Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Expanded-Bed Adsorption of Proteins Using Ion-Exchangers

Howard A. Chase[a]; Nicholas M. Draeger[a]
[a] DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, UNITED KINGDOM

To cite this Article Chase, Howard A. and Draeger, Nicholas M.(1992) 'Expanded-Bed Adsorption of Proteins Using Ion-Exchangers', Separation Science and Technology, 27: 14, 2021 — 2039
To link to this Article: DOI: 10.1080/01496399208019462
URL: http://dx.doi.org/10.1080/01496399208019462

SEPARATION SCIENCE AND TECHNOLOGY, **27**(14), pp. 2021–2039, 1992

Expanded-Bed Adsorption of Proteins Using Ion-Exchangers

HOWARD A. CHASE and NICHOLAS M. DRAEGER
DEPARTMENT OF CHEMICAL ENGINEERING
UNIVERSITY OF CAMBRIDGE
PEMBROKE STREET, CAMBRIDGE CB2 3RA, UNITED KINGDOM

Abstract

The use of expanded beds of ion-exchange adsorbents for the adsorption of proteins from feedstocks containing whole cells is described. Such feedstocks can be applied to the bed without prior removal of particulate material by centrifugation or filtration, thus showing considerable potential for this approach in simplifying downstream processing flow-sheets. Stable, expanded beds can be obtained using simple equipment adapted from that used for conventional packed bed adsorption and chromatography processes. Ion-exchange adsorbents are likely to be chosen for such separations as a result of the robustness of the ion-exchange groups to cleaning and sanitization protocols. Frontal analysis involving the measurement of breakthrough curves indicates that the adsorption performance of an expanded bed is similar to that achieved when the same amount of adsorbent is used in a packed configuration at the same volumetric flow-rate. In addition, the adsorption performance of an expanded bed of a cation exchanger was not diminished when adsorption was carried out in the presence of high concentrations of cells. However, the performance of similar beds of anion exchangers was found to be reduced under some conditions depending on the type of cells, their concentration, and their age. The results from the expanded bed adsorption experiments were consistent with those from batch adsorption which showed that the adsorption characteristics of the anion exchangers were diminished in the presence of cells in contrast to results with a cation-exchanger system where little deterioration in performance was noted. The results suggest that cation exchangers might be expected to be of more use for expanded bed adsorption in the presence of cells.

INTRODUCTION

The attractions of using an expanded bed of adsorbent beads for the direct adsorption of proteins from feedstocks containing whole cells or pieces of disrupted cells have been fully described in the recent literature (*1–10*). The principle of the method is demonstrated in Fig. 1. The application of particulate-containing feedstocks to conventional packed beds of

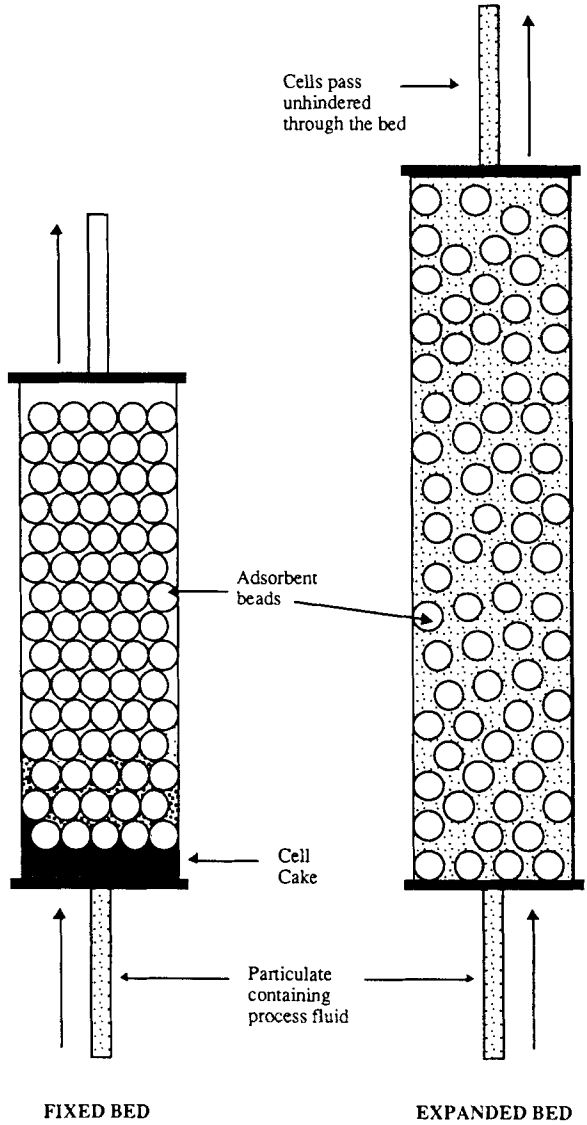


FIG. 1. Principle of the use of expanded beds for the adsorption of proteins in the presence of particulates. This schematic drawing (not to scale) demonstrates the trapping of particulates within the voids of a packed bed and the avoidance of such problems if an expanded bed is used instead.

adsorbent beads results in severe operational problems as a result of the trapping of particulate matter in the voids of the bed (Fig. 1, left). These problems may be manifested by an increase in the pressure drop across the bed and the formation of a plug of trapped solids at the inlet of the bed. However, when a bed of adsorbent beads is expanded in an unconstrained configuration by up-flow of liquid, particulate material is able to pass through the bed without becoming trapped (Fig. 1, right). Although Fig. 1 indicates a uniform particle size, the adsorbent particles used in the work described here have a significant distribution of particle sizes and some grading of the adsorbent occurs in the expanded bed, with the larger particles being located near the bottom of the bed and the smaller particles nearer the top. This grading of particle sizes contributes significantly to the stability of the expanded bed, thus removing the need to resort to complicated methods of expanded bed stabilization such as the application of a magnetic field to magnetically susceptible particles. The ability to be able to apply particulate-containing feedstocks directly to adsorbent systems may, in some circumstances, eliminate the need for prior removal of cells and/or cell debris by centrifugation or filtration, thus greatly reducing the complexity and associated costs of certain downstream processing flowsheets.

We have demonstrated that it is possible to establish a stable, expanded bed using adsorbents based on the agarose matrix Sepharose FF (*6–10*) without the need for special stabilization protocols such as the use of magnetic fields (*11*). The expansion of such beds follows simple characteristics that can adequately be described by the correlations of Richardson and Zaki (*12*). Residence time distributions have shown that flow through the bed is close to plug flow and that there is minimal circulation of adsorbent beads. Such conditions result in the expanded bed behaving as a quasi-packed bed, giving excellent adsorption performance very similar to that achieved when the same amount of adsorbent is used at the same linear flow velocity in a packed configuration.

Considerations governing the choice of suitable adsorbents for use in expanded bed procedures are similar to those to which any adsorbent selected for use in direct broth recovery techniques must comply. The porous structure of the bead must be such that it is resistant to fouling and degradation when operated in crude process liquids. More importantly, the ligand attached to the adsorbent must also be suitable for operation in crude process liquids and, in general, ideal ligands are not available. The choice of ligand is often complicated by the need to compromise between ligand stability and specificity for adsorbing species. The use of robust ligands, such as ion-exchange or hydrophobic interaction groups, often results in low capacities for the desired solute as a result of lack of

specificity for this compound. In addition, the relatively high ionic strength of some fermentation broths results in low capacities of ion exchangers used in direct broth extraction as the adsorption capacity of ion exchangers falls appreciably as ionic strength is increased. Conversely, the use of ligands with the requisite high specificity is often frustrated by the fragility of such ligands when used in the harsh operating cycles associated with the processing of crude feedstocks. Such problems are particularly acute when the highly selective ligands are proteins such as antibodies. Recent work has indicated that expanded beds of the highly selective adsorbent, Protein A Sepharose Fast Flow, show excellent adsorption performance in the presence of high concentrations of cells (*10*). However, the usefulness of such a system is compromised by the difficulties in sterilization of an adsorbent with proteinaceous ligands.

Ion-exchanger adsorbents based on the Sepharose Fast Flow matrix are completely stable during autoclaving (120°C, pH 7.0, 30 min) and during cleaning-in-place protocols using 2 *M* NaOH (*13*). Such stability is ideal for use of these adsorbents in direct extraction of proteins and peptides from fermentation broths despite the fact these adsorbents may not show a large, selective adsorption capacity for particular exocellular products. The aim of the work described here is to investigate further the influence of a number of types of cells on the adsorption properties of the anion exchanger Q-Sepharose FF and the cation exchanger S-Sepharose FF. This is followed by an investigation of the expanded bed adsorption performance of these adsorbents in the presence and absence of cells in order to demonstrate their potential usefulness in the direct extraction of proteins from cell broths.

EXPERIMENTAL

Ion-Exchange Adsorption Systems

In order to obtain a clear understanding of the performance of expanded beds with and without the presence of cells, model adsorption systems were investigated. The system chosen involves the adsorption of bovine serum albumin (BSA) (Sigma Chemical Co., Dorset, England) to the anion exchanger Q-Sepharose Fast Flow (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) with quaternary nitrogen groups and the cation exchanger S-Sepharose Fast Flow with sulfonic acid ligands. Adsorption experiments with Q-Sepharose FF and S-Sepharose FF were carried out in 0.1 *M* Tris/HCl buffer (pH 7.0) and 0.1 *M* sodium acetate buffer (pH 5.0). The physical properties of adsorbents based on the Sepharose FF matrix and their fluidization characteristic in expanded beds have been fully de-

scribed in our previous published work (*6–10*). In order to ascertain the effects of the presence of cells on the adsorption characteristics of these adsorbents, experiments were carried out with preparations of *Saccharomyces cerevisiae* (bakers' yeast), *Alcaligenes eutrophus,* and *Escherichia coli.* Suspensions of washed cells in the appropriate buffer for each adsorbent were used in these experiments, as opposed to using untreated fermentation broth containing cells, in order to be certain that any effects noticed were a direct and sole consequence of the presence of cells rather than being caused by the different physical properties of the broth or the presence of other unspecified compounds in the broth.

Measurement of Adsorption Isotherms in the Presence of Cells

A range of concentrations of BSA from 0.5 to 10 mg/mL were made up in separate flasks in 20 mL of 0.01 *M* Tris/HCl buffer, pH 7.0. A number of sets of flasks were made up in this way, each set of flasks containing a different concentration of cells ranging from 0 to 50 mg dry mass/mL. The adsorbent, in a 1:1 buffer slurry (1 mL in each test), was added to each flask. The flasks were then sealed, to prevent evaporation, and incubated with agitation in a 25°C water bath for between 50 and 70 hours to allow equilibrium to occur between the solid and liquid phases. After this period had elapsed, a 1.5-mL sample was removed from each flask in an Eppendorf centrifuge tube, and centrifuged to pellet adsorbent and cells. Controls were performed without added adsorbent, and with and without cells being present. The optical density of each supernatant was measured at 280 nm, and the reading was used to obtain the equilibrium concentration of protein in the liquid phase. Measurement of the optical density of the controls confirmed that there was, under these conditions, negligible release from the cells of material that absorbs light at 280 nm. The amount of protein adsorbed to the adsorbent was calculated by mass balance.

Packed and Expanded-Bed Procedures

Liquid was pumped through packed or expanded beds of adsorbents contained in columns with diameters of 2.2 and 3 cm. The column of diameter 2.2 cm was a standard glass chromatography column purchased from Amicon, Stonehouse, UK, fitted with an adjustable adapter with a 20-μm net. The column used for expanded bed adsorption experiments was a 3.0 × 30 cm glass column with a distributor consisting of a 5-mm glass inlet tube leading to a hemispherical flow distribution volume prior to a P160 sintered glass disk with a pore diameter of 160 μm (BDH Laboratory Apparatus Limited) which allowed unhindered passage of cells.

Particular care was taken to prevent any air bubbles from collecting behind the distributor as their presence was a common source of bed instability.

The adsorption of pure BSA to the ion exchangers was tested in packed and expanded bed configurations to examine and compare the efficiencies of the adsorption performance of the adsorbents under these conditions. In experiments in the absence of cells, the concentration of BSA in the outlet stream from the bed was followed by measuring optical adsorption at 280 nm, and this was used to measure the breakthrough curve. The layout of the apparatus used in these tests is described in full elsewhere (*6*, *8*, *9*). Two P-6000 (Pharmacia LKB Biotechnology) pumps were used in the system and could be controlled, either manually or automatically, using a personal computer interfaced to the system via a LCC 500 CI process controller (Pharmacia LKB Biotechnology). One of these pumps was used to pump buffer during equilibration and washing, and eluent during the elution stage, and the second pump was used to pump the adsorbate solution. The UV absorbance of the column outlet stream was measured at 280 nm with a UV-1 monitor (Pharmacia LKB Biotechnology). These signals were recorded on a chart recorder as well as being logged by a FPLC Manager software package (Pharmacia LKB Biotechnology).

Adsorbent slurry was washed in a filter with distilled water to remove the preservative liquor. The washed adsorbent was then poured into the bed until a suitable settled bed height was attained. The adsorbent was washed *in situ* initially with 0.01 *M* Tris/HCl buffer, and fines were elutriated by washing the bed with buffer at a linear flow velocity 1.5 times that which would be used subsequently for obtaining a stable expanded bed. The flow rate was then readjusted and washing with buffer continued until the bed had expanded to the required height and the top of the expanded bed remained at a constant level. When the bed was operated in the expanded configuration, the top adapter of the chromatography column was lowered to just above the surface of the expanded bed to avoid the presence of a column of liquid above the bed. During operation in a packed configuration, the top adapter was fitted with the standard net and lowered onto the surface of the bed. The bed was then ready for the application of adsorbate solution. Adsorbate was applied to the bed until the optical density of the liquid leaving the bed had approached that of the bed inlet stream. It was observed that at low levels of bed expansion, the height of the expanded bed dropped slightly during protein adsorption as a result of protein binding to the adsorbent, thus increasing its density. The flow rate through the bed was therefore increased gradually in order to maintain a constant extent of bed expansion.

When measurement of the breakthrough curve was complete, the system was switched to buffer delivery to wash excess adsorbate solution from the

column until the UV absorbance of the column outlet stream had decreased and leveled off. At this stage, the expanded bed was returned to a packed configuration by stopping flow through the bed and allowing the adsorbent to settle. This was followed by lowering the adapter onto the surface of the packed bed. This procedure was not necessary when the adsorption stage had been carried out in a packed bed configuration. Eluent was passed through the bed to remove adsorbed protein from the adsorbent. In general, a large peak of eluted protein was observed and elution was continued until the optical density had reached a steady, low value. The system was again switched to buffer delivery, and buffer was pumped through the column to remove excess eluent from the column. Reequililbration continued until the pH of the outlet stream had increased back to the original value at the beginning of the run.

For expanded bed adsorption experiments in the presence of cells, a modified procedure had to be adopted during the adsorption as the presence of particulates prevented the direct monitoring of the breakthrough curve of adsorbate by simple on-line measurement of the optical density at 280 nm. Adsorbate solution was pumped through the bed until an amount of adsorbate equal to the theoretical maximum adsorption capacity of the bed (as determined from the results of the batch isotherms) had been applied to the bed. The liquid leaving the expanded bed was collected in 10 mL fraction collector test tubes using a FRAC 300 fraction collector (Pharmacia LKB Biotechnology). The samples were centrifuged to pellet the cells, and the optical density of each clarified supernatant was measured at 280 nm. Control experiments confirmed that the measured optical density was an accurate measure of the level of adsorbate in these samples.

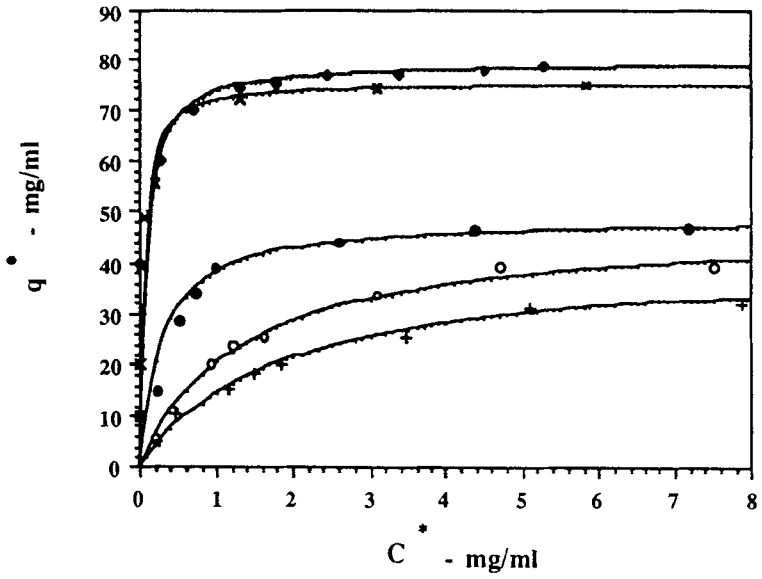
RESULTS AND DISCUSSION

Effect of Cells on Adsorption Characteristics

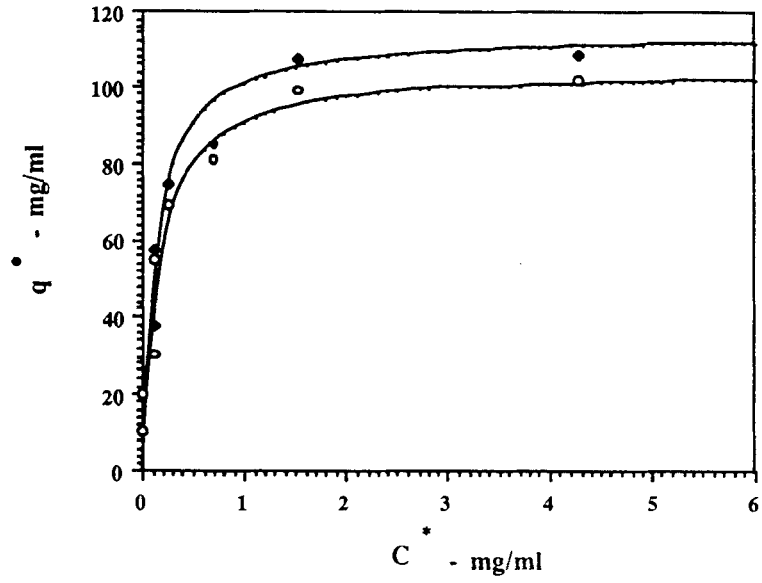
In order that any adsorbent can be used in direct broth extraction techniques, it is important to ascertain that the adsorption properties are not seriously impaired in the presence of cells. Experiments were carried out to determine the effect of cells on the equilibrium and adsorption characteristics of the two ion-exchange systems used in this study.

Equilibrium Adsorption Isotherms

The effects of the presence of cells of *S. cerevisiae* on the equilibrium characteristics of the adsorption of pure bovine serum albumin to the ion exchangers were determined by the measurement of adsorption isotherms. Figure 2(a) shows the results obtained with the adsorption of BSA to Q-



(a)



(b)

Sepharose Fast Flow which indicate a reduction in the adsorption capacity in the presence of cells. However, similar experiments studying the adsorption of BSA to S-Sepharose Fast Flow showed comparatively little alteration to the shape of the isotherms at a concentration of cells as high as 30 mg/mL (Fig. 2b). A more quantitative assessment of the results was performed by fitting the data to the Langmuir isotherm. In all cases, a good quantitative agreement was shown to an equation of the form (*14*):

$$q^* = \frac{q_m c^*}{(K_d + c^*)} \tag{1}$$

where q_m and K_d are the maximum capacity and the dissociation constant of the adsorption interaction, respectively. The effect of the presence of cells on the values of q_m and K_d are shown in Table 1. The values given in Table 1 demonstrate that at a cell concentration of 30 mg dry mass/mL, the maximum adsorption capacity of the cation exchanger (S-Sepharose Fast Flow) is reduced by only 8%, accompanied by a 13% increase in the dissociation constant. In the presence of the same concentration of cells, the equivalent values for the anion exchanger (Q-Sepharose FF) are altered more drastically, with a 49% decrease in the maximum capacity and an increase in the dissociation constant by almost two orders of magnitude. The capacities and dissociation constants of these ion exchangers for BSA in the absence of cells observed in these experiments are very similar to those reported previously (*15*, *16*).

A similar reduction of the capacity of Q Sepharose Fast Flow for BSA has also been observed in the presence of cells of *Alcaligenes eutrophus* (*13*) in which it was found that a cell concentration of 5 mg/mL reduced the value of the maximum capacity of the adsorbent for BSA from 80 to 66 mg/mL. The dissociation constant was observed to be increased from 0.02 to 3.5 mg/mL. The reason for the different influence of the effect of cells on the two types of ion exchanger almost certainly lies in the fact that the surfaces of the cells carry net negative charges at the pHs used in the adsorption experiments due to the presence of acidic polymers as com-

FIG. 2. Isotherms for the adsorption of BSA to ion exchangers in the presence of cells of *S. cerevisiae*. Isotherms were measured as described in the text for the adsorption of BSA to ion exchangers. (a) Adsorption to Q-Sepharose Fast Flow in 0.01 *M* Tris buffer, pH 7.0 at 25°C. The concentration of cells (mg dry mass/mL) present in each experiment were: 0 (♦); 5 (×); 10 (●); 30 (○); 50 (+). (b) Adsorption to S-Sepharose Fast Flow in 0.1 *M* acetate buffer, pH 5.0 at 25°C; (♦) 0 mg/mL; (○) 30 mg/mL. In all cases the solid line shows the best fit to a Langmuir-type isotherm, described in Eq. (1).

TABLE 1
Adsorption Equilibrium Characteristics of Ion Exchangers in the Presence of Cells[a]

Adsorbent	*S. cerevisiae* concentration (mg/mL)	q_m (mg/mL)	K_d (mg/mL)
Q-Sepharose Fast Flow	0	80	0.02
	5	75.2	0.05
	10	49.0	0.28
	30	47.6	1.31
	50	41.1	1.80
S-Sepharose Fast Flow	0	114	0.13
	30	105	0.15

ponents of the cell walls. Hence cells would be expected to bind to the positively charged anion exchangers but be repelled from the surface of negatively charged cation exchangers. The competition shown by the cells for the protein adsorption sites on the anion exchangers will depend on a number of factors including the charge density on the surface and the cell size.

The results of these batch experiments indicate that the available capacity for protein adsorption will be much more significantly reduced in the presence of cells for anion exchangers than for cation exchangers, suggesting that the latter type of exchanger may be more useful in direct extraction techniques. This hypothesis is put to the test in the next sections.

Comparison of Adsorption Breakthrough Profiles in Packed and Expanded Beds

A comparison was made between the efficiency of protein adsorption in a fixed and fluidized bed by examining the adsorption of BSA to Q-Sepharose FF in a 2.2-cm diameter column (Fig. 3). In these experiments, adsorbate was applied to the same amount of adsorbent at the same volumetric flow rate with the bed in either a packed or expanded configuration. It is apparent that when the adsorbent bed is expanded to approximately 3–4 times its settled bed height, there is little or no difference between the efficiency of protein adsorption in the fixed or fluidized bed modes. The breakthrough curves obtained for both modes of operation are virtually identical. It is, however, important to expand the bed gradually by passing buffer through it and to allow the expanded bed to stabilize before the start of the protein adsorption phase.

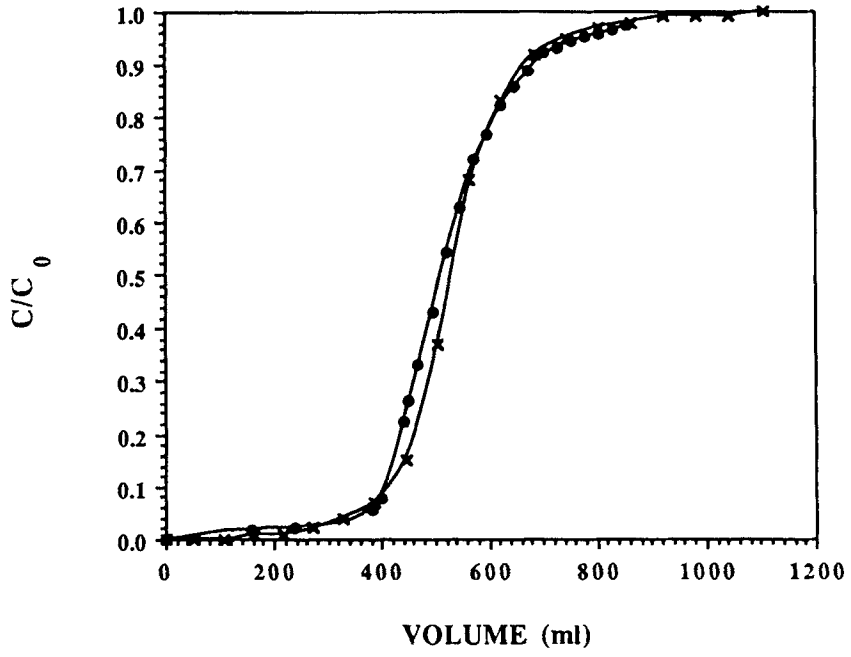


FIG. 3. Packed and expanded-bed adsorption of BSA onto Q-Sepharose Fast Flow. Breakthrough curves for the adsorption of a solution of 2.5 mg/mL BSA in 0.01 *M* Tris buffer, pH 7.0, to Q-Sepharose Fast Flow in a 2.2-cm column were measured with the bed in either an expanded or packed-bed configuration: (×) packed bed height = 4 cm; (●) expanded bed height ≈ 12 cm. Both experiments were carried out at a linear flow velocity of 1.6 cm/min (6 mL/min).

Similar agreement between the shapes of the breakthrough curves in packed and expanded beds has also been found in experiments studying the adsorption of BSA onto S-Sepharose Fast Flow. Hence the expanded bed appears to behave identically to the equivalent packed bed except that its interstitial voidage is greater, thus allowing the free passage of cells. This similarity has led us to suggest that models that have been developed to predict the performance of the adsorption phase in fixed-bed systems (*15*, *17*) can also be used to predict expanded bed adsorption performance provided that the greater voidage of the expanded bed is taken into account.

Expanded-Bed Adsorption in the Presence of Intact Cells

Previous work had shown that the performance of expanded-bed adsorption of BSA to Q-Sepharose FF was severely diminished in the presence of cells of *Alcaligenes eutrophus* (*9*). However, that work was carried

out with cells that were well into the stationary phase of growth and had accumulated granules of β-hydroxybutyrate within the cells. Hence, the effect of the age of *A. eutrophus* cells on expanded-bed adsorption of BSA onto Q-Sepharose FF was investigated as described in Fig. 4. A reduction in expanded-bed adsorption performance was again observed in the presence of cells when compared to experiments in the absence of cells. However, cells harvested in the exponential phase of growth appeared to have less effect on the effective capacity of the adsorbent, there being a reduction of only 2-fold in the capacity of the adsorbent as opposed to the 4-fold reduction observed in the tests with older cells harvested in the stationary phase. The extents of reduction of adsorbent capacity were consistent with the reduction in adsorbent capacity determined previously from equilib-

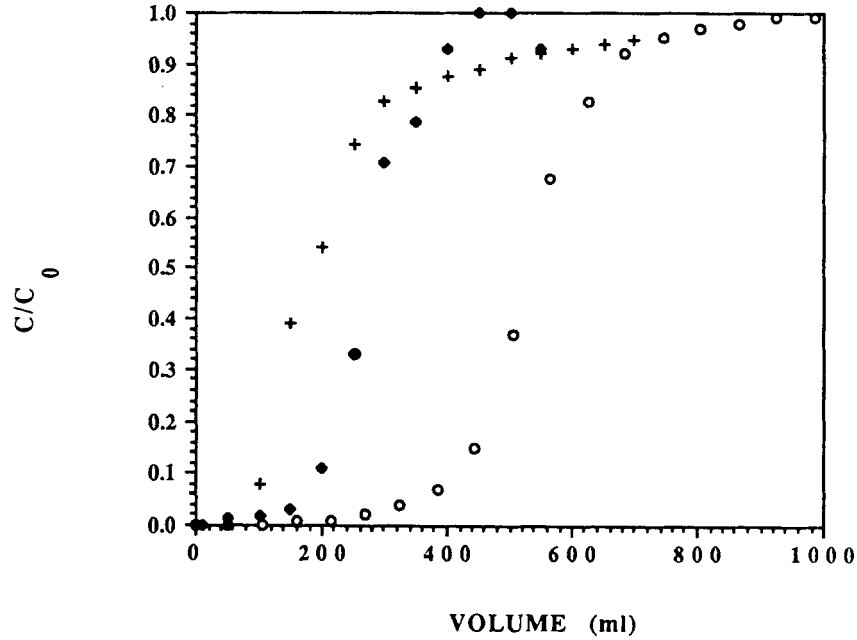


FIG. 4. The effect of the presence of *A. eutrophus* cells on the expanded-bed adsorption of BSA onto Q-Sepharose Fast Flow. Breakthrough curves were measured for the adsorption of a 2.5-mg/mL solution of BSA in various liquids onto an expanded bed of Q-Sepharose Fast Flow in a 2.2-cm diameter column. The height of the settled bed of adsorbent was 4 cm, and liquid was pumped through the bed to expand it to a height of 12 cm during measurement of the breakthrough curves. The solutions used were: (○) 0.01 *M* Tris buffer, pH 7.0; (◆) 0.01 *M* Tris/HCl buffer, pH 7.0, containing 5 mg/mL cells in their exponential phase; (+) 0.01 *M* Tris/HCl buffer, pH 7.0, containing 5 mg/mL cells in their stationary phase.

rium isotherm experiments in the presence of cells (*9*). Examination of the shapes of the breakthrough curves indicated that the rates of mass transfer also appeared to be reduced to a lesser degree in the presence of the exponential phase cells compared to the stationary phase cells. The reason for the less interference with protein adsorption shown by the exponential phase cells is not certain but is likely to be the result of differences in the surface properties of exponential and stationary phase cells. Examination of the morphologies of the cells during the various phases of growth reveals gross differences which are probably reflected in changes in the cell wall constituents which in turn affect the strength by which the cells interact with the ion exchanger.

Although adsorption performance was reduced in the presence of *A. eutrophus*, expanded-bed adsorption using anion exchangers still shows potential for use in direct broth extraction as, particularly in experiments with exponential phase cells, a considerable volume of cell/protein solution could be loaded onto the bed before breakthrough of BSA occurred, albeit at reduced capacity of the adsorbent for protein.

The expanded-bed adsorption performance of Q-Sepharose FF in the presence of different types of cells was also examined by testing the adsorption of BSA onto Q-Sepharose Fast Flow in the presence of 5 mg/mL of *E. coli* cells or *S. cerevisiae* (Fig. 5). In contrast to the results obtained with *A. eutrophus*, the effective capacity of the adsorbent appears to be affected only slightly by the presence of these cells in low concentration (5 mg/mL). Cells of *E. coli* were found to have somewhat less effect on the breakthrough profiles than cells of *S. cerevisiae*. The results for *S. cerevisiae* are entirely consistent with the finding from the isotherm experiments (Table 1) in which comparatively little decrease in adsorption performance was noted. It is perhaps surprising that *E. coli* cells have less effect on adsorption than those of *A. eutrophus* considering that these cells are also gram-negative and possess a net negative charge on their cell envelopes. However, reasons for the observed difference may include the larger size of the *E. coli* cells (up to twice as large in diameter and up to 4 times longer) and that the net negative charge on the cell envelope of the *E. coli* bacterium may be less than that of cells of *A. eutrophus*. Hence Q-Sepharose Fast Flow appears to show potential for use in direct extraction techniques when operated in the presence of low concentrations of cells.

Tests were carried out on the effect of higher cell concentrations on the adsorption performance of an adsorbent in a fluidized bed by adsorbing BSA to Q-Sepharose Fast Flow in the presence of varying concentrations of *S. cerevisiae* as shown in Fig. 6. As indicated from the equilibrium tests, the effective capacity of the adsorbent and the efficiency of the adsorption

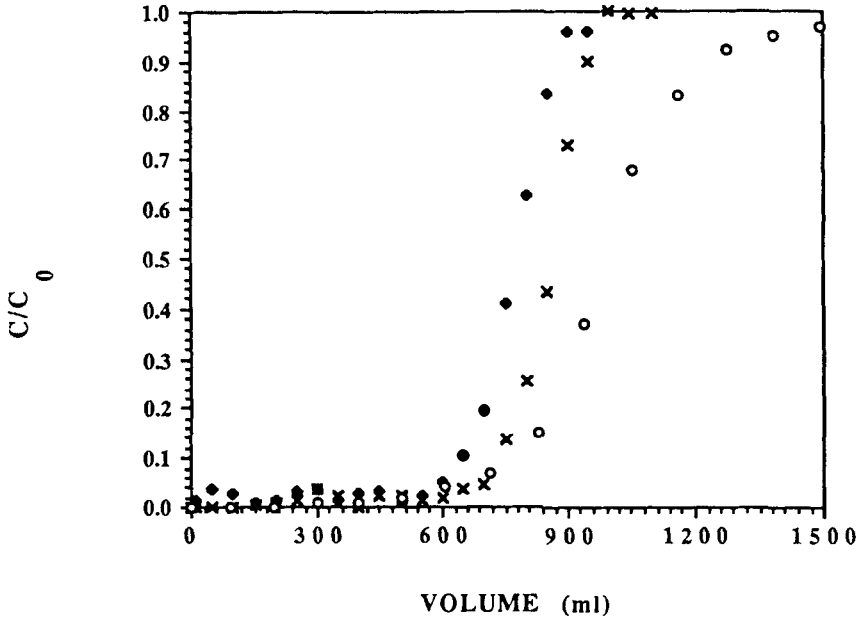


FIG. 5. The effect of cell type on the expanded-bed adsorption of BSA onto Q-Sepharose Fast Flow. Breakthrough curves were measured for the adsorption of a 2.5-mg/mL solution of BSA in various liquids onto an expanded bed of Q-Sepharose Fast Flow in a 3.0-cm diameter column. The height of the settled bed of adsorbent was 4 cm, and an appropriate flow rate was used to expand the bed to a height of 12 cm during measurement of the breakthrough curves. The solutions used were: (○) 0.01 *M* Tris/HCl buffer, pH 7.0, in the absence of cells; (×) 0.01 *M* Tris/HCl buffer, pH 7.0, containing 5 mg/mL *E. coli* cells; (◆) 0.01 *M* Tris/HCl buffer, pH 7.0, containing 5 mg/mL *S. cerevisiae* cells.

of protein to the adsorbent were reduced as the concentration of yeast cells increased. This decrease was again mirrored in the position of the breakthrough curves which showed a markedly decreased adsorption performance when the cell concentration was increased to 50 mg/mL. Hence the results agree with the conclusions of the batch studies. A qualitative inspection of the adsorbent after excess cells had been washed out showed that the adsorbent had become fouled and had turned a beige colour as a result of the binding of yeast cells to the adsorbent. An inspection of a sample of adsorbent particles under an optical microscope revealed that the Q-Sepharose Fast Flow particles appeared to have cells adhering to their surfaces. It appears, therefore, that on the basis of this evidence, Q-Sepharose is more suitable for direct extraction from broths containing cells at modest cell concentrations rather than high cell concentrations.

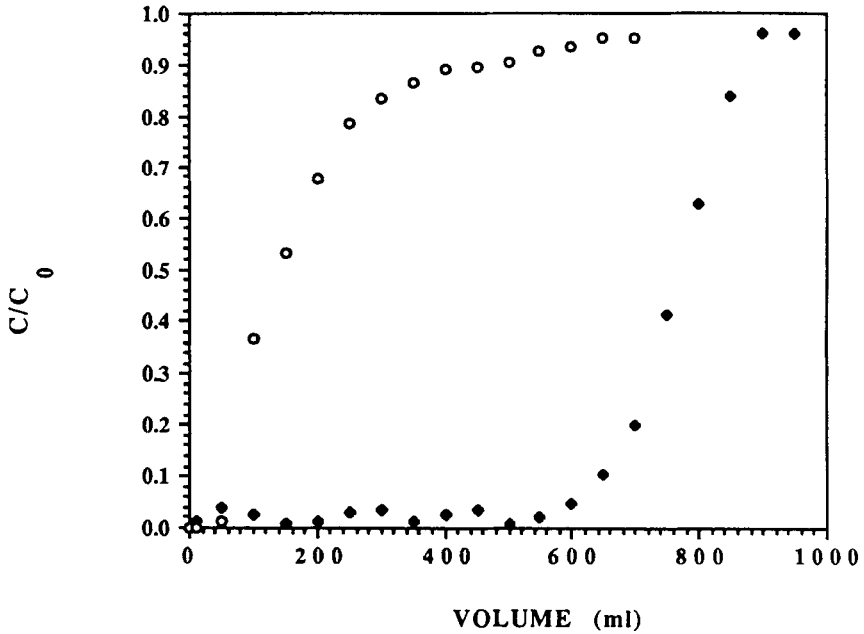


FIG. 6. The effect of the presence of yeast cells on the expanded-bed adsorption of BSA onto Q-Sepharose Fast Flow. Breakthrough curves were measured for the adsorption of a 2.5-mg/mL solution of BSA in various liquids onto an expanded bed of Q-Sepharose Fast Flow in a 3.0-cm diameter column. The height of the settled bed of adsorbent was 4 cm, and an appropriate flow rate was used to expand the bed to a height of 12 cm during measurement of the breakthrough curves. The solutions used were: (♦) 0.01 *M* Tris/HCl buffer, pH 7.0, containing 5 mg/mL cells; (○) 0.01 *M* Tris/HCl buffer, pH 7.1, containing 50 mg/mL cells.

Batch isotherm experiments had indicated that the adsorption of BSA to the cation exchanger S-Sepharose FF was not significantly affected by the presence of cells. This observation was confirmed by measurement of breakthrough curves for expanded bed adsorption. The results, shown in Fig. 7, indicate a slight reduction in adsorption performance in the presence of yeast cells at 5 mg/mL, but there is still a sharp breakthrough curve even in the presence of 30 mg/mL of cells, conditions under which the capacity of Q-Sepharose FF was severely reduced. From a qualitative inspection of the cation-exchange adsorbent after the adsorption runs, there appeared to be little or no fouling of the adsorbent by yeast cells. The adsorbent remained white, and inspection under an optical microscope showed that the S-Sepharose Fast Flow adsorbent appeared to be almost free of any cells adhering to the particles after washing. The results obtained

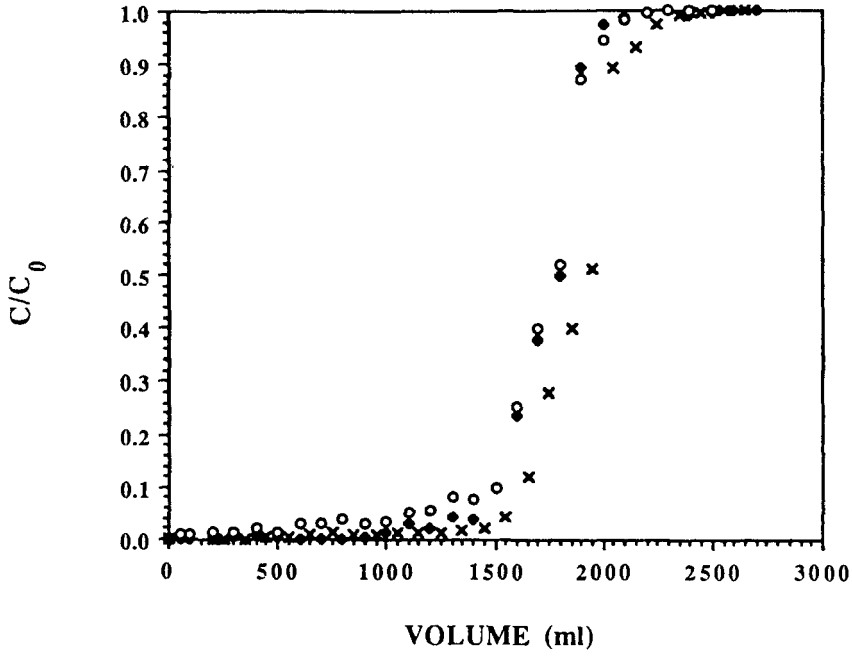


FIG. 7. The effect of the presence of yeast cells on the expanded-bed adsorption of BSA onto S-Sepharose Fast Flow. Breakthrough curves were measured for the adsorption of a 2.5-mg/mL solution of BSA in various liquids onto an expanded bed of S-Sepharose Fast Flow in a 3.0-cm diameter column. The height of the settled bed of adsorbent was 6 cm, and an appropriate flow rate was used to expand the bed to a height of 18 cm during measurement of the breakthrough curves. The solutions used were: (×) 0.02 *M* acetate buffer, pH 5.0, in the absence of cells; (♦) 0.02 *M* acetate buffer, pH 5.0, containing 5 mg/mL cells; (○) 0.02 *M* acetate buffer, pH 5.0, containing 30 mg/mL cells.

with expanded-bed adsorption of protein onto S-Sepharose Fast Flow were very similar to those obtained previously with the affinity system consisting of the adsorption of human immunoglobulin G to Protein A Sepharose Fast Flow. In the latter system the presence of cells had very little effect on the shape or position of the breakthrough curve obtained in expanded-bed adsorption experiments.

In general, no problems were observed associated with the physical aspects of the passage of cell suspensions through the expanded bed. Measurement of the cell concentration in the liquid that had flowed through the bed indicated that the hold-up of cells within the bed was negligible. The flow distribution systems used in these small-scale experiments were satisfactory as they allowed a stable expanded bed to be formed while

allowing the free passage of cells. However, alternative, more sophisticated flow distribution devices will be needed on scale up, and the design of these is currently under investigation.

At the end of the adsorption phase, it is necessary to wash particulates and nonadsorbed proteins out of the voids of the bed. This stage is also carried out with the bed in the expanded configuration in order to prevent the trapping of particulate material in the bed. Experiments described elsewhere (*9*, *10*) have indicated that when cell suspension is followed by a wash solution of greater density and viscosity (such as 25% glycerol in a suitable buffer), the stable front of this solution was found to be very effective in sweeping particulates from the bed without compromising bed stability. On the other hand, switching the liquid flow through the bed from a cell suspension to a less viscous and less dense wash buffer could result in destabilization of the expanded bed with undesirable mixing of the adsorbent beads and, in extreme cases, loss of adsorbent from the bed.

Following removal of cells from the expanded bed by washing, the adsorbed proteins can be eluted from the adsorbent by operation in either an expanded or a packed-bed configuration. The latter method may be indicated in circumstances where it is important to ensure that adsorbed proteins are eluted in the minimum volume or in situations where proteins are to be eluted selectively by using a gradient or a series of step changes in eluent composition.

The use of ion exchangers in direct broth extraction procedures is likely to require the thorough cleaning and regeneration of the adsorbent following each cycle of use. Protocols involving treatments with 2 *M* NaOH have been found to be highly effective at removing foulants from these adsorbent (*13*), and following such treatments the full adsorbent capacity is regenerated for use in a subsequent cycle of operation. It must be remembered that the studies described here have concentrated on the degree of fouling caused by whole cells suspended in simple buffer solutions, and that when the ion exchangers are used in actual direct broth extraction, they are likely to be fouled and have their adsorption performance diminished by other soluble components in the broth. Such reductions in adsorption capacity for the compound of interest will be highly system specific but must be fully assessed before a direct broth protocol is selected. They are, however, not a direct consequence of the use of expanded bed adsorption but arise from the use of comparatively nonselective adsorbents in a direct broth extraction procedure.

The success of expanded-bed purification depends crucially on the ability to be able to set up a stable, expanded bed. It is important to assess whether stable beds can be achieved in industrial columns with diameters many times greater than those used in this laboratory study. The ability to be

able to achieve an even distribution of flow across the whole surface of the bed has already been achieved in the design of columns intended for use in packed-bed chromatography on a large scale. However, it is necessary to make appropriate modifications to ensure that particulates will be able to pass through the flow distribution system.

We anticipate that the introduction of expanded-bed technology will have an important impact on certain downstream processing flow sheets as it will eliminate the need to include a step for the removal of particulates before a feedstock can be applied to a bed of adsorbent. The choice of a suitable adsorbent for use in an expanded-bed procedure, of the type described above, needs to be made with care; both the physical and adsorption properties of the adsorbent need to be considered. Adsorbents based on Sepharose FF are not ideal for use in direct broth expanded-bed adsorption as a result of the low density difference between the solid and liquid phases. This low difference, coupled with the high viscosities likely to be encountered with some bioprocess liquids, results in low values for the terminal velocity of the adsorbent particle in the liquid. This in turn requires operation of the expanded-bed adsorber at comparatively low flow rates in order to achieve the required degree of bed expansion. Such low flow rates may be unsuitable for industrial practice as a consequence of the extended period needed to load the feedstock onto the bed. It is important, therefore, that new adsorbents with densities higher than those used in this work be developed for use in expanded-bed adsorbers in order that adsorption can be performed at higher linear velocities. However alternative materials must maintain the generally good adsorption properties shown by adsorbents based on the Sepharose Fast Flow matrix.

CONCLUSIONS

This study of expanded beds of ion-exchange adsorbents, in model situations, has confirmed the potential of this mode of operation for the direct adsorption of protein feedstocks containing intact cells. Under these circumstances the expanded bed can be considered to be a direct analogy of a packed-bed system. With cation exchangers the adsorption performance is similar to that which would have been achieved if clarified material had been applied to the same amount of adsorbent in a packed configuration at the same volumetric flow rate. With anion exchangers, a loss of performance was noted which depended on the nature of the cell suspension. However, this loss of performance is not attributable to operation in the expanded-bed configuration but is a direct result of the presence of cells interfering with the adsorption of protein. We anticipate the introduction of expanded-bed purification protocols into a number of industrial-scale protein purification flow sheets in the near future.

NOTATION

BSA	bovine serum albumin
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
C	liquid phase concentration
c^*	equilibrium liquid concentration per unit bed volume
K_d	Langmuir isotherm constant (dissociation constant)
q	amount of adsorbate bound to adsorbent
q^*	amount of adsorbate bound to adsorbent at equilibrium with liquid phase
q_m	Langmuir isotherm constant (maximum adsorption capacity)
u	superficial liquid velocity
u_t	terminal velocity of adsorbent bead
ϵ	bed voidage

Acknowledgment

We would like to thank Pharmacia BioProcess Technology, Uppsala, Sweden, for the generous provision of chromatographic materials and instruments without which this work could not have been undertaken.

REFERENCES

1. C. M. Wells, K. Patel, and A. Lyddiatt, in *Separations for Biotechnology* (M. S. Verrall and M. J. Hudson, eds.), Ellis Horwood, Chichester, 1987, p. 217.
2. C. M. Wells, Ph.D. Thesis, University of Birmingham, 1989.
3. J. P. van der Wiel, Ph.D. Thesis, University of Delft, 1989.
4. J. P. van der Wiel and J. A. Wesselingh, in *Adsorption Science and Technology* (A. E. Rodrigues, M. D. LeVan, and D. Tondeur, eds.), Kluwer Academic Publishers, Dordrecht, 1989, p. 427.
5. N. B. Gibson and A. Lyddiatt, *Separations for Biotechnology 2*, (D. L. Pyle, ed.), Elsevier Science Publishers, Barking, England, 1990, p. 152.
6. N. M. Draeger and H. A. Chase, in *Advances in Separation Processes* (*I. Chem. E. Symp. Ser. 118*), Institution of Chemical Engineers, Rugby, England, 1990, p. 161.
7. N. M. Draeger and H. A. Chase, in *Separations for Biotechnology 2* (D. L. Pyle, ed.), Elsevier Science Publishers, Barking, England, 1990, p. 325.
8. N. M. Draeger and H. A. Chase, *Trans. Inst. Chem. Eng., C, 69,* 45 (1991).
9. N. M. Draeger and H. A. Chase, *Bioseparation, 2,* 67 (1991).
10. H. A. Chase and N. M. Draeger, *J. Chromatogr.*, Submitted for Publication.
11. M. Burns, *Biotech. Prog., 1,* 95 (1985).
12. J. F. Richardson and W. N. Zaki, *Trans. Inst. Chem. Eng., 32,* 35–53 (1954).
13. *Q and S Sepharose Fast Flow—Product Data Sheet,* Pharmacia LKB Biotechnology, Uppsala, Sweden.
14. H. A. Chase, *J. Chromatogr., 297,* 179 (1984).
15. G. L. Skidmore, B. J. Horstmann, and H. A. Chase, *Ibid., 498,* 113 (1990).
16. G. L. Skidmore and H. A. Chase, in *Ion Exchange for Industry* (M. Streat, ed.), Ellis Horwood, Chichester, England, 1988, p. 520.
17. B. J. Horstmann and H. A. Chase, *Chem. Eng. Res. Des., 67,* 243 (1989).