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A Note on the Resolution of Two Gaussian Peaks

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A Note on the Resolution of Two Gaussian Peaks

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Abstract

We consider the utility of the usual definition of the resolution of two peaks in chromatography in terms of the height of the valley between the two peaks. We show that over a wide range of relative amounts and bandwidths, the separation requirement $R = 1$ is very conservative.

There is a considerable literature on the definition of resolution in chromatography. In particular, the definition

$$R = \frac{\mu_2 - \mu_1}{2(s_1 + s_2)} \tag{1}$$

is a common one, where the μ_i are peak positions and the parameters s_i are standard deviations of the component peaks. The utility of R as defined in Eq. (1) is generally measured in terms of contaminant ratios, following Glueckauf (*1*). In many biochemical applications these ratios are of less importance than the visual resolution of the peaks, i.e., the biochemist wants to know the number of different substances in a given compound without necessarily physically separating them. It is interesting in this context to inquire as to how well R achieves separation, taking into account differences in peak areas and standard deviations.

In this note we show that R, as defined in Eq. (1), is insensitive to differences in peak concentrations and s_2/s_1 over a wide range of values of these parameters, when the concentration peaks are Gaussian. We will not discuss the more interesting problem of multipeak resolution in the present paper, except to say that it would be hard to do so without making possibly unrealistic simplifying assumptions about equality of peak areas and/or values of the μ_i (*2, 3*). We assume in what follows that the two concentration profiles have the form

$$c_1(x) = \frac{1}{\sqrt{2\pi}} \exp(-x^2/2), \qquad c_2(x) = \frac{m}{s\sqrt{2\pi}} \exp\left[-\frac{(x-\mu)^2}{2s^2}\right] \tag{2}$$

so that the first peak, which has area = 1, is centered at $x = 0$, and has $\sigma = 1$. The second peak, which has area = m which we can choose as ≤ 1, is centered at $x = \mu$, and has $\sigma = s$. The observable profile is then $c(x) = c_1(x) + c_2(x)$. We follow de Clerk and Buys (*4*) in relating resolution to the existence of an interpeak valley, and measure the quality of resolution by a parameter U defined to be $U = c_{max}/c_{min}$, where c_{min} is the minimum concentration between peaks where the minimum exists

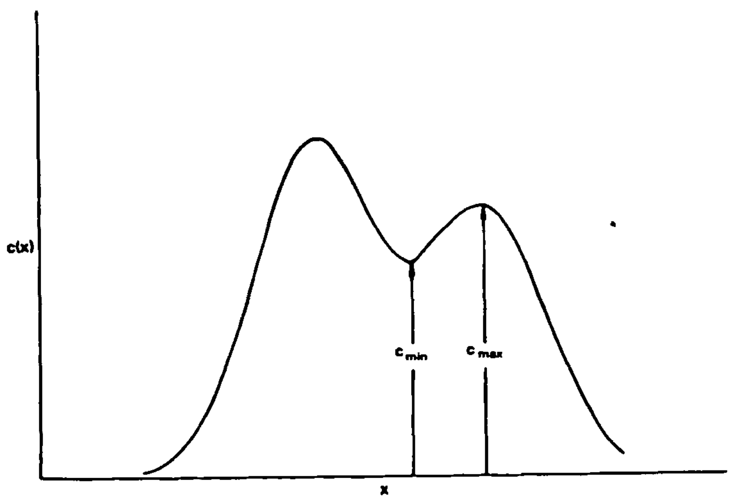

FIG. 1. Concentration profile with definition of parameters.

and c_{max} is the maximum concentration of the lower of the two peaks. These concentrations are illustrated in Fig. 1. The resolution now takes the somewhat simpler form

$$R = \frac{\mu}{2(s + 1)} \tag{3}$$

Let us first consider the equiconcentration case in which $m = 1$, and plot the parameter U as a function of s for fixed values of R. The resulting curves are shown in Fig. 2. Notice that if one fixes R and varies s, the mean of the second peak, μ, must also vary. This variation of a and μ jointly leads to the observed minima in the curves of U. When $R = 1$ the minimum is approximately 3.6, which guarantees clear recognizability in all cases. The curve of U for $R = 0.7$ has a minimum of approximately

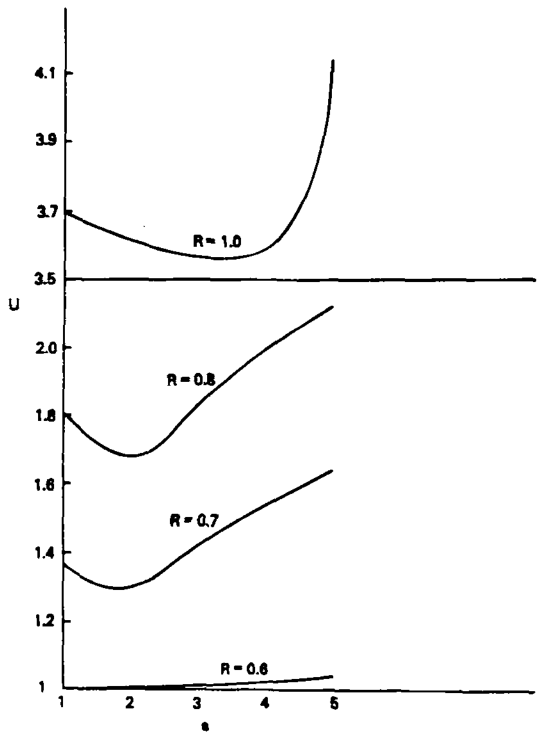

FIG. 2. Curves of U as a function of s for $m_1 = m_2 = 1$.

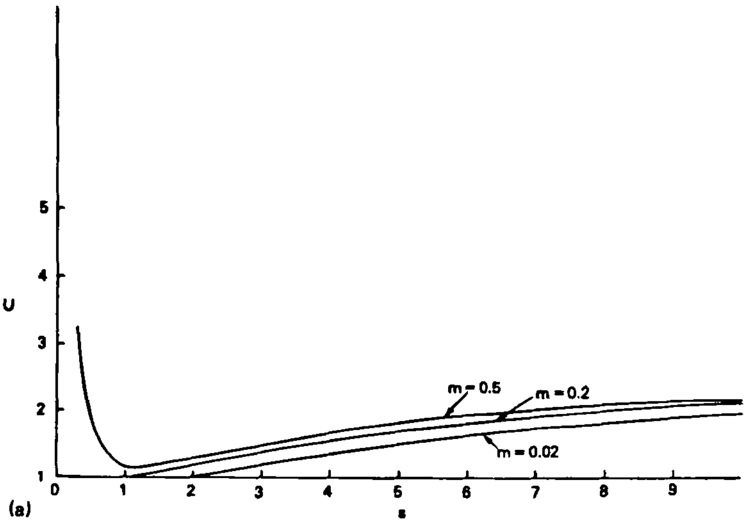

FIG. 3A. Curves of U as a function of s for different values of m for $R = 0.75$.

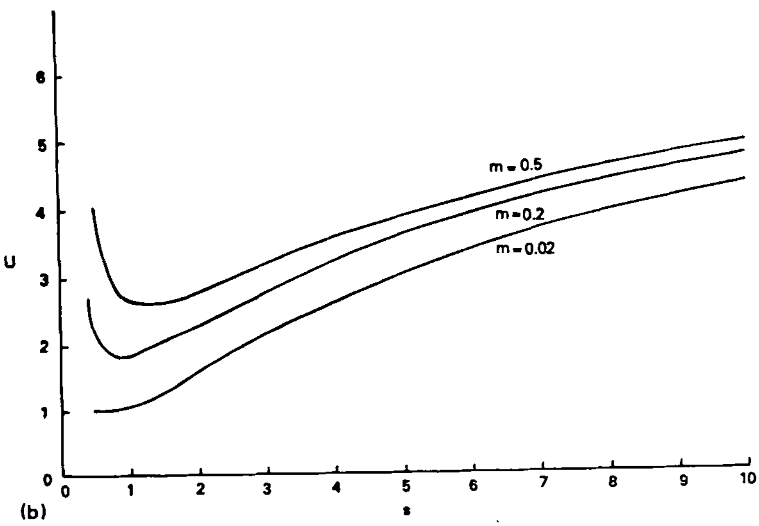

FIG. 3B. The same as Fig. 3A with $R = 1$.

1.3, which is a conservative lowest value of U that guarantees visual separation in the presence of data and baseline noise. The value of U increases considerably away from its minimum position, but for large values of s this corresponds to having a very low peak which may not be recognizable as such. However, such large differences in s are usually found in situations in which the Gaussian assumption is inappropriate.

Since the definition of R in Eq. (1) has no provision for including the relative values of m, there is an implication that it can be used as a separation criterion for all values of m. Without losing generality in the examination of this question, we can restrict ourselves to $m \leqslant 1$. In Figs. 3A and 3B we show curves of U as a function of s for $R = 0.75$ and $R = 1$ with different values of m. A clear separation is indicated for $m = 0.5$ and 0.2 (Fig. 1 has the curve for $m = 1$), and the curve for the extreme case $m =$ 0.02 is also quite close to the other two. For this last case one must

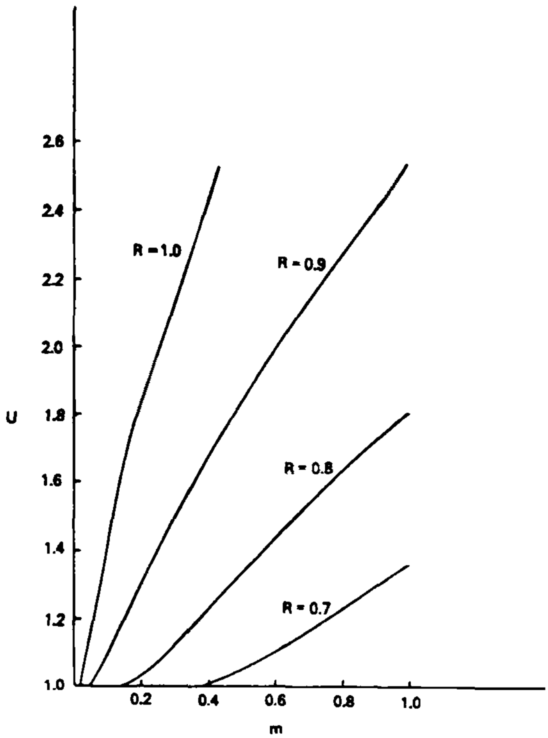

FIG. 4A. Curves of U as a function of m for a $R = 0.7, 0.8, 0.9$, and 1.0.

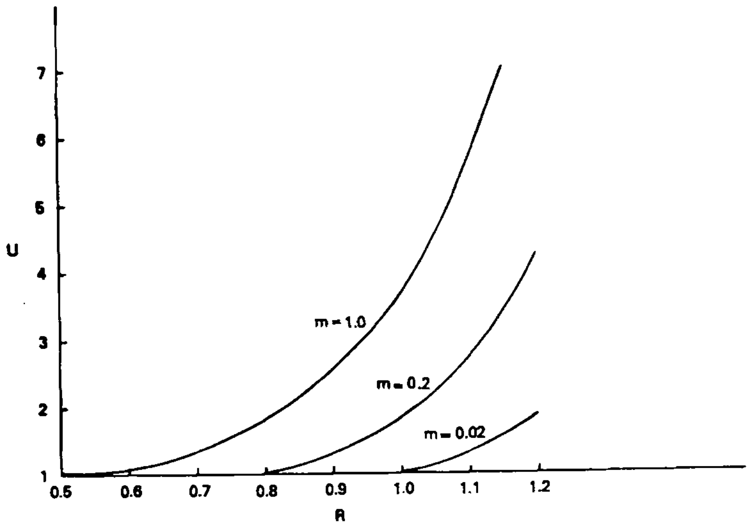

FIG. 4B. Curves of U as a function of R for $m = 0.02$, 0.2, and 1.

remember that the ratio of the maximum concentrations at the peaks, when the peaks are isolated, is m/s, which for $s \geqslant 1$ would probably not be measureable.

In Fig. 4A we show curves of U as a function of R for $s_1 = s_2 = 1$ for different values of m. If we define a critical value of resolution to be $U^* = 1.25$, then we see that for $m = 1$, the value of resolution that achieves this separation is $R \sim 0.67$, for $m = 0.2$ it is $R \sim 0.9$, and only for the unrealistically low value of $m = 0.02$ does the critical value of R exceed 1. Figure 4B contains the companion curves of U as a function of m for different values of R. If we again adopt the critical value $U^* = 1.25$, it follows from these curves that a resolution of $R = 0.7$ achieves this value for all $m \geqslant 0.83$, $R = 0.8$ for all $m \geqslant 0.41$, $R = 0.9$ for all $m \geqslant 0.17$, and $R = 1.0$ for all $m \geqslant 0.07$. Thus we see that in terms of the ratio U the requirement $R = 1$ is conservative over a wide range of values of m, and the curves in Fig. 3 suggest that this is also true over a wide range of relative variances.

There are presently many techniques for separation in two dimensions (*5*). For this case one can define a measure of resolution analogous to that given in Eq. (1). In ideal systems, i.e., systems with no concentration-dependent parameters and uniform fields, the contours of equal concentration for an isolated peak are elliptical, with a major axes equal to $\sigma_x\sqrt{2}$ and $\sigma_y\sqrt{2}$ in the x and y directions. The analog of resolution that might be considered is

$$R = \frac{1}{2}\left\{\left(\frac{x_2 - x_1}{\sigma_x + \sigma_x'}\right)^2 + \left(\frac{y_2 - y_1}{\sigma_y + \sigma_y'}\right)^2\right\}^{1/2} \tag{4}$$

where (x_i, y_i) are the peak centers and σ_x' and σ_y' belong to the second peak. A parameter U corresponding to this R can be defined by joining the centers of the two peaks and calculating the minimum concentration along that line. However, there are obvious deficiencies in the definition of resolution in Eq. (4), and an exploration of plausible alternatives as in (*6*) would seem to be desirable.

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