Fluorescence quenching of uric acid solubilized in bicontinuous microemulsion by nitrobenzene

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Abstract: Uric Acid is known to be practically insoluble in aqueous and alcoholic media. However, it exhibits a reasonable solubility in a Bicontinuous Microemulsion system – a 15-fold or more increase in solubility in this system compared to its solubility in water. The bicontinuous microemulsion is made up of three components – Dodecane-Surfactant-water. Uric acid solubilized in this system is quenched by nitrobenzene. The obtained fluorescence data do not obey the Stern-Volmer equation when plotted accordingly. Therefore, the modified Stern-Volmer equation was used to analyze the data. It was observed that only one third (1/3) of uric acid is accessible to quenching in this medium and the reaction is diffusion-limited. The Stern-Volmer quenching constant, K_{SV}, was calculated to be 130 M^{-1} and the fluorescence lifetime, τ_0, the quantum yield, ϕ, and the bimolecular quenching rate constant, k_q, were calculated as 10.6 nanoseconds, 0.06 and 1.23×10^{10} $M^{-1}s^{-1}$, respectively.

Keywords: bicontinuous microemulsion, fluorescence, quenching, uric acid.

Introduction

Uric acid is a heterocyclic compound that is the end product of the metabolic oxidation of purines[1]. Figure 1 shows the structure of uric acid. Its physiological properties are well known[2-6]. It is practically insoluble in water[4-6] hence its crystallization in joints of humans which results to gout and arthritic inflammations.
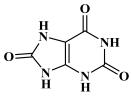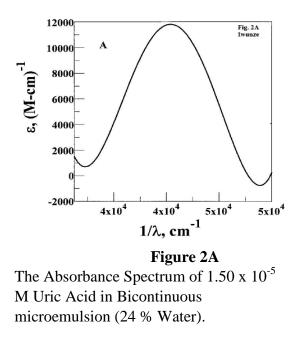

Figure 1 Structure of Uric Acid

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Despite these debilitating consequences on humans, uric acid has been observed as having some anti-oxidative properties[8-15]. A report on the contrary of this effect has been presented[16]. On the other hand, bicontinuous microemulsion is a thermodynamically stable fluid. It has been extensively studied and characterized[17-19]. It can solubilize both ionic and non-ionic solutes[20, 21]. It is visually observed that it can solubilize uric acid about 15 fold higher or more than water. The physico-chemical parameters for uric acid, to the author's knowledge, have not been documented. We therefore used nitrobenzene, a known good fluorescence quencher, to quench the fluorescence of uric acid in this medium to obtain its physico-chemical parameters using steady-state fluorescence and UV-Vis techniques.

Results And Discussion

The UV-Visible and the fluorescence spectra of 1.50×10^{-4} M uric acid are shown in Figure 2A and 2B. From Figure 2A the molar absorptivity, ε, and the wave number, $\bar{\nu}$, of the high and low energy of the spectrum were determined.

Figure 2A

The Absorbance Spectrum of 1.50×10^{-5} M Uric Acid in Bicontinuous microemulsion (24 % Water).
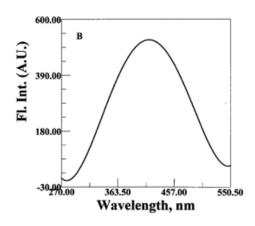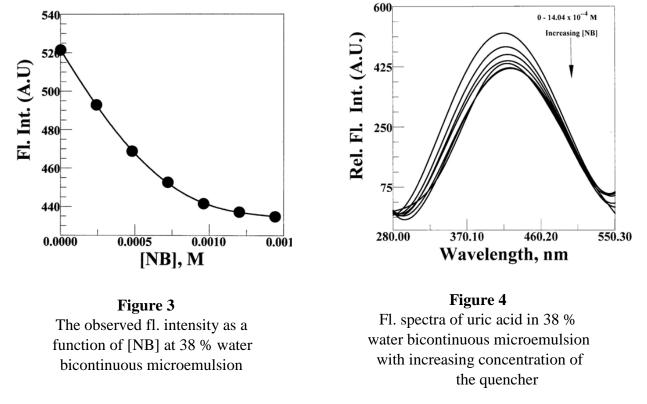
Figure 2 B

Fl. Spectrum of 1.449×10^{-4} M Uric Acid in Bicontinuous microemulsion (38 % Water).

These spectra show that uric acid in bicotinuous microemulsion exhibits an uncomplicated ground and excited state profile which makes it very convenient in the determination of its life time fluorescence. When the observed fluorescence intensity of uric acid is plotted as a function of the increasing quencher concentration, an exponentially decreasing curve is observed as it should be. This is shown in Figure 3.

The fluorescence spectra in the presence, I, and in the absence, I^0, of the quencher, NB, are shown in Figure 4.

Figure 3
The observed fl. intensity as a function of [NB] at 38 % water bicontinuous microemulsion

Figure 4
Fl. spectra of uric acid in 38 % water bicontinuous microemulsion with increasing concentration of the quencher

The obtained data were treated in accordance with the Stern-Volmer equation: $I^0/I = 1 + K[NB]$. A plot of I^0/I versus [NB], however, did not give a straight line curve rather it gave a concave down-ward curve as can be seen in Figure 5.
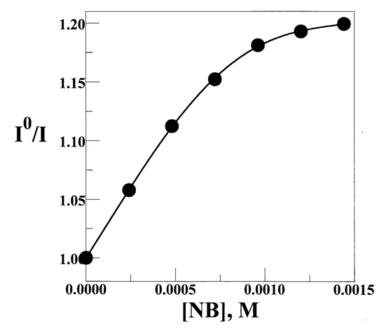

Figure 5 the Stern-Volmer plot of the observed fluorescence intensity as a function of the quencher concentration in 38 % water bicontinuous microemulsion

This concave down-ward plot implies that a fraction, f, of uric acid is not accessible to the quencher. However, when the modified Stern-Volmer equation, $I^0/\triangle I = 1/f + 1/fK[NB]$, was used, that is a plot of I^0/I versus $1/[NB]$, a linear plot was obtained as can be seen in Figure 6.
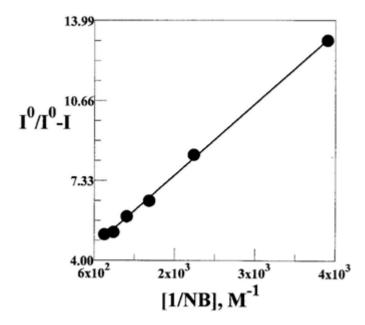

Figure 6   the modified Stern-Volmer plot for the quenching of uric acid by nitrobenzene in bicontinuous microemulsion

The fraction, f, is calculated as 0.34 from the intercept of this plot. The Stern-Volmer quenching constant, K which is the same as K_{SV} is calculated as 10^3 M^{-1}. This value was used with the Stern-Volmer quenching constant, K, to estimate k_q, the bimolecular quenching constant for the system under study as per the following known equation:

$$K_{SV} = \tau_0 k_q \qquad\qquad\qquad (1)$$

The τ_0 is estimated in the Florescence Lifetime Experiment in the experimental section. The value of k_q thus obtained is 1.23×10^{10} $M^{-1}s^{-1}$ which implies a diffusion-controlled reaction and it also agrees, within experimental error, with the value obtained using the equation $k_q = 2\pi NRD$ developed by Almgren and his co-workers[22] (2.0×10^{11} $M^{-1}s^{-1}$) for molecules undergoing a diffusion-controlled reaction in a bicontinuous microemulsion system. However, this equation is a modification of the Smoluchoski equation $k_d = 4\pi NRD$, where N is Avogadro's number, R the encounter radius which is approximated by the sum of the radii of the reacting molecules and D is the sum of their diffusion coefficients. In this work R for uric acid and NB were obtained using the relation $r = (3M/4\pi N\rho)^{1/3}$ where M is the molecular weight, N and ρ are the Avogadro's number and the density, respectively. The D in the Smoluchoski equation was determined using the Einstein-Stokes relation ($D = kT/6\pi r\eta$) where k, T and η are the Boltzmann constant, temperature in Kelvin scale and viscosity, respectively. The fluorescence lifetime for uric acid in bicontinuous microemulsion is calculated as 10.6 nanoseconds and its quantum yield is 0.06.

Table 1 lists the obtained physico-chemical parameters for uric acid obtained in bicontinuous microemulsion medium.

Table 1. The observed Physico-Chemical Parameters of Uric Acid in Bicontinuous Microemulsion

Parameter	Value	Unit
λ_{max} - Wavelength of Maximum Absorbance	250	nm
ε - Molar Absorptivity	1.18×10^4	$M^{-1} cm^{-1}$
K_{SV} - Stern-Volmer Quenching Constant	103	M^{-1}
τ_0 - Fluorescence Lifetime	0.62	ns
k_q - Bimolecular Quenching Rate Constant	2.1×10^{11}	$M^{-1} s^{-1}$
f - Fraction of Quenched Fluorophore	0.34	

Conclusion

It has been shown in this work that uric acid which is very insoluble in water can be solubilized in bicontinuous microemulsion. The bimolecular reaction in this medium is observed to be diffusion-controlled. The accessibility of uric acid in this medium is only 1/3 and the obtained
data is analyzed using the modified Stern-Volmer equation. The physico-chemical properties (bimolecular quenching rate constant, k_q , the quantum yield, ϕ, the fluorescence lifetime,τ_0, and its molar absorptivity, ε,) were determined by the combination of UV-Vis and steady-state fluorescence techniques.

Expermental Section

Chemicals

Uric Acid, reagent grade, was obtained from Fisher Scientific Co. didodecylammonium bromide, DDAB, was obtained in 98 % purity from Spectrum Chemical, nitro benzene, NB, 99 pure, was obtained from Aldrich Chemical Co. and dodecane, 99 + pure, was obtained from Alfa Aesar.

Methodology

Optical Measurements
All fluorescence measurements were performed using a Perkin Elmer's Luminescence Spectrophotometer, Model LS 50B. The wavelength of the excitation of uric acid was at 215.0 nm. At this excitation wavelength (215 nm), the fluorescence was observed at 420.00 nm. The excitation and emission slits were kept constant at 3.0 nm. In all experiments, unless otherwise specified, the concentration of uric acid was kept constant at 1.499×10^{-4} M and the

concentration of the quencher, nitrobenzene, was varied from 2.34×10^{-4} to 14.04×10^{-4} M. All experiments were conducted at room temperature ($25 \pm 0.2°C$). The fluorescence spectra from which the fluorescence intensities were obtained were uncorrected.

The absorptiometric experiments were performed using a Cary Spectrophotometer, model 1E, supplied by Varian Analytical Instruments. Absorptiometric spectrum of this compound whose λ_{max} is observed at 250 nm was obtained using a 1.0 cm cuvette. The molar absorptivity, ε, of uric acid in bicontinuous microemulsion was determined by plotting the ratio of the observed absorbance, A, taken at λ_{max} to the concentration, C, as a function of the respective wavelengths encompassing the absorbance spectrum in accordance with Beer-Lambert's law (A/bC). b, the solution thickness or light path length, was 1.0 cm. The ε was taken at the peak of the plot at which $\lambda = \lambda_{max}$.

Refractive Index Determination

The refractive index, n, of bicontinuous mcroemulsion used and that of the 0.10 M H_2SO_4 solutions were determined using the digital Abbe Leica Refractometer which was calibrated with triply distilled deionized water. The obtained refractive indices for these solutions were 1.39605 and 1.34323, respectively.

Viscosity Experiments

The viscosity of the used bicontinuous microemulsion (66.0 cP) was obtained using a computer-controlled Brookfield Viscometer, model RVDV II+

Quantum Yield

The quantum yield, ϕ, of uric acid was determined in accordance with the literature methodology[23] . Briefly, the solutions of the reference standard (quinine sulfate) and the uric acid were prepared to have approximately equal absorbancy, 0.16 and 0.14, respectively, and both were excited at the same wavelength (325 nm). In a review articles on quantum yields determination, Demas and Crobsy[24] comment that the quantum yield of quinine sulfate is constant between the excitation wavelength of 200 and 390 nm. The value of the quantum yield for this reference sample is recommended to be 0.54 and it was used in this work. The fluorescence emission spectrum of the uric acid solution was corrected for quantum yield determination. The observed fluorescence was corrected in accordance with equation 2[23]

$$\phi_u = \phi_s[A_1 n_1^2 / (A_2 n_2^2)] \tag{2}$$

In this equation, the subscripts 1 and 2 refer to the unknown sample (uric acid in bicontinuous microemulsion) and reference sample (quinine sulfate), respectively. The A refers to the area of the fluorescence band. Using the above equation, the fluorescence quantum yield for uric acid was determined as 0.06.

Fluorescence Lifetime

The fluorescence lifetime, τ_0, for uric acid in bicontinuous microemulsion was determined using the Strickler-Berg relation[25] given in equation 3.

$$1/\tau_0 = 2.88 \times 10^{-9} n^2 \langle \bar{v}_f^{-3} \rangle^{-1} \frac{g_l}{g_u} \int \varepsilon \, d\ln\bar{v} \tag{3}$$

In this equation n, $\bar{\nu}_f$ and $\bar{\nu}$ are the solvent (bicontinuous microemulsion) refractive index, the fluorescence wave number, taken at the center of gravity of the fluorescence spectrum, and the integrated wave number of the absorption band, respectively. As stated above, uric acid exhibits an uncomplicated single absorption band whose maximum centered at 250 nm. As a result of this, it is assumed that the population of molecules in upper, g_l, and lower, g_u, electronic states are in rapid equilibrium. Therefore, the ratio of molecules in the degenerate electronic states is taken to be unity. The integration in equation 3 was therefore done analytically by measuring the area enclosed under the absorbance envelope using the relation of Full Width at Half Maximum (FWHM) technique. In this case equation 3 is reduced to a much simpler relation given in equation 4:

$$1/\tau_0 = 2.88 \times 10^{-9} n^2 (\bar{\nu}_f^{-3})^{-1} \varepsilon \ln(\bar{\nu}_2/\bar{\nu}_1). \qquad (4)$$

subscripts 1 and 2 in this equation denote the wavenumber corresponding to the high and low end of the absorbance spectrum. Using the above equation, the fluorescence lifetime for uric acid in the medium is calculated as 10.6 nanoseconds. The quencher concentration varied from $2.0 \times 10^{-4} - 1.0 \times 10^{-3}$ M.

The bicontinuous microemulsion system is made up of the volume fraction of water in oil, $Q_w = W_v/(W_v + O_v)$, weight fraction of oil in water, $\alpha = O_{wt}/(O_{wt} + W_{wt})$ and weight fraction of surfactant in oil and water, $\gamma = S_{wt}/(S_{wt} + O_{wt} + W_{wt})$. In all these, the symbols denote: W = water, O = oil (dodecane), S = Surfactant (didodecylammonium bromide), wt = weight and v = volume. The value of the variables that make up the bicontinuous microemulsion of the combination used in this work is $Q_w = 0.38$, $\alpha = 0.55$ and $\gamma = 0.06$. In all the experiments, the density of the Bicontinuous microemulsion was taken as 0.866 g/mL and the density of uric acid (1.874 g/mL) was taken from Wolfram/Alpha compilation[26].

All chemicals were used as received and all experiments were conducted at room temperature (25 ± 0.2 °C).

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