RESEARCH ARTICLE

An insight to optical studies of acridine orange cationic dye within nanometer-sized microemulsions at fixed water content

Mousa Aliahmad [1], Abbas Rahdar [2*], Havva Esmailzaee ghale kohne [1], Bijan K. Paul [3]

[1] Department of Physics, University of Sistan and Baluchestan, Zahedan, Islamic Republic of Iran

[2] Department of Physics, University of Zabol, Zabol, Islamic Republic of Iran

[3] Department of Chemistry, Jadavpur University, Kolkata 700032, India

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ABSTRACT

Cationic dye Acridine orange (AO) has wide applications especially in biological fields such as analysis of lysosomal and mitochondria content by flow cytometry and so on. In the current work, spectroscopy of acridine orange (AO) dye at both low concentrations (m_{dye}/m_{water}=6.25×10^{-5}, 3.12×10^{-5}) and high concentrations (m_{dye}/m_{water}=0.002, 0.001) was studied in confined water nanodroplets within water/AOT/n-hexane microemulsions (MEs) at a constant water content (W= [Water]/[AOT]=10) and as a function of mass fraction of droplet (MFD) using absorption and fluorescence spectroscopic techniques. The absorption spectra of the dye at high concentrations of Acridine orange (AO) dye molecules showed that the absorption spectra of the samples deviated from Beer's law, and are broadened at larger MFD due to the interactions of AO dye molecules. The fluorescence spectrum investigated at two higher concentrations (0.002, 0.001) and low concentrations (6.25×10^{-5}, 3.12×10^{-5}). At high concentration of the dye, quenching of fluorescence intensity observed due to the accumulation of the dye molecules, coupled with a red shift with increasing MFD. However, in the lower concentration regime, enhancement of fluorescence intensity was observed with increasing MFD. The Stokes' shift of the dye for both high and low concentrations increased with MFD, but largely at high concentrations compared to that at low concentrations.

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INTRODUCTION

Reverse micelles and water/oil (W/O) microemulsions are dynamic nanoscopic aggregates of surfactant molecules, which have the ability to host hydrophilic components in organic solvents and provide a simple model for interactions in membranes [1]. Reverse micelles (RMs) are water-in-oil droplets stabilized by surfactant molecules in hydrocarbon solvents with charged groups pointing inward, and the tails in the bulk solvent could be used to control the extent of confinement symmetrically. The polarity, viscosity and the hydrogen bonding ability of water inside the pool and confined at the interface vary with the pool size W (W= [H_2O] /[surfactant]) [2-6].

The literature review indicates that the AOT microemulsions generate interfaces and nanometer-sized locations to entrap additives of hydrophilic substances such as fluorescent dyes leading to considerable modification of their photophysical properties compared to those in bulk media [7-14].

On the other hand, aggregates of organic dyes have been extensively studied and reported in the literature due to their application in many fields of science, such as photographic process, organic

* Corresponding Author Email: *a.rahdar@uoz.ac.ir*
a.rahdarnanophysics@gmail.com

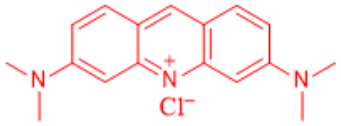

Fig.1. Schematic of AO

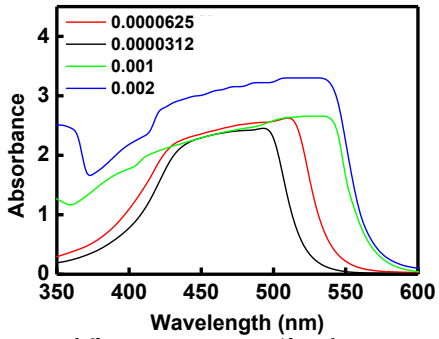

Fig. 2. Absorption spectra of AO in bulk water at different concentrations (from bottom concentrations are 3.12×10^{-5}, 6.25×10^{-5}, 0.001, and 0.002 at room temperature).

photoconductors, and photo-assisted processes in biological systems [3]. Therefore, the study of their optical properties including broad tunability, high quantum efficiency, and broad spectral bandwidth is an important issue to optimize their applications [3].

Acridine orange (AO, Fig.1), a metachromic dye, is used as a probe in study of micro-heterogeneous systems as well as to visualize biological compartments and measurements of pH gradient across the membrane due to their photo-physical and photo-chemical properties which strongly depend on the nature of the surrounding environment [6]. AO has also been used as a probe to investigate the interactions of small molecules with biological and bio-mimicking environments [7].

The photo-physics of various fluorescent dyes in water-in-oil, micro-emulsion has been extensively studied in the literature with a focus on the change of the non-polar bulk oil type and the molar ratio value of polar solvent (typically water) to surfactant. However, the effect of concentration of the dye in the water/AOT/hexane reverse micelle systems at a given water content is still lacking in the literature [1]. Therefore, in the present study, the photo-physics of the cationic dye AO has been investigated in the water/AOT/hexane reverse micelle at the water to the AOT molar ratio W= $[H_2O]$ /[AOT] = 10, and different MFD (mass fraction of a nano-droplet or mass fraction of dispersed phase) values thus changing the acridine orange concentration. For the purpose, we have employed absorption and fluorescence spectroscopic techniques.

According to literature reports the size and shape of W/O microemulsiones are affected by two parameters: (i) value of W (W = [water or polar solvent] / [surfactant]), which reflects the content of polar solvent within the reversed micelle system [1-2] and (ii) the mass fraction of the dispersed phase(mass fraction of water nano-droplet) (MFD = $(M_{disperded\ phase})/(M_{total})$) [8-9, 22-28].

EXPERIMENTAL

Materials

The dioctyl sodium sulfosuccinate (AOT, purity 99%), n-hexane (purity 95%) and acridine orange (purity 95%) have purchased from Sigma–Aldrich Chemical Co. and used without further purification.

Microemulsion preparation method

To prepare the AOT water nano-droplets containing different acridine orang concentrations, at first, a measured amount of acridine orange dye powder was dissolved in deionized water at a certain concentration in which the dye-to-water mass ratio was defined as concentration of the dye in the Aerosol-OT RM. Then the AOT micro-emulsions were prepared by mixing the required weight of the AOT surfactant, n-hexane oil and deionized water containing the different acridine orange concentrations based on the fixed molar ratio of water-to-AOT (W = 10) and finally the system was diluted with n-hexane based on the defined MFD at room temperature [8-9,20-21].

Instrumentation

The absorption spectra of the AOT RMs were carried out by using a UV-1650 PC spectrometer. The emission spectra of the samples at the excitation wavelength 400 nm were recorded with a Jasco FP-6200 spectrofluorimeter. The absorption and fluorescence spectra were properly background subtracted.

RESULT AND DISCUSSION

Absorption spectra

Fig. 2 depicts the absorption spectra of the AO dye at different concentrations in deionized bulk water at room temperature.

As seen from Fig. 2, broadening of the absorption spectra of the dye gradually increases with increasing AO concentration in bulk water along with a red shift. This can be assigned to the aggregation of dye molecules due to the increase in dye concentration in the liquid [8-18].

Figs.3–6 show the absorption spectra of AO fluorescent dye in the AOT MEs at concentrations of 0.002, 0.001,6.25×10^{-5} and 3.12×10^{-5} Mat W=10and different MFDs.

As evident from Fig.2, aqueous solutions of AO probe at high concentrations of 0.002,0.001 in bulk water showed one absorption band at~ 440 nm. Upon transfer of the dye into AOT MEs (Fig. 3 and 4), the absorption spectra of AO within microemulsions were different compared to that in bulk water. This is attributed to the effects of the microenvironment on the spectral properties of AO in water/AOT/n-hexane reverse micellar system due to the abnormal water properties in the water-in-oil microemulsions compared to bulk water [8-15].

As evident from Fig. 3and 4 by increasing MFD from 0.01 to 0.1, the absorption maximum of the acridine orange in the AOT droplet MEs showed a blue shift [8].

On the other hand,at low concentrations of 6.25×10^{-5} and 3.12×10^{-5}, the absorption maximum of acridine orange in AOT MEs at ~ 498 nm differed when compared to that of bulk water

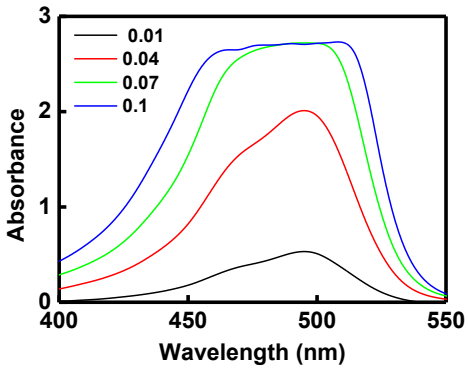

Fig. 3. Absorption spectra of AO (0.002) in AOT micro-emulsion at W=10 for the different droplet mass fraction (from bottom to up) MFD = 0.01, 0.04, 0.07 and 0.1 at RT.

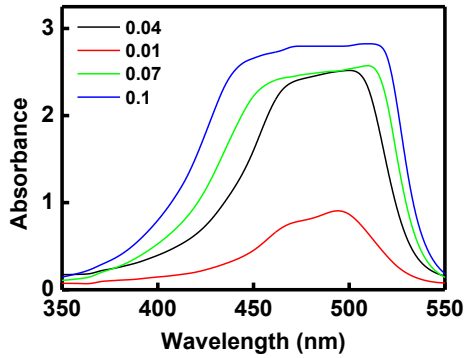

Fig. 4. Absorption spectra of AO (0.001) in AOT micro-emulsion at W=10 for different droplet mass fraction (from bottom to up) MFD = 0.01, 0.04, 0.07 and 0.1 at RT.

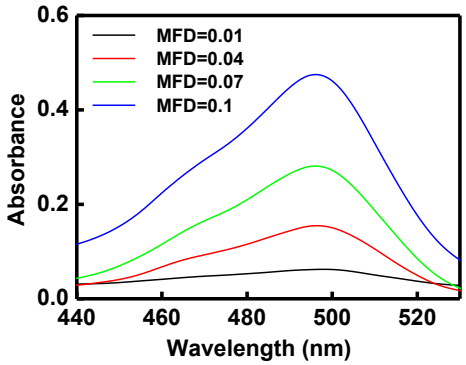

Fig. 5. Absorption spectra of AO (6.25×10^{-5}) in AOT micro-emulsion at W=10 for the different droplet mass fraction (from bottom to up) MFD = 0.01,0.04, 0.07 and 0.1 at RT.

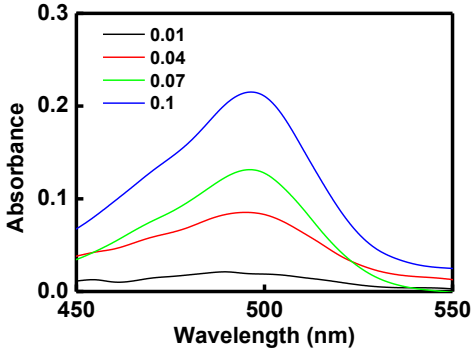

Fig. 6. Absorption spectra of AO (3.12×10^{-5}) in AOT micro-emulsion at W=10 for the different droplet mass fraction (from bottom to up) MFD = 0.01,0.04, 0.07 and 0.1 at RT.

at MFD = 0.1 (Figs. 5 and 6). Thus, from Figs. 3,4,5 and 6 the following conclusions can be made: (i) the absorption spectra of AO at high concentrations (0.002, 0.001) were wider than those in low concentrations (6.25×10^{-5}, 3.12×10^{-5}), (ii) the absorption spectra of the dye deviated wider than those in low concentrations, and (iii) the absorption spectra of the dye deviated from Beer's law at a larger MFD (MFD = 0.1) at high concentrations, whereas at low concentrations, the Beer's law was obeyed as a function of MFD [8-14]. The broadening of the absorption spectra and deviation from Beer's law in the AOT RMs that relies on the MFD parameter was attributed to the aggregation of the dye molecules of acridine orange at a high concentrations [8-9,14-15].

Variation of absorbance with MFD of AOT MEs containing different concentrations of AO is shown in Fig.7.

The variation of λ_{max} of absorbance of AO from Figs. 3-6 as a function of MFD in water/ AOT/n-hexane MEs containing different acridine orange concentrations is shown in Fig.8.

The results presented in Figs.7-8 indicate that variations in the absorption wavelength and intensity of AO at high concentrations (0.001 and 0.002) are more significant than those at low concentrations (6.25×10^{-5} and 3.12×10^{-5}) within AOT microemulsion.

On the other hand, our results indicated that the absorption wavelength and intensity of the dye within AOT microemulsionare different from those in bulk water.

These observations are explained based on the localization of the dye molecules in interfacial or core region of the water nano-droplets of microemulsion system that leads to the observed changes in the microenvironment around AcridineOrange within the water nano-droplet compared to that in the bulk phase of water [8-14].

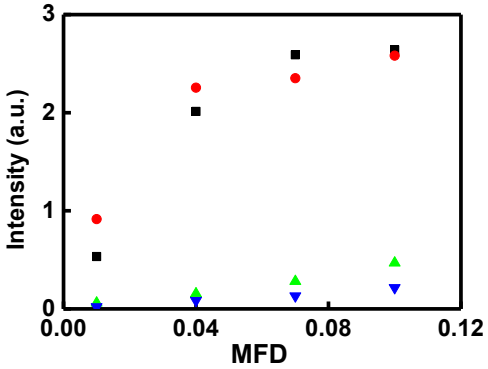

Fig. 7. Variation of absorbance of AO with droplet mass fraction of AOT MEs at different concentration of AO; square: 0.002, circle: 0.001, up triangle: 6.25×10^{-5}, down triangle:3.12×10^{-5} at W=10.

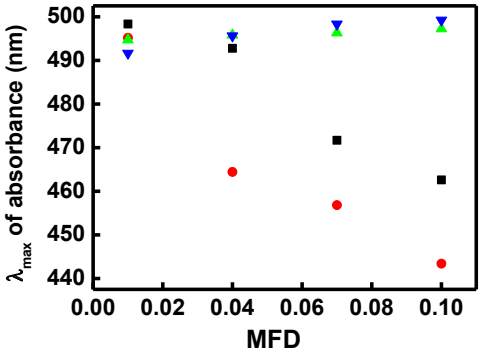

Fig. 8. Variation of absorbancewithMFD in AOT MEs containing different concentrations of AO; square: 0.002, circle: 0.001, up triangle: 6.25×10^{-5}, down triangle: 3.12×10^{-5} of W=10.

Emission spectra

The fluorescence spectra of AO dye in the bulk water are shown in Fig. 9.

Fig. 9 shows that the fluorescence spectrum of AO at a high concentration (0.002) in bulk water shows a structure less band at 642 nm.

The fluorescence spectra of AO in the AOT MEs at concentrations of 0.002, 0.001 ,6.25×10^{-5} and 3.12×10^{-5} M at W=10 are shown in Figs. 10-13, respectively.

As apparent from Figs.10-11, a comparison of the maximum fluorescence band of acridine orange at high concentrations of 0.001, 0.002showed that the fluorescence spectra of the dye in the water/ AOT/n-hexane MEs were shifted to the blue region and fluorescence intensity of AO decreased as MFD increased from 0.01 to 0.1.

In case of the fluorescence spectrum of acridineorange at low concentrations of 6.25×10^{-5} and 3.12×10^{-5} M, a comparison of the maximum fluorescence band of AO showed that the emission intensity of acridine orange in AOT MEs increased as a function of MFD (Fig. 12). It is well-known that the concentration of dye affects its fluorescence behavior [8-15,20-21].

Variation of fluorescence intensity of AO vs. MFD of AOT MEs containing different concentrations the acridineorange is shown in Fig. 14.

$\lambda_{max}$of fluorescence using Figs. 10-13 as a function of MFD in water/ AOT/n-hexane MEs containing different acridine orange concentrations is shown in Fig.15.

It is clear from the data in Figs. 14-15 that the changes in intensity and λ_{max} of fluorescence are most significant in a region where the MFD of the water/AOT/n-hexane microemulsionis relatively small and approached an extreme at larger MFDs.

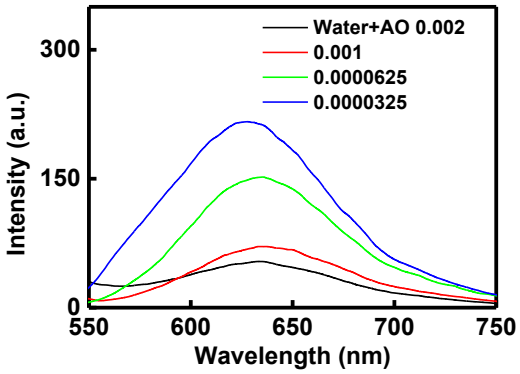

Fig. 9. Fluorescence spectra (λ_{ex} = 400 nm) of acridine orange in bulk water at various concentrations (from bottom to top: 0.002, 0.001, 6.25×10^{-5} and 3.12×10^{-5} at room temperature.

Table 1. The andfluorescence intensity of acridine orange in bulk water at various concentrations (0.002, 0.001, 6.25×10^{-5} and 3.12×10^{-5} at room temperature).

m_{dye}/m_{water}	λ_{max}(nm)	Fluorescence intensity(a.u.)
0.002	641.62	50.46
0.001	639.50	70.87
6.25×10^{-5}	635.96	125.06
3.12×10^{-5}	627.39	215.18

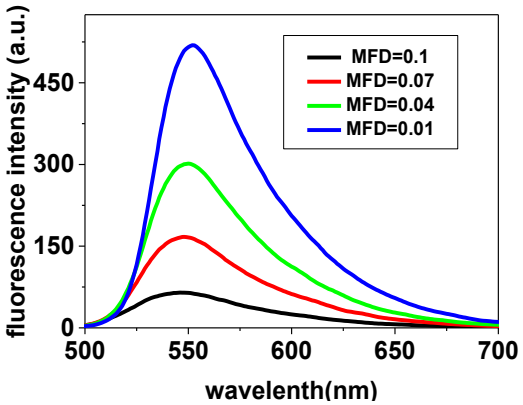

Fig. 10. Fluorescence spectra (λ_{ex} = 400 nm) of AO (0.002) in AOT MEs at W=10 at different MFD (from top to bottom MFD = 0.01, 0.04, 0.07 and 0.1 room temperature).

On the other hand, for dye at concentrations of 0.002, 0.001, the fluorescence intensity decreased as a function of MFD, while for dye at concentrations of 6.25×10^{-5}, 3.12×10^{-5} ,the fluorescence intensity is directly proportional to MFD. These results are in good agreement with former results, focusing on Rhodamine B within AOT microemulsion [8-9].

Decrease in fluorescence intensity of dye in high concentrations with MFD was attributed to increase in water droplet size within AOT microemulsion due to increase in droplet surface fluidity, whereas increases in fluorescence intensity of dye at low concentrations with MFD was attributed to decrease in water droplet size within AOT microemulsion due to increase in droplet surface rigidity [9, 21].

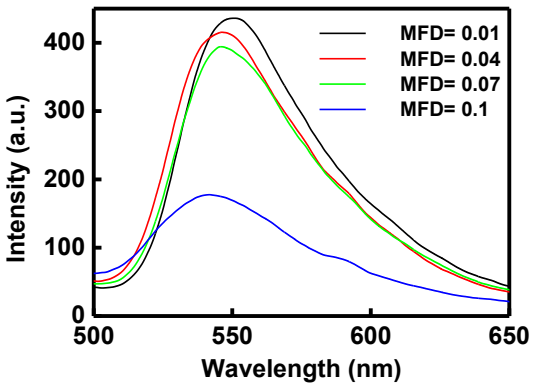

Fig. 11. Fluorescence emission spectra (λex = 400 NM) of AOT MEs containing acridine orange fluorescent dye at 0.001 concentrations at W=10 at different MFD (from top to bottom MFD = 0.01, 0.04, 0.07 and 0.1 room temperature).

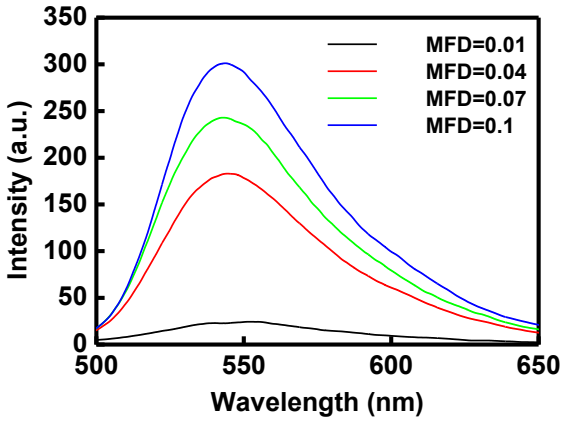

Fig. 12. Fluorescence spectra (λ_{ex} = 400 nm) of AO (6.25×10^{-5}) in AOT MEs at W=10 at the different MFD (from bottomto top MFD = 0.01.0.04.0.07 and 0.1 at room temperature).

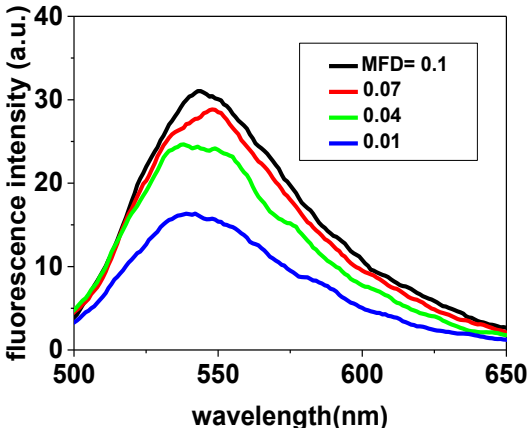

Fig. 13. Fluorescence spectra (λ_{ex} = 400 nm) of AO (3.12×10^{-5}) in AOT MEs at W=10 at the different MFD (from bottom to top MFD = 0.01, 0.04, 0.07 and 0.1 at room temperature).

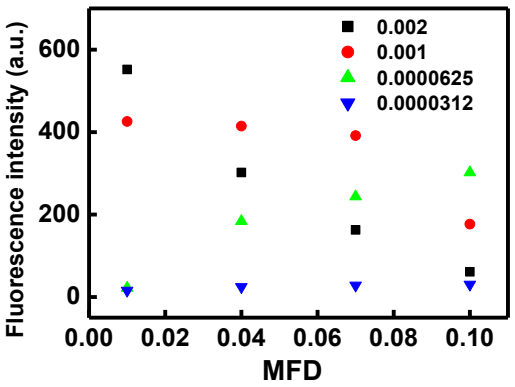

Fig. 14. Variation of fluorescence intensity versus droplet mass fraction of AOT MEs containing of AO in different concentrations; square: 0.002, circle:0.001, up triangle: 6.25×10^{-5} and down triangle: 3.12×10^{-5} of W=10.

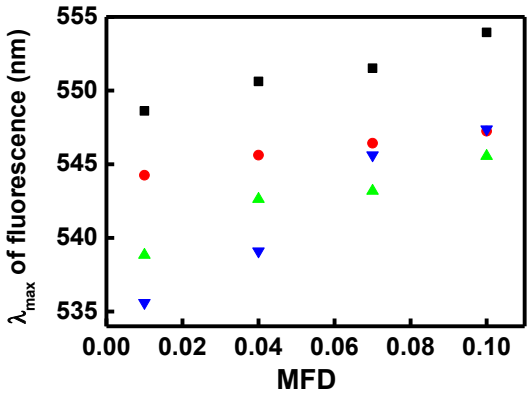

Fig. 15. Variation of the fluorescenceversus MFD in AOT MEs containing different concentrations ofacridine orange; square: 0.002, circle: 0.001, up triangle: 6.25×10^{-5} and down triangle: 3.12×10^{-5} of W=10.

In summary, these changes in the photo-physical properties of AO in AOT reversemicelle were assigned to change in droplet size due to change in the Acridine Orange concentration, hydration of the head groups of AOT surfactant, inter-nanodroplet interactions, inter-nanodroplet mass exchange rate [9-21].

It is also shown in Figs. 9- 15 that λ_{max} of AO at high concentrations (0.002, 0.001 M) and low concentration (6.25×10^{-5}, 3.12×10^{-5} M) in the AOT RM was 641, 639 nm and 635, 627nm respectively, which are still significantly shorter than that in bulk water. It can be explained by the fact that the acridine orange molecules are preferably localized at the oil–water interface, due to the attractive electrostatic interactions between the cationic dye molecules and the anionic sulfonate groups of AOT [8-9,2-20]. In other words, the shift in the fluorescence and absorption spectra of acridine orange within the nanometer-sized AOT micro emulsions compared to that in bulk water clearly revealed that the microenvironment around the dye molecules in AOT micro emulsions is significantly different than that in bulk water [8-9,2-20].On the other hand, the variation in the absorption and fluorescence emission maxima between MFD = 0.1 and MFD = 0.01 can also be attributed to the difference in the electronic ground-state dipole moment of AO in the AOT RMs than its electronic excited-state dipole moment [20].

Absorption (ν_a) and fluorescence (ν_f) band shift of AOT RMs based on quantum mechanical perturbation theory

The following equations, that is,$\nu_a - \nu_f = m_1 f(\varepsilon, n) +$ const. and $\nu_a + \nu_f = -m_2[f(\varepsilon, n) + 2g(n)] +$ const. are obtained from quantum mechanical perturbation theory of absorption (ν_a) and fluorescence (ν_f) band

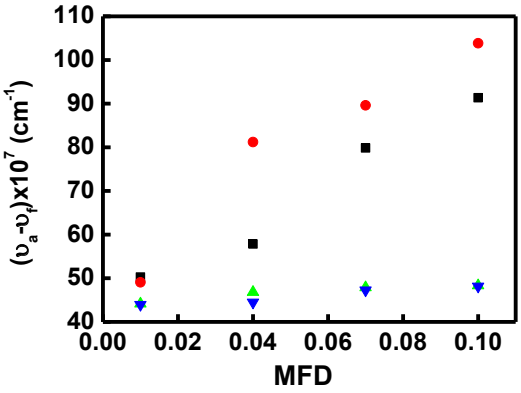

Fig.16. The (Stokes shift) (cm^{-1}) versus MFD of AO in AOT RMs at W=10 containing various concentrations of; square: 0.002, circle: 0.001, up triangle: 6.25×10^{-5} and down triangle: 3.12×10^{-5} M at RT.

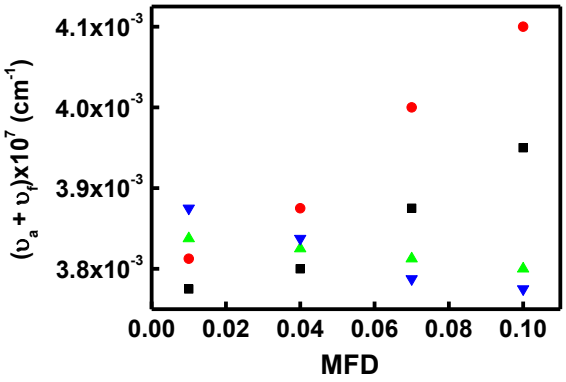

Fig. 17. The $\nu_a + \nu_f$ (cm^{-1}) versus MFD of AO in AOT MEs containing different concentrations of the dye; squared: 0.002, circle: 0.001, up triangle: 6.25×10^{-5} and down triangle: 3.12×10^{-5} at RT at W=10.

shift [20]. In AOT MEs various concentrations of AOwere used. In this case, the permittivity (ε) and refractive index (n) variations of the systems were negligible because the solvent type (here water) in this system was constant.

The spectral shifts $\nu_a - \nu_f$ (stokes shift), $\nu_a + \nu_f$ and the dipole moment ratio of the ground to excited state (μ_g/μ_e) of AO in the water/ AOT/n-hexane microemulsion as a function of MFD are also shown in Figs. 16-17, respectively.

It is clear from Fig. 16 for W=10 that the stokes shift of acridine orange at high concentrations of 0.002, 0.001 and low concentrations of 6.25×10^{-5}, 3.12×10^{-5} increases as a function of MFD within water/AOT/n-hexane MEs [8-9,20].

On other hand, these results indicate that variations of Stokes shift of acridine orange at high concentrations of 0.002, 0.001 with MFD occur to a greater extent compared to that with low concentrations (6.25×10^{-5}, 3.12×10^{-5}) of AO within water/AOT/n-hexane MEs. These results indicate that the surrounding environment of acridine orange at high concentrations change drastically between MFD = 0.01 and 0.1, but remains almost invariant with MFD at low concentrations of AO [8-9,20]. Variations of the Stokes' shift of AO with MFD within AOT microemulsion was attributed to the change in dipole moment ratio of acridine orange depending on its 5relative concentrations in the excited state and ground state with MFD in AOT RMs [8-10, 20].

The dipole moment ratio of the ground to excited state (μ_g/μ_e) of AO with MFD in the AOT RMs in W= 10 is shown in Fig.18.

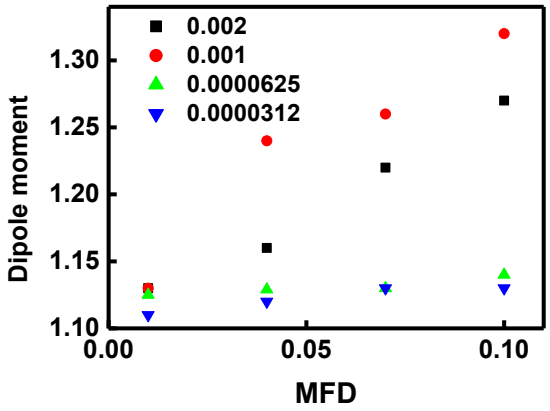

Fig.18. The dipole moment ratio of the ground to excited state versus droplet mass fraction of AOT MEs containing different acridine orange concentrations; squared: 0.002, circle: 0.001, up triangle: 6.25×10^{-5} and down triangle: 3.12×10^{-5} at RT at W=10.

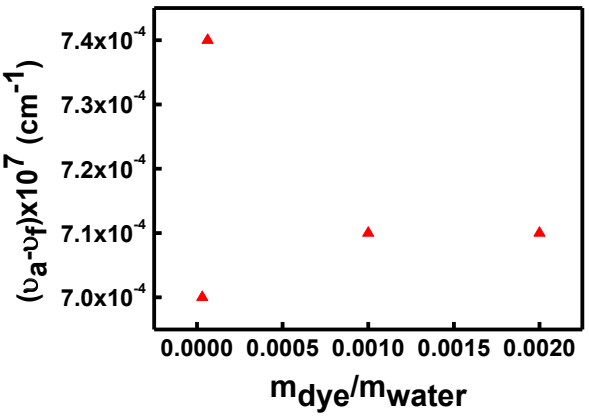

Fig. 19.(Stokes shift) (cm^{-1}) of AOin bulk water at different concentrationsof0.002, 0.001, 6.25×10^{-5} and 3.12×10^{-5} at RT.

As evident in Fig.18, the variation of the dipole moment ratio of the ground to excited state (μ_g/μ_e) of AO at high concentrations of 0.002 and 0.001is more significant than those at low concentrations of AO. So, the different band shifts in the emission and absorption spectrum of AO can be attributed to variation in the dipole moment ratio of the ground to excited state (μ_g/μ_e) of AO as a function of concentration of the dye with MFD in the AOT RMs [8-9,20].

The Stokes shift of acridine orange with MFD in bulk water is also shown in Fig.19.

The dipole moment ratio of the ground to excited state (μ_g/μ_e) of AO with MFD in the bulk water is shown in Fig.20.

Changes in dipole moment ratio of the ground to excited state and Stokes shift of AO at different concentrations in bulk water compared to those within AOT microemulsion were attributed to decrease in the extent of dye aggregation and increase in viscosity of environment within AOT microemulsion compared to that in bulk water [8-9,20].

CONCLUSION

In conclusion, the spectroscopic properties of Acridine Orange (AO) dye were studied in the water/AOT/n-hexane microemulsion at a fixed water content of 10 (W=[H2O]/[AOT]=10) using absorption and fluorescence spectroscopy. Transferring Acridine Orange dye from bulk water phase into the water/AOT/n-hexane microemulsion system induced variations in its spectroscopic properties. The Acridine Orange at high concentrations (m_{dye}/m_{water}=0.001, 0.002) in the AOT microemulsion exhibited a broad

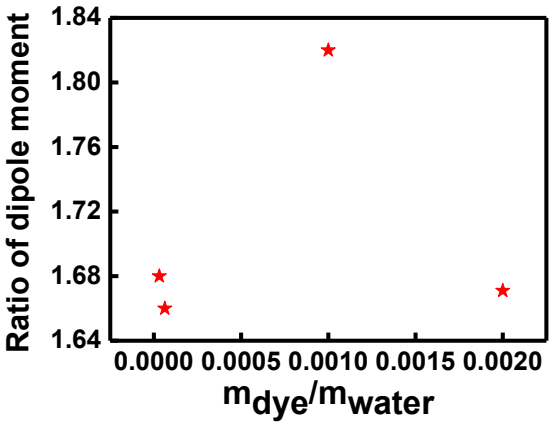

Fig.20.The dipole moments ratio of the ground to excited state of AO at different concentrations in bulk water; 0.002,0.001, 6.25×10^{-5} and 3.12×10^{-5} at RT.

absorption spectra and deviation from Beer's law and fluorescence self-quenching as a function of the mass fraction of droplet (MFD) that was assigned to the aggregation phenomenon of AO molecules. On the other hand, at lower concentrations of AO molecules ($m_{dye}/m_{water}=6.25\times10^{-5}$, 3.12×10^{-5}), the absorption intensity of dye increased with increasing MFD, and follows from Beer's Lambert. The intensity of emission of AO at the low concentrations increased with increasing MFD. variations of Stokes shift and the dipole moment ratio of the ground to excited state (μ_g/μ_e) of acridine orang dye at high concentrations of 0.002, 0.001 with MFD were more considerable than those of low concentrations 6.25×10^{-5}, 3.12×10^{-5} of dye within water/AOT/n-hexane MEs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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