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The Effect of Micelles on the Steady-State and Time-Resolved Fluorescence of Indole, 1-Methylindole, and 3-Methylindole in Aqueous Media

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3-Methylindole (skatole) is a component of animal waste and is, consequently, a primary component in odor problems arising in livestock management, notably swine production. The ability to probe and to exploit the interactions of 3-methylindole with micelles has important implications for monitoring and controlling odor problems. The effect of a surfactant (Brij-35) on the fluorescence properties of indole, 1-methylindole, and 3-methylindole in aqueous solutions is reported. Steady-state fluorescence spectra reveal a blue shift in the emission as the surfactant concentration is increased, while the absorption spectra are practically unaffected. Time-resolved fluorescence measurements reveal shorter average lifetimes for 3-methylindole (3-MI) as the Brij-35 concentration is increased. The fluorescence decay of 3-MI in water is described well by a single exponential, whereas, at the highest Brij-35 concentration, a triple exponential is necessary to describe the fluorescence decay. The contributions of each component in the fluorescence decay are used to determine the extent of 3-MI partitioning into the micelle phase. It is found that 93% of the 3-MI molecules partition into the micelle at the highest Brij-35 concentration used. The equilibrium constant for the association between the micelles and the 3-MI molecules is determined to be $2.6 \times 10^4\ M^{-1}$. In addition, the reduction of 3-MI in the vapor phase by addition of a dry surfactant, lecithin, is also demonstrated.

3-Methylindole (skatole) is an important component of animal waste. Because of its noxious odor, 3-methylindole is a primary problem in livestock management, especially in swine production.[1−7] In this article, we consider the effect of surfactants on the steady-state and time-resolved fluorescence of indoles in the aqueous and vapor phases. Micelles can be used as model, and perhaps practical, systems capable of trapping small molecules such as indoles. Understanding under what conditions 3-methylindole can be sequestered and developing means of detecting it are of great practical interest.

The fluorescence properties of indoles (Figure 1a−c) are extremely sensitive to the environment. This sensitivity arises from the presence of two closely spaced excited singlet states (Figure 2), which are traditionally denoted ^1L_a and ^1L_b.[8−15] The energy of the ^1L_b state is insensitive to the solvent, and it is the lower lying of the two excited singlet states in nonpolar solvents. In nonpolar solvents, ^1L_b is the emissive state.[16] On the other hand, the ^1L_a state interacts strongly with the solvent. In nonpolar media it lies above ^1L_b, but in polar media the ^1L_a state strongly interacts with the solvent and subsequently lowers its energy relative to that of ^1L_b, thus becoming the emissive or fluorescent state. Because of the interaction of these two excited states with their environment (and with each other), shifts of emission spectra to higher energies (blue shifts) are excellent signatures of the probe molecule moving to a nonpolar environment.[17,18] Also, as the indole moves to a nonpolar environment, the fluorescence lifetime shortens, due largely to the different radiative properties of the ^1L_b state with respect to those of the ^1L_a state[15] (Figure 2).

Organic molecules are generally hydrophobic in nature. The interior of micelles consists of a nonpolar region, which offers an ideal environment into which organic molecules may partition from the aqueous phase. Consequently, the micelle phase can induce substantial changes in the fluorescence properties of

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(16) This statement must be qualified. The ^1L_a and ^1L_b levels are typically closely spaced, so the lower state can thermally populate the higher one. Furthermore, on a fast (subpicosecond) time scale, the emission from the higher lying state is detectable. These two phenomena contribute to the low limiting anisotropy values of most indoles.[12]
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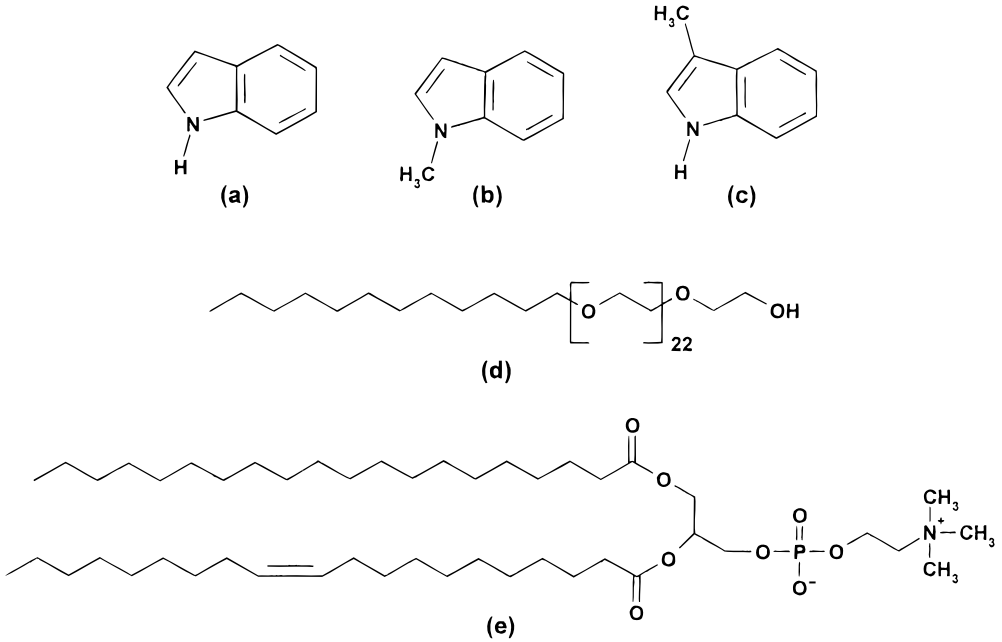

Figure 1. Structures of (a) indole, (b) 1-methylindole, (c) 3-methylindole, and (d) Brij-35 (poly(oxyethylene)23 lauryl ether). (e) A general chemical structure of lecithin (phosphatidylcholine). The long-chain fatty acid groups, by convention, occupy positions 1 and 2 of the glycerol bridge, while the phosphorylcholine headgroup occupies position 3. The hydrocarbon chains vary in length and saturation, with the more unsaturated chain in position 2, as represented by the double bond in the chain at this position.

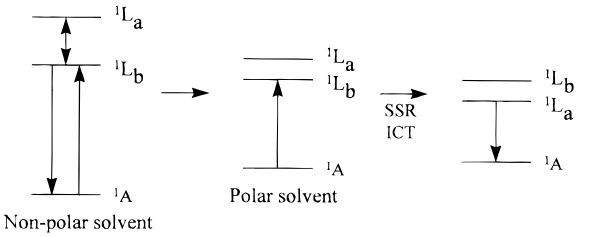

Figure 2. Representation of the two low-lying excited states of indole and its derivatives and the effect of solvent on the fluorescence. The figure is adapted from that in the article by Meech et al.[15] In nonpolar solvents, the ^1L_b state is the lower lying of the two excited states. Consequently, it is the fluorescent state. The ^1L_a state is very sensitive to solvent polarity, whereas the ^1L_b state is not. As the polarity of the solvent increases, the energy of the ^1L_a state lowers, and it can become the fluorescent state. The specific behavior of the fluorescence lifetimes of the ^1L_b and the ^1L_a states is determined largely by their radiative rates. Meech et al. note that the increase of fluorescence lifetime of 3-MI with increasing polarity cannot be attributed to the lowering of the energy of the ^1L_a state but must take into account nonspecific interactions with the solvent that can arise because the dipole moment of the ^1L_a state is greater than that of the ^1L_b state. The ability of the ^1L_a state to lower its energy in polar solvents is referred to as "solute−solvent interaction" (SSR). The ability of the ^1L_a state to interact with the solvent in such a way as to modify its radiative properties is referred to as "internal charge transfer" (ICT). These processes are denoted in the figure.

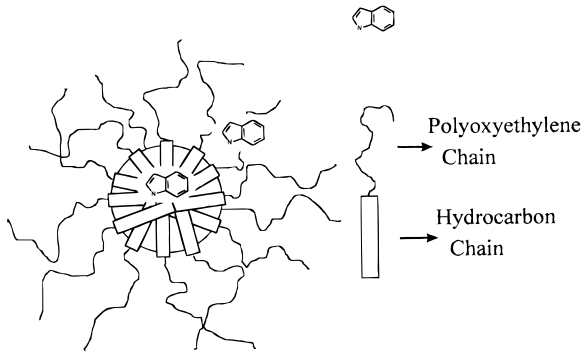

Figure 3. Schematic drawing of a Brij-35 micelle and possible depictions of the indole−micelle complex. The indole chromophore could be located within the nonpolar core of the micelle, solubilized between the inner core and the water phase in the hydrated oxyethylene phase or in the aqueous phase with no interaction with the micelle.

indoles. In the nonpolar micelle phase, the indole emits from the ^1L_b state, whose fluorescence will be blue shifted and shorter lived with respect to those of molecules in the polar aqueous phase having no interaction with the micelle and which emit from the ^1L_a state. The chromophore may also be located between the inner core of the micelle and the aqueous phase (water/micelle boundary),[20,21] which would be expected to afford different fluorescence behavior which is intermediate to those of the two just mentioned (Figure 3). The distinct fluorescence properties of the indoles in these different environments permit their association with the micelle to be quantified.

Given the biological importance of indoles, that indole is the chromophoric moiety of the amino acid tryptophan, and the considerable effort that has been devoted to understanding their fluorescence properties and exploiting these properties as probes of environment (e.g., see refs 22−25), it is remarkable that, with only a few exceptions of which we are aware (e.g., see refs 17−19 and 22), quantitative investigations of the interactions of indoles with micelles have been ignored.

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EXPERIMENTAL SECTION

Materials. Indole, 1-methylindole (1-MI), and 3-methylindole (3-MI) were obtained from Sigma Chemical Co. and were used without further purification. The neutral surfactant, poly(oxyethylene)23 lauryl ether (Brij-35, Figure 1d[20]), was also used as received. For Brij-35, micelles begin to form at a concentration of about 7×10^{-5} M[26,27] (the critical micelle concentration). The number of Brij-35 monomers that become incorporated into a single micelle is about 40 (the aggregation number).[27] Solutions were prepared using Nanopure water.

Lecithin, a phosphoglyceride, also referred to as phosphatidylcholine, was obtained from Sigma and was used as received. The particular lecithin used in our experiments was derived from soybeans. Lecithin consists of two long-chain fatty acid ester groups and a phosphorylcholine dipolar headgroup attached to a glycerol backbone (Figure 1e).[28] A lecithin solution was prepared by diluting 50 mg of solid lecithin with 2.0 mL of a 2:1 chloroform/methanol mixture[28] (25 mg/mL) and then diluting an aliquot of the 25 mg/mL solution to a final concentration of 0.025 mg/mL.

Sample Preparation. A 100 mL aqueous stock solution of each chromophore was prepared to have an optical density of 0.126 ± 0.009 at 280 nm. The concentration of the 3-MI in solution was ~1.48×10^{-5} M. Sonication was necessary to ensure that the chromophore was dissolved. One hundred milligrams of Brij-35 was dissolved in 4.0 mL of the chromophore stock solution to obtain a surfactant concentration of 2.1×10^{-2} M, followed by sonication for approximately 20 min. A 2.0 mL aliquot from the 2.1×10^{-2} M solution was diluted to 4.0 mL with the 3-MI aqueous stock solution to a concentration of 1.0×10^{-2} M Brij-35. This process of diluting each successive solution in half was repeated until the final dilutions for the indole and 1-MI solutions had a Brij-35 concentration of 8.1×10^{-5} M. For the 3-MI solutions, the final Brij concentration was 1.6×10^{-4} M. In all cases, the chromophore concentration was kept constant.

Sample preparations for the study of the effect of lecithin on the vapor of 3-MI were performed in the following manner. A 0.5 mL aliquot of the 9.8×10^{-5} M 3-MI/ethanol stock solution (6.4 μg of 3-MI) was placed into a sealed quartz cuvette with a syringe. Aliquots of the 0.025 mg/mL lecithin stock solution were added to the 3-MI sample in amounts of 0, 12.5, 25.0, and 37.5 μg. The solvent was then evaporated by passing argon over the sample.

Steady-State Measurements. Steady-state fluorescence measurements were performed using a Spex Fluoromax. All solutions were excited at 280 nm, and emission was monitored over a range of 300−500 nm. Absorption spectra from 250 to 350 nm were obtained for each of the 3-MI/Brij-35 solutions with a Perkin-Elmer Lambda 18 UV−visible spectrometer. For both fluorescence and absorption measurements, a 1 cm path length quartz cuvette was used.

The fluorescence measurements of the 3-MI vapor from the dry 3-MI/lecithin samples were performed on the same instrument as mentioned above. The 3-MI vapor was excited at 280 nm, and the emission was observed over a wavelength range from 290 to 330 nm. The integration time for each point on the spectrum was 3 s. The band-pass for both the excitation and emission monochromators was 4 nm. All measurements were performed at room temperature, approximately 23 °C. The emission of 3-MI vapor without the addition of lecithin was measured initially with subsequent measurements of the vapor from the three 3-MI/lecithin samples.

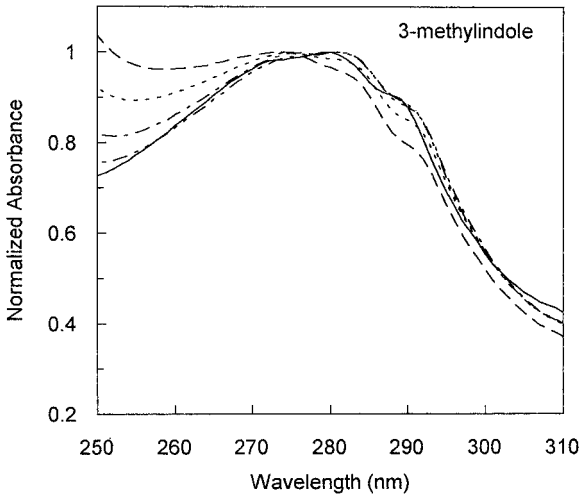

Figure 4. Absorbance spectra of 3-methylindole in Brij-35 micelles. The solid line is the absorbance in water without micelles. Concentration of Brij-35 in each broken line spectrum (from top to bottom): 2.1×10^{-2}, 1.0×10^{-2}, 5.2×10^{-3}, and 2.6×10^{-3} M. Below 2.6×10^{-3} M, the spectra were identical to that of water with no micelle.

Time-Resolved Measurements. Time-correlated single-photon counting was performed on the 3-MI aqueous solution and at the various Brij-35 concentrations. The apparatus is described elsewhere.[29] Fluorescence decays were obtained by collecting emission wavelengths >320 nm and using an excitation wavelength of 288 nm. The time window was 17 ns full-scale. All lifetime measurements were performed at 20.5 °C. The fluorescence lifetime obtained for 3-MI in water (8.5 ns) was comparable to that found in the literature.[15] Fluorescence decays were fit to a sum of not more than three exponentially decaying components:

$$F(t) = \sum A_i \exp(-t/\tau_i) \qquad (1)$$

where A_i is the preexponential factor representing the contribution to the decay curve of the component with lifetime τ_i. The preexponential factors, lifetimes, and χ^2 values were obtained from nonlinear least-squares fitting and global analysis using the program Spectra Solve.[30] An Instruments SA Inc. monochromator (8 nm band-pass) was used to obtain fluorescence decays at the blue (320 nm) and red (410 nm) edges of the 3-MI/Brij-35 fluorescence spectrum at the highest Brij-35 concentration.

RESULTS AND DISCUSSION

Steady-State Spectra. The absorbance maxima for the 3-MI solutions were practically unaffected by the presence of Brij-35 (Figure 4). The fluorescence spectrum of each chromophore studied was strongly dependent on the amount of Brij-35, and hence the amount of micelle, in solution. With the addition of micelle, the fluorescence spectra showed a blue shift in the

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Table 1. Steady-State Fluorescence Emission Maxima for Indole, 1-MI, and 3-MI

	λ_{max} (nm)		
[Brij-35] (M)	indole	1-MI	3-MI
0.0	346	355	367
8.1×10^{-5}	346	353	
1.6×10^{-4}	347	353	366
3.3×10^{-4}	346	355	365
6.5×10^{-4}	347	353	362
1.3×10^{-3}	343	353	358
2.6×10^{-3}	340	351	353
5.2×10^{-3}	338	348	351
1.0×10^{-2}	331	343	348
2.1×10^{-2}	328	336	342

emission maxima. The emission maxima of the indole and 1-MI showed less of a blue shift at the lower concentrations of Brij-35: no blue shift was apparent below concentrations of 6.5×10^{-4} M. On the other hand, 3-MI exhibited larger blue shifts in the emission maxima with the lower concentrations of Brij-35 (Table 1). Thus, 3-MI tends to have a greater affinity toward the nonpolar micelle than indole and 1-MI.

Time-Resolved Fluorescence. Time-resolved fluorescence was performed on the 3-MI solutions at the various Brij-35 concentrations. The 3-MI in water had a fluorescence lifetime of about 8.5 ns and fit very well to a single-exponential decay curve, $\chi^2 = 1.10$. As Brij-35 was added to the aqueous 3-MI solutions, the decay curves exhibited contributions from more than one component. Each component revealed the different environment from which fluorescence of the 3-MI is emitted. To verify which of the decay components was the result of emission from partitioning of 3-MI into the micelle phase, the red and blue edges of the emission band of a 3-MI/H_2O/Brij-35 mixture were studied with the use of a monochromator. The red edge decay resembled the decay of 3-MI in water and included the long component characteristic of emission in polar environments. The blue-edge decay exhibited the shorter components typical of 3-MI emission in nonpolar environments, and global analysis revealed the lack of any long component (Figure 5). We conclude that the long component of the fluorescence decay is due to 3-MI in water and the shorter components are due to 3-MI associated with micelle. Thus, 3-MI leaves the aqueous phase and partitions into the nonpolar micelle phase.

To obtain a more quantitative view of how much 3-MI remains in the aqueous phase, a global fit was performed using all decay curves, fixing the long component from water at 8100 ps, and fitting each to a triple exponential. A triple-exponential fit was chosen to take into account the three possible environments for the 3-MI: the aqueous phase, the nonpolar micelle phase, and the hydrated poly(oxyethylene) phase between the two (Figure 3). In the latter two cases, the indole can be considered to be partitioned into or associated with the micelle. The weight of the long component decreased dramatically with increasing micelle concentration (Table 2). At our highest surfactant concentration, the sum of the preexponential factors of the two short components of the fluorescence decay was ~0.93. Thus, 93% of the total fluorescence is composed of the two shorter components, which are attributed to the 3-MI associated with the micelle. The contribution of the 3-MI in the aqueous phase is 7%.

To obtain an initial estimate of the association (equilibrium) constant, K_{eq}, a Benesi−Hildebrand plot was made on the basis

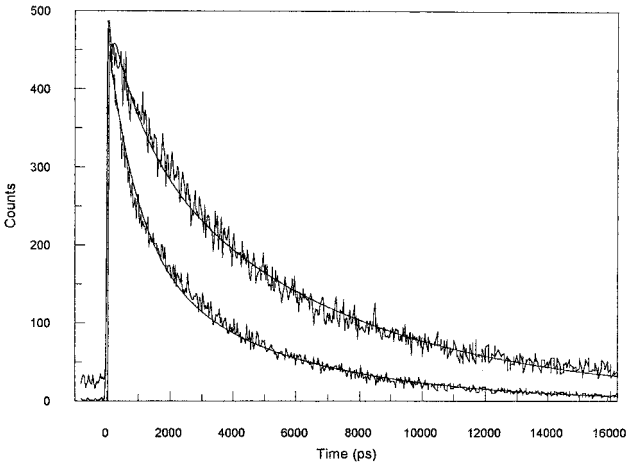

Figure 5. Time-resolved emission of the red (top decay) and the blue (lower decay) edges of a 3-MI/Brij-35 emission band ([Brij-35] = 2.1×10^{-2} M). Blue edge emission was collected at 320 nm, and red edge emission was collected at 410 nm. An 8 nm band-pass monochromator with a 1 mm slit was used. Global fitting parameters were used to fit each fluorescence decay. Lifetimes were fixed at 8100, 4671, and 955 ps, and the amplitudes were varied (eq 1). The blue edge can be fit to two components (the amplitude for the longest component is zero): $F(t) = 0.67 \exp(-t/955\text{ ps}) + 0.33 \exp(-t/4671\text{ ps})$, $\chi^2 = 1.3$. The red edge requires three components to be fit: $F(t) = 0.29 \exp(-t/955\text{ ps}) + 0.34 \exp(-t/4671\text{ ps}) + 0.37 \exp(-t/8100\text{ ps})$, $\chi^2 = 1.3$. The fluorescence decay from the blue edge is contaminated by some scattered light, against which we could not discriminate.

Table 2. Global Fitting Parameters of 3-MI/Brij-35 Solutions

[Brij-35] (M)	A_1	A_2	A_3
6.5×10^{-4}	0.81	0.13	0.06
1.3×10^{-3}	0.59	0.34	0.07
2.6×10^{-3}	0.41	0.52	0.07
5.2×10^{-3}	0.24	0.68	0.08
1.0×10^{-2}	0.13	0.76	0.11
2.1×10^{-2}	0.07	0.79	0.14
	$\tau_1 = 8100$ ps	$\tau_2 = 4671$ ps	$\tau_3 = 955$ ps

of a 1:1 3-MI/micelle association.[26,31,32] The equilibrium between 3-MI and a micelle is given by

$$3\text{MI} + \text{M} \rightleftharpoons 3\text{MI}\cdot\text{M} \qquad (2)$$

where M represents the micelle, and 3MI·M represents the 3-MI/micelle complex. The corresponding association constant is

$$K_{eq} = \frac{[3\text{MI}\cdot\text{M}]}{[3\text{MI}][\text{M}]} \qquad (3)$$

where the bracketed terms are the respective molar concentrations. The above expression can be rewritten in terms of initial known concentrations by substituting $[3\text{MI}]_0 - [3\text{MI}\cdot\text{M}]$ for [3MI] and $[\text{M}]_0 - [3\text{MI}\cdot\text{M}]$ for [M]; $[3\text{MI}]_0$ and $[\text{M}]_0$ are the initial concentrations of 3-MI and micelle, respectively:

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$$K_{eq} = \frac{[3MI{\cdot}M]}{([3MI]_0 - [3MI{\cdot}M])([M]_0 - [3MI{\cdot}M])} \quad (4)$$

We assume that the complex concentration is much less than the concentration of micelle. Therefore, $[M] \approx [M]_0$ and $[M]_0 - [3MI{\cdot}M] \approx [M]_0$. Substituting and solving for $1/[3MI{\cdot}M]$ gives

$$\frac{1}{[3MI{\cdot}M]} = \frac{1}{[3MI]_0} + \frac{1}{K_{eq}[3MI]_0[M]_0} \quad (5)$$

The association between 3-MI and a micelle is responsible for the blue-shifted emission spectra of the steady-state fluorescence and also for the increasing contribution of the short components in the time-correlated fluorescence decays. The preexponential factors of the short components are a measure of the contribution to the fluorescence from the 3-MI/micelle complex. Thus, the fluorescence intensity of the complex, 3MI·M, is proportional to the preexponential factor multiplied by its respective lifetime for each component of interest. In our case, concerning the triple-exponential global fits, the fluorescence intensity, F, of the complex is the sum of the two short-component fluorescence intensities:

$$F = A_2\tau_2 + A_3\tau_3 \propto [3MI{\cdot}M] \quad (6)$$

Using eq 6, eq 5 becomes

$$\frac{1}{F} = \frac{1}{[3MI]_0} + \frac{1}{K_{eq}[3MI]_0[M]_0} \quad (7)$$

A plot of $1/F$ vs $1/[M]_0$ should reveal a straight line for a 1:1 association between the micelle and 3-methylindole. $[M]_0$ for each solution was determined from the molar concentration of the Brij-35 and its aggregation number. The plot in Figure 6a shows our data with a linear correlation of 0.997. This suggests that our data indicate a 1:1 association. The association constant, K_{eq}, is calculated by dividing the intercept by the slope of the best-fit line through the data. K_{eq} in our case is 1.8×10^4 M^{-1}. The same procedure using the magnitude of the blue shift from the steady-state fluorescence data as a measure of the complex concentration in eq 7 also provided a K_{eq} of the same order of magnitude as previously determined.

The K_{eq} calculated above was based on the assumption that $[M] \approx [M]_0$. To obtain a better determination of the association constant without any assumptions, eq 4 was solved for the complex concentration, [3MI·M], and a nonlinear fitting procedure was employed to obtain K_{eq}:

$$[3MI{\cdot}M] = \frac{1}{2K_{eq}}\left(K_{eq}[3MI]_0 + K_{eq}[M]_0 + 1 - \sqrt{K_{eq}^2[3MI]_0^2 - 2K_{eq}^2[3MI]_0[M]_0 + 2K_{eq}[3MI]_0 + K_{eq}^2[M]_0^2 + 2K_{eq}[M]_0 + 1}\right) \quad (8)$$

In this case, because we previously showed that the association was 1:1, the complex concentration was calculated using the sum of the preexponential factors and the initial 3-MI concentration.

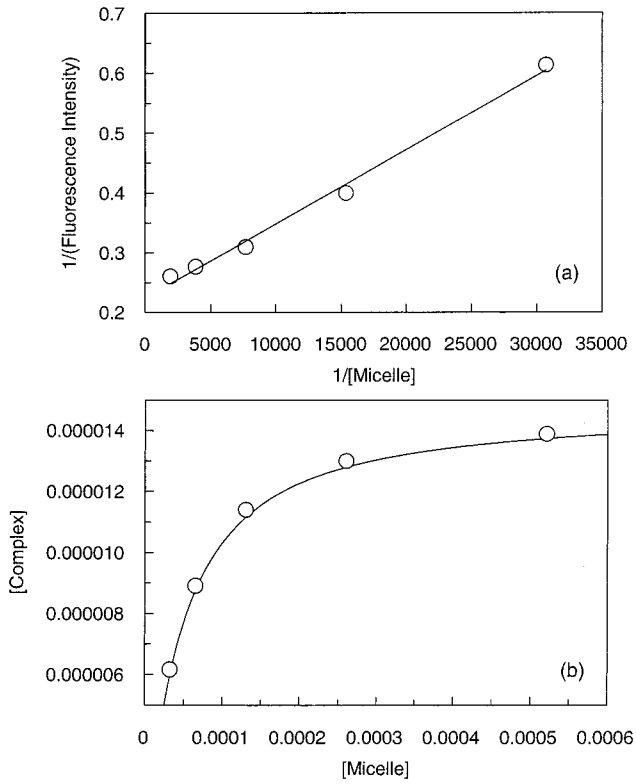

Figure 6. (a) Benesi−Hildebrand plot for the 3-MI/Brij-35 micelle complex with a linear correlation of 0.997. The value $1/F$ was determined using $F = A_2\tau_2 + A_3\tau_3$, where F is the fluorescence intensity of the chromophore that has partitioned into the micelle. A_2 and A_3 are the preexponential factors of the two shorter components in the triple-exponential global analysis; τ_2 and τ_3 are the respective lifetimes. The plot in (b) is the result of the nonlinear fit.

A plot of [3MI·M] vs $[M]_0$ was subject to a nonlinear fitting procedure, and K_{eq} was determined to be 2.6×10^4 M^{-1} (Figure 6b).

The partition coefficient, K_p, was determined to be 13.2 for our highest Brij-35 concentration, as calculated by

$$K_p = \frac{[3MI{\cdot}M]}{[3MI]_{aq}} \quad (9)$$

where $[3MI]_{aq}$ is the free uncomplexed 3-MI in solution. The concentrations were again determined from the preexponential factors from the time-correlated fluorescence decays and the initial 3-MI concentration.

In this example, the amplitudes and lifetimes obtained from the fluorescence decay profile are a direct measure of different probe environments and permit a straightforward evaluation of the association constant. Bound and unbound probes are immediately distinguished by the form of the fluorescence decay. If steady-state spectra are employed to construct a Benesi−Hildebrand plot, care must be taken to deconvolute the contributions of the bound and the unbound chromophores to the spectra.[33]

CONCLUSIONS

Indole, 1-MI, and 3-MI are hydrophobic in nature. Thus, when micelles are formed in aqueous solutions of these chromophores, they will partition into the micelle. We have shown that time-

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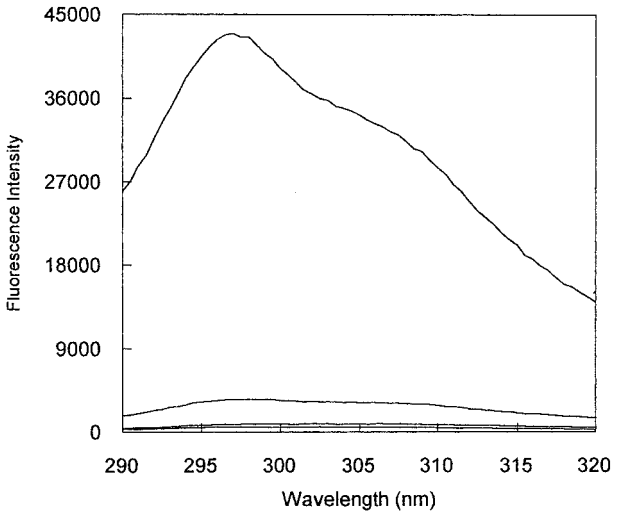

Figure 7. Fluorescence spectra of 3-methylindole vapor in equilibrium with its solid. The top spectrum is the emission of 3-MI vapor without lecithin added. The spectra of lower intensity are the emission of 3-MI vapor with the addition of dry lecithin. In each case, the 3-MI concentration was the same, 6.4 μg. For the spectra, from top to bottom, the amount of dry lecithin added was 0.0, 12.5, 25.0, and 37.5 μg, respectively. All fluorescence measurements were performed at room temperature, ~23 °C. Excitation wavelength, 280 nm; integration time, 3 s each point. The band-passes of the excitation and emission monochromators were 4 nm.

correlated single-photon counting is an effective technique for probing the chromophore/micelle interaction. The sensitivity of the indole fluorescence to the environment permits a quantitative measurement of the amount of chromophore that has associated with the micelle.

Of particular interest is the association of 3-methylindole (3-MI) with micelles. 3-MI is a volatile, noxious compound whose presence is particularly acute at swine production facilities.[1−7] 3-MI is produced in the intestine of pigs by microbial degradation of L-tryptophan and is thus one of the most common malodorous compounds found in swine manure. Along with other odorous compounds and gases formed from bacteria in the manure, 3-MI poses serious problems for the environment and the health of animals, workers, and surrounding communities.

Exploiting the interactions of 3-MI with micelles or other organized media may prove to be useful in addressing odor problems. Our results indicate an association constant to be on the order of 10^4 M^{-1} for 3-MI with Brij-35 micelles and thus demonstrate that 3-MI has a fairly high affinity for surfactant molecules. This affinity for surfactants suggests a means of reducing the amount of 3-MI that can escape into the vapor phase as an airborne contaminant. In closing, we note that 3-MI vapor is easily measured by fluorescence techniques (Figure 7) and that the experiments with model micelle systems discussed above led us to examine to what extent the 3-MI vapor is reduced by addition of a dry surfactant such as lecithin. Figure 7 demonstrates that the effect is dramatic. Notably, at the higher lecithin amounts used, the 3-MI *odor*, *as measured by nose*, was no longer detectable.

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