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Interaction of protoporphyrinix (PPIX) and 5-amino levulinic acid (ALA) in nanoemulsion

Abstract

Nanoemulsion is an interesting and unique fluid system in that it is used to solubilize both ionic and non-ionic molecules. Because of its uniqueness it has been used as a medium for drug delivery. It is therefore used in this work to study the interaction of Protoporphyrin (PPIX) and 5-Amino levulinic acid (ALA). While ALA is a distant precursor of PPIX, PPIX itself is not only a precursor of Heme but also a photosensitizer in the modality of Photodynamic Therapy (PDT). Both compounds are used use in PDT regimen. A steady-state fluorescence technique is used for the study of the interaction of these very important biological compounds. It is found that ALA quenches the fluorescence of PP IX in nanoemulsion. This observed quenching is diffusion controlled. The bimolecular quenching constant, kq, was determined as 2.86x 1010/M-s with an interaction constant, Ka, of 4.48 x 105 with the free energy of interaction, ΔGa of -32.234kJ/mole.

Keywords: interaction, nanoemulsion, protoporphyrin ix, 5-aminolevulinic acid, fluorescence

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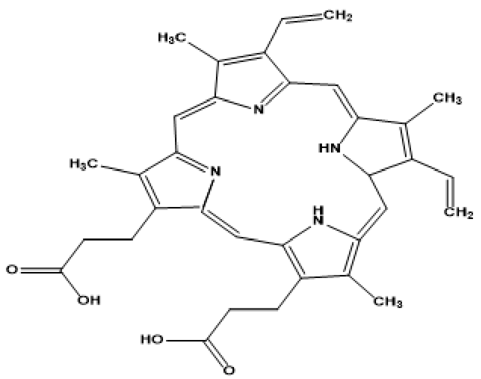
Introduction

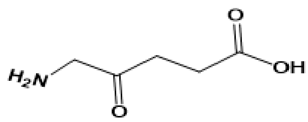
Nanoemulsion is a heterogeneous fluid system that is extensively used for drug delivery and solubilization of poorly soluble compounds.[1-6] It is made by dispersing oil in water using surface active agent (Surfactant) and a co-surfactant, usually a short chain alcohol, in appropriate ratios. PPIX and AlA are very important in biological processes including photodynamic therapy (PDT)[1-7] While ALA is a distant precursor of PP IX, PP IX itself, is a precursor of the Heme (oxygen carrier) found in mammals and plants. The scheme of the formation of any given Heme may be represented thus:

ALA → → → PP IX → → → HEM

The full and complete scheme of biosynthetic route of the ALA to Heme production is given in reference.[8] PPIX is poorly soluble in water[9-17] but it is freely soluble in Nanoemulsion, hence its use as a medium for studying its interaction with ALA using steady-state Fluorescence spectroscopy. Figure 1 shows the chemical structure of PP IX, ALA and the SEM image of Nanoemulsion.



Protoporphyrin IX



5-Aminolevulic acid

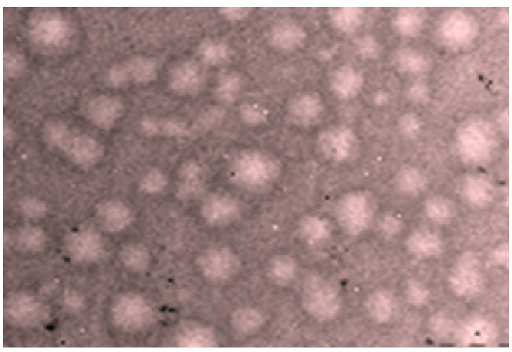


Figure 1 SEM Image of Nanoemulsion.

Experimental

Chemicals

All the chemicals used (tetradecane, surfactant (CTAB), 1-pentanol) were of analytical reagent grade obtained from Acros Chemicals and used as received without further purification.

Instrument

The Fluorescence spectra were obtained using Perkin Elmer's Luminescence Spectrophotometer, model LS 50 B

All solution was prepared using triply distilled deionized water from Photronix Reagent Grade water system.

Preparation of Nanoemulsion

The chemical composition of the Nanoemulsion used is given in Table 1.

Table 1 The chemical composition of the Nanoemulsion

Component	Wt., g	Percentage, %	Volume, mL
Water	174	76	174
CTAB (Surfactant)	12	5	12.63
Oil (Tetradecane)	14	6	18.25
Co-Surfactant (1-pentanol)	29.9	13	31.8

Literature methodology[14] was followed in the preparation of the Nanoemulsion used in this work. Briefly, a measured weight of 12.0 g of CTAB as added to 174 mL of water and the mixture formed a slurry. This slurry was mechanically stirred for about two or three minutes after which 18.25 mL of n-tetradecane were added to the slurry drop wise while the mixture is still being stirred. Thereafter 31.80 mL of n-pentanol were added, again dropwise. The stirring continued until the mixture became clear and translucent. The translucent solution was transferred to an ultrasonic sonicator where it was sonicated for about 10 minutes. The Nanoemulsion so prepared was found to be isotonic, clear, and translucent and was found to be stable for a considerable length of time.

Methodology

Stock solutions of 2.808 x 10-4 M PPIXS and 3.408 x 10-3 M ALA were prepared in Nanoemulsion solution. 0.40 mL aliquot of the PPIX stock solution were added to 10 5mL volumetric flasks. A measured volume of 2.0 mL of Nanoemulsion solution were added to each volumetric flask. The first flask contained no ALA and was diluted to the fiduciary mark with Nanoemulsion solution. Thereafter, aliquot volumes of the ALA sock solution were added to the rest of the flasks and the resulting mixture were carefully diluted the fiduciary mark with Nanoemulsion solution. The final concentrations were 2.246 x 10-4 M PPIX, and the concentration of ALA varied from 6.96 x 10-5 M to 6.96 x 10-4 M.

The fluorescence measurements were made by adding 3.0 mL of each solution to a 3.5-mL of a four-clear sided cuvette. The excitation was at 350 nm and the emission was observed at 695 nm. The instrument slit widths (excitation and emission) were kept constant at 5 nm.

Results and discussion

We show in Figure 2 the fluorescence spectra of PP IX without and with the quencher, Q, (5-Aminolevulinic acid-5-

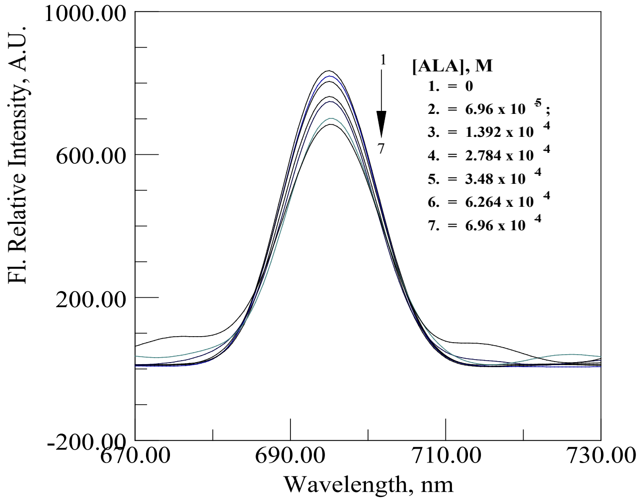


Figure 2 The fluorescence spectra of PPIX without and with different concentrations of ALA.

As can be seen, the fluorescence intensity of PP IX is decreased as the concentration of ALA increased

The Stern-Volmer equation (equation 1) is used to analyze the data obtained in Figure 2.

$$\frac{I^o}{I} = 1 + K_{sv}Q = 1 + k_q \tau Q \quad (1)$$

$$KSV = kq / \tau \quad (2)$$

In this equation I^o and I represent the fluorescence intensity of PP IX without and with quencher, respectively, and k_q is the bimolecular quenching rate constant. KSV is the Stern-Volmer quenching constant. τ and Q are, respectively, the fluorescence lifetime of PPIX and the concentration of ALA used as the quencher, Q.

A plot of $\frac{I^o}{I}$ versus Q gives a straight line as can be seen in Figure 3.

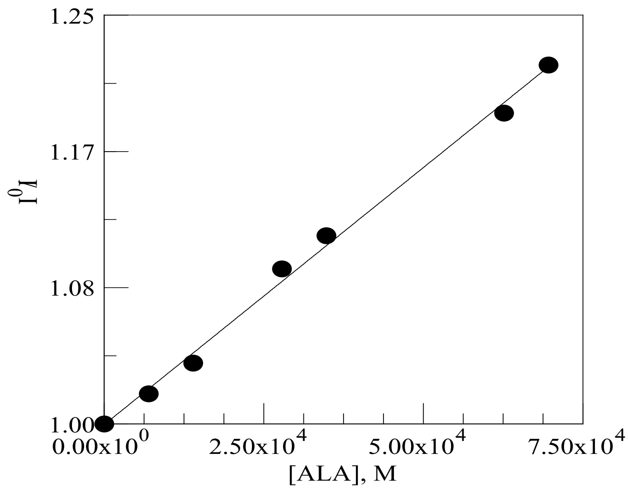


Figure 3 The KSV Plot of the PPIX-ALA Interaction.

From the slope of this plot, KSV was determined to be 314.44 and with the aid of the literature value of τ (15-18), kq was determined. A value of 2.86 x 1010/M-s was obtained. This value is consistent with a theoretically determined diffusion-controlled bimolecular reactions.[19]

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In other to determine the interaction constant and the ratio, n, of the molecules reacting, the equation due to Bai and his co-workers[20] shown in equation 3 was used.

“log(“ (I^o-I)/I)=”log”(K)+”nlog”([Q])

A linear plot was obtained as can be seen from Figure 4.

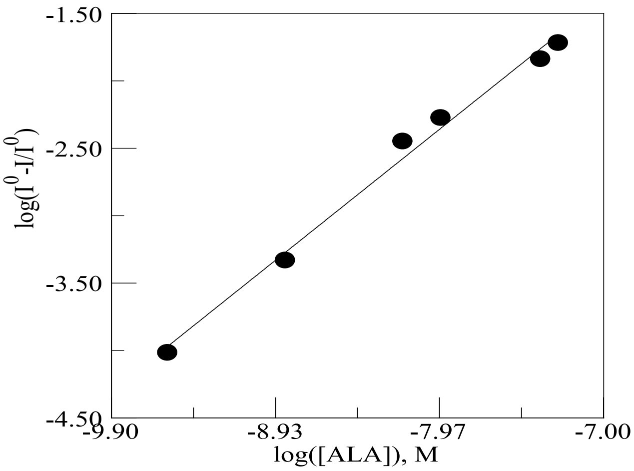

Figure 4 Plot of log(I°-I/I) versus log(Quencher).

Analysis using this equation gave Ka, the interaction constant of 4.48 x 105/mole. This value is approximately consistent with what was observed by other workers and the n is a 1:1 ratio.[21] The free energy, ΔG, of interaction was obtained using the relation of equation 3.

$$\Delta G = -RTlnK \qquad (3)$$

A free energy of the interaction was calculated as -32.24 kJ/mole indicating that the reaction is favorable and spontaneous as expected of all exergonic reactions we give in Table 2 the observed parameters of the reaction of PP IX and 5-aminolevulinic acid in Nanoemulsion.

Table 2 The Observed/Calculated parameters of the reaction Between PP IX and ALA in nano emulsion

Parameter	Value	Unit(s)
Stern-Volmer Quenching constant (KSV)	314.44	M-1
Bimolecular Quenching Constant (kq)	2.86 x 1010	M-1s-1
Interaction Constant (Ka)	4.48 x 105	mol-1
Free Energy of Interaction (ΔGa)	-32.24	kJ/mol
Interaction Ratio (n)	1:01	-

Conclusion

We have shown in this work that PPIX which is poorly soluble in water is freely soluble in Nanoemulsion. Steady-state fluorescence spectroscopy is used in this medium to study and to obtain relevant data regarding the interaction between two important biomolecules in PDT regimen, PPIX and ALA. It is further observed that the interaction is diffusion-controlled and quite exergonic. Consistent with the finds of other workers, the binding of ALA to PPIX is observed to be in the ration of 1:1 with a binding interaction constant, Ka, of 4.48 x 105/mol and free energy is interaction, Ga, of -32.24 kJ/mol.

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Conflicts of interest

The author hereby declares of having not conflict of interest in this article.

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