A Spectroscopic and Electrochemical Investigation of Interactions of Anticancer Uracil Derivatives with Cationic and Anionic Surfactants

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Summary: Interactions of 5-fluorouracil (5-FU), a commercially available anti-cancer drug and two other possibly anti-cancer actives, 2-thiouracil (2-TU) and 2,4-dithiouracil (DTU), with anionic sodium dodecyl sulphate (SDS) and cationic cetlytrimethyl ammonium bromide (CTAB) surfactants were studied using cyclic voltammetry and UV-Visible spectroscopic techniques. The results from both techniques asserted the formation of complex between the drugs and surfactants. In the pre-micellar concentrations, the binding was mainly due to the interactions between the surfactants monomers (electrostatic) and the drug molecules, while in the post-micellar region, drug was encapsulated within the micelle due to electrostatic as well as hydrophobic interactions. The UV-Visible spectroscopic data of the interaction between 5-fluorouracil and the surfactants exhibited an isobestic point which indicated the presence of equilibrium species in bulk and the micellar phase. Binding constant, partition coefficient between bulk and miceller phase, and the number of drug molecules incorporated per micelle were calculated.

Keywords: Micelles, Anticancer drugs, UV-Visible, Partition coefficient; Drug-surfactant complex, Binding Constant, Cyclic Voltammetry

Introduction

The effective usage of many potent anticancer drugs is limited due to their side effects, toxicity [1] and the non-availability of effective delivery systems. Designing new and effective delivery systems can play an important role in bringing down the therapeutic cost of cancer treatment as it is much more expensive [2] and time consuming to design and approve new drugs [3].

Surfactants are extensively used in pharmaceutical industry having applications as excipients, in drug solubilization and as drug carriers. Due to their uses in the pharmaceutical industry, surfactants have gathered the attention of many research groups working in the area of drug delivery and solubilization, having the added advantage of simulating the electrostatic and hydrophobic interactions of drugs with membranes[4-7], e.g. Chakraborty *et.al.,* shown that cationic micelles are used to estimate the interaction between NSAIDs and biomembranes [8]. In another study, Stephenson *et.al.,* shown the usage of surfactants for enhancing the solubilization of model drug compounds[9].The study of model systems of drugs and surfactants help to design better controlled drug delivery systems [10] which may help to utilize already established active drugs, which cannot be utilized otherwise due to their high and uncontrolled toxicity.

Controlling the toxicity and the dosage of anticancer drugs is an important research front [11, 12]. Controlled release methods such as lipid bilayers, liposomal nanospheres and vesicles [13], multifunctional nanoparticles [14], nano-forms of colloidal gels [15-19] are emerging as useful technologies to customise the release profile of different drugs. One of important methods is the use of aggregates of surfactants called micelles e.g., the anticancer drug, 5-fluorouracil (5-FU) can be loaded in a polymeric micelle and released in response to external stimuli such as pH and temperature [20]. The encapsulation of drugs in micelles and their release with the help of external stimuli at targeted site can maximize the bioavailability with relatively lower dosage [17, 18, 21]. Simulating a similar environment and studying model systems can be useful way to get fundamental insight in to the process of targeted drug delivery and can be treated as basis for further studies. 5-FU and its derivatives can be useful model drugs for improving our understanding of mechanism of interaction between drugs and the micellar carriers of different types [22].

5-FU is frequently used as a hydrophilic [23] model drug for testing various types of drug delivery systems [24-27]. Apart from its status as model drug, 5-FU has established therapeutic importance as a potent drug for the metastatic

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carcinoma of breast [24], treatment of solid tumours [26] and various types of cancer [28, 29], whereas thiouracils e.g. 2-thiouracil (2-TU) and 2,4-dithiouracil (DTU) are known as effective neoplastigen, tumorigen, carcinogen and teratogen agents. These cytotoxic anticancer drugs in comparison to other drug classes, present unique problems that primarily come from the side effects provoked by the drug attacking the target as well as healthy cells and the relative lack of specificity of their systemic bio-distribution. Their unfavourable pharmacokinetics compels the administration of high doses and imposes a rigorous schedule on patients for reaching the desired therapeutic effect. Hence, their clinical usage has been greatly limited over the past 50 years. With a view to overcome the toxicity associated with such drugs and improve their therapeutic value, we have used "surfactant micelles" as model drug carrier.

Surfactants are amphiphilic in nature and have the ability to form associative structures called micelles. Typically, the core of micelle is hydrophobic while the exposed surface of the micelle is hydrophilic in nature. The hydrophilic nature came from the ionic head groups of the surfactant monomers which make the surface of the micelle comparable to the surface of the membrane [30]. In other words, micelles can be used to simulate membrane structures and, their interaction with drugs can give useful information regarding the interaction of membrane with drugs. The extent to which a drug interacts with the surfactant can be described in terms of the electrostatic and hydrophobic effects depending upon the charge and surface area of drug/surfactant respectively. Drug-surfactant binding constant and micelle-water partition coefficient are useful parameters for the quantitative evaluation of the micellar effect on the properties of pharmaceutical drugs[31, 32], which in turn helps in understanding the drug structure-activity relationships as well as its interaction with the biological membranes [33].

In a previous study, we explored the DNA binding of some derivatives of uracils which showed that these derivatives have better binding as compared to the classical intercalator proflavin and clinically used chemotherapeutic agent epirubicin [34]. In this study, cyclic voltammetry supported by UV-Visible spectroscopy is used to explore the interaction between the surfactants micelles and the uracil derivatives.

Experimental

High purity (≥99%) chemicals 2-Thiouracil (2-TU), 2,4-dithiouracil (DTU), 5-fluorouracil (5-FU), sodiumdodecylsulphate (SDS), and cetyltrimethylammonium bromide (CTAB), were purchased from Sigma and were used as received without any further purification. Analytical grade ethanol was used to prepare stock solutions (2 mM). For cyclic voltammetry 1 mM working solutions were prepared by mixing 50% buffer and 50% stock solutions of the analytes. In case of UV-Vis spectroscopy, stock solutions were further diluted up to a concentration such that the wavelength of maximum absorbance of the band of interest did not exceed 1 and Beer-Lambert law could be obeyed.

Voltammetric experiments were performed using μAutolab running with GPES 4.9 software, Eco-Chemie, The Netherlands. Glassy carbon was used as working electrode. GCE of Digi-Ivi, USA with a 0.071 cm^2 area was used as working electrode. A Pt wire and saturated calomel electrode were used as counter and reference electrodes. Before each experiment, the surface of GCE was polished with alumina powder followed by thorough rinsing with distilled water. For reproducible experimental results the clean GC electrode was placed in supporting electrolyte solution and various cyclic voltammograms were recorded until the achievement of steady state baseline voltammogram. All the voltammetric experiments were conducted in a high purity argon atmosphere at room temperature (25±1°C). The pH measurements were carried out with a Crison micro pH 2001 pH-meter with an Ingold combined glass electrode. The work was done in media of different pH. The CVs were first recorded in pure solvent for getting baseline voltammograms and then the electrode system was shifted to solution. The CVs of the analyte solutions were compared with the blank solvent. Absorption spectra of the drug solutions containing surfactant in the range from pre-micellar to post-micellar concentrations were recorded on Shimadzu 1601 spectrophotometer. The concentration of the drug was kept constant in all cases. The path length of the cuvette was 1cm.

Results and discussion

Cyclic voltammetry (CV)

Interaction of uracil derivatives with CTAB

Cyclic voltammetry was used to experimentally observe the interactions between the model drugs 2-TU, 5-FU, and DTU with an anionic surfactant SDS and the cationic surfactant CTAB. The shape of the voltamogram clearly shows an electron transfer process. The cyclic voltamograms of 1mM solution of 5-FU is shown in Fig. 1a-c under different conditions while keeping the pH constant at

10.2. The Fig shows the impact of variation of the concentration of CTAB on cyclic voltammetry response of 5-FU. The hydrophobic adsorption of surfactant monomers on the electrode surface results in the formation of a hydrophilic and positively charged surface. The compactness of the charge on the surface of the electrode changes with the increasing concentration of surfactant. The result is the differentiating cyclic voltammetry responses in the pre-micellar, micellar and post-micellar phases. Fig. 1a shows premicellar concentration of CTAB at pH 10.2. Initially, addition of surfactant reduces the peak current with small positive shift of anodic peak. However, further addition of CTAB results in negative shift of anodic peak with increased peak current which almost restores the previous peak position. Fig. 1b shows the effects of micellar concentrations of the surfactant CTAB on the CV pattern of 5-FU. Clearly, a decrease in peak current and a positive shift in the anodic peak is evident in the Fig. 1b. Fig 1c shows the post-micellar concentrations of CTAB. In the micellar concentrations, a new peak can be clearly observed at 1.365 V at 8mM concentration of CTAB although it was entirely absent at 2 and 4mM concentrations of CTAB. However, a peak indication was found at 6mM concentration of CTAB. A significant increase in the peak current was also observed with increasing CTAB concentration in the post micellar phase when compared to the observed reduction in the peak currents in the pre-micellar and the micellar phases. However, the peak position remains almost unchanged at i.e., at 1.15 V.

The positive shift in the peak position (E_{pa} = 1.10 →1.16 V) with increasing concentration of CTAB in the pre-micellar concentrations stems from the initial adsorption of surfactant monomers on the electrode surface which provide an increased surface area of electrode for the possible electron transfer, dropping the peak current appreciably. Another possibility is the interaction of monomers of CTAB with 5-FU at the defined pH. Further, when the surfactant monomers get hydrophobically adsorbed on the surface of electrode, they practically reduce the distance between 5-FU and the electrode surface. This cause an initial drop in peak current as can be seen in the Fig. 1a.

As the concentration of surfactant is further increased, more and more surfactant monomers get adsorbed on the electrode surface, reorienting themselves, and covering relatively higher electrode surface. This causes an intermediate increase in the peak current and also a reversal of peak position; almost restoring the previous values. The observed anodic peak current is even more than the one observed for 5-FU without any addition of CTAB.

Further increase in the surfactant concentration in the post-micellar range, as shown in Fig. 1c, is exposed to the micellar effect. The self-association of micelle may lead to the solubilization/encapsulation of 5-FU make available the second nitrogen for oxidation which can be a possible reason for the appearance of a new peak at 6 and 8mM concentration of CTAB.

Fig. 2 shows the effect of different concentrations of CTAB on the electrochemical behavior of DTU at pH 7.0. Except for a single instance, when the concentration of CTAB is 10 mM, as shown in Fig. 2c, a decrease in peak current was observed in combination with a very small negative shift in the anodic peak voltage value. Overall, this trend indicates the facilitation of oxidation process with increasing surfactant concentration in the micellar, and the pre-micellar concentrations and most of the post micellar concentrations. The slow evolution of peak at 0.45 V can be explained by the possibility of solubilization of the DTU or otherwise by the possibility of the electrostatic interaction between the additive and the micelle.

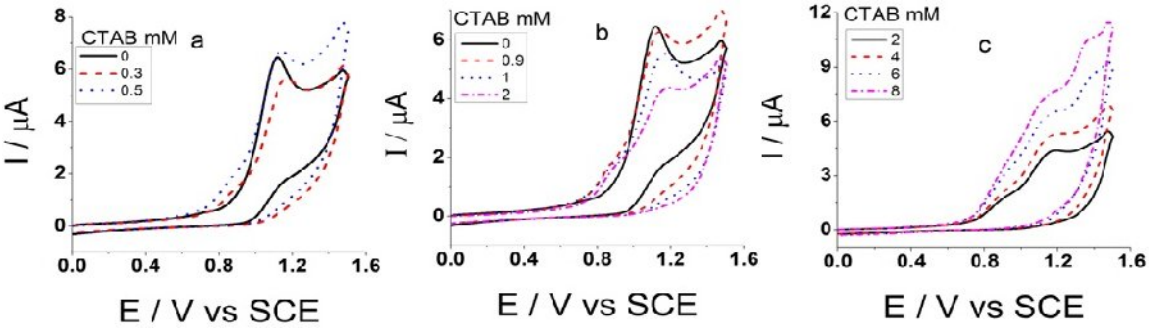

Fig. 1: CVs of 1 mM 5-FU at GCE obtained in pH 10.2 at 100 mV s^{-1} in the presence of (a) pre-micellar concentrations of CTAB (b) micellar concentration range of CTAB (0.9 mM–2mM) (c) post-micellar concentrations of CTAB (2 mM–8mM).

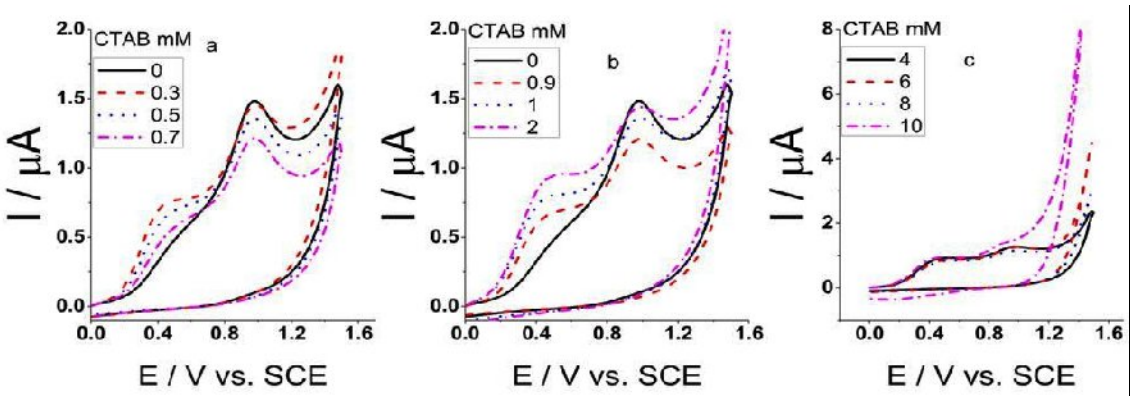

Fig. 2: Cyclic voltammograms of 1 mM DTU at GCE (in phosphate buffer (pH 7), scan rate 100 mV/s) in the presence of different (a) pre-micellar concentrations of CTAB (0.3 mM—0.7mM), (b) micellar range of concentrations of CTAB (c) post-micellar concentrations of CTAB.

Interaction of uracil derivatives with SDS

Observations were made showing the similar trend of change in current with the addition of anionic surfactant, SDS. The cyclic voltammograms of 5-FU, DTU and 2-TU in the presence of varying concentrations of SDS in micellar, pre and post micellar region were noted. With the addition of SDS (from 2 mM to 6 mM) in the pre-micellar region, the current intensity increased for 5-FU and decreased in the case of 2-TU and DTU. Neither shift in peak potential nor the appearance of a new peak was observed for 5-FU but 2-TU presented a new peak at lower potential due to electroactive product formation between 2-TU and SDS. SDS monomers get adsorbed on the electrode surface forming a negatively charged film that attracted the drug molecules i.e. 5-FU, DTU and 2-TU to the electrode via hydrogen bonding between the partial positive hydrogen attached directly to nitrogen atom and negatively charged sulphonate head group of SDS thus giving rise to increase of current intensity. Electron transfer is retarded by the screening of the electrode surface by higher SDS molecules resulting in the current decrease. The current amplification of 5-FU and 2-TU, in post micellar region, is attributed to the quitting of monomers from the electrode as micelles start forming at this concentration, thus making surface of electrode free for electron transfer. Due to the decrease in diffusion of the DTU molecules as these are now incorporated within the SDS micelle and diffuse along with it, diminution of peak current in post-micellar concentration is observed.

Electronic absorption spectroscopy

Interaction of uracils with CTAB

UV-Visible spectroscopy was also used for the investigation of interaction of uracil derivatives with SDS and CTAB. The changes in the electronic absorption spectra of 5-FU in the presence of varying concentration of CTAB in a medium buffered at pH 10.2 can be seen in Fig. 3. The analyte (5-FU) registered a couple of signals at 206 and 268 nm in the absence of CTAB. While in the presence of increasing concentration of CTAB in a fixed concentration of 5-FU, the signal at 206 nm showed increase in absorbance (hyperchromic effect) accompanied with red shift (bathochromic effect), generation of another signal at 296 nm and an isosbestic point. In contrast, the signal at λ= 268 nm exhibited hypochromic effect (decrease in absorbance) when the concentration of CTAB was increased in a solution containing constant amount of 5-FU. The appearance of a new peak at 296 nm is attributed to the 5-FU – CTAB adduct formation. The red shift of about 11 nm can be related to the interaction of 5-FU with the monomers and aggregates (micelles) of CTAB. The solubilizate is expected to compartmentalize in different zones i.e. the micellar core, stern layer or the surfactant-aqueous interface because solubilization is a dynamic process. 5-FU molecules will be encapsulated within the aggregates but their chromophores may be oriented towards solvent-micelle interface. Hence, the chromophore of 5-FU responsible for absorbance at 206 nm occupies solvent-micelle interface and thus this signal demonstrates hyperchromic effect with increase in CTAB concentration. Whereas, the peak of 5-FU at 268 nm exhibiting a decrease in absorbance with increasing concentration of CTAB suggests the chromophore responsible for the appearance of this signal to occupy the micellar core. Hence, the burial of 268 nm based chromophore of 5-FU in the micellar interior of CTAB leads to hypochromic effect. The isosbestic point shown in the inset of Fig. 3 can be related to the presence of two spectroscopically distinguishable forms of 5-FU in the micellar and ex-micellar phases. The electronic

absorption spectra of 2-TU and 2-DTU were also obtained in the presence of pre, micellar and post micellar concentrations of CTAB. DTU exhibited bathochromic shift of about 8 nm, whereas, 2-TU demonstrated a hypsochromic shift followed by bathochromic shift as the concentration of CTAB increased from pre-micellar to micellar concentration. The shift in the wavelength of maximum absorbance suggested drug-surfactant complex formation. The insertion of uracil derivatives in the micellar interior with their chromophores located at micelle- solvent interface absorb more light due to increase in local concentration. The characteristic spectral behavior of 2-TU in the presence of varying concentration of CTAB can be attributed to the switching of its interaction with the surfactant from electrostatic binding mode to hydrophic one. On the basis of the previous reports [35] 2-TU molecules are expected to form dimers in the presence of surfactant, which absorb UV-Vis light at lower wavelength as compared to drug molecules in monomeric form (in the absence of surfactant). Thus blue shift is observed in the presence of surfactant in pre-micellar concentration range. In post micellar concentration, the mode of interaction of 2-TU with CTAB changed as evidenced by the shift of signal to longer wavelength.

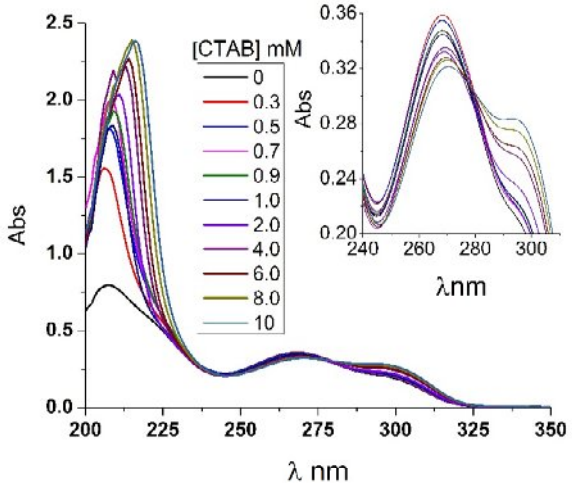

Fig. 3: UV-Vis spectra of 8×10^{-5} M 5-FU (shown in the wavelength window of 200 – 360 nm) in the absence and presence of varying concentration of CTAB (0.3 mM – 10mM). Inset shows the expanded form of the wavelength window of 245 – 320 nm.

In alkaline pH (10.2) the molecules of 5-FU are possible to lose proton from the –NH moiety thus resulting in the formation of di-anionic specie which will interact with the cationic head group of CTAB to form drug-CTAB complex of 1:2 stoichiometry. This electrostatic interaction causes red shift of λ_{max} and increase of absorption intensity with increasing surfactant concentration. The proposed mechanism of interaction between 5-FU and CTAB is presented in Fig. 4.

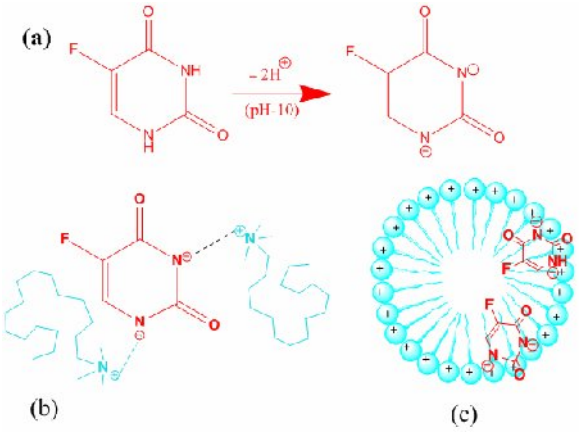

Fig. 4: (a) Formation of dianionic specie of 5-fluorouracil and proposed mechanism for the interaction of 5-FU di-anioninc specie with (b) CTAB monomers and (c) CTAB micelle.

Interaction of uracils with SDS

The interaction of 5-FU, 2-TU and DTU with SDS was also investigated by UV-Vis spectroscopy. The spectroscopic behavior of 5-FU changed in the presence of pre and post micellar concentrations of SDS. The signal of 5-FU at λ_{max} 205 nm showed pronounced variation in absorption intensity with increasing concentration of SDS. At 205 nm, the absorption intensity of 5-FU increased in the pre-micellar concentration range of SDS, then decreased as the micellar concentration approached, and finally increased again. However, the increase was still lower than the absorbance recorded without SDS. A slight hypsochromic shift of about 3 nm was also noticed. The diminution in absorption intensity can be related to the columbic repulsion of negatively charged 5-FU molecule and anionic head group of SDS. In micellar concentration range of SDS, 5-FU molecules get incorporated within the micelle due to the dominance of hydrophobic forces over repulsive electrostatic forces. The chromophore would be buried deep in the micellar core due to the repulsion of anionic 5-FU and negatively charged head group of SDS; hence, UV-Vis light will not be effectively absorbed. However, the peak of DTU at λ_{max} ~ 204 nm, is showing a slight increase in absorption intensity with increasing SDS concentration from

pre-micellar to micellar range which suggests a shallow insertion of the chromophore of the drug in the micellar interior.

Determination of binding constant (K_b)

From the variation of absorption intensity of uracil derivatives due to the influence of surfactants concentration, the extent of uracils-micelle interaction was quantified in terms of binding constant K_b [35]. The value of partition coefficient indicating the solubilization of uracils molecules between aqueous bulk phase and the micellar phase was also evaluated. The determination of these parameters is important for developing quantitative structure–activity relationships and understanding of their role in exerting biological actions. The value of binding constant was determined according to the reported methods [35, 36]. Drug and surfactant interaction can simply be represented by the following equilibrium;

Drug + Surfactant $\rightleftharpoons$ Drug-Surfactant complex

$$K_b = ([\text{Complex}])/([\text{Drug}][\text{Surfactant}]) \quad (1)$$

Representing the concentration of Complex by C_b

$$K_b = C_b/(([\text{drug}] - C_b)([\text{surfactant}] - C_b)) \quad (2)$$

Where, [drug] and [surfactant] are the analytical concentrations of drug and surfactant in the solution. According to the Beer Lambert law,

$$[drug] = A_0 / \varepsilon_{drug} \times \lambda \quad (3)$$

$$C_b = (A - A_0) / \varepsilon_b \times \lambda \quad (4)$$

where A and A_0 represent the absorbance of drug with and without surfactant and ε_{drug} and ε_b are the molar absorption coefficients of drug and complex, respectively, λ is the path length of the cuvette taken as 1cm.

By putting equations 3 and 4 in equation 2 one can obtain,

$$A_0/(A - A_0) = (\varepsilon_{drug})/(\varepsilon_b) + (\varepsilon_{drug})/(\varepsilon_b \times K_b) \times 1/[\text{surfactant}] \quad (5)$$

From the linear plot of $1/(A - A_0)$ vs. $1/[\text{surfactant}]$ the value binding constant K_b is obtained by dividing intercept over slope. The interaction strengths of 5-FU, DTU and 2-TU with CTAB and SDS quantified as K_b are listed in Table-1.

Table-1: Uracils-surfactants binding parameters as quantified by UV-Vis spectroscopy.

Parameters	2-TU		DTU		5-FU	
	CTAB	SDS	CTAB	SDS	CTAB	SDS
$K_b\ M^{-1}$	2.4×10^3	1.3×10^4	2.8×10^3	25.4	3.7×10^3	3.1×10^2
ΔG_b^o kJ/mol	-19.3	-23.5	-19.7	-8.01	-20.4	-14.2
$K_x \times10^5$	0.79	7.4	2.3	0.014	7.6	0.171
ΔG_x^o kJ/mol	-28.0	-33.5	-30.6	-18.0	-33.7	-24.2

Determination of partition coefficient (K_x)

Pseudo-phase model was used for the evaluation of partition coefficient. The ratio between the mole fraction of the drug in micellar and bulk aqueous phase is represented by K_x

$$(X^m_{\text{drug}})/(X^w_{\text{drug}}) = K_x \quad (6)$$

These mole fractions are related to the concentrations of the species involved in the solubilization system,

$$X^m_{\text{drug}} = C^m_{\text{drug}} /(C^m_{\text{drug}} + C^m_{\text{surfactant}}) \quad (7)$$

$$X^w_{\text{drug}} = C^w_{\text{drug}} /(C^w_{\text{drug}} + C^w_{\text{surfactant}} + n_w) \quad (8)$$

where, $C^w_{\text{surfactant}}$ and $C^m_{\text{surfactant}}$ denote surfactant concentration in the monomeric and micellar forms respectively. n_w indicates moles of water per dm^3=55.5M, In equation 8, the terms $C^w_{\text{surfactant}}$ and $C^m_{\text{surfactant}}$ being negligible compared to n_w can be neglected. By putting the values of X^m_{drug} and X^w_{drug} in eq. 6 the value of K_x is obtained as;

$$K_x = C^m_{\text{drug}} \times n_w/(C^m_{\text{drug}} + C^m_{\text{surfactant}})(C^w_{\text{drug}}) \quad (9)$$

where,

$$K_s = C^m_{\text{drug}} /(C^m_{\text{drug}} + C^m_{\text{surfactant}})(C^w_{\text{drug}}) \quad (10)$$

So, K_x is related to K_s as;

$$K_x = K_s \times n_w \quad (11)$$

The fraction of the associated drug may be represented as,

$$f = C^m_{\text{drug}} /[drug] \quad (12)$$

Below the CMC, this fraction f is equal to zero and above the CMC this fraction shows an increase with rise in surfactant concentration. Upon the approach of surfactant concentration to ∞, f

becomes equal to unity, as all added drug is solubilized in micelles. This fraction "f" can be calculated directly from the absorbance data using equation;

$$f = \Delta A / \Delta A_{\infty} \quad (13)$$

where, $\Delta A = A\text{-}A_w$, and $\Delta A_{\infty} = A_{\infty}\text{-} A_w$, A_{∞} is the absorbance of the drug completely bound to micelle.

Equation 10 can be written in linear form by using equations 12 and 13 as;

$$1/\Delta A = 1/\Delta A_{\infty} + 1/(K_s \Delta A_{\infty}) \times 1/((Cs+[drug]-CMC)) \quad (14)$$

where, $\Delta A = A - A_0$, $\Delta A_{\infty} = A_b - A_0$, Cs is the surfactant concentration above CMC, [drug] is the concentration of drug used, A and A_0 are the absorbance of drug with and without surfactant and A_b is the absorbance of surfactant bound drug. K_s was obtained from the ratio of intercept to slope of the plot of $1/\Delta A$ as a function of 1 / ([surfactant] + [drug]-CMC). On multiplication of K_s with molarity of water, the value of partition coefficient K_x was evaluated. The plot of $1/\Delta A$ as a function of 1 / ([CTAB] + [DTU] – CMC) can be seen in Fig. 4. Data obtained for the interaction of uracil derivatives with various surfactants are listed in Table-1.

The following equation was used for the calculation of Gibb's free energy of binding

$$\Delta G_b = -RT \times \ln K_b \quad (15)$$

At 298K the binding constant K_b was determined from the ratio of intercept to slope of the graph shown in Fig. 5.

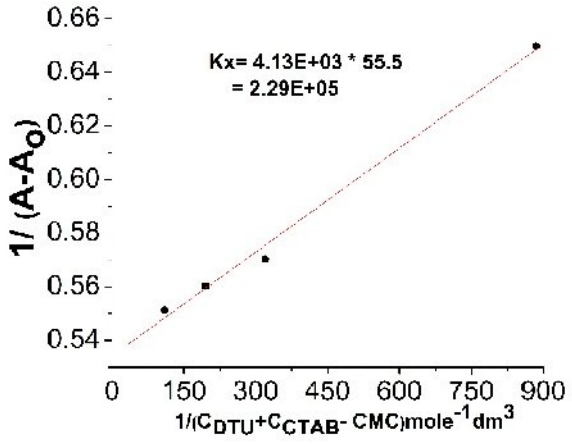

Fig. 5: Plot of 1 / ΔA vs. 1 / ([DTU] + [CTAB]-CMC) for DTU- CTAB interaction.

The free energy change of the transfer of drug molecules from aqueous bulk phase to the micellar phase was calculated using the equation given below;

$$\Delta Gx = -RT \times \ln K_x \quad (16)$$

The values ΔG_b, ΔG_x, K_b and K_x for uracil derivatives with CTAB and SDS are listed in Table-1. An examination of Table-1 shows stronger binding of 5-FU positively charged surfactant CTAB than 2-TU and DTU. The binding of 5-FU with the CTAB micelles investigated in alkaline medium of pH-10.2 started with electrostatic interaction and strengthened by hydrophobic forces that resulted in a higher value of binding constant as compared to the other two. The variation in the magnitude and mode of shift noticed in the electronic absorption spectra of different uracils in the presence of cationic surfactant revealed that the binding strengths and modes vary directly. Similar trend was observed for the interaction of uracils with negatively charged SDS micelles. 5-FU showed stronger binding propensity for SDS than 2-TU and DTU. Although, columbic repulsion should prevent the interaction of di-anionic 5-FU with negatively charged SDS micelle yet the dominance of hydrophobic interaction overcomes the columbic repulsive forces and thus K_b of 5-FU-SDS complexation is higher than 2-TU and DTU.

The partition coefficient, K_x, is an important thermodynamic parameter that shows the affinity of a drug for micellar phase in comparison to the bulk aqueous phase. Larger K_x value is indicative of greater partitioning of the drug molecules within the micellar and aqueous phase. The results of our experiments show that 5-FU has the highest partition coefficient as compared to other uracils. The solubilization behaviour is influenced by the charge on surfactant molecule and structure of the drug. Partition coefficient values obtained for aqueous/micelle - drug partitioning were found in the order: Aqueous/CTAB - drug > Aqueous/SDS – drug. With different surfactants, uracils showed the following order of partition coefficient, Surfactant-5-FU > Surfactant-DTU > Surfactant-2-TU. The lower polarity and longer aliphatic chain length of CTAB as compared to SDS support the drug molecules to encapsulate more easily in the micellar core; hence the partition coefficient value of CTAB is higher than SDS. The values of free energy change of drug-surfactant partitioning and binding showed trend similar to all other parameters discussed above. Negative value of ΔG_b for the interaction of 5-FU, DTU and 2-TU with CTAB and SDS micelles

represented the spontaneity of the interaction processes and varied in the sequence: 5-FU > DTU > 2-TU. ΔG_b or ΔG_x, also depends on the hydrophobicity of the surfactants thus more hydrophobic micellar interior offers more affinity for the hydrophobic drugs.

Determination of number of drug molecules per micelle (n)

For getting insights about the drug loading capacity within the surfactant micellar interior, approximate number of drug molecules per micelle was calculated using the following equation [37, 38];

$$n = C_m/M \quad (17)$$

where, C_m showing the concentration of the drug compartmentalized in the micelle was evaluated using equation;

$$C_m = A_0\text{-}A/\varepsilon_0 - \varepsilon_m \quad (18)$$

where, $\boldsymbol{\varepsilon_m}$ and $\boldsymbol{\varepsilon_0}$ represent the absorption coefficient with and without surfactant. $\boldsymbol{\varepsilon_0}$ is calculated from A_0 whereas $\boldsymbol{\varepsilon_m}$ is determined at higher concentration of surfactant i.e. above CMC when the absorbance of the drug-surfactant solution becomes almost constant. The aggregation number is determined by the equation given as;

$$M=(C_s\text{-}CMC)/N \quad (19)$$

where, C_s is the total surfactant concentration and N is the mean aggregation number of micelles at CMC in water. The CMC values of CTAB and SDS in aqueous solution are reported as 0.9 mM and 8.2 mM with mean aggregation number of 80 and 70 respectively [39, 40].

For a particular concentration of surfactant (C_s), higher value of "n" shows greater hydrophobicity of the drug [41]. The approximate number of 5-FU, 2-TU and DTU molecules per CTAB and SDS micelles are given in Tables-2 and 3 respectively. The data reveal that a maximum five molecules of 5-FU enter into a single CTAB micelle, whereas, three molecules of 2-TU and DTU incorporate per CTAB micelle. In case of SDS micelle, only one molecule of each drug could enter into a micelle. At higher post micellar concentration the number of micelles will be greater, although, drug molecules incorporated per micelle are small, yet the total amount compartmentalized per mole of surfactant will actually be high.

Table-2: Drug molecules incorporated per CTAB micelle and the corresponding data.

Drug	A_o	C_m mol dm^{-3}	ε_m M^{-1}cm^{-1}	M mol dm^{-3}	n (= C_m/M)
2-TU	0.92	15.8×10^{-5}	26.5×10^{3}	6.2×10^{-5}	3
DTU	0.56	18.4×10^{-5}	20.9×10^{3}	6.4×10^{-5}	3
5-FU	0.81	25.1×10^{-5}	16.6×10^{3}	6.2×10^{-5}	5

Table-3: Drug molecules incorporated per SDS micelle and the corresponding data.

Drug	A_o	C_m mol dm^{-3}	ε_m M^{-1}cm^{-1}	M mol dm^{-3}	n = C_m/M
DTU	0.63	1.7×10^{-5}	5.1×10^{3}	4.0×10^{-5}	1
5-FU	0.71	1.2×10^{-5}	0.8×10^{3}	1.1×10^{-5}	1

Conclusions

Interactions of three uracils with surfactants were successfully studied using cyclic voltammetry and UV-Visible spectroscopy. The solubilization of drug in surfactant micelle was indicated during cyclic voltammetry and later on confirmed by electronic spectroscopy. The number of uracils per micelle was higher in the case of cationic surfactant when compared to anionic SDS which showed that cationic surface of micelle in the case of CTAB is electrostatically favored by uracil drugs used in this study, which is also supported by CV profiles of the interactional study. The free energy of binding and free energy of partitioning showed a similar trend except for 2-TU. With different surfactants, uracils showed the following order of partition coefficient, Surfactant-5-FU > Surfactant-DTU > Surfactant-2-TU. The free energy of binding and partitioning varied in the sequence: 5-FU > DTU > 2-TU. These investigations are expected to provide useful insights about the loading of chemotherapeutic agents in micelles for enhancing the efficacy of cancer treatment.

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