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ANALYTICAL CHEMISTRY

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Volume 35
Pages 2166-72, December 1963

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▶ Techniques are described for evaluating the spectral sensitivity of the emission monochromator-phototube system and the spectral energy distribution of the excitation source–monochromator system of an Aminco-Kiers Spectrophosphorimeter, with fluorescence attachment. The treatment and use of sensitivity data for the correction of recorded spectra to units of relative energy or quanta are discussed. For this purpose, a computer program was written to correct the spectra and provide a print-out of the spectrum in units of relative quanta per unit frequency. Provision was made in the computer program for simultaneous integration of the corrected emission spectrum for use in calculation of quantum efficiencies. The quantum efficiencies for several compounds were determined using a comparative method making use of quinine sulfate as the reference standard.

APPLICATION OF LUMINESCENCE TECHNIQUES to analytical, physical, and organic chemical problems has increased recently, particularly now that commercial instruments are being used. The correction of emission spectra and the determination of quantum efficiencies are important in the study of photochemical reactions and of the mechanisms of fluorescence quenching and energy transfer in solution. An understanding of the origin of quenching is necessary when the phenomena of fluorescence or phosphorescence are used for quantitative analytical purposes. The measurement and comparison of quantum efficiencies for fluorescence *vs.* phosphorescence provide information concerning the nature of intersystem crossing (singlet-triplet transitions). Also, increased use of commercial apparatus will result in the publication of numerous luminescence spectra which should be corrected to be of greatest value to other workers.

Parker (*8*), in an excellent review, has recently discussed in detail the correction of fluorescence spectra and the measurement of fluorescence quantum efficiencies. He emphasized the calibration of the fluorescence monochromator-phototube combination, the correction of the emission spectra (to relative quanta per unit frequency interval *vs.* frequency), and the measurement of quantum efficiencies using reference compounds of known quantum efficiency. More recently, Parker (*7*) has discussed the calibration of a fluorescence monochromator in the ultraviolet region and has described the use of a fluorescent screen monitor for this purpose. The comparative method for measuring quantum efficiencies proposed by Bowen (*2, 3*) has been used with proper modification by Weber and Teale (*12*), and Melhuish (*4, 5*), as well as by Parker and Rees (*8*).

White, Ho, and Weimer (*13*) stressed the need to calibrate each instrument because sources, gratings, and phototubes perform differently from one spectrophotofluorometer to another. They describe methods for calibrating the Aminco-Bowman Spectrophotofluorometer. The emission monochromator was calibrated over the range 340 to 675 mμ using a previously calibrated Mazda projection bulb. The excitation monochromator (Hanovia xenon arc source) was calibrated by several methods including a chemical actinometer, a phototube of known response, and a thermopile. Although data have been published on the correction of fluorescence spectra, very little effort has been placed on the use of computers to reduce the tedium of such time consuming repetitive calculations.

In the present work, a relatively simple method for the calibration of an Aminco-Kiers Spectrophosphorimeter with fluorescence attachment is described. The emission monochromator (1-P-28 phototube) was calibrated over the entire region from about 220 mμ to 600 mμ using several sources of known spectral energy distribution. Using the calibrated emission monochromator, the spectral energy distribution curve for the excitation monochromator (Osram xenon arc source) was determined. A computer program was written to correct recorded spectra and provide a printout of the corrected spectrum in units of quanta per unit frequency interval *vs.* frequency. The computer program also provides for integration of the spectrum for use in calculating quantum efficiencies.

EXPERIMENTAL

Apparatus. An Aminco-Kiers Spectrophosphorimeter with fluorescence attachment was used throughout this study. The excitation monochromator contained an Osram xenon arc source and a 50- × 50-mm. grating (600 grooves per mm.) blazed for highest efficiency at 300 mμ in the first order. A 1P28 phototube was used with the emission monochromator which was identical to the excitation monochrometer except for a blaze wavelength of 500 mμ. Presentation of spectra from both monochromators was linear in wavelength. The spectral slit widths for these grating monochromators were essentially constant over the wavelength ranges studied.

Absorption spectra were determined with a Cary Model 14M spectrophotometer.

For calibration, a tungsten filament bulb operated at several different controlled voltages was used. Filament temperatures were measured with an optical pyrometer. Assuming black body radiation, the spectral energy distribution was calculated from Wien's law. For the ultraviolet region, a Hanovia quartz mercury-vapor arc lamp (lamp code SH) was uesd. The relative intensities of the numerous lines from this lamp were supplied by the manufacturer.

Spectra were recorded with a Mosely autograf drum-type X-Y recorder.

Corrections of the emission spectra were performed on an IBM 1620 computer.

Chemicals. Quinine sulfate (Matheson Coleman & Bell) was recrystallized twice from hot water acidified with sulfuric acid.

Anthracene (Eastman) was recrystallized from alcohol and the phenol (J. T. Baker, reagent grade), indole (Eastman), and Rhodamine B (Eastman) were used as supplied from the manufacturer.

Temperature was maintained at 25° ± 1° C. for all measurements. Assuming a temperature coefficient of emission intensity of 1 to 2%, variations introduced by temperature fluctuations probably did not exceed variations in instrument operation.

Calibration of Emission Monochromator (1-P-28 Phototube). The miniature tungsten filament lamp was placed inside the cell compartment so that the emission could pass directly through a pair of 0.5-mm. slits into the emission monochromator. The

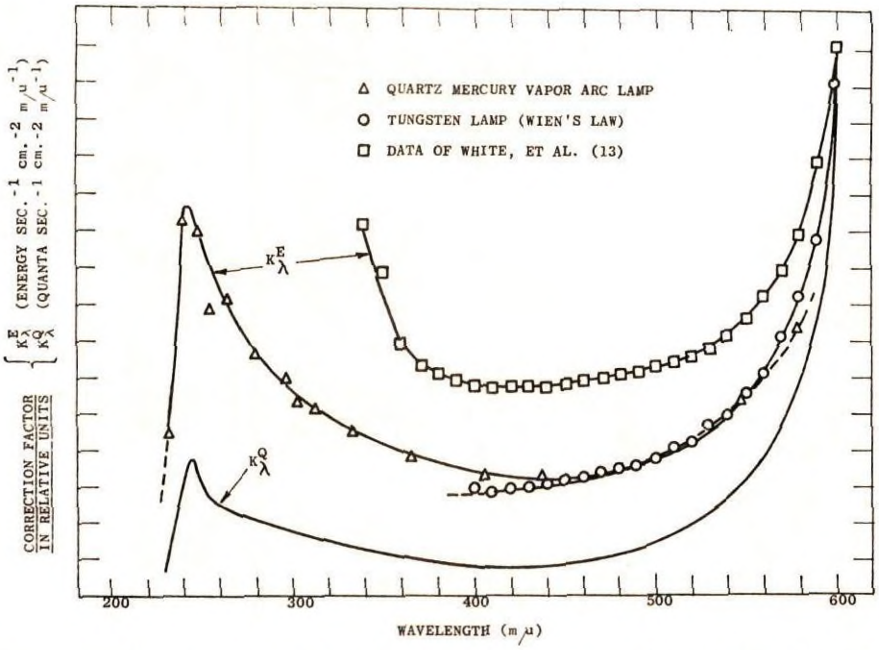

Figure 1. Correction factors for emission monochromator (1-P-28 phototube) in units of energy and quanta per unit wavelength interval

exit slit (photomultiplier slit) was also set at 0.5 mm. The output of the photomultiplier *vs.* wavelength was recorded on the X-Y recorder. From the measured filament temperature, the spectral energy distribution curve was calculated by means of Wien's law.

$$E_v = \frac{C_1\lambda^{-5}}{e^{C_2/\lambda T}} \qquad (1)$$

where

λ = wavelength in μ
T = temperature in °K.
$C_1 = \{5.673 \times 10^{-12}$ watt/(cm.^{2} K.°^{4})$\}$ $[(15/\pi^4)C_2^4]$
$C_2 = 1.4385 \times 10^4\ \mu$K.°

As the relative intensity of the emission from the tungsten lamp became small near 400 mμ, the useful spectral range was about 400 to 600 mμ. Beyond 600 mμ, the sensitivity of the 1P28 phototube diminished rapidly, which limited its use to wavelengths below 600 mμ. Correction factors were calculated at 10-mμ intervals by dividing the calculated relative energy by the recorded output of the photomultiplier:

$$K_\lambda^E = E/R \qquad (2)$$

where

E = energy flux per unit wavelength interval
R = recorded response of the monochromator-photomultiplier combination

Thus, multiplication of the recorded intensity at a given wavelength by K_λ^E converts the apparent intensity to a true relative energy. A correction factor for conversion to relative quanta per unit wavelength interval was obtained by multiplying by λ:

$$K_\lambda^Q = K_\lambda^E \cdot \lambda \qquad (3)$$

Presentation of spectra with an abscissa linear in frequency is preferred as the energetic relationship between individual bands (vibrational fine structure) then becomes linear. Therefore, for such presentation a factor must be included to account for the change in wavelength interval with change in frequency interval as shown by Parker and Rees (*8*):

$$\nu = c/\lambda;\ \frac{d\lambda}{d\nu} = -\frac{c}{\nu^2} = -\frac{\lambda^2}{c} \qquad (4)$$

$$\frac{dQ}{d\nu} = \frac{dQ}{d\lambda}\frac{d\lambda}{d\nu} = -\frac{dQ}{d\lambda}\cdot\frac{\lambda^2}{c} \qquad (5)$$

The correction factor then becomes:

$$K_\nu^Q = K_\lambda^E \cdot \lambda^3 \qquad (6)$$

Below 400 mμ, the monochromator-phototube response was determined by use of the Hanovia quartz mercury vapor lamp. The lamp was mounted above the cell compartment and the radiation reflected into the monochromator by placing a sheet of aluminum foil (pure, J. T. Baker) in the cell compartment. No correction was made for a slight variation of reflectivity *vs.* wavelength. The output of the photomultiplier was recorded as in the case of the tungsten lamp. Intensities of the emission bands were supplied by the manufacturer of the mercury vapor arc lamp. Although the relative energy distribution of a line source is expressed in terms of total relative energy emitted by the lines, these energies may be used for this calibration as though they were energies per unit wavelength interval (the spectral slit width of the monochromator; expressed in wavelength units, was constant *vs.* wavelength). Small exit slits (0.5 mm.) and small entrance slits (1 mm.) were used to reduce the intensity of the background emission (continuum) compared to that of the sharp mercury emission lines. In most cases the recorded intensity returned to essentially zero between the sharp emission lines. For the several weaker lines the background (continuum) was accounted for by drawing a base line to determine the peak intensity. The very weakest lines were not used for the calibration. Correction factors, *K*, were calculated according to the equations given above.

Emission monochromator-phototube correction factors for energy and quanta per unit wavelength interval are plotted in Figure 1. Correction factors for quanta per unit frequency interval are plotted in Figure 2. Data from the tungsten lamp and the mercury vapor arc lamp were normalized at 500 mμ, thus providing a continuous correction curve from about 240 to 600 mμ. Although the actual intensity of the

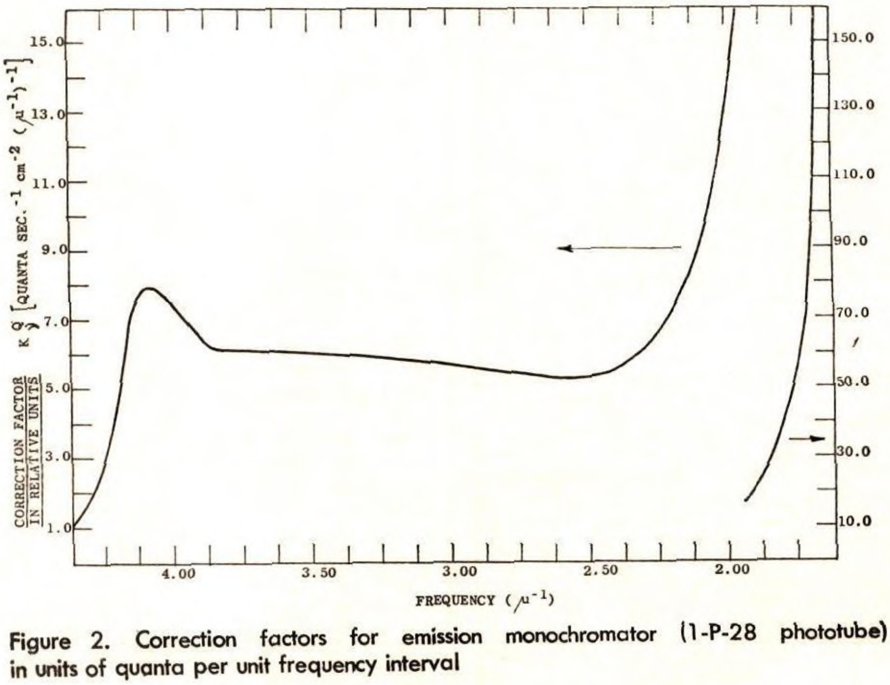

Figure 2. Correction factors for emission monochromator (1-P-28 phototube) in units of quanta per unit frequency interval

individual mercury vapor emission lines varied by several orders of magnitude, the resulting correction factors produced a rather smooth curve. For comparison, the data of White, Ho, and Weimer (*13*) were normalized and included in Figure 1. Their data extend to only 350 mμ at which point a sharp rise is noted. The low intensity from their tungsten lamp at 350 mμ (the Mazda lamp probably had an ordinary glass envelope) was likely the main reason for the sharp cut-off rather than absorption by the envelope of the phototube. The 1-P-28 phototubes are regularly used in spectrophotometers operating in the ultraviolet region of the spectrum and should show a reasonable response between 250 and 350 mμ. A 1-P-28 phototube has an S-5 response showing a maximum near 340 mμ according to manufacturer's data. Above 375 mμ, the data of White, Ho, and Weimer (*13*) agree rather well with the data shown in Figure 1. It is interesting that the response of the monochromator-phototube combination expressed in relative quanta per unit frequency interval approaches linearity between 350 and 500 mμ, the most frequently used region of the spectrum.

Calibration of the Excitation Monochromator (Osram Source). The excitation monochromator was calibrated by making use of the calibrated emission monochromator. Radiation from the excitation monochromator was scattered into the emission monochromator by placing a neutral reflecting or scattering material in the area of the cuvette holder. With narrow exit slits (0.5 mm.) on the emission monochromator the scattered radiation at intervals of 10 mμ was scanned with the emission monochromator and recorded. Several different materials were used to scatter the radiation, viz., a magnesium oxide screen, aluminum foil, quartz wool, and calcium carbonate. The recorded intensities were multiplied by the correction factor, K_λ^E, to obtain the emitted spectral energy distribution curve expressed in terms of energy per unit wavelength interval E_λ^E *vs.* wavelength (see Figure 3). The distribution of radiation from source and monochromator in terms of quanta per unit wavelength interval E_λ^Q was obtained by multiplying by K_λ^Q (or $E_\lambda^Q = E_\lambda^E \cdot \lambda$). Data obtained with the magnesium oxide reflector were chosen without correction for the change in coefficient of reflection with wavelength which varies by only several per cent over the range 250 to 500 mμ (*1, 11*). Although the other scattering materials behaved very much like the magnesium oxide, the magnesium oxide probably represents the best neutral diffuse reflector which is readily available.

The energy (or quanta) distribution curves may be used to correct excitation spectra or correct for differences in quanta of exciting radiation when the wavelengths of excitation are different for the reference standard and the unknown, whose quantum efficiency is being measured.

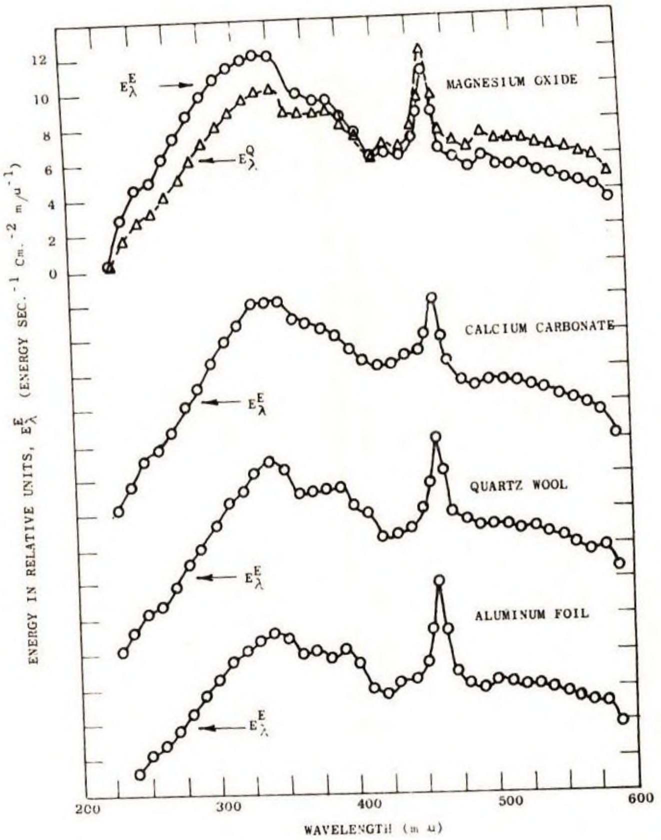

Figure 3. Spectral energy distribution for excitation monochromator (Osram xenon lamp)

Measurement of the Emission Spectrum. All spectra were obtained at solute concentrations representing linear response (emission intensity) *vs.* concentration. Under these conditions the undesirable inner-filter effects are minimized. Inner-filter effects have been thoroughly discussed by Parker and Rees (*9*) in a recent review. For very accurate quantum efficiency measurements, a correction for self-absorption of luminescence must be applied. In the case of certain compounds, this effect may be quite large if sufficiently dilute solutions are not used. In general, however, at very low concentrations the oxygen quenching constant will be much more significant than the self-quenching constant, necessitating the exclusion of oxygen during measurement. Therefore, oxygen was removed by degassing all solutions with high purity dry nitrogen and by continuously flushing the cell compartment with nitrogen.

Computer Processing and Correction of Spectra. Manual calculation of corrected intensities and replotting of the spectra is tedious. Modern computers are ideally suited for such time consuming calculations. A program was written for an IBM 1620 computer to process the spectral data and present the corrected data in a graphical form.

Intensities from the recorded spectra at 5-mμ intervals are punched on IBM cards. For very sharp peaks, shorter wavelength intervals are used. The correction factor K_λ^E at similar intervals of wavelength are also punched on IBM cards. An additional factor, λ^3 (see text above), is calculated for multiplication along with K_λ^E. After applying the correction factors, a parabola is fitted to the three points bracketing the peak to determine the maximum intensity and all of the intensities are normalized with respect to this computed maximum. The reciprocal of each wavelength value is calculated to yield frequency in units of reciprocal microns. The resulting table of normalized, corrected intensities and frequencies is used with a 4-point LaGrangian interpolation subroutine to compute the position for the ordinate character at intervals of 0.01 micron^{-1}. For use in quantum efficiency calculations, the ordinate values are summed to provide integrated area for graphical presentation of the spectrum. A pair of cards is punched for each point. The IBM printer uses the last 60 columns of each card of the pair to give 19 characters of identification and 101 positions for plotting. The first character of each card controls page skipping and space suppression on the printer and the next 19 may be used for identification, sequence, etc., but do not print. Successive pairs of cards represent an increment of the abscissa of 0.01 micron^{-1}. The integrated area (sum of ordinates) and normalization factor are printed last at the lower left hand corner of the spectrum. A suitable grid of scale characters is generated at the start, finish, and at each 10 increments of abscissa to give a finished appearance to the printed spectrum.

The Appendix gives the Fortran

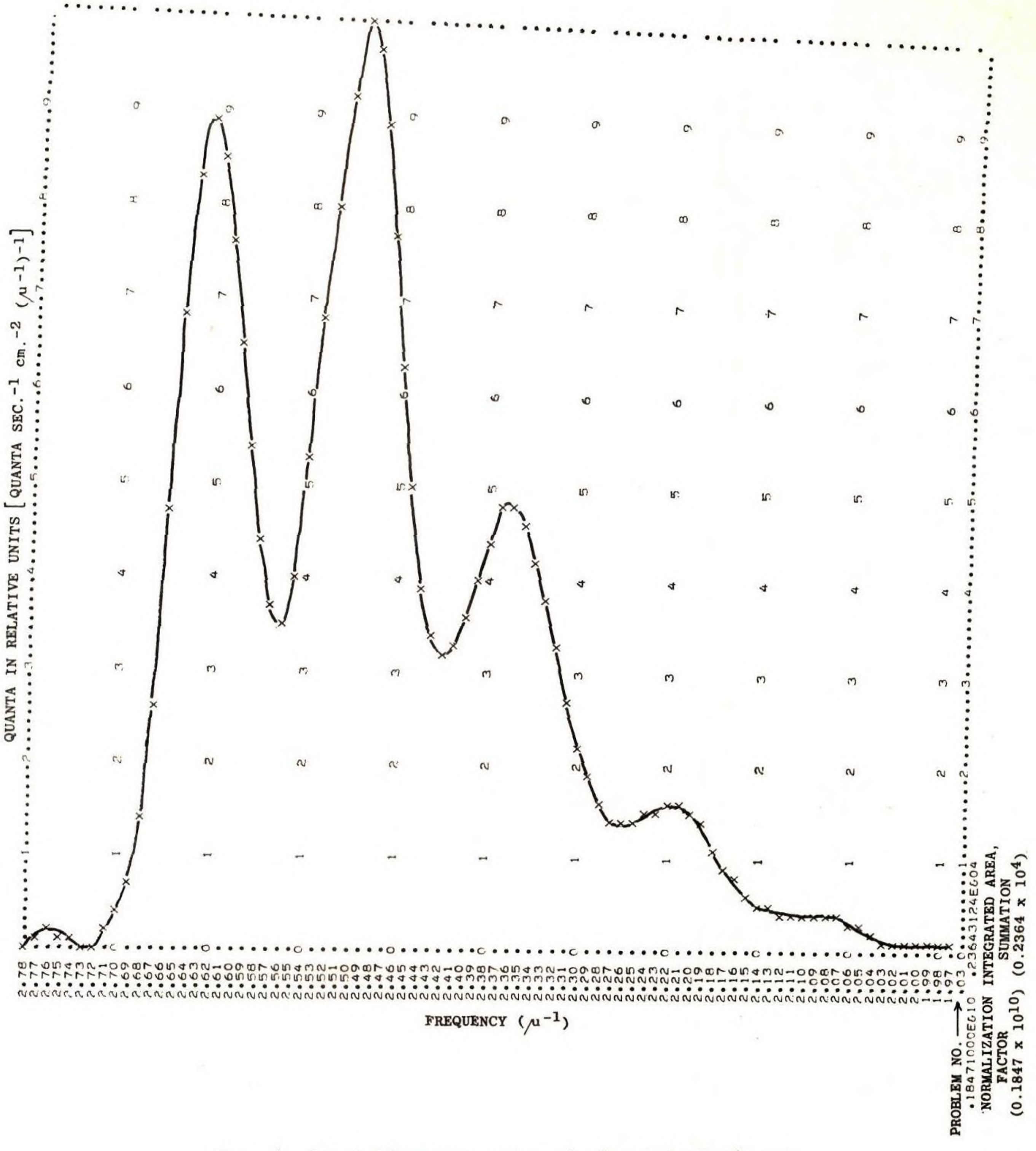

Figure 4. Corrected fluorescence spectrum of anthracene in units of quanta

listing (may be used on other computers) of the program with comments describing the function of each major division. The program uses a special subroutine (available upon request) to punch the cards which list as a graph. An example of a finished plot of the fluorescence spectrum of anthracene is shown in Figure 4.

RESULTS AND DISCUSSION

Quantum Efficiency Calculations. According to Parker and Rees (*8*), the total intensity of luminescence (F^Q for fluorescence intensity in quanta per second) is equal to the intensity of the absorbed energy times the quantum efficiency.

$$F^Q = \phi E^Q(1 - 10^{-\epsilon bc}) \qquad (7)$$

where

ϕ = quantum efficiency = $\frac{\text{quanta emitted}}{\text{quanta absorbed}}$
E = intensity of exciting radiation in quanta per second
ϵ = molar absorptivity (liters mole^{-1} cm.$^{-1}$)
b = optical path length in cm.
c = concentration in moles per liter

When the absorbing solute is reduced to low concentrations such that the per cent transmittance is high (region of linear emission intensity *vs.* concn. is when % $T \geq 95$), Equation 7 reduces to

$$F^Q = \phi E^Q\,(2.3\ \epsilon bc) \qquad (8)$$

When the same instrument and the same exciting wavelength is used the fluorescence intensities (integrated areas expressed in units of quanta) of two different compounds are related as follows:

$$\frac{F_2^Q}{F_1^Q} = \frac{E_2^Q\phi_2\epsilon_2bc_2}{E_1^Q\phi_1\epsilon_1bc_1} = \frac{\phi_2A_2}{\phi_1A_1} \qquad (9)$$

where

A = absorbance representative of the solution used for the luminescence intensity measurement
F = area of luminescence spectrum in units of quanta.

However, with the Aminco-Kiers instrument and certain other instruments having a separate excitation monochromator, the wavelength for maximum excitation may be selected for each different compound. In this case, the E^Q terms remain in the equation. If

Table I. Some Quantum Efficiencies Determined from Computer Processing of Fluorescence Data

(Quinine sulfate used as reference standard, ϕ = 0.55)

Compound	Max. (μ^{-1})	Concn. used (μg./ml.)	Quantum efficiency, ϕ This work[a]	Literature values	Reference
Anthracene, in ethanol	2.63, 2.50 2.37, 2.22	1.78	0.22	0.28	*(8)*
				0.26[b]	*(6)*
				0.27[b]	*(4)*
				0.27	*(7)*
Rhodamine B, in ethanol	1.75	0.372	0.76	0.69	*(8)*
				0.97	*(12)*
Phenol, in water	3.34	10.0	0.13	0.22	*(12)*
				0.19[c]	*(7)*
Indole, in water	2.90	1.00	0.24	0.45	*(12)*
Quinine sulfate, in water	2.14	1.00	...	0.55	

[a] Values not corrected for self-absorption.
[b] The recorded values, uncorrected for self-absorption, were 0.22 *(6)* and 0.228 *(4)*.
[c] In ethanol.

APPENDIX I

```
0̄7300 C       SPECTRO-PHOSPHORIMETER PROG. AD-
                 JUST, SCALE, PLOT AND INTEGRATE.
0̄7300 C       RCC-HVD-ALS. VERSION 11-19-62.
0̄7300         DIMENSION WAVL (75), CORDG (75), A(3, 4),
                 X(4), CRDG(4), D(4)
0̄7300         DIMENSION F(32)
0̄7300 C       READ TABLE OF CORRECTION FACTORS
                 (32 ITEMS) AND PROBLEM NO.
0̄7300         DO 5 I = 1, 32
0̄7312       5 READ 11, F(I)
0̄7396     100 BIGRD = 0.0
0̄7420         READ 10, PROBN
0̄7444 C       SET INDEX OF COMPUTED GO TO FOR
                 EXIT FROM INTERPOLATION TO 93.
0̄7444         KIF = 1
0̄7468 C       READ DATA, GET CORRECTION FACTOR
                 BY INTERPOLATION, SAVE LARGEST
                 RDG
0̄7468         DO 20 I = 1, 75
0̄7480         READ 10, WAVL(I), RDG, CORR
0̄7552         IF (WAVL(I) - 999.)23,24,24
0̄7644      23 FREQ = WAVL(I)
0̄7692         IF(FREQ -270.)12,13,13
0̄7760 C       UP TO 270.0 MILLIMICRONS, LINEAR IN-
                 TERPOLATION.
0̄7760      12 TEST = (270. - FREQ) /5.0
0̄7808         J2 = TEST
0̄7844         B = J2
0̄7880         J3 = 10 - J2
0̄7916         YRDG = F(J3) + (TEST - B)* (F(J3 - 1) -
                 F(J3))
0̄8084         GO TO 93
0̄8092 C       ABOVE 270.0, USE 4-POINT LAGRANGIAN
                 INTERPOLATION.
0̄8092      13 TEST = (FREQ -270.)/15.0
0̄8140         J2 = TEST
0̄8176         B = J2
0̄8212         J3 = J2 + 8
0̄8248         IF(TEST - B) 15,16,15
0̄8316      16 YRDG = F(J3 + 2)
0̄8364         GO TO 93
0̄8372      15 IF(J3 - 9)17,17,18
0̄8440      17 TEST = 270.
0̄8464         J3 = 9
0̄8488         GO TO 91
0̄8496      18 IF(J3 - 28)19,19,90
0̄8564      90 J3 = 28
0̄8588         TEST = 570.
0̄8612         GO TO 91
0̄8620      19 TEST = 255. + B* 15.0
0̄8668      91 DO 92 J = 1,4
0̄8680         J4 = J3 + J
0̄8716         CRDG(J) = F(J4)
0̄8788         X(J) = TEST
0̄8836      92 TEST = TEST + 15.0
0̄8908 C       GO TO INTERPOLATION. RETURN TO 93.
0̄8908         GO TO 75
0̄8916 C       MULTIPLY RDG BY CORR. FACTOR AND
                 CUBE OF WAVL(I). (NOW IN FREQ)
0̄8916      93 RDG = RDG * YRDG * FREQ * * 3
0̄8952 C       SAVE LARGEST CORRECTED RDG AND
                 INDEX RELATED TO IT (1-2).
0̄8952         IF(RDG - BIGRD) 20, 20, 22
0̄9020      22 BIGRD = RDG
0̄9044         J5 = 1 - 2
0̄9080      20 CORDG(I) = RDG
0̄9164 C       DATA ARE ALL IN. SET COMPUTED GO TO
                 FOR EXIT INTERPOLATION TO 94.
0̄9164      24 KIF = 2
0̄9188 C       INITIAL AREA UNDER CURVE = 0.0
0̄9188         SUMC = 0.0
0̄9212 C       SET UP MATRIX A FOR SOLUTION OF
                 PARABOLA THROUGH 3 POINTS WHICH
0̄9212 C       BRACKET BIGRD, THE MAXIMUM COR-
                 RECTED READING.
0̄9212         DO 30 L = 1,3
0̄9224         J1 = J5 + L
0̄9260         A(L,3) = 1000./WAVL(J1)
0̄9344         A(L,2) = A(L,3) * A(L,3)
0̄9452         A(L,4) = CORDG(J1)
0̄9524      30 A(L,1) = 1.0
0̄9608 C       SIMULTANEOUS EQUATION SOLUTION,
                 STATEMENTS THROUGH 31. ANS 1 N
                 A(L,4)
0̄9608         DO 31 I1 = 1,3
0̄9620         DIAG = A(I1, I1)
0̄9704         DO 32 J = I1,4
0̄9716      32 A(11,J) = A(11,J)/DIAG
0̄9908         K1 = 1
0̄9932      33 IF(K1 - I1)34,35,34
1̄0000      34 FELMT = A(K1,I1)
1̄0084         DO 36 J = I1,4
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APPENDIX I (*Continued*)

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1̄0096        36 A(K1,J) = A(K1,J) - FELMT* A(I1, J)
1̄0372        35 K1 = K1 + 1
1̄0408           IF(K1 - 3) 33,33,31
1̄0476        31 CONTINUE
1̄0512 C         COMPUTE MAX. VALUE OF PARABOLA.
                  SCALE CORDG(I) TO 100.0 MAX.
1̄0512 C         AND CONVERT WAVL(I) TO UNITS OF FRE-
                  QUENCY (RECIPROCAL CM.)
1̄0512           K = I - 1
1̄0548           FREQ = -0.5*A(3,4)/A(2,4)
1̄0608           BIGRD = A(1,4) - 0.25 * A(3,4) * A(3,4)/A(2,4)
1̄0692           CORR = 100.0/BIGRD
1̄0728           DO 25 I = 1,K
1̄0740           CORDG(I) = CORDG(I) * CORR
1̄0824        25 WAVL(I) = 1000./WAVL(I)
1̄0944 C         INITIALIZE PLOTF (GIVE IT PROBLEM NO
                  AND (-) SIGN TO INIALIZE CARD
1̄0944 C         NUMBER AND CAUSE FIRST LINE LEGEND
                  TO BE PUNCHED).
1̄0944           YRDG = -PROBN
1̄0980           YRDG = PLOTF (FREQ)
1̄1004 C         PICK INIT. FREQ FOR PLOT, AS N*0.02
                  JUST EQ. OR MORE THAN WAVL(1).
1̄1004           FREQ = WAVL(1)*50.0
1̄1040           I = FREQ
1̄1076           FREQ = I
1̄1112           FREQ = FREQ*0.02
1̄1148           IF(WAVL(1) - FREQ - 0.01)60,61,61
1̄1228        61 FREQ = FREQ + 0.02
1̄1264 C         FIND 4 BRACKETING VALUES OF WAVL(I).
                  PUT THEM, + CORRESPONDING VALUES
1̄1264 C           OF CORDG(I) INTO X AND CRDG AR-
                  RAYS USED BY INTERPOLATION R OUT-
                  LINE.
1̄1264        60 I = 0
1̄1288        50 I = I + 1
1̄1324           IF(WAVL(I) - 999.0)51,99,99
1̄1416        51 IF(WAVL(I) - FREQ)52,63,50
1̄1508        63 YRDG = CORDG(I)
1̄1556           GO TO 74
1̄1564        52 IF(1 - 3)65,65,68
1̄1632        65 DO 67 J = 1,4
1̄1644           X(J) = WAVL(J)
1̄1716        67 CRDG(J) = CORDG(J)
1̄1824 C         GO TO INTERPOLATION. RETURN TO 94.
1̄1824           GO TO 75
1̄1832        6S IF(I + 1 - K)80,81,81
1̄1912        81 DO 82 J = 1,4
1̄1924           J1 = K - 4 + J
1̄1972           X(J) = WAVL(J1)
1̄2044        82 CRDG(J) = CORDG(J1)
1̄2152           GO TO 75
1̄2160        80 DO 83 J = 1,4
1̄2172           J1 = I - 3 + J
1̄2220           X(J) = WAVL(J1)
1̄2292        83 CRDG(J) = CORDG(J1)
1̄2400 C         4-POINT LAGRANGIAN INTERPOLATION.
                ARGUMENT IS FREQ, X, CRDG. ANS, YRDG
1̄2400        75 YRDG = 0.0
1̄2424           DO 70 N = 1,4
1̄2436        70 D(N) = FREQ - X(N)
1̄2556           PN = D(1)*D(2)*D(3)*D(4)
1̄2616           DO 71 N = 1,4
1̄2628           DO 72 J = 1,4
1̄2640        72 D(J) = X(N) - X(J)
1̄2784           D(N) = 1.0
1̄2832           PI = D(1)*D(2)*D(3)*D(4)*(FREQ - X(N))
1̄2964           IF(P1)71,73,71
1̄3020        73 YRDG = CRDG(N)
1̄3068           GO TO 74
1̄3076        71 YRDG = YRDG + CRDG(N)*PN/PI
1̄3196 C         END OF INTERPOLATION. ALTERNATE
                  EXIT BY COMPUTED GO TO.
1̄3196        74 GO TO (93,94), KIF
1̄3272 C         SOME ANSWERS ARE SLIGHTLY NEGA-
                  TIVE. MAKE THEM 0.0
1̄327         94 IF(YRDG) 95,96,96
1̄3328        95 YRDG = 0.0
1̄3352 C         ACCUMULATE AREA UNDER CURVE.
1̄3352        96 SUMC = SUMC + YRDG
1̄3388 C         PLOT THE VALUE OF YRDG VS. FREQ.
1̄3388           YRDG = YRDG
1̄3412           YRDG = PLOTF (FREQ)
1̄3436 C         DECREMENT FREQ. AND RETURN TO 51
                  TO PLOT NEXT POINT.
1̄3436           FREQ = FREQ - 0.01
1̄3472           GO TO 51
1̄3480 C         SCALE PROBLEM NO. FOR PLOTTING SUB-
                  ROUTINE
1̄3480        99 PROBN = PROBN*0.01
1̄3516 C         GIVE PLOTF SIGNAL (-999.0) TO PUNCH
                  FINAL LEGEND AND PROB. NO.
1̄3516           YRDG = -999.0
1̄3552           YRDG = PLOTF (PROBN)
1̄3576 C         PUNCH THE SCALE FACTOR (BIGRD) AND
                  AREA (SUMC)
1̄3576           PUNCH 7, BIGRD, SUMC
1̄3612 C         RETURN TO START ANOTHER PROBLEM.
1̄3612           GO TO 100
1̄3620        10 FORMAT (F10.0,F10.1,F10.2)
1̄3652        11 FORMAT (4XF5.2)
1̄3690         7 FORMAT (1H2, 19X, E15.8, E15.8)
1̄3772           END
```

the quantum efficiency of one of the compounds is accurately known, the value for the second compound may be readily calculated.

$$\phi_2 = \phi_1 \cdot \frac{F_2^Q}{F_1^Q} \cdot \frac{A_1}{A_2} \cdot \frac{E_1^Q}{E_2^Q} \qquad (10)$$

Although it is generally held that ϕ is independent of the wavelength of exciting radiation (*10*), Equation 10 permits the use of selected wavelengths for maximum excitation provided that the spectral energy distribution of the excitation monochromator is determined.

Quantum efficiencies for several compounds were calculated by making use of the correction data, the computer program, and the above equations. Quinine sulfate was used as the reference compound throughout. Results are presented in Table I. The values for anthracene and Rhodamine B agree very well with the literature values. The low efficiency for phenol might be a reflection of the effect of pH which determines the decrease in phenol concentration as a result of conversion to the nonfluorescent phenolate ion. However, a more significant factor is self-absorption which is relatively large for anthracene, Rhodamine B, phenol, and indole. As seen in Table I, the values for anthracene, uncorrected for self-absorption, reported by Melhuish (*4, 6*) agree almost identically with the determined value. Similarly, correction for self-absorption in the case of the other compounds, may have yielded higher values. Fortunately, the quinine sulfate fluorescence spectrum does not overlap its absorption spectrum so that errors introduced by failure to correct for self-absorption are negligible. The extent of overlap of the emission and absorption spectra is an important point in the choice of a quantum efficiency reference standard.

CONCLUSIONS

The purpose of this paper was to present a convenient approach to calibration of luminescence equipment and to show how modern computers can be used for correction of the spectra used in the calculation of quantum efficiencies. Calibration data reported in this paper should not be applied to other instruments. Even though two instruments may be of the same manufacturer and model, the components (source lamp, phototube, electronics, etc.) may not function identically. For this reason, each separate instrument should be calibrated and if a component is changed it should be recalibrated.

ACKNOWLEDGMENT

The authors thank Esso Research Laboratories, Humble Oil and Refining Co., for permission to publish this material. Helpful discussions with P. Debye and D. P. Shoemaker are gratefully acknowledged.

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RECEIVED for review June 6, 1963. Accepted August 12, 1963. Division of Physical Chemistry, 145th Meeting, ACS, New York, September 1963.