Standard Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis

This standard is issued under the fixed designation E169; 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 are intended to provide general information on the techniques most often used in ultraviolet and visible quantitative analysis. The purpose is to render unnecessary the repetition of these descriptions of techniques in individual methods for quantitative analysis.

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

1.3 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.

2. Referenced Documents

2.1 ASTM Standards:

E131 Terminology Relating to Molecular Spectroscopy
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers
E387 Test Method for Estimating Stray Radiant Power Ratio of Dispersive Spectrophotometers by the Opaque Filter Method
E925 Practice for Monitoring the Calibration of Ultraviolet-Visible Spectrophotometers whose Spectral Bandwidth does not Exceed 2 nm
E958 Practice for Estimation of the Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers

2.2 Other References:

Note: Detailed discussion of the origin and validity of Beer’s law may be found in the books and articles listed in the bibliography at the end of these practices.

3. Summary of Practice

3.1 Quantitative ultraviolet and visible analyses are based upon the absorption law, known as Beer’s law. The units of this law are defined in Terminology E131. Beer’s law (Note 1) holds at a single wavelength and when applied to a single component sample it may be expressed in the following form (see Section 11):

\[ A = abc \]  

When applied to a mixture of \( n \) non-interacting components, it may be expressed as follows:

\[ A = a_1bc_1 + a_2bc_2 + \ldots + a_nbc_n \]

Note 1—Detailed discussion of the origin and validity of Beer’s law may be found in the books and articles listed in the bibliography at the end of these practices.

3.2 This practice describes the application of Beer’s law in typical spectrophotometric analytical applications. It also describes operating parameters that must be considered when using these techniques.

4. Significance and Use

4.1 These practices are a source of general information on the techniques of ultraviolet and visible quantitative analyses. They provide the user with background information that should help ensure the reliability of spectrophotometric measurements.

4.2 These practices are not intended as a substitute for a thorough understanding of any particular analytical method. It is the responsibility of the users to familiarize themselves with the critical details of a method and the proper operation of the available instrumentation.

5. Sample Preparation

5.1 Accurately weigh the specified amount of the sample (solid or liquid). Dissolve in the appropriate solvent and dilute to the specified volume in volumetric glassware of the required accuracy, ensuring that all appropriate temperature range tolerances are maintained. If needed, a dilution should be made with a calibrated pipet and volumetric flask, using adequate volumes for accuracy. With the availability of modern wide

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range electronic balances, (capable of reading kg quantities to four or five decimal places), gravimetric dilution should be considered as a more accurate alternative to volumetric, if available. Fill the absorption cell with the solution, and fill the comparison or blank cell with the pure solvent, at least 2x to 3x (if sufficient sample or solvent is available), before measuring.

5.2 The solution should be visibly clear, and free from particulate matter. However, there may still be present suspended particles not visible to the naked eye, and these will still scatter light by the Tyndall effect, causing a decrease in the measured intensity that increases as the wavelength decreases. Unless there is no alternative, absorbance should not be determined on turbid or light scattering samples. Any measurements performed on a light scattering solution are highly instrument specific and can be used only for comparative purposes in the same system.

   Note 2—To avoid the dilution step, the instrument may contain an automatic system which will allow adjustment of the path length of the measurement cell to optimize the measured absorbance.

6. Cell and Base-Line Checks

6.1 Clean and match the cells. Suggested cleaning procedures are presented in Practice E275.

6.2 Establish the base line of a recording double-beam spectrophotometer by scanning over the appropriate wavelength region with pure solvent in both cells. Determine apparent absorbance of the sample cell at each wavelength of interest. These absorbances are cell corrections that are subtracted from the absorbance of the sample solution at the corresponding wavelengths.

6.3 For single beam instruments, either use the same cell for pure solvent and sample measurements, use matched cells, or apply appropriate cell corrections.

6.4 On most software-controlled instruments, the cell corrections or the blank cell absorbance is stored in memory and automatically incorporated into the sample absorbance measurement.

6.5 An accurate determination of cell path length in the 1-cm range is not practical in most laboratories, and common practice is to purchase cells of known path length. Modern cell manufacturing techniques employed by a number of leading manufacturers can guarantee the path length of a 1-cm cell to ±0.01 mm or better.

7. Analytical Wavelengths and Photometry

7.1 Analytical wavelengths are those wavelengths at which absorbance readings are taken for use in calculations. These may include readings taken for purposes of background corrections. To minimize the effect of wavelength error, the analytical wavelengths are frequently chosen at absorption maxima, but this is not always necessary. If the wavelength accuracy of the spectrophotometer is such that the calculated uncertainty in the absorbance measurement is within acceptable limits at the extremes of this wavelength uncertainty range, then single point measurements on a slope can be used. For example, the use of isoabsorptive or isosbestic points is frequently useful.

   Note 3—If the sample matrix includes fluorescent compounds, the measured signal usually will contain a contribution from fluorescence.

7.2 Record the absorbance readings at the specified analytical wavelengths, operating the instrument in accordance with the recommendations of the manufacturer or Practice E275.

7.3 Absorbance values should be used only if they fall within the acceptably accurate range of the particular spectrophotometer and method employed. If the absorbance is too low, either use a longer absorption cell or prepare a new solution of higher concentration. If the absorbance is too high, use a shorter cell or make a quantitative dilution.4 If different cells are used, a new base-line must be obtained.

7.4 The precision and bias of the wavelength and photometric scales of the instrument must be adequate for the method being used. Procedures for checking precision and accuracy of these scales are presented in Practices E275 and E925.

8. Stray Radiant Energy (Stray Light)

8.1 The acceptable absorbance range for any given instrument will be governed not only by the specification of the spectrophotometer, but also by the condition at time of measurement.

8.2 Given that the measurement is fundamentally a difference in energy in an optical system, the factors affecting the measurement may include, but not be limited to: the output from the source(s), efficiency of the grating, cleanliness of the mirrors, etc.

8.3 Stray radiant energy in any instrument system may begin to cause a negative deviation error, long before the transmittance (absorbance) limit is reached. An effective estimation may be performed using Practice E387.

9. Resolution and Bandwidth

9.1 If the analytical method specifies a resolution or a spectral slit width, set the resolution of the instrument to the specified value. If the instrument has only a mechanical bandwidth indicator, use the information provided in the manufacturer’s literature to calculate the bandwidth that corresponds to the specified resolution.

   Note 4—The accuracy of resolution and mechanical bandwidth indicators can be determined using the procedure given in Practice E958.

9.2 If the analytical method does not state a required resolution or a bandwidth value but includes an illustrative spectrum, set the resolution or bandwidth of the instrument to obtain comparable data. As a rule of thumb, the resolution should be less than one-eighth of the bandwidth; thus for a peak of bandwidth 40 nm, the resolution should not exceed 5 nm.

9.3 If the method neither specifies resolution or bandwidth nor provides an illustrative spectrum, use the smallest resolution or bandwidth that yields an acceptable signal-to-noise ratio. Record this value for future reference.

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4 The errors associated with cell path lengths are significantly less than those generated by volumetric dilution, and therefore where possible, different path length cells should be used in preference to volumetric procedures.
10. Solvents and Solvent Effects

10.1 The ultraviolet absorption spectrum of a compound will vary in different solvents depending on the chemical structures involved. Non-polar solvents have the least effect on the absorption spectrum. Non-polar molecules in most instances are not affected in polar solvents. However, polar molecules in polar solvents may show marked differences in their spectra. Any interaction between solute and solvents leads to a broadening and change in structural resolution of the absorption bands. Ionic forms may be created in acidic or basic solutions. In addition, there are possible chemical reactions between solute and solvent, and also photochemical reactions arising from either room illumination or the short wavelengths in the beam of the spectrophotometer. It is important that the solvent used be specified in recording spectral data. (The change in spectra between acidic and basic conditions may sometimes be employed in multicomponent analysis.)

10.2 Reference solvent data is shown in Table 1. Availability of a particular solvent may be restricted by international agreement, and the users’ attention is directed to 1.3 of these practices. The short wavelength limit is approximate, and refers to the wavelength at which a 1-cm light path length gives an absorbance of unity.

10.3 Water, and 0.1 M solutions of hydrochloric acid, sulfuric acid, and sodium hydroxide also are commonly used as solvents. Buffered solutions, involving non-absorbing materials, are frequently used; both the composition of the buffer and the measured pH should be specified. Mixtures of 0.1 M di-hydrogen sodium phosphate and 0.1 M hydrogen di-sodium phosphate are useful in the 4.5 to 8.9 pH range. A table of non-absorbing buffers has been presented by Abbott (3).

11. Calculations

11.1 Quantitative analysis by ultraviolet spectrophotometry depends upon Beer’s law. The terms and symbols used are those defined in Terminology E131. According to Beer’s law:

\[ A = abc = (\varepsilon M) \times bc \]  

where:
- \( A \) = absorbance,
- \( a \) = absorptivity,
- \( b \) = cell length, cm,
- \( c \) = concentration, g/L,
- \( \varepsilon \) = molar absorptivity, and
- \( M \) = molecular weight.

11.1.1 In practice, a distinction must be made between \( c \), the concentration of the absorbing material in the cell at the time of observation, and the concentration of the absorbing material in the sample as received. This is here designated as a mass fraction \( C \) (g/g). The solution to be examined has a concentration of sample in solution, \( C_s \), which is in units of grams per litre.

\[ c = A/ab \] \hspace{1cm} (4)

\[ C = c/C_s = A/(abC_s) \] \hspace{1cm} (5)

11.2 If one or more dilutions are then made, the quantity called the dilution factor must be included. Dilution factor, \( f \), is the ratio of the final volume to the initial volume. If more than one dilution is performed, the dilution factor is the product of the factors from each dilution. If dilutions are made, the equation becomes the following:

\[ C = cf/C_s = A/(abC_s) \] \hspace{1cm} (6)

Note that \( c \) and \( C_s \), have the dimensions of grams per litre. If dilution is made, \( C_s \) is not the concentration in the cell at the time the absorbance is determined; the concentration in the cell is \( C_s/f \).

11.3 Chemical Calibration—The absorptivity of the absorbing material, the concentration of which it is desired to determine, is obtained by examination of a series of quantitative dilutions of a neat sample of this material. However, if no such neat sample is available, the best available material is used, or a value of the absorptivity is taken from the literature. Take care to specify this, by reporting values as “percentage against calibration material” or by noting that the accuracy of the analysis is dependent upon a published value of the absorptivity or molar absorptivity. (A reference must be cited.)

11.3.1 Some sample materials are highly fluorescent which can significantly reduce the measured absorbance. The effect of sample fluorescence may vary depending upon the spectrophotometer and wavelength chosen. Sample fluorescence may be a particular problem when using published absorptivity values.

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Note 5—Changes in the day-to-day values of resolution or bandwidth obtained with a given gain, or changes in signal-to-noise ratio at a given resolution, are indicative of present or potential problems. Increased resolution or a lowering of the S/N ratio may result from a lower output of the light source, deterioration of optical components, deposits on the windows of the cell compartment or on the inside wall of the reference cell, an absorbing impurity in the solvent, or a faulty electronic component.

### Table 1 Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Cutoff, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine</td>
<td>305</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>290</td>
</tr>
<tr>
<td>Benzene</td>
<td>280</td>
</tr>
<tr>
<td>N,N-Dimethylformamide</td>
<td>270</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>265</td>
</tr>
<tr>
<td>Methyl formate</td>
<td>260.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>245</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>235</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>220</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>215</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>210</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>210</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>210</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>&lt;210</td>
</tr>
<tr>
<td>Isooctane</td>
<td>&lt;210</td>
</tr>
</tbody>
</table>

* Procedures for special purification of solvents for further improvement in the wavelength limit are given in Refs (1, 2). Solvents of high purity for use in absorption spectroscopy also are available commercially.

* The boldface numbers in parentheses refer to a list of references at the end of this standard.
11.4 Types of Analyses (see Fig. 1):

11.4.1 One Component, No Background Correction:

\[ C = A \beta (abC_s) \]  

(7)

11.4.2 One Component, Simple Background Correction:

\[ C = \frac{(A_1 - A_2) \times f}{a_1bC_s} \]  

(8)

where the subscripts refer to analytical wavelengths. The term \( A_2 \) is the absorbance at the wavelength used for making a simple subtractive correction. It is usually selected from examination of the spectral curve of the reference material at a wavelength longer than that of \( A_1 \), preferably where \( a_2 \) is much less than \( a_1 \).

(1) One Component, No Background Correction.
(2) One Component, Simple Background Correction.
(3) One Component, with Slope-Type Background Correction.
(4) One Component, with Linear Background Correction.
(5) Two Components, with Overlapping Absorption for Only One Component.
(6) Two Components, with Mutually Overlapping Absorption.

FIG. 1 Plot of Components
11.4.3 One Component, with Slope-Type Background Correction:
\[
C = \frac{A_1 - \frac{[A_1 - A_3 + S(\lambda_3 - \lambda_1)]}{a_1bC_s}}{abC_s} 
\]

(9)

where:

\[S\] = slope between wavelengths 1 and 2 for the background.

11.4.3.1 The background absorption is usually not linear between the analytical wavelength and the wavelength at which a simple subtractive background correction may be obtained. When it is possible to determine the slope between wavelengths 1 and 2 by observation of the samples that do not contain the absorbing material that is to be determined, this may be used as a correction for the background absorption.

11.4.4 One Component, With Linear Background Correction:

11.4.4.1 The equation for the general case is as follows:
\[
C = \frac{A_1 - \frac{[A_1 - A_3] \times (\frac{\lambda_3 - \lambda_1}{\lambda_3 - \lambda_2})}{a_1bC_s}}{abC_s} 
\]

(10)

The absorptivity \(a\) is here the effective absorptivity as determined on a pure sample, using the corrections, and is somewhat lower than the true or absolute absorptivity.

11.4.4.2 This method is especially effective with materials that have sharp bands. Wavelengths 2 and 3 are selected to allow no contribution from component \(x\), selected to allow no contribution from component \(y\) as follows:
\[
A_{2x} = a_{2x}bC_s = a_{2x}bC_sC_{f}f 
\]

(14)

11.4.4.3 For the special case where the wavelength for \(A_1\) is exactly midway between the wavelengths for \(A_1\) and \(A_3\), the equation reduces to the following:
\[
C = \frac{A_1 - \frac{A_2 + A_3}{2}}{abC_s} 
\]

(11)

11.4.5 One Component, With Background Correction from Outside Data:
\[
C = \frac{(A - X) \times f}{abC_s} 
\]

(12)

where \(X\) is a background absorbance calculated from outside data.

11.4.5.1 This is a general case in which some empirical correction may be derived from data other than spectrophotometric, and is applied as an effective absorbance which is subtracted from the observed. As an example, the concentration of a known interfering material may be determined by titration, and the absorbance due to this calculated, and then subtracted.

11.4.6 Two Components, With No Overlapping Absorption—Apply the method in 11.4.1 twice, at the two analytical wavelengths. This is an almost impossible case, except when the relative concentrations of the two components are such that the product of absorptivity and concentration of one component at a given wavelength is more than 100 times the product for the other component, allowing the latter to be neglected.

11.4.7 Two Components, With Overlapping Absorption for Only One Component—Determine the component with no interference (component \(x\)) at an analytical wavelength, \(\lambda_1\), selected to allow no contribution from component \(y\) as follows:
\[
C_x = bA_1(a_1bC_s) 
\]

(13)

11.4.7.1 Calculate the contribution of this component to the observed absorbance at the other analytical wavelength, \(\lambda_2\), where both components are absorbing, as follows:
\[
C_{xy} = [A_2 - A_{2y}] \times f]a_{2x}bC_s 
\]

(15)

11.4.7.2 Calculate the concentration of component \(y\) as follows:
\[
C_y = \frac{[a_2A_1 - a_1A_2] f}{bC_s \times (a_1a_{2x} - a_1a_{2y})} 
\]

(16)

11.4.7.3 Calculate the concentration of component \(x\) as follows:
\[
C_x = \frac{[a_2A_1 - a_1A_2] f}{bC_s \times (a_1a_{2x} - a_1a_{2y})} 
\]

(17)

11.4.9 Inverted Matrix Method, for Two or More Components, With Mutually Overlapping Absorption—For information on the inverted matrix method, see Sections 10 and 17 of Practices E168.

11.5 Computerized Calculations—Newer instruments may perform automatically many of the calculations described in 11.4. The user should be aware of the algorithms used by the manufacturer. It is recommended that the user verify the reliability of computed results by periodically performing the calculations using the raw analytical data.

12. Presentation of Data

12.1 If absorption curves are to be presented with an analytical method, it is recommended that one of the following systems be used, with the wavelength (in nanometers) increasing linearly to the right:
\[
\log \varepsilon \text{ or } \log a \text{ plotted against } \lambda \\
A \text{ plotted against } \lambda \\
\varepsilon \times 10^n \text{ or } a \text{ plotted against } \lambda
\]

where the symbols are as defined in Terminology E131. Marking the analytical wavelengths and absorptivity values on the curve is suggested for clarity, or a separate table of analytical wavelengths and absorptivities may be used. (These data are helpful for others who may wish to use the method in a somewhat modified form.)

13. Keywords

13.1 molecular spectroscopy; quantitative analysis
REFERENCES


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(9) Mellon, M. G., Editor, Analytical Absorption Spectroscopy, John Wiley & Sons, N. Y.

