Designation: E578 – 07

StandardTest Method for Linearity of Fluorescence Measuring Systems[1]

This standard is issued under the fixed designation E578; 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 test method covers a procedure for evaluating the limits of the linearity of response with fluorescence intensity of fluorescence-measuring systems under operating conditions. Particular attention is given to slit widths, filters, and sample containers. This test method can be used to test the overall linearity under a wide variety of instrumental and sampling conditions. The results obtained apply only to the tested combination of slit width and filters, and the size, type and illumination of the sample cuvette, all of which must be stated in the report. The sources of nonlinearity may be the measuring electronics, excessive absorption of either the exciting or emitted radiation, or both, and the sample handling technique, particularly at low concentrations.

1.2 This test method has been applied to fluorescence-measuring systems utilizing continuous and low-energy excitation sources (for example, an excitation source of 450-W electrical input or less). There is no assurance that extremely intense illumination will not cause photodecomposition of the compounds suggested in this test method.[2] For this reason it is recommended that this test method not be indiscriminately employed with high-intensity light sources. It is not a test method to determine the linearity of response of other materials. If this test method is extended to employ other chemical substances, the principles within can be applied, but new material parameters, such as the concentration range of linearity, must be established. The user should be aware of the possibility that these other substances may undergo decomposition, or adsorption onto containers.

1.3 This test method has been applied to fluorescence-measuring systems utilizing a single detector, that is, a photomultiplier tube or a single photodiode. It has not been demonstrated if this method is effective for photo-array instruments such as those using a CCD or a diode array detector.

1.4 This test method is applicable to 10-mm pathlength cuvette formats and instruments covering a wavelength range within 190 to 900 nm. The use of other sample formats has not been established with this test method.

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

2. Summary of Test Method

2.1 This procedure is used for testing the linearity of fluorescence-measuring systems by using solutions of quinine sulfate dihydrate in sulfuric acid as standard test solutions. Other stable solutions which may be more suitable to the user can be employed (Note 1). The standard used to determine linearity should be stated in the report. The fluorescence of the test solution is measured in the measuring system with the cuvettes, slits, or filters that are to be employed in projected use.

NOTE 1—A substitute standard should have the following properties: (*1*) It should have a large quantum yield at very high dilution; (*2*) it should be stable to the exciting radiation during spectral measurements; (*3*) its fluorescence and its absorption spectra overlap should be small; (*4*) its quantum yield should not be strongly concentration dependent; and (*5*) it should have a broad emission spectrum, so that little error is introduced when wide slits are used.[3]

2.2 *Upper Limit of Linearity*—The fluorescence intensity of a series of standard solutions is measured, the resultant instrument readings are plotted against concentration on a log-log graph, and a smooth curve is drawn through the data points. The point (concentration) at which the upper end of the curve deviates by more than 5 % of the signal from the straight line (defined by the center region of the curve) is taken as the upper limit of linearity. The limit is expressed in micrograms per millilitre of quinine sulfate dihydrate.

NOTE 2—Absorption of the exciting radiation at high solute concentrations is dependent on instrument geometry and pathlength, and can result in fluorescence signal nonlinearity.

2.3 *Lower Limit of Linearity*—The lower limit of linearity is taken as the point (concentration) at which the lower end of the

[1] This test method 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.

Current edition approved March 1, 2007. Published March 2007. Originally approved in 1976. Last previous edition approved in 2001 as E578 – 01. DOI: 10.1520/E0578-07.

[2] Lukasiewicz, R. J., and Fitzgerald, J. M., *Analytical Chemistry*, ANCHA, Vol 45, 1973, p. 511.

[3] Gill, J. E., *Photochemistry and Photobiology*, PHCBA, Vol 9, 1969, p. 313.