Designation: E168 – 16

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

This standard is issued under the fixed designation E168; 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.

This standard has been approved for use by agencies of the U.S. Department of Defense.

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 practice does not purport to address all of the concerns associated with developing a new quantitative method. It is the responsibility of the developer to ensure that the results of the method fall in the desired range of precision and bias.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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, Note A4.7, Note A4.11, and Note A5.6.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E131 Terminology Relating to Molecular Spectroscopy

E334 Practice for General Techniques of Infrared Microanalysis

E932 Practice for Describing and Measuring Performance of Dispersive Infrared Spectrometers

E1252 Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis

E1421 Practice for Describing and Measuring Performance of Fourier Transform Mid-Infrared (FT-MIR) Spectrometers: Level Zero and Level One Tests

E1655 Practices for Infrared Multivariate Quantitative Analysis

3. Terminology

3.1 For definitions of terms and symbols, refer to Terminology E131.

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 E932. For Fourier Transform and dispersive instruments, also refer to Practices E1421 and E932 respectively, and for microanalysis with these instruments see Practice E334.

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 precision 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:

[1] These practices are under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and Separation Science and are the direct responsibility of Subcommittee E13.03 on Infrared and Near Infrared Spectroscopy.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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 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

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

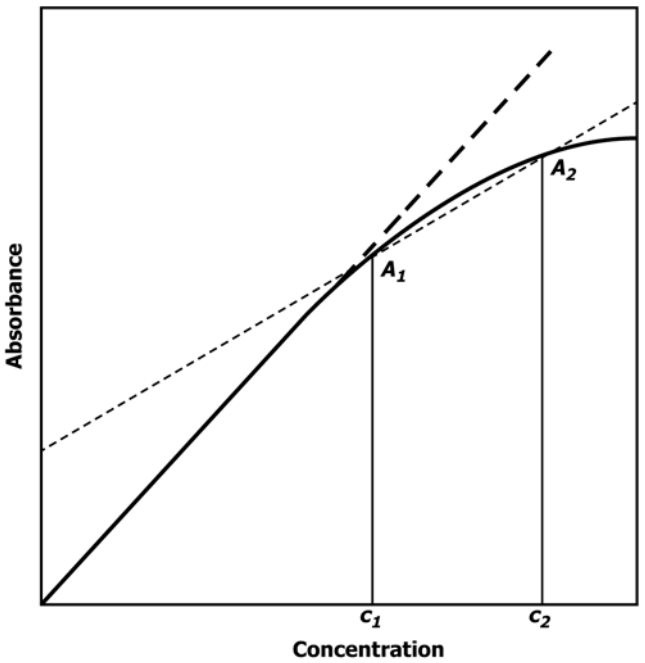


FIG. 1 An Analytical Working Curve

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)**.

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:

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

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:

$$A_1 = a_{11}bc_1 + a_{12}bc_2 + \cdots + a_{1n}bc_n \quad (4)$$

$$A_2 = a_{21}bc_2 + a_{22}bc_2 + \cdots + a_{2n}bc_n$$

.. ..

.. ..

$$A_i = a_{i1}bc_1 + a_{i2}bc_2 + \cdots + a_{in}bc_n$$

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 E131).

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.

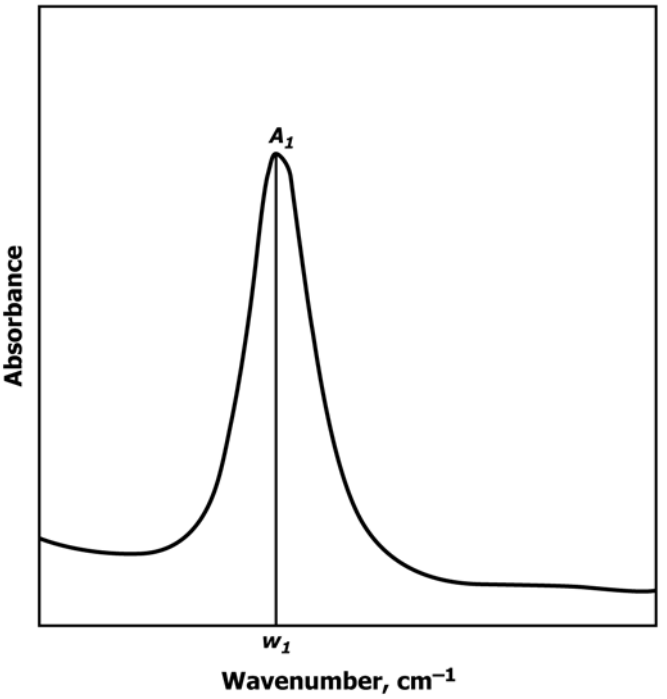


FIG. 2 A Zero-Absorbance Baseline

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

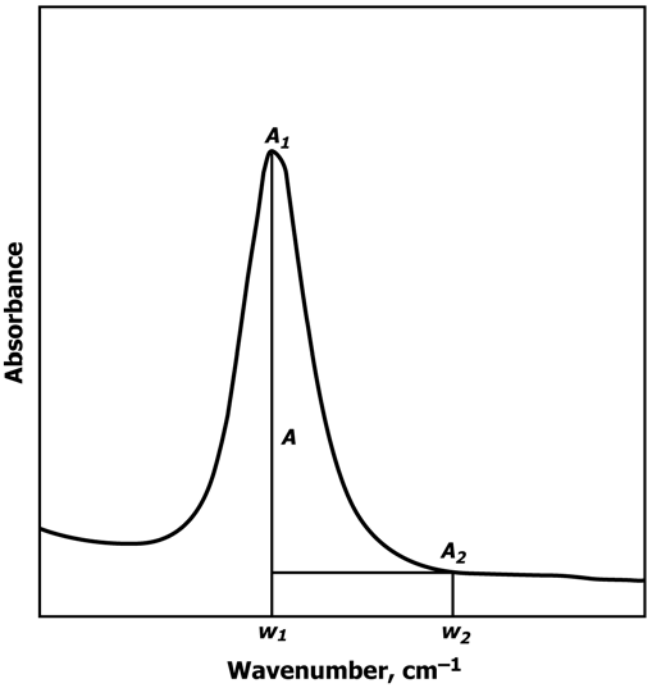


FIG. 3 A One-Point Baseline

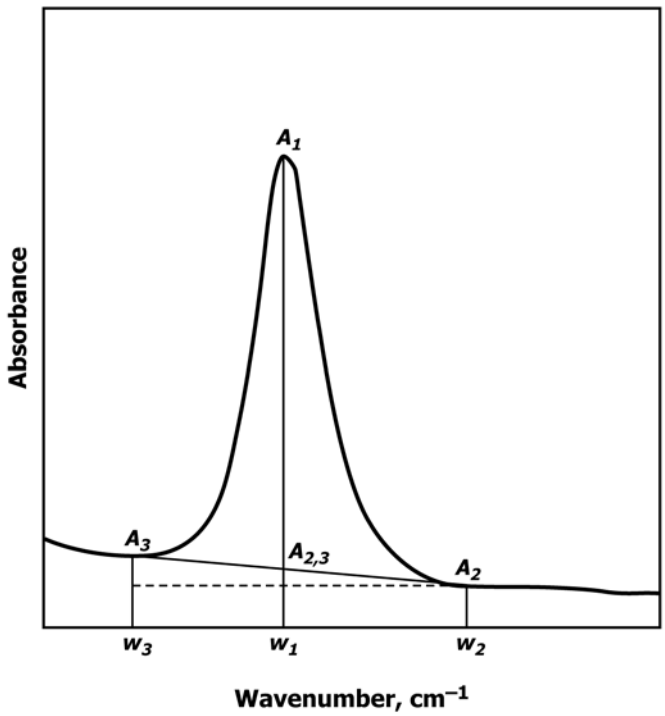


FIG. 4 A Two-Point Baseline

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 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.

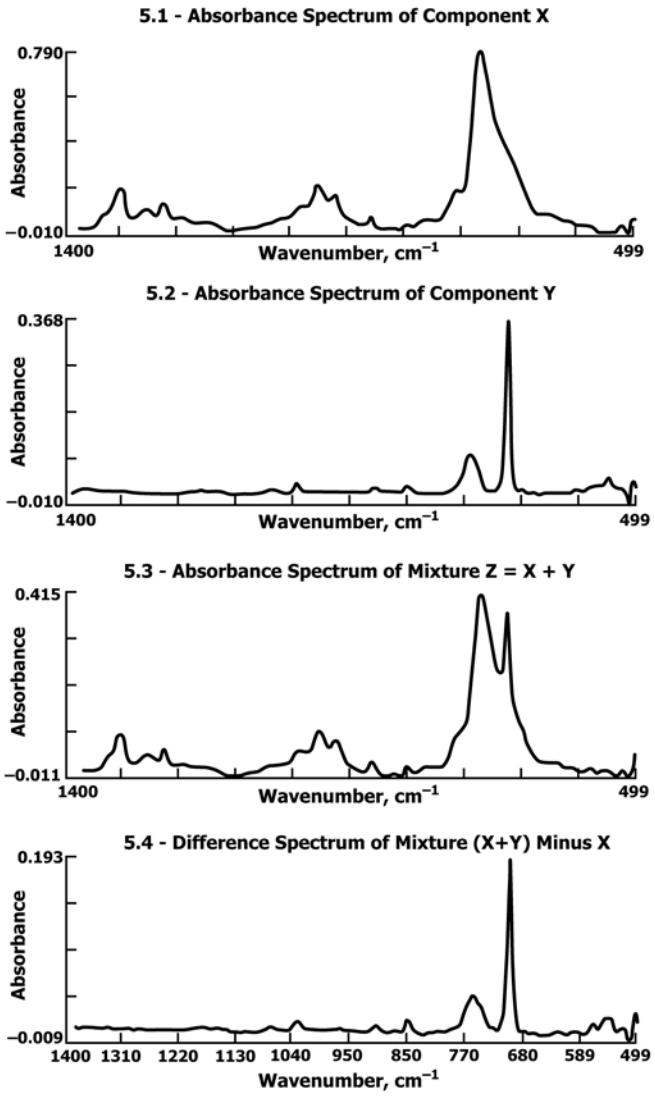
15.3.2 In Method 2, the analytical curve preparation does not allow for the possibility of band broadening for different components. This factor is more properly addressed by following Method 1 where the same diluent gas is employed for sample preparation and calibration. Low molecular weight gases frequently 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.



(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

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**).

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:

$$C_1 = A_1F_{11} + A_2F_{12} + \cdots + A_nF_{1n} \quad (5)$$

$$C_2 = A_1F_{21} + A_2F_{22} + \cdots + A_nF_{2n}$$

$$\cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots$$

$$\cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots$$

$$\cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots$$

$$C_n = A_1F_{n1} + A_2F_{n2} + \cdots + A_nF_{nn}$$

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**).

19.2 Determinate error is systematic error which can be attributed to definite causes. In quantitative infrared analyses, determinate error may arise from problems such as optical misalignment, photometric inaccuracy, stray radiant power, poor spectral resolution, improper sample handling, or deviations from Beer's law. Quantitative bias depends upon minimizing determinate error.

19.3 Indeterminate, or random, error arises from uncontrollable variables, and limits the precision with which measurement can be made. Often the major indeterminate errors are introduced by variation in sample positioning and errors in determining the baseline. However, if these are held constant, the major contributing indeterminant error frequently is detector noise, which is usually independent of signal. Therefore, the noise in transmittance units is independent of the amount of light reaching the detector. For a review of the sources of noise in Fourier transform instruments, see Ref (**11**) and Practice E1421.

19.4 For quantitative infrared spectrometry, the operative equation for determining concentration from transmittance measurements is Beer's law as follows:

$$A = -\log T = abc \tag{6}$$

$$c = A/ab \tag{7}$$

To determine the effect of random error (in the measurement of transmittance) (**1**, **12**, **15**) on the concentration, it is necessary to calculate the partial derivative as follows:

$$\frac{\delta c}{\delta T} = \frac{-\log e}{abT} = \frac{-0.434}{abT} \tag{8}$$

The standard deviation of the concentration s_c can be given by:

$$s_c = \left(\frac{0.434}{abT}\right) s_T \tag{9}$$

where s_T is the standard deviation of the transmittance measurement. The relative standard deviation of the concentration is:

$$\frac{s_c}{c} = \left(\frac{0.434}{\log T}\right)\left(\frac{s_T}{T}\right) \tag{10}$$

and the standard deviation of the transmittance is calculated from Eq A6.6 for a series of n measurements of T. s_T can be determined from the noise in the 100 % line since generally s_T will be independent of T.

20. Keywords

20.1 infrared spectroscopy; molecular spectroscopy; quantitative analysis

ANNEXES

(Mandatory Information)

A1. BASELINE PROCEDURES FOR COMPUTERIZED INSTRUMENTS

A1.1 Obtaining a Good Spectrum for Baseline Procedures

A1.1.1 There are two ways to get good (FT-IR) spectra. For the first method, three steps are necessary. *1*) Obtain both single-beam background and single-beam sample spectra. *2*) Ratio the single-beam sample to the single-beam background spectrum. This provides a spectrum in transmittance and requires conversion to absorbance. *3*) Convert to absorbance by computing the negative logarithm of the transmittance spectrum. The background can be that of the open beam, or an aperturing device, or an accessory, such as an ATR or gas cell or a liquid cell containing solvent used to dissolve the analyte.

NOTE A1.1—Dispersive duel-beam instruments perform the above by using the reference beam to obtain a background simultaneously. Hence, the reference beam should contain similar beam limiting accessories as

used in the sample beam. In some cases, for example with a gas cell, the above approach is impractical. The alternative is to run spectra of the sample and the empty accessory separately, then using the computer software to subtract the empty accessory from the sample. Keep in mind that these spectra must be in absorbance.

A1.1.2 The second method similar to A1.1 is frequently used when the spectrum of the sample material contains extraneous absorption features (for example, solvents or impurities). In this approach, the single-beam spectrum of the sample and the single-beam spectrum of the solvent or impurity are each ratioed against the single-beam background spectrum. Both transmittance spectra are converted to absorbance. The absorbance spectrum of the solvent or suspected impurity is then scaled by multiplying it by a factor chosen to minimize all spectral features caused by the solvent or impurity. The impurity or solvent spectrum is then subtracted from the sample spectrum. This paragraph describes in general how subtraction works with both FT-IR and dispersive spectra.

A1.1.3 For dispersive, optical-null instruments, the selection of instrumental settings or mode (for example, resolution, scanning region, etc.) are based on sample characteristics and the absorbance of the functional group being measured.

A1.1.4 In general, for both spectrometer types, spectral data are collected by the cell-in-cell-out method of 14.1. A baseline method is then used to obtain the actual quantitative data. These methods are demonstrated in Figs. 2-4.

A1.2 Calculation Procedure

A1.2.1 The calculation of data with one baseline point is discussed in 14.2.

A1.2.2 Automatic computation of peak absorbance with a two-point baseline is more subject to error. The calculations are based on the point-slope method, where the hypotenuse of a right triangle is the desired sloping baseline as shown in Fig. 4. The slope of the baseline may be either positive or negative. The peak absorbance is the result of the following:

$$A = (A_1 - A_2) - \frac{(A_3 - A_2)(w_1 - w_2)}{(w_3 - w_2)} \qquad \text{(A1.1)}$$

where:

A = corrected absorbance of the peak at w_1,
A_1 = uncorrected absorbance at w_1,
A_2 = baseline absorbance point at the lower wavenumber w_2, and
A_3 = baseline absorbance point at the higher wavenumber w_3.

A2. GENERAL CONSIDERATIONS FOR BAND AREA

A2.1 All data should be expressed in absorbance as a function of wavenumber.

A2.2 Band shape changes can cause peak-height data to be nonlinear. Band area, however, may remain essentially unaffected by the changes in shape of the band because band area is a function of the total number of absorbing centers in the sample. If the shape change is caused by changes in intermolecular forces, even band area may not be linear.

A2.3 Band area is calculated by integrating across bandwidth. Band area is advantageous when band shape undergoes change as a function of increasing concentration. Frequently, band area is found to be more accurate than peak-height measurements because one is, in effect, averaging multipoint data.

A2.4 When integrated area is used for quantitative analyses, the reliability of the results frequently depend on the baseline treatment selected. The accuracy by band area is often improved by limiting the range of absorbances. The wings contribute very little signal while contributing substantial uncertainties to the total area. A useful guideline is to limit the integration limits to absorbance values which are no smaller than 20 to 30 % of the peak absorbance.

A3. CALCULATION OF BAND AREA

A3.1 In reference to Fig. A3.1, when no baseline points are used for an area calculation, the area between lower and upper wave-number limits is the following:

$$I = A_{w4}\Delta + A_{w4+1}\Delta + \cdots + A_{w5-1}\Delta \quad \text{(A3.1)}$$

where:

I = integrated absorbance (area),
Δ = sampling interval in wavenumbers,
A = absorbance measured at the designated interval,
w_4 = lower wavenumber limit, and
w_5 = upper wavenumber limit.

The number of points in the sum is $np = (w4 - w5)/\Delta$.

A3.2 In reference to Fig. A3.2, for a one-point baseline treatment w_2 with a corresponding absorbance A_{w2}, the area I_1 is as follows:

$$I_1 = I_0 - I_{1b} \quad \text{(A3.2)}$$

$$I_1 = I_0 - (A_{w2})\,(w_5 - w_4)$$

$$I_1 = I_0 - [(\Delta_{w2}\ (np - 1))]$$

where I_0 is given by Eq A3.1.

A3.3 If a two-point baseline treatment is used with absorbances A_W2 at wavenumber w_2 and A_w3 at wavenumber w_3, as shown in Fig. A3.3, the formulation is as follows:

$$I_2 = I_0 - I_{2b} \quad \text{(A3.3)}$$

$$I_{2b} = \left[\sum_{j=1}^{np}\left(A_{w2} + \frac{(A_{w3} - A_{w2})\,(w_4 - w_2 + j\Delta)}{(w_3 - w_2)}\right)\right]\Delta \quad \text{(A3.4)}$$

and I_0 is given by Eq A3.1.

NOTE A3.1—The algorithms above are not the most accurate, but as Δ becomes smaller, all methods (that is, trapezoidal and Simpson's rule) approximate the same value.

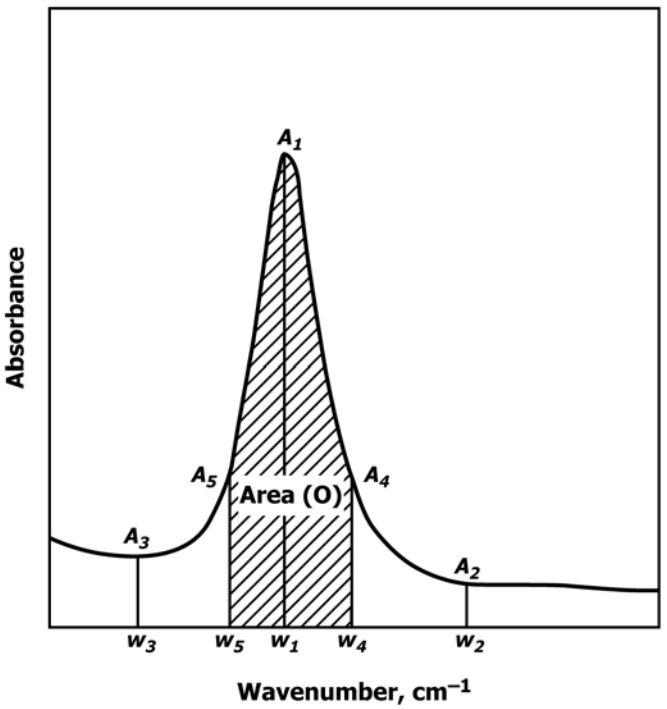


FIG. A3.1 Band Area With Zero-Absorbance Baseline

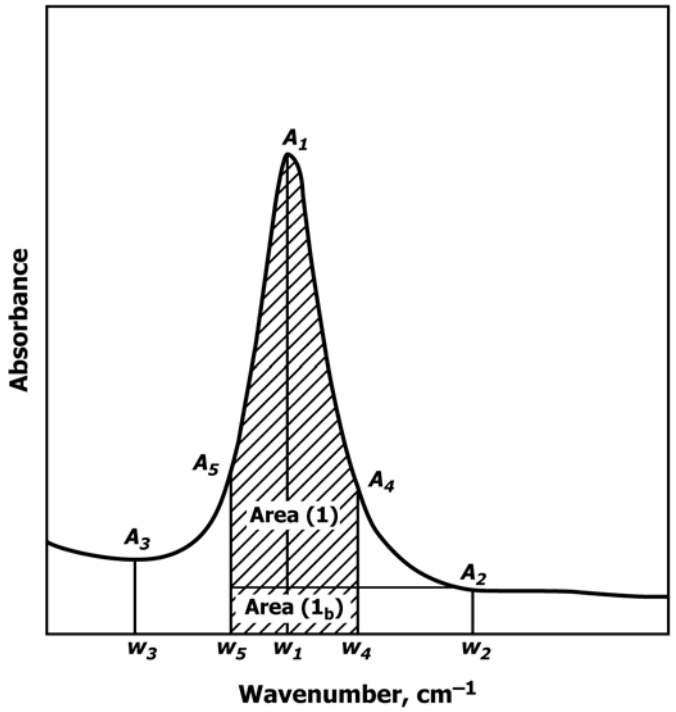


FIG. A3.2 Band Area With a One-Point Baseline

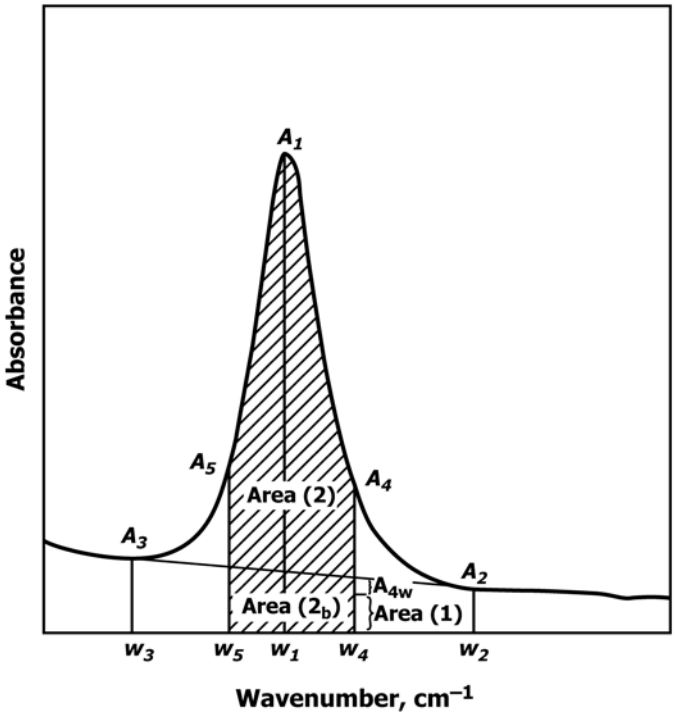


FIG. A3.3 Band Area With a Two-Point Baseline

A4. SAMPLE PREPARATION

A4.1 Where possible, solution techniques are recommended. However, other methods are discussed.

A4.2 *Liquids*—Liquids may be measured either neat or preferably, as solutions in a sealed cell of suitable path length (**15**).

A4.3 *Solution Techniques*:

A4.3.1 For liquids and solids, the following procedures are recommended but may not be all encompassing:

A4.3.2 Choose a solvent that has minimal absorbance at the analytical wavenumbers and that will completely dissolve the sample.

A4.3.3 Measure a specified amount of sample into a volumetric flask or other suitable container, and add some of the solvent to be used. The analyte must be completely dissolved.

A4.3.4 Allow for temperature equilibration to the same temperature as that used for the standards.

A4.3.5 Make up to volume or weight with solvent and mix thoroughly.

A4.3.6 Aqueous solutions can be analyzed using flat surface or circular ATR techniques.

NOTE A4.1—For other difficult solutes, such as elastomers and tars, it is frequently more convenient to roughly weigh the solute in a suitable container, add solvent from a graduated cylinder, dissolve, and run the analysis. The concentration is then obtained by doing a percent solids content on an aliquot from the remaining solution.

NOTE A4.2—Solvent influence or overlap on the absorption bands to be measured must be recognized and taken into account in the calculation.

NOTE A4.3—For best results, measure unknown sample solutions at concentrations that will place the analyte absorbances in the range of those used for calibration.

A4.4 *Solids by Mull Techniques* (**8, 12**):

A4.4.1 The following is the recommended procedure for preparing solids by mull techniques; however, other methods also may be appropriate. Average particle size of the sample should be reduced to less than the analytical wavelength. The particle size reduction is relatively easy for many samples without incurring any change in the sample. However, polymorphic changes, degradation, and other changes may occur during grinding. If such changes do occur and are not controlled, the method will not be valid. If changes are suspected, other techniques such as attenuated total reflectance (**12**) or diffuse reflectance (**11**) should be investigated.

A4.4.2 Weigh slightly more than the minimum amount of sample required, and then weigh the desired amount of an appropriate internal standard. Mix thoroughly.

NOTE A4.4—An appropriate internal standard (**7, 11, 12**) is a substance that (*a*) exhibits a band in a suitable region of the spectrum, and as close to the analyte's wavenumber as possible, (*b*) is not present in the sample, and (*c*) does not react chemically with or dissolve in the sample or mulling agent. An alternative to a separate internal standard is to use a band in the sample that does not change as the moiety of interest is varied. This approach is very useful in polymer analyses.

A4.4.3 Break up the mixture and distribute it over the mortar surface by gentle grinding with the pestle. Rub to an extremely fine powder by vigorous back and forth grinding until the mixture becomes caked and takes on a smooth, glossy, light-reflecting surface.

A4.4.4 Add one drop of mulling agent (see Note A4.5), grind with a rotary motion, using a smooth, hard mortar and pestle, until all of the mixture is picked up and suspended. The suspension should be viscous, not fluid.

NOTE A4.5—Mineral oil, fluorinated hydrocarbon, or appropriate substances can be used as the mulling agent. Matching the refractive indices as closely as possible gives best results.

A4.4.5 Transfer the sample to a clean, flat salt plate with a clean, rubber policeman or clean, plastic spatula.

NOTE A4.6—A discussion of salt plates and cell-window materials is covered in Practice E1252.

A4.4.6 Cover with a second clean, flat plate; squeeze and rotate to obtain the desired thickness and to remove all trapped air.

A4.4.7 Visually observe the scattering of light passing through the sample. As a guideline, the sample may appear slightly hazy, but objects on the far side should be distinguishable.

A4.5 *Solids By Pressed-Pellet Technique* (**8, 12**):

A4.5.1 Particle size of the sample should be reduced to less than the analytical wavelength. This is relatively easy for many samples without incurring any change in the sample. However, polymorphic changes, degradation, and other changes may occur during grinding. If such changes do happen and are not controlled, the method will not be valid.

A4.5.1.1 Grinding conditions must be established for the particular sample type since grinding severity can affect absorption-band intensity.

A4.5.1.2 Weigh the preground sample and powdered potassium bromide (KBr) or other alkali halide in specified amounts (sample weight should be about 1 % of the KBr weight; about 350 mg KBr is appropriate for a 13-mm disk to avoid interference fringes), and mix by hand in a mortar. This should be a mixing step rather than a grinding step.

A4.5.1.3 Place the mixture in an appropriate evacuable die, evacuate to at least 15 mm Hg and press at sufficient pressure to produce a transparent disk. Follow the manufacturer's recommendation as to pressure for a particular die. Other methods, such as minipress cells are also used, but these methods may not be as satisfactory due to crazing caused by trapped air and water vapor.

NOTE A4.7—**Precaution:** During pressing operations, place the die symmetrically in the press. Otherwise, the die may forcefully slip out of the press, causing personnel injury or damage to surrounding equipment. Some laboratories require safety shields in front of presses.

NOTE A4.8—Since the purity of alkali halide powders are not all the same, the same alkali halide powder should be used for the sample and blank.

NOTE A4.9—If a steel-ball mill is used, clean, dry, rust-free stainless steel vials and balls are recommended. A blank pellet (7) is made in the same manner as for the sample using the same ball-mill equipment, and procedures.

NOTE A4.10—Samples may undergo polymorphic changes or reactions such as oxidation, ion exchange, or salt formation with the alkali halide material. These events may also invalidate a method.

A4.6 *Gaseous Samples* (12, 15, 16, 17):

A4.6.1 Take care that samples do not affect cell windows, mirror, or interiors.

A4.6.1.1 Either fixed or variable path length cells can be used for gas analyses. Cells with path lengths from 1 cm to 20 m are readily available and other lengths can also be obtained.

A4.6.1.2 Cells can be filled through the use of a vacuum system coupled to a pressure gage for measurement of partial pressures.

A4.6.1.3 An alternative method for filling and using long path length cells is to have the cell incorporated into a closed-loop pumping system of known volume. A known (or unknown) sample can be injected from a gas syringe into the closed loop containing a diluent gas. The closed loop greatly reduces the time for mixing and helps to provide a homogeneous sample for analysis.

NOTE A4.11—**Precaution:** Safety considerations require the use of proper pressure reduction valves for metering gases into a sample bomb. Take precaution when mixing potentially hazardous or reactive gases.

NOTE A4.12—All measurements for calibration and analysis under a given analytical system should be at a single, reproducible total pressure, since the band intensity is a function of the partial pressure of the analyte, the total pressure of the sample, and the total pressure within the cell and the temperature.

NOTE A4.13—Mixing does not happen automatically. Attention must be paid to assure complete transfer and equilibrium mixing.

NOTE A4.14—Calibration mixtures should be cross-checked by an independent method.

A5. PREPARATION OF POLYMERS

A5.1 Polymeric materials are studied by a variety of techniques; the choice depends on the physical properties of the particular polymer. Thermoplastics are hot pressed to form films of a desired thickness. Cross-linked elastomers are often prepared by microtoming, while others, particularly reinforced materials, can be cold ground and pressed into a pellet using a suitable sintering agent such as KBr or made into a mull using a suitable suspending liquid such as mineral oil or fluorocarbons. Another method of forming a thin film is to cast from solvent. This is especially suitable for resins which either are not thermoplastic or break down at pressing temperatures. All of these techniques can be successfully used for quantitative or semiquantitative analysis. If the above methods are not satisfactory, either ATR (12) or diffuse reflectance (11) may be useful.

A5.2 Preparation of polymers by the hot-pressing technique requires a suitable hydraulic press with heated platens capable of reaching the softening point of the polymer. In some cases, water-cooled platens are also necessary to obtain the desired results. The pressing zone for a number of common polymers is given (18, 19, 20, 21). For quantitative results, the use of a template with an opening to accommodate the infrared beam is recommended. Typically templates from 0.3 to 6 mil (7.5 to 150 μm) are easily cut from metal shimstock. Sufficient powdered or pellets of polymer to fill the opening is placed in the template between two layers of aluminum foil which are then placed between two larger smooth backing plates. A small amount of pressure is applied while the polymer heats to the desired temperature, then pressure is increased rapidly to the desired value and released after a predetermined time. The template and the pressed film are quickly quenched (and dried gently, if necessary). Film thickness is determined by averaging multiple micrometer measurements of the sample area in the infrared beam. These measurements can then be used to calibrate the thickness with standard bands.

NOTE A5.1—Occasionally, a sinusoidal pattern is superimposed on a spectrum. This is referred to as fringing. The fringe pattern is caused by multiple internal reflections within the sample. This phenomenon can be reduced by placing fine (Grade 6/0) corundum paper between the first sheet of aluminum foil and the backing plate. The corundum paper causes a matte finish which scatters the reflected light diminishing the multiple internal reflections. This technique must be used cautiously because precision can be lessened by the matte finish (7).

NOTE A5.2—Aluminum foil or other metal in contact with the molten polymer may need a mold-release agent. Many releasing agents contain silicone polymers which can interfere with the peaks of interest. Wiping the surface with a solvent is sometimes effective, but use of cover plates coated with TFE-fluorocarbon or with a nonmigratory releasing agent is better.

NOTE A5.3—For very thin films, templates are less useful, and release-coated, flat metal plates can be used. Use smaller amounts of polymer to achieve thinner films.

NOTE A5.4—For quantitative determination of blended components, it is advisable to increase homogeneity by pressing a large sample, about 5 g, into a film. Cut this film and stack the pieces so outer edges will lie on center portions, and then repress. Select smaller samples from this blend for final pressing.

NOTE A5.5—Pressing techniques must be used with caution when investigating crystalline polymers, or polymers with other order-producing structures. These polymers are temperature sensitive. Some problems can be avoided by annealing under controlled conditions before quantitative measurements are made.

A5.3 *Microtoming* —This technique is used for cross-linked and hard elastomers. Hand-driven and automated microtomes are available where sample size and thickness are preselected for the analysis method. Temperature of the sample and knife should be controlled within the plastic range of the material (**22**).

A5.4 Friable polymers may be prepared for infrared examination by the pressed-pellet technique as in A4.5. Grinding at reduced temperatures allows the technique to be extended to soft or rubbery polymers. Avoid water contamination by condensation. Quantitative results are obtained by one of several methods: (*a*) using an internal standard band from one of the components, (*b*) adding a known quantity of an inert internal standard such as potassium thiocyanate, or (*c*) using a known concentration of polymer in the pellet matrix and measuring the thickness of the pellet.

A5.5 Friable polymers may also be prepared for infrared examination by the mull technique as in A4.4. For quantitative measurements, the best results are usually obtained by using an internal reference band, but adding an internal standard is an acceptable alternative.

A5.6 Polymers can be prepared as cast films. Selection of solvent is important since both solubility of the polymer and volatility of the solvent affect the resultant film (**23**). A film can be cast on the surface of an IR transparent material, or a free-standing film can be cast on the surface of glass, metal, water, TFE-fluorocarbon, polyethylene, or mercury (**Warning—**See Note A5.6.) The dried film is then mounted directly in the infrared beam. Uniform thickness is very difficult to achieve and best quantitative results are obtained by using a calibrated, preselected internal-thickness band.

NOTE A5.6—**Warning:** Mercury vapor is toxic. Exercise precaution when mercury is used.

NOTE A5.7—Polymer crystallinity and phase separation of blends commonly result as film drying progresses. Multiple areas should be sampled to test composition, and to provide average values.

A6. MORE ABOUT STATISTICS

A6.1 *The Gaussian Distribution and Confidence Intervals*:

A6.1.1 Often random error has a normal (Gaussian) distribution (**16,17**). This is generally true when there is no single dominant source of error. We can define a population as a hypothetical set of N observations from which the sample of observations actually obtained are taken. In a normally distributed population, the probability that the measured quantity has a value between x and $x + dx$ is given by $p(x)\,dx$ as follows:

$$p(x)dx = \frac{(2\pi)^{-1/2}}{\sigma}\exp\left[\frac{-(x-\mu)^2}{2\sigma^2}\right]dx \qquad (A6.1)$$

where μ is the true value of the measured quantity and σ is the true standard deviation of the measurements. σ is defined:

$$\sigma = \left[\frac{\sum_{i=1}^{n}(x_i-\mu)^2}{N}\right]^{1/2} \qquad (A6.2)$$

where N is the number of measurements. The standard deviation, σ, has the same units as the quantity being measured and expresses the degree of scatter among the measured quantities and is related to the precision of the measurements. The quantity σ^2 is called the variance and has additive properties. The overall variance of a multistep process is simply the sum of the variances of the individual steps if there is no correlation between the sources of variation of the individual steps. The square root of the overall variance yields the standard deviation for the whole process. To more fully visualize the utility of the standard deviation, the following substitution can be made:

$$y = \frac{(x-\mu)}{\sigma} \qquad (A6.3)$$

$$p(y)dy = (2\pi)\exp\frac{(-y^2)}{2}\,dy \qquad (A6.4)$$

The area under the distribution curve represents the probability that a measured value will be within a particular range, and the confidence interval placed upon a measured value can be given in terms of the standard deviation. For example, if the error is normally distributed, 68.26 % of the time the value of the single measurement will lie in the range between −σ and σ; 95.44 % of the time the measurement will lie between −2σ and 2σ; and 99.74 % of the time the measurement will lie between −3σ and 3σ.

A6.2 *Gaussian Distribution of a Sample*:

A6.2.1 In practice it is necessary to deal with a limited number of measurements, n (**14, 24, 25**) called a sample, which are obtained from the population. From these n observations, the arithmetic mean, $\bar{x}$, is taken as an estimate of the true population mean, μ, where:

$$\bar{X} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad (A6.5)$$

A6.2.2 The operational definition of the standard deviation must be modified for the sample, and it is given the symbol s, where:

$$s = \left[\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1} \right]^{1/2} \quad \text{(A6.6)}$$

s^2 is then the variance for the sample. The confidence interval of the mean for the sample is determined from the Student's t distribution as follows:

$$\mu = \bar{x} \pm \frac{ts}{n^{1/2}} \quad \text{(A6.7)}$$

Tables of Student's t distribution for appropriate significance levels and appropriate n values (or degrees of freedom, $n - 1$) are given elsewhere (**25-28**).

A6.3 *Significance Testing*:

A6.3.1 *The t-Test*—The t-test (**14, 25, 26**) is useful (*1*) for comparing the mean of an experimental study with an accepted value or (*2*) for comparing the means of two sets of data. In the first case t is defined (by rearranging Eq A6.7) as follows:

$$t = \frac{(\bar{x} - \mu)n^{1/2}}{s} \quad \text{(A6.8)}$$

where the symbols have their usual meanings. Slight modifications are necessary for the second case as follows:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s} \left(\frac{n_1 n_2}{n_1 + n_2} \right)^{1/2} \quad \text{(A6.9)}$$

$$s^2 = \frac{(n_1 - 1)s_1^{\,2} + (n^2 - 1)s_2^{\,2}}{n_1 + n_2 - 2} \quad \text{(A6.10)}$$

where the subscripts refer to the different data sets.

A6.3.2 The value of t calculated from the experimental data is compared to table values. It is clear that the value of t will become larger as the difference between $\bar{x}$ and μ (or $\bar{x}_1$ and $\bar{x}_2$) becomes larger, and large t-values imply statistically significant differences between the quantities being compared. The table value of t is chosen according to the significance level required and the number of degrees of freedom ($n - 1$ for the first case and $n_1 + n_2 - 2$ for the second case). If the calculated value of t equals the table value for the 95 % significance level, then only 5 % of the time would random error account for the difference in the quantities being compared.

A7. ADVANCED STATISTICAL METHODS

A7.1 Detailed descriptions of multilinear regression (also called inverse least-squares), principal components regression and partial least squares are discussed in Practices E1655. Other methods of multivariate analyses are briefly discussed below as classical least squares, cross-correlation, factor analysis, and analyses using derivative techniques. Because of the many possible variations of these methods and their relative complexity, only the general nature of these methods is presented.

A8. CLASSICAL LEAST-SQUARES METHODS

A8.1 When the quantitative equations relating A and c are written in the form of Beer's law (see Eq 4), then the least-squares analysis is a classical regression (often referred to in infrared spectroscopic literature as the K-matrix method) (**28-35**). Either univariate methods (that is, each reference spectrum contains only spectral features of one component in the spectral region of interest (**28, 29, 31**) or multivariate methods (that is, the calibration standards are mixtures of the pure components) (**30**) may be used. The univariate method is simply a special case of the multivariate technique. When the multivariate methods are employed, the least-squares analysis is a two-step analysis involving both a calibration step and an analysis step. The calibration step takes the m known calibration mixture spectra and estimates the pure component spectra by least-squares methods. The calibration equations are as follows:

$$A = KC + E \quad \text{(A8.1)}$$

where the A matrix is an $i \times m$ matrix of m calibration spectra at i wavenumbers, and C is an $n \times m$ matrix of the n known component concentrations for each of the m mixtures. K is the $i \times n$ matrix of the n pure component spectra at unit concentration and unit relative path length for each of the i wavenumbers. E is the $i \times m$ matrix of random noise or error in the absorbance values of the calibration spectra. K is estimated by least-squares methods as follows:

$$\hat{K} = AC' (CC')^{-1} \quad \text{(A8.2)}$$

where $\hat{K}$ is the least-squares estimate of K, the primes indicate transposed matrices and the superscript $^{-1}$ indicates the inverse of the matrix. The estimated K matrix, $\hat{K}$, is then used in the least-squares prediction of the unknown sample concentration as follows:

$$\hat{C} = \left(\hat{K}\hat{K}'\right)^{-1} \hat{K}' A \quad \text{(A8.3)}$$

where Ĉis the least-squares estimate of the unknown sample concentrations and A is now the unknown sample spectrum at i wavenumbers. In the univariate case, the K matrix is simply constructed directly from the measured pure component spectra. Baseline fitting of the sample spectrum can also be included in the solution of Ĉ in Eq A8.3 to allow for baseline variations between the calibration and sample spectra (**28, 29**). A weighted least-squares analysis may also be used to give greater emphasis to those spectral data with greater signal-to-noise ratios (**29, 30**). In Eq A8.2 and A8.3, the final number of simultaneous equations to be solved can be reduced to the number of components used in the analysis. This means that the dimension of the matrix which must be inverted is also the same as the number of components. Since this dimension is independent of the number of wavenumbers used in the analysis, the complexity of matrix inversion is also independent of the number of wavenumbers used. This favors the use of a large number of wavenumbers in the analysis which improves bias and precision and makes analyses possible even when the signal-to-noise ratios are less than one (**28**). Note that the use of a larger number of wavenumbers also increases the chance that the analysis will be compromised by an unknown component. The effect of an unknown component can be reduced if the algorithm is written to minimize the influence of those regions of the spectra that contain the unknown interferences (**29, 30**). The minimum number of calibration standards and wavenumbers necessary for the analysis is equal to the number of components.

A9. CROSS-CORRELATION METHODS

A9.1 The use of cross-correlation methods in infrared spectroscopy has recently been described (**20, 32, 33**). Cross-correlation techniques can evaluate the similarity between two spectra (for example, the reference and the sample) to determine quantitatively the amount of one contained in the other. If Beer's law is valid, then the value of the correlation function at zero displacement is a linear function of concentration. The mathematical details are given (**20, 32, 33**).

A10. ABSTRACT FACTOR METHODS

A10.1 Abstract factor methods utilize the concept of representing spectral data as a linear data set. Instead of using discrete data points representing changes in component concentration, factor analysis uses the entire spectrum of each standard and transforms the data into a linear representation of quantitative information. Factor abstract methods involve finding a set of principal factors (for example, eigenvectors or loadings) required to reproduce the calibration spectra. The relation between the scores of these principal factors for each calibration spectrum and the desired properties is determined by the application of standard linear regression methods. Analysis of an unknown sample is then accomplished by determining the scores of the principal factors for the sample spectrum. The relation between scores and concentrations determined from the sample data can then be used to yield the sample concentrations. The linear regression analysis may be carried out using any of a number of factor spaces (or basis sets) including Fourier and Taylor series or more commonly, principal components analysis (PCA or PCR) and partial least squares (PLS). Theories and application of factor analysis methods are presented in the literature (**9, 10, 21, 36**) and Practices E1655.

A11. DERIVATIVE METHODS

A11.1 Derivative methods are useful when the absorption band or bands being analyzed do not yield a suitable choice for a baseline as described in Sections 12 and 13. Derivative techniques have generally required that isolated spectral bands be used in the analysis although one least-squares full-spectrum derivative method has been described which allows for overlapping peaks (**31**). The derivative of a band or spectrum can often be calculated by taking the difference between absorbances in the spectrum at equally spaced wavenumbers. However, because derivatives increase spectral noise, mathematical smoothing (for example, Savitzky-Golay smoothing) is often used either before or after derivatives are calculated.

A11.1.1 *Use of First Derivatives*—There are three possible methods used in first-derivative analyses. These include using the magnitude of the positive first-derivative lobe, the magnitude of the negative-derivative lobe, or the difference between the two lobes. The ratio of any of the above quantities between corresponding sample and reference bands is assumed to be proportional to the ratio of sample and reference concentrations.

A11.1.2 *Use of Second Derivatives*—The second-derivative spectrum may be calculated from the first-derivative spectrum in the same manner that the first-derivative spectrum was calculated from the absorption spectrum. Generally the second-derivative spectrum has a positive lobe, a negative lobe, and a second positive lobe. The magnitude of any of these or their differences can be used in the quantitative analysis.

NOTE A11.1—Depending upon the specific algorithm used, the first derivative of the first derivative may not be exactly identical to the second derivative. This is not normally a problem, as long as a consistent approach is used.

A12. NOTES ON OPERATION OF NONCOMPUTERIZED, DISPERSIVE INSTRUMENTS

A12.1 For noncomputerized, dispersive instruments, the zero value of transmittance must be known in the spectral region where measurements are taken. Check the zero transmittance value at the analytical wavenumber by the following procedure:

A12.1.1 When increasingly thick samples are placed in the instrument, the absorption bands will bottom out on the chart paper at the effective zero transmittance as shown in Fig. A12.1. If the instrument does not record this point as zero transmittance, the value of T_0 is noted and transmittance values are corrected by subtracting the T_0 value before conversion to absorbance. Blocking the sample beam with an opaque object is sometimes necessary, but gives a less accurate zero. The true absorbance A is calculated by the following equation:

$$A = \log[(T_2 - T_0)/(T_1 - T_0)] \quad \text{(A12.1)}$$

where:

T_0 = transmittance with a very thick or concentrated sample in the beam (or with the beam blocked),
T_1 = transmittance obtained with the sample in the beam, and
T_2 = transmittance without the sample in the beam.

If $T_0 = 0$, then Eq A12.1 reduces to the following:

$$A = \log(T_2/T_1) \quad \text{(A12.2)}$$

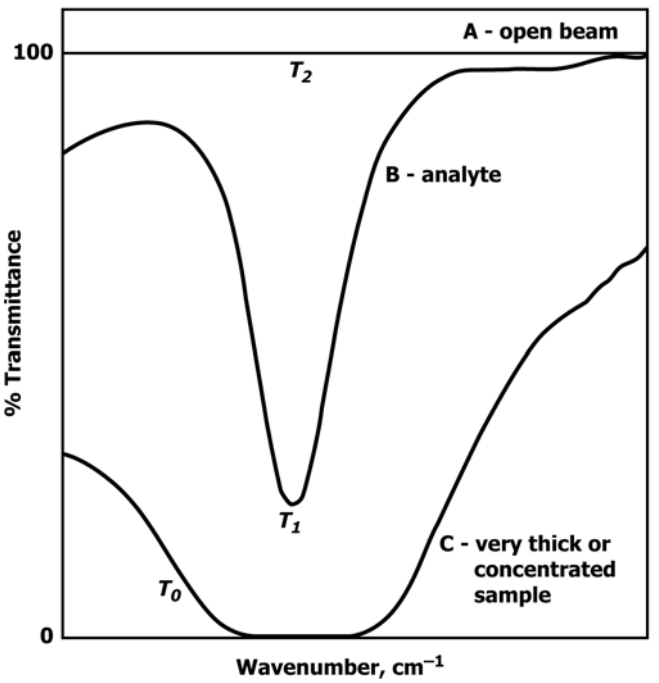


FIG. A12.1 Transmittance Zero Determination

A13. SETTING UP AN ANALYTICAL PROCEDURE

A13.1 Record the interval of the spectrum containing the band(s) to be measured. This interval should normally include at least one maximum and one minimum transmittance.

A13.2 For each of the analytical wavenumbers that are specified in the method make certain all peaks to be measured are in the desired absorbance range.

A13.3 For instruments without a computer, do the following at the selected wavenumbers:

A13.3.1 Measure the zero transmittance with a thick or concentrated sample (see Annex A12).

A13.3.2 Read the transmittance value T_1 for the sample (see Fig. A12.1).

A13.3.3 If possible, prepare a blank that is free of analyte, then measure T_2.

A13.3.4 Using a preselected baseline treatment, determine the analyte and baseline transmittances (see Note 3).

A13.3.5 Convert these transmittance values to absorbance and determine the analyte absorbance.

A13.4 For computerized data, do the following:

A13.4.1 Record the spectrum of the analyte sample.

A13.4.2 Record the spectrum of a blank if possible.

A13.4.3 Subtract the blank from the sample spectrum scaling appropriately.

A13.4.4 Using a preselected baseline treatment, determine the analyte absorbance.

NOTE A13.1—For dispersive instruments, a reference beam attenuator is useful for shifting the apparent zero absorbance for highly absorbing samples. For optical null instruments, changing the position of the attenuator will require readjustment of the gain for maximum sensitivity.

NOTE A13.2—Most computerized instruments have software for quantitative analysis which give step-by-step assistance in both calibration and analysis. The general procedure is, however, as outlined in A13.1 – A13.4.4.

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