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CORRECTION OF THE RESOLUTION FUNCTION FOR NON-IDEAL PEAKS

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SUMMARY

The resolution function is commonly used to describe the extent of separation between successive peaks in a chromatogram. However, the resolution is usually defined in such a way that it is applicable only to symmetrical (Gaussian) peaks. Moreover, the resolution does not provide a realistic estimate of the extent of separation between two peaks with greatly different areas. Nevertheless, the main advantage of the resolution is that its value can be predicted from retention and efficiency data for the individual peaks. Simple methods are described to correct the resolution function for (i) large variations in peak areas and (ii) peak asymmetry. The corrections are derived as modifications of the resolution equation. An important consequence of these modifications is that the resolution for a pair of peaks has two different values, one for each peak. The new resolution equations were evaluated using computer-generated (exponentially modified Gaussian) peak profiles. The effects of varying degrees of peak asymmetry and varying peak-area ratios were studied.

INTRODUCTION

The most common way to describe the extent of separation between two successive peaks, i and j, in a chromatogram is by the resolution (R_s), which is defined as

$$R_s = \frac{t_j - t_i}{{}^1\!/_2(w_i + w_j)} \tag{1}$$

where t is the retention time and w is the peak width. For Gaussian peaks the width is usually assumed to equal four times the standard deviation, σ, corresponding to the width of the peak at 13.5% (e^{-2}) of its height. This results in

$$R_s = \frac{t_j - t_i}{2(\sigma_i + \sigma_j)} \tag{2}$$

When eqn. 2 can be used to describe the extent of separation, the resolution can be predicted if the retention times and the standard deviations are known. For Gaussian peaks, the plate count, which is defined by

$$N_i = \left(\frac{t_i}{\sigma_i}\right)^2 \tag{3}$$

can be substituted for σ, yielding

$$R_s = \frac{t_j - t_i}{2t_i/\sqrt{N_i} + 2t_j/\sqrt{N_j}} \tag{4}$$

If, moreover, the plate count is the same for the two peaks ($N_i = N_j = N$), then

$$R_s = \frac{t_j - t_i}{t_i + t_j} \cdot \frac{\sqrt{N}}{2} \tag{5}$$

or, in terms of the capacity factor, k,

$$R_s = \frac{k_j - k_i}{2 + k_i + k_j} \cdot \frac{\sqrt{N}}{2} \tag{6}$$

Eqns. 5 and 6 are very important for chromatography. Under the assumption of equal N values, they allow the resolution to be calculated if the retention data (in terms of t or k) are known. This is especially important in two areas:

(i) *Column optimization.* Chromatographic theory allows the effects of operating parameters (e.g., flow-rate) and column characteristics (column length and diameter, particle size) to be predicted accurately. This allows the optimization of the column and operating parameters in order to obtain sufficient resolution in the shortest possible time, with the highest possible (detection) sensitivity, etc. (see, e.g., ref. 1, Chapter 7). Computer simulation[2] may be used to optimize the conditions. However, in order to optimize the resolution, it must be possible to calculate its value under varying conditions in practical (non-symmetrical peaks of different height) rather than theoretical (Gaussian peaks of equal height) situations.

(ii) *Selectivity optimization.* A number of methods have been developed especially for the optimization of chromatographic selectivity[1]. These interpretive methods rely on the observation that the retention of individual solutes can be predicted from a few experimental data. The quality of the separation in the entire chromatogram is a much more complicated function, which can be calculated from the retention data of the individual solutes if a sensible value for the resolution can be calculated from the retention data.

Alternative measures for the extent of separation between successive peaks have been suggested (see ref. 1, section 4.2). In particular, resolution criteria may be defined on the basis of peak-to-valley or valley-to-peak ratios. The main advantage of these empirical functions (abbreviated to P values) is that they are more generally valid for

chromatographic peaks of any shape or size, as long as the required characteristics (valley and peak heights) can be obtained from the chromatogram. A first major disadvantage is that there will be a threshold range in which peaks overlap severely, but not completely. In this range the resolution may differ significantly from zero, but no valley can be observed so that all P values equal zero. Secondly, for non-Gaussian peaks P values cannot be predicted on the basis of retention times and column efficiencies (plate counts), so that they cannot be used in the two important areas outlined above.

In this paper we shall try to define simple equations for calculating the resolution (R_s) in practical situations. In addition to retention and efficiency data, some information will necessarily be required on the size (for peak-height correction) or the asymmetry of the actual peaks, but one of the aims of this work was to keep the corrections as simple as possible and the number of additional parameters to a minimum.

THEORY

Large variations in peak areas

One factor that appears to affect the resolution between two successive peaks in a chromatogram, but which is not taken into account in any of the equations given in the Introduction, is the (relative) height or area of the two peaks (see, *e.g.*, ref. 3, Section 2.5). When two successive peaks have different heights (or areas), the relative overlap is larger for the smaller peak. The relative overlap can be found from the part of the peak area where the two solutes are eluted together (A_{ij}) and the total area of the peak. For example, for the first peak (i) in Fig. 1,

$$RO_i = \frac{A_{ij}}{A_i} \tag{7}$$

The second peak in Fig. 1 overlaps with both the first and third peaks, so that

$$^iRO_j = \frac{A_{ij}}{A_j} \tag{8}$$

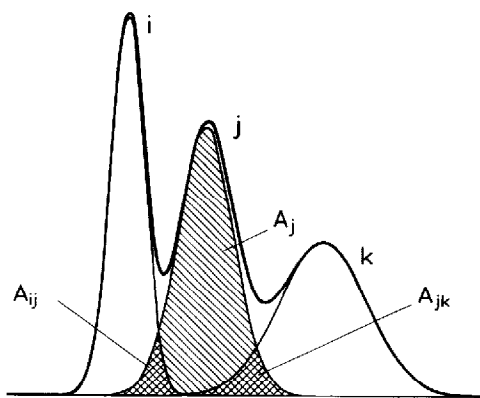

Fig. 1. Illustration of the relative peak overlap (RO).

and

$$^kRO_j = \frac{A_{jk}}{A_j} \tag{8a}$$

If the detection sensitivity is similar for the different peaks, then the relative overlap is a good indication of the extent to which solutes are separated. RO is not a practical resolution criterion, because it is very difficult to calculate its value from a chromatogram.

It is clear from eqns. 7 and 8 that RO will be different for peaks i and j if the areas A_i and A_j are different. This is different from the resolution, R_s, in the situation of eqns. 5 and 6. Both equations yield a single (absolute) value for each pair of peaks (i.e., $R_{s,ji} = -R_{s,ij}$). If we are to correct the resolution for the difference in height between successive peaks, then this symmetry will no longer be found. Instead, there will be two different resolution values describing the separation between two successive peaks: one describing the extent to which the first peak is separated ($R_{s,i}$) and one to characterize the separation of the second peak ($R_{s,j}$).

A Gaussian peak is described by

$$f_i(t) = h_i \exp -\frac{1}{2}\left(\frac{t - t_i}{\sigma_i}\right)^2 \tag{9}$$

where h_i is the height of the peak, t_i the retention time at the peak maximum and σ_i the standard deviation. The width of the peak is $4\sigma_i$ (i.e., $t - t_i = 2\sigma_i$) when $f_i(t)$ equals $h_i e^{-2} = 0.135h_i$. For a second peak (j) we find for the peak width at this height

$$h_i \exp(-2) = h_j \exp -\frac{1}{2}\left(\frac{t - t_j}{\sigma_j}\right)^2 \tag{10}$$

from which we find

$$\left(\frac{t - t_j}{\sigma_j}\right)^2 = 4 + 2 \ln(h_j/h_i) \tag{11}$$

or

$$t = t_j \pm \sigma_j\sqrt{4 + 2 \ln(h_j/h_i)} \tag{12}$$

so that the width of the second peak at 13.5% of the height of the first peak (indicated by the prefix i) is

$$^iw_j = 2\sigma_j\sqrt{4 + 2 \ln(h_j/h_i)} \tag{13}$$

We may now obtain the resolution of the first peak by applying eqn. 1 at $h = 0.135h_i$:

$$^iR_{s,ji} = \frac{t_j - t_i}{2\sigma_i + \sigma_j\sqrt{4 + 2 \ln(h_j/h_i)}} \tag{14}$$

If $h_i = h_j$, eqn. 14 reduces to eqn. 2. If we introduce the plate count (eqn. 3) into eqn. 14, we find

$$^iR_{s,ji} = \frac{(t_j - t_i)\sqrt{N_iN_j}}{2t_i\sqrt{N_j} + t_j\sqrt{N_i}\sqrt{4 + 2\ln(h_j/h_i)}} \tag{15}$$

and if $N_i = N_j = N$, then

$$^iR_{s,ji} = \frac{(t_j - t_i)\sqrt{N}}{2t_i + t_j\sqrt{4 + 2\ln(h_j/h_i)}} \tag{16}$$

An analogous argument for the second peak at $h = 0.135h_i$ leads to

$$^jR_{s,ji} = \frac{(t_j - t_i)\sqrt{N}}{t_i\sqrt{4 + 2\ln(h_i/h_j)} + 2t_j} \tag{16a}$$

According to eqns. 16 and 16a, the largest resolution value is obtained for the largest of the two peaks ($^iR_{s,ji} \geq {}^jR_{s,ji}$ if $h_i \geq h_j$ and *vice versa*). Eqn. 16 may be used in interpretive optimization procedures, where the retention surfaces are modelled as a function of the parameters to be optimized. For Gaussian functions there is no need to model the peak heights, as h will vary according to

$$h_i = \frac{h_i^0}{t_i} = \frac{h_i^0}{1 + k_i} \tag{17}$$

where h_i^0 is the height peak i would have if it were to be eluted at $t_i = t_0$ ($k_i = 0$). Only one experimental chromatogram is necessary to calculate h^0 values for all the peaks and eqn. 16 may be rewritten as

$$^iR_{s,ji} = \frac{(t_j - t_i)\sqrt{N}}{2t_i + t_j\sqrt{4 + 2\ln(h_j^0/h_i^0) + 2\ln(t_i/t_j)}}$$

$$= \frac{(k_j - k_i)\sqrt{N}}{2 + 2k_i + (1 + k_j)\sqrt{4 + 2\ln(h_j^0/h_i^0) + 2\ln\{(1 + k_i)/(1 + k_j)\}}} \tag{18}$$

The two separate criteria describing the resolution between two peaks may be used in different situations. If both i and j are relevant peaks in the chromatogram, then the lower value appears to be the more relevant. This implies that only the resolution for the smaller of two peaks should be considered. If, however, the analytical problem is to quantify the amount of solute corresponding to the larger peak and the small peak is a contaminant, the concentration of which is not relevant, then the resolution for the large peak is the relevant number.

Careful considerations are required for using corrected resolution values in criteria describing the quality of separation for complete chromatograms rather than for pairs of successive peaks (see ref. 1, p. 140).
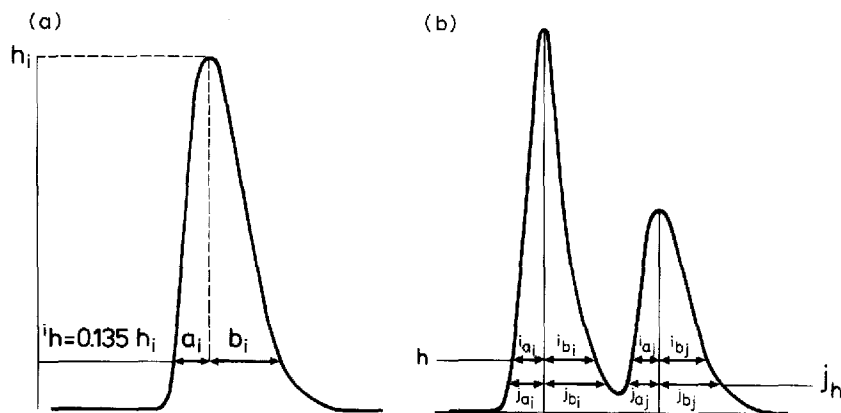
Fig. 2. (a) Illustration of the definition of the peak-width parameters a and b on the ascending and descending slope of a peak, respectively, at a certain fraction of the peak height (here 13.5%). (b) Illustration of the relevant parameters determining the resolution between two asymmetric peaks. $^{i}a_i$, $^{i}b_i$, $^{i}a_j$ and $^{i}b_j$ are measured at the reference height for peak i, i.e., $^{i}h = 0.135h_i$ and $^{j}a_i$, $^{j}b_i$, $^{j}a_j$ and $^{j}b_j$ are measured at the reference height for peak j, i.e., $^{j}h = 0.135h_j$.

Non-symmetrical peaks

For non-Gaussian peaks eqn. 2 is strictly invalid, but eqn. 1 may be used as long as the peaks are symmetrical. For non-symmetrical peaks $^{1}/_{2}w$ is not a good indication of the relevant peak width. Therefore, eqn. 1 may not be used. Instead[4], the relevant widths are those of the descending (end) slope of peak i (b_i) and the ascending (beginning) slope of peak j (a_j). This is illustrated in Fig. 2a, where it can be seen that a and b are defined on one slope of the peak, relative to the peak top. Parameters similar to a and b have been used to correct resolution values for the occurence of large, non-symmetrical peaks in a chromatogram[4]. For Gaussian peaks $a = b = {}^{1}/_{2}w$. In the general situation, in which the peaks are not Gaussians, we find

$$R_s = \frac{t_j - t_i}{b_i + a_j} \tag{19}$$

The parameter commonly used to characterize the degree of asymmetry of chromatographic peaks is the asymmetry factor, A_s, which is defined at a certain fraction, x, of the peak height as

$$A_s^x = \frac{b}{a} \tag{20}$$

At different values of x, not only will a and b be different, but their ratio may also change. Often, x is arbitrarily chosen to be 0.1, but $x = 0.135$ appears to be more logical (see above). However, when we define A_s at a certain fraction of the peak height, then the absolute height at which a and b are measured in the chromatogram will generally be different for each peak in the chromatogram. This is illustrated in Fig. 2b. At any given height in the chromatogram we can, in principle, measure a and b values.

If we take peak i as our reference peak (as indicated by the prefix before the symbols), then the obvious "observation height" is $0.135h_i$. At this height A_s can be calculated from eqn. 20 and the plate count from

$$^iN_i = 16\left(\frac{t_i}{^ia_i + {}^ib_i}\right)^2 \tag{21}$$

which for peak i itself is the conventional definition of N, but for all other peaks yields different values:

$$^iN_j = 16\left(\frac{t_j}{^ia_j + {}^ib_j}\right)^2 \tag{21a}$$

Eqn. 21a yields smaller N values ($^iN_j < {}^jN_j$) if peak j is larger than peak i and larger values ($^iN_j > {}^jN_j$) if peak j is smaller. Substitution of eqns. 20 and 21 in eqn. 19 yields

$$^iR_{s,ji} = \frac{(t_j - t_i)(1 + {}^iA_{s,i})(1 + {}^iA_{s,j})\sqrt{^iN_i^iN_j}}{4^iA_{s,i}t_i(1 + {}^iA_{s,j})\sqrt{^iN_j} + 4t_j(1 + {}^iA_{s,i})\sqrt{^iN_i}} \tag{22}$$

If the peaks are symmetrical ($A_s = 1$), eqn. 22 reduces to eqn. 4.

Eqn. 22 is fairly complex and it requires four parameters to obtain it from the chromatogram at the reference height (e.g., ^ia_i, ^ib_i, ^ia_j and ^ib_j). This is not very attractive and it may be difficult if the peaks are poorly resolved. Nevertheless, it has been demonstrated that relative peak widths (similar to a and b values) can be monitored during selectivity optimization procedures, yielding a separate surface next to the retention surfaces[4]. In the most dramatic situations, in which very large solvent or matrix peaks are present in the chromatogram, such a procedure should be followed. However, in situations in which the peaks are asymmetric and in which successive peaks differ significantly (e.g., by more than a factor of two) but not dramatically (e.g., by not more than a factor of ten) in height we should be looking for simplifications.

In practical situations we find it acceptable to require measurements of the width and the asymmetry of each individual peak, if these factors are to be taken into account. Hence, ^iN_i, $^iA_{s,i}$, ^jN_j and $^jA_{s,j}$ will usually be determined. However, the width and asymmetry of a peak at a reference height, determined by another peak (e.g., ^iN_j and $^iA_{s,j}$), will not usually be available. From this limited information the most sensible approximation is to assume the asymmetry factors to be independent of the height, i.e., in eqn. 20 A_s^x is assumed to be independent of x, but to correct for differences in the heights between successive peaks. In terms of eqn. 19 this leads to

$$^iR_s = \frac{t_j - t_i}{^ib_i + {}^ia_j} = \frac{t_j - t_i}{^ib_i + {}^ja_j\sqrt{1 + {}^1/_2 \ln(h_j/h_i)}} \tag{23}$$

With eqns. 20 and 21 we now find for the first peak

$$^{i}R_{s,ji} = \frac{(t_j - t_i)(1 + {}^{i}A_{s,i})(1 + {}^{j}A_{s,j})\sqrt{{}^{i}N_i^{j}N_j}}{4{}^{i}A_{s,i}t_i(1 + {}^{j}A_{s,j})\sqrt{{}^{j}N_j} + 4t_j(1 + {}^{i}A_{s,i})\sqrt{{}^{i}N_i}\sqrt{1 + {}^{1}/_{2}\ln(h_j/h_i)}} \quad (24)$$

The analogous equation for the second peak is

$$^{j}R_{s,ji} = \frac{(t_j - t_i)(1 + {}^{i}A_{s,i})(1 + {}^{j}A_{s,j})\sqrt{{}^{i}N_i^{j}N_j}}{4{}^{i}A_{s,i}t_i(1 + {}^{j}A_{s,j})\sqrt{{}^{j}N_j}\sqrt{1 + {}^{1}/_{2}\ln(h_j/h_i)} + 4t_j(1 + {}^{i}A_{s,i})\sqrt{{}^{i}N_i}} \quad (24a)$$

If, moreover, we assume that $^{i}N_i = {}^{j}N_j = N$ and that $^{i}A_{s,i} = {}^{j}A_{s,j} = A_s$, then we find a simple expression for a corrected resolution function for peak i:

$$^{i}R_{s,ji} = \frac{(t_j - t_i)(1 + A_s)\sqrt{N}}{4A_s t_i + 4t_j\sqrt{1 + {}^{1}/_{2}\ln(h_j/h_i)}} \quad (25a)$$

For the second peak (j) the corresponding equation is

$$^{j}R_{s,ji} = \frac{(t_j - t_i)(1 + A_s)\sqrt{N}}{4A_s t_i\sqrt{1 + {}^{1}/_{2}\ln(h_i/h_j)} + 4t_j} \quad (25b)$$

Eqns. 25a and b present a simple means of correcting for both the asymmetry of peaks and for variations in peak height. However, unlike eqns. 16 and 18, Eqn. 25a is not exact. If the two peaks considered show vastly different peak widths or asymmetry factors then, strictly, eqn. 22 should be applied.

For optimization purposes, eqns. 25a and b again represent two criteria for the separation between a pair of successive peaks, *i.e.*, one for each peak. Either or both of these may be used for optimization purposes in three different manners:

(1) correcting for differences in peak heights; this can be done if the concentrations in the sample are constant (*e.g.*, in quality control situations);

(2) assuming that $h_i = h_j$ if the concentrations are not constant;

(3) correcting for the largest possible difference in heights between peaks i and j, based on an expected range of possible solute concentrations. In this latter instance one should be aware of the increasingly approximate character of eqns. 25a and b with increasing peak-height ratios.

In the following evaluation the usefulness of the rigorous eqn. 22 and the simplified eqns. 24 and 25a and b will be examined.

EVALUATION PROCEDURE

In order to evaluate the applicability of the equations derived in this paper for characterizing the resolution in non-ideal situations, we used a series of computer-generated peak profiles. Exponentially modified Gaussian (EMG) functions provide a very accurate description of the true peak shape in analytical (reversed-phase) liquid

chromatography (LC). In other forms of chromatography the peak shapes may be different. For example, this will be the case in preparative LC. We have tested the present equations for EMG peaks, because of our intention to apply them for the purpose of optimizating analytical separations. This does not imply that use of the equations should be limited to EMG peaks. For other asymmetric peaks they will almost certainly yield more useful values than the conventional definition for R_s.

A series of EMG peaks were generated, with varying values for the time constant (τ) of the exponential decay[8]. The parameters of the Gaussian function were $t_{max} = 15$ min and $\sigma_t = 0.474$ min (corresponding to 1000 theoretical plates for $\tau = 0$) for the first peak. The values for τ were 0 (Gaussian peak), 1, 2, 3, 4 and 5 min. The t_{max} value of the second peak was increased from 15.8 to 19.0 min, while maintaining a plate count of 1000 for the Gaussian peak ($\sigma_t = t_{max}/\sqrt{1000}$). Different values for τ result in different values for the peak asymmetry, as is illustrated in Fig. 3. The asymmetry factor in this picture was obtained from the simulated peak profiles at a fraction of 13.5% of the peak height. It is seen that the present range of variation for τ corresponds to a range $1 \leqslant A_s < 3.23$. In practical chromatograms all peaks may have similar asymmetry factors, but large variations in A_s between successive peaks in a single chromatogram are also possible. For example, this is often the case in ion-pair chromatography with differently charged solutes. The situations examined in this paper were thought to provide the most severe tests for the proposed equations.

At the heart of the present evaluation method is a comparison between the values obtained from the modified resolution equations (eqns. 22, 24 and 25) and the relative overlap (eqns. 7 and 8), The relative overlap is thought to be the natural measure of chromatographic resolution. It is directly related to the extent to which a peak area can be used correctly for quantitative analysis, or the purity which can be obtained in a preparative separation. However, RO can only be determined if the individual peak profiles are known. In the present simulation study, the individual peak profiles are known so that a comparison can be made between the modified resolution functions and the relative overlap criterion. Ideally, a given value of RO should correspond to a unique value of the resolution, independent of the peak shape (asymmetry) and the peak-height ratio. If the calculated resolution value is plotted against RO, this ideal situation would lead to a single, monotonic line (*i.e.*, no minima or maxima) for all different conditions.

Further details of the evaluation procedure have been described elsewhere[9].
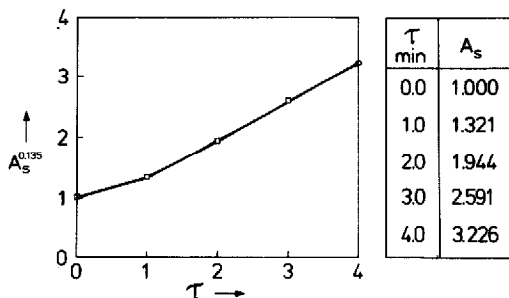

τ min	A_s
0.0	1.000
1.0	1.321
2.0	1.944
3.0	2.591
4.0	3.226

Fig. 3. Relationship between the exponential decay factor τ and the observed asymmetry factor at 13.5% of the peak height. Parameters for the Gaussian peak were $t_{max} = 15$ min and $\sigma_t = 0.474$ min (corresponding to $N = 1000$).

RESULTS

Fig. 4a shows the correlation between the resolution, calculated from eqn. 22, and the relative overlap for the first peak (RO_i) for situations in which the asymmetry of the first peak is varied. In this situation, the two peaks are of equal area. When the asymmetry of the first peak increases, its height decreases and therefore the ratio of peak heights also varies.

Along the horizontal axis, the difference in retention time between the peaks decreases from left to right, causing an increase in the relative overlap and a decrease in the calculated resolution. It is seen that there is little divergence between the different curves, so that the ideal situation of a single, monotonic relationship is approached. Only in the range where strong overlap occurs (*i.e.*, where the relative overlap becomes high and the resolution low) are different values for the resolution obtained for the peaks of different asymmetry with the same RO value. In this range a lower value for the resolution is obtained when the first peak becomes less asymmetrical.

Fig. 4b represents the same peak pairs as Fig. 4a, but both the resolution (eqn. 22) and the relative overlap are now calculated for the second peak. Because the tail of the first peak stretches far into (or even beyond) the second peak, the resolution of the latter is usually more difficult to determine, However, the different curves, corresponding to different asymmetry factors for the first peak, are still fairly close together.

In Fig. 5 the same peak pairs are characterized as in Fig. 4b, but the resolution is now calculated for the second peak with the approximated eqn. 24a. The results are seen to be virtually identical with those in Fig. 4b, and hence eqns. 24 and 24a are a good approximation of eqn. 22. This is of great practical value, because eqns. 24 and 24a only require the retention times, plate counts and asymmetry factors for each individual peak. The plate count may be obtained from the peak width at any given fraction of the peak height (in this case N has been obtained from eqn. 21, using the a and b values measured at 13.5% of the peak height). The asymmetry factors should
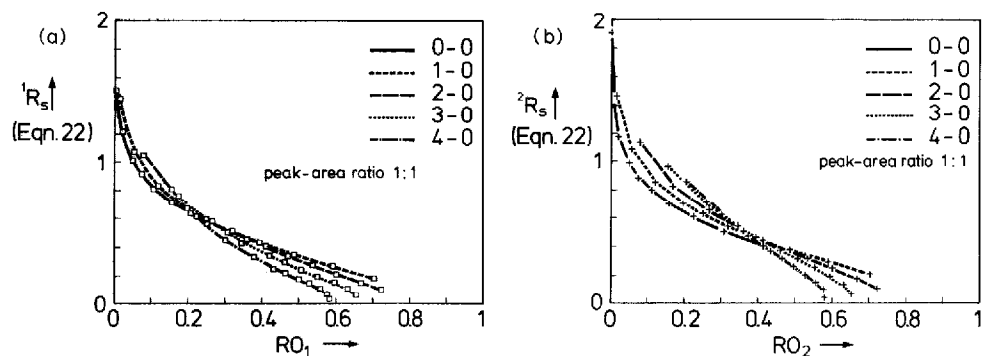

Fig. 4. Relationship between the relative peak overlap and the modified resolution calculated from eqn. 22 for two peaks of equal area. (a) Results for the first peak. (b) Results for the second peak. The exponential decay of the first peak was varied from $\tau = 0$ (———), $\tau = 1$ (---------), $\tau = 2$ (— — —), and $\tau = 3$ (· · · · ·) to $\tau = 4$ min (— · — · —). The second peak was symmetrical ($\tau = 0$). The retention time of the first peak was 15 min, that of the second peak was varied from 15.8 to 19 min. For both peaks the efficiency for the Gaussian function was taken to correspond to $N = 1000$.
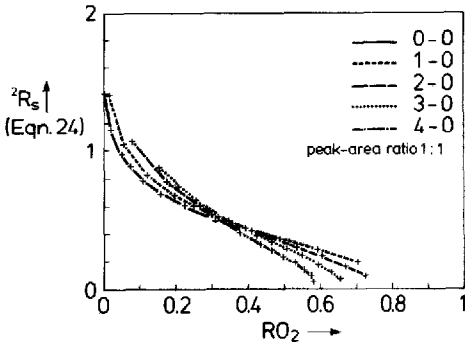
Fig. 5. As Fig. 4b, except that the modified resolution was calculated from eqn. 24a.

be obtained at 13.5% of the peak height. Eqn. 22 is much less attractive in practice, because it also requires the measurement of the peak width and asymmetry at the same fractions of the height of the preceding peak (and at the same fractions of the height of the following peak).

Fig. 6a shows that eqn. 25a is still a useful approximation in this instance. However, eqn. 25b (Fig. 6b) yields resolution values that are poorly correlated with RO. This is understandable, because eqn. 25b is based on the peak width and asymmetry of the second peak, which was taken to be symmetrical, while the asymmetry of the first peak increased. Naturally, poor results are obtained if a (strongly) asymmetric peak is assumed to be symmetrical. Nevertheless, this is exactly what is done when the conventional resolution equation is applied in this situation. Conventionally, both peaks are assumed to be symmetrical and the plate count of a symmetrical peak (*e.g.*, the second peak in the present peak pair) is used to calculate a value for N. Thus, a comparison of Figs. 4b, 5 and 6a with Fig. 6b serves to illustrate how much better it is to use the (approximated) modified resolution functions than it is to use the conventional resolution (eqn. 2).

It is important to realize that Fig. 4a and b show the resolution values each for one of the two peaks. Both the relative overlap and the modified resolution equations are based on the understanding that the true extent of separation in non-ideal
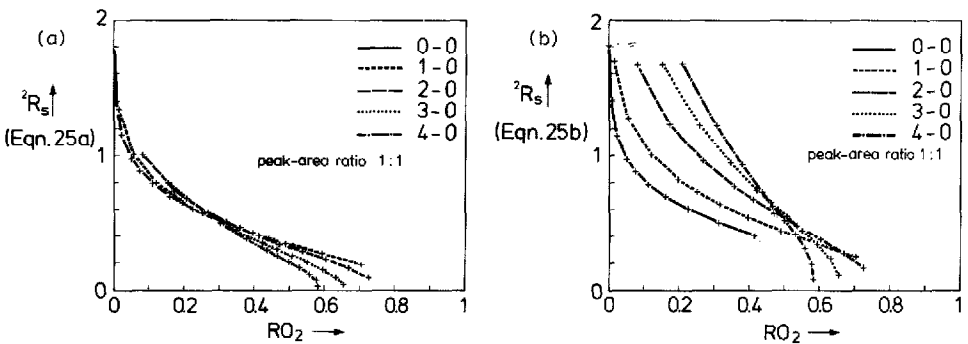

Fig. 6. As Fig. 4b, except that the modified resolution was calculated from (a) eqn. 25a and (b) eqn. 25b.

situations is different for the two peaks constituting a pair. However, because the areas of the two peaks are the same for the peak pairs used to make Fig. 4, the relative overlap is by definition the same for both peaks. Ideally, therefore, the curves in Fig. 4a and b should be identical, which is exactly true for the situation in which both peaks are Gaussian (solid lines). The extent of the deviation of the other curves from this line is a measure of the performance of eqn. 22.

In Fig. 7 we show two sets of curves for a situation which is similar to that in Fig. 4, except for the peak-area ratio. The area of the first peak is now four times larger than that of the second peak. As a result, the relative overlap is now much smaller for the first peak than it is for the second peak. This is seen in Fig. 7a. The highest possible RO for the first peak is 0.25. Increasing the asymmetry of the first peak is seen to have only a small effect on the relationship between the resolution calculated from eqn. 22 and RO. In Fig. 7b, which represents the data for the second peak, a different set of curves is observed, but again they are found close together, approaching the ideal situation.

Fig. 8 illustrates that eqn. 24a is still a reasonable approximation of eqn. 22 for the second (more difficult) peak in the situation of a 4:1 peak-area ratio. However, a larger variation is found between the different lines in Fig. 8 than in Fig. 7b. If the peak-area ratio becomes 8:1 (not shown), the difference between eqns. 22 and 24 becomes larger. For large peak-area ratios the former, less practical equation does need to be used. Eqn. 22 is essentially identical with the approach previously suggested for dealing with very large solvent or matrix peaks in a chromatogram[4].

Fig. 9 illustrates the effect of varying peak-area ratios. In Fig. 9a the modified resolution is calculated from eqn. 24a for the second in a pair of symmetrical peaks. In this situation the asymmetry factors are equal to 1 and eqn. 24a reduces to eqn. 16a. It can be seen from Fig. 9a that eqns. 16 and 16a perform very well in situations in which two Gaussian peaks of different areas occur.

When the asymmetry of the peaks increases, a less ideal picture of the calculated resolution vs. the relative overlap is obtained if eqn. 24 is used. For example, in Fig. 9b results are shown for a situation in which the first peak is asymmetrical ($\tau = 2$ min, i.e., $A_s^{0.125} \approx 2$) and the second peak is symmetrical ($\tau = 0$). Whereas the different curves tend to converge at the high-resolution end (i.e., at resolution values of 1 and higher, different resolution values may be calculated if the relative overlap (RO) of the
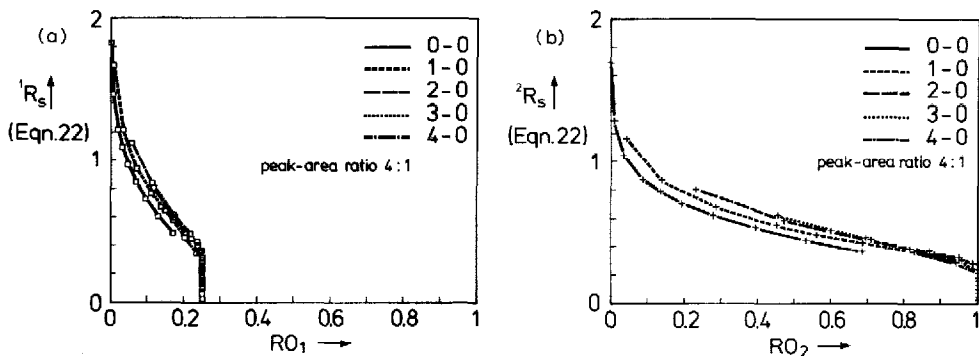

Fig. 7. As Fig. 4, except that the area of the first peak was four times larger than that of the second peak.
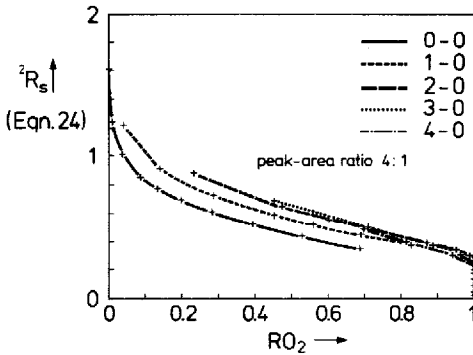
Fig. 8. As Fig. 7b, except that the modified resolution was calculated from eqn. 24a.

second peak is at a constant, high value. If $\tau = 1$ min, the results are in between those in Fig. 9a and b, whereas they become slightly worse is τ is increased further to 4 min (results not shown).

Fig. 9b is an indication of the limitations of eqns. 24 and 24a. If the peak-area ratio starts to differ significantly from 1, the peaks are strongly asymmetric and the resolution is small, than eqn. 24 becomes too much of an approximation and only eqn. 22 will yield good results.

DISCUSSION

We consider the performance of the modified resolution function as defined by eqn. 22, and closely approximated by eqns. 24 and 24a, to be highly satisfactory. Not only do the results compare very favourably with those that would be obtained with the conventional resolution function (compare, *e.g.*, Figs. 5 and 6b), the results
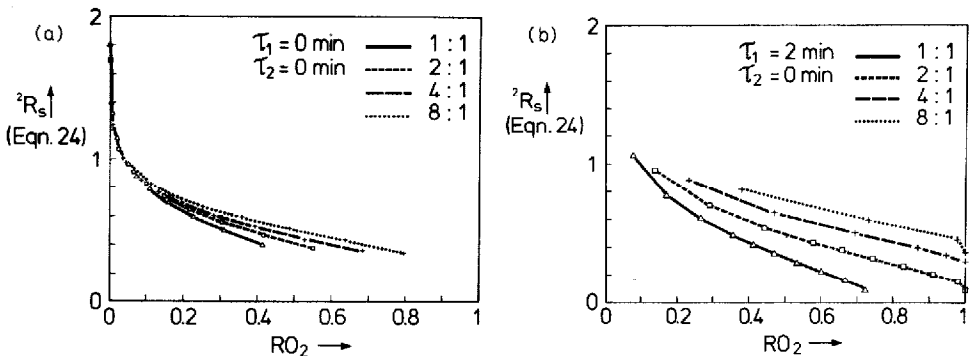

Fig. 9. Relationship between the relative peak overlap and the modified resolution calculated from eqn. 24a for the second of two peaks with different areas. (a) Both peaks symmetrical. (b) First peak asymmetric ($\tau = 2$ min, *i.e.*, $A_s^{0.135} \approx 2$). Peak-area ratios: 1:1 (———), 2:1 (- - - -), 4:1 (———), 8:1 (.........). The second peak was symmetrical ($\tau = 0$). The retention time of the first peak was 15 min, that of the second peak was varied from 15.8 to 19 min. For both peaks the efficiency for the Gaussian function was taken to correspond to $N = 1000$.

obtained with the present modified resolution function also compare favourably with other possible methods of characterizing the resolution[9]. Eqns. 24 and 24a can be used as a more practical alternative to eqn. 22 except in situations in which the peaks are asymmetric or of greatly different areas and the relative overlap is high (Fig. 9b). Eqn. 25 can be used if the plate counts and asymmetry factors are approximately constant throughout the chromatogram. Eqn. 16 can be used for Gaussian peaks of greatly different areas.

Another important consideration is that resolution values can be calculated from eqn. 24 if (i) the retention time (or capacity factor), (ii) the peak width at half-height (plate count) and (iii) the asymmetry factor at 13.5% of the peak height are known. It appears to be feasible to keep track of these three parameters for each individual peak during interpretive optimization procedures[1], so that the present approach can be used for this purpose. Empirical resolution functions, such as the ratio between the (average) peak height and the depth of the valley between peaks (peak-valley ratios) may be used to characterize the extent of separation, but cannot be used in interpretive optimization procedures.

A second advantage of the present resolution functions in the context of optimization procedures is that a value can be calculated, even for very strongly overlapping peaks. The relative overlap will yield a value of $RO = 1$ for the small peak, even if the degree of separation is small. The modified resolution for the small peak will still be different from zero, so that small improvements in separation may be exploited during optimization. However, this will require that the individual peak profiles be known, either from individual injections of each sample component or from the deconvolution of multi-channel data.

Whereas eqn. 24 was found to be an accurate approximation of eqn. 22, eqn. 25 cannot always be used. Eqn. 25 can be used if the plate heights and asymmetries of all peaks are (approximately) the same. However, if the asymmetry varies as dramatically as in Fig. 5, eqn. 25b, in which the smaller of the two asymmetry factors is used, yields very poor results (Fig. 5b). This problem will be even greater if the area of the first peak becomes larger than that of the second (not shown). In this situation the asymmetry factor of the least symmetrical peak may be used (see Fig. 5a), but we prefer to use the more complex eqn. 24 because (i) it is easier not to have to decide which of the two asymmetry factors (and plate counts) should be used and (ii) in order to make such a decision, the values for both peaks would usually need to be calculated anyway, so that there is no practical objection to using eqn. 24.

The present approach has a limitation in the calculation of the peak-height correction for the larger peak in a separation concerning peaks of different height. If a peak is more than e^2 (7.4) times larger than an adjacent peak, the square root in eqn. 24 becomes undefined. This is not a major problem, because it concerns the resolution for the large peak only. A reasonable value can be obtained by defining $\sqrt{1 + \frac{1}{2} \ln(h_j/h_i)}$ to be equal to zero if $h_j/h_i \leqslant e^{-2}$.

Chromatograms

In our opinion, we have provided a thorough, objective evaluation of the modified resolution functions in the systematic study described above. Fig. 10 is intended to allow the reader to form a subjective opinion from a visual inspection of a number of peak pairs, with a varying degree of asymmetry of the first peak (τ
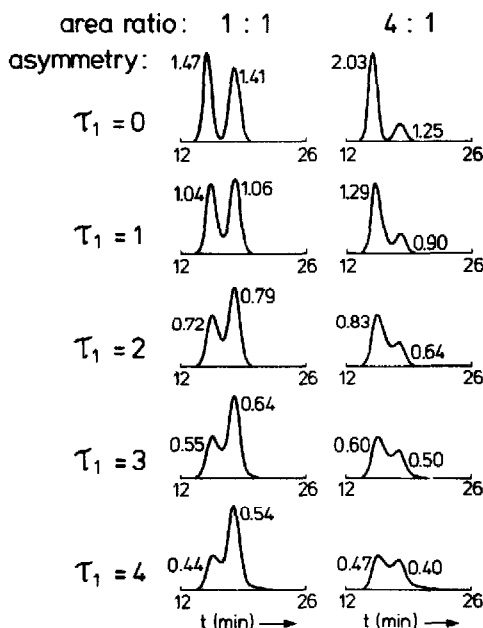
Fig. 10. Calculated resolution values (eqns. 24 and 24a) for each peak in ten different peak pairs. $t_1 = 15$ min, $t_2 = 18$ min. $N = 1000$ (for Gaussian peak). Exponential decay factors (τ) and peak-area ratios vary, as indicated. The conventional resolution value is 1.44 for all peak pairs.

increases from top to bottom) and for two different values of the peak-area ratio (1:1 in the first column, 4:1 in the second). For the Gaussian part of the first peak in each pair the retention time was 15 min and for the second 18 min. All peaks were calculated with a plate count of 1000 for the Gaussian peak. This implies that if the resolution is determined in the conventional way (eqn. 5), using the plate count as measured either from the second peak or from a standard injected separately, the first pair of peaks in Fig. 10 would have a resolution of 1.44. This value will decrease only slightly for asymmetric peaks owing to a marginal increase in the retention time of the peak maximum with increasing peak asymmetry (τ). For all peak pairs the conventional resolution function yields values close to 1.4.

In Fig. 10 the resolution values calculated from eqns. 24 and 24a are indicated for each peak. In the first pair (top left) both peaks are Gaussian. Because of the slightly different peak heights (equal area), the resolution is slightly higher than the conventional value for the first (larger) peak (1.47 instead of 1.44) and slightly lower for the second peak (1.41). However, in this situation there is very little difference between the conventional and modified resolution values.

If the peak-area ratio increases to 4:1 (top right), the modified resolution value increases to 2.03 for the first peak but decreases to 1.25 for the second, indicating that the separation of this pair is better for the first peak than it is for the second. To quantify small peaks next to large ones, a higher resolution is required than in the opposite situation.

When the asymmetry of the first peak increases for two peaks of equal area (top to bottom in the left column), the modified resolution starts to decrease and actually

becomes lower for the first peak than for the second. For a peak-area ratio of 4:1 (top to bottom in the left column) the loss in resolution is also larger for the first (asymmetric) peak.

The reader is invited to make a subjective judgement of the modified resolution values shown for each peak in Fig. 10, bearing in mind that the conventional resolution function yields a value of 1.4 for each of the peak pairs in this figure.

CONCLUSIONS

(1) The modified resolution functions presented in this paper can be used to characterize the resolution in non-ideal situations.

(2) The modified resolution function yields two values for the resolution of a pair of peaks, one for each peak. The largest value (best separation) will be obtained for the largest peak.

(3) A good correlation is obtained between the modified resolution and the relative peak overlap. Variations in the peak asymmetry and the relative peak area are shown to have a minor effect on this relationship. In this respect, the modified resolution behaves better than alternative peak-separation characteristics.

(4) The modified resolution function (eqn. 22) can be adequately approximated by a simpler equation (eqn. 24), which requires only the retention time, peak width and asymmetry factor of each individual peak.

(5) Because of the previous conclusion, the modified resolution function can be used in combination with interpretive optimization procedures.

(6) If the efficiency and the peak asymmetry are (roughly) constant for all peaks in the chromatogram, eqns. 25 and 25b may be used as a further approximation of eqn. 22. If this condition is not met, we recommend the use of eqns. 24 and 24a.

(7) For Gaussian peaks with different areas eqns. 16 and 16a provide a very simple and effective correction for the resolution

(8) If the peaks are strongly asymmetric the peak-area ratio is high and the resolution is low, the more complex eqn. 22 should be used, which requires monitoring of the peak width and asymmetry of each peak at a given fraction of its own height, in addition to that of its neighbours.

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