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Standard Guide for Fluorescence—Instrument Calibration and Qualification[1]

This standard is issued under the fixed designation E2719; 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 This guide (1)[2] lists the available materials and methods for each type of calibration or correction for fluorescence instruments (spectral emission correction, wavelength accuracy, and so forth) with a general description, the level of quality, precision and accuracy attainable, limitations, and useful references given for each entry.

1.2 The listed materials and methods are intended for the qualification of fluorometers as part of complying with regulatory and other quality assurance/quality control (QA/QC) requirements.

1.3 Precision and accuracy or uncertainty are given at a 1 σ confidence level and are approximated in cases where these values have not been well established.[3]

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

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[4]

E131 Terminology Relating to Molecular Spectroscopy

E388 Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers

E578 Test Method for Linearity of Fluorescence Measuring Systems

E579 Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution

3. Terminology

3.1 *Definitions* (2)*:*

3.1.1 *absorption coefficient (α), n*—a measure of absorption of radiant energy from an incident beam as it traverses an absorbing medium according to Bouguer's law, $I/I_o = e^{-\alpha b}$, where I and I_o are the transmitted and incident intensities, respectively, and b is the path length of the beam through the sample. **E131**

3.1.1.1 *Discussion*—Note that transmittance $T = I/I_o$ and absorbance $A = -\log T$.

3.1.2 *absorptivity (a), n*—the absorbance divided by the product of the concentration of the substance and the sample pathlength, $a = A/bc$. **E131**

3.1.3 *Beer-Lambert law, n*—relates the dependence of the absorbance (A) of a sample on its path length (see *absorption coefficient*, α) and concentration (c), such that $A = a\,bc$.

3.1.3.1 *Discussion*—Also called Beer's law or Beer-Lambert-Bouquer law. **E131**

3.1.4 *calibrated detector (CD), n*—optical radiation detector whose responsivity as a function of wavelength has been determined along with corresponding uncertainties (3).

3.1.5 *calibrated diffuse reflector (CR), n*—Lambertian reflector whose reflectance as a function of wavelength has been determined along with corresponding uncertainties (4).

[1] This guide is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and Separation Science and is the direct responsibility of Subcommittee E13.01 on Ultra-Violet, Visible, and Luminescence Spectroscopy.

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

[3] Certain commercial equipment, instruments, or materials are identified in this guide to foster understanding. Such identification does not imply recommendation or endorsement by ASTM International nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

[4] 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.

3.1.6 *calibrated optical radiation source (CS), n*—optical radiation source whose radiance as a function of wavelength has been determined along with corresponding uncertainties (**5, 6**).

3.1.7 *calibration, n*—set of procedures that establishes the relationship between quantities measured on an instrument and the corresponding values realized by standards.

3.1.8 *certified reference material (CRM), n*—material with properties of interest whose values and corresponding uncertainties have been certified by a standardizing group or organization. **E131**

3.1.9 *certified value, n*—value for which the certifying body has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by the certifying body (**7**).

3.1.10 *diffuse scatterer, n*—material that scatters optical radiation in multiple directions; this includes diffuse reflectors, which are often Lambertian, and scattering solutions, which are not Lambertian.

3.1.11 *fluorescence anisotropy (r), n*—measure of the degree of polarization of fluorescence, defined as $r = (I_{II} - I_{\perp})/(I_{II} + 2I_{\perp})$, where I_{II} and $I_{\perp}$ are the observed fluorescence intensities when the fluorometer's emission polarizer is oriented parallel and perpendicular, respectively, to the direction of the polarized excitation.

3.1.12 *fluorescence band, n*—region of a fluorescence spectrum in which the intensity passes through a maximum, usually corresponding to a discrete electronic transition.

3.1.13 *fluorescence lifetime, n*—parameter describing the time decay of the fluorescence intensity of a sample component; if a sample decays by first-order kinetics, this is the time required for its fluorescence intensity and corresponding excited state population to decrease to $1/e$ of its initial value.

3.1.14 *fluorescence quantum efficiency, n*—ratio of the number of fluorescence photons leaving an emitter to the number of photons absorbed.

3.1.15 *fluorescence quantum yield (Φ), n*—probability that a molecule or species will fluoresce once it has absorbed a photon.

3.1.15.1 *Discussion*—This quantity is an innate property of the species and is typically calculated for a sample as the ratio of the number of molecules that fluoresce to the number of molecules that absorbed.

3.1.16 *flux (or radiant flux or radiant power), n*—rate of propagation of radiant energy typically expressed in Watts.

3.1.17 *grating equation, n*—relationship between the angle of diffraction and wavelength of radiation incident on a grating, that is, $m\lambda = d(\sin\alpha + \sin\beta)$, where d is the groove spacing on the grating; α and β are the angles of the incident and diffracted wavefronts, respectively, relative to the grating normal; and m is the diffraction order, which is an integer (**8**).

3.1.18 *inner filter effects, n*—decrease in the measured quantum efficiency of a sample as a result of significant absorption of the excitation beam, reabsorption of the emission of the sample by itself, or both, and this causes the measured quantum efficiency to be dependent on the absorbance, concentration, and excitation and emission path lengths of the sample (**9, 10**).

3.1.19 *Lambertian reflector, n*—surface that reflects optical radiation according to Lambert's law, that is, the optical radiation is unpolarized and has a radiance that is isotropic or independent of viewing angle.

3.1.20 *limit of detection, n*—estimate of the lowest concentration of an analyte that can be measured with a given technique, often taken to be the analyte concentration with a measured signal-to-noise ratio of three.

3.1.21 *noise level, n*—peak-to-peak noise of a blank.

3.1.22 *photobleaching, n*—loss of emission or absorption intensity by a sample as a result of exposure to optical radiation.

3.1.22.1 *Discussion*—This loss can be reversible or irreversible with the latter typically referred to as photodegradation or photodecomposition.

3.1.23 *qualification, n*—process producing evidence that an instrument consistently yields measurements meeting required specifications and quality characteristics.

3.1.24 *quantum counter, n*—photoluminescent emitter with a quantum efficiency that is independent of excitation wavelength over a defined spectral range.

3.1.24.1 *Discussion*—When a quantum counter is combined with a detector to give a response proportional to the number of incident photons, the pair is called a quantum counter detector.

3.1.25 *quasi-absolute fluorescence intensity scale, n*—fluorescence intensity scale that has been normalized to the intensity of a fluorescent reference sample or artifact under a fixed set of instrumental and experimental conditions.

3.1.25.1 *Discussion*—This artifact should be known to yield a fluorescence intensity that is reproducible with time and between instruments under the fixed set of conditions.

3.1.26 *Raman scattering, n*—inelastic scattering of radiation (the wavelengths of the scattered and incident radiation are not equal) by a sample that occurs because of changes in the polarizability of the relevant bonds of a sample during a molecular vibration. (See Terminology E131, *Raman spectrum*.)

3.1.26.1 *Discussion*—The radiation being scattered does not have to be in resonance with electronic transitions in the sample, unlike fluorescence (**11**).

3.1.27 *Rayleigh scattering, n*—elastic scattering of radiation by a sample, that is, the scattered radiation has the same energy (same wavelength) as the incident radiation.

3.1.28 *responsivity, n*—ratio of the photocurrent output and the radiant power collected by an optical radiation detection system.

3.1.29 *sensitivity, n*—measure of an instrument's ability to detect an analyte under a particular set of conditions.

3.1.30 *spectral bandwidth (or spectral bandpass or resolution), n*—measure of the capability of a spectrometer to