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SOLID-MATRIX LUMINESCENCE ANALYSIS AND COUPLING SOLID-MATRIX LUMINESCENCE WITH SEPARATION METHODOLOGY

Final Technical Report

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Summary

Many significant advances were made over the past two years and five months. Several of the mechanistic aspects of solid-matrix phosphorescence (SMP) oxygen quenching were clarified. Interestingly, SMP quenching by oxygen diffusion occurs to little or no extent. Also, SMP moisture quenching on hydrophilic and partially hydrophobic papers was investigated. The SMP lifetimes of adsorbed phosphors were independent of the amount of the adsorbed moisture for phosphors with lifetimes up to 1 s. Novel methods were developed to coat Whatman No. 1 filter paper for the extraction of polycyclic aromatic hydrocarbons (PAH) from water. The coated papers were used for both the extraction and detection of PAH by solid-matrix fluorescence (SMF) and SMP at the fmol/mL levels.

A detailed study was undertaken to develop an understanding of some of important physicochemical interactions of phosphors in glucose glasses prepared from crystalline glucose and glucose melts. The moisture quenching of SMP, near-infrared spectrometry, differential scanning calorimetry, nuclear magnetic resonance spectrometry, and a polarity probe were used in the study. A model for SMP moisture quenching was developed, the effect of the anomeric percentages of α - and β -D-(+)-glucose on SMP was studied, and several SMP photophysical parameters were related to the interactions of the phosphors with the glucose glasses. Also, a comparative study of the SMP of phosphors in glucose glasses as a function of temperature was carried out. Activation energies acquired from SMP lifetime data were correlated with low-frequency vibrations and β -relaxation phenomena in the glucose glasses.

New photophysical methods were developed for the characterization of tetrol I-1 and (±)-anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPDE)-DNA Adducts with SMF and SMP. The SMF methods were based on fluorescence quenching of tetrol I-1 and the BPDE-DNA adducts. From these methods unique information was acquired for the quasi-intercalated BPDE-DNA adducts and the external form of the BPDE-DNA adducts. For SMP, the phosphorescence was enhanced by the heavy-atom effect and several photophysical parameters were used to in the characterization of tetrol I-1 and the BPDE-DNA adducts.

For liquid-liquid-liquid microextraction (LLLME), solution fluorescence spectrometry was employed along with data from LLLME to investigate the transfer processes in the three phase system. Also, it was shown that by using 1-octanol as an acceptor phase that the concentration of relatively large molecular-weight hydroxyl aromatics was increased considerably.

A. Introduction

In this report, the major results and conclusions of the research over the last two years and five months will be considered. The report discusses the mechanistic aspects of oxygen quenching of solid-matrix phosphorescence (SMP), mechanistic aspects of moisture quenching of SMP, interactions and methodology to investigate phosphors in glucose glasses, new methods for coating filter paper for solid-phase microextraction with solid-matrix fluorescence (SMF) and SMP detection, mechanistic consideration of the heavy-atom quenching of the SMF and the enhancement of SMP of benzo[a]pyrene-DNA adducts, and new developments in liquid-liquid-liquid microextraction.

B. Mechanistic Aspects of the Oxygen Quenching of the Solid-Matrix Phosphorescence of Perdeuterated Phenanthrene on Partially Hydrophobic Paper

In last renewal report, details of this part of the research were discussed. However, the manuscript cited was in review. It has now been published, and the manuscript gives considerable verbiage on the results and conclusions (1). One of the key conclusions was that SMP quenching by oxygen diffusion did not occur extensively, but that static quenching of SMP by oxygen was very significant. Also, some basic equations were developed that could be used to describe the quenching phenomena. In general, the methodology, results, conclusions and equations developed can be used for essentially any phosphor adsorbed on filter paper and similar solid matrices.

C. Moisture Quenching of Solid-Matrix Phosphorescence on Hydrophilic and Partially Hydrophobic Filter Papers

A detailed investigation was undertaken to determine the important parameters that were responsible for the SMP moisture quenching of phosphors adsorbed on Whatman No. 1

(hydrophilic) and Whatman 1PS (partially hydrophobic) papers (2). Several of the important parameters that cause SMP moisture quenching were elucidated. The three phosphors used were phenanthrene, perdeuterated phenanthrene, and benzo[e]pyrene. Both SMP lifetime and SMP intensity data were obtained over a wide range of adsorbed moisture. Also, moisture isotherms were obtained for the No. 1 and 1PS papers. The SMP lifetimes were **independent** of the amount of the adsorbed moisture on both the No. 1 and 1PS papers for phenanthrene and benzo[e]pyrene. However, the SMP lifetimes (in the range of 5 s) of perdeuterated phenanthrene on No. 1 and 1PS papers were dependent on the amount of adsorbed moisture for both papers. Thus, diffusional quenching did not occur for phosphors on No. 1 and 1PS papers with SMP lifetimes in the one second range. The changes in the SMP intensities as a function of adsorbed moisture for phenanthrene, benzo[e]pyrene, and perdeuterated phenanthrene on No. 1 paper could be modeled by a simple exponential function with phenanthrene and benzo[e]pyrene giving the better correlations compared to perdeuterated phenanthrene. The decrease in the SMP with moisture adsorption for the three phosphors adsorbed on 1PS paper did not correlate with the simple Stern-Volmer model and several other quenching models discussed in the literature. Thus, these data were fit to a relatively simple empirical equation. The quenching data showed that the SMP quenching phenomena for the three phosphors on No. 1 paper and 1PS paper were considerably different.

The investigation of adsorbed water was not only important for SMP, but for other researchers that have a fundamental interest in how moisture adsorbs to cellulose. The phosphors in this work could readily be employed to investigate the physical aspects of the adsorption of water to different sites in cellulose.

D. Methods for Coating Filter Paper for Solid-Phase Microextraction with Luminescence Detection and Characterization of Coated Filter Paper by Infrared Spectrometry

Novel methods were developed to coat Whatman No. 1 filter paper for the extraction of polycyclic aromatic hydrocarbons (PAHs) from water (3). The filter paper samples were coated using solutions of poly(hydrogenmethylsiloxane) or dichlorodimethylsilane. The coated papers were tested for the extraction of polar aromatic compounds and polycyclic aromatic compounds from aqueous solutions. The extracted compounds were detected by a combination of SMF and SMP. Also, the limits of detection and effective partition coefficients for the compounds were calculated for the coated papers. The limits of detection and effective partition coefficients were compared to those obtained with 1PS paper as an extraction medium. Both coated filter paper samples were shown to be more effective than Whatman 1PS paper for the extraction of polycyclic aromatic hydrocarbons. However, the paper treated with poly(hydrogenmethylsiloxane) gave the extraction properties and a limit of detection of 29 fmol/mL for benzo[e]pyrene. This contrasted with limits of detection of 153 and 77 fmol/mL with 1PS paper and the silane-treated paper, respectively. The paper coatings were also characterized by near- and mid-infrared reflectance spectroscopy. Both coating methods are simple procedurally and permit very reproducible data and low limits of detection to be obtained.

E. Interactions of Phosphors in Glucose Glasses for Solid-Matrix Phosphorescence

A detailed study was undertaken to develop an understanding of several of the important physicochemical interactions of phosphors in glucose glasses (4). This was important because we previously showed that glasses prepared from glucose melts had several advantages (5). For example, with the glucose melt, glasses could be prepared with pure methanol instead of a methanol:water solvent. This approach eliminated, or minimized water, which is a known

quencher. Also, the limits of detection of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), for example, were lowered by a factor of 3.4 for glasses prepared with glucose melts compared to the limit of detection obtained in glasses prepared from crystalline glucose.

Quenching of room-temperature solid-matrix phosphorescence (SMP), near-infrared spectrometry, differential scanning calorimetry, and a polarity probe were used to study the interactions of heterocyclic aromatic amines in glucose glasses prepared using crystalline glucose and a glucose melt. Because there was no simple method for the determination of moisture in glucose glasses, a near-infrared spectrometry method was developed to determine the wt% moisture in the glucose glasses. The wt% moisture of the glucose glasses was then related to phosphorescence intensities and lifetime ratios of the heterocyclic aromatic amines. The two heterocyclic aromatic amines used were PhIP and 2-amino-9H-pyrido[2,3-b]indole (AαC). These compounds are important in cancer research. For PhIP and AαC in glasses prepared from crystalline glucose, in addition to SMP intensities, the pre-exponential weighted mean lifetime (τ_M) and average lifetime (τ_A) were used in the data analysis. Also, it was necessary to use different SMP quenching models for the PhIP data and AαC data. By using the average lifetime for the SMP quenching data for AαC, it was possible to distinguish a matrix component and a dynamic component. One of the equations employed in the interpretation of the SMP quenching data is given by Eq. 2.

$$P_0/P = (\tau_{A0}/\tau)e^{K_m[Q]} \qquad (2)$$

In Eq. 2, P_0 is the SMP without moisture present, P is the SMP with moisture present, τ_{A0} is the average lifetime with no moisture present, τ is the average lifetime with moisture present, K_m is a matrix quenching constant (as opposed to a static quenching constant), and [Q] is the wt% moisture.

The glass transitions temperatures (T_g) were obtained from glucose glasses prepared with crystalline glucose and glucose melts with different amounts of water (4). The T_g values of carbohydrates decrease significantly as the water content increases, and T_g values gave useful information about the mechanical properties of the glasses. Generally, the SMP of either PhIP or AαC decreased with an increase in water content and a concomitant decrease in T_g. For example, the SMP of PhIP in glasses prepared from crystalline glucose decreased by almost half when the T_g changed from 23.8 to 20.4 °C. Because the glasses with the higher T_g are more rigid, deactivation of the triplet state of the phosphor by collisional and vibrational processes is less likely, and therefore the SMP intensities are higher. Much more research is needed to relate SMP to T_g values, water content, and viscosities of sugar glasses.

The micro-environmental polarity of glucose glasses was investigated by using pyrene as a polarity probe. The fluorescence characteristics of pyrene have been shown to be sensitive to the polarity of the surrounding environment. Typically an R value obtained from the fluorescence spectra of pyrene is used as a measure of polarity of the micro-environment. In this research, R values for pyrene in glucose glasses prepared from crystalline glucose and glucose melts were acquired. Also, to serve as a comparison, R values for several methanol:water solutions were obtained. In general, the R values for pyrene in the glucose glasses were greater that the R values for pyrene in methanol:water solutions. It was concluded that in the solid state there would be stronger interactions of pyrene in the glucose glasses compared pyrene in methanol:water solutions because of the close proximity of the glucose and water molecules to pyrene. The higher R values for pyrene in the glasses was most likely due to a greater reduction in symmetry of pyrene by interactions of hydroxyl groups from glucose and water.

Based on the results of this part of the research, the following model for the effect of water on glucose glass matrices is proposed. Due to random packing of glucose molecules , narrow channels of free space exist in the glasses. A certain fraction of the water molecules move through these channels, and these water molecules collide with the phosphor molecules and cause dynamic quenching of the SMP. Because phosphor molecules need to be held rigidly for SMP to be observed, the phosphor molecules do not diffuse. This type of quenching is different compared to solution fluorescence quenching whereby the fluorescent and quencher molecules show considerable motion and can readily collide. The other fraction of water molecules is randomly dispersed in the glucose matrix, and they disrupt the hydrogen bonding network in glucose. This weakens the glucose matrix and causes the matrix quenching of the SMP. The T_g values also support these conclusions. With water disrupting the hydrogen bonding network, the samples with the higher water contents have to be cooled to a lower temperature before a glass is formed. Thus, at the temperature at which the SMP is measured, the glasses with higher T_g values have higher viscosities and a more stable hydrogen bonding network compared to glasses with lower T_g values. This work represents the first study of the interactions between phosphor molecules in two types of glucose glasses. The results and the model developed are important for a fundamental understanding of the interactions in solid matrices and also for the development of new solid matrices. At this stage, the model is important, but it is incomplete. Additional research is needed to expand the model so a more complete understanding can be developed about the physicochemical interactions of the phosphors in sugars glasses.

F. Determination of the Anomeric Percentages of α- and β-D-(+)-Glucose in Glucose Glasses with Proton Nuclear Magnetic Resonance Spectrometry

No work was done previously in determining if the anomeric percentages of α - and β - D-(+) glucose in the glucose glasses prepared from crystalline glucose or the glasses prepared from the glucose melt affected the SMF and SMP of the lumiphors in these glasses. To determine the percentages of the anomers in the two types of glasses, we developed a proton nuclear magnetic resonance method that gave us the percentages of α - and β -D-glucose (6). Four sets of data were obtained, namely, glasses from crystalline glucose (no NaI), glasses from glucose melt (no NaI), glasses from crystalline glucose (10% NaI), glasses from glucose melt (10% NaI). NaI was added to the glasses because it has been used as a heavy-atom salt to enhance the SMP of PhIP and AαC (5). It was established from our NMR data that the anomeric percentages of α - and β -D-(+)-glucose in the two types of glasses, whether NaI was present or not, were essentially the same. Typical percentages were 45.4% of α -D-(+)-glucose and 54.6% β -D-(+)-glucose. It was concluded that differences in the SMF and SMP properties of lumiphors in the two types of glasses could not be attributed to the percentages of α and β anomers in the glasses.

G. A Comparative Study of the Solid-Matrix Phosphorescence of Heterocyclic Aromatic Amines in Glucose Glasses as a Function of Temperature

SMP intensities and lifetimes were obtained for PhIP and AαC as a function of temperature from room temperature to 93 K (7). The two solid matrices employed were glucose glasses prepared form crystalline glucose and glucose melts, both with and without the heavy-atom salt, NaI. The SMP intensities and lifetimes of PhIP and AαC in the two types of glasses without a heavy-atom salt were very sensitive to temperature changes. The SMP intensities of

PhIP and AαC in both glasses with NaI present did not change much with temperature. In fact, AαC gave essentially the same SMP in both glasses with 10% NaI at room temperature and at 93 K. Thus, basically these heavy-atom salt/glucose systems were able to achieve the maximum SMP signal at room temperature! The intensity-to-lifetime ratios showed that the triplet state formation efficiency of PhIP changed as a function of temperature for glasses prepared without NaI. This illustrated that the SMP was a function of both triplet state formation efficiency and phosphorescence lifetime. For glasses prepared with NaI, the triplet state formation efficiency of PhIP remained essentially constant with temperature. Thus, for this sample, the SMP intensity was only a function of SMP lifetime. Using the SMP lifetimes as a function of temperature, activation energies for the phosphors in the glasses were calculated. The SMP lifetime data fit a biexponential equation and two activation energies were obtained. The lower activation energy was related to low-frequency vibrations in the glucose matrix, and the higher activation energy was related to β-relaxation processes that occur in glucose glasses. At about 210 K, the β-relaxations ceased and only the low frequency vibrations were active at temperatures below 210 K. These results strongly suggest that if sugar glasses were prepared that do not show β-relaxation, then, most likely, loss of the nonradiative triplet state energy would not be a significant as with glasses that have β-relaxation phenomena. Thus, these glasses would have the potential of having enhanced SMP intensities.

H. New Photophysical Methods for the Characterization of Tetrol I-1 and (±)-anti-Benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPDE)-DNA Adducts with Solid-Matrix Fluorescence and Solid-Matrix Phosphorescence

Because of the results we obtained from DOE funding of the solid-matrix luminescence (SML) projects, previously we obtained eight years of funding from EPA to investigated the SML of (±)-anti-BPDE-DNA adducts. Recently, we had a proposal funded by NIH for three

years to investigate other PAH-DNA adducts and apply the SML methodology for the detection of PAH adducts in purified samples from humans. Under this DOE funding period, we explored the more fundamental aspects the effects of the heavy-salts, $TlNO_3$ and NaI, on the SML of tetrol I-1 and (±)-anti-BPDE-DNA adducts (8,9). The heavy-atom salts we investigated both quenched the SMF of tetrol I-1 and the (±)-anti-BPDE-DNA adducts (8) and enhanced the SMP of tetrol I-1 and the (±)-anti-BPDE-DNA adducts (9).

a. *SMF Quenching:* Several fluorescence quenching models were evaluated for both tetrol I-1 and the (±)-anti-BPDE-DNA adducts (8). The SMF quenching phenomena were quite different with the two salts for tetrol I-1 and the (±)-anti-BPDE-DNA adducts. Generally, with $TlNO_3$ as a quencher, a two-site model with two independent quenching sites was applicable to both the tetrol I-1 and the (±)-anti-BPDE-DNA adducts data. However, with the sodium iodide the SMF quenching data for tetrol I-1 were fit to the sphere of action model, but the (±)-anti-BPDE-DNA adducts SMF quenching data were qualitatively related to a BET isotherm. From the SMF quenching data, unique information was acquired for the quasi-intercalated BPDE-DNA adducts and the external form of the BPDE-DNA adducts. In addition, insights were obtained on how the adsorbed salts interacted with the solid matrix and with the tetrol I-1 and the (±)-anti-BPDE-DNA adducts. The SMF quenching with $TlNO_3$ and NaI has proven quite versatile in the characterization of the (±)-anti-BPDE-DNA adducts and tetrol I-1. Each salt provided a unique way to investigate the samples. With $TlNO_3$, SMF quenching information was obtained for both the quasi-intercalated and external forms of the(±)-anti-BPDE-DNA adducts. Comparison of the quenching data for the (±)-anti-BPDE-DNA adducts and tetrol I-1 revealed significant differences in how the adducts and tetrol I-1 interact with the solid matrix. Even though the quenching phenomena were complex, the models and equations developed

permitted the SMF data to be interpreted in a reasonable fashion. In this work, the DNA samples were modified at 1 adduct in 10^4 bases, and the fluorescence signals were very strong. Thus, the equations developed would have general applicability to samples of DNA modified at several different levels of (±)-anti-BPDE. It remains to be seen if the approaches developed could be used for human samples. However, other PAH-DNA adducts and PAH metabolites could easily be characterized by the SMF quenching methodology developed.

b. SMP Enhancement: Novel SMP methods were developed for the detection and characterization of the (±)-anti-BPDE-DNA adducts and tetrol I-1 by using the heavy-atom salts, $TlNO_3$ and NaI, to enhance the SMP (9). $TlNO_3$ was much more effective for enhancing the SMP of the (±)-anti-BPDE-DNA adducts and tetrol I-1. The amount of $TlNO_3$ adsorbed on the solid matrix was varied over a wide range, and SMP intensities, lifetimes, and spectra were acquired. Fundamental equations and calculated photophysical parameters were used to interpret the data and characterize the samples. The data indicated that there were two major populations of the (±)-anti-BPDE-DNA adducts and tetrol I-1 adsorbed on the solid matrix. However, because DNA was adsorbed so strongly to the solid matrix, the (±)-anti-BPDE-DNA adducts interacted in a more uniform manner with increasing amounts of $TlNO_3$. However, tetrol I-1 responded in a more random fashion with an increase in the amount of $TlNO_3$. Also, preexponential factors and the fractional contribution to the SMP decay curves showed that the fractional populations of tetrol I-1 and the (±)-anti-BPDE-DNA adducts were essentially independent of the amount of $TlNO_3$ adsorbed. The ln(SMP intensity) versus time plots for tetrol I-1 and the (±)-anti-BPDE-DNA adducts were quite different at times greater than 24 ms. The data indicated that DNA was held very rigidly to the solid which permitted $TlNO_3$ to interact with the (±)-anti-BPDE-DNA adducts in an orderly fashion. The ln(SMP intensity) versus time

plots for tetrol I-1 showed that tetrol I-1 interacted in a more random fashion with $TlNO_3$. The detection and characterization methods developed have general applicability PAH-DNA adducts and their metabolites.

I. Liquid-Liquid-Liquid Microextraction (LLLME)

This "simple" separation approach is inexpensive, enhances the concentration of the solute, and permits small molecular-weight solutes to be separated from DNA and proteins. It would be very effective in enhancing UV-visible or fluorescence signals from solutes separated by capillary electrophoresis (CE). Also, when used with SML, the signals would be improved considerably because the concentration of the solutes would be increased by LLLME. We have been using solution fluorescence to obtain mass-balance distribution data in the three phases (aqueous donor phase, organic phase in the pores of the fiber, and aqueous acceptor phase). Hydroxyl aromatics such as 1-hydroxypyrene (1-OH-Py) and 3-hydroxybenzo[a]pyrene (3-OH-B[a]P) are being used as model compounds because of their significance in the metabolism of PAH. One important breakthrough was demonstrating that 1-octanol can be used as the acceptor phase. This permits the enrichment factor for the hydroxyl aromatics to increase considerably (10). In other experiments, it was shown that with the relatively large hydroxyl aromatics investigated a considerable amount of solute was retained by the pores of the fiber. This occurred even when 1-octanol was the acceptor phase. It is clear from the literature that this aspect of LLLME has not been addressed adequately, and there is a need for fundamental studies to investigate this phenomenon and solute transfer processes in LLLME.

In work that was supported by DOE and EPA, we developed a CE method for the separation of tetrol in the presence of DNA and laser-induced fluorescence detection of the separated tetrol (11). The method developed is important not only because it is a unique and

rapid means of separating tetrol with DNA present, but the general CE approach will be used in conjunction with our experiments in LLLME.

J. Manuscripts and Presentations

The manuscripts published, in press, or in preparation are listed at the end of this report under **References**. References 1, 2, 3, 4, 6, 7, 8, 9, 10, and 11 were generated during this funding period. Over the funding period, eight presentations of the research were given at various meetings and universities by principal investigator and graduate students.

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