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The study of the quenching mechanism of hemoglobin by curcumin in nanoemulsion

Abstract

Steady state fluorescence spectroscopy was used to study the mechanism of electron transfer reaction between hemoglobin and curcumin (biologically active molecules) in nanoemulsion. Nanoemulsion is a thermodynamically stable heterogeneous system made-up of water, oil and a dispersing agent, usually a surfactant and a cosurfactant in appropriate ratios. The reaction is postulated as activation controlled and the requisite energies: Gibb's free energy, ΔG^{o}, (1.317 eV), the solvent reorganization energy, λ, (0.76 eV), the activation energy, $\Delta G^{\neq}$, (1.02 eV) and its attendant first order rate constant, k_{act}, (1.68 x 10^{11}/s) were determined. They were used to formulate a plausible electron transfer mechanism.

Keywords: nanoemulsion, activation-controlled, quenching, bimolecular, heterogeneous, electron-transfer

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Introduction

Nanoemulsion can be oil-in-water (o/w) or water-in-oil (w/o), a dispersing agent (surfactant) and a co-surfactant usually, a small chain alcohol. Nanoemulsion is thermodynamically stable, and it is known to solubilize both ionic and non-ionic molecules, and it is known not to be toxic. For this reason, it has been used for drug delivery, in pharmaceutical preparations and in dermatology. The synthesis, properties and functions of this unique system have been reviewed by numerous authors.[1–9] It is used in this work as a medium to study the mechanism of electron transfer reaction between hemoglobin and curcumin. Hemoglobin is an iron-containing protein of vital biological importance. It consists of four heme molecules, and it is known as an oxygen carrier from the respiratory organs to other tissues in the body. A succinct review of hemoglobin and its relevance in oxygen transport and other biological uses/activities is abundant in the literature.[10–13]

On the other hand, curcumin, a yellow pigmented phytochemical isolated from the rhizome of *Curcuma Longa L.* has been used in pharmacy and in medicine. In fact, it is used as a pro-drug. Among its multifarious uses are anticancer, anti-inflammation and antioxidant. The literature is replete of the uses and applications of this pro-drug.[14–18] The chemical structure of curcumin, the heme moiety that make-up the hemoglobin, and the SEM image of nanoemulsion is shown in Figure 1. To the author's knowledge, the mechanism, and the attendant energies of reaction between hemoglobin and curcumin in this unique medium (nanoemulsion) has not been reported, hence this study. Therefore, in this work, we used the steady state fluorometric method to obtain the relevant data that enables the formulation of the plausible reaction mechanism of these biologically important molecules in nanoemulsion.

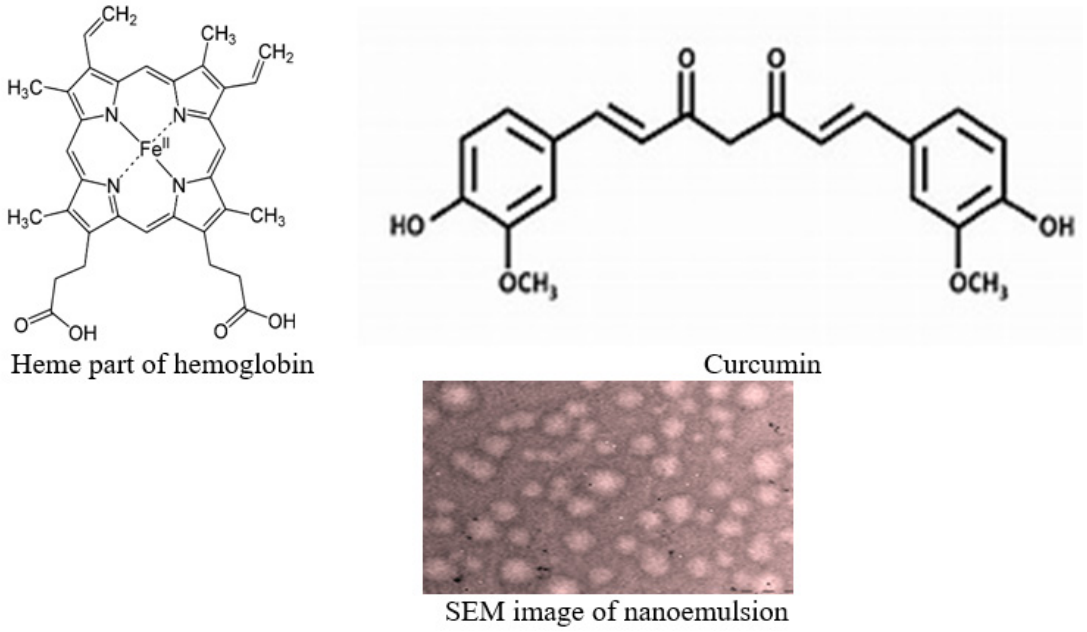

Figure 1 The chemical structure of Hem, curcumin and the SEM of nanoemulsion.

Experimental

Materials

Analytical reagent grade of curcumin, cetyltrimethyl ammonium bromide (CTAB) (surfactant), 1-pentanol as co-surfactant and tetradecane as oil, were obtained from Acros Chemicals. Lyophilized Horse Hemoglobin was obtained from Sigma chemical Co. These chemicals were used as obtained without further purification.

Instrument

Perkin Elmer luminescent Spectrophotometer, model LS 50B was used to obtain all fluorescence spectra for this work.

Preparation of nanoemulsion

Table 1 lists the chemical components used in the preparation of nanoemulsion that is used in this work. Briefly, 12.0 g of CTAB were added to 174.0 mL of distilled, deionized water, and vigorously stirred using magnetic stirrer. Then 18.25 mL of the oil (tetradecane) were gradually added to the water-surfactant mixture. While still stirring, a co-surfactant (1-pentanol) was added dropwise until 31.8 mL. It was observed that the resulting solution was clear and translucent. This solution was taken and sonicated for about 12.0 minutes and then mechanically stirred again for another 10.0 minutes. This clear and translucent solution is stored and used as needed. It was observed that the prepared nanoemulsion was stable for a considerable length of time.

Table 1 Lists all the chemical components used in the preparation of the nanoemulsion used in this work

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

Methodology

1.135 x 10^{-4} M hemoglobin and 3.7875 x 10^{-4} M curcumin stock solutions were prepared in 25 mL and 10 mL, volumetric flasks, respectively, using the prepared nanoemulsion. From these, a volume of 3.0 mL of nanoemulsion solution were added to 7 5.0 mL volumetric flasks. Into the first flask, 1 mL of hemoglobin solution was added and swilled for a thorough mixing. Thereafter, aliquot volumes of 0.2, 0.3, 0.4. 0.5 0. 0.6 and 0.8 mL of curcumin solution were gently and carefully added to flasks 2-7. These solutions were diluted to the fiduciary mark of the flasks with the nanoemulsion solution. The final concentration of hemoglobin was 2.27 x 10^{-5} M and that of curcumin varied from 1.515 x 10^{-5} M to 6.06 x 10^{-5} M. The fluorescence measurement of these solutions was made in a 4-sided quarts cuvette. The excitation wavelength was kept constant at 325 nm and the emission wavelength was observed at 647 nm.

Results and discussion

Shown in Figure 2 is the fluorescence spectra of hemoglobin with and without the quencher, curcumin. It can be seen that the fluorescence intensity of hemoglobin decreases as the quencher concentration is increased. The Stern-Volmer equation (equation 1) was used to analyze the data obtained from Figure 2.

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

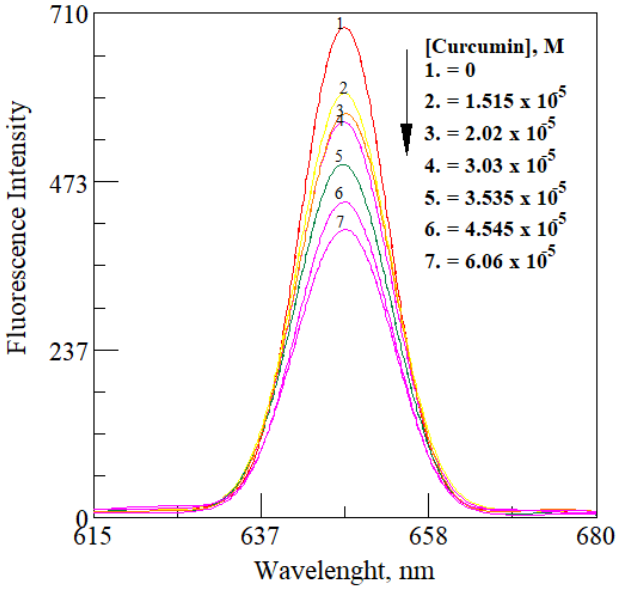

Figure 2 The fluorescent spectra of hemoglobin without and with difference concentrations of curcumin.

In these equations, I^o and I are the fluorescence intensity of hemoglobin in the absence of a quencher and in the presence of quencher, respectively. K_{sv} is the Stern-Volmer constant and k_q and τ^o are the bimolecular quenching rate constant and the fluorescence lifetime without any quencher, respectively. Figure 3 shows the Stern-Volmer plot of the ratio of the fluorescence Intensity observed at different concentrations of the curcumin concentration as per equation 1. This plot is quite linear with a slope of 11907.1216 M^{-1} which is taken as the Stern-Volmer constant, K_{SV}. With this, the k_q was calculated using equation 1. The literature value of 270.0 ps[19,20] was used for τ^o and k_q thus calculate is 4.41 x 10^{13}/M-s. This value is clearly three orders of magnitude higher than the value of diffusion-controlled reaction values ≈ 10^{10}/M-s.[21,22] This implies a ground-state complexation, and the quenching is static. Therefore, in other to effect electron transfer, this complex requires activation and hence the quenching can be treated as activation-controlled, which is assumed in this work. However, in other to fully understand this activation-controlled mechanism, it is necessary to determine the solvent reorganization energy, λ, the free energy change, ΔG^o, the activation energy, $G^{\pm}$ and the attendant activation rate constant, k_{eT}. We therefore proceed in the determination of these parameters.

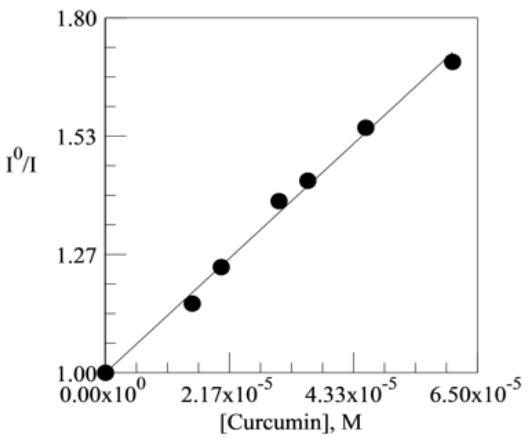

Figure 3 Plot of Io/I versus [curcumin].

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Determination of free energy of reaction, ΔG^o

In other to determine the free energy change of the reaction, we used the Realm-Weller relation[23,24] as given in equation 2.

$$\Delta G^{\circ} = E_{1/2}^{Ox} - E_{1/2}^{Red} - E_{o-o} - e^2 / 4\pi \varepsilon_o \varepsilon_s R_q \quad (2)$$

In this equation, $E_{1/2}^{Ox}$ and $E_{1/2}^{Red}$ are the electrochemical half-wave potential of the oxidized and the reduced reactants in the solution, which were obtained from literature, hemoglobin[4,25–30] and curcumin,[31] respectively, E_{o-o} is the zero-zero excitation energy of hemoglobin which were estimated using the relation given,[32,33] and R_q was approximated as the sum of the radii of the reactants, that is $R_A + R_D$ and were obtained from the literature.[34,35] e is electronic charge and ϵ_o and ϵ_s are the permittivity of vacuum and the dielectric constant of the medium (nanoemulsion),22,59 respectively.[36] The value of ΔG^o so determined using equation 2 is -1.317 eV. This value is in close agreement to an outer-sphere electron transfer reaction.[37–39]

Determination of solvent reorganization energy, λ_s

The Marcus dielectric continuum theory given in equation 3 is used to determine the solvent reorganization energy, λ, in nanoemulsion medium.

$$\lambda_s = \frac{e^2}{4\pi\varepsilon_0}\left(\frac{1}{2R_D}+\frac{1}{2R_A}-\frac{1}{R}\right)\left(\frac{1}{D_{op}}-\left(\frac{1}{D_S}\right)\right) \quad (3)$$

In this equation, R_A and R_D are the radii of the reactants, donor and acceptor, respectively, and R is the sum of the radii of the reactants. $D_{op} \approx$ the square of the refractive index, n, and D_s is the static dielectric constant. The values of these parameters are given in reference 36. When the appropriate values of the variables in equation 3 are inserted, the calculated λ is 0.76 eV. This value is in close agreement with values obtained by other workers.[40–44]

Determination of activation energy, ΔG±

We use the Marcus theory[40] to evaluate the activation energy of this reaction in nanoemulsion by using equation 4.

$$\Delta G^{\pm} = \left(\lambda_s + \Delta G^o\right)^2 \quad 4\lambda_s \quad (4)$$

With the values of ΔG^o and λ_s thus far calculated $\Delta G^{\pm}$ is then calculated. A value of 1.02 eV is obtained.

In other to calculate the rate constant for this activation-controlled reaction, the rate constant, k, taking into consideration that k_{act} is used interchangeably with k_{ET}.[44–49] We, therefore, use equation 5 to estimate the rate constant of the observed activation-controlled reaction of the system under study.

$$k_{act} = Ae\frac{-\Delta G}{k_B T} \quad (5)$$

In this equation A (the pre-exponential factor) is a term that include the equilibrium constant of the formation of the complex of the reactants, the electronic transmission coefficient, $k_{el,}$ and the frequency factor, ν_n. For an adiabatic reaction, as in the case in this study, k_{el} is approximated as $_1$. The value of ν_n has been estimated as 1 x 10^{13}/s.[49–52] The rate constant for this reaction is therefore, given in equation 6.

$$k = k_{el}\nu_n e\frac{-\Delta G}{k_B T} \quad (6)$$

With the values k_{el} and ν_n given above, equation 6 is similar to equation 5 and thus the value of the first order activation-controlled in nanoemulsion between hemoglobin and curcumin is calculated to be 1.68 x 10^{11}/s. The overall values of the parameter in this reaction are tabulated in Table 2. With the parameters thus obtained including those from the literature we postulate the following mechanism for the activation-controlled electron transfer in the hemoglobin-curcumin complex in nanoemulsion.

Table 2 The observed/calculated parameters of the ET reaction of hemoglobin and curcumin in nanoemulsion

Parameter	Value	Unit
k_q, Bimolecular quenching rate constant	11907.12	M^{-1}
ΔG° , Free energy of reaction	-1.317	eV
λ_s, Solvent reorganization energy	0.76	eV
ΔG±, Free energy of activation	0.102	eV
k_{act}, activation rate constant	1.68 x 1011	s-1

Conclusion

It has been shown in this work that the fluorescence of the hemoglobin-curcumin system is observed to be not only static but complexation reaction as the bimolecular quenching rate constant, k_q, obtained indicates This was explained by using the activated-controlled theory. The requisite energies obtained were used to formulate a plausible reaction mechanism for the hemoglobin-Curcumin complex solubilized in nano emulsion system. Furthermore, the observed quenching mechanism will enable an accurate prediction of the function of hemoglobin in the presence of curcumin. This is moreso since the observed fluorescence intensity of hemoglobin decrease with an increase in curcumin concentration. Also, both hemoglobin and curcumin are very active biomolecules and, an accurate prediction of the mechanism of their action becomes critical. This find also will generate further research work in chemosensory research field for detecting blood hemoglobin in a given solution of nano emulsion solution containing curcumin and its analogues.

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Conflicts of interest

The author declares that there is no conflicts of interest.

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