Buffer Action of Reversed Micelles
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The optical properties (absorption and fluorescence) of fluorescein sodium were investigated with changing the bulk pH value in aqueous, water-methanol mixed and dodecylammonium propionate reversed micellar solutions. A strong buffer action was found in the reversed micellar solutions. The pKₐ values for the equilibrium between fluorescein mono- and di-anions in the ground and excited singlet states highly decreased in the reversed micellar solutions compared with those in non-micellar solutions. The micellar buffer action strongly affected the initial rate of the 1,3-diphenylisobenzofuran oxidation caused by the photosensitization of fluorescein. The buffer action was closely related to the solubilized water content.

Introduction
Recently, reversed micellar systems are attracting a great attention of various workers [1-10] since they offer a hydrophilic environment in the interior of micelles in non-polar phase. Fendler et al. [1-5] have found many interesting chemical reactions in DAP reversed micellar solutions. We [8-10] investigated the DF oxidation by singlet oxygen produced by the photosensitization of dyes (F and pyrene) in DAP reversed micellar cyclohexanic solutions. The quantum yield of the reaction depended highly on the solubilized water concentration. In general, the micro environment inside reversed micelles provides us interesting reaction fields. The DAP molecule has the amine and propionate headgroups. These headgroups may be at or near the interior surface of DAP micelle to produce specific chemical environments.

In the present paper, the optical properties (absorption and fluorescence) of F were measured in the DAP reversed micellar solutions with solubilized water at various bulk pH's, and the buffer action of the micelles was investigated. The DF oxidation by singlet oxygen produced by the F photosensitization was also studied in connection with the micellar buffer action.

Materials and Methods
Cyclohexane, DAP and F obtained from Katayama Co. were guaranteed or extra pure reagents. DF purchased from Aldrich Chemical Co. was of special grade. Aqueous solutions (0.1 N) of NaOH and HCl, and (36 N) H₂SO₄ were purchased from Katayama Co. Laboratory-distilled water was further distilled from alkaline KMnO₄ solutions. Bulk pH (1–9) in aqueous and water-methanol mixed (1:1, v/v) solutions of F was controlled with 0.1 N NaOH and 0.1 N HCl, and was measured with a pH-meter (Hitachi-Horiba, type F-7 ss). The bulk pH’s 0.0, -1.0 and -2.0 were prepared using 36 N H₂SO₄ according to the Hammett’s acidity function [11].

Reversed micellar solution was prepared by dissolving DAP in cyclohexane or cyclohexanic solution containing DF at room temperature. The DAP cyclohexanic solution was stirred for ten minutes in the dark at 40 °C after adding powdered F. The F-solubilized DAP solution obtained was stirred again under the same conditions after adding definite amount of H₂O at various bulk pH’s.

The reaction mixture for photooxidation of DF in a quartz vessel (1 x 1 x 4 cm³) was irradiated with the yellow light (absorbed by F) isolated from a 150 W xenon lamp through a cut filter (colour glass filter, type V-Y 48, Toshiba Electric Co.) at 40 °C.

The absorption and fluorescence spectra were measured with a Hitachi type 124 spectrophotometer and a Hitachi type 203 spectrofluorimeter, respectively.

Results and Discussion
Fig. 1 shows the absorption and fluorescence spectra of F in aqueous, H₂O–MeOH mixed (1:1, v/v) and DAP reversed micellar solutions at various bulk pH’s. It is known that fluorescein takes various forms (di- and mono-anions, neutral molecule and cation) in aqueous solutions depending on the pH value. The pKₐ values for the equilibriums between di- and mono-anions, mono-anion and neutral molecule, and neutral molecule and cation are 6.7, 4.4

Abbreviations: F, fluorescein sodium; DF, 1,3-diphenylisobenzofuran; DAP, dodecylammonium propionate.
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Fig. 1. Absorption and fluorescence spectra of F at various bulk pH's. Concentrations of F and DAP, $3.6 \times 10^{-6}$ and $8.0 \times 10^{-2}$ M, respectively. [A] Curves 1, 2, 3, 4, 5 and 6, absorption spectra of F in aqueous solutions at pH's $\geq 7.0$, 6.0, 5.0, 4.0, 3.0 and 1.0, respectively; Curves 7 and 8, absorption spectra of F in DAP reversed micellar solutions containing 0.5 M H$_2$O at bulk pH's $\geq 3.0$ and $-2.0$, respectively; Curves a and b, fluorescence spectra of F under the same conditions for Curves 1 and 7, respectively.

In Fig. 1A, the absorption peaks of F in aqueous solutions were 489 nm in Curves 1 (pH $\geq 7.0$) and 2 (pH 6.0) (predominant form, di-anion), 455 and 477 nm in Curve 3 (pH 5.0) (predominant form, mono-anion), 435 nm in Curve 5 (pH 3.0) (predominant form, neutral molecule) and 435 nm in Curve 6 (pH 1.0) (predominant form, cation). The DAP reversed micelle provides a hydrophilic environment in its interior, and F is trapped inside the micelle. To study the ionic character inside the micelle using F as molecular indicator, 0.5 M H$_2$O at various bulk pH's was added to the F-solubilized DAP reversed micellar cyclohexanic solutions. The absorption spectra of F bound to DAP micelles had the same spectral shape and position above bulk pH 3.0 (Curve 7). This indicates that the predominant form of F was the di-anion in this bulk pH range. The absorption peak was red-shifted to 499 nm by the binding interaction between F and micelle. The absorption spectrum suggesting the predominance of the neutral molecule appeared at bulk pH $-2.0$ (Curve 8) with about 10 nm red-shift compared with Curve 3. The fluorescence spectra in aqueous and DAP micellar solutions are shown in Curves a and b, respectively. The fluorescence peak in DAP micellar solutions was also red-shifted by 10 nm compared with that in aqueous ones. The fluorescent species of F was the di-anion. In H$_2$O–MeOH (1:1, v/v) mixed solutions of F (Fig. 1B), the absorption peaks at various bulk pH's and the fluorescence peak of the di-anion appeared at longer wavelengths by 3–5 nm than the corresponding ones in aqueous solutions, respectively. The absorbances of the F di-anion band in various solutions are plotted against various bulk pH's as shown in Fig. 2. Curves 1 and 2 are the absorbance changes in aqueous and H$_2$O–MeOH (1:1, v/v) mixed solutions, respectively. Both curves decreased steeply with decreasing the pH value from the neutral. From Curves 1 and 2, the pK$_a$ values...
(in the ground state) for the equilibrium between the mono- and di-anions were evaluated to be 5.1 and 5.2 in aqueous and H$_2$O–MeOH mixed solutions, respectively. However, in DAP reversed micellar solutions, the absorbance exhibited a very gradual decrease up to about pH 2 with decreasing the pH value, then rapidly decreased. This rapid decrease began at higher pH’s in micellar solutions containing higher concentrations of H$_2$O. Curves 3 and 4 are the absorbances in micellar solutions containing 0.2 and 0.5 M H$_2$O, respectively. The pK$_a$ value in micellar solutions was evaluated to be -2.2 at 0.2 M H$_2$O and -0.3 at 0.5 M H$_2$O. According to Correll et al. [3], the size and aggregation number of DAP micelle depend on the solubilized water content. The solubilized water molecules below about 0.37 M are bound to the headgroups of the micelle and those above this concentration exist as free water inside the micelle. From Curves 3 and 4, the pK$_a$ value in micellar solutions with 0.5 M H$_2$O was larger than that in micellar solutions with 0.2 M H$_2$O.

The relative fluorescence intensity of F was measured at various bulk pH’s. Results obtained are shown in Fig. 3. Curves 1 and 2 are the fluorescence intensities in aqueous and H$_2$O–MeOH mixed solutions, respectively, and Curves 3 and 4 those in DAP reversed micellar solutions containing 0.2 and 0.5 M H$_2$O, respectively. The shape and position of these curves closely resembled the corresponding ones obtained in the absorbance–bulk pH relation shown in Fig. 2. From Curves 1, 2, 3 and 4, the pK$_a^*$ values (in the excited singlet state) for the equilibrium between the mono- and di-anion forms were evaluated to be 4.7, 4.8, -3.0 and -1.4 in aqueous, H$_2$O–MeOH mixed, 0.2 M H$_2$O micellar and 0.5 M H$_2$O micellar solutions, respectively. The ΔpK (pK$_a$–pK$_a^*$) value was 0.5 in aqueous solutions, 0.3 in H$_2$O–MeOH mixed solutions, 0.8 in 0.2 M H$_2$O DAP solutions and 1.1 in 0.5 M H$_2$O DAP solutions. The ΔpK values for F bound to the DAP micelles were larger than those for F in the non-micellar solutions.

As seen in Figs. 2 and 3, the pK$_a$ and pK$_a^*$ values shifted remarkably towards lower bulk pH’s. This may be caused by a strong buffer action inside the DAP micelle. One DAP molecule has an amine and a propionate headgroup. These headgroups seem to be at or near the interior surface of the DAP micelle. In most cases, the pK$_a$ value for the equilibrium of $-\text{NH}_2 + \text{H}^+ \rightleftharpoons -\text{NH}_3^+$ is 8~10 and that for the equilibrium of $-\text{COO}^- + \text{H}^+ \rightleftharpoons -\text{COOH}$ 2~5. Accordingly, it is considered that the amine headgroup is protonated below pH 8 in DAP micelles containing solubilized H$_2$O. The fluorescent species (di-anion) of F may be bound near the protonated amine headgroup. Nome et al. [5] reported that addition of acid to DAP micellar benzene solution containing 0.55 M H$_2$O resulted in neutralization of the propionate ion to propionic acid. We roughly evaluated the number of protons necessary for this neutralization using the aggregation number of the micelle and the water content per micelle which were obtained by Correll et al. [3], assuming that protons were supplied from HCl or H$_2$SO$_4$ in the solubilized water added.

![Graph showing relative fluorescence intensities of F at various bulk pH's.](image1.png)

**Fig. 3.** Relative fluorescence intensities of F at various bulk pH’s. Concentrations of F and DAP, 3.6 · 10$^{-6}$ and 8.0 · 10$^{-2}$ M, respectively; Curves 1 and 2, in aqueous and H$_2$O–MeOH (1:1, v/v) mixed solutions, respectively; Curves 3 and 4, in DAP reversed micellar solutions containing 0.2 and 0.5 M H$_2$O, respectively, temperature, 20 °C.

![Graph showing log of proton number per micelle, consumed by the protonation of the propionate, as a function of the bulk pH.](image2.png)

**Fig. 4.** The log of proton number per micelle, consumed by the protonation of the propionate, as a function of the bulk pH. Curves 1 and 2, 0.2 and 0.5 M H$_2$O, respectively; concentration of DAP, 8.0 · 10$^{-2}$ M; number of propionate/micelle, 8 at 0.2 M H$_2$O and 54 at 0.5 M H$_2$O. As to details unless otherwise stated, see the text.
Fig. 4 shows the log of the proton number per micelle as a function of the bulk pH. Since the number of the propionate per micelle was calculated to be 8 at 0.2 M H$_2$O and 54 at 0.5 M H$_2$O, the bulk pH where the nearly whole propionates were neutralized was determined to be about unity as shown by the arrows. This may explain the buffer action inside the DAP micelle above about bulk pH 2 as seen in Figs. 2 and 3. The buffer action below this bulk pH seems to vanish. Furthermore, the buffer action was stronger in the micelle containing 0.2 M H$_2$O (bound water region) than in the micelle containing 0.5 M H$_2$O (free water region) as seen in the above Figures.

Next, the initial rate of the DF oxidation was measured at various bulk pH's in H$_2$O-MeOH (1:1, v/v) mixed and DAP reversed micellar solutions in the presence of F and DF. DF was oxidized with the singlet oxygen generated by the photosensitization of F. Results obtained are shown in Fig. 5. The curves show the similar changes with the bulk pH to the corresponding curves shown in Figs. 2 and 3. This indicates that the molecular species of F responsible for the singlet oxygen production was the di-anion. Further, the rate in DAP micellar solutions strongly depended on the H$_2$O content in DAP micelles.

Fig. 5. The initial rate (R) of DF oxidation at various bulk pH's. Concentrations of F, DAP and DF, 3.6 $\cdot$ 10$^{-6}$, 8.0 $\cdot$ 10$^{-2}$ and 4.0 $\cdot$ 10$^{-5}$ M, respectively; Curves 1 and 2, DAP reversed micellar solutions containing 0.2 and 0.5 M H$_2$O, respectively; Curve 3, H$_2$O-MeOH (1:1, v/v) mixed solutions; temperature, 40 °C.

Fig. 6 shows the initial rate of DF oxidation as a function of H$_2$O concentration solubilized in DAP micelles at various bulk pH's. The rate decreased steeply with increasing the H$_2$O content in the bound water range (below about 0.4 M H$_2$O) followed by a gradual decrease in the free water range (above about 0.4 M H$_2$O). The decrease in the rate with increasing the H$_2$O concentration was more steep with lowering the bulk pH values. In the previous paper [13], we reported that the quantum yield for the singlet oxygen production by F photosensitization in DAP micellar solutions rapidly increased with decreasing the solubilized water content at the neutral pH. This increase in the quantum yield was attributed to the increase in the yield of the F phosphorescence state. The rapid increase with decreasing H$_2$O content observed in the bound water range (Fig. 6) may be also caused by the same reason.

In the present investigation, it was found that the interior of the DAP micelle had a strong buffer action in the wide bulk pH range and the pK$_a$ and pK$_{a}^{*}$ values for the equilibrium between mono- and di-anion of F highly decreased in DAP reversed micellar solutions. The buffer action observed is considered to be an important factor for chemical reactions inside DAP micelles.