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Research Article

3D Fluorescence Spectroscopic investigation on the interaction of Alizarin Red dye with Ionic Micelles

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Abstract:

We have explored the interaction of Alizarin red dye (Sodium salt of 1,2-dihydroxy-9,10-anthraquinone-3-sulfonic acid) with two ionic micelles, Sodiun dodecyl sulphate (SDS) and Cetyl trimethyl ammonium bromide (CTAB) using steady state and three dimensional (3D) fluorescence spectroscopy. Change in the absorption spectral profile of Alizarin red with the two different charged surfactants were measured and is due to the encapsulation of Alizarin red within the micellar microenvironment. Emission intensity is decreased and the wavelength is red shift at higher concentration in presence of SDS anionic micelles. In contrast, the wavelength is blue-shifted in presence of cationic micelle, CTAB indicating the strong interaction between Alizarin red and CTAB. The binding parameters (binding constant and binding sites) are determined from fluorescence emission intensity values using steady-state experiments. Binding of Alizarin red dye with both the ionic micelles occurred via, hydrophobic and electrostatic interaction.

Index Terms— Alizarin red, SDS, CTAB micelle, 3D fluorescence techniques

*1.*INTRODUCTION

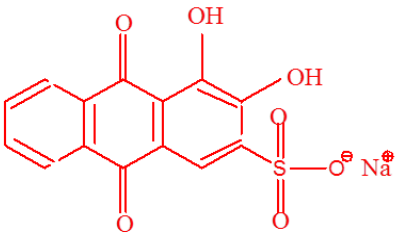
Numerous researchers have been interested in the interactions of dyes with surfactants and micelles. Micelles have recently attracted the attention of analytical chemists due to their increased selectivity and sensitivity, increased solubility of organic compounds in water, ability to catalyze reactions, and most importantly, the fact that their microenvironment changes in the presence of any probe. Micellars olubilization of hydrophobic substances is very useful in practise. When a surfactant is introduced at sub-micellar concentrations to an Alizarin red dye solution, both the surfactant monomer and the dye aggregates may interact, via electrostatic and/or hydrophobic interactions may occur [1]. We have focused on both the extent and the locations of solubilization. Spectral analysis can identify micellar binding sites and quantify local polarity surrounding the probe. Alizarin Red dye is a well-known biological staining agent and ESIPT probe. Surfactants are utilised in different dyeing processes, thus studying their interactions is important for industrial purposes [2]. Because of their complexity, understanding the nature and mechanism of dye-surfactant interactions is important and has been focus of

much research [3-6]. Studies have shown that chemical structure plays a major role in the interactions of dyes and surfactants [7-10]. Micellar systems can solubilize poorly soluble drugs, increasing their bioavailability, and therefore, used as drug carriers by encapsulation of the drugs, in order to ensure the transport to specific sites of action, to minimize drug degradation and loss, to prevent harmful side effects, thus improving the treatment efficacy [11]. The extent of interaction between the drugs and the surfactants can be best described by the hydrophobic effect and the electrostatic effect (primarily determined by the charge associated with the drug molecule as well as the surfactant molecules) [12,13]. Nowadays, the researchers are investigating new methods to develop efficient and cost effective technologies to remove environmentally harmful dyes from industrial effluents. In this regard, micellization is one of the methods to trap the hydrophobic dyes from the effluents [17].These changes have been earlier observed effectively by a number of techniques such as conductometry, fluorescence spectroscopy, spectrophotometry, potentiometry and voltammetry [18-22], where dye-surfactant interactions were found to be influenced by the charge, alkyl chain length of surfactants and the location of the substituents on the aromatic ring of the dye molecules. Photoinduced electron transfer operation in surfactant solutions is conceivably important for efficient energy conversion and storage since surfactant micelles assist to attain the separation of the photoproducts by hydrophilic-hydrophobic interactions of the products with the micellar interface [23-26]. Thus, the main goal of the present investigation is to study the influence of micelle environment on the Alizarin red dye molecules. Herein, we report the interaction of Alizarin red dye with two different micelles, SDS (Sodium dodecyl sulphate) and CTAB (Cetyl trimethyl ammonium bromide) using 3D fluorescence spectroscopy.

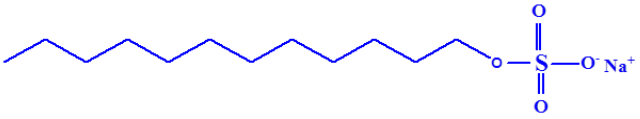
2 EXPERIMENTAL

2.1 MATERIALS AND METHODS

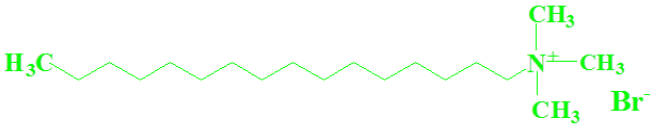
Alizarin red, SDS and CTAB were purchased from Sigma Aldrich chemicals were freshly prepared for each measurement. The absorption spectral measurements were carried out using Perkin Elmer Lambda spectrophotometer. The emission measurements were done with FP8500 JASCO spectrofluorometer Fluorescence emission spectra were recorded in the wavelength range 500-650nm by exciting probe at 517nm using a slit width of 5nm.The interaction of Alizarin Red with micelles was studied by means of fluorimetric titration. The titration was carried out in triple distilled water, by adding aliquots of surfactant ($2.4x10^{-3}$M) stock solution to a fixed amount of alizarin red ($1.5x10^{-5}$M) taken to obtain the molar ratio in the range 1to15.



(a) Structure of Alizarin Red.



(b) Sodium dodecyl sulphate (SDS)



(C) Cetyl trimethyl ammonium bromide (CTAB)

Chart 1. Molecular Structure of (a) Alizarin Red dye and surfactants (b) SDS and (c) CTAB

3 RESULTS AND DISCUSSION

The molecular structure of Alizarin red dye and the surfactants used in present study is given in Chart 1. Interaction studies between the dye and the charged surfactants in premicellar and post micellar conditions are carried out with the aid of absorption and 2D & 3D fluorescence spectroscopy. The binding sites and binding constants are determined from the emission intensity measurements.

3.1 Absorption Spectral features

The absorption spectrum of Alizarin Red in aqueous medium is given in Fig 1. The lowest electronically excited state of an unsubstituted anthraquinone lies in the UV- region, due to the n→π* character. Insteadπ→π*transition state becomes the lowest excited state when an anthraquinone is substituted with an OH group. The present probe molecule has two OH groups as substituents, which are having electron donating character, and the presence of sulfonate group makes the molecule hydrophilic (Chart 1(a)). The absorption spectrum of Alizarin Red dye showed maxima at 260, 333, 517 nm.The peak at 260nm corresponds to the n → π* transition and the peak at 517nm, is assigned to →π*, i.e., intramolecular charge transfer band from the substituent to the aromatic π system.

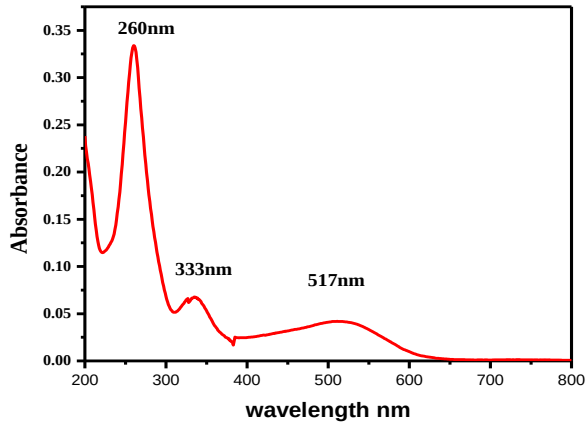


Fig. 1. Absorption spectrum of Alizarin Red dye in aqueous media.

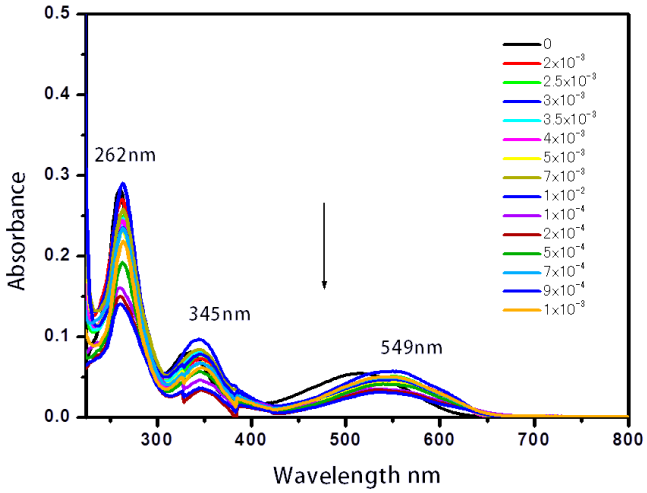


Fig. 2. Absorption spectrum of Alizarin Red dye in various concentration of CTAB.

UV-Visible absorption measurements were carried out to study the electronic interactions of Alizarin red with the micelle (CTAB,). The change in absorption spectrum of Alizarin Red with different concentration of CTAB is shown in Fig 2. The absorption maximum of Alizarin red in presence of CTAB at 517 nm is shifted towards the longer wavelength region (555 nm). It could be conception that aggregation of the cationic CTAB micelles with the anionic dyes defeats their mutual repulsion forces and thus favours the dye polymerization [7]. This red shift indicates that the alizarin red molecule is located in the polar periphery of the head group than in the bulk water environment.

Variation in the absorption maximum, λmax at 0.01mM Alizarin red dye as a function of CTAB and SDS concentrations is presented in Fig.3. In the pre-micellar concentration of the surfactant, the CTAB molecules agglomerate in presence of SO^{3-} anions of Alizarin red, generating an aggregate with pollutant removing properties. Consequently, in the pre-micellar condition, the electronic spectra could not be registered. Whereas in the post micellar concentration, bathochromic shift from 517 nm to 555 nm

was observed. This red shift can be attributed to the incorporation of the Alizarin red dye in the polar region of the CTAB micelles.

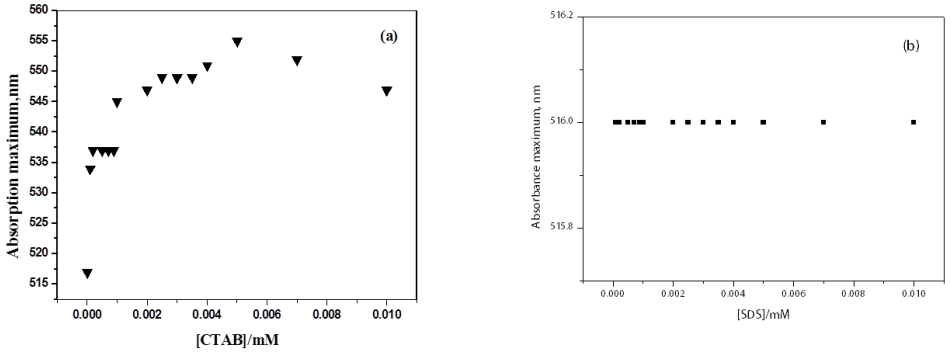


Fig. 3. Variation of the absorption maxima of Alizarin red with different concentration of (a) CTAB and (b) SDS

The experiments are repeated with the anionic micelle SDS. The absorption spectrum of Alizarin red dye with different concentration of SDS is shown in fig 4. There is no appreciable shift in the absorption maxima at 517nm and there is a slight increase in the absorbance, indicating the formation of a stable system. The results revealed that both the dye and the surfactants are anionic and the dye shuttles between the micelle and the bulk phase.

3.2. Determination of partition coefficient

Partition coefficient, Kx is a dominant parameter to determine the partition of dye between the micellar and the bulk water phases. Partition coefficient Kx can be calculated from the following equation [28].

$$\frac{1}{\Delta A} = \frac{1}{\Delta A^{\infty}} + \frac{1}{K_C \Delta A^{\infty}\left(C_{Dye} + C_{Sf} - CMC\right)}$$

$$K_C = \frac{C^m_{Dye}}{C^m_{Dye}\left(C^m_{Dye} + C^m_{Sf}\right)}$$

$$K_X = \frac{X^m_{Dye}}{X^w_{Dye}}$$

where X^m_{Dye} and X^w_{Dye} are the mole fraction of dye in micellar and aqueous phases.

K_c and K_x were obtained from the intercept and slope of the plot $1/\Delta A$ versus $1/(C_{Dye}+C_{Sf}-CMC)$. Using the absorbance intensity of the micelle-bound dye (in fig.5 and 6), partition coefficient K_x of Alizarin red with CTAB micelle was obtained as 34.91 and the value of K_c , partition constant is $0.2M^{-1}$. Similarly in SDS micellar environment, K_x of Alizarin red is 149.89 and K_c is $0.2041M^{-1}$. The calculated values of Kx and Kc are comparable with the reported literature values [28].

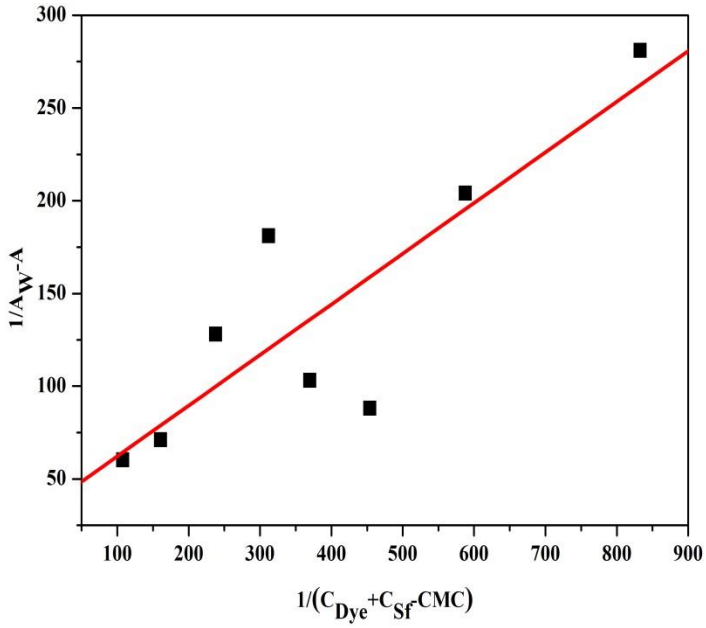


Fig. 5 . Plot of 1/ΔA versus 1/(C_{Dye}+C_{Sf}-CMC) for the interaction of Alizarin red with CTAB

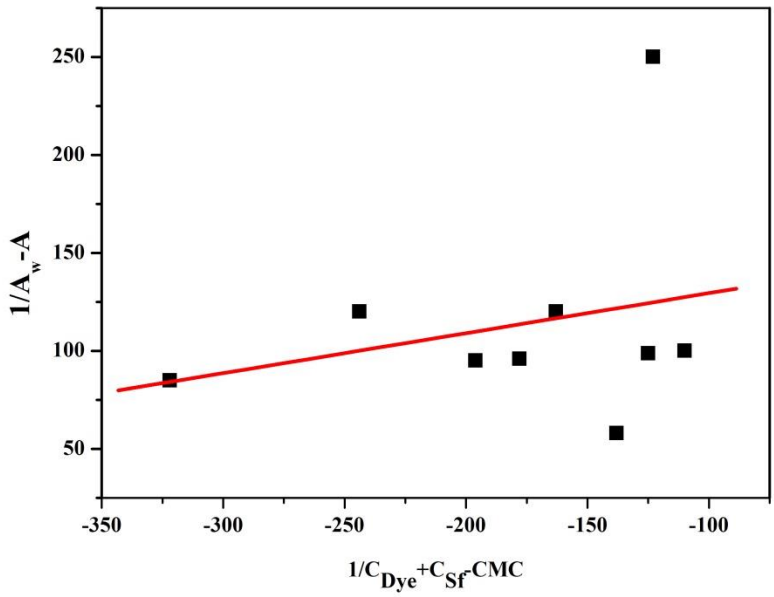


Fig.6. Plot of 1/ΔA versus 1/(C_{Dye}+C_{Sf}-CMC) for the interaction of Alizarin red with SDS

3.3. FLUORESCENCE SPECTRAL FEATURES

Emission spectrum of Alizarin Red in aqueous medium is given in Fig 7. On excitation at 515nm it showed two peaks at 585 and 623 nm. One belongs to the normal (N*) form and the other belongs to phototautomer (T) (Scheme 1), both forms highly emissive states and their spectral bands are well separated on the wavelength scale. In the excited state, proton transfer from OH group (1- position) to the carbonyl oxygen due to $\pi \rightarrow \pi^*$ charge transfer with great change in the molecular geometry will occur. So the OH group is more acidic and the carbonyl oxygen is more basic in the excited state than in ground state. This $\pi \rightarrow \pi^*$ band is assigned to the intramolecular charge transfer band from the substituent to aromatic system.

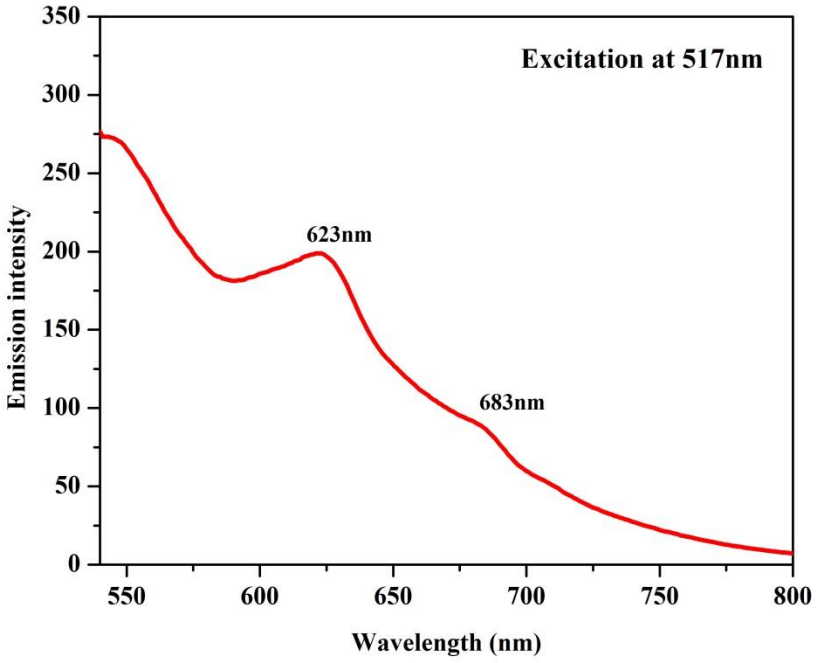
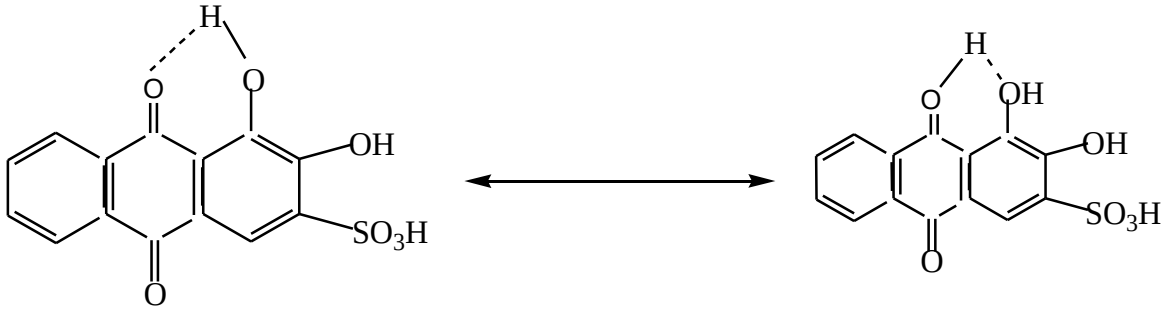


Fig.7. Emission spectrum of Alizarin Red dye in aqueous media (Excited at 517 nm).



Scheme1. Normal and phototautomer structures of Alizarin Red dye.

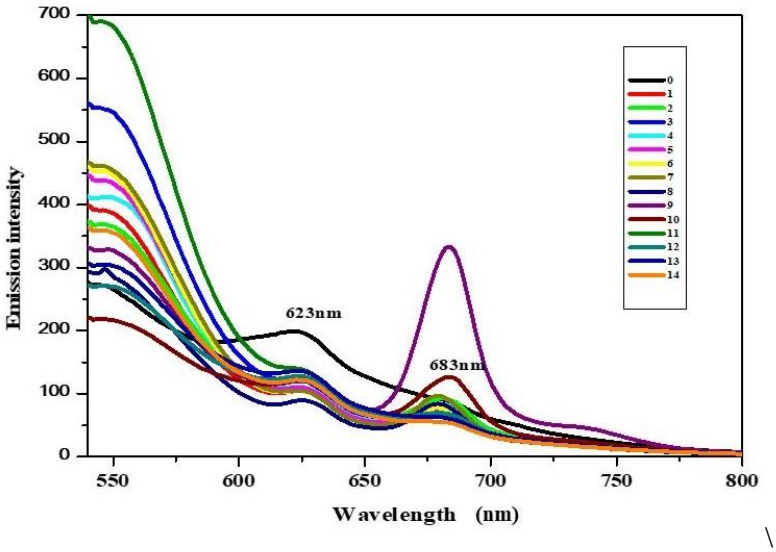


Fig. 8. Emission spectrum of Alizarin Red dye in different concentration of SDS (0, $2x10^{-3}$, $2.5x10^{-3}$, $3x10^{-3}$, $3.5x10^{-3}$, $4x10^{-3}$, $5x10^{-3}$, $7x10^{-3}$, $1x10^{-2}$, $1x10^{-4}$, $2x10^{-4}$, $5x10^{-4}$, $7x10^{-4}$, $9x10^{-4}$, $1x10^{-3}$M)

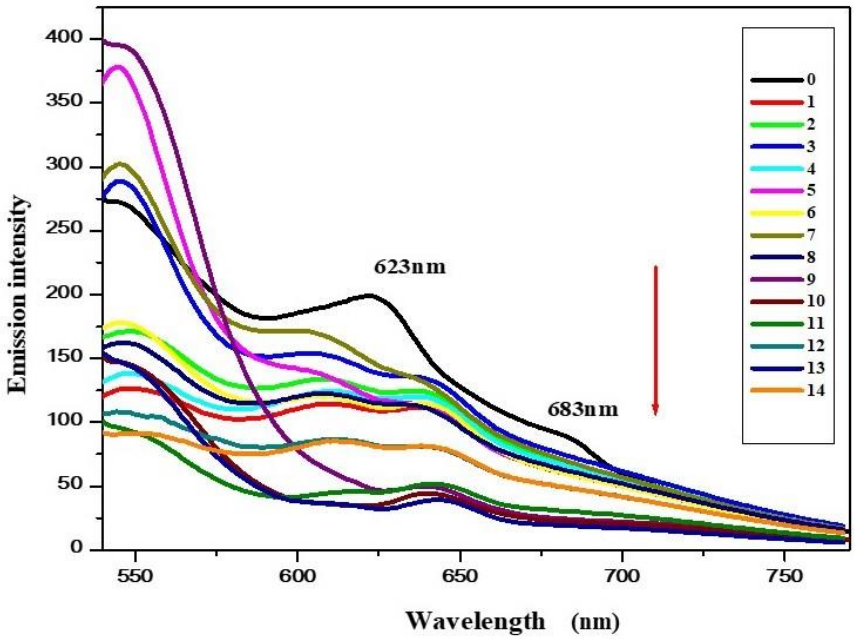


Fig.9. Emission spectrum of Alizarin Red dye in different concentration of CTAB(0, $2x10^{-3}$, $2.5x10^{-3}$, $3x10^{-3}$, $3.5x10^{-3}$, $4x10^{-3}$, $5x10^{-3}$, $7x10^{-3}$, $1x10^{-2}$, $1x10^{-4}$, $2x10^{-4}$, $5x10^{-4}$, $7x10^{-4}$, $9x10^{-4}$, $1x10^{-3}$M)

Emission spectrum of Alizarin Red dye in different concentration of SDS is shown in Fig 8. The magnitude of fluorescence intensity decreased on increasing the concentration of SDS. This decrease in fluorescence intensity exposes the fluorescence quenching of any photophysical process. The fluorescence intensity gradually decreases up to the concentration (7x10-3M) and further increase in the concentration leads to the fluorescence intensity increase. The change in emission intensity and red shift are due to the

predominant hydrophobic interaction of SDS with alizarin red dye molecule prefers to the cluster association with the polar region of SDS head group, resulting in red shift spectrum [16]. Emission spectrum of Alizarin Red dye in different concentration of CTAB is shown in Fig 9. Upon incremental addition of CTAB surfactants, the intensity gets decreased with a shift towards the lower wavelength region (blue shift). This is due to the hydrophobicity of the media for the presence of aromatic groups of dye and probe molecules were attracted towards from polar aqueous phase to the relatively non polar surface of micelles due to the electrostatic force of attraction.

3. DETERMINATION OF BINDING PARAMETERS

When small molecules bind independently to a set of equivalent sites on the macromolecules, belong to equation is used to describe the relationship between fluorescence intensity and the concentration of the quencher.

$$\log\frac{F_0 - F}{F} = \log K_A + n\log[\text{Surf}]$$

Plot of log $(F_0\text{-}F)/F$ *vs* log [Q] gives a straight line and the binding constant is $1.887M^{-1}$ and number of binding sites, n is 0.9428 (~1). From the results, it was observed that there is a strong binding interaction between Alizarin red and anionic micelles (SDS), though the dye/surfactant system is anionic, binding via., hydrophobic interaction. The value of 'n' is ~ 1 pointed out the existence of single binding site in anionic micelle for Alizarin red dye. In contrast, the binding constant is $0.1587M^{-1}$, number of binding sites n is ~1(0.7428) in the cationic micellar environment.

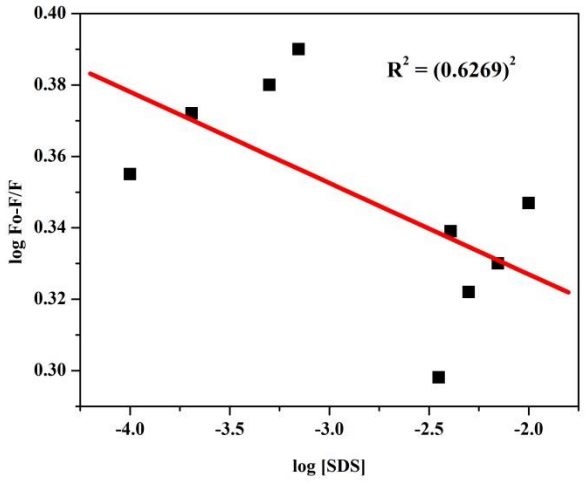


Fig.10. Binding plot for the interaction of Alizarin Red with SDS.

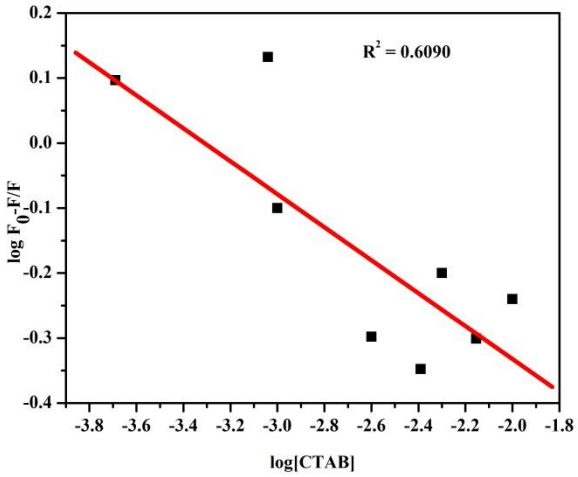


Fig.11. Binding plot for the interaction of Alizarin Red with CTAB.

4. 3D FLUORESCENCE SPECTRAL ANALYSIS

Three-dimensional fluorescence spectra have become a prominent fluorescence analysis technique in recent years [14]. The excitation wavelength, the emission wavelength and the fluorescence intensity can be used as the axes in order to investigate the information of the samples, and the contour spectra can also provide information about the location of the dyes present in the microenvironment [15]. It is well known that three-dimensional fluorescence spectrum can provide more detailed information about the change of the configuration of the biomolecules and also been used for qualitative analysis. Three dimensional spectra of Alizarin red in water is shown in Fig. 12. Two characteristics excitation/emission peaks for Alizarin red in water are identified on the fluorescence 3D spectrum with centers located at $\lambda_{ex}/\lambda_{em}$ = 500/610nm and $\lambda_{ex}/\lambda_{em}$= 515/680nm. Peak 1 and peak 2 corresponds to the spectral characteristic of Alizarin red in normal form and photo tautomer form when it is excited at 500 nm.

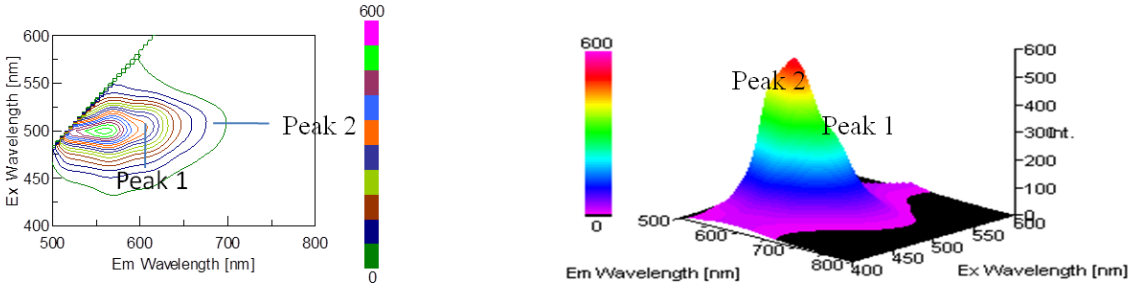


Fig.12. Three dimensional contour and emission spectra of Alizarin red in water.

4.2. ANALYSIS OF ALIZARIN RED IN CTAB

Figure 13 and 14 presents the contour and three dimensional spectra of Alizarin red in various concentrations of CTAB is shown below. Peak 1 $\lambda_{ex}/\lambda_{em}$= 430/585nm and peak 2 $\lambda_{ex}/\lambda_{em}$= 430/685nm are the two fluorescence peak. Peak A λex/λem= 550/550 → 600/600 is the Raleigh scattering peak. Upon, we increasing the concentration of CTAB, the two fluorescence peaks of Alizarin red (Peak 1 $\lambda_{ex}/\lambda_{em}$= 500/550nm and Peak 2 $\lambda_{ex}/\lambda_{em}$= 500/650) shift towards in the higher energy region (hypsochromic shift) was seen in fig 15. So due to the blue shift or hypsochromic shift, the probe molecule is located on the hydrophobic region of the micelle. Due to hydrophobicity of the media, probe molecule (Alizarin red dye) molecules were move from polar region to the nonpolar surface of cationic micelles(CTAB).

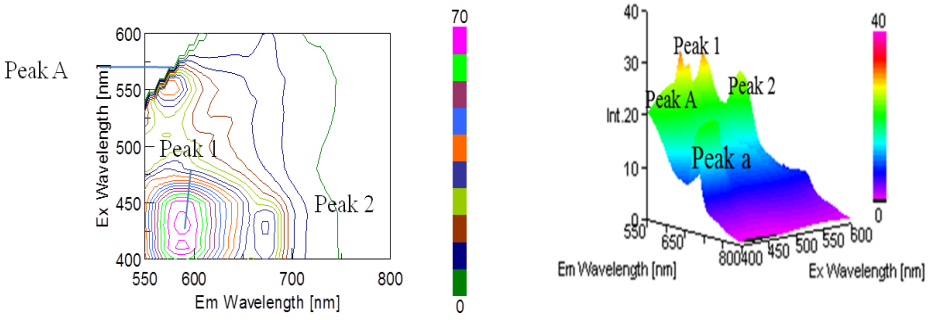


Fig.13. Contour and three dimensional emission spectra of Alizarin red in CTAB (2x10^{-3}M)

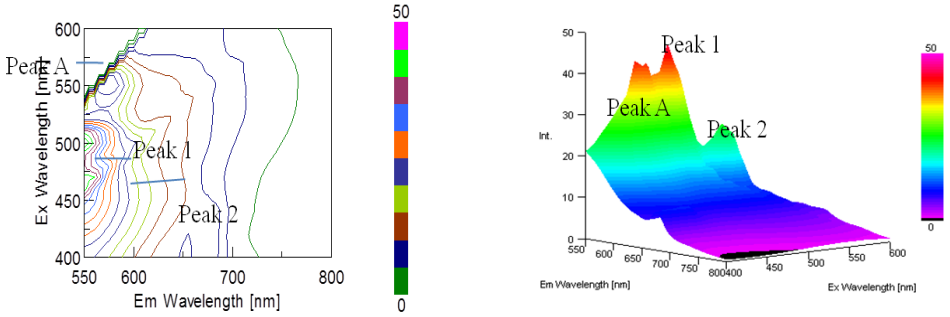


Fig.14. Contour and three dimensional emission spectra of Alizarin red in CTAB (1x10^{-2}M)

4.3. ANALYSIS OF ALIZARIN RED IN SDS

Figure 15 and 16 presents the contour and three dimensional spectra of Alizarin red in various concentrations of SDS is displayed below. Peak 1 $\lambda_{ex}/\lambda_{em}$= 500/620nm and peak 2 $\lambda_{ex}/\lambda_{em}$= 500/680 nm represents the two fluorescence peak. Peak A $\lambda_{ex}/\lambda_{em}$= 545/545 → 600/600nm is the Raleigh scattering peak. The fluorescence intensity of the peak 2 increases markedly (1x10^{-2}M) and the maximum emission wavelengths of the two peaks have obvious red shift following the addition of SDS indicating that the probe molecule is localized in the hydrophilic region of the anionic micelles (SDS).

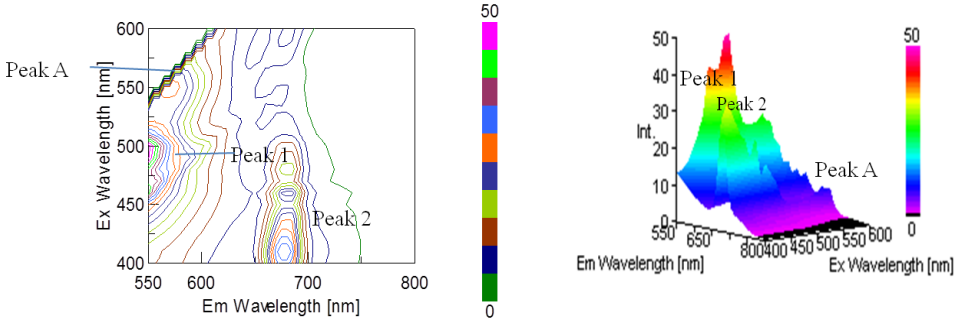


Fig.15. Contour and three dimensional emission spectra of Alizarin red in SDS ($2x10^{-3}$M)

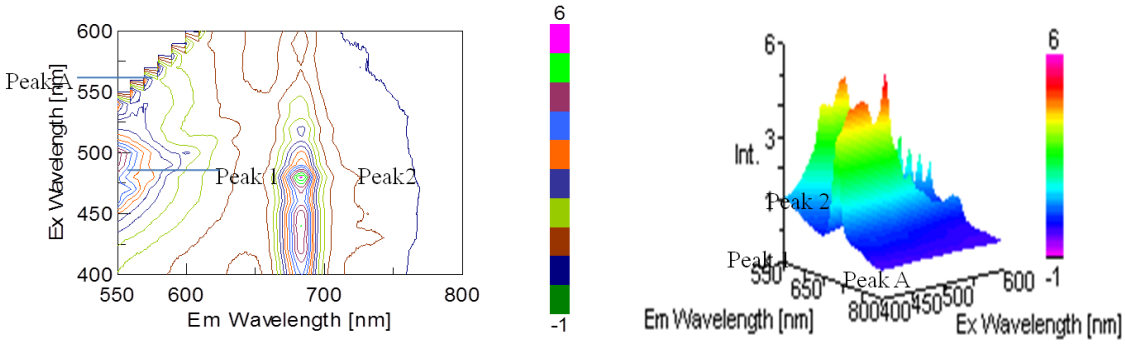


Fig.16. Contour and three dimensional emission spectra of Alizarin red in SDS ($1x10^{-2}$M)

5. CONCLUSION

In conclusion, the results reported the detailed information about the photophysical properties of spectroscopic probe like Alizarin Red, a molecule showing the ESIPT process in micelles (SDS and CTAB). In cationic (CTAB) micellar media, the decreased in the fluorescence intensity and the hypsochromic shift along with the contour map dictate the formation of Alizarin red dye-CTAB complex association with the hydrophobic tail region of the micelles. In anionic (SDS) micellar media, the decreased in the fluorescence intensity and the bathochromic shift in the contour map and three dimensional emission spectra shows that the probe molecule is localized on the polar hydrophilic region of the anionic micelles. The interaction of Dye with anionic micelle is stronger than the cationic micelle, because of higher electrostatic interaction between Alizarin red dye with SDS. In agreement, with emission intensity changes, the value of binding constant were found out and the number of binding site is ~ 1 indicate the existence of single binding site in micelles.

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