6-2001

Interactions between oxazine dyes and sodium dodecyl sulfate studied by molecular spectroscopy

Michael R. Webb
Union College - Schenectady, NY

INTERACTIONS BETWEEN OXAZINE DYES AND SODIUM DODECYL SULFATE STUDIED BY MOLECULAR SPECTROSCOPY

By

Michael R. Webb

****

Submitted in partial fulfillment

of the requirements for

Honors in the Department of Chemistry

UNION COLLEGE

June, 2001

Abstract

WEBB, MICHAEL R. Interactions between Oxazine Dyes and Sodium Dodecyl Sulfate Studied by Molecular Spectroscopy. Department of Chemistry, June 2001

We present the results of a fundamental investigation of how the presence of surfactant affects the self-interaction of cationic laser dyes in aqueous solution. Oxazine 1 and oxazine 170 are known to form non-fluorescent H-aggregates (π-stacked dimers). The interactions of each of these laser dyes with sodium dodecyl sulfate (SDS), a micelle forming, anionic surfactant, have been examined using UV-visible absorption and fluorescence spectroscopy, fluorescence anisotropy, and Rayleigh scattering. The colors of oxazine 170 solutions are visually distinguishable into three stages: solutions with no SDS, solutions with SDS below the cmc (8mM), and solutions with SDS concentrations above the cmc. This shows that there are interactions between the dye and the surfactant. The results of the UV-visible absorption and fluorescence spectroscopy, fluorescence anisotropy, and Rayleigh scattering show differences above and below the cmc, further indicating that there are different interactions taking place in these two environments.

Abstract

WEBB, MICHAEL R. Interactions between Oxazine Dyes and Sodium Dodecyl Sulfate Studied by Molecular Spectroscopy. Department of Chemistry, June 2001

We present the results of a fundamental investigation of how the presence of surfactant affects the self-interaction of cationic laser dyes in aqueous solution. Oxazine 1 and oxazine 170 are known to form non-fluorescent H-aggregates (π-stacked dimers). The interactions of each of these laser dyes with sodium dodecyl sulfate (SDS), a micelle forming, anionic surfactant, have been examined using UV-visible absorption and fluorescence spectroscopy, fluorescence anisotropy, and Rayleigh scattering. The colors of oxazine 170 solutions are visually distinguishable into three stages: solutions with no SDS, solutions with SDS below the cmc (8mM), and solutions with SDS concentrations above the cmc. This shows that there are interactions between the dye and the surfactant. The results of the UV-visible absorption and fluorescence spectroscopy, fluorescence anisotropy, and Rayleigh scattering show differences above and below the cmc, further indicating that there are different interactions taking place in these two environments.

Acknowledgements

I would like to thank the Union College Chemistry Department for giving me the opportunity to do this research and the education that was necessary to do it well. I would like to thank my research advisor, Professor Mary K. Carroll, for her guidance in both the research and writing aspects of this thesis. I would like to thank Dr. Katherine T. Alben and the Wadsworth Center for providing me with my first taste of research. I would like to thank my fellow chemistry majors for their support, advice, and entertainment throughout this project and the three years before that. Lastly, I would like to thank my family and friends for their support and encouragement.

Table of Contents

Table of Figures

Chapter 1

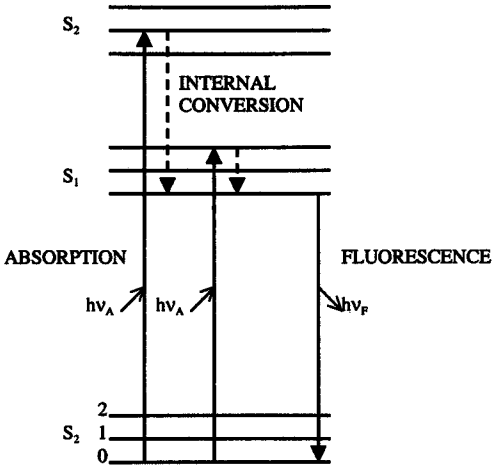
Introduction

1.1 Introduction to Spectroscopic Techniques

The spectroscopic techniques UV/Vis absorption, fluorescence, fluorescence anisotropy, and Rayleigh scattering were used in the research. The mechanisms behind these techniques are illustrated in figure 1-1 in a diagram known as a Jablonski diagram. The vertical scale in this diagram is energy. Photons are represented by curved arrows and energy state transitions are represented by straight arrows. UV/Vis absorption occurs when an atom or molecule absorbs light and uses the energy of this light to become electronically excited (to raise an electron to a higher energy orbital). Electrons in atoms and molecules can only exist in specific energy levels known as orbitals. Because these orbitals have specific energies associated with them and light exists in discrete packets (known as photons) with energy inversely proportional to their wavelength, only light of certain wavelengths (those wavelengths corresponding to the difference in energies between two orbitals) will be absorbed. The photons that cause these transitions generally have wavelengths in the ultraviolet (UV) or visible range. This process is shown by the leftmost upwards pointing arrow in figure 1-1.

Once an atom or molecule is electronically excited, it can lose this energy in several ways. One of these ways is by emitting photon through a process known as fluorescence, which occurs approximately 10^{-8} s after absorption (1). The emitted photon cannot have any more energy than the exciting photon. Some of the energy will be lost to vibrational relaxation before fluorescence occurs. This rapid (10^{-12} s) energy loss is called internal conversion (1). Because of this loss of energy and because the transition

.Figure 1-1: Jablonski Diagram
Adapted from Figure 1-3 from Lakowicz (1)



that releases a photon may not be to the lowest energy orbital, the photon emitted will ordinarily have lower energy than the photon that caused the excitation. This lower energy corresponds to a longer (redder) wavelength, so we say that fluorescence is red-shifted from absorbance.

When polarized light is used for excitation, the resulting emission is polarized. This polarization results from photoselection by the molecules based on their orientation relative to the direction of the polarized excitation (1). The emission can be depolarized due to rotation of the molecule during the approximately 10^{-8} seconds between absorption and emission (1). The degree of depolarization is an indication of how much rotation occurs in this brief time period. The amount of rotation is dependent on the solution's viscosity and the size and shape of the molecule. The degree of depolarization is generally given as the anisotropy (r). For the case that light polarized along the z axis and travelling along the x axis is used for excitation, anisotropy is defined

$$r = (I_z - I_y)/(I_z + I_y + I_x) \qquad (1.1)$$

where I_x, I_y, and I_z are the intensities of the portions of the fluorescence polarized along the x, y, and z axes, respectively. Since the radiation emitted by a fluorophore is symmetrically distributed along the z axis, $I_y = I_x$ (1). Because of this, anisotropy can be alternatively written

$$r = (I_z - I_y)/(I_z + 2I_y) \qquad (1.2)$$

These values needed for this claculation usually differ from the values that are measured directly because monochromators transmit vertically and horizontally polarized light with different efficiencies. We can define the directly measurable values in terms of I_x, I_y, and I_z by including three other terms. S_V and S_H are the selectivity of the detection system for

vertically and horizontally polarized light, respectively. K is the intensity ratio of vertically polarized excitation light to horizontally polarized excitation light. The measurable values are given in the form I_{HV}, where the first subscript indicates the orientation of the excitation polarizer and the second indicates the orientation of the emission polarizer.

$$I_{HH} = K\, S_H I_x = K\, S_H I_y \tag{1.3}$$

$$I_{HV} = K\, S_V I_y \tag{1.4}$$

$$I_{VV} = S_V I_z \tag{1.5}$$

$$I_{VH} = S_H I_x = S_H I_y \tag{1.6}$$

Combining equations (1.3) and (1.4) we find

$$I_{HV}/I_{HH} = (K\, S_V I_y)/(K\, S_H I_y) = S_V/S_H \tag{1.7}$$

We define a quantity called the G factor such that

$$G = S_V/S_H = I_{HV}/I_{VV} \tag{1.8}$$

We can also combine equations (1.5) and (1.6) to find

$$I_{VV}/I_{VH} = (S_V I_z)/(S_H I_y) = G(I_z/I_y) \tag{1.9}$$

Combining equations (1.9) and (1.2), we find

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}) \tag{1.10}$$

We have now found an equation to relate our experimental values to anisotropy (1).

Photons with energies not equal to the difference between orbital energy levels can also be absorbed and almost immediately reemitted. The emitted photons will be of the same wavelength as the absorbed ones, but will be scattered into a random direction. This process is known as Rayleigh scattering. Because the intensity of this type of

scattering is proportional to the dimensions of the scattering particles, it can be used to probe the size of molecular aggregates (2).

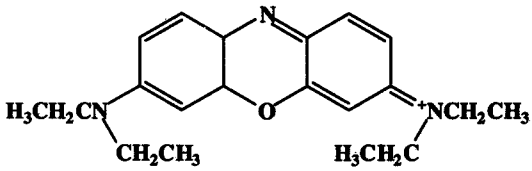
1.2 Oxazine 1 and Oxazine 170

The dyes oxazine 1 and oxazine 170 (structures shown in figure 1-2) were studied using the spectroscopic techniques discussed above. These dyes were chosen because they have chemical structures similar to methylene blue, a dye used in earlier research in this laboratory (3). In aqueous solution, these dyes tend to form dimers with different spectral characteristics than their monomers.

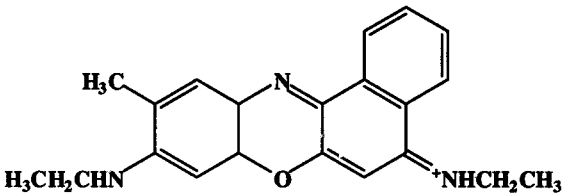
H and J aggregate dimers both consist of two dye molecules with parallel transition moments. The lowest excited state is split into two states, one with energy slightly higher than the usual state and one with energy slightly lower than the usual state (see figure 1-3) (4). Oxazine dyes tend to form H aggregates, which have the dimers stacked on top of each other like pieces of bread in a sandwich. In H aggregates, only transitions to the higher of the two states are allowed (4). This means that the light absorbed by these dimers to make this transition will be of higher energy, and therefore blue-shifted from the light absorbed by the monomer. For oxazine 1, the absorbance maxima occur at 654 nm for the monomer and 599 nm for the dimer. For Oxazine 170, the absorbance maxima occur at 623 nm for the monomer and 583 nm for the dimer (3). The monomers of both of these dyes fluoresce, but fluorescence is almost eliminated in the dimers due to rapid internal conversion to the lower energy state (3).

Figure 1-2: Structures of Dyes

Oxazine 1



Oxazine 170



Methylene Blue

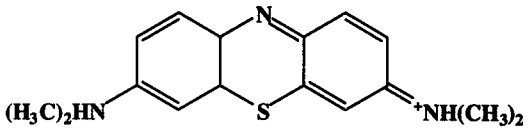
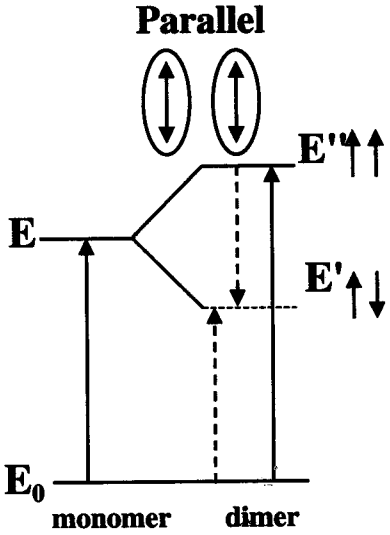


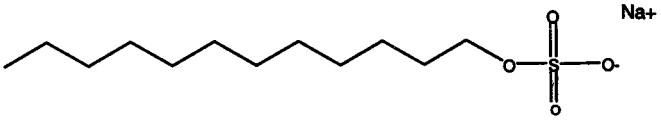
Figure 1-3: H-aggregate Splitting of Excited State
Adapted from Steinhurst and Owrutsky (4)



Surfactants, such as SDS, consist of polar, hydrophilic ends attached to long nonpolar organic chains (see the structure of SDS in figure 1-4). The presence of surfactants increases the free energy of a system by distorting the structure of the water molecules (5). Above a certain concentration, it becomes more energetically favorable for further surfactant molecules to cluster together than to remain separate. This is called the critical micelle concentration (cmc), and these clusters are called micelles. In aqueous solutions, micelles will usually form as spheres with the polar ends on their surfaces and the nonpolar organic chains on the inside. The organic micelle interiors will essentially become pockets of another solvent within the aqueous solution. Nonpolar molecules will tend to migrate into this region and will experience a much different environment there than they would in the bulk aqueous solution (6,7).

Dyes have been known to change spectral characteristics at the cmc and to alter the cmc (5). In this study we observed both the effects of the presence of the oxazine dyes on the cmc and the effects of SDS above and below the cmc on the spectral characteristics of the dyes. Based on their structures, it should not be surprising that SDS and the oxazine dyes readily interact. All three species have both organic and polar parts, so they can interact with each other through non-polar interactions, polar/ionic interactions, or both. Since we know that dye-dye interactions occur and change the spectral characteristics of oxazine 1 and oxazine 170, it should not be surprising that when dye-surfactant interactions occur, changes in spectral characteristics are observed. In addition to dye-dye and dye-solvent interactions, dye-surfactant and dye-micelle interactions are possible in solutions containing both dyes and surfactants (5). At concentrations below the normal cmc, surfactants and dyes can interact to form a mixed

Figure 1-4: Structure of SDS



micelle of the two species, lowering the observed cmc (7). Also below the cmc, dye-surfactant dimers can form (7). Since some of the oxazine monomers are now tied up in these dimers, spectral effects characteristic of the monomers decrease. These include fluorescence and absorption at the wavelengths characteristic of the monomers. Above the cmc, there are spectral changes due to deaggregation of the dye and a change in the molecular environment of the dye due to incorporation into the micelle interior (7). This red-shifts the absorption spectra (7).

1.3 Diode Lasers in Spectroscopy

For parts of the research presented in this thesis, we used a home-built instrument with a diode laser. Lasers have several properties that can make them well suited to being used as a light source in spectroscopy. They have several advantages over more traditional light sources such as lamps. Lasers are highly monochromatic (8), which gives them very good spectral resolution without use of a monochromator. They have good beam focusing ability (8), which gives them very good spatial resolution. Because they have a higher photon flux, they are able to increase sensitivity and lower the limit of detection of fluorescence and scattering measurements (9). Because fluorescence and scattering measurements are both measurements made against a background signal of nearly zero, increasing the intensity of the source increases the relative change in signal with an analyte present. It is this relative change that determines the sensitivity. Higher photon flux also shifts the dynamic range of absorbance measurements to higher concentrations (9). This is because a larger flux increases the signal against which

absorption is measured relative to without having much of an effect on the absolute amount of that signal that is absorbed. The relative change in signal is smaller, so there is less sensitivity. A large flux is necessary for a significant amount of light to pass through concentrated solutions, but lowered sensitivity is a disadvantage if you are dealing with dilute solutions. There are several other disadvantages of lasers that have prevented their widespread adoption. Most types of lasers are large, expensive, difficult to maintain, unstable, generally not tunable, and have limited lifetimes (8, 10).

Diode lasers do not possess some of these disadvantages. Diode lasers are compact, relatively inexpensive, low maintenance, highly stable, and have long lifetimes (8, 10, 11). They are compact and inexpensive enough for use in portable CD players. Output stability as low as <0.001% noise (8) and lifetimes on the order of 10 times as long as traditional lasers (50000 h *vs.* 5000 h) have been reported (10). Even so, there are still disadvantages to diode lasers. They are generally still limited to a single wavelength, and this wavelength is almost always in the near-IR or red region of the electromagnetic spectrum (10). While this wavelength range can be useful because it is unlikely that there will be background fluorescence interference, there are few analytes that fluoresce in this region (10).

1.4 Instrumental Design

Scattering measurements were performed with a home-built instrument using such a diode laser as a source (9, 12). This instrument is compact (about 7"x7"x3.5"), durable, and uses very little power. It is capable of measuring transmittance, scattering,

and fluorescence. Scattering and fluorescence measurements are made at an angle of 90 degrees to the diode laser source and transmittance measurements are made at an angle of 180 degrees to the diode laser. The detector for these measurements can either be a photodiode detector or a photomultiplier tube (PMT). Photodiodes are solid state devices that absorb photons and produce a current proportional to the intensity of the light. Like other solid state devices, they are reliable, small, and inexpensive (9). A photodiode is much less sensitive than a PMT, but the intensity of the laser source can help to offset this lack of sensitivity enough to make it a useful detector in this instrument. Neither of these detectors are wavelength specific, so line filters are needed to select wavelengths so that fluorescence and scattering signals can be differentiated. Line filters were chosen over monochromators because line filters are more easily integrated into compact instruments and are less expensive.

Chapter 2

Methods

Stock solutions of 30 μM oxazine 1 (Aldrich) and of 30 μM oxazine 170 (Aldrich) in deionized water were diluted to prepare all other oxazine solutions. These solutions were approximately 6 months old. Due to the tendency of dyes to adhere to the containers they are stored in, 30 μM actually represents the maximum concentrations; the actual concentrations are not precisely known (1). Stock solutions of 20 mM SDS (Sigma-Aldrich) were also prepared in deionized water.

Solutions used in these experiments were prepared using combinations of these three stock solutions and deionized water to dilute to a total volume of 3 mL. Where the dyes were used, they were present in a concentration of 3 μM. The experimental concentration of SDS ranged from 0 to 18 mM.

Fluorescence emission, fluorescence anisotropy, UV/Vis absorption, transmittance, and Rayleigh scattering measurements were performed on the sample solutions. Fluorescence measurements were taken using a PTI Quantmaster Fluorometer. Polystyrene cuvettes were employed. Excitation and emission slitwidths were set at 2 nm. The excitation wavelength was set at 644 nm for oxazine 1 solutions and 624 nm for oxazine 170 solutions. The emission spectra were recorded from 634 to 800 nm for oxazine 1 solutions and from 654 to 800 nm for oxazine 170 solutions.

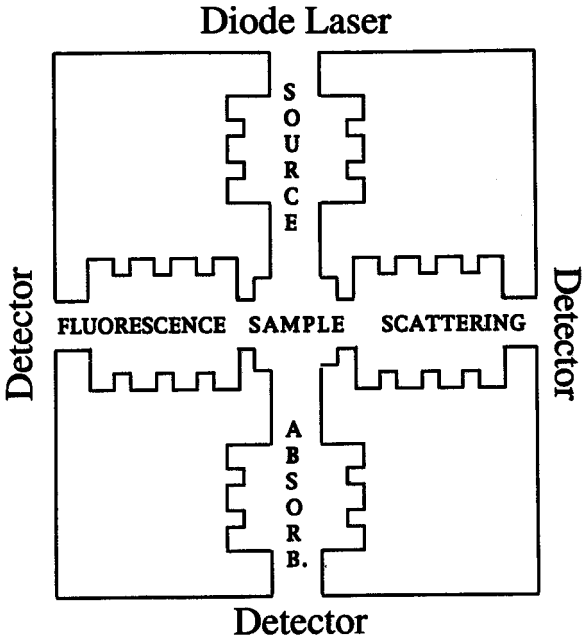
Fluorescence anisotropy measurements were taken with the same instrument. Quartz cuvettes were used for all anisotropy measurements to avoid anisotropy inherent in the polystyrene cuvettes. The measurements were taken with two different techniques. The first used polarizing filters (Edmund Scientific) oriented by hand and with the same excitation and emission wavelengths that were used for the fluorescence emission measurements. The reported anisotropy for these measurements is the mean of the

anisotropy measured over these wavelengths. The measurements were repeated using a second technique. This technique used motorized polarizing filters. The excitation wavelengths were the same as above. The emission intensity was measured for each sample once a second for a period of 60 seconds at a single wavelength. This wavelength was 670 nm for oxazine 170 and 648 nm for oxazine 1. The excitation and emission slitwidths were increased to 3 nm for measurements on oxazine 1 solutions. The excitation and emission slitwidths were increased to 5 nm for measurements on solutions of oxazine 170 with 6mM SDS or less. Due to the large increase in fluorescence intensity of oxazine 170 in the presence of SDS concentrations at and above the cmc, measurements on solutions with 8 mM SDS or greater were performed with excitation and emission slitwidths of 3 nm. Measurements on oxazine 170 solutions with SDS concentrations between 6 and 8 mM were performed both with 3 nm slitwidths and with 5 nm slitwidths. This was done to verify that the two sets of data were comparable.

Absorption spectra were taken on a Hewlett Packard HP8453 Diode Array Spectrophotometer. We measured the absorbance of the oxazine 1 solutions and of the oxazine 170 solutions from 400 to 800 nm. Polystyrene cuvettes were employed.

Rayleigh scattering measurements and transmittance measurements were taken with a home-built instrument (figure 2-1). The light source was a 670 nm diode laser. A lens in the source channel was used to focus the light on he sample. Transmittance was measured along a 180-degree path through the sample by a photodiode detector. A lens followed by a 671 nm line filter was placed along the path. The purpose of the line was to reduce the total light. Scattered light was measured along a 90-degree path through a quartz cuvette containing the sample, by a photomultiplier tube (PMT). A lens was

Figure 2-1: Instrumental Design



placed along the scattering path. For scattering measurements, the lens was followed by a 671 nm line filter to block fluorescence. The specifications and exact positions of these lenses are detailed in Andrew Leach's thesis (9).

Chapter 3

Results

3.1 Visual Observations & Absorbance Measurements

There was no obvious visual difference between the various oxazine 1/SDS solutions. They were all the same shade of light blue. There were obvious visual differences between some of the oxazine 170/SDS solutions. The oxazine 170 solutions containing 1.8 and 3.6 mM SDS were both purple. The solution containing 5.4 mM SDS was also purple, but was a slightly bluer shade. The solutions containing 7.2, 9.0, 10.8 mM SDS were all the same shade of blue. The solution without SDS was a slightly darker shade of blue.

Absorbance data for the oxazine 170 solutions correspond with these observations. The spectra of the solutions containing 7.2, 9.0, and 10.8 mM SDS had a peak at approximately 630 nm with a shoulder at shorter wavelengths. The spectra of the solutions with 1.8 and 3.6 mM SDS had one peak at approximately 575 nm. The spectrum of the solution with 5.4 mM SDS had peaks at both of these wavelengths; the peak at 575 nm was larger than the peak at 630 nm. The spectrum of the solution without SDS had a peak at approximately 620 nm with a shoulder extending to shorter wavelengths.

Despite the lack of obvious visual differences between the various oxazine 1/SDS solutions, there were significant differences in the absorption spectra. The absorption spectra of the various solutions all showed a peak at approximately 655 nm with a shoulder extending to shorter wavelengths. The shorter wavelength shoulder (approximately 600 nm) showed roughly the same absorbance for all the samples. The intensity of the 655 nm peak, decreased as the SDS concentration increased from 0 to 2 to

4 mM. After this, the absorbance increased and then leveled off for the solutions containing 8 and 10 mM SDS.

3.2 Fluorescence

The fluorescence spectra of the oxazine dyes also varied with SDS concentration. All the fluorescence spectra of oxazine 170 showed a peak at approximately 645 nm. As shown in figure 3-1, the intensity of the peak was relatively high in the absence of SDS. At concentrations of SDS below the cmc, the intensity dropped sharply to less than 10% of the original intensity. It then increased sharply between the solutions containing 5.4 mM and 7.2 mM SDS and continued to increase for the solution containing 9.0 mM and 10.8 mM SDS. For the solution containing 10.8 mM SDS, the intensity was three times as large as the original intensity.

The fluorescence spectra of oxazine 1 showed similar variations with SDS concentration. There was a single peak at approximately 670 nm. As shown in figure 3-2, the intensity of this peak decreased as the SDS concentration increased from 0 mM to 2 mM to 4 mM. The fluorescence intensity of the solution containing 4 mM SDS was approximately 20% of the original intensity. The drop was continuous for oxazine 1, not sudden as it was for oxazine 170. The intensity rose very sharply for the solution containing 6 mM SDS, then leveled off at about twice the original intensity for the solutions containing 8 mM, 10 mM, and 12 mM SDS. This suggests a cmc close to 6 mM for 3 μM oxazine 1 solutions.

Figure 3-1: Fluorescence of Oxazine 170 (3μM) / SDS Solutions

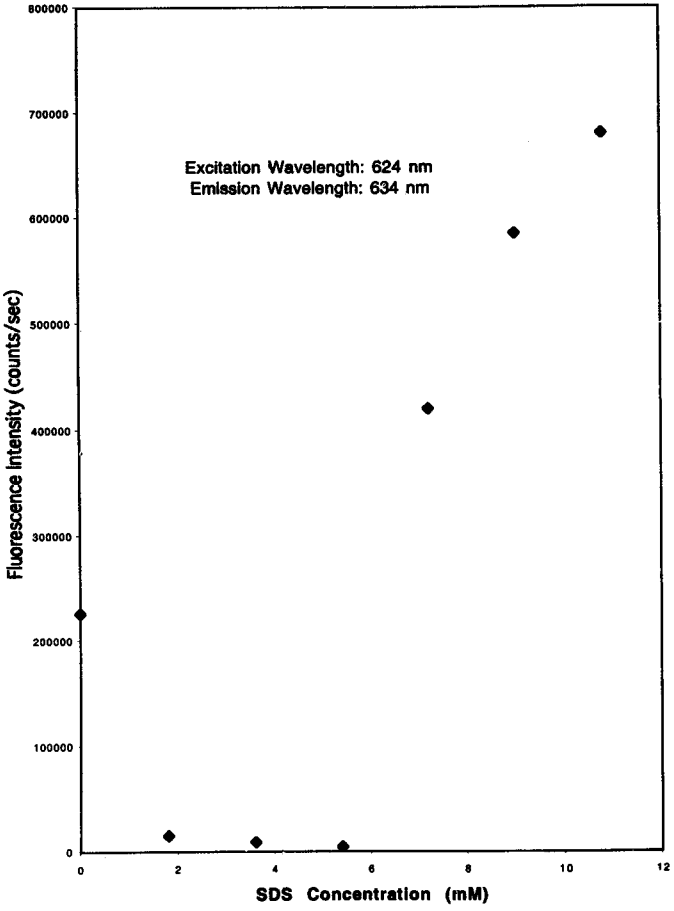
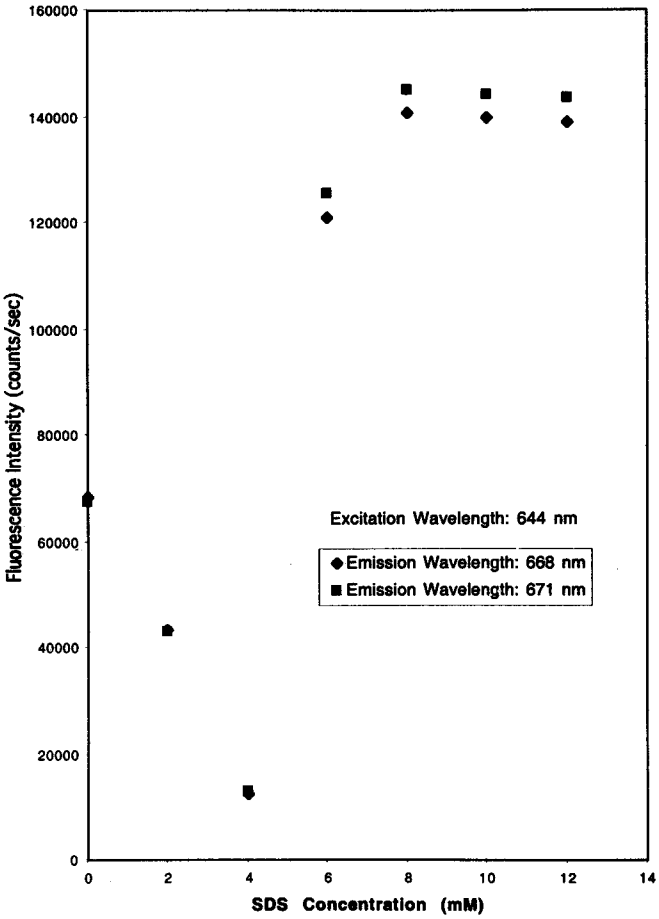


Figure 3-2: Fluorescence of Oxazine 1(3μM) / SDS Solutions



To help in the interpretation of these results, we also studied the affect of solvent polarity on fluorescence intensity. The solvent varied from pure DI water to 90% ethanol, 10% water by volume. Fluorescence intensities of oxazine 1 and oxazine 170 as a function of percent ethanol by volume are shown in figures 3-3 and 3-4, respectively. As these figures show, fluorescence intensity increases as % ethanol increases. The increase in fluorescence intensity between aqueous solution and 90% ethanol, 10% water solution is threefold for oxazine 170 and twofold for oxazine 1.

3.3 Transmittance and Scattering

The oxazine solutions' transmittance of the 671 nm diode laser light shows less variation with SDS concentration. For aqueous SDS solutions with no dye, the transmittance seemed to drop between 8 mM and 10 mM solutions, but the difference was small enough compared to the signal that no clear trend was seen. For aqueous solutions of oxazine 170 and SDS, the trend was also unclear.

Using the photodiode detector, we attempted to measure the scatter of aqueous solutions of SDS and aqueous solutions of SDS and oxazine 170. There was very little variation in the intensity of scatter detected and what scatter there was seemed to show a large degree of randomness. Experiments monitoring single samples over a period of 15 minutes showed that there was a decrease, then leveling off of scattering intensity detected over time. Because of this, we tried to allow time for the photodiode to stabilize before taking measurements. The randomness in the results seemed to decrease, but no trend was clear. We tried using quartz cuvettes instead of polystyrene and found that the

Figure 3-3: Fluorescence Intensity of Oxazine 1 as a Function of % Ethanol

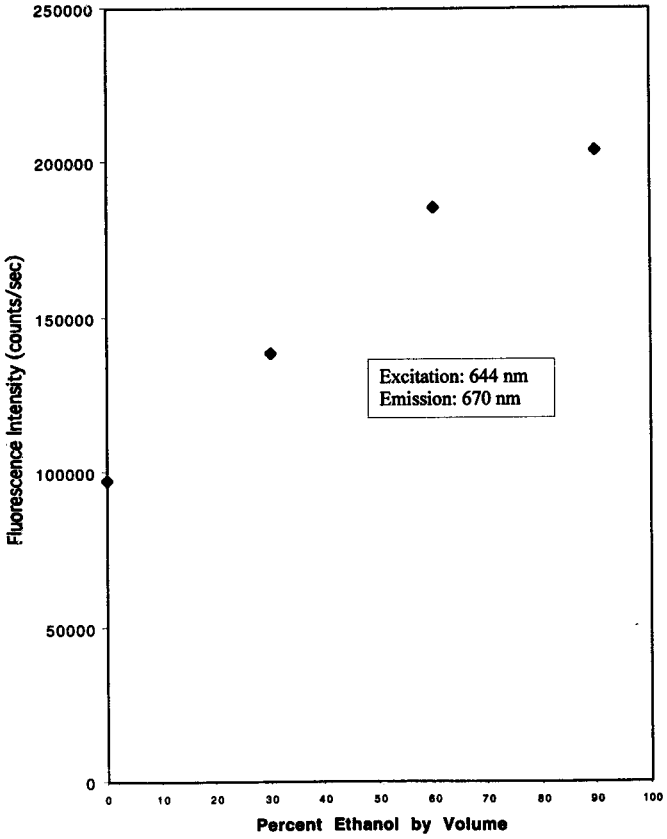
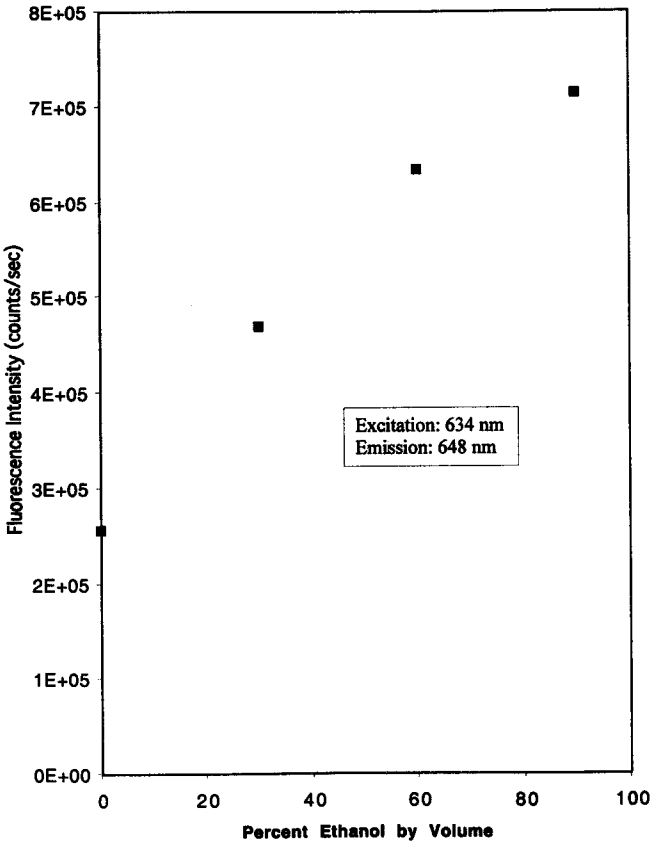


Figure 3-4: Fluorescence Intensity of Oxazine 170 as a Function of % Ethanol



trend was clearer, probably due to the lack of variations in quartz cuvettes that would have occurred in polystyrene cuvettes. The quartz cuvettes were rinsed with ethanol and several times with water between samples. We also tried filtering the samples. While this seemed to decrease the noise in measurements somewhat, it ultimately proved to be unnecessary.

To improve our results, we tried using a photomultiplier tube as our detector. We were then able to see the trend shown in figure 3-5. For samples containing SDS and no dye, the scattering intensity increased slightly with increasing SDS concentration until the cmc (8 mM) when it increased sharply. It then stayed fairly constant at this higher level. There was still a large amount of noise and a fairly large background, so we took steps to reduce the amount of external light penetrating the system and reaching the detector before measuring the scattering of the SDS/oxazine solutions.

The data acquired for solutions of SDS and oxazine 170 showed a much more obvious trend, as seen in figure 3-6. The scatter intensity was stronger for the solution containing 1.7 mM SDS than for the one with no SDS. After this, the intensity decreased with rising concentration of SDS through the solution containing 5.0 mM SDS. It then rose for the solutions containing 6.7 mM SDS and above. The intensity started to level off at higher concentrations, but was still increasing slightly at an SDS concentration of 18.0 mM.

The scattering intensity of oxazine 1/SDS solutions showed a similar trend, as shown in figure 3-7. Intensity dropped as SDS concentration increased up to an SDS concentration of 5.0 mM. The scattering intensity then rose sharply for the solution containing 6.7 mM SDS, rose again for the solution containing 8.3 mM SDS, and then

Figure 3-5: Scatter vs. SDS Concentration

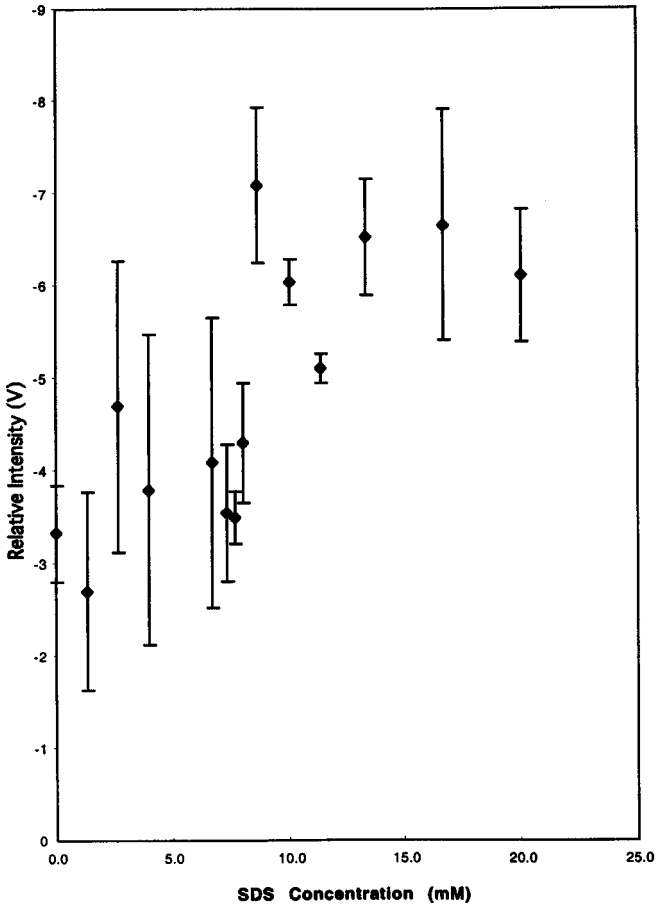


Figure 3-6: Scattering of Oxazine 170 (3μM) / SDS Solutions

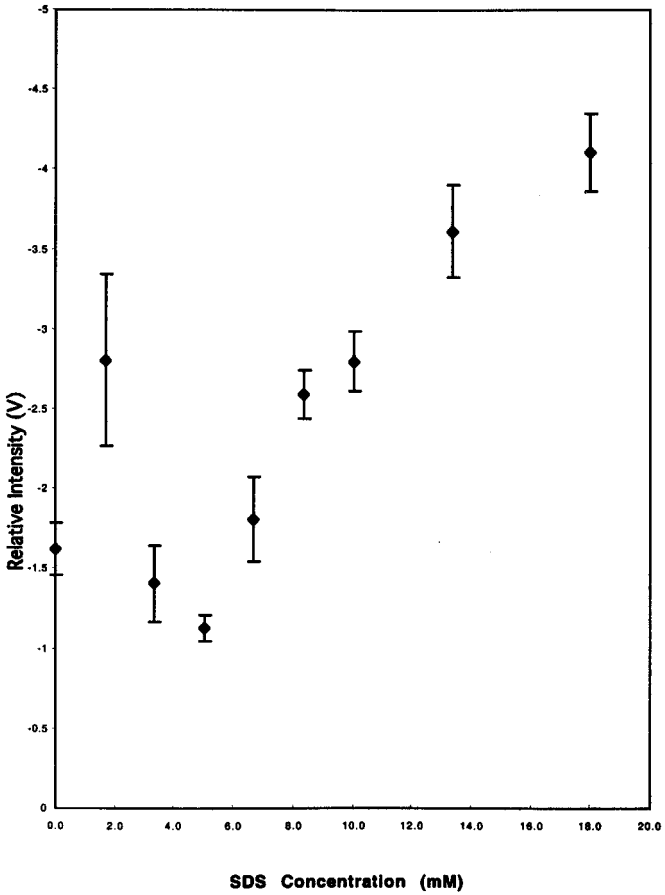
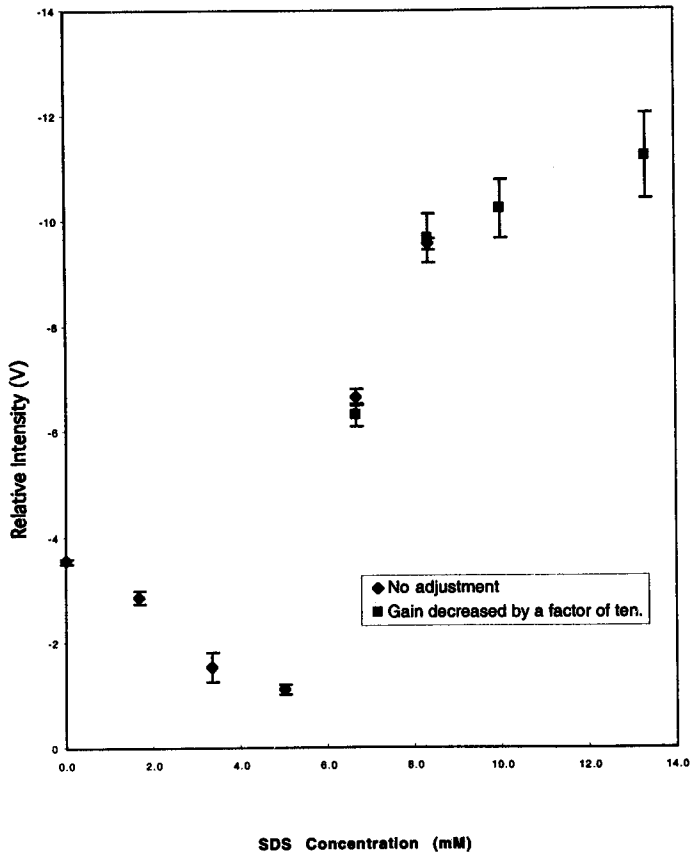


Figure 3-7: Scattering of Oxazine 1 (3 μM) / SDS Solutions



remained fairly constant for the remaining solutions (10.0 and 13.3 mM SDS). The signal-to-noise ratio was noticeably higher for the scattering of the oxazine 1/SDS solutions than the scattering of the oxazine 170/SDS solutions.

3.4 Anisotropy

The anisotropy of SDS/oxazine 170 solutions as a function of SDS concentration can be seen in figure 3-8. The anisotropy of the oxazine 170 solutions without SDS is 0.03. Increasing SDS concentration to 4mM has little effect on the anisotropy of the dye. Above this point, the anisotropy begins to increase and it increases sharply between the solutions containing 4 mM and 6 mM SDS. The anisotropy decreases slightly for the solutions containing 7.2 and 7.7 mM SDS, then increases again for the solution containing 8 mM SDS. It levels off at 0.09 for solutions of 10 mM SDS and above.

The anisotropy of SDS/oxazine 1 solutions shows a different trend, as seen in figure 3-9. Without SDS, the anisotropy of oxazine 1 is 0.11. At 2 mM SDS, it increases to 0.12. After this it drops off slowly, reaching 0.09 at 5 mM SDS before dropping sharply to 0.03 at 6 mM SDS. It then increases sharply before leveling off to a value of 0.14 at and above 8 mM SDS.

Figure 3-8: Anisotropy of Oxazine 170 (3 μM) / SDS Solutions

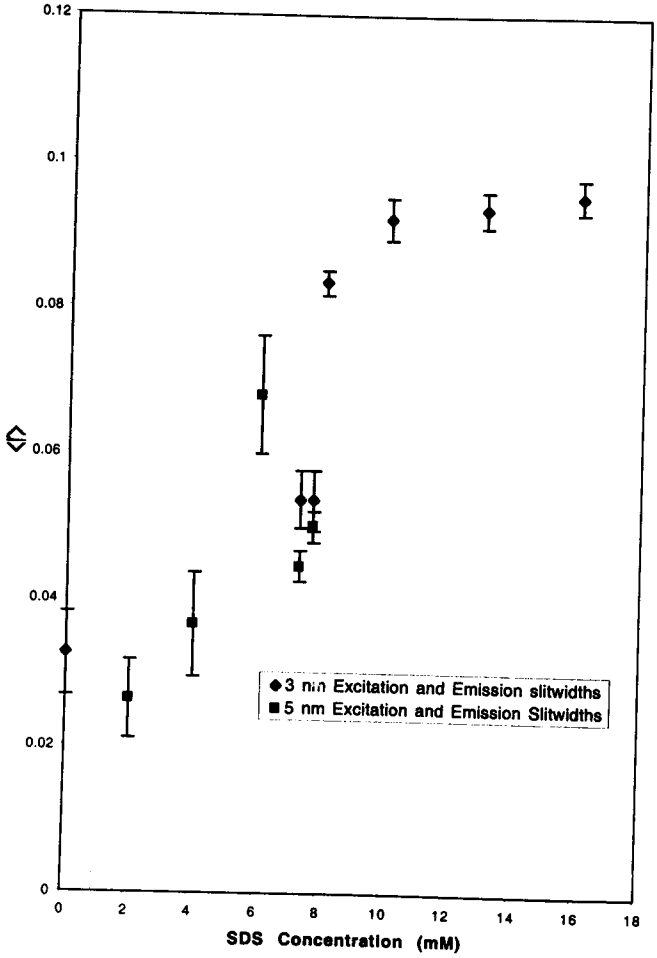
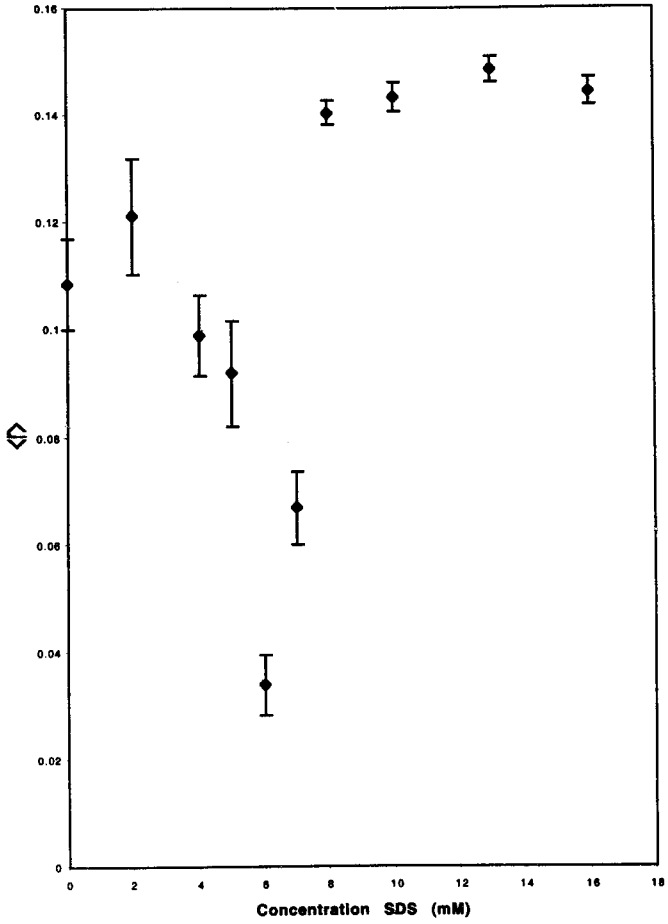


Figure 3-9 Anisotropy of Oxazine 1 (3 μM) / SDS Solutions



Chapter 4

Discussion

4.1 Visual Observations and UV/Vis Absorbance

Visual examination of the oxazine 170 / SDS solutions reveals evidence of different interactions between the dye and the surfactant above the cmc as compared with below it. This evidence is in the form of the solutions' colors. The oxazine 170 solutions that contain SDS at concentrations below the cmc are purple and the solutions with SDS concentrations above the cmc are blue. The cmc based on this color trend (approximately 6 mM) is lower than the cmc of SDS without dye in aqueous solutions (8 mM). This lowering of the cmc is also observable in the absorbance, fluorescence emission, scattering, and fluorescence anisotropy measurements for both dyes. This lowering of the cmc suggests that the dye and surfactant are forming mixed micelles (5).

The absorption spectra of the oxazine 170 / SDS solutions show that the variation in color is the result of variation in the relative sizes of the monomer and dimer absorbance peaks at 623 nm and 583 nm, respectively. For oxazine 170 solutions without SDS or with SDS concentrations below the cmc, the dimer absorbance is nearly constant. For these solutions, the monomer absorbance decreases with increasing SDS concentration. Above the cmc, the monomer absorbance increases to a value slightly higher than the monomer absorbance in the oxazine 170 solution without SDS, and the dimer absorbance begins to decrease with increasing SDS concentration.

While there is no obvious (visually detectable) evidence of different interactions above and below the cmc between oxazine 1 and SDS, there is such evidence in the absorption spectra. The absorbances of the monomer (at 654 nm) and the dimer (at 599 nm) vary with SDS concentration. As with the oxazine 170 / SDS absorption spectra, the

absorbance of the oxazine 1 monomer decreases with increasing SDS concentration below the cmc. Above the cmc, the monomer absorbance increases until it reaches a value slightly higher than the absorbance of the monomer in solutions containing no SDS. In the case of oxazine 1, however, there is no apparent decrease in dimer absorbance at high SDS concentrations.

This data shows that, below the cmc, the dye monomer concentrations decrease with increasing SDS concentration without increasing the dimer concentration significantly. This is due to an interaction between the SDS and dye, probably one in which they form ion pairs or clusters. Such a dye-surfactant salt could form a precipitate but with just 3 μM of the dye, the precipitate would be present in such low concentrations that it may be suspended in the solution and too dilute to make the solution cloudy (13). Above the cmc, the dye monomer concentrations increase, showing that the dye molecules have been released from the ion pairs or clusters in which they were bound. The new interaction between the dye and micelle is more favorable than the interaction between the dye and the SDS monomers or small aggregates that occurred below the cmc. In the case of oxazine 170, this interaction also competes favorably with dimer formation, as shown by the decreased dimer absorbance above the cmc.

As can be seen in figure 1-1, the oxazine 170 has one more ring than oxazine 1. Because oxazine 1 lacks the extra ring, the π–π interactions between oxazine monomers are weaker so that they compete less favorably with the electrostatic repulsion (4). The less favorable competition results in oxazine 1 species not forming dimers as favorably as oxazine 170 does. Because of this, dimer formation is not a significant competitor with

either dye-surfactant salt formation or dye-micelle interactions, as shown by the low absorbance of the oxazine 1 dimer (at 599 nm) at all observed concentrations of SDS.

4.2 Fluorescence

Similar trends in fluorescence intensity are observed for both dyes. Compared to solutions without SDS, dye solutions that contain SDS at concentrations below the cmc fluoresce weakly. At and above the cmc, the fluorescence intensities increase.

Since oxazine 1 and oxazine 170 dimers do not fluoresce significantly, fluorescence data can be used to investigate the monomer (3). We again see evidence that the monomer is tied up below the cmc. For oxazine 170 solutions, the fluorescence intensity drops sharply with 2mM SDS present. After this initial sharp drop, increasing concentration of SDS further causes the fluorescence intensity to decrease gradually until the cmc. This suggests, as the absorbance data does, that the free monomer is tied up in ion pairs or clusters. Above the cmc, the fluorescence intensity increases to more than three times that of the oxazine 170 solution without SDS. This shows that above the cmc the dye is freed from the interaction just discussed and that there is another interaction above the cmc. This new interaction increases the fluorescence intensity, which suggests that the interaction involves the interior of the micelles. It is known that the interior of micelles can enhance fluorescence of some dyes if the lower polarity of the micelle interior can stabilize the excited state (6). Experiments where the polarity of the solution was varied by varying the ratio of water to ethanol in the solvent showed that oxazine 170 fluorescence increases with decreasing polarity. Additionally, dyes incorporated into

micelle interiors are known to exhibit increased fluorescence because the micelle shields the dye from vibrational quenching by the hydrogen-bond structure of water, by water, or by other quenching species present in the bulk solution that are insoluble in micelles (6).

The fluorescence intensity of the oxazine 1 solutions follows similar trends with SDS concentration. The decrease in fluorescence intensity with increasing SDS concentration below the cmc is not as sharp as it is for oxazine 170, and the fluorescence intensity plateaus at approximately twice the intensity of the solution without SDS. Otherwise, the trends are the same, and my interpretations of the trends are the same, with the exception that a larger amount of SDS is required to tie up oxazine 1 than is required to tie up oxazine 170.

4.3 Scattering

The scattering data provides further evidence for these interactions. Since SDS scatters strongly and oxazine 1 and 170 do not, scattering can be used to probe the environment of SDS. As shown in figure 3-5, for solutions containing SDS but no dye, scattering is relatively constant until the cmc. We can not be sure that the scattering does not change because the error in the measurement is large compared to the trend. At the cmc, scattering increases sharply before leveling off above the cmc at a value approximately twice as high as scattering below the cmc. This shows that SDS micelles scatter more strongly than SDS monomers and small aggregates.

Scattering of oxazine 1 / SDS solutions as a function of SDS concentration follows a slightly different trend. This trend is easier to see because the relative amount

of noise in the measurement is much smaller, as is shown by the small error bars in figure 3-7. Below the cmc, scattering decreases with increasing SDS concentration. This may be an effect of the SDS on scattering that was masked by the large level of noise in the measurements of the scattering of SDS without dye present. Because SDS reduces surface tension, there are fewer small bubbles in the bulk of the solution. These bubbles contribute significantly to scatter, so the decrease in scattering may be due fewer bubbles being present. The low level of scattering suggests that any dye-surfactant aggregates formed below the cmc are relatively small. Scattering increases sharply at the cmc, then levels off above the cmc. This provides more evidence that, above the cmc, the SDS forms micelles preferentially to the SDS-dye ion pairs or clusters.

Scattering of oxazine 170 / SDS solutions as a function of SDS concentration shows a similar trend, as seen in figure 3-6. The general trend below the cmc is again one of decreasing scattering with increasing SDS concentration, but there is an initial spike in scattering with a small amount (2 mM) of SDS present. This spike may be due to scattering by some sort of dye-surfactant aggregate such as an ion pair. Above the cmc, scattering increases gradually with increasing SDS concentration, and the scattering intensity has not completely plateaued at an SDS concentration of 18 mM. This suggests that the formation of mixed micelles is not as favorable for the oxazine 170 / SDS solutions as it is for the oxazine 1 / SDS. Even at the cmc, enough of oxazine 170 is tied up in dimers that there is less monomer available to help form mixed micelles. Above the cmc, these dimers are broken apart because it is more favorable for the oxazine monomers to interact with the micelles.

4.4 Anisotropy

The observed change in anisotropy of oxazine 170 / SDS as a function of SDS concentration also provides evidence of different types of interactions above and below the cmc. Upon addition of SDS, the anisotropy initially appears to decreases slightly, but this decrease is approximately one standard deviation so it may not be significant. Anisotropy then increases gradually with increasing SDS concentration until the cmc. This is evidence of interactions with the SDS that reduce the dye's ability to rotate. A dye-surfactant ionic pair or cluster would result in such a decreased ability to rotate because of its larger size than an individual dye ion. The increase with increasing SDS concentration probably means that more SDS molecules join the clusters as the SDS concentration increases. Around the cmc, the anisotropy drops but remains higher than the anisotropy of a solution without SDS. This dip may be caused by the SDS forming micelles preferentially to ionic interactions with the dye. This could free up some of the dye molecules from their ionic interactions with the SDS monomers. Above the cmc, the anisotropy increases sharply before leveling off at two to three times the anisotropy of oxazine 1 with no SDS present. This shows that the dye is even less free to rotate. SDS micelles contain a large number of SDS monomers (around 70), so they rotate slowly. A dye molecule trapped within such a micelle would also rotate slowly, causing a high anisotropy.

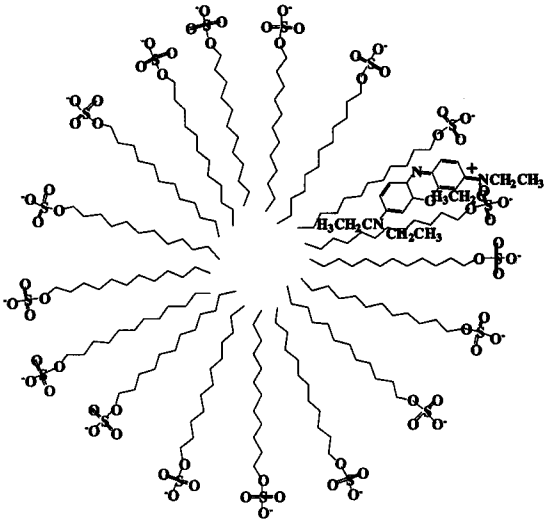
The anisotropy trend of oxazine 1 / SDS solutions is significantly different from the trend just discussed for oxazine 170. The anisotropy of a solution without SDS is three to four times as high as it was for the equivalent situation with oxazine 170. The

anisotropy appears to increase slightly in solutions with a relatively small amount of SDS (2 mM), but this increase may not be significant because it is small relative to the error in the measurements. After this, the anisotropy decreases gradually until the cmc. At the cmc the anisotropy decreases sharply. This dip may be caused by micelle formation competing strongly with dye-surfactant ion pair or cluster formation. The fact that the anisotropy is lower near the cmc than it is with no SDS present suggests that there is a third type of interaction in the case of oxazine 1. This interaction may involve dye molecules in the core of the micelle. To incorporate the nonpolar section of oxazine 1 in aqueous solution, water molecules have to form a fairly rigid structure around it. This structure may inhibit the rotation of the dye more than the less polar micelle interior would. Above the cmc the anisotropy increases sharply before it plateaus at a value above the anisotropy of the solution without SDS. The dye's lower ability to rotate above the cmc shows that it is within the micelle in a more rigid formation. This may be because the positively charged end of the dye is interacting with the negatively charged end of the SDS ions.

4.5 Conclusions

This data supports the conclusion that there are three distinct dye-surfactant interactions and that they are similar for both dyes. Without surfactant present, the dyes exist as a mix of monomers and dimers. At concentrations of 3 μM, the amount of oxazine 1 dimer present is very small, but there may be a significant amount of oxazine 170 dimer present. When SDS is present in concentrations below the cmc

Figure 4-1: Proposed Dye-Surfactant Interaction Above cmc



(approximately 6 mM when these dyes are present at 3μM concentration), the dye and surfactant appear to form insoluble ion pairs or clusters with each other. Below the cmc, the fraction of dye molecules in these clusters increases with increasing SDS concentration. This interaction does not significantly reduce the dimer concentration of either dye.

At the cmc, the dyes' fluorescence anisotropy trends suggest that a second dye-surfactant interaction replaces salt or cluster formation. This interaction allows the dyes to rotate more freely, which may be a sign that the dyes are incorporated into the micelle interior. Increased fluorescence intensity supports the conclusion that the dye is experiencing a less polar environment such as the micelle interior.

Above the cmc, a third dye-surfactant interaction replaces the earlier ones. This interaction is also between the dye and micelles. The relatively high fluorescence anisotropy indicates that the dye is more tightly bound. This may mean that the polar end of the dye molecule lies at or near the surface of the micelle and is held in place by ionic interactions. The high fluorescence intensity again indicates interaction with the micelle interiors. This suggests that the nonpolar sections of the dyes extend into the micelle interiors. This proposed interaction is shown in figure 4-1. This interaction competes successfully with oxazine 170 dimer formation and reduces the concentration of such dimers.

4.6 Future Work

Several questions remain regarding these proposed interactions. It is unclear why dye molecules would be incorporated in the interior but not the surface of the micelle at the cmc, but would interact with both above the cmc. The origin of the spike in scattering by oxazine 170 solutions at low SDS concentrations is also unclear. While it could be expected that the dye-surfactant salts that are forming would scatter significantly because of their size, it is unclear why these salts would do so at SDS concentrations of around 2 mM, but not at higher SDS concentrations. It remains to be explained why oxazine 1 solutions without SDS have three to four times the fluorescence anisotropy of oxazine 170 solutions without SDS. These results also provide little information about the size of the dye-surfactant salts. Tracking the peak fluorescence wavelength as a function of SDS concentration may reveal information about the dye's environments. In most polar molecules, the excited state is more polar than the ground state. Because of this, higher solvent polarity leads to an increased stabilization of the excited state relative to the ground state and a shift of the fluorescence spectrum to longer wavelengths in these molecules (6). We did not observe any large shift in wavelengths in our fluorescence measurements. Due to problems with the stability of the instrument, we were unable to measure the wavelengths to the precision required for a study of this type.

Fluorescence lifetime measurements may also help to answer some of these questions. Because fluorescence lifetime measurements measure the average time a molecule spends in the excited state, they can give a good indication of the molecule's environment. For example, since higher solvent polarity often leads to an increased

stabilization of the excited state relative to the ground state, higher solvent polarity would be expected to result in longer fluorescence lifetimes. If multiple fluorescence lifetimes are present for a given SDS concentration, this would show that fractions of the dye were in different environments. These measurements can also be combined with anisotropy measurements to calculate the rotational correlation time of the species (1). Since this is a measure of how quickly the species is moving, it could give us a better idea of the size of the ion pairs or clusters, and mixed micelles.

References

1) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy;* Plenum Press: New York, 1983.

2) D. A. Skoog, F. J. Holler, and T. A. Nieman, *Principles of Instrumental Analysis;* Harcourt Brace and Company: Philidelphia, 1998.

3) Wright, J. M., unpublished results.

4) D. A. Steinhurst and J. C. Owrutsky, *Second harmonic generation from oxazine dyes at the air/water interface;* Code 6111, U.S. Naval Research Laboratory: Washington, D.C.: 2000

5) M. J. Rosen, *Surfactants and Interfacial Phenomena;* John Wiley & Sons: New York, 1978.

6) J. Georges, Spectrochimica Acta Rev 13, 27 (1990).

7) M. E. Diaz Garcia and A. Sanz-Medel, Talanta 33, 255 (1986).

8) T. Imasaka, Talanta 28, 305 (1999).

9) Leach, A.M., unpublished results.

10) J. E. Melanson and C. A. Lucy, Analyst 125, 1049 (2000).

11) T. Imasaka, T. Higashijima, and N. Ishibashi, Analytica Chimica Acta 251, 191 (1991).

12) Unger, M., unpublished results.

13) M. K. Carroll, M. A. Unger, A. M. Leach, M. J. Morris, C. M. Ingersoll, F. V. Bright, Applied Spectroscopy 53, 780 (1999).