

Nanoemulsion mediated interaction of Hemoglobin and Curcumin

Abstract

Steady-state fluorescence spectroscopy was used to study the interaction between bioactive molecules, hemoglobin and curcumin in a nanoemulsion medium. It was observed that these molecules undergo a 1:1 binding with a favorable interaction. The free energy of interaction, ΔG° was calculated to be -25.18 kJ/mol and a binding constant of $2.593 \times 10^4 \text{ M}^{-1}$.

Keywords: hemoglobin, curcumin, binding ratio, free energy, nanoemulsion, bioactive, surfactant, oil

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Introduction

Nanoemulsion is a heterogeneous system of two immiscible liquids, for example, water and oil and dispersed and stabilized by a surface-active agent (surfactant) and a co-surfactant, usually a short chain alcohol. When these are mixed, and vigorously stirred and sonicated, a clear homogenous and isotonic liquid that is translucent is obtained. The mean droplet size of nanoemulsion is known to be between 50 and 200 nm.¹⁻⁴ A typical structure of nanoemulsion and its SEM image is shown in Figure 1.

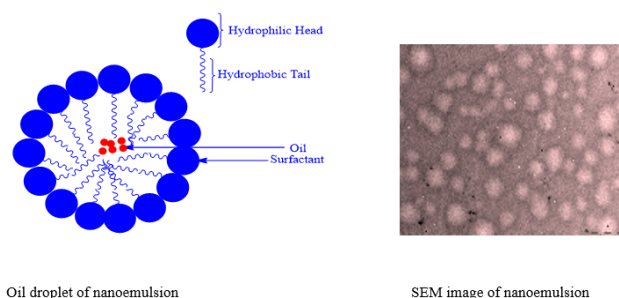


Figure 1 Oil droplet and SEM image of nanoemulsion.

Nanoemulsion has unique properties. It can solubilize both hydrophobic and lipophilic biomolecules.^{5,6} It is thermodynamically stable⁷ and it is known to be non-toxic.^{2,8,9} For these reasons it has been used as a drug delivery medium,¹⁰⁻¹⁴ in pharmacology formulations.¹⁵ In dermatology¹⁶ and in the food and cosmetic industries.¹⁷⁻²⁰ This is by no means exhaustive references for the formulation, uses and applications of nanoemulsion. However, the cited articles here and the reviews are the most recent citations in the literature for this system. As a result of its numerous uses and applications, nanoemulsion is chosen as medium to solubilize and study the interaction of hemoglobin and curcumin. These biomolecules are poorly soluble in water but are liberally soluble nanoemulsion. Hemoglobin (Hb) is an iron-containing biomolecule that contains four heme groups. A typical heme that contains iron and the 3-D structure of hemoglobin are shown in Figure 2.

Among the various activities and physiological uses of hemoglobin, the most important is its function as a blood transportation of molecular oxygen and carbon dioxide from the respiratory organ to other tissues of the body. A thorough review of the structure and relevant biological functions of hemoglobin is abundant in the literature.²¹⁻²⁷

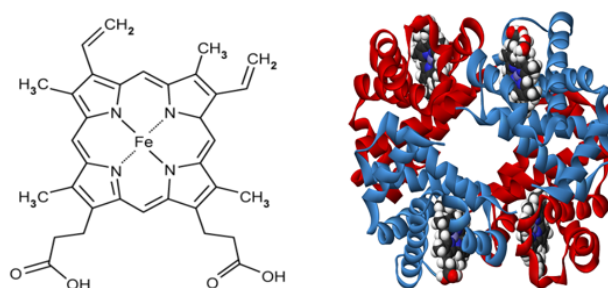


Figure 2 The Chemical structure of heme and the 3-D structure of hemoglobin

Similarly, curcumin (CUR) is a very vital bioactive prodrug. It is a polyphenolic phytochemical that is derived from *Curcuma Longa Lin* with the chemical structure shown in Figure 3.

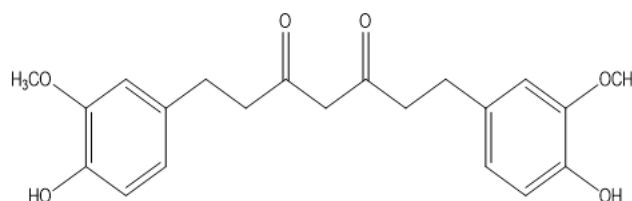


Figure 3 Chemical structure of curcumin.

Recent review articles detail the biological, physiological and medicinal uses of CUR.²⁸⁻³¹ The interaction and complexation of Hb and CUR has been studied in the aqueous solution. However, in this medium, it is mentioned of the poor solubility of CUR in water which limits its bioavailability and bioactivity which leads to some extent some ambiguity and certainty in the interpretation of the efficacy of this prodrug which is lipophilic. We have therefore studied this interaction in a nanoemulsion system in which these compounds (Hb and CUR) are soluble.

Experimental

Materials

Reagent grade curcumin, cetyltrimethylammonium bromide (CTAB) (surfactant), 1-pentanol (co-surfactant), and Tetradecane (Oil) were obtained from Acros Chemical. Lyophilized horse hemoglobin was purchased from Sigma Chemical Co. All these chemicals were used as obtained without further purification. Water used in all experiments in this work is triply distilled and deionized water from Photronix Reagent Water system.

Nanoemulsion preparation

In addition to the numerous articles and reviews in the literature and the reviews cited above in the introduction the preparation used in this work is as follows: 174 mL of water into which the 12.0 g of CTAB were added. After mixing and magnetically stirring, the solution a slurry of the surfactant and water was observed. To this slurry solution, 18.25 mL of oil (tetradecane) was added slowly with continuous stirring. The co-surfactant (1-pentanol) was then added dropwise until 31.8 mL have been added. At this juncture the entire solution turned clear and translucent. In the prepared nanoemulsion, the water is 76 %, Surfactant 5 %, co-surfactant 13 % and 6 % Oil. This system was stable for a considerable length of time.

The chemical composition used in the preparation of the used nanoemulsion are listed in Table 1.

Table 1 The composition of the prepared Nanoemulsion.

Component	Wt., g	Percentage, %	Volume, mL
Water	174.0	76.0	174.0
CTAB (Surfactant)	12.0	5.0	12.63
Oil (Tetradecane)	14.0	6.0	18.25
1-Pentanol (Co-Surfactant)	29.9	13.0	31.8

Table 2 Lists the observed/calculated parameters obtained in this work.

Parameter	Value	Unit
K_q	4.41×10^{13}	M^{-1}
K	2.593×10^4	M^{-1}
ΔG°	-25.18	kJ/mol
n (binding ratio)	1:1	

Methodology

A stock solution of 1.35×10^{-5} Hemoglobin and 3.7825×10^{-4} M Curcumin were prepared in 25.0 mL and 10 mL, volumetric flask, respectively. 7 5.0 mL volumetric flasks were thoroughly cleaned for solution preparation. Into all the flasks 3.0 mL nanoemulsion and 1.0 mL of hemoglobin were added. In flasks 2 through 7 curcumin volume ranges from 0.2, 0.3, 0.4, 0.5, 0.7 mL, respectively. The solutions were then diluted to the fiduciary mark with nanoemulsion. The overall concentration of hemoglobin is 2.27×10^{-5} M and that of curcumin varied from 1.515×10^{-5} to 6.06×10^{-5} M.

Instrumentation

The instrument used throughout this work is Perkin Elmer's Luminescence Spectrophotometer model LS 50B. The excitation wavelength was kept constant at 325.0 nm, and the emission wavelength was observed at 647 nm. The slit width of the excitation and emission was constant at 5.0 nm, respectively. The fluorescence measurement was done at room temperature in a 4-sided quartz cuvette.

Results and discussion

We show in Figure 4 the observed fluorescence spectra of hemoglobin with and without curcumin the quencher, Q.

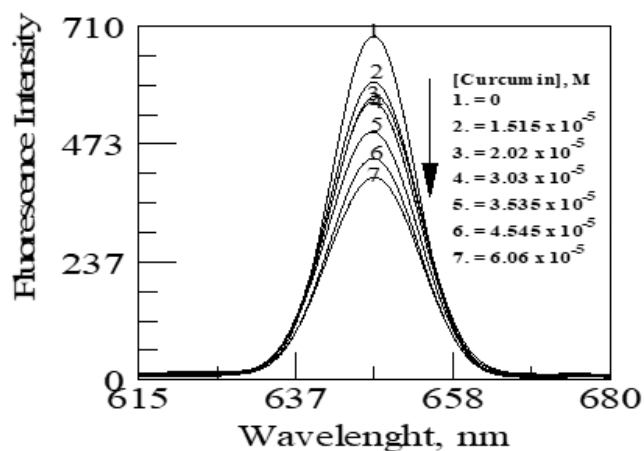


Figure 4 The Fluorescence Spectra of Hemoglobin with and without and with different concentration of curcumin.

As can be seen, the fluorescence of hemoglobin decreased as the concentration of curcumin increased. The intensity of spectra, when plotted with the corresponding concentration of curcumin, the quencher, Q, obeys the Stern-Volmer equation given in equation 1.

$$I^0/I = 1 + K_{SV}Q = 1 + k_q\tau^0Q \quad (1)$$

In this equation, I^0 and I are the fluorescence intensity of Hemoglobin without and with curcumin, respectively K_{SV} is the Stern-Volmer constant and k_q and τ^0 are the bimolecular quenching rate constant and the fluorescence lifetime of hemoglobin, without any quencher respectively. Equation 1 was used to plot the spectra of hemoglobin and curcumin. This is shown in Figure 5.

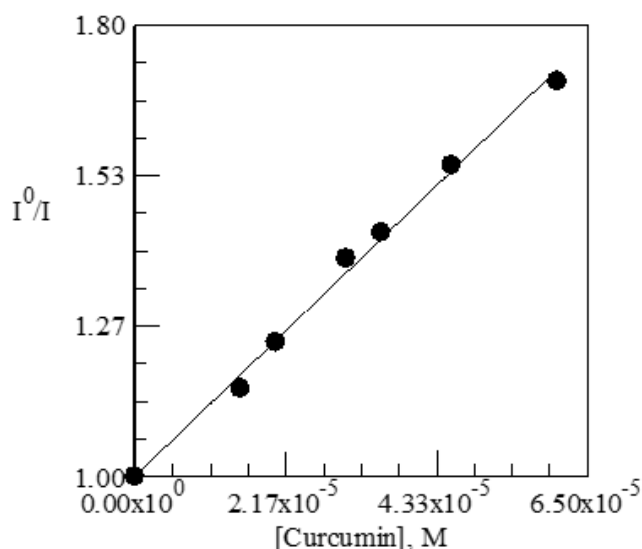


Figure 5 The Stern-Volmer plot in accordance with equation 1.

However, the interaction reaction between the reacting molecules was analyzed using Bai et al.³² formalism shown in equation 2.

$$\text{Log}[(I^0-I)/I] = \text{Log}(K) + n\text{Log}(Q) \quad (2)$$

Figure 6 is a plot obtained in accordance with equation 2:

In this equation, I^0 and I are the fluorescence intensity of Hemoglobin without and with Curcumin, respectively. K is the Association or Binding constant, and n is the binding ratio between the reacting molecules. As can be seen this plot, is quite linear and the extracted K value is $2.59 \times 10^4/\text{M}$ and n is $1.07 \approx 1.0$. This value of n is good agreement with the value obtained by other workers.³²

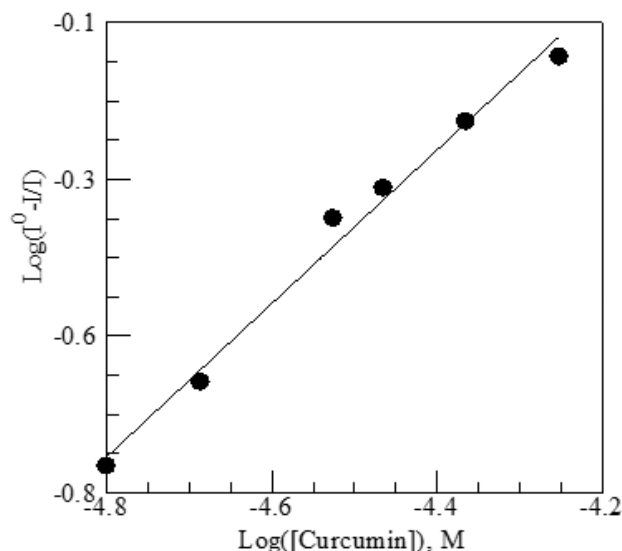


Figure 6 Log fluoresce intensity ration vs curcumin concentration.

With the K value of $2.593 \times 10^4/\text{M}$ the free energy of interaction was calculated using equation 3.

$$\Delta G^{\circ} = -RT \ln K \quad (3)$$

The value of ΔG° thus calculated is -25.18 kJ/mol . This very high exergonic free energy implies that the reaction between hemoglobin and curcumin in nanoemulsion is very favorable.

Conclusion

It has been shown in this work that hemoglobin interacts with curcumin favorably with high exergonic energy in nanoemulsion. The calculated binding or interaction ratio between these bioactive molecules is calculated to be 1:1 in good agreement with literature value.

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Conflicts of interest

Author declare there is no conflicts of interest.

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