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Absolute Spectrofluorometry

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The last 10 years has seen the increasing publication of the emission spectra of organic, inorganic and metal-chelate compounds, but there is no agreed method of presentation of such spectra. In the few cases where corrected emission spectra have been published, there is often no mention of the units used for the intensity coordinate or the method used for correcting spectra. A method of reporting absolute fluorescence spectra originally put forward in 1962 will be reexamined and improved. The two best known methods for calibrating spectrometers for absolute spectrofluorometry: (a) standard tungsten lamp, (b) quantum counter method, will be critically examined, and the limitations and possible improvements in accuracy will be proposed. The criteria for an emission standard will be examined and the use of emission standards for calibrating spectrofluorometers discussed. It is suggested that the distribution of emission standards to laboratories measuring corrected fluorescence spectra and the analysis and publication of the results should be done in the near future.

Key words: Actinometers; calibration of spectrofluorometers; detectors, absolute; fluorescence spectra, corrected; quantum counters; spectrofluorometers, design; spectrofluorometry, absolute; standard lamps; standards, fluorescence; thermopiles.

I. Introduction

Although fluorescence from organic molecules or molecular complexes has been used for many years as a method of trace analysis especially in biochemical and medical work, it was not until the introduction of commercial spectrofluorometers in the 1950's that fluorescence spectra began to appear in the literature in any great numbers. The selectivity and accuracy of analysis was greatly improved by measuring fluorescence spectra and also it became possible to recognize organic molecules in very dilute solution from their fluorescence spectra. The use of fluorescence spectra in many photophysical investigations such as determination of quantum efficiencies of fluorescence, studies on the configuration of ground and excited states, electronic energy transfer and so on, has become increasingly important in the last 10 or 15 years [1, 2].[1]

The emission spectrum recorded by a spectrofluorometer, unlike an absorption spectrum, is greatly distorted by the response function of the instrument (sensitivity as a function of wavelength), and by artefacts such as scattered light in the analyzing monochromator. The response function of the spectrofluorometer is determined by the transmission of the monochromator as a function of wavelength, the spectral sensitivity of the detector (usually a photomultiplier tube) and the variation in transmission and focusing properties of lenses (if used) with wavelength. The response function can only be reliably measured by using a source of known spectral energy distribution. Absolute spectrofluorometry, therefore, is mainly concerned with the problem of obtaining sources with accurately known spectral distributions.

Despite increasing publication of fluorescence spectra, especially in the last 10 years, it is disappointing to find that most spectra have not been corrected in any way and are thus of limited use to other workers. In the few cases where corrected spectra have been published, it seems customary to give the intensity "in arbitrary units" and provide no information on the dimensions of the units or the method used for correction. Those who publish fluorescence spectra should take note of a joint statement made by a number of investigators in 1962 [3] which stresses the importance of reporting the method of correction, subtracting "background fluorescence" and using appropriate units (e.g. quanta s^{-1} per unit wave number interval). This list has been extended by Demas and Crosby [4] to include those factors which are important when measuring quantum efficiencies of fluorescence from emission spectra.

In view of the fact that absolute calibration of spectrofluorometers is an exacting task requiring equipment not available in many laboratories, it would seem important to encourage the use of fluorescence emission standards. These can be used in three ways: (a) for presentation alongside the spectrum being investi-

[1] Figures in brackets indicate the literature references at the end of this paper.

gated so that the reader can correct the spectrum from the most recently known figures for the standard, (b) for calibrating spectrofluorometers, (c) for use in a ratio spectrofluorometer to give the ratio of the unknown to standard at each wavelength. Some standards have already been proposed [5, 6, 7], but new ones need to be found, especially in the ultraviolet and near infrared regions.

In this account of absolute spectrofluorometry, no attempt has been made to completely review the literature. The reader will find further references in the recent review article on photoluminescence quantum yields by Demas and Crosby [4]. Rather the emphasis will be on the experimental methods in absolute spectrofluorometry with a critical examination of methods of calibration, suggestions for improving accuracy and recommendations on nomenclature.

II. Definitions

A. Units

The recommended radiometric and photometric units as defined by the Système International d'Unitès (SI), are the joule (J), the watt (W) and the lumen (lm). The symbols and defining equations for quantities used in emission studies are:

radiant energy	$= Q$ (J)
radiant flux, φ	$= dQ/dt$ (W)
irradiance, E	$= d\varphi/dA$ (W m^{-2})
spectral irradiance, $E(\lambda)$	$= dE/d\lambda$ (W m^{-2} nm^{-1})

The spectral irradiance, $E(\lambda)$, is commonly used to record the output of black bodies and tungsten lamps. Unfortunately, there are no SI units for photon quantities and in this review we will use the nomenclature recently recommended by Muray, Nicodemus, and Wunderman [8]. The authors propose placing a "q" as a subscript on radiometric quantity to denote a photon quantity. Thus the photon units for some of the emission quantities would be:

photon energy	$= Q_q$ ($h\nu$)
photon flux, φ_q	$= dQ_q/dt$ ($h\nu$ s^{-1})
photon irradiance, E_q	$= d\varphi_q/dA$ ($h\nu$ s^{-1} m^{-2})
photon spectral irradiance, $E_q(\lambda)$	$= dE_q/d\lambda$ ($h\nu$ s^{-1} m^{-2} nm^{-1})
or $E_q(\tilde{\nu})$	$= dE_q/d\tilde{\nu}$ ($h\nu$ s^{-1} m^{-2} (cm^{-1})$^{-1}$)

In the past, the quantity $E_q(\tilde{\nu})$ has been variously labelled I, q, B, $Q(\tilde{\nu})$, etc., but the new symbol would appear to be less confusing and we recommend its adoption.

B. Presentation of Emission Spectra

The integrated corrected emission spectrum is proportional to the fluorescence efficiency. The most useful quantity for photochemists is the quantum fluorescence efficiency which is proportional to $\int_0^\infty E_q(\lambda)d\lambda$ or $\int_0^\infty E_q(\tilde{\nu})d\tilde{\nu}$. Thus the most useful spectra for publication are $E_q(\lambda)$ or $E_q(\tilde{\nu})$ spectra in which the maximum of the curve is given as $E_q(\tilde{\nu}) = 1.00$. Many workers report fluorescence spectra on a wavelength scale, but a wave number scale is to be preferred especially when vibrational structure is present. It is important to remember that the shapes and positions of the peaks of fluorescence spectra depend on the units used. This is illustrated in figure 1. Here $E(\lambda)$ and $E_q(\tilde{\nu})$ for quinine in 1 N H_2SO_4 are plotted on the same graph. The relation between the various units for emission spectra can be shown from the work of Ejder [9] to be:

$$\lambda^3 E(\lambda) = \lambda^2 E_q(\lambda) = \lambda E(\tilde{\nu}) = E_q(\tilde{\nu}).$$

Thus emission spectra can be readily transformed by multiplying by the appropriate power of λ; e.g. an $E_q(\tilde{\nu})$ spectrum can be converted to an $E_q(\lambda)$ spectrum by multiplying by λ^{-2}.

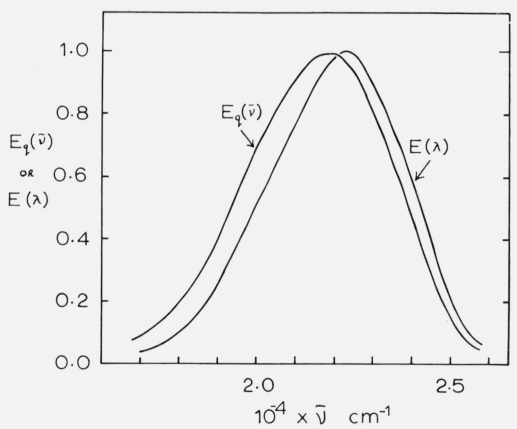

FIGURE 1. $E_q(\tilde{\nu})$ and $E(\lambda)$ spectra of quinine (10^{-4} M) in N H_2SO_4.

C. Glossary

The following abbreviations will be used in the following text, diagrams and tables:

FS = front surface
SV = side view
φ_f = quantum efficiency of fluorescence
OD = optical density or absorbance
PM = photomultiplier
M = monochromator
QC = quantum counter
TP = thermopile
$S(\tilde{\nu})$ = spectrofluorometer response function required to give $E_q(\tilde{\nu})$ emission spectra
λ = wavelength (nm)
$\tilde{\nu} = 1/\lambda$ = wave number (cm^{-1}).

III. Equipment

A. Excitation Sources

Undoubtedly the most popular exciting source for fluorescence is the high pressure xenon lamp in combination with a wide aperture grating monochromator. In order to get sufficient light at wavelengths shorter than 300 nm, it is common to use lamps of 450 watts or larger and these have the advantage of being somewhat more stable than the lower wattage lamps. If a very intense exciting source is needed, a medium or high pressure mercury lamp can be used with appropriate filters. When measuring the fluorescence spectra of concentrated solutions, powder or single crystals where front surface illumination is necessary (Sec. V), stray light from the exciting monochromator can be a problem, especially if the fluorescence emission from the sample is weak. Stray light can either be light appearing in the second or higher orders, or instrumental scatter of many wavelengths. This second type of instrumental scatter is easily demonstrated by placing the eye at the exit slit of the excitation monochromator when its wavelength is set in the UV region. Interference from second order stray light can be greatly reduced by using suitable nonfluorescent cutoff filters or bandpass filters [10]. Unfortunately few commercial spectrofluorometers have filter holders and it is necessary to have them fitted. Double grating monochromators will reduce instrumental scattered light to a large extent, but the throughput is also reduced. Good practical discussions on the use of filters and monochromators is given by Kortüm [11] and Parker [2].

The amount of stray light from a Gillieson type monochromator (as modified by Schroder [12]) when a mercury lamp is placed at the entrance slit is shown in figure 2, dashed line. The wavelength setting was 365 nm, the bandwidth was 20 nm and the 390 lines/mm grating was blazed at 350 nm. The light output was scanned with a Jarrell-Ash[2] 1/4 meter grating monochromator. The units are φ_q for the mercury lines and $E_q(\tilde{\nu})$ for the continuum. A Wratten 2 C filter (λ cutoff at 390 nm) was placed at the entrance slit of the 1/4 meter monochromator to eliminate errors due to scattering and multiple diffraction of the intense line in the analyzing monochromator (see Sec. III, B). The stray mercury lines may be further reduced by choosing a suitable bandpass filter for the exciting line.

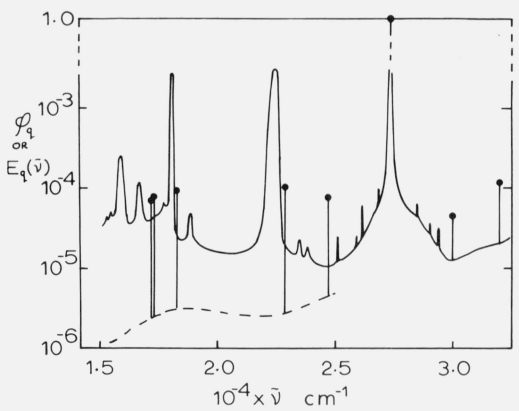

FIGURE 2. Stray light in excitation and analyzing monochromators. ● = mercury lines.

Dashed line: stray light from excitation monochromator set as 365 nm with a mercury lamp at entrance slit, and a 2c filter at exit slit.

Solid line: spurious peaks due to stray light in analyzing monochromator set at 365 nm, with a mercury lamp at the entrance slit.

B. Analyzing Monochromators

The most readily available and least expensive monochromator is the single grating instrument based on the Ebert-Fastie or Czerny-Turner mountings (fig. 3, b and c).

The Gillieson mounting (fig. 3a) is sometimes used in monochromators intended for excitation purposes. Most commercial instruments use single grating monochromators and provided one is aware of their limitations, they are adequate for measuring the rather broad fluorescence spectra given by organic molecules in solution. The advantages of grating monochromators over prism ones are: (a) linear wavelength dispersion, (b) high aperture and (c) easy interchange of gratings for different spectral regions. However, the grating monochromator generally has higher scattered light and the overlapping of orders can be a problem.

The most important source of stray light in a grating monochromator, particularly one of large aperture, is

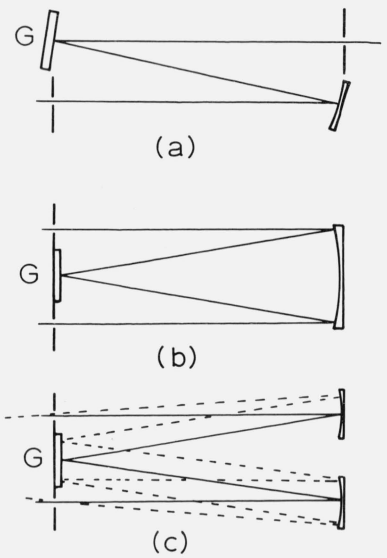

FIGURE 3. Types of grating monochromators: (a) Gillieson, (b) Ebert-Fastie, (c) Czerny-Turner. (G = plane grating).

[2] In order to adequately describe materials and experimental procedures, it was occasionally necessary to identify commercial products by manufacturer's name or label. In no instances does such identification imply endorsement by the National Bureau of Standards, nor does it imply that the particular product or equipment is necessarily the best available for that purpose.

multiple diffraction. One possible pathway for double diffraction is shown by the dashed lines in Figure 3c. This well known problem has not been quantitatively investigated until recently [12, 13]. Multiple-diffracted light can be reduced with a mask, but this reduces the transmission of the monochromator. An experimental determination of stray light in the Jarrell-Ash 1/4 meter grating monochromator with a grating blazed at 600 nm is shown in figure 2, solid line. The exciting wavelength was 365 nm and the baffle inside the monochromator was removed for these measurements. Many of the spurious peaks shown are due to multiple diffraction as may be proved by removing the exciting light with a Wratten 2 C filter; the peaks disappear, but the stray mercury lines from the excitation monochromator are not affected. This spurious light is particularly troublesome when scattered exciting light cannot be avoided as when measuring multicrystalline material from the FS or measuring solutions with a very low fluorescent intensity. In these cases it is advisable to remove the exciting light with a sharp cutoff filter and make corrections for any fluorescence absorbed by the filter. The minimizing and correction of stray light in spectrofluorometry will be further discussed in Section V, A.

C. Detectors and Amplifiers

The detector used in spectrofluorometry is the photomultiplier. End-window photomultipliers are to be preferred, since there is no grid in front of the photosurface to obstruct the narrow light beam from the exit slit of the analyzing monochromator. A quartz window photomultiplier with an S–20 photosurface has been found to have good sensitivity over the range 220 to 800 nm. A modern tube such as the EMI type 9698 QB has a very low dark current ($\sim 10^{-10}$A) and does not need cooling.

For fluorescence spectral measurements in the range 800 to 1100 nm, a cooled photomultiplier with and S–1 sensitivity curve is commonly used. It is possible that an uncooled P–I–N silicon diode may prove to be a more useful detector in this wavelength region [14], but unfortunately the surface areas of silicon photodiodes presently available are too small to be useful in spectrofluorometry.

For amplification of photocurrents of $\geq 10^{-12}$ A, solid state electrometers of the type described by Weinberger [15] are quite adequate and inexpensive. Selectable time constants ranging from 0.1–2 s. should be included in the amplifier to improve the signal-to-noise ratio. Photocurrents less than 10^{-12} A are best measured by photon counting. Not only is it possible to use simple amplifiers and discriminators [16, 17] but greater accuracy with shorter sampling times is achieved [18]. It should be noted that the photocathode should be at ground potential when the photomultiplier is used for photon counting to minimize noise due to current leakage from the photocathode.

D. Design of Spectrofluorometers

Parker [2] has discussed the design of spectrofluorometers and several instruments have been described in the literature [19, 22]. Few instruments, however, are completely satisfactory if they are required to perform a number of different functions, and we believe the following criteria should be examined before building a spectrofluorometer:

(a) There should be space around the sample so that heating and cooling baths, flow cells, liquid helium Dewars, etc., may be fitted.
(b) The instrument should be double beam in order to eliminate light source fluctuation and have the ability to measure excitation spectra.
(c) The sample should be capable of being observed from the front face and from the side.
(d) Excitation light source should be easily exchangeable.
(e) There should be space for fitting filters at the exit beam of the excitation monochromator and at the entrance slit of the analyzing monochromator.
(f) Space should be available for fitting chopping discs, e.g., for measuring phosphorescence spectra.
(g) The scanning speed should be accurately adjustable over a wide range.
(h) Amplifiers should have adjustable time constants to improve the signal-to-noise ratio.

A design used in this laboratory which meets all of these requirements is shown in figure 4. Three con-

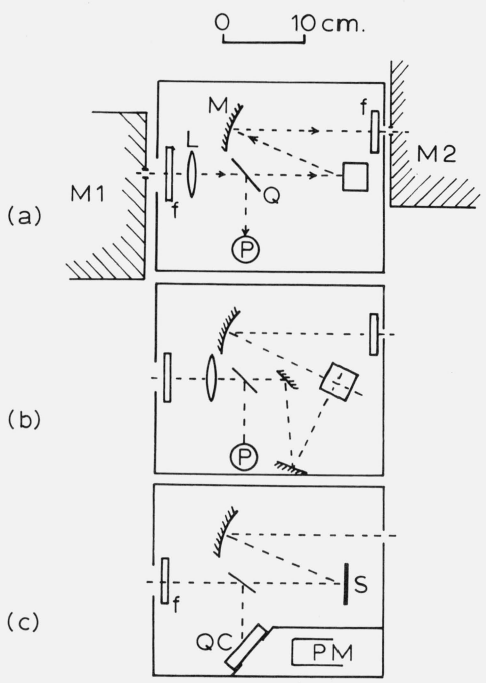

FIGURE 4. Spectrofluorometer design. M1, M2 = monochromators, Q = quartz beam splitter, f = filters, M = spherical aluminized mirror, P = photocell, PM = photomultiplier, QC = quantum counter, S = scatterer, L = quartz lens.

figurations are shown: (a) for measuring fluorescence spectra using FS excitation, (b) for measuring fluorescence spectra with SV and (c) configuration for calibrating the analyzing monochromator and photomultiplier, using the quantum counter method (Sec. IV, C). Light shields have not been shown on the diagram. Excitation spectra can be measured by replacing P in figure 4a with a quantum counter. The two signals from the monitoring photocell (or quantum counter photomultiplier) and the analyzing photomultiplier are amplified by electrometers and led to a ratio recorder of the type described by Haugen and Marcus [19]. Components are held rigidly on a series of optical benches contained in a hinged box between the two monochromators.

The spectrum recorded by spectrofluorometers is usually an uncorrected one plotted on a wavelength scale. The correction of the spectrum and replotting on a wave number scale is very laborious and instruments for the direct correction of spectra, as they are recorded, have been described [22, 23]. However these corrected curves must still be replotted if a wave number scale is required. The use of small, inexpensive on-line computers is becoming increasingly common and in the future it is likely that these will be used to correct both ordinates and plot the spectrum directly. The only additional equipment needed for the spectrofluorometer is a digital voltmeter with binary coded decimal (BCD) output and several stepping motors. Interfacing to a small computer such as the PDP–8 presents no problems.

IV. Calibration Methods

A. Introduction

The emission spectrum recorded by a simple spectrofluorometer, i.e., one without built-in automatic correction devices, is distorted not only by the scattering artefacts described in Section III, B, but also by the wavelength dependence of the detector and monochromator transmission. Only two calibration procedures for determining this response function have so far been developed. The first method depends on the assumption that a tungsten lamp operated at a color temperature, T_c, has a spectral distribution of a black body at T_c K multiplied by the emissivity ($\epsilon(\lambda)$) of tungsten:

$$E(\lambda) = \frac{8\pi hc\,\lambda^{-5}\,\epsilon(\lambda)}{[\exp(0.01438/\lambda T_c)]-1} \qquad (1)$$

where λ is in meters.

This emission standard is then used to calibrate the system as described in Section IV, B.

The second method makes use of an intense source of light and a monochromator. A detector of known response measures the watts (or quanta s^{-1}) emerging from the exit slit at various wavelengths. This calibrated source is then used to determine the response function of the spectrofluorometer. These two methods will be critically examined in Sections B and C, following.

It is important to note that the spectrofluorometer is not being calibrated under precisely the same conditions that pertain to the measuring of fluorescence spectra. Errors may arise if: (a) different areas of the grating or prism are illuminated; (b) different areas of the photomultiplier photocathode are illuminated; or (c) if the calibration curve depends on the plane of polarization of the light.

B. Tungsten Lamp Calibration

Vavilov [33], one of the first to measure a corrected fluorescense spectrum, made visual spectrometric comparisons between the output of a tungsten lamp of known color temperature and the radiant flux from a fluorescent solution. The greatest source of error in the use of tungsten lamps appears to be the uncertainty in the spectral emissivity ($\epsilon(\lambda)$) of tungsten. To overcome this error, Stair et al. [24] at the National Bureau of Standards, Washington, D.C., individually calibrate tungsten strip lamps against a high temperature black body, and it is probable that these lamps (type U90) are the most accurate spectral radiant sources presently available. The uncertainty in output ranges from a maximum of 8 percent at short wavelengths to 3 percent at long wavelengths [24]. A description of the use of a standard tungsten lamp is given by Christiansen and Ames [25]. Certain precautions must be taken in the use of standard tungsten lamps. The lamp housing dimensions, the position and the current used must be precisely as specified and the radiance must be taken normal to the tungsten strip from a small area in the middle.

The output of tungsten lamps is very low in the near UV region and care must be taken to correct for stray light by the filter subtraction method [25]. We have found in practice that it is not possible to use the NBS standard lamp below about 340 nm when calibrating a single grating monochromator and photomultiplier, owing to excessive stray light.

A good standard lamp, such as the NBS type U90 is expensive, difficult to use and has a limited lifetime. The usual practice is to compare the standard lamp against an inexpensive lamp which is used as a secondary standard. Such lamps are commercially available. Care must be taken with secondary standards that errors are not introduced by reflections from the glass envelope. Lippert et al. [5] made a secondary standard lamp which was filled with argon and backed by a horn-shaped absorber of the sort used in Raman spectroscopy.

C. Calibrated Emission Monochromator

The idea of taking a xenon lamp and monochromator and measuring the radiant flux from the exit slit in order to calibrate a monochromator and photomultiplier appears to have first been advanced by White et al. [26]. They used fluorescein in NaOH to detect the number of quanta s^{-1} emerging from the mono-

chromator over the wavelength range 250 to 300 nm. Melhuish [27] and Parker [28] describe the method in more detail. Since the accuracy of the method depends primarily on the detector used to measure the radiant flux, we will discuss detectors in more detail below (Sec. IV, D). A fixed fraction of the flux from the exit beam of the exciting monochromator is reflected [27] or scattered [28] into the analyzing monochromator and the photomultiplier current measured at each wavelength. Eastman [29] scatters the light into the analyzing monochromator with colloidal silica (Ludox) in order to have a geometry close to what will be used when measuring fluorescence spectra. In calculating the correction factor, $S(\tilde{\nu})$, it is important to allow for the wavelength dependence of the scatterer and the transmission characteristics of the quantum counter window and any beam splitters used in the optical system. For the apparatus used in this laboratory (fig. 4c),

$$S(\tilde{\nu}) = \frac{\varphi_q(\tilde{\nu})M(\tilde{\nu})W_1(\tilde{\nu})}{W_2(\tilde{\nu})\int_0^\infty R(\tilde{\nu})d\tilde{\nu}}$$

where $\varphi_q(\tilde{\nu})$ = relative number quanta s^{-1} observed by the detector
$M(\tilde{\nu})$ = reflectivity of scatterer
$W_1(\tilde{\nu})$ = transmittance of the quartz beam splitter
$W_2(\tilde{\nu})$ = transmittance of the quantum counter window
$R(\tilde{\nu})$ = recorder deflection

Equation (2) is strictly valid only if $S(\tilde{\nu})$ is constant over the bandwidth of slit used in the analyzing monochromator, typically 4 to 6 nm. A preliminary calibration is made using equation (2) to determine $S(\tilde{\nu})$. The correct calibration factor is then calculated from

$$S_{\text{corr.}}(\tilde{\nu}) = \frac{\varphi_q(\tilde{\nu})M(\tilde{\nu})W_1(\tilde{\nu})}{W_2(\tilde{\nu})\int_0^\infty [S(\tilde{\nu}_0)/S(\tilde{\nu})]R(\tilde{\nu})d\tilde{\nu}} \quad (3)$$

where $S(\tilde{\nu}_0)$ is the correction factor at the center of the scanned band. $S_{\text{corr.}}(\tilde{\nu})$ will usually differ from $S(\tilde{\nu})$ by not more than 2 or 3 percent, except in the region of 700 to 800 nm where the photomultiplier sensitivity changes very rapidly with wavelength. This procedure allows one to choose any slit width for the excitation and analyzing monochromator and is the most reliable method to use. Very little error is introduced if peak heights instead of $\int_0^\infty R(\tilde{\nu})d\tilde{\nu}$ are measured provided, (a) the bandwidth of the light leaving the excitation monochromator is 4 or 5 times less than the bandwidth of the analyzing monochromator and, (b) $S(\tilde{\nu})$ does not change rapidly over the bandwidth defined by the slit of the analyzing monochromator. Calibration by measuring peak height was used by Melhuish [27] and Parker [28], a procedure which was justified because $S(\tilde{\nu})$ did not change rapidly with wavelength over the wavelength range investigated.

It has been pointed out by Børresen and Parker [30] that any silica focusing lenses in the excitation monochromator system can cause calibration errors because a constant area of the scatterer receives varying amounts of the exciting flux depending on the wavelength, while the quantum counter measures the total photon flux in the beam.

The accuracy of this method of calibration rests on the accuracy of the response of the detector measuring $\varphi_q(\tilde{\nu})$ (Sec. IV, D) and on the accuracy to which the spectral reflectivity factor $M(\tilde{\nu})$ is known. Aluminum films were used in the calibration technique described previously [27] because if these are prepared by rapid evaporation at low pressure, the reflectivity is constant over a wide wavelength range [31]. The reflectivity of magnesium oxide seems to be less well known. Values published by Benford, Schwartz and Lloyd [32] were obtained using an integrating sphere. Multiple scattering in the sphere could result in a lower reflectivity at some wavelengths, than for a single scattering. We have recently found that the spectral reflectivity of fresh MgO (1 mm thick) to be the same as a freshly deposited aluminum film to within ± 1.5 percent from 340 to 800 nm (fig. 5). There was a slight decrease (~ 3 percent) at 300 nm, the shortest wavelength investigated. Good values of $M(\tilde{\nu})$ for single scattering from MgO are clearly needed, especially since the MgO scatterer is used in the determination of absolute quantum efficiencies of fluorescence [43].

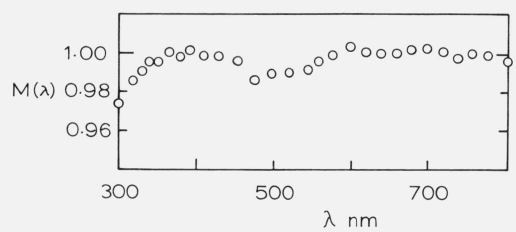

FIGURE 5. Reflectivity ($M(\lambda)$) of freshly deposited MgO versus a fresh rapidly deposited aluminum surface.

D. Detectors

The calibration of the emission monochromator by measuring the energy of quanta s^{-1} from the exit beam requires a detector with an accurately known spectral response. Three detectors have been used for this and each of these will be critically examined.

(1) Thermopiles: These detectors are coated with a layer which absorbs the radiation, causing the heating of a number of thermopiles. The thermoelectric current must be amplified with a high gain, low drift current amplifier. Thermopiles are generally compensated for changes in ambient temperature, but warm objects, such as the human body or a hot lamp housing, can cause changes in output. The absorbing coating on

thermopiles (lamp black or blackened gold) is often assumed to absorb all UV, visible and infrared light, but this is not true. The reflectivity of lamp black is known to increase in the UV region and so for a properly calibrated thermopile, the reflectivity of the actual coating on the junctions ought to be measured. The response curves of three thermopiles measured by Christiansen and Ames [25] differ considerably from each other (40–80 percent at 250 nm) so that it cannot be assumed that all thermopiles have a constant sensitivity over a wide wavelength range. The chief disadvantage of the thermopile is its low sensitivity. The measurement of the output of the emission monochromator becomes very difficult as a result of the narrow bandwidth required (1 to 3 nm) for calibration of the analyzing monochromator and photomultiplier. The wide wavelength response of a thermopile might actually be a disadvantage since stray infrared energy in the beam would also be detected. White et al. [26] and Rosen and Edelman [21] have used thermopiles for calibration purposes on the assumption of the flat energy response.

(2) Quantum Counters: Early investigations by Vavilov [33] showed that the energy efficiency of many dyes in solution was approximately proportional to the exciting wavelength (i.e. φ_f independent of λ) except near the absorption maximum. Further work by Anderson and Bird [34], Harrison and Leighton [35] and Bowen [36] showed that for many dyes, φ_f was independent of wavelength over a wide range and that such solutions could, therefore, be used to determine quanta s^{-1} in the UV and visible region by measuring the fluorescent flux from a fairly concentrated solution of the dye. Rhodamine B (4 g/l in ethylene glycol) was first tested and used as a wide range quantum counter by Melhuish [37]. An important innovation of this new quantum counter was to move the photomultiplier so that no directly transmitted light could be detected. It was now possible to reliably measure the quanta s^{-1} in a broad fluorescence band even if the tail of the band extended beyond 600 nm. The preferred geometry for a quantum counter is that of Parker [38] which not only avoids errors from directly transmitted light, but uses the full thickness of the quantum counter as a filter (fig. 4c). By comparing the rhodamine B quantum counter with a thermopile [27, 39] it has been shown that the response of the quantum counter is constant to within about ± 5 percent from 220 to 600 nm. Deviations which are observed may in fact be due to the thermopile and not the quantum counter [27]. Another method of testing a quantum counter is to use one for measuring excitation spectra. For an ideal quantum counter, the excitation spectrum should agree with the absorption spectrum, provided that φ_f is independent of λ and the OD of the fluorescent solution being measured does not exceed 0.05. A series of substances may be selected to cover a wide wavelength range and by limiting the excitation range to only one electronic band, the probability that φ_f changes with wavelength is reduced. Figure 6a shows the percent deviation of the excitation spectrum from the absorption spectrum for solutions of 2 amino-pyridine, quinine, 3 amino-phthalimide, proflavine, fluorescein, rhodamine B and methylene blue using a rhodamine B quantum counter in the apparatus shown in figure 6b. Solutions were freshly prepared since deterioration occurred when the solutions were exposed to light. The φ_f of quinine in 1 N H_2SO_4, for example, was 25 percent too low at 310 nm if left for three days in the light. The dashed line in figure 6a shows the comparison of a thermopile with the rhodamine B quantum counter [27].

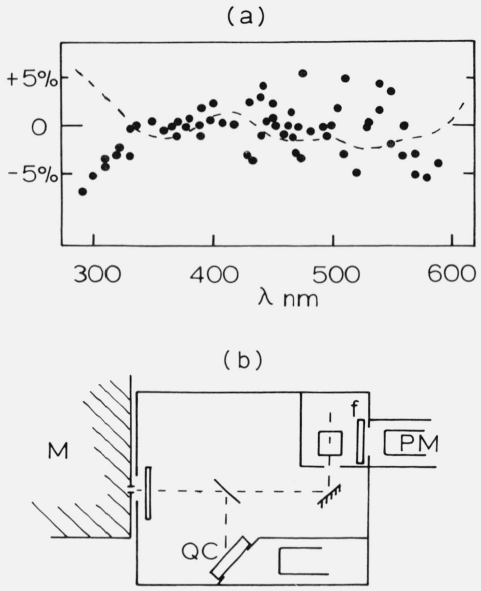

FIGURE 6. (a) Deviation of excitation spectra from absorption spectra ($\pm 3\%$).
(b) Apparatus for measuring excitation spectra.

Methylene blue has been used as a quantum counter by Seely [40]. A 1.5 g/l solution of methylene blue in ethylene glycol can be used from 520 to 700 nm, but it is about 50 times less sensitive than the rhodamine B quantum counter when both are used with an S–20 photomultiplier.

The advantage of the quantum counter over the thermopile is its high sensitivity. Moreover, if several different fluorescent compounds are used as quantum counters, all of which give the same response, we can be reasonably assured that we are actually measuring the quanta s^{-1} in the beam. The apparatus needed for calibrating an analyzing monochromator and photomultiplier using a quantum counter is illustrated in figure 4c. The excitation monochromator has a bandwidth of 1 to 2 nm and the analyzing monochromator a bandwidth of 4 to 8 nm. A filter cutting off at 350 nm is inserted at f (fig. 4c) when calibrating between 400 and 700 nm. The outputs from the amplifier connected to the quantum counter and the photomultiplier amplifier are fed to a ratio recorder. It is important to refer back to a particular wavelength from time to time, to correct for drift in the system.

(3) Actinometers: At least one author [26] has calibrated the output of an emission monochromator using the uranyl oxalate actinometer. The quantum yield of the more sensitive ferrioxalate actinometer has been measured as a function of wavelength by Parker and Hatchard [42] and could probably be used for calibration, although long exposure times would still be required. The wavelength response of the actinometer is not constant and its yield at different wavelengths must be measured against a detector such as the thermopile. Thus, the accuracy of the yield versus wavelength of the actinometer can be no better than the thermopile, which, as we have seen above, may not be very well known especially in the UV region.

E. Polarization Effects

The transmission of a grating monochromator depends on the direction of polarization of light incident on the entrance slit. Thus the correction factor, $S(\tilde{\nu})$, which has been measured for completely unpolarized light will not apply to plane polarized light. Figure 7 shows the correction curves for a Jarrell-Ash 1/4 meter monochromator (blaze = 600 nm) and a 9698–QB (S–20) photomultiplier. If the fluorescence spectrum being measured is not completely depolarized the correction factor $S(\tilde{\nu})$ obtained for unpolarized light cannot be used for correcting the spectrum. The true spectrum might be obtained using a polarizer at the entrance slit of the analyzing monochromator and recording $\parallel$ and $\perp$ spectra, correcting each by the appropriate $S(\tilde{\nu})$ curve and adding. It is also important to recognise that the $S(\tilde{\nu})$ depends on the plane of polarization when investigating polarization of emission spectra using a grating monochromator.

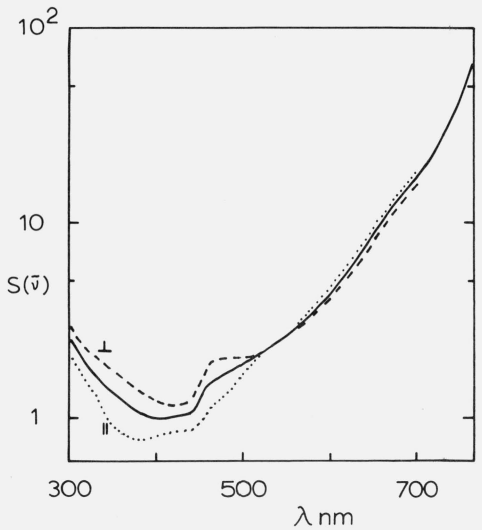

FIGURE 7. Correction curves $[S(\tilde{\nu})]$ for a grating monochromator and photomultiplier ——— = unpolarized light, ····· = polarization $\parallel$ to grating rulings, - - - - - = polarization $\perp$ to grating rulings.

The light from the exit slit of a grating monochromator is also polarized to some extent, the degree of polarization varying with the wavelength setting. Hence, in using this light to calibrate an analyzing monochromator as described in Section IV, C, it is important to depolarize the light by scattering off a magnesium oxide scatterer. The use of an aluminum reflector can sometimes give errors of 10 percent or more and, therefore, should not be used.

V. Methods of Measuring Spectra

A. Dilute Solutions

When the absorption spectrum overlaps the fluorescence spectrum, a true fluorescence spectrum, free from reabsorption effects, can only be obtained in dilute solution. It is important that the cell be blackened with matt black paint on three sides, if observation is from the front surface, or two sides, if viewed from the side. The use of side viewing reduces stray exciting light which is particularly important if the exciting wavelength is within the emission band. Stray light is due to the following causes:

(a) fluorescence of the solvent and cell material
(b) Raman and Rayleigh scattering by the solvent molecules
(c) scattering of light at interfaces or from dust in the solution.

Stray exciting light can often be reduced by filtering the solution to remove solid particles and this should be done as normal practice. Sometimes it is possible to choose a light filter which prevents stray exciting light from reaching the inlet slit of the analyzing monochromator, but does not absorb any of the fluorescence. Stray background light should always be checked after the measurement of the fluorescence spectrum by filling the cell with clean solvent and rescanning. Any stray light is then subtracted from the observed fluorescence spectrum.

Most spectrofluorometers measure fluorescence emitted at right angles to the exciting beam (SV mode). The decrease of fluorescence in these instruments as the concentration of the fluorescence is increased, sometimes attributed to concentration quenching, is actually a geometric effect caused by a shift in the region of absorption in the cell towards the front surface where it cannot be seen. Thus, at high concentrations of solute, the fluorescence must be observed from the same side as the exciting light enters (FS mode). FS viewing may also be used for dilute solutions; in fact, more fluorescence can be collected by the analyzing monochromator than in the SV mode. However, stray exciting light scattered from the front face of the cell will be much larger than in the SV mode. We have found that a 5×10^{-6} *M* (3 parts per million) solution of quinine in H_2SO_4 excited at 365 nm to be the lowest concentration that can be used in the FS mode before stray light becomes excessive.

Before correcting the fluorescence spectrum it is important to see if the refractive index of the solvent varies over the emission band scanned since the amount of light entering the slit of the analyzing monochromator from the fluorescent solution is proportional to n^2. For a fluorescent solute in benzene, for example, the photon irradiance increases by 3 percent from 434 to 588 nm as a result of dispersion in liquid benzene.

B. Concentrated Solutions and Crystals

When the absorption coefficient of the sample for the exciting light is so high that absorption is virtually complete in the first millimeter or less of the sample, fluorescence must be observed from the front surface. The scattering of exciting light into the analyzing monochromator now becomes a problem, especially if the sample is multicrystalline. Furthermore, it is not usually possible to measure a blank for crystalline samples. Although the use of a double monochromator for analyzing the fluorescence helps, the only real solution is to use a sharp cutoff filter to remove the exciting light. If the fluorescence emission falls within the region where the filter absorbs, the transmission of the filter must be measured and the observed fluorescence spectrum corrected for absorption by the filter.

A more serious error arises from reabsorption of fluorescence if the absorption and fluorescence spectra overlap. This error can be corrected by making use of the equation for reabsorption of fluorescence for the special case of front surface viewing [43]. For highly absorbing solutions where shifts in the region of absorption can be neglected, this equation becomes

$$dB(\lambda') = A f(\lambda') d\lambda' \alpha_\lambda (1 + \varphi K' + \varphi^2 K'' + \ldots) \int_0^l \exp[-(\alpha_\lambda + \alpha_{\lambda'} x) z] dz \qquad (4)$$

where a prime refers to fluorescence, unprimed to the exciting light,

A = constant
$f(\lambda')$ = true (corrected) fluorescence spectrum
$dB(\lambda')$ = observed (corrected) fluorescence spectrum
$\alpha = 2.303\,\epsilon C$ where ϵ = molar extinction coefficient and C = molar concentration
$x = \sec[\sin^{-1}(\sin \theta/n)]$ where θ = angle between the exciting beam ($\perp$ to the cell face) and the observing beam and n = refractive index of the solvent
l = thickness of the cell
z = distance from the front face
$K', K'', \ldots$ = reabsorption-emission factor [43]
φ = absolute quantum efficiency of fluorescence.

Equation (4) can be simplified further if absorption of the exciting light is sufficiently high that

$$\exp -(\alpha_\lambda + \alpha_{\lambda'} x) \ll 1$$

and it is assumed that all the K factors are equal. Then

$$dB(\lambda') = [A/(1 - \varphi K)]\,[\alpha_\lambda/(\alpha_\lambda + \alpha_{\lambda'} x)] f(\lambda')\, d\lambda'. \qquad (5)$$

Since the first term in square brackets is independent of λ, the observed spectrum $dB(\lambda')$ may be corrected by multiplying by $(\alpha_\lambda + \alpha_{\lambda'} x)/\alpha_\lambda$ at each wavelength.

Perylene in ethanol has a large overlap between absorption and fluorescence spectra and this compound has been used to test eq (5). The fluorescence spectra of 5×10^{-4} M and 10^{-6} M solutions of perylene in a 10 mm cell is shown in figure 8. The solution was excited and observed from the front surface. The spectrum was corrected using the rhodamine B quantum counter method (Sec. IV, C and D) using an MgO scatterer. The cell was blackened on 3 sides and corrections were made for stray light with solvent in the cell. The bandwidth of the analyzing monochromator was 1.5 nm. The spectrum of the 5×10^{-4} M solution, when corrected by eq (5) (Open circles, fig. 8) agrees well with the 10^{-6} M spectrum.

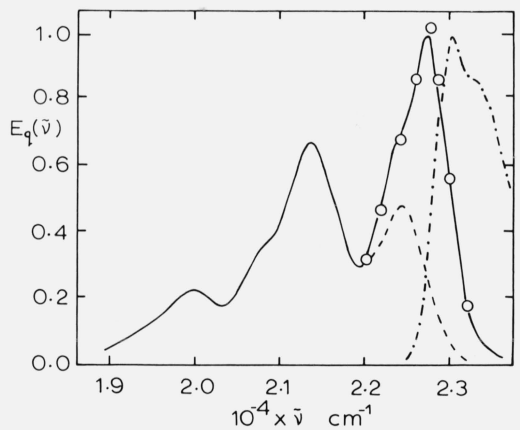

FIGURE 8. Fluorescence spectra of perylene in ethanol, $\lambda_e = 365$ nm, FS. ——$= 10^{-6}$ M, - - - - -$= 5 \times 10^{-4}$ M, - · - · - ·$=$ absorption spectrum.

By using a very thin cell and choosing an excitation wavelength where α_λ is as large as possible, the reabsorption effect can be minimised. Thus, 5×10^{-4} M perylene in a 0.2 mm-cell when excited at 404 nm gives a fluorescence spectrum almost identical ($\pm 3\%$) with the 10^{-6} M spectrum in the 1-cm cell.

C. Narrow Bands

The measurement of a fluorescence spectrum with narrow bands require narrow slits and a slow scanning speed. As a rough guide to the settings needed to record a spectrum without significant error, we may use

the equation of Petrash [44] which states that the error in a recorded band of Gaussian or dispersion shape is approximately proportional to

$$(\gamma^{-5} d\lambda/dt)^{4/9} \qquad (6)$$

where $d\lambda/dt$ = scan rate and γ = half-line width. Thus, if the time constant of the spectrofluorometer is adjusted so that the error in the recording is no greater than 1 percent, the scanning rate would have to be reduced nearly 100 times to record a band of one-tenth the width with the same accuracy. An incorrect setting of scanning rate or time constant is easily observed by stopping the scan on the steeply rising part of the band and observing any subsequent movement of the pen to a higher reading.

D. Reporting Corrected Fluorescence Spectra

The most useful fluorescence spectrum for publication is one measured in very dilute solution where errors due to reabsorption of fluorescence are negligible. However, there are several other factors which could influence the shape of the spectrum and, therefore, the author should give a complete description of the measuring conditions. These are listed below (see Ref. 3). Conditions (a) to (d) are the most important, but the complete list should be given if the spectrum is suggested as a standard.

(a) If a standard tungsten lamp is used for calibration, describe the origin and the accuracy of its spectral distribution. Were corrections made for scatter in the monochromator? (See Sec. IV, B.)
(b) If a standard fluorescent substance is used, give the author and list the factors (d) to (m) below.
(c) If a quantum counter, thermopile or actinometer is used for calibration, give full details of the detector. Describe the system used with particular reference to the spectral reflectance of the scatterer used.
(d) Clearly state the units used for the fluorescence flux; $E_q(\tilde{\nu})$ = quanta s^{-1} per unit wave number interval is preferred.
(e) Were corrections made for stray background light using the pure solvent? (See Sec. V, A.)
(f) State the geometry of the apparatus, whether front surface or side viewing and the dimensions of the cell.
(g) Give the bandwidth of the analyzing monochromator.
(h) The source, purity and concentration of solute should be stated.
(i) The solvent used and method of purification should be noted.
(j) The temperature should be given.
(k) Was the cell blackened on any of its faces?
(l) State the excitation wavelength and its spectral purity.
(m) Give φ_f, if known.

Some commercial spectrofluorometers record absolute fluorescence spectra. It is important to know what units are used for the fluorescence flux and convert to quanta s^{-1} per unit wave number interval, if necessary. These instruments use thermopiles or correction cams in order to automatically correct the emission spectrum and their accuracy is, therefore, uncertain. In reporting fluorescence spectra obtained with these instruments, it would seem desirable to include the spectrum of one or more standards.

E. Future Developments

If the time comes when there are a sufficient number of precisely known standards covering a wide wavelength range, a double beam spectrofluorometer, which takes the ratio of the fluorescence fluxes of the unknown and standard at such wavelength, would overcome the necessity of calibrating the analyzing monochromator and detector. If the observed ratio of unknown to the standard at the frequency is $R(\tilde{\nu})$, then

$$Eq\ (\tilde{\nu})_x = Eq\ (\tilde{\nu})_s \times R(\tilde{\nu})$$

where subscript x = unknown, and s = standard.

Lippert et al. [5] describe a double beam spectrofluorometer of this design using a 13 Hz chopping frequency. The advantage of a double beam system is that fluctuation in the exciting light or in the gain of the photomultiplier does not influence the recorded spectrum.

VI. Standards

A. Introduction

An absolute emission standard is ideally one which satisfies the following criteria:

(a) Broad wavelength range with no fine structure
(b) Small overlap between absorption and fluorescence spectra
(c) Easily obtained and purified
(d) Stable
(e) High φ_f
(f) Spectrum independent of exciting wavelength
(g) Clear, free from scattering centers
(h) Completely depolarized emission.

A standard with a broad emission band with no peaks is important so that the spectrum does not depend on the resolution of the analyzing monochromator. A high φ_f and a reasonably high OD at the exciting wavelength are both important to reduce errors due to stray light (Sec. V, A). A number of absolute standards have been proposed, notably by Lippert et al. [5], but the quinine spectrum is the only one measured by a number of authors. The new standard 2-amino pyridine proposed by Rusakowicz and Testa [45] covers approximately the same wavelength range as 2-naphthol and is to be preferred since it does not have the temperature effects shown by the latter.

The effect of reabsorption of fluorescence on the emission spectrum has been discussed in Section V, B. Although standards should be measured in very dilute solution where reabsorption has no effect, sometimes spectra are determined at high concentrations. It is important, therefore, to have some idea of what region of the standard spectrum might be affected by reabsorption. The upper limit of the error due to reabsorption is given by eq (5). The term $A=\alpha_\lambda/(\alpha_\lambda+\alpha_{\lambda'}x)$ has been included in tables 1 to 5 to show the wave number region where reabsorption errors can be expected.

B. Individual Standards

1. Quinine in sulfuric acid (fig. 9). Table 1 lists the determination since 1941 of the absolute fluorescence spectrum of quinine. Many of these figures have been taken from rather small diagrams and are therefore not accurate to better than ± 3 percent. All spectra have been converted to $E_q(\tilde{\nu})$ spectra using the appropriate conversion factors.

We have found that for a 10^{-4} *M* solution of quinine viewed from the FS, the spectrum is unaffected by: (a) varying the sulfuric acid concentration from 0.1 to 2 *N*, (b) varying the exciting wavelength from 260 to 390 nm, (c) measuring three different brands of quinine. Thus the differences between the published quinine spectra are almost certainly due to calibration errors. The largest deviations are found for those spectra determined from a tungsten lamp calibration.

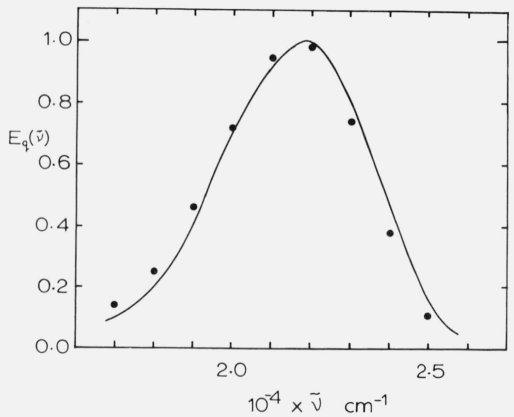

FIGURE 9. Quinine, 10^{-4} *M* in *N* H_2SO_4 (25 °C, $\lambda_e = 365$ nm). ● = Lippert [5].

TABLE 1. Absolute fluorescence spectrum of quinine in H_2SO_4 ($\lambda_e = 365$ nm)

$10^{-3}\times\tilde{\nu}$ cm^{-1}	A[a]	$E_q(\tilde{\nu})$										
25	0.95	0.07	0.105	0.105	0.135	0.105	0.15	0.10	0.16	0.14	0.15	0.152
24	.98	.36	.43	.375	.37	.375	.46	.37	.47	.46	.49	.480
23	1.00	.78	.80	.740	.79	.74	.80	.78	.80	.78	.83	.810
22		.99	.99	.980	.99	.98	1.00	.99	.99	.99	.99	1.00
21		.92	.91	.950	.92	.97	0.92	.97	.92	.97	.93	0.915
20		.56	.67	.715	.68	.75	.70	.74	.70	.77	.70	.710
19		.35	.40	.460	.40	.47	.42	.41	.42	.51	.42	.405
18		.20	.215	.240	.20	.26	.21	.21	.24	.26	.21	.200
17		.....	.104	.140	.09	.16	.10	.....	.11	.11	.08	.095
Ref.		47	6	5	48	7	27	21	46	29	22	41
Solvent		2*N*	2*N*	0.1*N*	0.1*N*	1*N*	1*N*	1*N*	0.5*N*	0.1*N*	.....	1*N*
Mode		*FS*	*FS*	*FS*	*SV*	*FS*	*SV*	*SV*	*SV*	*SV*	*SV*	*FS*
Conc(*M*)		10^{-5}	10^{-5}	10^{-3}	2×10^{-6}	5×10^{-3}	2×10^{-6}	10^{-5}	2×10^{-5}	.....		10^{-4}
Calibration[b]		*L*	*L*	*L*	*L*	*L*	*L*+*Q*	*T*	*Q*	*Q*	*Q*	*Q*

[a] $A=\alpha_\lambda/(\alpha_\lambda+\alpha_{\lambda'}x)$

[b] *L* = tungsten lamp calibration, *Q* = quantum counter calibration
T = thermopile calibration

2. 2-Naphthol (fig. 10). Lippert et al. [5] have published the absolute fluorescence spectrum of 2×10^{-4} *M* 2-naphthol in a 0.02 *N*, pH 4.62 acetate buffer at various temperatures. In using this standard, the temperature and the composition must be known, and the concentration and pH of the buffer must be exactly as specified by Lippert. Børresen [46] has published the absolute emission spectrum of a 10^{-4} *M*

solution of 2-naphthol using an instrument calibrated with the rhodamine B quantum counter. The results are given in table 2.

We have also checked the spectrum using the quantum counter method of calibration and found good agreement with Børresen's results, but rather poor agreement with Lippert.

3. 3-Amino phthalimide (fig. 11). A comparison of Lippert's spectrum with the absolute spectrum recently measured in this laboratory is given in table 3.

TABLE 2. Absolute fluorescence spectrum of 2-naphthol in 0.02 *N* acetate (pH 4.62 −4.68) ($\lambda_e = 313$ nm)

$10^{-3}\times\tilde{\nu}$ cm^{-1}	A[a]	$E_q(\tilde{\nu})$						
30	0.51	0.220	0.380	0.220	0.36	0.370	0.220	0.345
29	.93	.860	.900	.860	.90	.900	.860	.930
28.5	.99	.980	.995	.980	.97	.980	.980	1.00
28	1.00	.980	.955	.980	.97	.970	.985	.950
27		.745	.680	.740	.71	.745	.750	.715
26		.625	.590	.620	.62	.628	.630	.620
25		.780	.800	.770	.72	.750	.790	.825
24		.915	.930	.900	.80	.842	.930	.895
23		.810	.785	.795	.65	.630	.825	.670
22		.550	.515	.535	.41	.390	.565	.405
21		.300	.245	.295	.22	.224	.305	.235
20		.140	.092	.135	.095	.125	.145	.135
Ref.		5	49	5	46	41	5	41
Temp. (°C)		24	24	23	22.7	22.7	25	25
Conc. (M)		2×10^{-4}	10^{-5}	2×10^{-4}	10^{-4}	5×10^{-5}	2×10^{-4}	5×10^{-5}
Mode		FS	SV	FS	SV	FS	FS	FS
Calibration[b]		L	$Q+L$	L	Q	Q	L	Q

[a] $A = \alpha_\lambda/(\alpha_\lambda + \alpha_{\lambda'}x)$

[b] L = tungsten lamp calibration. Q = quantum counter calibration

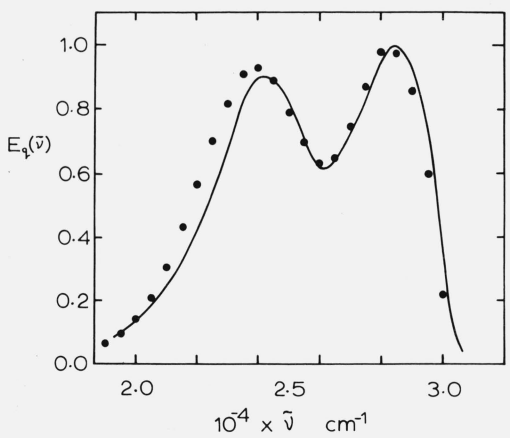

FIGURE 10. 2-Naphthol, 5×10^{-5} *M* in 0.02 *N* acetate, pH 4.65 (25 °C, λ_e = 313 nm). ● = Lippert [5].

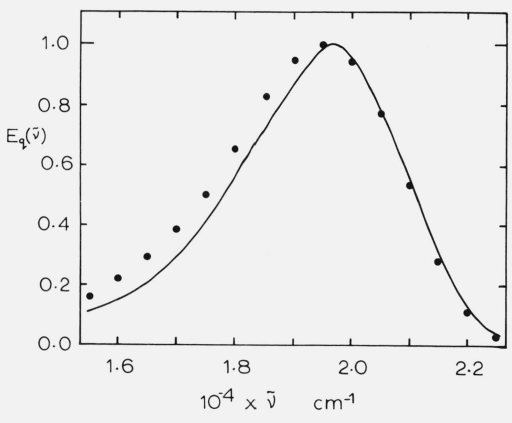

FIGURE 11. 3-Aminophthalimide, 2×10^{-4} *M* in *N* H_2SO_4 (25 °C, λ_e = 365 nm). ● = Lippert [5].

TABLE 3. Absolute fluorescence spectrum of 3-amino phthalimide in H_2SO_4 at 25 °C $\lambda_e = 365$ nm

$10^{-3} \times \tilde{\nu}$ cm^{-1}	A[a]	$E_q(\tilde{\nu})$	
23	0.97	0.01	—
22.5	.99	.03	0.04
22	1.00	.110	.125
21.5		.280	.308
21		.530	.560
20.5		.775	.790
20		.945	.955
19.5		1.000	.990
19		0.950	.875
18.5		.825	.725
18		.650	.565
17.5		.500	.402
17		.385	.290
16.5		.295	.207
16		.220	.151
15.5		.160	—
Ref.		5	41
Conc. (M)		5×10^{-4}	2×10^{-4}
Mode		FS	FS
Solvent		0.1 N	1 N
Calibration[b]		L	Q

[a] $A = \alpha_\lambda/(\alpha_\lambda + \alpha_{\lambda'}x)$

[b] L = tungsten lamp calibration, Q = quantum counter calibration

4. *m*-Dimethylamino nitrobenzene (DNB) (fig. 12). Lippert et al. [5] measured the absolute emission spectrum of DNB in 30 percent benzene and 70 percent hexane. Table 4 compares this spectrum with the one measured in this laboratory. The spectrofluorometer was calibrated with rhodamine B and methylene blue quantum counters. A disadvantage of this standard is its low φ_f and this, coupled with the fact that the instrumental sensitivity is decreasing in the region of the emission spectrum, results in a poor signal-to-noise ratio.

TABLE 4. Absolute fluorescence spectrum of *m*-dimethylamino nitrobenzene at 25 °C in 30 percent benzene, 70 percent hexane (λ_e = 365 nm)

$10^{-3} \times \tilde{\nu}$ cm^{-1}	A[a]	$E_q(\tilde{\nu})$	
22	0.93	0.03	0.10
21	.98	.235	.41
20	1.00	.610	.80
19		.935	1.00
18		.975	0.86
17		.825	.68
16		.615	.48
15		.405	.31
14		.235	.17
13		.110	—
Ref.		5	41
Conc. (M)		10^{-4}	5×10^{-4}
Mode		FS	FS
Calibration[b]		L	Q

[a] $A = \alpha_\lambda/(\alpha_\lambda + \alpha_{\lambda'}x)$

[b] L = tungsten lamp calibration, Q = quantum counter calibration

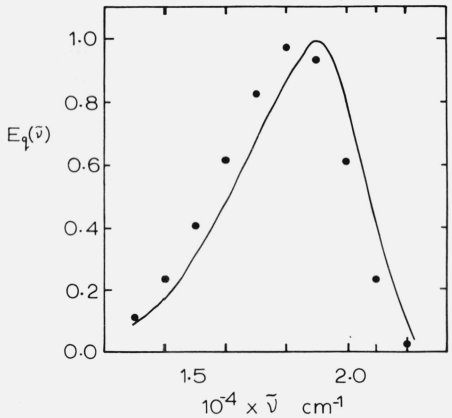

FIGURE 12. *m*-Dimethylamino-nitrobenzene, 5×10^{-4} M in 30 percent benzene, 70 percent hexane (25 °C, $\lambda_e = 365$ nm). ● = Lippert [5].

5. 2-Aminopyridine (fig. 13). The corrected fluorescence spectrum of 2-amino pyridine determined by Rusakowicz and Testa [45] using a tungsten lamp for calibration is given in table 5. The spectrum measured

TABLE 5. Absolute fluorescence spectrum of 2-amino-pyridine in H_2SO_4 at 25 °C

$10^{-3} \times \tilde{\nu}$ cm^{-1}	A[a]	$E_q(\tilde{\nu})$	
31	0.64	0.03	0.05
30.5	.82		.145
30	.94	.17	.325
29.5	.97		.540
29	1.00	.575	.686
28.5			.840
28		.93	.960
27.5			1.000
27		.99	0.980
26.5			.920
26		.805	.790
25.5			.660
25		.52	.530
24.5			.405
24		.26	.310
23.5			.222
23		.138	.148
22.5			.102
22		.068	.075
Ref.		45	41
Solvent		0.1 N	1 N
Conc.		10^{-5}	10^{-5}
Mode		SV	FS
Calibration[b]		L	Q
λ_e nm		285	313

[a] $A = \alpha_\lambda/(\alpha_\lambda + \alpha_{\lambda'}x)$

[b] L = tungsten lamp calibration, Q = quantum counter calibration

in this laboratory using the quantum counter method of calibration is in good agreement with their spectrum.

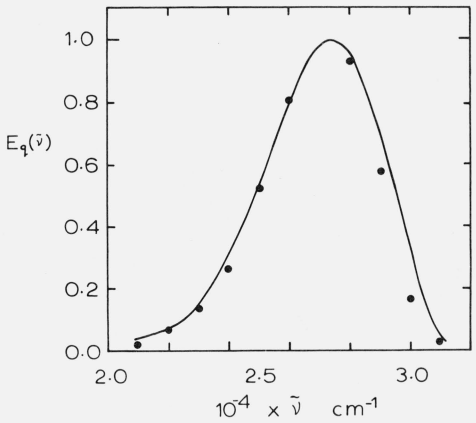

FIGURE 13. 2-Aminopyridine, 10^{-5} M in N H_2SO_4 (25 °C, $\lambda_e = 313$ nm). ● = Rusakowicz and Testa [45].

C. New Standards

The greatest lack, at present, is for standards in the ultraviolet and near infrared regions of the spectrum. Reisfeld et al. [50] recommend thallium activated potassium chloride as an emission standard in the UV region. For the red and near infrared regions, rhodamine B and methylene blue might be used since these substances have a high φ_f. Preliminary measurements in this laboratory (fig. 14) indicate that these two would cover the range 18,000 to 12,000 cm^{-1}.

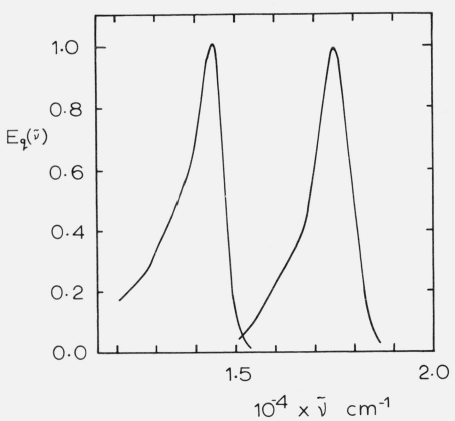

FIGURE 14. Rhodamine B, 5×10^{-6} M and methylene blue, 5×10^{-5} M in ethanol (25 °C, $\lambda_e = 436$ nm).

VII. References

[1] Birks, J. B., Photophysics' (John Wiley and Sons, Inc., New York, N.Y., 1970).

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