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Perturbations in the photophysical properties of isoxazole derivative of curcumin up on interaction with different anionic, cationic and non-ionic surfactants

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ABSTRACT

Surfactants are organized assemblies that are often used as models for biomembranes and they can also act as solubilizing agents for various hydrophobic drug molecules. Isoxazole derivative of curcumin (IOC) is a potential drug molecule whose biological efficacy is limited due to its low aqueous solubility. In the present work, different supramolecular confined environments have been utilized to enhance the aqueous solubility of IOC. Besides this, they have also been used as models for studying the perturbations in the photophysical properties of IOC upon encapsulation inside them. To serve this purpose, three different surfactants have been chosen from each category of cationic (CTAB: Cetyltrimethylammonium Bromide), anionic (SDS: Sodium Dodecylsulfate) and non-ionic (TX-100: Trition X-100) surfactants. In order to study the mechanism of interaction of IOC with the three different types of surfactant molecules, various steady-state and time-dependent spectroscopic techniques have been employed. The change in the optical properties of IOC, such as; red or blue shifts in the absorption or emission maxima and increase in the intensities has helped in gathering information about the successful encapsulation of IOC inside the surfactant micelles as well as the effect of different confined environments on the photophysics of IOC. The time-dependent stability of all the three micellar systems divulged with the help of UV–Visible spectroscopy has revealed that IOC-surfactant micellar systems are extremely stable in nature even after 3 days. The large value of partition coefficient and increment in the quantum yield values as well as average lifetime certainly indicates the partitioning of the IOC molecules inside the organized assemblies of all the three surfactant micellar systems. Moreover, the effect of salt (NaCl) concentration has also been investigated on the steady-state and time-dependent photophysical properties of IOC encapsulated surfactant nano-micellar system. It could be observed that the salt has no effect on the photophysics of IOC even at very high concentration. Hence, the information obtained from the present work opens door for a relatively new research area where novel drug formulations of IOC could be designed and developed that would help the mankind in the near future.

1. Introduction

Surfactants are organized assemblies that play a pivotal role in textile industry, chemical industry and pharmaceutical industry as an emulsifying, disintegrating and solubilizing agent.[1–4] Besides this, the micelles of surfactants are also used as models for biomembranes.[5] Surfactants are enormously used in the drug industry in order to increase the aqueous solubility of drugs, improve bioavailability, maintain drug stability along with control drug release and uptake.[6–7] Therefore, the knowledge to interaction mechanism of feebly soluble drug molecules with surfactants is of prior importance in order to design and develop drug formulations and delivery systems.[8] Surfactant molecules are amphiphilic in nature as they contain a hydrophilic polar head group as well as hydrophobic non-polar hydrocarbon chain. On the basis of their head groups, the surfactants can be classified as either ionic (anionic/ cationic) or neutral.[9] It is because of this amphiphilic nature that surfactants are capable of self-assembling in water so as to form micelles. The concentration at which the formation of this self-assembly occurs is known as critical micelle concentration (cmc).[10–11] At the cmc value, the surfactants are observed to experience drastic alterations in various physicochemical properties like surface tension, electromotive force, conductivity and so on.[12–13] Due to all these advantages, extensive research has been carried out in the recent times where different

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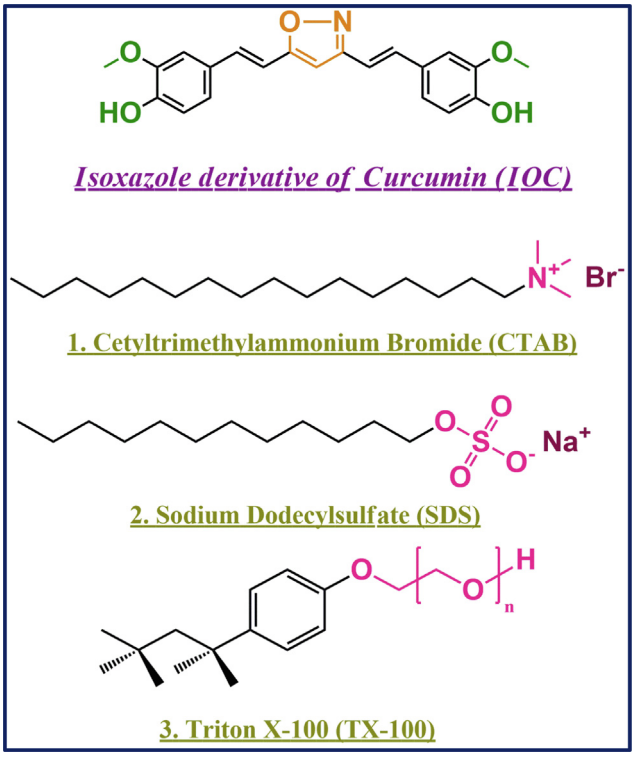
cationic, anionic, non-ionic and gemini surfactants have been utilized in enhancing the aqueous solubility and stability of various drug molecules and ionic liquids. Some of them are ceftriaxone sodium trihydrate, various antidiabetic drugs like: gliclazide, glyburide, glimepiride, glipizide, repaglinide, pioglitazone and rosiglitazone, and in a series of antibiotics including cloxacillin, cephalothin, cefotaxime, meropenem, and gentamicin.[5,14] Lee *et al.* have reported the utilization of surfactants (Tris and Trition-X 100) in enhancing the anti-bacterial activity of shikonin which is a highly liposoluble naphthoquinone pigment isolated from the roots of *L. erythororhizon*.[15] Additionally, there are numerous reports where surfactants have been employed to enhance the aqueous solubility of an extremely efficient drug named curcumin.[7,16–18]

Curcumin, a natural yellow medicinal pigment found in the roots of *Curcuma longa* has various beneficial pharmacological and biological applications like anti-inflammatory, antioxidant, anti-Alzheimer, antitumor, anticarcinogenic and free radical scavenger properties.[19–20] Despite all these applications, the biological efficacy of curcumin is hindered due to its extremely low aqueous solubility, rapid degradation at physiological conditions and low bioavailability.[21–22] To overcome these limitations, the scientific community has made several attempts to enhance the aqueous solubility of curcumin. One of the many approaches adopted by researchers is to entrap curcumin inside the core of micelles formed by surfactants. Surfactants from almost all the categories either independently or in combinations have been employed for this purpose such as; sodium dodecylsulfate (SDS, anionic surfactant),[18,23–24] Sodium dodecylbenzenesulfonate (SDBS, anionic surfactant),[25] Cetyltrimethylammonium bromide (CTAB, cationic surfactant),[16] cetyltrimethylammonium tosylate (CTAT, cationic surfactant),[26] Dodecyltrimethylammonium bromide (DTAB, cationic surfactant),[7,24] Triton X-100 (TX-100, non-ionic surfactant),[24] Tween-20 (non-ionic surfactant)[20,27] and many more. Kee *et al.* have reported that the alkaline hydrolysis of curcumin could be suppressed after encapsulation inside cationic micelles.[16] Despite all these efforts, the desired stability and bioavailability of curcumin could not be achieved by the scientific community. In the recent years, another approach that has been exploited by the researchers to extract the effectiveness of curcumin is to explore the structurally modified curcumin derivatives.[28]

One of the commonly investigated curcumin analogues is the isoxazole derivative of curcumin (IOC). IOC is the structurally modified curcumin derivative where the β-diketo group of curcumin molecule has been transformed into the five membered isoxazole ring (Scheme 1).[29] It has been reported that IOC possesses widespread biological applications like antitumor, anticancer, antioxidant, antimalarial, anti-proliferative, anti-Parkinson activities and many more.[28,30–34] IOC has also been reported to have modest inhibitory activity against COX-2 enzymes. [35] Although the aqueous solubility of IOC is greater than curcumin, the effectiveness of IOC is still largely compromised due to its low aqueous solubility. Therefore, keeping in mind the influence of surfactants in solubilizing curcumin, efforts have been made in the directions of enhancing the solubility of IOC using different types of surfactants. The information about the mechanism of interaction of IOC with different kinds of supramolecular confined environments would also help in understanding its mode of action inside biological entities and would also further help in designing and developing new formulations as well as drug delivery vehicles for IOC.

The present work aims in utilizing three different surfactants, one from each category of cationic (CTAB), anionic (SDS), and non-ionic (TX-100) surfactants in solubilizing poorly water-soluble drug, IOC. These surfactants have been selected in order to understand the effect of different supramolecular confined environments on the photophysics of IOC. The interaction mechanism of IOC with all the three surfactants have been studied using both steady-state and time-dependent spectroscopic techniques. The stability of the micelles formed in each case has been scrutinized by investigating the degradation profile which is achieved via recording of the absorption spectra at regular intervals of time. The thermodynamic parameters like change in Gibbs free energy, enthalpy and entropy have also been calculated for all the three kinds of nanomicellar systems. Besides this, the effect of salt (NaCl) on the photophysics of IOC encapsulated inside all the three types of nano-micellar systems, has also been scrutinized in depth. Further, the TCSPC studies have been deployed to study the excited state photophysical properties of IOC encapsulated inside different kinds of supramolecular confined environments.



Scheme 1. Chemical structures of Isoxazole derivative of Curcumin (IOC) along with the surfactants used in this study: Cetyltrimethylammonium bromide (CTAB), Sodium dodecylsulfate (SDS) and Triton X-100 (TX-100).

2. Experimental section

2.1. Materials

Curcumin, ethanol and hydroxylamine hydrochloride ($NH_2OH.HCl$) were purchased from Sigma Aldrich. Moreover, cetyltrimethylammonium bromide (CTAB), sodium dodecylsulfate (SDS) and Triton X-100 (TX-100) were also purchased from Sigma Aldrich. All the chemicals obtained were of analytical grade and were used without any further purification. The synthesis of isoxazole derivative of curcumin (IOC) was done by method reported in our previous studies.[29] In brief, 1 mmol of curcumin and 10 mL of ethanol were taken in a round bottom (RB) flask and the contents were allowed to stir for 15 mins. Subsequently, catalytic amount of acetic acid was added to the RB, followed by addition of 1.5 mmol of $NH_2OH.HCl$. Then, under N_2 atmosphere the reaction mixture was allowed to stir at 80℃ for 24 h and the thin layer chromatography (TLC) was used to study the progress of the reaction. Finally,

after the completion of the reaction, the contents of the RB were transferred to a beaker containing ice cold water which led to the formation of brown color precipitates. The crude formed was extracted via centrifugation and was recrystallized in methanol to obtain the pure form of IOC which was used for further studies. The stock solution of IOC was prepared in methanol whereas the stock solution of all the four surfactant solutions were prepared in potassium phosphate buffer solution of pH 7.4. HPLC grade water was used to prepare all the samples. In all the experiments the concentration of IOC was fixed at 10 μM.

2.2. Preparation of samples of IOC in different surfactant solutions

Firstly, stock solution of IOC was prepared in methanol. Then, the stock solutions of 0.1 M CTAB, 0.1 M SDS and 0.01 M TX-100 were prepared in 100 mM phosphate buffer solution of pH 7.4. In order to formulate the samples of IOC with surfactant, required amount of methanolic stock solution of IOC was added to vessels and the solvent was allowed to evaporate at room temperature. Then, after the formation of thin film, it was scratched from the vessel and requisite amount of surfactant solution was added to it. The resultant solution thus formed was introduced to ultrasonication for about 3 h. Following this, the samples were filtered through a 0.45 μ filter to get rid of any unbounded surfactant and IOC molecules. Finally, these samples were instantly used for further analyses.

2.3. Characterization techniques

The absorption spectra of IOC in the presence of different surfactant solutions were recorded using Shimadzu spectrophotometer (UV-2600) in the wavelength range of 200 nm – 800 nm. The quartz cuvettes having 1 cm path length were used for the measurements. The steady state emission and excitation spectra were recorded using Varian Carry Eclipse Fluorescence spectrophotometer in the wavelength range of 355 nm – 800 nm and from 200 nm to 450 nm; respectively. The samples of CTAB, SDS and TX-100 were excited at a wavelength of 338 nm, 336 nm and 339 nm; respectively. Similarly, for recording the excitation spectra, the emission wavelength was fixed at 408 nm, 430 nm and 408 nm; respectively. During all the measurements, the temperature was maintained at 25 °C by circulating the water through the thermostated cuvette holders and both the excitation and emission slits were kept at 5 nm throughout the measurements.

The fluorescence quantum yield of IOC in presence of CTAB, SDS and TX-100 solutions was calculated using the below written equation.[36]

$$'E_{Fs} = 'E_{FR}\left(\frac{A_S}{A_R}\right)\left(\frac{Abs_R}{Abs_S}\right)\left(\frac{\eta_S}{\eta_R}\right)^2 \quad (1)$$

In this equation, the subscripts "*S*" and "*R*" corresponds to sample solutions and reference sample; respectively. The symbols *ϕ, η, A, and Abs.* have been designated to the fluorescence quantum yield, refractive index, integrated area under the curve of emission spectra and absorption intensity at maxima; respectively. In this experiment, quinine sulfate in 0.1 N H_2SO_4 (which has quantum yield value of 0.546) has been used as reference.[37]

In order to conduct the experiments related to the picosecond resolved excited state lifetime measurements, the commercially available time-correlated single- photon counting (TCSPC) set up was used. This instrument has been provided by Edinburgh instruments, U.K. and it possesses MCP-PMT detectors. The picosecond resolved fluorescence transients could be recorded by employing a 375 nm picosecond pulsed laser diode as an excitation source with an instrument response function (IRF) of 75 ps. The decay patterns were obtained at the emission wavelength of 500 nm of the corresponding sample under investigation. During measurements, the emission polarizer angle was fixed at 55° while the excitation was vertically polarized. A long pass filter with cut-off at 400 nm was added in the emission channel to eliminate all the probability of the scattered excitation light. The obtained decay patterns were fitted using tri-exponential functions. The average lifetime of excited state of IOC molecule in the presence of different surfactant environments could be calculated using Eq. (2).[36]

$$\tau_{avg.} = a_1\tau_1 + a_2\tau_2 + a_3\tau_3 \quad (2)$$

Here, τ_1, τ_2 and τ_3 are the three components of the decay time of IOC in presence of different surfactant solutions and a_1, a_2 and a_3 represents their corresponding relative weightage.

The values of radiative and non-radiative decay rate constants could be determined using the Eq. (3).[36]

$$k_r = \frac{'E_{FF}}{\tau_{avg.}} \; and \; \frac{1}{\tau_{avg.}} = k_r + k_{nr} \quad (3)$$

Here, k_r corresponds to the rate constant for radiative process whereas k_{nr} represents non-radiative decay rate constant. The symbols τ_{avg} and ϕ_F corresponds to the average lifetime of the excited state of IOC molecule as determined by Eq. (2) and fluorescence quantum yield as calculated from Eq. (1); respectively.

All the measurements were carried out at 25 °C.

3. Results and discussion

The structure of isoxazole derivative of curcumin (IOC) along with all the surfactants used in the present study i.e., CTAB, SDS and TX-100 have been indicated in Scheme 1. In the present study, initially the characterization for the successful formation of micelles of IOC in the presence of different surfactants have been accomplished by using DLS and TEM analysis and the results obtained have been presented in the supplementary information (SI: Figure S1 and S2). Then after, the study of interaction mechanism has been achieved by using different steady-state and time-dependent spectroscopic techniques. The effect of temperature and salt (NaCl) on the photophysics of IOC has been evaluated in depth. The details of the results obtained along with the elaborated discussion and analysis follows below.

3.1. Steady state absorption and emission studies

In order to understand the effect of different microenvironment provided by different cationic, anionic and non-ionic surfactants, initially the absorption spectra of IOC (10 μM) in presence of CTAB (5 mM), SDS (20 mM) and TX-100 (1.5 mM) have been recorded. The overlay of all the three absorption spectra have been represented in supplementary information (SI: Figure S3a). In our previous studies, it has been reported that IOC in pure aqueous medium shows a main peak at 333 nm accompanied by a shoulder at 295 nm.[29] In general, the absorption spectra depicted in Figure S3a displays one main peak and one shoulder at around 295 nm. However, in case of TX-100 as surfactant, the peak at around 270 nm solely corresponds to that of TX-100. Moreover, it could be noticed that in presence of surfactants the main peak is red shifted. The IOC entrapped inside the micelles of CTAB, SDS and TX-100 manifests an absorption maxima at 338 nm, 336 nm and 339 nm; respectively. Thus, a red shift of 5 nm, 3 nm and 6 nm could be observed for CTAB, SDS and TX-100 micelles; respectively. This red shifted absorption spectra clearly demonstrates the presence of some specific interactions of IOC with all the three surfactant molecules. Further, to get fundamental understanding about the excited state photophysical properties of IOC

encapsulated inside different micelles, the emission spectra have been recorded and is presented in Figure S3b. The inset of the Figure S3a and S3b corresponds to the intensity magnified normalized emission spectra of the same samples. The emission spectra of pure IOC in an aqueous medium exhibits a maximum at 431 nm.[29] Whereas, in case of CTAB, SDS and TX-100, the emission maxima appears at 408 nm, 430 nm and 408 nm; respectively. An expected blue shift could be analyzed for all the three types of surfactants. The blue shift in the emission spectra illustrates the existence of IOC in comparatively more hydrophobic environment inside the surfactant aggregates. It has been reported by Sarkar *et al.* that upon increase in the solvent polarity, the absorption energy increases and the emission energy experiences a decrement. In other words, the absorption maxima exhibit a red shift whereas the emission spectra show a blue shift when the microenvironment around the probe becomes more and more non-polar in nature.[38] Depending on the extent of the blue shift, it could be easily said that the IOC molecules experience minimum hydrophobicity in the case of SDS micelles. Similar results have been obtained when curcumin was used as a fluorescence probe and was analyzed in the presence of CTAB, SDS and TX-100.[24,39]

Now, in order to investigate the interaction mechanism of IOC with different micellar environments, the absorption spectra of IOC at fixed concentration (10 μM) were recorded in the presence of increasing concentrations of CTAB, SDS and TX-100. The concentration of CTAB and SDS has been varied from 0 mM to 100 mM, whereas the concentration of TX-100 has been increased from 0 mM to 10 mM. The overlay of the absorption spectra in all the three type of surfactants have been presented in Fig. 1. The results indicate that in the absence of surfactants, the absorption peak is at 333 nm escorted by a shoulder at around 295 nm. But when the CTAB surfactant is added even in the minute quantity i.e., 0.4 mM, the absorption maxima shifts from 333 nm in pure aqueous medium to around 346 nm, in other words, a red shift of about 13 nm could be observed together with a new additional peak at around 400 nm. This new peak in case of cationic surfactant has also been reported for curcumin by Adhikary *et al.* where they have described that this peak is due to the presence of a small population of deprotonated curcumin at pH 7.4.[24] Further, when the concentration of CTAB is gradually increased up to its cmc (i.e., 0.92 mM to 1.0 mM), the absorption intensity is also found to increase which could be seen to get accompanied by a prominent peak at around 400 nm. But when the concentration of CTAB was further raised above the cmc value i.e., from 1 mM up to 100 mM, the shoulder at around 400 nm completely disappeared and this could be clearly manifested in the Fig. 1a. The absorption spectra after the cmc of CTAB depicted a blue shift in maxima from 346 nm to around 338 nm followed by a shoulder at around 295 nm. This could imply that after the cmc value, the IOC molecules are travelling from the bulk water phase towards more and more hydrophobic environment i.e., towards the core of the micelle. Additionally, in the case of SDS surfactant, after the addition of SDS in extremely low quantity i.e., 4 mM, the absorption maxima got shifted to 336 nm (Fig. 1c). Meanwhile, when the concentration of SDS has been further increased, only the absorption intensity increased and no effect could be observed on the absorption maxima. Moving forward, the absorption spectra of IOC in presence of non-ionic surfactant (TX-100) demonstrates a red shift from 333 nm to 339 nm (Fig. 1e). This information could assist us to deduce that after micellization in each one of the surfactants, TX-100 is capable of providing maximum hydrophobicity to the ground state of IOC molecules. Also, on increasing the concentration of TX-100 after cmc value, no alterations in the nature of absorption spectra could be observed and the only parameter that is getting effected is the absorption intensity. No shift in the absorption maxima could elucidate that the nature of interactions before and after micellization stages are quite similar in case of SDS and TX-100. Whereas it could be stated that the interaction mechanism of IOC is completely different for CTAB surfactant below and after its cmc value.

Moreover, the trend of augmentation in the absorption intensity upon increase in the surfactants' concentration could be easily understood with the help of the plot of absorbance versus concentration of surfactant. These graphs have been represented in the inset of their corresponding absorption spectra overlay (Fig. 1a, 1c and 1e). After the cmc value of CTAB, SDS and TX-100, it could be observed that the absorption intensity could not increase much, in fact a plateau could be declared at higher concentrations. This could imply that maximum solubilization of IOC by the surfactant molecules could be achieved with concentrations just above the cmc value and addition of the surfactant in the excess quantity has no significant impact on the solubility of hydrophobic drug IOC. The solubility of IOC in the presence of CTAB, SDS and TX-100 surfactant molecules has been calculated using the traditional Lambert-Beer's law. It has already been reported in the previously that the molar extinction coefficient of IOC in presence of methanol is around 4.521×10^4 $dm^3mol^{-1}cm^{-1}$.[29] Substituting this value in the formula, the maximum concentration of IOC inside the micellar aggregates has been found to be around 9.7 ± 0.2 μM, 9. 5 ± 0.2 μM and 9.3 ± 0.2 μM for CTAB, SDS and TX-100; respectively. Now, considering that the amount of IOC in all the formulations was initially fixed at 10 μM, these solubility values could account for a remarkable solubilization of around 97%, 95% and 93% for CTAB, SDS and TX-100; respectively Besides this, after comparing the solubilization tendencies of all the three types of surfactants, it could be conjectured that TX-100 provides minimum solubilization and CTAB provides maximum solubilization to the IOC molecules. For the sake of effortless understanding, all these values have been tabulated in the Table 1.

In order to understand the excited state photophysical properties of IOC encapsulated nano-micellar systems, one must record the steady-state emission spectra of the aforementioned systems. Therefore, the emission spectra of the IOC-surfactant micellar formulations were recorded in the range of 355 nm to 800 nm. The overlay of the emission spectra is represented in Fig. 1. In the absence of surfactants, the emission maxima of pure IOC could be obtained at around 431 nm.[29] In case of CTAB, even when minimal amount of CTAB is added (i.e., 0.4 mM), the emission maxima got shifted to 480 nm. A huge red shift of approximately 49 nm could be observed. This comprehensive red shift could possibly be due to the drastic enhancement in the micropolarity experienced by the IOC molecules which could probably be due to some sort of specific interactions amongst the two molecules in the premicellar state. Further, when the concentration of CTAB is around its cmc value, a broad emission maxima could be obtained ranging from 408 nm to 480 nm. Moreover, when the concentration of CTAB was substantially higher than its cmc value (i.e., from 1 mM to 100 mM), a comparatively sharper emission spectra could be obtained with the emission maxima at around 408 nm. This could imply that the IOC molecules are getting sifted from the stern layer towards the core of the micelles formed by the CTAB surfactant molecules. Based on the findings of absorption and emission spectra, it could simply be stated that below cmc the microenvironment encountered by the IOC molecules is polar in nature and beyond cmc it is hydrophobic in nature. The inset graph of the Fig. 1b depicts a plot between the fluorescence intensity of IOC at 408 nm and the concentration of CTAB in the formulations. The graph reveals that below the cmc of CTAB, the increase in the fluorescence intensity is quite rapid and after the cmc value the increase is gradual. When similar studies were performed with SDS surfactant, after addition of minimal amount of SDS solution, the emission maxima got blue shifted from 431 nm in pure aque-

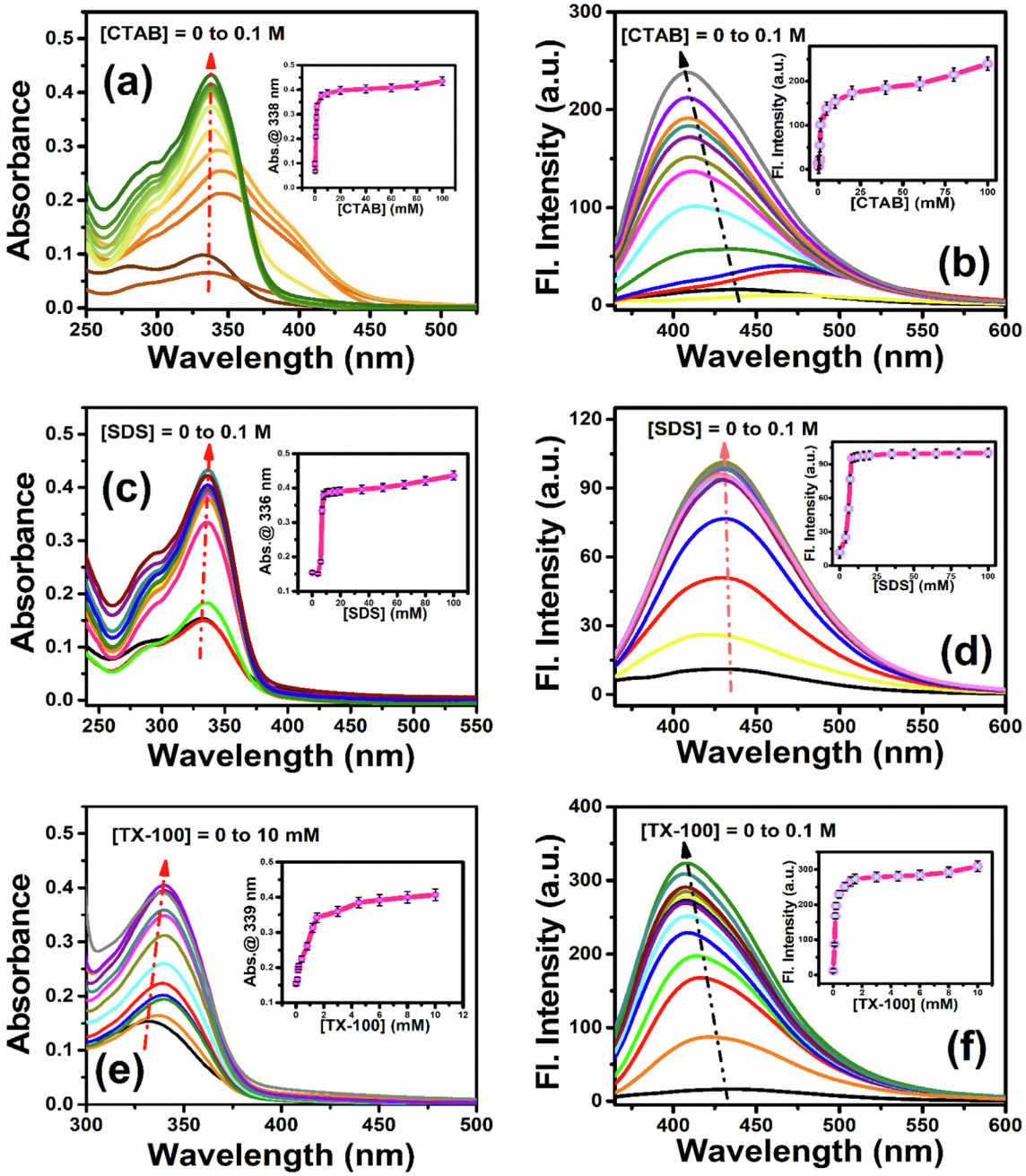


Fig. 1. The absorption spectra of IOC in the presence of increasing concentration of (a) CTAB (0 to 0.1 M), (c) SDS (0 to 0.1 M) and (e) TX-100 (0 to 0.01 M); respectively. The inset in each figure represents the absorbance (at absorption maxima) versus the concentration of surfactants. The emission spectra of IOC in the presence of increasing concentration of (b) CTAB (0 to 0.1 M), (d) SDS (0 to 0.1 M) and (f) TX-100 (0 to 0.01 M); respectively. The inset in each figure represents the variation of emission intensity with the increasing concentration of the surfactant.

ous medium to 425 nm in the presence of SDS surfactant. Whereas, when the concentration of SDS was further enhanced, the emission maxima got shifted from 425 nm to 430 nm. Thus, it could be stated that IOC molecules experience almost similar microenvironment upon increase in the SDS concentration. Another discrepancy in the emission spectra in the presence of SDS with

Table 1
Values of critical micellar concentration of different surfactants along with solubility, absorption maxima, emission maxima, Stokes' shift and $E_T(30)$ values of IOC in the presence of different surfactants. (Error within ± 5%).

Surfactant	cmc	Solubility (μM)	λmax (abs.) (nm)	λmax (emsn.) (nm)	Stokes'Shift (cm^{-1})	$E_T(30)$ (kcal/mol)
Water	–	3.5 ± 0.2	333	431	6828	63.1
CTAB	0.92 mM – 1 mM	9.7 ± 0.2	338	408	5076	40.80
SDS	8.2 mM	9.5 ± 0.2	336	430	6506	62.91
TX-100	0.22 mM – 0.24 mM	9.3 ± 0.2	339	408	4989	40.80

that to CTAB is that; in case of SDS, the emission intensity attains a plateau just above its cmc value. The inset of Fig. 1d certainly helps us to clearly analyze the trend of emission intensity with the concentration of SDS with the saturation concentration of SDS for IOC reaching at 10 mM. Furthermore, the Fig. 1f shows the emission spectra of IOC in the presence of increasing concentration of TX-100 surfactant. Here, even with a slight addition of TX-100 solution, the emission maxima could display a blue shift and at the saturated concentration of TX-100 (i.e., 100 mM), the emission maxima got shifted to 408 nm. This blue shift of 23 nm suggests that the IOC molecules are traveling from the bulk water phase to the hydrophobic core of the TX-100 micelle. Moreover, it could also be noticed that in the presence of low concentrations of TX-100, the emission spectra are quite broad and it keeps on becoming sharper as the concentration of TX-100 increases. This sharpness could indicate that the rotational and vibrational modes of the IOC molecules are getting restricted and the IOC molecules are residing in more compact hydrophobic microenvironment. For the sake of clarity, a graph has been plotted between emission intensity @408 nm on *y*-axis and concentration of TX-100 on the *x*-axis and it has been displayed in the inset of Fig. 1f. Furthermore, the trend in the emission maxima for all the three types of surfactants follows the order: CTAB (408 nm) ~ TX-100 (408 nm) < SDS (430 nm). This information aids us to infer that the IOC molecules experiences almost similar hydrophobic microenvironment in case of CTAB and TX-100 micelles whereas the SDS surfactant aggregates provides the most hydrophilic environments. Based on these findings, it could also be inferred that in case of the CTAB and TX-100 micelles, the IOC molecules are present in the palisade layer whereas in the case of SDS micelles, the IOC molecules are present in the stern layer of the micelles which felicitates strong interaction with the water molecules. Similar results have also been reported when curcumin has been investigated as the fluorescent probe inside these supramolecular confined environments.[24] In addition, a comparison between the absorption and emission maxima of IOC in the presence of these surfactants with those inside some organic solvents and water could help us to get an insight about the resemblance of the microenvironments. It could be analyzed that the microenvironment experienced by the IOC molecules in the presence of CTAB and TX-100 is similar to that of aprotic solvent like Tetrahydrofuran whereas that in the presence of SDS molecules is analogous to that of polar protic solvents like methanol and water.[29]

Now, in order to accumulate more details about the extent of reorganization of the local environment of the IOC molecules, the fluorescence Stokes shift has been calculated for all the three surfactants. The obtained values in case of pure aqueous medium as well as for the three surfactants have been tabulated in Table 1. The value of Stokes shift of IOC in the absence of surfactants is around 6828 cm^{-1} whereas the values obtained in case of CTAB and TX-100 were 5076 cm^{-1} and 4989 cm^{-1}; respectively and that acquired for SDS micellar system is 6506 cm^{-1}. Therefore, it is clearly evident that the local microenvironment experienced by the IOC molecules in the presence of all the three surfactants is definitely less polar as the values of Stokes shift have decreased and it could be stated that the SDS surfactant could provide a rather polar microenvironment to the IOC molecules as compared to the other two surfactant assemblies, further confirming that in case of SDS, IOC molecules are present in the stern layer of the micelle.

Now, in order to gather information about the nature of the emitting species, the fluorescence excitation spectra have been recorded in the presence of all the three surfactants. The overlay has been represented in the supporting information (SI: Figure S3c). The maxima in case of CTAB, SDS and TX-100 nano-micellar systems could be obtained at 338 nm, 339 nm and 341 nm; respectively. Also, the nature of the absorption and fluorescence excitation spectra is almost similar in nature where both possesses two peaks i.e., one main peak around 338 nm accompanied by a shoulder at around 295 nm. Thus, it could be easily interpreted that the there is only one emitting specie in the different IOC-surfactant nano-micellar formulations and it responds distinctively in presence of different microenvironments.

Furthermore, the effect of excitation wavelength on the emission spectra and vice versa have also been investigated on all the three IOC-surfactant formulations. The overlay of both red and blue end wavelengths on both the fluorescence excitation as well as emission spectra have been represented in the Supplementary Information (SI: Figure S4). From the figure, it could be easily seen that the effect of excitation wavelength on emission spectra and emission wavelength on the excitation spectra is almost negligible. This could further confirm that there is only one specie i.e., IOC, responsible for the fluorescence spectra.

3.2. Determination of $E_T(30)$ values

A more precise information about the micropolarity of the environment experienced by the IOC molecules in presence of different cationic, anionic and non-ionic surfactant could be obtained by determining the solvent polarity parameter ($E_T(30)$). This could be executed by studying the changes occurring in the spectral properties of the fluorescent probe molecule in the presence of different environments of known polarity. Thus, the emission spectra of IOC have been recorded in the presence of varying ratios of different 1,4-Dioxane/ water mixtures. The overlay of the emission spectra of IOC in presence of different mixtures has been illustrated in the supplementary information (SI: Figure S5). The $E_T(30)$ values obtained for CTAB, SDS and TX-100 micellar systems are around 40.80 kcal/ mol, 62.91 kcal/ mol and 40.80 kcal/ mol; respectively (Table 1). From the values of Table 1, it could also be analyzed that the increment in the values of Stokes shift is in accordance with the increase in the micropolarity parameter. These results echo with the results obtained in our previous study where the Stokes shift of IOC in presence of various solvents increases with the polarity of the solvent.[29]

3.3. Determination of partition coefficient

In order to check the efficiency of a newly studied system as a potential drug delivery vehicle, one must have some quantitative knowledge about the penetration of the drug inside the micelle and this could be efficiently done by calculating the partition coefficient. In this, the fluorescence spectra of different samples containing a fixed amount of IOC (10 μM) and variable concentration of surfactants (definitely above cmc) were recorded and the values of fluorescence intensities of different samples were substituted in the equation mentioned below.

$$\frac{I_\infty - I_0}{I_t - I_0} = 1 + \frac{[\text{Water}]}{k_p} \times \frac{1}{[\text{Surfactant}]}$$

Here, I_o, I_t and I_∞ are the fluorescence intensities of IOC in the presence of bulk water, at various concentrations of surfactant and at concentration where the fluorescence intensity becomes saturated. The value of partition coefficient could be obtained by plotting the graph between $(I_\infty - I_o)/(I_t$ and $I_0)$ and $[\text{Surfactant}]^{-1}$ (Fig. 2). Based on the slope of these graphs, the value of partition coefficients at 25 °C could be calculated to be around 1.63×10^4, 1.55×10^4 and 2.133×10^5 for CTAB, SDS and TX-100 nano-micellar systems; respectively. These values of partition coefficient are considered to be quite high for such newly studied systems. Also, it could be observed that the value of K_p in case of TX-100 micelles is almost 10 times higher than those obtained in the case of CTAB or SDS. This

information benefits us to presume that maximum extent of penetration of the IOC molecules from the bulk water phase towards the core of the micelle could be achieved in case of the non-ionic surfactant i.e., TX-100.

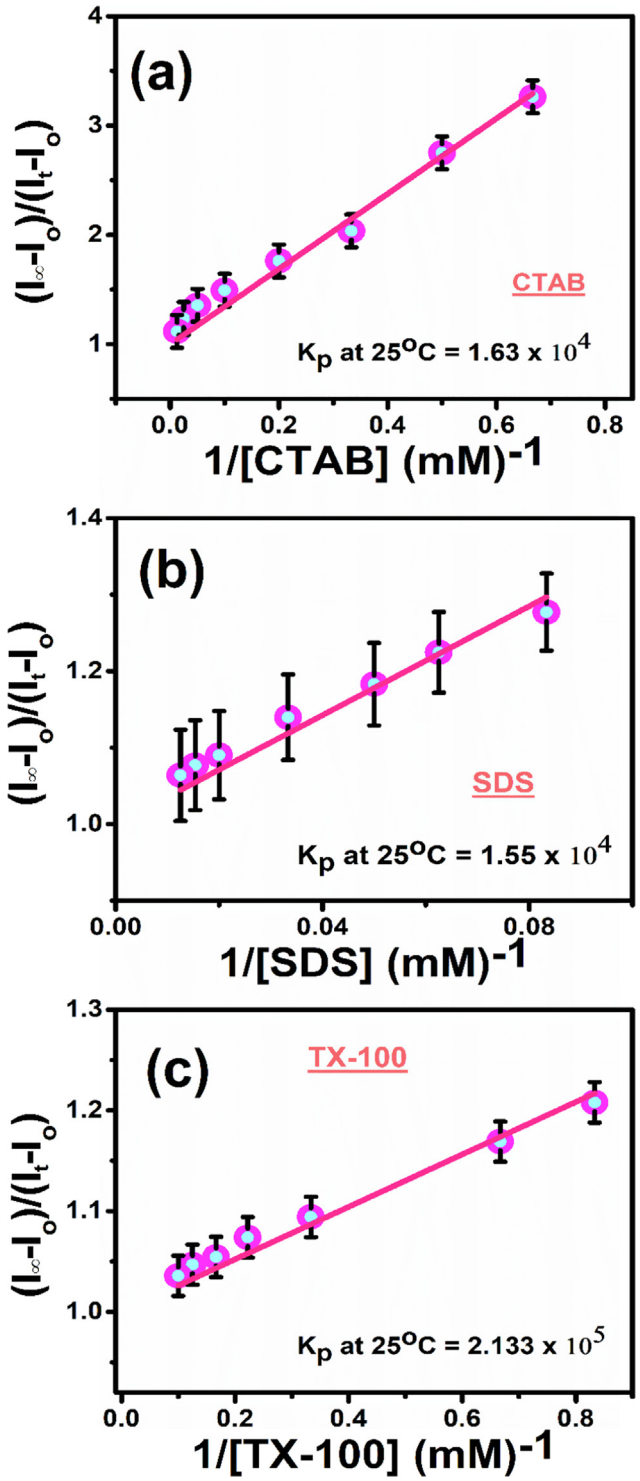


Fig. 2. Plot of $(I_\infty - I_o)/(I_t - I_o)$ vs. 1/[surfactant] for the determination of partition coefficient of IOC in the presence of (a) CTAB, (c) SDS and (e) TX-100 micelles; respectively.

3.4. Stability of IOC-Surfactant nano-micellar system

The prolonged stability of a newly designed nano-formulation is utterly important criteria which must be fulfilled so that it could be utilized as a drug delivery vehicle. The stability of different IOC-surfactant nano-micellar formulations has been evaluated by studying the degradation profile via UV–Visible spectroscopy by recording the absorption spectra of all the three formulations at regular intervals of time up to 3 days. The overlay of the absorption spectra for all the three formulations have been represented in Fig. 3. It can be easily identified from the figure that the stability of the IOC-surfactant nano-micellar system is maximum in case of non-ionic surfactant i.e., TX-100. The rate of degradation of the formulations has been determined for all the samples by plotting a graph between % change in the absorption intensity at maxima versus time and it has been depicted in the inset of Fig. 3a, 3b and 3c for CTAB, SDS and TX-100; respectively. The overall rate of degradation of the micellar system of CTAB, SDS and TX-100 could be calculated to be around 6.8%, 3.06% and 3.28%; respectively. Such low values of the rate of degradation for nano-micellar formulations correspond to the extraordinary stability of IOC encapsulated surfactants. However, the time-dependent absorption spectra of IOC inside CTAB and SDS micellar solutions shows some variation with time. In the case of CTAB surfactant, after 24 h an additional peak could be seen to appear at around 400 nm. This peak at around 400 nm in case of CTAB surfactant has also been obtained when CTAB was added to IOC in minute quantities (below its cmc value). Similarly, a shift in the shoulder peak from 295 nm to 280 nm along with slight increase in the absorption intensity could be noticed for the IOC-SDS micellar system. These observations indicate that with prolonged duration of time, some additional specific interactions might be occurring between IOC and CTAB/ SDS surfactants. However, no signs of such interactions could be observed in case of non-ionic surfactant (TX-100).

3.5. Effect of temperature on micellar systems

The study of effect of temperature on a newly pioneered system is highly necessary because it gives the information about the types of interactions occurring amongst the two constituents at different temperatures. The temperature dependent studies also help in analyzing the thermal stability of the system and changes occurring in the interaction mechanism at elevated temperatures. The substantial interaction forces that could occur between the small organic molecules and macromolecules are electrostatic interactions, hydrogen bonding interactions, hydrophobic forces, and van der Waals' forces.[40] Ross *et. al.* have reported the thermodynamic rules to estimate the type of interactions between the small organic molecule and macromolecules.[41] In the present work, the effect of temperature on the IOC-surfactant nano-formulations has been studied using fluorescence spectroscopy. The temperature has been varied from 5 °C to 80 °C for CTAB and TX-100 and in the range of 5 °C to 95 °C in case of SDS. The overlay of temperature effect in the entire above mentioned temperature range for all the three micellar systems have been shown in Fig. 4. It can be clearly seen from this figure that the fluorescence intensity is definitely decreasing with increase in the temperature for the three surfactant micellar systems. This implies that the forces of interactions that were responsible for holding the IOC molecules inside the micelles are definitely been torn down. Increase in temperature could only weaken the forces of interactions that were responsible for keeping the micellar system intact. Due to this, the number of IOC molecules bounded with surfactant molecules could be seen to be decreasing as the IOC molecules were moving out of the micelles as the micelle gets ruptured at

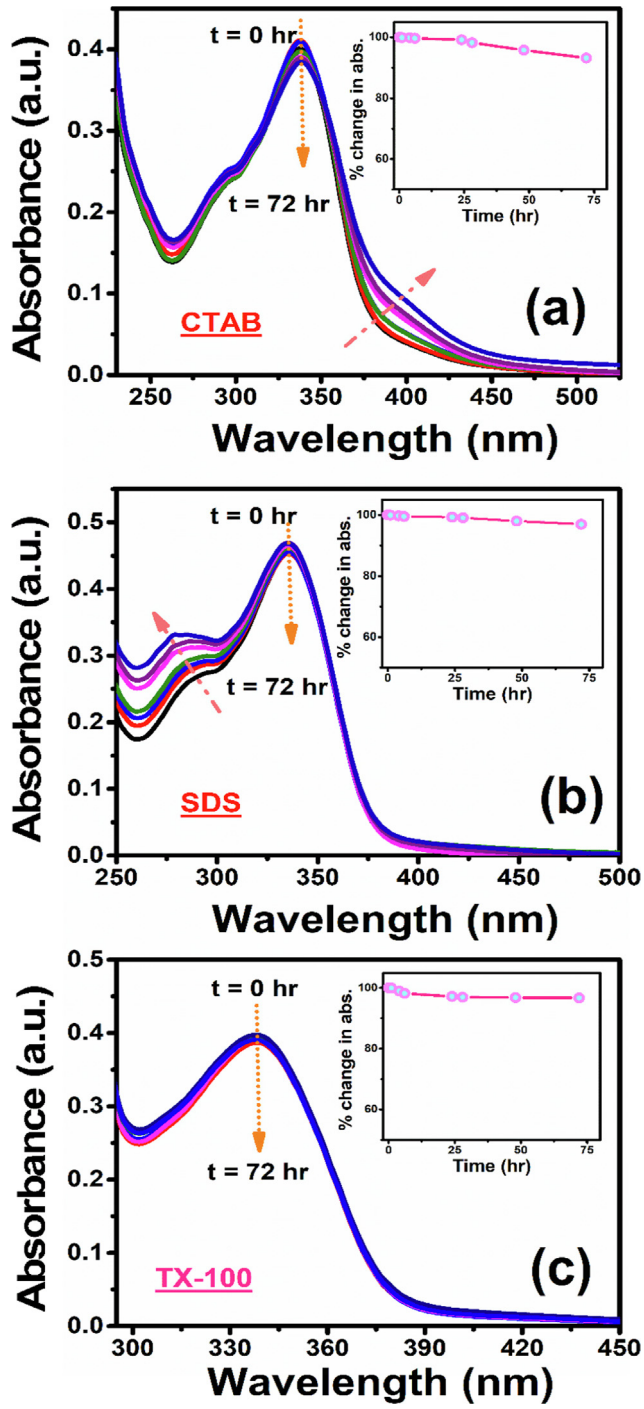


Fig. 3. The overlay of the absorption spectra of IOC recorded at regular intervals of time, in the presence of (a) CTAB, (b) SDS and (c) TX-100; respectively. The inset in each figure represents the percentage change in the absorbance value (at absorption maxima) versus time.

higher temperatures and this could account for the decrease in the emission intensity. The inset of Fig. 4 which is a plot between emission intensity versus temperature, provides a clear picture of the effect of temperature on the IOC molecules encapsulated in the surfactant micelles.

Moreover, to get an insight about the nature of interactions between the IOC molecules and various surfactant molecules and also about the feasibility of formation of the micellar systems, the calculations for thermodynamic parameter is essential. In order to calculate the thermodynamic parameters like change in Gibbs free energy (ΔG), enthalpy change (ΔH) and entropy change (ΔS); initially the values of partition coefficient were calculated at different temperatures. The temperature values chosen for this experiment were in the range of 20 °C–60 °C at an interval of 5 °C. The overlay of the graph for calculation of partition coefficient at each temperature in case of all the three surfactants has been represented in the supplementary information (SI: Figure S6). Following this, the values of change in Gibbs free energy at each temperature were calculated using the equation represented below.

$$\Delta G = -RTln(k_p)$$

In this equation, "R" represents the universal gas constant, "T" is the absolute temperature (in Kelvin) and K_p is the value of partition coefficient at temperature T. The value of change in Gibbs free energy at 25 °C for CTAB, SDS and TX-100 were found to be around -24.035 kJ mol^{-1}, -24 kJ mol^{-1} and -21.33 kJ mol^{-1}; respectively. Based on these values, it could be easily stated that the process of micelle formation in all the three cases is highly spontaneous in nature. The values of other two mentioned thermodynamic parameters could be calculated using the van't Hoff equation described below.

$$\ln K_p = -\left(\frac{\Delta H}{R}\right)\frac{1}{T} + \frac{\Delta S}{R}$$

In the above-mentioned equation, the terms have their usual meanings as discussed previously. Using this equation, a graph could be plotted between ln K_p on the *y*-axis versus 1/T on the *x*-axis as shown in Fig. 4b, 4d and 4f for CTAB, SDS and TX-100; respectively. The values of ΔH and ΔS could be obtained from slope and intercept of this straight-line graph; respectively. The value of change in enthalpy (ΔH) for CTAB, SDS and TX-100 were found to be around -14.973 kJ mol^{-1}, -0.65 kJ mol^{-1} and -10.5 kJ mol^{-1}; respectively, whereas the values for change in entropy (ΔS) could be calculated to be around 30.57 J K^{-1} mol^{-1}, 78.024 J K^{-1}mol^{-1} and 65.51 J K^{-1}-mol^{-1} for CTAB, SDS and TX-100; respectively. All the corresponding thermodynamic parameters at their respective temperatures are tabulated in Table 2. Based on these calculated values, the following findings could easily be postulated.

- Such high negative values of change in Gibbs free energy suggests that the process of micellization is highly spontaneous in nature.
- As indicated by the negative value of change in enthalpy, this process is exothermic in nature.
- Such high positive values of change in entropy could reveal that the process of micellization for all the three surfactants is both entropy-driven as well as enthalpy driven.

3.6. Fluorescence quantum yield and time-resolved fluorescence studies

The fluorescence quantum yield for IOC has been calculated in the presence of all the three surfactants at varying concentrations and the values have been arranged in Table 3. This table clearly signifies that the value of fluorescence quantum yield (ϕ_x) increases with increase in the concentration of CTAB and TX-100 but the concentration of SDS has almost negligible effect. Further, it could be seen that the values of fluorescence quantum yield are relatively higher in case of non-ionic surfactant i.e., TX-100 with almost 60-fold increment as compared to that obtained in the pure aqueous medium. These observations are in agreement with our previous solvent dependent studies where it has been inferred that the fluorescence quantum yield of IOC is maximum in case of non-polar and polar aprotic solvents.[29] In addition, with the intentions of accumulating knowledge about the impact of different kinds of

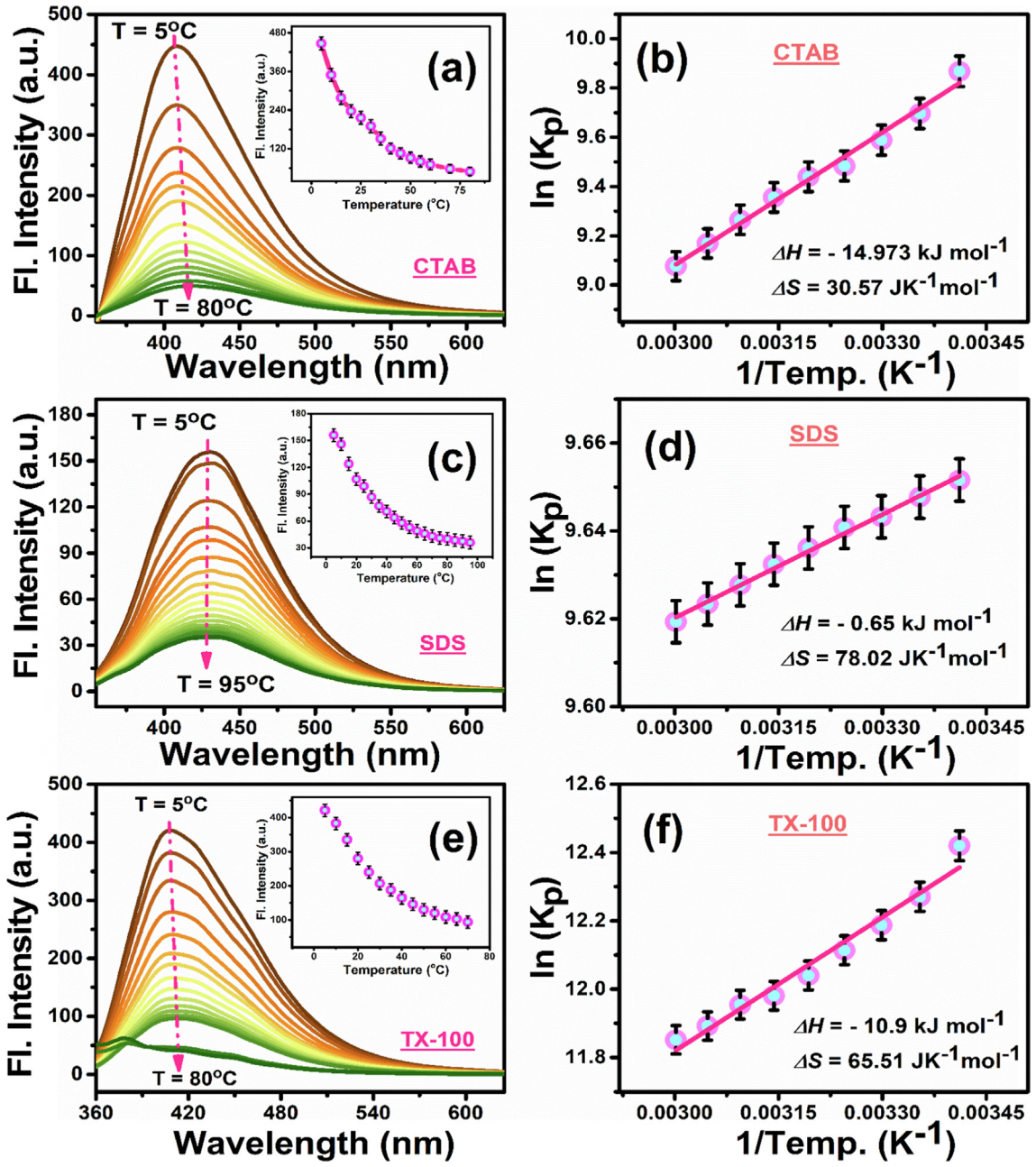


Fig. 4. The overlay of the temperature-dependent emission spectra of IOC in the presence of (a) CTAB, (c) SDS and (d) TX-100; respectively. The inset in each figure represents the variation of emission intensity with the increasing temperature. The van't Hoff plot of IOC at different temperatures in the presence of (b) CTAB, (d) SDS and (f) TX-100 micelles; respectively.

surfactant micellar systems on the excited state photophysics of IOC, picosecond resolved fluorescence decay transients of IOC have been recorded at increasing concentration of CTAB, SDS and TX-100. The overlay of the concentration dependent excited state decay patterns of IOC encapsulated micellar systems have been illustrated in Fig. 5. The decay patterns have been fitted using tri-exponential function and the values obtained for the three decay components along with their relative contributions have been tabulated in Table 3. It could be noted that the value of first component is in the range of 60 ps to100 ps, the second component ranges from 500 ps to 750 ps whereas the third component varies from 1.4 ns to 2.5 ns. The average lifetime of IOC in aqueous solution has been found to be around 100 ps. However, when the concentration of CTAB was increased from 0.5 mM to 50 mM, initially the average lifetime of IOC did not change much but got elevated to 163 ps when the concentration of CTAB was 50 mM. In a similar manner, when the concentration of SDS and TX-100 were varied in the range of 5 mM–50 mM and 0.1 mM–50 mM; respectively, it was found that while the SDS micelles could only escalate the average lifetime up to 115 ps, the TX-100 micelles could enhance the $\tau_{avg.}$ to around 160 ps even at the lowest concentration of 0.1 mM. Moreover, the contribution of second and third component was found to be comparatively higher in the case of TX-100 than in the cases of CTAB or SDS. Thus, it could be inferred that the aliphatic anionic surfactant (SDS) is less efficient in elevating the average lifetime of IOC than the cationic (CTAB) and non-ionic (TX-100) surfactants. Furthermore, from the values obtained for the fluorescence quantum yield and average lifetime, the values

Table 2
Change in partition coefficient and Gibbs free energy of IOC in the presence of different surfactants at varying temperature. (Error within ± 5%).

Surfactant	CTAB[a]		SDS[b]		TX-100[c]	
Temp. (K)	K_p ($\times 10^4$)	ΔG (kJ/mol)	K_p ($\times 10^4$)	ΔG (kJ/mol)	K_p ($\times 10^4$)	ΔG (kJ/mol)
293.15	1.93	−24.05	1.554	−23.52	24.76	−30.27
298.15	1.63	−24.04	1.548	−23.91	21.33	−30.42
303.15	1.46	−24.17	1.541	−24.30	19.63	−30.72
308.15	1.31	−24.30	1.537	−24.70	18.23	−31.03
313.15	1.26	−24.58	1.531	−25.09	16.94	−31.35
318.15	1.16	−24.75	1.525	−25.48	15.96	−31.69
323.15	1.06	−24.89	1.518	−25.87	15.55	−32.12
328.15	0.96	−25.02	1.511	−26.25	14.61	−32.45
333.15	0.87	−25.14	1.505	−26.64	14.03	−32.83

[a] In the above-mentioned temperature range for CTAB surfactant; ΔH = -14.97 kJ/mol and ΔS = 30.57 $JK^{-1}mol^{-1}$.
[b] In the above-mentioned temperature range for SDS surfactant; ΔH = -0.65 kJ/mol and ΔS = 78.02 $JK^{-1}mol^{-1}$.
[c] In the above-mentioned temperature range for TX-100 surfactant; ΔH = -10.9 kJ/mol and ΔS = 65.51 $JK^{-1}mol^{-1}$.

of decay rate constants for the radiative and non-radiative processes could be calculated using Eq. (3) and the values have been tabulated in Table 3. It is evident that an increase in the concentration of any of the surfactant does not have much effect on the value of k_r but the values of k_{nr} definitely get decreased. Also, the non-radiative process predominates in the process of deactivation of the excited state of IOC than the radiative ones.

3.7. Effect of salt concentration

In order to study the effect of salt on the photophysical properties of IOC-surfactant nano-micellar system, the concentration of IOC was fixed at 10 μM and the concentrations of CTAB, SDS and TX-100 were maintained at 5 mM, 20 mM and 1.5 mM; respectively. Meanwhile, the concentration of salt; i.e., sodium chloride, was varied up to 2 M. A total of six solutions were prepared in which the concentration of NaCl was altered as 0 mM, 0.05 M, 0.1 M, 0.5 M, 1 M and 2 M; respectively. While preparing the samples, something unusual was sighted happening in case of SDS. In the IOC-SDS micellar system, at higher concentrations of NaCl (greater than 0.5 M), thick white precipitates could be observed. Kratsey *et al.* have reported that NaCl and SDS are immiscible in the micellar state and they have called it as the salting out effect of SDS in the presence of NaCl.[42] It has been observed that the concentration of NaCl has an insignificant and unnoticeable change in the absorption spectra for all the three nano-micellar systems in terms of both nature of the spectra and the absorbance value. Based on these observations, it could easily be said that in the studied concentration range, salt (NaCl) has no effect on the ground state photophysics of the IOC molecules encapsulated inside the surfactant micelles. But in order to check its effect on the excited state properties on the IOC molecule, the steady-state emission spectra was recorded in the wavelength range of 355 nm to 800 nm. The overlay of the emission spectra depicting the effect of NaCl on the IOC-surfactant nano-micellar system has been represented in Fig. 6. It could be easily noticed from this figure that the fluorescence intensity monotonically increased on increasing the concentration of salt in all the three micellar systems. This increase in the emission intensity could be ascribed to the salting-in effect. Further, in order to get a clear picture of the effect of NaCl on the excited state photophysical properties of IOC-surfactant micellar system, the time-dependent excited state lifetime measurement experiments were performed. In the case of CTAB and TX-100, the concentration of NaCl was maintained at 1 M, whereas in the case of SDS it was fixed at 0.1 M (due to insolubility at higher concentration of NaCl). The overlay of the decay transients in the presence and absence of salt have been represented in Fig. 6b, 6d and 6f for CTAB, SDS and TX-100; respectively. The values of the fitting parameters for all the decay parameters have been arranged in Table 3. It could be observed that while the addition of 1 M NaCl to 5 mM CTAB solution increased the average lifetime of IOC from 96 ps to 138 ps and the addition of 0.1 M NaCl to 20 mM SDS solution had very minimal effect on the average lifetime (108 ps to 127 ps). Similarly, the addition of salt has negligible effect on the $\tau_{avg.}$ value of IOC encapsulated inside TX-100 micelles. Thus, it could be concluded that salt has no effect on the excited state photophysics of IOC in presence of non-ionic surfactants (TX-100). In contrast, the introduction of salt in the presence of cationic surfac-

Table 3
Picosecond resolved decay parameters, fluorescence quantum yield, and radiative and non-radiative decay rate constants of IOC in the presence of aqueous solution with increasing concentrations of surfactants. (Experimental error ± 6%).

Surfactants	Concentration	τ_1 (ps) (%a_1)	τ_2 (ps) (%a_2)	τ_3 (ps) (%a_3)	$\tau_{avg.}$ (ps)	χ^2	ϕ_x	k_r (s^{-1}) ($\times 10^8$)	k_{nr} (s^{-1}) ($\times 10^9$)
Water	–	64.01 (96.5)	690.2 (2.46)	2123.1 (0.98)	99.72	1.095	0.0066	0.66	9.96
CTAB	0.5 mM	68.9 (96.91)	535.8 (2.01)	1455.7 (1.04)	92.82	1.045	0.0207	2.42	10.55
	5 mM	74.7 (97.38)	539.9 (1.57)	1405.9 (1.04)	95.94	1.065	0.0216	2.25	10.20
	10 mM	76.1 (97.82)	629.7 (1.08)	1517.5 (1.09)	97.78	1.026	0.0229	2.34	9.99
	50 mM	90.7 (95.12)	723.4 (2.44)	2424.7 (2.44)	163.09	1.039	0.0241	1.48	5.98
	5 mM + 1 M NaCl	90.1 (95.12)	750.6 (3.66)	2028.3 (1.22)	137.90	1.067	0.0355	2.57	6.99
SDS	5 mM	75.8 (96.84)	578.1 (2.11)	1821.9 (1.05)	104.73	1.001	0.0173	1.65	9.38
	20 mM	76.9 (96.7)	584.2 (2.20)	1878.4 (1.10)	107.87	1.023	0.0172	1.60	9.11
	35 mM	78 (96.55)	592.2 (2.33)	1995.8 (1.11)	111.27	0.998	0.0172	1.54	8.83
	50 mM	81.1 (96.62)	612.1 (2.25)	1998.3 (1.12)	114.52	1.025	0.0172	1.50	8.58
	20 mM + 0.1 M NaCl	83 (95.35)	661.8 (3.49)	2101.2 (1.16)	126.67	1.007	0.0172	1.53	8.81
TX-100	0.1 mM	94.6 (91.25)	447.6 (6.25)	1824.1 (2.5)	159.87	1.005	0.0380	2.37	6.02
	1.5 mM	95.4 (92.5)	463.5 (5.01)	1912.1 (2.5)	159.21	1.083	0.0392	2.46	6.03
	10 mM	96 (92.66)	480.3 (5.15)	2124.1(2.19)	160.16	1.081	0.0396	2.47	5.99
	50 mM	95.9 (92.28)	475.7 (5.06)	1857.7 (2.66)	161.89	1.068	0.0401	2.47	5.93
	1.5 mM + 1 M NaCl	97.2 (91.27)	503.7 (6.71)	1929.1 (2.01)	161.40	1.092	0.0462	2.86	5.91

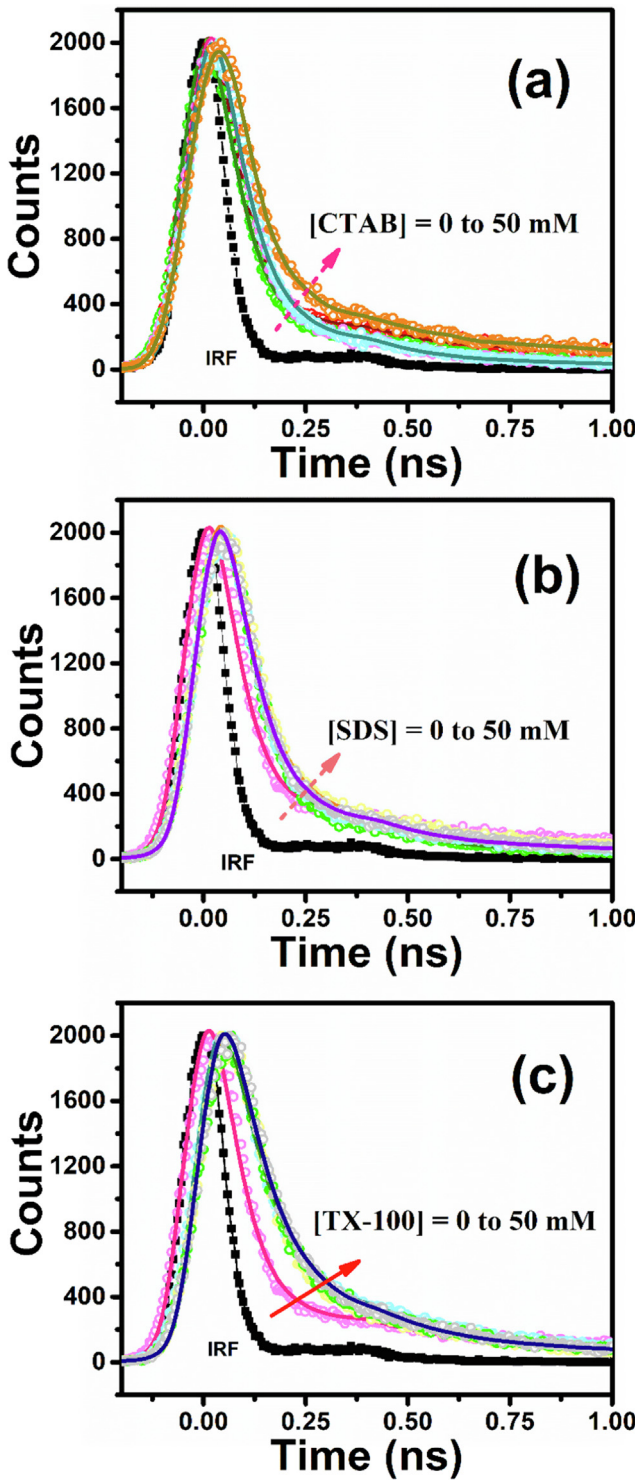


Fig. 5. The overlay of the picosecond resolved decay patterns of IOC in the presence of increasing concentrations of (a) CTAB, (b) SDS and (c) TX-100 micelles; respectively.

tants could slow down the process of decay of the excited state of the IOC molecules.

In addition, the fluorescence quantum yield of all the three micellar system were calculated in the presence of salt and the values have been arranged in Table 3. It could be seen that although the addition of salt increased the fluorescence quantum yield of IOC in case of CTAB and TX-100, it had almost negligible effect in the case of SDS. Based on this data, the radiative (k_r) and non-radiative (k_r) decay rate constants were also determined and the values have been tabulated in Table 3. It could be analyzed that when CTAB and TX-100 were used as surfactants, the value of k_r increased and that of k_{nr} decreased whereas in case of SDS, there was no significant alterations in the values of both the rate constants. It has been already been established in literature that salts are good candidate for the hydrogen bond making as well as breaking substances.[19] Also, the major contribution in the non-radiative processes is from the H-bonding between the IOC and the surfactant molecules. After the addition of salt, there is a possibility of formation of new H-bond network which perturbs the already existing H-bonding. Hence, as a result, the value of non-radiative decay rate constant decreases.

In order to understand the stability of the three nano-micellar formulations in the presence of salt, the time-dependent absorption spectra of the three above-mentioned samples have been recorded up to 3 days. The overlay of the degradation profiles has been represented in the Fig. 7. In the case of CTAB surfactant, no variation in the intensity of the main peak could be observed, indicating the magnificent stability in the presence of cationic surfactant. In addition to this, the peak at around 400 nm (which could be noticed in the absence of salt) was completely absent following the addition of NaCl. Moreover, in the case of IOC-SDS-NaCl micellar system, the absorbance value of the main peak remained unaltered, whereas the absorption intensity of the peaks in the range of 270 nm – 285 nm kept getting decreased. The peaks in the later mentioned range is due to the interaction of the micellar system with NaCl only and the decrement might be due to the salting-out effect of SDS in the presence of NaCl.[42] Besides this, as represented in the Fig. 7e, the addition of NaCl to the IOC-TX-100 surfactant micellar solution seemed further enhanced the aqueous stability of the nano-formulation. Thus, it could be inferred that the addition of NaCl (even at higher concentration) had absolutely no effect on the stability of the nano-micellar systems and therefore, these formulations could be further investigated as potential drug delivery vehicles.

The binding interactions in the IOC-surfactant micellar system in the presence of NaCl could be further explored by studying the effect of temperature on the micelles formed with the samples at the aforementioned concentrations. The overlay of emission spectra of IOC encapsulated surfactant micelles in the presence of 1 M/ 0.1 M NaCl at different temperatures is shown in Fig. 7b, 7d and 7f, for CTAB, SDS and TX-100; respectively with the insets describing the plot between the fluorescence intensity versus temperature. Finally, in the case of all the three surfactants in the presence of NaCl, the only change in the temperature-dependent emission spectra that could be observed was the decrease in the fluorescence intensity on increasing the temperature. Hence, it could be stated that salt had almost negligible effect on the thermal stability of the IOC encapsulated surfactant micelles.

4. Conclusion

In the present work, the aqueous solubility of the potential hydrophobic drug molecule termed as Isoxazole derivative of curcumin (IOC) has been successfully enhanced by using surfactants of various surface charges. The surfactants chosen for this purpose are CTAB, SDS and TX-100 picking up one from each category of cationic, anionic and non-ionic surfactants. The steady state spectroscopic techniques have revealed that the mechanism of interaction is entirely different for different kinds of surfactants. The absorption and emission maxima as well as the intensities are completely dependent on the microenvironment provided by various surfactants. The maximum partition of the IOC molecules from the bulk water phase to palisade layer of the micelles could be

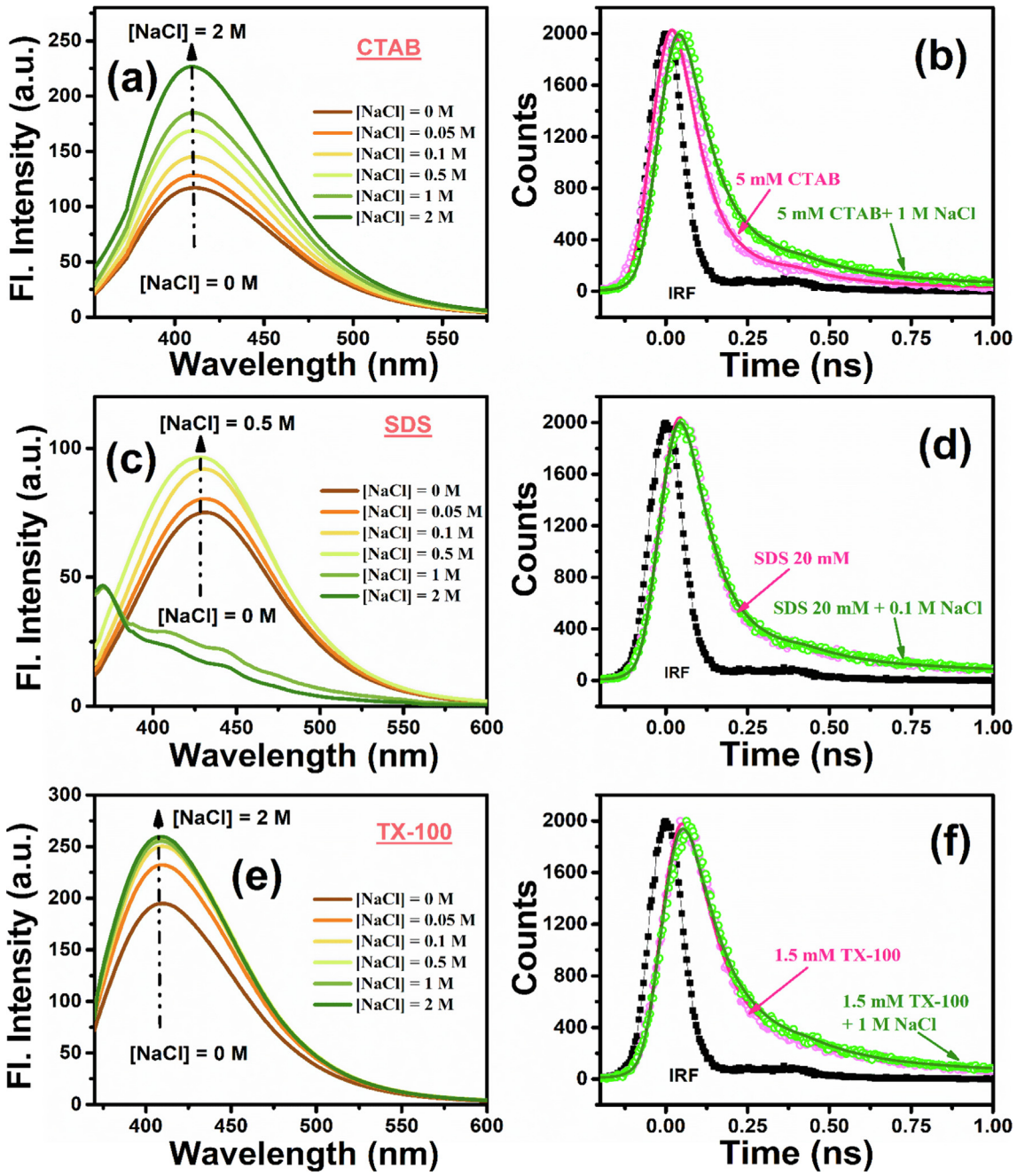


Fig. 6. The effect of increasing salt (NaCl) concentration on the emission spectra of IOC in the presence of (a) CTAB, (c) SDS and (e) TX-100 micelles; respectively. The effect of addition of salt (NaCl) on the picosecond resolved decay patterns of IOC in the presence of (b) CTAB, (d) SDS and (f) TX-100 micelles; respectively.

found in case of non-ionic surfactant i.e., TX-100. The low degradation rate constants and high negative values of change in Gibbs free energy for all the three surfactants certainly reveals that the process of micellization for all the surfactants is highly spontaneous in nature and once formed they are extremely stable. The effect of salt concentration has also been examined on the photophysical properties of IOC inside all the three nano-micellar systems. The time-dependent studies have revealed that the addition of salt increases the fluorescence quantum yield as well as average lifetime of the IOC molecules encapsulated inside surfactant micelles. These outcomes have helped us concluding that the surfactants are very well efficient in enhancing the aqueous solubility of IOC and also form stable formulations (in terms of time-dependent and temperature-dependent stability in the presence as well as absence of salt). Thus, this work opens a wide area of research where the design and development of new drug formulations and delivery vehicles could be done using different kinds of surfactants, polymers, liposomes and many more.

CRediT authorship contribution statement

Manisha Sharma: Writing - original draft, Formal analysis, Methodology, Conceptualization, Investigation, Writing - review & editing. **Swati Rani:** Validation, Formal analysis. **Subho Mozumdar:** Conceptualization, Resources, Validation, Writing - review & editing, Supervision.

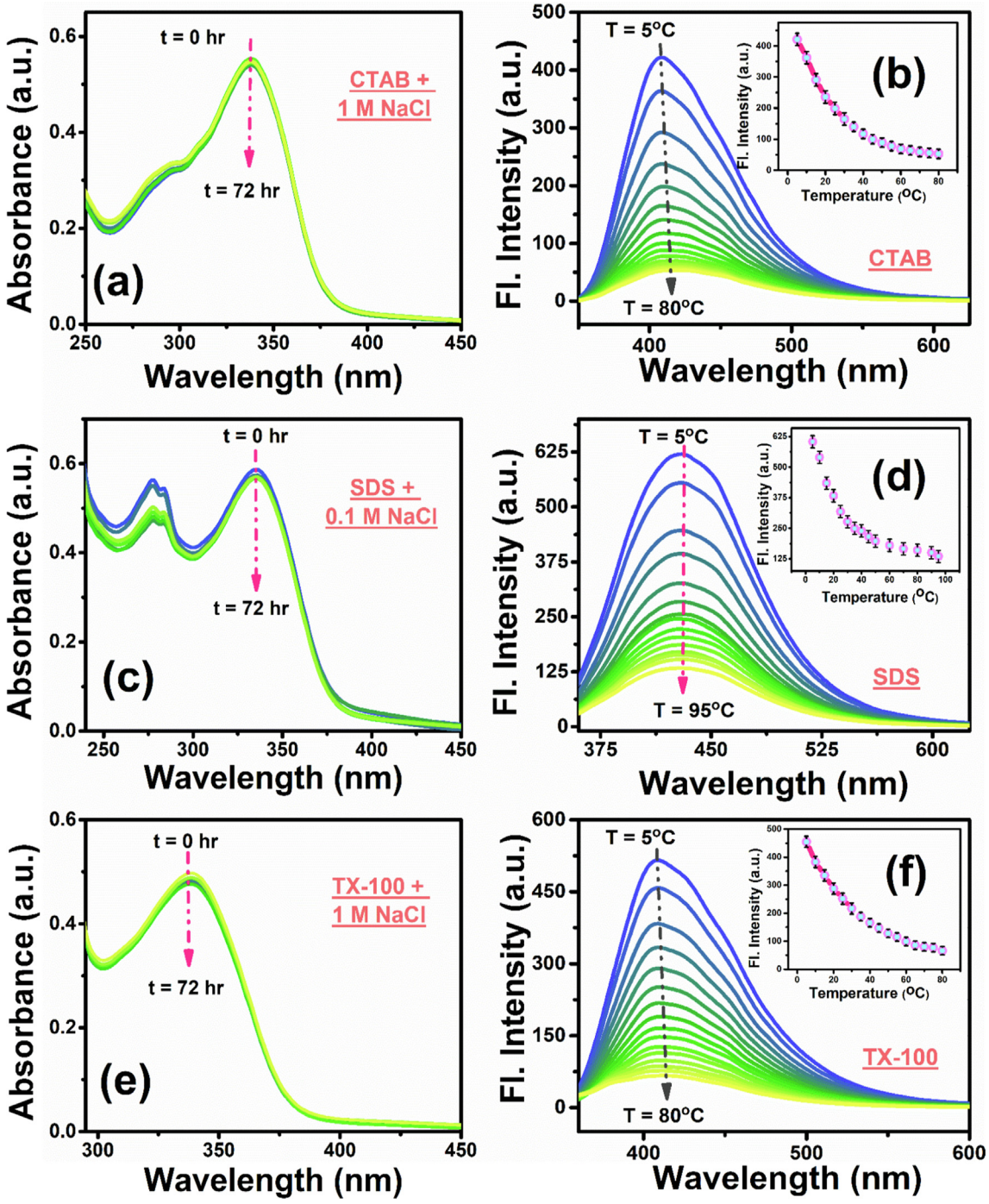


Fig. 7. The overlay of the absorption spectra of IOC recorded at regular intervals of time, in the presence of (a) CTAB, (c) SDS and (e) TX-100 micelles; respectively with the addition of NaCl. The overlay of the temperature-dependent emission spectra of IOC in the presence of (b) CTAB, (d) SDS and (f) TX-100 micelles; respectively with the addition of NaCl. The inset in each figure represents the variation in the emission intensity of IOC encapsulated surfactant micelles in the presence of NaCl with the increasing temperature.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2021.116981.

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