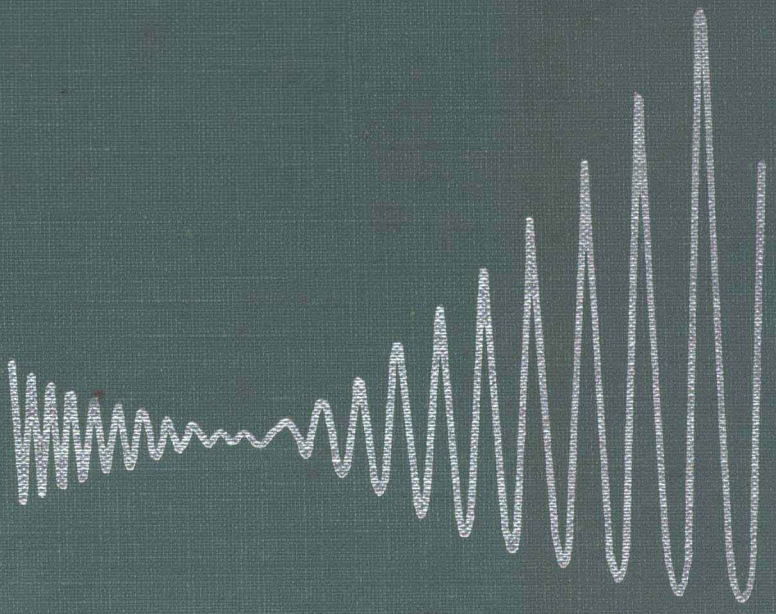
Topics in . . .

Chemical Instrumentation—II

GALEN W. EWING
Editor



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Chemical Instrumentation—II

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GALEN W. EWING
Editor

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Chemical Instrumentation—II

FOREWORD

In this second volume of reprinted articles from the "Topics in Chemical Instrumentation" column in THE JOURNAL OF CHEMICAL EDUCATION, all articles from January 1970 through November 1975 are included. They have been ordered, not chronologically, but by subject matter.

As in the previous volume, the printing has been done by photo-offset from reprints of the individual articles. The authors have been offered an opportunity to add supplemental material, and a number have done so.

The illustrations inserted as space fillers between articles are reproduced from woodcuts appearing in the 13th English edition of Ganot's *"Éléments de Physique,"* published by Wm. Wood and Co., New York, 1890. The captions are mine.

Increased printing costs have prevented the JOURNAL from underwriting this volume, and its publication was in jeopardy for some time. The Books Department of the American Chemical Society, under the direction of Robert F. Gould, was fortunately in a position to step in, so that the book finally appears under ACS auspices.

I wish to acknowledge with heartfelt thanks the continued vigorous support of the editor of the JOURNAL, Dr. W. T. Lippincott, and of the business manager, Frank J. Altschul, Jr., and particularly I wish to thank R. F. Gould, whose personal interest made the publication of this book possible.

Galen W. Ewing

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Edited by GALEN W. EWING, Seton Hall University, So. Orange, N. J. 07079

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LXXXIV. A Review of Wavelength Calibration Methods for Visible-Range Photoelectric Spectrophotometers*

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INTRODUCTION

It is well known that in accurate spectrophotometry, eare must be taken to calibrate the instrument properly. This typically may include use of standards and calibration procedures for setting the zero line and the 100 percent line, and for checking the photometric scale and the wavelength scale. In this paper we review several methods for the calibration of the wavelength scales of photoelectric spectrophotometers for the visible spectral range, nominally 400–700 nm. In most cases the same or similar methods may be applied to wavelength calibration in the near ultraviolet and the near infrared spectral regions but this extension is not specifically considered in this paper.

In practical spectrophotometry the monochromator section of the instrument is not capable of isolating a single wavelength of light from the continuous spectrum emitted by the source. Rather, a finite band of radiation is passed by the monochromator. The problem in wavelength calibration is to determine which wavelength within this finite band best describes the position of the total band. In order to understand how the different wavelength-calibration techniques allow determination of this representative wavelength, we first describe this finite band of radiation in a qualitative sense. This is considered in the following section. The actual techniques of wavelength calibration are then discussed.

SLIT DISTRIBUTIONS AND THEIR EFFECTS ON SPECTROPHOTOMETRIC DATA

Slit Distributions

The monochromator has an entrance and an exit slit, and together these determine the slit distribution of the instrument. The entrance slit serves to focus the source on the dispersing element (grating or prism) and adjusts the size of the image at the plane of the exit slit so long as all intermediate optics such as lenses and mirrors are unchanged. The exit slit fixes the dimensions through which the image must pass. The image width in wavelength units may be equal to, less than, or greater than the exit-slit width in the same units. In each of these cases a different slit distribution results. Adding the assumption that the radiation is of equal intensity (more properly called *irradiance*) throughout the image, we follow the analysis of Buc and Stearns *(1)* and Hogness *et al. (2)* for symmetrical slit distributions.

Referring to Fig. 1a, let ABCD be the image of a monochromatic line at the exit plane and let EFGH be the dimensions of the exit slit. The numbers 1, 2, 3, etc., correspond to positions of the wavelength setting. We now plot the transmittance of the system as a function of the position of the image of the entrance slit as it passes over

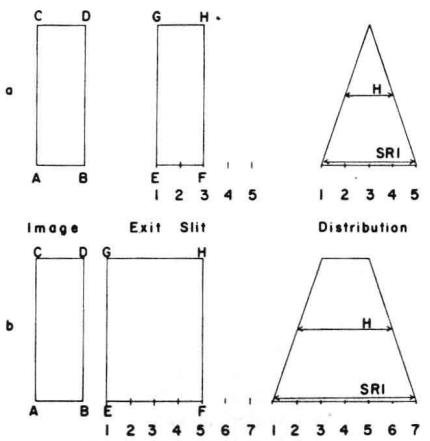
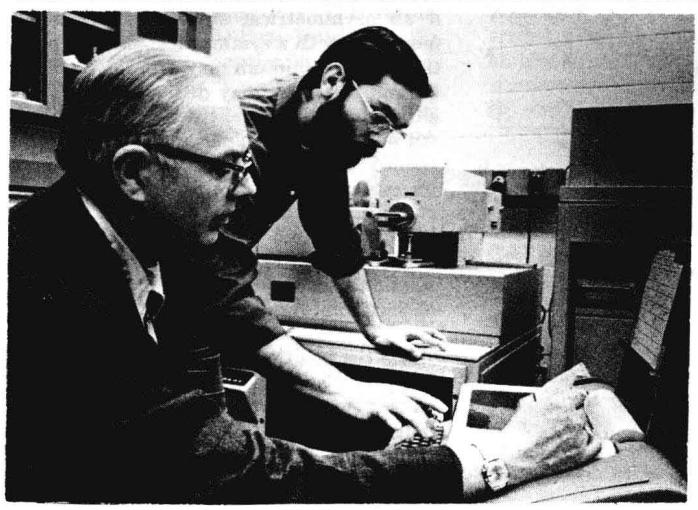


Fig. 1. Slit distribution functions.

* *Contribution No. 57 from The Rensselaer Color Measurement Laboratory*



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the exit slit. Up to the point when the leading edge BD of the image reaches position 1, no light is transmitted by the exit slit. When BD reaches point 2, half of the total light is passed, and at point 3 all of the light in the image passes through the exit slit. As the image reaches point 4 the transmittance of the system again falls to one half, and at point 5 it falls to zero. This plot of transmittance versus the wavelength describing the position of the image is the slit distribution for the monochromator.

Generally, slits are characterized only by their width. The spectral slit width, H, is defined as the difference in wavelength between the points where the transmittance is one half the maximum. We also define the spectral region isolated, SRI, as the sum of the image width and the exit-slit width in wavelength units. In Fig. 1 the SRI corresponds to the distance from points 1 to 5. In this first example, the image size and exit-slit size were chosen to be equal. This is the ideal situation and it yields a triangular, symmetrical slit distribution. In this case there is a single wavelength of maximum transmittance. Also in this case the spectral slit width is exactly one half the spectral region isolated.

The more general case occurs when the image size and exit-slit size are not identical. For the next example, assume that the image is one-half the size of the exit slit. Following the same type of analysis as before, we obtain the distribution in Fig. 1b. The slit distribution is now trapezoidal with a width equal to the distance between points 2 and 6 and a spectral region isolated equal to the distance between points 1 and 7. Note that the SRI is no longer twice the slit width and that there is a range of wavelengths over which the transmittance is a maximum. If we reverse this case so that the image size is twice the exit-slit size, the same distribution is obtained.

The limiting case is obtained if either the image width approaches zero for a finite exit-slit size, or the exit-slit width approaches zero for a finite image size. In either of these cases, the slit distribution is rectangular and the slit width and spectral region isolated are equal. This model does not include the increase in diffraction which occurs as the slits are narrowed. The diffracted light can be considered as either stray light in the instrument, or as a secondary effect which broadens the slit distribution.

Effect of Slit Distribution on Photometric Values

The effect that the slit distribution has on photometric values (transmittance or reflectance) can now be considered. We first discuss the error produced by increasing the width of the slit distribution. Assume that there are two symmetrical rectangular slits, one infinitely narrow and the other of finite slit width, both having the same wavelength position. Also assume that over the wavelength range encompassed by the second slit, the transmittance of the optics in the instrument, the irradiance of the light source and the responsivity of the photodetector are all constant. We now compare the measured photometric value obtained with the infinitely narrow slit to that obtained with the finite slit for several types of samples having idealized absorptance* curves.

The simplest case is the straight-line curve for a sample of constant absorptance. The photometric value obtained by using each slit is simply the area under the true absorptance curve divided by the area under the 100 percent curve obtained by removing the sample. Fig. 2a demonstrates that there is no photometric error involved in widening the slit for such a neutral curve. Both the narrow and the finite slit yield the same correct result.

The next case is a straight line absorptance curve of arbitrary slope. Referring to Fig. 2b, it can be seen that the narrow and finite slits again yield the correct photometric values. In use of the wide slit, the added area *abc* exactly compensates for the area lost *ab'c'* so that the correct value is obtained. Therefore, so long as the straight-line portion of the curve extends over a larger wavelength range than the spectral slit width, any symmetrical slit distribution yields the correct photometric reading.

Next let us consider the case where there is a change in the slope of the absorptance curve, particularly the presence of a maximum or a minimum. We assume a triangular transmittance minimum and see in Fig. 3a the effect of slit width on the photometric value recorded at the minimum point. With the infinitely narrow slit the photometric value is 50% in the example, but with the wide slit (which we chose to have a width equal to the width of the minimum) there is a photometric error. Since the area *abcb'a'* equals the area *bcb'* the recorded photometric value is 75%. Notice that the photometric value has increased in this case. If the example had been a transmittance maximum, the photometric value would have decreased. The result is that increasing slit width tends to "round off" the true absorptance curve.

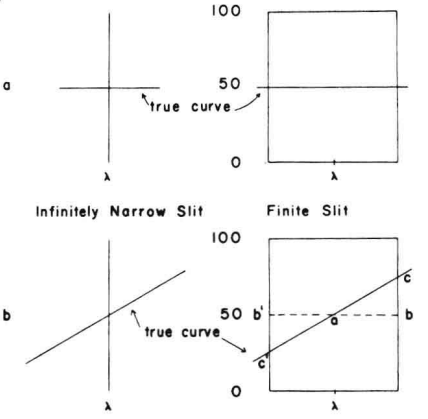

Fig. 2. Effect of slit distribution on photometric values for linear spectral curves.

An extreme case of photometric error occurs when there is a regular cycle of maxima and minima and one complete cycle equals the slit width. In this case no maxima or minima are observed and the observed photometric value is just the average of the true values. This is illustrated in Fig. 3b.

It has been shown here how the measured transmittance or reflectance varies with slit width for a rectangular slit. The same variation holds for any symmetrical slit function. However, the actual values for a given curvature also depend on the shape of the slit distribution. Rectangular, trapezoidal, and triangular slit distributions of equal width will yield different photometric values for a given maximum or minimum.

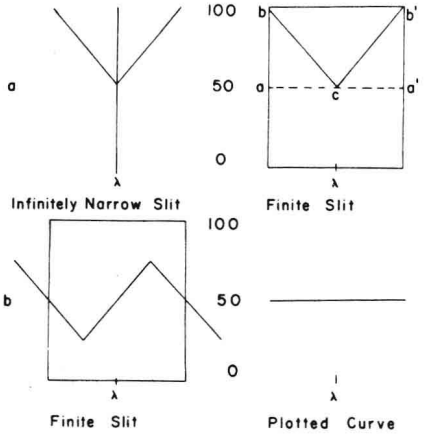

Fig. 3. Effect of slit distribution on photometric values at absorptance maxima and minima.

Our findings can be summarized as follows: the photometric error introduced by the use of finite symmetrical slits depends on the curvature of the absorptance curve and does not depend on the slope of the curve. Unfortunately, it is the points of great curvature (maxima or minima) that we often wish to use for wavelength calibration. If the calibration point is a symmetrical minimum or maximum, the effect of a finite symmetrical slit distribution will be simply to raise or lower the calibration point. The wavelength position of the calibration point will not be shifted. However, if an asymmetrical absorptance is being measured with a symmetrical slit, the position of the minimum may actually be shifted and this shift may depend on both the slit width and the slit distribution shape. An additional problem arises if the minimum is actually composed of two or more unresolved minima. In this case changes in slit width will shift the position of the calibration point as the resolution changes. A good example of this is the transmittance minimum of the familiar didymium filter at 585.0 nm. As shown in Fig. 4, decreasing the slit width resolves this minimum and shifts the position of the calibration wavelength. Calibration points which are subject to resolution effects are strongly dependent on slit width.

* *We use* absorptance *(one minus the transmittance) to avoid the complications of transformation to a logarithmic function such as* absorbance.

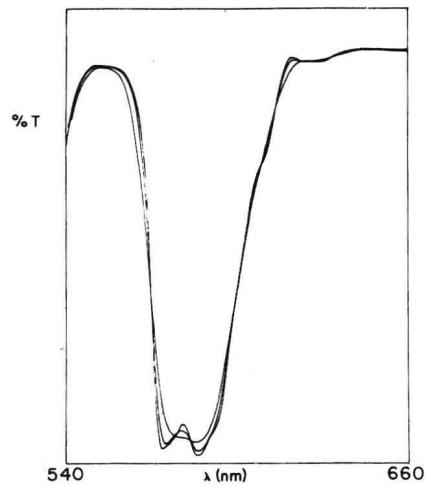

Fig. 4. Effect of slit width on the spectral transmission curve of a didymium filter, as measured at spectral slit widths of 2.5, 5, and 10 nm on a Zeiss DMC-25 spectrophotometer. *(26)*

The wavelength position of a symmetrical slit may be defined in a number of ways. If we assume that all instrumental factors (source, optics, detector) are constant over the slit interval, the wavelength position of the slit may be defined in any of the following ways (Fig. 5):

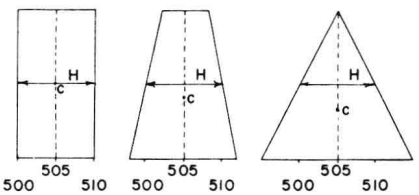

Fig. 5. Various methods of defining the position of the wavelength scale. C is the centroid.

1. As the midpoint between the one-half transmittance points
2. As the midpoint of the spectral region isolated
3. As the wavelength that passes through the centroid of the slit distribution. Note that only for the triangular slit distribution is the wavelength unambiguously defined as the wavelength of maximum transmittance.

Unfortunately, a perfectly symmetrical slit distribution is probably a rarity in wide-slit spectrophotometers. This results from the fact that the lamp irradiance, the transmittance of the optics, and the photodetector responsivity are all generally a function of wavelength. We now define an "effective" slit distribution as the transmittance of the slits (τ_s), weighted by the transmittance of all other optics (τ_o), the irradiance of the source *(E)*, and the responsivity of the detector *(R)*, all as functions of wavelength over the spectral slit interval. The signal *(S)* from the photodetector is

$$S(\lambda) = \tau_s(\lambda) \cdot \tau_0(\lambda) \cdot E(\lambda) \cdot R(\lambda)$$

It would take either precisely constant contributions for each of these factors or a fortuitous cancelling of effects for the resulting distribution $S(\lambda)$ to be symmetrical.

Suppose that there is a rectangular slit function centered at 510 nm with a width equal to 20 nm and the transmittance of the optics and the irradiance of the source are constant over the spectral slit interval. If the spectral responsivity of the detector were a straight-line function that fell to zero at 520 nm from a maximum at 500 nm, the resulting effective slit distribution would be that given in Fig. 6. This, of course, is an extreme example, but it illustrates the effect that instrumental variables may have on the effective slit distribution *(3)*.

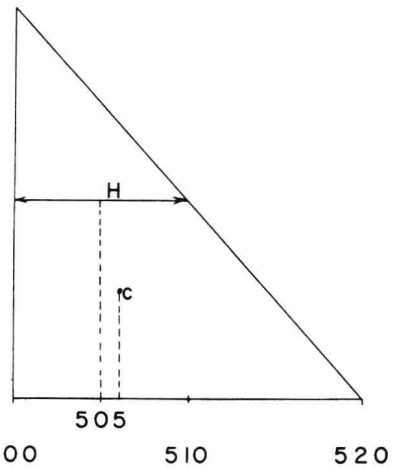

Fig. 6. An extreme example of an asymmetrical slit function.

The next question is, what is the wavelength position of this slit? The wavelength of maximum transmittance is 500 nm, the midpoint of the one-half transmittance points is 505 nm, the midpoint of the spectral region isolated is 510 nm, and the wavelength through the centroid is still another value. Which one defines the position of the slit correctly? There is no answer.

In addition to the problem of wavelength definition, an asymmetrical slit produces the same types of photometric errors as a symmetrical slit. It shows resolution effects with multiple peaks and rounding-off of the true curve at points of great curvature. With an asymmetrical slit distribution, the wavelength position of a symmetrical or an asymmetrical minimum or maximum may be shifted.

In this brief discussion it has been impossible to go into the mathematics of slit-error correction. This topic is discussed in an introductory way by Forsythe *(4)* and at a more advanced level by Hardy and Young *(5)* and by Eberhardt *(6)*.

METHODS OF WAVELENGTH CALIBRATION

Absorbing Filters

The most common method of wavelength calibration for spectrophotometers with wide slit widths is the use of calibrated absorbing filters. Several types have been described in the literature, including glass and liquid filters which have narrow absorptance bands, and pairs of filters which have spectral absorptance curves which cross at a unique wavelength position.

Standard filters with sharp absorptance bands consist in the main of solutions of salts of rare-earth metals, or these same salts dispersed in glass. Among the filters that have been suggested in the literature are samarium and neodymium chloride in solution *(7)*, holmium oxide, both in solution and in glass *(8, 9, 10)*, and didymium (a mixture of praseodymium and neodymium oxides) in glass *(11, 12)*. The latter standard has been the most widely used and is obtainable from the National Bureau of Standards (NBS) calibrated for the wavelength positions of its transmission minima. The holmium oxide glass may also be obtained from the NBS, calibrated for spectral slit widths of less than 2 nm.

Since the didymium-glass filter is in such widespread use, it will be described fully. This filter shows several transmittance minima across the visible and near-infrared spectrum which may be used for wavelength calibration. The NBS originally found the wavelength positions of these minima on Gibson photoelectric and König-Martens visual spectrophotometers with slit widths of 4, 8, 10 and 20 nm. Thus the determination is subject to any wavelength error that might have been present in these reference instruments. In other words, the determination of these wavelength positions was empirical rather than absolute. Therefore, an uncertainty in the wavelength positions is introduced. The NBS reports the wavelength positions to 0.1 nm but states that the uncertainty in these positions with 10 nm slit widths is ±1 nm in the visible range for filters which they have calibrated individually against their standards. The uncertainty in uncalibrated didymium filters is probably about the same since there has been little batch to batch variation in these filters as manufactured. (However, very early didymium glasses differed in composition from those now available, leading to some differences in curve shapes.)

The method of wavelength calibration using these filters has several disadvantages. Since several of the minima are composed of multiple absorptions, the calibration points are strongly dependent on slit width. Also, since the absorptance bands are generally asymmetrical, the points of minimum transmittance are dependent on the asymmetry of the slit distribution. In effect, this means that the calibration is only truly reliable on spectrophotometers with slit distributions identical to those of the Gibson and König-Martens instruments. Actually, these errors are probably small enough to be within the stated uncertainty in the wavelengths of the minima as long as the data for the correct slit width of the test instrument are used. Determining the actual calibration position is another difficulty with this method because the rate of change of transmittance is rather small near a minimum.

On the other hand, the method holds many advantages. First, it is relatively inexpensive. The calibration can be done quickly, in the mode of operation in which samples are actually measured. The results do not depend strongly on temperature or on the exact positioning of the filter in the

sample beam of the spectrophotometer. Although this method gives no direct information on the slit width and distribution shape, one learns through experience to recognize when the slit width is too large or too small from the appearance of the curve at certain points. For example, referring again to Fig. 4, the 585.0 nm minimum becomes more rounded at slit widths greater than 10 nm and the shoulder at 625 nm becomes a slight minimum at slit widths less than 10 nm.

The use of absorbing filters whose curves cross at some point was suggested by Sanghi and Parthasarathy *(13, 14)*. They originally employed the isosbestic point of an acid-base indicator as the position for wavelength calibration. This is the point in the spectrum where the curves of a single indicator at two different values of pH cross. For instance, at 469.1 nm solutions of methyl orange have the same transmittance in the pH range from 2.6 to 5.0. Later, the same authors suggested *(14)* that the difficulties in using liquid filters could be avoided by simply using pairs of solid colored filters whose curves cross at some unique point.

To implement the method one must find, on a spectrophotometer which has been carefully calibrated, the wavelength position at which the transmittance values are identical for two colored filters. Then to calibrate another instrument, one simply finds the wavelength position that gives the same value of transmittance for each of the two filters. Comparison with the calibrated values completes the determination. The problem here is that the method is still empirical. Any random or systematic errors in the original measurements to determine the crossover points will influence all later calibrations. Additionally, the original calibration, depending on the choice of filters, may or may not be strongly influenced by the slit parameters. So again there may be the situation where the calibration is strictly valid only for instruments with slit distributions identical to those of the reference instrument.

Again the advantages are simplicity and low cost. The original authors gave no estimate of the uncertainty of the measurements but it is probably about the same as for the didymium-filter method. It may be less if the filters are chosen so that they have straight-line curves of large slope at the crossover point. In this case the precision with which the position can be determined is greater than for a minimum where the photometric value changes slowly. Also, the dependence on the slit distribution is much less for straight-line absorptances than for strong curvature. Depending on the choice of filters, this method may or may not be sensitive to temperature and positioning in the sample beam.

Line Spectra

Many investigators *(2, 15–20)* have reported on the use of line spectra for wavelength calibration. The method generally entails replacing the light source in the spectrophotometer with a line source such as a mercury arc, and determining the signal from the detector as a function of wavelength. Either the position of maximum response or that determined by extrapolation from the readings on either side of the peak response is taken as the wavelength position of the slit. This position can then be compared with the position of the spectrum line which may be found in the "MIT Wavelength Tables" *(21)* or other compilations. If more than one spectrum line passes the exit slit, the wavelength position for calibration is taken to be the average of the wavelengths of the individual lines, weighted by their relative intensities. The method is semi-empirical since the wavelength positions of the lines were originally determined experimentally. However, they are known to such great precision (usually better than 0.001 nm) that for spectrophotometric purposes they may be taken to be absolute values.

For spectrophotometers with narrow slits this is generally the method of choice. With instruments having wider slits, however, significant errors may occur. If we again make the assumptions that the source irradiance is constant and that the slit distribution is symmetrical, this method gives the correct wavelength position of the slit. But if this is not true an error will occur. Replacing the original source with a line source removes the effect of the wavelength-dependent spectral irradiance of the lamp on the effective slit distribution and thus on the wavelength position of the slit. In other words, the slit distribution is changed by changing light sources and this is not corrected for in the determination. Also, there is the question of what is the correct definition of wavelength for an asymmetrical slit. In this case the method gives the wavelength of maximum response.

This method has the advantages that it is "absolute," simple to apply and temperature independent. For narrow slits the uncertainty is quite small. For spectrophotometers with wider slits, however, this method is unsatisfactory and large errors may occur because of slit asymmetry.

Combination of Line Spectra and Absorbing Filters

In this method, suggested by Van den Akker *(22)*, the problem of the effect of slit parameters on calibration with line spectra is avoided. Following his analysis, let the light passing the exit slit have wavelengths from λ_1 to λ_2. Let the irradiance of the light source be E, the transmittance of the optics (including slits) be τ_g, the true transmittance of the filter (the reflectance of the sample may be substituted) be τ and the relative responsivity of the detector be R, all as a function of wavelength between λ_1 and λ_2. Then the measured photometric value is given by:

$$\bar{\tau} = \int_{\lambda_1}^{\lambda_2} E\tau_g R\tau \, d\lambda \Big/ \int_{\lambda_1}^{\lambda_2} E\tau_g R \, d\lambda$$

If we now attempt to choose a wavelength to represent the position of this slit, it can be seen that there is none which will give a measured value of transmittance that agrees with those obtained with monochromatic light for all possible types of samples.

Van den Akker assumed that since most transmittance or reflectance spectra of colored materials are not strongly curved, they could be represented as linear over the wavelength region from λ_1 to λ_2. In this case the transmittance can be expressed by a straight line equation:

$$\tau = a + b\lambda$$

The equation for the measured transmittance is then

$$\bar{\tau} = a + b\left[\int_{\lambda_1}^{\lambda_2} E\tau_g R\lambda \, d\lambda \Big/ \int_{\lambda_1}^{\lambda_2} E\tau_g R \, d\lambda\right]$$

There can then be found a wavelength, λ_e, such that

$$\lambda_e = \int_{\lambda_1}^{\lambda_2} E\tau_g R\lambda \, d\lambda \Big/ \int_{\lambda_1}^{\lambda_2} E\tau_g R \, d\lambda$$

for all possible linear curves, independent of the sign or value of the slope. In the use of this effective wavelength of the slit, there are only small errors for typical curves of colored samples, but rather large errors at points of strong curvature such as sharp maxima and minima. It should also be pointed out that since E, τ_g, and R are generally not constant, the effective wavelength will not be the midpoint between λ_1 and λ_2.

This method avoids the errors caused by slit parameters. Slit width and shape for a symmetrical slit distribution do not affect the photometric values of linear curves. Slit asymmetry is simply ignored by the way in which the wavelength position is defined. The method yields the position of the slit which gives the same value for the transmittance of a linear absorptance curve as is obtained with monochromatic light.

The application of this analysis is as follows. One obtains a line source such as a mercury arc which may be placed directly in the lamp house of the spectrophotometer. At the wavelengths of emission of this source, the transmittance of a filter with a steep positive or negative slope is measured. One then finds the wavelength position that yields the same transmittance value with the same filter, but with the continuous source in place. To complete the analysis, this wavelength position is compared with the wavelength of emission of the spectrum line.

Since the measurements with the line source and the continuous source are both done in the same spectrophotometer, there is no problem of dependence of the measurements on the calibration of a reference instrument. The uncertainty therefore depends on the slope of the curve at the calibration point and on the photometric accuracy of the instrument. For a curve with a slope of 1.0% per nanometer the uncertainty is 0.1 nm if the photometric scale is accurate to 0.1%. This represents a great improvement over the methods previously described. Sufficient care in the selection of the filters should eliminate any problems of temperature or filter-position dependence.

The greatest disadvantage is that the method holds only for linear curves. In other words, we have traded slit dependence for curvature dependence. This means that this method may be applied with a minimum of error to samples with

only gradual curvature. It is less satisfactory for analytical spectrophotometry which requires greater accuracy at peak positions, since the effective wavelength of the band for a linear absorptance is not necessarily the same as the effective wavelength of the band for a peak absorptance.

Interference Filters

Heidt and Bosley *(23)* reported a method of wavelength calibration employing an interference filter. The filter consists of two plates of optically flat glass which have a thin layer of air between them. The transparent glass is at least ¼ inch thick to keep distortion to a minimum, and the inside faces of the plates are half-silvered or half-aluminized to increase the intensity of the interference. To keep the spacing between the plates equal at all points, a soft metal foil (such as gold leaf) is used as a spacer. The filter assembly is illustrated in Fig. 7.

The thickness of the foil is selected so that there are several orders of interference fringes across the part of the spectrum to be calibrated. Let the thickness of the air

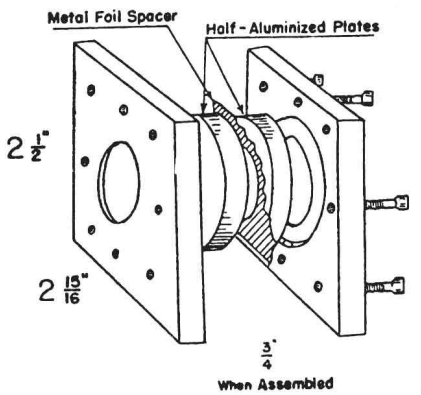

Fig. 7. Interference-filter assembly [after Heidt and Bolsey *(23)*].

space be d; then the positions of the calibration points are given by:

$$\tau_N = 2d/N$$

where N is an integer for absorptance maxima and is an integer plus one-half for absorptance minima. The value of d can be selected to give many calibration points across the visible spectrum. Fig. 8 gives curves for two such interference filters run on a General Electric-Hardy spectrophotometer with 10-nm slits. In the upper curve, the cycle of the calibrator equals the slit width, so that the photometric error has completely eliminated the maxima and minima in the 400 nm wavelength region. Therefore, the slit width of the instrument places an additional restraint on the selection of the thickness. For the General Electric instrument a thickness equal to 8 μm was found to be satisfactory.

Once the interference filter is prepared, it is calibrated by the comparison of the positions of its maxima and minima with the positions of line spectra run on the same instrument. To complete the calibration one simply compares the wavelength positions of the maxima and minima with their calibrated values. The only major uncertainty in the method arises from the location of the calibration points, since the

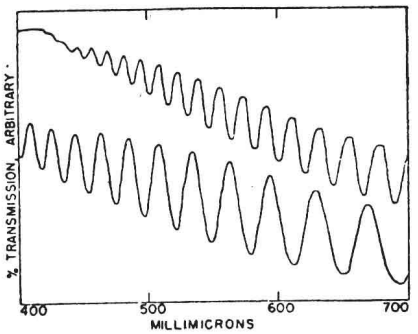

Fig. 8. Transmittance of two interference filters as measured on a General Electric-Hardy spectrophotometer [after Heidt and Bolsey *(23)*].

curve does not change rapidly at maxima and minima. The authors compared their calibration method with the use of line spectra on a Cary 14 spectrophotometer, with very narrow slits, and found no large differences in the ultraviolet or visible wavelength ranges.

Slit parameters will affect this method slightly. The limitation on the thickness that slit width causes has already been pointed out. Since the maxima and minima are nearly symmetrical, the positions of these calibration points are not affected by slit width or shape as long as the slit distribution is symmetrical. Asymmetrical slits, on the other hand, shift the positions of the maxima and minima.

The method is dependent on temperature if it affects the filter thickness d by affecting the mechanical parts of the assembly. This is probably a small effect. The position of the filter may be somewhat more critical. If the filter is not perpendicular to the beam, the path length is increased and the wavelength positions of the calibration points are systematically shifted. Due to the difficulties of fabrication and calibration inherent in this method, it has not been popular for wavelength calibration.

Retardation Plates

Buc and Stearns *(1, 24)* suggested a method of wavelength calibration employing a retardation plate. They used a calibrator consisting of a Nicol prism polarizer, a retardation plate, and a Nicol prism analyzer to provide a series of known points for wavelength calibration. (Spectrophotometers utilizing the Martens photometer, in which a polarized beam is already generated, do not require the Nicol prisms; the General Electric-Hardy instrument is of this type.) A retardation plate is a transparent plate of a birefringent material such as quartz. When plane-polarized light from the first Nicol prism falls on the retardation plate, it is split into two orthogonal plane-polarized beams. Since the birefringent material has a different refractive index for these two beams, the light from each beam does not reach the rear surface of the plate at the same time. The slower beam is retarded with respect to the faster beam. If the slower beam arrives at the rear surface an integral number of wavelengths behind the faster beam, the two will be in phase and the calibrator will show a transmission maximum. That is, it behaves as a full-wave plate. If the two beams arrive at the rear surface a half wave out of phase, then the plate is a half-wave plate and a transmission minimum results. If the two beams are one fourth or three fourths of a wave out of phase, an average transmission point occurs. In most spectrophotometers the average transmission points are at 25.0 percent transmittance. On instruments such as the General Electric-Hardy which use Martens photometers, the average transmittance is 17.1 percent.

The birefringence (extraordinary refractive index minus the ordinary refractive index) varies with wavelength. Therefore as the wavelength region is scanned a series of maxima, minima and average transmission points is plotted. A typical plot of a retardation plate calibrator for a General Electric-Hardy instrument is shown in Fig. 9. The major advantage of the retardation-plate method is that the positions of the calibration points may be calculated directly from the properties of the calibrator. Thus it is not an empirical method. All that is needed is the temperature, the birefringence, and the thickness of the retardation plate. The temperature in the sample compartment is easily measured. The thickness is found by interferometry, and the birefringence of quartz can be found in the *International Critical Tables (25)* or other similar compilations. A short computer program can then be written to yield the exact locations of the calibration points.

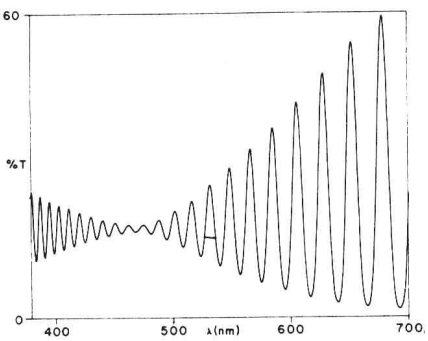

Fig. 9. Transmittance of a quartz retardation plate 1.7364 mm thick measured on a General Electric-Hardy (new model) spectrophotometer.

To apply these theoretical calibration points it is necessary to make an assumption as to the proper definition of the position of the slit. Buc and Stearns chose to follow Van den Akker's definition of the effective wavelength. That is, the wavelength position of the slit is the wavelength which gives the same photometric values as are obtained with monochromatic light for all linear curves. In this method, the calculation has replaced Van den Akker's line spectra and the retardation plate has replaced the linear absorption filters. Since the curve approximates a straight line when it passes through the average transmission points, these are the best positions for wavelength calibration. To complete the calibration one simply compares these average transmission wavelengths with the calculated values of wavelength.

The original authors also used retardation plates to study the effect of the width and shape of the slit function on measured photometric values. They were able to calculate the true transmission curves for their calibrators and compare them with the measured transmission curves obtained

with an assumed effective slit distribution. That distribution was trapezoidal and symmetrical, of arbitrary width and shape. They allowed the slit width, H, to vary and were able to see its effect on photometric values. They also introduced a shape factor, f, which they defined as the width at maximum transmittance divided by the slit width. By varying the shape factor between zero (triangular) and one (rectangular), the effect of shape on photometric values was observed.

Buc and Stearns were then able to construct calibration curves which allow one to determine the slit width and the shape of the slit distribution, as long as it may be assumed to be symmetrical. Since two quantities are to be determined, it takes more than one measurement to provide the data for the determination. Also the determination must be done for one wavelength area at a time. The photometric values of absorptance minima are found for three different retardation plates and the four combinations of these same retardation plates taken more than one at a time. The photometric values are then plotted against the thickness of the retarders in the beam. Data from this curve plus the calibration curves are sufficient to define the width and shape of the slit function.

The wavelength positions of the calibration points are not dependent on slit width or distribution shape for symmetrical slits, since the average transmission points are in straight-line areas of the curve. Asymmetry, however, will affect the calibration points. To determine how great an effect this may be, the authors assumed an extreme case of an asymmetrical slit distribution (Fig. 6) and calculated its effect on the position of an average transmission point. They found that the difference between the wavelength positions of an average transmission point for the assumed asymmetric slit distribution and for a triangular symmetric distribution of equal width and having its centroid at the same wavelength is less than 1 percent of the slit width if the transmission of the absorptance maximum is less than 26 percent of the average transmission. A different condition applies to Martens-photometer instruments. This condition can be met by properly selecting the thickness of the retardation plate. The end result is that for any slit distribution the wavelength position of the slit can be determined within 1 percent of its width. For a slit of 10 nm width this would give an uncertainty of less than 0.1 nm.

In summary, this method does not depend on the width or shape of the slit distribution for symmetrical slit functions, and the effect of asymmetrical slit functions can be minimized. However, there are several disadvantages to the method. The primary one is that quartz retardation plates and Nicol prisms are expensive. The plate must be perpendicular to the beam; and if it is to be used for slit width and shape calibration, its angular position is also critical. The calibration is temperature dependent through the change in the refractive indices of quartz with temperature. This is not a large effect, however, and only requires that the temperature of the instrument be held near the temperature for which the calibration points were calculated. The complexity of the method is probably its greatest disadvantage for most workers.

CONCLUSIONS

Of the several methods of wavelength calibration discussed here, none can be selected as the best possible method for all situations. Each must be considered in relation to the particular instrument being used, the desired accuracy of the wavelength calibration, and the types of samples to be measured. It should be remembered that no method is absolutely correct under all possible circumstances, and that different calibration methods will give different results.

However, certain methods are generally more suitable than others for a given set of circumstances. For analytical or theoretical spectrophotometry, the accuracy of the wavelength positions of sharp absorptances is very important. Instruments for these purposes generally have narrow slit widths. Under these circumstances, the line-spectra method is the most useful. The interference-filter method may also prove useful for some applications of this type of spectrophotometry (and indeed it is widely practiced using solid films instead of air spacings between glass in the infrared spectral region). For instruments with wide slits and in cases where the samples do not exhibit great curvature, the methods of Van den Akker or Buc and Stearns may be used. For this type of work, these methods offer a highly accurate and consistent method of calibration at the expense of somewhat greater difficulty in application. In situations where extreme accuracy is not important, the absorbing filter methods may be used. They offer a rapid, inexpensive and simple analysis at the sacrifice of accuracy.

Under special circumstances, certain characteristics of the various methods other than accuracy and bandpass limitations become important. For example, in a recording spectrophotometer it is often advantageous to be able to record the instrument wavelength calibration as a curve superimposed on a sample curve. This requires that the calibration method be capable of being run at any time without change in instrument set-up. The absorbing filter, interference, and retardation plate methods allow this, while the line spectra and line spectra plus absorbing filter methods do not. Similarly, if the calibration is to be repeated frequently these same methods are much quicker than methods requiring instrument changes. Particularly in the field of spectrophotometric color measurement, one is as concerned with the precision of measurement as with its accuracy. The ability to follow the day-to-day variation of the wavelength calibration of these instruments is an important advantage of methods which can be applied by simple insertion of a calibrator into the beam of the instrument. In non-recording spectrophotometers and in cases where small fluctuations in the wavelength scale are of little concern, this difference in the methods is not so important.

The interference filter and retardation plate methods have an additional unique advantage. Both of these methods allow the experimenter to tailor the calibration to his specific needs. That is, the wavelengths of the calibration can be controlled and the calibrator can be selected to give results compatible with the slit width employed in the instrument. Thus, if one wants a calibration at 617 nm for a bandpass of 8 nm, a calibrator can be designed to meet that specification. With the line spectra, absorbing filters, and line spectra plus absorbing filters methods, one is limited by the available spectrum lines and filters.

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LXXVII. Instrumentation for Fluorescence and Phosphorescence

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Molecules and atoms can absorb ultraviolet and visible electromagnetic radiation and be raised to excited electronic states. The reverse process, transition from an excited electronic state to the ground electronic state with emission of radiation, is called luminescence. Sir G. G. Stokes identified the phenomenon of fluorescence in 1852 *(1)*. In the past several years there have been many advances in luminescence instrumentation, and work on the application of the corresponding methods to chemical systems continues to increase. This article is concerned with molecular photoluminescence, wherein photons of electromagnetic radiation excite molecules, and includes coverage of modern instrumentation. Bioluminescence, chemiluminescence, electroluminescence, atomic fluorescence, and ancillary instrumentation will not be considered.

Theory

Figure 1 shows some molecular electronic energy levels with superimposed vibrational states. Absorption originates from a low vibrational level of the ground electronic state for a solute molecule surrounded by solvent molecules. Absorption terminates when the solute molecule arrives in any one of several possible vibrational levels in an excited electronic state that is still surrounded by the ground state equilibrium of the solvent molecules. Within 10^{-12} seconds, the electronically excited solute molecule relaxes to the lowest vibrational level of the lowest excited singlet state; and the solvent molecules reorient themselves to a state of equilibrium compatible with the new molecular polarity. The vibrational and solvent relaxation processes are accompanied by a loss of thermal energy. Emission can then occur from the lowest vibrational level of the lowest excited singlet state, dropping the solute molecule into one of a number of possible vibrational levels of the ground electronic state, still surrounded by the excited ground state equilibrium for the solvent molecules. This emission of electromagnetic radiation by the solute molecules is called *fluorescence* and almost always occurs from the lowest vibrational level of the lowest excited singlet level in a molecule. According to the Pauli exclusion principle, spins of two electrons in the same orbital are opposite to one another (paired). A molecule with an even number of electrons has all electrons paired and is said to be in a singlet state. Whether the molecule is in the ground state or excited state, as long as the electrons are paired, the molecule is in a singlet state *(2,3)*.

The decay time of fluorescence is of the same order of magnitude as the lifetime of an excited singlet state, 10^{-9} to 10^{-7} seconds. Based on spectroscopic selection rules, the transition from an excited singlet to a singlet ground state is spin-allowed and thus highly probable. After fluorescence emission, vibrational relaxation and solvent reorientations occur and the molecule finally arrives in a ground state equilibrium configuration *(3)*. As Figure 1 shows, other processes can compete with fluorescence and the excited molecule can lose energy by other mechanistic paths such as internal conversion and intersystem crossing. Internal conversion is a radiationless process whereby a molecule passes from a higher to a lower electronic state without emission of a photon. This process is not well understood; it occurs by direct vibrational coupling between electronic states and by quantum mechanical tunneling *(3)*. When a radia-

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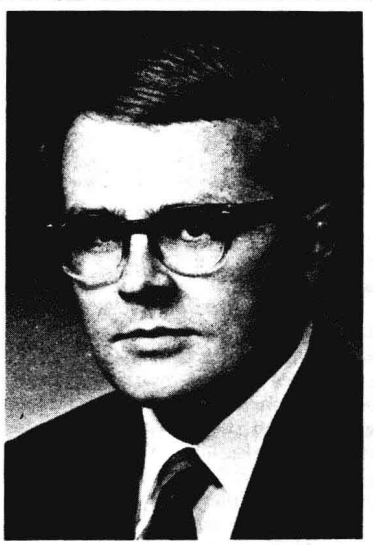

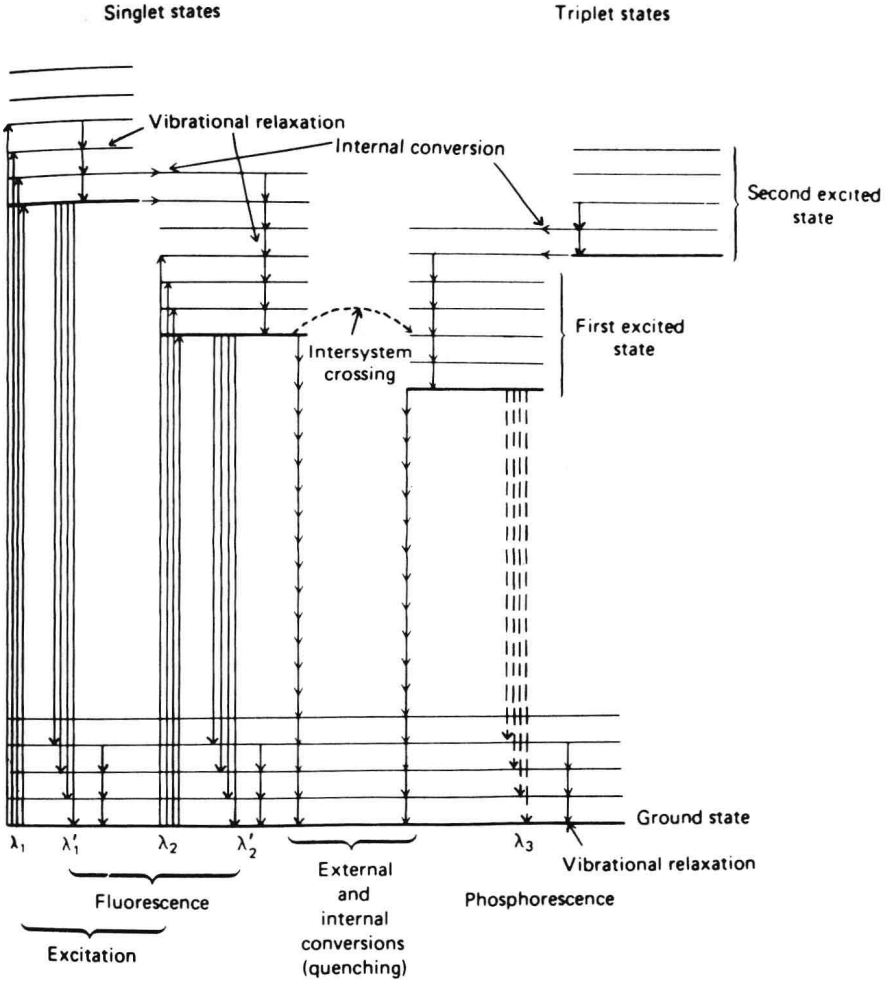

Figure 1. Partial energy level diagram for a photoluminescent system. (Holt, Rinehart and Winston, Inc., *(2)*.)

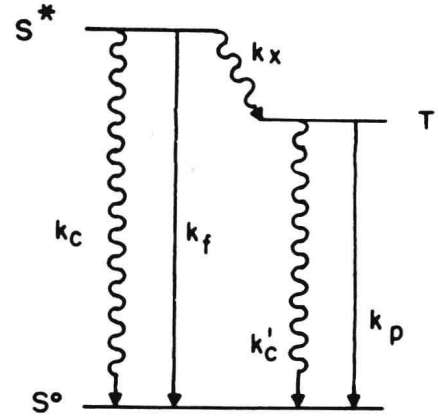

Figure 2. Summary of rate constants for excited state processes: S_0, ground state; S^*, lowest excited singlet state; T, lowest triplet state; k_c, internal conversion for lowest excited singlet state; k_c', internal conversion between lowest triplet state and ground state; k_p, phosphorescence; k_x intersystem crossing. Wavy lines indicate nonradiative transitions. (Taken from Ref. *(6)*; Wiley-Interscience.)

tionless transfer occurs between the first excited singlet state to the lowest excited triplet state, it is known as intersystem crossing (Figure 1). The mechanism for this process involves vibrational coupling between the excited singlet and triplet states. (The multiplicity of a spectroscopic state is given by the equation: $M = 2S + 1$, where S is the vector sum of the spin quantum number s of the unpaired electrons. Hence, a triplet state is one in which all the electrons in the molecule are paired except two, or $S = 1$. In a singlet state $S = 0$; all electrons are paired). If intersystem crossing is favored over fluorescence or internal conversion to the ground state, the molecule can pass from the lowest singlet state to a triplet state. Then the molecule will undergo a vibrational relaxation and solvent reorientation to arrive at the lowest vibrational level of the lowest excited triplet state. From this state, electromagnetic radiation can be emitted, or internal conversion can occur. If a radiative transition occurs, it is called *phosphorescence*. Since phosphorescence originates from the lowest triplet state, its decay time is similar to the lifetime of the triplet state, 10^{-4} to 10 seconds. The transition from a triplet excited state to a singlet ground state is a forbidden transition as it involves a change in spin; a transition of this type is highly improbable. This fact gives a triplet electronic state its long lifetime. The long lifetime greatly increases the probability of collisional transfer of energy with solvent molecules which is very efficient in solution at room temperature and is often the main pathway for loss of triplet state excitation energy. Because of this, phosphorescence is rarely observed at room temperature but can be easily observed by dissolving the solute in a solvent that freezes to form a rigid glass at the temperature of liquid nitrogen (77°K). EPA, consisting of a mixture of ethyl ether, isopentane and ethyl alcohol in a ratio of 5:5:2 can be used as this rigid glass medium. Other solvents that have been used are described by Guilbault *(4)*.

The efficiency of luminescence can be discussed in terms of the quantum yield, which is the ratio of the number of molecules that undergo luminescence to the total number of excited molecules. The maximum value of the quantum yield is one; it can be related to rate constants for excited state processes. The quantum yield for fluorescence is given by

$$\phi_f = \frac{k_f}{k_f + k_c + k_x}$$

If k_f is much larger than k_c and k_x, then the fluorescence quantum yield will be high approaching unity. Figure 2 summarizes these rate constants. The quantum yield for phosphorescence depends upon other competitive process and is given by

$$\phi_p = \frac{k_p}{k_p + k_c'} \times \frac{k_x}{k_f + k_c + k_x}$$

In the absence of photochemical reactions such as self-decomposition, the equation $\phi_f + \phi_p = 1$ is valid showing the complementary nature of fluorescence and phosphorescence *(5,6)*.

Because the molecules relax to lower vibrational levels in the excited state and because of the solvent reorientation in the excited state and ground state, the electromagnetic radiation corresponding to fluorescence is of lower energy than the exciting radiation, and therefore appears at longer wavelengths. Phosphorescence is influenced by vibrational relaxation and solvent reorientations in the excited singlet state, the triplet state, and the ground state, as well as intersystem crossing. Accordingly, the electromagnetic energy corresponding to phosphorescence is of still lower energy than fluorescence and will appear at longer wavelengths.

For analytical work, such as determining the concentration of a fluorescing species in a sample, the quantitative relationship between the total fluorescence intensity and concentration is given below *(7)*.

$$F = I_0(1 - 10^{-\epsilon bc})\phi_F \qquad (1)$$

where

F = total fluorescence intensity, quanta per second
I_0 = intensity of exciting light, quanta per second
c = concentration of solution
b = cell length
ϵ = molar absorptivity
ϕ_F = quantum efficiency of fluorescence

Equation 1 can be written in the following form *(8)*:

$$F = I_0\left[2.3\,\epsilon bc - \frac{(2.3\,\epsilon bc)^2}{2} + \frac{(2.3\,\epsilon bc)^3}{3!} - \cdots \frac{(-2.3\,\epsilon bc)^n}{n!}\right]\phi_F \qquad (2)$$

When ϵbc is less than about 0.05, equation 2 simplifies to

$$F = I_0(2.3\,\epsilon bc)\,\phi_F \qquad (3)$$

A similar expression can be written for phosphorescence *(9)*:

$$P = I_0(2.3\epsilon bc)\phi_p \qquad (4)$$

It is evident from Equations 3 and 4 that the amount of fluorescence or phosphorescence is directly proportional to concentration in dilute solutions. At high concentrations, this proportionality no longer holds. Because the luminescence emission becomes dependent upon the higher terms in the power series, the "inner filter" effect has to be considered. This effect occurs at high concentrations because the solution in the back part of the sample cell does not receive the same intensity of excitation energy as the front part of the sample cell since the intervening solution has acted as an "inner filter" by absorbing most of the exciting light. The solution as a whole is not uniformly excited and there will be a reduction in luminescence emission. In addition, the luminescent light may also be absorbed by another compound in the solution. Furthermore, if the excitation and emission spectra of a sample overlap, then the luminescence emission can be absorbed by the sample itself. Thus non-uniform excitation of the sample and absorption of emitted radiation by the sample causes a decrease in luminescence emission. The linear range of the relative phosphorescence intensity extends farther than for relative fluorescence intensity. This occurs mainly for three reasons: (1) at 77° K the probability of collisional deactivation is greatly reduced, (2) self-absorption is usually reduced because the phosphorescence emission spectrum is farther removed from the excitation spectrum as compared to fluorescence, and (3) the sample cell used for phosphorimetry is small, approximately 0.10 cm in diameter. The small diameter allows for more uniform excitation of the sample at higher concentrations.

Phosphorimetry in many cases is more sensitive than fluorometry. Equations 3 and 4 show that the luminescence intensity will increase proportionately with the intensity of exciting light. In phosphorimetry, emission is not observed during excitation because a rotating shutter phosphoroscope is used which allows periodic excitation of the sample and periodic out-of-phase measurement of phosphorescence (Figure 3). Because of this, it is possible to excite molecules with a more intense excitation source. Also, by use of the phosphoroscope, phosphorescence emission is measured in the absence of fluorescence and scattered incident light, making it easier to amplify the signal as the comparison is done against a "black" background. In fluorometry, also, slits are generally used to minimize scattered light and thus the intensity of exciting light is decreased.

Both fluorometry and phosphorimetry are very sensitive as well as moderately selective. The selectivity of both is illustrated by excitation resolution and emission resolution. In excitation resolution, the excitation wavelength is varied to selectively excite different molecules in a mixture. In emission resolution, the emission wavelength is varied to preferentially measure the emission from different molecules in a mixture. In addition, with phosphorimetry, phosphoroscopic resolution and time-resolved phosphorimetry may be carried out. By measuring the emission from a mixture of two phosphorescent compounds after termination of exciting radiation, and at a time sufficient for the fast decaying species to disappear, the phosphorescence of the slow decaying species may be measured. This is called phosphoroscopic resolution because the phosphoroscope speed is adjusted in order to measure the slower decaying species. In time-resolved phosphorimetry, the concentration of two similar phosphorescent compounds is determined by recording the logarithm of the phosphorescence signal from a mixture versus time after termination of excitation. From this recording, the individual phosphorescence from both compounds can be determined and related to concentration *(4)*.

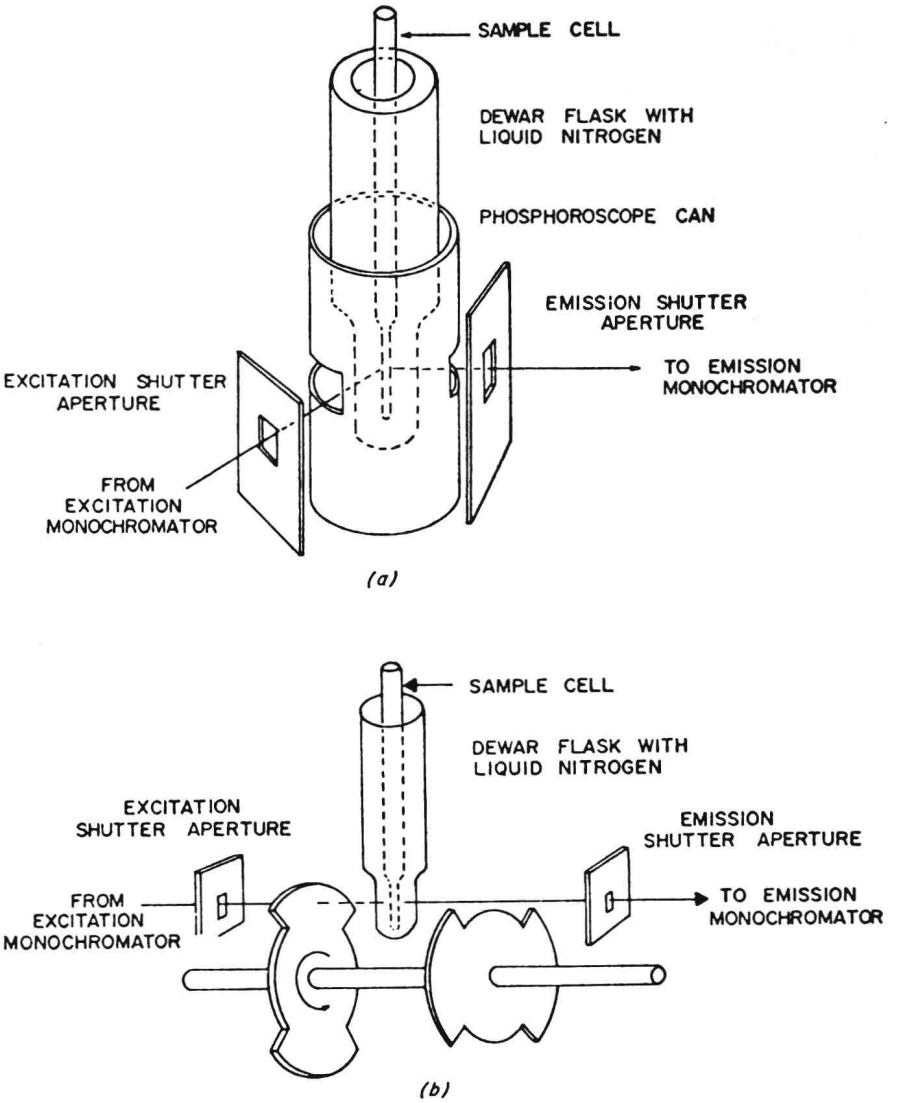

Figure 3. Schematic representation of phosphoroscope interaction with radiation: (a) rotating can device (b) Becquerel-type device. (Analytical Chemistry *(23)*.)

Fundamental Instrumentation

The instrumentation required to produce and measure fluorescence comprises a light source, a means of producing a restricted band of radiation of just the right frequency to excite the sample, and a second similar monochromatic device that allows only the emitted fluorescent radiation to pass to a detector. This is indicated in Figure 4. As excitation of molecules to higher electronic states take place in the ultraviolet region, the usual light sources for fluorometry must be rich in ultraviolet light; mercury lamps or xenon arcs are appropriate. The desired restricted band of radiation is obtained either by means of filters or monochromators. The detector can be a photocell or photomultiplier and the readout may appear directly on a meter dial, a recorder, or an oscilloscope.

In terms of "building blocks," the equipment for fluorometry is quite similar

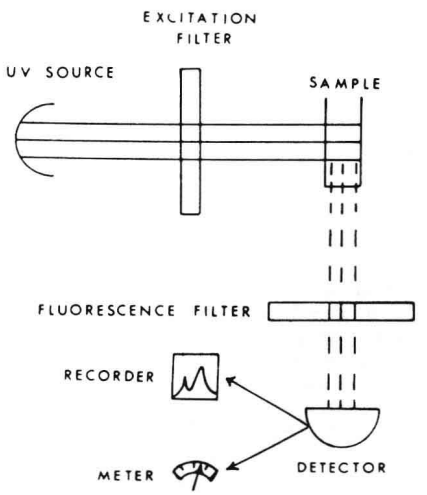

Figure 4. Block diagram of the components of a typical fluorometer.

to that employed for spectrophotometry. Most commercial equipment for fluorometry is designed by the manufacturer to use the same components such as filters, photocells, photomultipliers, monochromators, amplifiers, etc., that he employs in his spectrophotometers or colorimeters. Because these basic units have been described and reviewed in this series such components will not be described in this article.

The great sensitivity of luminescence analysis comes about because the sensitivity is proportional not only to the concentration of the sample and the length of the light path, but also to the intensity of the light source and the sensitivity of the photometer. Even though much more intense light sources are used in luminescence analysis, the major increase in sensitivity comes about because the detector is directly measuring the luminescent radiation, which can be amplified much more readily than is the case for the difference or ratio between the incident and transmitted radiation that is involved in spectrophotometry.

Quantitative measurements are made in the conventional manner, for example, by preparing a calibration curve (intensity of fluorescence vs. concentration) or by the method of standard addition. Once the right wavelengths for the excitation and emission are known, filter instruments serve quite well for fluorometry. Using the same light source and detector, filter instruments may show a greater sensitivity than comparable instruments employing monochromators, if there is greater light intensity available for excitation. The filter instruments are generally called fluorometers or fluorophotometers if they employ a photomultiplier detector. Instruments with continuously variable wavelengths for both excitation and emission, that is, instruments with two monochromators, are called spectrofluorophotometers or fluorescence spectrometers. A tabular comparison of the filter fluorometers and fluorescence spectrometers is provided at the end of the article.

In using the fluorescence spectrometer, the following procedure is commonly followed. If the sample shows fluorescence in the visible region (and the instrument is so designed that it can be observed visually) the excitation monochromator is varied until fluorescence occurs. The excitation monochromator is then set at any point within the excitation wavelength band and the emission monochromator is allowed to scan, recording the emission spectrum. The emission monochromator is then set at the wavelength at which maximum fluorescence occurred, and the excitation monochromator is now allowed to scan and the excitation spectrum is recorded. In turn, the final emission spectrum is obtained by setting the excitation monochromator at the maximum excitation wavelength and again scanning with the emission monochromator. Should it be impossible to observe the sample fluorescence visually, one can observe the maximum deflection of a photometer, by manually varying the excitation and emission monochromators. Also, one can obtain the conventional absorption spectrum of the compound by scanning the excitation monochromator through the absorption wavelengths with the emission monochromator set at the maximum wavelength of the emission spectrum. In theory, the excitation and absorption spectra should be identical. However, the excitation and absorption spectra usually are not exactly superimposable because of the non-constant intensity of the source and response of the phototube as the wavelength is varied. By spectrofluorometric means, excitation spectra of solutions 10^{-3} more dilute (compared to normal spectrophotometers) may be measured if the compound shows appreciable fluorescence.

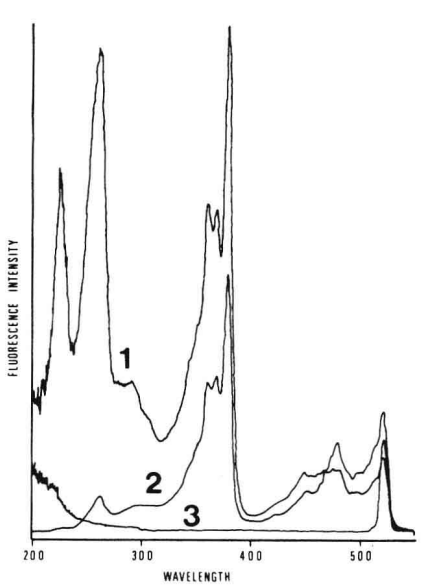

Figure 5. A. Room temperature Spectra of Naptho[2,3-d]-2-selena-1,3-diazole in cyclohexane measured on a Farrand MK-1-spectrofluorometer. 1) Corrected excitation spectrum; 2) Uncorrected excitation spectrum; 3) Corrected excitation spectrum of solvent blank: Conditions: emission 520 nm; 2 nm exciter bandpass; 5 nm analyzer bandpass; 3-73 filter in sample chamber: Note the substantial increase in sensitivity in the lower UV range for the corrected spectrum as well as the reversal in relative peak heights at 360 and 368 nm in the corrected and uncorrected spectra.

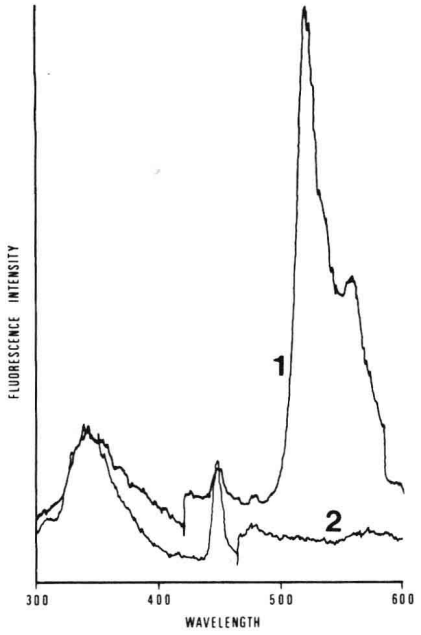

Figure 5. B. Emission spectra. 1) Naphtho[2,3-d]selena-diazole with 225 nm excitation under identical conditions as Figure 5A, except that the filter was removed; 2) Solvent blank

Typical excitation and fluorescence spectra, measured with a Farrand spectrofluorophotometer, are shown in Figure 5. Because instruments of this type generally measure the characteristics of the sample without a continuous comparison to a reference standard, the instrument has the same major drawbacks which appear in attempting to record absorption spectra with a single beam instrument *(10)*. Thus, if the light source is unstable and varies in intensity, it can cause the appearance of false peaks in the spectrum. Equally as serious as the fluctuations in the light source is the variation of the sensitivity of the phototube with regard to wavelength, as shown in Figure 6. For example, in recording the fluorescence spectrum with an R212 photomultiplier tube, if the sample actually has a strong fluorescence emission at 500 nm and a less intense emission at 400 nm, the 500 nm emission could appear to be the weaker one because the tube shows a 50% higher output for 400 nm radiation. In addition, the light source for the excitation must show considerable intensity at the desired excitation wavelength. Quinine shows fluorescence with excitation at a strong resonance line in the mercury spectrum. Accordingly, a fluorometer using a mercury source may show considerable sensitivity for this material, but less sensitivity for a more strongly fluorescent compound which is excited more effectively with another excitation line. This caution must be considered in selection of the light source for fluorescence measurements. Xenon lamp sources tend to show a linear intensity throughout their spectral range and are preferred for use with monochromator instruments. Mercury sources or special "fluorescent lamps" are commonly used in filter instruments. The light output of a xenon and mercury lamp are compared in Figure 7.

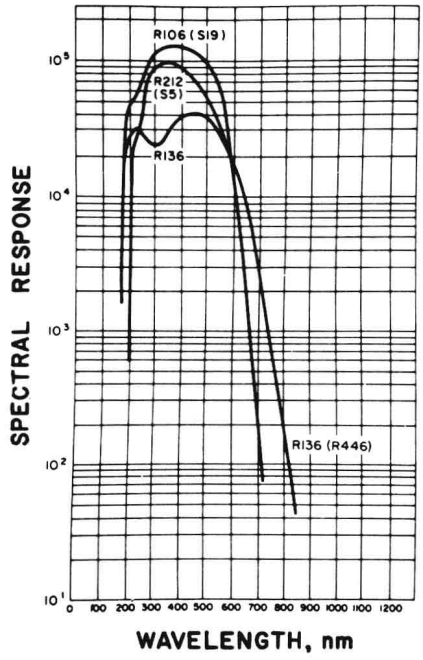

Figure 6. Photomultiplier response curves. (Courtesy of Perkin-Elmer Corp.).

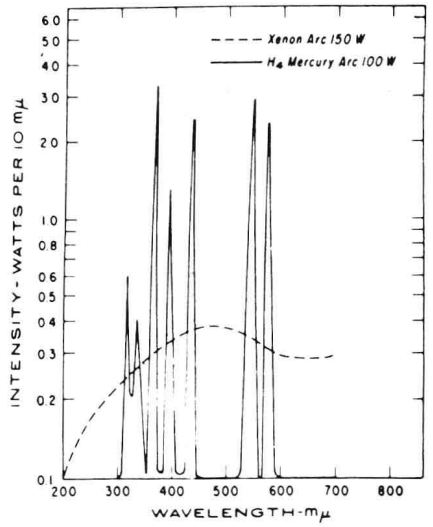

Figure 7. Spectral characteristics of the 150-watt xenon arc and Pyrex-jacketed 100-watt H-4 mercury lamp. (J. Chem. Educ. *(11).*)

Commercial Instruments

Commercial spectrofluorophotometers which allow the scanning of excitation and emission spectra, and filter fluorometers have been previously described in this series *(11)*. Certain of these instruments are still offered commercially, and some models have been improved while keeping their same basic design. Accordingly, only two conventional spectrofluorophotometers and one filter fluorometer are described in this article. Phosphorescence accessories and pulse instrumentation are also described.

Fluorescence Instrumentation

The spectrofluorophotometers offered by the Perkin-Elmer Corporation are representative respectively of the conventional simple spectrofluorophotometers and more complex instrumentation required to correct for non-constant source intensity and non-constant detector response throughout the ultraviolet-visible spectral region.

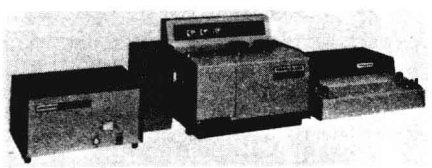

Figure 8. Perkin-Elmer Model 204 Fluorescence Spectrophotometer.

An example of the first type is the Model 204 Fluorescence Spectrophotometer (shown in Figure 8) whose functional layout is shown in Figure 9. The system is designed to provide qualitative spectral emission and excitation data as well as quantitative fluorescence measurements with good sensitivity, stability and convenience. It consists of a 150-watt xenon source, grating monochromators for both excitation and emission, with a sample compartment in between, a photomultiplier detector to provide the signal for a 5-in. meter readout or a recorder. The bandpass of both monochromators is fixed at 10 nm. The sample compartment accommodates a holder for four standard cells. A number

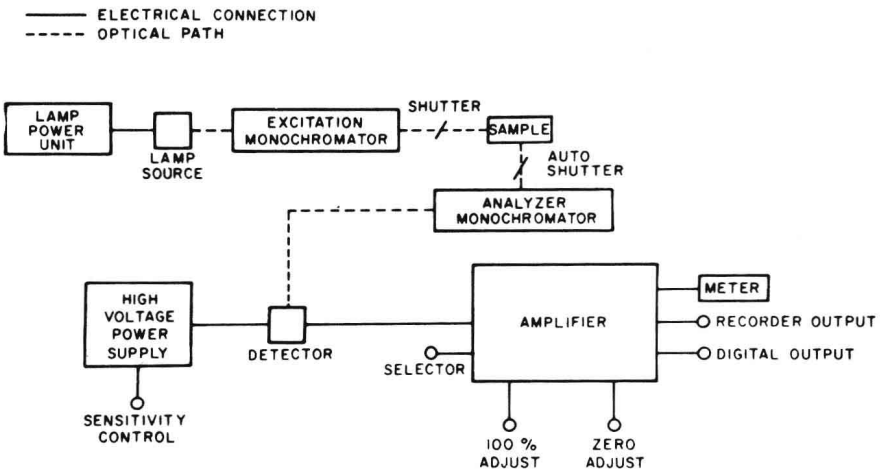

Figure 9. Functional layout of the Perkin-Elmer Model 204 Fluorescence Spectrophotometer.

of accessories including micro cells, flow cells, temperature control and test tube holder, and Technicon interface accessories are optionally available.

The Model MPF-4 fluorescence spectrophotometer is pictured in Figure 10. Figure 11 shows an optical diagram for the Model MPF-3 fluorescence spectrophotometer. An earlier instrument in the same MPF series; the MPF-4 instrument employs essentially the same Rhodamine B type of spectral correction system. As can be seen in Figure 11, the instrument employs two grating monochromators for excitation and emission, respectively. A portion of the radiation from the xenon lamp is reflected by a beam splitter onto a detector photocell. The source light then illuminates the sample (in the sample compartment) and the emitted fluorescent radiation is detect-

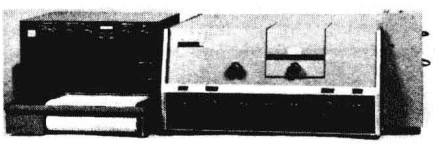

Figure 10. Perkin-Elmer MPF-4 Fluorescence Spectrophotometer.

ed in turn by a second detector photocell. A difference amplifier measures the ratio of the difference in intensity between the sample and the reference circuits. Correction for the excitation spectrum is made by recording the ratio of the sample detector output signal to that of a reference detector which monitors the emission of a quantum counter. The quantum counter consists of a concentrated solution of Rhodamine B in a triangular shaped cell. The quantum counter will absorb all the incident radiation and convert it into a proportional number of emitted quanta of fluorescent radiation. Rhodamine B is used as a quantum counter because it has the property of maintaining a constant ratio of quanta absorbed from 200 to 600 nm to quanta emitted at 630 nm. The fluorescent radiation from Rhodamine B is passed through a 630 nm filter, onto a detector. The detected signal is fed to the reference amplifier, and the sample fluorescence is directed through the emission monochromator to the sample photomultiplier. The amplified sample signal is electronically ratioed to the reference signal and recorded. A 25-tap pre-programmed potentiometer is coupled to the wavelength drive of the excitation monochromator to alter the sample amplifier gain to compensate for the non-constant reflectance of the beam splitter and for energy changes due to polarization from the monochromator. When the excitation system is properly adjusted, the excitation spectrum of a sample of Rhodamine B run against the Rhodamine B reference cell will give a response of relative intensity versus wavelength flat to within ±5%.

For correction of emission spectra, the emission monochromator is treated separately. A flat response curve for the detector output is obtained by preadjustment of another 25-tap potentiometer coupled to the emission monochromator. One way to preadjust the emission system is to use a tungsten filament lamp that has been calibrated against a National Bureau of Standards spectral radiance reference lamp. The output of the lamp is measured directly through the emission monochromator. The 25-tap potentiometer coupled to the emission monochromator is adjusted to match the calibrated values of the tungsten lamp. Another way to preadjust the emission system is to obtain the spectrum of the xenon source by scanning the excitation monochromator with a concentrated solution of Rhodamine B in the sample position. This gives the true relative intensity versus wavelength of the source emission because the Rhodamine B acts as a detector and emits fluorescent radiation of 630 nm which is directly proportional to the quantum intensity of exciting light. To adjust the corrected emission system, both the excitation and emission monochromators are scanned synchronously using the Rhodamine B sample, and the trimming potentiometers of the emission corrector are adjusted so that the spectrum obtained is identical to that of the xenon source. At wavelengths beyond 600 nm, the corrected emission system is adjusted using a calibrated National Bureau of Standards lamp. The Model MPF-4 uses an AC optical and electrical system to provide high stability and automatic gain control which serves in conjunction with the Rhodamine B reference detector to automatically adjust dynode voltage to maintain constant amplifier gain regardless of the variations in source energy. This provides better pen response in the short wavelengths (200 to 240 nm) where source energy is relatively low, and