## Separation Science and Technology

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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**To cite this Article** Boyde, T. R. C.(1971) 'Separative Resolution', Separation Science and Technology, 6: 6, 771 — 780
**To link to this Article: DOI:** 10.1080/01496397108066950
**URL:** http://dx.doi.org/10.1080/01496397108066950

SEPARATION SCIENCE, 6(6), pp. 771–780, December, 1971

# Separative Resolution

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## Summary

Resolution refers to the separation between two components of a mixture and no more (at any one time). The definition must be unidirectional since the separation between two components may not be mutually good. New indices of resolution are proposed, based upon these principles.

In all manner of separation processes, whether on an analytical or a preparative scale, there is a need for generally acceptable measures of effectiveness. Those in use may be grouped according to whether they indicate the qualities of the apparatus and the experimental conditions, the actual degree of separation achieved, or a function of product quality and yield.

Among examples of the first class are, e.g., in gas chromatography, the number of theoretical plates in a column and the selectivity of the stationary phase for a given separation. Given these, it is possible to calculate the degree of separation which should be achieved, i.e., to predict a value for a parameter of the second class. It has been too frequently overlooked that such predictions may not be fulfilled in practice, and it is simply this fact which makes it inevitable that efficiency will continue to be assessed in terms of the degree of separation or "resolution" actually observed.

Many different definitions have been proposed for parameters of the second class. Naturally, some have been based on an analogy with optical resolution. This leads to the definition of a Boolean quantity, and, if taken strictly, to the neglect of nearly all the quantitative data that may be available in a particular case (*1*). The optical analogy may be applied in other ways, however. Thus, for example, it would appear to be misused when an analytical electrophoresis technique is described as being of high resolution because it yields more "bands" than another. Apart from the obvious comment that the bands do not necessarily represent different components of the original mixture, one may also point out that resolution may be less in some parts of the pattern and improved in others. This shows that resolution must refer to how well an individual component is separated from its neighbors—a better justified application of the optical analogy. Again, it has been said that a preparative technique cannot be expected to yield a resolution comparable with the analytical scale procedure from which it was developed (e.g., Ref. *2*). In reality the two are not being compared on the same basis—different definitions of resolution are being used, consciously or not. The clean separation of zones as visualized in the analytical procedure is illusory.

The alternative is to use a quantitative expression for the degree of separation or resolution. Perhaps the most widely known is that defined by the International Union of Pure and Applied Chemistry Commission on Nomenclature in Gas Chromatography, i.e., $R$ = peak separation ÷ mean peak width $[R = 2\Delta z/(w_1 + w_2)]$. Here $\Delta z$ refers to the distance between the peak maxima, and $w_1$ and $w_2$ are the widths of the two peaks concerned. Peak width is defined as the interval between the baseline intercepts of the tangents to the points of inflection on each limb of the distribution curve, and in the case of a Gaussian curve is equal to four times the standard deviation. Throughout this paper the symbol $R$ is used in this sense only. Other suggested definitions of resolution are due to Haarhoff and Pretorius (*3*) ($= 2R$), and Brennan and Kemball (*4*), the intention apparently being to define resolution $= \Delta z - \frac{1}{2}(w_1 + w_2)$. Zhukhovitskii and Turkel'taub (*5*) review the application of several similar parameters of this kind. In common with other Russian authors, they define peak width as the width between points at which $y = y_{\max}/e$. For a Gaussian curve this corresponds to a peak width of $2(2)^{1/2}\sigma$. They define $K = \Delta z/(2)^{1/2}\sigma$ [i.e., $= 2(2)^{1/2}R$], assuming identical peak widths for adjacent peaks, and $K_1 = \Delta z/2(2)^{1/2}(\sigma_1 + \sigma_2)$, i.e., $K_1 = R/(2)^{1/2} = K/4$. According to the Russian reviewers, these parameters are "sensitive" to the mole ratio of the components being separated, but this can only refer to the difficulty of making appropriate measurements from the tracing of two imperfectly resolved and unequal peaks for which independent assays are not available. Certainly they regard this "sensitivity" as a disadvantage.

An alternative to such empirical indices is to consider the degree of impurity of one or both components. Glueckauf (*6*) uses fractional impurity as a standard of resolution rather than as a measure of resolution achieved. His well-known diagram relates relative retention to the number of theoretical plates required to obtain a given fractional impurity ($\eta$) in both components, i.e., the cut between the components is to be made at a point such that both exhibit the same degree of contamination. One of the conclusions of Glueckauf's theory is that it is easier to achieve a given separation the more unequal are the amounts of the two components. The derivation, however, depends upon the validity of the approximation ($m$ = mole number)

$$\eta_2 = \Delta m_1/(m_2 - \Delta m_2) \approx \Delta m_1/m_2$$

i.e., its validity is restricted to low mutual contamination, or, more generally and more precisely, to the condition

$$\eta \ll m_1/m_2$$

where $m_1$ is the mole number of the component present in a smaller total amount. For example, Glueckauf's treatment yields an absurd result if $m_1 = 0.001m_2$ and the desired level of $\eta$ is set at 0.001, (a not too uncommon practical situation).

Haarhoff and Pretorius (*3*) attempt to improve on the Glueckauf treatment by using $\eta_{1:1}$ (the ratio of contaminant to main component which would exist in each of the adjacent peaks considered, *if* there were equal total amounts of the two components) as a measure of separation achieved. They also give a diagram relating this to their "resolution" parameter (a linear absorption isotherm is assumed).

Parameters of the third class inevitably connect product quality and yield in a more or less arbitrary way. Rietema (*7*) reviews the topic and lays down criteria for a satisfactory index of performance, including, naturally, that the index number should increase with improvement of quality at constant yield. However, it is inevitable that a small decline in quality combined with a substantial increase in yield should also lead to an increase in index number. If such a loss in quality is unacceptable, then the performance index is failing in its purpose, showing that such indices must be of limited scope. Zhukhovitskii and Turkel'taub (*5*) have likewise concluded that over-all indices of performance carry hidden dangers and that it is best to consider separately all the various aspects of the suitability of a technique for a particular separation. Rony's (*8, 9*) recently introduced index, the "extent of separation" ($\xi$) is reviewed in more detail below. It is actually an index of the third class, subject to the same defects and limitations as other indices of this type, and when applied to the simple two-component case it is identical with that introduced by Rietema (*7*).

## THE CONCEPT OF RESOLUTION

It is necessary now to examine more closely the concept of resolution so as to find out what can and can not be achieved in defining an index. This will serve also to illustrate the objections to parameters of the third class and will lead to a consideration of the desirable features of an index.

We have stated above that resolution can only be understood in a precise fashion when it refers to the separation of a component from its neighbors. Consider further three components, A, B, and C, eluted in sequence, say, from a chromatographic column. Let us suppose that the elution volume of B is altered by some change of experimental conditions without affecting the elution volume of A or C, or the profile of any of the three peaks. Then B must be eluted closer to one of its neighbors and further from the other. Is it possible to say whether the resolution is better or worse, over-all? If the resolution of B from A is worse, that of B from C is better and vice versa, and to define an over-all resolution is to make a value judgment upon the relative importance of contamination of B by A, or by C. If one takes the view that resolution is maximized by minimizing the total contamination, this is equivalent to attaching equal weight to the two contaminants, and this or any other predetermined relative weighting may be unacceptable in practice.

The unavoidable conclusion is that "resolution" must refer to the separation of two components only. The restriction to "neighbors" is actually unnecessary.

Rony gives the following expression for calculating the "extent of separation" of two components, 1 and 2.

$$\xi = \text{abs}\left[\frac{n_{22}}{n_2^0} - \frac{n_{12}}{n_1^0}\right]$$

Where $n_1^0$ and $n_2^0$ are the number of moles of 1 and 2, respectively, in the original mixture, and $n_{ij}$ is the number of moles of $i$ in region $j$ at

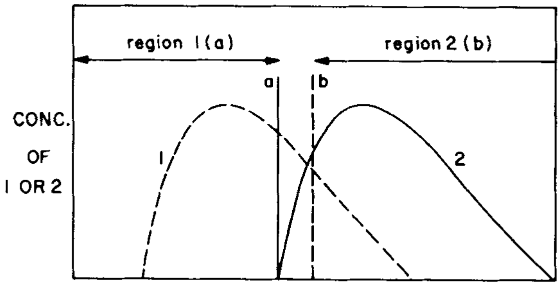

FIG. 1. For explanation see text. Two components, A and B, are eluted as shown: *a* and *b* represent cut points for dividing the eluate into two regions (*8*, *9*). Thus if point *a* is chosen, everything to the left of this is Region 1, and everything to the right is Region 2, In terms of per cent of total A or B, the composition of the regions is: cut point *a*, Region 1 66% A and 0% B, Region 2 34% A and 100% B; cut point *b*, Region 1 81% A and 8% B, Region 2 19% A and 92% B.

the conclusion of the separation process. The number obtained for $\xi$ varies with the boundary chosen between Regions 1 and 2. Thus in Fig. 1, if the cut is made at Point *a*, $\xi = 0.66 - 0 = 0.66$. If the cut is made at Point *b*, we have $\xi = 0.81 - 0.8 = 0.73$. Thus *b* is a more favorable point of "cut" than *a*, in fact, some point in the vicinity of *b* corresponds to $\xi_{\text{opt}}$.

It is claimed as a virtue of Rony's index that it is invariant to a permutation of the component indices $i$, or the region indices $j$ (*8*). As a corollary, the single number describing the quality of separation achieved may conceal startling differences between the two regions (Fig. 1, legend). Also, a higher value of $\xi$ can be obtained for a case which must ordinarily be considered a worse separation and will quite certainly be so considered if the object of the procedure is to obtain 1 in the highest possible yield, but at all costs free from 2 (Fig. 1, legend). Thus as a practical guide $\xi$ has failed us in two distinct ways. In the special case where the two distribution profiles are mirror images of each other, a cut corresponding to $\xi_{\text{opt}}$ does divide the regions such that each contains complementary proportions of 1 and 2, but it remains true that $\xi$ fails as a practical guide because neither $\xi_{\text{opt}}$ nor any other value of $\xi$ can be relied on as indicating the best point of cut. In addition, one must point out that $\xi_{\text{opt}}$ would not form a good basis for comparing two techniques for a particular separation, unless it could be guaranteed that the elution profiles were similarly related to each other in the two cases.

We conclude, therefore, that not only is "resolution" to be confined to a consideration of the separation of two zones only, but that it must be unidirectional. We may obtain a given resolution of A from B: it does *not* follow that the resolution of B from A is equally good, and indeed it will *not* be equally good (or bad) except in special or idealized cases. There seems no alternative to selecting a first component as (transiently perhaps) of greatest interest and defining resolution in terms of the degree of overlap of the distribution profile of the second component with the first. It is proposed that the use of the phrase "the resolution of A from B" should be taken as identifying A as the "component of greatest interest" for the time being.

An outstanding problem is how to express the degree of overlap, having regard for the following:

(1) The Gaussian curve is rejected as a standard distribution profile, since few experimental profiles follow it exactly. Likewise no other theoretical model is acceptable in general.

(2) Nevertheless, the Gaussian and other probability distribution curves have features whose force we must accept, notably that at any given distance $x$ from the peak of the distribution curve, $y(x) > 0$. Any choice made in defining zone or peak width is arbitrary: to justify the choice one must argue quantitatively in terms of the proportion of the component which lies outside the set limits.

Therefore, we suggest that overlap can only be considered by arbitrarily setting limits for the zone and then determining the total amount of B within these limits by analysis or by making reasonable assumptions concerning the distribution profile of B. One must be explicit about how the amount of B is estimated and the limits set for A are not to be constrained by any notions of symmetry or propriety. One might select $\pm 2\sigma$ from the mode, *or* 80% symmetrically distributed about the mode, *or* the leading 75%, *or* $+2\sigma$ to $-1\sigma$, etc. It should be commonplace to consider explicitly the relationship between the "cut" chosen for A and the degree of contamination by B.

The features desirable in an index of resolution are reviewed extensively by Rietema (*7*) and by Rony (*8*). The discussion above indicates that many of the "desirable" features are actually false friends. From the above and the reviews quoted, one may assemble a list of desiderata.

(1) Refers to two components at a time only.
(2) Unidirectional.

(3) Free choice of "cut." Index independent of recovery.

(4) For many purposes the index should be independent of the initial ratio or total quantities of the components referred to, as long as the physical behavior of both components is unchanged. Highly significant information may then result from studies of the actual effect of variations in the total or relative amounts of the components upon the value of the index.

(5) Nevertheless, easy conversion to actual composition of fractions is desirable, and it will be as well if the same principle can accommodate unequal amounts of the components.

(6) Applicable to any kind of separation process.

(7) Same principle open to use whether or not there are independent assays for the two components.

(8) Value lies between 0 and 1.

(9) Increasing number corresponds to improved performance.

The additional feature recommended by Rony—susceptibility to analysis for optimum conditions—is not further considered in this paper, only because it is felt that the assumptions demanded concerning the results of separative processes are unwarranted in most cases. The philosophy of the present work is that, no matter how cogent the arguments for various theories of separative processes, they are never exactly, and rarely even approximately, borne out. Optimization becomes appropriate when empirical functions are available to describe particular processes.

## PROPOSALS

It is proposed to use the symbol $\eta$ for "contaminant ratio," defined as the ratio of (named) impurity to the component of greatest interest in a given fraction. It may often be more convenient to use the "enrichment ratio" ($\theta \equiv 1 - \eta$). The identifiers suggested below may be used with either.

The substances referred to may be given as inferior characters—thus $\eta_{\mathrm{A,B}}$ refers to the resolution of A from B, i.e., to the amount of B present in a preparation of A. It may be indicated as a superior character whether the values have been corrected for the total amount of B being greater or less than that of A (the correction factor being total A/total B), $\eta^u$ meaning "uncorrected," $\eta^e$ meaning "equalized." Further detail may be given as inferior or superior characters outside brackets. Thus the basis of comparison may be given as an inferior character—*m* for moles, *w* for weight, *E* for absorbancies only, and it should be indicated in superior characters what "cut" of A was used—whether, for example, the peak shape was plotted and the tangents to the inflection points drawn to give a triangle, $(\eta)^t$; whether a given number of fractions was taken, $(\eta)^{1-12}$, or a given boiling point range, $(\eta)^{297-300°}$, or a given percentage, $(\eta)^{0-95\%}$, or whether some more complicated law was followed. If an independent assay was not available, then the basis for estimating the amount of B present in A should be given also, e.g., *G* for Gaussian curve.

A fully elaborated symbolic representation might be, $(\eta^e{}_{A,B})_E{}^{t,G} = 0.01$, corresponding to $(\theta^e{}_{A,B})_E{}^{t,G} = 0.99$. This would indicate that in the fraction of A taken for calculation (that defined by drawing the conventional triangle), the optical density due to contaminating B was 1% of that due to A on the basis of an assumed Gaussian distribution curve for B and after correction for different total amounts of A and B. Such a full symbolic representation will rarely be required. Usually most of the information will be given in the text, explicitly or otherwise, and the full system should only be used where necessary to avoid error or for emphasis.

It is appreciated that when using uncorrected indices, or if the intended purification procedure made matters worse instead of better, values of $\eta$ may be greater than 1 and hence values of $\theta$ less than zero. Thus No. 8 of the listed desiderata is not ideally fulfilled, but $\theta = 1$ ($\eta = 0$) always represents perfect separation. No. 9 is fulfilled by the index $\theta$.

## DISCUSSION

Figure 2 shows how $\theta$ is related to some existing resolution parameters. The relationship to the parameters proposed by Haarhoff and Pretorius and by Zhukovitskii and Turkel'taub is obviously very similar, since they differ from *R* only by a constant factor.

The theoretical model used—two overlapping Gaussian distribution curves—has been the most popular because the Gaussian distribution should be approximated by extensive countercurrent distribution, ideal chromatography (*10*), and some other separation methods. The diagram given should be of service in that it allows one to enter with a value of *R* or $\xi_{opt}$ and read off $\theta$, or vice versa. Note, however, that this is only for a very restricted model. If the parameters proposed here are to be

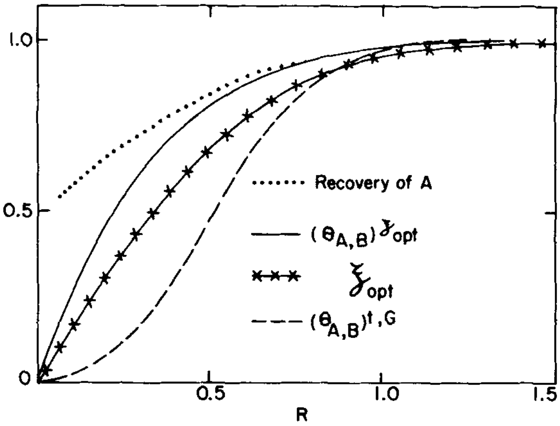

FIG. 2. The situation envisaged is overlapping Gaussian elution curves of identical height and width. $R$ is calculated from the formula $2\Delta z/(w_1 + w_2)$. The other parameters are calculated from values for the area under a Gaussian curve given in Ref. *12* and are plotted against the corresponding values of $R$. $(\theta_{A,B})^{t,G}$ has been calculated from the area under Curve B lying between $\pm 2\sigma$ from the maximum of Curve A, the recovery of A being 95.4%. The remaining curves all refer to a common model situation, i.e., the "cut" between the two elution curves being made at $\xi_{opt}$ (*9*).

used to their full capacity, one must be prepared to use other models, and abandon them altogether in many instances. As $\theta$ and $\eta$ are intended chiefly for practical use, it is to a degree inappropriate that one should consider them in relation to theoretical models at all.

The parameter $R$ is simple and elegant. No doubt, and quite properly, it will continue to be used. It would, for example, be laborious and pointless to attempt to use $\theta$ in the case of gas chromatographic separations with $R$ appreciably greater than 1. However, it is already well recognized that the definition of $R$ makes it unsatisfactory for use with asymmetrical peaks and it has certainly not found favor with practitioners of other techniques who must often be content with very imperfect results. We also suggest that $\eta$ and $\theta$ will be useful in the field of preparative gas chromatography, and perhaps in analytical work wherever peaks are ill-resolved, or asymmetrical, or both, and especially where the determination of each of the components is independent of the other.

Rony's proposed index, $\xi$, the "extent of separation," has been used by him in a powerful and far-reaching analysis of the efficiency of separation procedures (*8, 9, 11*). This is work which will stand on its merits, but it is necessary to emphasize that there are dangers in this approach. In using an index such as $\xi$, its nature must always be borne in mind. Thus the question, "Where is the optimum location of a *single cut* between two elution peaks such that each component has the maximum purity?" (*9*), should not be asked at all. The "extent of separation" is a compromise between recovery and purity, and to locate a cut at $\xi_{\text{opt}}$ is to ensure that neither component is isolated in maximum purity.

It is not claimed that $\eta$ and $\theta$ must replace $\xi$, or $R$, or other parameters of the second and third classes, or that it is no longer necessary to consider separations theory and parameters of the first class. It is the contention, however, that the proposed indices, or something very like them, must be used alongside the others and must take pre-eminence in evaluating or planning actual separations, particularly when there is information available from pilot experiments. Considerations of yield and recovery obviously already influence the choice of experimental conditions. The present work may be helpful in making clear the nature of resolution, and in providing properly defined indices to work with. We expect that diagrams relating recovery to $\theta$ will be widely used in assessing the results of separation experiments in the future.

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*Received by editor March 3, 1971*