Designation: E 168 – 99

Standard Practices for
General Techniques of Infrared Quantitative Analysis[1]

This standard is issued under the fixed designation E 168; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These practices cover the techniques most often used in infrared quantitative analysis. Practices associated with the collection and analysis of data on a computer are included as well as practices that do not use a computer.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements appear in Section 6 and Note A4.7, Note A4.11, and Note A5.6.

2. Referenced Documents

2.1 *ASTM Standards:*
E 131 Terminology Relating to Molecular Spectroscopy[2]
E 334 Practice for General Techniques of Infrared Microanalysis[2]
E 932 Practice for Describing and Measuring Performance of Dispersive Infrared Spectrometers[2]
E 1252 Practice for General Techniques for Qualitative Infrared Analysis[2]
E 1421 Practice for Describing and Measuring Performance of Fourier Transform Infrared (FT-IR) Spectrometers: Level Zero and Level One Tests[2]
E 1655 Practices for Infrared Multivariate Quantitative Analysis[2]

3. Terminology

3.1 For definitions of terms and symbols, refer to Terminology E 131.

4. Significance and Use

4.1 These practices are intended for all infrared spectroscopists. For novices, these practices will serve as an overview of preparation, operation, and calculation techniques. For experienced persons, these practices will serve as a review when seldom-used techniques are needed.

5. Apparatus

5.1 The infrared techniques described here assume that the equipment is of at least the usual commercial quality and meets the standard specifications of the manufacturer. For dispersive instruments, also refer to Practice E 932. For Fourier Transform and dispersive instruments, also refer to Practices E 1421 and E 932 respectively, and for microanalysis with these instruments see Practice E 334.

5.2 In developing a spectroscopic method, it is the responsibility of the originator to describe the instrumentation and the performance required to duplicate the repeatability and bias of a method. It is necessary to specify this performance in terms that can be used by others in applications of the method.

6. Hazards

6.1 Users of these practices must be aware that there are inherent dangers associated with the use of electrical instrumentation, infrared cells, solvents, and other chemicals, and that these practices cannot and will not substitute for a practical knowledge of the instrument, cells, and chemicals used in a particular analysis.

7. Considerations for Quantitative Infrared Measurements

7.1 Quantitative infrared analysis is commonly done with grating, filter, prism, or interferometer instruments. The following guidelines for setting up an analytical procedure are appropriate:

7.1.1 Always operate the instrument in the most stable and reproducible conditions attainable. This includes instrument warm-up time, sample temperature equilibration, and exact reproduction of instrument performance tests for both standards and samples. After calibration, use equivalent settings for analyses. For all infrared instruments, refer to the manufacturer's recommendations for the instrument settings. After calibration, use these same settings for analysis.

7.1.2 The absorbance values at analytical wavenumbers should fall within the acceptably accurate range of the particular spectrometer used. In general, a single absorbance measurement will have the best signal-to-noise ratio when it is in the range from 0.3 to 0.8 absorbance units (AU) **(1)**.[3] The

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[1] These practices are under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and are the direct responsibility of Subcommittee E13.03 on Infrared Spectroscopy.
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[2] *Annual Book of ASTM Standards*, Vol 03.06.

[3] The boldface numbers in parentheses refer to the list of references at the end of these practices.

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sensitivity of Fourier transform (FT-IR) spectrometers is such that lower absorbance values can be used quite effectively, provided that the baseline can be estimated accurately (see Section 12). Absorbances greater than 0.8 AU should be avoided wherever possible because of the possibility of instrumentally-caused non-linearity, both for dispersive (2) and FT-IR (3,4) spectrometers. Variation of the concentration and sample path length can be used to adjust absorbance values into the optimum range. When multiple components are determined in a particular sample, it is acceptable to use absorbance values outside the optimum range, (5) however, absorbances greater than 1.5 AU should be avoided (2-4). Weaker absorption bands of high concentration components may be selected to provide absorbance values within the optimal range.

7.1.3 The most accurate analytical methods are implemented with samples in solution. With liquid samples that are not exceptionally viscous, best results are obtained if the cell is not moved after the first sample is introduced into the instrument (the fixed-cell method). The reason is that sample cell position is difficult to reproduce accurately by insertion into typical cell holders. Suitable fittings and tubes can be attached to the cell to allow sample changing in a flow-through manner. When it is not practical to use a flow-through cell, the cell should fit tightly in the holder so that lateral and tilting motions are restricted.

7.1.4 Unless there is reason to suspect deposition on or contamination of the cell from the samples, it is generally preferable to wash out the current sample with the next sample, if sufficient sample is available. The volume of sample used to flush the cell should be at least five times (and preferably more, for example, 20 times) the volume between the sample inlet and cell exit points.

7.1.5 For some bands, the wavenumber of the maximum absorbance changes as a function of concentration. Similarly, the position of the baseline points may change with concentration. Selection of baseline points must be done carefully to account for the shift of the absorbance maximum. The question arises whether it is preferable to measure absorbances at fixed wavenumber locations or at the observed maximum of the analytical band. The best approach is empirical testing of both the fixed point and the tracking methods of evaluation.

7.1.6 Whenever possible, working directly in absorbance is preferable. That is, either the instrument or associated data processor makes the necessary conversion from transmittance to absorbance. If spectra cannot be obtained in absorbance, then Eq A12.1 and A12.2 in Annex A12 can be used to convert the data.

7.1.7 Use spectral regions offering the most information on the analyte. Select analytical wavenumbers where the component has a relatively large absorptivity. In addition, other analytes should have minimal effect on the measured absorbance.

7.1.8 The performance of the spectrometer should be sufficiently good to give adequate linearity of response for the desired range of concentrations. The signal-to-noise ratio, S/N, should be acceptable for the desired precision.

7.1.9 Select analytical wavenumbers such that the linearity of the absorbance-concentration relationship is least affected by molecular interaction, dispersion in refractive index, and spectrometer nonlinearity.

8. Theory for a Single-Compound Analysis

8.1 Quantitative spectrometry is based on the Beer-Bouguer-Lambert (henceforth referred to as Beer's) law, which is expressed for the one component case as:

$$A = abc \tag{1}$$

where:
A = absorbance of the sample at a specified wavenumber,
a = absorptivity of the component at this wavenumber,
b = sample path length, and
c = concentration of the component.

Since spectrometers measure transmittance, T, of the radiation through a sample, it is necessary to convert T to A as follows:

$$A = -\log T = -\log \frac{P}{P_0} \tag{2}$$

where:
P_0 = input radiant power at the sample, and
P = radiant power transmitted through the sample.

9. Calibration for a Single-Component Determination

9.1 Proper sample preparation is essential to quantitative analysis. See Annex A4.

9.1.1 Quantitative analysis has two distinct parts: calibration and analysis. For a simple one-component analysis, select an appropriate solvent that is essentially free from interfering absorptions at the analytical wavenumber.

9.1.2 For calibration, measure the absorbances, A, of the analyte solutions at several known concentrations, c. Absorptivities, a, are then calculated, using Eq 1 with the baseline corrections as described in Sections 12-14. Alternatively, the absorbances, A, of a single solution in several cells of different, but accurately known, path lengths may be measured; however, interaction effects will not be elucidated in this fashion.

9.1.3 Calculate the average of the several a values for future use, or draw an analytical working curve by graphing absorbance versus concentration for a constant path length as demonstrated in Fig. 1. Use the linear part of the curve to calculate a. The calculation of a where curvature is present will be discussed in 18.1 and 18.2.

NOTE 1—In practice, the calibration curve may not have a y intercept of zero. This could be due to a variety of factors including, but not limited to, incompletely resolved analyte bands, reflection losses, and solvent interferences. It is important that the method used to calculate the calibration curve not force the y intercept to be zero.

9.1.4 For analysis, dissolve the unknown in the solvent, measure the absorbance, A, and determine the concentration, c, of the analyte graphically or by calculation. Convert this concentration in solution to the concentration in the unknown sample.

9.1.5 Both analysis time and chance of error are less if the concentrations of the unknowns and the cell path length are kept the same over a series of analyses, and the concentrations of the calibration solutions have bracketed the expected high and low values of the unknown solutions (6, 7).
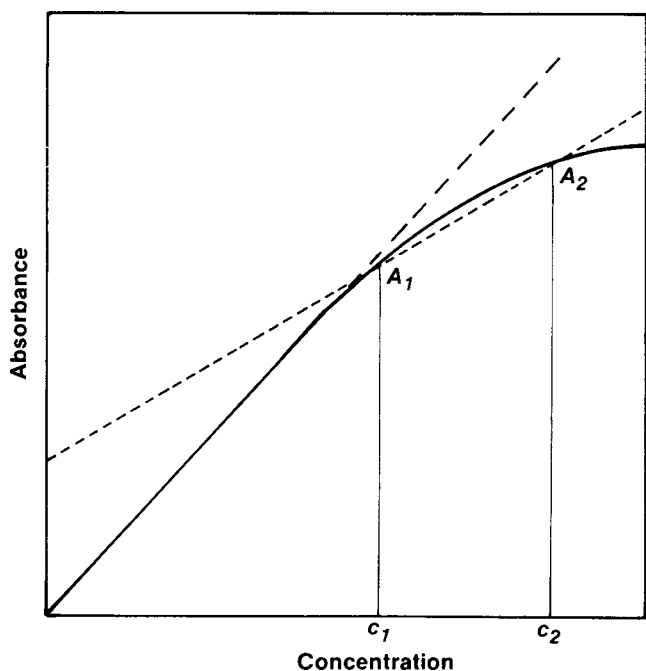
FIG. 1 An Analytical Working Curve

10. Theory for Multicomponent Analysis

10.1 Beer's law is expressed for a mixture of n independently absorbing components at a single path length and single wavenumber as:

$$A = a_1bc_1 + a_2bc_2 + \cdots + a_nbc_n \qquad (3)$$

Eq 3 defines an absorbance at a wavenumber as being due to the sum of the independent contributions of each component. In order to solve for the n component concentrations, n independent equations containing n absorbance measurements at n wavenumbers are necessary. This is expressed for constant path length as follows:

$$A_1 = a_{11}bc_1 + a_{12}bc_2 + \cdots + a_{1n}bc_n \qquad (4)$$
$$A_2 = a_{21}bc_2 + a_{22}bc_2 + \cdots + a_{2n}bc_n$$
$$\quad .. \quad \ldots\ldots \qquad \ldots\ldots$$
$$\quad .. \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots$$
$$\quad .. \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots$$
$$A_i = a_{i1}bc_1 + a_{i2}bc_2 + \cdots + a_{in}bc_n$$

where:
A_i = total absorbance at wavenumber i,
a_{in} = absorptivity at the wavenumber i of component n,
b = path length of the cell in which the mixture is sampled, and
c_n = concentration of component n in the mixture.

10.2 During calibration, concentrations c_n are known, and baseline corrected absorbances A are measured. The experimental absorptivity-path length products $a_{in}b$ are then calculated (see Note 2). During analysis, the absorptivity-path length products $a_{in}b$ are known, and the absorbances A are measured. The unknown concentrations are then calculated (see Section 17). Therefore, accurate calibration generally requires that experimental absorptivity values be obtained from at least n standards. The following requirements must be met:

10.2.1 The number of standards must be equal to or greater than the number of analytes, n, and

10.2.2 The number of analytical wavenumbers, i, must be equal to or greater than the number of independent components, n.

Note 2—All absorbance conversions use transmittance (that is, the decimal value), not percent transmittance. Regardless of form (that is, decimal or percent), the term transmittance refers to the term P/P_0 of Eq 2, and should not be called transmission. (See Terminology E 131).

10.3 The first requirement allows the analyst to use more than the minimum number of standards. Over-determination of standards permits error estimation in the analytical result. The second requirement allows the use of more than the minimum number of peaks for specifying a chemical system, where at least one distinctive band is selected for each component (7-10).

10.4 The procedures used in multicomponent analysis will be discussed further in the following section which is also an introduction to general solution phase analyses.

11. Multicomponent Solution Analysis

11.1 For the quantitative analysis of mixtures, Eq 4 is applicable. The absorptivities a_{in} of the n components of the mixture at the ith analytical wavenumber are determined from absorbance measurements made on each component taken individually. These absorbances must be measured under conditions (sample path length, temperature, pressure, and solvent) identical to those used for the unknowns, and they should be corrected for baselines as discussed in Sections 12-14. Absorbance measurements are made with concentrations of the analyte bracketing the amounts expected in the unknown samples.

11.2 Where possible, prepare samples as dilute solutions and place in cells of appropriate path lengths (typically 0.2 to 1.0 mm). Use lower concentrations in longer path length cells rather than higher concentrations in shorter path length cells to obtain absorbance values in the 0.3 to 0.8 range. Lower concentrations will minimize nonlinear effects due to dispersion (that is, change of refractive index with wavenumber). Where freedom from intermolecular effects is uncertain or where intermolecular effects are known to be present, calibration must be based on measurements taken from synthetic mixtures of all components as described in 15.1.2.

11.3 Dissolve a known weight of a pure component in a suitable infrared solvent. Measure the absorbance at all analytical wavenumbers and correct for baselines as discussed in Sections 12-14. Repeat this procedure for several concentrations covering the range of concentrations expected in the samples to be analyzed, remembering that concentrations of components must be linearly independent. Plot absorbance versus concentration. Similarly, construct analytical curves for this component at each of the other analytical wavenumbers. Repeat this procedure for each of the n components. Thus, there are i plots for each component, or a total of $i \times n$ analytical curves, each yielding one of the values of $a_{in}b$.

11.4 The number of standard mixtures required is at least equal to n, the number of components. For each analytical wavenumber, there will be a set of at least n equations in n

unknowns. The n sets of equations can be solved directly for the values of $a_{in}b$. If more than n synthetic mixtures are used as standards, a least-squares procedure can be used to calculate the values of $a_{in}b$. To repeat, in order to obtain information about errors, at least one more mixture than the number of analytes is needed.

12. Baselines in General

12.1 Any quantitative method depends on the choice of a reproducible baseline. The correction of raw data for baseline absorbance is important in some methods. The guiding factor in baseline selection is the reproducibility of the results. Methods used for drawing baselines with computerized instruments are similar in most ways to those for data recorded on chart paper. Where differences exist, they will be explained in Annex A1.

13. Single Wavenumber Measurement

13.1 A technique known as the "cell-in-cell-out" method is often used in single-beam infrared work. In this method, a blank (that is, solvent in cell, potassium bromide (KBr) pellet, or other substrate) is measured at a fixed wavenumber and then the analyte readings are recorded (7). In the simplest cell-in-cell-out method, a zero absorbance baseline is used (see Fig. 2). If the spectrum cannot be obtained in absorbance, the absorbance is calculated as in Eq A12.1 where $T_2 = 1.0$ and T_1 = transmittance at the analyte wavenumber (1, 6) (see Note 2).

14. Baseline Method (7)

14.1 The cell-in-cell-out technique was the method of choice for early single-beam infrared instruments. After the advent of double-beam dispersive spectrometers, the baseline method has been the method of choice. Portions of the data around the base of the bands are picked as baseline references. There are two common variations.

14.2 When one baseline point is chosen, the value of an absorbance minimum, A_2, is subtracted from the absorbance maximum, A_1, as demonstrated in Fig. 3. The point of minimum absorbance is adjacent to or at least in the vicinity of the band under evaluation.

14.3 Two points may be needed if the band of interest is superimposed on a sloping background. Manually a line is drawn from one side to the other as in Fig. 4. The absorbance of the band is calculated as the value at the peak maximum A_1 minus the baseline absorbance minimum A_{23}. An inappropriate choice of baseline in this situation may have deleterious effects on the accuracy of the final calculation.

NOTE 3—The above baseline correction procedure should be performed only if the spectrum is plotted in absorbance units. When the spectrum is plotted in transmittance, the two baseline transmittances and the transmittance at the analytical wavenumber should be converted to absorbance. The corrected baseline absorbance can be calculated by Eq A12.1 in Annex A12. Conversion to absorbance is required because a sloping linear baseline in transmittance becomes curved in absorbance.

15. Nonsolution Analyses

15.1 *Liquids*:

15.1.1 Analyzing a liquid mixture without the use of a diluting solvent is sometimes complicated by intermolecular forces. An absorption band may undergo intensity changes or frequency shifts, or both, relative to the same absorption band of the component in solution. The absorbance contribution of a component in a mixture can seldom be calculated from its absorbance measured in the pure state. It is desirable to determine the absorptivities from known mixtures having proportions near those of the samples.

15.1.2 Prepare mixtures having known concentrations of the various components covering the expected ranges. Measure baseline corrected absorbances at each of the wavelengths chosen for the analysis and substitute them (along with the
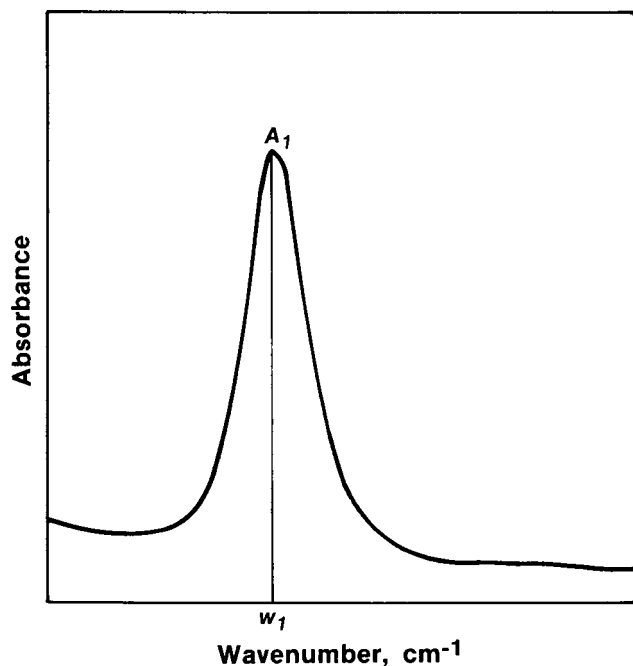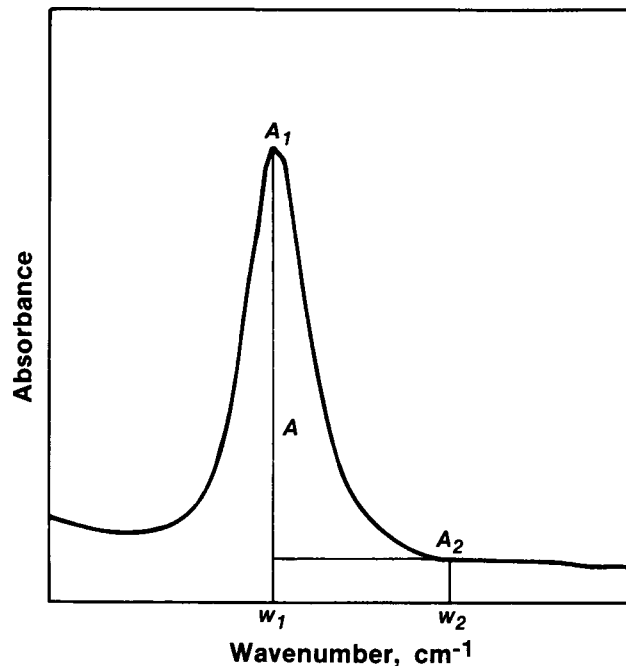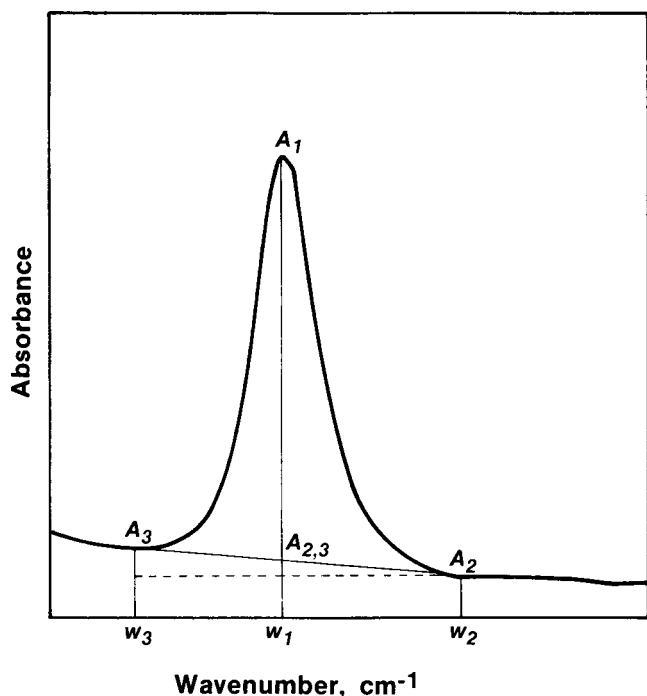

FIG. 2 A Zero-Absorbance Baseline

FIG. 3 A One-Point Baseline

FIG. 4 A Two-Point Baseline

known concentrations) in Eq 4. Solve for the absorptivity-path length products, $a_{in}b$ directly from the set of n simultaneous equations, or use a multivariant method (see Annex A8) if sufficient data are available.

15.1.3 If the concentrations in the unknowns vary widely, calculation of a second set of the $a_{in}b$ products is recommended. A second set may be necessary due to the presence of intermolecular influences, and the differences in the values of the absorptivities thus determined will indicate the extent of these influences.

15.1.4 A single set of absorptivities may not suffice to analyze mixtures throughout all possible concentration ranges of the components, in which case, narrowing the range of concentrations is recommended.

15.1.5 Since the $a_{in}b$ products are calculated directly in this procedure, it is not necessary to plot analytical curves.

15.2 *Solids*:

15.2.1 For cast films, pressed films, or pellets, follow the same general procedure as for liquids (see 15.1). Measure the thickness of each film and apply a proportional correction for deviations from standard thickness.

NOTE 4—The spectra of films and pellets can be complicated by the presence of a fringe pattern. For pellets and films, follow the suggestions in A4.5.1.2 and Note A5.1, respectively. A fringe pattern is undesirable because analyte absorbance values can be altered by its presence.

15.2.2 In cases where all components of a mixture are determined to a total of 100 %, it is usually sufficient to determine only the ratios of absorbances. In such cases, it is not necessary to know the thickness of the sample layer; it is only necessary to know the ratio of the components. However, a knowledge of the thickness is needed to determine the presence of impurities because the total then will be less than 100 %.

15.2.3 The above procedure for films is also used with powders prepared as mulls. Measurement of thickness can be

accomplished by an internal standard technique as described in A4.4.2. This involves the addition to the sample of a known weight ratio of a compound having an absorption band of known absorptivity that does not overlap the bands of the sample.

15.2.4 When powders are measured as pressed plates or pellets, analytical curves are prepared in the same manner as solutions, see Sections 9 and 11.

15.3 *Gases*:

15.3.1 All calibration measurements for a given analysis must be made at a fixed total pressure. This pressure must be equal to the total pressure employed in the analysis. An analysis may be set up in either of two ways:

15.3.1.1 *Method 1*—A fixed sample pressure is established that is a fraction of the total pressure obtained by addition of a nonabsorbing diluent gas.

15.3.1.2 *Method 2*—A fixed sample pressure is used as the total pressure. Analytical curves are prepared by introducing a pure component at various measured pressures which bracket the expected component pressures in the sample. A diluent gas is then added to bring the total pressure up to the established value.
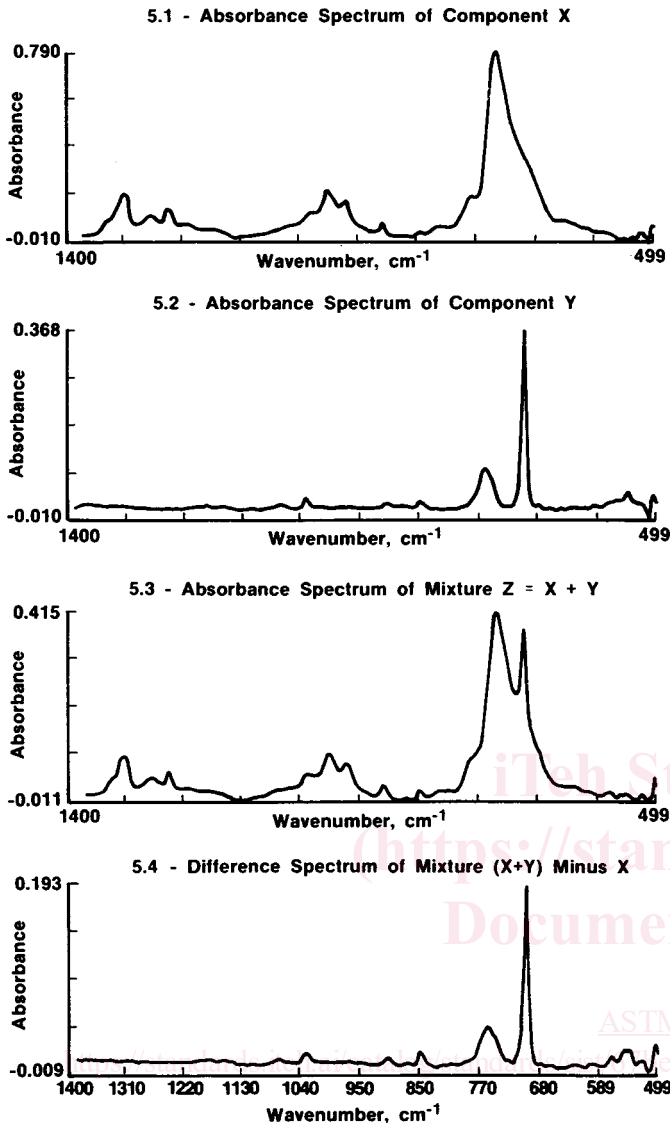
15.3.2 In Method 2, the analytical curve preparation does not allow for the possibility of band broadening for different components. This factor is more properly addressed by following Method 1 where the same diluent gas is employed for sample preparation and calibration. Low molecular weight gases frequently produce very strong, sharp absorption features. Addition of a diluent gas and use of pressure less than atmospheric may be necessary. Absorbances are measured for each standard at the wavenumbers selected for analysis. Where possible, integrated absorbances (see Annex A3) are preferred to offset the effect of small pressure variations. The absorbances are plotted against the partial pressures (or mole fractions) to produce analytical curves.

16. Difference Method

16.1 Spectral subtraction using a computer is a common practice in qualitative infrared analysis. This technique is also used to perform quantitative infrared analyses. The advantage of spectral subtraction (the difference method) is that small concentration differences can be measured with greater accuracy than is possible on superimposed bands.

16.2 A generalized procedure follows and is illustrated in Fig. 5. All spectra are obtained using samples of well characterized path length and concentration. Fig. 5(*c*) shows the spectrum of Z, an unknown mixture containing components X and Y. Using a subtraction routine, the spectrum of X is removed using the isolated, in this case higher, wave-number bands of X as a guide **(11)**. The concentration of Y is ascertained from Fig. 5(*d*) by reference to an analytical curve or by calculation as described in 9.1.3.

16.3 The same result is achieved with a noncomputerized double-beam spectrometer by placing sample X in the reference beam, and the unknown mixture in the sample beam. If the sample and reference are in solution, a variable path length cell can be used in the reference beam to remove spectral contributions due to X **(7, 12)**.

5.1 - Absorbance Spectrum of Component X

5.2 - Absorbance Spectrum of Component Y

5.3 - Absorbance Spectrum of Mixture Z = X + Y

5.4 - Difference Spectrum of Mixture (X+Y) Minus X

(a) Absorbance Spectrum of Component *X*
(b) Absorbance Spectrum of Component *Y*
(c) Absorbance Spectrum of Mixture *Z = X + Y*
(d) Difference Spectrum of Mixture *(X + Y) – X*

FIG. 5 An Example of Difference Spectroscopy

17. Calculation Methods

17.1 *Matrix Inversion*:

17.1.1 After the values of the $a_{in}b$ products have been determined for a given set of *n* components, according to 10.2, substitute the numerical values into Eq 4. Solve the *n* equations for concentrations, c_n, in terms of the baseline corrected absorbances, A_n, by matrix inversion **(6)**. The inverted equations will have the following form:

$$C_1 = A_1 F_{11} + A_2 F_{12} + \cdots + \quad A_n F_{1n} \qquad (5)$$
$$C_2 = A_1 F_{21} + A_2 F_{22} + \cdots + A_n F_{2n}$$
$$\begin{array}{ccccc} .. & ..... & ..... & .... & ..... \\ .. & ..... & ..... & .... & ..... \\ .. & ..... & ..... & .... & ..... \end{array}$$
$$C_n = A_1 F_{n1} + A_2 F_{n2} + \cdots + A_n F_{nn}$$

where F_{in} are the inverted coefficients. Thereafter calculation of individual sample concentration is simply done by substituting the measured absorbance values, A_n, in the equations.

17.2 Matrix inversion is a convenient method to calculate concentrations from the simultaneous equations presented in Eq 4. Programs for solving simultaneous linear equations using matrix-inversion techniques are available on many programmable calculators and computers and are contained in most commercial quantitative analysis programs. Classical least squares regression (CLS) is simply a sophisticated method of matrix inversion (see Annex A8).

18. Correction for Curvature in Beer's Law Plots

18.1 In some cases, the analytical curve of one or more analytes of a mixture will exhibit curvature to such an extent that the value of the slope may differ significantly between low and high concentrations. Two methods are acceptable: a non-linear regression using a computer or graphical method as immediately explained. If the graphical method (see 9.1.3) is used, and if the concentrations of analytes fall in the linear and low range, then the values of the slope for the linear range can be used. However, if the concentration is in the higher range, a correction is necessary. The following method is recommended:

18.1.1 The concentration of the component under consideration ranges in the sample between c_1 and c_2 in Fig. 1. Draw a straight line between A_1 and A_2. The slope of this line is the value of $a_{in}b$ that is used in Eq 4. The intercept of this line with the absorbance axis yield the value of a correction term, A_0, which must be subtracted from the measured absorbance of the sample at the analytical wavenumber of the analyte. This subtracted result is substituted for A_2 in Eq 4 at this analytical wavenumber. If the concentration of the component under scrutiny should happen to fall outside the range c_1 to c_2, it will be necessary to repeat the above procedure to determine the slope and intercept for the new concentration range.

18.2 In some binary mixtures, pure bands representing the individual components are not present. However, single bands or groups of bands, as intensities or area, can be ratioed and plotted to the known concentrations **(13)**. These calibration curves are almost always curved, but as explained in Ref. **(13)**, curved absorbance/concentration plots are not a problem since numerous computer programs are available for non-linear regression analysis.

19. General Considerations for Statistical Evaluation

19.1 The statistical evaluation of experimental data and the parameters necessary for reporting statistical confidence are described in this section and in Annex A6. The reliability of an experimentally measured quantity is an important factor which must be considered in evaluating any experimental technique. This reliability can be described by two terms: precision and bias. The precision of a technique refers to the reproducibility of replicate measurements; the bias represents the degree to which the measured quantity approaches the true value. The sources of experimental error limiting bias or precision, or both, are broadly classified as determinate or indeterminate error **(1, 14, 15)**.