

BIOSEPARATIONS:

By the year 2000, the world biochemical market will reach an estimated \$40–100 billion¹. Bioprocess engineering is a vital (but currently weak) link between lab discoveries and the fulfillment of this commercialization potential. To strengthen the link, researchers are exploring biochemically based separations, especially for high-value-added chemicals. Biochemical approaches—including liquid-liquid partitioning—promise readily scaleable, economical separation of large biomolecules, as well as an answer to industry's need for better statistical, mechanical, and thermodynamic models and measurements for bio-separations.

One bioprocess in particular—two-phase aqueous partitioning—has great potential as an economical separation method for biochemical products. It offers the potential for strict product quality control, as well².

With many potential applications in the food and pharmaceutical industries for such things as enriching soybean and corn endosperm proteins and harvesting lysozymes for use in artificial blood, partitioning serves three main roles:

- concentrating dilute solutions of biological substances of interest;
- purifying enzymes and other proteins; and
- extractive bioconversion³.

Based on partitioning the components of an organic mixture between two immiscible (or partly miscible) solvents, this energy-efficient method

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uses water as the solvent in dealing with biological materials^{3,4}.

In such a two-phase aqueous system, incompatible polymers segregate in the water to form two phases. While it doesn't work for some small molecules such as amino acids (which distribute themselves evenly in the two phases), such a system suits large biomolecules. These partition unevenly to form various kinds of colloids⁵⁻⁷—and provide a convenient handle for separations (see figure 1).

Calculating the Partition Coefficient

A useful parameter for characterizing the partition of component *i* of a mixture in a two-phase matrix is the partition coefficient $K_i = C_{it}/C_{ib}$, where C_{it} and C_{ib} are the concentrations of the partitioned substance in the top and bottom phases, respectively.

According to Bronsted⁸ the following relationship exists for the partition coefficient K_i :

$$K_i = \exp(M_i \lambda / kT)$$

where

- M_i = molecular weight of the partitioned substance (*i*)
- k = Boltzmann constant
- T = absolute temperature
- λ = a constant related to the two-phase system's characteristics and other properties of the partitioned substance

This exponential relation implies that for large molecules, a small change in λ significantly influences partitioning behavior.

In general, the partition coefficient for a soluble substance is a function of the following:

- properties of the two phases;
- properties of the sample; and
- temperature.

Note, however, that the coefficient remains independent of the total volume of the system. Therefore, the partition coefficients for large-scale processes will be equal to the values obtained in lab-scale experiments.

Calculating the Partition Ratio

The partition ratio G_i is the ratio between the amount of substance *i* in

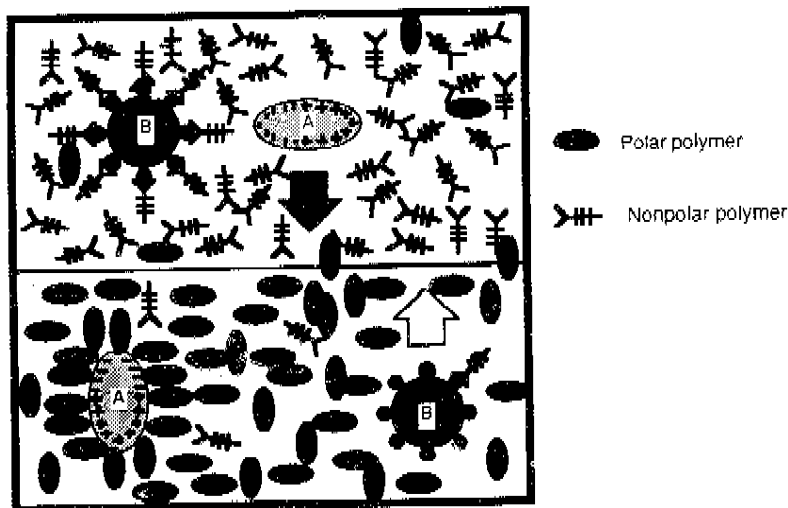


Figure 1. Partition of mixture containing macromolecules A and B between two polymer phases.

DESIGN AND ENGINEERING OF PARTITIONING SYSTEMS

the top and bottom phases, and is written as

$$G_i = (C_{it}/C_{ib})(V_t/V_b) \text{ or } G_i = K_i(V_t/V_b)$$

where C_i is the concentration of the solute i and V is volume. Subscripts t and b denote top and bottom phases.

For efficient large-scale separation of components p and q of a mixture of biological macromolecules, partition ratios G_p and G_q must have suitable values. The best separation occurs when

$$G_p G_q = 1 \text{ or } K_p K_q (V_t/V_b)^2 = 1$$

Another vital parameter, the time required for phase separation, depends on both the viscosities and the density differences between the two phases. Near the critical point, settling time is long, due to the small density difference. Far from the critical point, settling time is also long, in this instance due to high phase-viscosity. The shortest settling times occur at intermediate polymer phase compositions. One can also enhance set-

ling rate by adding a salt, a third polymer, or an electric field.

Column Design

Macromolecules and other biological substances with differing partition coefficients can be separated either in a single-stage (batch or continuous) or multistage apparatus^{9,10}.

For industrial-scale partitioning, multistage separations rely on a liquid-liquid partitioning column. In this arrangement, a pump feeds a heavy polymer phase into the top of the column and a lighter phase into the bottom of the column. These immiscible phases move in opposite directions through alternating mixing and separation stages (see figure 2).

The mixing stages effect mass transfer; settling stages allow for phase separation. To perform a separation, the operator introduces a sample in one of two ways: either by feeding it along with one of the two polymer phases, or by injecting it directly into one of the mixing or settling stages.

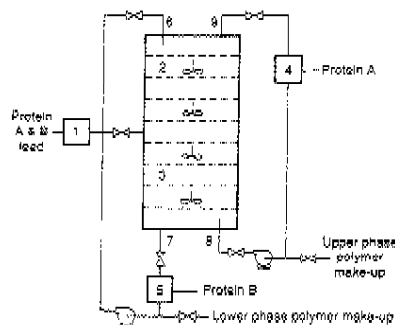
An alternative multistage system

consists of a series of stages separated by filter plates. Mixing and separating take place in the same stage. The lighter phase enters from the bottom of the column and moves upward while the heavy phase is kept stationary. After allowing mixing and phase separation to occur, the system pumps a volume of the lighter phase into the column from below. This influx pushes an equal volume of light phase through the filter and into the stage above, where it migrates up through the stationary phase.

Mathematical Models of Partitioning

Given that ten or more variables affect a biological substance's partition coefficient¹¹, qualitative and quantitative understanding of this process presents a formidable task. And although a vast amount of scattered data on protein partition coefficients have been published^{3,12}, these data apply to systems that are thermodynamically unspecified and inconsistent among experiments.

Predictive models that illuminate



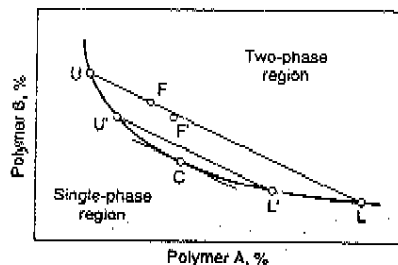
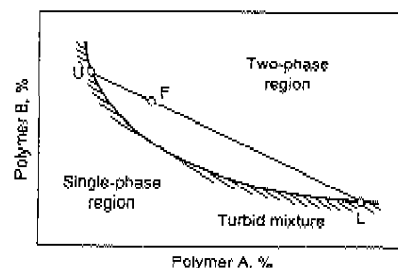
LEGEND

- 1 Feed tank
- 2 Stirring stage
- 3 Settling stage
- 4 Protein A purification unit
- 5 Protein B purification unit
- 6 Lower polymer phase in
- 7 Lower polymer phase out
- 8 Upper polymer phase in
- 9 Upper polymer phase out

Figure 2. Multistage partitioning with alternating mixing and separating stages.

Figure 3. Phase diagram of a mixture of two polymers and water. Mixtures having compositions represented by points above the binodal curve will separate into two phases; mixtures represented by points below the binodal curve exist as a single phase. A system with total composition F separates into upper phase U and lower phase L , with the ratio of the volumes of the two phases approximately equal to the ratio of the distances FL and FU on the connecting line. Similarly, a system with total composition F' separates into phases U' and L' . At critical point C , the two liquid phases become identical.

Figure 4. An aqueous solution of polydisperse polymers A and B . Shaded area indicates regions in which mixtures will be somewhat turbid, due to incomplete solubility of certain molecular weights of B in A and vice versa.



the interrelationships among system variables should primarily predict the phase diagram of the polymers (see figure 3). But such models are complicated by the polydispersity of the polymers and by the effect of salt ions in enhancing the partition of charged proteins¹³. Interactions between the polymers and the proteins further complicate matters.

One can, however, calculate phase diagrams of two-phase aqueous systems from the equality relationship between the components' chemical potentials. To understand multicomponent interactions, the modeler systematically rearranges them into binary interactions for calculation.

A difficulty in predicting aqueous chemical potentials stems from the common assumption that polymers in aqueous two-phase systems are monodisperse as single components^{14,15}. Polymers have a molecular weight distribution, so treating them as single components is not always satisfactory.

Mansoori and Ely's phase equilibrium model for polydisperse solutions¹⁷, modified and extended to polydisperse polymer aqueous solutions, takes the molecular weight distribution into account. It leads to the following equations for chemical potentials of systems characterized as polydisperse polymer 1/polydisperse polymer 2/water:

$$\frac{\mu_{1i} - \mu_{1i}^*}{RT} = \ln m_{1i} + \xi m_{1i} + \alpha M_{1i} (m_{1j_1} + m_{2j_2})$$

$$\frac{\mu_{2i} - \mu_{2i}^*}{RT} = \ln m_{2i} + \delta m_{2i} + \beta M_{2i} (m_{1j_1} + m_{2j_2})$$

$$\frac{\mu_w - \mu_w^*}{RT} = -\frac{1}{m_w} \left[m_1 + m_2 + \frac{\xi}{2} m_1^2 f_1 + \frac{\delta}{2} m_2^2 f_2 + (\beta m_2 \bar{M}_2 + \alpha m_1 \bar{M}_1)(m_{1j_1} + m_{2j_2}) \right]$$

where

$$f_1 = \int F_1^2(l) dl$$

$$j_1 = \int \left[F_1^2(l) / M_{1i} \right] dl$$

In the equations above,

$$m_i = \sum m_{ij}, \bar{M}_i = \sum m_{ij} M_{ij} / m_i$$

and m_{ij} 's are the molalities of the i^{th} molecular-weight fraction of polymers 1 and 2. M_{ij} 's are the ratios of molar volumes of the i^{th} fraction of

polymers 1 and 2 with respect to water (w), respectively.

The chemical potentials in the working conditions and reference state are denoted as μ and μ^* , respectively. α, β, δ , and ξ are interaction terms for polymer-polymer and polymer-water systems.

The molecular weight distribution functions of the two polymers in the two phases can be expressed as $F_1(l, \sigma_1, \eta_1)$ and $F_2(l, \sigma_2, \eta_2)$, where σ_1 and σ_2 are variances and η_1 and η_2 are mean molecular weights of the two polymers, respectively. (J_2, f_2, m_2 , and M_2 are defined similarly to J_1, f_1 , etc.)

Using the "equality of chemical potentials" algorithm for continuous mixture phase equilibria^{17,18}, we can calculate equilibrium compositions of the two phases.

If the biomolecule in the two-phase system is considered as another polymer molecule, and assuming the large biomolecule bears a net charge Z_{BMM} , then:

$$\mu_{\text{BMM}} = \mu_{\text{polymer}} + Z_{\text{BMM}} F \phi_{\text{phase}}$$

In this expression, μ_{polymer} is the chemical potential of a polymer in the solution, F is the Faraday number, and ϕ_{phase} is the electrostatic potential. The second term in this equation contributes to partitioning when there is an electrostatic potential difference between the two phases. As a result, the contribution of macromolecular charge to its partition coefficient is as follows:

$$\Delta K_{\text{BMM}} = \exp(-Z_{\text{BMM}} F \Delta \phi / RT)$$

Since each phase in equilibrium must be electrically neutral, $\Delta \phi$ between the two phases must satisfy the electroneutrality conditions, which vary for each two-phase partitioning system. Ions present in the two phases and, possibly, ionization of the biomolecules affect the electroneutrality conditions of a two-phase system.

Research Needs and Prospects

A recent National Bureau of Standards workshop drew a clear consensus that more experimental data are essential to develop predictive partitioning models. Care in choosing the systems for study will ensure that the number of measurements is kept low¹⁹. For example, using molalities at the critical point of the solution, one can calculate interaction param-

eters between polymers and water and between the polymers themselves using some binary polymer-water mixture data²⁰.

Assuming adequate R & D funding and planning, partition-based bioseparations are likely to find increasing use in biotechnology product development, due to the system's biocompatibility, amenability to scale-up, and favorable economics compared with other bioseparation techniques.

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