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Analytical Efficiency in Chromatography. I. Qualitative Efficiency

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Abstract

The basic definition of qualitative analytical efficiency has been evaluated in the light of recent advances in automated numerical analysis. The low resolution region $R < 1.0$ is explored theoretically, and it is shown that significant improvement results by analysis of the first derivative instead of the actual concentration curve.

INTRODUCTION

The definition of analytical efficiency allows for some degree of arbitrariness, a circumstance which is reflected in the wide variety of such functions proposed in the past. Some uniformity has resulted from the proposals of the IUPAC Committee (*1*) and the resolution functions R_s and R, defined by

$$R_s = \frac{x_2 - x_1}{2(\sigma_1 + \sigma_2)} \tag{1}$$

and

$$R = \frac{x_2 - x_1}{4\sigma_1} \tag{2}$$

appear to be well-established as criteria of merit for the over-all efficiency of an analytical column. The symbols x_i and σ_i refer, respectively, to the position of the peak maximum and the standard deviation of the ith peak. Despite its general acceptance, however, it is felt that the advent of new techniques for analyzing chromatograms have created the need for a re-examination of the basic definition of analytical efficiency.

The theory of efficiency in chromatography may be thought of as consisting of three parts, viz., (a) the definition of the efficiency function (or resolution function); (b) the relation of the function to the column parameters and their interpretation in terms of the fundamental physicochemical mechanisms underlying the chromatographic process, and (c) optimization of the efficiency with respect to these parameters. Several recent studies (e.g., *2*, *3*) have dealt with the last two aspects; in the present work the accent is on the first and in particular on the relationship between efficiency and analyzing device. Special consideration will be given to the low resolution region since it is in this region that new analyzing techniques are being developed to unravel the fine structure of complex chromatograms. The importance of better analyzing methods is illustrated by the fact that an increase in resolution from $R = 1.0$ to $R = 0.5$ leads to a reduction in the required column length of an analytical column by a factor of 4 while a similar decrease in plate height by a factor of 2 would only result in a reduction factor of 2.

Another shortcoming of the resolution function as defined above is the fact that it applies strictly only to two identical peaks. Several attempts to extend the resolution concept to nonequimolar mixtures are recorded in the literature. Glueckauf's original formulation (*4*) showed the unexpected feature of an increase in resolution with nonequimolar mixtures. This was substantiated by the work of Haarhoff (*5*) and Said (*6*) who independently showed, however, that Glueckauf had overestimated the effect. In addition Haarhoff suggested a new criterion for the resolution of nonequimolar peaks which predicted the opposite behavior.

The purpose of the present study, then, is to systematize and extend these theories to the low resolution region and to emphasize the intimate relationship which exists between the definition of the analytical efficiency and the peak analyzing device. No special attention will be given

to the influence of differing peak widths on efficiencies since σ_1 and σ_2 are usually approximately equal for small R values.

PEAK CHARACTERISTICS AND THE DEFINITION OF ANALYTICAL EFFICIENCY

The incompletely resolved components of the solute mixture arriving at the column outlet may be analyzed in two distinct ways, each with its own criterion for analytical efficiency. In the first place, the mixture may pass through a detector (e.g., flame ionization, electron capture, catharometer) and a chromatogram recorded. Second, actual fractions of the mixture may be removed for subsequent analysis by other techniques (e.g., Ir or mass spectrometry). These two methods will be termed direct and indirect analysis, respectively, each of which may be further subdivided according to its qualitative or quantitative nature. In indirect analysis the criteria for efficiency is usually equivalent but for direct analysis they require separate attention. In the present paper qualitative analysis only will be considered.

Elution curves are conveniently described in terms of their moments, the first three being sufficient to characterize Gaussian distributions These symmetrical peak forms will be the only type considered in thi. study. The elution curve for Gaussian curves is given by

$$C_i = \frac{m_i}{(2\pi\sigma_i^2)^{1/2}} \exp\left[-\frac{(x - x_i)^2}{2\sigma_i^2}\right] \tag{3}$$

where C_i = concentration of ith component in arbitrary units, x = axial distance coordinate in arbitrary units, x_i = position of distribution mean of the ith component, m_i = area of peak (assumed in this work to be proportional to the mass of the ith component), and σ_i = standard deviation of the ith peak.

$$m = m_2/m_1 \tag{4}$$

and

$$s = \sigma_2/\sigma_1 \tag{5}$$

respectively represent the molar and the peak width ratios. It may be assumed, without loss of generality, that $m_2 \geq m_1$.

Following Glueckauf (4), the minor portions Δm_1 and Δm_2 are defined

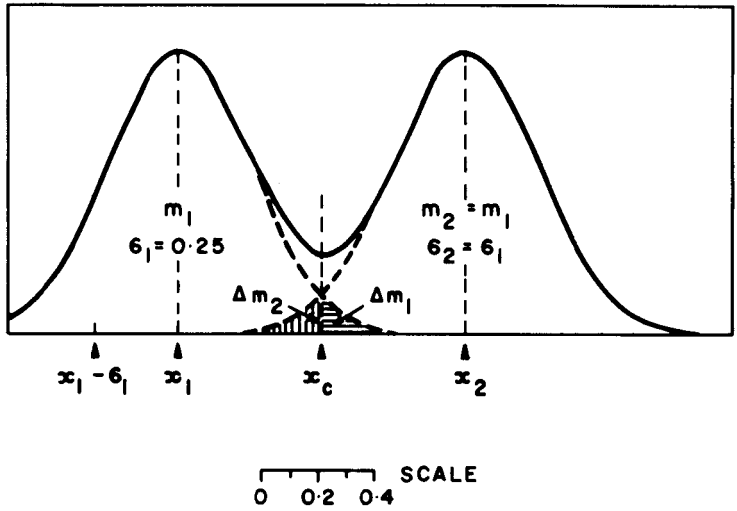

FIG. 1. The component and total concentration curves for two peaks with $m = s = 1$ ($R = 1$).

as in Fig. 1 and the impurity ratios η_1 and η_2 by

$$\eta_1 = \Delta m_2/(m_1 - \Delta m_1) \tag{6}$$

$$\eta_2 = \Delta m_1/(m_2 - \Delta m_2) \tag{7}$$

Let

$$Z(x) = \frac{1}{(2\pi)^{1/2}} \exp\left(-\frac{x^2}{2}\right) \tag{8}$$

and

$$Z^*(x) = \int_{-\infty}^{x} Z(t)\, dt \tag{9}$$

respectively denote the probability function and its first integral. The relations between $Z^*(x)$ and the more generally known and tabulated functions, viz., the normal probability integral

$$N^*(x) = \int_{-x}^{x} Z(t)\, dt \tag{10}$$

and the error function

$$\text{erf}\ (x) = \frac{2}{(\pi)^{1/2}} \int_0^x \exp\ (-t^2)\ dt \tag{11}$$

are given by

$$Z^*(x) = \tfrac{1}{2}\{1 + N^*(x)\} = \frac{1}{2}\left\{1 + \text{erf}\left(\frac{x}{\sqrt{2}}\right)\right\} \tag{12}$$

The minor portions now follow as

$$\Delta m_1 = \int_{x_c}^{\infty} C_1(x)\ dx = m_1\{1 - Z^*(X_1)\} \tag{13}$$

and

$$\Delta m_2 = \int_{-\infty}^{x_c} C_2(x)\ dx = m_2\{1 - Z^*(X_2)\} \tag{14}$$

where

$$X_1 = (x_c - x_1)/\sigma_1 \tag{15}$$

and

$$X_2 = (x_2 - x_c)/\sigma_2 \tag{16}$$

Equation (16) may also be rewritten in terms of the resolution function R_s. One then has

$$X_2 = \frac{2(1 + s)R_s - X_1}{s} \tag{17}$$

The term "resolution function" requires some clarification. The expressions resulting from the definition of the efficiency functions may be inconvenient to apply in practice and for this reason a related function, the resolution function, is generally used. The latter may be defined as any function which is in a 1–1 correspondence with the analytical efficiency. In particular this implies that it should exhibit extrema at identical values of the independent variables, a requirement which is obviously of importance in optimization. A more practical restriction on the resolution function is that it should be a function only of parameters which may be easily measured. In this respect the moments of the distribution are particularly convenient. As will be evident from the next paragraphs, resolution may usually be expressed in terms of the respective ratios of the zeroth and second moments, i.e., m and s. However, the mathematical difficulties encountered in generalizing from the $m = s = 1$

case, to $m \neq 1$ and $s \neq 1$ may defeat the original intention of restricting the resolution function to a simple expression. In what follows the $m = s = 1$ case will always be solved first. The generalization is then effected by computing the ratio R_{ms}^2/R^2 which is equal to the factor by which the number of plates is altered to obtain a separation of the same efficiency as that characterized by R for the $m = s = 1$ case. (In some cases the comparison will be taken relative to R_s.)

INDIRECT ANALYSIS

In indirect analysis the aim usually is to obtain both fractions in as pure a state as possible, irrespective of whether a qualitative or quantitative analysis is to be made. The cut should therefore be made in such a way that the percentage impurities in the two fractions are the same. This is, of course, Glueckauf's original criterion. The case where only one component has to be regained at a specified purity or where more than one cut has to be made will not be considered. A useful graphical procedure for handling such situations has been worked out by Said (*6*).

It follows directly from Eqs. (6), (7), (13), (14), and (15) and the condition $\eta_1 = \eta_2 = \eta$ that the cut point may be found by solving for X_1 from

$$m^2 = \frac{\{1 - Z^*(X_1)\}Z^*(X_1)}{\{1 - Z^*(X_2)\}Z^*(X_2)} \tag{18}$$

The corresponding equation, derived by Glueckauf and used by Said,

$$m^2 = \frac{1 - Z^*(X_1)}{1 - Z^*(X_2)} \tag{19}$$

was derived under the assumption that $\Delta m_1 \ll m_1$ and $\Delta m_2 \ll m_2$. This simplification is unnecessary and may indeed lead to erroneous results for large values of m where the cut point becomes markedly displaced from the midpoint. Equation (18) will now be considered for the three cases ($m = s = 1$), ($m = 1$, $s \neq 1$), and ($m \neq 1$, $s \neq 1$). In all these cases the impurity ratio will be taken as a measure of the analytical efficiency

$$E_i = \eta \tag{20}$$

(1). $m = s = 1$. For $m = 1$ the solution of Eq. (18) follows directly

as

$$X_1 = X_2 \tag{21}$$

Combining Eqs. (17) and (21), and setting $s = 1$, one obtains

$$X_1 = 2R \tag{22}$$

or

$$x_c = \frac{x_1 + x_2}{2} \tag{23}$$

which merely confirms the intuitively expected result that the cut point should be located midway between the peak maxima. Inserting this value of X_1 in Eq. (6), the expression for E_i is found as

$$E_i = \frac{1 - Z^*(2R)}{Z^*(2R)} \tag{24}$$

(2). $m = 1$, $s \neq 1$. Since Eq. (21) is also an exact solution for this case, one has, by using Eq. (17)

$$X_1 = 2R_{1s} \tag{25}$$

or

$$x_c = \frac{x_2 + sx_1}{1 + s} \tag{26}$$

so that E_i is given by

$$E_i = \frac{1 - Z^*(2R_{1s})}{Z^*(2R_{1s})} \tag{27}$$

and

$$\left(\frac{R_{1s}}{R}\right)^2 = \frac{4}{(1 + s)^2} \tag{28}$$

The functional relationship between R and E_i and between R_s and E_i are identical so that the same graph may be used for both cases. Graphs of η vs. R_s are common in the chromatographic literature (*4*) and will therefore not be reproduced here.

(3). $m \neq 1$, $s \neq 1$. It is unfortunately impossible to find exact analytical expressions for X_1 (or x_c) from Eq. (18). The latter may therefore be solved either numerically or approximate solutions may be sought. Said's analysis (*6*) of Glueckauf's results (*4*) indicate how misleading such approximations can be.

After investigating several methods of approximation, the procedure developed by Haarhoff (*7*) was found to give the best results. This method uses the approximation (*8*)

$$1 - Z^*(x) \underset{\infty}{\overset{R}{\rightarrow}} \frac{1}{(2\pi)^{1/2}x} \exp\left(-\frac{x^2}{2}\right) \tag{29}$$

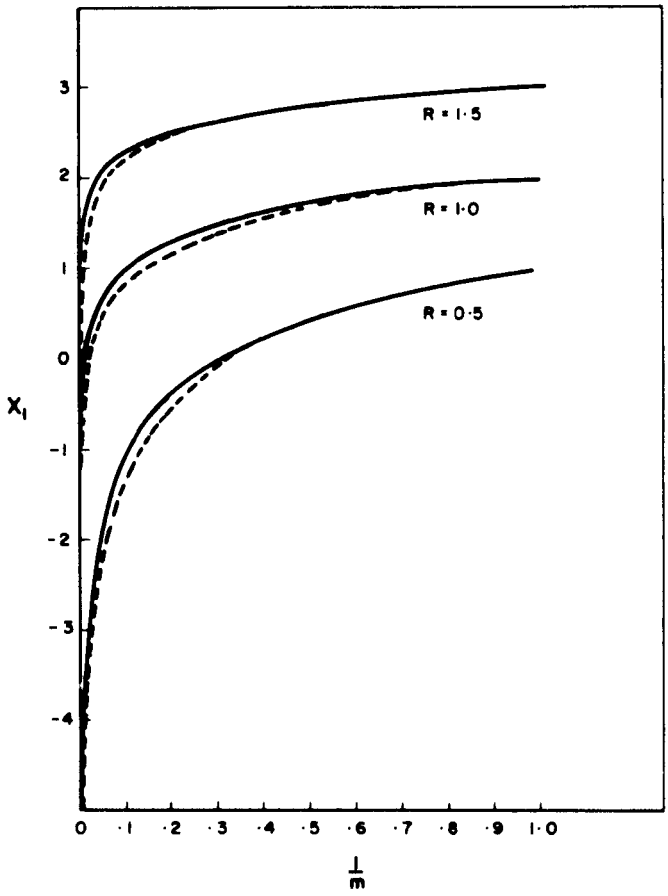

FIG. 2. Cut points for $\eta_1 = \eta_2$ vs. $1/m$ according to Eq. (30) compared with the exact values from Eq. (18).

to determine the limiting value of b_1 in

$$X_1 = \frac{4R}{1+s} - \frac{b_1}{R} \qquad \text{when} \quad R \to \infty \tag{30}$$

b_1 is found to be given by

$$b_1 = (s/2) \ln m \tag{31}$$

The results obtained by using Eq. (31) are compared in Fig. 2 with the exact values obtained by solving Eq. (18) numerically. An unexpected result is that the approximation remains valid even for small R values. For this reason the above mathematical procedure will be extensively used in the work which follows. Figure 2 should be useful for the determination of the cut point for indirect analysis as well as preparative work.

An expression for the minimum value of the resolution R_{ms} required for a separation with the same efficiency as that required for the corresponding case where $m = s = 1$ can be obtained by solving for R_{ms} in terms of R from the equation

$$\frac{m\{1 - Z^*(X_2)\}}{Z^*(X_1)} = \frac{1 - Z^*(2R)}{Z^*(2R)} \tag{32}$$

where X_1 satisfies Eq. (30) with $R = R_{ms}$. An approximate analytical solution is difficult to obtain, and such a solution would not be very interesting either, since Haarhoff (*7*) has demonstrated by numerical computation that R_{ms} decreases only slightly with increasing m (for s near unity). For larger values of s the effect is more pronounced, but since the case $s = 1$ is practically the most important, and taking into consideration the increasing difficulty of locating the cut position accurately, a good practical rule would be to take E_i as independent of molar ratio in indirect analysis. This statement will, of course, only remain valid as long as the amount of matter contained in the smaller fraction remains adequate for the subsequent analysis.

DIRECT ANALYSIS

In direct qualitative analysis the aim is to detect the presence of peaks, usually by means of their peak maxima. It will be shown, however, that this procedure is restricted (for $m = s = 1$) to $R > 0.5$ and that detection based on inflection points may be advantageous. These

two operating points will be dealt with separately since they require different efficiency criteria.

OPERATING POINT = PEAK MAXIMUM ($x_c = x_1$)

The existence of two maxima is usually inferred from the existence of a minimum point in the total concentration curve. This analyzer will therefore be termed the minimum point analyzer.

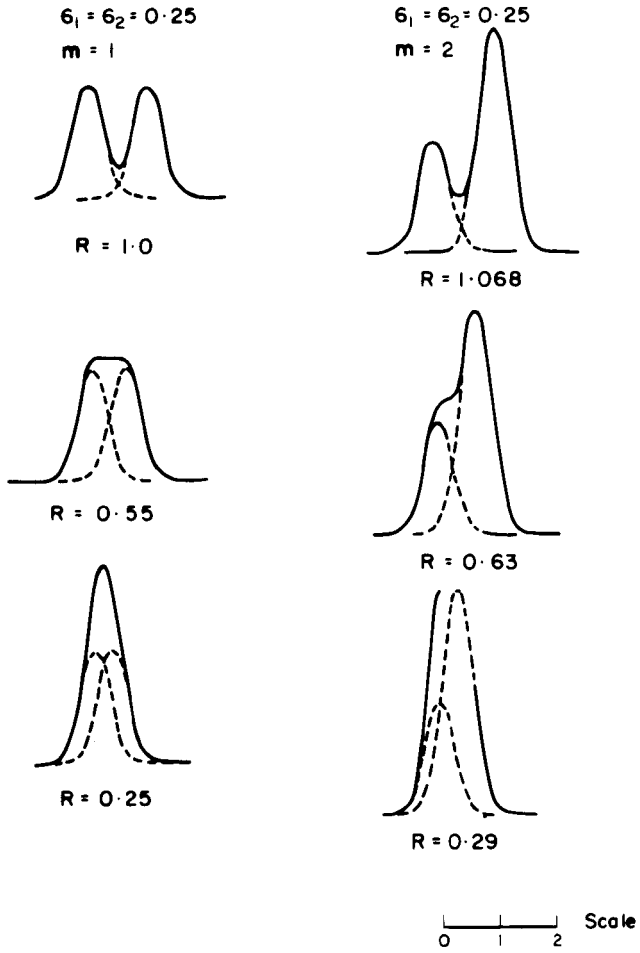

FIG. 3. Illustration of the characteristics of the total concentration curve at low R values.

$$m = s = 1$$

As is evident from Fig. 3, the valley between two identical Gaussian peaks becomes more shallow as R decreases until inversion occurs at a certain value of $R = R_t$. This transition value presents a natural threshold value for resolution and is given by that value R_t which satisfies the equation

$$\left\{\frac{\partial^2(C_1 + C_2)}{\partial x^2}\right\}_{x=2R\sigma} = 0 \tag{33}$$

where $2R\sigma$ is the position of the minimum defined by $\partial(C_1 + C_2)/\partial x = 0$. Solution of Eq. (33) yields $R_t = 0.5$. The separate peaks can no longer be distinguished by means of the peak maximum criterion at $R \leq R_t$.

$$m \neq 1, s = 1$$

This case is complicated by the observation that inversion occurs at values of R where the two peaks may still be visually distinguished although an analyzer operating on the minimum point in the total concentration curve has ceased to function (see Fig. 5). This is at least partly due to the ability of the eye to distinguish two peaks by their inflection points, and visual analysis is therefore more appropriately discussed in the next section. As an indication of the deleterious effect of an increase of mass ratio on the resolution, the variation of R_t with m has been computed and is given in Fig. 4. The computational details are analogous to those used in computing quantitative efficiencies and are discussed more fully in a separate paper (*9*). Briefly, the cut point for $\Delta m_1 = \Delta m_2$ is found to coincide (to a good approximation) to that for the minimum for $s = 1$. The approximation is made that the impurity Δm_2 needed to level the valley on the side of the first peak equals that needed for the equimolar case, i.e., at $R = 0.5$. The value $R = R_t$ corresponding to this Δm_2 is then the required threshold value. R_t is seen to increase rapidly with increasing m, $R_t = 1.0$ being reached at a molar ratio of about 40. In the above-mentioned paper the R_{ms} value is shown to be approximately given by

$$R_{ms} = \frac{sR_{1s} + (R_{1s}^2 + \frac{1}{2}\ln m)^{1/2}}{1 + s} \tag{34}$$

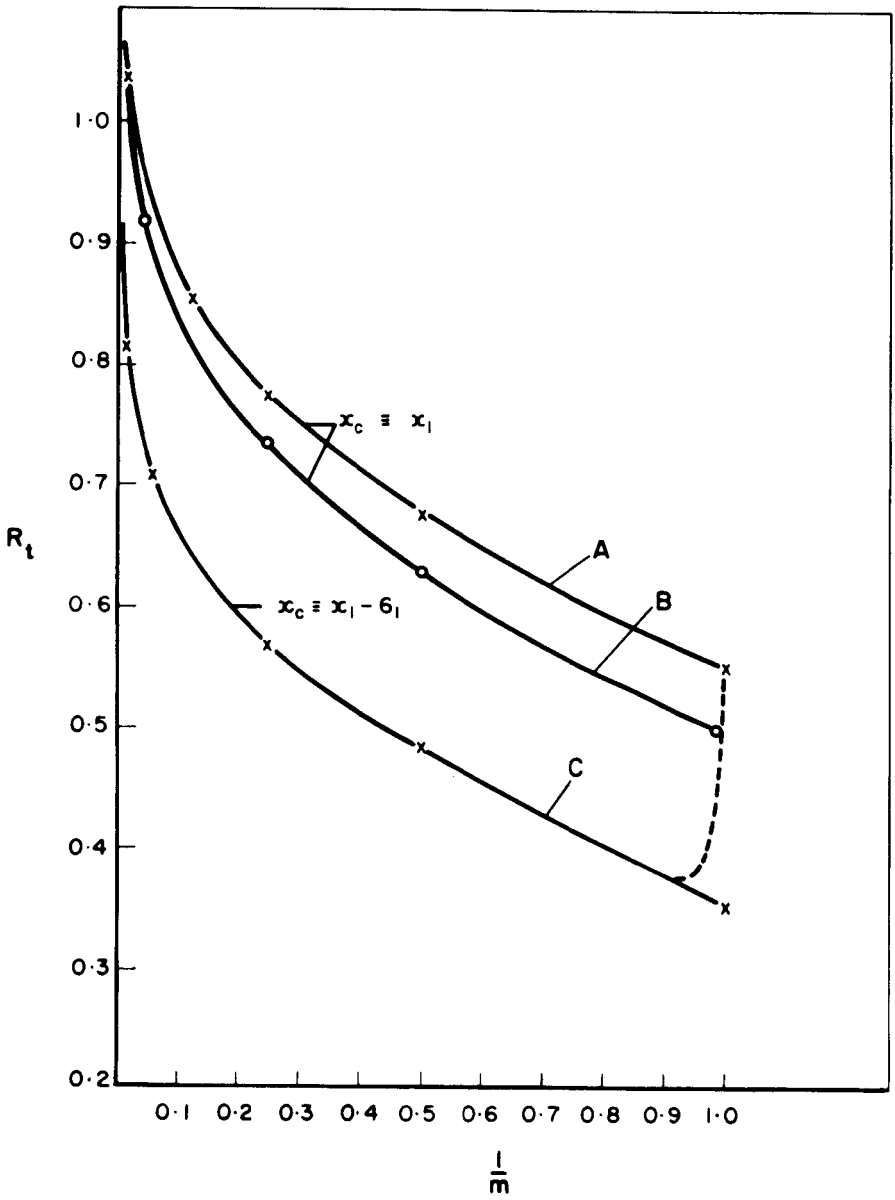

FIG. 4. Threshold values R_t vs. $1/m$ for direct analysis. Curves A and C refer to analyzers operating on the minimum and inflection points, respectively. Curve B is the improved approximation for the minimum point analyzer. The dotted line shows the probable behavior of an inflection point analyzer in the immediate vicinity of the point $m = 1$.

OPERATING POINT = INFLECTION POINT

This criterion applies to automatic analyzers which are able to detect the two positive maxima in the first derivative of the total concentration curve (see Fig. 5). An interesting feature of these curves is the fact that

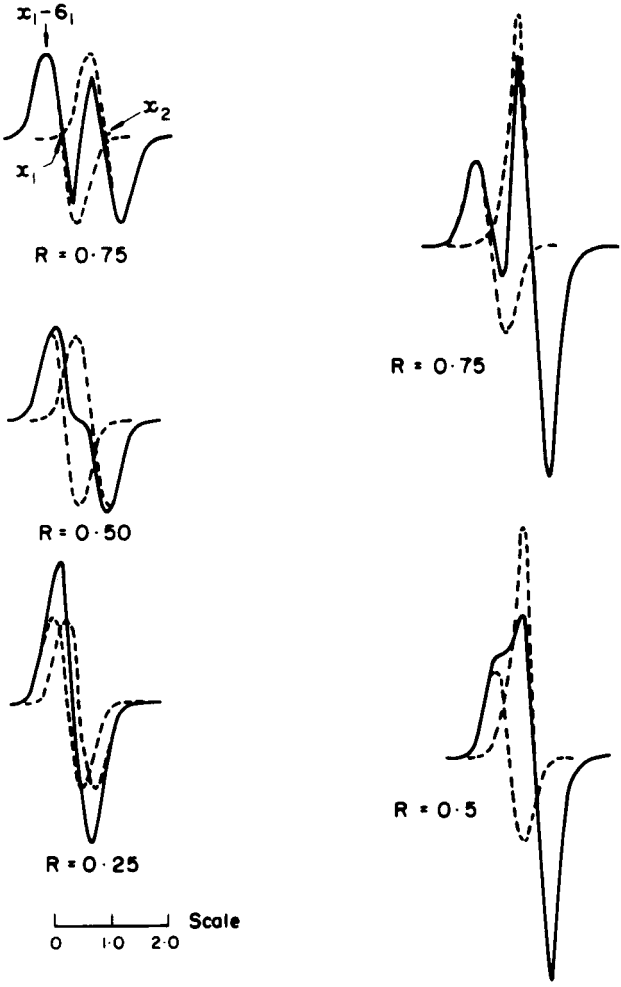

FIG. 5. Characteristics of the total first derivative curve for $m = s = 1$ (left-hand column) and $m = 4$, $s = 1$ (right-hand column). $\sigma_1 = \sigma_2 = 0.25$.

the second peak is lost completely at $R = 0.5$ when m equals unity. For m slightly larger than unity, the second peak is detectable in principle to $R \sim 0.35$. The latter value was arrived at by assuming the two peaks to be discernable until the valley between them, at $x = x_1 - \sigma_1/2$, becomes level with the total height at $x_1 - \sigma_1$, i.e., where

$$\left\{\frac{\partial(C_1 + C_2)}{\partial x}\right\}_{x=x_1-\sigma_1} = \left\{\frac{\partial(C_1 + C_2)}{\partial x}\right\}_{x=x_1-\sigma_1/2} \tag{35}$$

Let the value of R which satisfies Eq. (36) be denoted by R_t. It then follows from Eqs. (3), (17), (21), and (35) that R_t is found by solving the following equation:

$$s^2 \exp\left(-\tfrac{1}{2}\right) + \frac{m}{s}(4R_t + 1) \exp\left\{-\frac{1}{2s^2}(4R_t + 1)^2\right\}$$

$$-\frac{s^2}{2}\exp\left(-\tfrac{1}{8}\right) + \frac{m}{s}(4R_t + \tfrac{1}{2}) \exp\left\{-\frac{1}{2s^2}(4R_t + \tfrac{1}{2})^2\right\} = 0 \tag{36}$$

Equation (36) was solved graphically for various m values. The results are given in Fig. 4. A comparison of the two operating points clearly shows the superiority of the inflection point analyzer since there is a constant difference of $\Delta R_t \sim 0.15$ over the whole range of m-values considered. (As remarked earlier, this does not apply to the point $m = 1$ and in practice one would expect a behavior such as that indicated by the dotted line in the vicinity of this point). The above approximation actually underestimates the efficiency of the inflection point analyzer since the maximum in the first derivative curve moves to values in excess of $x_1 - \sigma_1/2$ as R decreases. If the same approximation is applied to the minimum point analyzer, the difference becomes $\Delta R_t \sim 0.2$ as indicated in Fig. 4.

A general definition of the qualitative inflection point efficiency has still to be formulated. The following definition is suggested. "Let the efficiency of the qualitative inflection point analyzer be defined as the ratio of the concentration of the first component to that of the second at the peak maximum of the first." On the basis of this definition the value of $R = R_{ms}$ which is required to resolve nonidentical peaks with the same efficiency as their identical counterparts may be computed. From Eqs. (3) and (17) and the above definition it follows that R_{ms} is found

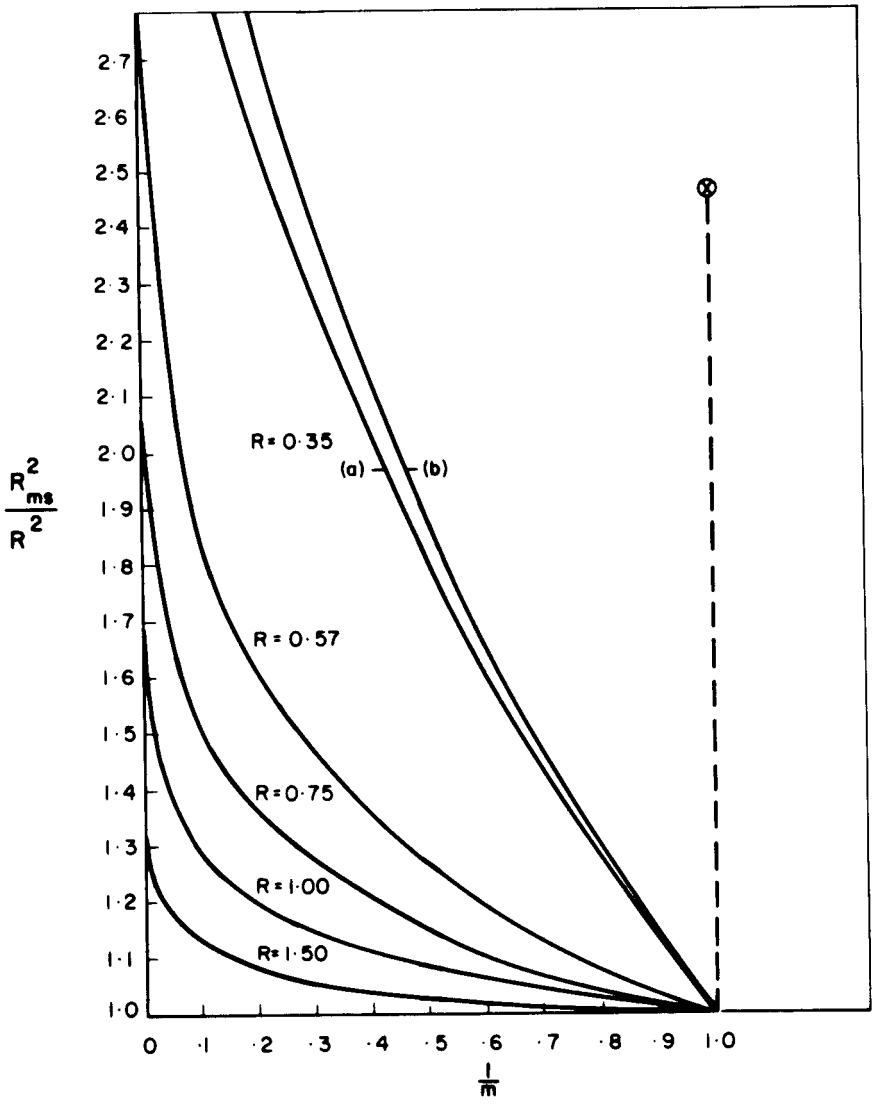

FIG. 6. Plate number ratio vs. $1/m$ for direct qualitative analysis according to Eq. (38). Curves (a) and (b) were computed from Eq. (38) and Fig. 4, respectively, and illustrate the correspondence between the two criteria for the efficiency. The dotted line shows the behavior at $m = 1$.

from

$$E_d = \frac{s}{m} \exp\left(\frac{8R_{ms}^2}{s^2}\right) = \exp\,(8R^2)$$

i.e.,

$$\frac{R_{ms}^2}{R^2} = s^2 + \frac{s^2}{8R^2} \ln \frac{m}{s} \qquad (37)$$

Equation (37) is of the required form since the ratio R_{ms}^2/R^2 gives directly the ratio of the number of theoretical plates required for the analysis. This is shown graphically in Fig. 6. In the same figure the present criterion for efficiency is compared to that which led to Eq. (36). The agreement is considered satisfactory to justify the use of the much simpler definition.

DISCUSSION

In the preceding sections two methods of detecting the presence of peaks have been compared, viz., that operating directly on the total concentration curve and that which analyses the first derivative of the chromatogram. The latter type has been shown to be appreciably more sensitive than the former. It may also be noted that the inflection point analyzer automatically distinguishes between asymmetry and the presence of a second peak since the former would not give rise to additional inflection points. For direct analysis there is a marked decrease of efficiency with increasing m; for indirect analysis there is a small increase of efficiency but this is probably negligible in practice.

The results may be applied as follows: From a knowledge of the optimum parameters for analytical efficiency a column is designed to yield a satisfactory resolution value for the $m = 1$ case in excess of the appropriate R_t value. The increase in the number of plates for the $m \neq 1$ case may then be found from Eq. (34) for the minimum point analyzer. All the foregoing conclusions regarding the relative merits of the two analyzers depend on the validity of the implicit assumption that the total concentration and first derivative curves may be measured with the same accuracy. The degree to which this assumption holds good has still to be ascertained.

SYMBOLS

b_1 convenient parameter, Eq. (31)
C_i concentration of the ith component

E_d	analytical efficiency for direct analysis (Eq. 37)
E_i	analytical efficiency for indirect analysis (Eq. 20)
m	$= m_2/m_1$, molar ratio
m_i	$(i = 1, 2)$ area of ith peak (proportional to number of moles of ith component)
$N^*(x)$	normal probability integral, Eq. (10)
R	resolution function for equimolar mixtures with $\sigma_1 = \sigma_2$
R_s	resolution function
R_{1s}	resolution function R_s for mixtures with $m = 1, s \neq 1$
R_{ms}	resolution function R_s for mixtures with $m \neq 1, s \neq 1$
R_t	threshold value for resolution
s	$= \sigma_2/\sigma_1$, peak width ratio
X_1	$= (x_c - x_1)/\sigma_1$, convenient parameter Eq. (15)
X_2	$= (x_2 - x_c)/\sigma_2$, convenient parameter Eq. (16)
x	axial distance coordinate
x_c	position of cut point
x_i	$(i = 1, 2)$ position of maximum of ith peak (also position of mean for symmetrical peaks)
$Z(x)$	normal probability distribution function, Eq. (8)
$Z^*(x)$	first integral of $Z(x)$, Eq. (9)

Greek Letters

η	impurity fraction
η_i	$(i = 1, 2)$ impurity fraction of component i
σ_i	$(i = 1, 2)$ standard deviation of ith peak

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