Synthesis of Long Chain Malachite Green Leuco Base (LMGH) and Its Spectroscopic Study in Organic Solvents and Ionic Micellar Solutions

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(Received September 2, 1994)

The synthesis of long chain malachite green leuco base (LMGH) is reported. The absorption spectra of LMGH in different solvents seem to be independent of the solvent polarity (dielectric constant), while the emission spectra are dependent on it. Slight fluorescence self-quenching of LMGH in methanolic solution appears at a concentration of ca. 4×10^{-5} mol dm^{-3} and it becomes the dominant factor above 2×10^{-4} mol dm^{-3}. The effect of the long chain on the basicity of LMGH relative to that of malachite green leuco base (MGH) can be revealed by the fluorescence quenching of both compounds by Fe^{3+}. The quenching process obeys a static type of mechanism, which is followed by an electron transfer of LMGH to Fe^{3+}. The fluorescence quenching efficiency of micellized LMGH by Fe^{3+} is very dependent on the charge of the micelle, and is most effective in sodium dodecyl sulfate (SDS) micelle. The color appearance of the dye from LMGH upon mixing with Fe^{3+}, is easily observed in methanol, but not in SDS micelle. The critical micelle concentrations (cmc) of anionic (SDS) and cationic (hexadecyltrimethylammonium chloride, CTAC) micelles have been estimated using LMGH as a probe.

The ionization potential of malachite green leuco base (MGH) is similar to that of *N,N*-dimethyl-*p*-toluidine (7.33 eV).[1] Along with the easy production of MGH radical cation, which upon further oxidation gives a stable, intense, brilliant green colored cation, this low value makes MGH of current interest to many chemists in different fields.

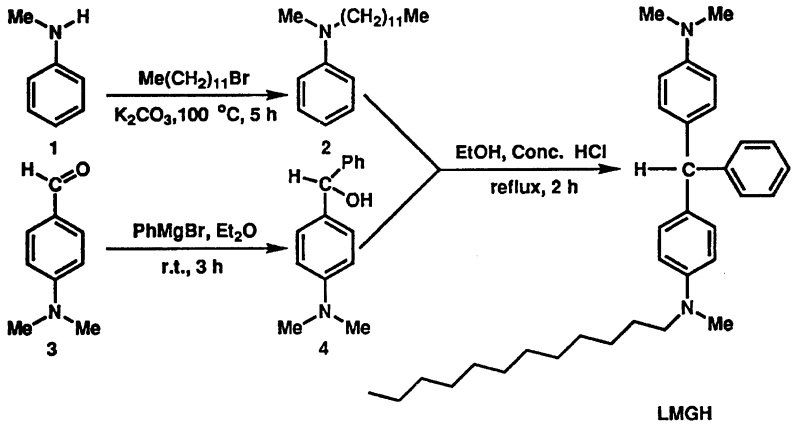
In recent years, efforts have been made to find potential applications of MGH as a photosensitive material and as carbonless paper.[2,3] Examination of the more recent reports shows that numerous elements are able to associate with MGH. This enables elements such as Au, Ag, Hg, Pd, Fe, Co, Ni, Zn, Cu, Mn, Al, and I^- to be detected spectrophotometrically.[4] In clinical chemistry, MGH is employed in medical diagnoses for occult blood detection and for microestimation of glucose, hemoglobin, and other heme compounds.[5] In addition, MGH is potentially applicable as a radiation dosimeter for γ radiation.[6]

It seems that these applications are mainly due to the inherent photochemical nature of MGH. Recently, a few reports have appeared on the photochemistry of MGH in organic solvents.[7—9] Aqueous surfactant solutions continue to attract a great deal of current attention. When surfactants associate spontaneously in water to form colloidal agglomerates, their microheterogeneous character can significantly alter the physicochemical properties of many systems.[10,11] Such changes have been widely studied by using fluorescence techniques.[11—13] The photochemistry as well as the localization of a chromophore having alkyl substituent in micellar solution seem to depend on the length of the alkyl chain.[14—16]

Therefore, long chain malachite green leuco base (LMGH) was designed; its expected ionization potential would be lower than that of MGH. Also, LMGH fulfills the requirement of being very hydrophobic, and hence in aqueous micellar solutions it will be associated exclusively with the micellar phase. In the present study, we report the synthesis (Scheme 1) and the spectral characterization of LMGH. The color appearance of the dye from LMGH as well as the effect of micellar system on the fluorescence quenching of LMGH by Fe^{3+} were also studied.

Experimental

General. Sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium chloride (CTAC) were used to prepare anionic and cationic micelles, respectively, using distilled water. Organic solvents were of spectrophotometric grade and all other chemicals were at least reagent grade. ^{1}H NMR spectra were run on a GE QE-300 spectrometer and all chemical shifts are reported as δ values relative to the residual protons of the deuterated solvent (δ=7.25, $CDCl_3$). Microanalyses were performed by the microanalytical laboratory of the Faculty of Pharmaceutical Sciences (Kyoto University). Mass spectra (70 eV) were determined. Merck



Scheme 1.

silica gel 60 (70—230 mesh) was used for product purification. All reactions were performed in oven-dried glassware and under an inert atmosphere (N_2 or Ar). Absorption spectra were recorded on a U-3410 Hitachi spectrophotometer. Quartz cells with the path length of 1 and 10 mm were used. Corrected fluorescence spectra were measured on a Rf-5000 Shimadzu spectrofluorometer and recorded at 90° angle using a 10 mm quartz cell. Excitation was always at 286 nm. Water and methanol stock solutions of $FeCl_3 \cdot 6H_2O$ were prepared. Micellar solution of LMGH was obtained by using appropriate volumes of methanol stock solution (2.28×10^{-3} mol dm^{-3}). Alcohols may serve as co-surfactants[17—21] by populating the micellar interface, which usually improves the micelle stability. All measurements were made at room temperature on aerated solutions.

Synthesis. Compounds (**2**, **4**), were synthesized (Scheme 1) by modified procedures previously reported,[22,23] and were used without further purification.

***N*-Methyl-*N*-phenyldodecylamine (2).**[22] A mixture of *N*-methylaniline (**1**) (3.22 g, 30 mmol), 1-bromododecane **2** (4.98 g, 20 mmol), and anhydrous potassium carbonate (2.76 g, 20 mmol) was stirred for 5 h at 100 °C. The reaction mixture was cooled, and mixed with water, neutralized with dil HCl, and extracted with ether. After the usual work-up, the residue was purified using column chromatography (silica gel, hexane followed by chloroform) to give **2** (3.6 g; 65%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ=0.87 (t, 3H), 1.3 (s, 18H), 1.7 (m, 2H), 2.85 (s, 3H), 3.2 (t, 2H), 6.4—7.3 (m, 5H).

4-(*N*,*N*-Dimethylamino)benzhydrol (4).[23] Into a solution of phenylmagnesium bromide (37 mmol) in ether (50 ml), a solution of 4-(*N*,*N*-dimethylamino)benzaldehyde (**3**) (5 g, 33.5 mmol) in ether (80 ml) was added over a period of 2 h at room temperature. After stirring for an additional 1 h, the reaction mixture was poured into an excess of saturated aqueous ammonium chloride. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure to afford **4** as a solid product in quantitative yield: ^{1}H NMR ($CDCl_3$) δ=2.5 (s, 1H), 2.87 (s, 6H), 5.67 (s, 1H), 6.5—7.4 (m, 9H).

4-(*N*,*N*-Dimethylamino)-4′-(*N*-dodecyl-*N*-methylamino)triphenylmethane (LMGH). A mixture of **2** (4.13 g, 15 mmol), **4** (5 g, 22 mmol), ethanol (50 ml), and concentrated HCl (2 ml) was refluxed with stirring for 2 h. The reaction mixture was cooled and alkalized with aqueous NaOH. The mixture was extracted with dichloromethane, then the combined organic extracts were dried (K_2CO_3) and evaporated. Silica-gel column chromatography of the residue with benzene as an eluent yielded the product **LMGH** (5.22 g, 72%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ=0.94 (t, J=6.6 Hz, 3H), 1.32 (s, 18H), 1.59 (m, 2H), 2.93 (s, 3H), 2.95 (s, 3H), 3.30 (t, J=7.5 Hz, 2H), 5.42 (s, 1H), 6.64—6.73 (2d, J=8.7 Hz, 4H), 6.99—7.06 (2d, J=8.7 Hz, 4H), 7.18—7.33 (m, 5H); MS m/z (rel intensity) 485 (M^+, 53), 484 (M^+, 57), 329 (100), 165 (35). Found: C, 83.97; H, 10.17; N, 5.71%. Calcd for $C_{34}H_{48}N_2$: C, 84.24; H, 9.98; N, 5.78%.

Results and Discussion

Synthesis and Spectral Properties of LMGH in Organic Solvents. The material 4-(*N*,*N*-Dimethylamino)-4′-(*N*-dodecyl-*N*-methylamino)triphenylmethane (**LMGH**) was prepared in good yield by the condensation of *N*-methyl-*N*-phenyldodecylamine (**2**)[22] with 4-(*N*,*N*-dimethylamino)benzhydrol (**4**)[23] (Scheme 1).

The UV spectral data of LMGH in various solvents showed insignificant changes. The solvent polarity (dielectric constant), however, markedly affected the emission spectra of LMGH and caused bathochromic shift {emission λ_{max}=341.6 nm in hexane (D=1.88) and emission λ_{max}=356.8 nm in acetonitrile (D=37.5)}, indicating that the excited state is more polar than the ground state.[24] This effect, together with the high molar absorptivity (ca. 35000) of LMGH, suggests a π–π^* transition. As can be seen in Table 1, increasing LMGH concentration in methanolic solution gives a negative deviation from the Lambert–Beer law above a concentration of 4×10^{-4} mol dm^{-3}. However, insignificant change in the absorption and/or emission λ_{max} is observed, indicating the abscence of LMGH molecular aggregation, owing to its non-planarity. Representative absorption and fluorescence spectra of LMGH in acetonitrile (1×10^{-5} mol dm^{-3}) are shown in Fig. 1.

Fluorescence Quenching of LMGH in Methanol. Figure 2 shows the effect of LMGH con-

Table 1. Effect of LMGH Concentration on the Absorption and Emission Spectra in Methanolic Solutions

Concentration / mol dm^{-3}	UV_{max}/nm (absorbance)	Fluorescence max/nm
4×10^{-6}	267 (0.13)	354.4
2×10^{-5}	266 (0.66)	354.4
4×10^{-5}	266 (1.31)	354.4
6×10^{-5}	266 (1.94)	354.4
2×10^{-4}	267 (6.77)	354.4
4×10^{-4}	267 (13.08)	353.4
8×10^{-4}	267 (22.03)	352.4
1×10^{-3}	269 (23.78)	352.4

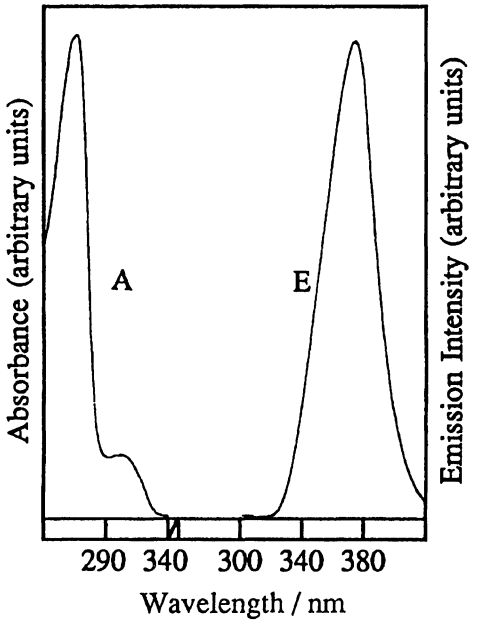


Fig. 1. Absorption (A) and emission (E) spectra of LMGH (1×10^{-5} mol dm^{-3}) in acetonitrile.

centration on the fluorescence intensity. The LMGH fluorescence intensity in methanol increases linearly with LMGH concentration up to 2×10^{-5} mol dm^{-3}, followed by a curvature increase until 2×10^{-4} mol dm^{-3}, and then decreases with further increase of the LMGH concentration. This behavior can be explained in terms of concentration quenching, which implies that some effects in the fluorescent molecule facilitate radiationless transitions resulting in quenching. In concentrated solutions, the fluorescence emitted is partly reabsorbed on passing through the solution; this is particularly noticeable for the shorter wavelength maxima, where there is a partial overlap between fluorescence emission and absorption bands.[24] Such an effect of reabsorption is demonstrated in Fig. 1 for the absorption and fluorescence spectra of LMGH. Therefore, up to 2×10^{-5} mol dm^{-3}, the concentration of LMGH is low enough to avoid quenching; however, upon increasing the concentration the quenching takes place.

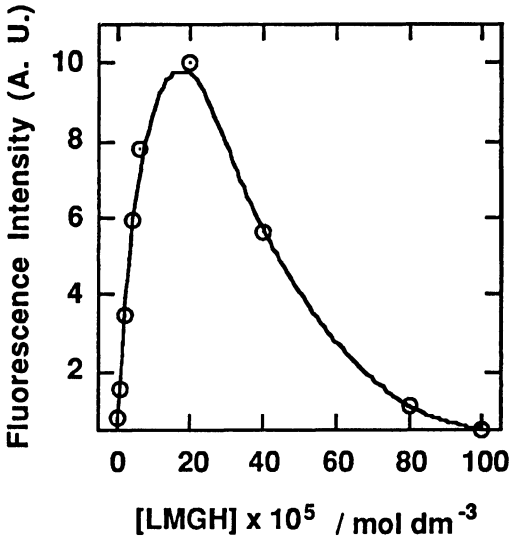


Fig. 2. Effect of LMGH concentration on the emission intensity in methanol.

Fluorescence quenching of LMGH and MGH by Fe^{3+} were investigated in methanol using the Stern–Volmer equation,[25] where (I_0) and (I) represent the fluorescence intensities in the absence and presence of the quencher, respectively, [Q] is the concentration of quencher, and K_{SV} is the Stern–Volmer constant.

$$I_0/I = 1 + K_{SV}[Q]. \quad (1)$$

Thus, to gain some insight into the effect of the long alkyl chain on the quenching rate of LMGH relative to that of MGH, we performed two quenching experiments for both compounds in methanol under the same conditions of concentration (2×10^{-5} mol dm^{-3}), spectral band width, and room temperature. $FeCl_3\cdot6H_2O$ was chosen as the quencher, due to its negligible extinction coefficient at the excitation wavelength (286 nm) and its high electron affinity. As shown in Fig. 3, the quenching efficiency of LMGH was always higher than that of MGH. This result may reflect the effect of the long alkyl chain, which increases the amine basicity and consequently enhances the electron donor ability of LMGH over that of MGH. Also the Stern–Volmer plots in both cases are not linear, indicating a static type of mechanism via ground-state complex formation, a fact of which is also confirmed by the changes of the absorption spectrum[26] of LMGH and/or MGH upon adding Fe^{3+}. Similarly, and after dark reaction, color (Fig. 4) was observed at the rate of ca. 5×10^{-5} s^{-1} at 27 °C using 1.14×10^{-5} mol dm^{-3} of LMGH and excess of [Fe^{3+}] in methanol. A plausible electron transfer mechanism for the complex and the resulted dye formations is shown in Scheme 2, i.e., upon mixing Fe^{3+} with LMGH, a ground-state complex formation takes place, followed by fast electron transfer from the LMGH-moiety to Fe^{3+} to give LMGH radical cation. This affords slowly a stable green LMG cation by further electron

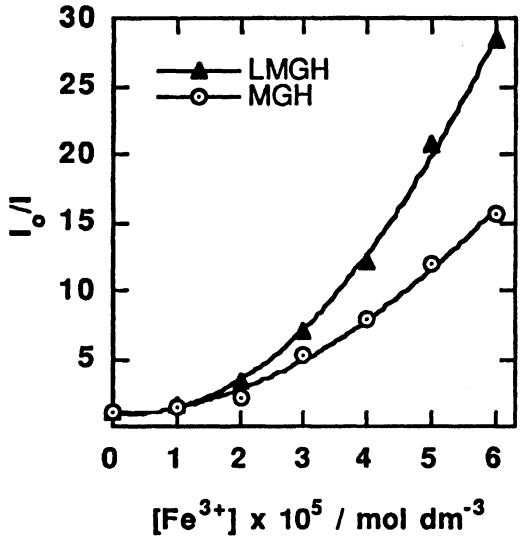


Fig. 3. Fluorescence quenching of LMGH and MGH (2×10^{-5} mol dm^{-3}) by [Fe^{3+}] in methanol.

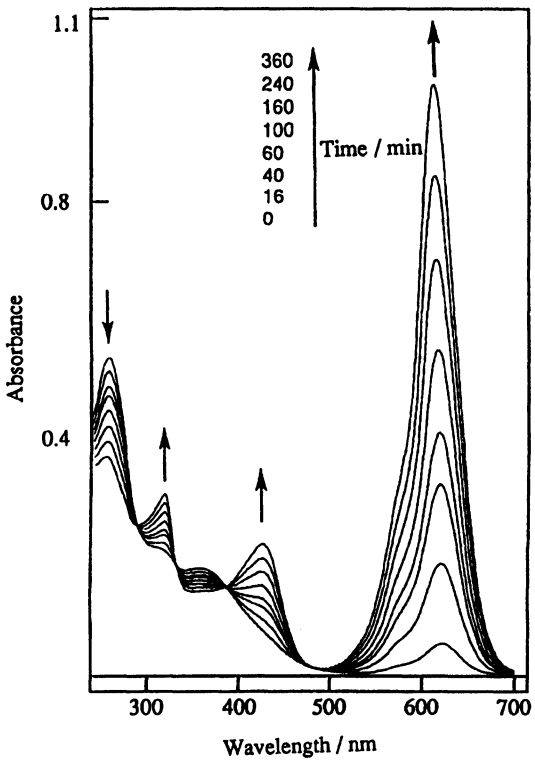
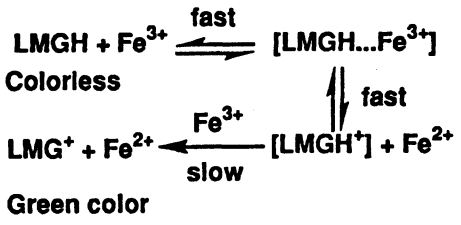


Fig. 4. Time-dependent absorption spectra of a methanol solution containing LMGH (1.14×10^{-5} mol dm^{-3}) and excess of [Fe^{3+}] (6×10^{-5} mol dm^{-3}) at 27 °C.



Scheme 2.

transfer.

We have found that the fluorscence quenching of LMGH and/or MGH using Fe^{3+} as the quencher in methanol is time-independent, though the color formation is dependent. Therefore, we suggest that the complex formation step is the quenching one. The above mechanism is supported by the appearance of a stable green color at λ_{max} 622 nm, which is characteristic for malachite green dye (MG).[27] The disappearance of the green color upon mixing the colored solution with aqueous NaOH is also characteristic for MG as pH sensitive.[28] Moreover, the clear formation of isosbestic points, as shown in Fig. 4, indicates no side reaction during LMGH quenching, which is also obtained using different concentrations of Fe^{3+}.

Micellization of LMGH. In order to determine whether LMGH is adsorbed on the surface or micellized with the micelle, we measured the fluorescence intensity of LMGH incorporated into micelles (SDS and CTAC, 0.1 mol dm^{-3}) of various compositions in comparison with those of aqueous methanolic solutions (90%) as shown in Fig. 5. For all compositions in micellar solutions, the fluorescence intensity of LMGH is enhanced over those in aqueous methanol. Also it is worth noting that the fluorescence intensity of LMGH in CTAC is enhanced over that in SDS, which may reflect the solubility effect due to the difference in the micellar aggregation numbers (105 for CTAC and 62 for SDS).[29] Since the micellar interior is aprotic and the micellar interface is highly protic, the fluorescence intensity should be higher if LMGH is micellized and remoted from the headgroup region than if it is adsorbed in a place where it has access to the water molecules. Therefore, we suggest that the LMGH chromophoric part is away from

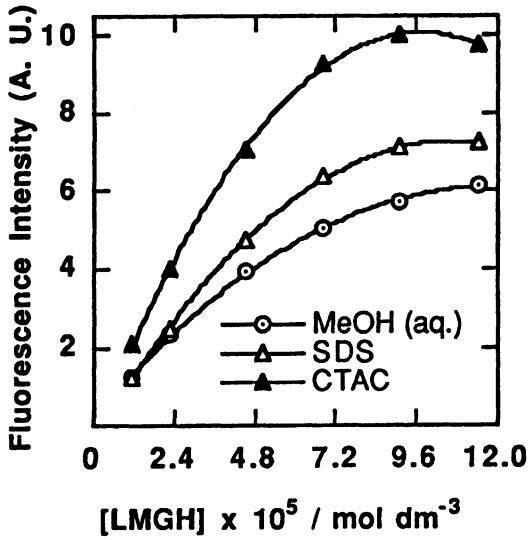


Fig. 5. Fluorescence intensity of LMGH in aqueous methanol (90%), SDS (0.1 mol dm^{-3}), and CTAC (0.1 mol dm^{-3}) as a function of LMGH concentration.

the ionic headgroup region of the micelle and stays in the hydrophobic one of the micellar interior.

In another approach we have checked the micellization of LMGH (1.38×10^{-5} mol dm^{-3}) at different concentrations of SDS and CTAC to assess whether or not LMGH is a fluorescent probe for detergent solutions. Interestingly, the fluorescence intensity of LMGH in SDS (Fig. 6) and CTAC (Fig. 7) increases as the surfactant concentration increases, then began to saturate at the point near the cmc[29] of both SDS (8.1×10^{-3} mol dm^{-3}) and CTAC (1.4×10^{-3} mol dm^{-3}) at which each LMGH molecule started to occupy a complete micelle or, in other words, when there were enough molecules in solution to build a complete micelle around every LMGH molecule. However, MGH micellization under the same conditions as above is accompanied by a substantial decrease in the fluorescence intensity of MGH as the surfactant concentration increases. This behavior may be attributed to the adsorption of MGH on the micellar interface or buried in the agglomerate state. Thus, we conclude that each LMGH molecule must promptly become located in a separate micelle.

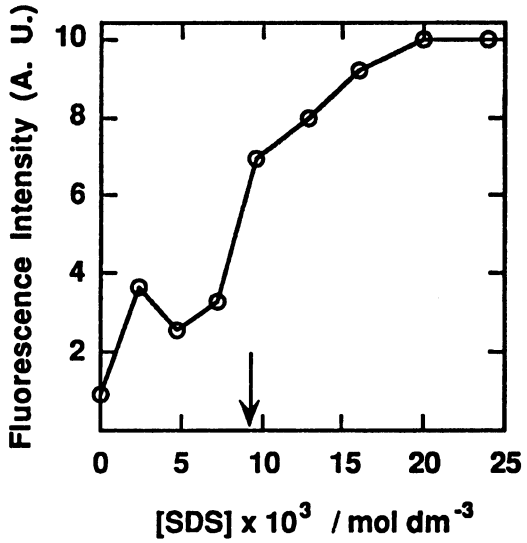


Fig. 6. Effect of increasing SDS concentration on the emission intensity of LMGH (1.38×10^{-5} mol dm^{-3}). Arrow indicates the critical micelle concentration (cmc).

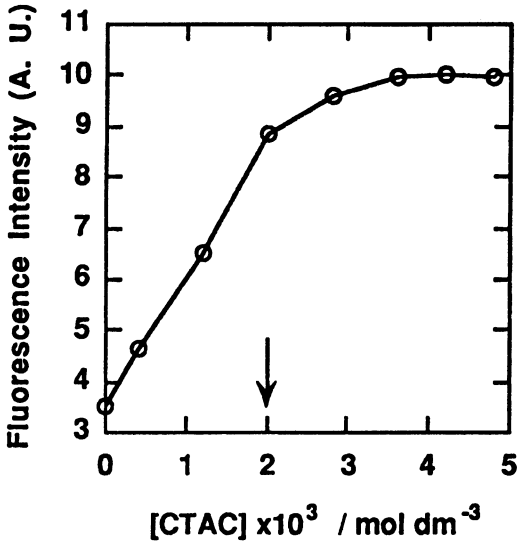


Fig. 7. Effect of increasing CTAC concentration on the emission intensity of LMGH (1.38×10^{-5} mol dm^{-3}). Arrow indicates the critical micelle concentration (cmc).

During LMGH micellization with SDS, we observed a decrease in the fluorescence intensity (Fig. 6) which is probably due to a premicellar aggregation or the formation of "LMGH-rich micelles". The premicellar aggregrates of LMGH deaggregated rapidly when more detergent was used. There is better hydrophobic stabilization of LMGH by the surfactant hydrocarbon chains and a more favorable entropy change in micelles compared with premicellar aggregates. Similar premicellar aggregation has previously been reported.[30]

Quenching studies of LMGH (1.38×10^{-5} mol dm^{-3}) fluorescence by Fe^{3+} in anionic and cationic (SDS and CTAC, 0.01 mol dm^{-3}) micelles and in methanolic solution were investigated. Expectedly, the quenching efficiency (Fig. 8) was enhanced markedly in SDS over that in methanol, but it was not affected in CTAC. This arises from absorption of Fe^{3+} on the SDS micelle surface in place of the regular counterion.[11,31] This leads to abnormally high concentrations of reactants at the micelle surface. However, in the case of the CTAC micelle a complete inhibition of the complex formation (Scheme 2) was suggested due to electrostatic repulsion of the quencher by the micelle from the vicinity of LMGH.

In SDS, the quenching efficiency is sharply increased at low concentrations of Fe^{3+}, while at higher ones it is

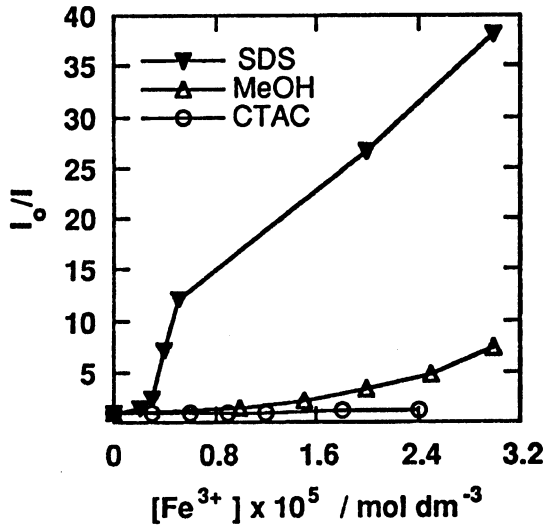


Fig. 8. Fluorescence quenching of LMGH (1.38×10^{-5} mol dm^{-3}) by [Fe^{3+}] in methanol, SDS (0.01 mol dm^{-3}), and CTAC (0.01 mol dm^{-3}).

moderately increased. This behavior seems to be due to the ionic strength effects on both the kinetics and the critical micelle concentration (cmc). Through its effects on the cmc, an increase in the ionic strength is expected to increase the number of micelles, so that the number of Fe^{3+} ions per micelle increases more slowly than $[Fe^{3+}]$.[32] This phenomenon is confirmed by the absence of such behavior in methanolic solution. Though the color formation could be observed easily in methanol (Fig. 4), the formation of that one in SDS could not be observed. This is due to the physical separation of the micelle as a result of LMGH micellization, which would prevent a LMGH radical cation from being further oxidized by another Fe^{3+}.

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