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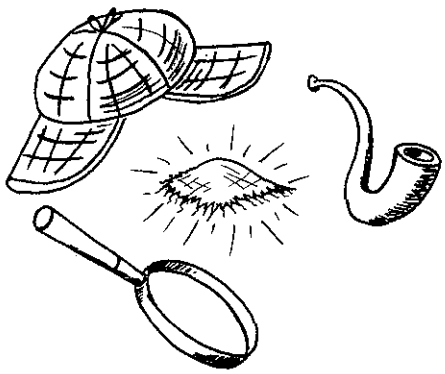
PHOSPHORESCENCE PROVIDES THE CLUES

by S. DiGregorio and R. Kaminski

You shudder, raising your collar up around your ears. It is cold in these marshes. It is always cold in these marshes and the dampness has a peculiar knack of working its way inside your clothing. The darkness makes every cautious step a challenge as you try to keep your feet on the path and out of the watery bogs to either side. Through the drifting haze that surrounds you, a light appears in the distance. It might be a house, you think at first—but then it begins to move, wandering like a sailboat tacking desperately over a foggy sea. As it comes closer, you gradually recognize the shape of a man, his arms flailing wildly—his skin, his whole form, a mass of glowing fire. Again you shudder, realizing that this apparition has been blamed for the murders committed in the area over the past weeks. Now it stands there blocking your path. You freeze—and suddenly the beast rushes by, knocking you into the bog. But, in his haste, a piece of that luminescent skin is snagged and torn away by the branch of a tree. That single shred of evidence, in the expert hands of your friend Sherlock Holmes, will be enough to identify this beast. After he has pulled you out of the bog, Holmes's deductions lead him to the beast's lair—a dusty room in an old boardinghouse. When he flicks off the light, there, suspended in midair, is a glowing shirt "treated with phosphorus." Thus, through his knowledge of phosphorescence, Holmes is able to strip away the mystery from the Scarlet Claw.

In movies and literature, detectives often exploit the most esoteric bits of trivia to solve their cases. For Holmes the critical clue was material familiar even to children who have played with trinkets and paints radiant with this cold light. Still, it is surprising how little has been done until now to transform this curio into an effective tool. The word phosphor comes from the Greek "phos," light, and



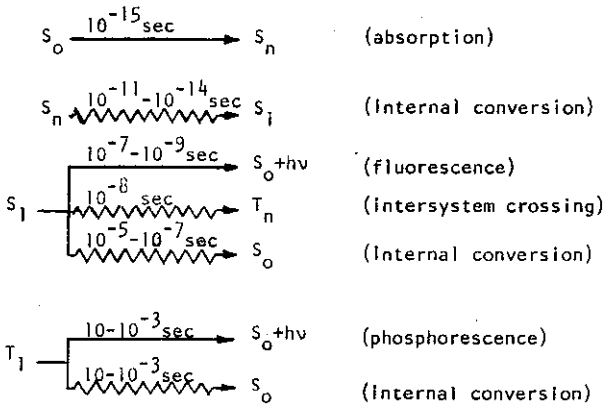
"phero," I bear. Though originally it referred to any material that glowed in the dark (justifying Holmes's use of the word "phosphorus") and has been applied to a chaotic array of nimbi and scintillations, in the last few decades its meaning has undergone constant refinement. Phosphorescence, or afterglow, is a special case of a general class of phenomena known as luminescence which includes bioluminescence, the emission of light by chemical processes (chemiluminescence) inside living organisms such as lightning bugs, and fluorescence, the emission of light by a material during exposure to an outside source of radiation such as the sun or a lamp. Ostensibly, phosphorescence differs from the latter in that the sample continues to emit light long after the exciting radiation has been removed.

Luminescence, in all its various forms, has always been a diverting and entertaining phenomenon. From the time man learned to speak well enough to pass on his ideas, he has pondered over these extraordinary things that seemed to have captured a small piece of the sun. Rabbinical tradition, for instance, would have us believe Noah carried a luminous stone in the Ark and that stone shone brighter by night than by day, allowing him to distinguish between the two when the sun and moon were hidden by clouds during the forty days of rain.

Another ancient, though undateable, tale comes out of India. There, it is said, one cobra out of every twenty carries a luminous stone in its mouth to attract fireflies which it then proceeds to eat with undisguised relish. These stones were called "Naja Kallu" and more than one colonial Englishman has testified to having seen them.

The first verifiably authentic reference to luminescence does not appear until 1500 B.C. when glowworms were mentioned in the Chinese *Shih Ching* or *Book of Odes*. Thereafter, chemiluminescence is encountered in the works of Aristotle. Pliny the Elder, who had the misfortune to perish in the eruption of Mt. Vesuvius in 79 A.D., catalogued, in his *Historia Naturalis*, a host of luminescent phenomena including glowworms, the lantern fish, a luminous mollusc, as well as luminous wood and fungus.

Still, we are, as Sherlock Holmes was, primarily interested in phosphorescence and, though Cellini writes of a diamond that would shine after being exposed to light (1568) and Paracelsus (1490-1555) discusses a method for extracting what appears to be the element phosphorus from urine, it was not until 1603 that a substance exhibiting afterglow was finally prepared. In that year, an Italian cobbler named Vincenzo Cascariolo (who found time between pairs of shoes to dabble in alchemy) synthesized what he called "lapis solaris" or sunstone. Cascariolo claimed this material, a local variety of barium sulphate laced with sulfur, "imbibed" the light of the sun and waited till after dark to emit it. This substance later became famous as the Bolognian Stone and alchemists received it with tremendous enthusiasm. Though they nurtured the hope that this was the long-sought philosophers' stone and its action would transform the baser metals into gold, we have no record of how they managed to deal with their disappointment.



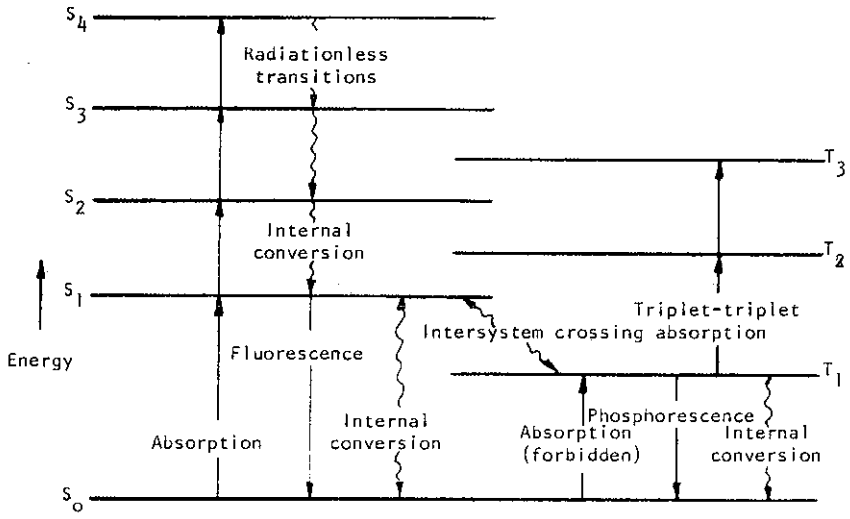


Fig 1 Energy level diagram for a molecule or atom. S refers to singlet and T to triplet states. Subscript numbers identify individual states with S_0 as the ground State.

The transition from wizardry and folklore to systematic scientific investigation, however, did not occur until 1713 when Francisco Maria Zanotti began to examine the emission and absorption spectra of phosphors, concluding that the color of emitted light was a characteristic of the individual material. Then, in 1858, Edmond Becquerel invented the phosphoroscope, a mechanical device consisting of two slotted discs with a common rotational axis. By placing a sample between the discs and alternating the position of the slits, he was able to excite and observe phosphorescence when the discs were spun. This device aided Becquerel in studies of intensity, spectra, and temperature effects which eventually led him to propose the exponential law of decay.

Fluorescence and Phosphorescence

As with any phenomenon with a long history, luminescence has become a victim of the words chosen to describe it. Over the years, the terms phosphorescence and fluorescence, for example, have been freely applied to all things that glowed in the dark, no matter what process was originally responsible for making them glow. Thus, references occur to phosphorescent fish, fungus, corpses, and other types of chemiluminescence.

Yet, by the middle of the 20th century it was known that fluorescence and phosphorescence were actually two of the routes an excited atom or molecule can take to return to the ground state. To trace the itinerary of such a trip, we can consult the road map in Fig 1.

A molecule or atom begins its journey in the singlet* ground state S_0 when it absorbs a photon and is elevated into one of the excited singlet states in the manifold S_1, S_2, . . . S_n. In the event that the excited states of S_2 through S_n are reached, the molecule will surrender some of its energy in the form of heat (internal conversion), as it cascades down to the S_1 state. From the S_1 level, three possible routes can ultimately return the molecule to the ground state S_0:

1. An internal conversion can again take place with energy released as heat.
2. A photon may be ejected, its energy corresponding to the difference in energy between the excited state of the molecule and the ground state. The amount of time the molecule spends in the excited state is typically 10^{-8} seconds, and the emitted radiation is termined "fluorescence." Generally, fluorescence occurs from the lowest vibrational excited state of S_1 to one of the various vibrational states that form the fine structure of S_0.
3. A molecule in an excited state may drop into a lower energy state T_n by a variant of internal conversion referred to as "intersystem crossing." The excited states of T are triplet states which result from a change in spin of one of the valence electrons of the molecule. While transitions between different excited T states may easily occur with the absorption or liberation of energy, transitions between a T and S state are quantum mechanically "forbidden." But this is misleading. Forbidden transitions do occasionally happen, the molecule dwelling in the T or metastable state for a long period of time before dropping to a lower S state. If a photon is released during the forbidden transition, "phosphorescence" results. Phosphorescence is then spectrally characteristic of a triplet state to singlet state transition, the excursion between excitation and emission taking anywhere from 10^{-7} to many seconds.

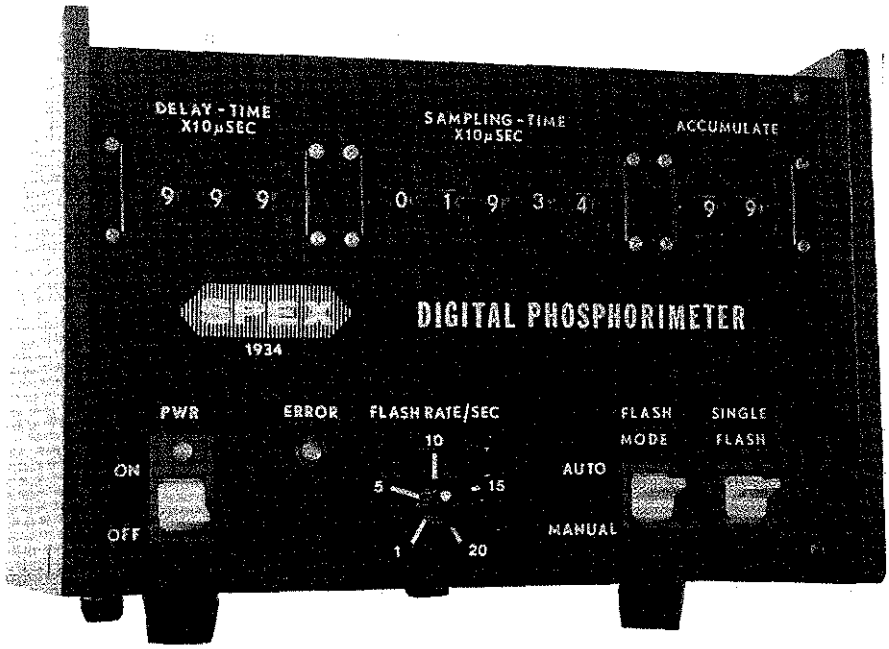
Incidentally, long emission delays may also result from other mechanisms. An alternative to the forbidden transition from a T to a lower S state is the process of "delayed" fluorescence, whereby the excited molecule in a T state reverts to an excited S state through the intersystem crossing route. Subsequent transition from the excited S_1 state to the S_0 state can now produce fluorescence. The resulting emission spectrum appears to be identical to a conventional fluorescence spectrum, but with a longer delay introduced while the molecule detoured through the T state.

Although fluorescence and phosphorescence are both powerful molecular probes, the added dimension of time makes phosphorescence uniquely suited for this pursuit. Phosphorescence, for instance, may well interfere with fluorescence spectra

*Singlet and triplet designations usually refer to magnetic (Zeeman) splitting of spectral lines. Singlet lines remain unaffected by a magnetic field. Triplets split into three components.

rendering analysis, at best, difficult. However, the extended lifetime of phosphorescent emissions insures that, if we are patient enough to wait longer than 10^{-8} seconds, the fluorescence will have vanished. This permits the gathering of data normally inaccessible or hopelessly obscured.

Despite some inconsistencies and, perhaps, confusion in certain definitions of fluorescence, delayed fluorescence, and phosphorescence, all may fortunately be studied with the Spex Phosphorimeter. It is a unique and pragmatic device, treating the phenomena according to lifetime without regard to the processes involved. No longer can phosphorescence be relegated to a subservient role among techniques for examining complex molecular structures. And to take advantage of this a relatively simple procedure suffices. The sample is exposed to a source of radiation and then that source is removed. After a selected interval of time the emission or excitation spectrum is scanned and recorded. Alternately, the wavelength is kept constant and the length of the delay before emission is observed is varied so a decay curve can be calculated which traces the intensity of the phosphorescence with time. Either method will provide substantial information about the substance.



MODEL 1934 PULSED-LAMP PHOSPHORIMETER

Phosphorimeters, Past and Present

From Becquerel to Winefordner (1) advances in technology were evidenced by better excitation sources, emission detectors, and monochromators. But the ability to control the crucial delay before sampling remained stubbornly unchanged and dimensionally shallow. Slotted discs and rotating cans limited to around 1 msec delay are still common.

Propelling phosphorimetry out of the 19th century, the new Spex 1934 Phosphorimeter accessory to our 1902 FLUOROLOG spectrofluorometer (2,3) reaches well past the limit of the old technique, deep into the microsecond range. Gone are the continuous lamp and mechanical chopper. A pulsed source and fast, digital, signal processing circuitry carefully interfaced to the FLUOROLOG, comprise the new instrument which is based on the design described by Fisher and Winefordner.

Essentially, the Spex Phosphorimeter is a programmed drone which relies on the FLUOROLOG's monochromators and data facilities. Not only does it trigger the flash, but it also gates the counter output before sampling the emission. It can then continue to repeat that sequence, accumulating the data for introduction into the FLUOROLOG. The best part of this scheme is that the analyst can choose all the variables: delay time, sampling time, flash frequency, and number of cycles over which the data are accumulated.

The heart of the system is a xenon lamp having a flash duration of 3 μsec at ½ peak amplitude (Fig 2). The output of this lamp is optically monitored by the phosphorimeter circuitry, and each time the lamp flashes, this circuitry establishes how long to wait before sampling. Then, based on the setting of a two-digit switch with a range from 1 to 99, the emission data is either passed on to the FLUOROLOG or accumulated over another cycle.

Delays may be set as small as 1 μsec or as large as 10 msec, with the option of 1 or 10 μsec steps provided by a multiplier switch. Data gathering is thus enhanced by the ability to creep extremely close to the excitation pulse. (Below 10 μsec some correction may be required as the afterglow of the light pulse becomes significant.) Duration of the sampling time is adjustable in 10 μsec steps from 10 μsec to 999,990 μsec—a full five orders of magnitude. The lamp itself operates either in a single flash mode without interfering with the relationship between the other variables (sampling, delay and accumulation), or it can be set to flash from 1 to 20 times per second automatically. And, to keep all the settings consistent with one another, an error message light warns when too high a flash rate and too long a sampling time would lead to overlapping, the flash recurring before the sample time has expired.

When the FLUOROLOG is in the scan mode, the Phosphorimeter supplies it with intensity data that may be plotted against wavelength by the FLUOROLOG's recorder. On the other hand, decay rates can readily be calculated from the information displayed on the FLUOROLOG's data register.

Phosphorescence Emission Spectra

Once an analyst begins to focus attention upon a particular phosphor, there are two significant sources of interference that may disguise the characteristics of the spectra and mask pertinent lines. No such problems plague the Spex Phosphorimeter. Not only can the fluorescence of a sample be rejected, but the spectrum of any one phosphor contained in a mixture of phosphors may be enhanced on the basis of its lifetime.

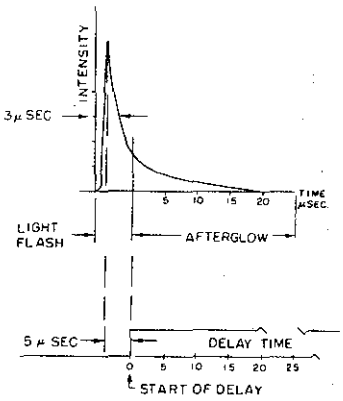


Fig 2 Xenon pulsed lamp curve and timing sequence.

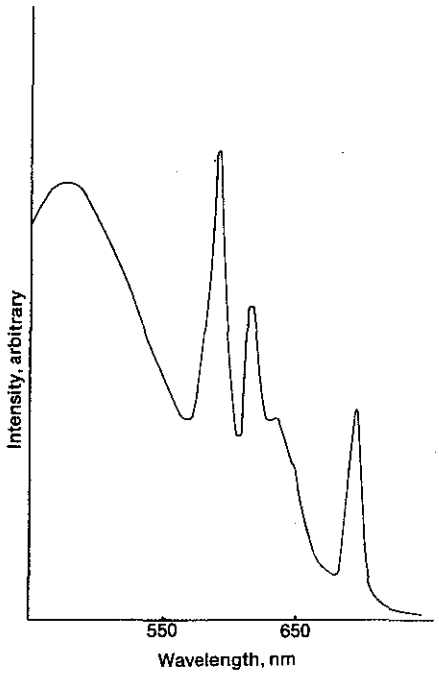


Fig 3a Emission scan of $EuCl_3$ in a solution of Zn-protoporphyrin obtained on the FLUOROLOG with a 450 watt cw xenon lamp. The broad background of the porphyrin fluorescence effectively masks the europium phosphorescence bands.

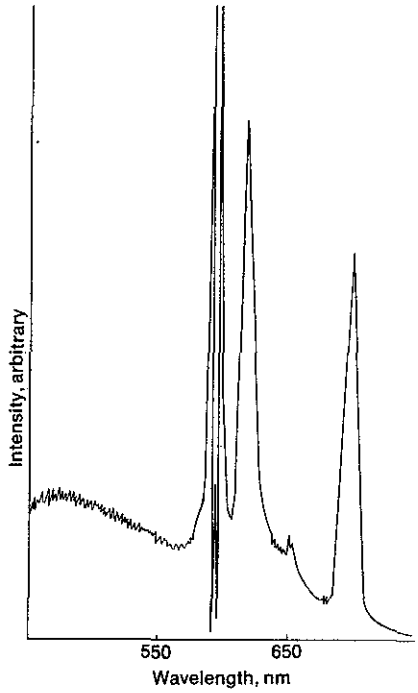


Fig 3b The pulsed lamp supplied with the Phosphorimeter has replaced the cw lamp, but no delay has yet been inserted between excitation and sampling. Still, the europium phosphorescence is enhanced. Note the wraparound feature of the FLUOROLOG in the main peak.

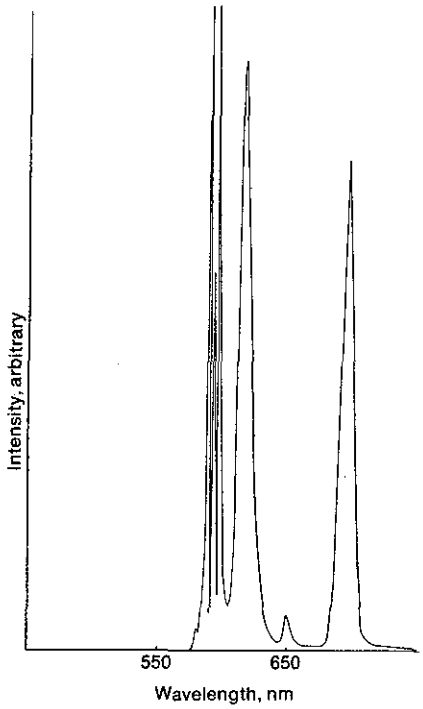


Fig 3c A 30μsec delay is introduced and the fluorescence is now extinct.

Any delay longer than 1 μsec will automatically discriminate against fluorescence. Fig 3a is a typical fluorescence spectrum taken with a cw lamp and no delay between excitation and emission measurement. The sample is a mixture of a fluorescing and a phosphorescing substance. Interference between the two tends to smudge the spectral fingerprint. Fig 3b shows what happens when the cw lamp is replaced by the Phosphorimeter's pulsed source. Even though no delay was interspersed, the fluorescence background contracted appreciably and the characteristic peaks of the phosphorescent substance begin to stand out. Finally, in Fig 3c, with a 30 μsec delay, the fluorescence can be seen to have followed the wooly mammoth into extinction, leaving only a finely resolved phosphorescence spectrum.

When a sample is encountered that contains more than one phosphor, it may be possible to enhance the emission of one of them so it dominates the spectrum. Suppose, for instance, that the sample has two components of disparate lifetimes. A short delay and sampling time will favor the quickly decaying phosphor, inhibiting the slower one from accumulating a significant signal. On the other hand, increasing the delay to a point where phosphorescence of the first component has considerably decayed will reduce its contribution and result in a spectrum dominated by the more persistent phosphor. The spectra of $EuCl_3$ and $TbCl_3$ in separate solutions illustrate this effect (Fig 4). The spectra for a mixture of the two is shown in Fig 5. As the delay is increased, the contribution from the short-lived $EuCl_3$ is seen to wane until the $TbCl_3$ spectral features eventually prevail.

When only a single phosphor, or the integral spectrum of all the phosphors in a mixture is sought, a short delay time (about 10 μsec) and a long sampling time (up to twice the lifetime of the slowest decaying phosphor) are optimum. Of course, the flash rate should be adjusted so the interval between flashes is slightly longer than the sum of the delay and sampling times.

Lifetime Determinations

Perhaps the most revolutionary consequence of adding the dimension of time to spectrofluorometry is this Phosphorimeter's talent for tracking the decay rate of a phosphor. This enormously valuable parameter has significant applications:

1. Once the individual lifetimes of phosphors have been obtained, subsequent determinations of decay rates for a mixture of phosphors provide a potent method of qualitative, as well as quantitative, analysis.
2. Lifetimes are the predominant factor governing the selection of materials for cathode-ray tube and other persistent optical displays.
3. The metastable states responsible for phosphorescence also form an energy transfer mechanism that plays a prominent role in photochemistry. An understanding of the lifetimes of these states may be the key to an optimal photochemical reaction.

Becquerel's ground-breaking observations on the way phosphorescence varies systematically with time have evolved into a technique for determining lifetimes of phosphors by measuring emission after different delays.

Since it is now known that the decay process is first order, the phosphorescence intensity, I, evolves according to the following equation:

$$dI/dt = -kI \qquad [1]$$

Here k is a rate constant identified as the inverse of the average lifetime of the particular phosphor ($k = 1/T$). Integration of equation 1 yields

$$I = I_0 e^{-kt} = I_0 e^{-t/T} \qquad [2]$$

where I_0 represents the intensity at $t=0$. Obviously, when $t=T$ the intensity will have decayed by a factor of 1/e.

If we now take the logarithm of equation 2, we get

$$\log I = -2.3\, t/T + \log I_0 \qquad [3]$$

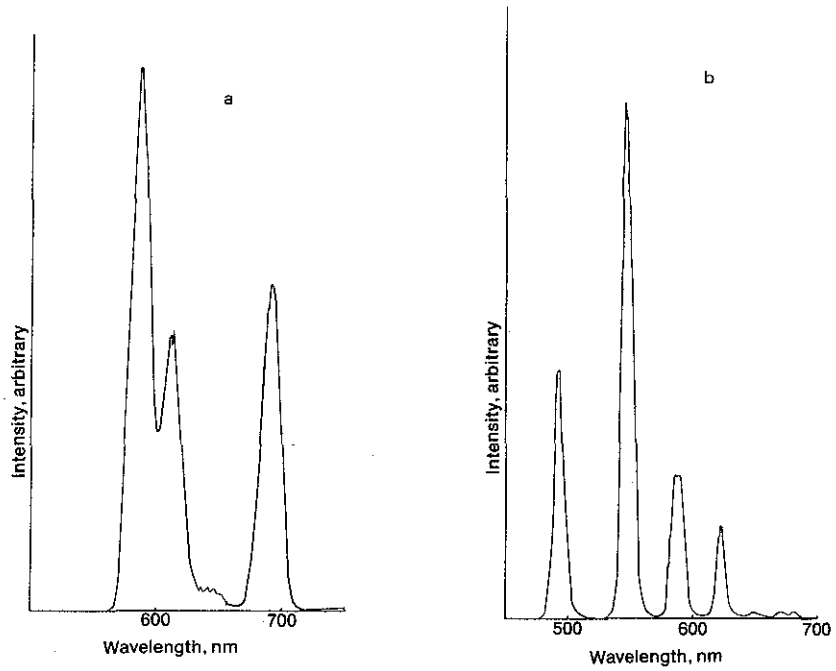


Fig 4 Spectra of $EuCl_3$(a) and $TbCl_3$(b). Excitation wavelength was 395 nm, sample time 1 msec, delay time 30 μsec.

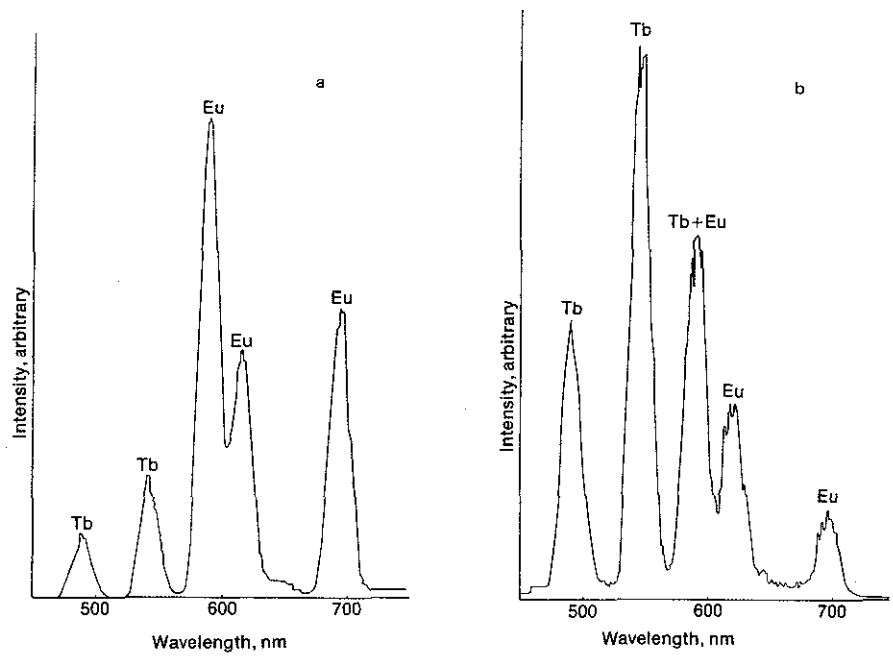


Fig 5 Emission spectra of a mixture of $EuCl_3$ and $TbCl_3$ for delay times of 10 μsec(a) and 400 μsec(b) illustrate a technique for enhancing one component at the expense of another on the basis of lifetimes.

After differentiation, this becomes

$$T = -2.3\ dt/d(\log I) \qquad [4]$$

Equation 3 has the same form as the straight line

$$y = kx + \text{Constant} \qquad [5]$$

and so the slope of the linear portion of a plot of log I vs t will provide the negative inverse of the lifetime (T) for the excited state responsible for the observed phosphorescence emission. In a practical sense, T is determined from the time required for the intensity to change by one decade divided by 2.3.

Perhaps the best way to accent how the wide dynamic range and innumerable selections for decay and sample times available on the Phosphorimeter surround even the most persistent phosphor is to group decay periods into three categories: moderate, fast, and slow.

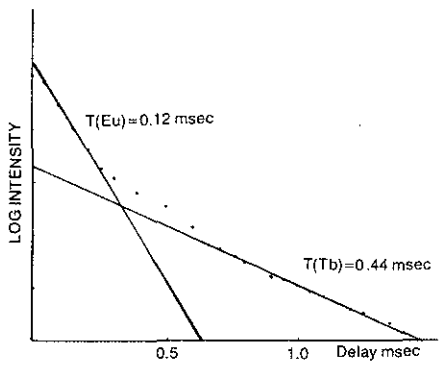


Fig 6 Decay curve for a mixture of $EuCl_3$ and $TbCl_3$ clearly demonstrates the break between contributions of each. Lifetimes are calculated from the slope of each component. This method is applicable to phosphors with fast or moderate persistence (T=10-10,000 μsec).

Moderate decay rates are those with lifetimes between 100 and 10,000 μsec. A direct decay curve of individual phosphors or mixtures in this category can be acquired by stepping through the delay range from 10 μsec to 10 msec with the sampling time set to a value that is short compared with the lifetime of the phosphor (about 10%). (Since the delay is exponential, longer values of sampling time tend to distort the curve, necessitating mathematical corrections to the data.) The lifetime can be computed directly from equation 4 or reconstructed from the slope of the plotted logarithmic curve. Fig 6 is a decay curve for a mixture of $TbCl_3$ and $EuCl_3$ which clearly shows the break between the contributions of the two components.

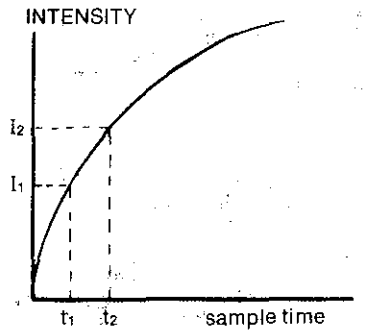


Fig 7a For long-lived phosphors, intensity may be plotted against sample time while holding the delay constant.

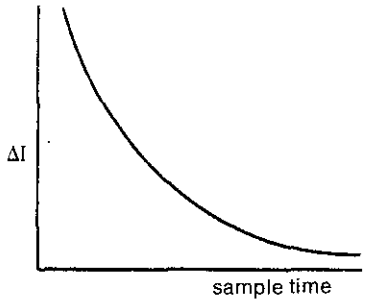


Fig 7b Plotting the change in intensity with time yields a decay curve.

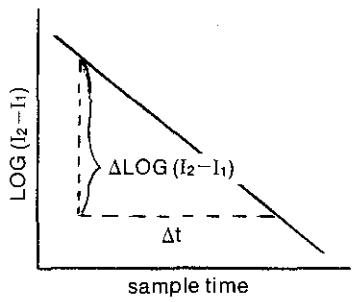


Fig 7c Finally, a plot of the logarithm of the change in intensity reveals the lifetime of the phosphor from $T=-2.3\Delta t/[\Delta \log (I_2-I_1)]$.

Fast decay rates (T=10-100 μsec) are accessible with the short delay range of the Phosphorimeter which encompasses times between 1 and 100 μsec. However, the minimum sampling time provided is 10 μsec and unless the change of intensity during the sample time is taken into account, a decay curve plotted directly from this data for phosphors in the short-band portion of this range will not produce a perfectly exponential function. The experimenter need only be a bit more careful here.

Slow decay rates have lifetimes from 10 msec to several seconds. Although the longest delay time available is 10 msec, lifetimes above this are measurable by varying the sample time. When the delay is set to an arbitrary constant, say 10 msec, and the intensity is examined as a function of sampling time, the results will be similar to Fig 7a. A decay curve (Fig 7b) can now be generated from this data by plotting the change in intensity between sets of points ($\Delta I=I_2-I_1$) against the mean value of each successive sampling-time point. If the sample time intervals (t_2-t_1) are all equal, ΔI is actually the derivative of the original curve and reflects the difference between successive values of I in the first plot. If, finally, log(ΔI) is plotted (Fig 7c), the lifetime of the phosphor may be determined from the slope as explained earlier.

An alternative to this method is to continue measuring the intensity as a function of sampling time until a definite plateau is reached. The final value of I on this plateau is equivalent to I_0 and if the value I_0-I_0/e is now located on the curve, the corresponding magnitude of t will equal

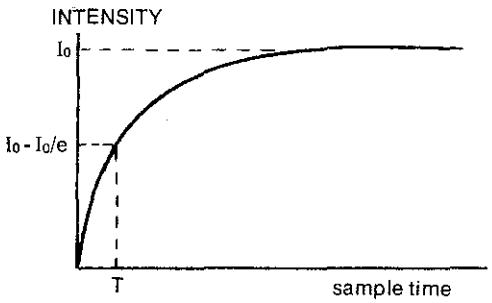


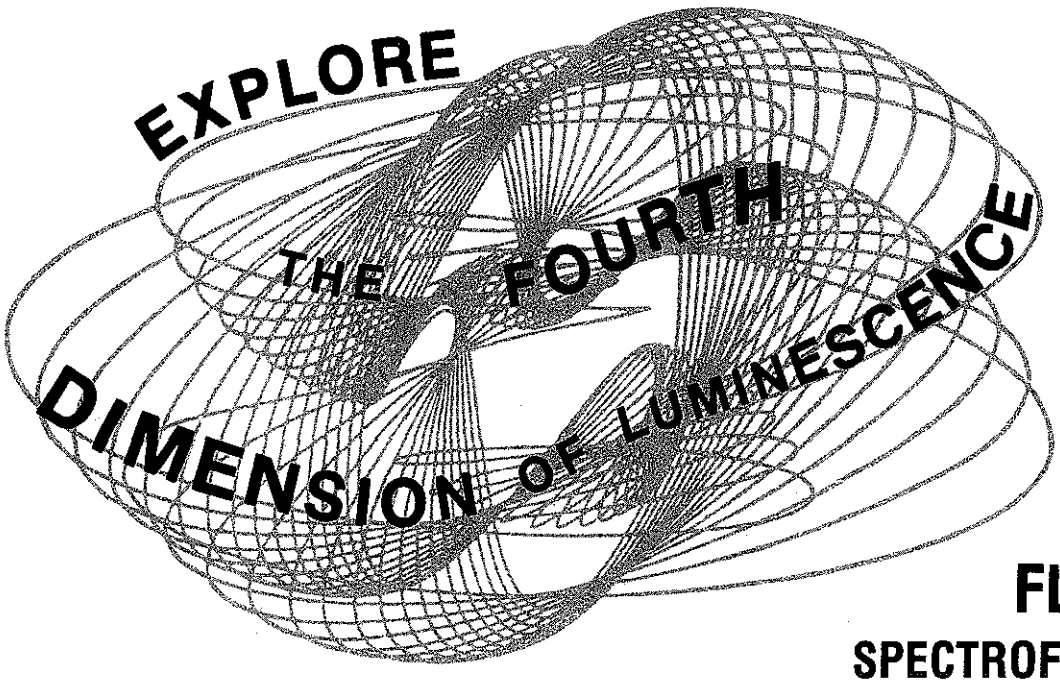
Fig 8 An alternate method for determining lifetimes of long-lived phosphors. If the plot in Fig 7a is continued until it reaches a plateau, the maximum value of the intensity is equivalent to I_0. By locating I_0-I_0/e in the curve, the corresponding time coordinate is T, the lifetime.

the lifetime of the phosphor (equation 2). The results of such a determination are shown in Fig 8.

Even as we illustrate these applications, it must be kept in mind that the Phosphorimeter is not prejudiced in favor of any particular definition of phosphorescence. On the contrary, the virtue of its design encourages the study of any phenomenon exhibiting time-dependent emission, irrespective of the mechanism responsible for the delay. Just as the adventure of the Scarlet Claw was only one of many cases unscrambled by Sherlock Holmes, fluorescence rejection is but one of many suitable challenges for this new Phosphorimeter. Its prowess can be demonstrated on your samples in our laboratory on request.

REFERENCES

1. R.P. Fisher, J.D. Winefordner, Anal. Chem., **44**, 948 (1972)
2. "Spectrofluorometry Moves Forward With the Fluorolog," *The Spex Speaker, XX*, No. 4, 1 (1975)
3. "Spex Fluorolog: Some Illuminating Aspects," *The Spex Speaker, XXI*, No. 3, 1 (1976)



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The responsiveness of the FLUOROLOG to those unique situations so often encountered in the lab is embodied in its operation. Three detectors (Emission, Reference and Transmission) function individually or in combined modes: E, R, E/R, E/(R-T), T/R, LOG R/T. Twin double monochromators (f/4, 0.22 m Czerny-Turner with subtractive dispersion) scan at speeds from 0.1 to 10 nm/sec and step (0.1 to 10 nm/step) in time intervals from 0.1 to 500 sec, or after a selected amount of excitation radiation has reached the reference detector. Output is digital and analog.

Isolate time-dependent emissions with as little as 1 μsec between excitation and emission. A pulsed xenon flash-lamp combined with high-precision processing circuitry has created an extremely versatile and accomodating instrument. Delays are variable from 1 μsec to 10msec; sampling times from 10 μsec to 0.9 sec, with data accumulated up to 99 flashes.

UNSURPASSED FOR HANDLING EASE AND ACCURACY IN:

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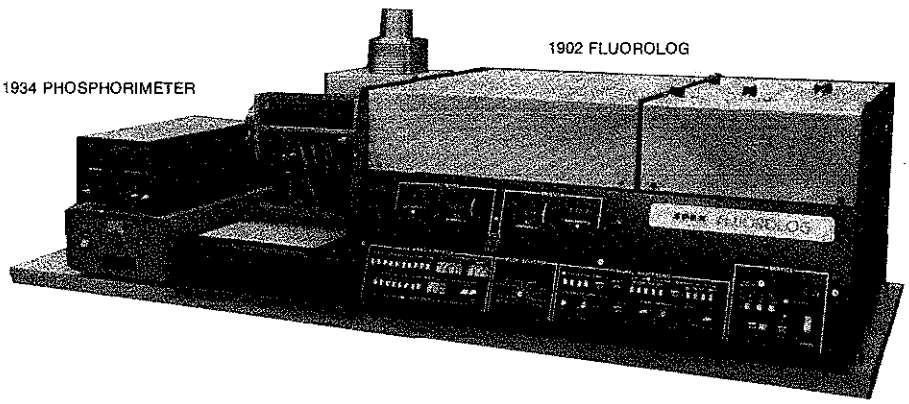
.. TIME RESOLUTIONS OF A MIXTURE OF COMPONENTS
.. IMPLEMENTING PHOSPHORESCENT IONS AS ENVIRONMENTAL PROBES



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.. BOX-CAR INTEGRATION BY DIGITAL ELECTRONICS
.. LARGE, REMOVABLE SAMPLE COMPARTMENT
.. MODULAR CONSTRUCTION FOR FLEXIBILITY WITH SOURCES AND DETECTORS

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