

The fluorescence study of the quenching of nanoemulsion by protoporphyrin IX (PPIX)

Abstract

Nanoemulsion is a unique and versatile fluid that solubilizes both ionic and non-ionic molecules. It has been used extensively for drug delivery. Nanoemulsion, prepared with water-oil-surfactant and co-surfactant, is fluorescent at or the near IR region of electromagnetic spectrum. However, when protoporphyrin (PPIX), a useful photosensitizer in PDT modality, and a poorly water-soluble compound, is introduced into the nanoemulsion medium, the observed fluorescence of the nanoemulsion is quenched. We have used the steady-state fluorescence technique to study the mechanism of this quenching. It is observed that the interaction between nanoemulsion and PPIX is 1:1 and the interaction constant, K_a , is about $2.37 \times 10^5/\text{mol}$ and the Free energy of interactions, G_a , is -30.66 kJ/mol .

Keywords: nanoemulsion, fluorescence, porphyrinix, complexation, binding constant, bimolecular quenching constant

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Abbreviations

CTAB, cetyltrimethyl ammonium bromide; ΔG_a , free energy of association; K_a , association constant; K_{SV} , stern-volmer constant; k_q , bimolecular quenching constant; NEM, nanoemulsion; PDT, Photodynamic therapy; PPIX, protoporphyrin IX; o/w, oil-in-water

Introduction

Nanoemulsion is a heterogeneous fluid system that is extensively used for drug delivery.¹⁻⁷ It is also used to solubilize both ionic and non-ionic molecules. It is also found useful in food industry⁸ It is a colloidal system made by dispersing oil in water with a dispersing agent, surfactant, and co surfactant, usually a short chain alcohol, in appropriate ratios. The dispersing agent used in this work is cetyltrimethylammonium bromide (CTAB). The composition of the nanoemulsion used in this work is listed in Table 1 Nano emulsion fluoresces intensely at or near the near-IR region³⁻⁹ of the electromagnetic spectrum – a region that is most useful for biological analysis. On the other hand, protoporphyrin, PPIX, is a poorly water-soluble¹⁰⁻¹⁵ photosensitizer but it is quite soluble in nanoemulsion. However, when PPIX is mixed with nanoemulsion the fluorescence intensity of the nanoemulsion is decreased. To the best knowledge of the author, there has not been any study in the association or complexation of nanoemulsion with PPIX. We have, therefore, used the steady-state fluorescence technique to study the observed mechanism of this quenching. Figure 1 shows the SEM image of the prepared nanoemulsion and the structure of PPIX.

Table 1 Composition of the prepared 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

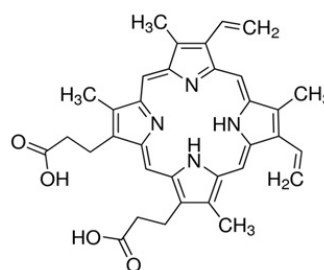
Experimental

Chemicals

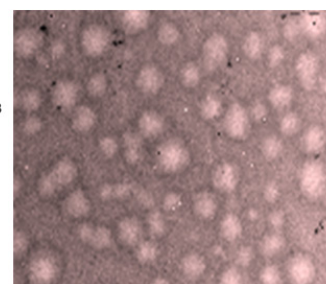
The entire chemical used (tetradecane (Oil), surfactant (CTAB), 1-pentanol) were of analytical reagent standard and were obtained from Acros Chemicals and used without further purification.

Instrument

The Fluorescence spectra were obtained using Perkin Elmer's Luminescence Spectrophotometer, model LS 50 B. All solutions were prepared using triply distilled deionized water from Photronix Reagent Grade water system.



Structure of PPIX



SEM Image of Nanoemulsion

Figure 1 Chemical structure of PPIX and SEM image of Nanoemulsion (NEM).

Methodology

All fluorescence data were obtained in a four-sided cuvette. The excitation wavelength was at 400 nm and the emission was observed at 795 nm. The instrument slit width (excitation and emission) were kept constant at 3.5 nm.

Preparation of nanoemulsion

The preparation of the nanoemulsion used in this work followed the literature methodology.¹⁴ 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 (oil) were added to the slurry dropwise 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. The nanoemulsion so prepared may be said to be oil in water (o/w) nanoemulsion. The chemical compositions used for preparation of the used nanoemulsion are shown in Table 1

Methodology

All solutions were made in the prepared nanoemulsion. Different aliquots were used in dissolving and diluting different amounts of PPIX in 5.0 mL volumetric flasks. Ten flasks were used and the first flask contained no PPIX and the rest contained PPIX concentration that varied from 6.45×10^{-7} M to 3.24×10^{-6} M PPIX.

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

We show in Figure 2 the fluorescence spectra of nanoemulsion with different concentration of PPIX As can be seen in this figure, the fluorescence intensity of nanoemulsion decreases with an increase in PPIX concentration. This observed phenomenon is consistent with molecular quenching experiments. We show in Figure 3 the ratio of the observed fluorescence intensity without, I_0 and with, I , of the PPIX concentration in accordance with the Stern-Vollmer equation:

$$I^0 / I = 1 + K_{SV} [Q] = 1 + k_q \tau_o [Q] \tag{1}$$

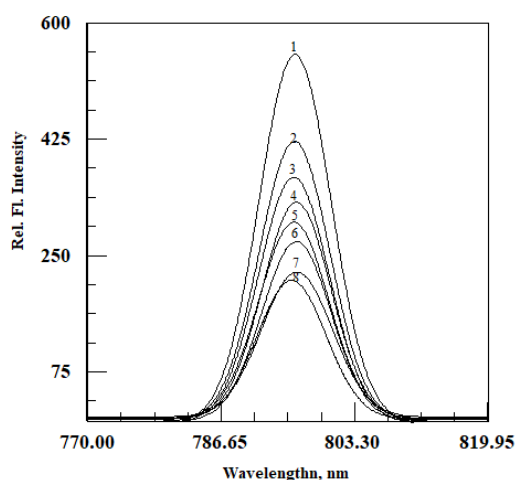


Figure 2 The Fluorescence spectra of Nanoemulsion with and without PPIX. 1. = 0 (see methodology in text), 2. = 6.45×10^{-7} , 3 = 9.073×10^{-7} , 4. = 1.1664×10^{-6} , 5. = 1.296×10^{-6} , 6. 1.944×10^{-6} , 7. = 2.8572×10^{-6} , 8. = 3.24×10^{-6}

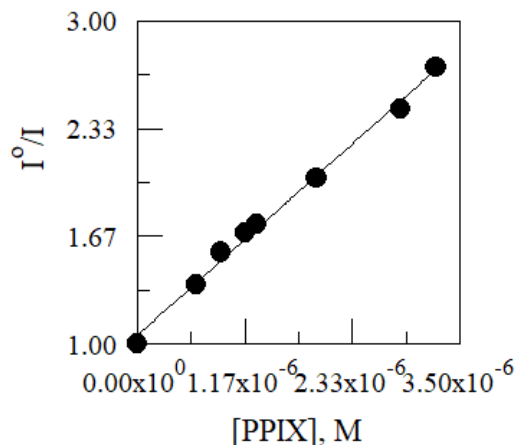


Figure 3 The stern-vollmer Plot from the data of Figure 2.

This plot is quite linear with a correlation coefficient of 0.998, implying that the observed data obeys the Stern-Volmer equation very well. In this equation, k_q and τ are the bimolecular quenching constant and the fluorescence lifetime of nanoemulsion without a quencher, respectively. K_{SV} and Q are, respectively, the Stern-Volmer constant the quencher concentration, where Q is the PPIX in this study.

From the relation in equation 1 above, $K_{SV} = k_q \tau_o$. We have approximated the lifetime, τ_o , of nanoemulsion to be 4.0 ns^{16-18} to obtain k_q value of $1.22 \times 10^{14} \text{ M}^{-1}\text{s}^{-1}$. This value is about five orders of magnitude larger than the k_q in diffusion-limited quenching of most biomolecules obtained in water which is about $2.0 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$. We then assume that the observed quenching of nanoemulsion by PPIX is by collision and subsequent ground-state complex formation. For this reason, use is made of the equation developed by Bai and his co-workers equation given in equation 2²⁴. This equation has been used successfully by other workers^{19,22-24} to further analyze and obtain some relevant data for this quenching experiment.

$$\text{Log}(I^0 - I / I) = \text{log}(K) + n \text{log}(Q) \tag{2}$$

In this equation, K and Q are the binding constant and quencher, respectively, n is the binding or complexation ratio. I and I^0 are the fluorescence intensity with and without quencher. When the obtained fluorescence data were plotted according to this equation, a linear curve is obtained as can be seen in Figure 4.

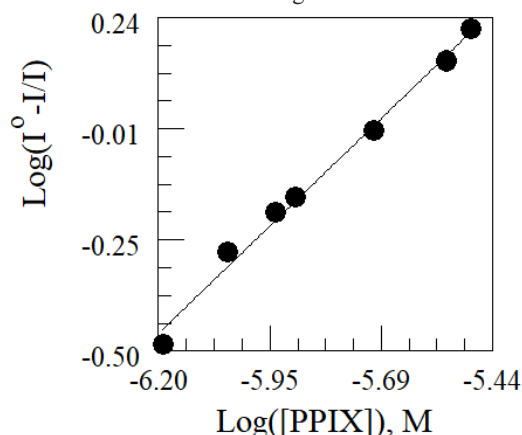


Figure 4 Plot of Fluorescence data according to Bai Equation.

From intercept and slope of the plot, the values of K and n were determined as $2.37 \times 10^5 / \text{mol}$ and $0.941 \approx 1$, respectively. The free energy of interaction, ΔG_a was calculated using the relation:

$$\Delta G = -RT \ln K. \text{ A value of } -30. \text{ kJ/mol}$$

We list in Table 2, the relevant values of the quenching reaction of nanoemulsion by PPIX.

Table 2 Parametric values obtained in the quenching of Nanoemulsion by PPIX

Parameter	Value	Unit
KSV (Stern-Volmer Quenching constant)	4.89×10^5	M^{-1}
k_q (bimolecular quenching rate constant)	1.22×10^{14}	$\text{M}^{-1}\text{s}^{-1}$
K (Binding Constant)	2.37×10^5	mole^{-1}
n	1	
ΔG	-30.66	kJ/mole

Conclusion

We have shown in this work that nanoemulsion is fluorescent and the fluorescence intensity is quenched by PPIX. The observed

quenching is static, collisional and complexation with a binding ration of 1:1. The interaction constant was determined as $2.37 \times 10^5/\text{mol}$. This interaction is very exergonic and spontaneous with a free energy of -30.66 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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