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Standard Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis¹

This standard is issued under the fixed designation E 169; 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 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of whoever uses this standard to consult and establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

- E 131 Terminology Relating to Molecular Spectroscopy²
- E 168 Practices for General Techniques of Infrared Quantitative Analysis²
- E 275 Practice for Describing and Measuring the Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers²
- E 925 Practice for Periodic Calibration of Narrow Band-Pass Spectrophotometers²
- E 958 Practice for Measuring Practical Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers²

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 E 131. 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 10):

$$A = abc$$

When applied to a mixture of n noninteracting components, it may be expressed as follows:

$$A = a_1bc_1 + a_2bc_2 + \dots + a_nbc_n$$

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 user to familiarize him or herself 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. (Solvent and flask should be approximately the same temperature as the spectrophotometer). If needed, a dilution should be made with a calibrated pipet and volumetric flask, using adequate volumes for accuracy. Fill the absorption cell with the solution, and fill the comparison or blank cell with the pure solvent.

6. Cell and Base-Line Checks

6.1 Clean and match the cells. Suggested cleaning procedures are presented in Practice E 275.

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 For some newer 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

¹ These practices are under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and are the direct responsibility of Subcommittee E13.01 on Ultraviolet and Visible Spectroscopy.

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² Annual Book of ASTM Standards, Vol 14.01.

common practice is to purchase cells of known path length.³ A check on path length matching, however, may be made by measuring the absorbance of a strongly absorbing solution ($A \sim 0.9$) versus pure solvent, and then emptying, cleaning, reloading fresh solvent and sample into the other cells, and remeasuring the absorbance. Similarly, the absorbance of the sample in a series of cells to be used in an analysis can be measured versus pure solvent in a given reference cell.

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. The analytical wavelengths are frequently chosen at absorption maxima, but this is not always necessary. For example, the use of isoabsorptive or isosbestic points is frequently useful.

7.2 On manually operated spectrophotometers, record the absorbance readings at the specified analytical wavelengths, operating the instrument in accordance with the recommendations of the manufacturer or Practice E 275. On automatic recording spectrophotometers, record the absorbance readings from the chart at the analytical wavelengths. For some newer instruments, the absorbance may be displayed on a suitable output device (for example, a CRT, DVM, etc.), or used directly in calculations.

7.2.1 The wavelength region scanned may be somewhat greater than that required to contain only the analytical wavelengths. Take care to start the chart and scanning drives exactly together when using variable slit instruments. End the scan well before the slit has opened fully.

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. 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 E 275 and E 925.

8. Spectral Band Width and Slit Width

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

NOTE 2—The accuracy of spectral band width and mechanical slit width indicators can be determined using the procedure given in Practice E 958.

8.2 If the analytical method specifies a mechanical slit width for a particular type of instrument and the same type

of instrument is being used, set the slit width to the specified values. If a different type of instrument is being used and information is available from which the spectral slit width of both types of instruments can be calculated, adjust the instrument settings to obtain a spectral slit width equal to the one calculated from the specified mechanical slit width.

8.3 If the analytical method does not state a spectral band width or a slit width value but includes a spectrum illustrating adequate resolution, set the spectral band width or slit width of the instrument to obtain comparable resolution.

8.4 If the method neither specifies spectral band width or slit width nor provides an illustrative spectrum, use the smallest spectral band width or slit width that yields an acceptable signal-to-noise ratio. Record this value for future reference.

NOTE 3—Changes in the day-to-day values of spectral band width or slit width obtained with a given gain, or changes in gain required to obtain a given spectral band width or slit width, are indicative of present or potential problems. Increased spectral band width or gain 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.

9. Solvents and Solvent Effects

9.1 The ultraviolet absorption spectrum of a compound will vary in different solvents depending on the chemical structures involved. Nonpolar solvents have the least effect on the absorption spectrum. Nonpolar 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 multi-component analysis.)

9.2 Common commercially available solvents of "spectroscopic purity" are listed in Table 1. The short wavelength limit is approximate, and refers to the wavelength at which a 1-cm light path length gives an absorbance of unity.

9.3 Water, and 0.1 *N* solutions of hydrochloric acid, sulfuric acid, and sodium hydroxide also are commonly used as solvents. Buffered solutions, involving nonabsorbing materials, are frequently used; both the composition of the buffer and the measured pH should be specified. Mixtures of 0.1 *N* dihydrogen sodium phosphate and 0.1 *N* hydrogen disodium phosphate are useful in the 4.5 to 8.9 pH range. A table of nonabsorbing buffers has been presented by Abbott (8).⁴

10. Calculations

10.1 Quantitative analysis by ultraviolet spectropho-

³ A 1-cm cell having its pathlength certified to 0.005 cm is available as Standard Reference Material 932 from National Bureau of Standards (NBS), Department of Commerce, Washington, DC 20234.

⁴ The boldface numbers in parentheses refer to the list of references at the end of this standard.