SEPARATION BY SORPTION

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Many processes for manufacturing biochemicals have costs that are dominated by the expense of purification. Fermentation products are diluted by water and contaminated by debris, salts, proteins, and a variety of compounds that may have properties quite similar to those of the desired material. Purification usually starts with some way to increase the concentration of the product so that large volumes of water need not be handled during the more selective steps. Processes such as solvent extraction and ion exchange can accomplish severalfold concentration and considerable purification. Separation of the product from molecules with similar properties can be very difficult. This chapter will cover two methods that are in large-scale use: column procedures for vapor-phase adsorption of water and liquid chromatography.

Liquid chromatography (LC) has gained a prominent position in separations of biological molecules during the last 20 years. This is particularly true for analytical-scale separations, where availability of the necessary instrumentation and chromatography supports has made this a widely used method (Regnier, 1983). Development of high-performance, preparative-
scale LC separations is also proceeding. Systems capable of separating up to 1 kg/hr or more of product are available from several manufacturers.

The production of high fructose corn syrup can involve large-scale LC to upgrade fructose content from that attainable by enzyme conversion (typically 42–45%) by a partial separation of the glucose from the fructose (Antrim et al., 1979). A commercial system with countercurrent extraction scheme is the Universal Oil Products (UOP) Sarex Process (Wankat, 1982). The fructose is blended with high fructose corn syrup to obtain a 55% fructose product. This product is similar to cane sugar in sweetness and is used in many soft drinks.

Purification of antibiotics, amino acids, and other high-value products is amenable to LC separations. Engineering is needed to overcome the decreases in resolution, product concentration, and separation rate normally encountered in scaling up an LC separation. The factors that must be considered include the type of absorbent or support, its packing characteristics, particle size, column length and diameter, and methods for ensuring uniform sample introduction and collection.

Another recent development is an adsorption process using corn to remove water from 190 proof ethanol vapors to obtain a substantially water-free product used as a gasoline octane booster. Distillation of ethanol from water still requires 15,000–30,000 Btu/gal. New drying technology to decrease this input is reducing ethanol cost.

The impact of energy cost for separating water from 2,3-butanediol is even greater. In this case, the discovery and development of an energy efficient separation technique for water from butanediol would greatly enhance the economic viability of obtaining this diol from renewable resources.

1. ADSORPTION USING POLYSACCHARIDES

The distillation of ethanol fermentation broth to 190 proof alcohol (92.4% by weight ethanol) is readily achieved (Katzen et al., 1980). Distillation above this concentration becomes more difficult and disproportionately more energy intensive because of the characteristics of the ethanol–water equilibrium curve and the existence of an azeotrope (95.6% by weight ethanol) at atmospheric pressure (Ladisch and Dyck, 1979). One approach to obtaining a substantially water-free product is to pass 190 proof vapor over an appropriate adsorbent (Garg and Auskaiatus, 1983; Ladisch et al., 1984). A system using corn grits has recently been developed (Voloch et al., 1980; Hong et al., 1982; Ladisch et al., 1984).

The process consists of first drying corn grits packed in a stationary bed. Air, CO₂, or N₂ containing less than 0.015 mol fraction water is used at 80–100°C to dry the grits to a moisture of 2% or lower. Once the grits are dried and the adsorption bed is heated to 80°C or higher (i.e., above the dew point
of 190 proof alcohol), adsorption is initiated. The 190 proof ethanol vapors are passed upflow through the column at a superficial velocity of 0.5–1 cm/sec. The water adsorbs on the corn grits, and the water-free ethanol vapors pass through the column. The heat of adsorption at these conditions is about 1200 Btu/lb water adsorbed (Rebar et al., 1984), and hence, a significant temperature rise occurs in the bed. At conditions of practical interest, the adsorption is characterized by a combined wave front where the temperature and concentration waves elute together (Ladisch et al., 1984a). Once breakthrough of the concentration (and temperature) waves begin to occur, the flow of vapor to the column is stopped.

Desorption is carried out by passing air, CO₂, or N₂ in a direction countercurrent to that used for adsorption. The moisture content of the regenerating gas must be less than 0.015 mol fraction water if satisfactory results are to be obtained. The gas is preheated to above 80°C before being passed through the column. Once the grits return to their initial moisture and temperature, the regeneration gas is shut off. The column is then ready for the next adsorption cycle.

Of particular interest is the selectivity of corn (separation factor of α = 1750) (Rebar et al., 1984), since only a negligible amount of ethanol is adsorbed (Hong et al., 1982). The polysaccharides in corn grits responsible for the adsorptive characteristics of the grits are starch, cellulose, and hemicellulose (Hong et al., 1982). Pure cellulose has been shown to have water sorption characteristics similar to starch. Dry biomass materials including agricultural residues and wood chips are also suitable adsorbents. These characteristics, together with the ability of corn to dehydrate other alcohols (Ladisch et al., 1984a; Ladisch and Tsao, 1982), suggest that polysaccharides may find unique applications in the separation of water from volatile fermentation products.

2. COLUMN DESIGN FACTORS IN LIQUID CHROMATOGRAPHY

The translation of a bench scale separation to a production scale must systematically consider column packing, sample introduction, dispersion phenomena, column capacity, particle size and velocity effects, and first estimation of separation costs. The discussion below of these key factors is based on experience with LC over spherical supports using water as the eluent. Parts are taken from a recently published review (Ladisch et al., 1984b).

2.1. Column Packing

Column packing materials and supports in large-scale use will typically be larger (40–300 μm diameter) than analytical scale supports (5–30 μm diameter). The size distribution of a commercial scale support (such as an ion
exchange resin) is often broader than an analytical grade support. If the support has a density that is greater than that of the eluent (water), significant fractionation of the support can occur during the packing of a large column (e.g., 2 ft in diameter and 10 ft long). This situation (illustrated in an exaggerated manner in Fig. 9.1) is undesirable since it can cause sections of different void fractions to be formed. As a consequence, dispersion of a solute band moving down the column will be enhanced by the mixing caused by areas of different porosity in the bed.

Several measures can be taken to minimize fractionation of the packing material. The packing material can have a narrow size distribution. Attempts could also be made during support manufacture to control the density of the support to be close to that of the liquid used during the packing procedure. Minimizing differences in the rates of settling of different size particles will minimize fractionation. The effect of particle size on the terminal velocity, \( \nu_t \), of a particle in a viscous fluid is illustrated by (Bird et al. 1960):

\[
\nu_t = \frac{2R^2(\rho_r - \rho)g}{9\mu} \quad \text{for} \quad \frac{D_r \rho}{\mu} < 0.1
\]  

(9.1)

where \( R \) is particle radius; \( \rho_r \) and \( \rho \) are the densities of the particle and fluid, respectively, and \( \mu \) is the viscosity. The settling velocity is proportional to the square of the radius. Hence, if the difference, \( \rho_r - \rho \), is significant, significant differences in \( \nu_t \) will occur for particles having different values of \( R \).

The density of the liquid could be adjusted (by temperature changes and dissolution of solutes) to be close to the density of the particles. Although these approaches may be feasible for aqueous systems where ion exchange resins (\( \rho \approx 1 \)) are used, inorganic-based supports with a relatively high
density require very rapid pumping of support slurries into the column to minimize fractionation due to settling effects. Although this procedure is workable for analytical or preparative scale columns, it is more difficult to do on a commercial scale given the large volumes required.

2.2. Column: Particle Diameter Ratio

The average, particle diameter for supports used on an analytical scale typically range from 5 to 30 μm. The column diameter to particle diameter ratios for analytical columns inside diameter (i.d.) of 2–8 mm are on the order of 100–300. As the separation is scaled-up (in the case of ion exchange type supports), the average particle size also tends to be larger (40–300 μm) due to operating limits on pressure drop as well as support cost.

The cost of a commercial chromatographic grade resin is about $5–50/kg. In comparison, an analytical type resin costs about $100–400/kg (Anonymous, 1984). The analytical resin costs reflect the much smaller particle size (less than 20 μm) and narrower particle size distributions for these resins as compared to a commercial grade resin.

The choice of column diameter is important in view of known differences in void fraction in the packing of a spherical support material as a function of radial distance from the column wall (Cohen and Metzner, 1981). The void fraction at the wall is 1, and then decreases to the average value of the bed (typically in the range of 0.3–0.5) as the center of the column is approached. At least 40 particle diameters (away from the wall) are required for an average value to be attained. The higher void fraction near the wall allows the possibility of sample "fingering," with sample movement in the axial direction being more likely to occur along the walls of the column than in the bulk of the column packing. This, in effect, is another form of dispersion that decreases column efficiency since the sample no longer resembles a plug.

2.3. Sample Loading

After the column has been packed and equilibrated with the eluent, the sample is loaded. The loading of the sample must be carried out in a manner that allows the sample volume to be introduced as a plug with as little mixing with the eluent as possible. This is facilitated by a properly designed distributor consisting of a porous plate that has a sufficient pressure drop to distribute an incoming liquid feed evenly (see Fig. 9.2). The distributor plate is situated directly upon the column packing material to minimize mixing of the sample with the eluent, which would otherwise occur if a dead volume were present. Again, at the outlet the objective is to minimize mixing due to extra column effects. Extra column effects for analytical systems are discussed elsewhere (DiCesare et al., 1981). These effects also impact the efficiency of preparative and commercial scale separation systems.
2.4. Dispersion: The Concept of Plate Height

Once the sample is introduced into the column (preferably as a "plug"), the tailing edge is washed through the distributor and onto the column by the eluant. A sample having a volume of 0.01-0.5% of the column void volume is typical of analytical scale chromatography for which the following analysis is applicable.

A sample plug pushed through the column by the eluant will have a tendency to disperse. As it elutes, it may give a gaussian-shaped peak as illustrated in Figure 9.3. The average solute concentration (i.e., total solute-eluant volume corresponding to the peak width) is typically reduced by a factor of 3-20-fold relative to the inlet sample concentrations. The width of this peak at the base is $4\sigma$, and at half-height is $2.35\sigma$. The number of plates, defined in terms of $\sigma$ is

$$N = \frac{L}{H} = \frac{L^2}{\sigma^2} \quad (9.2)$$

where $\sigma = \sqrt{\frac{HL}{W}}$, $N$ is the plate count, and $H$ is the plate height. $L$ is usually defined as the column length, although this is not strictly correct.

![Figure 9.3. Gaussian-shaped peak as applied to chromatography.](image-url)
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(Martin and Syng, 1941; Knox, 1973; Jacobson, 1982). Rearrangement and expression in terms of \( t_w \), the time required for the peak to elute after the sample is injected, and \( t_{0.5} \), the peak width in units of time, gives

\[
N = \frac{L^2}{(W/4)^2} = 16 \left( \frac{t_w}{t_{0.5}} \right)^2 \tag{9.3}
\]

Since it is sometimes difficult to measure the peak width at the base, the width at half-height \( (t_{0.5}) \) is used instead (at half-height, \( W = 2.35\sigma \)). The plate height, \( H \), is obtained from 9.4 by dividing the length of the column:

\[
H = \frac{L}{N} = \frac{L t_{0.5}^{1/2}}{5.54 t_w^{1/2}} \tag{9.4}
\]

A high plate count (or small plate height) is not necessarily synonymous with good resolution. Indeed, commercial scale columns with observed plate counts of 30–100 can give close to baseline separation of two- and three-component mixtures. A good separation depends on the combined factors of (1) plate height (a measure of sample dispersion inside the column) and (2) differences in the capacity of the packing to dynamically absorb or retain (and then desorb) two or more components as they pass through the column (i.e., a difference in capacity factors).

2.5. Capacity Factor (\( k' \))

The capacity factor, \( k' \), for a single component (i.e., solute 1) is defined as

\[
k' = \frac{\text{Moles of solute 1 in stationary phase}}{\text{Moles of solute 1 in mobile phase}} = K \frac{V_s}{V_m} = \frac{X_s}{X_m} \frac{V_s}{V_m} \tag{9.5}
\]

where \( V_s \) and \( V_m \) are the volumes of the sample in the stationary and mobile phases, respectively, \( X_s \) and \( X_m \) are the concentrations of the solute in the stationary and mobile phases, respectively, and \( K \) is the distribution coefficient between the two phases.

The elution of a solute is characterized by the retention volume, \( V_r \):

\[
V_r = V_m + KV_s \tag{9.6}
\]

When Eq. (9.6) is combined with Eq. (9.5), it gives

\[
k' = \frac{V_r}{V_m} - 1 \tag{9.7}
\]
where $V_s$ is the retention volume of the solute peak (corresponds to retention time, $t_s$) and $V_0$ is equivalent to the column void volume (measured by retention time, $t_0$, of an excluded component). Consequently, the relation for the capacity factor becomes

$$k' = \frac{t_s - t_0}{t_0}$$

(9.8)

The void volume, $V_0$, can be determined by injecting blue dextran (M.W. = 2,000,000), which is excluded from penetrating most ion exchange supports because of its size.

The capacity factor, $k'$, is often taken as an intrinsic property of the support. In fact, it can be shown that $k'$ is a function of $\epsilon$, the column void fraction, as well as $K$, the distribution coefficient (which is a property of the support) (Jacobson, 1982; Ghin and Chang, 1982). It can also be shown that $k'$ will increase with decreasing void fractions. This provides some rationale for the increase in resolution obtained in systems that mechanically compact the support after packing. An example is the Elf Aquitaine Series 300 LC (anonymous, 1983) in which a piston is used to compress the packing material in the axial direction. Good resolution of 0.80 kg of androstenedione from 0.20 kg of androstadienedione is reported over a column containing 40 kg of Merck silica H 60 at a linear eluent velocity of about 0.70 cm/min. The separation requires 6–7 hr.

2.6. Resolution

The resolution factor, $R_s$, is a measure of how well two bands are resolved. It is defined and calculated from the equation

$$R_s = \frac{(t_{s2} - t_{s1})}{\frac{1}{2}(t_{s1} + t_{s2})}$$

(9.9)

where $t_{s2}$ and $t_{s1}$ are the retention times of the bands (solute in units of time, and $t_{s1}$ and $t_{s2}$ are the peak widths at baseline of these bands in units of time.

According to Snyder and Kirkland (1979), the capacity factor, the number of theoretical plates, and the resolution factor are related by

$$R_s = \frac{1}{4} (a - 1) \sqrt{N} \left( \frac{k'}{1 + k'} \right)$$

(9.10)

This relationship is derived as follows (for bands 1 and 2) from Eq. (9.8):

$$t_{s1} = t_0 (1 + \epsilon)$$

(9.11)
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\[ t_{e2} = t_0 (1 + k_j) \]  
(9.12)

Assuming that \( t_{e1} = t_{e2} \) and substituting these relationships into Eq. (9.9) gives

\[ R_s = \frac{t_0 (k_j - k'_j)}{t_{e1}} \]  
(9.13)

Since

\[ N = 16 \left( \frac{L_s}{L_c} \right)^2 \]  
(9.14)

for band 1,

\[ t_{e1} = \frac{4t_0}{\sqrt{N}} = \frac{4t_0 (1 + k'_1)}{\sqrt{N}} \]  
(9.15)

Substituting this result into Eq. (9.13) gives

\[ R_s = \frac{(k_j - k'_j) \sqrt{N}}{4(1 + k'_1)} = \frac{1}{4} \left( \frac{k_j}{k'_1} - 1 \right) \sqrt{N} \left( \frac{k'_1}{1 + k'_1} \right) \]  
(9.16)

The definition of the separation factor \( \alpha = k'_1/k'_j \) thus results in Eq. (9.10), where \( k' \) represents the average values of the capacity factors of the two bands and where \( N \) in Eq. (9.16) represents the simple average of the plate counts for components 1 and 2. A resolution factor of 0.9 or higher gives a first indication of a suitable separation.

An example of a modest plate count, combined with a support having the appropriate capacity factors for two components, is given below. Let \( N = 80 \) and \( k'_j = 1.25 \) and \( k'_1 = 2.1 \) (which gives \( k' = 1.675 \)). In this case, a first approximation of resolution can be obtained from Eq. (9.10). Substituting in the values gives

\[ R_s = \frac{[2.1/1.25] - 1}{4} \sqrt{80} \left( \frac{1.675}{1 + 1.675} \right) = 0.952 \]

A resolution of 0.9 or higher typically indicates acceptable performance on a large scale. This numerical example illustrates that the absence of a high plate count (1000-50,000/m) normally associated with analytical columns does not necessarily indicate a poor resolution of the two components.
2.7. Scale-Up: Particle Size, Eluent Rate, and Sample Size

The diffusion coefficient, $D$, affects separation efficiency if this parameter is significantly different for the solutes being separated. Pieri et al. suggest use of the Wilke-Chang equation to estimate $D$:

$$
D = \frac{7.4 \times 10^{-12} T \sqrt{\phi} M_{\text{eluent}}}{\eta V_{\text{solute}}} \quad (9.17)
$$

where $T$ is temperature (°K), $\phi$ is the eluent association factor; $M_{\text{eluent}}$ is the eluent molecular weight in grams, $\eta$ is the eluent viscosity in MPa sec, and $V_{\text{solute}}$ is the solute molar volume. In many LC separations the solutes being fractionated are similar. For example, in a pheromone separation, two of the components are diastereomers, and the other solutes have similar values of $D$ (Pieri et al., 1983). Based on their experience with the reverse phase separation of pheromones, Pieri et al. (1983) report that the key scale-up parameters are, in fact, the operational variables of particle size, eluent rate, and sample volume.

For linear chromatography (i.e., sample concentration is in a range that corresponds to a linear isotherm), the relationship between the maximum sample volume that can be injected, $V_{s,i}$, and the volume of the mobile phase inside the column, $V_m$ (i.e., void volume) for $R_s > 1.3$ is (Gareil et al., 1983) as follows:

$$
V_{s,i} = V_m \left( k_i (\alpha - 1) - \frac{1}{\sqrt{N}} \left( 2 + k_i + \alpha k_i \right) \right) \quad (9.18)
$$

where $\alpha = k_1/k_1'$ and the other parameters are as defined previously for an analytical system. Thus, determination of $k_1'$, $k_2$, $N$, and $V_m$ from an analytical injection allows estimation of $V_{s,i}$. For $R_s = 1$, Pieri et al. (1983) present the expression

$$
V_{s,i} = V_m \left( (\alpha - 1)k_1' - \frac{1.25}{\sqrt{N}} \left( 2 + k_1' + \alpha k_1' \right) \right) \quad (9.19)
$$

Thus, for example, if $\alpha = 1.675$ for $k_1' = 1.25$, $k_2 = 2.1$, and $N = 80$, the maximum allowable sample volume, $V_{s,i}$, for $R_s = 1$ would be estimated at

$$
V_{s,i} = V_m \left( (1.675 - 1)(1.25) - \frac{1.25}{\sqrt{80}} (2 + 1.25 + 2.1) \right) = 0.0961 V_m
$$

Equations (9.18) or (9.19) will predict that $V_{s,i} > V_m$ if $k_1$, $k_2$, and $\alpha$ are large enough. The maximum sample injection volume can be larger than the void volume if the adsorption of one component is so strong as to be almost
irreversible. Elution of the adsorbed component may then require a different eluant or different operating conditions, which will cause the component to desorb. In the limit, the separation can then be characterized as an adsorption process with loading and regeneration cycles rather than as liquid chromatography.

If the second term on the right-hand side of Eqs. (9.18) and (9.19) is large, these equations reduce to

$$V_{i,i} = V_n k';(a - 1)$$  \hspace{2cm} (9.20)

This will typically occur if the $N > 1000$.

A material balance gives the amount of product $Q_{i,n}$ recovered. By definition, Eqs. (9.18)–(9.20) are developed for $R_e \geq 1$. Hence, all of the solute (for component $n$ given by $Q_{i,n}$) originally injected is theoretically recovered, and $Q_{i,n}$ is simply

$$Q_{i,n} = C_{i,n} V_{i,i}$$  \hspace{2cm} (9.21)

where $C_{i,n}$ is the solute concentration of component $n$ in the sample injected. This result is of limited use if full recovery of a solute is not achieved. In practice, recovery and purity will be determined experimentally, with relationships of the type given in Eqs. (9.19) and (9.20) giving only a first indication of the sample loading that might be possible. The treatment of deviations from ideal conditions, often encountered in production scale LC, is discussed later in this chapter.

The development of a process-scale LC separation often results from analytical scale data obtained under analytical scale conditions. Hence, the average particle diameter may be quite small (less than 20 μm) relative to those practical on a larger scale (> 50 μm). Factors that currently limit use of smaller particle sizes on a large scale include pressure drop limitations and untested operational stability under industrial conditions. However, these limitations are minor relative to the current lack of availability of commercial quantities of appropriately sized supports at a reasonable cost, a situation that is expected to change in the near future.

Current practice is to increase particle size as the scale of separation increases. This must be considered in estimating column productivity, since the plate count is a function of both particle size and the linear velocity of the eluant (Pieri et al., 1983). The relationship is given by

$$N = \frac{D^n}{m} \cdot \frac{L}{u d_p^{1+n}}$$  \hspace{2cm} (9.22)

where $n, m$ are empirical constants, $D$ the diffusion coefficient, $L$ is the column length, $u$ is the eluant linear velocity, and $d_p$ is the particle diameter.
This result is obtained from the Snyder equation

\[ h = \frac{L}{N \sigma_p} \quad \text{(9.23)} \]

where

\[ h = \frac{L}{N \sigma_p} \quad \text{(9.24)} \]

and

\[ \phi^\ast = \left( \frac{u_d \sigma_r}{D} \right)^{n} \quad \text{(9.25)} \]

Combining Eqs. (9.23)–(9.25) and rearranging gives Eq. (9.22). Values of \( n \) in Eq. (9.23) range from 0.4 to 0.6, with 0.5 assumed by Pieri et al. (1983). If the plate height is specified to be constant and the particle size increases upon scale-up in a specified manner, either column length and/or eluant velocity must be adjusted. For example, let \( N = 80 \) with suitable analytical scale results being obtained for \( d_{p,A} = 50 \, \mu m \) at \( u = 2 \, \text{cm/min} \) and \( L = 60 \, \text{cm} \). A large-scale column is to be packed with the same type of support, except that \( d_{p} = 200 \, \mu m \) \((= 0.02 \, \text{cm})\). Assuming \( n = 0.3 \), where \( N \) for the analytical and large-scale columns are equal, Eq. (9.22) gives

\[ \frac{D_5^{0.5}}{m} \frac{L_A}{u_A^{0.5} d_{p,A}^{1.5}} = \frac{D_x^{0.5}}{m} \frac{L_x}{u_x^{0.5} d_{p,x}^{1.5}} \quad \text{(9.26)} \]

where subscripts \( A \) and \( x \) denote analytical and process scales, respectively. At the same temperature for both scales, Eq. (9.26) reduces to

\[ \frac{L_x}{u_x^{0.5}} = \left( \frac{L_A}{u_A^{0.5}} \right) \left( \frac{d_{p,x}}{d_{p,A}} \right)^{1.5} \quad \text{(9.27)} \]

For this particular example, \( L_x/u_x^{0.5} = (42.4)(8) = 339.2 \). Hence, at the same linear velocity as used on the analytical scale \((i.e., u_x = u_A = 2 \, \text{cm/min})\), \( L_x = 480 \, \text{cm} \). The plate height \( [H = L/N] \), see Eq. (9.4) is thus estimated to be increased by a factor of 8 when the particle size is increased by a factor of 4. If a 300-\( \mu m \) particle (instead of 200 \( \mu m \)) were to be used on a large scale, the plate height would be increased by a factor of 14.7 over the analytical scale case.

Other variations can be (estimated) from Eq. (9.27), including the effect of reducing eluant linear velocity. Operating temperature may also become a factor, since raising the temperature would decrease \( L_x \) by increasing \( D \) [see Eq. (9.17)] due to viscosity as well as direct temperature effects on
the diffusion coefficient. Calculation of under nonideal conditions (i.e., skewed peaks) is more difficult and is addressed later in this chapter.

The guidelines summarized in this section should be used with caution given constraining conditions inherent in the semiempirical equations presented. Nevertheless, experience shows these relationships to be helpful in obtaining a first estimate of column size and throughput upon scale-up.

2.8. Productivity

The productivity, $P$, of a column for each cycle depends on the acceptable product purity. As an example, let us consider separation of a two-component mixture containing equal parts of component 1 and 2 at a concentration of $X_1$ (weight fraction) of each in a total sample volume of $V_t$. A product of specified purity of component 2 is desired. In this case, the volume of product, $V_{p,2}$, obtained having the desired purity has an average concentration of $X_{p,2}$ (note, typically $X_{p,2} < X_{1,2}$). The yield, $Y$, is then

$$Y = \frac{X_{p,2}V_{p,2}}{X_{1,2}V_t} \quad (9.28)$$

The weight of support, $W_s$ (actual wet weight), is known for this column. The productivity, $P$, for this cycle is

$$P = \frac{X_{p,2}V_{p,2}}{W_s} \quad (9.29)$$

If both components constitute a desirable product, the productivity would, of course, be higher:

$$P = \frac{X_{p,2}V_{p,2} + X_{p,1}V_{p,1}}{W_s} \quad (9.30)$$

The product fractions that are not of the desired purity must be reprocessed if they are to be recovered at the desired purity. The amount of product recovered for each cycle is determined by experiment.

If the productivity does not change during the operational life of the support, the average productivity $\bar{P}$, is the same as $P$. If productivity decreases as the support ages, the average productivity, $P$, is $P = \frac{P_{in}}{L}$, where $P_{in}$ is the total weight of product obtained over $L$ number of cycles. The relationship of productivity to resin cost is described later in this chapter.

2.9. Deviations from Ideal Conditions

The preceding description is based on a system in which the components elute in the form of gaussian peaks and in which the sample volume is a small
fraction of the overall total column volume. In cases of practical interest, the components being separated may elute as skewed (non-Gaussian) peaks and the sample volume may, in fact, occupy 10–40% of the column void fraction. The engineering fundamentals for such cases are not as well developed as for the ideal case and would seem to deserve further attention. An empirical approach, however, can still be used to carry out a preliminary analysis of column performance. The second central moment of a chromatographic peak, \( \mu_2 \), is the variance, \( \sigma^2 \) (Kucera, 1965). Let \( \mu_k \) be the \( k \)th central moment of a function \( c(t) \) defined by (Kucera, 1965):

\[
\mu_k = \frac{1}{m_0} \int_{m_{\text{io}}}^{m} (t - \mu'_1)^k \, c(t) \, dt \tag{9.31}
\]

where the \( k \)th moment of function \( C(t) \) is given by

\[
\mu'_k = \frac{m_k}{m_0} \tag{9.32}
\]

and

\[
m_k = \int_{m_{\text{io}}}^{m} t^k \, C(t) \, dt \tag{9.33}
\]

The parameters for time, \( t \), and the concentration, \( C(t) \), represent the ordinate and abscissa, respectively, of a chromatographic peak for a single component under isocratic (constant flow) conditions. Integration of the area under the chromatographic peak gives

\[
m_0 = \int_{m_{\text{io}}}^{m} C(t) \, dt \tag{9.34}
\]

The first moment, \( \mu'_1 \), of the curve is

\[
\mu'_1 = \frac{\int_{m_{\text{io}}}^{m} t \, C(t) \, dt}{m_0} \tag{9.35}
\]

Numerical values of \( t \) and \( C(t) \) are determined experimentally. Numerical integration using Eqs. (9.31), (9.34), and (9.35) [with \( k = 2 \) in Eq. (9.31)] gives the value of \( \sigma^2 \). The plate count can then be estimated using Eq. (9.2).

The effect of large sample volumes on the shape of the peak has been presented by Barford et al. (1978). They demonstrated that the observed resolution, \( R'_s \), is a function of sample volume as given by

\[
\frac{1}{R'_s} = \frac{1}{R_s} + \left( \frac{V}{\Delta V_R} \right) \tag{9.36}
\]
where \( R_s \) is the resolution obtained for a small sample volume (i.e., \( V \to 0 \)), \( V \) is the sample volume, and

\[
\Delta V_R = V_{R,2} - V_{R,1}
\]  
(9.37)

where \( V_{R,2} \) is the observed retention volume for component 2, and \( V_{R,1} \) is the retention volume for the first component. Hence, if a separation system is characterized with respect to an analytical scale (small sample volume application), a first estimate of sample loading or resolution can be obtained.

### 2.10. Preliminary Cost Estimate

A major factor in LC separations is the cost of the support or adsorbent. This cost relative to the quantity of product obtained is given by Ladisch et al., (1984b):

\[
C_S = \frac{S}{\bar{F} \eta l}
\]  
(9.38)

where

- \( C_S \) = product cost due to support (\$/kg product)
- \( S \) = direct cost of support (\$/kg support)
- \( \bar{F} \) = average productivity (\( \text{kg product} / \text{kg support} \cdot \text{cycle} \))
- \( \eta \) = turnaround time (cycles/hour)
- \( l \) = operational life of the support (hours) = \( \eta \)
- \( \eta \), not including time during storage or regeneration
- \( L \) = support life in cycles

One cycle refers to the interval between when the sample is injected and the last eluant is collected immediately before injecting the next sample. The turnaround time, \( \eta \), refers to the fraction of the cycle (or number of cycles) completed in 1 hr. The productivity, \( \bar{F} \), is expressed in terms of the dry weight of product obtained at a specified purity. The cost of the support refers to the actual weight as supplied at a cost \( S \). The operational life is the time elapsed under use (rather than storage) conditions before the support is discarded.

The product obtained per kilogram support is given by \( \bar{F} \eta l \). However, \( \bar{F} \) will be a function of the manner in which the support loses its operational stability or capacity. If the loss is catastrophic after a certain time, \( t_1 \), then

\[
\bar{F} = P(t) = \text{constant at } t < t_1
\]

\[
\bar{F} = P(t) = 0 \text{ at } t > t_1
\]  
(9.39)
Consequently, \( \bar{P} \) is the same for each cycle, \( l \). If the loss is a first-order decay process approximated by

\[
P(t) = P_o \exp(-lt/c)
\]  

(9.40)

then the total amount of product obtained over a number of cycles, \( L \), is

\[
P_{\text{tot}} = P_o \int_{0}^{L} e^{-lt} \, dl = P_o \gamma (1 - e^{-L/c})
\]  

(9.41)

where \( \gamma \) is the "time" constant (in cycles) for the loss in productivity. The value of \( \gamma^{-1} \) is the slope of the line obtained by plotting \( \ln [P(t)/P_o] \) versus \( t \), while treating \( L / t \) as a continuous parameter. This approach is approximate since the loss in productivity is expressed in terms of a discrete variable (i.e., \( L \)), rather than a continuous variable (i.e., \( t \)). This reflects the fact that in a chromatographic process the loss in productivity would be measured for each cycle rather than continuously. Based on Eq. (9.41), the average productivity, in this case, is

\[
\bar{P} = \frac{P_{\text{tot}}}{L}
\]  

(9.42)

If the support is periodically regenerated, the average productivity becomes

\[
\bar{P} = \frac{P_{\text{tot},R}}{L_R}
\]  

(9.43)

where \( L_R \) is the number of cycles between regenerations, and \( P_{\text{tot},R} \) is the total product obtained between regenerations. The productivity of the support is assumed to return to \( P_o \) after each regeneration.

The cost, \( C_s \), is asymptotic with respect to \( \bar{P} \) (Fig. 9.4a). The relationship in Eq. (9.38) can be expressed linearly by plotting \( \bar{P}^{-1} \) versus \( C_s \) (Fig. 9.4b). The parameter \( \bar{P}^{-1} \) represents the amount of support required for a certain level of productivity. The quantity \( S/\eta \) reflects the support cost per cycle.

If a support costing \$10/kg and having a 2000-hr operation life is used in a system in which a sample is injected every 2.5 hr (\( \eta = 0.4 \)) with \( \bar{P} = 0.02 \) kg product \cdot (kg support)^{-1} \cdot (cycle)^{-1}, the support cost is 1.25 cents \cdot (kg support)^{-1} \cdot (cycle)^{-1} and the product cost, \( C_s \), is \$0.625/kg product. At \( t = 8000 \) hr, this becomes \$0.156/kg product. At \( t = 2000 \) hr, \( S = \$500/kg \) support, and \( \bar{P} = 0.02 \) at \( \eta = 0.4 \), the cost \( C_s \) is \$31.25/kg product. These examples show how Eq. (9.29) and Figure 9.4 might be useful in making a first estimate of the impact of support cost, capacity, and operational stability on product cost.

The cost of regenerating a support is given by

\[
C_R = \frac{R}{\bar{P}} \left( \frac{L}{L_R} \right)
\]  

(9.44)
Separation by Sorption

\[ C_S = \frac{\bar{P}}{\bar{S}} \]

where \( S \) = support cost and \( \bar{S} \) = support life

\( \frac{\bar{S}}{\bar{W}} = 1.0 \)
\( \frac{\bar{S}}{\bar{W}} = 0.5 \)
\( \frac{\bar{S}}{\bar{W}} = 0.1 \)

Figure 9.4. Cost functions for estimating support cost: (a) plot of Eq. (9.37) and (b) inverse plot of Eq. (9.37).

where \( R \) = regenerant cost in dollars/((kilogram support)(cycle)) and the other parameters are as defined previously. The ratio \( L/L_R \) represents the number of regenerations carried out at regular intervals over the life of the support.

The eluant cost is given by

\[ C_E = \frac{M}{\bar{P}} \]  \hspace{1cm} (9.45)

where \( M \) = solvent cost in dollars/((kilogram support)(cycle)).

The combined cost is then

\[ C_{tot} = C_S + C_R + C_E \]  \hspace{1cm} (9.46)
This should be useful in obtaining a first estimate of a separation cost once a product of satisfactory purity has been attained.

3. SUMMARY

The scale-up of an LC separation requires significant bench and pilot scale developmental effort even if an excellent analytical scale separation is already defined. Equations have been presented that can be used to estimate size, cost, and productivity of a large-scale system based on analytical scale results. Proper definition of operational variables is a key factor in a technically successful scale-up effort. These variables include eluant flowrate, particle size, sample loading, and appropriate column packing procedures. The main objective, in all cases, is to minimize dispersion for a given set of conditions. Although technical feasibility is a necessary condition for commercial applicability, economic feasibility is the sufficient condition. Methods for obtaining a first estimate of separation costs are also described. The combination of technical and economic analyses presented here allows a first estimate of LC scale-up specifications to be made.

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