CONVENIENT AND SIMPLE METHODS FOR THE OBSERVATION OF PHOSPHORESCENCE IN FLUID SOLUTIONS. INTERNAL AND EXTERNAL HEAVY ATOM AND MICELLAR EFFECTS

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Abstract—Phosphorescence of organic molecules in fluid solutions may be conveniently and readily observed under certain conditions. If $k_p$ (radiative phosphorescence rate constant) is $\geq 10^{-5}$ s$^{-1}$, then in the absence of photoexcitation phosphorescence is observable upon N$_2$ purging. For example, nitrogen purged, acetonitrile solutions of bromo and dibromonaphthalene display readily observable phosphorescence as a result of internal heavy atom enhancement of $\Phi_p$ and $k_p$. External heavy atom enhancement of $k_p$ (CH$_2$BrCH$_2$Br solvent) of aromatic hydrocarbons even allows observation of phosphorescence from these compounds in N$_2$ purged fluid solutions. Although bromonaphthalenes are not significantly phosphorescent in N$_2$ purged aqueous solution, phosphorescence is readily observed in N$_2$ purged detergent (HDTBr, HDTCl, and SDS) solutions above the critical micelle concentration.

The general factors which determine whether phosphorescence is "readily" observable in fluid solution are briefly discussed and the results are interpreted in light of these factors.

INTRODUCTION

At 77 K, phosphorescence is a commonly observed phenomenon following photoexcitation of organic compounds (Kasha, 1947; Lower and El-Sayed, 1966). The acceptance of the identity of the phosphorescent state as the triplet state of organic molecules was not general for nearly two decades after the classical papers of Lewis and Kasha and of Terenin (Lewis and Kasha, 1944; Terenin, 1943). In spite of the observation of phosphorescence of biacetyl in fluid solution (Lewis and Kasha, 1945; Bäckström and Sandros, 1962, 1960), this molecule was commonly viewed as a pathological exception to the striking and persistent failure to observe phosphorescence from organic molecules in fluid solution. The elegant flash spectroscopy of Porter (Porter and Windsor, 1958; Porter and Wilkinson, 1961) provided convincing evidence for the existence of the triplet state in fluid solution, but the problem of why phosphorescence was "quenched" (implication: $\Phi_p < 10^{-4}$) in fluid solutions remained unsolved. Although simple specific bimolecular deactivation by adventitious diffusional quenchers such as O$_2$ and solvent impurities qualitatively rationalizes the absence of phosphorescence, even in the middle 1960's more complicated interpretations such as phonon involvement (Kellogg and Schwenker, 1964) and coupling of molecular rotation with electronic motion (Porter and Stief, 1962) continued to be proposed as alternative explanations.

After several scattered reports of the observation of very weak phosphorescence of molecules other than biacetyl in fluid solutions near room temperature (Bäckström and Sandros, 1960; Parker and Hatchard, 1962), a breakthrough was published by Parker and Joyce (Parker and Joyce, 1969) who demonstrated that the combination of an "unreactive solvent" (e.g. perfluorocarbons), low concentration of oxygen, and careful solvent and solute purification allows the ready observation of phosphorescences whose quantum efficiency approached that observed at 77 K! Shortly afterwards, numerous reports followed and demonstrated that phosphorescence of organic molecules in fluid solution was a viable means of studying photochemical reaction mechanisms (Clark et al., 1969; Saltiel et al., 1970). However, there may still be a feeling among those photochemists who employ emission spectroscopy mainly as an analytical tool, that the general observation of phosphorescence in fluid media requires very special or exotic solvents (e.g. fluorocarbons), elaborate or unconventional purification procedures, and specially designed ultrasensitive equipment.

The purpose of this paper is to help to eradicate this misconception by demonstrating that phosphorescence in fluid solution is, in fact, readily observable in common and useful solvents (e.g. acetonitrile) and requires only (a) simple N$_2$ gas purging, (b) attention to concentrations and (c) conventional purification schemes. Of further interest and importance to biochemists interested in luminescence techniques, we report that phosphorescence of simple molecules such as halonaphthalenes may be observed in micellar solutions (models for membrane solutions). Only a few examples are known of the use of triplets as probes of biologically relevant systems (Saviotti and Galley, 1974; Imakubo and Kai, 1977; Cherry and Schneider, 1976). Hopefully, the results reported here will serve to stimulate the application of phosphorescence spectroscopy for fluid solutions, in particular to areas involving the study of the structure and dynamics of macromolecules.
MATERIALS AND METHODS

Naphthalene and 1,4-dibromonaphthalene were crystallized six times from 95% ethanol, dried overnight and then sublimed twice. Triphenylene was recrystallized six times from 95%, pure, H$_2$SO$_4$. The water was distilled twice. Acetonitrile (Matheson, Coleman and Bell or Eastman; Spectro grade) was used without further purification. Ethylene bromide (Aldrich) was treated with conc. H$_2$SO$_4$ until the acid layer was almost colorless. The bromide was then washed three times with NaHCO$_3$, three times with water and then dried over MgSO$_4$. The resulting material was passed through a neutral alumina column and then twice distilled. The detergents (HDTCl and HDTBr, Eastman; SDS, Eastman or Pierce) were purified by washing four times with ether, followed by dissolution in boiling ethanol. Ether was added to the cooled ethanol solutions until they became slightly cloudy. Ethanol was then added in small amounts until the solutions just became clear. The resulting solution was kept in a refrigerator until precipitation occurred and the precipitate was collected by filtration. This procedure was repeated four times. The final precipitate collected was dried in vacuo overnight.

The N$_2$ purging method employed in this study basically involves vigorous bubbling of a sample through a small (less than 1 mm) glass capillary followed by immediate capping of the cell. The effectiveness of the degassing depends on (a) the purity of the N$_2$ gas, (b) the rate of bubbling, (c) the prevention of air leakage after thorough degassing has been achieved. For example, for non-detergent solutions an ordinary quartz fluorescence cuvette equipped with a snug Teflon stopper is appropriate. The cell is filled to two-thirds or one-half capacity with sample solution and then vigorously (>1 bubble/s) purged with N$_2$. A disposable pipette (Beckman pasteur) may be employed and should be set so that its tip is ~1 mm from the bottom of the cell. Bubbling for the order of 10 min is usually sufficient, but an unknown system should be tested empirically to determine whether shorter or longer times are more appropriate. If the solvent is volatile, cooling to 0°C usually retards evaporation to the point that concentration changes are insignificant. Although simple to execute, the success of this procedure depends on the ability to stopper the sample quickly. It thus serves best when only qualitative information is desired (i.e. is there phosphorescence or not?).

A more satisfactory and quantitative method involves the use of a fluorescence cell that has been modified by attachment of a Teflon stop cock to replace the cap. Nitrogen was bubbled through samples by means of a very thin capillary that was inserted through the stop cock (open position) with the tip as close to the cell bottom as possible (~1 mm). The samples were bubbled vigorously as possible (rate limited by capillary size) for ~15 min. The bubbling may be terminated by simply snapping the capillary by turning the stopcock or by closing the stop cock immediately as the capillary is withdrawn. For degassing detergent solutions (foam!) a long stem (~10 cm) is recommended as is a bubble breaker bulb.

Although conventional N$_2$ (Airco, Grade 4.5 TM) is satisfactory, we found that Union Carbide-Linde, "oxygen-free" grade was superior (i.e. phosphorescence intensities were 2-3 times greater with the latter source). In the case of aromatic hydrocarbons in ethylene dibromide best results were achieved by vacuum degassing (5 cycles) rather than by N$_2$ purging.

Luminescence spectra were recorded on a Hitachi-Perkin Elmer fluorimeter (MPF-2A or 3L, equipped with Hamamatsu 777 phototube). The spectra reported are uncorrected. Bands in the spectrum that were established as being due to scattering or overtones are not shown in the spectra reported.

RESULTS

The photoluminescence of 1,4-dibromonaphthalene (as well as that of 1- and 2-bromonaphthalene) is readily observable in N$_2$ purged acetonitrile solution (Fig. 1). From a comparison with data from low temperature emission, the observed spectral distributions
of the observed bands correspond to fluorescence (~340 nm) and phosphorescence (~530 nm).

The phosphorescence of N₂-purged, purified acetonitrile solutions of aromatic hydrocarbons such as naphthalene and triphenylene is too weak for detection by a conventional fluorimeter. In vacuum degassed, purified ethylene bromide, however, phosphorescence is readily observable from these aromatic hydrocarbons (Fig. 2).

The phosphorescence of bromonaphthalenes is exceedingly weak in N₂-purged aqueous solutions. Addition of detergent (hexadecyltrimethylammonium bromide, HDTBr, hexadecyltrimethylammonium chloride, HDTCI or sodium dodecysulfate, SDS) results in the readily measurable phosphorescence, the latter being detectable even in the absence of N₂ purging (Figs. 3, 4 and 5).

The intensity of both the fluorescence and phosphorescence (Fig. 6) of 1,4-dibromonaphthalene showed critical micelle concentration (CMC) behavior (Fendler and Fendler, 1975). The phosphorescence of 1,4-dibromonaphthalene could not be observed in aerated aqueous solution. The latter observation provides strong evidence that micelle formation and micellar sequestering of the naphthalene is responsible for the observation of phosphorescence.

Kinetic model for interpreting phosphorescence efficiency

The quantum yield of phosphorescence \( \Phi_p \) is given by the deceptively simple expression:

\[
\Phi_p = \Phi_o \frac{k_p}{k_d + k_q[Q]} \tag{1}
\]

where \( \Phi_o \) is the quantum yield for intersystem crossing from \( S_1 \) to \( T_1 \) and \( k_q[Q] \) is the "effective" pseudounimolecular decay of \( T_1 \) induced by a quencher \( Q \). Equation 1 assumes a wavelength independence of \( \Phi_p \). It is important to note that \( k_d \) and \( k_q[Q] \) are
more generally a sum of quenching terms, i.e.
\[ k_d = \sum_i k_{d,i} \quad \text{and} \quad k_{q}[Q_j] = \sum_i k_{q,i}[Q_j] \] (2)

where \( k_{d,i} \) is a particular unimolecular decay constant and \( k_{q,i}[Q_j] \) is the specific contribution of quencher \( Q_j \) to the total pseudo-unimolecular quenching. It is also crucial to recognize that a term \( k_{q}[Q_j] \) may become important for very long triplet lifetimes at high intensities if \( Q_j = T^* \) in other words, under conditions such that triplet-triplet annihilation is significant, Eq. 1 must be rewritten in a more general form as:
\[ \Phi_p = \Phi_u \frac{k_p}{k_p + k_o + k_{q}[Q] + k_{q}[Q]^2} \] (3)

Discussion of the terms determining \( \Phi_p \)

Consideration of Eq. 3 reveals the factors that are generally involved in determining whether phosphorescence may be observed under any experimental conditions. We consider seriatim the impact of each of these factors on the magnitude of \( \Phi_p \).

(1) The intersystem quantum efficiency, \( \Phi_u \). This term represents the probability that an absorbed photon which excites \( S \) will produce a triplet state. \( \Phi_u \) is not a molecular constant, but depends on experimental conditions (Wilkinson, 1975). In particular, if \( S \) is subject to reactions or quenching, \( \Phi_u \) will be correspondingly decreased as will \( \Phi_p \). We shall assume that chemical deactivation of \( S \) can be identified and eliminated and shall consider only physical deactivation of \( S \). The formation of excimers and exciplexes (Forster, 1969; Birks, 1970) constitutes a general and commonly encountered means of deactivating \( S \). Such a process is concentration dependent, so that \( \Phi_u \) may be a function of solute (phosphorescer) concentration.

Importantly, \( \Phi_u \) may be increased by variation of structure or of reaction conditions. If a substituent increases the relative probability of \( S \rightarrow T \) conversion, \( \Phi_u \) will increase. The most common method is to enhance \( \Phi_u \) relative to other deactivation pathways available to \( S \). Quite often the same factors which enhance \( \Phi_u \) (e.g. heavy atom effects) also enhance \( k_p \), (vide infra).

(2) The radiative rate constant, \( k_p \). The radiative rate, \( k_p \), generally depends on the extent of spin orbit coupling available to \( T \). The \( k_p \) values for organic molecules range from \( \sim 10^2 \text{ s}^{-1} \) for molecules possessing heavy atoms affixed to the emitting lumiphore or possessing lowest energy \( n, \pi^* \) states, to \( \sim 10^{-1} \text{ s}^{-1} \) for aromatic hydrocarbons (McGlynn, 1969). The magnitude of \( k_p \) may be increased by external heavy atom perturbation (Kasha, 1952; Karvarnos et al., 1971). Under the assumption that \( \Phi_p \geq 10^{-3} \) is required for "ready" observation of phosphorescence by conventional spectrophotometers, it is obvious that favorable conditions exist only when \( k_p \) is \( 10^{-3} \times (k_d + k_{q}[Q] + k_{q}[Q]^2) \), even if \( \Phi_u \sim 1 \).

(3) The inherent unimolecular decay of \( T \), \( k_d \). The magnitude of \( k_d \) depends strongly on molecular structure and temperature. For the molecules employed in this study, photoreactions are inefficient and assumed to be negligible. There is no general way to handle the contribution of \( k_d \) to \( \Phi_p \), except to use model compounds to calibrate expectations, or to make direct measurements of \( \tau_f = k_d^{-1} \). Note that \( k_d \) includes "solvent quenching" in the broad sense that nonreaction interactions of triplets with solvent may determine the magnitude of \( k_d \).

The factors which enhance \( \Phi_u \) and maximize \( k_p \) may also lead to an enhancement of \( k_d \) by (a) introducing new chemical pathways for triplet deactivation or (b) by enhancing intersystem crossing back to \( S \) (Cowan and Koziar, 1977).

(4) The pseudo-unimolecular quenching term, \( k_{q}[Q] \). This term represents the contribution of all specific
diffusional bimolecular quenching by a ground state species, Q. Q may be an adventitious quencher, residual oxygen, or the ground state of the phosphorescing molecule itself. The latter point has not been generally appreciated in the earlier literature. Self-quenching of triplets is a well established phenomenon (Porter and Ledger, 1972; Wolf et al., 1977; Takemura et al., 1976) and may, in some cases, provide a limit to $\Phi_p$.

(5) The pseudo bimolecular quenching term, $k_q[Q]^2$.
At relatively high intensities, the interaction and annihilation of two triplets may determine $\Phi_p$, i.e. when $k_q[Q]^2 = k_p[T_1]^2$ and this term is $> 10^3 k_p$.
Numerous examples of triplet–triplet annihilation appear in the literature (Yekta and Turro, 1972). Even for conventional spectrophotometers which are not considered to possess “intense” light sources, this term may become significant for exceedingly long lived triplets (e.g. aromatic hydrocarbons).

**DISCUSSION**

From the above analysis, solute concentration and light intensity, in addition to $k_p$ and $k_a(Q)$, can be seen to play a dominant role in determining $\Phi_p$. Many studies in the past have tended to focus on the $k_a(Q)$ factor only in an attempt to optimize $\Phi_p$. Let us consider the results depicted in Figs. 1–5 in terms of the model for $\Phi_p$ described above.

**Halonaphthalenes and aromatic hydrocarbons in acetonitrile**

The ready observation of phosphorescence from fluid solutions of the halonaphthalenes is the result of an optimization of $\Phi_p$, $k_p$, and a minimization of $k_a(Q)$. For aromatic hydrocarbons, $\Phi_p$ is variable and often much less than unity (Wilkinson, 1975). However, replacement of H with Br usually causes $\Phi_p$ to approach unity by enhancing $k_a$ selectivity. The heavy atom also enhances $k_q$. From low temperature studies, $k_q \sim 10^{-1} \text{s}^{-1}$ for the bromonaphthalenes (Karvarnos et al., 1971). Thus, for $\Phi_p$ to be $\geq 10^{-3}$, $k_q[Q]$ must be $\leq 10^4 \text{s}^{-1}$ (we assume that $k_q$ is negligible). If $k_q \sim 10^{10} \text{M}^{-1} \text{s}^{-1}$, i.e. the fastest allowable rate for diffusional bimolecular quenching, then a value of $[Q] < 10^{-8} \text{M}$ is required for the “ready” observation of phosphorescence. Evidently, simple purging with N$_2$ gas is sufficient to reduce the concentration of O$_2$ below this limit in acetonitrile, and phosphorescence of bromonaphthalenes is observable. If $k_q < 10^{10} \text{M}^{-1} \text{s}^{-1}$, a higher concentration of quencher can be tolerated.

Inclusion of a heavy atom can be detrimental to $\Phi_p$ if $k_a$ is greatly enhanced relative to $k_p$. This would be the case if $k_a$ were more sensitive to the influence of heavy atoms than $k_p$, or if the heavy atom introduced fast chemical deactivation pathways.

In the case of aromatic hydrocarbons, $k_q \sim 10^{-1} \text{s}^{-1}$. This requires $[Q] < 10^{-8} \text{M}$ to assure the “ready” observation of phosphorescence. It is thus apparent why phosphorescence of aromatic hydrocarbons is not observed in acetonitrile as the result of simple N$_2$ purging, i.e. the residual [O$_2$] is probably $> 10^{-6} \text{M}$. More elaborate purification procedures demonstrate that it is possible to observe phosphorescence from aromatic hydrocarbons in fluid solution (Tsai and Robinson, 1968). It may also be that triplet–triplet annihilation or decay from excited states determines the magnitude of $\Phi_p$ for aromatic hydrocarbons.

**Halonaphthalenes and aromatic hydrocarbons in ethylene bromide**

Vacuum degassing allows observation of the phosphorescence of both halonaphthalenes and aromatic hydrocarbons in ethylene bromide (Fig. 2) but not in acetonitrile. The question arises as to whether $\Phi_p$, $k_p$ or $k_a(Q)$ is affected relative to acetonitrile. Although $\Phi_p$ certainly increases upon proceeding from acetonitrile to ethylene dibromide, this cannot be a major factor since $\Phi_p$ is ~0.7 for naphthalene and ~0.9 for triphenylene (Wilkinson, 1975) so that an increase of less than a factor of two is possible. We do not believe that ethylene bromide as solvent suppresses the $k_a(Q)$ term in Eq. 1. More likely, $k_p$ is increased in magnitude. This supposition is supported by other experiments concerned with “heavy atom” induced enhancement of phosphorescence (Vander Donckt et al., 1973; McGlynn et al., 1969; Karvarnos et al., 1971).

**Halonaphthalenes and aromatic hydrocarbons in micellar solutions**

The “ready” observation of phosphorescence of halonaphthalenes is a remarkable observation (its detection, although weak, is possible even in aerated micellar solution).

In Fig. 6, it is seen that both fluorescence and phosphorescence of 1,4-dibromonaphthalene (DBN) show critical micellar behavior in HDTBr solutions. The intensity of DBN fluorescence is essentially independent of aeration or nitrogen purging, but the phosphorescence of DBN is very sensitive to aeration or nitrogen purging, even though a weak phosphorescence is observed in aerated solutions near and above the CMC. The CMC behavior of DBN luminescence may depend both on enhanced solubility of DBN at above the CMC and (for phosphorescence) on reduced sensitivity to quenching. The CMC value observed (~3 mM) is comparable to literature values (Mukerjee and Mysels, 1971). These results indicate that the phosphorescer does not leave its micellar environment on the time scale of 1 ms (our lifetime measured for 1,4-dibromonaphthalene phosphorescence) since only very weak phosphorescence is observed in pure water. It also requires an unusual “protection” of $T_1$ from O$_2$ as a quencher. The basis of this “protection mechanism” is not yet clear since evidence exists that O$_2$ can penetrate and exist in micelles (Hautala, et al., 1973; Dorrence and Hunter, 1972; Wallace and Thomas, 1973). It should also be noted that compartmentalization of the phosphorescer suppresses bimolecular quenching mechanisms such as triplet–triplet annihilation and self-quenching. Unless the occurrence of O$_2$ and a phosphorescer in...
the same micelle causes peculiar behavior with respect to quenching, we take these results to cast doubt on our earlier interpretation (Hautala et al., 1973) that O_2 is preferentially sequestered into micelles from the aqueous phase.

We have been unable to detect phosphorescence from N_2 purged micellar solutions of naphthalene, triphenylene and pyrene. The lack of phosphorescence dominates emission. Reaction of T requires that a quenching mechanism occur which from N_2 purged micellar solutions of naphthalene, to quenching, we take these results to cast doubt on the same micelle causes peculiar behavior with respect to O_2 quenching, or water (that penetrates the micelle interior) may be important for HDTCI and HDTBr micellar solutions. Thomas has been able to induce phosphorescence from aromatic hydrocarbons in micellar solutions by the clever application of thallous ion in anionic micellar solutions (Thomas, et al., 1977). Evidently the thallous ion increases k_p (i.e. serves as a heavy atom perturber) or decreases k_q [Q] (i.e. by suppressing quenching by O_2 or some other species).

CONCLUSION

The "ready" observation of phosphorescence from organic molecules at room temperature in fluid solution is neither a phenomenon displayed by "odd ball" structures nor an experiment requiring extraordinary or unconventional precautions, purification, or equipment. In the absence of specific reaction with solvent and/or unimolecular reactions from T, conditions discussed in this paper should allow the "ready" observation of phosphorescence for many organic compounds. The impact of "purity" of a sample or solution has relevance only with respect to a specific reaction with solvent or unconventional precautions, purification, or equipment. From the work reported here, it can be concluded that conventional purification, a careful analysis and selection of the experimental conditions is a proper strategy for observation of phosphorescence in fluid solution. The use of phosphorescence probes for the examination of micellar and macromolecular systems of biological interest is apparent and offer a rich potential for future investigations.

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