

TABLE 4.0-1. Cell Disintegration Techniques<sup>a</sup>

Method	Technique	Principle	Stress on Product	Cost	Examples
Chemical	Osmotic shock	Osmotic rupture of membrane	Gentle	Cheap	Rupture of red blood cells
	Enzyme digestion	Cell wall digested, providing disruption	Gentle	Expensive	<i>Micrococcus lysodeikticus</i> treated with egg lysosome
	Solubilization	Detergents solubilize cell membrane	Gentle	Moderate-expensive	Bile salts acting on <i>E. coli</i>
	Lipid dissolution	Organic solvent dissolves in cell wall, and so destabilizes it	Moderate	Cheap	Toluene disruption of yeast
	Alkali treatment	Saponification of lipids solubilizes membrane	Harsh	Cheap	
	Homogenization (blade type)	Cells chopped in Waring blender	Moderate	Moderate	Animal tissue and cells
Mechanical	Grinding	Cells ruptured by grinding with abrasives	Moderate	Cheap	
	Ultrasonication	Cells broken with ultrasonic cavitation	Harsh	Expensive	Cell suspension at least on a small scale
	Homogenization (orifice type)	Cells forced through small hole are broken by shear	Harsh	Moderate	Large scale treatment of cell suspensions, except of bacteria
	Crushing in ball mill	Cells crushed between glass or steel balls	Harsh	Cheap	Large scale treatment of cell suspensions and plant cells

Based in part on Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, 1982.

## Osmotic Shock

The simplest of the three major chemical methods is osmotic shock. This is nothing more than dumping a given volume of cells into pure water—often about twice the volume of cells. The cells swell because they contain solutes which cause an osmotic flow of water into the cells. In some cases, they swell so much that they burst. Their contents, released into the surrounding solution, can now be separated using the methods in following chapters. The susceptibility of cells to lysis depends strongly on their type. Red blood cells are easily lysed. Animal cells often can be lysed, but only after animal tissue has been mechanically minced or homogenized, as described in Section 4.3. Plant cells are much more difficult to lyse, for their cell walls often contain strong woody material which is relatively impermeable to osmotic flow.

The osmotic flow involved comes from the osmotic pressure, which is surprisingly large. To estimate the size of this pressure, we turn to the definition of chemical equilibrium, which states that the chemical potential of water  $\mu_{\text{H}_2\text{O}}$  must be constant

$$\mu_{\text{H}_2\text{O}} \text{ (outside)} = \mu_{\text{H}_2\text{O}} \text{ (inside)} \quad (4.2-1)$$

The chemical potential of the pure water outside must include a reference value and a pressure correction; the corresponding potential inside involves a reference value, pressure correction, and a correction for solution concentration. For an ideal incompressible solution, these corrections convert Eq. (4.2-1) into the following:

$$\mu_{\text{H}_2\text{O}}^0 + \bar{V}_{\text{H}_2\text{O}} P_{\text{out}} = \mu_{\text{H}_2\text{O}}^0 + \bar{V}_{\text{H}_2\text{O}} P_{\text{in}} + RT \ln(1 - x_1) \quad (4.2-2)$$

where  $\mu_{\text{H}_2\text{O}}^0$  is the reference value,  $\bar{V}_{\text{H}_2\text{O}}$  is the partial molar volume of water, and  $x_1$  is the total mole fraction of solutes inside the cell. If the cell's contents are a dilute solution,  $\bar{V}_{\text{H}_2\text{O}}$  is almost equal to the molar volume of water  $\tilde{V}_{\text{H}_2\text{O}}$ , and  $x_1$  is small. Thus

$$\begin{aligned} P_{\text{out}} - P_{\text{in}} &= \frac{RT}{\bar{V}_{\text{H}_2\text{O}}} \ln(1 - x_1) \\ &= \frac{RT}{\tilde{V}_{\text{H}_2\text{O}}} (-x_1 - \dots) \\ &= -RTc_1 + \dots \end{aligned} \quad (4.2-3)$$

This relation is called van't Hoff's law.

Thus at equilibrium, the pressure outside the cell must be less than that inside. If this is not the case, then water will flow into the cell and potentially lyse the cell. We can use Eq. (4.2-3) to estimate the size of the pressure. Many cells have a solute concentration roughly equivalent to 0.1 M NaCl, or 0.2 M solutes. Thus from the preceding,

$$\begin{aligned}
 p_{\text{out}} - p_{\text{in}} &= -RTc_1 \\
 &= -82 \frac{\text{cm}^3 \text{ atm}(300^\circ\text{K})}{\text{g mol}^\circ\text{K}} \frac{0.2 \text{ g mol}}{1000 \text{ cm}} \\
 &= -5 \text{ atm}
 \end{aligned} \tag{4.2-4}$$

This osmotic pressure is, for us, always surprisingly large. This large pressure is what can lead to cell rupture.

## Solubilization

The second major method of chemically rupturing cells is solubilization by detergents. Typically, a concentrated detergent solution is added to about half the solution's volume of cells. The detergent disrupts the cell membrane. The resulting suspension can be centrifuged to remove cell fragments, and then run through an adsorption column or an extractor to isolate the product.

The reason that solubilization is effective lies in detergent chemistry. This chemistry depends on detergent structures like those in Figure 4.2-1. All these structures have a hydrophilic portion, which is often ionic, and a hydrophobic part, which is frequently a hydrocarbon. As a result, these detergents are all amphipathic, capable of interacting with both water and lipid.

This amphipathic nature holds whether the detergents are anionic, cationic, or nonionic. All types are illustrated in the figure. Sodium dodecylsulfate (SDS), which is the example given, is the most widely studied of the anionic detergents. The anionic materials also include the soaps, which are the salts of fatty acids. Because soaps depend on a carboxylic acid group, they are effective detergents only at high pH, where this group remains ionized. They are ineffective in hard water, where calcium ions can react with them to form insoluble precipitates.

The disadvantages of conventional soaps can be avoided by replacing the carboxylic acid group with a sulfate. The sulfate in most current laundry

---

## CHAPTER 2

---

# Filtration and Microfiltration

Filtration separates solids from a liquid by forcing the liquid through a solid support or filter medium. It is a straightforward procedure for well defined crystals. However, the small size and deformability of micro-organisms make filtration of fermentation beers or other biological solutions considerably more complicated. In fact, if we attempt to filter these beers using purely conventional technology, the filtration is often too slow to be practical.

As a result, we have two goals in this chapter. First, we must explain in simple terms how conventional filtration is accomplished. Second, we must describe how these procedures are modified for bioseparations. These modifications center on the properties of the cake of solids which accumulates on the filter medium. Only rarely will the solids be removed without additives. Frequently, the solids will be aggregated and removed with another solid, like diatomaceous earth, added as a "filter aid." Sometimes, solid accumulation will be minimal because of rapid "cross flow," tangential across the surface of the filter medium. It is these modifications which make filtration of biologically produced feeds non-routine.

In this chapter, we discuss in detail only filtrations where cake formation is significant. These filtrations usually involve larger particles or microorganisms, and are called conventional filtration. Filtrations where cross flow is dominant are effective for smaller microorganisms and so are called "microfiltration." These are deferred to Chapter 9, where they are discussed in parallel with more similar processes.

We describe the equipment for conventional filtration in Section 2.1. This equipment is used to remove biomass, amorphous precipitates, or crystalline products. Many biological precipitates, which accumulate as dense impermeable cakes, require the unconventional procedures discussed in Section 2.2. The general theory of conventional constant pressure filtration is presented in Section 2.3 and extended to continuous rotary filters in Section 2.4. This theory of filtration is concerned with filtration fluxes and ignores subjects like filtrate clarity and optimization. Laboratory tests which address these questions are covered in Section 2.5. A synopsis of microfiltration is given in Section 2.6.

## ~~2.1.~~ EQUIPMENT FOR CONVENTIONAL FILTRATION

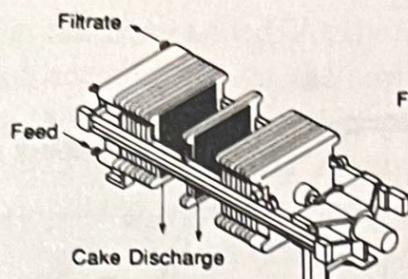
Equipment for filtration varies widely, from the conventional plate-and-frame filter press to rotary vacuum filters. Four types of small filters are illustrated in Figure 2.1-1. The most common, the plate-and-frame press shown in Figure 2.1-1a, contains caulked metal septa and a recessed cake space. Other types have flushed plates that can be used with a paper or cloth filter medium separated by open frames where the cake is formed. This type of filter is used where a relatively dry cake discharge is desired. It should not be used where there are toxic fumes or biohazards.

The other three filters in Figure 2.1-1 are enclosed, and hence can be used with aerosols or biohazards. Because their use is labor intensive, they are used only for relatively small scale work.

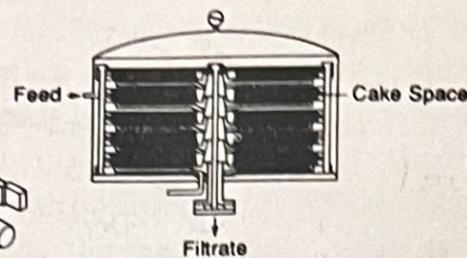
The horizontal plate filter shown in Figure 2.1-1b is useful for small scale separations. Filtration occurs only from the top of each plate (the bottom side of the cake) so that even with intermittent operation, the deposited cake remains in place. In designs like that shown, the filter leaf assembly is cleaned and the filter medium is added outside of the filter housing. In other variations, the cake may be removed with a sluicing nozzle or discharged by rapidly rotating the leaves.

The vertical leaf filter illustrated in Figure 2.1-1c requires only a small floor area but must have sufficient headroom for removal of the leaves and

*Open, useful  
for dry cake  
discharge, not  
for toxic fumes  
or biohazards*

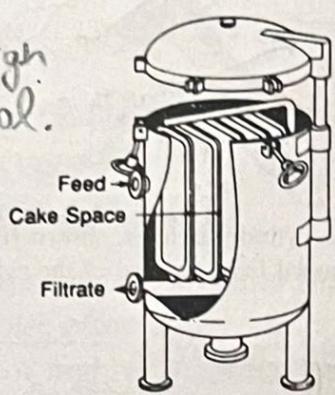


a) Plate and Frame



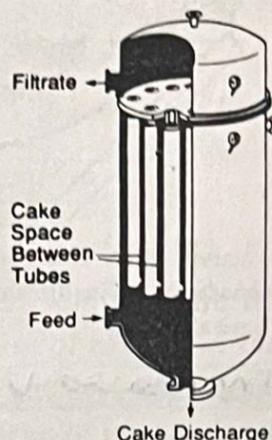
b) Horizontal Plate

*Relatively high  
filtration area.*



c) Vertical Leaf

*Enclosed can be  
used for aerosols  
& biohazards*



d) Candle Type

*Labor intensive  
hence small scale  
work*

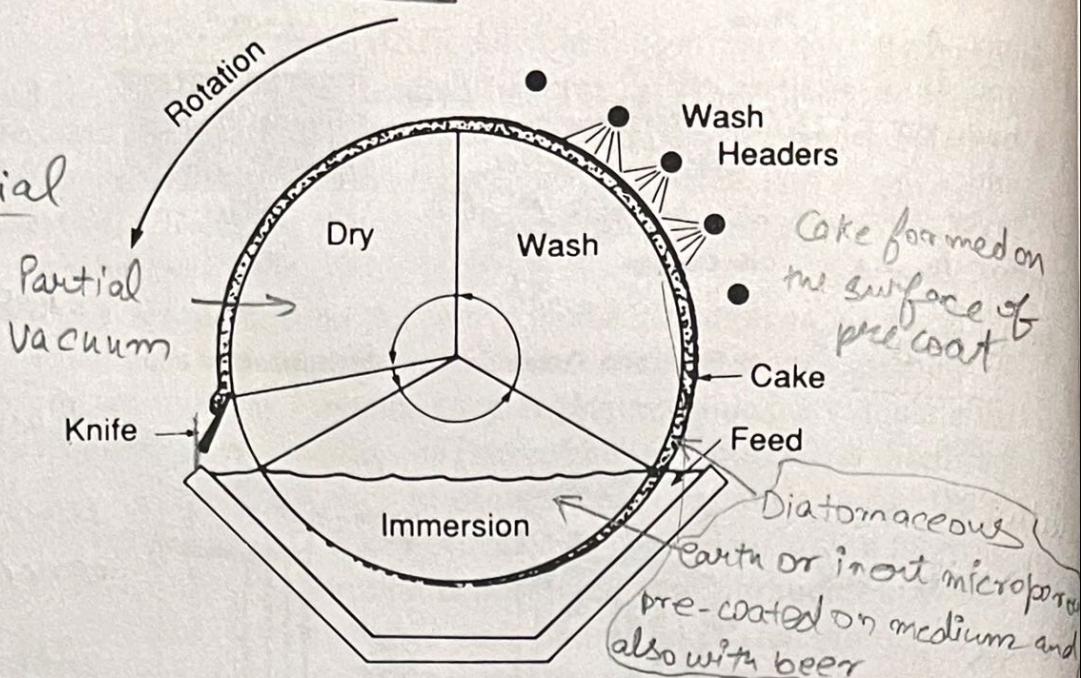
**Figure 2.1-1.** Small filters. The conventional plate-and-frame filter, shown in (a), is less common for bioseparations than the three other types, which are enclosed. These other types are labor intensive, and so used only for small scale filtrations.

the cake. It has a relatively high filtration area per volume. The tubes on the candle type vertical tank filter, shown in Figure 2.1-1d, are suspended from a tube sheet. Filter cake is formed on the external surface of the tubes and filtrate flows through the deposited cake into the head for discharge. The tubes are cleaned by backwashing.

We now turn from these small scale, enclosed filters to that workhorse of bioseparations, the rotary vacuum filter. Such filters are common for large scale operations whenever the solids are difficult to filter. They are used extensively for large scale commercial operations. Because they are often automated, they have a lower labor cost.

The designs for rotary vacuum filters vary widely, but are exemplified by that shown schematically in Figure 2.1-2. The filter includes a rotating drum, shown from the end. Pressure outside the drum is atmospheric, but

For commercial use:



**Figure 2.1-2.** Rotary vacuum filter. This continuous cylindrical filter, shown from the end, is the workhorse for bioseparations. The filtrate is removed from the end of the cylindrical drum. The times shown are typical values.

Disadvantage: Increased Vol. of solids

pressure inside the drum is a partial vacuum. The drum is partially submerged in the beer and rotates at a low speed during the operation. Liquid is sucked through the filter cloth and solids are retained on the surface of the drum, forming the cake. When the cake rotates out of the beer, it is washed, dried, and removed.

It is the step of solid removal, shown here with a knife, which varies most widely in industrial designs. Methods developed over the years include (1) a simple knife or doctor blade supplemented by an air blowback through the filter medium; (2) a string discharge also assisted by the release of vacuum and a small blowback; (3) a continuous belt discharge where the filter medium is not caulked to the filter drum but moves around a series of rolls; and (4) the continuous rotary vacuum precoat filter.

The idea of a filter precoated with some other medium is the first point where bioseparations become different from more conventional technology. After all, this synopsis of equipment is common to many texts, but the idea of precoating is not. What is precoating?

Essentially, precoating is the application of a layer of diatomaceous earth or some other inert microporous material to the filter medium itself. This layer is often thick. This coated filter is used with a beer to which still more

inert material has been added. As the drum rotates, a thin layer of biomass and filter aid accumulates on the surface of the precoat and continues to build during the cake formation segment of the cycle. This cake is washed and dewatered; then, a slowly advancing doctor blade shaves off a thin layer of accumulated biomass, thereby exposing a fresh surface of precoat for the next cycle. The depth of cut by the doctor blade depends on the penetration of the filtered particles. Vacuum is maintained throughout the entire filtration cycle. When the bed of precoat is depleted, a new layer is applied and further filtration proceeds. The result is a kind of dynamic filter which remains effective much longer than more conventional filters. Its disadvantage is an increased volume of solids.

### Challenges:

- Highly compressible filter cake
- High - non newtonian vis cosit y

## 2.2. PRETREATMENT

Fermentation beers and other biological solutions are notoriously hard to filter. They are often hard to filter because of high, non-Newtonian viscosity or because of highly compressible filter cakes. These cakes can deform into an impermeable mat. This is especially true of mycelial microorganisms, but may apply to any bacterial broth.

As a result, these biological feeds often require pretreatment. In the following paragraphs, we discuss three such treatments: heating, coagulation and flocculation, and adsorption on filter aids. Parenthetically, these treatments are also useful for centrifugation and sedimentation.

### Heating

The simplest pretreatment—and the least expensive—is to heat the feed. Such heating can not only improve the feed's handling characteristics, but also may pasteurize it. The chief constraint of this approach is the thermal stability of the product.

Why heating is effective is often chemically complicated. As an example, we consider a dilute ovalbumin solution. If we heat this solution, we will denature this protein and make it much easier to filter. In other words, we are taking a dilute solution of egg white, boiling it, and winding up with threads of cooked egg. The cooked egg threads will be much easier to filter than the original solution, but the ovalbumin has been irreversibly altered.

## Coagulation and Flocculation

A second pretreatment method is the addition of electrolytes to promote coagulation and flocculation in the initial solution. Useful agents range from simple electrolytes through acids and bases to synthetic polyelectrolytes.

Simple electrolytes act by screening the electrostatic repulsion which commonly exists between colloidal particles. When this electrostatic repulsion is reduced, attractive London-van der Waals forces predominate. The

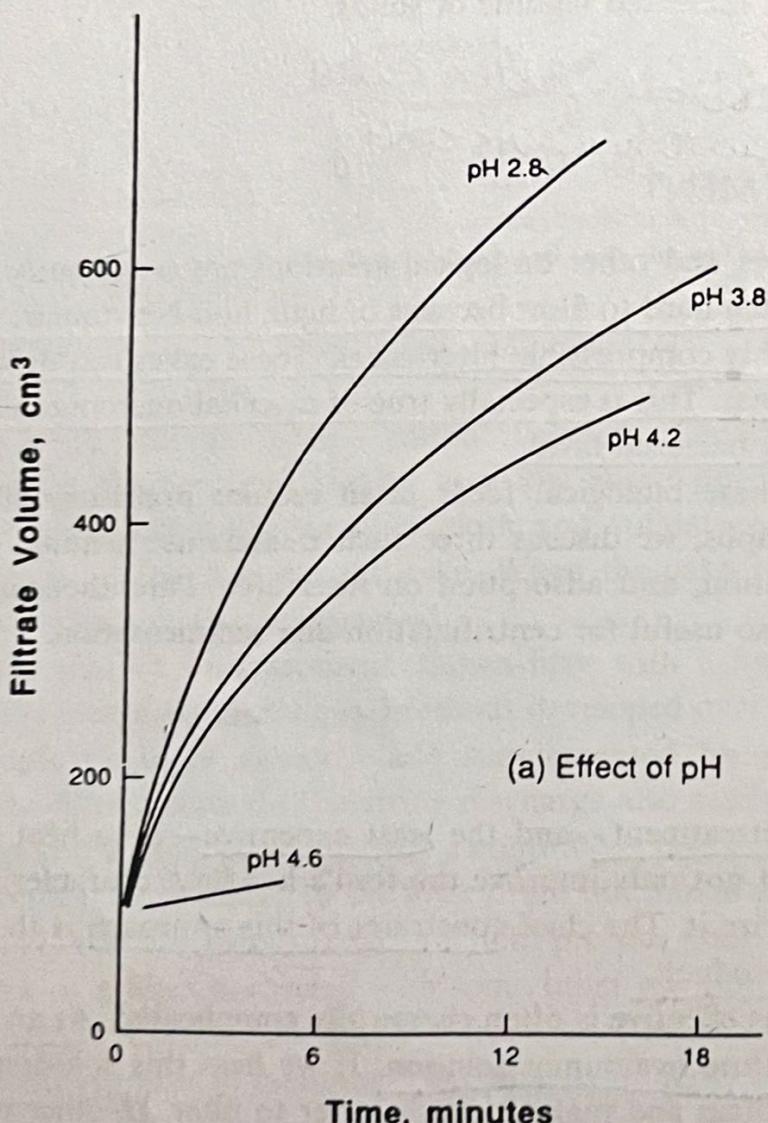


Figure 2.2-1. The effect on filtrate volume of pH and filter aid. For *Streptomyces griseus*, lowering the pH or adding filter aid accelerates the filtration. (Data from S. Shirato and S. Esumi, *J. Ferm. Tech. (Japan)*, 41, 87, 1963.)

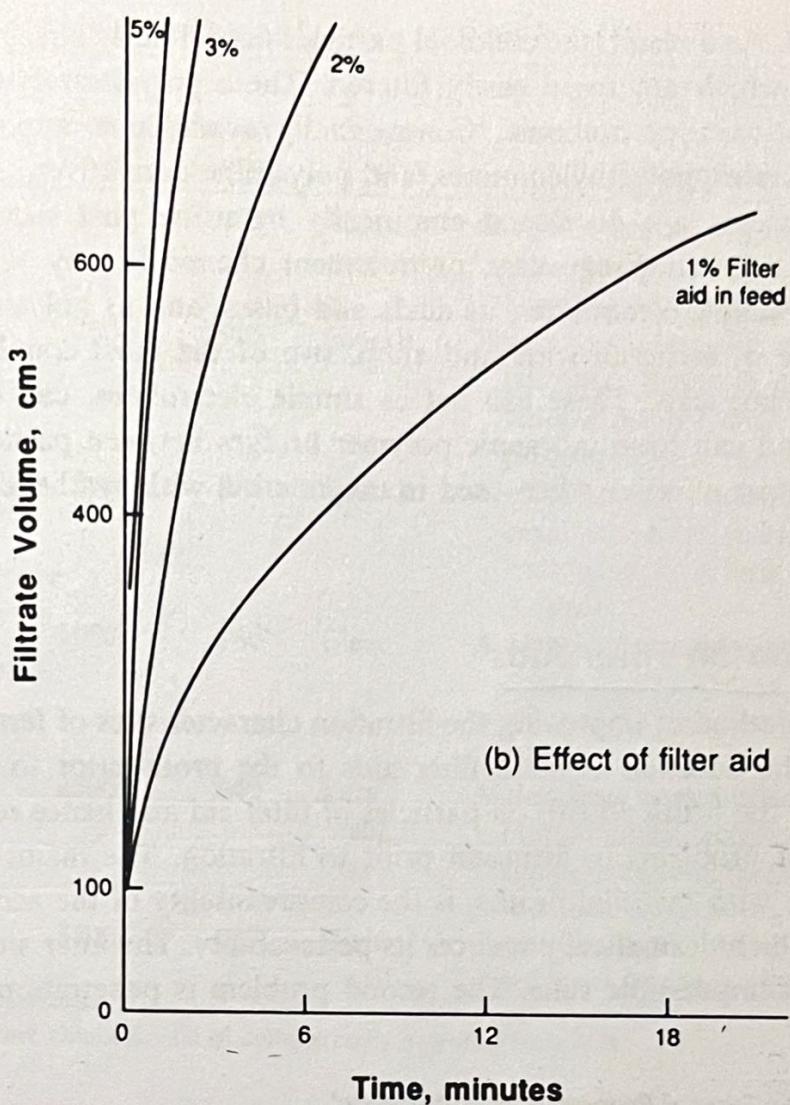


Figure 2.2-1. (Continued)

colloids can then coagulate as larger, denser particles which are more easily filtered.

Acid and bases change the pH and hence the charge on the particles. If this charge is reduced, the particles may coagulate and hence become easier to filter; if the charge is increased, the particles may coagulate still less, and the solution can become more difficult to filter. An example of the effect of pH on filtration rate is given in Figure 2.2-1a.

Synthetic polyelectrolytes added as pretreatments can both reduce electrostatic repulsion and adsorb on adjacent particles, forming bridges be-

tween them. As a result, the colloidal particles flocculate as large, less dense aggregates which are more easily filtered. These polyelectrolytes can be anionic, cationic, or nonionic. Commercially available examples include polyacrylamides, polyethylenimines, and polyamine derivatives.

Pretreatments are developed empirically by using past successes and failures as a guide. Frequently, pretreatment chemicals may act simultaneously as simple electrolytes, as acids and bases, and as polyelectrolytes. This is true of ferric chloride and alum, two of the most common water treatment chemicals. These can act as simple electrolytes, can buffer the solution, and can form inorganic polymer bridges between particles. They are often most effective when used in conjunction with synthetic polyelectrolytes.

### Adsorption on Filter Aids

The third method of improving the filtration characteristics of fermentation broths is the addition of solid filter aids to the broth prior to filtration. Colloids in the broth adsorb on particles of filter aid and hence reduce two of the chief problems in filtration prior to filtration. The major problem, particularly with mycelial broths, is the compressibility of the accumulated biomass, which dramatically reduces its permeability. The filter aid effects a much less compressible cake. The second problem is penetration of small

TABLE 2.2-1. Typical Properties of Filter Aids<sup>a</sup>

Grade	Density (kg/m <sup>3</sup> )		pH	Water Adsorption (%)	Relative Flow Rate
	Dry	Wet			
<i>Diatomaceous Earths</i>					
Standard					
Super-Cel	130	280	7.0	260	200
512 Hyflo					
Super-Cel	140	280	10.0	250	500
535	190	280	10.0	250	1400
560	310	320	10.0	220	7500
<i>Perlites</i>					
Terracel 300	110	260	7.5	—	300
Terracel 500	130	240	7.5	—	900

<sup>a</sup>These data are taken from Johns-Manville brochures.

TABLE 2.2-2. Filter Aid Classification<sup>a</sup>

Grade	Relative Flow Rate	Relative Clarity	Visual Clarity	Applications
elatom FP-2	100	1000	Bright sparkle	Beer, oils, pectins, sugar, wine, vinegar, alcohol, citric acid, gelatin, lard, tallow, polymers
elatom FP-4	200	995	Sparkle	Beer, gelatin, fats, oils, cider, lacquers, petroleum products, pectins, sugar, vinegar, alcohol, citric acid, phosphoric acid, cane sugar, lube oil
elatom FW-6	300	986	Sparkle	Antibiotics, beer, caustics, chemicals, cider, enamels, gelatin, glue fruit juices, kelp, lard, oils, petroleum products
elatom FW-20	1000	960	Clear	Acids, citrates, tallow, water, chemicals, fruit juices, kelp, grape juice, apple juice, hemp oil, fuel oil, oils
elatom FW-50	2500	940	Very slight veil	Algins, wort beer, antibiotics, casein, pressed juices, lacquers, polymers, syrups, sorghum, tallow, varnish, water, titanium, corn gluten, citric juices
elatom FW-80	5500	927	Slight veil	Algins, antibiotics, biochemicals, polymers, resins, water, wort beer

These properties are characteristic of commercially available filter aids.

particles, such as fragmented mycelium or bacterial cells, into the precoat layer in a rotary vacuum filter. This penetration can blind the pores of the precoat matrix.

The two types of filter aids which have been found most effective for pretreatments are the diatomaceous earths and the perlites. The diatomaceous earths are the skeletal remains of tiny aquatic plants deposited centuries ago. The perlites are volcanic rock processed to yield an expanded form. Properties of both these filter aids are given in Table 2.2-1, and typical applications are presented in Table 2.2-2. For special applications where silica-containing materials are undesirable, ground wood pulp and starch are sometimes used. The vast improvements effected by the addition of filter aids is illustrated by the data in Figure 2.2-1b.

There can be disadvantages to using filter aids to enhance filtration. For example, the data in Table 2.2-2 show that as particle size increases, flow

increases but filtrate clarity decreases. As a second example, some products like the aminoglycoside antibiotics may irreversibly bind to diatomaceous earth. These examples underscore the importance of experiments in evaluating the use of any pretreatment for filtration.

### 2.3. GENERAL THEORY FOR FILTRATION

We now turn to the mathematical description of filtration. We do so in two steps. First, in this section, we develop general equations for conventional filtration, both for compressible and incompressible cakes. Second, in Section 2.4, we apply these equations to the rotary vacuum filter, for this filter is the most common type in large scale bioseparations. In both of these sections, we will assume that we are using equipment like that described in Section 2.1 and have a feed stream pretreated by the methods of Section 2.2.

Our general theory involves three steps. First, we need to review the equations of the fluid mechanics which are important for filtration, a subject conveniently described as Darcy's law. Second, we solve these equations for incompressible cakes, which is the simplest case. Third, we solve them for compressible cakes, which is the case common for bioseparations.

#### Darcy's Law

Darcy's law relates the flow rate through a porous bed of solids to the pressure drop causing that flow

$$v = \frac{\kappa \Delta p}{\mu \ell} \quad (2.3-1)$$

where  $v$  is the velocity of the liquid,  $\kappa$  is a proportionality constant usually called the Darcy's law permeability of the bed,  $\Delta p$  is the pressure drop across the bed of thickness  $\ell$ , and  $\mu$  is the viscosity of the liquid. Like Ohm's law, Eq. (2.3-1) states that the flow is directly proportional to the potential  $\Delta p$  and inversely proportional to the resistance ( $\ell/\kappa$ ). Strictly speaking, Darcy's law holds only when

$$\left| \frac{dv \rho}{\mu(1 - \epsilon)} \right| < 5 \quad (2.3-2)$$

where  $d$  is the particle size or pore diameter in the filter cake,  $\rho$  is the liquid

density, and  $\epsilon$  is the void fraction in the cake. For biological separations, the dimensionless quantity  $[dv \rho / \mu(1 - \epsilon)]$ , called the Reynolds number, almost always easily obeys this inequality.

For a batch filtration, we may rewrite the velocity as

$$\boxed{v = \frac{1}{A} \frac{dV}{dt}} \quad (2.3-3)$$

where  $V$  is the total volume of filtrate and  $t$  is the time. We may also rewrite the resistance ( $\ell/k$ ) to include explicitly the contributions of cake and filter medium

$$\boxed{\frac{\ell}{k} = R_M + R_C} \quad (2.3-4)$$

where  $R_M$  is the resistance of the filter medium and  $R_C$  is the resistance of the accumulated biomass.

Combining Eqs. (2.3-1), (2.3-3), and (2.3-4), we find the basic differential equation for filtration at constant pressure drop

$$\boxed{\frac{1}{A} \frac{dV}{dt} = \frac{\Delta p}{\mu(R_M + R_C)}} \quad (2.3-5)$$

The medium's resistance  $R_M$  is a constant, independent of the cake. In contrast, the cake's resistance  $R_C$  varies with the amount filtered  $V$ . The exact nature of this variation depends on whether the cake is incompressible or not.

## Incompressible Cakes

If the cake is incompressible, the cake thickness is directly proportional to the filtrate volume and inversely proportional to the filter area. As a result, the cake's resistance  $R_C$  is described by the equation

$$\boxed{R_C = \alpha \rho_0 \left( \frac{V}{A} \right)} \quad (2.3-6)$$

where  $\alpha$  represents the specific cake resistance and  $\rho_0$  is the mass of cake solids per volume of filtrate. This implies that  $\alpha$  has the dimensions of length per mass. Substitution of Eq. (2.3-6) into Eq. (2.3-5) yields

$$\boxed{\frac{1}{A} \frac{dV}{dt} = \frac{\Delta p}{\mu [\alpha \rho_0 (V/A) + R_M]}} \quad (2.3-7)$$

This equation is subject to the initial condition

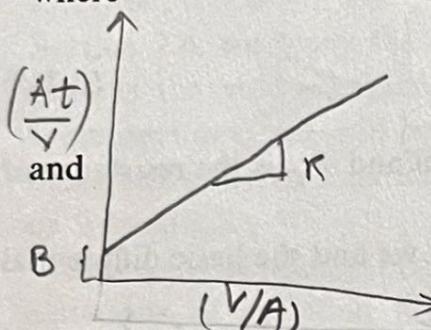
$$t = 0, \quad V = 0 \quad (2.3-8)$$

This condition says that at the start of the experiment, no solution has been filtered.

Equation (2.3-7) is easily integrated and rearranged to give

$$\left( \frac{At}{V} \right) = K \left( \frac{V}{A} \right) + B \quad (2.3-9)$$

where



$$K = \frac{\mu \alpha \rho_0}{2 \Delta p} \quad (2.3-10)$$

$$B = \frac{\mu R_M}{\Delta p} \quad (2.3-11)$$

Thus a plot of  $(At/V)$  versus  $(V/A)$  should be linear. The slope  $K$  is a function of the pressure drop  $\Delta p$  and of the properties of the cake, represented by  $\alpha$  and  $\rho_0$ . The intercept  $B$  should be independent of the properties of the cake, but it is proportional to the medium's resistance  $R_M$ . Often, this medium resistance is insignificant, so Eq. (2.3-9) becomes simpler still:

$B \rightarrow$  neglect  
As  $B$  is constant

$$\Rightarrow t = \left( \frac{\mu \alpha \rho_0}{2 \Delta p} \right) \left( \frac{V}{A} \right)^2$$

*thickness of cake for a given volume*

↓
↑
(2.3-12)

These equations, valid for incompressible cakes, will be tested in the examples given later in the section.

### Compressible Cakes

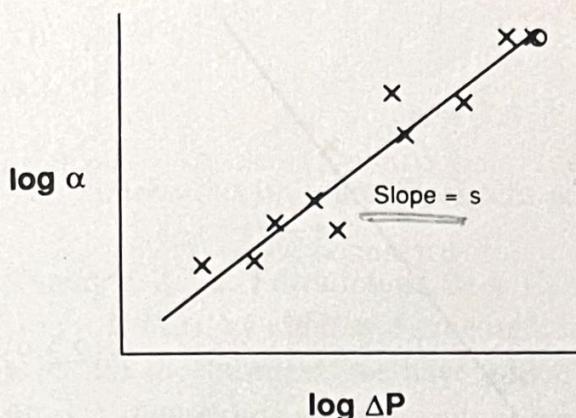
Unfortunately, almost all cakes formed of biological materials are compressible, and so cannot be described with the simple analysis just outlined. As these cakes compress, filtration rates drop. This in turn results in compromised economics.

To estimate the effects of compressibility, we assume that the cake resistance  $\alpha$  is a function of the pressure drop:

$\alpha \rightarrow$  for compressible cake it depends on  $\Delta p$

$$\alpha = \alpha' (\Delta p)^s \quad (2.3-13)$$

↑  
constant



**Figure 2.3-1.** Cake resistance versus pressure drop. The cake resistance often increases as the pressure drop across the cake increases. Such an increase, due to the cake's compressibility, is easily described by an exponent  $s$ .

where  $\alpha'$  is a constant related largely to the size and shape of the particles forming the cake, and  $s$ , the cake compressibility, varies from zero for a rigid, incompressible cake to near one for a highly compressible cake. In practice,  $s$  ranges from 0.1–0.8. Values of  $s$  and  $\alpha'$  are most easily determined by plotting the logarithm of  $\alpha$  versus the logarithm of  $\Delta p$ , as shown in Figure 2.3-1. When values of  $s$  are high, one should consider pretreating the feed with filter aids, as described in Section 2.2.

Fortunately, the fact that a cake is compressible does not change the basic result for a constant pressure filtration, given by Eq. (2.3-9). It does mean that the value of  $K$  measured with this equation may be a more complex function of  $\Delta p$  than first expected. It should not change the value of  $B$ , which remains inversely proportional to pressure.

The implications of these results are illustrated by the examples which follow.

~~class~~

**Example 2.3-1. Streptomyces Filtration from an Erythromycin Broth.** Using a test filter, we find the following data for a broth containing the antibiotic erythromycin and added filter aid:

Filtration Time (sec)	Volume of Filtrate (liters)
5	0.040
10	0.055
20	0.080
30	0.095

The filter leaf has a total area of  $0.1 \text{ ft}^2$  and the filtrate has a viscosity of  $1.1 \text{ cP}$ . The pressure drop is 20 in. of mercury and the feed contains  $0.015 \text{ kg dry cake per liter}$ .

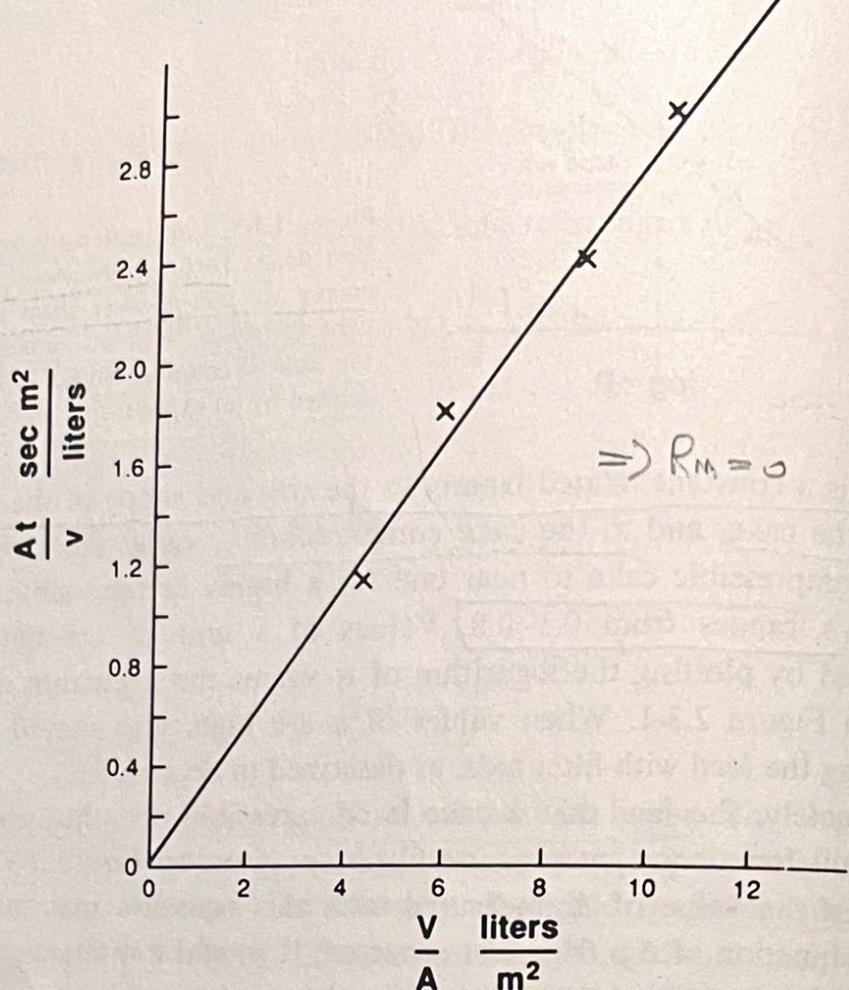
Determine the specific cake resistance  $\alpha$  and the medium resistance  $R_M$ .

$$A = 0.1 \text{ ft}^2$$

$$\mu = 1.1 \text{ cP}$$

$$\Delta P = 20 \text{ in}$$

$$P_0 = 0.015 \text{ kg/l}$$



**Figure 2.3-2.** Filtration of erythromycin broth. The near zero intercept on this plot implies that the resistance of the filter medium is not important. The slope describes the ever increasing resistance of the filter cake.

**Solution.** Following Eq. (2.3-9), we plot  $(At/V)$  versus  $(V/A)$ , as shown in Figure 2.3-2. The intercept on this plot is essentially zero, so  $R_M$  is zero: The medium has no significant resistance.

From the data and Eq. (2.3-10), we see from the slope of the data that

$$t \left( \frac{A}{V} \right)^2 = \frac{\mu \alpha \rho_0}{2 \Delta p}$$

$$29 \frac{\text{sec}}{\text{cm}^2} = \frac{\left( \frac{0.011 \text{ g}}{\text{cm sec}} \right) \alpha \left( \frac{15 \times 10^{-3} \text{ g}}{\text{cm}^3} \right)}{2 \left( 20 \text{ in. Hg} \frac{3.39 \times 10^4 \text{ g}}{\text{cm sec}^2 \text{ in. Hg}} \right)}$$

This gives

$$\alpha = 2.4 \times 10^{11} \text{ cm/g.}$$

The dimensions of  $\alpha$  are consistent with its definition.

Important

~~A~~ **Example 2.3-2. Filtration of Beer Containing Protease.** We have a suspension of *Bacillus subtilis* fermented to produce the enzyme protease. To separate the biomass, we have added 1.3 times the biomass of a Celatom filter aid, yielding a beer containing 3.6 wt% solids, with a viscosity of 6.6 cP. With a Buchner funnel 5 cm in diameter attached to an aspirator, we have found that we can filter 100 cm<sup>3</sup> of this beer in 24 min. However, previous studies with this type of beer have had a compressible cake with  $s$  equal to 2/3.

We now need to filter 3000 liters of this material in one of the pilot plant's plate-and-frame filter presses. This press has 15 frames, each of area 3520 cm<sup>2</sup>. The spacing between these frames can be made large. This is an advantage, because we can then filter all the beer without emptying the press, and hence reduce the risk of contamination. The resistance of the filters themselves is much smaller than the filter cake, and the total pressure drop which can be used is 65 psi.

- (a) How long will it take to filter this beer at 50 psi?
- (b) How long will it take at half this pressure drop?

*Solution.* We first need to calculate the properties of the filter cake. Because the filter medium has a negligible resistance, we can combine Eqs. (2.3-12) and (2.3-13) to find

$$t = \frac{\mu \alpha' \rho_0}{2 \Delta p^{1-s}} \left( \frac{V}{A} \right)^2$$

Inserting the values given

$$24 \text{ min} = \frac{\mu \alpha' \rho_0}{2(14.7 \text{ psi})^{1/3}} \left( \frac{100 \text{ cm}^3}{\pi/4(5 \text{ cm})^2} \right)^2$$

$$\mu \alpha' \rho_0 = 4.53 \text{ min psi}^{1/3}/\text{cm}^2$$

The units for  $\mu \alpha' \rho_0$  are unusual but convenient.

(a) We now can use this same equation to find the time:

$$t = \frac{\mu \alpha' \rho_0}{2 \Delta p^{1-s}} \left( \frac{V}{A} \right)^2$$

$$= \frac{4.53 \frac{\text{min psi}^{1/3}}{\text{cm}^2} \frac{\text{hr}}{60 \text{ min}}}{2(50 \text{ psi})^{1/3}} \left( \frac{3000 \text{ liters}}{2 \times 15 \times 3520 \text{ cm}^2} \frac{1000 \text{ cm}^3}{\text{liter}} \right)^2$$

$$= 8.2 \text{ hr}$$

Note that the viscosity and beer concentration do not explicitly enter this calculation; they appear only as the product  $\mu \alpha' \rho_0$ .

(b) If the pressure drop is cut in half, the same equation gives

$$t = 10.4 \text{ hr}$$

The time increases only slightly because the cake is so compressible.

**Example 2.3-3. Filtration of Incompressible Steroid Crystals.** We have filtered a slurry of sitosterol at constant pressure through a filtration medium consisting of a screen support mounted across the end of a Pyrex pipe. We find that the resistance of this new medium is negligible. We also find the following data in a laboratory test:

Weight of crystals	62 g
Pressure of filtration	15 psi
Filter diameter	5.08 cm
Cake depth	12.5 cm
Cake volume	253.3 cm <sup>3</sup>
Filtration time	163 min

The cake is essentially incompressible.

On the basis of this laboratory test, predict the number of frames (30 in.  $\times$  30 in.  $\times$  1 in. thick) needed for a plate-and-frame press (cf. Fig. 2.1-1a). Estimate the time required to filter a 63 kg batch of steroid. In these calculations, assume that the feed pump will deliver 10 psi and that the filtrate from the press must be raised against the equivalent of 15 ft head.

*Solution.* To design this filter, we first recognize that filtration occurs on both sides of the frame. As a result, the area per frame is

$$2 \times \left( 30 \text{ in.} \frac{2.54 \text{ cm}}{\text{in.}} \right)^2 = 1.16 \times 10^4 \text{ cm}^2$$

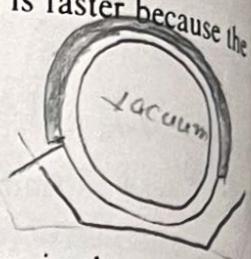
30

Again, from Eq. (2.3-12),

$$\begin{aligned} t &= \left( \frac{\mu \alpha}{2 \rho_0} \right) \frac{(\rho_0 V)^2}{\Delta p(A)^2} \\ &= \frac{261 \text{ min(psi) cm}^4}{g^2} \frac{(63,000 \text{ g})^2}{3.5 \text{ psi} [18(1.16 \times 10^4 \text{ cm}^2)]^2} \\ &= 6.8 \text{ min} \end{aligned}$$

Even though the pressure drop is smaller, the filtration is faster because the area is much larger.

*Same fig  
as 2.1-2*



## 2.4. CONTINUOUS ROTARY FILTERS

One very common filtration method in bioseparations is the continuous rotary filter. This method is used for more large scale antibiotic processing than all others combined. It is the preferred choice whenever the filtration is slow and difficult.

We analyze the operation of these units in this section. As shown in Figure 2.1-2, a filtration cycle on this filter consists of three chief steps:

1. cake formation,
2. cake washing to remove either valuable or unwanted solutes, and
3. cake discharge.

Although the type of discharge selected is important to the overall success of the operation, it does not affect the size of the filter. It is not discussed here, but is detailed in more specialized monographs. The analysis of the other two steps of this cycle is given in the following paragraphs.

### Cake Formation

We first consider the cake formation which begins as the rotating drum first drops into the broth. We assume that the resistance of the filter medium  $R_M$  is negligible, so that we can use Eq. (2.3-5), the basic expression for filtration:

$$\frac{1}{A} \frac{dV}{dt} = \frac{\Delta P}{\mu(R_c + R_m)}$$

(Start)

$$\frac{1}{A} \frac{dV}{dt} = \frac{\Delta p}{\mu R_c}$$

$R_M \rightarrow 0$

(2.4-1)

As before, this is subject to the initial condition that

$$t = 0, \quad V = 0 \quad (2.4-2)$$

We rewrite  $R_c$  for a compressible cake by combining Eqs. (2.3-6) and (2.3-13):

$$\begin{aligned} R_c &= \alpha \rho_0 \left( \frac{V}{A} \right) & \alpha = \alpha' (\Delta p)^s \\ &= \alpha' \rho_0 \left( \frac{V}{A} \right) (\Delta p)^s \end{aligned} \quad (2.4-3)$$

By combining Eq. (2.4-1) with Eq. (2.4-3), we can integrate over that part of the drum's revolution where the cake is forming to find

$$\begin{aligned} t = 0 \rightarrow t = t_f & \quad \text{(Handwritten note)} \\ V = 0 \rightarrow V = V_f & \quad \text{(Handwritten note)} \end{aligned}$$

$$t_f = \frac{\mu \alpha' \rho_0}{2(\Delta p)^{1-s}} \left( \frac{V_f}{A} \right)^2 \quad (2.4-4)$$

where  $t_f$  is the cake formation time and  $V_f$  is the volume of filtrate collected during that period. This relationship is sometimes written in terms of the cycle time  $t_c$ , given by

$$t_f = \beta t_c \quad (2.4-5)$$

where  $\beta$  is the fraction of time that the filter is submerged, that is, the fraction of the cycle devoted to cake formation. Combining this with Eq. (2.4-4), we find a relation for filtration flux expressed in terms of cycle time:

$$\frac{\text{flux}}{\text{time}} \rightarrow \frac{V_f}{At_c} = \left[ \frac{2\beta(\Delta p)^{1-s}}{\mu \alpha' \rho_0 t_c} \right]^{1/2} \quad (2.4-6)$$

We clearly see that cake formation can be altered by varying either the total cycle time  $t_c$  or the fraction of the total cycle time devoted to cake formation  $\beta$ . In addition, we note that at constant  $\beta$ , the filtration flux is inversely proportional to the square root of the cycle time.

## Cake Washing

After formation, the cake contains a significant amount of solute-rich liquid broth. This broth is usually removed by washing the cake. The washing has two functions. First, it displaces the solute-rich broth trapped in pores in

~~34~~~~Class~~

**Example 2.4-1. Filtration of *Streptomyces erytheus* Beer.** We want to filter 15,000 liter/hr of a beer containing erythromycin using a rotary vacuum filter originally purchased for another product. Our filter has a cycle time of 50 sec and an area of 37.2 m<sup>2</sup>. It operates under a vacuum of 20 in. Hg. The pretreated broth forms an incompressible cake with the resistance in Example 2.3-1:

$$K = \left( \frac{\mu \alpha \rho_0}{2 \Delta p} \right) = \frac{29 \text{ sec}}{\text{cm}^2}$$

We want to wash the cake until only 1% of the retained solubles is left, and we expect that the washing efficiency will be 70% and that 1% of the filtrate is retained.

- (a) Calculate the filtration time per cycle.
- ✓ (b) Find the washing time.

*Solution.* (a) We first find the volume of filtrate which must be removed per cycle

$$V_f = \frac{15,000 \text{ liters}}{\text{hr}} \left( \frac{\text{hr}}{3600 \text{ sec}} \right) 50 \text{ sec} \\ = 208 \text{ liters}$$

We now rearrange Eq. (2.3-12) or (2.4-4) to find the filtration time:

$$t_f = \frac{\mu \alpha' \rho_0}{2(\Delta p)^{1-s}} \left( \frac{V_f}{A} \right)^2$$

For an incompressible cake, s = 0 and α' = α; thus

$$t_f = \frac{\mu \alpha \rho_0}{2 \Delta p} \left( \frac{V_f}{A} \right)^2 \\ = \frac{29 \text{ sec}}{\text{cm}^2} \left( \frac{208 \times 10^3 \text{ cm}^3}{37.2 \times 10^4 \text{ cm}^2} \right)^2 \\ = 9 \text{ sec}$$

This is about 1/5 of the cycle time.

TABLE 2.5-2. Typical Vacuum Filtration Rates<sup>a</sup>

Product	Microorganisms	Filtration Rate (liters/hr m <sup>2</sup> )
Kanamycin	<i>S. kanamycetius</i>	~ 0.7
Lincomycin	<i>S. lincolnensis</i>	2.6–3.8
Erythromycin	<i>S. erytheus</i>	2.9–5.7
Neomycin	<i>S. fradise</i>	~ 1.1
Penicillin	—	12–16
Protease	<i>B. subtilis</i>	0.9–3.7

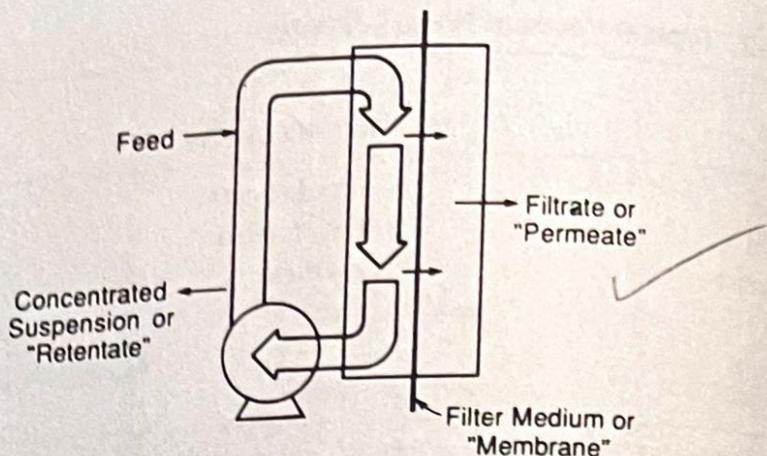
<sup>a</sup>All values are for vacuum precoat filtration. Values with a filter leaf agree closely with full scale filters.

To make a filter leaf test, we first precoat the filter leaf with the selected filter aid. We then place the leaf under vacuum and submerge it in the broth for the time selected for cake formation. During this cake formation, we agitate the broth to give a uniform mixture during the entire test period. After cake formation, we withdraw the leaf and, still under vacuum, allow it to drain for the allotted time. We then submerge the leaf in the wash liquid for the desired washing period, remove it, and dry the accumulated cake. We then advance the cake ring shown in Figure 2.5-3 to expose the proper thickness of cake and filter aid, and cut off this exposed cake. As a result, we have a fresh precoat surface with which to repeat the test. Measurements of cake mass and filtrate volume are the data needed for the large scale process design implied by the earlier sections of this chapter. Typical values found from these tests are given in Table 2.5-2. Try the test yourself—it works well.

## 2.6. MICROFILTRATION

At this point, we may wish that we could filter without a filter cake. After all, most of the pressure drop in conventional filtration comes from the cake. When the cake is compressible, the pressure drop is not even linearly related to the flow and, hence, is a significant complication, both for filtration analysis and for filter design. Filtration without a cake might offer real advantages.

Filtration without a cake is part of the process called microfiltration. As Figure 2.6-1 suggests, it is achieved by using a large cross flow tangential to



**Figure 2.6-1.** A schematic of microfiltration. The feed solution is rapidly recycled past the filter medium—the membrane—to minimize any cake formation. A detailed analysis of this and parallel separations is given in Chapter 9.

the surface of the filter itself. At the same time, the flow normal to the filter is relatively small. The large ratio of cross flow to flow through the filter minimizes the accumulation of cake.

Microfiltration is used to concentrate insolubles. It is like ultrafiltration, and is analyzed with similar equations. Parallel equations are used to describe electrophoresis and electrodialysis. As a result, we have deferred a detailed discussion of microfiltration to Chapter 9, where we will describe it along with these purification methods.

At the same time, we want to give a brief synopsis of microfiltration here, just to strengthen the comparison with conventional filtration. This synopsis centers on three points: the filter itself, the requirements for purging, and the filter geometry. Each will be discussed in the following text.

The key part of the microfiltration is the filter medium itself, or as it is more commonly called, the membrane. These membranes are thin and microporous. The pores are small and highly monodisperse; they must retain the particles being filtered, but quickly pass liquid and smaller solutes. As a result of the small pores, the membrane has a low Darcy's law permeability and a high resistance to flow.

These features mean that a microfiltration membrane is different from a more conventional filter medium. Such a conventional medium has a high Darcy's law permeability, that is, a low resistance to flow. Indeed, in the examples of the previous sections, the resistance of the filter medium was often negligible. This is not the case for microfiltration.

The second central point for microfiltration is the necessity of periodic purging. In most cases, the initial microfiltration feed is a highly dilute suspension. As liquid is removed, the suspension becomes concentrated. Rapidly pumping this suspension, which is necessary to sustain cross flow, is increasingly difficult, for its viscosity skyrockets. As a result, the concentrated suspension must be discharged, even when it contains much more liquid than that in a conventional filter cake. As a result, microfiltration may be followed by conventional filtration or centrifugation to more completely separate the insolubles from the solution.

The final central point for microfiltration is the shape of the filter. In microfiltration, the flow through the membrane is slower than the flow through a conventional filter cake. Because the flow per area is so slow, microfilters are designed with a larger filter area per filter volume than conventional filters. Three designs are common: the plate-and-frame, the spiral wound module, and the hollow fiber module. The plate-and-frame filter is like that in Figure 2.1-1a, but with a microfiltration membrane replacing the conventional filter medium. These devices have a modest surface area per volume, but do not often plug and are easy to clean. Moreover, if one piece of membrane fails, it is easily located and replaced.

The spiral wound and hollow fiber modules have a much larger area per volume, but plug more easily. The spiral wound device is like a large envelope filled with feed which is filtered through the envelope's wall. However, the envelope is loosely rolled, and the filtrate is collected from the end of the roll. The hollow fiber module is like a small shell-and-tube heat exchanger in which the tubes are microporous fiber membranes. The suspension being filtered flows down the fibers' interior—the fibers' lumen—and filtrate is collected from the outside of the fibers on the shell side. Both the spiral wound and the hollow fiber filters are often discarded when even part of the filter fails.

This synopsis of microfiltration describes how the process occurs and what the equipment will look like. It defers a more detailed discussion to Chapter 9. Take our advice; wait patiently; it will be clearer then.

Learn:  
drawbacks of  
membrane filtration

### Membrane materials

## 2.7. CONCLUSIONS

- ① cellulose 80% } porosity.
- ② Polypropylene 35%
- ③ Polycarbonate 3%

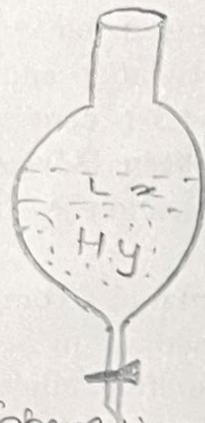
Filtration is the best established and most versatile method for removing insolubles from dilute streams like fermentation broths. While filtration has been successfully used for blood fractionation, its value for protein sep-

660.63  
BELIB  
N88

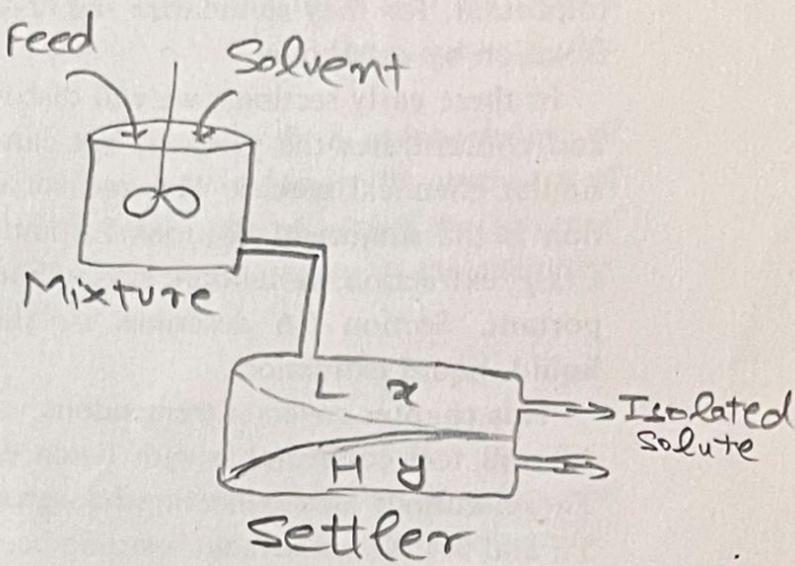
Lakshminath Bezbaroa Central Library  
Indian Institute of Technology Guwahati  
ACC. No....124606.....  
Date.....14/11/18.....

## CHAPTER 5

### Extraction



Separating funnel



In the first chapter of this book, we suggested that biological separations can be regarded as a four step process. The first step is to remove insoluble materials from a feed, which frequently is a fermentation beer. The second step is to isolate the product from the resulting dilute solution, producing a more concentrated solution. The third step is to purify the product, removing other species which may be similar. The fourth and final step is a final purification, which we call "polishing."

This chapter is the first of two concerned with the second step, product isolation. While many separation processes are used for this isolation, two are common: extraction and adsorption. This chapter emphasizes extraction, which for 50 years has been the more important of the two. This past importance reflects the central role of antibiotics in biochemical separations. As products other than antibiotics gain large markets, other separation processes may become more important. For now, extraction remains a major method of product isolation.

Our discussion begins in Section 5.1 with a review of the chemistry responsible for extraction. This chemistry is tremendously simplified because the solutions are commonly dilute. We then turn to the analysis of

batch extractions in Section 5.2. This analysis is simple, and so provides an easy route to the vocabulary used to describe extraction. In Section 5.3, we describe repeated extractions in a staged cascade. Such cascades are common engineering practice, for they are basic to other separations like distillation. In Section 5.4, we discuss extractions by differential contacting. Such differential contacting leads to the idea of mass transfer coefficients, which are like modified reaction rate constants. These four sections are important, for they summarize the engineering ideas required for product isolation by extraction.

In these early sections, we will discover that extraction not only isolates and concentrates the product, but can begin to purify it, removing other similar chemical species. This idea of simultaneous isolation and purification is the subject of Sections 5.5 and 5.6. Section 5.5 is concerned with Craig extraction, a laboratory method which has been industrially important. Section 5.6 describes isolation and purification by fractional liquid-liquid extraction.

This chapter covers a tremendous variety of material in a compact way. All will feel comfortable with batch extractions described in Section 5.2. Those without an engineering background should carefully study Sections 5.3 and 5.4. Those without a strong background in chemistry should closely read Section 5.1. Those who already have some experience will find Sections 5.5 and 5.6 most useful.

## 5.1. THE CHEMISTRY OF EXTRACTION

The extraction processes described later in this chapter take advantage of the partitioning of a solute between immiscible liquid phases. For example, citric acid is more soluble in methyl amyl ketone than in water at pH 4. Penicillin dissolves more readily in amyl acetate than in water at pH 5.5. Catalase has a higher concentration in polyethylene glycol rich solutions than in dextran rich solutions. In each of these three cases, the solute concentration increases in the first liquid phase as a result of depletion from the second liquid phase.

This partitioning is conveniently summarized as a partition coefficient  $K$

$x \rightarrow$  Solute conc. in lighter phase (L)

$$y \rightarrow \text{ " " " heavier phase (H)} K = \frac{x}{y} \quad (5.1-1)$$

where  $x$  is the solute concentration in the lighter liquid  $L$ , and  $y$  is the same solute's concentration in the heavier liquid phase  $H$ . In many cases, the light phase will be an organic solvent, and the heavier phase will be water. Often, the values of  $K$  will be constant independent of solute concentration for a given solvent pair. A constant  $K$  reflects the fact that most biochemical extractions take place in dilute solution.

Values of  $K$  must be found by experiment. From the selection of values in Table 5.1-1, we see that values scatter, without obvious trends. However, the trends are there, skeletons of chemical generalizations that are hidden yet familiar. It is these chemical skeletons which are the subject of this section.

In discussing these values of  $K$ , we begin with a quick review of thermodynamic equilibria. We next discuss manipulating the chemistry of the solutes, which is often hard. We then describe changing the solvents' chemistry, which is easier. The results supply a perspective of the chemistry responsible for extraction.

## Basic Equations

The values of  $K$  in Table 5.1-1 are based on the fact that the chemical potential  $\mu$  of the solute at equilibrium is constant and equal in each phase:

$$\mu(H) = \mu(L) \quad (5.1-2)$$

or

$$\mu^0(H) + RT \ln y = \mu^0(L) + RT \ln x \quad (5.1-3)$$

where the  $\mu^0$ 's are chemical potentials in standard reference states. Equation (5.1-3) is easily rearranged:

$$\frac{x}{y} = K = \exp\left(\frac{\mu^0(H) - \mu^0(L)}{RT}\right) \quad (5.1-4)$$

The logarithm of the partition coefficient is proportional to a difference in chemical potentials in the standard states.

To gain a qualitative understanding of Eq. (5.1-4), we imagine a small amount of light solvent  $L$  in equilibrium with excess heavy solvent  $H$ . Because  $H$  is present in excess, the chemical potential  $\mu(H)$  of the solute in this solvent is fixed, as shown in Figure 5.1-1. That in the light solvent  $\mu(L)$

TABLE 5.1-1. A Selection of Distribution Coefficients<sup>a</sup>

Extraction

Type	Solute	Solvent <sup>b</sup>	$K^c$	Remarks
Amino acids	Glycine	n-butanol	0.01	25°C
	Alanine	n-butanol	0.02	
	Lysine	n-butanol	0.2	
	Glutamic acid	n-butanol	0.07	
	$\alpha$ -aminobutyric acid	n-butanol	0.02	
	$\alpha$ -aminocaproic acid	n-butanol	0.3	
Antibiotics	Celesticetin	n-butanol	110	
	Cycloheximide	Methylene chloride	23	
	Erythromycin	Amyl acetate	120	
			0.04	
	Lincomycin	n-butanol	0.17	at pH 4.2
	Gramicidin	Benzene	0.6	
		Chloroform-methanol	17	
	Novobiocin	Butyl acetate	100	at pH 7.0
			0.01	at pH 10.5
Proteins	Penicillin F	Amyl acetate	32	at pH 4.0
			0.06	at pH 6.0
	Penicillin K	Amyl acetate	12	at pH 4.0
			0.1	at pH 6.0
	Glucose isomerase	PEG 1550/potassium phosphate	3	
	Fumarase	PEG 1550/potassium phosphate	0.2	
	Catalase	PEG/crude dextran	3	

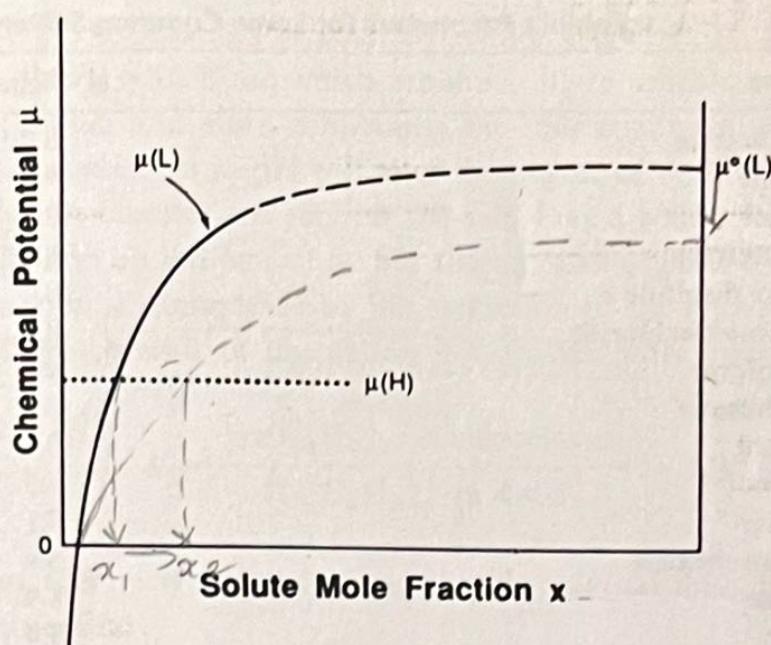
<sup>a</sup>Taken from the following: H. Hustedt, K. H. Kronen, and M. R. Kula, Large scale purification of enzymes by liquid extraction, in *Utilisation des enzymes en technologie alimentaire*, P. Dupfeux (ed.), Symp. Int'l, Versailles, France, May 5-7, 1982; M. Souders, G. J. Pierotti, and C. L. Dunn, Penicillin, *Chem. Eng. Prog. Symp. Ser.*, 66(100), 41 (1970); and P. A. Belter, personal files.

<sup>b</sup>Versus water except as indicated.

<sup>c</sup>Concentration in mol/liter in the light phase *L* divided by that in the heavy phase *H*.

varies with concentration  $x$  as shown. In principle, as the concentration  $x$  gets large,  $\mu(L)$  will approach  $\mu^0(L)$ ; in practice,  $\mu(L)$  may be limited by solubility, as suggested by the dashed line. As  $x$  goes to zero,  $\mu(L)$  goes to negative infinity. The intersection of  $\mu(L)$  and  $\mu(H)$  gives the concentration  $x$  present in *L*.

We can now see how changes in *L* will change  $x$ . If we change *L* so that its standard energy  $\mu^0(L)$  is raised, we lift the entire curve for  $\mu(L)$  and thus decrease the equilibrium value of  $x$ . If we reduce  $\mu^0(L)$ , we will increase the value of  $x$ .



**Figure 5.1-1.** Solute chemical potential versus concentration. The solute's chemical potential is the basis of extraction. Equilibrium occurs when the values in the feed ( $H$ ) and solvent ( $L$ ) phases are equal.

But how can we change  $\mu^0(L)$ ? We have two routes: changes in the solvent or changes in the solute. These are discussed next.

### Changes in Solvent

The most obvious way to change  $\mu^0(L)$  is to choose a different extraction solvent. There is no reliable thermodynamic theory which can quantitatively predict the best choice, but there are several which can serve as a qualitative guide. We discuss one of these, the concept of solubility parameter.

According to this theory, the partition coefficient  $K$  can be written as

$$\ln K = \frac{\mu^0(H) - \mu^0(L)}{RT}$$

$$= \frac{\bar{V}_H(\delta_A - \delta_H)^2 - \bar{V}_L(\delta_A - \delta_L)^2}{(RT \bar{V}_A)} \quad (5.1-5)$$

where the  $\bar{V}_i$  are the partial molar volumes of the heavy solvent  $H$ , the light solvent  $L$ , and the solute  $A$ , respectively, and the  $\delta_i$  are the corresponding solubility parameters. Some of these parameters, listed in the surprising

TABLE 5.1-2. Solubility Parameters for Some Common Solvents<sup>a</sup>

Solvent	$\delta$ (cal <sup>1/2</sup> cm <sup>-3/2</sup> )
Amyl acetate	8.0
Benzene	9.2
Butanol	13.6
Butyl acetate	8.5
Carbon disulfide	10.0
Carbon tetrachloride	8.6
Chloroform	9.2
Cyclohexane	8.2
Hexanol	10.7
Acetone	7.5
Pentane	7.1
Perfluorohexane	5.9
Toluene	8.9
Water	9.4

<sup>a</sup> Taken from J. H. Hildebrand, J. M. Prausnitz, and R. L. Scott, *Regular and Related Solutions*, Van Nostrand, New York, 1970; and from the *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 1986.

units of cal<sup>1/2</sup>/cm<sup>3/2</sup>, are given in Table 5.1-2. When no single solvent has the desired solubility parameter, mixed solvents can be effective.

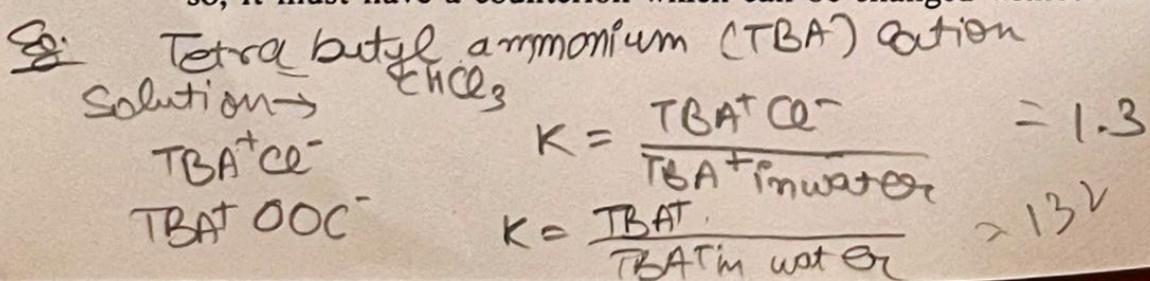
To use this theory, we just make an extraction with two solvents whose  $\delta_i$  are known. We use this experiment to find  $\delta_A$ , the solute's solubility parameter. We then can estimate the partition coefficients for new solvents from their values of  $\delta_i$ . Such estimates are not quantitatively reliable, but they can be a good guide for new experiments.

### Changes in Solute via Ion Pairs

In many cases, we will not be able to change the extraction solvent. Alternatives may be expensive, volatile, flammable, or biohazardous. In these cases, we may be able to improve the extraction by changing the solute.

Changes in the solute may initially seem impossible. Surely, the solute is what we must isolate, and changing it chemically may destroy it. After all, if we are trying to separate lincomycin, it will no longer be lincomycin if we change it.

Changes in the solute depend on the fact that the solute can be ionic. If so, it must have a counterion which can be changed without changing the



solute itself. Two changes in counterions are useful. First, the counterions can be replaced with those which are much more soluble in the extraction solvent. Second, and more commonly, the solute may be weakly acidic or basic, so that changes in pH will alter the degree of its ionization.

Changes in counterions rely on the fact that a solute which is ionic in water will form an ion pair of no net charge in an organic solvent. Such ion pair formation is exemplified by the extraction of the tetrabutylammonium cation. If the chloride of this cation is extracted with chloroform, we find

$$K = \frac{[\text{N}(\text{C}_4\text{H}_9)_4^+ \text{ in chloroform}]}{[\text{N}(\text{C}_4\text{H}_9)_4^+ \text{ in water}]} = 1.3 \quad (5.1-6)$$

If sodium acetate is added to this chloride solution and the extraction is repeated, we find

$$K = \frac{[\text{N}(\text{C}_4\text{H}_9)_4^+ \text{ in chloroform}]}{[\text{N}(\text{C}_4\text{H}_9)_4^+ \text{ in water}]} = 132 \quad (5.1-7)$$

In the first case, we extract a dilute solution of  $\text{N}(\text{C}_4\text{H}_9)_4^+\text{Cl}^-$  ion pairs; but in the second, we obtain a more concentrated solution of  $\text{CH}_3\text{COO}^-\text{N}(\text{C}_4\text{H}_9)_4^+$  ion pairs.

Such ion pair extractions require choosing organic soluble counterions. Possibilities, listed in Table 5.1-3, produce what some call "greasy salts." Such salts are an underused method of improving extractions.

*A Doubt*

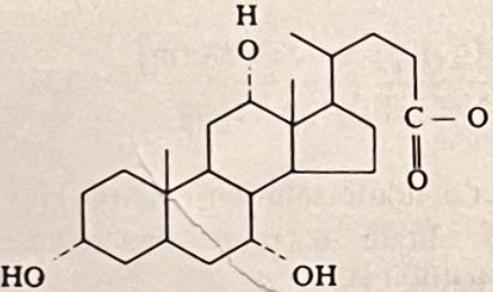
### Changes in Solute via pH

Many of the solutes which we want to isolate are weak acids or bases whose extraction can be dramatically altered by changes in pH. We will develop the analysis for weak acids; that for weak bases is analogous. A weak acid can partly ionize in water, but it will not ionize significantly in organic solvents. The apparent partition coefficient lumps both ionized and un-ionized forms, i.e.,

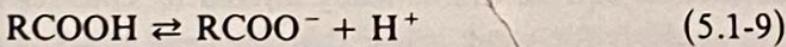
$$K = \frac{[\text{RCOOH}]_L}{[\text{RCOOH}]_H + [\text{RCOO}^-]_H} \quad (5.1-8)$$

where  $[\text{R}]_L$  and  $[\text{R}]_H$  are solute concentrations in the light organic and the heavy aqueous phases, respectively. The concentrations of un-ionized and

TABLE 5.1-3. Typical Counterions for Ion Paired Extractions

Ion	Chemical Structure	Rem
Acetate	$\text{CH}_3\text{COO}^-$	Simple; not soluble in organic solvent
Butyrate	$\text{CH}_3(\text{CH}_2)_2\text{COO}^-$	More soluble in organic solvent than previous ion
Tetrabutylammonium	$(\text{C}_4\text{H}_9)_4\text{N}^+$	A solid choice
Hexadecyltributylammonium	$\text{CH}_3(\text{CH}_2)_{15}(\text{C}_4\text{H}_9)_3\text{N}^+$	May form micell
Perfluorooctanoate	$\text{CF}_3(\text{CF}_2)_6\text{COO}^-$	May stay ionic in organic solvent
Dodecanoate	$\text{CH}_3(\text{CH}_2)_{10}\text{COO}^-$	May form micell
Linoleate	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=(\text{CH}_2)_7\text{COO}^-$	May form liquid crystals
Cholate		Based on bile acids
Tetraphenylboride	$\text{B}(\text{C}_6\text{H}_5)_4^-$	Can degrade in many solvents

ionized solutes in water are subject to the equilibrium



so that

$$K_a = \frac{[\text{RCOO}^-]_H [\text{H}^+]_H}{[\text{RCOOH}]_H} \quad (5.1-10)$$

where  $K_a$ , the association constant of the weak acid, is usually tabulated in units of moles per liter. In some idealized experiments,  $[\text{RCOO}^-]_H$  and  $[\text{H}^+]_H$  may be closely related, but in practice,  $[\text{H}^+]_H$  is treated as an independent variable. As such, Eqs. (5.1-8) and (5.1-10) can be combined to

give

$$K = \frac{K_i}{1 + K_a/[H^+]_H} \quad (5.1-11)$$

where the "intrinsic partition coefficient"  $K_i$  is given by

$$K_i = \frac{[RCOOH]_L}{[RCOOH]_H} \quad (5.1-12)$$

Combining Eqs. (5.1-11) and (5.1-12) leads to

$$\log_{10} \left[ \left( \frac{K_i}{K} \right) - 1 \right] = \text{pH} - \text{p}K_a \quad (5.1-13)$$

where the  $\text{p}K_a$  ( $= -\log_{10} K_a$ ) values are given in the literature. The corresponding result for weak bases is

$$\log_{10} \left[ \left( \frac{K_i}{K} \right) - 1 \right] = \text{p}K_b - \text{pH} \quad (5.1-14)$$

These results show how the partition coefficient of a weak acid or base can be manipulated by changing the concentration of  $[H^+]$  in the aqueous solution.

Values of  $K_a$  for a variety of solutes of biological interest are given in Table 5.1-4. The changes in partition coefficients implied by this table can be used to purify as well as isolate a solute of interest. For example, for solutes A and B, the selectivity of the separation is given by

$$\beta = \left( \frac{K_i(A)}{K_i(B)} \right) \left( \frac{1 + K_a(B)/[H^+]_H}{1 + K_a(A)/[H^+]_H} \right) \quad (5.1-15)$$

In some cases, the selectivity at moderate pH is much better than that at extreme pH. This and other characteristics of the partition coefficient are illustrated in the following examples.

**Example 5.1-1. The Partition Coefficient of Weak Acid.** A partially hydrolyzed sugar believed to be monoacidic is extracted from water into 1-hexanol. It has  $K$  values of 6.4 and 0.22 mol/liter at pH 4.0 and 5.8, respectively. What is the value of  $K$  at pH 7.0?

*Solution.* From the values given, we can use Eq. (5.1-13) to find

$$K_i = 11.7$$

$$K_a = 8.3 \times 10^{-5} \text{ mol/liter}$$

From these same equations, at pH 7.0,

$$K = \frac{11.7}{[1 + 8.3 \times 10^{-5}/10^{-7}]} = 0.014$$

This assumes that the compound does not ionize further.

**Example 5.1-2. Separation of Penicillins.** For the system water–amyl acetate, penicillin *K* and penicillin *F* have values of  $K_i$  of 215 and 131, respectively. They have  $pK_a$ 's of 2.77 and 3.51. If penicillin *F* is the desired product, will an extraction at pH 3.0 give a purer product than one at pH 4.0?

*Solution.* From the values given, we can use Eq. (5.1-11) or (5.1-13) to find the following values for each of the components:

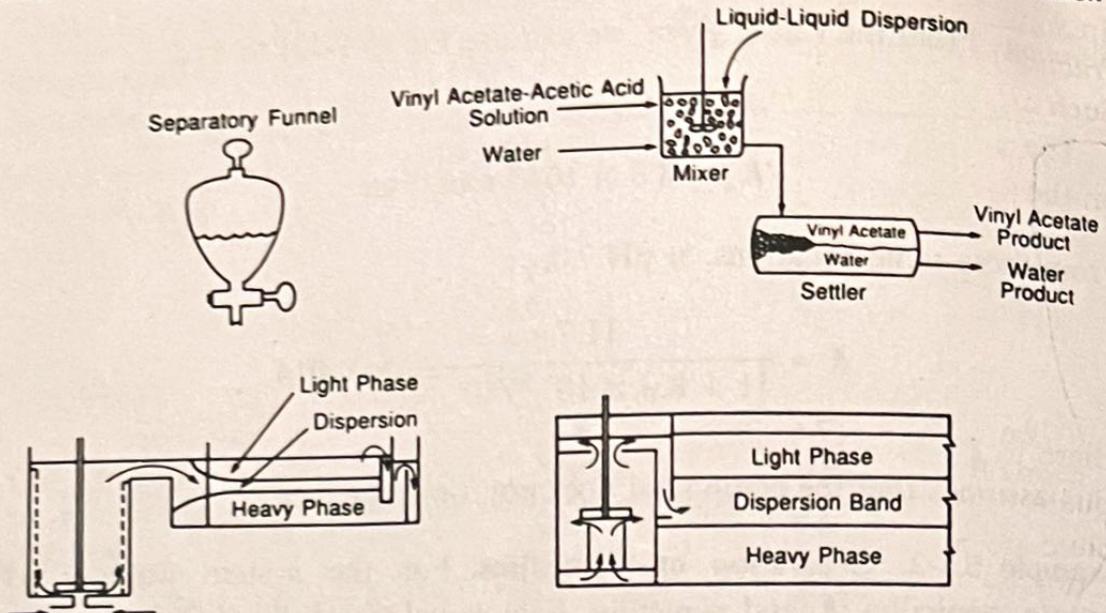
	$K_K$	$K_F$
pH 3	80	100
pH 4	12	32

Thus  $\beta = 1.3$  at pH 3, but  $\beta = 2.7$  at pH 4. The extraction at pH 4 may require more stages to isolate penicillin *F*, but the isolated solute will be purer.

## 5.2. BATCH EXTRACTIONS

In a batch extraction, a solution containing the desired solute is mixed with a solvent immiscible with the solution. In the common case, the initial solution is dilute and aqueous, and the immiscible solvent is organic. The solute dissolves in the new organic solvent until its concentrations in water and organic are in equilibrium.

The equipment in which the extraction occurs reflects the scale of the operation. For small scale extractions, the classical choice is a separatory



**Figure 5.2-1.** Extractors. Separatory funnels are the simplest laboratory device; mixer-settlers of various designs are often used for large scales.

funnel like that shown in Figure 5.2-1. For larger scale extractions, a variety of devices are used, as suggested by the figure. All allow the two fluids to be mixed together and then separated.

## Analytical Methods

We want to calculate the concentrations at equilibrium from the concentrations in the initial feed. To do so, we need two relations: an equilibrium constraint and a mass balance on the solute. The equilibrium constraint for dilute solution will often be

$$\left( \begin{array}{l} \text{solute concentration} \\ \text{in light phase } L \end{array} \right) \propto \left( \begin{array}{l} \text{solute concentration} \\ \text{in heavy phase } H \end{array} \right)$$

$$\underline{x = Ky} \quad (5.2-1)$$

where  $x$  is the product concentration in the extraction solvent  $L$ ,  $y$  is the product concentration in the feed solvent  $H$ , and  $K$  is an equilibrium constant. The extraction solvent is called  $L$  because as an organic material it is usually the lighter of the two. The feed solvent is called  $H$  because it is commonly water, and hence the heavier.

The units of the concentrations  $x$  and  $y$  can vary, which will change the values of  $K$ . For example,  $x$  and  $y$  can be in moles/liter or mole fractions.

In some cases, one concentration will be in one kind of unit (e.g., a mole fraction) and the other will be in some other unit (e.g., a partial pressure). Such an annoying variety of units is not difficult, but requires care.

The second of the two relations required for extraction is a mass balance on the solute

$$\begin{aligned} \text{solute into} \\ \text{the extraction} \end{aligned} = \begin{aligned} \text{solute out of} \\ \text{the extraction} \end{aligned} \\ Hy_F + Lx (= 0) = Hy + Lx \quad (5.2-2)$$

where  $y_F$  is the solute concentration in the heavy feed. Note that this equation tactfully assumes that the extraction solvent initially contains no solute and that both  $L$  and  $H$  are constant.

We can combine Eqs. (5.2-1) and (5.2-2) to find the concentrations after the extraction:

$$x = \frac{Ky_F}{1 + E} \quad (5.2-3)$$

$$y = \frac{y_F}{1 + E} \quad (5.2-4)$$

where the quantity  $E$ , called the extraction factor, is given by

$$E = \frac{KL}{H} \quad (5.2-5)$$

Obviously, if  $K$  is large, most of the solute winds up in the extraction solvent. Other useful quantities are also easily found. For example, the fraction extracted  $p$  is

$$p = \frac{Lx}{Hy_F} = \frac{E}{1 + E} \quad (5.2-6)$$

The quantity  $p$  will be useful in the examples that follow.

### ~~Graphical Methods~~

Batch extraction problems can also be solved by graphical methods. To be sure, such a solution for the preceding specific case is trivial because the analytic method is so straightforward. However, such graphical methods are

## 5.2 Batch Extractions

the "equilibrium line." The mass balance is referred to as the "operating line." You should remember that they are nothing more than energy and mass balances, respectively.

**Example 5.2-1. A Batch Steroid Extraction.** Water containing 6.8 mg/liter of a steroid is extracted with initially pure methylene dichloride. The equilibrium constant for the steroid is 170 and the ratio of water to solvent is 82. What is the concentration in the organic after the extraction? What fraction of the steroid has been removed?

*Solution.* From the values given, the extraction factor  $E$  is

$$E = \frac{KL}{H} = \frac{170}{82} = 2.07$$

From Eq. (5.1-3), we find

$$\begin{aligned}x &= \frac{Ky_F}{1 + E} = \frac{170(6.8)}{1 + 2.07} \\&= 377 \text{ mg/liter}\end{aligned}$$

From Eq. (5.2-6), the fraction extracted  $p$  is

$$\begin{aligned}p &= \frac{E}{1 + E} = \frac{2.07}{1 + 2.07} \\&= 0.67\end{aligned}$$

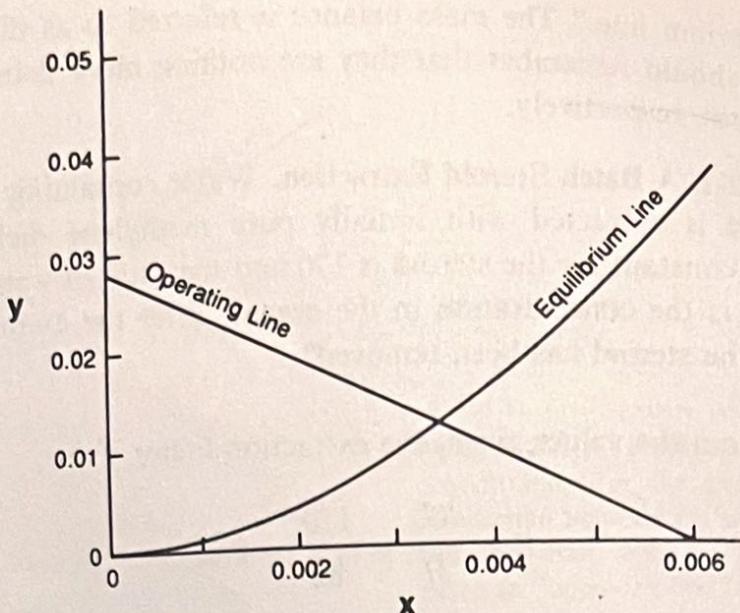
Even though the equilibrium constant is large, the fraction extracted is modest because there is so much more water than organic.

**Example 5.2-2. Amino Acid Stripping.** For a nonessential amino acid, the equilibrium relation between toluene and pure water is

$$x^2 = (0.001 \text{ mol/liter})y$$

We plan to contact 4.7 liters of toluene containing 0.006 M amino acid with 1 liter of water. What fraction of the amino acid can we extract?

*Solution.* Because the equilibrium is not in the form of Eq. (5.2-1), we cannot use the usual analysis in Eq. (5.2-6). Instead, we plot the equilibrium relation, as shown by Figure 5.2-3. We also plot the mass balance or



**Figure 5.2-3.** Amino acid stripping. This graph, used in solving Example 5.2-2, illustrates the simple ideas for batch extraction.

operating line, which is

$$y = 4.7(0.006 - x)$$

The intersection of these lines gives the desired concentration

$$y = 0.012 \text{ mol/liter}$$

The fraction extracted  $p$  is then

$$\begin{aligned} p &= \frac{Hy}{Lx_F} = \frac{1.0(0.012)}{4.7(0.006)} \\ &= 0.43 \end{aligned}$$

A good exercise is to calculate the additional amount removed in a second extraction with 1 liter of water. Such repeated extractions are the subject of the next section.

### 5.3. STAGED EXTRACTIONS

This section is concerned with extractions which are repeated again and again to isolate a desired product. The repeated extractions take place in a chain or cascade of separation equipment; the elements of this cascade are