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Study of the dynamic binding capacity of two anion exchangers using bovine serum albumin as a model protein

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SUMMARY

The dynamic binding capacity (DBC) of two anion exchangers (particle sizes 30 and 90 μm) were evaluated using bovine serum albumin as a model protein. The DBC showed a Langmuirian dependence on protein concentration. In general, the DBC decreased with increased loading flow-rate, but this dependence was insignificant with long (10- and 20-cm) columns. The DBC of the 30-μm anion exchanger was only slightly dependent on flow-rate even in short (5-cm) columns. The breakthrough curves were generally steeper with narrow columns and with the 30-μm material.

INTRODUCTION

In preparative chromatography, the maximum throughput is important for economic reasons; one would like to load as much protein in as little time as possible. As suggested by Chase[1], frontal analysis can be used to evaluate the dynamic binding capacity (DBC) of a chromatographic matrix. The protein is continuously loaded onto the column at a particular flow-rate until breakthrough of the feed stream occurs. The amount of protein that has been applied at this point is the DBC. If one continues to feed the protein through the column, a breakthrough curve is generated, its slope representing the rate of increase in protein concentration in the effluent. The sharpness of such a curve provides a measure of the binding efficiency and a clue to the kinetics involved. This approach is far better than performing batch studies, as it represents real operating conditions.

The DBC for a particular protein is dependent on many factors[2]: the nature of the gel matrix[3,4], the flow-rate at which the protein is applied to the column, the column dimensions (*i.e.*, column diameter and bed height), protein size, film, pore and overall diffusion constants of the protein and the nature of the adsorption isotherm of the protein. Regnier and co-workers[4–6] have collected extensive data on various parameters that affect the intrinsic loading capacity of silica-based materials

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for both reversed-phase and anion-exchange chromatography of proteins. Protein binding depended on the accessible surface binding sites rather than the total surface area, and the pore size affected not only the loading capacity but also the resolution of the proteins. An extensive review on ion-exchange chromatography was published by Helfferich[7]. Despite recent advances in the theoretical understanding of chromatographic processes[8–13], protein chromatography still remains largely empirical. Recently, Kopaciewicz *et al.*[14] studied the effects of particle, pore and protein sizes on the binding capacity of silica-based media. In their study, both static and dynamic experiments were carried out. They found that the DBC was inversely proportional to flow-rate and particle size, and the magnitude of these relationships depended strongly on the pore size of the media. The purpose of this work was to study experimentally the effect of loading flow-rate, column dimensions, gel particle size and initial concentration on the DBC of two agarose-based media for a protein.

EXPERIMENTAL

Materials

Bovine serum albumin (BSA) (fraction V) was purchased from Miles Diagnostics. The two anion exchangers tested were Q Sepharose Fast Flow (FF) and Q Sepharose High Performance (HP) (Pharmacia). Both of these gels are 6% cross-linked agarose-based media with similar pore-size distributions (they both have exclusion limit of 4×10^6 daltons). Their average bead sizes are 90 and 30 μm, respectively. In addition, they share similar chemical properties with $-CH_2N^+(CH_3)_3$ as the ion exchange group. Analytical-reagent grade Tris–HCl was obtained from Sigma, and the fast protein liquid chromatographic (FPLC) system and HR columns (Pharmacia) were used throughout this study.

Methods

The DBC of Q Sepharose Fast Flow and Q Sepharose High Performance were evaluated using BSA as a model protein. The DBC is defined as the sample load at which the sample concentration of the effluent, monitored by UV absorbance at 280 nm, is 10% of the input stream. This is expressed as milligrams of BSA per millilitre of packed gel. The slopes of the breakthrough curves are approximated by the straight line connecting 10% and 90% of maximum deflection of the UV trace. Columns of I.D. 5 and 10 mm were packed at bed heights of 5, 10 and 20 cm under a linear flow-rate of 5 cm/min. BSA, dissolved in 50 m*M* Tris–HCl, pH 7.0 at 10 mg/ml, was fed through the gel matrices at linear flow-rates from 0.25 to 4.0 cm/min. The DBC was determined as a function of four parameters: the linear sample loading flow-rate (F), the column diameter (D), the packed bed height (H) and the average particle size of the gel. Experiments were performed according to the combinations of F, H and D listed in Table I, and two data points were collected for each combination.

The DBC was also determined as a function of initial protein concentration according to Table II. A 5 mm I.D. column packed with 90 μm FF material to a height of 10 cm was used for this study. BSA at concentrations from 1 to 10 mg/ml was loaded onto the column at 1.0 or 4.0 cm/min.

All experiments were performed at room temperature. To avoid systematic effects due to uncontrolled variables such as small temperature fluctuations, the order of the experiments was randomized.

TABLE I

AVERAGE VALUES OF DBC FOR ALL EXPERIMENTAL COMBINATIONS OF *F*, *H*, *D*, AND PARTICLE SIZE

Linear velocity, F (cm/min)	*Bed height, H (cm)*	*Column diameter, D (mm)*	*DBC (mg BSA/ml packed gel)*	
			30 μm	*90 μm*
0.25	5	5	86.3	68.7
0.50	5	5	86.7	68.0
1.00	5	5	85.6	66.3
2.00	5	5	84.6	64.7
4.00	5	5	84.6	56.2
0.25	5	10	59.6	66.5
0.50	5	10	57.2	65.3
1.00	5	10	60.0	63.2
2.00	5	10	50.0	59.6
4.00	5	10	57.3	45.6
0.25	10	5	77.1	72.1
0.50	10	5	77.7	71.4
1.00	10	5	78.0	70.3
2.00	10	5	76.4	69.1
4.00	10	5	76.0	66.0
0.25	10	10	71.6	61.1
0.50	10	10	70.7	58.8
1.00	10	10	71.3	60.0
2.00	10	10	69.1	56.8
4.00	10	10	68.1	56.7
0.25	20	5	72.2	67.4
0.50	20	5	72.9	67.3
1.00	20	5	72.4	66.3
2.00	20	5	71.7	66.8
4.00	20	5	72.2	66.5
0.25	20	10	64.7	62.9
0.50	20	10	63.6	62.2
1.00	20	10	63.2	61.8
2.00	20	10	61.5	61.8
4.00	20	10	62.0	60.9

RESULTS AND DISCUSSION

Effect of gel particle size

In Fig. 1, DBC is plotted against linear flow-rate. Each line is associated with a particular column dimension. The DBC of both media are similar. The 30-μm HP material showed only a very small dependence on flow-rate even in the 5-cm column. The 90-μm material showed a substantial decrease in the DBC with flow-rate only in the 5-cm column.

TABLE II

DBC VALUES AS A FUNCTION OF INITIAL PROTEIN CONCENTRATION TESTED WITH A 10 cm × 5 mm I.D. COLUMN USING THE 90-μm BEADS

Initial concentration (mg/ml)	*DBC (mg BSA/ml packed gel)*	
	0.2 ml/min	*0.8 ml/min*
1	50.5	53.0
2	55.6	56.2
4	61.4	59.8
6	71.3	65.8
8	72.0	69.1
10	74.4	72.4

The sharpness of the breakthrough curves is presented in Fig. 2 in the same manner as in Fig. 1. This is expressed as the rate of increase in the effluent concentration with respect to the amount of BSA applied. The 30-μm material achieves a steeper breakthrough slope, and hence a more efficient binding of protein, than the

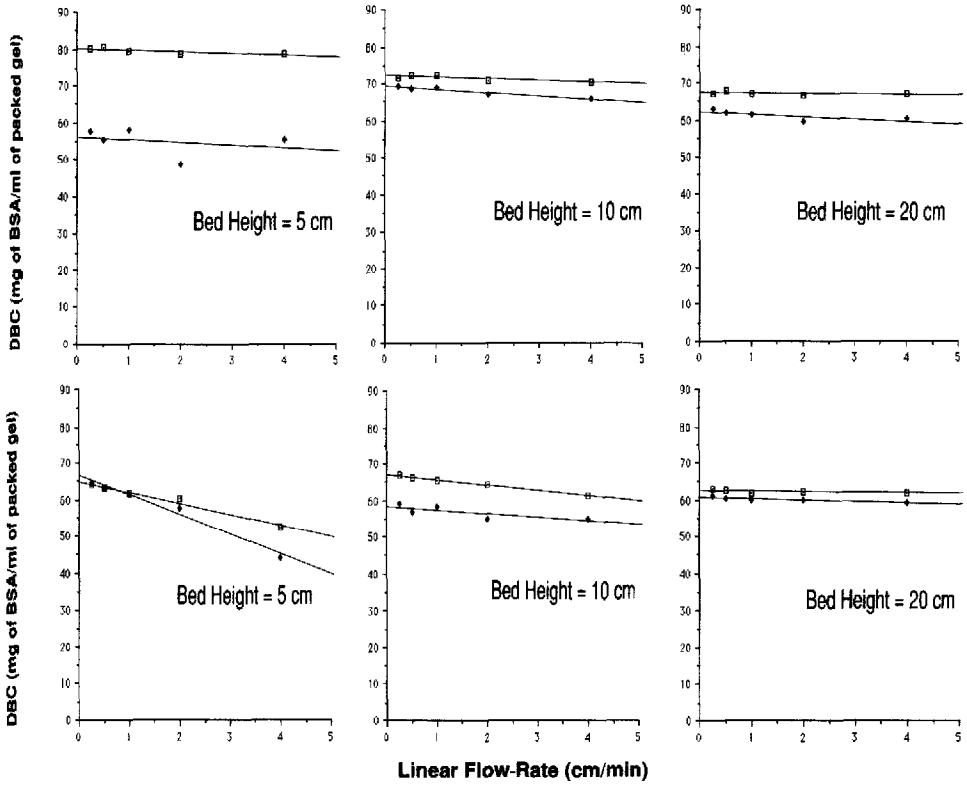

Fig. 1. DBC *vs.* linear flow-rate for (top) the 30-μm gel and (bottom) the 90-μm gel. □ = 5 mm I.D.; ◆ = 10 mm I.D.

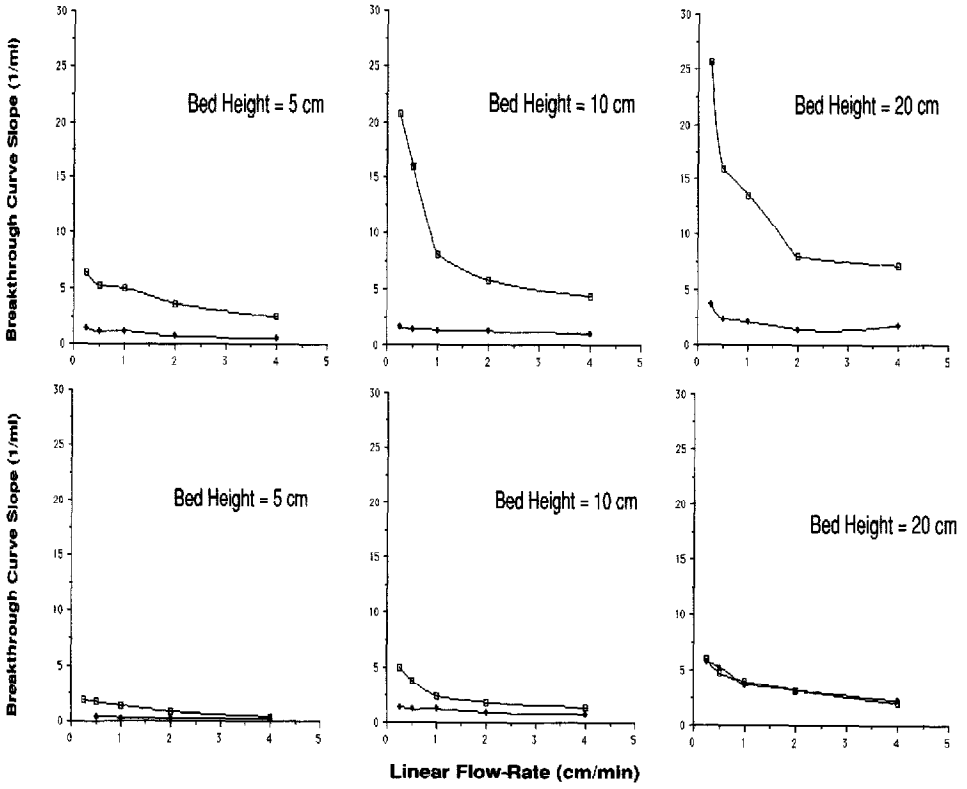

Fig. 2. Breakthrough curve slope *vs.* linear flow-rate for (top) the 30-μm gel and (bottom) the 90-μm gel. □ = 5 mm I.D.; ◆ = 10 mm I.D.

90-μm beads. The rate of protein adsorption, particularly for large proteins, is improved when small gel particles are used[15]. This is due to the increase in surface area and shorter intraparticle diffusion path. Assuming a two-phase model, the increase in surface area facilitates film diffusion and results in a higher DBC. The shorter pore diffusion path allows a faster adsorption of protein and thus a higher adsorption efficiency.

Coupling effect of bed height and linear flow-rate

In Fig. 1, the DBC of the 90-μm material becomes less dependent on the flow-rate as the bed height increases from 5 to 20 cm. When the bed height reaches 20 cm, one can increase the loading flow-rate 16-fold (from 0.25 to 4.0 cm/min) with only a slight decrease in the DBC. As for the 30-μm material, the DBC has very little dependence on the flow-rate at all the bed heights tested. In addition, steeper breakthroughs are associated with a lower loading flow-rate for both media, as shown in Fig. 2.

In general, an increase in linear flow-rate corresponds to a decrease in the DBC. This is largely due to the mass-transfer resistance experienced by the protein. At high loading flow-rates, not enough time is allowed for pore diffusion, and poor utilization

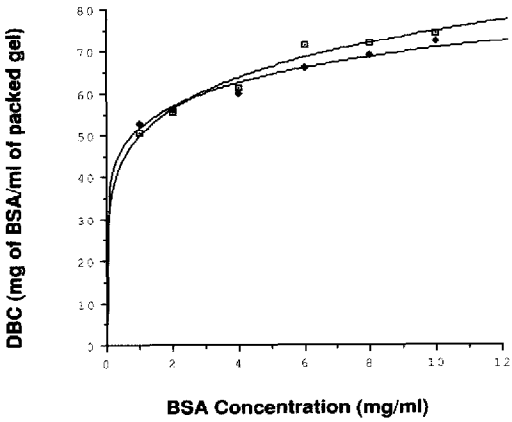

Fig. 3. DBC *vs.* initial BSA concentration at different flow-rates. □ = 1.0 cm/min; ◆ = 4.0 cm/min.

of the gel bed results. For the 90-μm FF material, we found that the effect of linear flow-rate on the DBC decreases with increasing bed height. A longer gel bed allows for a longer residence time, and therefore more time for pore diffusion to be completed. When using larger gel particles, a long gel bed is recommended to maximize throughput in the sample loading stage. Naturally, when using a long column, the tradeoffs are a higher operating back-pressure and an increased separation time.

Effect of protein concentration

In Fig. 3, the DBC of the 90-μm FF material is plotted against protein concentration for two loading flow-rates. These two curves are Langmuirian. If batch adsorption of BSA on an ion exchanger is of the Langmuir type, the dynamic adsorption of this protein may exhibit a deviation from Langmuirian behavior owing to the kinetic effect[16]. In this instance, a 10-cm bed was used to guarantee efficient gel utilization, which explains the similarity of the curves at two different flow-rates.

Effect of column diameter

A higher DBC and a steeper breakthrough are associated with the 5-mm I.D. columns. We attribute this effect of column diameter to an uneven radial distribution of protein during sample loading.

CONCLUSIONS

When using smaller particles (HP medium, 30 μm), the DBC hardly depends on the loading flow-rate even in a short column. Also, steeper breakthroughs are associated with smaller particles. The tradeoff here is a higher operating pressure.

A long gel bed can compensate for the effect of flow-rate on the DBC when larger gel particles (FF medium, 90 μm) are used. The tradeoffs here are a higher operating pressure and a longer separation time owing to the long column.

Dynamic adsorptions at different flow-rates display similar Langmuirian behavior if efficient mass transfer is maintained.

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