentration can destroy the hydration layer on the surface of a protein, enhancing the hydrophobic interactions between ligand and protein, such as in the situation of hydrophobic interaction chromatography (HIC). However, the ligand density on HCIC adsorbents is usually higher than those on HIC adsorbents, which certainly strengthens the hydrophobic interactions and reduces the dependence of protein binding on the salt concentration. Therefore, in comparison with adsorbents for HIC, HCIC adsorbents show some "salt-independent" properties, as mentioned by Burton and Harding,[7] but this description is not so exact. It is better to use the term "salt-tolerant" instead of "salt-independent", because it can be concluded only that salt has a relatively slight impact on protein binding to HCIC adsorbents. Moreover, the salt concentration can also influence the repulsive electrostatic interactions under the acidic elution conditions in HCIC. On the whole, high salt concentrations would shield the electrostatic interactions and weaken the electrostatic repulsion between the HCIC ligand and the protein. Therefore, in additon to the control of pH, salt

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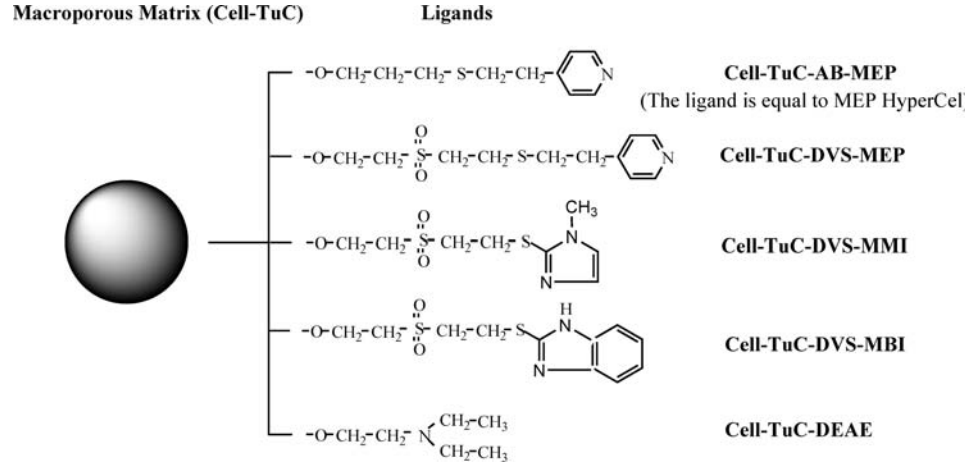

Figure 1. Structures of the four HCIC adsorbents and the ion-exchange adsorbent Cell-TuC-DEAE, all of which have same macroporous cellulose matrix, Cell-TuC.

addition could also play an important role in screening the HCIC process for protein purification. However, information on this aspect is quite limited in the literature, which is not propitious for the applications of HCIC. Gao et al.[13,14] reported the influences of salt on the protein adsorption of mixed-mode adsorbents with benzylamine as the ligand, and a specific salt concentration could be found to result in a minimum adsorption capacity. Although benzylamine shows a dual-mode binding, it is not a HCIC ligand.

In this work, with the immunoglobulin of egg yolk (IgY) as the model antibody, the adsorption behavior on four laboratory-made HCIC adsorbents (Cell-TuC-AB-MEP, Cell-TuC-DVS-MEP, Cell-TuC-DVS-MMI, and Cell-TuC-DVS-MBI) has been studied. The HCIC adsorbents were prepared using Cell-TuC as the macroporous cellulose composite matrix and three mercaptoheterocycles [4-mercaptoethylpyridine hydrochloride (MEP), 2-mercapto-1-methylimidazole (MMI), and 2-mercaptobenzimidazole (MBI)] as functional ligands, as published previously.[15,16] The adsorption isotherms and kinetics were investigated at various salt concentrations, and the behavioral differences of the ligands are discussed. The pore diffusion model was used to calculate the total effective diffusivity (D'_e) for further analysis. The influences of salt concentration on the adsorption behavior and mechanism are discussed.

Experimental Section

Materials. The HCIC adsorbents (Cell-TuC-AB-MEP, Cell-TuC-DVS-MEP, Cell-TuC-DVS-MMI, and Cell-TuC-DVS-MBI) were prepared as in our previous work,[16] and the structures of the different ligands are shown in Figure 1. The ion-exchange adsorbent Cell-TuC-DEAE, which has the same matrix as the HCIC adsorbents, was prepared as in previous work.[17] MEP HyperCel was kindly provided as a gift from the Pall Corporation (East Hills, NY). Immunoglobulin of egg yolk (IgY, 180 kDa, pI = 5.0−5.3) was purchased from AGS Biological Products Co., Ltd. (Changxing, China). Other reagents were of analytical reagent grade and purchased locally.

Protein Adsorption Equilibrium Experiments. The adsorbents were equilibrated with an appropriate buffer, after which 0.1 g samples of the drained adsorbents were added to 7 mL aliquots of 20 mM phosphate/citric acid buffer (pH 7.0) containing protein at concentrations of (0.125, 0.25, 0.5, 1.0, 2.0, and 4) mg·mL^{-1} and various concentrations of $(NH_4)_2SO_4$. The mixtures were kept at 25 °C for 10 h in a shaking incubator (180 rpm). After the adsorption process reached equilibrium, the adsorbents were separated by centrifugation (4000·*g*) and decanting, and the supernatants were analyzed with a spectrophotometer (Ultrospec 3300 pro, Amersham Biosciences, Uppsala, Sweden) at a wavelength of 280 nm to determining the protein concentrations. The amount of adsorbed protein was calculated using the mass balance equation,

$$q^* = \frac{(c_0 - c^*)\cdot v\cdot \rho_s}{m} \tag{1}$$

where q^* and c^* are the equilibrium adsorption capacity [mg·(mL of adsorbent)$^{-1}$] and equilibrium protein concentration in the liquid phase (mg·mL^{-1}), respectively, c_0 is the initial protein concentration (mg·mL^{-1}), v is the volume of protein solution (mL), and m and ρ_s are the mass and sedimented density of the adsorbents used in the experiments, respectively.

The adsorption equilibrium was fitted to the Langmuir equation,

$$q = \frac{q_m c^*}{k_d + c^*} \tag{2}$$

where q_m is the saturated adsorption capacity [mg·(mL of adsorbent)$^{-1}$] and k_d is the dissociation constant (mg·mL^{-1}).

Adsorption Kinetics Experiments. Measurements of the kinetics of adsorption of IgY onto the adsorbents as a function of salt concentration were performed as published previously.[18] Typically, 1.807 g of drained adsorbent (volume of particles = 1 mL) was mixed with 25 mL of 1 mg·mL^{-1} IgY in the 20 mM phosphate/citric acid buffer (pH 7.0) containing a particular amount of added $(NH_4)_2SO_4$. The experiments were conducted in a shaking incubator at 180 rpm and 25 °C. At various time intervals, 100 μL of solution was removed, and the protein concentration was determined with a spectrophotometer (Ultrospec3300 pro, Amersham Biosciences, Uppsala, Sweden) at 280 nm, after which the sample was returned to the flask within 30 s.

Determination of Hydrodynamic Diameter and ζ Potential of the Protein. The hydrodynamic diameter and ζ potential of IgY were measured using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, U.K.). The protein was dissolved in the 20 mM phosphate/citric acid buffer (pH 7) to a concentration of 1 mg·mL^{-1} with a defined amount of added

$(NH_4)_2SO_4$, and then the solution was filtered using a 0.1 μm filter. Each measurement was carried out five times at 25 °C, and the average value was obtained.

Adsorption Kinetics Model. In this work, the pore diffusion model was used to describe the adsorption kinetics. The governing continuity equation for the intraparticle mass transfer by pore diffusion is[19,20]

$$\varepsilon_p \frac{\partial c_p}{\partial t} + \frac{\partial q}{\partial t} = \frac{D'_e}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial c_p}{\partial r}\right) \quad (3)$$

where D'_e is the total effective diffusivity, which includes the diffusion present in the pore and on the surface of the adsorbents, r is the radial distance, c_p is the protein concentration in the pore, and ε_p is the effective particle porosity, which can be determined from the literature.[21] The value of ε_p for IgY in the composite particle (Cell-TuC) is 0.478.

The protein adsorption is assumed to be at equilibrium and expressed by the Langmuir isotherm (eq 2). Thus, eq 3 can be transformed into the expression

$$\left[\varepsilon_p + \frac{q_m k_d}{(k_d + c_p)^2}\right]\frac{\partial c_p}{\partial t} = \frac{D'_e}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial c_p}{\partial r}\right) \quad (4)$$

In the batch adsorption system, the mass transfer of protein from the bulk solution to the outer surface of the adsorbent is expressed as

$$\frac{dc}{dt} = -\frac{3k_f}{r_p}\frac{v_p}{v}(c - c_p|_{r=r_p}) \quad (5)$$

The initial conditions for c and c_p are

$$c|_{t=0} = c_0 \quad (6a)$$

$$c_p|_{t=0} = 0 \quad (6b)$$

and the boundary conditions at $r = 0$ and $r = r_p$ are

$$\frac{\partial c_p}{\partial r}_{r=0} = 0 \quad (6c)$$

$$\frac{\partial c_p}{\partial r}_{r=r_p} = \frac{k_f}{D'_e}(c - c_p|_{r=r_p}) \quad (6d)$$

In eqs 5 and 6d, k_f is the film mass-transfer coefficient, r_p is the radius of the adsorbent particles, v_p is the volume of adsorbents, and v is the volume of protein solution. k_f can be estimated as follows:[19]

$$k_f = \frac{2D_{AB}}{d_p} + 0.31\left(\frac{\mu}{\rho D_{AB}}\right)^{-2/3}\left(\frac{\Delta\rho\mu g}{\rho^2}\right)^{1/3} \quad (7)$$

where d_p is the mean particle diameter, μ is the liquid viscosity, ρ is the liquid density, and $\Delta\rho$ is the density difference between solid and liquid phases. The diffusion coefficient (D_{AB}) of IgY in free solution is 3.9×10^{-11} m^2·s^{-1} at 25 °C.[22]

The adsorption kinetics model was solved by the orthogonal collocation method with the main equation and the initial and boundary conditions. The number of collocation points in the radial direction of the adsorbent was set at 15, and the total effective diffusivity was determined at different salt concentrations.

Results and Discussion

Adsorption Equilibrium of IgY on the HCIC Adsorbents. The addition of salt to the solution has a great effect on the hydration layer of protein, which would certainly influence the hydrophobic contact area on the protein surface. A high salt concentration can also thin the double electric layer and thus shield the electrostatic force. It has been found that the hydrophobic ligands could bind on the surface of the protein to form a stable complex along with the release of water molecules from the contact area.[23] Since the hydrophobic and electrostatic interactions dominate the binding essence of HCIC, the adsorption of IgY onto the HCIC adsorbents would be influenced more or less by salt addition.

The experimental data for IgY adsorption onto the HCIC adsorbents at various $(NH_4)_2SO_4$ concentrations at pH 7 are given in Table 1. Figures 2 to 6 show the isotherm adsorption curves. The q_m and k_d data are summarized in Figures 7 and 8, respectively. It was found that the saturated adsorption capacities of all of the adsorbents tested increase linearly with an increase in salt concentration from (0 to 0.8) M, while the k_d values decrease gradually with salt addition. As for Cell-TuC-DVS-MMI and Cell-TuC-DVS-MBI, the value of q_m reaches almost 190 mg·(mL of adsorbent)$^{-1}$ at a salt concentration of 0.8 M, with growth rates of (37.4 and 43.6) %, respectively. The decrease in the dissociation constant k_d indicates stronger binding affinity at higher salt concentration. The results verified the important effect of the salt on the protein adsorption of HCIC adsorbents, as mentioned above. The addition of ammonium sulfate could release water molecules from the protein surface and enlarge the hydrophobic contact area, resulting in an enhanced thiophilic and hydrophobic interaction and thereby improving the adsorption ability of the HCIC adsorbents.

Figure 7 shows that the increases in q_m for all of the adsorbents tested have similar slopes, but the absolute values show significant differences among adsorbents. This might be explained by the individual ligand structures of the different adsorbents, as shown in Figure 1. MEP HyperCel is one of a few commercial adsorbents for HCIC, provided by Pall Biosepara. The other four adsorbents were developed recently by our laboratory using different activation processes and various functional ligands.[15] Two activation methods [allyl bromide (AB) and divinyl sulfone (DVS)] and three kinds of mercapto heterocyclic ligands (MEP, MMI, and MBI) were used to form different ligand structures. MEP HyperCel and Cell-TuC-AB-MEP have the same functional ligand and similar ligand density but different matrix structures. Therefore, as shown in Figures 7 and 8, Cell-TuC-AB-MEP and MEP HyperCel have almost identical values of q_m and k_d. In comparison with Cell-TuC-AB-MEP and MEP HyperCel, Cell-TuC-DVS-MEP has the same main ligand (MEP) but a slight difference in the spacing arm because of the sulfone group, which significantly increases the adsorption capacity under the conditions tested. The results indicate that the sulfone group on the spacing arm might improve the thiophilic interaction with the antibody, resulting in higher adsorption capacity. Moreover, Cell-TuC-DVS-MBI and Cell-TuC-DVS-MMI show the highest adsorption capacities as a result of the strong hydrophobicity of the imidazole and benzimidazole groups. The results demonstrate that the hydro-

Table 1. Experimental Data for IgY Adsorption onto HCIC Adsorbents at Different $(NH_4)_2SO_4$ Concentrations at pH 7

	0 M		0.2 M		0.4 M		0.6 M		0.8 M	
	c	*q*	*c*	*q*	*c*	*q*	*c*	*q*	*c*	*q*
adsorbent	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}
Cell-TuC-AB-MEP	0.103	10.489	0.057	11.375	0.042	9.855	0.095	22.109	0.149	28.237
	0.198	19.290	0.145	21.603	0.074	18.121	0.183	40.846	0.220	46.401
	0.542	36.977	0.497	45.798	0.325	42.114	0.494	65.836	0.412	76.050
	1.200	59.537	1.1015	75.106	0.869	80.378	1.107	92.990	1.032	101.221
	2.088	71.962	2.034	87.004	1.693	93.745	1.983	109.829	1.658	114.443
	3.179	77.925	2.907	93.389	2.758	103.011	3.057	118.045	2.893	126.090
Cell-TuC-DVS-MEP	0.078	13.126	0.046	14.169	0.039	16.921	0.046	18.531	0.082	18.951
	0.1278	30.493	0.076	32.379	0.063	28.758	0.088	37.643	0.080	35.956
	0.412	58.001	0.357	69.826	0.237	71.855	0.302	77.877	0.178	76.199
	1.082	86.256	0.945	98.339	0.768	102.409	0.832	109.942	0.747	121.333
	1.876	96.421	1.874	110.496	1.585	120.251	1.819	134.439	1.383	137.176
	3.065	105.628	2.827	119.321	2.581	128.782	2.931	141.720	2.505	145.674
Cell-TuC-DVS-MMI	0.089	12.620	0.031	15.456	0.035	16.295	0.039	19.485	0.049	23.517
	0.153	24.064	0.081	31.656	0.049	31.787	0.079	39.618	0.071	34.055
	0.553	55.679	0.300	64.907	0.198	70.806	0.235	81.168	0.160	74.680
	1.114	83.807	0.903	93.187	0.659	100.441	0.887	125.393	0.722	134.752
	1.945	98.467	1.786	122.715	1.557	135.242	1.693	148.701	1.547	157.220
	2.838	107.517	2.750	130.486	2.577	143.847	2.640	163.314	2.509	172.122
Cell-TuC-DVS-MBI	0.016	19.629	0.031	16.579	0.026	19.271	0.036	20.619	0.061	23.030
	0.072	31.876	0.099	32.650	0.042	29.650	0.062	37.015	0.065	36.845
	0.289	58.258	0.337	80.129	0.217	71.083	0.262	84.489	0.203	87.704
	0.817	97.100	0.937	106.637	0.648	107.536	0.836	121.491	0.674	119.173
	1.576	109.958	1.871	123.930	1.513	132.558	1.652	145.121	1.408	155.748
	2.556	120.930	2.541	131.147	2.531	142.410	2.771	162.166	2.383	173.399
MEP HyperCel	0.099	4.176	0.067	8.827			0.063	9.759		
	0.163	12.870	0.115	21.418			0.074	25.105		
	0.321	28.481	0.276	37.416			0.198	51.693		
	0.672	48.972	0.595	58.767			0.502	74.029		
	1.589	70.697	1.411	82.452			1.318	104.720		
	3.523	82.760	3.399	94.798			3.389	116.451		

phobic interactions dominate the protein binding for HCIC. Comparison of the k_d values for Cell-TuC-DVS-MBI and Cell-TuC-DVS-MMI shows that k_d for Cell-TuC-DVS-MBI is much smaller than that for Cell-TuC-DVS-MMI; this is due to the stronger hydrophobic interaction with the benzene ring on MBI. However, the two adsorbents show similar k_d values when salt is added, as shown in Figure 8. These results indicate that salt addition reduces the differences in hydrophobicity of the ligands, especially at high salt concentrations. For the HCIC process, some amount of salt in the solution would promote protein binding.

Adsorption Kinetics of IgY on the HCIC Adsorbents. On the basis of the data for the adsorption isotherm, the maximum adsorption ability could be obtained for the thermodynamic equilibrium under the corresponding conditions. As mentioned above, the addition of ammonium sulfate could favorably enhance the adsorption ability of IgY on the HCIC adsorbents. However, the chromatographic separation of protein in a column is a dynamic procedure. The diffusion properties in the pores of the adsorbent have an essential impact on the dynamic adsorption, which should be explored further for potential applications. Chang and Lenhoff[24] investigated the adsorption kinetics of lysozyme on six kinds of cation exchangers. It was found that the effective pore diffusivity (D_e) of most of their cation exchangers increased as the salt concentration increased, but those of some cation exchangers decreased. Gao et al.[18] measured the adsorption kinetics of BSA on mixed-mode adsorbents, and a maximum value of D_e was found among different salt concentrations. Hence, the influence of salt on the dynamic adsorption of protein varied for different kinds of adsorbents. In the present work, the effects on the HCIC adsorbents were studied. Table 2 gives the uptake data for HCIC and DEAE adsorbents at various $(NH_4)_2SO_4$ concentrations at

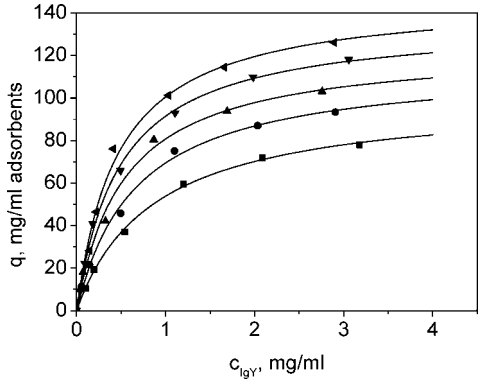

Figure 2. Isotherm adsorption of IgY on Cell-TuC-AB-MEP at pH 7 as a function of $(NH_4)_2SO_4$ concentration: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M; left-pointing triangles, 0.8 M.

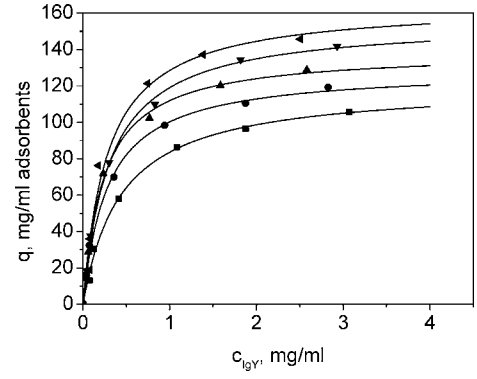

Figure 3. Isotherm adsorption of IgY on Cell-TuC-DVS-MEP at pH 7 as a function of $(NH_4)_2SO_4$ concentration: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M; left-pointing triangles, 0.8 M.

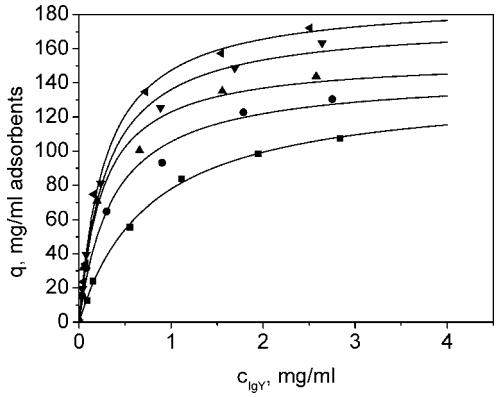

Figure 4. Isotherm adsorption of IgY on Cell-TuC-DVS-MMI at pH 7 as a function of $(NH_4)_2SO_4$ concentration: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M; left-pointing triangles, 0.8 M.

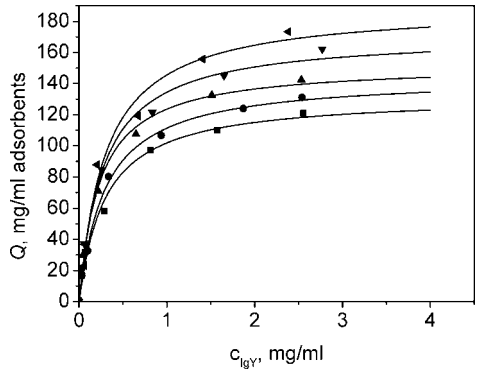

Figure 5. Isotherm adsorption of IgY on Cell-TuC-DVS-MBI at pH 7 as a function of $(NH_4)_2SO_4$ concentration: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M; left-pointing triangles, 0.8 M.

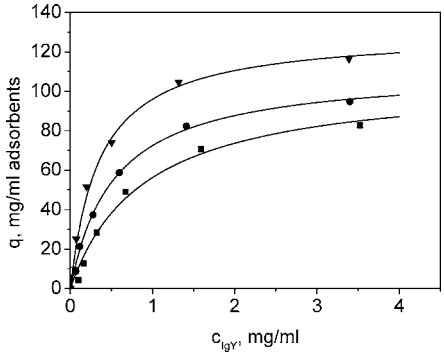

Figure 6. Isotherm adsorption of IgY on MEP HyperCel at pH 7 as a function of $(NH_4)_2SO_4$ concentration: ■, 0 M; ●, 0.2 M; ▼, 0.6 M.

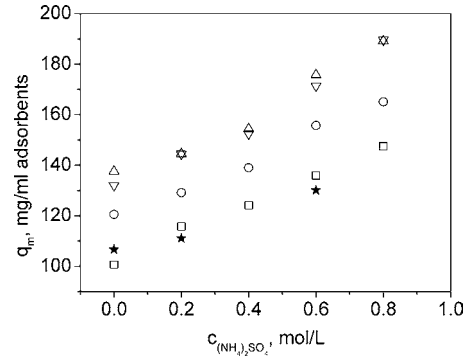

Figure 7. Effect of $(NH_4)_2SO_4$ concentration on the maximum binding capacity (q_m) of IgY on adsorbents at pH 7: □, Cell-TuC-AB-MEP; ○, Cell-TuC-DVS-MEP; △, Cell-TuC-DVS-MMI; ▽, Cell-TuC-DVS-MBI; ★, MEP HyperCel.

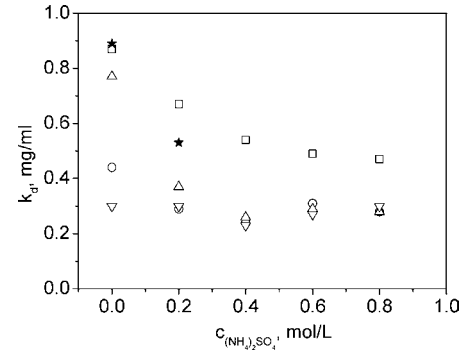

Figure 8. Effect of $(NH_4)_2SO_4$ concentration on the dissociation coefficient (k_d) of IgY on adsorbents at pH 7: □, Cell-TuC-AB-MEP; ○, Cell-TuC-DVS-MEP; △, Cell-TuC-DVS-MMI; ▽, Cell-TuC-DVS-MBI; ★, MEP HyperCel.

pH 7. Figures 9 to 12 show the adsorption uptake curves. The pore diffusion model was used to calculate the total effective diffusivity (D'_e), and the data are shown in Figure 13. In general, a maximum value of D'_e was found at an ammonium sulfate concentration of 0.2 M for all of the HCIC adsorbents tested.

Analysis of the Salt-Promoted Adsorption Mechanism. In order to explore the mechanism of the interesting tendency of D'_e, the hydrodynamic diameter and ζ potential of IgY, representing the size and charge of IgY in the solution, respectively, were determined as a function of salt concentration. Normally, a protein in aqueous solution is covered with a hydrated layer due to the ionic and hydrophilic groups on its surface, so the hydrodynamic diameter is the proper parameter for describing the size of a protein in a real solution environment and is suitable for representing the diffusion in the pores. On the other hand, the charge properties of the protein surface can be described by the ζ potential. As we know, the ζ potential is the potential of an imaginary shear plane around a charged particle, and it depends on both the surface charge of the particle and the environmental conditions (e.g., pH, ion concentration) that could determine the electrostatic interactions between charged surfaces in solution. As shown in Figure 14, the hydrodynamic diameter and ζ potential of IgY changed significantly as a function of salt concentration. The hydrodynamic diameter of IgY decreased from about (20.0 to 16.9) nm as the concentration of ammonium sulfate increases from (0 to 0.8) M. This result indicates that the hydration layer on the protein surface could be distinctly reduced by the increase in salt concentration, and thus, the hydrophobic contact area should be easily exposed for inducing the hydrophobic interactions. Martenson[25] also reported the decrease in the hydrodynamic diameters of several proteins upon the addition of salt. On the other hand, with the increase in ammonium sulfate concentration from (0 to 0.8) M, the ζ potential of IgY decreased dramatically from about −6 mV to near zero. A lower ζ potential of the protein means some amount of shielding effect on the electrostatic interactions, which could also favor the hydrophobic interaction between protein and the HCIC ligands.

Here the influences of salt addition on the adsorption kinetics might be analyzed in detail. First, the protein size (hydrodynamic diameter) decreases distinctly with increasing salt concentration, which can improve the protein diffusion in the pores of the adsorbent. Second, the increase in the number of water molecules released and the hydrophobic contact area with salt addition would enhance the hydrophobic interactions between proteins and the HCIC ligands, which favors surface diffusion. Third, the electrostatic force (corresponding to the ζ potential)

Table 2. Experimental Data for IgY Uptake onto HCIC and DEAE Adsorbents at Different $(NH_4)_2SO_4$ Concentrations at pH 7

	0 M		0.2 M		0.4 M		0.6 M	
	t		t		t		t	
adsorbent	min	c/c_0	min	c/c_0	min	c/c_0	min	c/c_0
Cell-TuC-AB-MEP	0	1	0	1	0	1	0	1
	10	0.692	10	0.667	10	0.673	10	0.685
	20	0.604	20	0.557	20	0.548	20	0.570
	30	0.534	30	0.502	30	0.475	30	0.507
	40	0.477	40	0.467	40	0.428	40	0.456
	50	0.453	50	0.428	50	0.404	50	0.442
	60	0.438	60	0.413	60	0.366	60	0.435
	70	0.423	70	0.385	70	0.354	70	0.419
	80	0.407	80	0.375	80	0.339	80	0.396
	90	0.402	90	0.365	90	0.331	90	0.377
	100	0.390	100	0.344	100	0.317	100	0.371
Cell-TuC-DVS-MEP	0	1	0	1	0	1	0	1
	10	0.682	10	0.645	10	0.672	10	0.686
	20	0.586	20	0.531	20	0.532	20	0.559
	30	0.522	30	0.466	30	0.469	30	0.497
	40	0.485	40	0.418	40	0.412	40	0.454
	50	0.457	50	0.389	50	0.377	50	0.418
	60	0.442	60	0.368	60	0.352	60	0.403
	70	0.434	70	0.342	70	0.339	70	0.393
	80	0.426	80	0.325	80	0.322	80	0.362
	90	0.434	90	0.313	90	0.306	90	0.351
	100	0.411	100	0.304	100	0.296	100	0.352
Cell-TuC-DVS-MMI	0	1	0	1	0	1	0	1
	10	0.719	10	0.605	10	0.598	10	0.651
	20	0.582	20	0.490	20	0.466	20	0.526
	30	0.504	30	0.425	30	0.402	30	0.451
	40	0.443	40	0.383	40	0.351	40	0.405
	50	0.417	50	0.341	50	0.306	50	0.403
	60	0.384	60	0.305	60	0.290	60	0.345
	70	0.380	70	0.276	70	0.265	70	0.324
	80	0.376	80	0.260	80	0.249	80	0.310
	90	0.344	90	0.249	90	0.241	90	0.288
	100	0.339	100	0.232	100	0.238	100	0.284
Cell-TuC-DVS-MBI	0	1	0	1	0	1	0	1
	10	0.630	10	0.654	10	0.637	10	0.677
	20	0.507	20	0.545	20	0.525	20	0.557
	30	0.468	30	0.493	30	0.455	30	0.488
	40	0.426	40	0.435	40	0.406	40	0.444
	50	0.412	50	0.385	50	0.358	50	0.418
	60	0.381	60	0.356	60	0.344	60	0.400
	70	0.373	70	0.328	70	0.322	70	0.381
	80	0.367	80	0.313	80	0.306	80	0.374
	90	0.360	90	0.302	90	0.300	90	0.354
	100	0.361	100	0.286	100	0.283	100	0.348
Cell-TuC-DEAE	0	1						
	10	0.563						
	20	0.482						
	30	0.479						
	40	0.399						
	50	0.379						
	70	0.357						
	80	0.335						
	90	0.334						
	100	0.334						

decreases with salt addition, which would weaken the electrostatic repulsion between protein molecules and enhance the congregation tendency of protein due to the hydrophobic interaction, hydrogen bonding, and van der Waals forces. This would reduce the diffusion of proteins in the solution.[26,27] Fourth, the increase in salt concentration would slightly increase the viscosity of the solution and more or less hinder protein diffusion.[28] The change in D'_e as a function of salt concentration might be determined comprehensively by the factors mentioned above. When the concentration of ammonium sulfate increased from (0 to 0.2) M, the hydrodynamic diameter of IgY decreased significantly from (20.0 to 17.8) nm while the ζ potential of IgY remained the same, as shown in Figure 14. Therefore, the reduction of protein size and the degree of exposure of the hydrophobic contact are on the protein surface would enhance the pore diffusion in the adsorbent, resulting in an increase in D'_e. When the concentration of ammonium sulfate increased further from (0.2 to 0.8) M, the ζ potential of IgY changed significantly from about −6.0 mV to near zero, while the

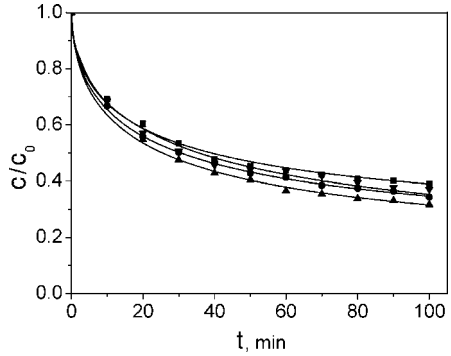

Figure 9. Adsorption kinetics curves of IgY on Cell-TuC-AB-MEP at pH 7 at different $(NH_4)_2SO_4$ concentrations: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M.

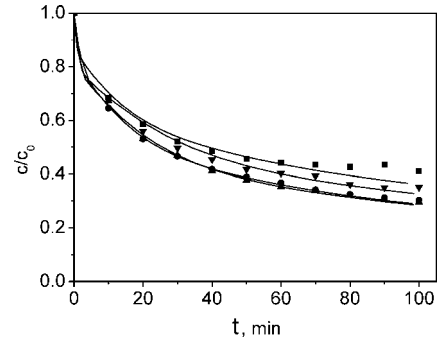

Figure 10. Adsorption kinetics curves of IgY on Cell-TuC-DVS-MEP at pH 7 at different $(NH_4)_2SO_4$ concentrations: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M.

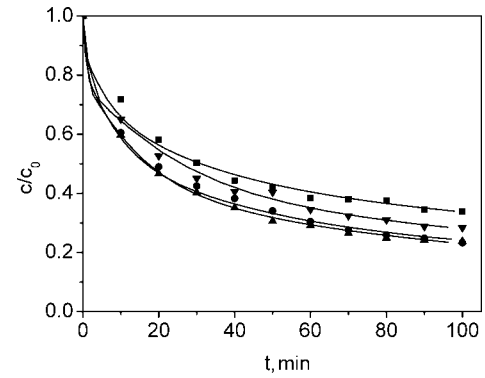

Figure 11. Adsorption kinetics curves of IgY on Cell-TuC-DVS-MMI at pH 7 at different $(NH_4)_2SO_4$ concentrations: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M.

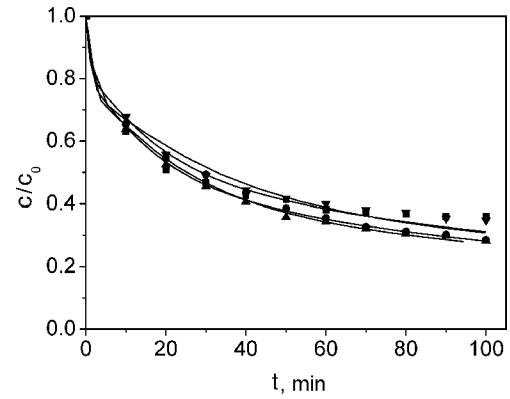

Figure 12. Adsorption kinetics curves of IgY on Cell-TuC-DVS-MBI at pH 7 at different $(NH_4)_2SO_4$ concentrations: ■, 0 M; ●, 0.2 M; ▲, 0.4 M; ▼, 0.6 M.

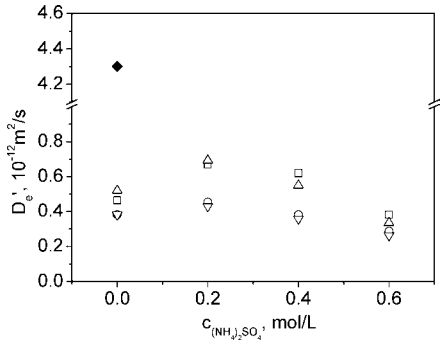

Figure 13. Effect of $(NH_4)_2SO_4$ concentration on the total effective pore diffusivity of IgY on four HCIC adsorbents and Cell-TuC-DEAE at pH 7: □, Cell-TuC-AB-MEP; ○, Cell-TuC-DVS-MEP; △, Cell-TuC-DVS-MMI; ▽, Cell-TuC-DVS-MBI; ◆, Cell-TuC-DEAE.

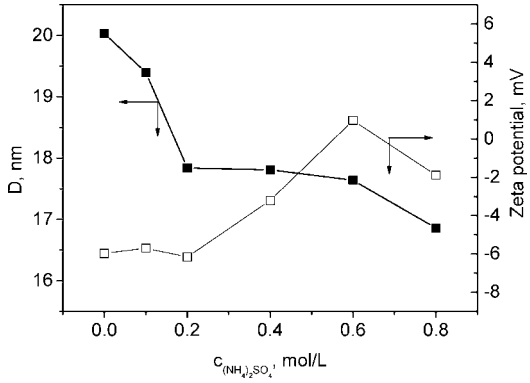

Figure 14. Hydrodynamic diameter and ζ potential of IgY at pH 7 as functions of $(NH_4)_2SO_4$ concentration: ■, hydrodynamic diameter; □, ζ potential.

hydrodynamic diameter of IgY decreased slightly from (17.8 to 16.9) nm. The impairment or loss of electrostatic interactions and the increment in the viscosity of solution would aggravate the diffusion resistance in the pore and cause D_e' to decrease. Therefore, the results indicate a salt-promoted HCIC process. At the appropriate salt concentration (0.2 M ammonium sulfate in the present case), the addition of salt not only improves the adsorption equilibrium capacity but also enhances the protein diffusion and benefits the dynamic adsorption.

It should be noted that most values of D_e' for the four HCIC adsorbents tested in the present work are relatively low, in the range (0.2 to 0.8) $\times$ 10^{-12} $m^2 \cdot s^{-1}$. The D_e' value for Cell-TuC-DEAE, an ion exchanger with the same matrix prepared previously,[16] is about 4.3 $\times$ 10^{-12} $m^2 \cdot s^{-1}$, which is 10-fold higher than that of the HCIC adsorbents. In comparison with the long-distance electrostatic interactions in the ion exchanger, the hydrophobic and thiophilic interaction for HCIC is relative weak and slow, so the reaction resistance might play an important role in the total effective diffusion. Therefore, for HCIC, use of a relatively low operation velocity should be considered for effective adsorption.

Conclusions

In this study, the adsorption equilibrium and adsorption kinetics of IgY on HCIC adsorbents as a function salt concentration has been evaluated by adsorption isotherms and the pore diffusion model. It was found that the adsorption capacities increased linearly with an increase of salt concentration from (0 to 0.8) M. The molecular structure of the HCIC ligand dominates the differences in adsorption capacity for the various HCIC adsorbents. The hydrophobic interactions between IgY and the HCIC ligands play the most important role in the adsorption process, which could be enhanced by the addition of salt. On the basis of the adsorption kinetics experiments, a maximum value of D_e' was found at a concentration of 0.2 M ammonium sulfate. In addition, the hydrodynamic diameters and ζ potentials of IgY at various salt concentrations were also determined and used to evaluate and understand the influences of salt addition on the adsorption kinetics. The results indicate that an appropriate salt addition to the solution could reduce the protein size and promote the exposure of the hydrophobic contact area on the protein surface, resulting in an improvement in protein diffusion in the pore of the HCIC adsorbents. Therefore, a proper amount of salt addition is recommended for the salt-promoted HCIC process. In addition, in comparison with an anion exchanger, the pore diffusion coefficient in the HCIC adsorbents is relatively low, indicating that a relatively low operation velocity should be considered for effective adsorption in the HCIC process.

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