PHASE RESOLVED PHOSPHORIMETRY

By

JOHN JAD MOUSA

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John Jad Mousa

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The phase and frequency characteristics of several organic phosphors at 77°K, excited by a continuum source of sinusoidally varying intensity, have been studied with emphasis upon the change in these characteristics with the frequency of source modulation and the lifetime of the phosphorescence. The phase characteristics have been used as the basis of a new analytical technique, phase resolved phosphorimetry.

A lock-in amplifier was employed as a phase sensitive detector and a xenon arc lamp as the excitation source. The phasing out or nulling out of signals with a certain phase relationship to a reference signal has been used to demonstrate that emission and excitation spectra of molecules which show severe overlap can be phase resolved into the

spectra of the individual components. The phase resolution was accomplished by adjustment of the reference signal phase angle, called the phase method, and by varying the frequency of modulation at a constant reference phase setting, called the frequency method. The phase resolution of fluorescence and scattered light from phosphorescence emission was also demonstrated. The quantitative analysis of synthetic binary mixtures by phase resolution was shown to be feasible.

CHAPTER I

INTRODUCTION

The first reported use of the phosphorescence of organic molecules as a means of chemical analysis was in a study by Keirs, Britt and Wentworth in 1957 [1]. These authors discussed some of the experimental and theoretical aspects of phosphorimetric analysis and demonstrated that mixtures of phosphors could be resolved, in some cases, spectrally, by differences in their excitation and emission spectra, and in other cases, temporally, by differences in their respective luminescence lifetimes. Since this work appeared, other workers have developed theories and experimental systems which provided the basis for the exploitation of the phosphorescence lifetime as a method for the resolution and analysis of mixtures of organic phosphors. The efforts in this area include the work of St. John and Winefordner [2] who used a continuously operated continuum source coupled with a DC read-out and a logarithmic converter to resolve the decay curves of a mixture of long-lived phosphors. Later, Hollifield and Winefordner [3] described a unique single disk mechanical phosphoroscope instrument for the measurement of phosphorescence intensities at several intervals along a decay curve. O'Haver

and Winefordner [4, 5] developed the theoretical basis for both mechanically time resolved and pulsed source, time resolved phosphorimetry. The advantages of a pulsed source, time resolved system for phosphorimetry were discussed by Winefordner [6] and the subsequent experimental work by Fisher and Winefordner [7] demonstrated the actual application of these ideas to the resolution and analysis of synthetic binary mixtures of phosphorescent species. This same technique, with improvements in instrumentation, was employed by O'Donnell, Harbaugh and Winefordner in several studies for the determination of phosphorescence lifetimes [8], and the resolution and analysis of mixtures of halogenated biphenyls [9].

Time resolved phosphorimetry is a stroboscopic technique which uses a repetitive, short duration, high intensity flash of exciting radiation, coupled with the sampling of the luminescence signal during selected time intervals after the termination of the exciting light. Gated detectors or signal averagers are used to monitor either portions of or the entire decay curve. Time resolved determination of fluorescence lifetimes is a more difficult experimental technique because of the extremely short fluorescence lifetimes (in the nanosecond region). The experimental difficulties associated with high intensity, short duration sources and fast response detectors had to be surmounted so that this technique could be used successfully. Advances in technology and instrumentation have overcome most of these problems in recent years.

Previously, fluorescence lifetime measurements were made with phase or modulation fluorometers. Both phase and modulation fluorometers employed a continuously operated excitation source whose intensity is modulated sinusoidally at a high frequency (in the MHz range). The result is the excitation of a sinusoidally varying luminescence from the sample. Phase fluorometers usually consist of some type of a phase delay or phase changer in the signal read-out system and a phase sensitive detector which compares the phase of the fluorescence signal to that of a reference signal derived from the excitation source. In modulation fluorometers, both DC and AC detection systems are used to measure the DC level and AC component of the luminescence signal and to compare this to the same ratio for the exciting radiation. The amplitude and phase of the luminescence signal is a function of the frequency of modulation and the lifetime of the luminescence. By measuring the phase shift or degree of modulation of the signal, fluorescence lifetimes can be calculated. A review by Birks and Munro [10] covers the historical development of phase, modulation and time resolved fluorometers describing some of the experimental systems employed and giving the theoretical basis for each technique. Modulation of the exciting light intensity has been accomplished in several ways. Kerr cells were used in one of the earliest instruments [11]. Electro-optical devices, such as crystals of KD_2PO_4 which are based upon the Pockels effect, were used by Müller et al.

[12]. Ultrasonic gratings which use the standing wave produced in a liquid medium to modulate the light intensity were used by several workers [13, 14, 15, 16, 17], including Bailey and Rollefson [18], who used the first modern instrument. Direct high frequency modulation of electrical discharges has also been used successfully [19, 20, 21]. Phase delays were introduced by means of variable optical paths [11, 14, 15], different lengths of delay cables [13] or calibrated electrical RC phase changers [16, 17, 18, 19, 20].

Phase or modulation fluorometers have been used primarily to study single exponential decay processes when lifetime measurements were desired. The analysis of complex decays by the phase fluorometer was complex and difficult. Birks and Munro [10] give reference to several fluorometers which operated at several different frequencies, but it is pointed out that none of these were used to study complex decays. The possibility of obtaining the Fourier transform of the exponential decay process by phase measurements at different frequencies was also mentioned. Schmillen [22] performed fluorescence decay time measurements on hydrocarbon crystals and obtained evidence for several exponential decay processes occurring in anthracene. This evidence was obtained by performing phase measurements and Fourier analysis of the decay processes. In 1968, Doi and Toshinai [23] published a theoretical paper which involved the evaluation of transient phenomena, such as fast decaying

phosphor luminescences, by their frequency characteristics. Applying a Fourier transformation to exponential build-up and decay processes, they derived expressions which were equivalent to the equations describing the phase and amplitude behavior of luminescent species under sinusoidal excitation as in a phase fluorometer. In a later paper [24], these same authors applied their theory to the determination of the amplitude transfer characteristics of inorganic, red-emitting phosphors. The parameter which they labeled as the amplitude transfer characteristic corresponded to the parameter called the degree of modulation in the theory of phase and modulation fluorometers. Although in their theoretical paper, these workers predicted a phase shift parameter which they called the phase transfer characteristic, no attempt at measuring this parameter was made in their experimental work. This phase parameter corresponded to the phase shift parameter in the theory of the phase fluorometer. Experimentally, their work involved the excitation of phosphors with a sinusoidally modulated electron beam, although they stated that the technique and principles involved could just as well be applied to optical excitation of luminescent species. The phenomenon of the phase shift of fluorescence has been used in several cases to study the kinetics of fluorescence quenching [25, 26]. In a later paper, Veslova, Cherkasov and Shirokov [27] demonstrated the resolution and recording of individual fluorescent spectra from each of two luminescent

centers with overlapping emission spectra by means of a modulated light source and a phase sensitive detector. The use of a lock-in amplifier to determine the phase shift of luminescence during a chemical reaction by the null or quadrature technique has also been reported [28].

Using the different phase and amplitude relationships of luminescence signals from species with different lifetimes, the purpose of this research project was the investigation and evaluation of these relationships in analytical phosphorimetry for the resolution and analysis of mixtures of organic molecules. This new analytical method will be termed phase resolved luminescence spectrometry. The objective of the investigation was not the construction of the optimum experimental system, but rather the demonstration of the principles involved, showing the potentialities and weaknesses of this new technique as a method of resolution in phosphorimetry. The molecules studied were selected on the basis of the strength of their native phosphorescence signals and because their lifetimes were known via time resolved determinations.

CHAPTER II

THEORY

Phosphorescence Theory

A detailed review of the theory and background of the phenomenon of phosphorescence will not be attempted in this work as several good sources of information on theory and instrumentation are available [29, 30, 31, 32, 33]. A simplified review of the basics of phosphorescence theory is useful, however, so as to properly lay the foundation for the theory of the phase resolution technique. Taking account of only electronic transitions, consider a simple, unsaturated organic molecule. In general, the pair of electrons occupying the highest filled molecular orbital will have opposite directions for their spin angular momentum. This situation gives rise to a singlet electronic state as the ground energy level for the molecule. Upon the absorption of the appropriate energy of radiation, an electron from this highest filled orbital may be promoted to the next highest unfilled molecular orbital. If the direction of the spin angular momentum of the electron remains the same, the molecule is said to be in the first excited singlet state. Once the molecule is in this excited singlet level, it may undergo several processes.

These processes are: (i) deactivation to the ground singlet level with the emission of radiation, a process called fluorescence; (ii) deactivation by radiationless means through collisions and vibrational energy dissipation called internal conversion; and (iii) intersystem crossing, in which the spin angular momentum becomes reversed in direction and the molecule is said to be in the first excited triplet state. From this triplet level, the molecule may again undergo radiationless or radiational deactivation to the ground singlet state as well as back intersystem crossing to the first excited singlet level. The emission of radiation accompanying a transition from the first excited triplet state to the ground singlet state is called phosphorescence. Thus, the phosphorescence is produced via excitation of the molecule to the first excited singlet level followed by intersystem crossing. Because the transition between the triplet level and the singlet ground state is a spin-forbidden transition, the lifetime of the first excited triplet state and the decay time of the phosphorescence are quite long as compared to the lifetime of the first excited singlet state and the decay time of the fluorescence. The phosphorescence emission may have a decay time ranging from 10^{-3} to 100s. On the other hand, fluorescence lifetimes are generally of the order of 10^{-9} to 10^{-8}s. Because of the long-lived nature of the triplet level, it is very susceptible to radiationless quenching due to collisions with neighboring molecules.

For this reason, phosphorescence is rarely observed in liquid solutions, but is usually observed at low temperatures in rigid matrices. A diagram illustrating the various processes described here is presented in Figure (1).

The mean lifetime of the phosphorescence is related to the various triplet level deactivation processes by the following equations:

$$\tau_P = \frac{1}{K} \tag{1}$$

where

$$K = k_P + k_Q + k_{NR} + k_{ST} \ . \tag{2}$$

In these expressions, τ_P is the mean lifetime of the first excited triplet level and K is the sum of the rate constants for radiationless vibronic deactivation (k_{NR}), quenching deactivation by collisions with impurities or solvent molecules (k_Q), back intersystems crossing to the first excited singlet (k_{ST}) and radiational deactivation (k_P). At low temperatures and in rigid media the quenching and back intersystem crossing term are very small, and only the vibrational radiationless processes and the radiative processes are significant.

The intensity of the phosphorescence decreases upon the termination of the exciting light according to the following exponential relationship,

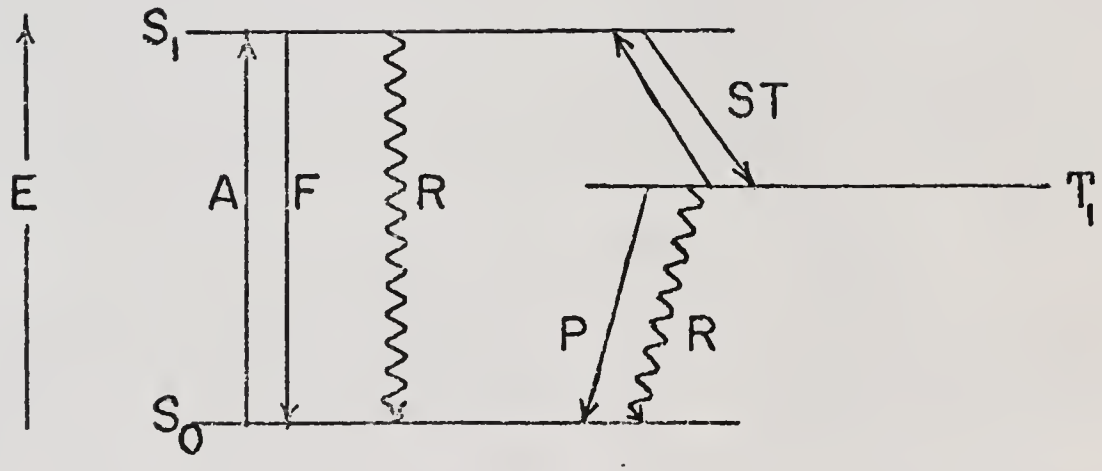


Figure 1. Energy level diagram showing relationship between fluorescence and phosphorescence processes.

S_o = ground singlet level.
S_1 = first excited singlet level.
T_1 = first excited triplet level.
A = absorption.
F = fluorescence.
P = phosphorescence.
ST = intersystem crossing.
R = radiationless deactivation.

$$I_P = I_P^o \exp(-t/\tau_P). \qquad (3)$$

Here I_P is the phosphorescence intensity at any time, t, after the termination of the exciting light, and I_P^o is the intensity of the phosphorescence due to the steady state excitation of the molecule. The mean phosphorescence lifetime, τ_P, is then the time it takes for the phosphorescence to drop to 1/e of its initial steady state value.

The lifetime of the phosphorescence is influenced by the nature of the molecular orbitals involved in the various transitions, i.e., whether the emitting triplet state is $n-\pi^*$ or $\pi-\pi^*$ in character. Usually molecules in which $n-\pi^*$ triplet states are involved in the transitions exhibit shorter phosphorescence lifetimes than those where $\pi-\pi^*$ triplet states are involved. In some cases, heavy atom substituents on aromatic molecules can shorten the lifetimes considerably. In addition, lifetimes can be affected by the solvent characteristics.

Quantitative phosphorescence measurements are based upon the relationship that the steady state intensity, I_P^o, of the phosphorescence emitted is proportional to the intensity, I_A, of the exciting light absorbed by the molecule and the quantum efficiency, Y_P. The quantum efficiency is defined as the ratio of the number of photons emitted per the number of photons absorbed (actually the energy efficiency should be used but would only change the following equations by a constant factor and so is not used here).

$$I_P^o = I_A Y_P. \tag{4}$$

The intensity absorbed can be expressed in terms of the intensity, I_o, of the exciting light striking the sample and the intensity, I_T, of light transmitted by the sample.

$$I_A = (I_o - I_T). \tag{5}$$

Making use of the Beer-Lambert Law, $I_T = I_o\ 10^{-\varepsilon bc}$, where ε is the molar absorptivity coefficient, b is the thickness of the absorbing layer, and c is the concentration, then from Equations (4) and (5)

$$I_P^o = Y_P I_o (1-10^{-\varepsilon bc}). \tag{6}$$

Expanding Equation (6) as a power series and assuming an absorbance ($A=\varepsilon bc$) of less than 0.01, then Equation (6) becomes,

$$I_P^o = Y_P I_o (2.3)\varepsilon bc \tag{7}$$

or

$$I_P^o = k_P I_o \tag{8}$$

where k_P is equal to $(2.3)\ \varepsilon bc Y_P$. This equation indicates that in sufficiently dilute solutions there exists a linear relationship between concentration and the intensity of the phosphorescence [1, 34].

Because quantitative measurements upon mixtures of compounds in solution will be attempted in this work, it is important to mention here a physical phenomenon known as the

"inner filter effect." If one considers a two-component mixture of A and B, all of the exciting radiation intensity will not be available for the excitation of component A because of the presence of component B. Thus, component B acts as a "filter" for the excitation radiation. There is of course the same type of "inner filter effect" of component A upon component B. Zander [34] and St. John and Winefordner [2] discussed and derived equations for the evaluation of this effect. The inner filter effect can introduce an error in the determination of the concentration of one of the components in the mixture especially if standards of the pure compound are used. The inner filter effect is especially acute when one of the components of the mixture has a much higher concentration or absorptivity than the other component. This effect can be minimized by using dilute solutions and mixtures of compounds of similar absorptivity and concentration. On the other hand, a correction factor may be calculated [2]. For the solutions used in this work, the concentration of the components was adjusted so that the inner filter effect could be minimized.

Theory of Phase and Frequency Characteristics of Luminescence

The following derivation of the equations describing the phase and frequency behavior of luminescence will follow the procedure of Birks and Munro [10] in their development of the equations for phase and modulation fluorometry. The

use of a periodic exciting light will be assumed initially, but this condition is in no way necessary to obtain the final equations describing the phase and frequency characteristics of the luminescence, as they can be independently obtained via Fourier transformation of the time dependency of luminescence law as described by Doi and Toshinai [23].

The phosphorescence intensity as a function of time, $I_p(t)$, will be a function of the time dependence of the exciting light, $I_o(t)$, and the phosphorescence decay behavior, $i_p(t)$. Although only phosphorescence will be discussed here, the expression below should also apply generally for fluorescent as well as phosphorescent species. The phosphorescence intensity can be expressed by a convolution integral as in Equation (9).

$$I_p(t) = \int_o^\infty i_p(t') \cdot I_o(t-t')dt'. \qquad (9)$$

In this expression, $I_p(t)$ is the phosphorescence intensity at any time t and $i_p(t')$ is the value of the exponential decay function at a time t' relative to the start of the decay. $I_o(t-t')$ is the value of the exciting light function at the start of the decay process. The product of the exciting light function and the decay function is integrated over the interval from $t' = 0$ to $t' = \infty$.

Assuming a periodic character for the exciting radiation, $I_o(t)$ can be expressed as a Fourier series,

$$I_o(t) = k_o(1+\Sigma_j k_j \exp[i(\omega_j t+\phi_j)]), \qquad (10)$$

where k_o is an arbitrary constant and ω_j, k_j and ϕ_j are

respectively the angular frequency, relative amplitude and phase of the j^{th} component. Taking the phosphorescence lifetime as τ_P, the decay behavior of the phosphorescence can be expressed as:

$$i_P(t) = i_P^o \exp(-t/\tau_P). \tag{11}$$

Now, substituting $I_o(t-t')$ and $i_P(t')$, Equation (9) becomes,

$$I_P(t) = \int_o^\infty i_P^o \exp(-t'/\tau_P) \cdot k_o(1+\Sigma_j k_j \exp[i(\omega_j(t-t')+\phi_j)])dt', \tag{12}$$

which reduces to

$$I_P(t) = k_o i_P^o \tau_P \{1+\Sigma_j \left(\frac{1}{1+i\omega_j\tau_P}\right) k_j \exp[i(\omega_j t+\phi_j)]\}. \tag{13}$$

Equation (13) consists of a Fourier series similar to $I_o(t)$ in which the phases and amplitudes of the various components depend upon those of the same frequency components in $I_o(t)$. Equations (10) and (13) are similar except for the constant multiplicative term $i_P^o\tau_P$, and the function $(1+i\omega_j\tau_P)^{-1}$. This second function affects the amplitude of the various components of $I_P(t)$ and introduces a phase shift with respect to $I_o(t)$. The vector amplitude $\vec{m}_j$ and the phase Θ_j of the j^{th} component in $I_P(t)$ relative to the equivalent quantities in $I_o(t)$ are given by

$$\vec{m}_j = (1+i\omega_j\tau_P)^{-1} \tag{14}$$

and

$$\Theta_j = \tan^{-1}(\omega_j\tau_P). \tag{15}$$

The absolute value of the relative amplitude, m_j, is given by

$$m_j = (1+\omega_j^2\tau_P^2)^{-\frac{1}{2}} \tag{16}$$

or

$$m_j = (1+4\pi^2 f_j^2\tau_P^2)^{-\frac{1}{2}}, \tag{17}$$

where f_j is the linear frequency and is equal to $\omega_j/2\pi$. If a sinusoidal character for the exciting radiation is assumed, then the subscripts j may be dropped because only the terms where j = 1 will be considered.

The parameter, m_j, which we shall call the degree of modulation, is essentially a measure of the amplitude of the AC component of the luminescence signal with respect to the amplitude of the AC component of the exciting radiation. It is seen that the value of this parameter depends upon the frequency of modulation and the lifetime of the phosphorescence. In Figures (2a) and (2b) a graphical illustration is given of the variation of m_j with modulation frequency and phosphorescence lifetimes in the millisecond range. At low modulation frequencies, the value of m_j approaches unity, which essentially is the point at which the maximum signal level is observed, and gradually approaches a limiting value of zero as the frequency is increased. If the exciting radiation is modulated at a sufficiently high frequency, the figures illustrate the greater signal amplitude of short-lived phosphors over long-lived phosphors which can be expected. This behavior can be used

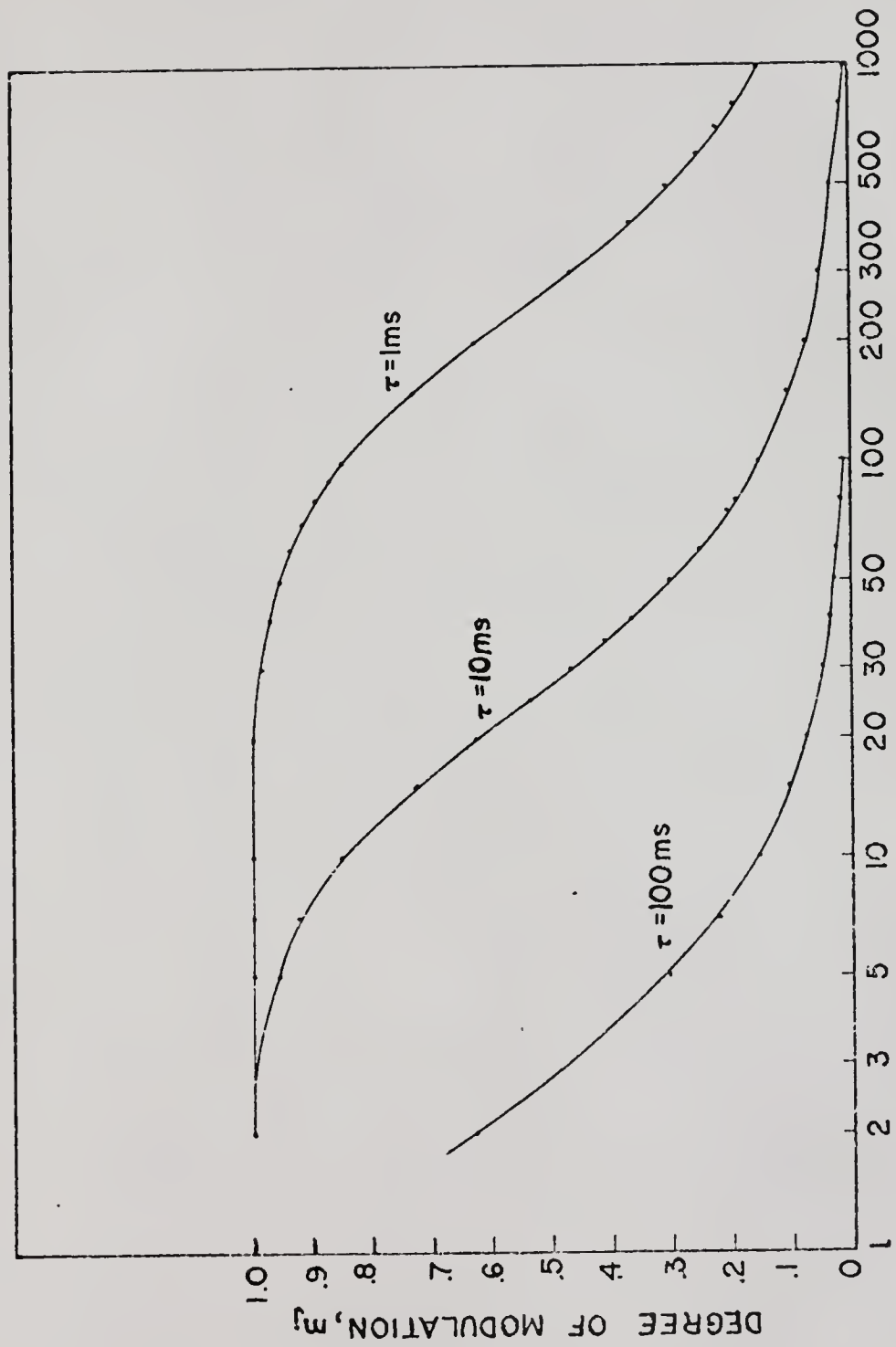


Figure 2a. Semi-logarithmic plot of theoretical variation of degree of modulation, m_j, with the frequency of modulation.

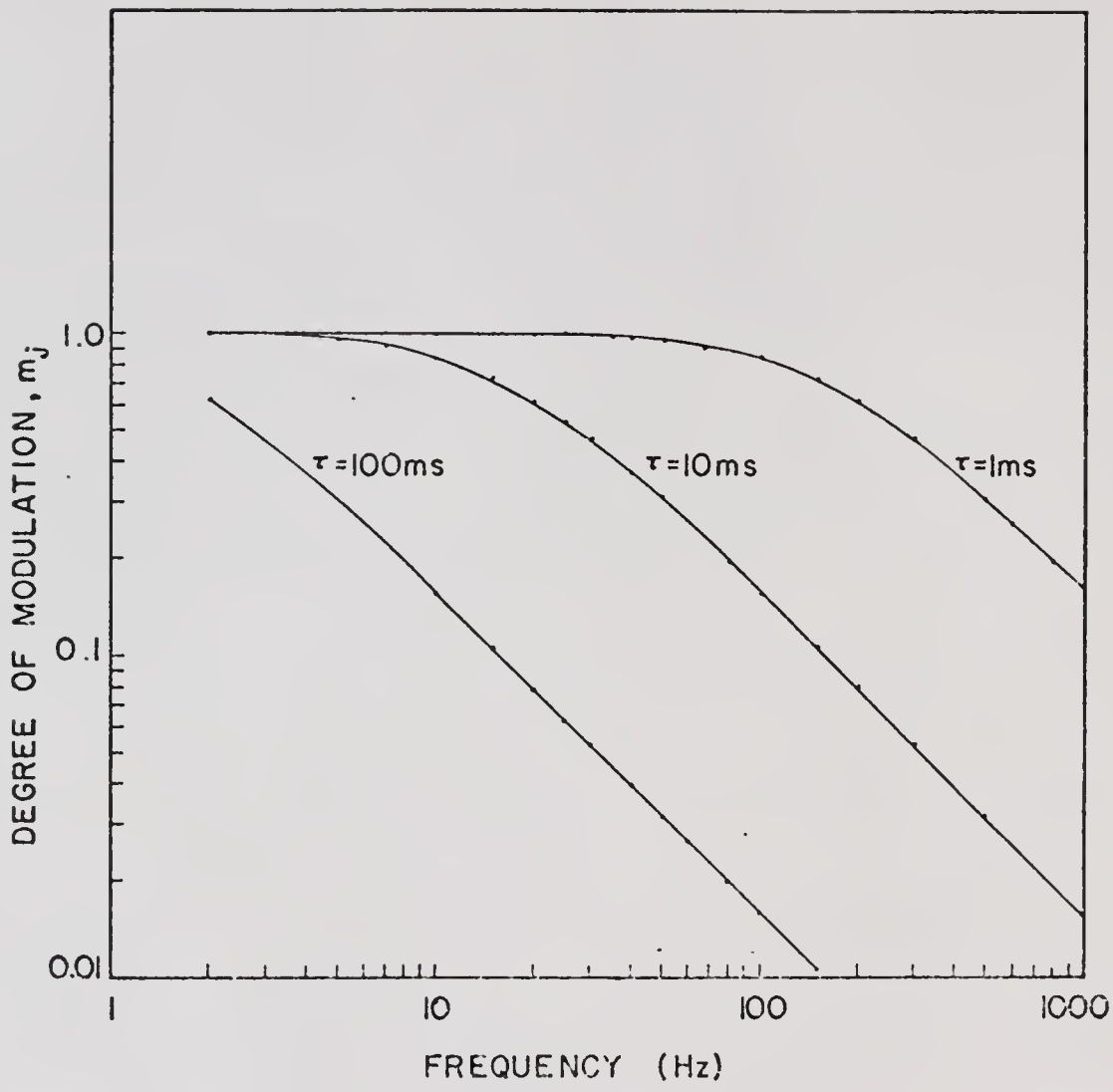


Figure 2b. Logarithmic plot of theoretical variation of degree of modulation, m_j, with the frequency of modulation.

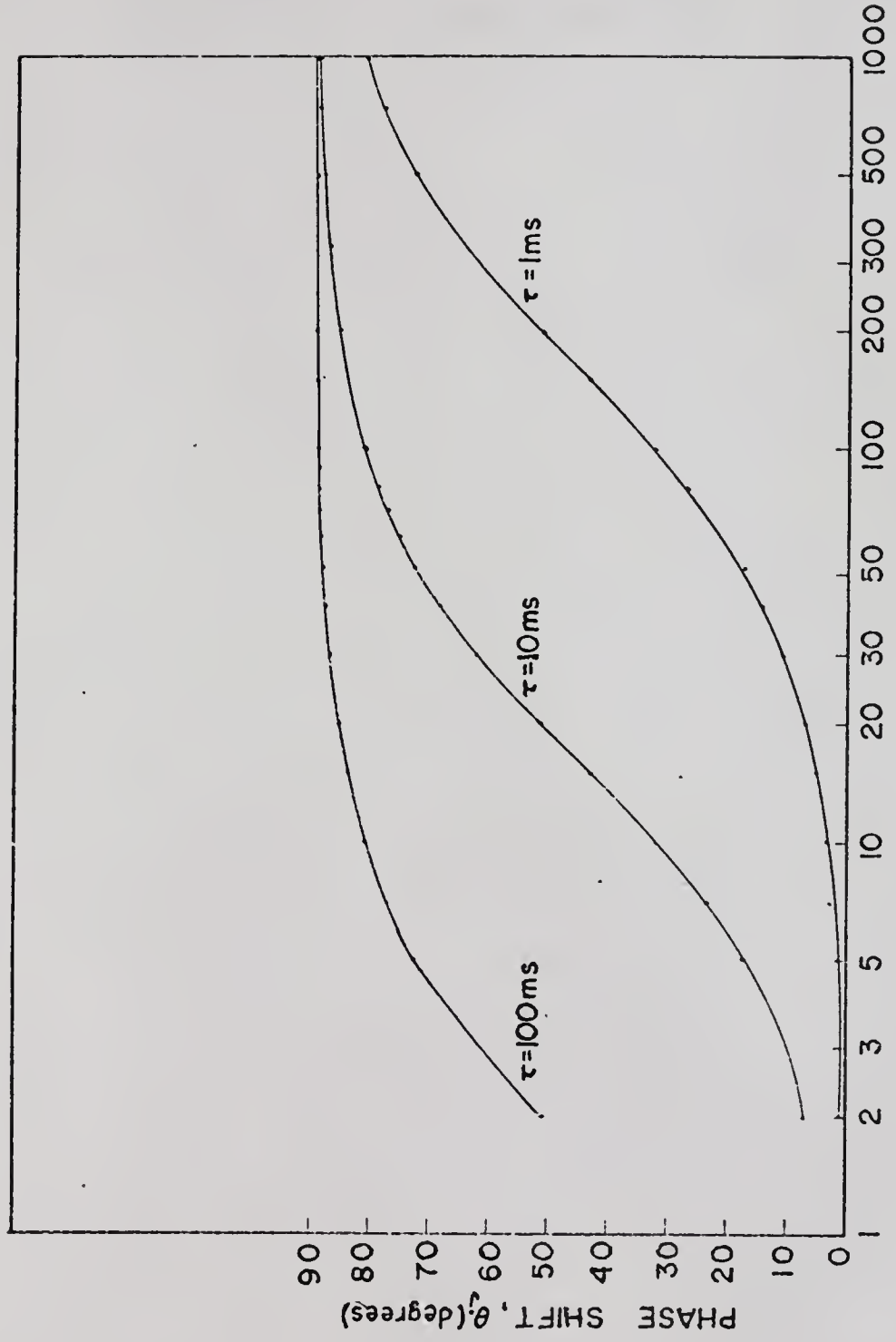


Figure 3. Theoretical variation of phase shift angle, Θ_j, with the frequency of modulation.

advantageously when short-lived phosphors are to be determined in the presence of long-lived background interferences.

The phase shift angle, Θ_j, is also a function of frequency and lifetime as illustrated in Figure (3). The phase angle is measured with respect to the phase angle of the exciting light and, as the figure shows, varies from 0 to $\pi/2$ radians or 0 to 90 degrees. At low frequencies, the luminescence signal is essentially in phase with the exciting light and as the frequency increases, the luminescence signal becomes nearly 90° out of phase. At a constant frequency setting, the phase shift angle, Θ_j, will vary according to the phosphorescence lifetime of the molecule.

Phase Resolved Phosphorimetry

In phosphorimetric analyses, it is frequently the case that the emission and excitation spectra of similar molecules overlap severely. Thus, an analysis of one of the components in a mixture of similar species could be subject to severe interference. However, in many cases, the phosphorescence lifetimes for these same molecules are quite different. Therefore, analytical techniques which exploit the lifetime of the phosphorescence for the resolution of the signals from similar phosphors should be quite useful.

In the preceding section, it was shown that a molecule which is capable of luminescence, when excited with light of a periodic intensity, will emit a luminescence with a periodic variation in intensity. The amplitude and phase of this luminescence with respect to these same parameters in the excitation light will be functions of the frequency of modulation and the lifetime of the luminescence. This statement applies, of course, to all the different types of luminescence which a molecule is capable of emitting. Thus, for a molecule which displays fluorescence as well as phosphorescence one would expect to observe a periodic signal from both of these phenomena. In standard phosphorimetry, where a rotating can phosphoroscope is used, no fluorescence emission is observed and only the phosphorescence is measured. In time resolved phosphorimetry, the fluorescence is eliminated by gating the detector to observe the signal after a delay time, during which the fluorescence has decayed completely. In phase resolved phosphorimetry, however, this is not the case as no phosphoroscope is used, and the fluorescence emission must also be considered.

The exciting light function can be expressed as the sum of a constant intensity term and a sinusoidally varying intensity term,

$$I_o = I_o' + I_o'' \cos \omega t. \qquad (18)$$

The amplitude of this function is I_o'' and its phase has a value of zero. The expression for the emitted luminescence,

according to Equations (13), (14) and (15), will have the same form as Equation (18) and can be represented as

$$I_L = k_L I_o' + m_L k_L I_o'' \cos(\omega t - \theta_L), \quad (19)$$

where k_L is a constant factor taking into account the quantum efficiency and concentration factors, m_L is the degree of modulation with respect to I_o and θ_L is the phase shift angle.

The total intensity from the sample, I_T, will be made up of the phosphorescence intensity, I_P, the fluorescence intensity, I_F, and a scattered or stray light component I_S.

$$I_T = I_P + I_F + I_S. \quad (20)$$

These individual intensities upon excitation with a sinusoidal excitation radiation are given by

$$I_P = k_P I_o' + m_P k_P I_o'' \cos(\omega t - \theta_P), \quad (21)$$

$$I_F = k_F I_o' + m_F k_F I_o'' \cos(\omega t - \theta_F) \quad (22)$$

and

$$I_S = k_S I_o' + m_S k_S I_o'' \cos(\omega t - \theta_S), \quad (23)$$

where $k_P = (2.3) Y_P \varepsilon bc$ and $k_F = (2.3) Y_F \varepsilon bc$. Here, Y_P and Y_F are the relative quantum efficiencies for phosphorescence and fluorescence respectively. The parameter, k_S, is that fraction of the excitation radiation detected as stray light or scatter. The degree of modulation of the fluorescence

and phosphorescence is given by m_F and m_P respectively, and the relative phase shift by θ_F and θ_P. The scattered light will also have a degree of modulation, m_S, and a phase shift, θ_S, which should be almost the same as that of the exciting light. For the purposes of the following discussion, it will be assumed that the stray light is negligible, and the total luminescence intensity will be given by

$$I_T = k_P I_o' + m_P k_P I_o'' \cos(\omega t - \theta_P) + k_F I_o' + m_F k_F I_o'' \cos(\omega t - \theta_F) \quad (24)$$

and, rearranging,

$$I_T = (k_P I_o' + k_F I_o') + m_P k_P I_o'' \cos(\omega t - \theta_P) + m_F k_F I_o'' \cos(\omega t - \theta_F). \quad (25)$$

Thus, the total luminescence intensity will be given by a constant term, $(k_P I_o' + k_F I_o')$, and an AC component due to the sum of the AC components of the fluorescence and phosphorescence. Depending upon the frequency of modulation, the parameters m_P and θ_P, and m_F and θ_F can be widely different. In the frequency range to be covered in this work (2 to 1000 Hz), the value of m_F is equal to unity and the phase shift parameter is zero. These fluorescence parameters do not begin to vary until the MHz frequency range is approached. Thus, under the conditions of low frequency operation, the fluorescence AC term will be insensitive to variations in frequency and will have the same phase as the exciting light. In phase resolved phosphorimetry, a frequency and phase

selective detection system is employed; thus only the AC terms of the luminescence intensity will be observed. Equation (25) thus reduces to

$$I_T = m_P k_P I_o'' \cos(\omega t - \Theta_P) + k_F I_o'' \cos \omega t. \qquad (26)$$

With the proper choice of excitation and emission wavelengths and by using molecules which are strongly phosphorescent, the fluorescence terms in Equation (22) can be made negligible and only the phosphorescence observed. In many cases, this selectivity cannot be achieved and the fluorescence must still be considered. For the sake of simplicity, it will be assumed that for the molecules used in this study, the above condition holds and only the phosphorescence is observed. Thus, Equation (26) can be rewritten as

$$I_P = m_P I_P^o \cos(\omega t - \Theta_P), \qquad (27)$$

where $I_P^o = k_P I_o''$.

In this work, a lock-in amplifier detection system is employed. The principle of operation of this system is that only AC signals having the same frequency as a reference signal are selectively demodulated and amplified. The phase of this internal reference signal can be adjusted so as to give maximum response to a signal having the same frequency and phase characteristics. The output DC signal is related to input AC signal by the following relationship

$$E_{(out)} = \gamma \cdot \bar{E}_{(in)} \cdot \cos(\phi_R - \phi_{(in)}), \qquad (28)$$

where $\bar{E}_{(in)}$ is the average input voltage, γ is an amplification

factor and $(\phi_R-\phi_{(in)})$ is the phase difference between the reference signal and the input signal. In normal operation, ϕ_R is made equal to $\phi_{(in)}$ and the maximum signal is obtained. If $\phi_R = 90°+\phi_{(in)}$, then the cosine term goes to zero and no output signal is observed. This condition is called the null or quadrature condition.

Consider now a phosphorescence signal $E_{P(in)}$ from a solution containing one phosphorescent species applied to the input of a lock-in amplifier. The output signal will be given by

$$E_{P(out)} = \gamma \cdot \bar{E}_{P(in)} \cdot \cos(\phi_R-\phi_P), \qquad (29)$$

where ϕ_P is the instrumentally measured phase angle of the phosphorescence signal with respect to the reference signal. It is related back to Θ_P which is the phase shift with respect to the excitation light. Consider now the situation when a signal from a binary mixture is considered

$$E_P = \gamma[\bar{E}_{P(1)} \cdot \cos(\phi_R-\phi_1)+\bar{E}_{P(2)} \cdot \cos(\phi_R-\phi_2)] \qquad (30)$$

where ϕ_1 and ϕ_2 are the relative phases of phosphorescent components 1 and 2, respectively, and $\bar{E}_{P(1)}$ and $\bar{E}_{P(2)}$ are the average input signals from components 1 and 2. The phase resolution of this signal into its two components can be accomplished in two ways. One of these methods shall be called the phase method. This technique makes use of the fact that if the phase of the reference signal ϕ_R is made equal to $90° \pm \phi_{1 \text{ or } 2}$, then the signal from one of the

components may be "nulled out" and only the signal from the other component measured. For example, if $\phi_R = 90°+\phi_1$, then

$$E_P = \gamma[\bar{E}_{P(1)} \cdot \cos(90°+\phi_1-\phi_1)+\bar{E}_{P(2)} \cdot \cos(90°+\phi_1-\phi_2)]$$

$$= \gamma \cdot \bar{E}_{P(2)} \cdot \cos(90°+\phi_1-\phi_2)$$

$$E_P = \gamma \cdot \bar{E}_{P(2)} \cdot \sin(\phi_2-\phi_1) \qquad (31)$$

or, if $\phi_R = 90°+\phi_2$, then

$$E_P = \gamma[\bar{E}_{P(1)} \cdot \cos(90°+\phi_2-\phi_1)+\bar{E}_{P(2)} \cdot \cos(90°+\phi_2-\phi_2)]$$

$$= \gamma \cdot \bar{E}_{P(1)} \cdot \cos(90°+\phi_2-\phi_1)$$

$$E_P = \gamma \cdot \bar{E}_{P(1)} \cdot \sin(\phi_1-\phi_2). \qquad (32)$$

It is observed that the output signal becomes proportional to the sine of the phase angle difference between the two components. Therefore, the best results are obtained when the difference in phase angles is the greatest so that the sine term is maximized. It should be noted that one of the sine terms can be negative; this would give rise to a negative output signal on the lock-in amplifier. This presents no problem as a 180° shift of the reference phase can be used to give a positive output signal. The phase technique is utilized at a fixed frequency, and therefore the magnitudes of the signals, $\bar{E}_{P(1)}$ and $\bar{E}_{P(2)}$, will be affected by their respective modulation parameter, m, at that particular frequency.

The other resolution method shall be called the frequency method. This is based upon the fact that the phase angle, Θ, is related to the frequency of modulation by $\Theta = \tan \omega\tau$. Therefore, if we set the reference phase angle at a constant value, ϕ_R, there will be a certain frequency at which $\phi_R = 90° \pm \phi_1$, and another frequency at which $\phi_R = 90° \pm \phi_2$. If, for example, the frequency is such that $\phi_R = 90° + \phi_1$, then the same situation exists as before in the phase resolution technique, i.e.,

$$E_P = \gamma \cdot \bar{E}_{P(1)} \cdot \sin(\phi_2 - \phi_1) \text{ at } \omega_1, \tag{33}$$

and vice versa if $\phi_R = 90° + \phi_2$, then

$$E_P = \gamma \cdot \bar{E}_{P(2)} \cdot \sin(\phi_1 - \phi_2) \text{ at } \omega_2. \tag{34}$$

The same sine relationship holds as before, but, in this case, the measurements are made at two different frequencies and so the magnitudes of $\bar{E}_{P(1)}$ and $\bar{E}_{P(2)}$ are also affected. This effect upon the degree of modulation with a change in frequency can place a limit on the utility of this method, if the frequencies are such that the amplitudes are greatly reduced.

An expression for an AC signal composed of a phosphorescent and fluorescent emission is therefore given by

$$E_L \doteq \gamma[\bar{E}_P \cdot \cos(\phi_R - \phi_P) + \bar{E}_F \cdot \cos(\phi_R - \phi_F)]. \tag{35}$$

This equation describes the output signal from the detector when a single species which fluoresces and phosphoresces is

present. The phase resolution technique can be applied in this situation to resolve overlapping or interfering fluorescence and phosphorescence emission spectra. In these situations, the fluorescence emission will have a phase angle, ϕ_F, which will be identical to the phase angle for the exciting radiation. Setting ϕ_R equal to $90°+\phi_F$ will phase out the fluorescence and scattered exciting light, and only the phosphorescence will be observed.

CHAPTER III

EXPERIMENTAL

Reagents

Reagents used without further purification were: 2-bromobiphenyl, 3-bromobiphenyl, 4-bromobiphenyl, 4,4'-dibromobiphenyl and 4-iodobiphenyl (Pfaltz and Bauer, Flushing, N.Y.); 4,4'-bisdimethylaminobenzophenone and 4'-hydroxybutyrophenone (J. T. Baker, Phillipsburg, N.J.); anthraquinone (sublimed) (Eastman Organic Chemicals, Rochester, N.Y.); benzophenone (Fisher Scientific Co., Fair Lawn, N.J.).

The solvent used for this study was ethyl alcohol obtained by special distillation of 95% v/v ethanol (U.S. Industrial Chemicals Co., New York, N.Y.). Absolute alcohol was obtained by a modification of the procedure described by Lund and Bjerrum [35]. The procedure depends upon the reactions:

$$Mg(s)+2C_2H_5OH = H_2+Mg(OC_2H_5)_2$$

$$Mg(OC_2H_5)_2+2H_2O = Mg(OH)_2(s)+2C_2H_5OH$$

These reactions proceed readily when activated by iodine. In the modified procedure, 1.5 l of 95% ethancl is placed in a 2 l round bottom flask and approximately 10g of

magnesium turnings and several grams of iodine are added. Upon warming the reaction mixture, evolution of hydrogen gas commences and the mixture is gently refluxed for approximately 6 hours. During the reflux period the water is removed as magnesium hydroxide and the iodine is reduced to iodide. After refluxing, the ethanol is slowly distilled and the middle 70% of the distillate is collected. Low phosphorescence background and a high degree of rigid, clear glass formation are observed for the absolute ethanol produced in this procedure.

Instrumental System

The experimental system employed in this work consists of several basic components. First, a continuum light source whose intensity may be modulated in a periodic manner; secondly, a means of selecting the appropriate wavelengths of light for excitation of the sample and observation of the luminescence emission, and thirdly, a phase and frequency selective detector in the signal measurement system. A DC detection system is also used at certain times to obtain modulation data. These components are very similar to the types of instrumentation used by the workers in the field of phase and modulation fluorometry as described in the introduction to this work. The aim of this work, as far as the instrumentation was concerned, was to construct and assemble the simplest analytically useable experimental apparatus. In the

following sections, each of these basic components of the instrumental apparatus will be discussed. A block diagram of the instrumental system is given in Figure (4).

The source of excitation radiation in this work was a 150 W xenon arc lamp (type 901C-11, Hanovia Lamp Div., Conrad Precision, Ind., Newark, N.J.). The source was enclosed in a lamp housing (Schoeffel Inst. Corp., Westwood, N.J.) with an adjustable condensing lens attachment for focussing the source radiation upon the entrance slit of a monochromator. The normal operating conditions for this lamp call for operation at 20V and 7.5A. The current for the operation of the source was provided by a Harrison 6268A DC power supply (Hewlett-Packard, Palo Alto, Calif.) operated in its constant current mode. The lamp was started using a starter as described by Zweidinger and Winefordner [36]. A schematic diagram of the starting and modulation circuit is given in Figure (5). The circuit consists of two sections; a starting section designed for normal start-up of the lamp and transfer of control to the modulation section, and the modulation section itself which is designed to keep the arc operating at a constant intensity level and to add on a sinusoidal variation in intensity.

These two sections were designed such that the lamp could be started with the modulation circuit switched out, thereby preventing destruction of the semiconductor devices by the high energy AC pulse used to start the lamp. To start the lamp, the Harrison 6268A DC power supply was

Figure 4. Block diagram of instrumental system.

A = 0-40V, 0-30A DC power supply (Harrison 6268A).
B = starter circuit [36].
C = 0-100V, 0-0.2A DC power supply (Harrison 6116A).
D = summing operational amplifier and current booster.
E = modulation circuit.
F = excitation and emission monochromators.
G = photomultiplier tube and housing.
H = high voltage power supply.
I = load resistors.
J = differential amplifier (Tektronix 1A7A).
K = amplifier (optional) (PAR 211).
L = lock-in amplifier.
M = strip-chart recorder (optional).
N = x-y recorder (optional).
P = xenon arc lamp.
S = sample compartment.

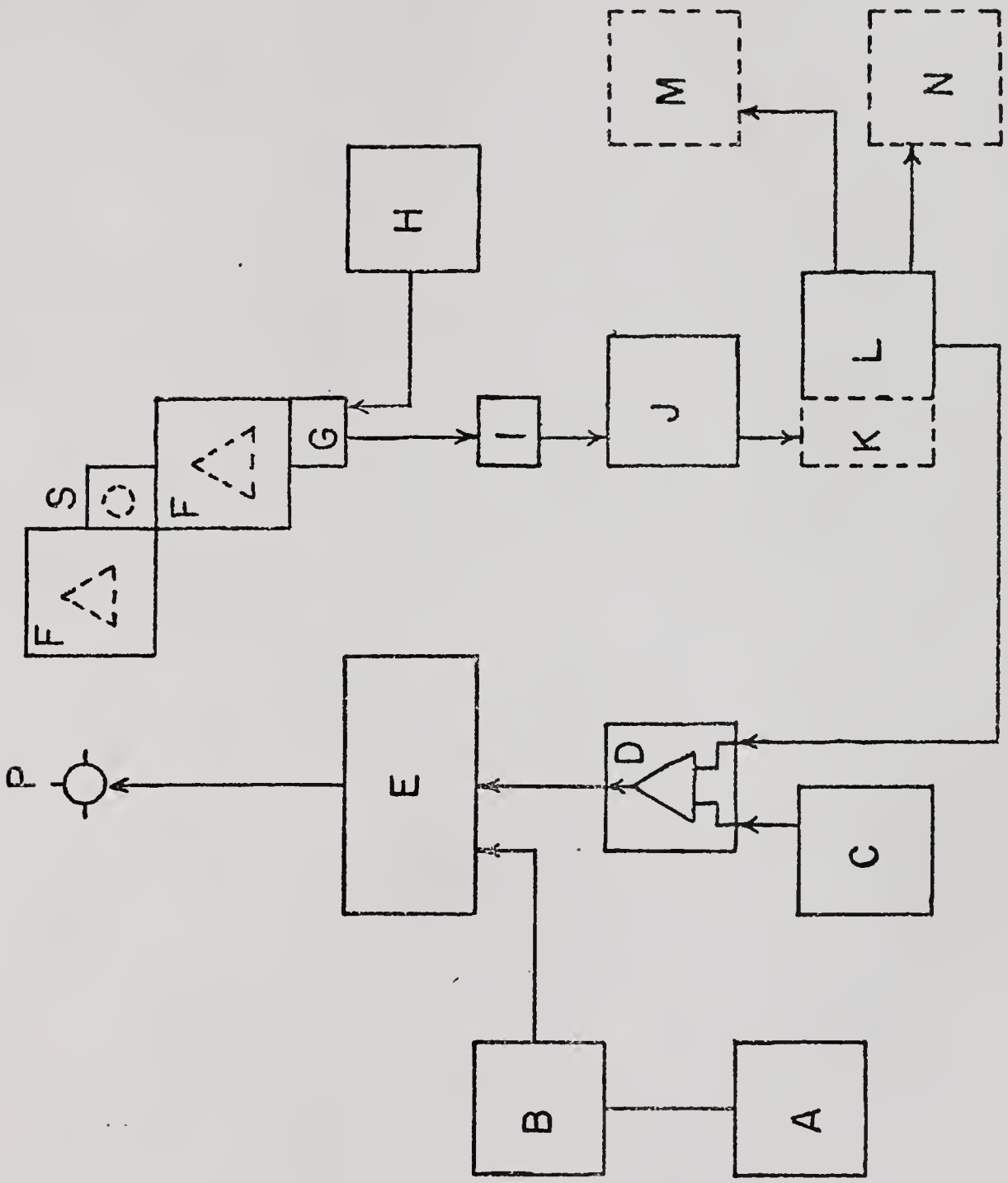
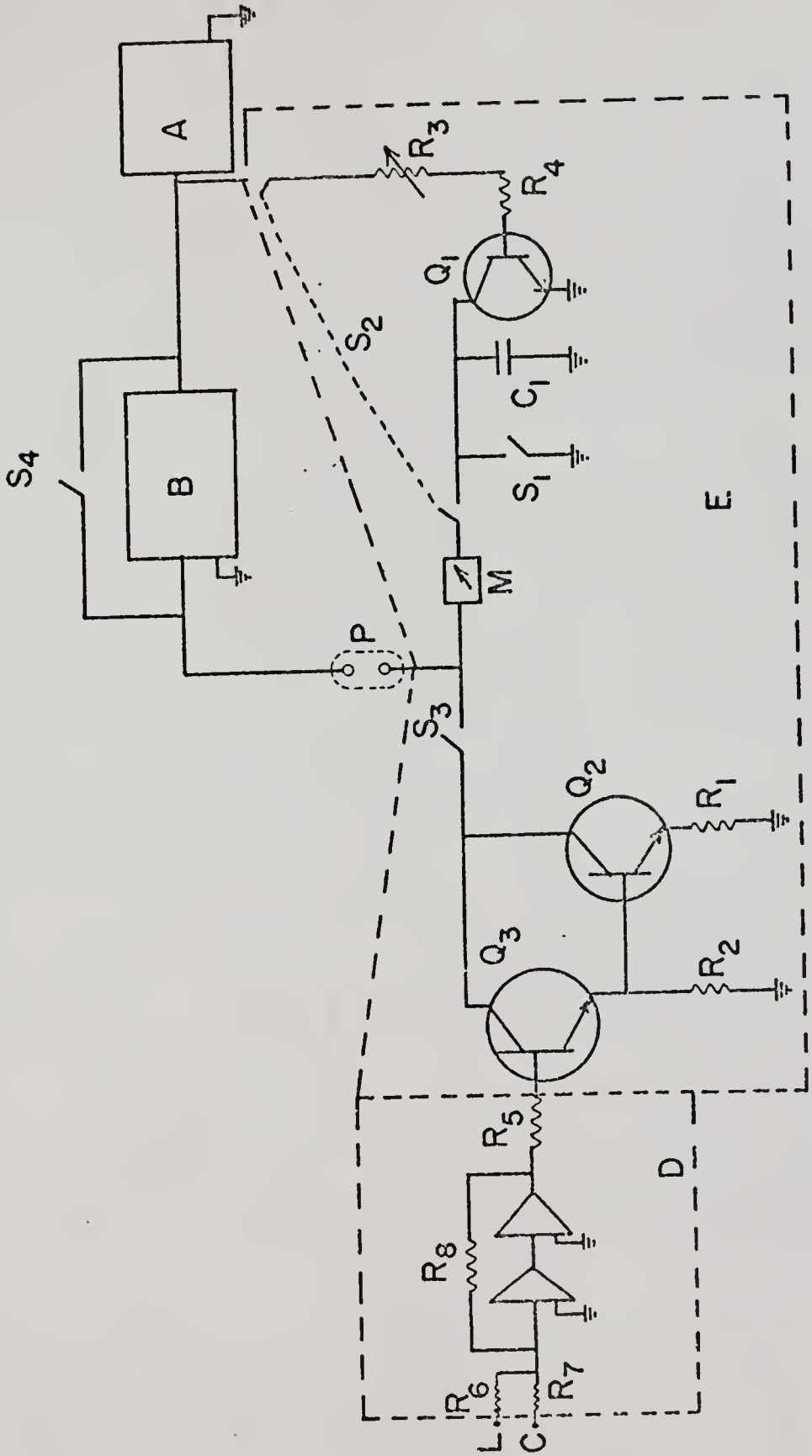

M
N
H
L
J
K
I
G
S
F
F
P
E
D
C
B
A

Figure 5. Schematic diagram of starting and modulation circuit.

C_1 = 4000 MFD, 40 WVDC.
Q_1 = 2N5881.
Q_2 = 2N5885.
Q_3 = 2N1479.
R_1 = 1Ω ± 5%, 240 W.
R_2 = 1 KΩ ± 5%.
R_3 = 0-5 KΩ ± 5%.
R_4 = 100Ω ± 5%.
R_5 = 1 KΩ ± 5%.
R_6 = 10 KΩ ± 5%.
R_7 = 100 KΩ ± 5%.
R_8 = 100 KΩ ± 5%.

S_1 = SPST switch.
S_2 = DPST switch.
S_3 = SPST switch.
S_4 = SPST heavy duty switch.
M = Simpson 0-10A DC ammeter.
A,B,C,D,E,L = (See Figure (4)).


A
B
S_4
S_2
R_3
R_4
Q_1
C_1
S_1
E
M
P
S_3
Q_2
R_1
Q_3
R_2
R_5
D
R_8
R_6
R_7
L
C

turned on and the voltage setting on its front face adjusted to 34V. Switches S_3 and S_4 were open and switches S_2 and S_1 were closed. In this configuration, the modulation circuit is switched out of the circuit, and the shorting jumper on the starter is removed. Closing S_1 essentially shorts the controlling power transistor Q_1 during the starting of the lamp and prevents its destruction. The starter is switched on momentarily until the lamp ignites and then is switched off. The current control on the DC power supply is set at a value of about 8.5A and should not need to be reset during the operation of the apparatus. The current level set here should be high enough so as not to become the current limiting factor whenever the lamp is modulated. When the lamp is started and current is flowing through the shorting switch S_1, the variable resistor R_3 is adjusted to its minimum resistance. This makes R_4 the controlling resistor for the base current into Q_1. Switch S_1 is then opened, and current begins to flow through Q_1. The large capacitor C_1 is used as a ballast to ease transfer of current control to Q_1 when the circuit is broken as S_1 is opened. A high current should not be allowed to flow through Q_1 for a considerable length of time due to its power dissipation requirements, and thus the resistance R_3 is increased to lower the current to approximately 4A as monitored on meter M. Next, the DC power supply (Harrison Model 6116A Hewlett-Packard, Palo Alto, Calif.) which will supply a constant DC current level to the lamp modulation current is

turned on and the voltage level set at 2V. The output voltage from this supply together with a sinusoidal signal to be added later is applied to the input of a summing operational amplifier and current booster (Model EUW-19, Heath Co., Benton Harbor, Mich.) where the voltages are amplified and added. The output of this amplifier is used to supply the base current to transistor Q_3 which is in a modified Darlington configuration with power transistor Q_2. The output of Q_3 drives the base of Q_2 which in turn controls the current flowing through the lamp circuit and load resistor R_1. After the DC power supply is set at 2V, switch S_3 is closed and current begins to flow through Q_2 and R_1. The voltage level of the DC power supply is then increased gradually while the resistance R_3 is simultaneously increased so as to decrease the current flowing through Q_1. When 6V are being supplied by the DC power supply and R_3 is at its maximum setting, there should be approximately 2A flowing through meter M. At this time, S_2 is opened and the current comes under the complete control of the modulation circuit. The DC voltage can now be set at a convenient level to supply the current desired through the lamp.

The modulation signal is provided by the internal oscillator of the lock-in amplifier to be used as a detector. This is similar to the experimental set-up used by Phillips to modulate microwave powered discharges [28]. The sinusoidal signal is taken from the reference in/out jack of a

PAR Model 220 lock-in amplifier (Princeton Applied Research, Princeton, N.J.) and applied to the input of the summing amplifier along with the DC voltage. The feedback arrangement of the resistors R_6, R_7 and R_8 allows for a X10 amplification of the sinusoidal signal but no amplification of the DC level. The signal level available at the reference in/out jack can be adjusted and varied from 1V rms to 0.5 mV rms. This signal level is adjusted until the desired degree of modulation is obtained in the exciting radiation.

When the modulation circuit is in operation, the starter is switched out of the circuit by closing switch S_4. This is performed so as to remove any high frequency limitations on the amplitude of modulation which may be introduced by the RC circuits in the starter.

In operation, the modulation circuit described performs satisfactorily. The degree of modulation is selected so as to place the peak lamp current value within the current level set by the front controls of the Harrison 6268A power supply. Too high a modulation causes instability and results in the extinguishing of the lamp. The lower current value also is limited, especially in operation at the lower frequencies. Too low a current level will also cause instability in the lamp output. Of course, the degree of modulation possible will be affected by the value of the DC voltage supplied along with the modulation signal. This DC voltage will determine the constant current level for the lamp operation, and the superimposed AC signal will be

centered at this value. In this work, it has been possible to modulate the light intensity up to 75% with no resulting instabilities. The degree of modulation was found to be constant from 1 Hz through approximately 1000 Hz. Above 1000 Hz, the amplitude of the lamp modulation began to decrease. This decrease is probably due to the power supply limitations on how fast the current may be modulated.

A note of caution should be introduced here about the effect of the starting pulse for the lamp upon electronic components nearby. It was found early in this investigation that this high energy pulse could destroy sensitive FET components in the lock-in amplifier. Thus, whenever the lamp was started all connecting cables to all the inputs of the lock-in amplifier were removed, and the lock-in turned completely off.

The optical system consists of an Aminco SPF Spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) which has been modified for the present studies. The usual lamp housing was removed and a slit holder with slits was attached at the entrance of the excitation monochromator. The light from the source was focused on this entrance slit as stated previously. Although the excitation and emission monochromators are baffled to reduce stray light, it was found necessary to add an extra baffle in the interior of the excitation monochromator to reduce stray light even further. The gratings used in the monochromators were blazed at 300 nm

for the excitation monochromator and at 500 nm for the emission monochromator. The usual slit holder at the exit slit of the excitation monochromator was modified to hold a 1" by 1" square Corning 7-54 filter. This filter prevented stray visible radiation from entering the sample compartment. This slit holder was designed so that various slit widths could be placed at the exit of the excitation monochromator. The appropriate slit width was selected by positioning the proper slit in front of the sample compartment. The sample compartment was the standard Aminco phosphorescence cell compartment with the rotating can phosphoroscope removed.

The sample solution was contained in a quartz sample cell (5 mm od x 3 mm id) 25 cm long which was held in place via a modified Varian A-60A NMR spinner assembly (Varian Instruments, Palo Alto, Calif.) as described by Lukasiewicz, et al. [37]. The tube was placed into a liquid nitrogen dewar which fitted into the Aminco sample cell assembly. In these experiments, the sample tube was not rotated. The luminescence emission from the sample was detected at the exit slit of the emission monochromator by an RCA 1P21 photomultiplier tube (American Instrument Co., Silver Spring, Md.) which was operated at a constant voltage of 700V supplied by a Heath high voltage power supply (Model EU-42A, Heath Co., Benton Harbor, Mich.). The signal from the photomultiplier was directed to a load resistor box which converted the signal to a voltage. The output from the load

box was then applied to the input of a high gain, differential amplifier (Type 1A7A, Tektronix, Inc., Portland, Ore.). The output of this amplifier was then applied to both the input of a lock-in amplifier (Model 220, Princeton Applied Research, Princeton, N.J.) and the input of a DC electrometer (Model 610BR, Keithley Instr., Inc., Cleveland, Ohio). The DC electrometer was used to measure the average DC level of the signal whenever modulation measurements were required. The lock-in amplifier measured the average AC component of the incoming signal. The lock-in amplifier was equipped with a variable frequency control and a calibrated, adjustable phase shifter. A phase quadrant switch allowed shifting the phase of the internal reference by 90°, 180° or 270°. The output of the lock-in amplifier was displayed either on a Moseley x-y recorder (F. L. Moseley, Pasadena, Calif.) or a Sargent Model TR strip chart recorder (E. H. Sargent & Co., Chicago, Ill.). For the phase resolution measurements, an additional preamplifier (PAR Model 211, Princeton Applied Research, Princeton, N.J.) was placed between the lock-in and the 1A7A amplifier. This provided an additional measure of sensitivity.

Procedure

The modulation behavior for the selected phosphorescent species was determined by measuring the AC component of the luminescence signal while changing the frequency of modulation of the exciting light. The parameter m (the degree of

modulation) was measured by comparing the ratio of the AC to DC component in the luminescence signal (AC_L/DC_L) to the same ratio (AC_S/DC_S) in the excitation light.

$$m = (AC_L/DC_L)/(AC_S/DC_S). \qquad (36)$$

The excitation radiation was sampled by setting the excitation and emission monochromators to a wavelength in the visible region, say 450 nm, and measuring a portion of the scattered radiation from the sample cell. When the scatter signal was being measured, the 7-54 filter was shifted from its position and a narrow 1mm slit was put into position in front of the sample cell compartment. The exit slit from the cell compartment was also reduced to 1mm in order to limit the intensity of the scatter signal. The DC component was measured on the DC electrometer and the average AC component by the lock-in amplifier. The DC component of the luminescence signal was measured at 50 Hz as were also the AC and DC components of the excitation light. Lifetime values by the modulation method were calculated from the data at 50 Hz.

Phase shift angle information was obtained by measuring the instrumental phase angle of the exciting radiation on the lock-in amplifier and comparing this value to the value of the instrumental phase angle of the luminescence signal. The instrumental phase angle of an input signal is taken to be equivalent to the instrumental phase angle of the reference signal which gives peak response to that input

signal. This peak reference phase angle is determined by rotating the quadrant switch and phase dial on the lock-in amplifier so that a null output signal is obtained for the input signal. Rotating the quadrant switch by 90° allows one to measure the peak signal and determine its phase angle. The same narrow slits, etc., as before were used when measuring the scatter signal to determine the relative phase angle of the exciting radiation. This measurement was made at every frequency at which the phase angle for the luminescence was determined. Thus, the absolute phase shift angle, Θ, presented in this work represents the difference angle between the instrumental phase angle of the exciting light and the instrumental phase angle of the luminescence signal. The lifetime values evaluated by this phase angle method were calculated from the data at 50 Hz. In both the modulation and phase measurements, the read-out system did not include the PAR 211 preamplifier or the x-y recorder.

Spectral resolution of the overlapping luminescence spectra of two phosphors was accomplished by two methods: the phase method and the frequency method. The phase method involved the measurement of the instrumental phase angles for standards of each of the two compounds in the mixture. The phase quadrant and phase shifter dials on the lock-in amplifier were then set at $90° + \phi_1$, where ϕ_1 is the phase angle of one of the compounds. The spectrum of the mixture of components was then plotted at this phase angle setting and frequency. The phase dials were then set so that the reference phase angle was equal to $90° + \phi_2$, and the mixture spectrum was again obtained. In these measurements, the frequency of modulation is kept constant.

The frequency method involved setting the phase dial and quadrant switch at $90° + \phi_S$, where ϕ_S is some selected phase angle. One of the standards is then run and the frequency of modulation is varied until the phase angle for this compound, ϕ_1, becomes equal to ϕ_S. A zero output signal reading will be obtained for this standard at this frequency, f_1. A standard solution for the other compound in the mixture is then measured, and the frequency, f_2, at which its output signal goes to zero is noted. The spectrum of the mixture is then determined at f_1 and f_2, keeping the phase angle setting constant.

For the quantitative measurements with the phase method, standards of concentration range near to that of the samples to be determined are measured as above in the spectral measurements, and the peak instrumental phase angle is determined for each of the compounds. For maximum accuracy, the most sensitive scale settings are used to determine the null point for the signals. However, the sensitivity which can be used in practice is limited by the noise and fluctuations in the signal. Once the peak phase angles for each of the components in the mixture are known, the phase dial is set as before at $90° \pm \phi_1$, where ϕ_1 is the peak phase angle of one of the components. A standard analytical curve is determined for the other component at this phase setting. Next, the reference phase is set at $90° \pm \phi_2$, and an analytical curve is measured for the other component. The signal from the binary mixture is then determined at

both phase angle settings, and the concentration of each component determined from the appropriate analytical curves.

In the frequency method experiments, the reference phase angle, ϕ_R, is set at a constant value which is equal to 90° + ϕ_S, where ϕ_S is some selected phase angle. A standard solution for each of the components in the mixture is measured, and the frequency at the quadrature or null point is noted for each standard. The analytical curve for component 2 is determined at frequency f_1, where the signal from component 1 is nulled out. Conversely, standards for component 1 are measured at the frequency f_2, where the signal from component 2 is nulled out. Again the concentration of each component is determined from the appropriate analytical curves.

In many of the phase resolution experiments, a negative signal is often obtained for one of the component signals. This signal can be made positive by changing the reference quadrature switch by 180°.

When phasing out a fluorescence signal, the phase angle of the fluorescence signal is taken to be the same as that for a scatter signal. The phase angle, ϕ_{SR}, for the exciting light is measured and the reference phase angle set at 90° ± ϕ_{SR}. The spectrum of the compound is then determined at this phase setting.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Experiments

The first experiments conducted involved the evaluation of the experimental system and the selection of appropriate analytical conditions. During the preliminary stages of data taking, it was observed that the amplitude response of the PAR lock-in amplifier was not constant with frequency. This behavior was confirmed by an experiment in which the response of the lock-in to a scatter signal was measured versus the frequency of modulation. The results are given in Figure (6). This unequal response over the frequency range is to be expected because the gain of the lock-in amplifier varies over the several frequency ranges, as listed in the operation manual. For this work, the gain was determined to give peak response in the 10 to 100 Hz range. For the sake of convenience and accuracy when determining modulation parameters and calculating lifetimes, the response at several frequencies was assigned correction factors which ranged from 0.894 at 2 Hz to 1.09 at 1000 Hz. These correction factors were calculated on the basis of the response at 50 Hz having a correction factor of 1.00.

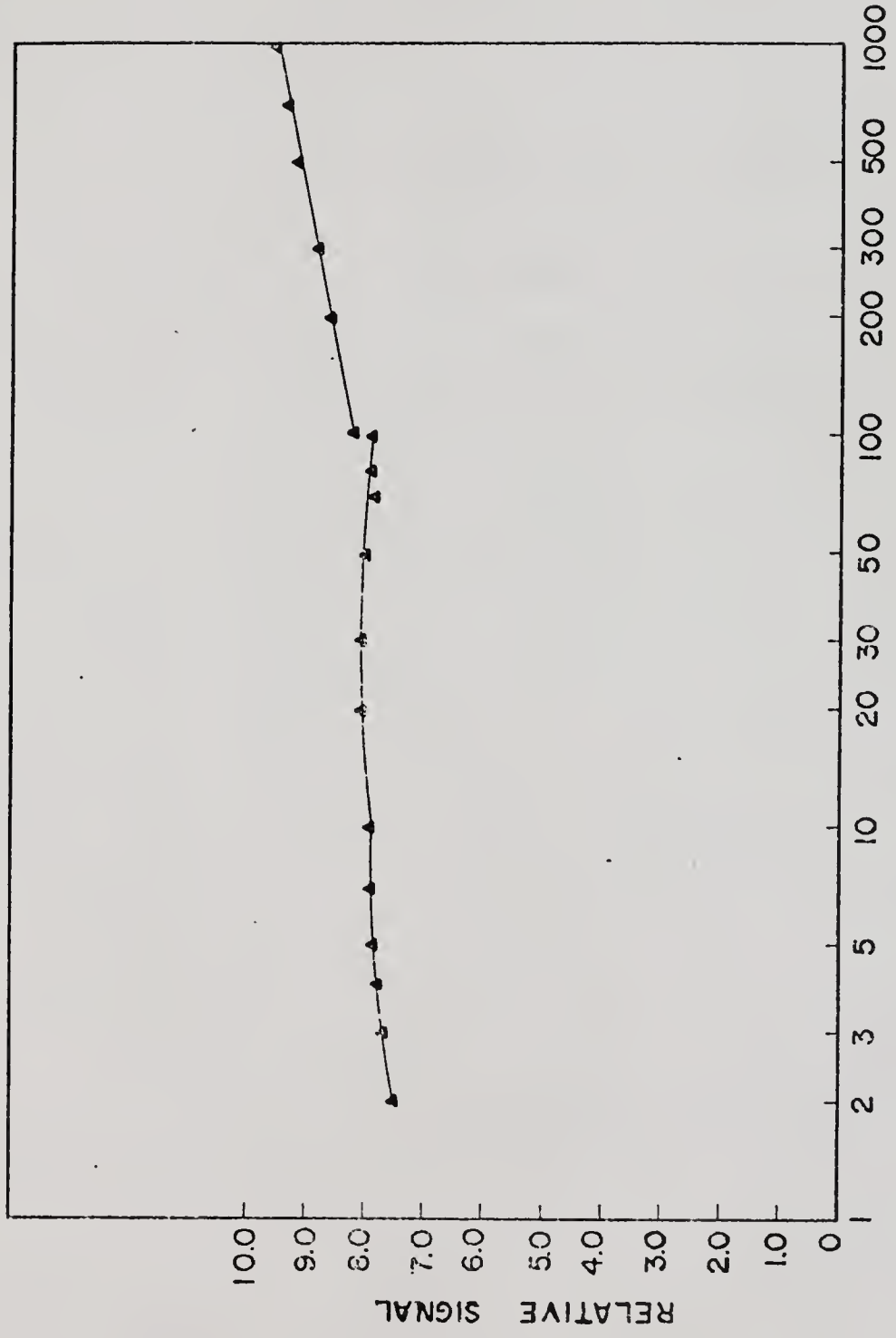


Figure 6. Variation of lock-in amplifier response to a scatter signal with the frequency of modulation.

The phase response of the lock-in amplifier versus frequency was also varying. This characteristic is illustrated in Figure (7), which is a plot of the relative instrumental phase angle for a scatter signal versus frequency. In this case, the phase angle of the scattered light was always measured whenever a phase measurement on a signal was desired.

The percent of modulation of the exciting light is defined as the ratio of the AC to the DC component of the exciting radiation times 100%. Most of the experiments in this work were conducted with a source modulation of approximately 53%. This degree of modulation was found to produce a stable output from the lamp and preserved its life. For some of the spectral resolution experiments, a degree of modulation of 75% was used. It should be obvious that the greater the percent of modulation of the exciting light, the more sensitive will be the measurement of the phosphorescence. However, this work was not concerned with limits of detection but demonstration of a principle, and thus the degree of modulation which gave the most stable signal was used.

Measurements of a scatter signal were also made to determine the effect of changing the load resistance upon the measured phase angle. It was observed that at frequencies below 500 Hz, the load resistance had little effect upon the value of the instrumental phase angle. The gain setting on the 1A7A amplifier appeared to produce a change of the phase angle at the high gain settings. The use of these high gain scales was avoided when phase measurements were

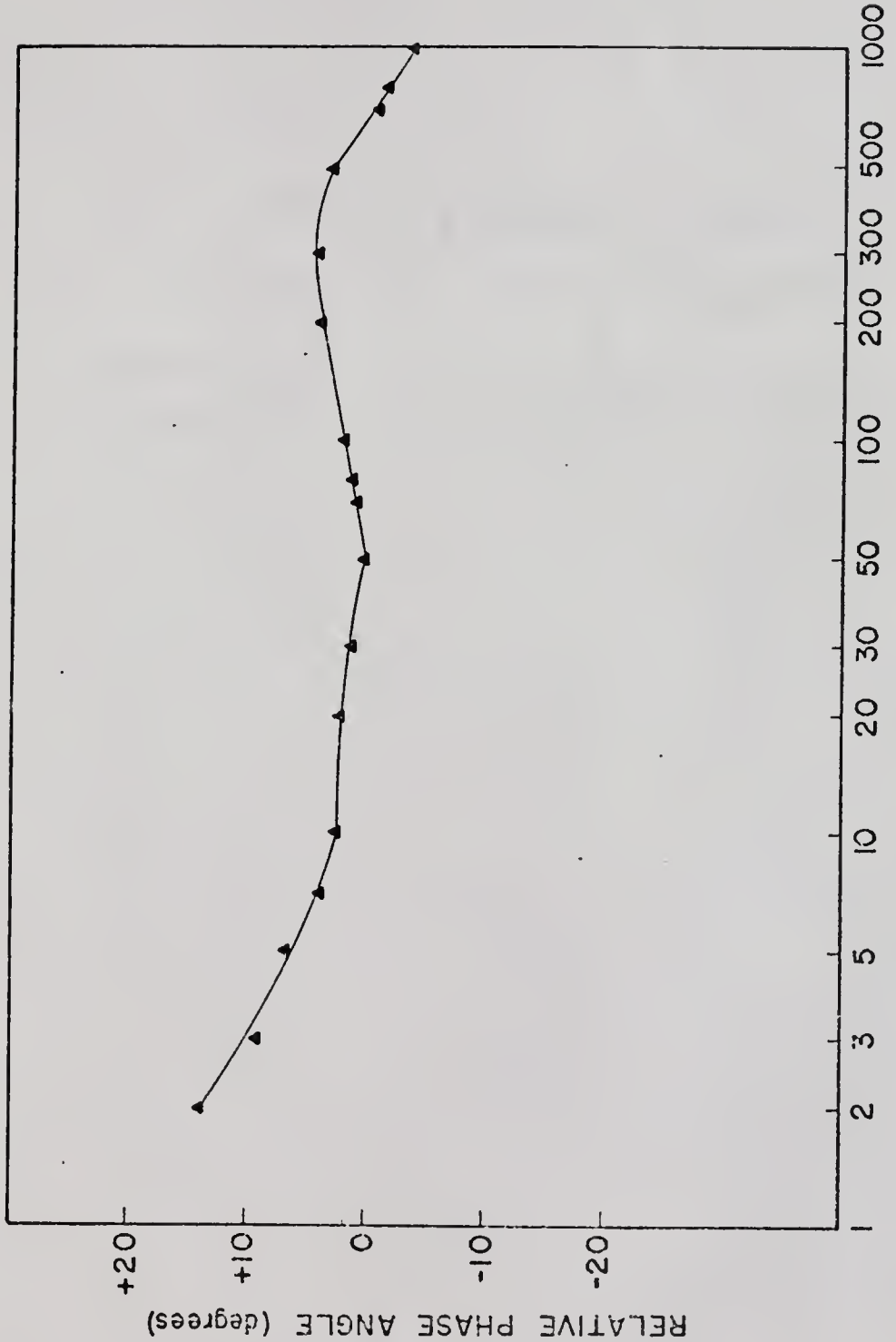


Figure 7. Variation of instrumental phase angle for a scatter signal with the frequency of modulation.

taken and greater use was made of changing the load resistor. For the initial measurements, the PAR Model 211 preamplifier was not used in the system. When this preamplifier was employed, its gain was set at times (x 10) ten and not varied. The phase angles for the scatter signal appeared to vary considerably with the gain setting on this amplifier.

As mentioned before, the solvent used in these studies was absolute ethanol. During the first experiments, it became apparent that the condition of requiring a glassy, uncracked matrix for measurement of phase and modulation was critical. Phase angle values could vary as much as 6° if the sample was cracked rather than clear. This variation could possibly be due to an increase in scattered light from a cracked sample over a clear sample. It was also noted that the relative signal increased when the sample was cracked.

The considerations which went into the selection of the molecules to be studied in this work were based upon their relative phosphorescence intensity, lack of substantial fluorescence emission, and the availability of lifetime information for the molecules. In a paper by O'Donnell, Harbaugh and Winefordner [9], the lifetimes of several arylketones and halogenated biphenyls [8] were reported. Because this present work was concerned with the observation of phase and modulation characteristics for molecules of varying lifetimes and because of the availability of several arylketones and halogenated biphenyls in this laboratory, several of these

molecules with a sizable range in lifetimes were selected. Additionally, because lifetime data and quantitative analysis data were available for mixtures of halogenated biphenyls, it was decided to use these same types of molecules as a means of comparing the phase resolution technique with the time resolved technique previously used.

The variation of the phosphorescence signal with modulation frequency is illustrated in Figure (8) for some of the molecules measured. In all cases, the measurements were made at the peak phosphorescence excitation and emission wavelength. The curves for benzophenone and 4-bromobiphenyl followed the expected behavior for molecules of their respective lifetimes, with a leveling off at low frequencies and a linear portion extending to higher frequencies. The curve for 4-hydroxybutyrophenone displays a deviation in slope at the higher frequencies. The curve for 4,4'-bis-dimethylaminobenzophenone seems quite anomalous. Benzophenone is known to show strong phosphorescence and no fluorescence. In the case of the 4-bromobiphenyl, again the phosphorescence is strong, but a fluorescence emission is also observed. By choosing the appropriate excitation and emission wavelengths, the fluorescence was reduced considerably. Traces of this fluorescence could possibly account for the slight upward deviation of the curve at the very high frequencies. For 4-hydroxybutyrophenone, selection of wavelengths was also employed, but in this case, a stronger fluorescence is present, and the deviation occurs at lower frequencies.

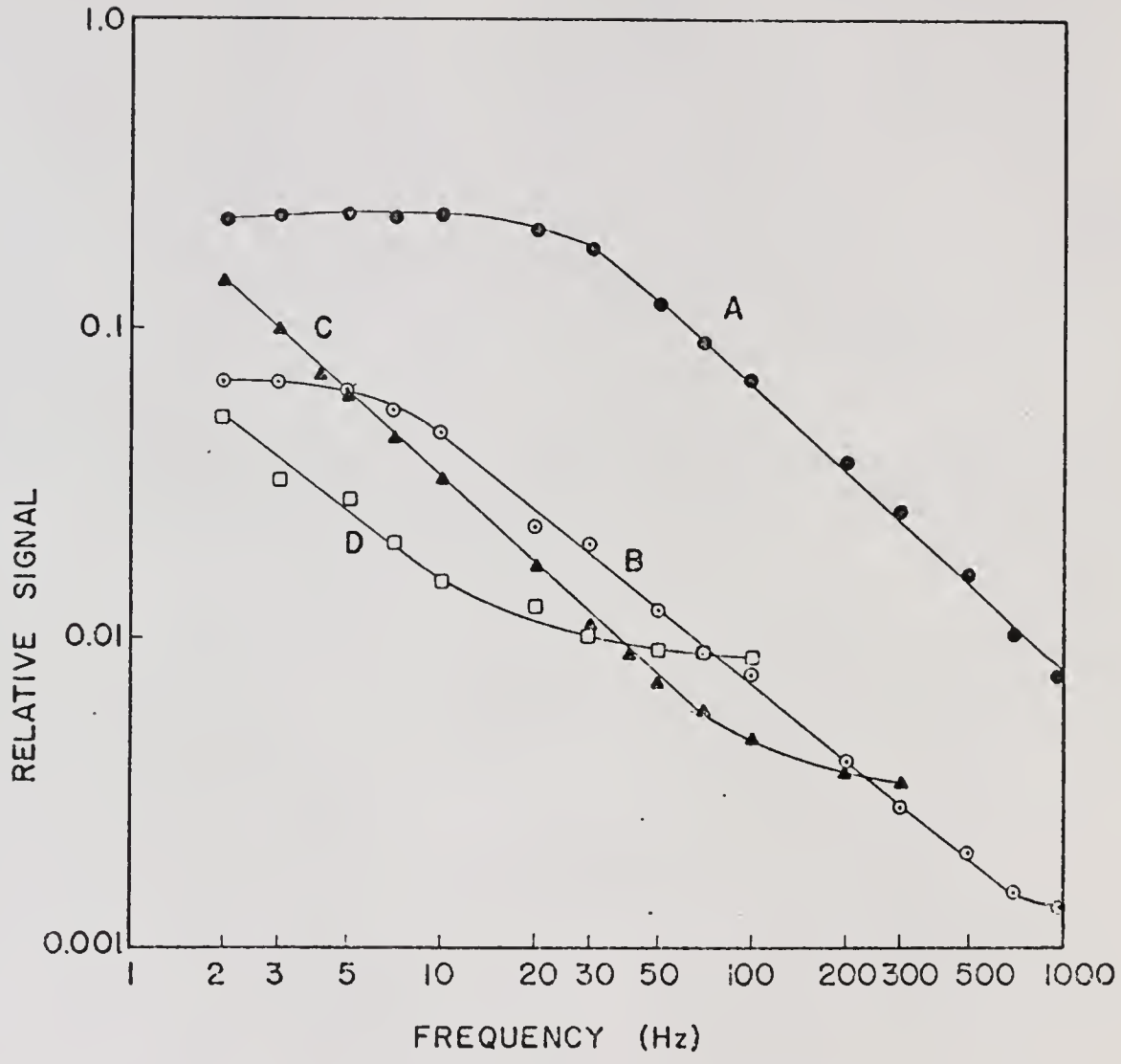


Figure 8. Variation of phosphorescence intensity with the frequency of modulation.

A = benzophenone.

B = 4-bromobiphenyl.

C = 4-hydroxybutyrophenone.

D = 4,4'-bisdimethylaminobenzophenone.

The rapid leveling off of the 4,4'-bisdimethylaminobenzophenone (4,4'-DMAB) curve is due to the fact that the fluorescence and phosphorescence emission spectra for this compound overlap severely.

The behavior of the 4,4'-DMAB illustrates one of the major limitations of the experimental technique and theory of phase resolution in phosphorimetry. Because the total luminescence emission is observed, a molecule which shows strong fluorescence and weak phosphorescence and/or which shows severe overlap of the fluorescence and phosphorescence emission spectra results in a severe deviation in the signal versus frequency curve. This, of course, limits the determination of accurate modulation factors and phase angles, and subsequently an accurate lifetime value cannot be determined. In quantitative measurements, the presence of the fluorescence emission places a lower limit to the sensitivity of the phase resolved phosphorescence technique because in this experimental technique, only one signal can be phased out at any one time. Thus, in resolving two phosphorescent species, the limit of detection would be limited by a constant fluorescence signal. If the fluorescence signal is weak, however, the phase settings used in the experiment could reduce the fluorescence signal and improve the analysis. In order to simplify the measurements of modulation and phase parameters, the molecules selected for further study were limited to those in which the fluorescence interference was small.

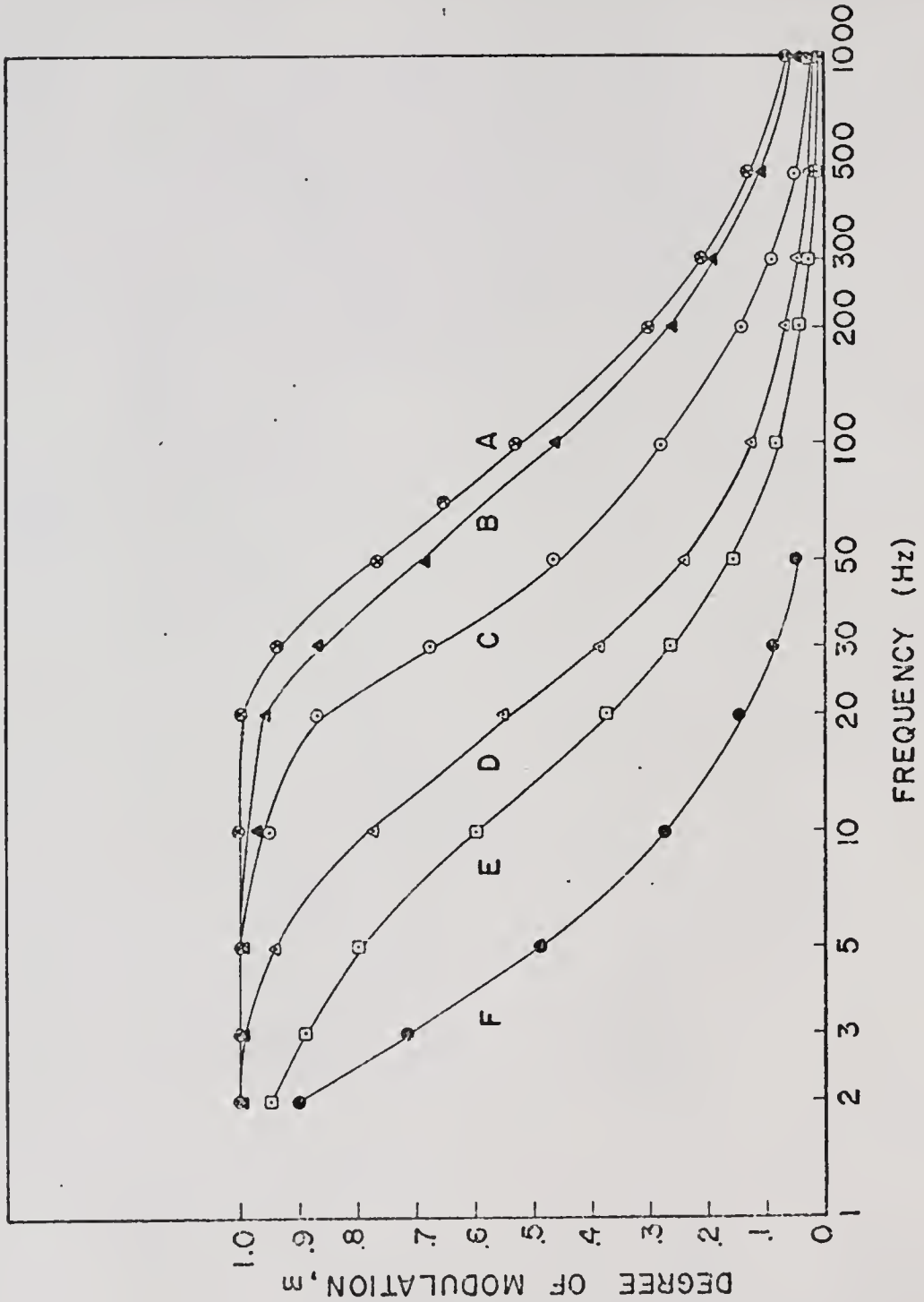
Phase and Modulation Characteristics

A determination of the degree of modulation parameter, m, and the phase angle parameter, Θ, was made for a series of molecules. The results are summarized in Figures (9) and (10). The modulation curves for these molecules show the expected variation of amplitude with modulation frequency. It is observed that the phosphorescence from long lifetime molecules decreases in intensity more rapidly than the phosphorescence from short lifetime molecules. All the curves tend to approach a maximum value for m of 1.0 at low frequencies and approach a value of zero as the frequency is increased. The lifetime of these molecules may be calculated in theory from the value of m at any frequency using Equation (17). However, the most accurate values of the degree of modulation are obtained in that portion of the curve with the greatest slope. Therefore, the lifetime values were determined at 50 Hz because the measurement of the source AC and DC levels was performed at this frequency.

The interesting aspect of these modulation curves is that they illustrate enhanced response for a short lifetime molecule over that for a very long lifetime molecule. This is especially important when the background phosphorescence from the solvent is of a long-lived nature. The curves also show a disadvantage of this experimental technique in that only signals from phosphorescent species with lifetimes shorter than approximately 50 ms can be measured easily. This disadvantage arises due to the difficulty of making measurements

Figure 9. Variation of experimentally determined degree of modulation, m, with the frequency of modulation.

A = anthraquinone, τ = 3.0 ms.
B = 4-iodobiphenyl, τ = 3.5 ms.
C = benzophenone, τ = 6.0 ms.
D = 4,4'-dibromobiphenyl, τ = 12. ms.
E = 4-bromobiphenyl, τ = 17. ms.
F = 3-bromobiphenyl, τ = 55. ms.


DEGREE OF MODULATION, m
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
5
10
20
30
50
100
200
300
500
1000
FREQUENCY (Hz)
A
B
C
D
E
F

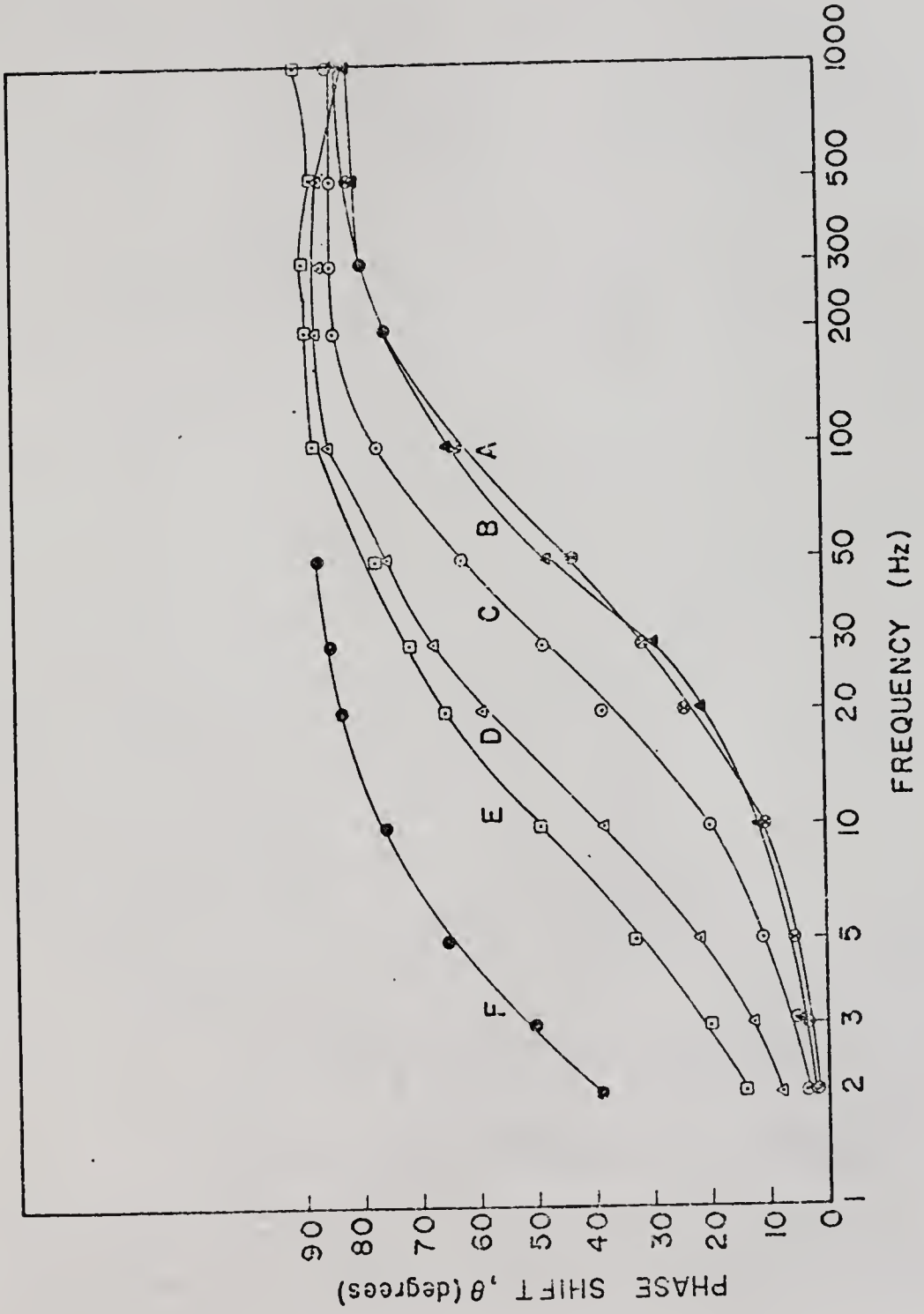
at frequencies below 10 Hz. These difficulties are due to low frequency fluctuations in the signal, due to bubbling in the liquid nitrogen dewar, and the necessity of using long time constants when making the measurement.

The phase shift angle measurements demonstrate the expected behavior for molecules of different lifetimes. Here the curves show how the phase shift for the luminescence signal varies from 0° at low frequencies to 90° at high frequencies. It should be noted here that the error in these measurements is greatest at the very low (<10 Hz) and high frequencies (>100 Hz). At the low frequencies, bubbling noise made the determination of null signals very difficult. At the high frequencies, the determination of the phase angle became difficult because of the low signal levels involved and the increased possibility of observing scattered and fluorescence radiation. The leveling off of these curves at approximately 85° could be attributed to these factors as well as to the characteristics of the lock-in amplifier at these higher frequencies. Preliminary determinations of the phase shift angles in this region were improved somewhat when the 7-54 filter was used in the excitation path. This seemed to signify some visible stray light contribution at the higher frequencies.

Lifetime data can be calculated from the phase angle value at any frequency, but here again, as in the modulation experiment, the most accurate values are those which lie on that portion of the curve with the greatest slope. The

Figure 10. Variation of experimentally determined phase shift angle, Θ, with the frequency of modulation.

A = anthraquinone, τ = 3.0 ms.
B = 4-iodobiphenyl, τ = 3.5 ms.
C = benzophenone, τ = 6.0 ms.
D = 4,4'-dibromobiphenyl, τ = 12. ms.
E = 4-bromobiphenyl, τ = 17. ms.
F = 3-bromobiphenyl, τ = 55. ms.


PHASE SHIFT, θ (degrees)
0
10
20
30
40
50
60
70
80
90
1
2
3
5
10
20
30
50
100
200
300
500
1000
FREQUENCY (Hz)
A
B
C
D
E
F

lifetimes were calculated from the data at 50 Hz. Table (1) lists the lifetimes calculated from the phase and modulation data and compares these to the lifetimes reported by time resolved phosphorimetry. The lifetimes were calculated from the phase data using the equation

$$\Theta = \tan^{-1} 2\pi f\tau. \qquad (37)$$

The results in Table (1) seem to indicate good agreement within the experimental error between the phase and modulation data. The exception is the 4-bromobiphenyl which shows a wide spread in values. This deviation could be due to indeterminate errors for that particular compound, which include such things as errors in reading the meter on the DC electrometer when modulation data were required, and failure to obtain accurate quadrature or null points, thereby resulting in bad phase angle data.

Phase Resolved Phosphorescence of Synthetic Mixtures

Using the information provided by the phase and frequency characteristics for these molecules, phase resolution experiments were now feasible. The selection of optimum experimental conditions for phase resolution studies involved the selection of the molecules to be studied and the optimum instrumental conditions. When one of the components of a mixture is nulled (phased) out, the resulting signal from the other component is proportional to the sine of the difference in phase angles for the two

TABLE 1

PHOSPHORESCENCE LIFETIMES FROM PHASE AND MODULATION DATA[a]

	Lifetime (ms)[b]		
MOLECULE	PHASE[c]	MODULATION[c]	TIME RESOLVED[d]
Benzophenone	5.9	6.1	7.
Anthraquinone	3.0	2.7	3.6
4-Iodobiphenyl	3.5	3.3	3.2
4-Bromobiphenyl	14.	20.	17.
3-Bromobiphenyl	55.	59.	58.
4,4'-Dibromobiphenyl	12.	13.	12.

a. Solvent: ethanol.

b. Relative errors in lifetimes are $\pm$ 10%.

c. All values calculated from measurements at 50 Hz.

d. Data taken from [8, 9].

components; therefore, the mixtures used were selected with consideration of their phase angle difference at a particular frequency. The operating frequency was also important as it would determine the relative maximum signal which could be expected from the compounds and the value of the phase angles. Referring to Figure (10), it can be observed that the maximum difference in phase angle for the molecules studied occurs in the frequency range between approximately 10 and 100 Hz. The ease of measurement would favor the use of the higher frequency end of this scale. However, for some of the long-lived phosphors, the degree of modulation drops to 0.1 or less of its original value in this higher frequency range. As a compromise choice, the operating frequency was chosen as 25 Hz for the spectral and quantitative measurements involving phase resolved phosphorimetry.

The compounds for the spectral studies were selected on the basis of their phase angles and the shape of their respective emission and excitation spectra. A mixture of benzophenone and 4-bromobiphenyl was chosen because there was approximately 25° difference in their phase angles and the spectral characteristics of each were quite different. It should be stated here that both of these molecules have excitation bands which overlap and are indistinguishable. Thus, only the emission spectra are reported in this experiment. The results are given in Figure (11). Curve A is the spectrum of the mixture measured at its peak phase

Figure 11. Phase resolved emission spectrum of a mixture of 2.2×10^{-5}M benzophenone and 2.9×10^{-5}M 4-bromobiphenyl at 25 Hz. Excitation wavelength is 275 nm.

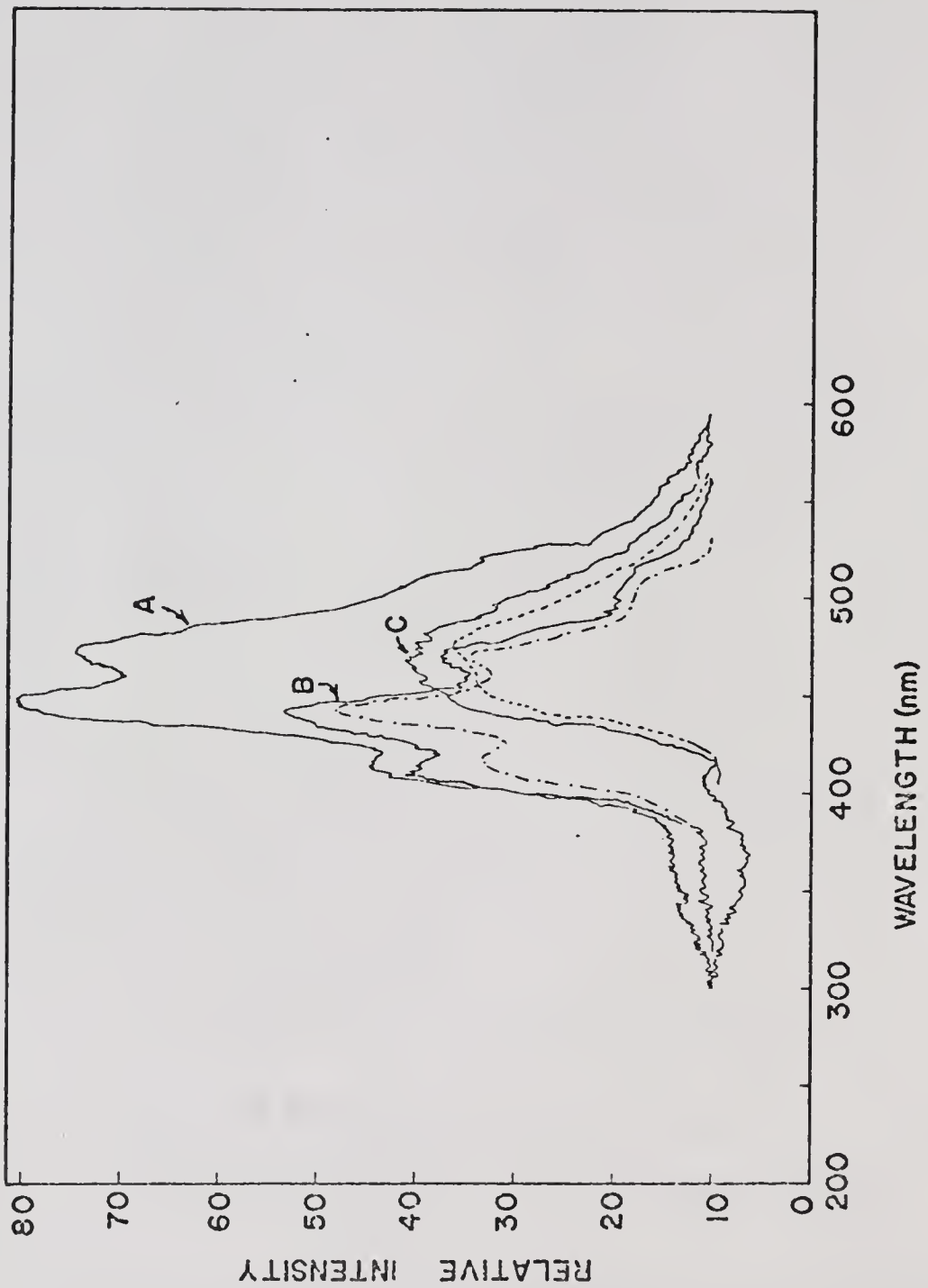
A = mixture spectrum at peak phase angle, $\phi_R = 270° + 39°$.

B = mixture spectrum, $\phi_R = 0° + 26°$.

C = mixture spectrum, $\phi_R = 180° + 52°$.

(—·—) = spectrum of benzophenone standard, $\phi_R = 270° + 52°$.

(---) = spectrum of 4-bromobiphenyl standard, $\phi_R = 270° + 26°$.


RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B
C

setting which in this case lies between the phase angles for each of the components. Then by setting the reference phase setting on the lock-in amplifier so that the signal from 4-bromobiphenyl is 90° out of phase with the reference signal, the spectrum of the mixture is again determined and curve B results. Vice versa, if the reference phase angle is set such that the signal from the benzophenone is phased out, the spectrum of the 4-bromobiphenyl, curve C, results. Thus, the spectrum of the mixture has been resolved into its two major components.

In certain situations, the spectra of the components do not completely overlap either in the excitation or emission spectra. In these cases, it should be possible to resolve the components spectrally. However, if one component emits a much stronger phosphorescence, the spectrum of the other component may be completely hidden. A good example of this problem is given in Figure (12). This is a spectrum of a mixture of anthraquinone and 4-bromobiphenyl. The spectrum resembles the spectrum of the anthraquinone in both its emission and excitation spectral details. If a higher amplification setting is used and the emission monochromator is placed at 475 nm, the resulting excitation spectrum is seen in Figure (13) as curve A. A shoulder is observed on the anthraquinone excitation peak which represents the excitation peak of the 4-bromobiphenyl. Exciting at this shoulder peak results in emission spectrum B which resembles that of the

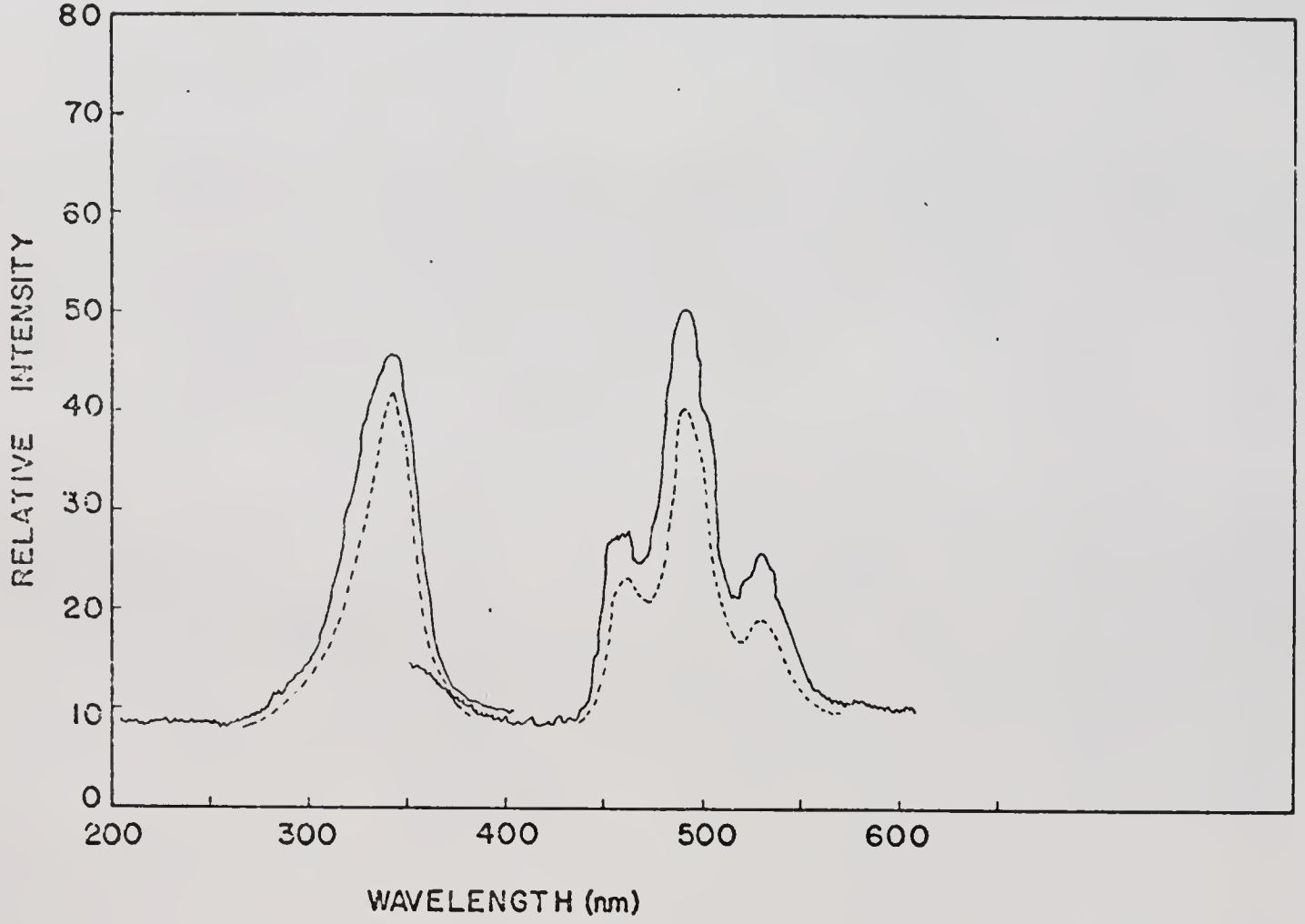


Figure 12. Excitation and emission spectra of mixture of 7.5×10^{-5}M anthraquinone and 2.9×10^{-5}M 4-bromobiphenyl at 25 Hz.

(---) = spectrum of anthraquinone standard, ϕ_R = 270° + 70°.

Figure 13. Phase resolved excitation and emission spectra of a mixture of 7.5×10^{-5}M anthraquinone and 2.9×10^{-5}M 4-bromobiphenyl at 25 Hz.

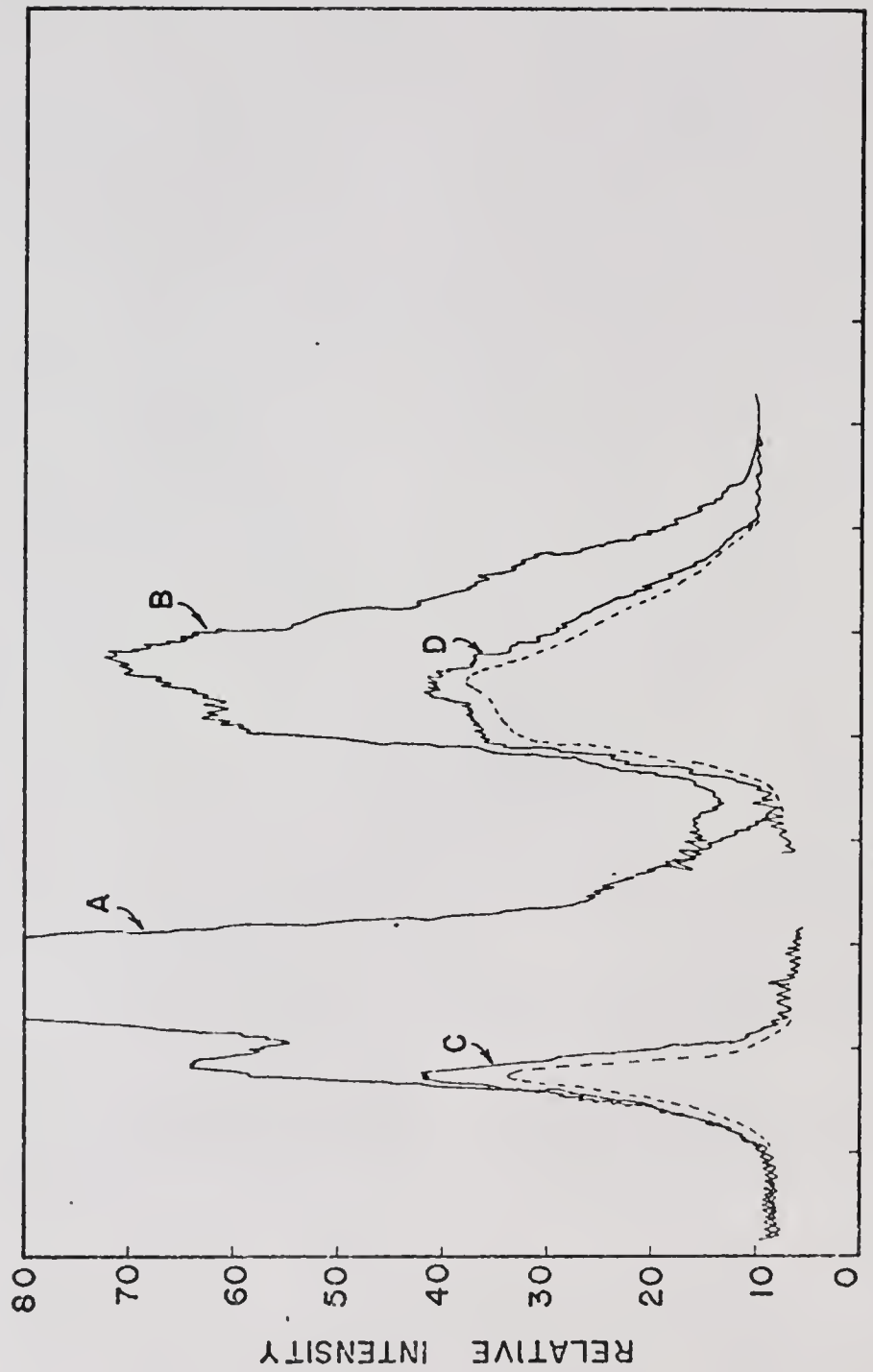
A = excitation spectrum of mixture, emission wavelength = 475 nm, $\phi_R = 270° + 70°$.

B = emission spectrum of mixture, excitation wavelength = 275 nm, $\phi_R = 270° + 70°$.

C = excitation spectrum of mixture, emission wavelength = 475 nm, $\phi_R = 180° + 70°$.

D = emission spectrum of mixture, excitation wavelength = 275 nm, $\phi_R = 180° + 70°$.

(---) = excitation and emission spectra of 4-bromobiphenyl standard, $\phi_R = 270° + 26°$.


RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B
C
D

4-bromobiphenyl alone. An even more impressive separation can be made by phasing out the signal from the anthraquinone and recording the resulting spectrum. The result is shown in the excitation and emission spectra, C and D, respectively. Emission spectrum D more closely resembles the emission spectrum of 4-bromobiphenyl.

An alternate method of resolution is called the frequency method and the use of this technique is illustrated in Figure (14). Here again, the mixture of benzophenone and 4-bromobiphenyl was selected. In this experiment, however, the reference phase angle was set at a value 90° away from a selected phase angle ϕ_S. This phase angle was selected by consideration of the modulation and phase curves given in Figures (9) and (10). From previous measurements, it was known that the instrumental phase angle of the exciting light was approximately 90° + 270° on the lock-in amplifier. Setting the reference phase angle constant at 40° + 270° would give maximum response to a molecule having its absolute phase shift angle at around 50° + 270°. This corresponds to a horizontal line at 50° on the Θ versus frequency diagram, Figure (10). Observation of the intersection point with the phase curves for 4-bromobiphenyl and benzophenone shows that these points occur at approximately 10 Hz and 35 Hz. This means that if the reference phase angle is set at 40° + 180° and the frequency of modulation adjusted to near 10 Hz, the signal from the 4-bromobiphenyl would be phased out.

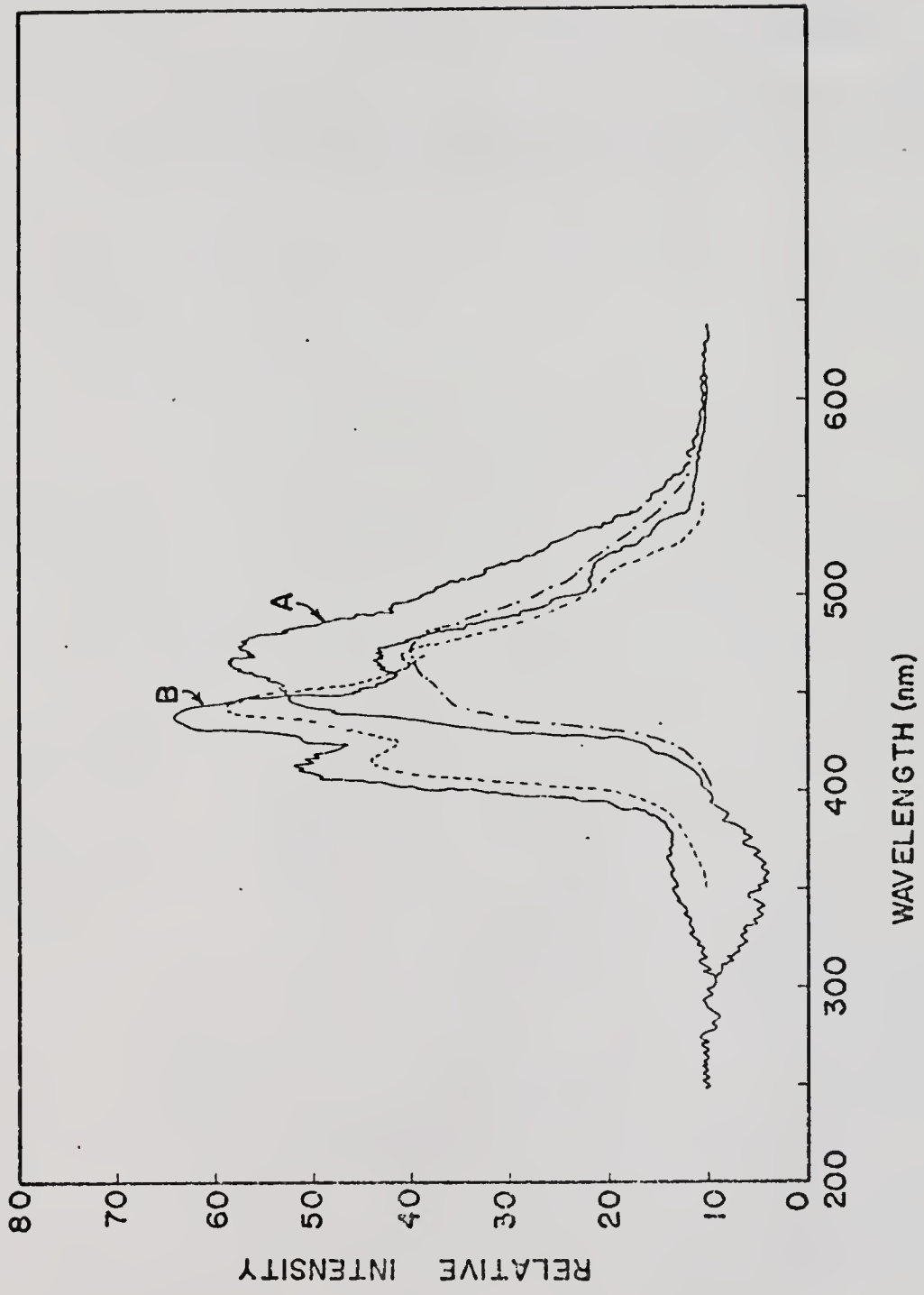
Figure 14. Frequency method resolved emission spectrum of a mixture of 2.2×10^{-5}M benzophenone and 2.9×10^{-5}M 4-bromobiphenyl. Excitation wavelength is 275 nm.

A = emission spectrum of mixture, frequency = 37.5 Hz, $\phi_R = 180° + 40°$.

B = emission spectrum of mixture, frequency = 14.7 Hz, $\phi_R = 180° + 40°$.

(---) = emission spectrum of benzophenone standard.

(—·—) = emission spectrum of 4-bromobiphenyl standard.


RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B

Similarly, if the frequency is adjusted to near 32 Hz, the signal from the benzophenone would be phased out. The results of this experiment are shown in Figure (14). The spectrum of the mixture determined at 13.1 Hz shows only the spectrum of the benzophenone, B. At 37.5 Hz, the spectrum, A, of the biphenyl results.

In both the phase and frequency techniques, when one component is phased out, the signal from the other component may have a negative value. Because the meter on the lock-in amplifier measures only positive signals, and for the sake of convenience when recording signals on the strip chart recorder, the reference phase angle is shifted 180° in order to give a positive signal. This positive and negative relationship between the signals from each component is illustrated in Figures (15) and (16). The zero level on these figures is set near mid-scale on the intensity axis. These figures show the spectrum of the benzophenone and 4-bromobiphenyl mixture at a much lower concentration than before. The peak centered at 365 nm is a solvent fluorescence peak. In Figure (15), curve A is the spectrum of the mixture with the biphenyl component phased out. Curve B shows the spectrum of a 4-bromobiphenyl standard at this same phase setting. In Figure (16), curve A is the emission spectrum of the biphenyl component with the benzophenone phased out. Curve B illustrates the signal from a benzophenone standard at this same phase setting.

Figure 15. Phase resolved emission spectrum of a mixture of 4.5×10^{-6}M benzophenone and 5.8×10^{-6}M 4-bromobiphenyl at 25 Hz and $\phi_R = 0° + 26°$. Excitation wavelength is 275 nm.

A = mixture spectrum with the signal from 4-bromobiphenyl phased out.

B = emission spectrum of 4-bromobiphenyl standard.

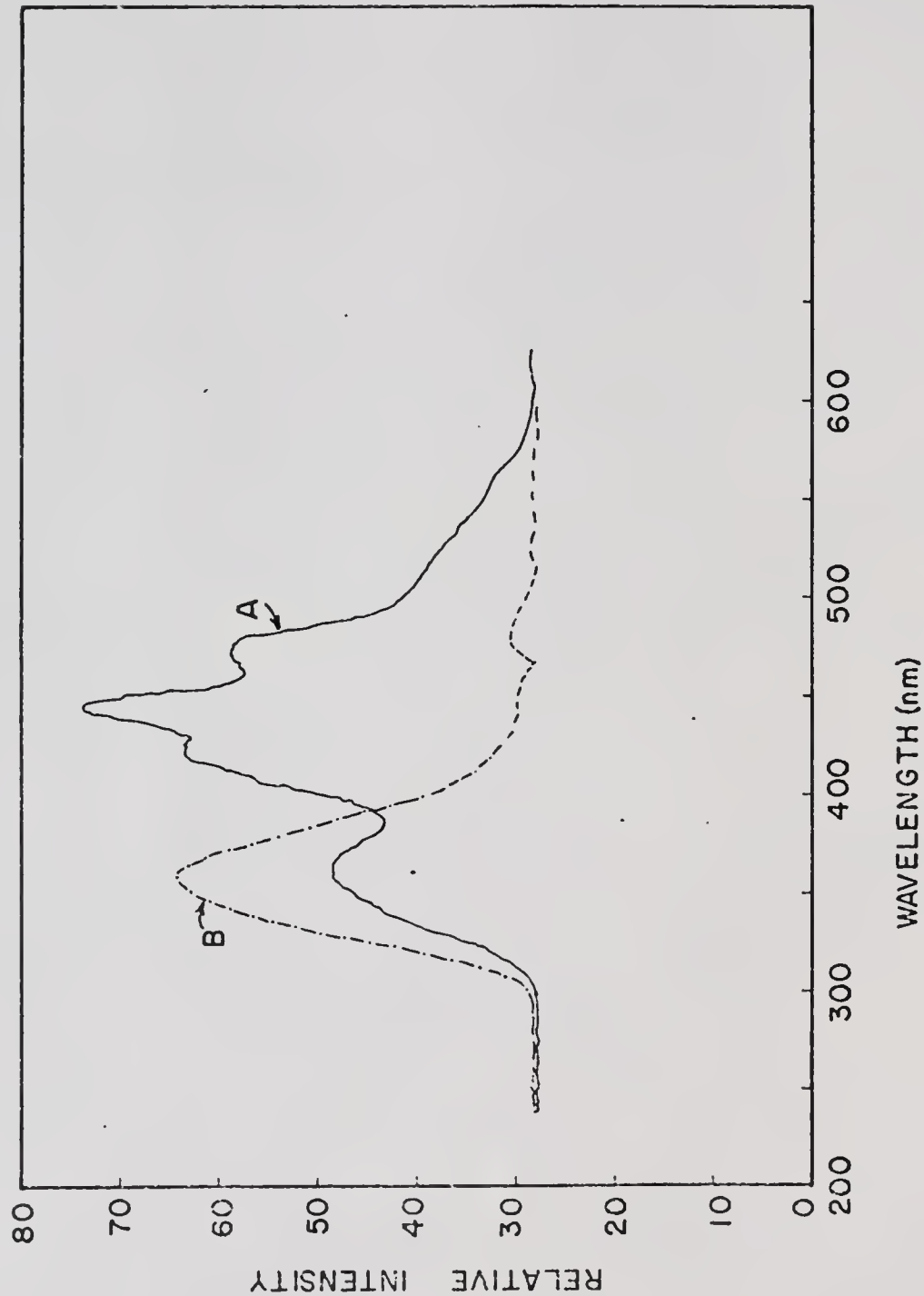
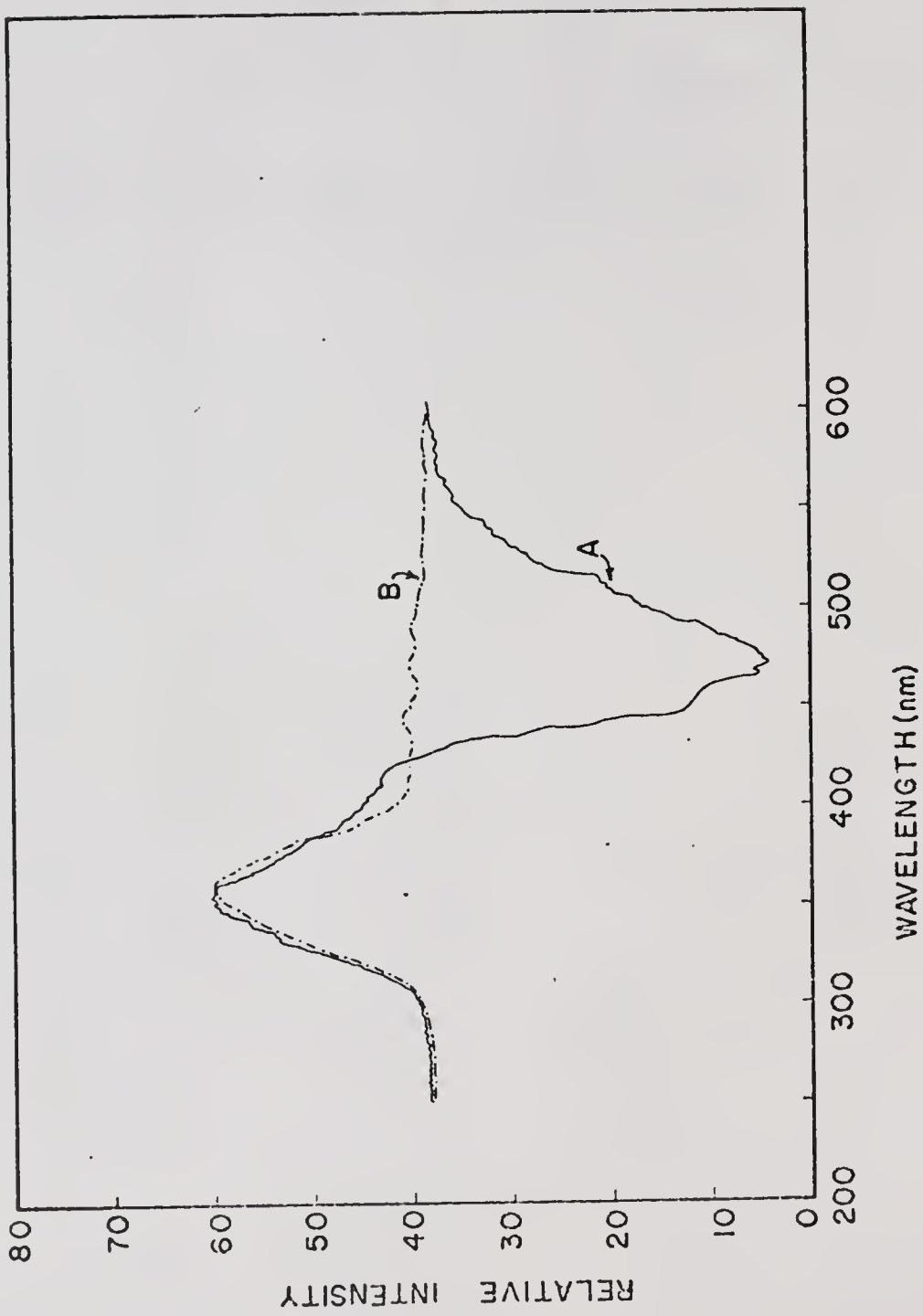

RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B

Figure 16. Phase resolved emission spectrum of a mixture of 4.5×10^{-6}M benzophenone and 5.8×10^{-6}M 4-bromobiphenyl at 25 Hz and $\phi_R = 0° + 52°$. Excitation wavelength is 275 nm.

A = mixture spectrum with the signal from benzophenone phased out.

B = emission spectrum of benzophenone standard.


RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B

As mentioned earlier, the fluorescence emission from a sample may also be phased out by proper selection of the reference phase angle. An example of this ability is given in Figures (17) and (18). In Figure (17), the excitation and emission spectrum is given of a solution of 2-bromobiphenyl determined at the reference phase angle which gave the peak response for the phosphorescence emission. Details A and C are the excitation and phosphorescence emission spectrum for this solution. Note that an off-scale intense fluorescence emission, B, is also present. Because in the frequency range of operation in this technique, the fluorescence should have the same phase and frequency characteristics as the source radiation, measuring the scattered light phase angle, ϕ_{SR}, and setting the reference phase angle at $90° \pm \phi_{SR}$ should phase out this fluorescence emission. The results are illustrated in Figure (18). Note here the residual fluorescence signal B and the undistorted phosphorescence excitation and emission spectra, A and C, respectively.

In the preceding examples of spectral resolution, the phase angles of the individual components were determined through the use of pure standard solutions. However, in some cases where the excitation and emission spectra do not completely overlap, these individual phase measurements can be made directly on the mixture through wavelength selection, if either the excitation or emission spectra are widely different, or by measurements on the wavelength

Figure 17. Excitation and emission spectra of 2.1×10^{-3}M 2-bromobiphenyl at 50 Hz and $\phi_R = 270° + 68°$.

A = excitation spectrum emission wavelength = 465 nm.

B = fluorescence emission peak, excitation wavelength = 275 nm.

C = phosphorescence emission peak, excitation wavelength = 275 nm.

(---) = phosphorescence emission spectrum of 2-bromobiphenyl standard.

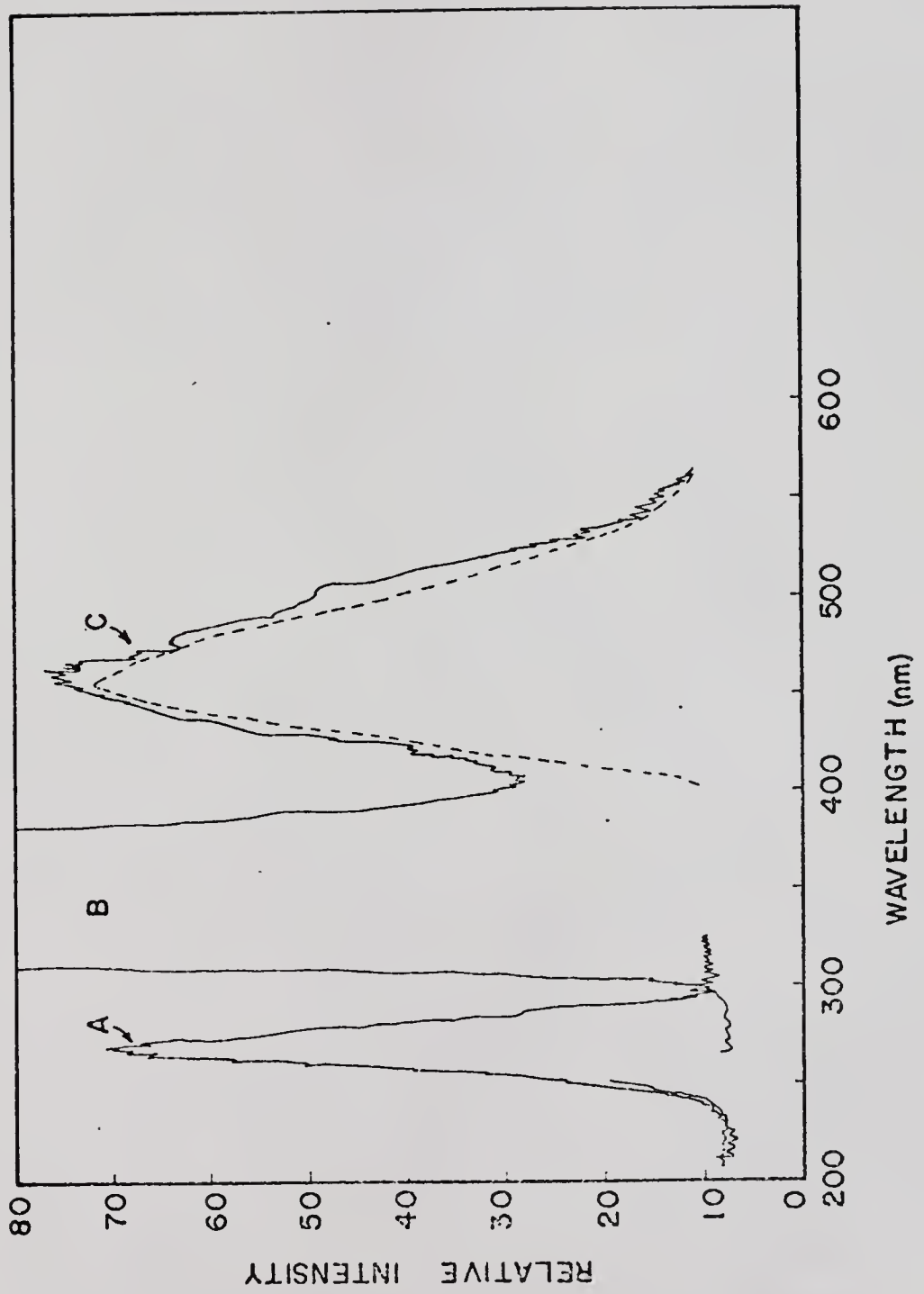
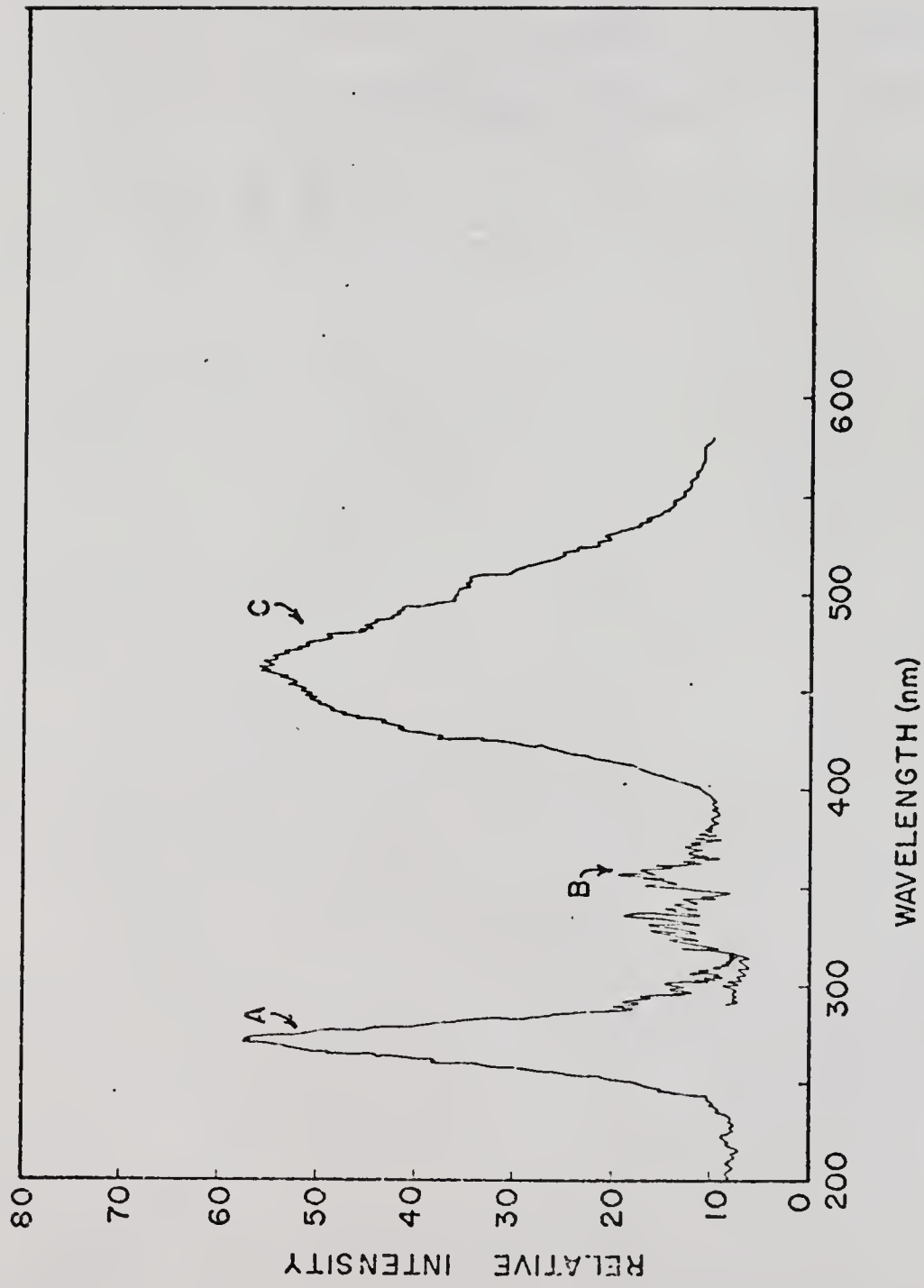

RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B
C

Figure 18. Phase resolved excitation and emission spectra of 2.1×10^{-3}M 2-bromobiphenyl at 50 Hz and $\phi_R = 180° + 95.3°$.

A = excitation spectrum, emission wavelength = 465 nm.

B = residual fluorescence emission, excitation wavelength = 270 nm.

C = phosphorescence emission spectrum, excitation wavelength = 270 nm.


RELATIVE INTENSITY
80
70
60
50
40
30
20
10
0
200
300
400
500
600
WAVELENGTH (nm)
A
B
C

edges of the spectrum where the signal from one component predominates.

Both the phase and frequency methods of phase resolution could, in principle, be applied to quantitative measurements. In this work, the emphasis was placed upon the phase method because it required measurements at only one set frequency. In the frequency technique, measurements had to be made at two frequencies. The accurate resetting of the frequency values was more difficult to achieve with the experimental apparatus used here than the resetting of reference phase angle values. Also, the selection of an appropriate phase angle setting in the frequency technique was more critical because the null frequencies established by this phase angle setting had a great effect upon the signal obtained from each component. This was because the modulation factor, m, also depends upon the frequency of modulation. An example of a quantitative resolution by the frequency method will be given later.

Referring to the Equations (31) and (32), it is observed that the signal obtained from the remaining component of a binary mixture when one component is nulled out is reduced proportionally to the sine of the phase angle difference. This reduction of signal is demonstrated in Figure (19) which shows the analytical curves obtained for standard solutions of two compounds of a binary mixture when determined at their peak signal phase angle and at the phase angle where the signal from one of the components is

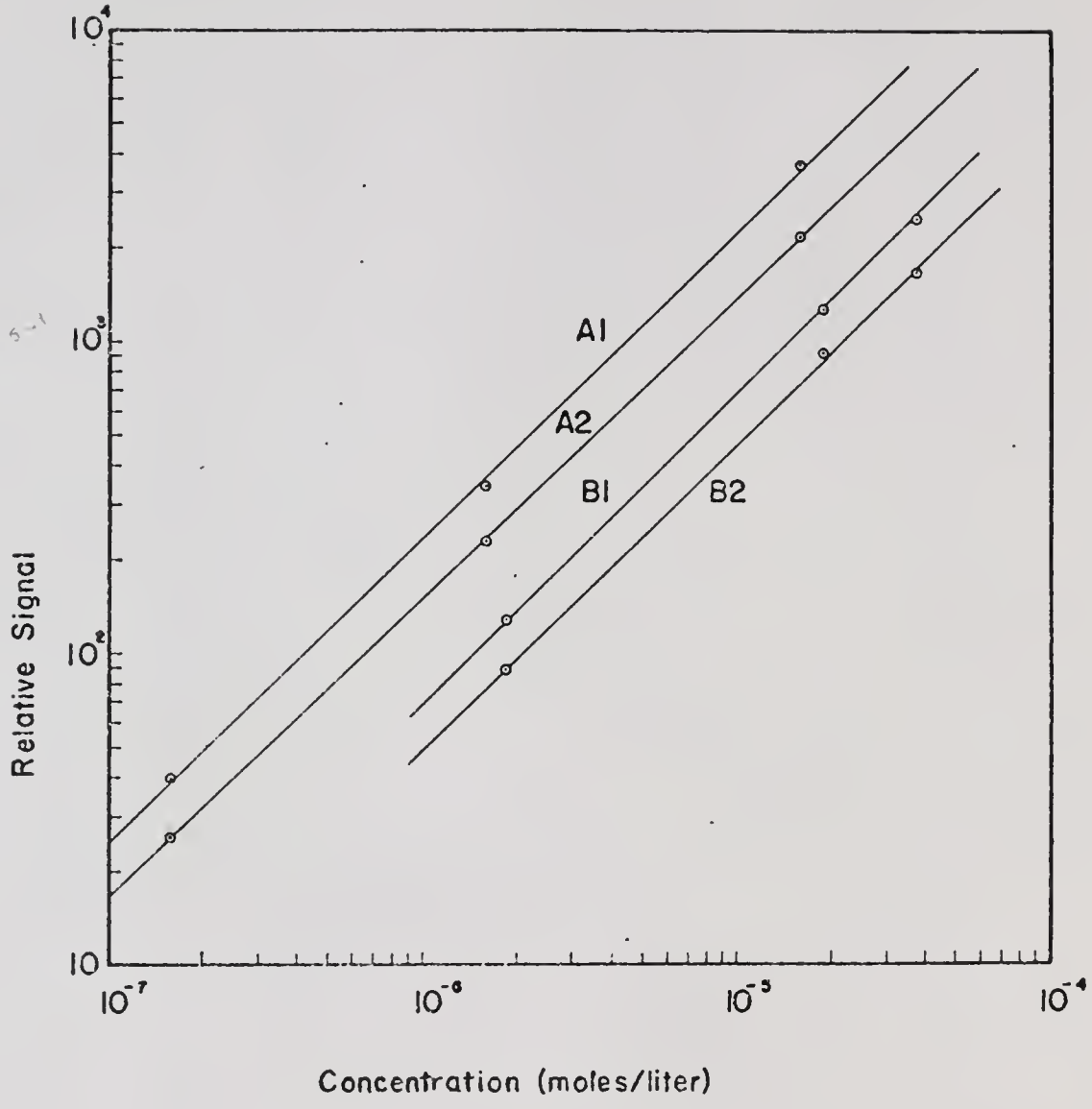
Figure 19. Analytical curves for 4-bromobiphenyl and 4-iodobiphenyl at the peak phase setting for each molecule and at the phase setting where one component is phased out.

A1 = analytical curve for 4-iodobiphenyl, $\phi_R = 270° + 55°$.

A2 = analytical curve for 4-iodobiphenyl, $\phi_R = 0° + 12.5°$.

B1 = analytical curve for 4-bromobiphenyl, $\phi_R = 270° + 12.5°$.

B2 = analytical curve for 4-bromobiphenyl, $\phi_R = 180° + 55°$.


A1
A2
B1
B2
Relative Signal
Concentration (moles/liter)

phased out. Calculating the ratio of the values for the relative signal at the same phase setting for each pair of curves, yields an average value of 0.68 for the 4-bromobiphenyl curves and 0.62 for the 4-iodobiphenyl. Taking the sine of the difference phase angles, in this case 42.5°, yields a value of 0.676. These values seem to agree within experimental error. The results of analyses performed upon several binary mixtures are given in Tables (2) through (4). These results indicate that the best data are obtained when the concentration of each of the compounds in the mixture is less than 10^{-5} M. This seems to suggest that an inner filter effect could be operative at the higher concentrations. Also, depending upon the pair of compounds selected for the mixture, a ten times greater concentration of a strongly phosphorescent species in the presence of a weaker phosphorescent species seems to introduce large errors in the determination of the less phosphorescent species. This is evident in Table (2) in which the strongly phosphorescent 4-iodobiphenyl is present along with the weaker phosphorescent 4-bromobiphenyl. The cause of this phenomenon is probably the inability to completely phase out a strong signal and to detect a weak signal in the presence of a large noise component. It should be noted here that the noise component is proportional to the sum of the signals from both components and the phasing out of one component signal does not phase out the noise associated with it.

TABLE 2

PHASE RESOLUTION OF BINARY MIXTURE I
BY THE PHASE METHOD[a,b,c]

MIXTURE I	LIFETIMES (ms)	PEAK PHASE ANGLE (degrees)
A: 4-Iodobiphenyl	3.5	270 + 55.0
B: 4-Bromobiphenyl	17.	270 + 12.5

	CONCENTRATION ADDED (M)	PHASE ANGLE SETTING (degrees)	CONCENTRATION FOUND (M)	PERCENT ERROR
A:	8.0×10^{-7}	0 + 12.5	8.3×10^{-7}	+3.8
B:	1.86×10^{-6}	180 + 55.0	1.80×10^{-6}	-3.3
A:	8.0×10^{-6}	0 + 12.5	8.0×10^{-6}	0
B:	1.86×10^{-5}	180 + 55.0	1.77×10^{-5}	-4.8
A:	1.60×10^{-5}	0 + 12.5	1.60×10^{-5}	0
B:	1.86×10^{-6}	180 + 55.0	1.20×10^{-6}	-36

a. All measurements performed at 275 nm excitation wavelength and 475 nm emission wavelength.

b. All measurements performed at 25 Hz.

c. Degree of source modulation = 53%.

TABLE 3

PHASE RESOLUTION OF BINARY MIXTURE II
BY THE PHASE METHOD[a,b,c]

MIXTURE II	LIFETIMES (ms)	PEAK PHASE ANGLE (degrees)
A: 4,4'-Dibromobiphenyl	12.	270 + 29.8
B: 4-Iodobiphenyl	3.5	270 + 67.0

	CONCENTRATION ADDED (M)	PHASE ANGLE SETTING (degrees)	CONCENTRATION FOUND (M)	PERCENT ERROR
A:	1.83×10^{-5}	180 + 67.0	1.80×10^{-5}	-13.
B:	1.89×10^{-5}	0 + 29.8	1.65×10^{-5}	- 1.6
A:	3.66×10^{-6}	180 + 67.0	3.90×10^{-6}	+ 6.6
B:	3.78×10^{-6}	0 + 29.8	3.55×10^{-6}	- 6.4
A:	1.83×10^{-5}	180 + 67.0	1.90×10^{-5}	+ 3.8
B:	3.78×10^{-6}	0 + 29.8	3.60×10^{-6}	- 4.8
A:	3.66×10^{-6}	180 + 67.0	3.60×10^{-6}	- 1.6
B:	1.89×10^{-5}	0 + 29.8	1.95×10^{-5}	+ 3.2

a. All measurements performed at 275 nm excitation wavelength and 475 nm emission wavelength.

b. All measurements performed at 25 Hz.

c. Degree of source modulation = 53%.

TABLE 4

PHASE RESOLUTION OF BINARY MIXTURE III
BY THE PHASE METHOD[a,b,c]

MIXTURE III	LIFETIMES (ms)	PEAK PHASE ANGLE (degrees)
A: Benzophenone	6.0	270 + 53.0
B: 4-Bromobiphenyl	17.0	270 + 27.5

	CONCENTRATION ADDED (M)	PHASE ANGLE SETTING (degrees)	CONCENTRATION FOUND (M)	PERCENT ERROR
A:	4.19×10^{-6}	0 + 27.5	4.0×10^{-6}	-4.6
B:	6.52×10^{-6}	180 + 53.0	6.9×10^{-6}	+5.8
A:	8.38×10^{-7}	0 + 27.5	7.6×10^{-7}	-9.3
B:	1.30×10^{-6}	180 + 53.0	1.4×10^{-6}	+7.7

a. All measurements performed at 275 nm excitation wavelength and 475 nm emission wavelength.

b. All measurements performed at 25 Hz.

c. Degree of source modulation = 53%.

The precision obtainable using the instrumentation in this work is illustrated in Table (5). From the data, it is observed that the relative standard deviation expected is of the same order as the percent error in the analysis of the binary mixtures. The principal sources of noise in this experiment are bubbling noise in the liquid nitrogen coolant and electronic noise from the amplifiers. Of these two, the bubbling noise is especially bad at the low frequencies of operation.

An example of the frequency technique for the analysis of a binary mixture is given in Table (6). Compared with the data (Table (4)) obtained for a comparable mixture by the phase technique, the percent error in the frequency method appears to be greater. It should be noted that the signal level for the 4-bromobiphenyl is lower here because the measurements are made at 37.5 Hz instead of 25 Hz as in the phase technique. The lower signal levels resulted in relatively more noise and thus poorer precision.

TABLE 5

PRECISION MEASUREMENTS ON TWO BINARY MIXTURES[a,b,c]

MIXTURE I	A:	1.3×10^{-5} M	4-Bromobiphenyl
	B:	3.8×10^{-6} M	4-Iodobiphenyl
MIXTURE 2	A:	2.6×10^{-6} M	4-Bromobiphenyl
	B:	2.1×10^{-6} M	Benzophenone

MIXTURE	PHASE SETTING (degrees)	NUMBER OF DETERMINATIONS	PERCENT RSD[d]
I	A: 180 + 69.3	10	5.9
	B: 0 + 29.4	10	10.
II	A: 180 + 52.3	10	7.4
	B: 0 + 24.3	10	8.9

a. All measurements performed at 275 nm excitation wavelength and 475 nm emission wavelength.

b. All measurements performed at 25 Hz.

c. Degree of source modulation = 53%.

d. RSD = relative standard deviation.

TABLE 6

PHASE RESOLUTION OF A BINARY MIXTURE BY THE FREQUENCY METHOD[a,b]

	MIXTURE	LIFETIMES (ms)
A:	Benzophenone	6.0
B:	4-Bromobiphenyl	17.

	CONCENTRATION ADDED (M)	FREQUENCY (Hz)	PHASE ANGLE SETTING (degrees)	CONCENTRATION FOUND (M)	PERCENT ERROR
A:	4.50×10^{-6}	13.1	0 + 40.0	4.9×10^{-6}	+8.9
B:	5.84×10^{-6}	37.5	180 + 40.0	5.3×10^{-6}	-9.3

a. All measurements performed at 275 nm excitation wavelength and 475 nm emission wavelength.

b. Degree of source modulation = 75%.

CHAPTER V

SUMMARY AND FUTURE WORK

The frequency and phase characteristics of several phosphorescent species have been studied with emphasis upon the change in these characteristics with the frequency of modulation and the lifetime of the phosphorescence. A phase sensitive detector has been employed to study the possibilities of phase resolution as an analytical tool in phosphorimetry. It was shown that emission and excitation spectra of molecules which show severe overlap can be phase or frequency resolved into the spectra of the individual components. Fluorescence emission was also phase resolved from phosphorescence emission. These spectral resolutions were demonstrated with solutions of pure compounds and synthetic binary mixtures. The quantitative analysis of synthetic binary mixtures by the phase resolution technique was also shown to be feasible.

Future work should include several of the following ideas. First, major improvements of the instrumentation should be attempted with emphasis upon the use of better monochromators and better phase detection systems. The use of conductive cooling devices for freezing the sample should be considered as a means of reducing the major source

of noise--bubbling in the liquid nitrogen. High energy modulated light sources should be investigated which can yield a higher degree of modulation and which can be modulated at higher frequencies. The higher frequencies of modulation should enable the study of fluorescence as well as phosphorescence emission. The possibility of obtaining the inverse Fourier transform of the frequency and phase characteristics obtained in this technique, as a means of obtaining the original decay characteristics of the luminescence, should also be considered. Use of other periodic waveforms such as square-waves for the modulation of the light source, coupled with measurements at the harmonic frequencies present should be investigated as a means of improving the versatility of this technique for the analysis of mixtures. The use of phosphoroscope chopping and source modulation at different frequencies together with detection at the sum or difference frequencies should be investigated as a means of eliminating fluorescence and scattered light interference.

LIST OF REFERENCES

1. R. J. Keirs, R. D. Britt, Jr., and W. E. Wentworth, Anal. Chem., 29, 202 (1957).

2. P. A. St. John and J. D. Winefordner, Anal. Chem., 39, 500 (1967).

3. H. C. Hollifield and J. D. Winefordner, Chemical Instrumentation, 1, 341 (1969).

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

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

6. J. D. Winefordner, Accts. Chem. Res., 2, 361 (1969).

7. R. P. Fisher and J. D. Winefordner, Anal. Chem., 44, 948 (1972).

8. C. M. O'Donnell, K. F. Harbaugh and J. D. Winefordner, Anal. Chem., 45, 381 (1973).

9. C. M. O'Donnell, K. F. Harbaugh and J. D. Winefordner, Anal. Chem. 45, 609 (1973).

10. J. B. Birks and I. H. Munro, in G. Porter, Ed., Progress in Reaction Kinetics, 4, Pergamon Press Ltd., London, 1967.

11. E. Gaviola, Z. Physik, 35, 748 (1926).

12. A. Müller, R. Lumry and H. Kokubun, Rev. Sci, Inst., 36, 1214 (1965).

13. S. H. Liebson, M. E. Bishop and J. O. Elliott, Phys. Rev., 80, 907 (1950).

14. O. Maercks, Z. Physik, 109, 685 (1938).

15. L. A. Tumerman, J. Phys. U.S.S.R., 4, 151 (1941).

16. E. A. Bailey and G. K. Rollefson, J. Chem. Phys., 21 (8), 1315 (1953).

17. A. Schmillen, Z. Physik, 135, 294 (1953).

18. R. D. Spencer, W. M. Vaughn and G. Weber, in E. C. Lim, Ed., Molecular Luminescence, W. A. Benjamin, Inc., New York, N.Y., 1969.

19. J. B. Birks and W. A. Little, Proc. Phys. Soc., A66, 921 (1953).

20. J. B. Birks and D. J. Dyson, J. Sci. Inst., 38, 282 (1961).

21. T. D. S. Hamilton, Proc. Phys. Soc., 78, 743 (1961).

22. A. Schmillen, in H. P. Kallmann and G. M. Spruch, Eds., Luminescence of Organic and Inorganic Materials, John Wiley and Sons, Inc., New York, N.Y., 1962.

23. K. Doi and A. Toshinai, Jap. J. Appl. Phys., 7 (12), 1504 (1968).

24. K. Doi and A. Toshinai, Appl. Opt., 9 (12), 2762 (1970).

25. W. R. Ware, J. Am. Chem. Soc., 83, 4374 (1961).

26. W. R. Ware, J. Phys. Chem., 66, 455 (1962).

27. T. V. Veslova, A. S. Cherkasov and V. I. Shirokov, Opt. Spectrosc., 29, 617 (1970).

28. L. F. Phillips, Rev. Sci. Inst., 42, 1078 (1971).

29. M. Kasha, Rad. Res. Suppl., 2, 243 (1960).

30. J. D. Winefordner, S. G. Schulman and T. C. O'Haver, Luminescence Spectrometry in Analytical Chemistry, John Wiley and Sons, New York, N.Y., 1972.

31. C. A. Parker, Photoluminescence of Solutions, Elsevier, New York, N.Y., 1972.

32. S. P. McGlynn, T. Azumi and M. Kinoshita, Molecular Spectroscopy of the Triplet State, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1969.

33. R. S. Becker, Theory and Interpretation of Fluorescence and Phosphorescence, John Wiley and Sons, Inc., New York, N.Y., 1969.

34. M. Zander, Phosphorimetry, Academic Press, New York, N.Y., 1968.

35. H. Lund and J. Bjerrum, Ber., 64B, 210 (1931).

36. R. Zweidinger and J. D. Winefordner, Anal. Chem., 42, 643 (1970).

37. R. J. Lukasiewicz, P. A. Rozynes, L. B. Sanders and J. D. Winefordner, Anal. Chem., 44, 237 (1972).

BIOGRAPHICAL SKETCH

John Jad Mousa was born on September 24, 1948, at Jacksonville, Florida. He attended public schools in Corpus Christi, Texas, and in June 1966 he graduated with honors from Roy Miller High School in Corpus Christi. He attended Del Mar College in Corpus Christi for two years and in June 1970 received the degree of Bachelor of Science *summa cum laude* from the University of Houston at Houston, Texas. Since September 1970, he has worked toward the degree of Doctor of Philosophy at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James D. Winefordner, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Roger G. Bates
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Gerhard M. Schmid
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John E. Singley
Professor of Environmental Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Willis B. Person

Willis B. Person
Professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1973

Dean, Graduate School