PROBLEM

Lysozyme is to be purified by cation exchange adsorption in the batch mode from 1000 litres of egg white protein solution (total protein concentration = 10 g/1). Lysozyme makes up 5% of egg white proteins on a weight basis. Preliminary laboratory-scale lysozyme binding experiments were carried out using two cation exchange adsorbents and the free and bound concentrations determined are shown below:

Lysozyme concentration in solution (g/l)	Adsorbent #1 Bound lysozyme concentration (g/l)	Adsorbent #2 Bound lysozyme concentration (g/l)
0.2	15	10
0.4	18	15
0.6	20	20
0.8	22	25

Comment on the nature of the adsorption isotherms. Which adsorbent will be better for the above mentioned separation?

PROBLEM

A hormone is being recovered from 10 litres of a biological fluid by affinity adsorption. The adsorption follows linear isotherm and the concentration of the hormone in the fluid is 0.01 g/l. 80% of the hormone could be adsorbed in the batch mode by 10 ml of affinity adsorbent. How much hormone would be adsorbed if 50 ml of adsorbent were used?

Problem

Ex 6.4-1 Lactate dehydrogenase adsorption. This enzyme is being adsorbed in a fixed bed 1.3 m long, packed with modified cellulose. The bed is 7 cm in diameter and has a void fraction of 0.30. A diuted feed containing 1.7mg/L of the enzyme is being fed to this bed; at this dilution, the enzyme is adsorbed according to linear isotherm: q (mg/cm^3) = 38 y(mg/cm^3

The velocity in the bed produces breakthrough in 6.4 h and the bed is exhausted in 10 hr. Calculate the following

(a) Length of the adsorption zone at breakthrough

(b) Length of equilibrium zone at breakthrough

(c) Fraction of the bed's capacity which is being used

Example 6.4-2. Cephalosporin Adsorption. We are studying the adsorption of this antibiotic on a weakly anionic resin. From batch experiments, we find that

$$q(\text{g/liter resin}) = 32(y(\text{g/liter solution}))^{1/3}$$

We now want to measure adsorption in a fixed bed.

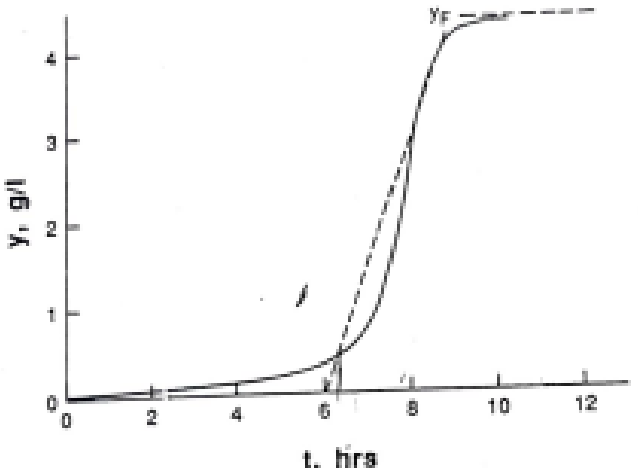
The bed which we choose is 1.0 m long and 3.0 cm in diameter, with a bed density of 0.67 cm^3 resin/cm^3 bed. Our feed solution contains 4.3 g/liter of the antibiotic. When we use a superficial bed velocity of 2 m/hr,

we obtain the breakthrough curve shown in Figure 6.4-4. Answer the following:

(a) Calculate how much of the feed is lost if we stop the adsorption when $y = 0.4$ g/liter.

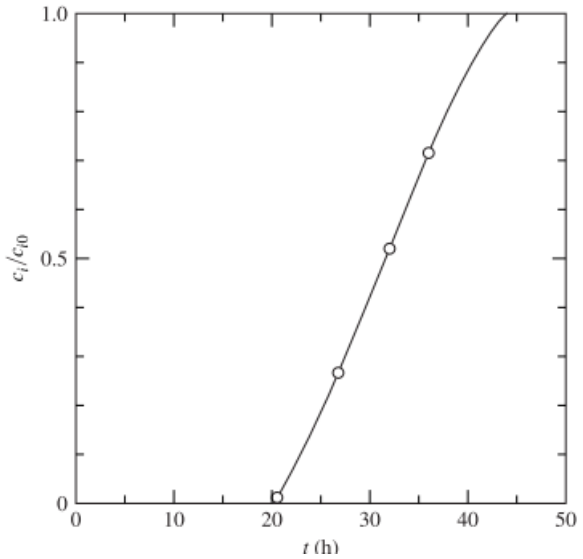
(b) Estimate what fraction of the bed's capacity is used at this breakthrough. Assume the bed is exhausted when $y = 4.0$ g/liter.

(c) Estimate the rate constant in the bed.



Problem

The breakthrough data given in Table were obtained for the adsorption of a pharmaceutical product in a laboratory column (5 cm diameter × 15 cm high) at a feed flow rate of 400 ml/h and feed concentration of 0.75 U/liter, where U is units of biological activity of the pharmaceutical product. It is desired to scale up the process to operate in a column 30 cm high. What break-point time can be expected in the 30 cm high column?



t (h)	c_i (U/liter)
20.5	0.01
26.7	0.17
32.0	0.39
36.0	0.53

Ref 2, (Harrison) Ex 7.6

Problem

Stages in Triglyceride chromatography. HPLC of stearic-oleic-steric glyceride gives a peak leaving the column after 85 minutes. The wdth of this peak when concentration is half the maximum is 5 minutes. Estimate the no. of hypothetical stages in this column.

Problem

Chromatography of Bovine Serum Albumin. 10 g of this albumin is eluted from an 80 liter Sephadex column which has a void fraction of 0.4. The concentration in the column peaks after 470 liters are eluted; this maximum concentration is 1.8% of that originally in the column.

Estimate:

(a) The equilibrium constant for binding the albumin to the Sephadex;

(b) The number of stages in the column; and

(c) The concentration profile in the column.

Problem

Aspartame isomer separation. Racemic amino acids can be separated using l proline attached with silanes to the surface of silica gel. In one of the experiments, aspartame isomers give the following results:

	Peak time (t0, minutes)	Peak spread (t0σ, minutes)
d aspartame	62	3
l aspartame	71	6

These results were obtained with a 25 cm column, 0.41 cm in diameter, filled with 0.62 volume fraction of 45 X 10^{-4} cm silica gel spheres. The flow in the column is 2.0 cm^3/minutes. Find the apparent rate of constant k for this separation. Compare these rate constants with those expected from the mass transfer correlation

$$\frac{k}{v} = 1.17\left(\frac{dv}{\nu}\right)^{-0.42}\left(\frac{\nu}{D}\right)^{-0.67}$$

In which d is the packing diameter, v is then solvent velocity, v is the kinematic viscosity, D is the diffusion coefficient, equal to about 0.7 X 10^{-5} cm^2/second for aspartame under these conditions.

Belter Ex 7.4-1

Problem

- Fumarase chromatography. 10 g of the enzyme fumarase are being purified in an ion exchange column. At a velocity of 30 cm/hr, the peak in the concentration exists the column in 93 minutes and the standard deviation of this peak is given as 12 minutes.

(a)How long must we purify for a 90% yield?

(b)If we increase the flow to 60 cm/hr, how long must we run for this same yield if the process is controlled by diffusion and reaction?

(c)How long must we wat if the process is controlled by mass transfer?

(d)How long must we wait if Taylor dispersion controls?

(e)How long must we wait if the column actually contains equilibrium stages?

Problem

Transferrin desalting. A dilute feed in which 80% of the total solute is transferring and 20% behaves like sodium chloride is to be desalted on a dextran gel column. Operating the column at 10 cm/hr gives the following results:

	Peak time (t0, minutes)	t0σ, minutes
Void volume	27	
Transferrin	41	4
Salts	88	4

A complete separation is obtained under these conditions. To obtain more product, we plan to increase the velocity through the column. We find that the peak t0 varies inversely with velocity, but that the parameter σ varies with the square root of velocity. What is the maximum velocity and the time which will give a 99% yield of transferring which is 98% pure?

Problem

A column 20 cm long, with an internal diameter of 5 cm, gives sufficient purification to merit scale-up. The column produces 3.2 g of purified protein per cycle, and a cycle takes 6 h, from equilibration through regeneration.

(a) To design a throughput of 10 g/h, what are the new column's dimensions if the superficial velocity is held constant and it is assumed that the gradient and particle size are not changed on scale-up?

(b) If available standard column diameters are 20 and 25 cm. What bed depths would apply to each of these columns?

Harrison Ex 7.8 & 7.9

Problem

- Lincomycin A and B are chromatographed through a bed of cellulose beads with a solvent system of butanol, acetic acid, and water. The total volume of the bed is 100 liters and void fraction is 0.35. The where yo is the initial concentration caused by the pulse and V is the volume of the upper tank. equilibrium constants K have been previously measured as 10.2 and 11.8 for A and B, respectively. Estimate the volume of eluate at which the peak will occur for each of these materials.

- In the process described in the problem above, we observe that 2.2% and 15.8% of the lincomycin A were collected at eluate volumes of 600 and 650 liters, respectively.

(a)Determine the quantity of eluate that must be collected to obtain a yield of 95% of lincomycin A.

(b) If the composition of the original mixture was 85% A and 15% B, determine the purity of the A rich product obtained in part (a). Assume sigma for both solutes is the same.

Problem

- Two solutes have a linear equilibrium with the stationary phase. Their equilibrium con- stants are 6.5 and 6.6, respectively. The task is to separate 20 ml of the mixture on a column 5 cm in diameter. The flow rate is 10 ml/min. Determine the minimum column length required to just separate the two compounds, with $\varepsilon = 0.3$.
- It is desired to scale up the throughput by a factor of 150 for a linear gra- dient ion exchange chromatography of a product protein from the laboratory to the plant. The condi- tions for the laboratory chromatography are the fol- lowing: 1.0 cm bed diameter (ID) × 20 cm bed height, 20 μm particle size, and 30 cm/h superficial velocity. The particle size of the same type of ion exchange resin available for the plant operation is 40 μm. Two columns are available in the plant: one column 14.0 cm diameter (ID) × 50 cm high, and another column 18.0 cm diameter (ID) × 50 cm high. To keep the resolution for the chromatography constant in the plant, which column should be used? For this column, what should be the resin bed height and the superficial velocity and what do you esti- mate the pressure drop to be? The viscosity of the mobile phase is 1.0 cp, and the void fraction for resin in the plant column is 0.33.

Problems

Salting out potassium nitrate. We plan to mix 1 liter of 2 M KCl with 1 liter of 8 M $NaNO_3$. How much KNO_3 will precipitate? Approximate solubility products useful in this problem are: KCl, 10; KNO_3, 1.5; NaCl, 35 and $NaNO_3$, 100. Each is given in $(mol/l)^2$;each implies an ideal solution, which is a significant approximation.

Salting out BSA. By experiment, we find that the solubility of this albumin is about 12 and 0.006 g/liter at ammonium sulfate concentrations of 2.5 and 3.5 M, respectively. What is its solubility at 3.0 M $(NH_4)_2SO_4$?

Yeast Dehydrogenase Purification. Two of these dehydrogenase have the precipitation rate constants.

$Ka = 5.0 \times 10^{57}/\text{second } e^{-380\ KJ/RT}$

$Kb = 4.2 \times 10^{64}/\text{second } e^{-415\ KJ/RT}$

You have solution containing equal activities of each enzymes. What will the activity be after 10 minutes at 20 ^{o}C? What will it be after 10 minutes at 50 ^{o}C?

Catalase precipitation. We want to precipitate catalase from an enzyme mixture which includes cholesterol oxidase, our desired product. Catalase has molecular weight of 250,000, a precipitate density of 1.3 g/cm^3, a diameter in solution of 10.4 nm, and a diffusion coefficient of 4.1 X 10^{-7} cm^2/second. It is to be precipitated by a sudden pH change in a 100 liter tank stirred with a 0.1 hp motor. The feed concentration is 0.2 g/liter and the sticking coefficient is about 0.05 sec. Estimate the following:
(a)The time over which diffusion will limit growth;
(b)The concentration of particles at the end of this time; and
(c)The time required to grow 100 μm particles for centrifugation.

Assume that nucleation is fast and flocculation is negligible, and that the solution has properties close to those of water.

Problems

The transport of ovalbumin. Imagine a solution of 25^{o}C containing 0.004 g/cm^{3} of ovalbumin, a protein of molecular weight 45,000. The solution is buffered at a pH of 3.5 and has the velocity close to water, 8.9 X 10^{3} g/cm sec. Under these conditions, the protein has a charge of +10, a diffusion coefficient of 7.8 X10^{-7} cm^{2}/sec, and a sedimentation coefficient of 3.5 X 10^{-13} seconds.

(a) Estimate the diameter of the protein
(b) What are the flux and velocity when this protein diffuses from the solution across a 1 cm film into pure water?
(c) What is the protein's velocity under the influence of gravity?
(d) What are the flux and velocity due to a force of 1 volt/cm?

Two oxidases, one of which is specific for testosterone, are to be separated by gel electrophoresis at 4°C. Within the gel, these enzymes have diffusion coefficients of 1.15×10^{-7} and 3.20×10^{-7} cm^2/sec, respectively. At the pH chosen, they have charges of +7 and +1, respectively. If we use a field of 1.8 V/cm, how long will it take until the two enzymes are separated by a distance of 1 cm?

You want to separate α_1 globulin and α_2 globulin by' membrane electrophoresis with mobilities of $-5.1*10^5$ & $-4.1*10^5$ cm^2/v sec respectively You plan to use a flow opposed to an electric field of 0.82 V/cm such that one protein will move against the flow but the second will be swept along with it. What flow should you use?

The surface concentration for chymotrypsin ultrafiltration. We are carryin out the ultrafiltration of chymotrypsin in a spiral wound module at a rate of 1.3×10^{-3} cm/sec (28 gal/ft^2 day). The solution concentration is 0.44 wt%, the protein's diffusion coefficient is 9.5×10^{-7} cm^2/sec, and the boundary layer is about 180×10^{-4} cm thick. How high is the surface concentration?

Oxidase purification by gel electrophoresis. Two oxidases one of which is specific for testosterone, are to be separated by gel electrophoresis at 4 ^{o}C. Within the gel, these enzymes have diffusion coefficient of 1.15×10^{-7} and 3.20×10^{-7} cm$^{2/}$sec, respectively. At the pH chosen, they have charges of +7 and +1, respectively. If we use a field of 1.8V/cm, how long will it take until the two enzymes are separated by a distance of 1 cm?

Problems

Characterizing an ammonium sulfate crystallization. A continuous crystallization vessel containing 100 liters of ammonium sulfate slurry is fed with 50 liter per hr of supersaturated solution. The withdrawal rate of product slurry is also 50 liters per hr. A nucleation rate B of 7.18×10^7 nuclei /liter hr, and a growth rate G of 0.056 mm/hr are expected. Determine the following:

(a) The dominant crystal size,
(b) The number of crystal equal to or smaller than this size,
(c) The fraction of crystals in this range, and
(d) The product slurry concentration.

In these calculations, assume cubic crystals with a density of $1.769 g/cm^3$.

10 kg of adipic acid is slurried in 13.1 kg of water and heated to. 90°C to solubilize the acid. The solution is then filtered to remove insoluble impurities. During the heating and filtration, 10% of the water is evaporated. The clarified solution is cooled to 35°C and filtered. The solubility at 35°C is 0.05 kg acid per kg water. Determine the weight of crystals recovered in this operation.

A mixture of stigmasterol and sitosterol weighing 2040 kg is divided into two fractions by crystallization. The original mixture contains 86.3% stigmasterol. The recovered crystals are 96.6% stigmasterol and weigh 1137 kg. The solids in the liquor contain 74.6% stigmasterol, found by evaporation to dryness. Calculate β

As part of a fundamental study of crystallization, you have mounted a single crystal of this antibiotic in a clean, supersaturated solution which flows past the crystal at an adjustable rate. By examining it microscopically, you find that the crystal grows at a rate almost independent of crystal size. At 10% supersaturation, this rate is 0.02 cm/hr at a flow of 5.0 cm/sec and is 0.04 cm/hr when the flow is six times larger. From mass transfer correlations, you expect that the mass transfer coefficient varies with the square root of the fiuid velocity. Estimate k and κ from these observations.