$$\bar{c}_N(p) = 1 - p\bar{t} + \frac{(-N)(-N-1)}{2}\left(\frac{p\bar{t}}{N}\right)^2 \quad (6.108)$$

where we have neglected the higher order terms of the expansion. The second moment of the output curve can now be calculated from Eq. (6.108) and Eq. (6.89):

$$m_2 = \frac{N(N+1)\bar{t}^2}{N^2} = \frac{(N+1)}{N}\bar{t}^2 \quad (6.109)$$

Since the second central moment is equal to

$$\mu_2' = \sigma_t^2 = \mu_2 - \mu_1^2 \quad (6.110)$$

the variance of the tracer output, σ_t^2, is given by

$$\sigma_t^2 = \left(\bar{t}^2 + \frac{\bar{t}^2}{N}\right) - \bar{t}^2 = \frac{\bar{t}^2}{N} \quad (6.111)$$

This result also applies to plate theory, provided the plate count is large enough for the exiting peak (i.e., the chromatogram in response to a pulse input) to be Gaussian. For such a case the plate count can be expressed as the squared average retention time (the squared first moment) divided by the variance (the second central moment):

$$N_p = \frac{t_r^2}{\sigma_t^2} \quad (6.112)$$

and H can be written as

$$H = \frac{L\sigma_t^2}{t_r^2} \quad (6.113)$$

Strictly speaking, plate theory is applicable only when the exiting peaks are Guassian. However, plate theory can also provide useful guidelines for the optimal choice of operating conditions under other, less ideal circumstances, such as those often encountered in preparative chromatography. Moreover, the concept of plate height can be very useful in the interpretation of chromatographic results and in the diagnosis of chromatographic columns. The height equivalent of a theoretical plate is also a useful measure of column efficiency.

For plate theory to be useful in predicting how a particular operating variable will affect the chromatographic process, we must be able to relate N_p or H to that operating variable. Understanding the dependence of plate height on flow velocity is particularly important. The relationship between H and u is expressed by various *plate height equations*. We will now describe a plate height equation derived from the moment method.

Combining Eqs. (6.91), (6.93), and (6.110) we can write, for a short pulse (i.e., $t_0 \cong 0$):

$$\frac{L\sigma_t^2}{t_r^2} = \frac{2L^2}{u_i}\frac{\left\{\delta_1 + \frac{D_L}{\varepsilon}(1+\delta_0)^2\frac{1}{u_i^2}\right\}}{(L/u_i)^2\{1+\delta_0\}} \quad (6.114)$$

The assumption of a single rate-limiting step has been substantiated in practice. For example, in the case of anti-arsanilic acid monoclonal antibodies immobilized to controlled-pore glass, good agreement between experimental and theoretical breakthrough curves was obtained using the pore diffusion equations with $N_{t,pore} = 8$ (Problem 6.14).

Plate Theory

Although the differential mass balances of Section 6.4.2.2 may represent the most rigorous and accurate description of chromatography, alternative models do exist. In fact, the first mathematical model of chromatography, published by Martin and Synge in the *Biochemistry Journal* in 1941, takes a very different approach. This description, known as plate theory, models the column as a series of discrete, well-mixed stages. Plate theory is analogous to the "tanks-in-series" model for a nonideal flow reactor. The number of stages is a direct measure of axial dispersion and mass-transfer resistance in the system. Although plate theory is not a rigorous description of chromatography, it can be very useful for elucidating key factors that govern the efficiency and performance of chromatographic columns. Thus, the plate theory approach has been widely used, and many variations of Martin and Synge's original treatment have appeared over the years. The following presentation of plate theory combines the original concept with some of these more recent developments.

Assuming the column is comprised of individual stages, or plates, we can write

$$H = \frac{L}{N_p} \tag{6.105}$$

where H is the *height equivalent of a theoretical plate* (HETP), L is the column length, and N_p is called the *theoretical plate number*, the *number of theoretical plates*, or simply the *plate count of the column.*

A general expression for the plate count in terms of moments can be obtained by considering flow through N equal-size ideal stirred tanks (the tanks-in-series model common to reaction engineering). If a pulse of inert tracer is injected into the first vessel, the output of tracer is described by the residence time distribution of the fluid, $\xi(t)$[19]. Thus for an impulse input, $\bar{c}(p) = \bar{\xi}(p)$, where $\bar{c}(p)$ is the Laplacian of the exiting tracer concentration. The Laplace transform of a material balance on the jth tank in the series gives

$$\frac{\bar{c}_j(p)}{\bar{c}_{j-1}(p)} = \frac{1}{1 + p(\bar{t}/N)} \tag{6.106}$$

where $\bar{t}$ is the total mean residence time in the N-tank series. Eq. (6.106) indicates that

$$\bar{c}_N(p) = \bar{\xi}(p) = (1 + p\bar{t}/N)^{-N} \tag{6.107}$$

If N is sufficiently large, Eqn (6.107) can be rewritten as

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(19) O. Levenspiel, *Chemical Reaction Engineering, Second Edition* (John Wiley & Sons, New York, 1972), pp. 253-325.

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Therefore,

$$H = \frac{\left(\frac{u_i}{L}\right)^2\left\{\frac{2L^2}{u_i^3}\frac{D_L}{\varepsilon}(1+\delta_0)^2+\frac{2L^2\delta_1}{u_i}\right\}}{(1+\delta_0)^2} \tag{6.115}$$

$$H = \frac{2D_L}{\varepsilon u_i}+\frac{2u_i\delta_1}{(1+\delta_0)^2} \tag{6.116}$$

$$H = \frac{2D_L}{\varepsilon u_i}+\frac{\frac{2(1-\varepsilon)}{\varepsilon}\left[\frac{\rho_p(K_Lq_{max})^2}{k_f}+\frac{R^2}{15}(\beta+\rho_pK_Lq_{max})^2\left(\frac{1}{D_{eff}}+\frac{5}{k_sR}\right)\right]u_i}{\left[1+\left(\frac{1-\varepsilon}{\varepsilon}\right)\beta+\left(\frac{1-\varepsilon}{\varepsilon}\right)\rho_p(K_Lq_{max})\right]^2} \tag{6.117}$$

In terms of the superficial velocity, u_s, H becomes

$$H = \frac{2D_L}{u_s}+\frac{2(1-\varepsilon)\left[\frac{\rho_p(K_Lq_{max})^2}{k_f}+\frac{R^2}{15}(\beta+\rho_pK_Lq_{max})^2\left(\frac{1}{D_{eff}}+\frac{5}{k_sR}\right)\right]u_s}{[\varepsilon+(1-\varepsilon)\beta+(1-\varepsilon)\rho_p(K_Lq_{max})]^2} \tag{6.118}$$

Because of its complexity, Eq. (6.118) is not very convenient to use for the interpretation of experimental results of the analysis of column performance. A simpler form of Eq. (6.118) is

$$H = \frac{2D_L}{u_s}+\frac{2[K'/k_d+(\alpha-\varepsilon+K')^2/K_{OL}a_p]u_s}{(\alpha+K')^2} \tag{6.119}$$

where k_d is a modified desorption rate constant [$k_d = k_r/q_{max} = k_f/(K_Lq_{max})$] and

$K' = (1-\varepsilon)\rho_p(K_Lq_{max})$ = (maximum bound solute)/(column volume) · K_L

$\alpha = \varepsilon+(1-\varepsilon)\beta$ = fraction of column volume available to solute

$K_{OL}a_p = \left[\frac{R^2}{15(1-\varepsilon)}\left(\frac{1}{D_{eff}}+\frac{5}{k_sR}\right)\right]^{-1}$ = (column mass transfer coefficient) · (particle surface area).

If we again relate the dispersion coefficient to the molecular diffusivity and the superficial velocity [Eq. (6.97)], Eq. (6.119) becomes

$$H = 2l+\frac{2\eta D_{mol}}{u_s}+\frac{2[K'/k_d+(\alpha-\varepsilon+K')^2/K_{OL}a_p]u_s}{(\alpha+K')^2} \tag{6.120}$$

If the slight velocity dependence of k_s is neglected, Eq. (6.120) assumes the same form as the so-called *Van Deemter equation*[20], written in simplified form as

$$H = A+\frac{B}{u}+Cu \tag{6.121}$$

where

(20) J.J. Van Deemter, F.J. Zuiderweg, and A. Klinkenberg, *Chem. Eng. Sci.*, **5**, 271 (1956).

$$A = 2l, \quad B = 2\eta D_{mol}, \quad C = 2\frac{[K'/k_d + (\alpha - \varepsilon + K')^2/K_{OL}a_p]}{(\alpha + K')^2} \tag{6.122}$$

The influence of molecular diffusion in liquid chromatography is usually quite small; hence, a suitable plate height equation for liquid chromatography is

$$H = 2l + \frac{2[K'/k_d + (\alpha - \varepsilon + K')^2/K_{OL}a_p]u_s}{(\alpha + K')^2} \tag{6.123}$$

Alternatively, in terms of the axial Peclet number (Eq. 6.99),

$$H = \frac{2d_p}{Pe_{axial}} + \frac{2[K'/k_d + (\alpha - \varepsilon + K')^2/K_{OL}a_p]u_s}{(\alpha + K')^2} \tag{6.124}$$

Eqs. (6.123) and (6.124) predict a linear dependence between H and u_s, which, as shown in Figure 6.34, is typical for chromatographic separations of proteins.

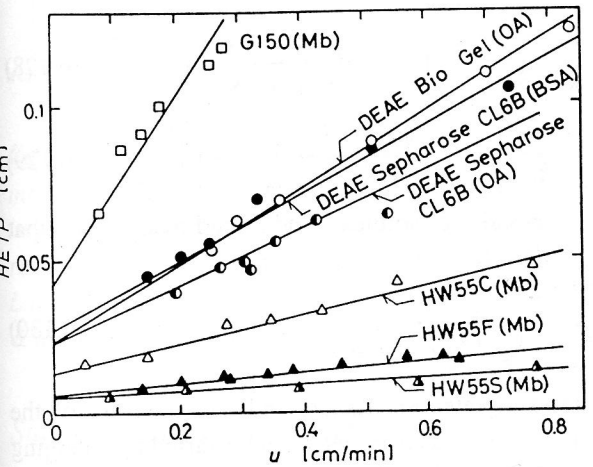

Figure 6.34. HETP versus linear mobile-phase velocity us for various gel filtration columns (G150, HWFFC, and HW55F) and for ion-exchange gel columns at high ionic strength (DEAE Bio Gel and DEAE Sepharose). Mb, OA, and BSA: myoglobin, ovalbumin, and bovine serum albumin, respectively. [From S. Yamamoto et al., Ion Exchange Chromatography of Proteins, Marcel Dekker, Inc., New York, 1988.]

Plate theory is also useful for evaluating the resolution of a chromatographic separation. Specifically, for two closely spaced peaks the resolution (Eq. 6.58) can be approximated as the product of three terms that reflect the *selectivity*, *efficiency*, and *capacity* of the chromatographic system. To derive this expression, we begin with Eq. (6.58) for the resolution of two adjacent peaks:

$$R_s = \frac{t_{r,2} - t_{r,1}}{(1/2)(w_1 + w_2)} \tag{6.58}$$

For Gaussian peaks, the baseline peak widths can be related to the standard deviation, σ_t, by $w = 4\sigma_t$ (Fig. 6.35). Assuming that the peaks have the same width, Eq. (6.58) becomes

$$R_s = \frac{t_{r,2} - t_{r,1}}{4\sigma_t} = \frac{1}{4}\frac{t_{r,1}}{\sigma_t}\left(\frac{t_{r,2} - t_{r,1}}{t_{r,1}}\right) \tag{6.125}$$

Combining Eq. (6.125) with the general definition of the plate count [Eq. (6.112)] yields

$$R_s = \frac{\sqrt{N_p}}{4}\left(\frac{t_{r,2} - t_{r,1}}{t_{r,1}}\right) \tag{6.126}$$

We now introduce the *retention factor* (also referred to in the literature as the *capacity factor*), k_i, defined for species *i* as

$$t_{r,i} = t_0(1 + k_i) \tag{6.127}$$

Therefore, Eq. (6.126) can be expanded as

$$R_s = \frac{\sqrt{N_p}}{4}\left[\frac{t_0(1+k_2) - t_0(1+k_1)}{t_0(1+k_1)}\right] = \frac{\sqrt{N_p}}{4}\left[\frac{k_2 - k_1}{1+k_1}\right] \tag{6.128}$$

$$R_s = \frac{\sqrt{N_p}}{4}\left(\frac{k_1}{1+k_1}\right)\left(\frac{k_2 - k_1}{k_1}\right) = \frac{\sqrt{N_p}}{4}\left(\frac{k_1}{1+k_1}\right)\left(\frac{k_2}{k_1} - 1\right) \tag{6.129}$$

Finally, defining the *separation factor* $\alpha = k_2/k_1$ for solutes 1 and 2, and recognizing that $k_1 \cong k_2 \cong k'$ (the average retention factor), leads to

$$R_s = \underbrace{\left(\frac{\sqrt{N_p}}{4}\right)}_{\text{(Efficiency)}} \cdot \underbrace{\left(\frac{k'}{1+k'}\right)}_{\text{(Retention)}} \cdot \underbrace{(\alpha - 1)}_{\text{(Selectivity)}} \tag{6.130}$$

The *selectivity* is related to the ratio of retention factors and is a measure of the chromatographic system's discriminatory power. This parameter can be varied by changing the composition of the mobile and/or stationary phases. The *retention* measures the fraction of eluate present in the stationary phase and therefore expresses the retentive power of the system. The average retention factor k′, and hence the retention, is related to the *solvent strength*, i.e., the ability of the solvent to provide a small or large k′ value (strong solvents give small k′ values; weak solvents, large k′ values)[21]. The *efficiency* measures the relative narrowness of the peaks, and is governed by the column length L and the various factors affecting H (Eqs. (6.105) and (6.123)]. The different ways in which each of the parameters of Eq. (6.130) affects resolution are shown graphically in Figure 6.35.

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(21) For listings of solvent strengths and further discussion of resolution in liquid chromatography, see L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography* (John Wiley & Sons, New York, 1974).