Synthesis and Study of Micelles and Trihydroxy Tetraphenylporphyrin

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The development of photodynamic therapy involves the use of porphyrin drugs, which requires the further understanding and characterization of the porphyrins. This study focuses on the quenching of trihydroxy tetraphenylporphyrin ($TPP(OH)_3$) by 9,10-*anthraquinone*-2-sulfonate (AQS) in solutions of cetyl trimethylammonium bromide (CTAB), 12-3-12 Gemini surfactant, and 12-2-12 Gemini surfactant. The Stern-Volmer quenching constants were determined and showed no correlation between each of the solution environments. The results indicate that the trihydroxy tetraphenylporphyrin best interacted with the 12-3-12 Gemini surfactant followed by CTAB, and finally the 12-2-12 Gemini surfactant. This contributes to the knowledge of the trihydroxy tetraphenylporphyrin and will hopefully serve to better the understanding and development of photodynamic therapy.

Introduction

Porphyrins have been used in photodynamic therapy (PDT) because of their known nature to localize within tumor cells, and their ability to readily absorb light and generate singlet oxygen.[1] Although it has already been used in medicine, there is a paucity of data on the porphyrins interacting with micellar media

One of the first porphyrins studied was tetrasulfonated tetraphenylporphyrin (TPPS). The porphyrin under study here is trihydroxy tetraphenylporphyrin $TPP(OH)_3$). TPPS and $TPP(OH)_3$ are relatively similar in structures. Therefore, some preliminary work on TPPS was done in this research to demonstrate the competency in the methods and experimental portions of this study.

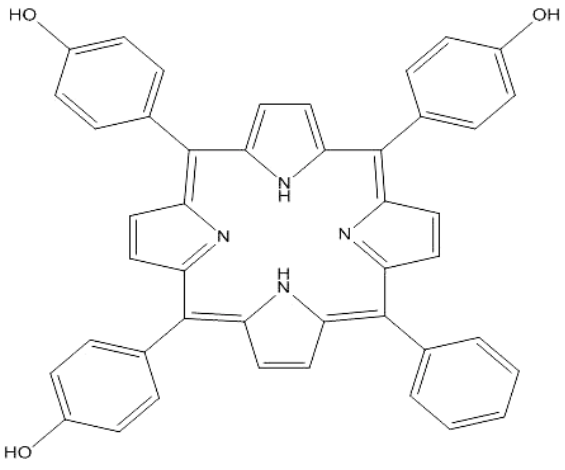

Figure 1: Trihyrdoxy tetraphenyl tetraporphyrin

[1]Gerhardt, Sarah A.; Lewis, James W.; Kilger, David S.; Zhang, Jin Z.; Simonis, Ursula. *J. Phys. Chem.*

It is known that porphyrins are highly polarizable[2] and has been reported that asymmetric porphyrins may have preferable characteristics for PDT purposes.[3] Vermathen *et. al.* study focuses on making one side of the porphyrin more polar and the other side more nonpolar. This may be beneficial in PDT. By having such a polarity characteristics, Vermathen *et. al.* believe the porphyrin can insert itself across the membrane of the targeted cell and destroy the entire cell rather than just rupturing the membrane of the targeted cell. With these polarity specifications, making different structure of the porphyrins becomes more difficult.

$TPP(OH)_3$ is structurally complimentary to the mono-substituted porphyrins studied by Vermathen *et. al.* In studying $TPP(OH)_3$, it is hoped that valuable contributions to these polarity concepts of porphyrins are made and will advance the uses of porphyrins in medicine.

Experimental Section

Porphyrin Synthesis. Trihydroxy tetraphenylporphyrin ($TPP(OH)_3$) was synthesized via the modified Adler-Longo method and specific protocols established by Bonar-Law *et. al*.[4] with minor adjustments to yield more trihydroxy tetraphenylporphyrin: With constant stirring, 0.214g of hyrdoxybanzaldehyde, 0.027g of benzaldehyde, and 140μL of fresh pyrrole were added to synthesis pot of 200mL of aqueous sodium dodecylsulfate (SDS), with a blanket of steadily flowing nitrogen to shield from oxygen. With the addition of these, the solution transitions from a transparent pale yellow solution to a transparent deep red-orange solution. After 30 minutes of constant stirring, a warmed 10mL solution of tetrachloroquinone (TCQ) in THF was added. Upon this addition, the transparent deep red solution immediately became am opaque purple-brown solution. The reaction was left open to air overnight with constant stirring, (oxygen from air functions as a reactant).

The resulting suspension was poured into a separatory funnel and washed with 200mL of ethyl acetate (EtOAc), 10mL of potassium hydroxide (2 Molar) (KOH), 20mL of potassium chloride (3 Molar) (KCl), and 20mL of potassium phosphate(1 Molar) at pH 7. The reaction flask was rinsed out with 100mL of de-ionized water (dH_2O). The aqueous layer was further washed with 100mL of EtOAc; the resulting organic layer was combined with the previously collected organic layer. The combined organic layers were subjected to further washes with 200mL of dH_2O. The final organic layer was extracted and dried with excess amounts of sodium sulfate (Na_2SO_4). After 30 minutes, the Na_2SO_4 was filtered off through a glass frit and the filtrate was collected and transferred into a round-bottom flask for evaporation via the Roto-vap; the filtrate was evaporated down to ~10-15mL.

The final product was transferred onto a fine silica gel column (mesh size 270 to 400). The porphyrins were initially flushed down the column with 3% THF in CH_2Cl_2. The concentration of THF was increased (but did not exceed 12%), to assure that all retrievable products were flushed off the column. Fractions containing products were

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[2] Kano, Kanji; Fukuda, Kazuya; Wakami, Hideo; Nishiyabu, Ryuhei; Pasternack, Robert F. *J. Am. Chem. Soc.* **2000,** *122,* 7495.

[3] Vermathen, Martina ; Louie, Elizabeth A.; Chodosh, Adam B. ; Ried, Sandra ; Simonis, Ursula. *Langmuir,* **2000,** *16,* 210-221.

[4] Bonar-Law, Richard P. *J. Org. Chem.* **1996,** *61*, 3629-3630.

transferred into small round-bottom flasks and evaporated down to ~5-10mL each. The highly concentrated fractions were then tested against a standard, (TPPS) for assurance of purity and identification. Like fractions were combined together. Once identified by R_f values, all fractions were evaporated down to dry solids via the Roto-vap, and were left to further air dry. Some fractions remained a thick oil consistency in the drying process; these received and addition of acetone and were transferred onto a watch glass and left to air-dry.

^{1}H NMR. The $TPP(OH)_3$ was thoroughly characterized by NMR. A few crystals of the product was transferred into an NMR tube and diluted with ~1mL acetone-d6. The spectrum was obtained through a high field 500MHz ^{1}H NMR.

UV-vis. UV samples of $TPP(OH)_3$ in the presence of several different surfactants, (CTAB, 12-3-12, and 12-2-12) were run on an Agilent 8453 diode array spectrophotometer. The Soret bands of the various samples were used as a general focal point for the fluorescence spectra and to discern whether the porphyrin was a monomeric or oligmeric in the micelles. The lower intensity Q bands were also recorded. Some samples required the use of a 0.10cm path length cell.

Fluorescence Quenching. Fluorescence samples were prepared in ~5mL test tubes and spectral analysis was done with BioTek Synergy Mx Fluorescence plate reader, sodium 2-sulfonated, 9, 10-anthraquinone (AQS) serving as the quencher. The excitation wavelength was either 410nm or 525nm for the numerous experiments. The emission wavelength range was set to 580nm to 780nm for nearly all experiments, furthermore, fluorescence quenching experiments were done for TPPS with CTAB, DTAB, and 12-3-12 as standards and were compared to known results.[5]

1.65mg $TPP(OH)_3$ was dissolved in 5mL of reagent grade acetone to make a stock solution of 0.50mM $TPP(OH)_3$. 0.5mL of the 0.50mM $TPP(OH)_3$ in acetone was transferred into a 5mL glass vial, and warmed in a hot water bath to strip away the acetone. $TPP(OH)_3$ was then dissolved with 1.25mL of 40mM surfactant and 1.25mL of dH_2O. 0.10mL aliquots of the $TPP(OH)_3$-surfactant solution is transferred into a series of 5mL test tubes and further dosed with 0.4mL of 40mM surfactant, varying concentrations of AQS (10mM stock solution made with dH_2O), and varying amounts of dH_2O (to achieve a final volume of 1mL). These samples were then transferred directly into desired wells of a 96-well plate in 0.20mL aliquots. For each surfactant, the series of samples consisted of ~6 different concentrations of AQS ranging from 0.00mM to no more than 2.50mM; see Table 1.

[5] Richelli, Fernanda; Stevanin, Daniela; Jori, Guilio. *Photochemistry and Photobioloy,* **1988,** *45,* 13-18.

Sample	Vol. of $TPP(OH)_3$ in 20mM 12-3-12	Vol. of 40mM 12-3-12	Vol. of 10mM AQS	Vol. of dH_2O	Total Vol.
1	0.10mL	0.40mL	0.00mL	0.50mL	1.00mL
2	0.10mL	0.40mL	0.05mL	0.45mL	1.00mL
3	0.10mL	0.40mL	0.10mL	0.40mL	1.00mL
4	0.10mL	0.40mL	0.15mL	0.35mL	1.00mL
5	0.10mL	0.40mL	0.20mL	0.30mL	1.00mL
6	0.10mL	0.40mL	0.25mL	0.25mL	1.00mL

Table 1: Sample preparation information for fluorescence quenching.

The fluorescence quenching data was used to generate Stern-Volmer plots, from which the quenching constants, K_{SV}, were determined for each $TPP(OH)_3$-surfactant interaction. K_{SV} values infer something about the porphyrin sampling and position near the interface of the micelles.

Results

See Figure 2 for ^1H NMR spectrum of $TPP(OH)_3$ which was synthesized in this research. The spectrum was compared to a spectrum obtained for $TPP(OH)_3$ obtained in previous work done by Steve Bachofer and an undergraduate and J.Geoff Allen in 1999. The two spectra have resonances at the same chemical shift values.

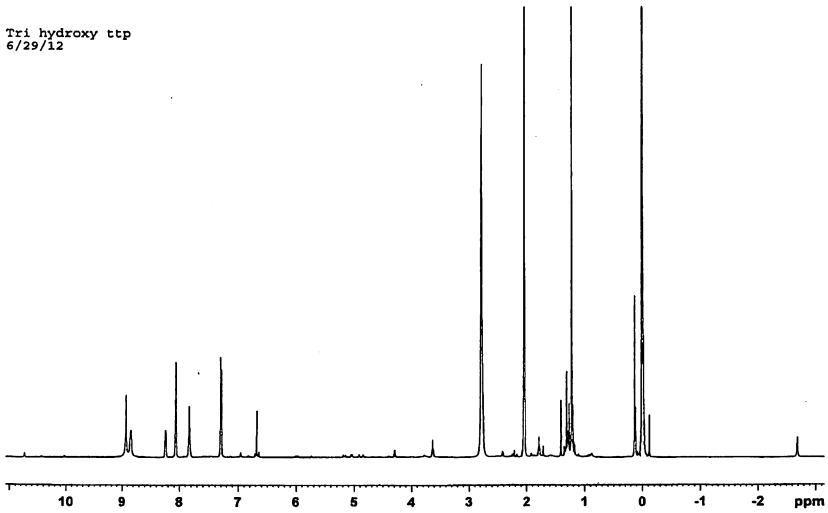

Figure 2: ^1H NMR spectral analysis of $TPP(OH)_3$.

δ (ppm)	Splitting
-2.75	Singlet
6.71	Singlet
7.31	Doublet
7.84	Doublet
8.06	Doublet
8.25	Doublet
8.88	Doublet

Table 2: ^1H NMR experimental values for $TPP(OH)_3$

The Soret bands were used to narrow the emission range that would be focused on in the fluorescence quenching experiments should the range of 580nm to 780nm not work. UV-vis spectral analysis was also used to characterize $TPP(OH)_3$ based on its Soret bands and accompanying Q bands.

The Soret band for $TPP(OH)_3$ with 12-3-12 gemini surfactant was λ_{soret} = 423nm; the Q bands were λ_Q = 520nm, 560nm, and 605nm. For these samples the porphyrin to surfactant concentration ratio was 1:120, 1: 80, and 1:40

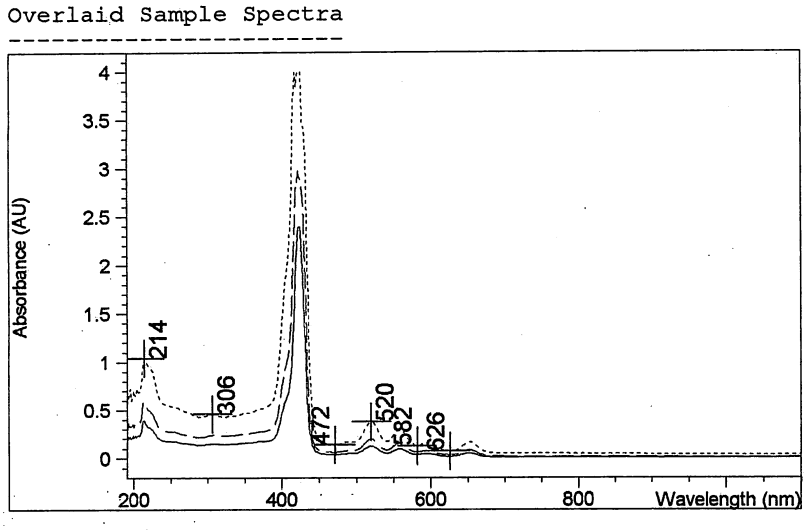

Figure 3: Overlaid UV-vis spectra for $TPP(OH)_3$ and 12-3-12 gemini surfactant.

The data retrieved from the fluorescence quenching experiment were used to generate a Stern-Volmer plot which can be seen in Figure 5. A linear line is observed as expected. The slope of this linear line is the quenching constant, K_{SV}. The fluorescence quenching experiments of TPPS with CTAB surfactant and 2AQS as the quencher yield a K_{SV} value equal to $0.33mM^{-1}$ which matches the reported values of Richelli.[6] Table 3 gives the K_{SV} values for $TPP(OH)_3$ in the presence of 12-2-12 gemini surfactant, 12-3-12 gemini surfactant, and CTAB.

Surfactant	Ksv
12-2-12	$1.50mM^{-1}$
12-3-12	$6.90mM^{-1}$
CTAB	$0.83mM^{-1}$

Table 3: K_{SV} values for $TPP(OH)_3$.

[6] Richelli, Fernanda, *et. al. Photochemistry and Photobioloy,* **1988,** *45,* 13-18.

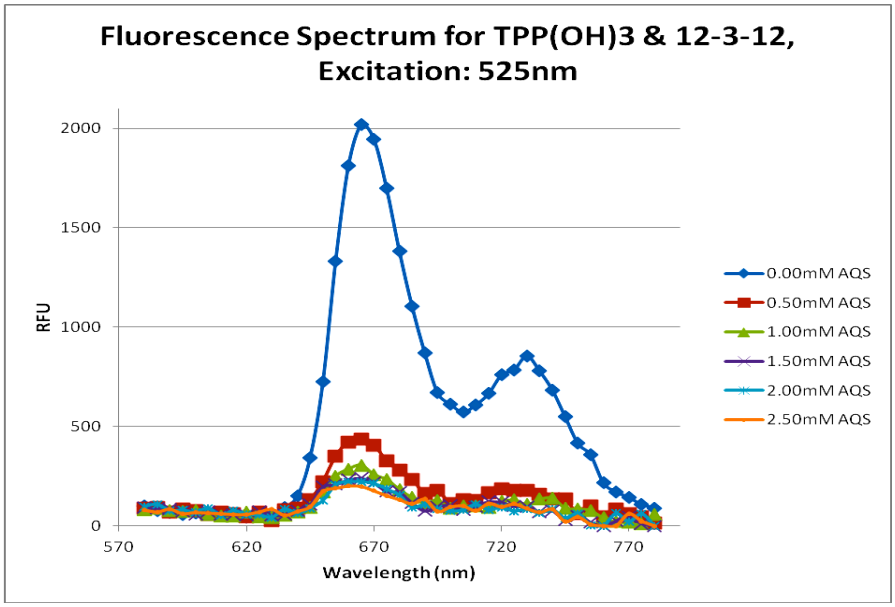

Figure 4: Overlaid fluorescence spectral analysis of $TPP(OH)_3$ with 12-3-12 and AQS.

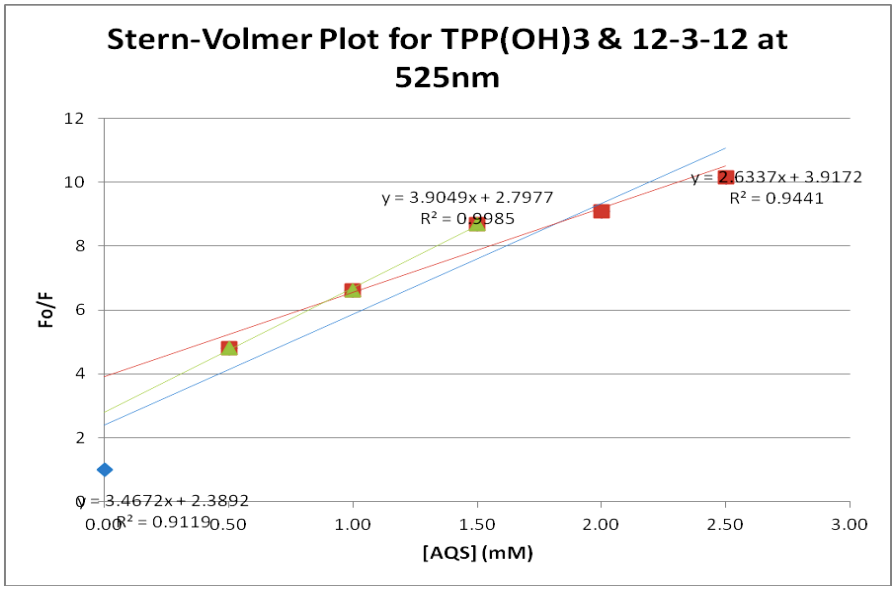

Figure 5: Stern-Volmer plot generated from fluorescence data.

Discussion

The chemical shift values obtained from the ^1H NMR spectrum confirm that $TPP(OH)_3$ was synthesized. The peak at δ = -2.75ppm indicates that there are hydrogen atoms that have been highly shielded due to its location within the π-cloud. The nitrogen

atoms that form the N-H bond are responsible for the position of the protons in the highly shielded environment. The remaining chemical shifts (δ = 6.71ppm, 7.31ppm, 7.84ppm, 8.06ppm, 8.25ppm, and 8.88ppm) account for the hydrogen atoms located on the phenyl portions of the porphyrin. The nice clean peaks indicate that the $TPP(OH)_3$ was successfully isolated and purified.

The Soret band obtained for $TPP(OH)_3$ was as expected for the tetraphenylporphyrin. The Soret wavelength for the spectra are comparable to the single hydroxy substituted TPP reported by Gerhardt and coworkers. The unique Q bands determined for $TPP(OH)_3$ suggest that it occurs as a monomer, rather than an oligomer like the commercial porphyrin product Photofrin® used in PDT. This then poses a question of which one can perform optimally as the photosensitizer in PDT, an oligomer or a monomer. However, this research does not explore this aspect.

The Stern-Volmer plots generated for $TPP(OH)_3$ in the presence of the varying surfactants contain multiple linear regressions which included slightly different sets of concentration range of the AQS, but remained in the range that was primarily selected for the experiments. This was done to account for and determine if a certain threshold of [AQS] was reached, or if the [AQS] was too low for proper analysis. For each case where it was found that the [AQS] was either too low or too high, a new [AQS] range was chosen or smaller incremental differences between consecutive samples were set for a new quenching experiment.

The quenching constants determined for $TPP(OH)_3$ suggest that this particular porphyrin best interacted with the 12-3-12 gemini surfactant at the interface. The 12-2-12 gemini surfactant did not interact quite as well as the 12-3-12 gemini surfactant, but did so more successfully than the CTAB did.

It is uncertain what makes the 12-3-12 gemini surfactant for more interactive with $TPP(OH)_3$ than the 12-2-12 gemini surfactant; perhaps the additional methane plays a far more significant role than is realized. Future studies on this aspect may be beneficial towards the understanding of porphyrins, specifically the $TPP(OH)_3$.

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