Bioseparation Basics

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Bioseparations are critical to the field of biochemical engineering. Each bioseparation process must be tailored to separate, purify, or recover the desired bioproduct. This article gives an introductory overview of filtration and chromatography — two key bioseparation unit operations.

Modern biotechnology is built on genetic engineering — the genetic manipulation of organisms to produce commercial products. Bioproducts are derived by extraction from plants or animals or by synthesis in bioreactors containing cells or enzymes.

Bioproducts are sold for their chemical activity: methanol for its solvent activity, ethanol for its neurological activity (or as a fuel), penicillin for its antibacterial activity, streptokinase (an enzyme) for its blood-clot-dissolving activity, hexose isomerase for its sugar-converting activity, and whole *Bacillus thuringiensis* cells for their insecticide activity, to name a few very different examples. The wide variety represented by this tiny list suggests that bioseparations must encompass a correspondingly wide variety of methods. The choice of separation method depends on the nature of the product, as well as purity, yield, and — most importantly — activity requirements.

Bioproducts have unique properties. For bioseparation purposes, important properties include thermal stability, solubility, diffusivity, charge, and isoelectric pH, among others. A considerable amount of process planning is based on the lability, or susceptibility to change, of most bioproducts. Temperature, pH, and concentration must be maintained within specific ranges to assure product bioactivity.

Purification of bioproducts by a bioseparations process typically involves a long sequence of steps, and each step requires the use of one or more unit operations, such as filtration, extraction, chromatography, and drying. This article provides an overview of bioseparation and discusses the basic principles and theory of two key unit operations — filtration and chromatography.

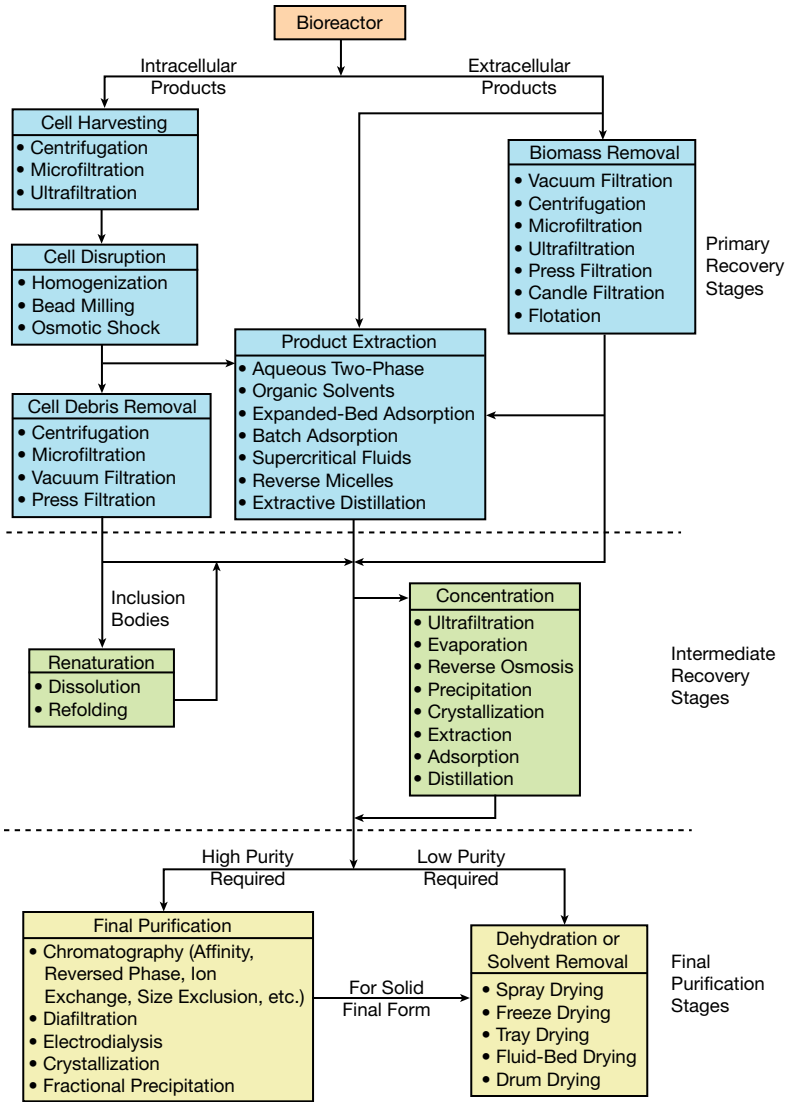
Developing a sequence of bioseparations

The development of a flowsheet for the recovery and purification of a biological product is a creative process that draws on the engineer's experience and imagination. Attempts have been made to capture that experience in computer software *(1–4)* and automate, to some extent, the process synthesis tasks.

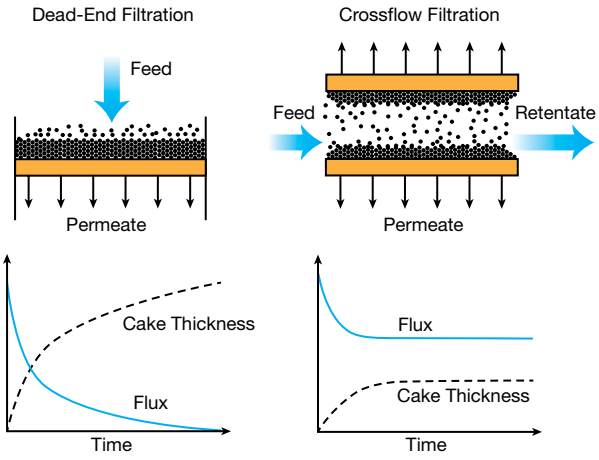
Experienced engineers rely heavily on certain rules of thumb, or heuristics, for putting together the skeleton of a recovery and purification process *(5)*. A few such heuristics include:

- Remove the most plentiful impurities first.
- Remove the easiest-to-remove impurities first.
- Make the most difficult and expensive separations last.
- Select processes that make use of the greatest differences between the properties of the product and those of its impurities.
- Select and sequence processes that exploit different separation driving forces.

Figure 1 depicts the bioseparation steps that may be involved in a product recovery process. The primary recovery stages are mainly concerned with separating the product from cells or cell debris; these stages follow the rule of removing the most plentiful impurities first (usually water). The intermediate recovery stages concentrate the product by an operation (*e.g.,* ultrafiltration, evaporation, reverse osmosis, etc.) that depends on the nature of the product. Occasionally, it is necessary to perform a protein-refolding step if the product is a protein formed within the cells' inclusion bodies (IBs), which are insoluble aggregates sometimes formed when recombinant proteins are produced by cells. The final purification stages follow the rule of doing



▲ **Figure 1.** A bioproduct recovery process typically involves some combination of these steps and operations. Source: Adapted from *(6)*.



◀ **Figure 2.** In dead-end filtration, the thickness of the solids buildup increases and the permeate flux decreases with time, ultimately reaching zero. In crossflow filtration, the feed can contain either a soluble or a solid solute, which becomes concentrated at the membrane surface; the permeate flux reaches a constant value at steady state. Source: Adapted from *(6)*.

the most difficult and expensive separations last; these stages sometimes require the use of separation driving forces that are different from each other (*e.g.,* charge difference, size difference, or solubility difference) to attain the required purity. Selection and sequencing of unit operations is based on the properties of the product, the properties of the impurities, and the properties of the producing microorganisms, cells, or tissues.

Most bioprocesses, especially those employed in the production of high-value, low-volume products, operate in batch mode. Conversely, continuous bioseparation processes are utilized in the production of commodity biochemicals, such as organic acids and biofuels.

Two of the most common unit operations in bioseparation processes are filtration and liquid chromatography.

Filtration

Filtration separates particulate or solute components according to size when a fluid suspension or solution flows through a porous medium under a pressure differential. There are two broad categories of filtration, which differ according to the direction of the fluid feed in relation to the filter medium (Figure 2). In conventional, or dead-end, filtration, the fluid flows perpendicular to the medium, depositing a cake of solids on the filter medium. In crossflow filtration (also called tangential-flow filtration), the fluid flows parallel to the medium, minimizing the buildup of solids on the medium.

Filtration is often used in the early stages of bioproduct purification, in keeping with the process design heuristic — remove the most plentiful impurities first. At the start of purification, the desired bioproduct is usually present in a large volume of aqueous solution, and it is desirable to reduce the volume as soon as possible to reduce the scale, and thus the cost, of subsequent processing operations. Filtration is an effective means of accomplishing volume reduction.

Conventional filtration is typically used when a product has been secreted from cells, and the cells must be removed to obtain the product that is dissolved in liquid. Antibiotics and steroids are often processed using

conventional filtration to remove the cells. Conventional filtration is also commonly used for sterile filtration in biopharmaceutical production.

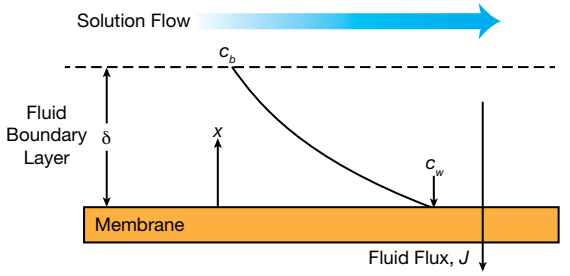
Crossflow filtration has been used in a wide variety of applications, including the separation of cells from a product that has been secreted, the removal of cell debris from cells that have been lysed (broken apart), the concentration of protein solutions, the exchange or removal of salts in a protein solution, and the removal of viruses from protein solutions.

As illustrated in Figure 2, in crossflow filtration, the fluid flows parallel to the membrane surface, resulting in constant permeate flux at steady state. The membrane used for crossflow filtration depends on whether the component being filtered is soluble or insoluble.

When dissolved species such as proteins are being filtered, ultrafiltration membranes are generally employed. The ultrafiltration membrane is selected so that the species of interest will not pass through it. The retained species is carried to the surface of the membrane by the convective flow of fluid and builds up on the membrane surface. The concentration of the species can be so high that it precipitates on the membrane surface, further impeding the flow of fluid through the membrane. The resulting layer of solids on the membrane surface is called a gel layer. In addition, even without precipitation, the increased osmolarity near the membrane surface creates a solvent flow that opposes the applied transmembrane pressure.

When suspended (insoluble) particles are present, the particles carried to the membrane form a cake at the surface. For these applications, microfiltration membranes are generally utilized. These membranes let dissolved components pass through, but retain particles larger than a certain size. In some instances, such as in the crossflow filtration of cells that have been ruptured, the layer at the membrane surface contains both suspended particles and precipitated solutes.

Crossflow filtration of dissolved species is illustrated in Figure 3. For a solute that is rejected by the membrane, there will be a concentration gradient of this solute across a stagnant boundary layer next to the surface of the membrane. The increase in solute concentration at the membrane surface (c_w) compared to that in the bulk solution (c_b) is known as concentration polarization.



▲ **Figure 3.** In this representation of the boundary layer in crossflow filtration with a dissolved solute in the feed, a solution flows parallel to the membrane surface, and fluid flows through the membrane. A stagnant boundary layer forms next to the membrane surface, creating a gradient in the concentration, *c*, of solute. Source: Adapted from *(6)*.

At steady state, the rate of convective mass transfer of solute toward the membrane surface must be equal to the rate of mass transfer of solute by diffusion away from the membrane surface, which is described by *(7)*:

$$Jc = -D\frac{dc}{dx} \tag{1}$$

where J is the transmembrane fluid flux, c is the concentration of the solute, and D is the diffusion coefficient of the solute. For a boundary layer thickness of δ:

$$J = \left(\frac{D}{\delta}\right)\ln\left(\frac{c_w}{c_b}\right) \tag{2}$$

which can also be written as:

$$\frac{c_w}{c_b} = \exp\left(\frac{J\delta}{D}\right) \tag{3}$$

The term D/δ can also be defined as the mass-transfer coefficient, k. The ratio c_w/c_b is sometimes called the polarization modulus and indicates the extent of concentration polarization. Equation 3 indicates that the polarization modulus is particularly sensitive to changes in J, δ, and D because of the exponential functionality involved. For high-molecular-weight solutes (low D) and membranes with high solvent permeability (high J), concentration polarization can become severe, with $c_w/c_b > 10$.

Correlations have been developed for the mass-transfer coefficient. For laminar flow, boundary layer theory has been applied to yield analytical solutions (known as the Leveque or Graetz solutions) for k *(8)*:

$$k = 0.816\left(\gamma_w\frac{D^2}{L}\right)^{\frac{1}{3}} \tag{4}$$

where γ_w is the fluid shear rate at the membrane surface and L is the length of the flow channel over the membrane. The dimensionless constant 0.816 is applicable for the gel-polarized condition of concentration polarization where the solute concentration at the wall is constant.

For a rectangular slit of height $2h$ and bulk stream velocity u_b:

$$\gamma_w = \frac{3u_b}{h} \tag{5}$$

and for a circular tube of diameter d:

$$\gamma_w = \frac{8u_b}{d} \tag{6}$$

For turbulent flow, empirical correlations have been developed for the mass-transfer coefficient. These correlations are based on dimensional analysis of the equation of change for the conservation of mass for forced-convection mass transfer in a closed channel, which gives the Sherwood number, *Sh (9)*:

$$Sh = \frac{k \times d_h}{D} = f(Re, Sc, L/d_h) \quad (7)$$

where *Re* is the Reynolds number:

$$Re = \frac{d_h u_b \rho}{\mu} \quad (7a)$$

Sc is the Schmidt number:

$$Sc = \frac{\mu}{\rho D} \quad (7b)$$

d_h is the equivalent diameter of the channel:

$$d_h = 4\left(\frac{\text{cross-sectional area}}{\text{wetted perimeter}}\right) \quad (7c)$$

u_b is the bulk stream velocity, ρ is the density of the fluid, and μ is the viscosity of the fluid.

Nomenclature

c	= concentration of solute (M, or kg/m^3)
C	= chemical species in the mobile phase
CS	= chemical species adsorbed to an adsorption site
d	= diameter of tube (m)
d_h	= equivalent diameter of channel (m)
D	= diffusion coefficient (m^2/s)
h	= half-height of rectangular flow channel (cm)
J	= transmembrane flux (m^3/m^2-s)
k	= mass-transfer coefficient (= D/δ) (m/s)
K_{eq}	= equilibrium constant (units vary)
L	= length of flow channel (m)
q_i	= concentration of separand i in the stationary phase averaged over an adsorbent particle (M, or kg/m^3)
Re	= Reynolds number (= $d_h u_b \rho/\mu$) (dimensionless)
Sc	= Schmidt number (= $\mu/\rho D$) (dimensionless)
Sh	= Sherwood number (= $k \times d_h/D$) (dimensionless)
S_{tot}	= total concentration of adsorbent sites (kg/m^3)
t	= time (s)
u_b	= bulk fluid velocity (m/s)
u_i	= effective velocity of solute i (m/s)
v	= mobile phase superficial velocity (flowrate divided by empty column cross-sectional area) (m/s)
x	= distance (m)

Greek Letters

γ_w	= shear rate at membrane surface (s^{-1})
δ	= boundary layer thickness (m)
ε	= column void fraction (dimensionless)
μ	= viscosity of fluid (kg/m-s)
ρ	= density of fluid (kg/m^3)

An example of a correlation for *Sh* is *(10)*:

$$Sh = 0.082 Re^{0.69} Sc^{0.33} \quad (8)$$

Example 1: Protein ultrafiltration

Ultrafiltration of a protein solution at constant volume is achieved by the addition of water or buffer to the feed in an operation called diafiltration. The flow channels for this system are tubes that are 0.1 cm in diameter and 100 cm long. The protein, which has a molecular weight of 490,000, has a diffusion coefficient (D) of 1.2×10^{-7} cm^2/s. The solution has a viscosity of 1.2 cP and a density of 1.1 g/cm^3. The system is capable of operating at a bulk stream velocity of 300 cm/s. We want to determine the polarization modulus for a transmembrane flux (J) of 45 L/m^2-h.

We can determine c_w/c_b from Eq. 3 if we know $J\delta/D = J/k$. The transmembrane flux is given as J = 45 L/m^2-h. We can determine k from either Eq. 4 or Eqs. 7 and 8, depending on whether the flow is laminar or turbulent, which requires calculating the Reynolds number:

$$Re = \frac{d_h u_b \rho}{\mu} = \frac{0.1 \text{ cm} \times 300 \text{ cm/s} \times 1.1 \text{ g/cm}^3}{0.012 \text{ g/cm-s}} = 2{,}750$$

In the Reynolds number calculation, the equivalent diameter of the channel is the same as the diameter of the tubes. The flow is turbulent since the Reynolds number is greater than 2,100. For turbulent flow, the Schmidt number is:

$$Sc = \frac{\mu}{\rho D} = \frac{0.012 \text{ g/cm-s}}{1.1 \text{ g/cm}^3 \times \left(1.2\times10^{-7} \text{ cm}^2/\text{s}\right)} = 9.09\times10^4$$

From Eqs. 7 and 8:

$$k = \frac{DSh}{d_h} = \frac{D}{d_h}(0.082) Re^{0.69} Sc^{0.33}$$

$$= \left(\frac{1.2\times10^{-7} \text{ cm}^2/\text{s}}{0.1 \text{ cm}}\right) \times 0.082 \times \left(2{,}750^{0.69}\right) \times \left(90{,}900^{0.33}\right)$$

$$= 1.01\times10^{-3} \text{ cm/s}$$

The polarization modulus can be estimated from Eq. 3:

$$\frac{c_w}{c_b} = \exp\left(\frac{J\delta}{D}\right) = \exp\left(\frac{J}{k}\right)$$

$$= \exp\left(\frac{45 \frac{\text{L}}{\text{m}^2\text{-h}} \times 10^3 \frac{\text{cm}^3}{\text{L}} \times \frac{1 \text{ m}^2}{10^4 \text{ cm}^2} \times \frac{1 \text{ h}}{3{,}600 \text{ s}}}{1.01\times10^{-3} \frac{\text{cm}}{\text{s}}}\right) = 3.45$$

Thus, there is a significant increase in protein concentration at the membrane surface.

For situations where the concentration is so great it becomes the limiting resistance to flow, it may be necessary

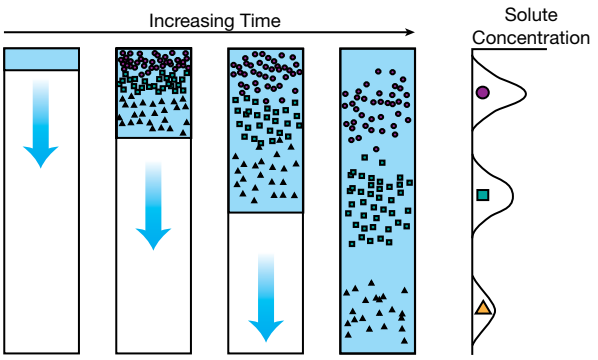
to increase the crossflow velocity to reduce the boundary layer thickness, thereby increasing the mass-transfer coefficient (k). Increasing the velocity may necessitate a more-expensive pump and may lead to higher energy costs, which need to be considered when determining the optimal design.

Liquid chromatography

Liquid chromatography is a type of adsorption process that is based on the affinity of various soluble molecules for specific types of solids. In adsorption, there are typically three groups of solutes: those that do not adsorb to the stationary phase (sometimes called flow through); those that adsorb and then are subsequently recovered by stripping; and those solutes that are nearly irreversibly bound and can only be removed by regenerating the adsorbent, which usually destroys the solutes. In chromatography, multiple solutes can be separated from each other and the target product solute can be recovered at the end of the process step. The term adsorption is used both as the name of the unit operation and to describe the physical adherence of a solute to an adsorbent resin in chromatography. In chromatography, soluble molecules in a liquid phase flow past a resin, or stationary phase. The stationary phase is typically a packed bed in a column. Since the liquid flows past the solid, it is referred to as the mobile phase.

One method commonly used in chromatography is elution chromatography, illustrated in Figure 4. A liquid feed stream containing a mixture of solutes is introduced at the inlet of a column packed with the stationary phase. After the introduction of the feed, an elution solvent is fed continuously to the column, carrying the solutes, which adsorb in separate zones over the length of the column.

In on-off chromatography *(11)*, the stationary phase is generally saturated with the target solute before the elution solvent is introduced. The elution solvent causes the solute to transfer into the mobile phase, and it leaves the column with that stream.



▲ **Figure 4.** A feed stream that contains three solutes is introduced to a column packed with a stationary phase. An elution solvent then flows through the column, and the three solutes separate as a result of differing affinities for the stationary phase. Source: Adapted from *(6)*.

The adsorption of a chemical species to a resin surface or ligand can be represented by the equilibrium reaction:

$$C + S \xleftrightarrow{K_{eq}} CS \tag{9}$$

where C is the dissolved chemical species, S is an adsorption site, CS is the chemical bound to the site, and K_{eq} is the equilibrium constant governing the reaction. The equilibrium constant for this adsorption is:

$$K_{eq} = \frac{[CS]}{[C][S]} \tag{10}$$

Consider the three assumptions inherent in this representation of the adsorption reaction. First, it is completely reversible, and the chemical's solution properties and solution state are not altered by its interaction with the adsorption site. Second, chemicals bind to sites in a one-to-one fashion, and only bind to sites. In other words, the binding is specific, and there is no nonspecific binding or interaction between molecules on the surface. Third, there is only one mode of binding to the site; all binding is equal and described by a single value for K_{eq}. Despite these limiting assumptions, this model often serves as a very accurate description of adsorption in liquid chromatography.

In many cases, the concentration of adsorption sites is much larger than the concentration of dissolved chemical species ([S] >> [C]), and the equilibrium expression becomes:

$$K_{eq} = \frac{[CS]}{[C]} \tag{11}$$

or

$$[CS] = K_{eq}[C] \tag{12}$$

This is known as linear equilibrium. Although the linear isotherm approximation is commonly used in analytical liquid chromatography, it is less useful for preparative, or industrial-scale, chromatography.

The most efficient chromatography operation uses all the adsorption sites available. The concentration of unoccupied sites is not easily measured, but the total number of sites (S_{tot}) is:

$$S_{tot} = [CS] + [S] \tag{13}$$

Combining this with the general expression for K_{eq} (Eq. 10) gives:

$$[CS] = \frac{K_{eq} S_{tot}[C]}{1 + K_{eq}[C]} \tag{14}$$

This is the well-known Langmuir isotherm. If the value of K_{eq}[C] in the denominator is much less than one, a form of the linear adsorption equation is recovered, and Eq. 14 becomes Eq. 11. When K_{eq}[C] is much larger than one, [CS] is equal to S_{tot}, that is, the adsorption sites are saturated.

The Langmuir isotherm (a plot of Eq. 14) has a linear slope near the low-concentration limit and plateaus as the resin becomes saturated (Figure 5). Equilibrium isotherms that are concave downward are considered to be favorable for adsorption. The Langmuir isotherm has often been used to correlate equilibrium adsorption data for proteins. This isotherm is especially important for preparative and industrial chromatography. The adsorption of various species from a complex mixture is now seen as influenced not only by the equilibrium constants, which are species-specific, but also by the total binding capacity of the resin (S_{tot}), which is more specific to the resin than to the chemical species.

A basic understanding of the dynamic behavior of adsorption processes is essential for the design and optimization of large-scale chromatography processes. The development of a mass balance is the starting point for analyzing fixed-bed adsorption. Simplifications of this mass balance lead to analytical solutions that give insight into the adsorption process. Since the overall pattern of mass transfer is governed by the form of the equilibrium relationship, the main features of the dynamic behavior can be understood without performing detailed calculations *(12)*. Thus, this analysis of the mass balance for adsorption assumes equilibrium locally, and for simplicity ignores the dispersion term, which allows us to focus on the velocity at which a solute traverses the column, u_i.

Using an equilibrium isotherm relationship of the form $q_i = f(c_i)$, the analysis assuming local equilibrium and no dispersion leads to:

$$u_i = \frac{v}{\varepsilon + (1-\varepsilon) q_i'(c_i)} \tag{15}$$

where c_i is the concentration of separand i in the mobile phase, $[C]_i$; q_i is the concentration of separand i in the stationary phase averaged over an adsorbent particle, $[CS]_i$; q_i' is dq_i/dc_i; ε is the void fraction (mobile phase volume divided by total column volume); and v is the mobile phase superficial velocity (the flowrate divided by the cross-sectional area of the empty column).

For preparative chromatography processes where high adsorbent loadings are desired, the equilibrium is nonlinear and generally the Langmuir isotherm is applicable. Figure 5 shows that for the Langmuir isotherm, $q_i'(c_i)$ decreases with concentration, so that the effective solute velocity u_i increases with concentration according to Eq. 15. For the solute at the front that advances through the fixed bed, the concentration change is not continuous, and finite differences rather than differentials must be used for the change in q_i with c_i, as follows:

$$u_i = \frac{v}{\varepsilon + (1-\varepsilon)\dfrac{\Delta q_i}{\Delta c_i}} \tag{16}$$

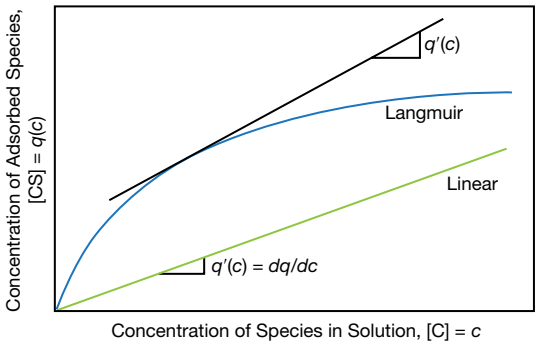
The resulting velocity of the solute front is called the shock-wave velocity, as the mathematics describing this phenomenon are similar to those describing acoustic waves and ocean waves.

In cases where the equilibrium isotherm is favorable (*i.e.,* concave downward, as for the Langmuir isotherm), a shock wave is self-sharpening, in that low concentrations in the leading edge of the wave are slowed, while higher concentrations that lag the center of the wave move at higher velocity. This is evident from Eq. 16 for the effective velocity of the solute: as c_i decreases, $\Delta q_i/\Delta c_i$ increases and u_i decreases. Therefore, while resistance to mass transfer and axial mixing act to broaden the shock wave, the adsorption equilibrium acts to maintain the sharp step in solute concentration.

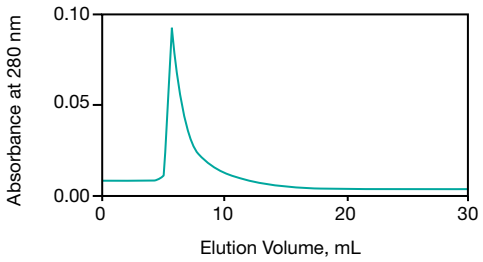
Example 2: Protein chromatography

In a laboratory experiment, a cell extract containing a fusion protein (L-asparaginase-atrial natriuretic peptide) was fed to a column of L-asparagine agarose affinity adsorbent *(13)*. The column was washed to remove nonadsorbed proteins, and then the fusion protein was eluted from the adsorbent in the isocratic mode (*i.e.,* constant composition) using 0.2 M sodium chloride.

During the elution, an optical detector at the column exit measured the absorbance at 280 nm to monitor the protein concentration. The elution profile in Figure 6 shows a sharp shock front, which is in agreement with a Langmuir isotherm. The diffuse trailing wave can be explained by Figure 5 for a Langmuir isotherm and Eq. 16 for the effective velocity of the protein, which applies since the concentration is continuous. Low concentrations that trail the front give



▲ **Figure 5.** Equilibrium isotherms that are concave downward, such as the Langmuir isotherm, indicate favorable adsorption. Source: Adapted from *(6)*.



▲ **Figure 6.** This is an example elution profile for the chromatographic purification of the L-asparaginase-atrial natriuretic peptide fusion protein on a column of L-asparagine agarose (1 cm × 4.5 cm). The fusion protein adsorbed to the column after a cell extract flowed through the column. After the column was washed, the fusion protein was eluted using 0.2 M sodium chloride in sodium phosphate buffer (10 mM, pH 7.5). The arrow indicates the start of the elution. Source: Adapted from *(13)*.

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higher $q_i'(c_i)$ and thus lower u_i, resulting in a broadening trail behind the sharp front.

When chromatography to purify a protein is scaled up from the laboratory scale, knowledge of the laboratory elution profile, such as Figure 6, is helpful in interpreting the large-scale elution profile. Significant deviation from the laboratory elution profile could indicate poor packing of the adsorbent particles or poor distribution by the inlet liquid header. For the protein chromatography shown in Figure 6, it is expected upon scaleup that there will still be a broad trailing wave in the elution after the peak, because the Langmuir equilibrium isotherm continues to govern the adsorption behavior. To quantify the deviation from the laboratory elution, it may be necessary to do further analysis of the large-scale elution, such as the determination of the height of a theoretical plate (HETP) in the column *(6)*.

Closing thoughts

The field of bioseparations has developed into a major segment of biochemical engineering. Because each new bioproduct has unique features, the work to develop new bioseparation processes will continue to be both interesting and challenging. In order for chemical engineers to model and design bioseparation processes, an understanding of the basic principles and theory is important — for example, how to control the effect of concentration polarization during ultrafiltration, or why separations by chromatography are strongly influenced by the equilibrium isotherm. CEP

To Learn More

This article is based on the AIChE instructor-led course that the author teaches, Bioseparations: Principles, Applications, and Scale-Up (CH401). This two-day course provides a more-detailed look at the most important unit operations in industrial bioseparation processes. Students will learn how to avoid mistakes when analyzing and interpreting bioseparation data, and how to select appropriate equipment and techniques for successful scaleup of bioseparation applications.

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