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Indeterminate Errors in the Measurement of Chromatographic Peaks*

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Summary

There are four independent sources of indeterminate error in the measurement of peaks by height and width: placing the base line, measuring the height, measuring the intermediate height, and measuring the width. Perimeter methods, i.e., planimeter and cutting and weighing, have similar errors arising from placing the base line, tracing the peak outline, obtaining a reading, and, for cutting and weighing, variability of the paper thickness. In general, these errors depend on peak shape and peak area. The relative error in area decreases with increasing peak area. In most cases there is an optimum peak shape which gives minimum error. Although many factors affect the optimum shape, it frequently is in the range of 2 to 10 for the ratio of height to width at half-height.

The measurement of the area under a Gaussian- (or near Gaussian-)shaped peak is a common operation in a modern laboratory. In particular, such measurements form a major part of gas-chromatographic analyses and in a typical isothermal analysis the peaks may vary from sharp, thin spikes near the beginning of the analysis

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to peaks so broad as to be almost indistinguishable from the base line at the end of an analysis. This program originated out of studies in programmed-temperature gas chromatography, which includes among its advantages the production of peaks of nearly constant peak shape throughout the whole analysis. It is therefore possible that all components of a given sample could be eluted as peaks having an optimum shape for area measurement.

Many factors affect the precision and accuracy of integration of the area under a peak, and a number of theoretical and experimental studies have been reported (*1–8*). Several of these have involved comparisons of different methods of measuring peak areas, and others have been associated with studies of the over-all precision of the chromatographic process. The present paper is limited to a theoretical consideration of the indeterminate errors (and their relation to peak shape) that occur in the principal manual methods for integrating Gaussian peaks: measurement of height and width at some fraction of height, use of a planimeter, and cutting out the peak and weighing the paper. The last two techniques have certain similarities in that they both depend on tracing the perimeter.

As Harris and Habgood pointed out (*9*), the precision in an area measured for a chromatographic peak by the height-width method depends on the precision with which the height and width of the peak can be measured. As long as the relative error of these two measurements is favorable, that is, the absolute values for the height and width are large, then the precision in area will be good. Conversely, peaks having a small absolute value for either height or width will have areas measured with poor precision.

The precision in an area measured for a chromatographic peak by either the planimeter or the cutting and weighing method depends on the precision with which the peak perimeter can be traced. Both methods will involve some degree of wandering from the perimeter during the tracing operation. For a given degree of wander during the tracing operation, the precision in area will be best for peaks of minimum perimeter. Conversely, the precision in area will be poor for either sharp or flat peaks, both of which have large perimeters.

In other words, under isothermal conditions both rapidly eluted and strongly retained gas-chromatographic sample components have undesirable height-to-width ratios. At some retention value an optimum shape for area determination is to be expected.

HEIGHT-WIDTH METHOD

The measurement of peak area by the height-width method requires four separate operations and measurements. First a base line under the peak must be located and drawn. There will be an error associated with this operation, the standard deviation of which we will designate ΔB. Next the peak height h is measured from this base line. The standard deviation of the error for this operation is designated Δh. Third, the position of an intermediate height is located. This intermediate height y is chosen to be some fraction r of the total height h, that is $r = y/h$. The standard deviation of the error in locating the position of y is designated Δy. Finally, the peak width w_r is measured at the position y. The standard deviation of this measurement is designated Δw. Each of the four basic measurement errors is illustrated in Fig. 1.

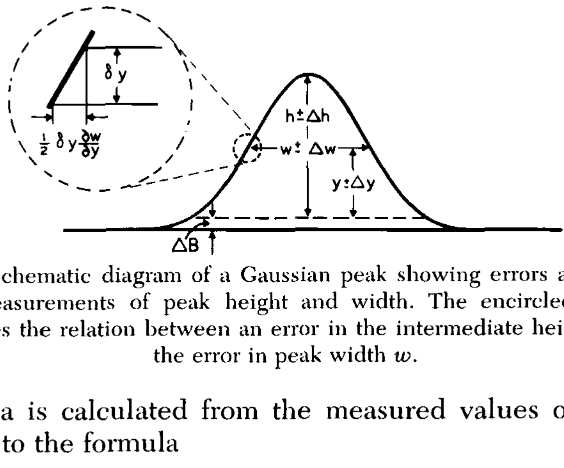

FIG. 1. Schematic diagram of a Gaussian peak showing errors associated with measurements of peak height and width. The encircled portion illustrates the relation between an error in the intermediate height y and the error in peak width w.

The area is calculated from the measured values of h and w_r according to the formula

$$A = C_r h w_r \qquad (1)$$

where C_r is a constant for a given r (6). The value of C_r is given by

$$C_r = \tfrac{1}{2}\sqrt{\pi/\ln(1/r)} \qquad (2)$$

The enlarged portion of Fig. 1 shows that an error in the establishment of the intermediate height y, even though due to an error in height measurement or base-line placement, introduces an error in the determination of width. If this error in y is δy (where the symbol δ is used to distinguish from the error Δy arising in the actual measurement of y), then the resultant error in the over-all width is

$$\delta w = \delta y(\partial w/\partial y) \tag{3}$$

Each of the four basic errors will contribute to the error for the calculated area of a peak. The total effect of these four sources of error is obtained by adding them in a statistical sense.

Error in Placing the Base Line

The error in determining the base line affects the area directly through the value of h and indirectly through the value of w_r used in Eq. (1). The direct error in h from this source is equal to ΔB. The value of w_r is affected according to Eq. (3) by the error in y that results from the base-line uncertainty ΔB. This error arises in two ways. For example, placing the base line too low (Fig. 2) will result

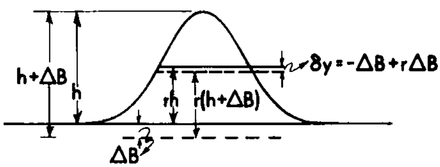

FIG. 2. Schematic diagram of a Gaussian peak, indicating the error caused in the intermediate height for height-width measurements due to the base line being drawn too low by an amount ΔB.

in an error in h, δh, equal to ΔB and will cause the intermediate height to be measured from too low a position. For this reason the value of y will be too small by an amount ΔB, that is, will contain an error $-\Delta B$. On the other hand, the measured value of h is too large by an amount ΔB, and hence the distance y to be marked off will be large by the amount $r\ \Delta B$. Thus the error in y directly resulting from this error ΔB in the base line is given by

$$\delta y_B = -\Delta B(1 - r) \tag{4}$$

If the base line were drawn too high by an amount ΔB, the errors in h and y would be $-\Delta B$ and $+\Delta B(1 - r)$.

The area is affected by the errors in the values of height and width according to the general relationship

$$\Delta A = (\partial A/\partial w)\ \delta w + (\partial A/\partial h)\ \delta h \tag{5}$$

Or, in relative terms, a combination of Eqs. (1) and (5) yields

$$\Delta A/A = (\delta w/w_r) + (\delta h/h) \tag{6}$$

If Eq. (4) is substituted in (3) the error in width due to the base-line uncertainty ΔB is

$$\delta w = -\Delta B(1 - r)(\partial w/\partial y)_{rh} \tag{7}$$

From the Gaussian formula expressed by

$$w_r = 2\sigma(2 \ln h/y)^{1/2} \tag{8}$$

where σ is the standard deviation of the Gaussian curve, the partial derivative of Eq. (7) can be obtained:

$$(\partial w/\partial y)_{rh} = -\sqrt{2}\ \sigma/rh(\ln 1/r)^{1/2} \tag{9}$$

Substitute Eqs. (7), (8), and (9) in (6), noting that δh is equal to $+\Delta B$ in this case, and the expression for the relative error in peak area arising from base-line uncertainty ($\Delta A_B/A$) becomes

$$\Delta A_B/A = (\Delta B/h)\{1 + [(1-r)/2r \ln(1/r)]\} \tag{10}$$

Error in Measurement of Height

Error in measuring height also affects the area, both directly through the value of h and indirectly through the value of w_r used in Eq. (1). The argument here is similar to that used for the base-line error, with the important exception that now the base line is assumed to be established and we are concerned only with the measurement of height from this base line. Hence the error carried into the y determination, δy_h, is $r\ \Delta h$, compared with $-\Delta B(1 - r)$ in the previous case. As a consequence, through Eqs. (3), (6), (8), and (9) the expression for the relative error in area ($\Delta A_h/A$) due to an error in the height measurement is

$$\Delta A_h/A = (\Delta h/h)\{1 - 1/[2 \ln 1/r]\} \tag{11}$$

Error in the Measurement of Intermediate Height

Since the height h is now established, the error incurred in measuring the intermediate height y can affect the area only indirectly through the value of w_r used in Eq. (1). Hence in this instance δh in Eq. (6) is equal to zero. By the arguments previously presented, the resulting expression for the relative error in peak area ($\Delta A_y/A$) due to an error in measuring the intermediate height is

$$\Delta A_y/A = -\Delta y/2\ rh \ln 1/r \tag{12}$$

Error in the Measurement of Width

The error arising in the measurement of the width w_r affects the area only directly through the value of w_r used in Eq. (1). Thus the relative error in peak area ($\Delta A_w/A$), as a result of an error in the width measurement, is

$$\Delta A_w/A = \Delta w/w_r \tag{13}$$

Total Error in Peak Area

The four basic errors arising from the measurement operations are independent and will add as variances. The total relative error in peak area, therefore, is expressed by

$$\Delta A/A = \sqrt{(\Delta A_B/A)^2 + (\Delta A_h/A)^2 + (\Delta A_y/A)^2 + (\Delta A_w/A)^2} \tag{14}$$

Sources of Measurement Errors

In general, the independent errors described in the preceding section may be related to one or more of the following:

1. Deciding where to draw the base line or between which points to make measurements. The uncertainty here arises from irregularities in the recorder trace.
2. Correctly placing the ruler vertical to, or parallel to, the base line as required in the particular operation.
3. Correctly observing the reading of the ruler in relation to the points at which measurements are made.

All irregularities in the recorded trace may be treated as noise and may conveniently be divided into high- and low-frequency noise. High-frequency noise has a period that is short compared with the width of the peak. There is then some possibility of smoothing the trace before making measurements. Low-frequency noise has a period that is long compared with peak width and is an erratic base-line drift.

The precision with which the base line can be drawn, and hence the magnitude of ΔB, depends on both the high- and the low-frequency noise. High-frequency noise requires smoothing the base line on either side of the peak and thereby introduces an uncertainty. Low-frequency noise or base-line drift means that the true base line is less likely to be a straight line between the limiting wings of the peak.

The precision with which the peak height h can be measured from the chosen base line depends on the noise in the region of the peak maximum. In particular, that noise with a frequency comparable to the peak width limits the precision in locating the true peak maximum. In addition, the precision in measuring the height depends on the proper placement of the ruler and on the error incurred in the measurement observation or reading of the ruler.

The precision with which the intermediate height y can be located depends upon the proper vertical and horizontal placement of the ruler. In addition, there will be an error in reading and marking off the required distance and then an error in placing the ruler for the width measurement at the indicated value of y. Some compensation of errors may occur in these last two steps. Note that the operation of locating the intermediate height from the established base line is independent of noise that may be present in the peak trace.

The precision with which the peak width w_r can be measured depends on the ruler being placed parallel to the base line. In addition, there is uncertainty in locating the true sides of the peak under noise conditions. High-frequency noise is of primary significance here. Even without noise there is error in reading the ruler.

Finally, sharp peaks present a special problem in regard to noise. The existence of all but the highest-frequency noise in the trace of sharp peaks is virtually lost to the observer. Therefore, whether the height and width (and thus the area) or just the height of the sharp peak is being measured, the results appear deceptively precise. As already noted, the merging of peaks of any shape with noise of similar frequency becomes a problem. However, with peaks of flat or even of intermediate shape the operator is usually aware of interfering noise and can interpret the results accordingly. This may not be possible with sharp peaks that give the appearance of being ideally Gaussian.

Reading Error

Reading error arising out of the measurement of distances between two lines is inherent in all measurement operations except base-line placement. It therefore warrants close examination.

The various measurement operations may each be considered to involve measuring the distance between two lines with a ruler—in the case of h and y between two parallel lines and in the case of

w between two inclined lines with the slopes decreasing as the peak becomes flatter.

A minimum value of the reading error would be expected when measuring the distance between two well-defined parallel lines (Fig. 3A). The error would increase to infinity as the angle be-

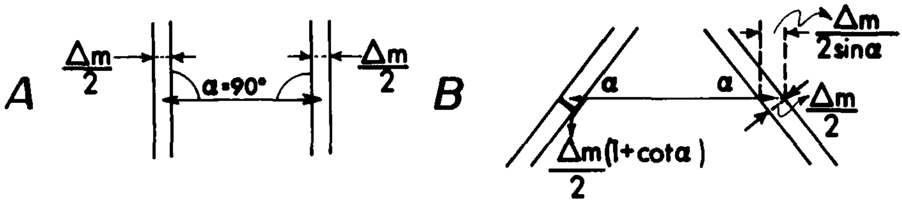

FIG. 3. Schematic representation of reading error. (A) Reading error Δm arising in a measurement between parallel lines. (B) Reading error arising in a measurement between sloping lines. By simple geometry the expected reading error is $\Delta m/\sin\alpha$ (the right part of the figure); the quantity $\Delta m(1 + \cot\alpha)$ (left part of the figure) gives a better fit with experiment.

tween the lines and the ruler decreased from 90° to 0° (Fig. 3B). This would be analogous to measuring the widths of symmetrical peaks varying in shape from sharp to flat.

A relation between the error and the degree of inclination between the lines and the ruler can be derived by considering Figs. 3A and 3B. Assume each line to have a band of uncertainty of width $\Delta m/2$. The uncertainty for a measurement between sloping lines each of which forms an angle α with the ruler might then be expected to be

$$\Delta m_\alpha = \Delta m/\sin\alpha \tag{15}$$

or the equivalent expression

$$\Delta m_\alpha = \Delta m(1 + \cot^2\alpha)^{1/2} \tag{16}$$

A preliminary investigation (*10*) of the standard deviation of measurements between two lines as a function of the angle α as defined in Fig. 3B showed a better fit to a simpler relationship than that of Eq. (16), namely,

$$\Delta m_\alpha = \Delta m(1 + \cot\alpha) \tag{17}$$

Equation (17) suggests that the uncertainty increases somewhat faster with decreasing values of α than would be expected by the construction on the right side of Fig. 3B and the uncertainty would be better represented by the heavy line shown in the left part of this figure. Equation (17) is used to relate the observational error in the width measurement to the observational error associated with the height measurement.

Noting that

$$\cot \alpha = -\tfrac{1}{2}(dw/dy) \tag{18}$$

and using Equation (9), we obtain for a Gaussian curve

$$\Delta m_\alpha = \Delta m\{1 + [\sigma/rh(2 \ln 1/r)^{1/2}]\} \tag{19}$$

The Limiting Case

Under ideal conditions of noise-free Gaussian peaks a minimum error in area will result from the observational error in each measurement operation. This therefore constitutes the limiting case in area determination by the height-width method. The minimum error in measuring h is equal to Δm, and the minimum error in measuring w_r is given by Eq. (19). We assume both the minimum error in base-line placement and the minimum error in locating the intermediate height to be equal to Δm.

Making appropriate substitutions for the various terms of Eq. (14) leads to the following expression for the total relative error in peak area for the limiting case:

$$\frac{\Delta A}{A} = \sqrt{\left(\frac{\Delta m}{h}\right)^2 \left[1 + \frac{(1-r)}{2r \ln 1/r}\right]^2 + \left(\frac{\Delta m}{h}\right)^2 \left[1 - \frac{1}{2 \ln 1/r}\right]^2 + \left[\frac{-\Delta m}{2rh \ln 1/r}\right]^2 + \left[\frac{\Delta m\ \{1 + [\sigma/rh(2 \ln 1/r)^{1/2}]\}}{2\sigma(2 \ln 1/r)^{1/2}}\right]^2} \tag{20}$$

which may be rearranged to the more simplified and general expression

$$\frac{\Delta A}{A\ \Delta m} = \frac{1}{h}\sqrt{\left[1 + \frac{(1-r)}{2r \ln 1/r}\right]^2 + \left[1 - \frac{1}{2 \ln 1/r}\right]^2 + \left[\frac{-1}{2r \ln 1/r}\right]^2 + \left[\frac{1}{w_r} + \frac{1}{4r \ln 1/r}\right]^2} \tag{21}$$

The four terms under the square-root sign refer, as before, to the four errors ΔB, Δh, Δy, and Δw.

By use of Eq. (21) the quantity $\Delta A/A\ \Delta m$, which is the relative error in area per unit observational error, can be calculated for various situations. For example, with a particular value of r, the fractional height at which the width is measured, and a given peak area A, it is possible to calculate this relative error as a function of peak shape, h/w, since h and w_r are related to the area through Eqs. (1) and (2). In Fig. 4A the relative error $\Delta A/A\ \Delta m$ is plotted against $h/w_{1/2}$ for r equal to 0.5 and various values of the peak area. The abscissa scale is reversed so that the sharp (early) peaks in an isothermal chromatogram are to the left. For each peak area the relative error is a minimum at some optimum peak shape—in this case, at $h/w_{1/2}$ equal to 2 to 3.

The preliminary experiments (10) indicate a value of Δm of or a little below 0.01 cm for careful observers. Taking Δm to be 0.01 cm means that the numerical values of the ordinate in Fig. 4 may also be interpreted as the actual percentage error in area $\Delta A/A$.

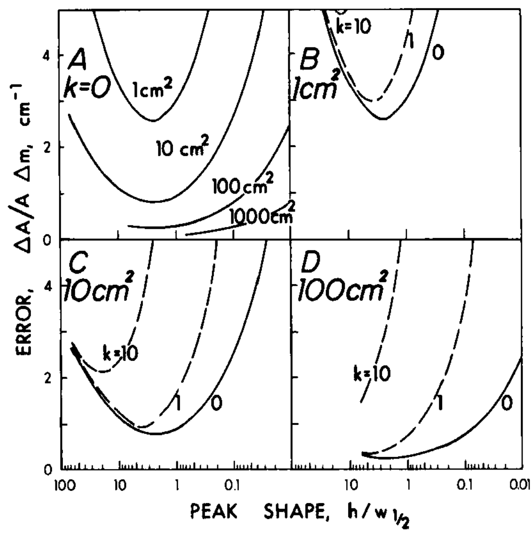

FIG. 4. Relative error per unit of observational error as a function of peak shape for the limiting case of Eq. (21). The curves in A are for peaks of 1, 10, 100, and 1000 cm² area, whose widths are measured at 0.5 of the peak height. The measurement errors ΔB, Δh, and Δy are assumed to be equal to Δm, and the measurement error Δw is assumed to be equal to $\Delta m(1 + \cot \alpha)$. In B, C, and D the solid lines are reproduced from part A and the dashed lines illustrate the effect on relative error when base-line uncertainty ΔB increases with increasing peak width according to Eq. (22) and k equal to 1 to 10.

Increasing Base-Line Uncertainty with Increasing Peak Width

So far we have taken the base-line uncertainty ΔB to be the same for peaks of all widths. As already noted, the base-line uncertainty will increase with an increase in low-frequency noise and base-line drift. We might expect that any particular low-frequency noise would give an increase in base-line uncertainty as peaks broaden. We suggest that the uncertainty might increase according to the square root of the peak width, and that a suitable function for ΔB might be·

$$\Delta B = \Delta B^{\circ}(1 + k\sqrt{w_{1/2}}) \qquad (22)$$

where k is a constant and $w_{1/2}$ is the peak width measured at half-height.

The limiting case where ΔB° is equal to Δm in practice might correspond to a chromatogram with negligible short-term noise but some low-frequency noise or drift.

Assigning a value of 10 to k, for example, and taking ΔB° equal to 0.01 cm would mean that a peak with a 15-cm width at half-height would have a ΔB value of about 0.3 cm, which is approximately 1% of the base width. Substituting Eq. (22) in (10) and (21) produces the broken curves of Fig. 4.

Figures 4B, 4C, and 4D illustrate the profound effect that increasing base-line uncertainty [Eq. (22)] has on the errors for areas of 1, 10, and 100 cm^2. Flat peaks (low $h/w_{1/2}$ values) are affected to the greatest degree. In addition, these three parts of the figure show that the optimum peak shape becomes sharper as the parameter k increases.

Relative Importance of the Individual Measurement Errors

Figure 5 shows the relative importance of each of the four measurement errors ΔB, Δh, Δy, and Δw for the limiting conditions. In parts A, B, and C of Fig. 5 the relative error in area per unit observational error is given for each measurement error assumed to be present by itself; that is, all the other terms in Eq. (21) are assumed to be zero. The relative error in area due to the error in measuring the width is of considerable importance for sharp peaks. This importance diminishes as the peak flattens and then increases at the

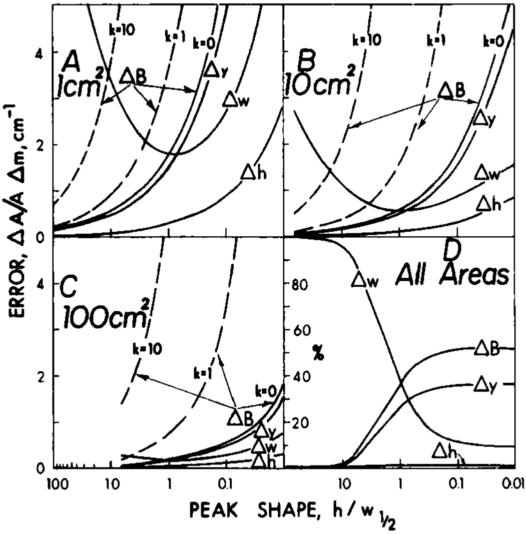

FIG. 5. Individual and relative contributions of the four measurement errors ΔB, Δh, Δy, and Δw to the relative error per unit of observational error as a function of peak shape for the limiting conditions of Eqs. (21) and (22). In A, B, C the solid lines show the relative error due to each of the four measurement errors if each were to be the only measurement error present; the dashed lines show the effect of ΔB increasing with peak width according to Eq. (22). D shows the relative importance of each measurement error to the over-all error, that is, each squared term in Eq. (21) expressed as a percentage of the total relative error for the limiting case. The base-line-placement error ΔB is assumed independent of peak width ($k = 0$).

point where the observational error of reading between the sloping peak sides begins to increase dramatically (see the Δw curve of Fig. 5).

The other errors ΔB, Δh, and Δy are comparatively unimportant for sharp peaks. As the peaks flatten, however, the relative errors in area due to these three quantities rise markedly, particularly that due to base-line uncertainty ΔB. The increase in relative error due to ΔB is even more pronounced when allowance is made for the functional increase in base-line uncertainty of Eq. (22). The dashed curves of Figs. 5A, 5B, and 5C illustrate this effect for k equal to 1 and to 10. Since the curves of Figs. 5A, 5B, and 5C apply to any value of Δm, they may be used to calculate the terms in Eq. (21) for cases in which the individual errors ΔB, Δh, etc., are different from Δm.

Finally, considering again the total expression for $\Delta A/A\ \Delta m$ used in Fig. 4, Fig. 5D shows the relative importance of each squared term as a percentage of the total. Although this relationship depends upon r (Fig. 5D is only for $r=0.5$), the curves are independent of peak area.

The relationships that have been developed here, and that include the parameter r, permit an analysis to determine the optimum value of r, that is, the best fractional height at which to measure the peak width. Such an analysis, which is being prepared in a separate publication, should be somewhat more extensive than that published by Said and Robinson (*6*), who, considering only the uncertainty in the height measurement and its effect on the computed area, concluded that the width should be measured at $1/e$ of the height.

PERIMETER METHODS

Two integration methods, cutting and weighing and planimetry, have been grouped as perimeter methods because of their basic similarity. Both require four separate operations. First a base line must be located and drawn under the peak. The standard deviation of this error we shall designate ΔB as before. Next the outline of the peak must be traced or cut. This will result in a band of uncertainty around the edge whose area is equal to the peak perimeter P times the width of the band designated as $\overline{\Delta m}$. Third, the planimeter or balance will be read with a reading error, of standard deviation ΔR. Finally the peak area is calculated from the instrument reading according to the formula

$$A=fR \tag{23}$$

where R is the planimeter or balance reading and f is the necessary conversion factor. For cutting and weighing only, variation in paper thickness is equivalent to an indeterminate error in f, Δf. The factor f will be implicit when a calibration graph is used to express the instrument reading directly in weight, concentration, or percentage units.

The value of f is obtained from instrument readings of known areas, and inaccuracies arising in the determination will result in a determinate error in the conversion factor. This determinate error in f should not be confused with indeterminate errors under consideration in this paper.

Error in Placing the Base Line

The error in placing the base line is essentially the same as described in the height-width method. For perimeter methods it is convenient to treat this error as a narrow strip of length 6σ across the bottom of the peak, as shown in Fig. 6. The relative error in

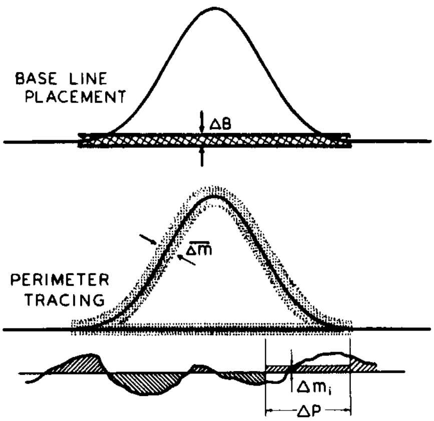

FIG. 6. Schematic diagram of a Gaussian peak indicating the errors associated with perimeter methods of peak integration. Upper, error due to uncertainty in base-line placement. Lower, error arising from tracing or cutting around the perimeter; a detailed view of the tracing of a section of the perimeter is shown at the bottom.

area ($\Delta A_B/A$) resulting from this base-line uncertainty can then be expressed as

$$\Delta A_B/A = \Delta B 6\sigma/\sqrt{2\pi}h\sigma = (6/\sqrt{2\pi})\Delta B/h \qquad (24)$$

Error in Tracing the Peak Outline

The error incurred in tracing or cutting the peak perimeter (Fig. 6) may be considered as the area of a band of uncertainty of width $\overline{\Delta m}$ around the perimeter P. Examined in greater detail, as indicated at the bottom of the figure, the net error for any single tracing is a succession of small errors which will partially cancel each other as the trace deviates first to one side, then to the other, of the true line. Consider the perimeter as divided into n increments each of length ΔP; ΔP is taken just large enough that the direction and magnitude of the average deviation Δm_i over a given increment is independent of that of the preceding and following increments. The net area of the band of uncertainty around the perimeter is the algebraic sum of the areas of all such increments:

$$\Delta A = \sum^{n} \Delta P \, \Delta m_i = n \, \Delta P \sum \Delta m_i/n = P \, \Delta m_{av} \tag{25}$$

Because the values of Δm_i are assumed to be indeterminate and hence to have equal probabilities of being positive or negative, the sum of all values of Δm_i, and hence also Δm_{av}, should approach zero given a sufficiently large sample, that is, as n becomes large. For the finite length of any given perimeter, Δm_{av} may be considered as the average of a limited sample n drawn from an infinite population the average of which would be zero. We are interested in the standard deviation of the Δm_{av} values that would be obtained by successive repetitions of the measurements on one peak. Calling this standard deviation of the mean $\overline{\Delta m}$, we may show by the usual statistical methods (*11*) that $\overline{\Delta m}$ is inversely proportional to $\sqrt{n}$. Since n is proportional to P, we have

$$\overline{\Delta m} = \overline{\Delta m}^\circ / \sqrt{P} \tag{26}$$

where $\overline{\Delta m}^\circ$ is the standard deviation of the mean per unit length of perimeter. Substituting $\overline{\Delta m}$ for Δm_{av} in Eq. (25) and expressing the error relative to the area yields

$$\Delta A_T/A = \overline{\Delta m}^\circ \sqrt{P}/A \tag{27}$$

A simple approximation to the perimeter of a Gaussian curve can be made using the triangle formed by drawing tangents to the inflection points. The base width of such a triangle is 4σ, and the perimeter is simply the sum of the base and the two sides. Making the approximation that the area of the triangle equals that of the Gaussian peak leads to the following expression for the perimeter:

$$P = 4\sigma + 2\sqrt{4\sigma^2 + (\pi/2)h^2} \tag{28}$$

Combining Eqs. (27) and (28) gives the expression for relative error in area due to the tracing operation:

$$\Delta A_T/A = [4\sigma + 2\sqrt{4\sigma^2 + (\pi/2)h^2}]^{1/2}[\overline{\Delta m}^\circ]/\sqrt{2\pi}\, h\sigma \tag{29}$$

Other Indeterminate Errors

The error in reading the instrument, whether planimeter or balance, is in part analogous to the observational error Δm described for the height-width method. The relative error in area resulting from the instrument reading error can be expressed simply as

$$\Delta A_R/A = \Delta R/R \tag{30}$$

The magnitude of ΔR is unknown and will involve the pole arm setting of the planimeter, type of balance, etc. A balance is inherently so sensitive that, particularly for small peaks, $\Delta R/R$ will be much smaller with a balance than with a planimeter. For either planimeter or balance, the value of $\Delta R/R$ is independent of peak shape for peaks of constant area.

In the case of the cutting-and-weighing method the error due to nonuniformity in paper thickness must also be included in a total error expression. As already indicated, it is convenient to treat this error as an error $\overline{\Delta f}$ in the conversion factor f. By arguments directly analogous to those used in the development of $\overline{\Delta m}$ in the previous section,

$$\overline{\Delta f} = \overline{\Delta f}^\circ/\sqrt{A} \tag{31}$$

where $\overline{\Delta f}^\circ$ is the standard deviation in the calibration factor as it would be determined from a large number of measurements of separate unit weights of chart paper. This gives the relative error in area due to variations in paper thickness:

$$\Delta A_f/A = \overline{\Delta f}^\circ/f\sqrt{A} \tag{32}$$

The magnitude of $\overline{\Delta f}^\circ$ depends only on variability in the paper.

The General Error Equation for Perimeter Methods

The errors due to base-line placement, perimeter tracing, instrument reading, and paper thickness variation are random in origin and occur independently of each other. Consequently the total indeterminate error in the calculated area of a peak is obtained by adding their variances. For the planimeter method the total relative error in area can be written in general form as

$$\Delta A/A = \sqrt{(\Delta A_B/A)^2 + (\Delta A_T/A)^2 + (\Delta A_R/A)^2} \tag{33}$$

The total relative error in area for the cutting and weighing method can be written as

$$\Delta A/A = \sqrt{(\Delta A_B/A)^2 + (\Delta A_T/A)^2 + (\Delta A_R/A)^2 + (\Delta A_f/A)^2} \quad (34)$$

The relative magnitudes of the individual terms are much less obvious than was true for the height-width analysis, where, at least for the limiting case, all errors could be related to the basic reading error Δm. For a further analysis of the perimeter errors we have chosen to ignore the effects of the reading error ΔR and of paper thickness $\overline{\Delta f}$. These effects are independent of peak shape (although not of peak area) and at least in some cases should be minor. By considering only ΔB and $\overline{\Delta m}$ we arrive at a simple expression for investigating peak shape that is applicable to both perimeter methods.

$$\Delta A/A = \sqrt{(\Delta A_B/A)^2 + (\Delta A_T/A)^2} \quad (35)$$

Rewriting Eq. (35) in terms of (24) and (29) gives

$$\Delta A/A = \sqrt{(18\ \Delta B^2/\pi h^2) + \{[4\sigma + 2\sqrt{4\sigma^2 + (\pi/2)h^2}]\,[\overline{\Delta m}^\circ]^2/2\pi h^2\sigma^2\}} \quad (36)$$

Figures 7A, 7B, and 7C are plots of Eq. (36) as a function of peak shape for peaks of 1, 10, and 100 cm² area. In each of these three figures the base-line uncertainty ΔB is assigned the same value of 0.01 cm and is assumed to be independent of peak width. Since the magnitude of $\overline{\Delta m}^\circ$ is unknown, it is assigned values ranging from 0 to 0.1 cm to cover all cases of practical interest. As $\overline{\Delta m}^\circ$ approaches zero, the second term of Eq. (36) vanishes, and, in the limit, the relative error in area is that due to base-line placement.

Figures 7A, 7B, and 7C show that, depending on the value of $\overline{\Delta m}^\circ$ other than zero, optimum peak shapes exist for perimeter methods. For all three areas the $h/w_{1/2}$ value for the optimum shape decreases (i.e., the peaks flatten) as $\overline{\Delta m}^\circ$ decreases. It appears likely that the optimum shape for the perimeter methods is in the range of 1 to 10 for the height-to-width ratio. Optimum peak shape is more clearly defined for peaks of small area than for those of large area. In addition, and as would be expected, a given value of $\overline{\Delta m}^\circ$ induces a much larger relative error in area with peaks of small area than with peaks of large area.

Figure 7D illustrates the effect of varying base-line uncertainty for peaks of constant area (10 cm²) with a unit band-width uncer-

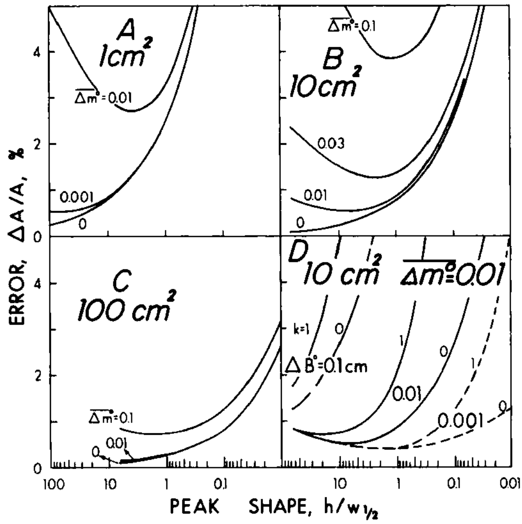

FIG. 7. Relative error as a function of peak shape for the limiting case of Eq. (35) for perimeter methods. The curves in A, B, and C are for peaks of 1, 10, and 100 cm² area with various values of $\overline{\Delta m}^\circ$ and base-line uncertainty $\Delta B^\circ = 0.01$ cm. (Errors due to instrument reading and paper thickness variation are not included.) D shows the effect of changes in base-line uncertainty through variation in both ΔB° and k of Eq. (22).

tainty $\overline{\Delta m}^\circ$ of 0.01 cm. A range of base-line uncertainties both with and without an increase due to increasing peak width is illustrated. As base-line uncertainty increases, the optimum peak becomes sharper.

This paper has been limited to the principal manual methods for measuring peak areas, and a detailed analysis of the indeterminate errors in the measurement of areas by mechanical or electronic integrators has not been attempted. No integrator can eliminate the base-line error and a $\Delta A_B/A$ term will always be present. In addition, there will be the equivalent of a reading error of magnitude depending on the sensitivity and range of the integrator.

Experimental measurements are in progress to test the relationships given in this paper and to determine the values of the appropriate parameters.

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