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Measures of Resolution for Multicomponent Systems in One and Two Dimensions with Application to Pore Gradient Electrophoresis

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Abstract

Several criteria are suggested for measuring the effectiveness of separation systems (chromatography, electrophoresis, etc.) in one and two dimensions in the presence or absence of gradients. A major point is that resolution is truncated so that if two peaks are separated by more than some sufficient amount, ΔS, their effective separation remains constant. This eliminates overemphasis on large separations which tend to obscure less well-separated peaks. It is shown that the qualitative behavior of at least two of the suggested class of criteria are similar, and that the criteria are insensitive to arbitrary parameters over a wide range. Illustrative examples are given from one- and two-dimensional gel electrophoresis, including the effects of pore gradients and/or ionic detergents (e.g., sodium dodecyl sulfate).

INTRODUCTION

Two techniques are generally used to characterize resolution in chromatographic and other chemical separation systems. The first is through

an analysis of the separation of two peaks relative to their bandwidths (*1–3*), and the second is through some variant of counting the number of peaks in a given distance or time (*4–7*). Some work has been done in extending the first approach to nonuniform chromatographic systems (*8, 9*), but there appears to be no comparable generalization of the second approach in the literature. There is some utility in such a generalization since peak widths will generally vary across a nonuniform field. A second extension of the concept of resolution in chromatography that has not been studied in any detail is that of resolution of multicomponent systems in two-dimensional systems. This is useful in evaluating two-dimensional electrophoresis-chromatography of amino acids, peptide mapping or "finger printing," and the numerous methods recently developed for macromolecular mapping (see Ref. *10* for review). Since it is possible to design pore gradients in such systems as polyacrylamide gel electrophoresis, it would seem useful to have some criterion for evaluating the utility of these systems compared with the use of gels with uniform concentration.

In this paper we suggest several such criteria that have potential applicability to separation techniques in one and two dimensions. In later parts of this paper we apply some of the ideas to gel electrophoresis, because the physical chemistry of this process has been carefully elucidated and is readily described in mathematical terms (*11–13*).

SUGGESTED CRITERIA OF RESOLUTION

We may consider two situations, the first in which peak broadening is small, uniform, and can be neglected, and the second in which peak broadening is indeed an important effect in limiting resolution. In the first case, in analytical systems the results of a separation can be expressed as the position of the points corresponding to the species. For such a system it is desireable to have the points spaced as far apart as possible. In the second case one would attempt to maximize a suitable generalization of the familiar resolution function. Although the first case is unrealistic, it is useful to get some insight into an ideal or limiting case.

We assume, as the condition of the experiment, that a field is applied for a certain amount of time following which it is switched off and peak positions are recorded. In one dimension, therefore, the separation process leads to a set of N positions, $y_1, y_2, \ldots, y_N$, and the corresponding set of distances $L_1 = y_2 - y_1$, $L_2 = y_3, \ldots, L_{N-1} = y_N - y_{N-1}$.

Let us further introduce a length L^* such that when the distance between any two peaks exceeds L^* the peaks will be said to be separated. Then the criterion of how well a separation system works will be a function $f(L_1, L_2, \ldots, L_{N-1})$ which we require to have the two properties:

1. $f(L_1, L_2, \ldots, L_{N-1})$ increases when any $L_j < L^*$ increases leaving the remaining distances unchanged.
2. $f(L_1, L_2, \ldots, L_{N-1})$ is unchanged when any $L_j > L^*$ is changed in such a way that it remains $\geq L^*$, provided that the remaining L_j are unchanged.

Obviously, there are an infinite number of possible functions that satisfy the two stated conditions. We may specialize our requirements further by assuming that $f(\mathbf{L})$ can be factored as

$$f(\mathbf{L}) = g(L_1) + g(L_2) + \cdots + g(L_{N-1}) \tag{1}$$

which, together with the two specifications above, implies that $g(L)$ satisfies

$$\frac{dg}{dL} > 0 \text{ for } L < L^*, \qquad \frac{dg}{dL} = 0 \quad \text{for} \quad L > L^* \tag{2}$$

The simplest candidate function $g(L)$ satisfying these requirements is

$$g_1(L) = \frac{1}{N-1}\left(\frac{L}{L^*}\right)^+ \tag{3}$$

where the symbolism x^+ is used for

$$x^+ = \begin{cases} x & \text{for} \quad x < 1 \\ 1 & \text{for} \quad x > 1 \end{cases} \tag{4}$$

The function $f(\mathbf{L})$ resulting from this choice of $g(L)$ has the property that $f(\mathbf{L}) = 1$ when all of the L's are $> L^*$ and $f(L) < 1$ when any L is $< L^*$. A second possible choice for $g(L)$ with the properties specified in Eq. (2) is

$$g_2(L) = \frac{1}{N-1}\left(\frac{L}{L^*}\right)^+ \left\{-\ln\left[\left(\frac{L}{L^*}\right)^+\right] + 1\right\} \tag{5}$$

This is an analog of the entropy of the set of L's [the resulting $f(\mathbf{L})$ also has the property that $f < 1$]. The two criteria given above will be

denoted by

$$\bar{L} = \frac{1}{N-1} \sum_{i=1}^{N-1} g_1(L_i) \tag{6a}$$

$$\bar{E} = \frac{1}{N-1} \sum_{i=1}^{N-1} g_2(L_i) \tag{6b}$$

The separation criteria in Eq. (6) can be generalized in two directions. The first is the inclusion of band spreading. A simple, but not unique, way of doing this is to use the classical resolution formula. Let σ_i be the standard deviation of the ith peak, and let R_i be the resolution peaks i and $i + 1$, i.e.,

$$R_i = L_i/[2(\sigma_i + \sigma_{i+1})] \tag{7}$$

Since the criterion $R = 1$ leads to good qualitative resultion, a useful measure of multipeak resolution is the quantity

$$\bar{R} = \frac{1}{N-1} \sum_{i=1}^{N-1} R_i^{+} \tag{8}$$

A second generalization of these ideas is to separation techniques in two dimensions. Since peak positions are not uniquely ordered in two dimensions as they are in one, we define the L_i to be the distance between peak i and its nearest neighbor. With this definition we can use the results of Eqs. (1)–(8) in two dimensions except that $N - 1$ is to be replaced by N since there is no longer a natural order. To generalize Eq. (7) we must specify what is meant by σ_i. For this purpose we note that there will in general be two values of peak widths, $\sigma_i(x)$ and $\sigma_i(y)$, in the two directions. There is some arbitrariness in choosing σ_i from these but one plausible choice is

$$\sigma_i = [\sigma_i^2(x) + \sigma_i^2(y)]^{1/2} \tag{9}$$

which we retain in the remainder of this work. With this specification we can use Eq. (8) as a measure of separation in two dimensions.

GEL ELECTROPHORESIS

The ideas developed in the last section will now be applied to gel electrophoresis. In one dimension with a constant gel concentration we let T be the gel concentration and let x be the space coordinate along the column satisfying $0 \leq x \leq L$. Let k_R be the retardation coefficient for a

molecule defined by the relation*

$$M = M_0 \exp(-k_R T) \tag{10}$$

where M is the mobility at gel concentration T, and M_0 is the mobility in the absence of a gel. The velocity (mobility × voltage/length) and diffusion coefficients are assumed to be related to gel concentration as

$$v = V_0 \exp(-k_R T) \tag{11a}$$

$$D = D_0 \exp(-a k_R T) \tag{11b}$$

where a is a dimensionless constant and V_0 and D_0 are the velocity and diffusion constants at zero gel concentration, respectively.

When T is a constant, a peak of zero width initially at $t = 0$ will be at

$$x(t) = vt \tag{12}$$

at time t and the variance of the normalized concentration curve is just

$$\sigma^2(t) = \int_{-\infty}^{\infty} x^2 c(x, t)\, dx - \left[\int_{-\infty}^{\infty} xc(x, t)\, dx\right]^2 = 2Dt \tag{13}$$

Both of these results are valid in the absence of microheterogeneity of the gel or the protein.

When $T(x)$ is a linear gradient of the form

$$T(x) = T_0 + (T_1 - T_0)(x/L) \tag{14}$$

so that $T(0) = T_0$, $T(L) = T_1$, we assume that the form of the relations between M, v, and D and $T(x)$ remains that given in Eqs. (10) and (11). When the dimensionless parameter $D_0/(LV_0)$ is small, it has been shown (*13*, *14*) that to a good approximation $x(t)$ and $\sigma^2(t)$ are given by

$$x(t) = \frac{L}{k_R(T_1 - T_0)} \ln\left[1 + \frac{k_R(T_1 - T_0)}{L} V_0 t\right] \tag{15}$$

$$\sigma^2(t) = \frac{2D_0}{LV_0} \frac{L^3}{k_R(T_1 - T_0)(3 - a)} \frac{1}{\left[1 + \dfrac{k_R(T_1 - T_0)}{L} V_0 t\right]^2} \times \left[\left(1 + \frac{k_R(T_1 - T_0)}{L} V_0 t\right)^{3-a} - 1\right] \tag{16}$$

*The parameter k_R here corresponds to K_R ln 10, using the customary definition of retardation coefficient (*11*).

One-Dimensional Fractionation

We consider the application of the preceding ideas to a set of 25 molecular species. All measurements will be with reference to the maximum length of the column, i.e., we take $L = 1$ without loss of generality. In two dimensions the column lengths are chosen equal in both directions. The k_R take on one of the five values 0.02, 0.04, 0.06, 0.08, and 0.10; V_0 takes the values 0.2, 0.4, 0.6, 0.8, and 1.0; and $D_0 = 10^{-3}$. Thus we have a mixture of size isomers and charge isomers. In Fig. 1 we show several curves of the average value $\bar{L}$ (Eq. 6a) for electrophoretic separation in one dimension. Peak positions and band-widths will be calculated on the assumption that the separation is carried on for as long as it takes the fastest moving species to reach the end of the column. Because of this, only the relative values of V are required. The parameters are $a = 1$, and the four values of L^* are 0.04, 0.06, 0.08, and 0.10. A constant gel concentration, T, is assumed and the curves are plotted as a function of this

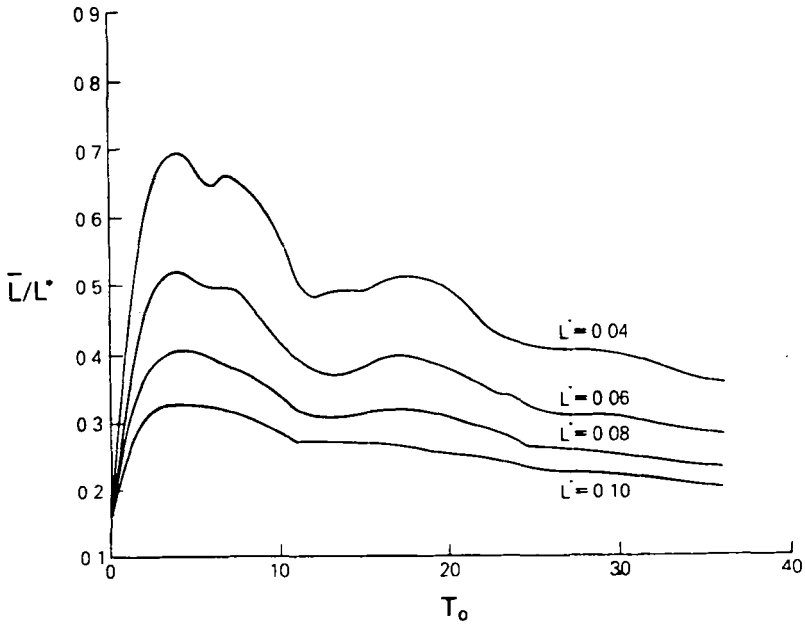

FIG. 1A. Curves of $\bar{L}$ as a function of uniform gel concentration T_0 for different values of L^*. The total column length is $L = 1$ in these units.

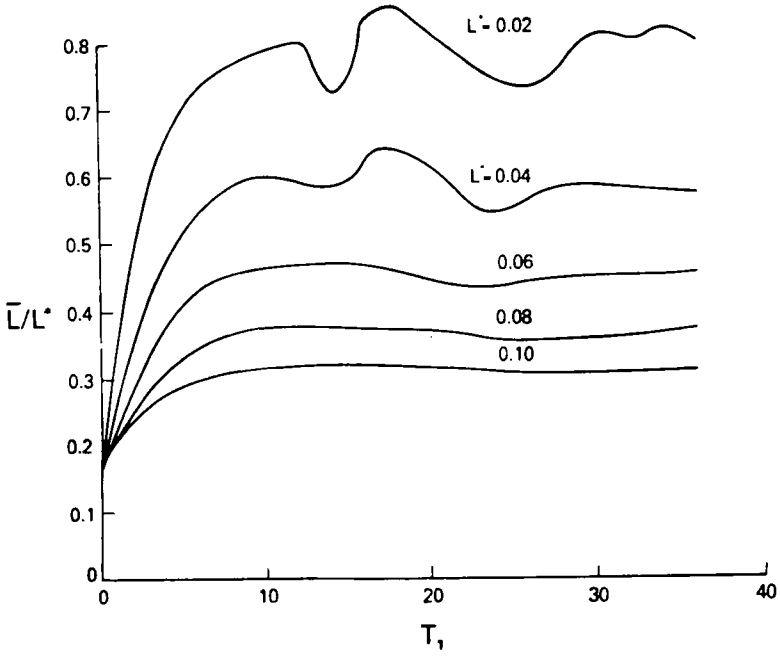

FIG. 1B. Curves of $\bar{L}$ with a gel gradient, plotted against T_1, when $T_0 = 0$.

concentration. For L^* very small, L is identically equal to 1 since all species are separated. In the opposite limit, $L^* = L$, the column length, none of the species are completely separated, and the value of the average of the L_i/L is just equal to $(x_{25} - x_1)/(24L)$. This value is independent of the location of the intermediate peaks and therefore this type of average cannot be considered a satisfactory measure of separation. The curves shown in Fig. 1A are plotted as a function of the constant gel concentration T and all show similar qualitative behavior over the range of L^* considered. The erratic fluctuations in $\bar{L}$ are not important and result from the use of a function with a discontinuous derivative to express resolution. Figure 1B shows the same behavior for the case of a pore gradient when $T_0 = 0$; $\bar{L}$ is plotted as a function of T_1. The results in Fig. 1 lead us to conclude that the choice of L^* is not critical provided that it is neither too large nor too small.

In Fig. 2 we present analogous curves when there is significant peak spreading. Figure 2A shows curves of $\bar{R}$ for constant gel concentration

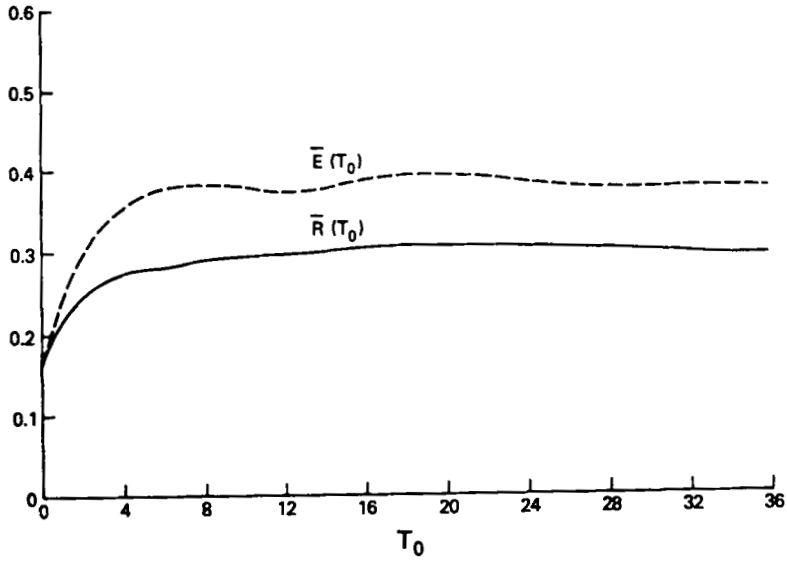

FIG. 2A. Comparison of $\bar{R}(T_0)$ and $\bar{E}(T_0)$ as a function of uniform gel concentration T_0.

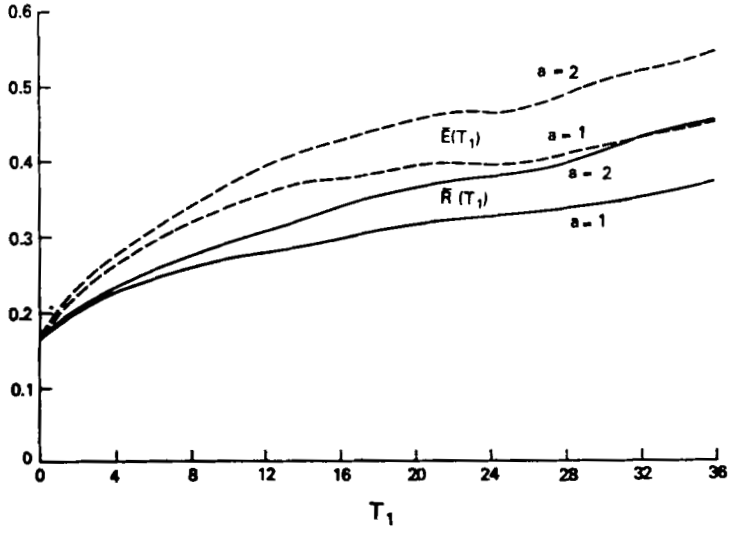

FIG. 2B. Comparison of $\bar{R}(T_1)$ and $\bar{E}(T_1)$ in the case of a gradient, with two values of a for different dependence of bandspreading on gel concentration. The concentration at the base of the column is $T_0 = 0$.

and the entropy analog, $\bar{E}$. Both criteria indicate that any gel concentration over 8% gives roughly the same degree of resolution. In Fig. 2B we make the same comparison when a gradient is present, including, in addition, the effect of varying the dependence of diffusion constant on the gel concentration by means of the parameter a. Again, both criteria ($\bar{R}$ and $\bar{E}$) lead to the conclusion that the steepest possible gradient should be used to achieve the best separation. In Fig. 3 we show curves of $\bar{R}$ for the case of a gradient for three different gel concentrations (T_0) at $x = 0$. As can be observed there is some slight gain to be had by having as high an initial ($x = 0$) gel concentration as possible, but the separation criterion is not very sensitive to this parameter.

Two-Dimensional Fractionation

In our discussion of the two-dimensional case we follow the convention that the gel concentrations in the first dimension go from T_0 to T_1 and in the second dimension go from T_0' to T_1'.

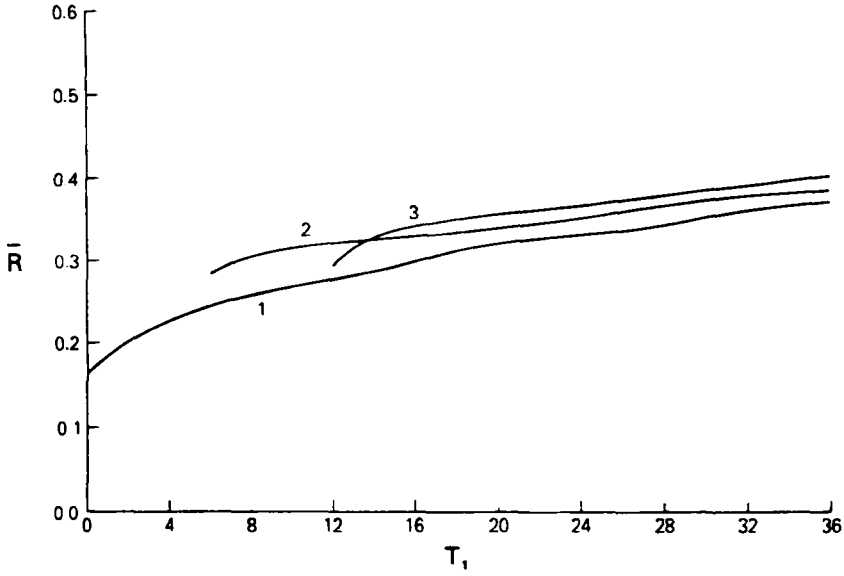

FIG. 3. Curves of $\bar{R}(T_1)$ for a gel gradient for three values of T_0. 1: $T_0 = 0$. 2: $T_0 = 6$. 3: $T_0 = 12$.

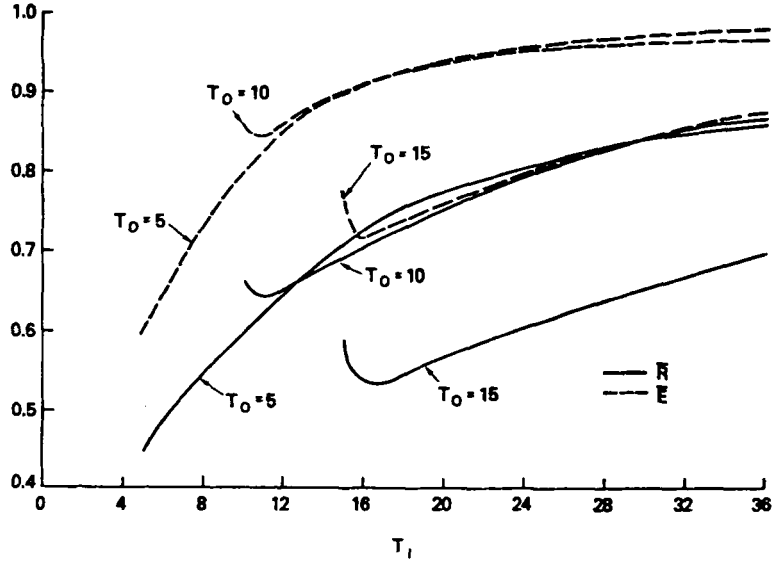

FIG. 4. Curves of $\bar{R}$ and $\bar{E}$ for two-dimensional gel electrophoresis as a function of T_1' for a constant gel concentration in the first direction.

We show several curves of $\bar{R}$ and $\bar{E}$ for the two-dimensional case in Fig. 4. A constant gel concentration, T_0, is used in the first direction and a $0 - T_1'$ linear gradient is assumed for the second direction. The agreement between the two criteria is evident and leads us to conclude that either criterion will lead to closely similar sets of parameters. Figures 5A and 5B give some indication of the improvement in performance that can be gained through the use of sodium dodecyl sulfate (SDS) in the second direction (*10*, *15*, *16*), thus allowing (ideally) for pure size separation. In Fig. 5A we compare two basic cases, the first corresponding to a constant gel concentration in the first direction and a gradient in the second, and the second case consisting of a constant gel concentration in the first direction, followed by treatment with SDS and a gradient in the second direction. The $\bar{R}$ criterion is plotted as a function of T_1'. Curves 1 and 2 show $\bar{R}$ without the use of SDS, and curves 3 and 4 include treatment with SDS. The considerable improvement in separation by use of SDS in the second direction is obvious from these curves. Figure 5B shows curves of $\bar{R}$ against T_0' for constant gel concentrations in both directions,

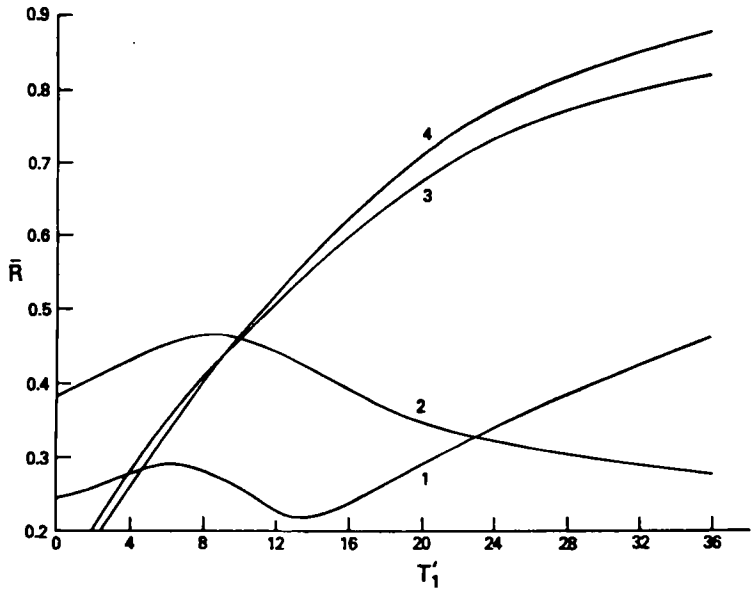

FIG. 5A. A comparison of two dimensional electrophoresis with and without SDS by means of $\bar{R}(T_1')$. Curve 1 represents a constant concentration of 5% in the first direction, a $0 - T_1'$ gradient in the second, and no SDS. Curve 2 gives the same function for a constant 10% gel in the first direction. Curve 3 assumes a 5% gel in the first direction followed by treatment with SDS and a $0 - T_1'$ gradient in the second. Curve 4 is for a 10% gel in the first direction and no SDS pore gradient in the second.

using the values of $T_0 = 5$ and 10 in the first direction and various values of T_0' in the second. A comparison of Figs. 5A and 5B indicates that the constant gel concentration system does a better job at separating the species than does the system with a constant concentration and a gel gradient, provided that SDS is used.

We have also made calculations of $\bar{L}$ for perpendicular pore gradient electrophoresis in which the amount of migration in the first direction determines the constant gel concentration in the second direction (*10*). The results of our calculations indicate that this separation technique is ineffective compared to constant gel concentrations in both directions, the average distances on a dimensionless scale being less than 0.1 in all cases.

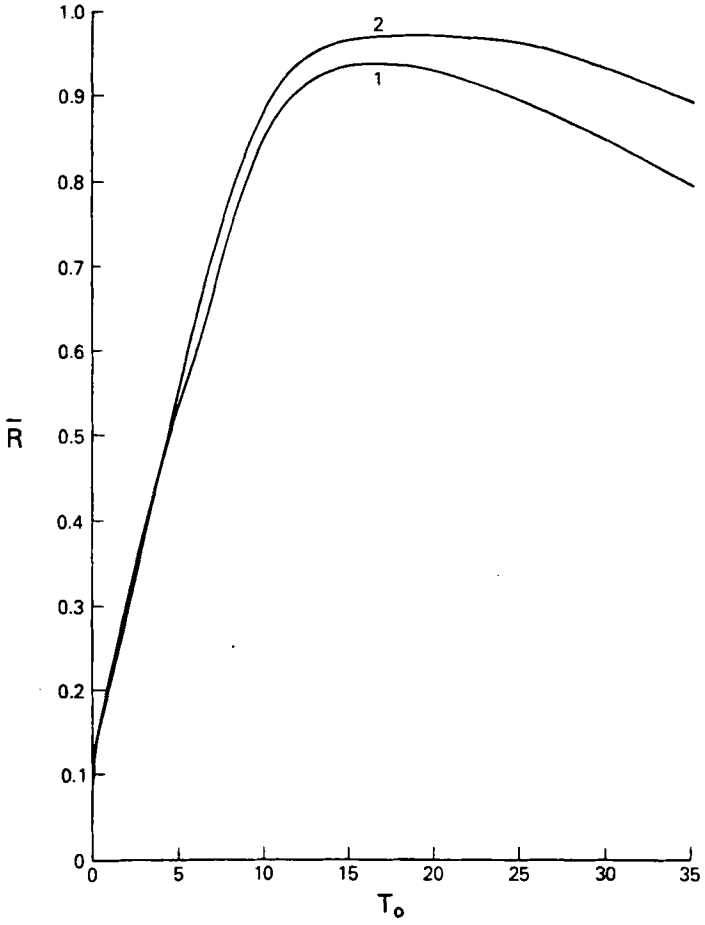

FIG. 5B. Curves of $\bar{R}(T_0')$ for a constant T_0'% gel in the second direction and (1) a constant 5% gel in the first direction, ($T_0 = 5$), or (2) a constant 10% gel in the first direction ($T_0 = 10$). The use of SDS is assumed.

DISCUSSION

We have compared several criteria for evaluating the effectiveness of separations by one- and two-dimensional analytical systems, with or without a gradient and with or without equalization of free mobility by SDS. Fortunately, it appears that the evaluation criteria are not critical, and the optimal parameters predicted by all criteria within the class defined are very similar. In order to use these criteria, however, it is necessary to know something about the molecular species to be separated. An interesting extension of the present work would be to assume a known probability distribution of the parameters of each molecular species rather than the parameters directly. Another generalization would be to consider the effects of size and charge microheterogeneity. The results here can be readily generalized to consider the use of isoelectric focusing as one of the dimensions of fractionation. Superficially, this is analogous to the use of $T_0 = T_1 = 0$, i.e., pure "charge" fractionation.

Some of the results shown in Figs. 1 to 5 may appear to be contrary to intuition and should be significant in the design of experiments. For the arbitrary grid of 25 (V_0, k_R) pairs that have been chosen, we see that there is little to be gained by use of a gradient steeper than $T_1 - T_0 = 10$, and we note that the $\%T$ in other dimension should be as far removed as possible from the average of T_0 and T_1.

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