An Absolute Spectrofluorometer

A new instrument has been developed for simple determinations of absolute fluorescence spectra.

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Fluorescence, the property possessed by some substances of emitting light upon exposure to external radiation, has been utilized extensively in theoretical physics and chemistry (*1*). At present, measurements of fluorescence are most widely used for quantitative chemical analysis, and analytical procedures based on such measurements are unusually sensitive and specific.

The technique of fluorescence analysis was first developed in the mid-1930's, when it was found that vitamins were particularly suited to this form of analysis. Growth of the technique was slow but steady until photomultiplier tubes came into general use, in the late 1940's. These tubes allow light to be measured at extremely low intensities—a prerequisite for the full exploitation of fluorescence measurements. At the same time, biochemical studies, previously centered on the more gross constituents of organisms, were directed toward the minor constituents such as hormones, enzymes, and trace elements. Since then there has been a rapid increase in the use of fluorescence techniques. Udenfriend has recently written an excellent summary of fluorescence measurements in biology and medicine (*2*). The range of applications of fluorescence analytical techniques in industrial and general chemistry is indicated in a review by White (*3*). A central library and clearing house for information for the analytical uses of fluorescence has now been established, and well over 6000 abstracted and indexed references are currently available (*4*).

An interesting offshoot from the analytical applications is the use of fluorescent dyes as tracers, for example, in studies of mass flow of water in bays and harbors. In such studies, fluorescent materials with very unusual excitation and emission spectra are used, and with proper selection of instrumental settings, concentrations as low as one part in one hundred billion may be continuously recorded, with negligible background interference (*5*). Similar fluorescent materials which are soluble in oil have also been found and are being used to mark product interfaces in petroleum-product pipe lines (*6*).

In a more precise definition, fluorescence is expressed as the immediate (in the order of 10^{-8} second) emission of light from a molecule after it has absorbed radiation. When a molecule absorbs a quantum of radiation in the "electronic" region of the spectrum (2000 to 8000 Å), an electron is elevated from the ground state to an excited state. The electron may then return to the ground state by a number of different competing processes. If its return to the ground state is direct, there is an accompanying emission of a photon of light. In most systems other than gases, the return to the ground state is a two-step process. The electron first falls by nonradiative processes to the lowest vibrational level of the excited state, and then returns to the ground state.

The fluorescence of a material in a given environment may be specified by its quantum efficiency, its excitation spectrum, its emission spectrum, and by the lifetime of the excited state. Quantum efficiency (ϕ) is the ratio of photons emitted to photons absorbed. Quantum efficiency varies from zero, for nonfluorescent materials, to nearly 100 percent. The excitation spectrum is a plot of intensity of emitted light as a function of the wavelength of the exciting light. Where the quantum content of the exciting light is held constant, the excitation spectrum of a system containing a single fluorescent molecular species is usually proportional to the number of quanta absorbed, hence proportional to a plot of the extinction coefficient (*7*). The emission spectrum is a plot of intensity of emitted light as a function of the wavelength of the emitted light. The shape of the emission spectrum is usually independent of the excitation wavelength (*8*). For analytical purposes, fluorescence lifetime is generally not measured. In highly viscous systems containing large fluorescent molecules, the lifetime of the fluorescence may be short compared with the time required for molecular disorientation by Brownian movement. Under these conditions, the emitted light may be polarized and nonsymmetrical with respect to the sample. The utility of such measurements in determinations of molecular conformation and the techniques for determining quantum efficiency have been discussed (*1*).

Fundamental Equations

The fundamental relationship defining fluorescence in dilute solution may be expressed as (*9*)

$$F = 2.3\, I_q\, ECD\phi \qquad (1)$$

where F is the total fluorescence intensity, in quanta per second, in all directions; I_q is the intensity of exciting light, in quanta per second; E is the molecular extinction coefficient; C is the concentration of fluorescent material; D represents optical depth; and ϕ, quantum efficiency.

Excitation and emission spectra may be more readily visualized in terms of the differential form of Eq. 1:

$$\frac{dF}{d\lambda_{EM}} = 2.3\, I_q\, ECD\, \frac{d\phi}{d\lambda_{EM}} \qquad (2)$$

where $dF/d\lambda_{EM}$ is the total fluorescence intensity in quanta per unit emission bandwidth, and $d\phi/d\lambda_{EM}$ is the quantum efficiency per unit emission bandwidth. The operation of a fluorometer is more easily understood if Eq. 2 is expressed as

$$F_A = f_3\,(\lambda_{EM})\frac{dF}{d\lambda_{EM}} = K_3\, f_3\,(\lambda_{EM})\, I_q\, EC\frac{d\phi}{d\lambda_{EM}} \qquad (3)$$

were F_A is the indicated fluorescence, at some instrumental setting; $f_3\,(\lambda_{EM})$ relates indicated fluorescence to actual fluorescence (it accounts for limited

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angular pick-up of the light detector, and for variation in the sensitivity of the light detector as a function of emitted wavelength); and K_3 represents an instrumental constant which contains the effective optical depth, and various unit conversion factors.

Filter Fluorometer

The filter fluorometer is used primarily for repetitive, quantitative chemical determinations. In this class of instruments, excitation and emission wavelengths are chosen by color filters. Udenfriend has described ten filter fluorometers which are available commercially (*2*); probably about 10,000 such instruments are in service today. They vary widely with respect to light sources, available filters, sensitivity, and types of samples accepted, but in general are very satisfactory for precise microchemical determinations.

For the filter fluorometer, or for any spectrofluorometer operated at constant excitation and emission wavelengths, Eq. 3 is reduced to

$$F_A = K_4 C \qquad (4)$$

The constant, K_4, may be established by calibration with a standard. The dial or meter reading is proportional to concentration. Measurements may be extended to extremely low concentrations by increasing the sensitivity of the light detector or the intensity of the light source. In spectrophotometry, for comparison, concentration is related to the loss of light, as light passes through a solution. This is a differential measurement, which tends to accentuate the instability of the instrument. As an example, at 99 percent transmission, a 0.1 percent instrumental instability in a spectrophotometer introduces a 10 percent uncertainty in concentration. Fluorometry may be readily used to determine concentration in a solution with a 99.999 percent transmission.

Equation 3 also provides an explanation for instrumental specificity. In spectrophotometric techniques, substances with appreciable extinction coefficients at the same wavelength as that chosen for the unknown will interfere with the results. With a fluorescence technique, the interfering substance, besides having an appreciable extinction coefficient at the excitation wavelength chosen for the unknown, must also emit appreciable fluorescence at the emission wavelength chosen for the unknown.

Uncorrected Spectrofluorometer

Instruments in which monochrometers are used to establish excitation and emission wavelengths, and which have no special compensatory features, are termed "uncorrected" spectrofluorometers. They are widely used in the larger research and analytical laboratories; eight different models which are available commercially have been described by Udenfriend (*2*). Most of the instruments in service are direct descendants of the instrument developed by Bowman *et al.* (*10*). These instruments (Fig. 1) consist basically of a continuous light source of high intensity, usually a xenon arc lamp; a photomultiplier with associated amplifier; and two monochromators. One monochromator is located between the light source and the sample (excitation) and the other between the sample and the photomultiplier tube (emission). By setting the emission monochromator at a wavelength at or near the emission peak of a compound and scanning the excitation monochromator, the excitation spectrum may be obtained. Reversing the process gives the emission spectrum. For quantitative analysis, one selects the desired excitation and emission wavelengths and compares the relative fluorescence intensities of standard and unknown samples, as with a filter fluorometer.

The term "uncorrected" is applied to this type of spectrofluorometer because the excitation and emission spectra presented are a combination of the true spectra of a compound and various instrumental artifacts. With reference to Eq. 3, when excitation spectra are plotted no attempt is made to hold I_q constant.

Excitation spectra are hence a composite of the true excitation spectra, the spectral distribution of lamp output with wavelength, and monochromator efficiency with wavelength. When emission spectra are plotted no correction for f_3 (λ_{EM}) is made, hence emission spectra are a composite of the true emission spectra, the spectral distribution of sensitivity of the light detector, and monochromaor efficiency with wavelength. In certain regions of the spectrum, these instrumental factors become dominant. In practice, because of the individual nature of the light sources and photomultiplier tubes, no two uncorrected spectrofluorometers will present the same spectra on the same compound. Even individual instruments will vary as the light source ages or is changed.

Despite these drawbacks, the uncorrected spectrofluorometer can be put to many uses. The instruments may be used for quantitative analyses with the convenience that wavelengths can be dialed (instead of filters having to be changed). They may be used for comparisons of spectra within one laboratory and rough comparisons of spectra with other laboratories.

The specificity of an analysis may often be assured by comparison of spectra of final extracts with standards. If no standard is available, emission spectra obtained at a variety of exciting wavelengths may be compared. If such emission spectra are *not* similar, the presence of more than one molecular species is indicated (*8*). Comparison of excitation spectra at different emission wavelengths will yield similar data (*7*).

In the development of procedures eventually aimed at quantitation with relatively inexpensive filter fluorometers, an uncorrected spectrofluorometer is of advantage. The visual presentation of spectra is more than sufficient to allow selection of optimum filters for the filter fluorometer.

Absolute Spectrofluorometer

For some time there has been a need for an instrument which would allow the precise and simple determination of the characteristics of a fluorescent material in a form which could be easily transferred from laboratory to laboratory. Such an instrument should be capable of (i) determining the quantum efficiency of an unknown material, and (ii) recording excitation and emission spectra in a manner which has easily definable physical significance.

In addition to the obvious value of being able to transfer such information exactly, these absolute measurements would be of immediate use within a single laboratory. The literature is full of relative studies of solvent-solute interaction and the action of "quenching" materials. The significance of such studies would have been much greater if a simple means had been available to establish the actual value of the quantum efficiency.

Absolute excitation spectra may usually be converted to the corresponding extinction coefficient spectra, as obtained by spectrophotometric measure-

ments. This is particularly useful in several situations. First, it is useful where sensitivity is important—for example, where only small quantities of the sample are available. Absorption spectra may be obtained from a sample with a concentration as low as one ten-thousandth that required in a spectrophotometer. Second, studies may be deliberately made at high dilutions, where intermolecular effects are minimized. Third, it is valuable where specificity is a problem. Only those materials which are fluorescent and whose fluorescence emission falls within the emission band selected will be measured. All others will be rejected. The whole body of theory governing absorption of light may thus be brought to bear, with extraordinary sensitivity and specificity, on the characterization of fluorescent materials.

Absolute emission spectra will be useful in the characterization of materials, as more knowledge accumulates. They should also prove to be of immediate use for study of the distribution of the energy levels in the unexcited (or ground level) state of molecules (see *9*).

Workers in a number of laboratories have prepared correction tables for uncorrected spectrofluorometers, or have modified commercial instruments to obtain corrected or partially corrected spectra. Manual correction of exciting energy as a function of wavelength has been carried out by comparison with standard lamps, and by the use of chemical actinometers, calibrated light detectors, thermopiles and bolometers, and quantum counters. The sensitivity of the detector and monochromator as a function of wavelength has been evaluated by means of standard lamps, and by transferring energy from a previously standardized excitation monochromator. There are many references to such work (*9, 11*).

Automatic correction of excitation spectra is accomplished in the instrument of Parker by means of "quantum counter" (*12*). In this instrument, a fixed fraction of the light falling on the sample also falls on a special cell containing a high concentration of a fluorescent material. Over a considerable range of exciting wavelengths, all incident energy is absorbed and a fixed quantum fraction re-emitted at essentially a fixed wavelength. A portion of this emitted beam is then allowed to fall alternately on the same photomultiplier that is used to measure the fluorescence emission from the sample. Signals due to the light from the quantum counter and to the sample are electrically separated, and used to operate a ratio recorder. Since the light emitted by the quantum counter is proportional to the quanta falling on the sample, automatically corrected excitation spectra are obtained. Essentially the same type of correction has been reported in which a fluorescent screen is used (*13*). Correction of emission spectra by the use of a cam and optical attenuator has also been reported (see *14*).

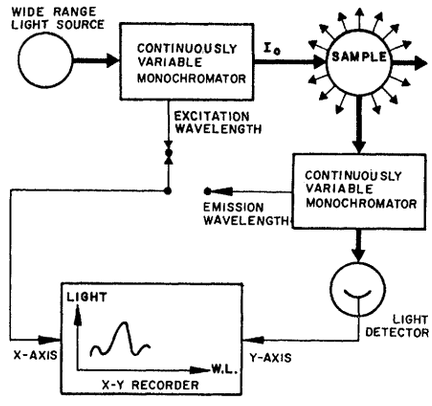

Fig. 1. The uncorrected spectrofluorometer.

In an instrument (*15*) described by Slavin (*16*), both excitation and emission spectra are corrected automatically. Excitation spectra are corrected to constant energy (rather than constant quanta) by means of a thermocouple which continuously monitors a fraction of the light falling on the sample and adjusts the excitation monochromator slits to keep this energy constant. Emission spectra are corrected by an electrical cam synchronized with the emission monochromator, via a slit servo on the emission monochromator.

A New Instrument

Quantum counters have several disadvantages in that they provide incomplete coverage of the wavelength range of interest, their properties tend to change drastically with temperature, and they are subject to aging problems. Fluorescent screens have the same problems.

Energy control schemes in which a thermocouple and slit servo are used alter the pattern of light falling on the sample as the slits operate and hence affect the optical characteristics of the sample cell. The operator has no knowledge of the bandwidth of the excitation monochromator, as the sample is scanned. The instrument operates at all wavelengths at the noise level and scanning speed dictated by the operator's bandwidth requirement at the portion of the spectrum scanned which has the least energy.

In the Turner model 210 "Spectro" (Fig. 2), a new correction method (*17*) is used which is in many ways a cross between the quantum counter and the thermocouple methods, and it embodies many of the best features of both (*18*). The instrument is shown diagrammatically in Fig. 3. To the left of the dashed line in this diagram, the equipment is essentially identical with that used in the highly efficient uncorrected spectrofluorometers already described. Bandwidths are set to the operator's requirements and are fixed. There is no loss of efficiency as spectra are scanned.

When this instrument is used as an absolute spectrofluorometer, a photomultiplier alternately receives two beams of light. One beam is from the fluorescent sample, the other is from a fixed wavelength reference-lamp whose intensity is automatically adjusted to match the energy of the exciting light falling on the sample. The beam from the reference lamp is further attenuated to match the efficiency of the photomultiplier and the emission monochromator as a function of emission wavelength. It follows that (i) with the emission monochromator fixed and the excitation monochromator scanning, the excitation spectrum thus presented is corrected to constant excitation energy; and (ii), with the excitation monochromator fixed and the emission monochromator scanning, the emission spectrum is presented in terms of the units for which the attenuator is calibrated (quanta per unit bandwidth).

A fluorescent lamp is used as the reference lamp since its color does not vary with intensity. A bolometer is used to compare the excitation of the sample with the intensity of the reference lamp, since it is sensitive only to energy and is not dependent on wavelength. A conventional optical density wedge is a suitable reference beam attenuator, since the reference beam is at a fixed wavelength. The position of this wedge is controlled by a cam, individually cut to match the combined characteristics of the emission monochromator and photomultiplier used.

Method of Operation

The detailed operation of the individual blocks of equipment will now be considered with respect to Eq. 3, the basic equation for presentation of fluorescence spectra.

The bolometer alternately receives two beams of light. One beam, at an energy level, I_{EX}, is an adjustable fraction of the exciting light falling on the sample. The second beam is an adjustable fraction of the light from the reference lamp. When the energy content of the two beams is equal, the output of the bolometer is zero. Unbalance generates a small a-c voltage, at the alternation frequency of the light. This a-c voltage is amplified about 10^6 times and converted to d-c by a commutator, which is synchronized with the alternation of the light beams and used to correct the power to the reference lamp. The extraordinarily high amplification used ensures that the two beams are maintained at equal intensity to a high degree of accuracy. Since the bolometer is used only to compare and not to measure, balance is independent of the sensitivity and temperature of the bolometer. The resultant equation is

$$I_{R1} = K_5 I_{EX} \tag{5}$$

where I_{R1} is the energy incident on the sample.

Since the energy content of a quantum of light varies inversely with the wavelength, Eq. 5 may be rewritten in terms of the number of exciting quanta I_q, and exciting wavelength, λ_{EX}, with an appropriate change in the constant:

$$I_{R1} = \frac{K_6 I_q}{\lambda_{EX}} \tag{6}$$

The photomultiplier receives sequentially no light, light from the sample, and light from the reference lamp. With reference to Eq. 3, the electrical output of the photomultiplier changes in response to the beam derived from the sample by F_A. The intensity of the second beam is proportional to I_{R1} from Eq. 6 multiplied by the attenuation factor entered by the wedge filter and cam system. Since the color of this second beam is fixed, the photomultiplier response to the reference beam is

$$V_R = K_7 f_A(\lambda_{EM}) \frac{I_q}{\lambda_{EX}} \tag{7}$$

where V_R is the change in electrical output of the photomultiplier, when it is illuminated by the reference beam, and $f_A(\lambda_{EM})$ is the attenuation factor entered by the wedge filter and cam system.

The recorder system, described later, presents the ratio of F_A to V_R. The basic equation governing the position θ, of the recorder pen may be obtained by combining Eqs. 3 and 7

$$\theta = \frac{F_A}{V_R} = K_8 \frac{f_3(\lambda_{EM})}{f_A(\lambda_{EM})} \lambda_{EX} EC \frac{d\phi}{d\lambda_{EM}} \tag{8}$$

When $f_3(\lambda_{EM})$ and $f_A(\lambda_{EM})$ are made proportional, Eq. 8 is reduced to Eq. 9

$$\theta = K_9 \lambda_{EX} EC \frac{d\phi}{d\lambda_{EM}} \tag{9}$$

This equation is the fundamental equation of the instrument. It can be used to define both the nature of excitation spectra and emission spectra which may be obtained, and the method to be used for determination of quantum efficiency.

Excitation spectra are obtained by plotting θ as a function of the excitation wavelength, λ_{EX}, at a fixed value of λ_{EM}. With λ_{EM} fixed, $d\phi/d\lambda_{EM}$ is a constant yielding

$$\theta = K_{10} \lambda_{EX} CEf(\lambda_{EX}) \tag{10}$$

The relation between extinction coefficient spectra, $Ef(\lambda_{EX})$, and excitation spectra is show by Eq. 10. In those cases where the extinction coefficient is known at any wavelength, the constant K_{10} may be evaluated. Extinction spectra may then be determined by dividing the excitation spectra by the wavelength and K_{10}. Relative absorption spectra, often of value in characterizing an unknown, may be determined simply by dividing the excitation spectra by wavelength.

Emission spectra are obtained by plotting θ as a function of the emission wavelength, λ_{EM}, at a fixed value of λ_{EX}. With reference to Eq. 9, it may be seen that with λ_{EX} fixed, E automatically assumes a fixed value. The relation between the emission spectra, in quanta per unit bandwidth as a function of emission wavelength, and the spectra obtained may be seen by inspection of Eq. 9. Where the quantum efficiency is known and the extinction coefficient is known, at wavelength λ_{EX}, the constant K_9 may be determined. This is done by measuring the area under the recorded spectrum, and replacing $d\phi/d\lambda_{EM}$ with ϕ (see Eq. 11, below). Emission spectra may then be determined by dividing the recorded spectra by the term $K_9 \lambda_{EX} EC$. Relative emission spectra are plotted directly.

Typical excitation and emission spectra obtained with this instrument on anthracene are shown in Fig. 4. Figure 5 shows emission spectra of quinine sulfate at concentrations of one and ten parts per billion, and the Raman spectrum of the solvent (water).

The equation for determining quantum efficiency is derived from Eq. 9 by taking the area, A, under the recorded spectra. This is the same as integrating Eq. 9:

$$A = K_{11} \lambda_{EX} EC\phi \tag{11}$$

To obtain the quantum efficiency of an unknown material, it is necessary to standardize the instrument by running a curve on a material of known quantum efficiency; many such standards exist (*10*, *19*). The extinction coefficients of both the known and unknown substances are then determined at the appropriate (but not necessarily identical) wavelengths. With the subscript S used to denote the standard, and U to denote the unknown, the equation for determining quantum efficiency becomes:

$$\phi_U = \phi_S \frac{A_U K_{12S} \lambda_{EXS} E_S C_S}{A_S K_{12U} \lambda_{EXU} E_U C_U} \tag{12}$$

In the Turner model 210, K_{12} may be varied in accurately known steps over a wide range. Since, from Eq. 12, the ratio of the sensitivity on the standard to sensitivity on the unknown, G, is all that need be known, Eq. 12 becomes

$$\phi_U = \phi_S G \frac{A_U \lambda_{EXS} E_S C_S}{A_S \lambda_{EXU} E_U C_U} \tag{13}$$

Use as a Spectrophotometer

For many studies of absolute fluorescence, the absorption properties of the substance being analyzed must be known. The model 210 may be used as a ratio-recording, dual-beam, dual-monochromator spectrophotometer, recording directly either optical density or percent transmission. The characteristics of the instrument when used as a spectrophotometer have been specifically tailored to the needs of fluorescence analysis.

Conventional spectrophotometers introduce into absorption spectra a little-recognized error, the fluorescence artifact (*20*). In taking the absorption

spectrum of a fluorescent compound, not only the true transmitted beam, but a portion of the emitted fluorescent light reaches the detector. Unless provision is made for eliminating this fluorescent light, the indicated percent transmission is higher than it should be, with large errors occurring at low values. In well-designed spectrophotometers, this artifact is minimized but not eliminated by arranging the detection optics to view the smallest practical solid angle since the transmitted beam is collimated and the fluorescent light is not. Complete elimination of this artifact requires a monochromator both between the sample and the detector and between the light source and sample. A direct arrangement of this type is very sensitive to minor optical errors and misalignment in the sample cell and holder.

The availability of two monochromators designed to operate on opposite sides of the sample and an excess of light (xenon arc lamp) suggested that deliberate diffusion of the transmitted beam would reduce alignment problems. Accordingly, in the model 210 a diffuse screen (*17*) is placed beyond the sample. This screen is treated as a light source for a second set of light-gathering optics for the second monochromator.

The xenon arc lamp, in addition to providing the intensity necessary for deliberate loss of light in the diffuser screen system, also has the advantage of yielding useful amounts of energy over the entire wavelength range of the instrument. There is no necessity for the usual change of lamps in the middle of a scan (at about 3500 Å).

Fluorescence measurements are strictly linear only when it may be assumed that the sample has essentially 100 percent transmission. From a practical standpoint, 95 percent transmission and higher is adequate. Transmission and fluorescence measurements do not, therefore, overlap well in concentrations required for measurements unless provision is made for long absorption cells. Provision was therefore made for 10-cm absorption cells.

Luminescence

Consideration of the correction utilized in fluorescent emission spectra shows that the instrument possesses the basic characteristics required for recording of corrected emission spectra of primary sources, such as lamps and chemiluminescent systems.

When the Mode Selector is in the Luminescence positions, the reference lamp is held at a fixed intensity by a vacuum-type photocell and a null-balance electronic system. Under these conditions, the neutral density optical wedge still enters the required correction for emission monochromator and photomultiplier efficiency as a function of emission wavelength, but with regard to a fixed reference level rather than exciting energy level. Thus, emission spectra of stable primary light sources may be recorded.

Recording Computer

The characteristics of any instrument are greatly affected by the recording system used. The overall system may be considered in terms of four parameters related to the final recording. These are static accuracy, dynamic accuracy, random noise, and recording time. In the model 210 static accuracy is primarily related to such factors as amplifier linearity, potentiometer linearity, gear backlash, and servo system dead zone. It is in large part dependent on the quality of the components used, and has been held to about 1 percent

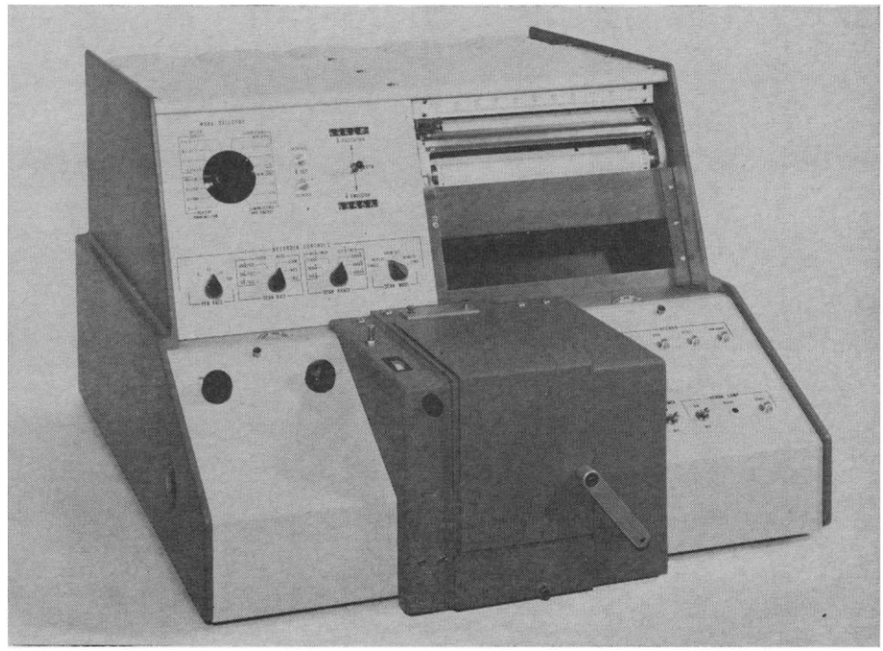

Fig. 2. The Turner model 210 "Spectro."

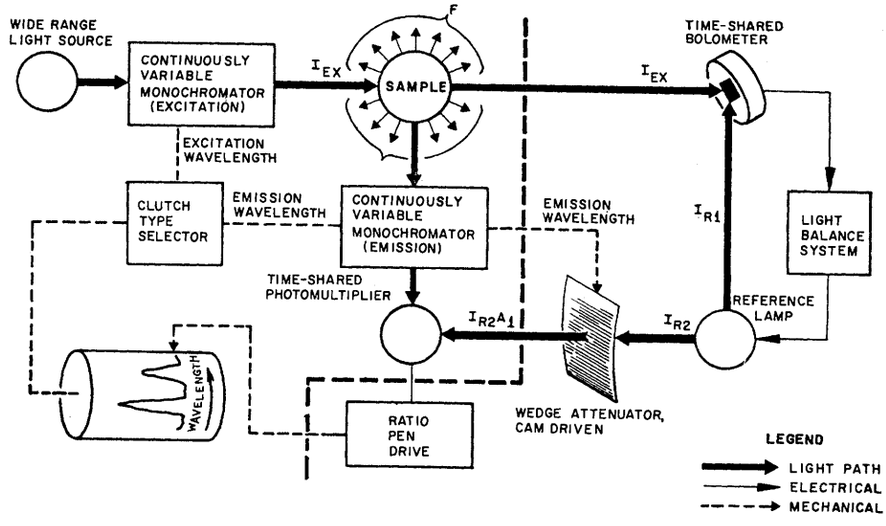

Fig. 3. Schematic representation of the model 210.

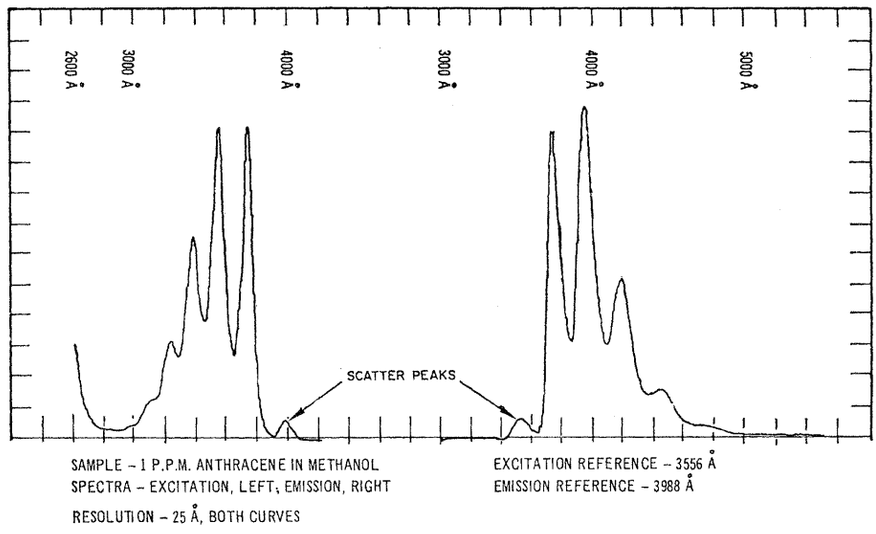

Fig. 4. Excitation and emission spectra for anthracene.

of full scale. Static accuracy is quite independent of the other three characteristics.

Dynamic accuracy in a two-dimensional plot may be considered as the maximum absolute distance between a point on the recorded curve and the curve which would have been recorded at infinitely slow speed, with noise being ignored. Random noise is the uncertainty in knowledge of any point in the plot. Recording time is the length of time required to abstract information from a sample. These last three characteristics are interrelated and must be considered in terms of the physical nature of the sample and measuring system.

Random noise will be considered first. At low light intensity, inherent noise of the photomultiplier predominates. At higher intensities, statistical variation in the number of photons received by the photocathode predominates. Both sources of noise are, however, similar in nature and extend over a very broad range of frequencies. Information on the sample is contained in the ratio of the excess of two electrical signals alternately available from the photomultiplier over dark current. In this instrument, the first step in the control of random noise is to maintain constant the broad-band noise of the output of the photomultiplier. The high frequency output of the photomultiplier (well above signal frequency) is separated out. This output is predominantly noise. It is rectified, compared with a standard voltage, and the resultant error voltage is used to control the dynode voltage, hence gain of the photomultiplier. During one portion of each cycle, the photomultiplier receives no light. During this period, dark current is compensated for by setting the output of the amplifier following the photomultiplier at zero. This is accomplished by a commutator synchronized with the cycle of light falling on the photomultiplier. The two electrical signals are separated out by the same commutator. The resultant voltages are thus related to the excess of reference voltage over dark current and excess of signal voltage over dark current. Since all information required exists in the ratio of these signals, changing the gain of the photomultiplier has no effect.

The reference voltage is inverted in polarity and applied across a precision potentiometer, whose wiper position is proportional to pen position. The voltage at the wiper is compared to the signal voltage. The error drives the pen servo motor to balance, thus causing the pen position to be equal to the ratio of the signal and reference voltages. In addition, a voltage proportional to pen velocity is combined in such a way that it retards the return of the pen to balance. Because of this velocity term, the pen servo system may be described as a conventional first order or velocity-damped servo system. Since noise at the input is constant, and the system is velocity-damped, random motion of the pen will remain constant. Since the error voltage for any given pen error is related to the magnitude of the reference voltage, the speed of the pen response depends directly on the reference voltage and varies over a wide range.

Dynamic accuracy depends on maintaining low the time rate of change of the function which the pen is following with respect to the speed of the pen response. Since the function being determined is fluorescence versus wavelength, it is permissible to vary the wavelength scan rate in order to alter the time rate of change of the function.

Maintaining dynamic accuracy is achieved in two steps. When the pen is not moving (area of constant fluorescence), scan speed must be limited so that if fluorescence changes, the pen will have time to respond. This is ac-

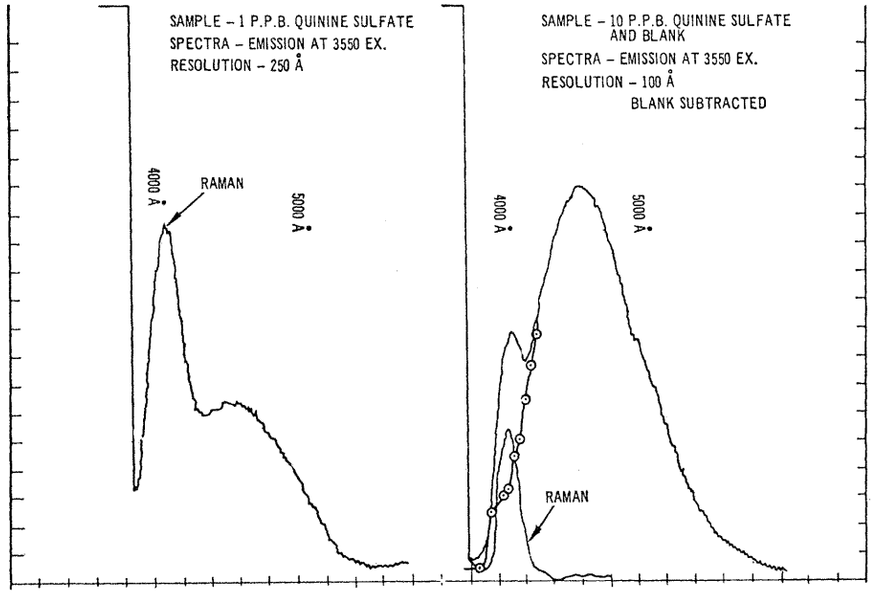

Fig. 5. Emission spectra for quinne sulfate (1 ppb, one part in 10^9).

complished by setting a maximum wavelength scan rate which is proportional to the reference voltage previously mentioned. The lower the reference voltage, the lower the rate of pen response and the lower the wavelength scan rate. When the pen is moving and is under the control of the velocity-damped servo system, its error is proportional to its velocity and inversely proportional to its response speed. To control this form of error, the reference voltage is compared with a voltage proportional to absolute pen speed. When the pen speed exceeds the reference voltage, wavelength scan rate is reduced.

The operator selects the maximum random noise which he feels is acceptable for his purpose by setting the Pen Rate control (Fig. 2). He then sets the maximum permissible error by setting the Scan Rate control. In the Med. position, for example, maximum error is 2 percent of full scale and 10 Å in wavelength. Recording of the spectra will then proceed at the maximum rate consistent with selected noise and selected maximum error.

Fixed scanning rates, without regard for error, are provided for special purposes, such as repeat scanning of rate reactions. They are selected by setting the Scan Rate control to the Fixed position.

Special Features

Grating monochromators transmit light of higher order in addition to the light desired. For example, a grating monochromator set to pass 6000-Å light will pass a very appreciable amount of 3000-Å light. This effect has been noted many times, and has been the source of considerable confusion. Reference (*21*) is typical of publications noting this effect.

In the model 210, blocking filters are automatically inserted in both the excitation and emission monochromators to eliminate this artifact. The filters are inserted by a two-step cam mechanism, as a function of wavelength. Since there is essentially no transmission in either monochromator below 1800 Å, the first filter is not required until 3600 Å. At 3600 Å, soft glass which eliminates all light below about 3200 Å is moved into place. At about 6100 Å, a Schott OG-3 filter, 1 mm thick, which eliminates all light below about 5600 Å is moved into place. Thus, second-order transmission over the entire wavelength range of 1900 to 11,000 Å is eliminated.

The recorder is of novel design, containing many of the best features of both drum and strip-chart recorders. When recording spectra, the recorder operates as a normal drum recorder, with the advantages of inherently accurate synchronization with wavelength and ease of repeat scanning.

Chart paper is supplied in a continuous roll, which is stored *inside* the drum. Paper feeds out through a slot over a sprocket-type guide roll around the drum, and under a clamping bar which is also used for tearing off completed recordings. After completion of a recording, paper change is a one-handed operation. Release the clamping bar; pull the recording off, under the bar. When the next section of paper has been pulled into place, clamp the bar and tear off the completed recording.

The recorder may also be used for recording as a function of time. For this type of operation, the drum is held in a fixed position. The paper feeds out of the drum and down over a writing platen to a take-up spool. A special cover snaps over the open right-end of the drum. This cover contains a motor which drives the guide roll at the desired speed.

Provision for control of the temperature of the fluorescent sample is supplied by passing temperature controlled water from an external source through a heat exchanger in intimate contact with the sample cell holder.

References and Notes

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