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Mediterranean Journal of Chemistry **2018**, 6(6), 68-74

Interaction of curcumin with berberine hydrochloride in Nanoemulsion

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Abstract: Curcumin, a phytochemical, has various pharmacological uses so also is berberine hydrochloride, a phytochemical alkaloid. These chemicals are extensively used in many bodily ailments, ranging from cancer, obesity and attendant diabetes including glaucoma to anti-inflammation. However, these compounds are quite insoluble in an aqueous system, which limits their bioavailability but they are liberally soluble in nanoemulsion. Nanoemulsion is a nano-sized oil droplet that is dispersed in water using a surface-active agent (surfactant), oil and a short chain alcohol. An investigation of the interaction between these pharmacologically important compounds was carried out in this medium using steady-state fluorescence and UV-Vis spectroscopic techniques. The data obtained showed that this interaction led to the quenching of curcumin fluorescence by berberine hydrochloride with a bi-molecular quenching rate constant of 2.839 x 10^{13}/M-s and an interaction constant of 3.63 x 110^{3} in a 1:1 molar complexation ratio. Using the Forster mechanism, the estimated Forster radius for this interaction (R_o) is estimated to be about 27.70 Å and the interaction distance (r) between these reacting molecules is calculated to be about 31.40 Å.

Keywords: Phytochemical, Curcumin, Berberine hydrochloride, nanoemulsion, surfactant, bi-molecular, quenching rate constant, complexation, Forster radius.

Introduction

Curcumin,1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadine-3,5-dione is a yellow pigmented phytochemical isolated from the rhizome of *curcuma longa* L. Curcumin exists in two forms, The Diketone form and the keto-enol forms. These structures are shown in Fig. 1.

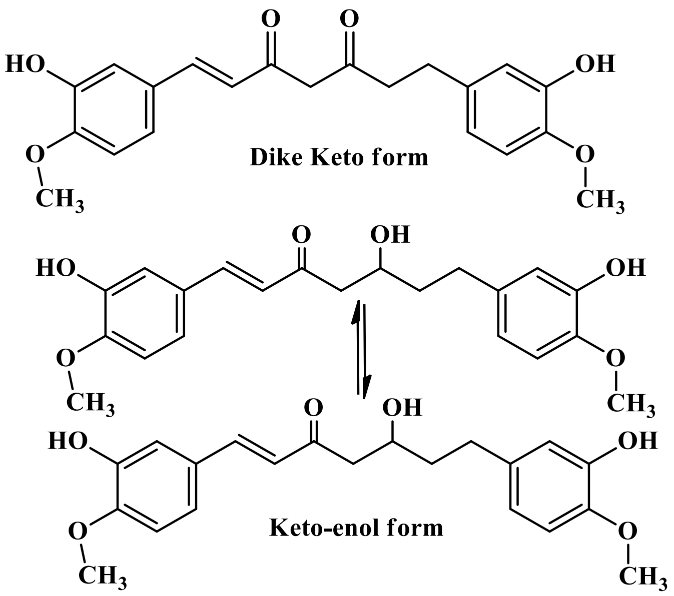

Figure 1. Chemical Structure of Curcumin

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DOI: http://dx.doi.org/10.13171/mjc71/01805211015-iwunze

Received April 8, 2018
Accepted, April 18, 2018
Published May 21, 2018

It is known to possess numerous pharmacological properties that include anti-oxidative activity [1-7] and chemo- and therapeutic activity [8-14]. A more recent mini-review of its medicinal chemistry has been given [15]. On the other hand, berberine hydrochloride (BHCl) is a quaternary ammonium salt. It is an alkaloid usually obtained in such plants as *berberis vulgaris* and *berberis arista*, among others. Its chemical structure is shown in Fig. 2.

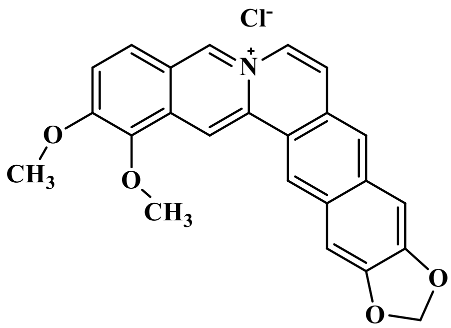

Figure 2. Chemical Structure of Berberine Hydrochloride

The internet and literature studies are replete with the use of this molecule for both pharmacological and application to ameliorate several human ailments that range from cancer, diabetes, obesity and attendant glaucoma [16-21].

Although these compounds are quite useful in ameliorating many human ailments, they are insoluble in an aqueous system, which limits their bioavailability, but is found to be liberally soluble in nanoemulsion – a system of nano-sized oil droplets dispersed in water with a surfactant and a co-surfactant, usually a short chain alcohol. This medium is therefore used to solubilize these water-insoluble compounds which enable to study of their interaction. The SEM image of the prepared nanoemulsion is shown in Fig. 3.

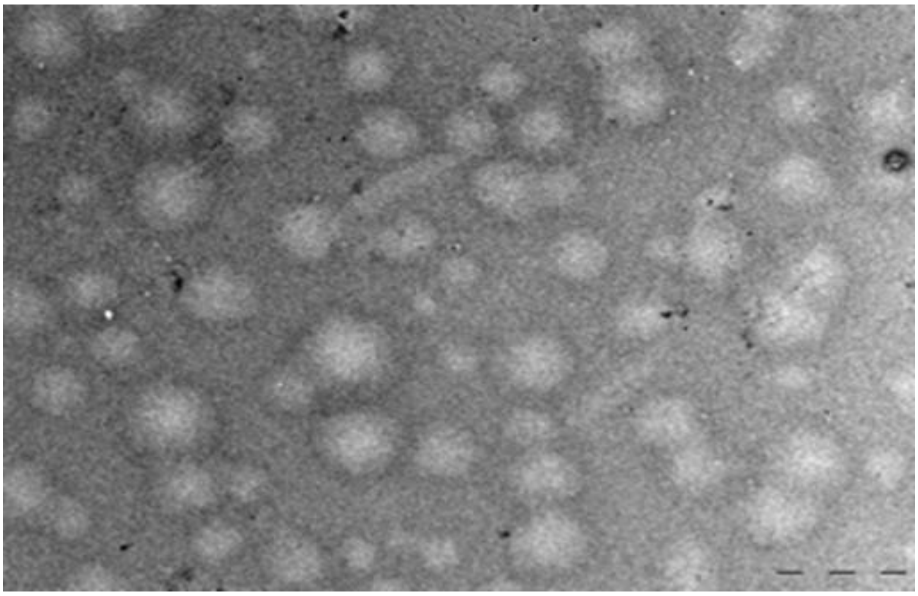

Figure 3. The SEM Image of Nanoemulsion

Experimental

Chemical

Analytical reagent grade tetradecane, 1-pentanol of 99 % purity, cetyltrimethylammonium bromide (CTAB) as surfactant and curcumin were obtained from Across Chemicals. All chemicals were used as received.

Instrumentation

The fluorescence spectra used in this work were obtained from Perkin Elmer's luminescence spectrophotometer, model LS 50B and the absorptiometric spectra were obtained using Cary spectrophotometer, model 1E supplied by Varian Analytical Instrument Co.

Methodology

All the fluorescence spectra were obtained in a four-sided 1-cm cuvette. The excitation and emission slit widths were kept constant at 5.0 nm. The fluorescence spectra of curcumin and those of the complex solutions were excited at 382 nm and the emission was observed at 514 nm.

The absorbance of the curcumin and berberine hydrochloride was obtained in a two-clear sided cuvette of 1.0 cm.

The used solutions were prepared by introducing 1.0 mL of curcumin stock solution (1.74×10^{-4} M) to 10 5.0 mL volumetric flasks. Into the first flask, a volume of 0.4 mL of berberine hydrochloride was added and thereafter 0.2 mL increments of this solution were added to the rest of the flasks. These were then diluted to the fiduciary mark of the 5.0 ml volumetric flask with the nanoemulsion solution. The concentration of the solution so prepared varied from 0 to 1.032×10^{-4} M BHCl and the curcumin concentration was kept constant at 3.48×10^{-5} M.

All experiments were performed at room temperature, 25 ± 0.2 °C.

Nanoemulsion Preparation

Appropriate weight of CTAB and a measured volume of water were mixed together. Into this mixture, a known volume of n-tetradecane as oil was added with vigorous stirring. A calculated volume of co-surfactant (1-pentanol) was added drop-wise with vigorous mechanical stirring for about five minutes or until the mixture become clear. The resulting solution is then transferred to the ultrasonic bath where it was sonicated for an additional 7 – 10 more minutes. The solution so prepared was isotonic, clear and translucent. This solution was stable for a considerable length of time. We show in Table 1 the composition of the prepared nanoemulsion.

Table 1. The Composition of the Prepared Nanoemulsion.

Component	Weight, g	Volume, mL	Percentage, %
Water	174.0	174.0	76.0
CTAB (Surfactant)	12 0	12.63	5.0
Oil (1-tetradecane)	14.0	18.25	6.0
Co-surfactant (1-pentanol)	29.90	31.80	13.0

Results and Discussion

BHCl is very weakly fluorescent in aqueous solution [22-24]. This is also our observation of this molecule in nanoemulsion. On the other hand, curcumin, although a solvent dependent fluorophore [25-29] exhibits a remarkable fluorescence in nanoemulsion. Fig. 4 shows the relative fluorescence intensity of these two molecules in nanoemulsion.

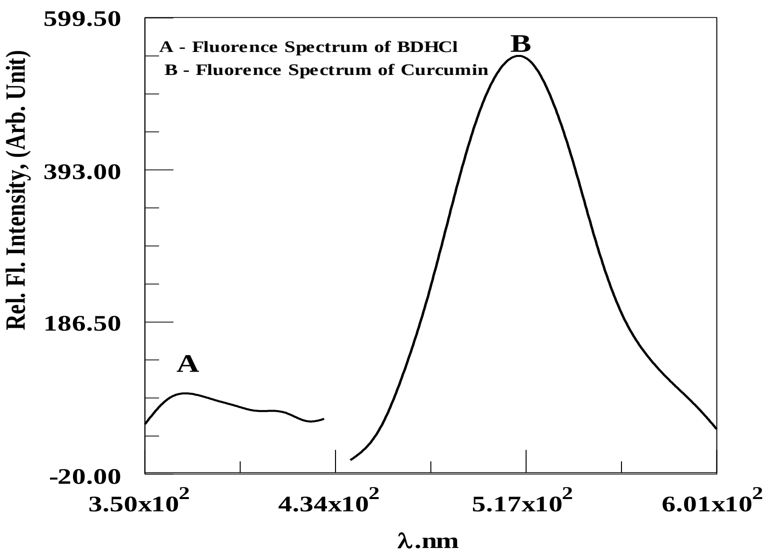

Figure 4. Comparison of the emission spectra of BHCl and Curcumin

However, it is observed that BHCl quenches the fluorescence of curcumin upon interaction in nanoemulsion solution as can be seen in Fig. 5.

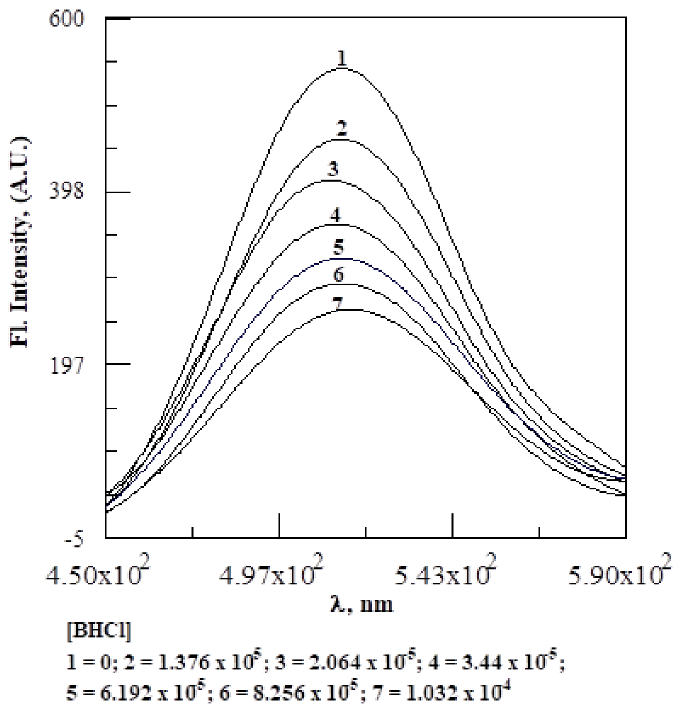

Figure 5. Fluorescence spectra of Curcumin-BHCI complex

It can be seen in this figure that the fluorescence of curcumin decreases with an increase in BHCl concentration. The data from this figure was analyzed using the Stern-Volmer equation shown in equation 1.

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

In this equation, k_q, τ_o and [Q] are the bi-molecular quenching rate constant, the fluorescence lifetime of curcumin and the quencher, BHCl, respectively, and the K_{SV} is the Stern-Volmer constant. A τ_o value of 347 ps was taken from the literature [30, 31] and it was used for all subsequent calculations. As per equation 1, a plot of I^0/I versus the quencher concentration gave a fairly good linear curve as can be seen in Fig. 6.

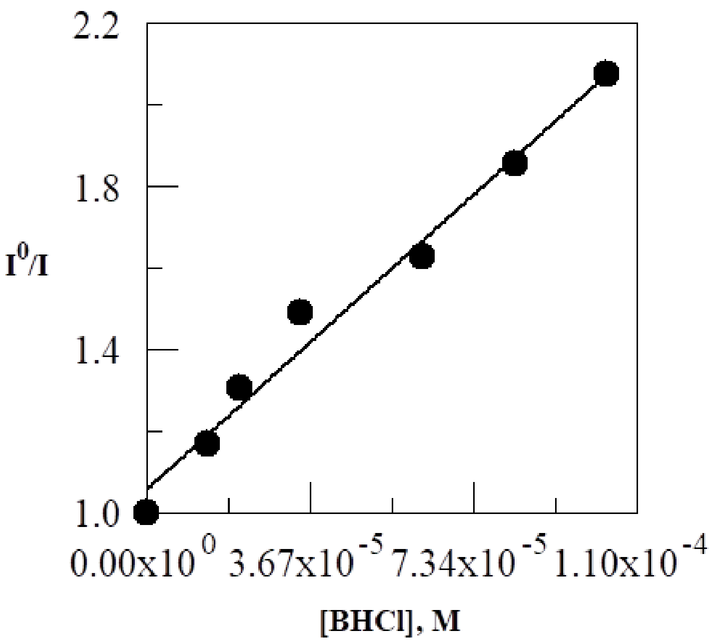

Figure 6. Stern-Volmer Plot of the quenching of the Curcumin Fluorescence by BHCI.

The slope of this plot was used to obtain a value of 2.84 x 10^{13}/M-s for kq.

Since the objective of this work is to characterize the interaction of curcumin and BHCl in nanoemulsion an interaction constant was determined using equation 2 that is due to Feng and his co-workers [32].

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

K and n are the interaction constant and binding ratio between BHCl and Curcumin, respectively. Plotting the fluorescence data in accordance with equation 2 gave a linear curve as can be seen in Fig. 7.

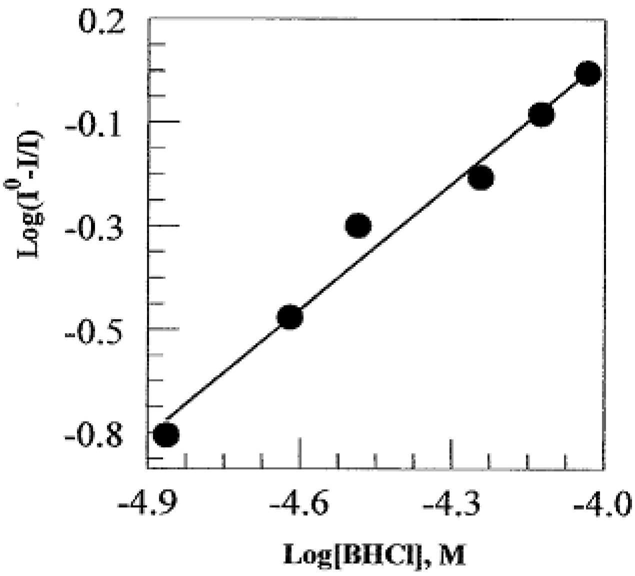

Figure 7. Plot of log(I^0-I/I) versus log([BHCl]

This plot gave an intercept of log 3.56 and a slope of 0.9. This indicates a binding constant of 3.63 x 10^3 and a binding ratio of approximately 1:1. With the calculated K value, the free energy of interaction is calculated using equation 3.

$$\Delta G = -RT\ln K \quad (3)$$

A value of -20.31 kJ/mol was obtained. This very exergonic free energy indicates a favorable and spontaneous interaction between these two molecules and possible resonance energy transfer between them. Fig. 8 shows the absorbance spectrum of BHCl and the emission spectrum of curcumin. As can be seen, there is a reasonable overlap between these spectra.

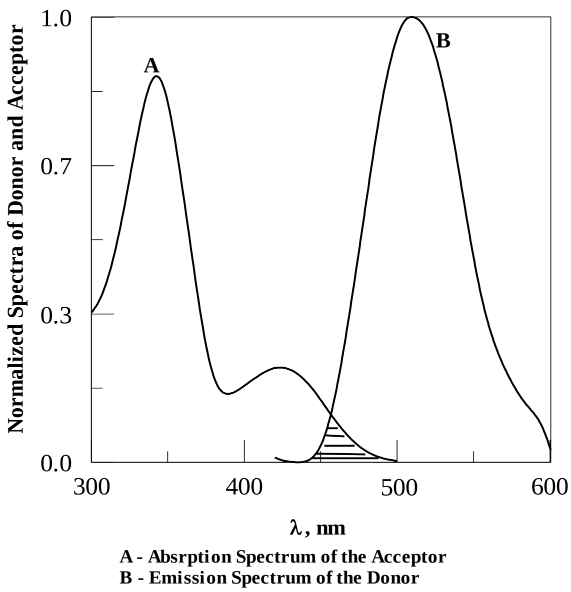

Figure 8. Spectra of the Acceptor and Donor.

The Förster Energy Transfer, FRET, mechanism which has received numerous attention, detailing its importance in chemical and biological reactions, principles and applications *inter alia*, the transfer energy efficiency, E, the overlap integral, J, the Förster radius, R_o, and the reactants distance of nearest approach, r, and the rate constant of the energy transfer, k_T, [33-42] is used to interpret the observations made in this work. The energy transfer efficiency between the donor molecule (curcumin) and the acceptor molecule (BHCl) that results in this exergonic free energy is calculated using equation 4 [43]

$$E = 1 - I/I^0 \tag{4}$$

The I in this equation is the observed fluorescence intensity at 1:1 complexation between the donor and acceptor molecules and I^0 is the fluorescence intensity of the donor in the absence of the acceptor. A value of 0.32 as transfer efficiency was obtained. Using this value, the Förster radius, R_o, and the distance, r, between the donor and acceptor were determined using equation 6.

$$E = (R_o/R_o + r)^6 \tag{5}$$

R^6_o in this equation is given by the relation in equation 6.

$$R^6_o = 8.8 \times 10^{-25} K^2 n^{-4} \phi J \tag{6}$$

K^2, the orientation factor, whose value has been established as 2/3 [43] and the average value of 0.104 of ϕ, the quantum yield of curcumin, is taken from the literature [26, 27, 30, 31, 45]. The refractive index of 1.2361 used was taken from ref. 44.

Determination of J

From the literature, *loc. cit.* the equation for the determination of the overlap integral is

$$J = \int_0^\infty I^0 \ \varepsilon_A(\lambda)\lambda^4 d\lambda \tag{7}$$

The ε_A (molar absorptivity) of the donor, 1.45 x 10^4/M-cm is taken from refs. 46 and 47. I^0 541.20 is the experimentally determined the donor fluorescence intensity. With these values, the spectral overlap, integrated from 430 nm to 500 nm as shown in the shaded area of Fig. 8, was calculated. The obtained value is 1.89 x 10^{-14} cm/M. This value is within the range of J values in the literature [43]. The energy transfer rate is obtained by using equation 8 *loc. cit.*

$$k_T = 1 - (I_{da}/I_d) \tag{8}$$

In this equation, I_{da} is taken to be the fluorescence intensity at the 1:1 complexation of the donor and the acceptor and I_d is the fluorescence intensity of the donor in the absence of the acceptor. Using this equation, the resonance energy transfer rate of 1.36 x 10^9/s is calculated.

Table 2 lists the parameters determined in this work.

Table II. Determine Curcumin-Berberine hydrochloride Complexation Parameters.

Parameter	Value	Unit
kq (bi-molecular quenching constant)	2.83 x 10^{13}	$M^{-1}s^{-1}$
K (Complex Equilibrium Constant)	3.63 x 10^3	
n (Binding Ratio)	1:1	
J (Overlap Integral)	1.9 x 10^{-14}	cm^3/M
E (Energy Transfer Efficiency)	0.32	
Ro (Forster Radius)	27.71	Å
r (Distance Between the Reactants)	31.4	Å
k_T (Energy Transfer Rate Constant)	1.36 x 109	s^{-1}

Conclusion

We have shown in this work that BHCl forms a 1:1 complexation ratio with curcumin. BHCl which is weakly fluorescent both in an aqueous system and in nanoemulsion quenches the fluorescence intensity of curcumin. The bi-molecular quenching constant and the Interaction constant were determined as 2.84 x10^{13}/M-s and 3.63 x10^3. A spectral overlap between BHCl and curcumin was observed leading to a resonance energy transfer efficiency of 0.32. The calculated Förster radius and the distance of closeness approach between the donor and acceptor molecules are 27.71 Å and 31.40 Å, respectively.

Acknowledgement

The author is grateful to the Chemistry Department of Morgan State University for her support of this work. The author is also very grateful to Prof. Michael McCaffery of the Johns Hopkins University for his help in obtaining the SEM image of the nanoemulsion.

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