Spectral Characteristics and Photosensitization Effect on TiO$_2$ of Fluorescein in AOT Reversed Micelles

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The microenvironment in reverse micelles composed of water, aerosol OT (AOT, sodium bis(2-ethylhexyl)sulfosuccinate), and isooctane has an important effect on the spectral properties of fluorescein (FL). The absorption peaks of FL red-shifted by about 10 nm from the corresponding positions in aqueous solution. Moreover, the absorption extinction coefficient of FL in reversed micelles increased with the water content, $\omega$. The fluorescence of FL was more effectively quenched in AOT reversed micelles than in aqueous solution. In addition, aggregation occurred for FL in reversed micelles, which hindered the photo-induced electron transfer from the excited singlet of FL to the conduction band of TiO$_2$.

Key Words: fluorescein; TiO$_2$ nanoparticles; reversed micelles; microenvironmental effect; fluorescence quenching; electron transfer; aggregation.

1. INTRODUCTION

Nanometer-sized semiconductors have attracted growing interest in the past decade (1–4). Various methods for preparation of ultrafine particles have been reported. To stabilize and regulate the size of the particles, ultrasmall semiconductor particles can be generated in reverse micelles, which provide a unique microheterogeneous system (5, 6). In the system, isolation of water-soluble reactants in different water pools limits the extent of interaction to a degree that the growth of the resulting products is highly controlled.

Metal oxide semiconductors have been widely used as photocatalysts. However, they have one severe defect; that is, their photoactivity is limited to the UV region. Hence, photosensitization using organic dyes is very important for improving the visible light response of large bandgap semiconductors. Reversed micelles can also provide a favorable microenvironment for the photosensitization process. Although the photosensitization of colloidal TiO$_2$ particles in water-in-oil (w/o) microemulsion by organic dyes has been studied in our laboratory (7) and by others (8), there are still some problems that need to be further clarified. For example, because of the special microenvironmental effects of micelles, the organic dye structure may be changed, which may in turn influence the efficiency of the photosensitization. With this view, we selected fluorescein (FL; see Scheme 1), an xanthene dye, as a probe for studying the photochemical behavior of the dye–TiO$_2$ system in AOT (sodium bis(2-ethylhexyl)sulfosuccinate) reversed micelles. FL has been widely used in dye laser (9), solar energy conversion (10–12), and photosensitized reactions (12–15). In the present work, special attention was given to the effects of microenvironment on the state of the organic dye and the interaction with TiO$_2$ nanoparticles. Interestingly, it was found that the spectral properties of FL in reversed micelles are drastically different from those in an aqueous solution, and the fluorescence quenching of fluorescein by TiO$_2$ is more effective in the monomer state than in the aggregation state.

2. EXPERIMENTAL

2.1. Materials

The TiO$_2$ particles were prepared by hydrolysis of Ti(O–i–C$_3$H$_7$)$_4$ (purchased from Aldrich Chemical Co.) in reverse micelles consisting of water pools in AOT as surfactant and isooctane as hydrophobic continuous phase, according to

$$\text{Ti(O–i–C}_3\text{H}_7\text{)}_4 + 2\text{H}_2\text{O} \rightarrow \text{TiO}_2 + 4\text{i–C}_3\text{H}_7\text{OH}. \ [1]$$

The procedure was as follows: 2.23 g AOT was dissolved in 50 ml isooctane ([AOT] = 0.1 M), and water containing 0.2 M H$^+$ (HCl or HClO$_4$) was added to maintain a desired $\omega$ ($\omega = [\text{H}_2\text{O}]/[\text{surfactant}]$). The solution was ice-cooled and stirred. After 30 min, 1.2 ml of a fresh 0.3 M Ti(O–i–C$_3$H$_7$)$_4$ solution in anhydrous isooctane was added dropwise to the above solution under vigorous stirring at 0°C and kept stirring for 3 h. Thus prepared, TiO$_2$ particles can be stable for 1–2 months. It is noteworthy that the stability of the prepared TiO$_2$ nanoparticles in reverse micelles is related to the local acidity of the water pools and to the water content, $\omega$.

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All other chemicals used in the experiments were analytical grade reagents, and doubly distilled deionized water was used.

2.2. Apparatus and Measurements

Absorption spectra were recorded on a Hewlett-Packard 8451A diode array UV-Vis spectrophotometer by using 1-cm path length cells.

Emission spectra were measured with a Hitachi MPF-4 fluorescence spectrophotometer and a Perkin-Elmer LS-05 fluorescence spectrophotometer with a computer for data collection and reduction. The fluorescence lifetime measurements were carried out on a multiplexed, time-correlated, single-photon counting spectrophotometer (HORIBA, Model NAES-1100) excited using the pulsed light of a hydrogen lamp which can generate a half-maximum stable pulse width of less than 2 ns in the range 200–700 nm. Fluorescence lifetimes of 200 ps to several hundred microseconds can be accurately recorded with this spectrophotometer.

All measurements were performed at room temperature (298 ± 1 K) unless specified in the text.

3. RESULTS AND DISCUSSION

3.1. Spectral Variation of FL with Environment

Figure 1 shows the absorption of fluorescein in bulk water and in AOT reversed micelles. Both in bulk water and in reverse micelles, FL exhibited two absorption peaks, but the peak positions were different. The absorption peaks of FL in reversed micelles red-shifted by about 10 nm from that in bulk water. In addition, as shown in Fig.1, the absorption intensity of FL in reversed micelles increased with increasing water pool content, \( \omega \). This is further verified by Fig. 2, which shows the changes in absorbance at 487 nm for FL of various concentrations in aqueous solution and in reversed micelles. Moreover, from Fig. 1 it can be seen that the two absorption peaks of FL in reversed micelles are at 458 and at 487 nm, respectively. The increased magnitude of the two peaks with increasing \( \omega \) is different. Similar changes in emission spectra were also observed. As shown in Fig. 3, the fluorescence intensity of FL also increased with increasing \( \omega \). It can be concluded from these experiments that the spectral properties of FL are sensitive to changes in its surrounding.

As stated above, the absorption of FL in reversed micelles is different from that in bulk water. This means that water in small water pools has a character different from that in bulk water and has been verified by various techniques, such as NMR, molecular probe, and ESR spin probe (16). The effective polarity, acidity, and microscopic viscosity of the surfactant-entrapped “water pools” are expected to be substantially different from those in bulk water. In addition, the absorption and emission intensities of FL in reversed micelles varied with water content. This is an indication that
us to vary the concentration of TiO₂ in a w/o system of the same nature.

Figure 4 shows the absorption spectra of TiO₂ with various concentrations in AOT reversed micelles. The spectra were made after subtraction of the background spectrum of the TiO₂-lacking w/o system. Clearly, the absorption spectra show the onset of absorption (determined by the linear extrapolation of the steep part of the UV absorption toward the base line (18)), λₐₒ = 350 nm, corresponding to a band-gap energy of $E_g = 3.52$ eV. It can be seen that this onset of absorption does not change upon dilution, although the optical density is altered. The particle size can be estimated from the onset of the absorption according to the following relationship between the bandgap shift ($\Delta E_g$) and the radius ($R$) of size-quantified particles (19),

$$\Delta E_g = \frac{h^2}{8\mu R^2} - \frac{1.8 \epsilon^2}{\epsilon R}$$

where $h$ is the Planck constant, $\mu$ is the reduced mass of the exciton, and $\epsilon$ is the dielectric constant of the semiconductor. As a approximation, $\mu = 1.63 m_e$ ($m_e$ is the electron rest mass), and $\epsilon = 184$ (20). Considering that the bandgap of bulk TiO₂ is 3.2 eV for anatase (21) and 3.0 eV for rutile (22), the diameter of the prepared particles is estimated to be 1.4–1.7 nm. This value is slightly larger than that reported in Ref. (8). Considering that the weight density of TiO₂ is about 4 g/cm³, the agglomeration number of the prepared TiO₂ particles is estimated to be 43–77.

The water pool in the above w/o system is acidic and can be decreased by mixing the w/o TiO₂-containing microemulsion with the 0.1 M AOT solution containing NaOH of a certain concentration (of course, the $\omega$ of the microemulsion

3.2. Characterization of TiO₂ Nanoparticles in Reversed Micelles

Metals, semiconductors, and other inorganic crystallites generated in reverse micelles reveal a correlation between the size of the particles and the size of the micelles, which, in turn, depends linearly on the water-to-surfactant molar ratio, $\omega$ (17). That is, particle size increases with increasing water content.

To prepare reversed micelles including a high concentration of TiO₂ particles with a size substantially smaller than the water pool, a novel method was developed. The primary step was the hydrolysis of Ti(O-i-C₃H₇)₄ in a w/o system with a desired $\omega$. The resulting TiO₂-containing w/o system could be diluted with a w/o system of similar composition (identical $\omega$ ratio and H⁺ concentration). This step enabled
is identical) because the contents in water pools can be exchanged by collision. In some cases, this process is necessary to investigating the properties of TiO₂ particles.

### 3.3. Interaction of FL with TiO₂ Nanoparticles in Reversed Micelles

Figure 5 shows the absorption spectra of FL in reversed micelles with or without the presence of TiO₂ nanoparticles. Clearly, in this case the absorption of FL exhibited only one main peak, which was different from that in Fig. 1. The spectral difference between them is attributed to the variation in the acidity of the water pool. In Fig. 5 the water pool was acidic, while in Fig. 1 it was nearly neutral. As we know, FL is a very unstable dye; its structure changes easily with the pH value of its surroundings. Therefore, the absorption spectrum of FL is naturally susceptible to the pH value. This aspect has been studied by Shen et al. (23). With the addition of TiO₂ (Fig. 5), the main absorption peak decreases slightly. Meanwhile, an extended absorption at longer wavelengths (>460 nm) occurs. Therefore, it is certain that a strong interaction between FL and TiO₂ in the ground state causes the change in the electronic state of FL. In an acidic environment, the surface of TiO₂ nanoparticles has a positive charge. Because of its molecular polarity, FL can be adsorbed on the surface of TiO₂ by electrostatic interaction. On the other hand, due to the restriction of the micelles’ “cage effect,” the interaction between FL and TiO₂ will also be enhanced.

The addition of TiO₂ not only caused the change in absorption of FL but also had an effect on the emission of FL. As shown in Fig. 6, in reversed micelles containing an acidic water pool (pH 0.8), FL exhibited two emission peaks, one at 480 nm and the other at 520 nm. Therefore, the emission spectrum of FL in this case can be resolved into two parts. With the addition of TiO₂, the fluorescence intensity of FL decreases. However, the influence by TiO₂ is different for the two emission peaks. That is, the emission at 520 nm is much more influenced by TiO₂, as seen in Fig. 7. The two emission peaks should correspond to the two existing states of FL in the reversed micelles. Compared with the emission of FL in aqueous solution (Fig. 8), it is certain that the emission peak at 520 nm comes from the monomer state of FL. For FL in diluted aqueous solutions, only the monomer state existed. In reversed micelles, we think the emission peak at 480 nm is from the aggregation state (dimer). Because of the steric confinement in the reversed micelles, it is very possible for FL to aggregate. In the present work, the emission from the aggregated state blue-shifts by about 40 nm compared to that from the monomer. As we know, aggregation will cause the absorption to red-shift or blue-shift, which is determined by the molecular orientation in aggregation (24). If the aggregation resulted from the parallel interaction between the molecules (i.e., there are parallel transition dipoles among the aggregated molecules), blue-shift occurs. Nevertheless, the blue-shift band corresponding to the aggregation state in absorption spectra was not observed in the present experiment (Fig. 5). It may be that...
the amount of the aggregated molecules in the system was relatively small, and in such a case the absorption spectrum was not as sensitive as the fluorescence spectrum to the changes in the molecular state. An analysis of the composition of the w/o system will help us to understand this point. Using time-resolved fluorescence probing, Lang and Jada (25) have studied the structure of AOT reverse micelles. On the basis of their results for an AOT w/o system, similar to that of the present experiment, the radius of the water pool, $R_w$, and the surface area per AOT molecule at the surface of the water pool, $\sigma$, are about 40 Å and 50 Å$^2$, respectively. So the molar concentration of micelles, $[m]$, can be calculated by the form of $[m] = [\text{surfactant}] \sigma / 4\pi R_w^2$, which is $2.5 \times 10^{-4}$ mol/L. Considering the FL concentration of $1 \times 10^{-5}$ mol/L, the dye occupies 4% of the micelles. If FL molecules are randomly distributed over the micelles, that is, with a Poisson distribution, the probability of a FL molecule in a single water pool is 3.84%, and that of two or more molecules in a single water pool is 0.16%. So, in the case of $[\text{FL}] = 1 \times 10^{-5}$ mol/L, the concentration of FL dimer must be less than $4 \times 10^{-7}$ mol/L. It is difficult to detect by means of absorption spectrum.

In addition, it is expected that the existing state of FL in reverse micelles is related to the water content, $\omega$. This is confirmed by the experimental results. As shown in Fig. 8, the monomer was indeed increased with increasing water content, $\omega$. From Fig. 8 one can also see that the fluorescence emission peak of FL in reversed micelles red-shifts with regard to that in water solution. It has been widely recognized that the emission characteristics of many fluorophores are very sensitive to the solvent polarity (25). For example, the fluorescence maximum shifts to longer wavelengths with increasing solvent polarity. This is due to the solute–solvent, dipole–dipole reorientation of the polar solvent molecules about the increased (relative to the ground state) dipole moment of the excited state. The negative charge of AOT molecules forms a spherical electrostatic field around the water pool, which causes an increase in polarity of the microenvironment. For the fluorophores solubilized in the water pool, a red shift would occur naturally.

There are two possible mechanisms for the fluorescence quenching, i.e., energy transfer and electron injection (26). The absorption of TiO$_2$ is in the UV region (Fig. 4), while the emission of FL is in the visible region (Fig. 6). Hence, there is no spectral coverage between them. According to the laws of energy, it is impossible for the fluorescence quenching to be caused by energy transfer. In essence, the fluorescence quenching is attributed to injection of an electron from the excited singlet of FL to the conduction band of TiO$_2$. This can be determined by the potential discrepancy between the excited singlet of FL to the conduction band of TiO$_2$. This can be determined by the potential discrepancy between them. For FL, $E_{ox} = 0.91 \text{ V (vs Ag/AgCl, MeOH)}$, $E_s = 1.9 \text{ eV}$, and $\epsilon_v = -(E_{ox} - E_s + 0.35) - 4.51 = -3.25 \text{ eV (27)}$. While $E_{cb}(\text{TiO}_2) = -4.2 \text{ eV (28)}$, $\eta = \epsilon_v - E_{cb}(\text{TiO}_2) > 0$, which acts as “driving force” for the electron injection from the excited singlet of FL to the conduction band of TiO$_2$.

Long ago, Kautsky and Merkel (29) demonstrated experimentally that aggregation of dyes facilitated their action as
photophysical sensitizers in photochemical reactions, at the same time diminishing fluorescence efficiency. However, as seen in Figs. 6 and 7, the emission at 520 nm is more influenced by TiO$_2$. This indicates that FL in the monomer state favors the electron injection process, which causes the fluorescence quenching, more than FL in the aggregated state. Of course, if the micellar radius is decreased, the interaction between the aggregated FL and TiO$_2$ nanoparticles will be enhanced. This can be seen from Fig. 9. Nevertheless, one point should be noted, even though the fluorescence quenching at 480 nm is obvious in such a case, the quenching efficiency at 520 nm is still higher.

Reverse micelles provide a unique microenvironment and serve as compartmentalized media for chemical reactions; therefore, more effective photosensitization of TiO$_2$ particles in w/o microemulsions is expected. This is verified by the comparison of the fluorescence quenching of FL in reverse micelles with that in water/ethanol solution, in which the water content is the same as that in the w/o system. As shown in Fig. 10, the quenching in reversed micelles is more efficient.

As stated before, FL can be adsorbed easily onto the surface of TiO$_2$ nanoparticles owing to the electrostatic interaction and the concentration effect of micelles. Measurements of fluorescence lifetime show that, in reverse micelles with and without the presence of TiO$_2$ nanoparticles, the lifetimes of FL are constant, ca. 3.5 ns. This means that the fluorescence quenching of FL by TiO$_2$ is caused by the adsorption between them. In such a case the adsorbance ($C_a$) of FL at a given concentration ($C_0$) is proportional to the surface area of TiO$_2$ colloids, which in turn is proportional to the concentration of TiO$_2$ ([TiO$_2$]),

$$C_a = K[TiO_2].$$  \[3\]

where $K$ is the apparent adsorption constant of FL on TiO$_2$. Because of the electron transfer, the fluorescence emission of the adsorbed FL is very weak. The fluorescence of adsorbed FL can be ignored. That is, the observed fluorescence intensity of FL ($I$) is determined by the concentration of free FL in the water pool:

$$I = K'(C_0 - K[TiO_2]).$$  \[4\]

Thus, the relative fluorescence intensity ($I/I_0$) can be deduced as

$$\frac{I}{I_0} = 1 - \frac{K}{K'C_0}[TiO_2],$$  \[5\]

where $I_0$ is the fluorescence intensity in the absence of TiO$_2$. A linear dependence of $I/I_0$ on the concentration of TiO$_2$ colloids with a slope equal to $-K/K'C_0$ would be expected. This is indeed confirmed by the linearity of the plots in Figs. 7 and 10. The value of the slope can be used to evaluate the fluorescence quenching efficiency of FL by TiO$_2$ colloids: the smaller the slope, the higher the quenching efficiency. Therefore, from Figs. 7 and 10, one can see that the quench-
ing efficiency by TiO\textsubscript{2} for FL in reversed micelles increases with decreasing water content, \( \omega \); moreover, it is noteworthy that the quenching efficiency by TiO\textsubscript{2} in reversed micelles is much higher than that in bulk solution. The slopes of the two lines in Fig. 10 are \(-70.4\) and \(-316.3\), respectively, indicating that the quenching efficiency in reversed micelles is nearly 4.5 times higher than that in bulk solution.

These results reveal an obvious “cage effect” in reversed micellar systems. Owing to this effect, spectral changes for FL occurred, and the fluorescence quenching efficiency by TiO\textsubscript{2} nanoparticles was improved.

### 4. CONCLUSIONS

FL in AOT reversed micelles has optical properties that differ considerably from those of FL in aqueous solution. Compared to that in aqueous solution, the absorption peak of FL in reverse micelles red-shifts by 10 nm. The water in reverse micelles is drastically different from that in bulk aqueous solution. Furthermore, the absorption intensity of FL in reverse micelles containing varying water contents increases with increasing \( \omega \). These characteristics of FL in different environments indicate that FL may be used as a spectrometric probe for studying the reversed micellar microstructure. In the present work, TiO\textsubscript{2} nanoparticles were synthesized in AOT reversed micelles. The photosensitization of TiO\textsubscript{2} by FL in reverse micelles was more effective than that in bulk solution. In addition, aggregation occurred for FL in reverse micelles, and the extent of aggregation increased with decreasing \( \omega \). FL in the monomer state favored the photosensitization process over that in the aggregation state.

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