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ORIGINAL PAPER

Enhancement of Curcumin Fluorescence by Ascorbic Acid in Bicontinuous Microemulsion

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Abstract Steady-state fluorescence spectro-photometric technique is used in this work to determine the chemical parameters of the complex formed between curcumin and ascorbic acid in bicontinuous microemuslion (Bμen). The Bμen liquid used is made up of a four-components system (water–oil-surfactant and co-surfactant (1-pentanol)) in the ratio of 42.11:13.7:21.34:22.85 % w/w. The oil and surfactant used are tetradecane and cetyltrimethylammonium bromide. Curcumin is known to have low solubility in water, but liberally soluble in Bμen, hence the use of Bμen in this study. The observed fluorescence intensity of curcumin was enhanced by introduction of ascorbic acid to the curcumin solution. The increase in the fluorescence intensity showed a very good linearity with a regression coefficient of 0.9974. The association constant, K_a, that resulted between curcumin and ascorbic acid was calculated as 2.15×10^4 with the free energy of association, ΔG_a, of −24.71 kJ/mol. The ratio of the complex that was formed by these two molecules was determined as 1:1.

Keywords Bicontinuous microemulsion · Curcumin · Ascorbic acid · Fluorescence

Introduction

Microemulsion is an optically clear and thermodynamically stable isotropic liquid that has been found application in the food industry, pharmacology and enhanced oil recovery. Perhaps the best definition of microemulsion is that given by Lindman [1]. It is a liquid made up of dispersed oil in water or vice versa and stabilized by an amphiphile (surfactant). The phase diagram and structure of microemulsion have been exhaustively discussed in the literature [2–7]. On the other hand bicontinunuous microemuslion (Bμem) is an extension of the microemulsion with the exception that in Bμem both the oil and water are continuous with a surfactant (and sometimes with a short chain alcohol as co-surfactant) forming a layer between the oil and water phases. Gart and his co-workers [8] and other workers have shown conclusively, using data obtained from many physico-chemical techniques, that Bμem spans the range of 25 to 65 % by weight of water. Like the microemulsion system, the phase diagram and structure of Bμem have been given in literature [9–14]. However, we show in Fig. 1 the over-simplified structure of this unique liquid. The unique property of Bμem lies in the fact that because it contains both hydrophobic and hydrophilic phases, the ionic molecules can be solubilized in the hydrophilic phase while the non-ionic solutes are solubilized in the oil phase [15]. Like microemulsion, it has also been found to be an excellent delivery medium for many different kinds of drugs [16–18]. In addition, Bμem has been used in several applications ranging from a medium for chemical synthesis to its use in chemical and biological analysis [19–26]. Both curcumin and ascorbic acid are pharmacologically active molecules. They are also known to be anti-ROS (reactive oxygen species). While ascorbic acid, a naturally occurring organic compound has been extensively used as vitamin C, it is known as an anti-oxidant [27–29]. On the other hand, curcumin, a phytochemical, has been used for many therapeutic and pharmacological activities including its role as anti-ROS [30–32]. The chemical structures of these anti-ROS compounds are shown in Fig. 2.

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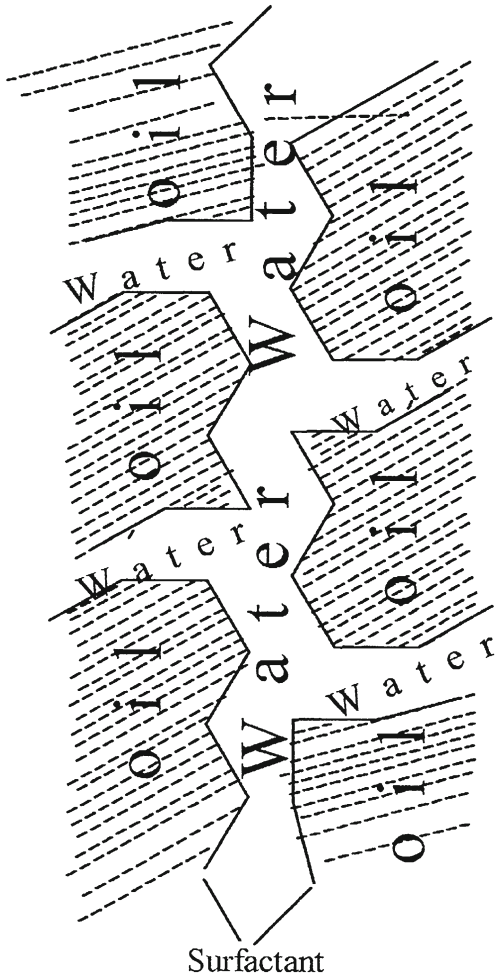


Fig. 1 The over-simplified structure of bicontinuous micoemulsion

To the author's knowledge, the complexation of these ROS molecules has neither been observed nor studied hitherto. Therefore the determination of this complex and its physico-chemical parameters determination are the themes of this work.

Methodology

Chemicals Cetyltrimethylammonium bromide (CTAB) and tetradecane both of 99+ % purity were obtained from Acros Organics. Reagent grade 1-pentanol was obtained from Sigma-Aldrich chemicals. These chemicals were used as obtained.

Instrumentation The main instrument used in this work is the Luminescence Spectrophotometer, model LS 50B supplied by Perkin Elmer.

All the fluorescence spectra were obtained in a four-sided 1-cm cuvette. The excitation and emission slit widths were kept constant at 6.0 nm. Curcumin and all the complex solutions were excited at 348 nm and the emission was observed between 528 and 529 nm.

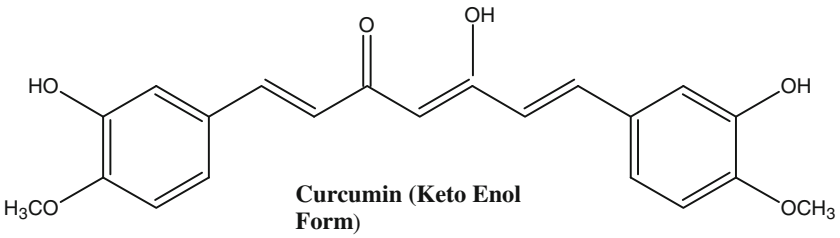
The water used in all experiments was triply distilled and deionized through the reagent grade water system supplied by Photronix.

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

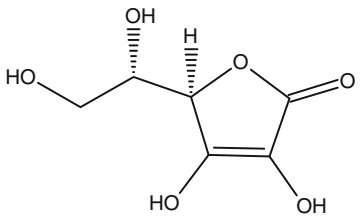
Results and Discussion

I show in Fig. 3a the fluorescence spectra of curcumin in the presence and absence of ascorbic acid. Figure 3b is the corresponding fluorescence intensity of the observed spectra. It can

Fig. 2 The chemical structures of curcumin and ascorbic acid



Curcumin (Keto Enol Form)



L-Ascorbic Acid

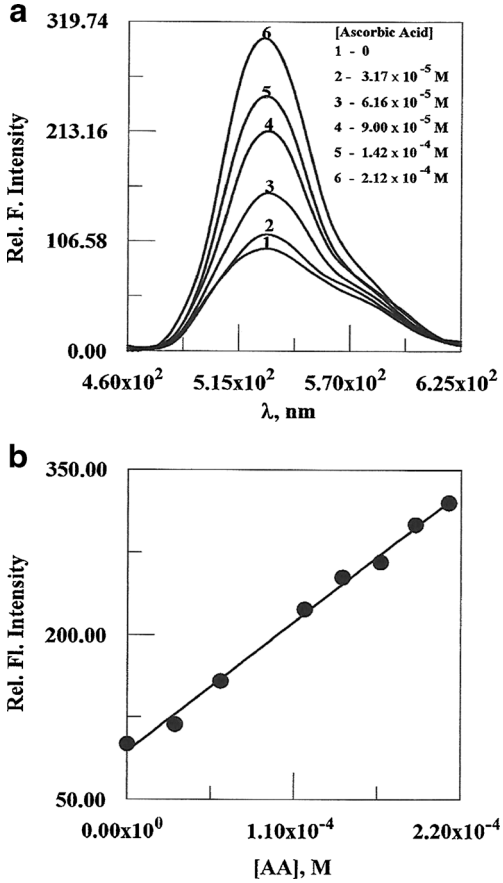


Fig. 3 **a** The Fluorescence spectra of curcumin with and without ascorbic acid. **b** The Relative Fl. Intensity of the Curcumin as a function of the ascorbic acid

be seen that the fluorescence intensity of curcumin increases as the concentration of ascorbic acid is increased. When this observed intensity is plotted as a function of ascorbic acid concentration, a linear plot is obtained with a correlation coefficient of 0.9974. As a result of this observation, curcumin

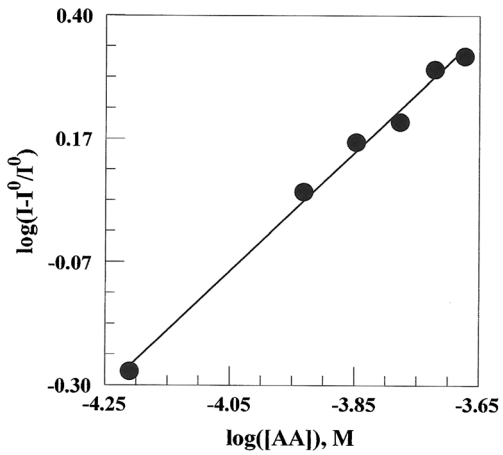


Fig. 4 Plot of $\log(I\text{-}I^0/I^0)$ versus the log([Ascorbic Acid])

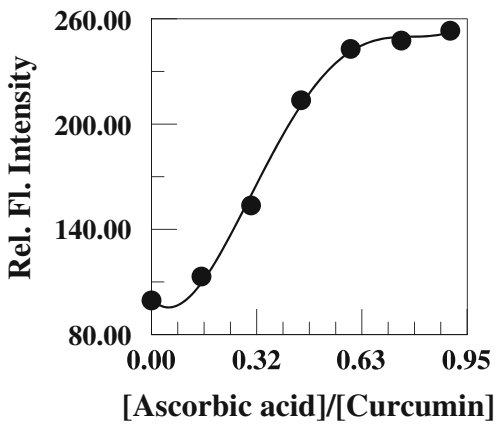


Fig. 5 Plot of the Rel. Fl. Intensity as a function of the molar ratio of ascorbic acid and curcumin

may be used as a biosensor for the determination of ascorbic acid.

Analysis of Data

We consider a typical complexation reaction:

$$A + nB \rightleftarrows X \quad (1)$$

$$K = \frac{[X]}{[A][B]^n} \quad (2)$$

X= the concentration of the complex and $[A]=[A_o]-[x]$ $[B]=[B_o]-[X]$.

However, $[X] << [A_o]$ and $<< [B_o]$. As a result, Eq. 2 becomes Eq. 3.

$$K = \frac{[X]}{[A_o][B_o]} \quad (3)$$

We can relate the concentration of the complex to the observed fluorescence intensity, I. But I is the sum of the fluorescence intensity of the complex and that of the $[A_o]$. The fluorescence intensity of [Ao] = I^0, then the fluorescence of the complex becomes $I\text{-}I^0$. In light of this, Eq. 3 can be re-written in terms of fluorescence intensity as $K[B_o]^n = \frac{I-I^0}{I^0}$

Table 1 The Observed/calculated parameters for the association of curcumin and ascorbic acid

Parameter	Value
K_a(Association constant)	2.15×10^4
ΔG_a(Free energy of association)	−24.71 kJ/mol
n (Binding ratio)	1.0

If we take the logarithm of both sides of the last relation we get the flowing result through which we can determine the value of n and K (K_a)

$$\mathrm{Log}\left(\frac{I-I^0}{I^0}\right) = \mathrm{Log}(K) + n\mathrm{Log}\left([B]_0\right) \quad (4)$$

Notice that this is analogous to equation due to Feng et al. [33]. The difference being the fact that these workers developed an equation for quenching process. Here the development is for enhancement.

A plot of $\mathrm{Log}\left(\frac{I-I^0}{I^0}\right)$ versus $\mathrm{Log}\left([B]_0\right)$ will give a straight line whose slope, in accordance with Eq. 4 will give the value of n, the binding ratio between A and B. The intercept will be used to calculate the binding/association constant, K_a.

Equation 4 was used to construct a plot shown in Fig. 4. As can be seen, this plot is quite linear with a correlation coefficient of 0.9974. The n determined from the slope is 1.08 which is approximately 1.0 and the K_a calculated is 2.15×10^4. The energy of association, ΔG_a, was calculated using the relation: $\Delta G_a = -RT\ln K$. The value thus obtained is −24.71 kJ/mol

The obtained fluorescence data were also used to plot the molar ratio of [ascorbic acid] to [curcumin]. The resultant plot is shown in Fig. 5. A plateau was observed when the concentration ratio of ascorbic acid to curcumin concentration is about 0.8 which is ≈1.0. This confirms what was obtained using Eq. 4.

The parameters obtained for curcumin-ascorbic acid complex is tabulated in Table 1.

Conclusion

It has been shown in this work that the presence of ascorbic acid enhances the fluorescence intensity of curcumin in a bicontinuous microemulsion medium. The observed fluorescence intensity is linear with an increase in the concentration of ascorbic acid. This opens up a possibility of using curcumin as a biosensor for ascorbic acid. The K_a obtained is $2.15\times b\ 10^4$. The binding ratio between curcumin and ascorbic acid is 1:1. The observed free energy of association is quite high (−24.71 kJ/mol) implying that the complex formed is not only spontaneous but quite stable. The relevant obtained parameters are given.

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