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Solvent Effects in Room Temperature Phosphorescence

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Room temperature phosphorescence (RTP) analysis is a technique in which solutions containing organic phosphors are applied to filter paper and dried in the absence of oxygen. Adsorption to the paper inhibits molecular vibrations and promotes phosphorescence. Although the solvent must be removed by volatilization before phosphorescence can occur, it appears that the nature of the solvent affects the intensity of the resulting phosphorescence. We examined the room temperature phosphorescence of p-aminobenzoic acid which had been dissolved in water, organic solvents, or mixtures of water and organic solvents. It was found that solvent volatility had little correlation with RTP intensity and that solvents with high dielectric constants produced more intense signals.
INDEX DESCRIPTORS: Room Temperature Phosphorescence, luminescence, p-aminobenzoic acid

Luminescence is a branch of spectroscopy which deals with the visible and near ultraviolet spectrum (180 - 800 nm). Fluorescence and phosphorescence are two techniques of luminescence spectroscopy which involve the detection and analysis of light during the transition from a high molecular energy state (excited state) to a lower molecular energy state (electronic ground state).

Phosphorescence is the emission of a photon during deactivation from the triplet state to the ground state. Since the transition from the triplet state to the ground state is forbidden, the electron will exist there much longer ($10^{-6} - 10$ sec) than the excited singlet state ($10^{-9} - 10^{-7}$ sec). This makes the triplet state susceptible to collisional deactivation. This process competes with phosphorescence and must be eliminated.

HISTORICAL DEVELOPMENT

Although fluorescence spectroscopy has found many practical applications in chemical analysis [1,2], the development of phosphorimetric techniques has come about very slowly. As early as 1888, phosphorescence was noticed in solid solutions of organic dyes [3], yet, the first report which focused on Room Temperature Phosphorescence (RTP) was not made until 1958 [4]. In the 1960's, the technique of supercooling the molecules with liquid nitrogen was introduced and became known as low temperature phosphorescence (LTP). LTP allowed for a rigid support which lessened collisional deactivation, yet the cryogenic conditions made the analyses difficult, time-consuming, and greatly limited in use. However, the "rediscovery" of RTP in 1972 [5] has opened many frontiers for application of this analytical technique.

APPLICATIONS

Room temperature phosphorescence has developed into an important new analytical method due to the fact that it is simple, rapid, cost-effective, sensitive, and can be combined with other techniques [6]. Enormous potential for RTP can be found in the areas of environmental, industrial, clinical, and pharmaceutical analysis. Already, it has been used to detect and measure air pollution, tar in cigarette smoke, pesticides, drugs in blood samples, and components of shale oil and coal [6,7,8].

COMPONENTS OF RTP

Two criteria are important to consider when selecting a phosphor. First, the compound must have a reasonable quantum yield at low temperatures. This means that a measurable number of molecules must undergo phosphorescence as compared to the total number which absorb light. Most compounds which meet this criterion are highly conjugated and have at least one aromatic ring [9]. Second, strong RTP signals are usually exhibited by compounds which are either highly polar or ionic [10]. However, intense signals from nonpolar aromatic compounds are possible with the addition of ions of heavy atoms such as thallium, cesium, and iodine. This "heavy atom effect" enhances phosphorescence by increasing the quantum yield.

The choice of the sample substrate is important in limiting collisional deactivation. A rigid medium is needed to immobilize the excited triplet state molecules and prevent them from colliding with each other and deactivating to the ground state. RTP has been studied using a wide variety of sample substrates such as cellulose filter paper, silica gel, asbestos, sodium acetate, and liquid micelle solutions. Of these, the most common substrate is the cellulose filter paper. Filter paper consists of cotton cellulose, a linear polymer of 2,000 - 9,000 beta-glucose units. Each unit has three hydroxyl groups ($-OH$) available for hydrogen bonding. Cellulose molecules group together in the paper in bundles known as microfibrils. In some areas of the microfibril, the cellulose chains align in an orderly crystalline structure, while other areas of the same microfibril have a disordered arrangement of cellulose molecules. These disordered areas, known as accessible regions, are responsible for the chemical activity of the paper by being the "holes" where the phosphor can enter and bond with the cellulose hydroxyl groups.

Dry cellulose is hygroscopic which means that the paper will absorb water from the air. Water is taken up primarily in the accessible regions of the filter paper. Three different types of water are distinguishable in the cotton fibers. These are adsorbed water, abosrbed water, and imbibed water. Adsorbed water is bound to the hydroxyl groups of the cellulose and cannot be removed by drying. Absorbed water is loosely held in the pores of the pater and has no effect on the individual fibers. Imbibed water is held by occlusion (adhesion to the fibers), can be removed by drying, and reintroduced by humidity in the air or addition of a solution [11].

ASSESSMENT

The RTP method of sample preparation using filter paper has several advantages. It is known for its speed and simplicity, the filter paper substrate is available from commercial suppliers, and it does not require long preparation [6,12]. After practice, the entire procedure for one trial can be accomplished in 10-20 minutes (depending on drying time). Filter paper has been successfully used for the widest variety of organic compounds and generally yields the best RTP results. Although it has excellent features, filter paper also has two distinct problems. It is extremely sensitive to moisture quenching and it gives off phosphorescence background emission which interferes

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with the RTP signal [13].

In order to observe any RTP signal from compounds adsorbed on paper, the paper must be thoroughly dried [7,14]. Once the sample is exposed to the air, the ambient humidity causes quenching which is the reduction of the phosphorescent signal due to collisional deactivation [9,12-14]. After a very short period of time (a few seconds to a minute), the signal can become undetectable. An increase in the humidity of the air causes a noticeable increase in the amount of quenching [9]. It has been concluded that the water molecules compete with the phosphor for bonding sites in the paper's accessible regions, thus increasing collisional deactivation [15]. Disruption of hydrogen bonding by moisture also allows oxygen to enter near the phosphor [15]. Oxygen is a very efficient quenching agent of the excited triplet state [1]. Interaction between the triplet state oxygen (its natural ground state) and a molecule in the excited triplet state (phosphor) results in collisional deactivation for the phosphor and the production of excited singlet-state oxygen [15]. Thus, the intensity and the lifetime of the phosphorescent signal are decreased.

INTRODUCTION TO THE PROBLEM

For all the progress made in the study of RTP, many areas still remain to be explained. One of these, the role of the solvent in room temperature phosphorescence was the focus of this research. In previous studies [16,17], the choice of the solvent seemed to affect the phosphorescent signal. Yet, this influence was not very well understood. Possible explanations centered around solvent properties such as hydrogen bonding or water solubility and the role they played in RTP measurements.

DEVELOPMENT OF THE THEORY

On the basis of previous studies, ideas were formulated to explain possible results and the effect they would have on the role of the solvent in room temperature phosphorescence. It was assumed that the solvent did affect RTP measurements and that some property of either the solvent or filter paper was responsible.

The primary aim of this research was to establish the need for imbibed water on the filter paper prior to drying. It is believed that the imbibed water allows for the movement of the cellulose chains, thus making space available for the entry of the phosphor molecules into the paper fibers and their further distribution into the accessible regions of the paper where bonding with the paper can occur.

It was expected that the results would show that imbibed water was needed for a strong RTP signal. This would be supported only if water containing solutions resulted in signals with an intensity significantly larger than that of the blank.

In previous studies [17], samples dissolved in heptane, chloroform, acetone and methylene chloride resulted in signals significantly lower than samples dissolved in water or water/ethanol mixtures. Based on this research, it would be expected that volatile solvents such as methylene chloride and acetone would result in low phosphorescent signals since they evaporate quickly.

An alternative outcome might have been that good signals were obtained from those solvents with boiling points near or greater than water, such as 1-propanol, and 2-butanone. This result would have indicated that these solvents were able to carry the phosphor to the accessible regions of the paper. The fact that some time (a minute or so) was needed for this process might explain why the more volatile solvents have not produced measurable signals.

Another possibility would be that water insoluble solvents such as carbon tetrachloride and methylene chloride would result in low signal intensities, indicating that water soluble solvents are needed to penetrate the accessible regions of the filter paper.

SOLVENT STUDIES

In order to test these ideas, the room temperature phosphorescence of para-aminobenzoic acid (PABA) was studied with these solvents alone - phenol, 1-propanol, methanol, acetone, 2-butanone, methylene chloride, and carbon tetrachloride - and in various mixtures with water. The physical properties of these solvents are shown in Table 1. Research [10,18-20] has shown that para-aminobenzoic acid gives very reproducible phosphorescent signals. This factor along with availability and solubility made PABA the phosphor of choice for the solvent studies.

Instrumentation

RTP intensity measurements were obtained on an Aminco-Bowman spectrophotofluorimeter equipped with a xenon arc light source, a rotating cylinder phosphoroscope, a solid sample holder, and a photomultiplier tube detector. The photomultiplier tube slit was set

Table 1: Physical Properties of Solvents

Solvent	Boiling Point (°C)	Hydrogen Bonding Ability	Water Solubility (parts/100 part H_2O)	Dieletric Constant E (at °C)
Methanol	64.7	yes	∞	32.63 (25)
Acetone	56.5	yes	∞	20.70 (25)
1-Propanol	97.8	yes	∞	20.1 (25)
2-Butanone	72.1	no	35	18.51 (20)
Phenol	181	yes	8	9.78 (60)
Methylene Chloride	40	no	1	9.08 (20)
Carbon Tetrachloride	77	no	0.08	2.24 (20)
Water	100	yes	—	78.54 (20)

Table 2: k Values for P-aminobenzoic Acid in a Nitrogen Atmosphere
Percent Solvent

Solvent	100%	80%	60%	40%	20%
Methanol	17.4	50.7	51.7	57.6	76.5
Acetone	1.54	34.6	65.5	74.2	76.4
1-Propanol	0.74	25.4	34.6	71.0	76.7
2-Butanone	0.30	9.37	22.0	47.8	76.2
Phenol	0	—	—	—	—
Methylene Chloride	0.27	—	—	—	—
Carbon Tetrachloride	0.29	—	—	—	—
Water	50.6	76.5	*	*	*

—No value (water insoluble)
* Value solvent dependent.

at 5 mm and the excitation and emission slits were excluded. The excitation wavelength was 300 nm and the emission wavelength was 428 nm.

Regents

The solvents of the best available quality were used: acetone, methanol, methylene chloride, and carbon tetrachloride were spectrophotometric grade, while the 1-propanol, 2-butanone, and phenol were ACS certified grade. Distilled water was used for the solvent dilutions. The solvents and PABA were used without any further purification and the nitrogen gas was dried by passing through a drying chamber filled with Drierite.

Procedure

A circle of Schleicher and Schuell 507 filter paper was placed in a glovebag and dried overnight in a nitrogen atmosphere. A paper hole punch was used to cut a small paper disc (65 mm diameter) and 4.0 uL of solution were deposited onto the disc with a micro-syringe. An alligator clip was used to hold and transport the paper to a glovebag where it was dried for 10 minutes under a nitrogen atmosphere. The sample was placed in the holder and the phosphorescent signal was measured while the sample was flushed with dry nitrogen until a stable signal was obtained.

The stock (100%) solvent solutions were prepared to approximately 100 ppm concentration by dissolving about 10 mg of PABA in a 100 ml volumetric flask containing the desired solvent. Solvent solutions containing 80%, 60%, 40%, and 20% solvent were then prepared by transferring aliquots of the stock solution with pipets to 25 ml volumetric flasks and diluting with distilled water. The stock solvent solutions were dried with silicon dioxide dessicant to remove any traces of water. Blanks were prepared from the solvent solutions dried on filter paper and their phosphorescent signal was subtracted from each solvent solution reading.

Calculations

For each solvent solution studied, a k value was calculated. This constant describes the linear relationship between phosphorescence intensity (I) and sample concentration (c, in ppm) [21,22]:

$$I = kc$$
$$k = I/c$$

This calculation was needed in order to compensate for the small variations in solution concentrations due to dilution effects and slightly differing amounts of phosphor. The k values were compared to determine what effect the solvents had on room temperature phosphorescence measurements. A lower k value indicated that less phosphorescence was detected. It is desirable to find the highest possible k value, thus achieving optimum sensitivity and finding the largest phosphorescent signal.

RESULTS

When studying the k values for PABA in a nitrogen atmosphere (Table 2), some definite patterns are observed. The most obvious result is that as the percentage water content increases, the k value also increases. This increase in not steady, rather it seems to jump. The largest "jumps," though, seem to follow this pattern:

methanol	0-20%,
acetone	20-40%,
1-propanol	40-60%,
2-butanone	60-80%.

When these jumps occur, the k values for the solvent change from below that of water (k = 50.6) to above this value. Also, surprisingly, all the 20% solvent solutions level out at k = 76.5, which is much higher than the value of water! The linear dynamic range of PABA in both methanol and acetone was 120-40 ppm. However it is possible that the "jumps" are due to deviations in the linear range of PABA in mixed solvents. The results of acetone were very different than what was expected. As evidenced, the k values for acetone were relatively large, especially when compared to other volatile solvents such as methylene chloride and carbon tetrachloride.

FURTHER STUDY

Other areas of study included the technique of double spotting solvent/wavelength interactions, and solvent volatility tests.

Double Spotting

The study of this technique concerns itself with optimizing RTP

signals. Double spotting is a method in which one solvent (containing the phosphor) is syringed onto the filter paper and dried. A second solvent is then added and the paper redried. This method is generally used for solvents which are either miscible or only slightly miscible in each other. Methylene chloride/water mixtures were studied using this technique. In some trials, the methylene chloride was the first solvent spotted, while in others it was water. The method of adding water followed by methylene chloride gave higher signals than that of methylene chloride followed by water, however the signals were extremely erratic and not at all reproducible. The relative standard deviation for double spotting was about 25% as compared to less than 2% for our previously outlined procedure. A possible explanation for this technique's lack of reproducibility is that the addition of the second solvent causes the spreading out and removal of some of the phosphor. As discussed earlier, the phosphor interacts with the cellulose and is held in the paper fibers. The addition of the solvent would have the effect of breaking some of these bonds and thus causing the phosphor to be replaced by the solvent during the redrying of the paper.

Changes in Wavelength

A study [16] has shown that by changing the excitation and emission wavelengths, optimum signals from various solvents can be observed. In our study, changing the wavelengths by one or two nanometers did increase the phosphorescent signal, but also increased the background interference. After subtraction of this background, the resulting signal was on occasion equal to, but generally lower than that at the normal wavelength setting.

CONCLUSIONS

The solvent does indeed have a role in room temperature phosphorescence. This role cannot be explained sufficiently by the concept of solvent volatility. Carbon tetrachloride, methylene chloride and acetone are quite volatile solvents, yet they give very different results. The k value for acetone is 5 times as large as the k value for either methylene chloride or carbon tetrachloride. The solvent with a boiling point near water, 1-propanol, resulted in half as large a k value as acetone and only about 1/20th that of methanol. Likewise, phenol, under these assumptions, would have given good RTP measurements, however, no measurable k value was observed. Therefore, some property other than volatility must be used to explain the results of acetone.

It can be concluded that not every solvent can carry the phosphor equally to the accessible regions of the paper. Also from the results, it is clear that water/solvent interactions are important and result in larger phosphorescent signals than just the solvents alone. Yet, for the 20% solvents, this "solvent effect" disappeared and the k value leveled out.

These general conclusions lead to the question of imbibed water and its importance in room temperature phosphorescence. Imbibed water enhances the phosphorescent signal. This can be evidenced by the very large k values for water-containing solutions as compared to those without water; however, the 100% solvents, especially methanol and acetone, also gave large values. These results lead to the consideration of an explanation based on solvent/paper interactions, solvent/water interactions, imbibed water, and possible atmospheric effects.

The two present theories concerning the phosphor/support interactions are that of hydrogen bonding [6,11,23-25] and swelling [16].

The hydrogen bonding theory is the traditional explanation, developed to explain the main interaction between the hydroxyl groups and the phosphor. It is based on studies done with silanized paper [9] which indicated that by reducing the number of hydroxyl groups on the filter paper causing reduction of hydrogen bonding interactions, the signal intensity was also reduced. Also observed was the increase in quenching which resulted when the humidity increased. This result indicated that moisture was competing with the phosphor for hydrogen bonding sites thus increasing the movement of the phosphor and chances of collisional deactivation. Another study [25] showed that compounds with the potential for strong hydrogen bonding interactions provided more intense RTP signals than similar compounds without these polar functional groups. The most current theory [24], has expanded on this simple hydrogen bonding notion by describing the solid surfaces that induce RTP behave as either proton donors, proton acceptors, or both simultaneously.

Lately, studies [16,26] have questioned the validity of the hydrogen bonding theory as the sole explanation for the interaction between the paper and the phosphor. Most phosphors have the ability to hydrogen bond, but strong phosphorescence has been detected in the absence of hydrogen bonding interactions [27,28]. One study [26] explained this by saying that other substances such as sugars and salts which had been added to the phosphor either inhibited the motion of the phosphor molecule or plugged the channels and interstices of the matrix, thus providing protection from quenching.

This ambiguity concerning the hydrogen bonding theory was the motivation which resulted in the matrix isolation mechanism (swelling theory). The basis for this theory is that cellulose has gel properties and it swells in the presence of polar solvents such as water [29]. The swelling results from hydrogen bonding between water molecules and the hydroxyl groups of the cellulose [29]. This opens new areas in the fiber where more water can enter, thus enabling the phosphor to penetrate into the submicroscopic pores. Upon drying, the fibers collapse and the water/cellulose bonds are replaced by cellulose/cellulose bonds [29], which causes the phosphor to become trapped in the fiber. Therefore, as the swelling ability of the solvent increases, the phosphorescent intensity should also increase in the following order (best to worst): water > methanol > propanol > acetone. It was the researcher's contention that acetone does not swell cellulose and was vaporized prior to analysis, thus resulting in its low intensity. It was also their contention that good results should be found with the following compounds which swell cellulose: thiourea, resorcinol, and phenol. Our studies have definitely shown that phenol does not give good RTP results. In conclusion, our research has brought the idea of cellulose swelling as the sole cause of the phosphor-paper interaction into question, especially in the explanation of our results using phenol and acetone.

From our studies, it was concluded that imbibed water, solvent/water interactions, and solvent/paper interactions all had an effect on room temperature phosphorescence measurements. From this evidence and these conclusions, a general explanation of what occurs during the RTP procedure will be developed and used in formulating a theory to explain the role of the solvent.

Drying the paper in an inert atmosphere such as nitrogen or helium has the effect of removing the absorbed water held in the pores and the imbibed water from the fibers. The atmosphere prevents any quenching and also opens the fibers. The solvent, upon addition, carries the phosphor into the fibers where the phosphor molecules compete with the solvent molecules for hydrogen bonding sites. Since polar and ionic phosphors have strong hydrogen bonding capabilities, they compete better which results in greater phosphor bonding and a larger signal. Redrying of the sample causes the majority of the solvent to evaporate, thus favoring the phosphor's bonding while the remaining solvent bonds in either the pores or fibers.

For RTP, water is needed initially because it easily penetrates the fibers, yet it must be subsequently driven off in order to detect any measurable signal. The same is true for the other solvents. They aid in the movement of the phosphor into the fibers, however, too much remaining solvent will limit the number of bonding sites available to the phosphor and thus decrease the signal. This solvent/phosphor

competition can be used to understand why solvents such as methylene chloride and carbon tetrachloride do not give good RTP measurements. Both are very volatile solvents and neither hydrogen bonds. Therefore, they evaporate rapidly, but do not carry the phosphor very far into the fiber. It is likely that water still remains in the fiber after drying, which is probably why the 100% water solution does not produce the highest phosphorescent signals.

These conclusions lead to the importance of the dielectric constant of the solvent. A high dielectric constant means that the solvent is better able to penetrate into the fibers and hydrogen bond there, thus carrying in more phosphor and resulting in a high signal. The order of the dielectric constants (Table 1) matches that of the k values for the 100% solutions (Table 2). As the percent water in the solvent mixtures increase, "jumps" are seen where the mixture's k values become greater than that of water. This also follows the order given by the dielectric constants. Then, when the water content rises to 80%, the solvent effect becomes negligible and the k values level off. In conclusion, no simple explanation of solvent effects on room temperature phosphorescence can be made. The answer seems to lie in the various influences of volatility, hydrogen bonding ability and the solvent's dielectric constant. From evidence gained in this study, it is recommended that future work with room temperature phosphorescence uses solvents that have high dielectric constants or that the solvent mixtures be diluted to 80% water content thus minimizing the effects of the solvent.

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