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The Extent of Separation: Applications to Elution Chromatography

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Summary

The fundamental equations for calculating the extent of separation in binary elution chromatographic systems are derived. At a specific instant of time, the elution curve in such systems is characterized by two quantities: the optimum cut-point location and the optimum extent of separation. Equations relating the optimum extent of separation to the resolution and to the extent of separation for a single equilibrium stage are given. A number of figures obtained from computer examples illustrate the use of the theoretical results. A new type of component detector—a mobile detector—is proposed.

INTRODUCTION

In a previous article in this series, a universal separation index—ξ, the extent of separation—was proposed and mathematically described (*1*). A number of claims were made for the index, such as, for example, that it was normalized, dimensionless, and easy to calculate and that it applied to any type of separation system, any concentration profile, any initial level of component purity, and any final level of component purification. To substantiate some of these claims, the extent of separation will now be applied to a binary (i.e., two migrating components) chromatographic system.

The theoretical treatment that is most closely related to the present one was given by Glueckauf more than a decade ago (*2*). He concluded that the optimum location of the cut between two elution peaks was at the geometric mean of the peak migration distances,

$$z^G_{opt} = t\sqrt{V_{1eff}V_{2eff}} \quad (1)$$

where V_{1eff} and V_{2eff} are the effective migration velocities of components 1 and 2, respectively. Glueckauf further concluded that the impurity fraction, η, was dependent upon the initial mole ratio of the two components in the mixture,

$$\eta = \frac{2}{(n_1^0/n_2^0) + (n_2^0/n_1^0)}\,\eta_{1:1} \quad (2)$$

n_i^0 is the total amount of component i present within the chromatographic system. Although these equations are generally accepted and have become part of the established chromatographic literature (3–5), one must seriously question their validity. For example, if either V_{1eff} or V_{2eff} were equal to zero, z^G_{opt} would also have a value of zero, a result that is incorrect.

This article will be concerned with four questions: (1) Where is

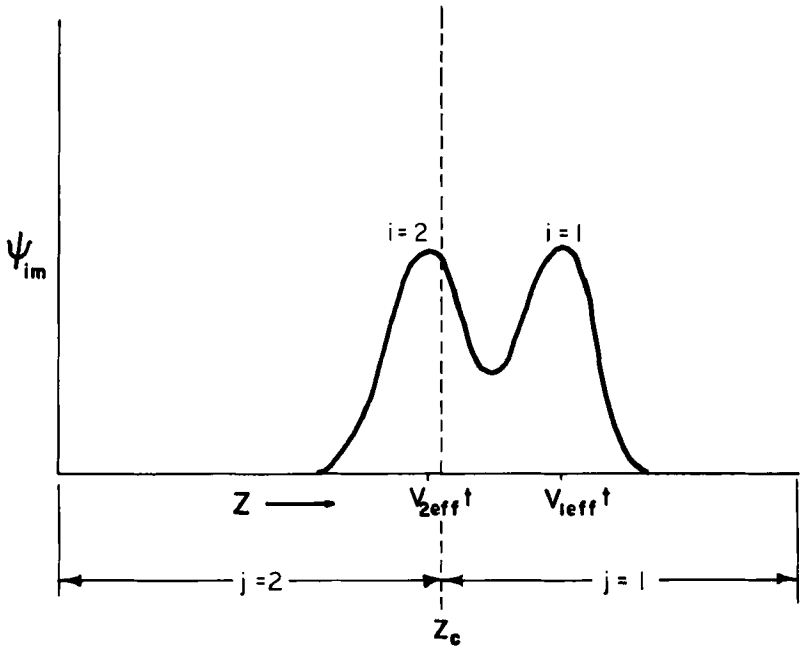

FIG. 1. Elution curve output (from a binary elution chromatographic system) as a function of the axial distance z for a given instant of time t.

the optimum location of a *single cut* between two elution peaks such that each component has the maximum purity (Fig. 1)? (2) How does the extent of separation of a pair of elution peaks vary with the initial mole ratio of the two components? (3) Can a rate of separation be defined for chromatographic systems? (4) Are there relationships between ξ for chromatographic systems and ξ for a single equilibrium stage? To answer these questions, the fundamental equation for the extent of separation for a pair of Gaussian peaks will be derived and differentiated to determine the optimum cut-point location, z_{opt}. The optimum extent of separation, ξ_{opt}, will be computed at this point and then differentiated to determine the optimum values of the two distribution coefficients, K_1 and K_2. Next, ξ_{opt} will be differentiated with respect to time to determine the rate of separation, r^S. Finally, ξ_{opt} will be compared with the previously obtained formula for the extent of separation for a single equilibrium stage (*1*),

$$\xi_{ss} = \text{abs}\left[\frac{1}{1+K_1} - \frac{1}{1+K_2}\right] \tag{3}$$

A number of computer examples will illustrate the use of the derived equations.

EXTENT OF SEPARATION

Let us consider a binary chromatographic system in which two components ($i = 1, 2$) each distribute between a mobile and a stationary phase. The normalized concentration of component i (which is initially injected as an instantaneous pulse at $z = 0$ and $t = 0$) in the mobile phase (m) as a function of distance (z) and time (t) is given by (*6*)

$$\psi_{im}(z, t) = \frac{L}{\sigma_i(t)\sqrt{2\pi}} \exp\left\{-\frac{(z - V_{ieff}t)^2}{2\sigma_i(t)^2}\right\} \tag{4}$$

where the subscript i represents component i; L is the length of the chromatographic "column"; $\sigma_i(t)$ is the standard deviation of the elution peak; V_{ieff} is the effective molar velocity of the elution peak,

$$V_{ieff} = \frac{v_m}{1+K_i} \tag{5}$$

v_m is the molar velocity of the mobile phase; K_i is the distribution coefficient,

$$K_i = \frac{n_{is}}{n_{im}} \tag{6}$$

n_{im} and n_{is} are the number of moles of i in the mobile and stationary phases, respectively; $\psi_{im}(z, t)$ and $c_{im}(z, t)$ are the normalized and actual concentrations of component i in the mobile phase, respectively,

$$\psi_{im}(z, t) = \frac{c_{im}(z, t)}{c_{im}^0} = \frac{AL(1 + K_i)}{n_i^0} c_{im}(z, t) \tag{7}$$

and A is the total cross-sectional area of the apparatus. It is assumed here that A, L, v_m, and K_i are all constant.

To calculate the extent of separation, we must first define the two regions $j = 1, 2$; as shown in Fig. 1, they are distinguished by a cut point z_c. This point could, for example, correspond to the place where a piece of filter paper is cut to optimally separate two chromatographic spots (see Fig. 1 of Ref. *1*). The total amount of component i in region 2 is given by

$$Y_{i2} = \frac{n_{i2}}{n_i^0} = \frac{1}{2}\left\{\text{erf}\left(\frac{z_c - V_{ieff}t}{\sigma_i\sqrt{2}}\right) + 1\right\} \tag{8}$$

which is obtained from Eq. (4) by integrating with respect to z from $z = 0$ to $z = z_c$ [the segregation fraction Y_{i2} has been discussed previously (*1*)]. The quantity erf(x) represents the error function of the argument x and is given by

$$\text{erf}(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-\theta^2}\, d\theta \tag{9}$$

which has the properties

$$\text{erf}(-x) = -\text{erf}(x) \qquad \text{erf}(0) = 0 \qquad \text{erf}(\infty) = 1$$
$$\text{abs}[\text{erf}(x)] = \text{erf}(\text{abs}[x]) \tag{10}$$

A table of error functions as well as a simplified computer program for calculating them are given elsewhere (*6*). The extent of separation can now be calculated according to the formula (*1*)

$$\xi = \text{abs}[Y_{22} - Y_{12}] \tag{11}$$

so that

$$\xi = \tfrac{1}{2} \text{ abs } \left[\text{erf} \left(\frac{z_c - V_{2\text{eff}} t}{\sigma_2 \sqrt{2}} \right) - \text{erf} \left(\frac{z_c - V_{1\text{eff}} t}{\sigma_1 \sqrt{2}} \right) \right] \tag{12}$$

OPTIMUM CUT-POINT LOCATION

The optimum cut-point location can be obtained if we differentiate ξ with respect to z_c and set the resulting derivative equal to zero:

$$\frac{\partial \xi}{\partial z_c} = \frac{1}{\sqrt{2\pi}} \text{ abs } \left[\frac{1}{\sigma_2} \exp \left\{ - \frac{(z_c - V_{2\text{eff}} t)^2}{2\sigma_2^2} \right\} - \frac{1}{\sigma_1} \exp \left\{ - \frac{(z_c - V_{1\text{eff}} t)^2}{2\sigma_1^2} \right\} \right] = 0 \tag{13}$$

If we assume that $\sigma_1 = \sigma_2 = \sigma$, Eq. (13) simplifies to

$$(z_c - V_{2\text{eff}} t)^2 = (z_c - V_{1\text{eff}} t)^2 \tag{14}$$

This equation has two different solutions:

$$a. \quad z_c - V_{2\text{eff}} t = +(z_c - V_{1\text{eff}} t) \tag{15}$$

$$b. \quad z_c - V_{2\text{eff}} t = -(z_c - V_{1\text{eff}} t) \tag{16}$$

The first solution corresponds to a minimum in ξ, whereas the second is the desired optimum,

$$z_{\text{opt}} = \frac{V_{1\text{eff}} t + V_{2\text{eff}} t}{2} \tag{17}$$

From Eq. (12), we can calculate the value of ξ at the optimum cut point,

$$\xi_{\text{opt}} = \text{abs } \left[\text{erf} \left(\frac{V_{1\text{eff}} t - V_{2\text{eff}} t}{\sigma \sqrt{8}} \right) \right] = \text{erf} \left(\text{abs } \left[\frac{V_{1\text{eff}} t - V_{2\text{eff}} t}{\sigma \sqrt{8}} \right] \right) \tag{18}$$

For large values of the argument x,

$$x = \frac{V_{1\text{eff}} t - V_{2\text{eff}} t}{\sigma \sqrt{8}} \tag{19}$$

it is more convenient to employ the complementary extent of separation,

$$\psi_{opt} = 1 - \xi_{opt} = \text{erfc}\left(\text{abs}\left[\frac{V_{1eff}t - V_{2eff}t}{\sigma\sqrt{8}}\right]\right) \quad (20)$$

where erfc(x) is the complementary error function,

$$\text{erfc}(x) = 1 - \text{erf}(x) \quad (21)$$

OPTIMUM DISTRIBUTION COEFFICIENTS

We must still inquire, for what *absolute values* of the distribution coefficients K_i is the "best" separation achieved? An answer can be obtained if we rewrite ξ_{opt} in Eq. (18) as

$$\xi_{opt} = \text{erf}(\text{abs}[x]) = \text{erf}\left\{\text{abs}\left[\frac{v_m t}{\sigma\sqrt{8}}\left(\frac{1}{1+K_1} - \frac{1}{1+K_2}\right)\right]\right\} \quad (22)$$

define K_2 in terms of K_1 and α, the quotient of the distribution coefficients,

$$\alpha = \frac{K_2}{K_1} \quad (23)$$

differentiate Eq. (22) with respect to K_1, and finally set the resulting derivative equal to zero,

$$\frac{\partial \xi_{opt}}{\partial K_1} = 0 \quad (24)$$

Equation (24) reduces to the problem of finding the value of K_1 for which

$$\frac{\partial}{\partial K_1}\left[\frac{1}{1+K_1} - \frac{1}{1+\alpha K_1}\right] = 0 \quad (25)$$

This problem has been previously solved (*1*), so we can conclude that *the optimum conditions that apply for a single equilibrium stage also apply for a two-phase chromatographic system.*

RATE OF SEPARATION

If we write σ, the standard deviation, as

$$\sigma = \sqrt{2\,Dt} \quad (26)$$

where D is a pseudo diffusion coefficient (cm²/sec), Eq. (22) can be differentiated with respect to time to yield the rate of separation,

$$r^S = \frac{\partial \xi_{opt}}{\partial t} = \frac{v_m}{\sigma \sqrt{8\pi}} e^{-x^2} \text{ abs} \left[\frac{1}{1+K_1} - \frac{1}{1+K_2}\right] \tag{27}$$

Since σ is proportional to $t^{1/2}$, the rate of separation is infinite at $t = 0$. When $x \leqq 0.20$, Eq. (22) can be approximated by

$$\xi_{opt} \approx \frac{v_m t}{\sigma \sqrt{2\pi}} \text{ abs} \left[\frac{1}{1+K_1} - \frac{1}{1+K_2}\right] \tag{28}$$

so the rate of separation becomes

$$r^S \approx \frac{v_m}{\sigma \sqrt{8\pi}} \text{ abs} \left[\frac{1}{1+K_1} - \frac{1}{1+K_2}\right] \tag{29}$$

COMPARISON TO A SINGLE EQUILIBRIUM STAGE

One of the most important characteristics of ξ_{opt} is its close relationship to the extent of separation for a single equilibrium stage [Eq. (3)]. Thus Eqs. (22) and (28), respectively, simplify to

$$\xi_{opt} = \text{erf}\left(\frac{v_m t}{\sigma \sqrt{8}} \xi_{ss}\right) \tag{30}$$

$$\xi_{opt} \approx \frac{v_m t}{\sigma \sqrt{2\pi}} \xi_{ss} \qquad (x \leqq 0.20) \tag{31}$$

COMPARISON TO THE RESOLUTION

Huber has indicated that the term "resolution" applies only for peaks of the same height (7). Therefore, with the restriction $n_1^0 = n_2^0$, the resolution equation employed by Giddings (8),

$$\text{Rs} = \frac{\Delta z}{4\sigma} \tag{32}$$

can be written in terms of x, ξ_{ss}, or ξ_{opt}:

$$\text{Rs} = \text{abs}\left[\frac{V_{1eff}t - V_{2eff}t}{4\sigma}\right] = \frac{\text{abs}[x]}{\sqrt{2}} = \frac{v_m t}{4\sigma} \xi_{ss} \tag{33}$$

$$\xi_{opt} = \text{erf}(\text{Rs } \sqrt{2}) \tag{34}$$

When $x \leqq 0.20$, Eq. (34) simplifies to

$$\xi_{opt} \approx \text{Rs} \sqrt{\frac{8}{\pi}} \tag{35}$$

DISCUSSION

For a binary elution chromatographic system in which the standard deviations of the two peaks are the same ($\sigma_1 = \sigma_2$), the main conclusions of this article can be summarized as follows:

a. The optimum cut-point location, z_{opt}, is at the arithmetic mean of the peak migration distances; it is independent of the magnitude of the initial mole ratio, n_1^0/n_2^0 [Eq. (17)].

b. The extent of separation for a single equilibrium stage, ξ_{ss}, and the optimum extent of separation, ξ_{opt}, are both independent of the magnitude of the initial mole ratio [Eqs. (3) and (18)].

c. The optimum extent of separation is closely related to ξ_{ss} [Eq. (30)]; for slight separations ($x \leqq 0.20$), ξ_{opt} is directly proportional to ξ_{ss} [Eq. (31)].

d. The optimum conditions that apply for a single equilibrium stage also apply for a chromatographic system [Eq. (25)].

e. The optimum extent of separation is closely related to the resolution, Rs [Eq. (34)].

Perhaps the most controversial of the above conclusions is *b*; since this may be one of the most important characteristics of a universal separation index, it is worthwhile to consider it in greater detail. Consider two different single equilibrium stages (I and II) each containing identical components 1 and 2 and assume that the respective distribution coefficients are $K_1 = 10^{-6}$ and $K_2 = 10^6$. For stage I, let us further assume that $n_1^0 = n_2^0 = 1$ mole, whereas for stage II, $n_1^0 = 10^{-6}$ and $n_2^0 = 10^6$ moles. In view of the similarities between a chromatographic system and a single equilibrium stage, any conclusions that apply to the latter also apply to the former.

Stage I is a situation that is frequently encountered in treatments of separation problems. Since both components are weighted equally, this situation can be employed to check all characteristics of a separation index except the effect of the initial mole ratio. In this case the mole fraction of impurity in components 1 and 2 changes from an initial value of $X_2^0 = X_1^0 = 0.50$ to a final value of $X_{21} = X_{12} = 10^{-6}$. The extent of separation for this process is $\xi_{ss} = 0.999998$. We can conclude that an excellent separation has occurred, since each component has been isolated in its respective region with a purity of 99.9999%.

In stage II the extent of separation is also 0.999998. In this case,

however, the impurity mole fraction in component 2 is reduced from $X_1^0 = 10^{-12}$ initially to a value of $X_{12} = 10^{-18}$ Similarly, component 1 is *enriched* from a mole fraction of $X_1^0 = 10^{-12}$ to $X_{11} = 10^{-6}$. Component 1 is still not pure, but it has experienced a millionfold enrichment. An excellent separation has occurred in this case also, since $Y_{11} = Y_{22} = 0.999999$; i.e., 99.9999% of each component is located in its respective region.

The point here is that while we might intuitively expect stage I to correspond to a higher extent of separation than stage II, *purity and separation are not equivalent terms.* Separation is related to the isolation of a pair of components, while purity is better characterized by the individual mole fractions, X_{ij}, or perhaps by an overall parameter such as the extent of purity, ξ^P,

$$\begin{aligned}\xi^P &\equiv \text{abs det} \begin{vmatrix} X_{11} & X_{12} \\ X_{21} & X_{22} \end{vmatrix} \\ &= \text{abs}[X_{11} + X_{22} - 1] = \text{abs}[1 - X_{12} - X_{21}] \\ &= \text{abs}[X_{11} - X_{12}] = \text{abs}[X_{22} - X_{21}] \end{aligned} \tag{36}$$

In stage I, $\xi^P = 0.999998$, whereas in stage II, $\xi^P = 10^{-6}$, so the two situations are quite different from a purity standpoint. We conclude that *when a comparison of the separation of a pair of components in different separation systems is made, it should be done on the basis of identical initial mole ratios.*

EXAMPLES

Several of the preceding equations have been programmed on a computer to demonstrate the application of the extent of reaction to chromatographic systems. Listings of the computer programs and a more detailed discussion of their use are given elsewhere (*6*).

Figures 2 through 5 represent Eq. (4) for components 1 and 2 and give ξ_{opt} for nine different time values. The overall range of z in the figures has been subdivided into 100 equal intervals (labeled $N = 1$ through $N = 100$). The scale in the vicinity of the two peaks has been expanded to include $\sqrt{8}$ standard deviations on either side of the peak maxima, $V_{1eff}t$ and $V_{2eff}t$. The parameters employed in this calculation are $K_1 = 0.20$ (dimensionless), $K_2 = 0.22$ (dimensionless), $\alpha = 1.10$, $v_m = 10$ cm/sec, $L = 100$ cm, $\sigma^2 = 0.165t$ cm^2, and $c_{1m}^0 = c_{2m}^0 = 8.27 \times 10^{-6}$ mole/cm^3 mobile phase. In Fig. 2 the

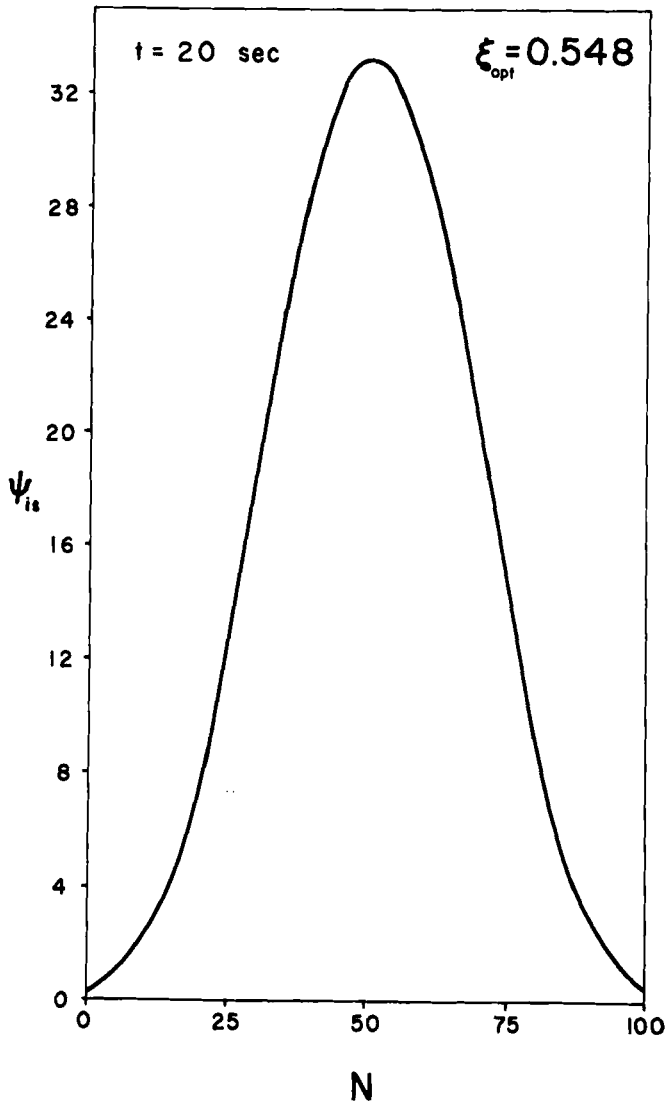

FIG. 2. Elution profile at $t = 20$ sec.

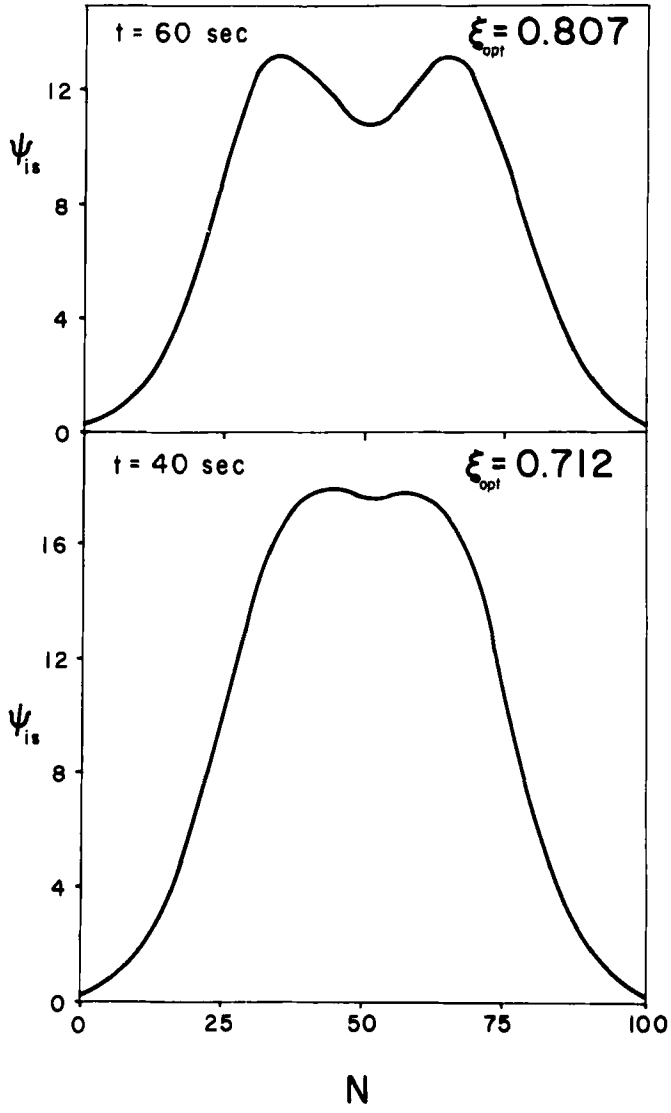

FIG. 3. Elution profiles at $t = 40$ and 60 sec.

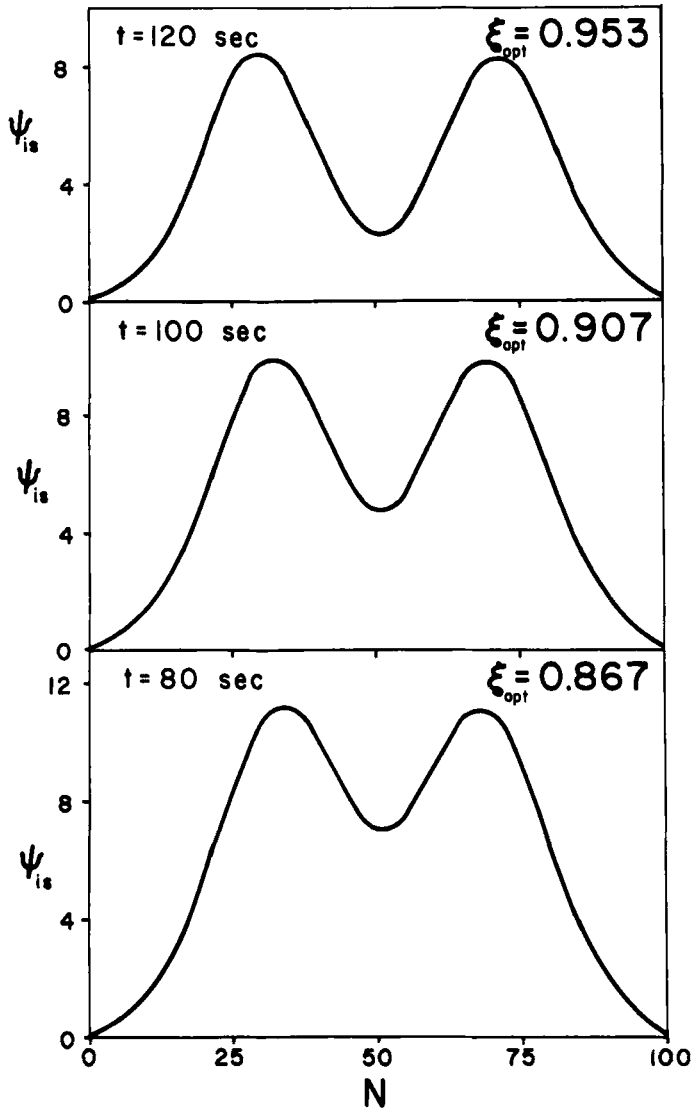

FIG. 4. Elution profiles at $t = 80$, 100, and 120 sec.

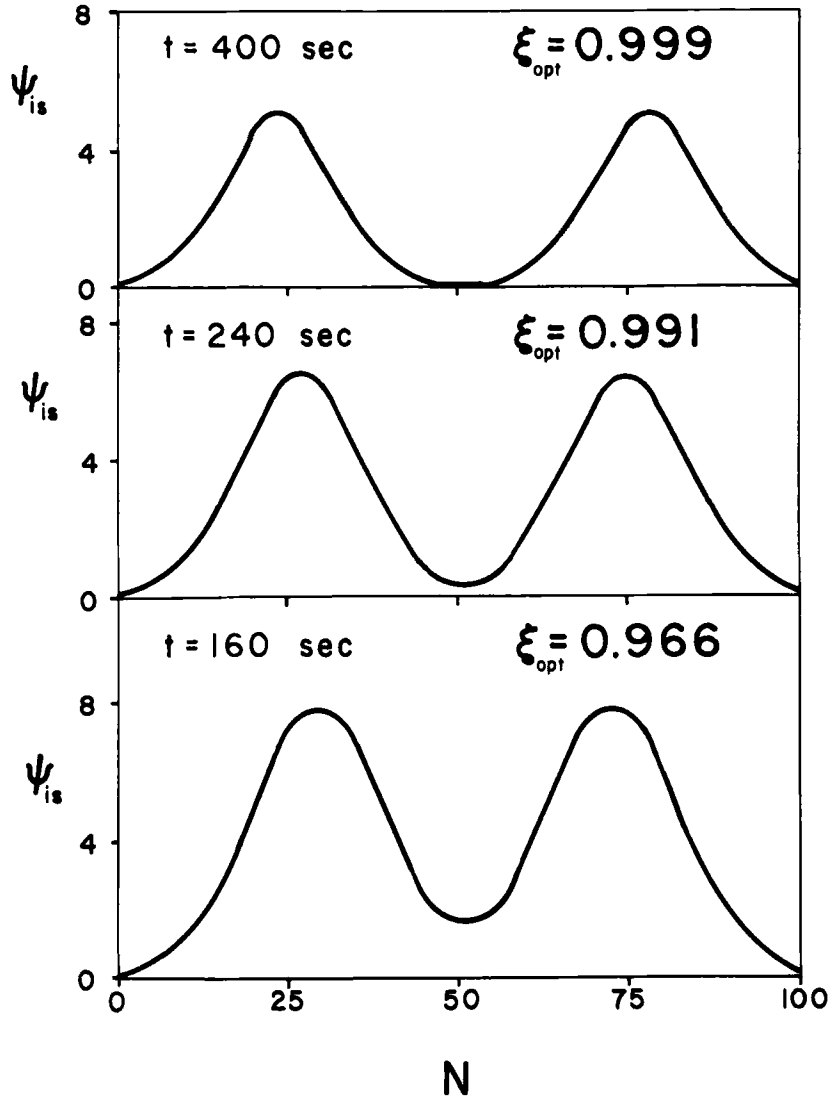

FIG. 5. Elution profiles at $t = 160$, 240, and 400 sec.

separation is substantial ($\xi_{opt} = 0.548$) despite the fact that resolution into two separate peaks has not yet occurred. The curve at $t = 120$ sec corresponds approximately to the condition Rs = 1. Base-line separation, according to Fig. 5, occurs at $\xi_{opt} = 0.995$ and corresponds to a high degree of component purity.

In Fig. 6, the extent of separation, ξ, is calculated as a function of the location of the cut point z_c for five different time values. The same parameters are employed as given above. Clearly, the optimum value of ξ occurs if the cut is made at $\frac{1}{2}(V_{1eff}t + V_{2eff}t)$, which in the figure corresponds to $N = 51$.

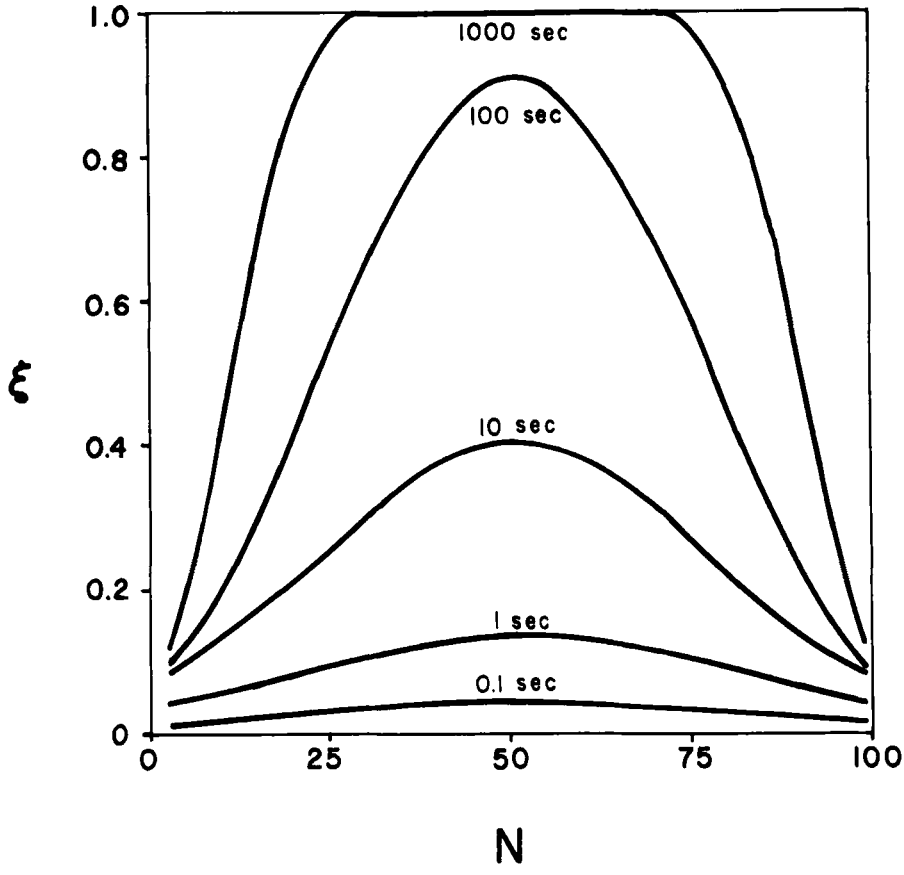

FIG. 6. The extent of separation, ξ, as a function of z_c. These curves demonstrate that the optimum value of ξ is located at $N = 51$.

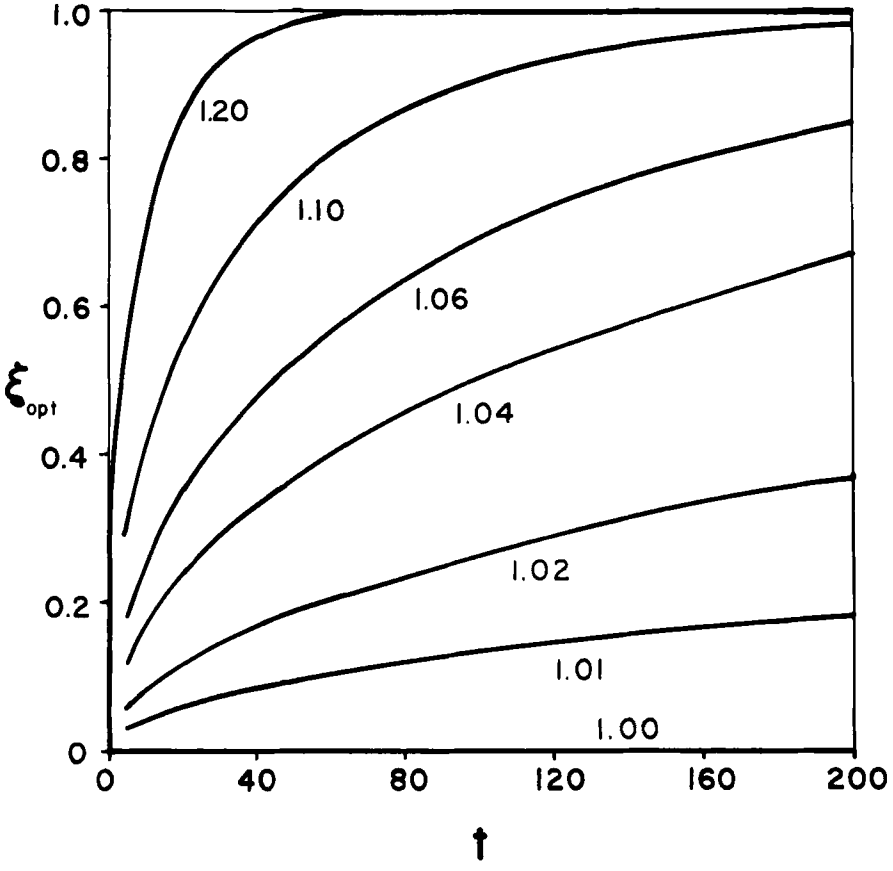

FIG. 7. The optimum extent of separation, ξ_{opt}, as a function of the value of the partition coefficient, κ_2.

In Fig. 7, ξ_{opt} is calculated as a function of time for seven different values of the partition coefficient, κ_2 (cm^3 mobile phase/cm^3 stationary phase).

$$K_2 = 0.20\kappa_2 \tag{37}$$

The remaining parameters are the same as above.

The calculation given in Fig. 8 is similar to that of Fig. 7 with the exception that $K_2 = 0.24$ and component 2—the slower moving one—is given a head start of 25 cm. Note how the extent of separation passes through a minimum, $\xi_{opt} = 0$, at $t = 93$ sec as component 1

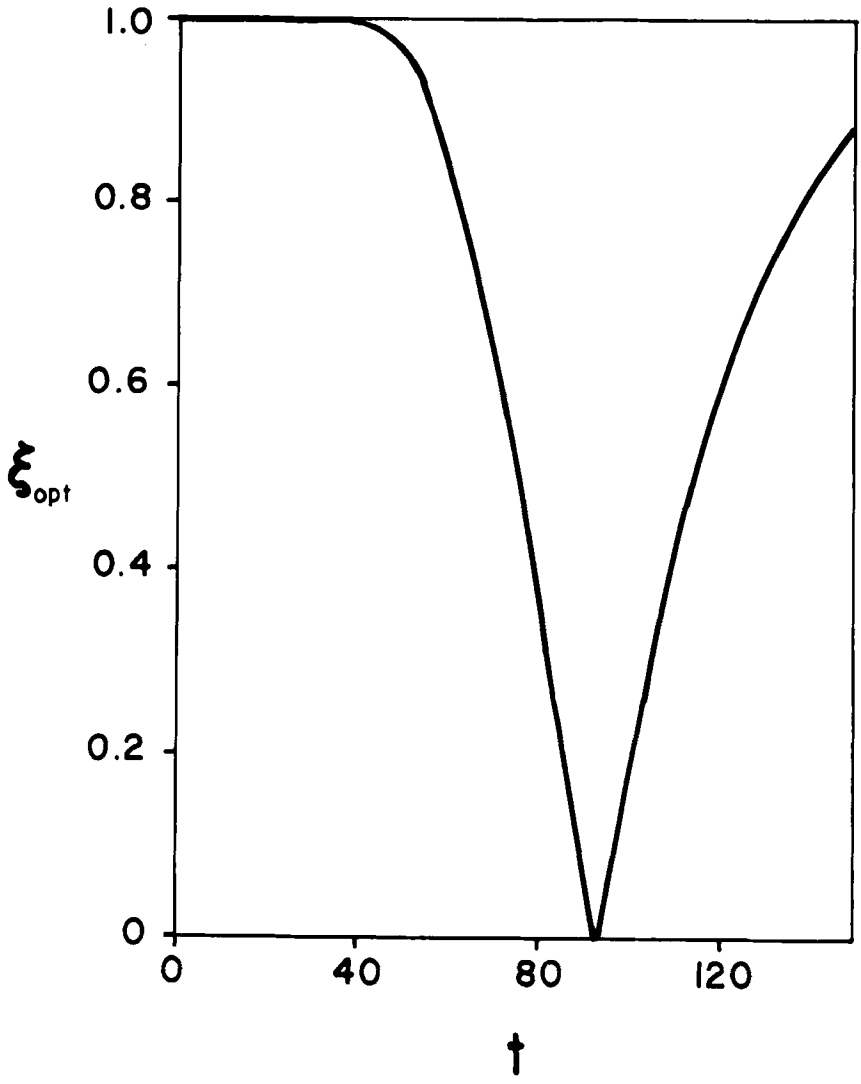

FIG. 8. Simulation of a mobile detector. This curve demonstrates how ξ_{opt} passes through zero as component 1 first overtakes and then passes component 2.

first overtakes and then passes component 2. If both components are given head starts of z_1^0 and z_2^0, the time at which $\xi_{opt} = 0$ is given by

$$t = \frac{z_2^0 - z_1^0}{V_{1eff} - V_{2eff}} = \frac{z_2^0 - z_1^0}{v_m \xi_{ss}} \tag{38}$$

This behavior has at least one experimental consequence: If component 1 were present in very small amounts ($n_1^0 \ll n_2^0$) but served as an indicator whenever it was in the vicinity of component 2, it would act as a *mobile detector*. The detected signal (such as fluorescence, a color change, or a broadening of an NMR signal) would reach a maximum value at the time given by Eq. (38).

List of Symbols

A	total cross-sectional area of chromatographic apparatus (cm^2)
c	concentration ($moles/cm^3$)
D	pseudo diffusion coefficient (cm^2/sec)
K	distribution coefficient
L	length of chromatographic column (cm)
n	total number of moles (moles)
N	interval number
r^S	rate of separation (sec^{-1})
Rs	resolution
t	time (sec)
v	molar velocity of phase (cm/sec)
V	molar velocity of component (cm/sec)
x	argument of error function
X	mole fraction
Y	segregation fraction
z	axial distance in chromatographic apparatus (cm)
α	quotient of the distribution coefficients
η	impurity fraction
$\eta_{1:1}$	impurity fraction for equimolar mixture
θ	integration variable
κ	partition coefficient ($moles/cm^3$ stationary phase: $moles/cm^3$ mobile phase)
ξ	extent of separation
ξ_{opt}	optimum extent of separation
ξ^P	extent of purity

ξ_{ss} extent of separation for a single equilibrium stage
σ standard deviation
ψ normalized concentration
ψ_{opt} optimum complementary extent of separation

Superscripts

0 initial value

Subscripts

1 component 1
2 component 2
c cut point
i component i
$i$eff effective value for component i
ij component i in region j
im component i in mobile phase
is component i in stationary phase
m mobile phase
opt optimum value

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