Time-Resolved Phosphorimetry

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Phosphorescence and fluorescence are both processes in which radiation is emitted by a molecule following an initial excitation. Both forms of photoluminescence are the basis of analytical methods of great utility, but methods based on phosphorescence measurements are particularly versatile and extremely sensitive. This Account deals with the principles and the instrumentation involved, as well as with the applications of time-resolved phosphorimetry.

Phosphorescence and fluorescence are usually discussed with reference to a scheme, shown in Figure 1, originated by Jablonski[1] and put on a firm footing by Lewis and Kasha.[2] Absorption of a photon populates any of a number of excited singlet states. For most molecules in condensed phase, the fluorescence spectra are independent of the frequency of the exciting light. This indicates that regardless of the level to which the molecule is initially excited, it relaxes to some common level before emission. This common level is, of course, the lowest vibrational level of the first excited singlet state, S_1. The relaxation processes, known as "internal conversions," are very rapid between singlet levels, S_2, S_3. . . and S_1 and generally quite slow between S_1 and S_0. The fluorescence yield, defined as the number of photons emitted in the fluorescence process divided by the number of photons absorbed, is often less than 1, thus indicating the presence of another process depleting the excited singlet population. A vast amount of evidence of different sorts shows that another relaxation pathway is crossing to the triplet manifold. This process, called "intersystem crossing," is forbidden to first order, since it does not conserve spin, but is made allowed by spin–orbit coupling. It occurs at a rate which is competitive with fluorescence, *i.e.*, with a half-life of 10^{-7} to 10^{-9} sec. The emission from the triplet manifold, phosphorescence, typically has a half-life of 1 to 10 sec. Phosphorescence also originates from the lowest vibrational level and so occurs at longer wavelengths than either fluorescence or absorption.

Phosphorescence spectra are characteristic of the emitting molecule with an intensity that is directly proportional to the concentration of the absorbing species (assuming that the absorbance of the sample solution is less than about 0.04). This is the basis of the analytical method called "phosphorimetry."[3] The organic system to be analyzed is cooled to liquid nitrogen temperature to minimize collisional quenching processes and thermal activation of the molecule in the triplet state back to the excited singlet state. By using the appropriate solvent,[3] clear rigid glasses can be obtained. By means of a mechanical rotating can or disk phosphoroscope to modulate the exciting radiation to the sample out of phase with the measurement of phosphorescence, the phosphorescence can be measured in the absence of fluorescence and of scattered incident light. Thus it is possible to measure extremely low concentrations of many organic molecules, *e.g.*, the limits of detection of many drugs is in the range of 0.0001 to 1 μg/ml. By means of selective excitation of species and selective measurement of the phosphorescence, it is often possible to obtain great specificity as well as sensitivity of analyses.

If two or more molecules possess similar absorption and phosphorescence spectra, it is still possible to measure concentrations of species in complex mixtures if the species of concern possess different phosphorescence lifetimes. In this technique, the phosphorescence signal is measured a short time interval after excitation is terminated. By proper choice of the time delay (*i.e.*, the time between end of excitation and start of measurement), it is possible to resolve the phosphorescence signals of a complex mixture. It is this method, time-resolved phosphorimetry, which we shall now investigate.

Phosphorescence Lifetime, τ

The phosphorescence of any organic phosphor persists for some time after termination of excitation. For an organic phosphor in a rigid organic glass at low temperature and at a low concentration, the decay of phosphorescence intensity, I_t, as a function of time, t, after termination of excitation is given by eq 1, where I_0 is

$$I_t = I_0 e^{-t/\tau} \quad (1)$$

the intensity of phosphorescence at time $t = 0$ and τ is the mean phosphorescence lifetime. Since most phosphorescence lifetimes are of the order of seconds, they can be simply measured by recording the photodetector output as a function of time, t. The mean phosphorescence lifetime, τ, for an organic phosphor in rigid organic glasses at low concentrations is given[3] by eq 2,

$$\tau = 1/K \quad (2)$$

where K is the total pseudo-first-order rate constant for deactivation of the triplet level by radiational and nonradiational means. Therefore τ is expected to be a function of both the *specific molecule* (phosphor) and the *specific environment and temperature* (solvent type, im-

(1) A. Jablonski, *Z. Physik.*, **94**, 38 (1935).
(2) G. N. Lewis and M. J. Kasha, *J. Amer. Chem. Soc.*, **66**, 2100 (1944).
(3) W. J. McCarthy and J. D. Winefordner, *J. Chem. Educ.*, **44** 136 (1967).

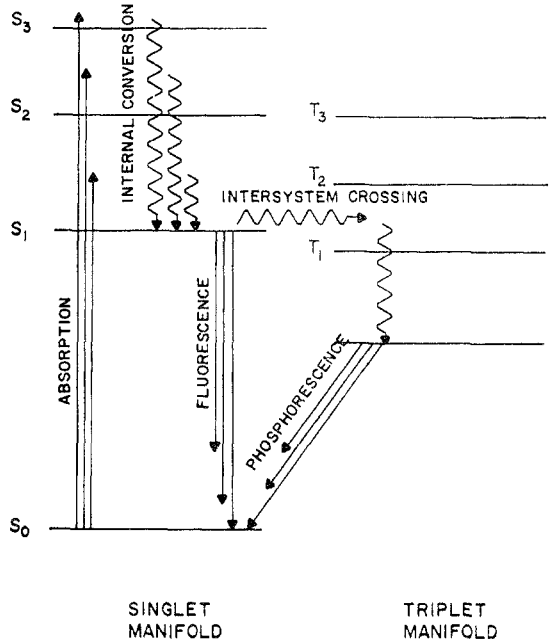

Figure 1. Energy level diagram to represent fluorescence and phosphorescence.

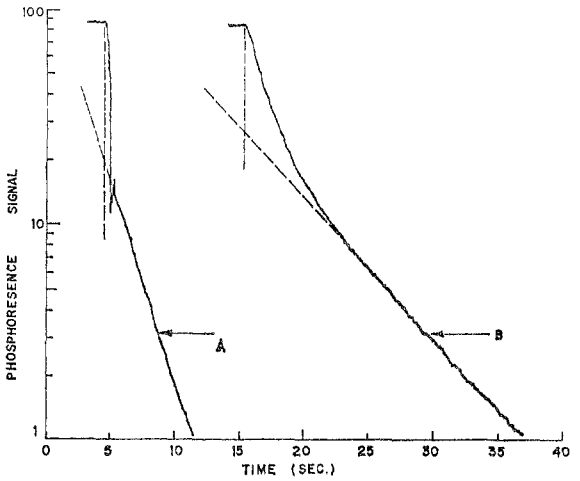

Figure 2. (A) Logarithmic decay curve for benzoic acid (3.00×10^{-4} M, τ = 2.4 sec)-benzaldehyde (6.45×10^{-5} M, τ <0.1 sec); (B) logarithmic decay curve for tryptophan (6.04×10^{-6} M, τ = 6.4 sec)-tyrosine (5.11×10^{-6} M, τ = 1.4 sec).

purities present, etc.). Phosphorescence lifetimes are quite short for carbonyl compounds as well as substituted aromatic hydrocarbons containing a heavy atom. The phosphorescence lifetimes for aromatic hydrocarbons decrease as the phosphorescence transition shifts to longer wavelengths since the triplet-to-singlet intersystem crossing rate increases rapidly. The mean phosphorescence lifetimes are essentially independent of the absorber concentration, and so for a given solvent and temperature, τ is characteristic of only the substance of concern. Therefore τ values are useful for identification. The lifetimes of most organic phosphors vary little with solvent temperatures if $T \lesssim 77$°K but considerably if $T \gtrsim 200$°K.

Methods of Time Resolution

Use of Mechanical Chopping. In these methods, the rotating can or Becquerel disk[4] phosphoroscopes are used to modulate out of phase the exciting radiation and the measurement of the emitted radiation (see following section). Two techniques, which have been used to achieve time resolution for quantitative analysis, are the *graphical method* and the *phosphoroscopic resolution method.*

The Graphical Method. In this technique, the decrease in the logarithm of the phosphorescence intensity *vs.* time after termination of excitation is monitored directly. This technique is quite similar to the use of a semilogarithmic plot of disintegration rate *vs.* time after termination of excitation of isotopes differing in half-lives for measuring radioactive isotopic species and their concentrations.[5] St. John and Winefordner[6] used a commercial fluorimeter with phosphoroscopic attachment and a logarithmic converter system. Their system was linear over three decades of intensity for lifetimes as short as 0.5 sec. Using this instrument, the intensity signal for the slow decaying species was found by extrapolation of the linear portion of the curve at large t to $t = 0$, and the intensity signal for the fast decaying species was found by subtraction of the slow decaying signal from the mixture at $t = 0$. The concentrations of the slow and fast decaying species were then read directly from analytical working curves prepared using the pure phosphors at different concentration levels. Of course, if the slow and fast decaying species absorb appreciably in the regions of excitation, a correction for this has to be made.

In Figure 2, logarithmic decay curves for mixtures of benzoic acid–benzaldehyde and tryptophan–tyrosine are given.[6] St. John and Winefordner[6] measured synthetic mixtures of those species (benzoic acid 3.00×10^{-4} M to 3.00×10^{-5} M and benzaldehyde 6.45×10^{-5} M to 2.03×10^{-4} M; tryptophan 6.04×10^{-6} M to 2.53×10^{-6} M and tyrosine 5.32×10^{-6} M to 4.73×10^{-6} M) and obtained recoveries with relative errors of about 3%. This error was primarily a result of sample cell positioning error and of location of the intersection point of the semilogarithmic plot. The mixture of tryptophan–tyrosine was easily resolvable[6] even though the ratio of decay times was only 4.6. The concentration ratio of the components in a two-component mixture can vary considerably if the analyst is willing to sacrifice sensitivity and accuracy of measurement of one or both of the components.

Several *other uses* of the measurement system should be mentioned. Phosphorescence decay times greater than 0.2 sec can be simply determined by measuring the slope of the logarithmic decay curve for the component of concern. It is also simple to check the purity of any phosphorescent molecule by verifying the linearity of the logarithmic decay curve. It should, however, be pointed out that nonexponential decays have been ob-

(4) J. D. Winefordner, W. J. McCarthy, and P. A. St. John, *Methods Biochemical Anal.*, 15, 369 (1967); M. Zander, "Phosphorimetry," Academic Press, New York, N. Y., 1968.
(5) G. E. Boyd, *Anal. Chem.*, 21, 335 (1949).
(6) P. A. St. John and J. D. Winefordner, *ibid.*, 39, 500 (1967).

served for some pure organic phosphors.[7] For example, St. John and Winefordner[6] have failed to obtain exponential decays for high-purity benzyl alcohol and toluene in absolute ethanol.

The Phosphoroscopic Resolution Method. Keirs, Britt, and Wentworth[8] first described the technique called phosphoroscopic resolution in which differences in phosphorescence lifetimes were utilized as a means of selective analysis. Phosphors differing in decay times were resolved by means of variation of the phosphoroscope shutter delay time (time between the termination of excitation and observation of emission). They were able to minimize the phosphorescence due to rapidly decaying phosphors while measuring the phosphorescence of slowly decaying phosphors with little loss of sensitivity. The separation of two phosphors with very short decay times was very much less sensitive and applicable only over a very limited range of concentration. More recently, Hollifield and Winefordner[9] have described a versatile, modular single disk phosphoroscope of variable speed for phosphoroscopic resolution of several complex mixtures of organic molecules.

Keirs, Britt, and Wentworth[8] were able to resolve a mixture of acetophenone (τ = 0.008 sec, concentrations 9.0×10^{-5} to 9.0×10^{-4} M) and benzophenone (τ = 0.006 sec, concentrations 3.5×10^{-6} to 3.5×10^{-5} M) using a laboratory-constructed phosphorescence instrument. A Becquerel phosphoroscope consisting of two motors (1200 and 300 rpm) and two chopper blades (ten and two slots) were used to obtain resolution times of 0.001 and 0.02 sec, respectively. By making measurements with the two choppers and by solving simultaneous equations, they obtained accuracies of about 90–110%.

Hollifield and Winefordner[9] using the single disk phosphorescence instrument (the same chopper modulated the exciting radiation and the emitted radiation) with a nine-slot Becquerel disk and a 15,000 rpm variable speed motor were able to resolve phosphoroscopically several complex mixtures using two shutter speeds. All measurements were performed with the excitation and emission monochromators set at the wavelengths corresponding to *maximum* overlap of the spectra of the two components of each mixture. Triplicate measurements were made for each binary mixture, and all results were corrected for solvent background and exciting light absorption by the other component. The two synthetic mixtures determined by phosphoroscopic resolution were: 4-bromoacetophenone (τ = 0.004 sec, concentrations 2.5×10^{-5} to 5.0×10^{-4} M) and 2,4-dibromoacetophenone (τ = 0.008 sec, concentrations 1.8×10^{-5} to 7.6×10^{-6} M); and benzoic acid (τ = 2.4 sec, concentrations 4.1×10^{-5} to 8.0×10^{-6} M) and benzaldehyde (τ = 0.02 sec, concentrations 4.7×10^{-5} to 9.0×10^{-6} M). It should be noted that compounds with *equal decay times* cannot be resolved by any method. Recoveries similar to those obtained by Keirs, Britt, and Wentworth[8] were obtained. The precision of measurement was about 10% or less due to instrument noise and variation in shutter speed. These experiments indicated that it was possible to analyze certain binary mixtures at small concentration by phosphoroscopic resolution provided their decay times were sufficiently different. The results obtained for the binary mixtures[9] indicated that the largest errors occurred for the situation where one component was at quite low concentration in the presence of a large excess of the second component or where the decay times were quite similar.

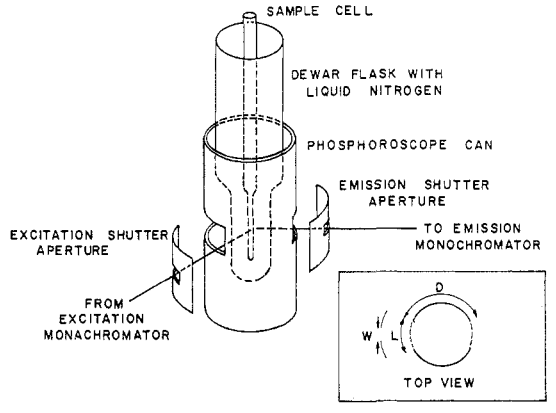

Figure 3. Schematic diagram of rotating can phosphoroscope.

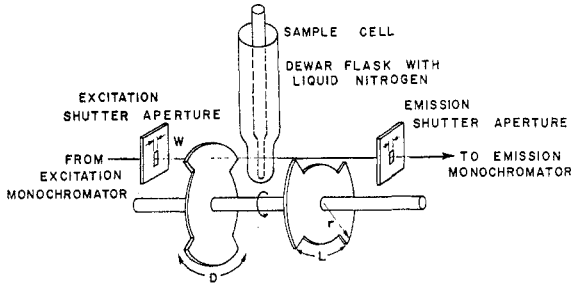

Figure 4. Schematic diagram of rotating disk phosphoroscope.

Influence of Instrumental Design on Measured Phosphorescence Intensity

Design of Mechanical Phosphoroscope.[10] The physical arrangements of the mechanical rotating shutters in two typical spectrophosphorimeters are shown in Figures 3 and 4. The rotating can phosphoroscope, shown in Figure 3, is the type used in the phosphoroscope attachment for several commercial spectrophotofluorimeters. Radiation from the excitation monochromator passes through the excitation shutter aperture and onto the rotating can. When the can is positioned so that

(7) T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, **39**, 1186 (1963); G. Von Förster, *ibid.*, **40**, 2059 (1964); H. Sternlicht, G. C. Nieman, and G. W. Robinson, *ibid.*, **38**, 1326 (1963).

(8) R. J. Keirs, R. D. Britt, and W. E. Wentworth, *Anal. Chem.*, **29**, 602 (1957).

(9) H. C. Hollifield and J. D. Winefordner, *Chem. Instr.*, in press.

(10) T. C. O'Haver and J. D. Winefordner, *Anal. Chem.*, **38**, 602 (1966).

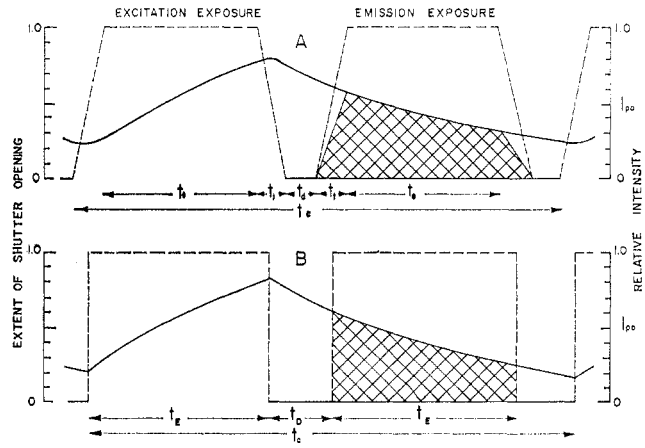

Figure 5. Graphical representation of operation of a phosphoroscope: (A) true representation; (B) approximate representation. Key to symbols and lines on diagram (see text for discussion of symbols): t_e, exposure time; t_d, shutter delay time; t_t, shutter transit time; t_c, time for one cycle of excitation and observation; $t_E = t_c + t_t$; $t_D = t_d + t_t$. (- - -) Extent of opening of excitation shutter apertures according to left-hand vertical coordinate. The value zero means completely closed; 1/2 means half-way open; 1.0 means completely open, etc. (· · ·) Extent of opening of the emission shutter aperture according to left-hand vertical coordinate. (—) Instantaneous phosphorescence intensity of the sample at any time according to the right-hand coordinate. I_{p0}, phosphorescence intensity which would be measured if the excitation and emission shutter apertures were opened for a length of time long enough to allow the phosphorescence intensity to reach a steady-state value; I_{px}, phosphorescence intensity at point X; P_p, integrated phosphorescence intensity measured by the detection system for one cycle of excitation and observation (area under heavy solid line).

one of the openings is aligned withthe excitation shutter aperture, the excitation radiation falls on the phosphorescent species in the sample and excites phosphorescence. At this instant, the emission shutter aperture is blocked by the phosphoroscope can so that fluorescence and scattered excitation radiation will not be detected. As the phosphoroscope can continues to rotate, the excitation shutter aperture is blocked. Fluorescence and scattered excitation radiation decay to a negligible amount almost instantly, and only phosphorescence remains. When the phosphoroscope can has rotated so that its other opening is aligned with the emission shutter aperture, the phosphorescence radiation passes into the emission monochromator and photodetector.

The rotating disk phosphoroscope shown in Figure 4 has been used primarily in laboratory-constructed phosphorimeters. Two notched disks are attached to a rotating shaft. When excitation radiation is passing through the excitation shutter aperture and the open portion of the excitation shutter disk, the other disk is aligned so that its closed portion is blocking the emission shutter aperture. Because the two disks rotate together, the sample is alternately excited and observed.

O'Haver and Winefordner,[10] using the approximation shown in Figure 5B for the operation of the phosphoroscope, have derived an intensity experiment for the observed phosphorescence per revolution (per cycle) of the

Table I

Characteristics of Typical Rotating Can[a] and Typical Rotating Disk (Becquerel)[b] Phosphoroscopes

	Can type[c]		Disk type[d]	
	Real time, sec	Normalized time[e]	Real time, sec	Normalized time
t_c	4.3×10^{-3}	1.00	4.3×10^{-3}	1.00
t_e	1.5×10^{-3}	0.35	1.9×10^{-3}	0.45
t_t	1.3×10^{-4}	0.03	2.7×10^{-5}	0.0063
t_d	3.9×10^{-4}	0.09	2.7×10^{-5}	0.0063

[a] Rotating can phosphoroscope used on Aminco phosphoroscope attachment. [b] Laboratory constructed. [c] Dimensions in Figure 3: $r = 1.18$ cm; $W = 0.1$ cm; $L = 1.4$ cm; speed of rotation, M, 7000 rpm. [d] Dimensions in Figure 4: $r = 5.1$ cm; $W = 0.1$ cm; $L = 7.3$ cm, $\delta = 0.2$ (δ is the overlap, *i.e.*, the distance in centimeters behind which the shutter edge which opens the emission shutter aperture follows the shutter edge which closes the excitation shutter aperture); speed of rotation = 7000 rpm. [e] Normalized time = t/t_c.

phosphoroscope. However several assumptions are required to simplify the resulting expression. First, the excitation radiation intensity is proportional to the extent of opening of the excitation shutter aperture. Second, the fraction of the instantaneous intensity of the phosphorescence radiation striking the emission shutter aperture which is received by the photodetector is proportional to the extent of opening of the emission shutter aperture. Third, the extent of opening of both excitation and emission shutter apertures varies linearly with time during opening and closing.

Under the above conditions, the phosphorescence intensity per cycle, P_p (P_p is area under the solid line in Figure 5B), in relative intensity units is given by eq 3,

$$P_p = \frac{\tau I_{p0}[\exp(-t_D/\tau)][1-\exp(-t_E/\tau)]^2}{[1 - \exp(-t_c/\tau)]} \quad (3)$$

where τ is the mean phosphorescence lifetime and all other terms are defined in the caption of Figure 5. Since the maximum observed integrated phosphorescence per cycle, P_c, in intensity units if the excitation and emission shutters stay open continuously is given by eq 4, the observation efficiency factor, α, which is de-

$$P_c = I_{p0}t_c \quad (4)$$

fined as the ratio of P_p to P_c is given by eq 5. The

$$\alpha = \frac{\tau[\exp(-t_D/\tau)][1 - \exp(-t_E/\tau)]^2}{t_c[1 - \exp(-t_c/\tau)]} \quad (5)$$

parameter α is just the ratio of the measured signal when using a phosphoroscope to that obtained when using continuous excitation. Plots of α *vs.* τ/t_c for a typical rotating can phosphoroscope and a typical rotating disk (Becquerel) phosphoroscope (see Table I for characteristics of phosphoroscopes) are given in Figure 6. The usefulness of the equation for α and the plots in Figure 6 is that the instrumental signal obtained by commercial phosphorimetric instrumentation for phosphorescence in which an integrating direct current is used is *directly proportional* to the value of α.

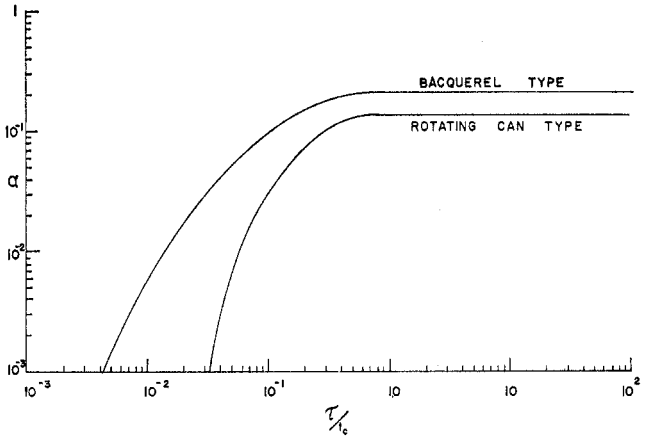

Figure 6. Calculated α values as a function of τ/t_c (from eq 17) for a typical rotating can type phosphoroscope and a rotating disk type phosphoroscope (see Table I for characteristics of phosphoroscopes).

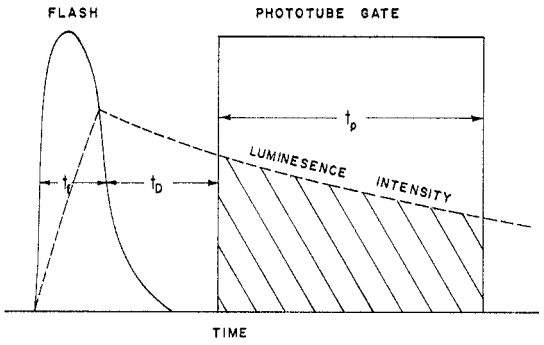

Figure 7. Simplified diagram of events taking place during one cycle of sample excitation and observation in a typical pulse system (see text of paper for definitions of t_f, t_D, and t_p) (time and intensity axes are not to scale but only indicate the events occurring).

Several simplifying limiting cases can be considered. If $\tau/t_c \ll 1$ and $\tau/t_E \ll 1$, then

$$\alpha \simeq \frac{\tau \exp(-t_D/\tau)}{t_c} \quad (6)$$

and so the measured signal is independent of t_E. If $\tau/t_c > 100$ and if $\tau/t_E > 100$, then

$$\alpha \simeq (t_E/t_c)^2 \quad (7)$$

which is generally the case for *organic phosphors* measured using either disk or can phosphoroscopes with characteristics similar to those in Table I. For this case, the measured signal is independent of t_D and τ, and *time-resolved phosphorimetry is not practical.*

In time-resolved phosphorimetry using the phosphoroscopic resolution method, the value of α for a given phosphor is varied by means of varying the rotation speed, θ, and sometimes by varying the number of openings in the can or disk, n. By using two different phosphoroscopic conditions to give a large α for both phosphors in a binary mixture and to give a small α for one and a large α for the other, it is possible to resolve a complex mixture. It is not necessary to know the absolute value of α for each species under the two phosphoroscopic conditions. Thus by means of plots such as in Figure 6, it is possible to predict the success of phosphoroscopic resolution. It is, of course, important that the *shutter speeds be accurately reproduced* when measuring the pure solutions for the analytical working curves and the unknown mixtures of phosphors. This is most simply achieved by using two synchronous motors or one synchronous motor with a pulley system.

In order to achieve *phosphoroscopic resolution* of two phosphors with relative errors less than 10%, $\alpha_A/\alpha_B \gtrsim 10$ at the slow shutter speed. Of course, if $\alpha_A/\alpha_B > 100$ at the slow shutter speed, then A can be measured essentially in the absence of B.

When using the *graphical method* of time-resolved phosphorimetry, it is important that α/I_{p0} be as large and as different as possible for all phosphors of interest, and so it is essential for versatility that t_c be made as small as possible by using large values of θ and n.

Design of Pulsing System.[11] The equations derived by O'Haver and Winefordner[10] for mechanical choppers have been extended by O'Haver and Winefordner[11] to apply to measurement systems using pulsed flash tubes for excitation and pulsed multiplier phototubes for observation of sample luminescence. Pulsed systems have not yet been used for analytical studies but should have great utility and so and will be briefly discussed in this paper. The equations to be given are valuable for evaluating any given pulsed system for analytical applications, for predicting the optimum instrumental conditions for measurement, and for comparing the pulsing system with the mechanical system.

A simplified diagram of the events taking place during one cycle of sample excitation and observation in a typical pulsed system is given in Figure 7. The sample luminescence is excited by a very short, intense burst of light from the flashtube. The half-intensity width of the flash is called t_f. The intensity of the sample luminescence (dotted line) increases during the flash, reaches a maximum, and then decays exponentially after the flash. If the flash intensity were maintained at its peak value for an indefinite time, the sample luminescence would eventually reach a steady-state value, called I_{p0}, which is proportional to the peak flash intensity. At a time t_D, the delay time, after the end of the excitation flash, a high-voltage negative gate is applied to the multiplier phototube dynode chain, activating it for a time t_p (the multiplier phototube gate width). At some time after the trailing edge of the multiplier phototube gate, another cycle of excitation and observation occurs. The number of such cycles occurring per second is called the pulse repetition frequency, f. In a pulse system, t_D, t_p, t_f, and f are all independently adjustable, within certain limits.

The integrated liminescence (since pulsing systems are much faster than mechanical chopping systems, it is

(11) T. C. O'Haver and J. D. Winefordner, *Anal. Chem.*, 38, 1258 (1966).

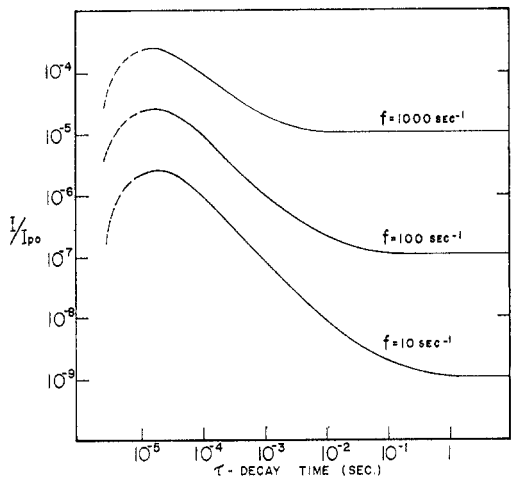

Figure 8. Plot of relative response (ratio of integrated intensity, I, to the steady-state integrated intensity, I_{p0}) *vs.* sample decay time, τ, for three different pulse repetition frequencies, f (solid lines were calculated using equations in text; dotted lines indicate expected trend although equations in text are not strictly valid for these regions). The parameters used to calculate the curves are as follows: $t_f = 10^{-6}$ sec; $t_p = 10^{-5}$ sec; $t_D = 10^{-5}$ sec.

possible to use the former for fluorescence as well as phosphorescence measurements, and thus the general term, luminescence, is used) intensity observed per cycle, P_p, is the area under the curve of luminescence intensity *vs.* time during the photomultiplier gate and is shown as the shaded area in Figure 7. If the excitation pulse width, t_f, is small compared to τ, *e.g.*, if $t_f = 10^{-6}$ sec and if τ is greater than 10^{-5} sec, then the integrated phosphorescence intensity observed per unit time is given by eq 8.

$$I = fP_p = I_{p0}\frac{ft_f[\exp(-t_D/\tau)][1 - \exp(t_p/\tau)]}{1 - \exp(-1/f\tau)} \quad (8)$$

In Figure 8, I/I_{p0} (corresponds to α for mechanical phosphoroscopes) is plotted as a function of τ for three values of the repetition frequency, f. Note the peak value of I which indicates that there is an *optimum experimental condition for any luminescent molecule.* The values of the parameters t_D, t_p, and t_f are given in the caption of Figure 8.

There are several *limiting cases* of interest. If $\tau \gg 1/f$, t_f, t_D, and t_p, then eq 8 becomes

$$I \cong I_{p0}f^2t_ft_p \quad (9)$$

which corresponds to the *flat portion on the right of Figure 8. Most organic phosphors should be described by eq 9.* If $\tau < 1/f$ and $\tau > t_f$, then

$$I \cong I_{p0}ft_f[\exp(-t_D/\tau)][1 - \exp(-t_p/\tau)] \quad (10)$$

which corresponds to the *center and left portion of the curve in Figure 8.* Equation 10 is generally applicable to organic phosphors with $\tau < 10^{-3}$ sec and is especially suitable for organic phosphors (and fluorphors) with τ values between 10^{-3} and 10^{-5} sec.

Comparison of Electronic and Mechanical Chopping Devices. Electronic choppers or pulsed techniques have not yet been used for analytical studies but should have several real advantages over mechanical chopping techniques for time-resolved phosphorimetry. For example, with organic phosphors (or fluorophors) of *much shorter lifetime,* it is possible by means of special electronic measurement techniques to measure species with lifetimes as short as a few nanoseconds. In addition, much greater specificity of measurement can be obtained using the pulsing method. As can be seen from Figure 8, the relative response for long decay times (eq 9 is valid) is much less than for short decay times (eq 10 is valid). Therefore, the ratio of sample phosphorescence to interferent phosphorescence can be increased with electronic pulsing depending on the difference in the lifetimes of the two species. For example, if the two species are designated A and B (B is the interferent), then the intensity ratio I_A/I_B, assuming A is described by eq 10 and B by eq 9, *i.e.*, A is a fast decayer and B is a slow decayer, is given by eq 11. As a practical example, suppose $\tau_A = 10^{-3}$ sec, $\tau_B = 1$ sec, and $f = 10$ Hz. For this case, $I_A/I_B = 100(I_{p0})_A/(I_{p0})_B$, which is 100 times greater than would be expected if $\tau_A = \tau_B = 1$ sec. An even greater increase would result if $\tau_A < 10^{-3}$ sec.

$$\frac{I_A}{I_B} = \frac{(I_{p0})_A}{(I_{p0})_B}\frac{1}{\tau_A f} \quad (11)$$

It is of interest now to compare the mechanical chopping system to the flash tube system described above. Assuming the commercial phosphoroscope system described in Table I, we find for the above hypothetical mixture of A and B, $I_A/I_B = 0.53(I_{p0})_A/(I_{p0})_B$, which indicates that the sample-to-interferent intensity ratio is almost halved (I for the mechanical phosphoroscope is simply P_p times the modulation frequency of the phosphoroscope). Thus for this hypothetical case we would expect to obtain a 200-fold increase in I_A/I_B by using the pulsed system rather than the mechanical chopper system. Of course, for very short lifetimes ($\tau < 10^{-3}$ sec for the mechanical choppers given in Table I and $\tau < 10^{-5}$ sec for the pulsed system given in the caption to Figure 7), the response falls off very rapidly. This, of course, *provides rejection of interference from fluorescence and incident scattered light.*

Therefore the major advantage of pulsed systems over mechanical systems is the possibility of selecting experimental conditions so that the species of interest is being measured at the peak shown in Figure 8. In addition, changes in τ of the sample (due to temperature variations, self-quenching, solvent quenching, or quenching by foreign substances) would then result in less change in I than if τ were on the sloping portion of the curve in Figure 8. This would result in higher accuracy and more nearly linear analytical curves. The value of τ at which the maximum I occurs, τ_{opt}, is given by eq 12. The value of I at τ_{opt}, I_{peak}, is found by sub-

$$\tau_{opt} = t_p/\ln[(t_D + t_p)/t_D] \quad (12)$$

stituting eq 12 into eq 8. For the curves in Figure 8, eq 12 predicts that τ_{opt} is 1.5×10^{-5} sec and $I_{peak} = 2.5 \times 10 \times 10^{-7}f$. Since the decay time of a molecule will be a constant for any given experimental condition, *i.e.*, it is difficult to adjust τ to give τ_{opt}, and since τ_{opt} is a function of t_D and t_p, the maximum in the I *vs.* τ curve can be made to occur at τ_{opt} of any given sample (except a sample obeying eq 9) by adjusting t_D and t_p. For instance, if t_p is fixed, then the optimum value of t_D, *i.e.*, $t_{D,opt}$, may be found by solving eq 12 for t_D, and so

$$\tau_{D,opt} = t_p/\exp[(t_p/\tau) - 1] \quad (13)$$

The use of pulsing systems for time-resolved phosphorimetry seems to have many advantages over mechanical systems for analytical studies. It is hoped that commercial equipment to allow such measurements will soon be available.

Current Measurement System. The phototube output when using modulation or pulsing techniques can be measured using either an *integrating meter* or a *gated electronic* system. In the former, the average direct current signal from the photodetector is measured (the response time of the electrometer readout system is much longer than $1/f$ of the mechanical or electronic chopper). In the latter, the photodetector signal is *only* measured during the "on" time, *i.e.*, the photodetector output is only measured during time t_E when using a mechanical chopper (cross-hatched area in Figure 5) and during time t_p when using an electronic chopper (cross-hatched area in Figure 7). Since $t_p \ll 1/f$ when using the gated system with electronic chopping, whereas $t_E \sim 1/f = t_c$ when using the gated system with mechanical chopping, we would expect a considerable reduction of the dark current shot noise which is only measured during time t_p. In addition, by using pulsing techniques, it is possible to obtain about the same average power of luminescence radiation as when using modulation or continuous excitation techniques. Therefore a great improvement of the signal-to-noise ratio should result by using the gated system with electronic chopping or pulsing. In addition if photon counting techniques[12] are used, it is possible to discriminate against cosmic ray pulses, dynode chain pulses, leakage current, and stray pickup which further improves the signal-to-noise ratio.

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The Techniques of Flash Vacuum Pyrolysis. The Cyclopentadienyl Radical and Its Dimer

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The chemistry of organic free radicals, which are important reactive intermediates in numerous chemical reactions, has primarily been based on product and steady-state kinetic studies. Even though these indirect methods have led to an important and substantial body of information, it is often necessary and desirable to use more direct techniques. For example, the technique of flash photolysis, whereby radicals are generated photolytically by an intense flash of light and then immediately observed spectroscopically, has been very useful for the direct observation of radicals and of their elementary reactions. However, it is obviously limited to those radicals which can be generated photochemically. Flash vacuum pyrolysis (FVP) is an alternative and somewhat similar method which has special value for the study of thermal free radicals and related intermediates.

Flash vacuum pyrolysis is characterized by the use of low pressures and short contact times. The choice of these conditions has two important consequences. Because of the low pressures employed, typically in the range 10^{-2} to 10^{-4} torr, radical–molecule reactions in the oven are improbable with FVP. One can therefore study the primary pyrolytic intermediates uncomplicated by further chain or bimolecular radical–molecule reactions. The use of short contact times, generally in the range of 1 to 20 msec, permits a significant fraction of the radicals produced to survive the pyrolysis and emerge from the oven. Thus, FVP serves as a convenient and general method for generating free radicals or other reactive thermal intermediates for subsequent experiments.

The thermal fragments emerging from the oven can be studied by a variety of physical and chemical techniques. The pyrolysate may be passed directly into the ionization chamber of a mass spectrometer to obtain the mass and to establish the radical or molecule nature of a particular fragment. The fragment intensity as a function of the pyrolysis temperature (temperature profile) gives information about its ease of formation and stability. The only bimolecular processes usually observed under FVP conditions are radical–radical reactions which can also be studied mass spectrometrically. The ionization potentials of the fragments, when combined with the appropriate appearance potentials and thermochemical data, afford estimates of their heats of formation.

Detailed spectroscopic studies of the pyrolysis prod-