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15 February 1960

Colloidal Silica as a Standard for Measuring Absolute Fluorescence Yield

Abstract. The use of colloidal silica as a standard for fluorescence efficiency measurements is proposed. Its advantages over glycogen as a standard are availability, purity, and stability. Absolute fluorescence yields are reported for a number of substances and compared with values in the literature.

Most methods for determining the absolute quantum yield of fluorescence involve either evaluation of the absolute geometry of the apparatus or compensation for it by comparison with a standard scatterer. The latter is usually accomplished by comparing emission from fluorescent solutions with radiation scattered by a magnesium oxide plate, under conditions of identical geometry. Both relative-scattering methods and absolute-geometry methods are tedious and suffer from poor reproducibility (generally 20 percent relative).

Recently, Weber and Teale (*1*) published a novel method for determining absolute quantum yields of fluorescence. Their method was based on a comparison of the radiation emitted by fluorescent solutions with that scattered by a solution of macromolecules, attenuating the incident radiation to the same extent. The macromolecules behave as pure dipolar scatterers of the incident radiation, and after correction for polarization of the scattered radiation, are equivalent to a material of unit quantum efficiency. By comparison of fluorescent solutions with solutions of the standard scatterer, under identical conditions, the following equation can be derived for the quantum efficiency:

$$q = \frac{[dF/dE]_{\lambda_0}[3 + P_f]\, f(\lambda_0)}{[dS/dE]_{\lambda_0}[3 + P_s]\, f(\Delta\lambda)} \quad (1)$$

where dF/dE is the slope of a plot of fluorescent intensity against absorbance for the compound under investigation, dS/dE is the slope of a plot of intensity of scattered radiation against apparent absorbance for the standard scatterer, P_f is the polarization of fluorescence, P_s is the polarization of the scattered radiation, and $f(\lambda_0)/f(\Delta\lambda)$ is a correction factor for the difference in detector response to the wavelengths of the scattered radiation and the fluorescent radiation. Thus, the only experimental data necessary to determine fluorescence efficiency are plots of emission versus absorbance for the compound under investigation and the standard scattering material. By using an experimental apparatus not much different from a simple fluorometer, it was possible for Weber and Teale to obtain quantum yields reproducible to about 7 percent.

The standard scattering material used by Weber and Teale was an aqueous solution of glycogen. Although this material gave reproducible quantum yields, we felt that it was not generally desirable as a standard scatterer because of three inherent disadvantages: (i) the material is not readily available in pure form; (ii) since glycogen is naturally occurring, contamination by small amounts of impurities is possible; (iii) the solutions are not stable over a long period of time. The purpose of the present investigation was to find an acceptable substitute for glycogen that is readily available, free from possible contamination, and stable. After a survey of several possible materials, we chose a suspension of colloidal silica in water, sold under the trade name of Ludox (*2*). This material is completely satisfactory in terms of the above criteria, and it gives quantum yields reproducible to about 7 percent relative, agreeing with the values reported by Weber and Teale to within 0.02 quantum yield, on the average.

The apparatus used was essentially that of Weber and Teale with the following minor modifications. The source of radiation was an AH-6 mercury arc. The 3650 and 3130 line groups were isolated with filters as described by Kasha (*3*). The detector in all instances was an RCA 6217 photomultiplier tube, with an integrating screen of 10^{-3} *M* rhodamine B in ethylene glycol. A Heathkit "Multimeter" was used to measure the photocurrent.

Ludox, the radiation-scattering medium used for this work, is colloidal silica (light-scattering grade GC-3414-.85). The molecular weight of this material compared favorably with the molecular weight of the glycogen used by Weber and Teale (1 to 2 $\times$ 10^6 and 2 to 9 $\times$ 10^6, respectively). It had a uniform spherical particle size of 10 to 15 mμ (diameter). A plot of apparent absorbance against reciprocal fourth power of the wavelength gave a straight line.

Baker reagent-grade benzene and U.S.P. ethanol were used throughout. Phillips Petroleum research-grade hexane was used. The integrating screen used Allied Chemical and Dye XH-P grade ethylene glycol (synthetic and nonfluorescent).

Anthracene (Eastman Special Grade) gave a violet fluorescence and was used without further purification. Naphthalene (Eastman White-Label) was purified by distillation and subsequent recrystallization from alcohol. Fluorescein (Eastman Tech.) was acetylated and recrystallized from alcohol until it was colorless. Pure fluorescein was produced by saponification of the purified acetate. Eosin (Eastman Tech.) was purified in a manner similar to fluorescein except that the acetate was recrystallized from benzene. Acriflavin (National Aniline High-grade) was dissolved in bicarbonate solution and precipitated as the hydrobromide. DuPont technical Koch acid (1-naphthol-3,6,8-trisulfonic acid), sodium salt, was recrystallized four times from 10-percent sodium chloride solution.

Table 1. Comparison of quantum yield values.

Compound	Solvent	Quantum yield		
		This report	Weber and Teale	Other works
Acriflavin	Water	0.56	0.54	0.40 (*5*)
Anthracene	Benzene	0.31	0.29	0.24 (*6*)
Anthracene	Ethanol	0.30	0.30	
Naphthalene	Hexane	0.13	0.10	
Naphthalene	Ethanol	0.13	0.12	
Fluorescein	0.1 N NaOH	0.93	0.93	0.72 (*5*), 0.80 (*7*), 0.79 (*8*)
Eosin	0.1 N NaOH	0.20	0.19	0.15 (*7*)
Koch acid, sodium salt	Water	0.14	0.15	

Table 1 compares the quantum yields obtained in this study with those of Weber and Teale as well as with the results of other workers. The values obtained by using Ludox as a standard scatterer show agreement to within 0.02 quantum yield on the average. This corresponds to an average agreement of about 6 percent, the approximate uncertainty associated with a given result.

Generally, glycogen and/or Ludox give quantum yields somewhat higher than those obtained by other workers. The lower values of other workers probably can be traced to one of the following experimental difficulties: improper evaluation of the geometry of the system, materials of questionable purity, or measurements on solutions having too great a concentration (*4*).

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21 December 1959

On the Presumed Sterile Induction of Plant Tumors

Abstract. A cooperative reinvestigation of the possibility that filtrates of crown-gall bacterial cultures can induce autonomous tumors in plants has been conducted. The results indicate that the neoplasms formed after treatment do not fulfill the criteria for crown gall since they are not autonomous.

The isolation and characterization of the "tumor-inducing principle" involved in the transformation of normal plant cells into autonomous crown-gall tumor cells (*1*) is important to an understanding of the neoplastic alteration of plants. Klein (*2*) reported that tumor-inducing principle activity for tomato plants was found in filtrates of media containing plant sap in which crown-gall bacteria had grown. This report was confirmed by Manigault *et al.* (*3*) with geranium plants and was extended by Klein and Knupp (*4*) to disks of carrot phloem and by Bender and Brucker (*5*) to tomato, *Datura*, sunflower, and *Kalanchöe* plants.

On methodological and experimental grounds, the report of Klein (*2*) and, on conceptual grounds, the studies of Manigault *et al.* (*3*) were questioned by Braun and Stonier (*6*). Bopp, in a personal communication, reported inconclusive results in attempting to repeat the work of Manigault *et al.*, while Braun and Stonier obtained negative results in three rather large series of experiments. Thomas and Klein (*7*) reported positive results on carrot phloem disks with test solutions produced from precipitated and dialyzed culture media.

In view of the potential importance of the positive reports and the controversy which existed concerning the interpretation of those results, it was felt advisable to join in a cooperative investigation of the problem. This report covers the research conducted by us over a 6-month period, with full access to all available research notes and information.

The positive results on tomato reported by Klein (*2*) were obtained following passage of relatively large volumes of metabolite solutions (about 150 ml) through a single porcelain filter candle having a maximum pore diameter of 1.7 μ. It is clear from results obtained in the cooperative effort as well as from other types of information that this report must be rejected because of bacterial contamination of the test solutions. In this instance the methodological error involved testing too small an aliquot of the total filtrate for sterility. It was found, for example, that even when culture fluids were subjected to centrifugation for 25 minutes prior to filtration through a single candle, the filtrate contained small numbers of bacteria. Of 25 1-ml samples of filtrate tested in one experiment, two samples were contaminated with demonstrably virulent crown-gall bacteria. An equal number of samples of a filtrate obtained by passage through two filter candles of increasing fineness were, on the other hand, uncontaminated. In no instance was it possible to obtain tumors on tomato plants when sterile preparations were applied according to the method described by Klein (*2*).

Kalanchöe, tomato, and *Datura* plants, as well as the phloem tissue of carrot roots, were treated with purified test solutions prepared according to the methods of Thomas and Klein (*7*). There was no response when these preparations were introduced on three successive days into wounded stems of *Kalanchöe* plants. Within 48 to 72 hours after the start of the experiments, localized "swellings" were observed at the points of inoculation in tomato and *Datura*. These did not continue to enlarge beyond a week, a time limit which is characteristic of self-limiting growths resulting from localized areas of irritation.

Similar swellings were observed in tomato but not in *Datura* when wounded controls were treated at the cut stem end above the wound with 1-percent indole-3-butyric acid in lanolin. Wounded control plants showed typical healing responses. Intact plants treated with preparations from media in which avirulent bacteria had grown showed little or no response. On the other hand, tumors initiated by virulent bacteria were still actively growing and had reached massive size after a period of 5 weeks. Disks of carrot phloem tissue treated with these preparations formed small, raised overgrowths which were in no instance of the size or vigor of tumors induced by virulent bacteria. Because of the great variability in the responses of carrot disks treated with test preparations, several different test plants should be examined in such studies.

Since the preparations developed by Thomas and Klein with the use of carrot phloem as a test object did not induce the formation of autonomous neoplasms either on carrot phloem disks or when applied to stems of intact plants of three highly susceptible species, these preparations cannot, on the basis of evidence available, be considered to be the tumor-inducing principle. The concept that sterile induction of crown gall on carrot had been accomplished was derived primarily from etiological considerations in which the timing of the responses, the nature of the pre- and post-treatments, the virulence of the bacteria, and variations in the methods used to obtain active preparations were given paramount consideration. These criteria are not considered adequate for a demonstration of the sterile induction of a crown-gall tumor.

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4 February 1960