The Solution Properties of Amphiphilic Drugs and their Interactions with Surfactants and Human Serum Albumin

Islamabad

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Muhammad Usman

Department of Chemistry
Quaid-i-Azam University
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In The Name of Allah
The Most Compassionate
The Most Merciful
Dedicated
To
Islam,
Religion of Peace and
Justice
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All praises are for Allah almighty who enabled me to accomplish this task successfully. All loves and affections are for his last Prophet Hazrat Muhammad (Sallallah o alaihi wa sallam) each and every moment of whose life is a tower of light to show us what is right and what is wrong in life.

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MUHAMMAD USMAN
This dissertation reports physicochemical behaviour of some amphiphilic drugs as well as their interaction with anionogenic tensides (ionic surfactants) and Human serum albumin (HSA). A detail study of self aggregation of these drugs i.e. Clindamycine Phosphate (CLN), Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ), Dexamethasone Sodium Phosphate (DSP), Pefloxacin Mesylate (PFL), Citalopram HBr (CIT), Fluphenazine 2HCl (FLP), Trifluperazine 2HCl (TRF) and Certizine 2HCl (CRT) has been worked out.

Surface tension and specific conductivity were measured to calculate the critical micelle concentration (CMC) of drugs and in this way their surface and thermodynamic parameters have been estimated. Surface activity was studied by measuring surface parameters i.e. surface pressure, $\Pi$, surface excess concentration, $\Gamma$, area per molecule of drug and standard Gibbs free energy of adsorption, $\Delta G_{ads}^{\circ}$. The electrical conductivity was measured as a function of concentration in the temperature range of 293-323K and CMC was determined. Consequently thermodynamic parameters like standard free energy of micellization, $\Delta G_m^{\circ}$, standard enthalpy of micellization, $\Delta H_m^{\circ}$ and standard entropy of micellization, $\Delta S_m^{\circ}$ were computed using closed association model.

Aggregation properties of some structurally related drugs trifluperazine and fluphenazine and Quinacrine and Chloroquine have also been brought under study. For the most of drugs, association was found to be both enthalpy as well as entropy driven. Dexamethasone sodium phosphate, however, undergoes open association rather than micellization.

We have also studied interaction of amphiphilic drugs with anionic surfactants sodium dodecyl sulfate (SDS) and cationic surfactant cetyltrimethyl ammonium bromide (CTAB). Aqueous micellar solutions of these surfactants were used for solublization of these drugs. The change of CMC of surfactant due to drug was determined by UV/Visible spectroscopy and conductivity method. UV/Visible spectroscopy was used to check the qualitative and quantitative features of this interaction and to calculate
partition coefficient (Kx), free energy of partition and number of drug molecules per micelle while conductivity method is helpful to calculate different thermodynamic parameters.

The complexation of amphiphilic drugs with HSA at physiological conditions (pH 3.0 and 7.4) have also been analyzed by using UV/Visible spectroscopy, fluorescence spectroscopy and dynamic laser light scattering. In this way values of drug-protein binding constant, number of binding sites and hydrodynamic radii were calculated and discussed in detail.
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1.1 Surfactants

A surfactant or amphiphile is a substance that can adsorb at interface of the system and decrease interfacial free energy. The term interface is used to indicate the border between two phases that are immiscible and surface is the one where one phase is air or gas.

The minimum work for creation of interface is called the interfacial free energy. The surface tension is actually the interfacial free energy per unit area. The surfactant adsorbs at interfaces of system and brings a notable change into the work to expand interfaces. Surfactants, in most of cases lessen interfacial tension, however there are case where they are used to augment it [1, 2].

1.2. Amphipathic or amphiphilic structure of surfactants

Surfactants have specific structure having a group that shows repugnance for solvent, called lyophobic group, and the other being strongly irresistible for solvent called lyophilic group. The structure having lyophilic as well as lyophobic group in a same compound is known as amphiphilic, diphillic or amphipathic structure. Such nature of surfactants may be regareded as “split personality”. It is exactly this nature of surfactant molecules that underlines their tendency to gather at interfaces. If water is being used as a solvent then lyophilic part may be regarded as hydrophilic and lyophobic part as hydrophobic one. The polar or hydrophilic part is referred to as the head group and non-polar or hydrophobic as the tail. The polar or hydrophilic part may have either positive or negative charge, giving rise to cationic or anionic surfactants respectively, or may be composed of polyoxyethylene chain, as in the most of the nonionic surfactants. The nonionic or the hydrophobic portion of the molecule is usually a flexible hydrocarbon chain although many compounds especially those of biological interest, may have aromatic hydrophobic group. An example of amphiphilic compound having dual nature
The existence of hydrophilic as well as hydrophobic moieties in the same molecule confers it dual character responsible for the phenomenon of surface activity, micellization and solublization. As a class these substances, which include soaps and detergents, can be called association colloids, a name indicating their tendency to associate in solution, forming particle of colloidal dimensions. Owing to their tendency to become adsorbed at interface, they are often called surface-active agents or surfactants [1-3].

When the surfactants are added into water, their hydrophobic groups being water-hating increase the free energy of system due to distortion of water structure. Now less work is required to bring surfactant molecules, than water molecule, to the surface. The presence of hydrophilic group, however, does not let the molecule being completely expelled from the water as a separate phase, since that would require desolvation of the hydrophilic groups. The amphiphilic or amphipathic molecules, therefore, when present at low concentration, get accumulated at surface and cause reduction in free energy [1].

On the basis of charge surfactants are classified as;

(1) Ionic surfactants         (2) Non ionic surfactants

1.3. Ionic surfactants

These are the ones having either positive, negative or both charges on the surface active part of molecule. They have further three sub classes depending upon charge

(i) Anionic surfactants      (ii) Cationic surfactants     (iii) Zwitterionic surfactants:

1.3.1. Anionic surfactants:

In anionic surfactant the surface-active specie has negative charge, e.g.

Sodium dodecyl sulphate (SDS)  \( \text{CH}_3 (\text{CH}_2)_{11} \text{SO}_4^- \text{Na}^+ \)

Potassium laurate               \( \text{CH}_3 (\text{CH}_2)_{10} \text{COO}^- \text{K}^+ \)
1.3.2. Cationic Surfactants

In cationic surfactants the surface-active portion contains positive charge, e.g.

Cetyl trimethyl ammonium bromide $\text{CH}_3 (\text{CH}_2)_{15} \text{N}^+ \text{CH}_3) \text{Br}^-$

Dodecylamine hydrochloride $\text{CH}_3 (\text{CH}_2)_{11} \text{N}^+\text{H}_3 \text{Cl}^-$

1.3.3. Zwitterionic Surfactants

In such surfactants surface-active portion may bear both positive and negative charge, e.g.

Long chain amino acids $\text{R}^+\text{NH}_2 \text{CH} \text{CH}_2 \text{COO}^-$

1.4. Nonionic Surfactants

Such surfactants carry no charge on surface-active portion, e.g.

Polyoxyethylene monohexadecyl ether $\text{CH}_3 (\text{CH}_2)_{15} (\text{OCH}_2\text{CH}_2)_{21} \text{OH}$

On the basis of their sources surfactants may be classified as

(i) Natural Surfactants  (ii) Artificial Surfactants

1.5. Natural Surfactants

These are naturally occurring compounds that exhibit surfactant like properties e.g. Proteins, lipids, cholesterol, bile acid, glycolipid, pulmonary surfactant (lung surfactant), Phosphatides (lecithin, dialkylglycerylphosphorylcholine):

\[
\begin{align*}
\text{CH}_2\text{OCOR}_1 \\
\text{CH}_2\text{OPO} (\text{OH}) \text{OCH}_2\text{CH}_2 \text{N}^+ (\text{CH}_3)_3 \text{OH}^-
\end{align*}
\]

where R1 and R2 represent fatty acid residue.
The lecithins are believed to be effective for shipping of water insoluble compounds in vivo. Cholic acid and deoxycholic acids are the most important naturally occurring bile acids, which also behaves as association colloids.

1.6. Artificial Surfactants

These are synthetic or man made surfactants e.g. Cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulphate (SDS).

A special class of surfactants comprise of drugs (ionic or non ionic)

1.7. Drugs

A large number of drugs also behave like surfactants including phenothiazine derivatives, e.g. chlorpromazine, diphenylmethane derivative e.g. diphenylhydramine and tricyclic antidepressants e.g. amitriptyline [2].

Figure 1.1. Structure of a typical amphiphilic drug (Trifluperazine 2HCl).

1.8. Properties of surfactants/amphiphiles

Surfactants distort water structure and raise free energy of solution. The system, however, has natural tendency to minimize its free energy. To satisfy this natural desire the system may undergo.

- Adsorption
- Micellization

1.8.1. Adsorption of Surfactants

At low surfactant concentration free energy of solution may be lessened by expelling their hydrophobic parts out of water in such a way that their hydrophilic parts remain attached to the solution surface. The adsorption has been studied to determine;
• The concentration of surfactant at interface (The performance of surfactant in many interfacial processes i.e. foaming, detergency, emulsification etc depends on interfacial concentration of surfactants)

• The orientation of surfactants at interface

• The free energy change in system, $\Delta G$, enthalpy change, $\Delta H$ and entropy change, $\Delta S$, during adsorption

One of the most commonly measured properties of surfactant related to their adsorption at air/solution interface is reduction in surface tension.

1.8.1.1. Surface Tension

It is the minimum amount of work required to expand the interface by unit area. It is the measure of interfacial free energy per unit area of boundary between liquid and air above it [1].

1.8.1.2. Reduction of Surface tension

Reduction in surface tension depends directly on the replacement of solvent molecules at interface by those of surfactant. It is one of the most fundamental interfacial phenomena. The surface molecules of liquid have greater magnitude of potential energy than those in the interior and the work required to bring molecules at surface is equal to difference in potential energy of surface and bulk molecules and is the measure of surface free energy per unit area, or surface tension [1, 2].
1.8.2. Micellization

Interfacial adsorption is a way to diminish free energy of solution but there is a limit of concentration to which adsorption may occur. After having reached this limit, no more adsorption is possible and energetically unfavourable contact between hydrophobic part and water may be further avoided by self-aggregation of surfactant molecules within bulk of solution. These aggregates are called micelles, the simplest form of association colloid, and process is called micellization [2, 4, 5].

Micelle formation takes place under influence of noncovalent forces e.g. hydrogen bonding, ionic bond, hydrophobic and van der Waals interaction [6]. Importance of micellization is not only because large number of interfacial processes e.g. detergency, solubilization etc takes place due to micelles but it also affects those ones that have no direct relation with micelles for example reduction of surface or interfacial tension.
Model 1.2. Dynamic equilibrium between surfactant molecules adsorbed at surface and in the interior.

1.8.2.1. The critical micelle concentration (CMC)

The concentration at which micelle formation just starts is called critical micelle concentration (CMC) [1]. It is a narrow range of concentration, rather than having a sharp value, within which solution properties of amphiphiles undergo sudden change in their magnitudes. Both CMC and properties of aggregates are governed by several factors including affinity and size of counterion, size of head group, the length of hydrocarbon chain, ionic strength, and pH [4, 5, 7].

Micellization is a complex process so it is very difficult to pinpoint the concentration where it actually does start. A struggle to find an easy way available to identify the exact value of CMC is, therefore, still in progress. Fortunately, a number of physical properties such as surface tension, electrical conductivity, osmotic pressure, light scattering, refractive index, detergency, solublization, and charge density show
sharp change in magnitude at CMC and they, therefore, can be used to detect CMC.

Model 1.3. Formation of micelle [8]

Figure 1.2: Plots of physical properties versus concentration of amphiphile for detection of CMC.
1.8.2.2. How and why micellization occur?

Surfactant undergoes micellization in aqueous solution because the reduction of hydrocarbon-water interface is energetically favored [7]. At the start of micellization, initially two amphiphilic molecules join their hydrophobic parts under influence of hydrophobic forces to form a dimer, on next stages trimers and tetramers are formed. Subsequently more and more surfactant molecules join them to build a larger micelle.

![Model 1.4](image)

**Model 1.4.** Various steps involved in the micelle formation

The inception of micellization is the result of two competing factors. Transfer of hydrocarbon chains from water to oil like interior of micelle drives micellization while repulsion between head groups of ionic surfactants as they come close together opposes it. The balance between factors favoring and opposing micellization will decide the possibility and extent to which micellization may occur [1, 5].

1.8.2.3. Micellar structure and shape

Since micellization is a dynamic process it will be unrealistic to regard micelles as rigid structures. It is, therefore, edifying to consider average micellar shape [1, 2]. In aqueous solution of surfactant having concentration not far above the CMC, in absence of
additives, the shape of micelle is roughly considered to be spherical having hydrophobic parts in interior region surrounded by hydrated hydrophilic groups and bound water.

The radius of spherical micelle is equal to length of fully lengthened hydrophobic chains, which are more expanded in micelles than in normal state. In ionic micelles, hydrophilic groups carry some charge and somewhat more than one half of counter ions are associated with them in form of electrical double layer. In hydrocarbon medium structure of micelle is similar but reversed.

The size, shape, and aggregation number of micelle depend on temperature, concentration, additives and structure of surfactant monomers. The structure of micelle may thus vary from spherical, rod or disk like to lamellar. The micelle become non spherical at concentration ten time higher than CMC. Surfactant molecules, at least in some cases, form extended parallel bimolecular sheets with individual molecules oriented at right angle to plane of sheet. In concentrated solution, micelle may acquire cylindrical shape, the lyophilic groups forming surface of cylinder and the lyophobic ones comprise its interior [1, 2].

1.8.2.4. Micellar aggregation number

The number of surfactant molecules in a micelle is called aggregation number. It is decided by the dissimilarity between surfactant and solvent. It, thus, increases with increase in hydrophobic character of surfactants. The addition of neutral electrolyte to ionic surfactant solution causes aggregation number to increase, which is probably due to compression of electrical double layer surrounding the ionic heads and decreasing repulsion between them. In this way more surfactant molecules are permitted to be present in micelle [1, 2]. Micellar solutions are assumed to be monodisperse (all micelles having same size and same aggregation numbers) so that unnecessary complications may be avoided [10].

1.8.3. Factors affecting CMC

The factors on which CMC markedly depends are

- The structure of surfactant
- Presence of electrolyte
- Presence of organic additives
- Temperature
Model 1.5. Schematic structure of amphiphiles and of their self-assembled supramolecular aggregates [9].

1.8.3.1. Structure of surfactants

Generally CMC decreases as hydrophobic character of surfactant increases.

1.8.3.1.1. Effect of hydrophobic group

The increase in number of carbon atoms in unbranched hydrocarbon chain leads to decrease in CMC. However, for the chain greater than 16, this rule no longer holds possibly due to coiling of chains in solutions [2]. The value of CMC usually becomes one half on addition of each methylene group to straight hydrophobic chain attached to single terminal hydrophilic group. However, in nonionic surfactants each methylene group reduces CMC to one-tenth of its original value. A phenyl group, being part of hydrophobic group, is considered equivalent to about three and half-methylene group. If hydrocarbon chain is branched, the carbon atom on which branching occurs appears to exhibit one half of effect than that on straight chain. The presence of sp2 hybridized carbon (in case of C=C bond) causes CMC to increase. The value of CMC is higher for cis isomer than for the trans one. Significant increase in CMC is observed when polar group is added to hydrophobic chain. The carbon atoms between non-polar group and hydrophilic group have one half the effects on CMC than in absence of polar group. The CMC will decrease if hydrocarbon based hydrophobic group is replaced by fluorocarbon based hydrophobic one. However, replacement of terminal methyl group by the trifluoromethyl group enhances the CMC [1, 2].

1.8.3.1.2. Effect of hydrophilic group

The surfactants having same hydrophobic but different hydrophilic groups have pronounced difference between their CMC values [2]. The aqueous solution of nonionic surfactants has much lower CMC than that of ionics with same number of carbon atoms. The CMC of zwitterionics and ionics is almost same provided they have same number of carbon atoms. The CMC will increase if hydrophilic group is shifted from terminal to more critical position. In this case hydrophobic group act as if it had become branched at the position of hydrophilic group, with carbon atoms at shorter end of chain having half
of their usual effect on CMC. The CMC is higher if charge on hydrophilic group is closer to α-carbon of hydrophobic group or when more than one hydrophilic groups are attached to hydrophobic group [1, 2].

1.8.3.2. Effect of counterion binding

The increase in degree of counterion binding, in aqueous solution causes CMC to decrease. The extent of binding of counterion increases with increase in polarizability and valence while it decreases with increase in hydrated radius [1]. In conventional ionic surfactant a change in counterion to one with greater polarizability or valence leads to a decrease in CMC and aggregation number. The size of counterion is also deciding factor, an increase in CMC is observed with increase in hydrated radius [2].

1.8.3.3. Effect of electrolyte on CMC

The CMC decreases in presence of electrolytes. The effect on various types of surfactants in increasing order is given as under

Anionics, cationics > zwitterionics > nonionics

The effect of concentration of electrolyte on CMC, for anionics and cationics, is given by the equation.

$$\log \text{CCMC} = -a \log C_i + b$$  \hspace{1cm} (1.1)

where $a$ and $b$ are constants for a given ionic head at a particular temperature and $C_i$ is total monovalent counterion concentration in moles per litre. The decrease in CMC in these cases is due to decrease in thickness of ionic atmosphere around ionic head groups and consequent decrease in repulsion between them.

For nonionics and zwitterionics above relation doesn’t hold. Instead following equation is better to be used,

$$\log \text{CCMC} = -K C_s + \text{constant}$$  \hspace{1cm} (1.2)

where $K$ is constant for particular surfactant, electrolyte and temperature and $C_s$ is concentration of electrolyte in moles per litre. In case of nonionics and zwitterionics the change in CMC due to the addition of electrolyte is not because of its effect on
hydrophilic group but it is attributed to “salting in” or “salting out” effect of hydrophobic groups in aqueous solution. The water-ion interactions, in presence of electrolyte, change the magnitude of work required to create volume in water to accommodate a nonpolar solute. Consequently, activity coefficient of solute is changed. If required work is increased due to presence of electrolyte then activity coefficient of solute is increased and solute is salted out; if required work is decreased the solute is salted in. The CMC decreases in cases of “salting out” effect and increases in case of “salting in” effect. Total effect of electrolyte is the approximate sum of its effects on the various parts of solute molecules in contact with aqueous phase. Since the hydrophilic parts, in both monomers and micelles, are in contact with aqueous phase, thus the effect of electrolyte on it, in both phases, is cancelled out. It is, therefore, the hydrophobic portion that is most likely to be affected by the presence of electrolytes.

The effect of cations and anions is additive and depends on the hydrated radius of ions, that is, lyotropic number; the magnitude of effect directly depends on radius of hydrated ion [1].

1.8.3.4. Effect of organic additives

The marked change in CMC is observed in aqueous solution of surfactant due to presence of small amount of organic additives. On the basis of effects produced by these materials on CMC, they are classified as

(i) Class I Materials   (ii) Class II Materials

1.8.3.4.1. Class I Materials

They are generally polar organic compounds such as alcohols and amides. Their effect on CMC is visible at much lower concentration than Class II Materials. Shorter chain members of this class are adsorbed at the outer portion of micelle at micelle-water interface, while the longer ones are probably adsorbed in outer portion of core. In case of ionic surfactants, adsorption in this fashion reduces repulsion between ionic heads and decreases the work required for micellization and consequently reducing the CMC.

Straight chain additives depress CMC to greater extent than branched ones. The length of hydrophobic group directly affects the depression in CMC till its length
approximates that of surfactant. It is because the molecules most effective in reducing the CMC are solubilized in the outer region of micelle core and are under lateral pressure, which tends to push them into micellar core. This pressure increases with cross sectional area of molecules. The straight chain molecules have smaller cross sectional area than the branched ones and are facing less lateral pressure. They, therefore, have greater tendency to reside in outer portion of core and consequently reduces CMC to greater extent. Another factor may be that interaction between hydrophobic part of straight chain additives and surfactants is greater in case of branched ones and surfactant molecules. The molecules having greater affinity for water due to hydrogen bonding will produce greater depression in CMC than those having less tendency of hydrogen bonding because the greater attraction of the former with water will counter balance the lateral pressure and will enable them to remain in outer region of micellar core. Very short chain polar compounds (dioxane and ethanol), at low concentration, are adsorbed at the surface of micelle and cause very small decrease in CMC [1].

1.8.3.4.2. Class II Materials

These materials change CMC at much higher concentration than the members of class I. They change the CMC by modifying interaction of water with surfactant molecules or their micelles by bringing a change in the structure of water, its dielectric constant or its solubility parameters (cohesive energy density). They include urea, formamide, N-methylacetamide, guanidinium salts, short chain alcohols, dioxane, ethylene glycol and other polyhydric alcohols such as fructose and xylose.

Urea, formamide and guanidinium salts tend to increase the CMC of surfactants in aqueous solution because of their disruption of water structure. This may enhance the degree of hydration of hydrophilic heads, which opposes micellization, and cause the CMC to increase. They, and other water structure breaker, also increase the CMC by decreasing entropy effect accompanying the micellization. The structure promoters for water such as xylose and fructose decrease the CMC due to the similar reasons.

Dioxane, ethylene glycol and short chain alcohols at high bulk concentration increase solubility of surfactants in water and thus increase the CMC. We can also offer an alternative explanation for action of these compounds that they reduce dielectric
constant of water therefore mutual repulsion of ionic heads in micelle increases and thus opposing micellization and increasing the CMC [1].

1.8.3.5. Effect of temperature

The effect of temperature on CMC is complex. The CMC first decreases with temperature to a minimum value and then increases. According to M. J. Rosen [1] the increase in temperature causes decreased hydration of hydrophilic heads, which favors micellization. Increase in temperature also causes disruption of structured water around hydrophobic ends, an effect that disfavors micellization. However according to Farida Akhtar et.al.[11], for ionic surfactants, increase in temperature increases, the degree of hydrophobic dehydration which favors micellization and the degree of hydrophilic hydration decreases which disfavors micellization. Partial dehydration of polar heads cause gradual increase in repulsion between them thus making micellization difficult and increasing the CMC, disruption in structured water around hydrophobic groups also increases with temperature. The relative magnitudes of two opposing factors will decide whether CMC will increase or decrease in particular temperature range. For ionics the minimum in CMC appears around 298K while for non ionic at 323K. For bivalent metal alkyl sulphate CMC is independent of temperature [1].

1.8.4. Solublization by solution of surfactants

Organized assemblies of surfactants have immense application in daily life. The micelles produced by surfactants enhance solubility of insoluble or sparingly soluble organic compounds. This increase in solubility is because hydrophobic parts of solute are incorporated into inner part of micelle [12].

This is what is known as solublization, one of the important properties of surfactant related to micellization. It is defined as the spontaneous dissolving of a substance by reversible interaction with micelle to form thermodynamically stable isotropic solution. Although this process is useful for both soluble and insoluble materials. From practical point of view this phenomenon is applied to dissolve materials that are normally insoluble, although it is equally useful to enhance solubility of less soluble ones [1].
1.8.4.1. Why does solublization occur?

The micelles are composed of a hydrophilic surface and a hydrophobic core in water media. This specific structure enables it to establish chemical interaction with either hydrophilic or lipophilic molecules. These aggregates exhibit an interfacial region separating the polar bulk aqueous phase from hydrocarbon like interior [12].

1.8.4.2. Applications of solublization

Major practical importance of solublization in aqueous media addresses following areas

- In the synthesis of product containing water insoluble ingredients, here it can replace the use of organic solvents or co solvents.
- In detergency, to remove oily dirt.
- In micellar catalysis.
- In emulsion polymerization being an important factor in initiation step.
- Dry cleaning involves solublization into nonaqueous media.
- In drug delivery.
- In enhanced oil recovery.
- To decontaminate polluted water [13].

The plot between solubility of insoluble material versus surfactant concentration exhibits that solubility is very slight before CMC while it rises abruptly after CMC. This indicates that solublization is a micellar phenomenon.
1.8.4.3. Locus of solublization

The nature of material solublized and type of interaction between surfactant and solublizate decides exact location of solublization. Solublization is believed to take place at number of different sites in micelle as made clear in model 1.6.

1. On surface of micelle
2. Between hydrophilic head groups (in case of polyoxyethylenated surfactant)
3. In outer core of micellar interior (between hydrophilic head groups and first few carbon atoms in the hydrophobic portion)
4. In the micellar core.

Model 1.6. Various possible loci of solublization [8]
Saturated aliphatic and alicyclic hydrocarbons, in aqueous solution, are solublized, in inner core of micelle. The hydrocarbons, to be easily polarized for example short chain arenes initially absorb at micellar surface of cationic surfactant. On further addition, however, they get incorporated either deep into palisade layer or be accommodated in the core of micelle. The polarizability of π-electron cloud of aromatic ring count for their initial adsorption at surface of micelle. The polar molecules of large size such as alcohols having long chains or polar dye stuff are solublized, in aqueous medium, in palisade layer with their polar group oriented toward micellar surface and nonpolar portion toward its interior. Interaction between polar groups of solublizate and surfactant is presumably hydrogen bonding or dipole-dipole interaction. Less polar and long chain compounds penetrate deep into palisade layer than more polar and short chain ones. In water solution polar molecules of small size, are adsorbed at the surface or solublized below but very near to the surface inside palisade layer. The locus of solublization remain unchanged when shape of micelle changes due to increase in concentration [1].

1.8.4.4. Factors affecting extent of solublization

1.8.4.4.1. Structure of the surfactant

The interior part of micelle and the deep palisade layer is the possible accommodation for the long chain polar compounds and hydrocarbons. The larger the size of micelle, the greater the amount of material solublized. Therefore, any factor that increases diameter or aggregation number of surfactant enhances degree of solublization. Since dissimilarity between surfactant and solvent increases aggregation number. Therefore, an increase in length of hydrocarbon chain causes increased solublization of hydrocarbon interior of micelle [1]. Bivalent metal alkyl sulphates have greater aggregation number and thus higher solublizing power than corresponding sodium salt. Branched chain surfactant has less solublizing power than those having straight chain because of their shorter effective chain length. Similarly unsaturated soaps have less solublizing power for hydrocarbons than saturated soaps. Nonionic surfactants are better solublizing agents than ionics, in dilute solution, because of having low CMC. Generally the tendency of polar and non polar molecules to be solublized in micellar core decreases
in following order.

Nonionics > cationics > anionics

The greater solublizing power of cationic as compared to that of anionics is because of loose packing of nonionic surfactant molecules in micelle[1].

1.8.4.4.2. Structure of the solublizate

Crystalline solids get solublized to lesser extent than do liquid surfactant molecules of similar structure, perhaps due to latent heat of fusion. The extent of solublization of aliphatic and alkyl aryl hydrocarbons appear to decrease if chain length, unsaturation or cyclization increases provided only one ring is formed. Extent of solublization decrease with increasing molecular size of condensed aromatic hydrocarbons. Solubility remains almost same whether solublizate has straight chain or branching [1]. The depth of penetration in palisade layer varies with changing structure of polar solublizate. For a, more or less, spherical micelle, less space is available for solublization as micelle is deeply penetrated. Thus the polar compounds that are adsorbed at surface of micelle are solublized to greater extent than nonpolar ones, which are more deeply penetrated.

1.8.4.4.3. Effect of electrolyte

While adding the small amount of neutral electrolyte to aqueous solution of ionic surfactants the extent of solublization of nonpolar solublizate to be solublized in inner portion of core usually increases and that of polar ones to be solublized in outer portion of palisade layer decreases. The addition of electrolyte decreases repulsion between ionic heads thereby decreasing CMC and increasing aggregation number and volume of micelle. The increase in solublization of nonpolar compounds is due to increase in volume of micelle and the decrease in that of polar ones is due to close packing of palisade layer owing to decrease in repulsion between polar heads and consequent decrease in space available for their solublization[1].

1.8.4.4.4. Effect of monomeric organic additives

The presence of hydrocarbon in micelle, after being solublized, helps the polar compounds to be more solublized. The swelling of micelle due to solublized
hydrocarbons makes more space available for polar compounds in palisade layer. The solublization of polar compounds conversely, increases solublization of hydrocarbons. The polar compounds having longer chain but lesser hydrogen bonding with water are more capable to increase solublization of hydrocarbons. The reason for above-mentioned phenomenon is that the compounds having long chain and low polarity are able to penetrate deeply in micelle and thus they expand the micelle and enable more compounds to be accommodated in it [1].

1.8.4.4.5. Effect of polymeric organic additives

The compounds that exist as macromolecule, such as synthetic polymers and natural polymers i.e. starch, proteins, cellulose derivatives, interact with surfactants. In this way a complex is formed between surfactants and macromolecules in which surfactant molecules are adsorbed onto the macromolecules mainly due to electrostatic and hydrophobic interactions. Some times, polymer- surfactant complex, at high surfactant concentration, has greater tendency to solublize others than pure surfactant and solublization takes place even below CMC. Thus, if macromolecule of proper structure is added to surfactant solution its solublization power is enhanced [1].

1.8.4.4.6. Effect of temperature

The temperature increases the extent of solublization of both polar and nonpolar solublizate into solution of ionic surfactants. It is, possibly, due to thermal agitations that make possible the inclusion of large amount of solublizate into micelle. The percentage increase in amount of solublize at high temperature is inversely proportional to that at lower temperature.

For nonionic polyoxyethylenated surfactants, effect of temperature depends on the nature of solublizate. The solublization of non polar solublizate to be solublized in inner core of micelle increases with temperature. This rapid increase in solublization power near cloud point is due to rapid increase in aggregation number. However, in case of polar material, to be incorporated in palisade layer, the extent of solublization first increases with temperature due to thermal agitation and then decreases because of dehydration and tighter coiling of polyoxyethylene chains, decreasing the available space for solublizate. Near the cloud point amount of solublized material, particularly for short
chain compounds that are solublized near micellar surface, decreases markedly [1].

1.8.5. Interaction of drugs with surfactants

A drug, in wider sense, is any substance that alters normal body function [14]. The heterogeneous media (micelles, lipid bilayer vesicles, and biomembranes) when interacts with drugs induces changes in some of their physicochemical properties (solubility, spectroscopic and acid-base properties). These changes are monitored to quantify the degree of drug/micelle interaction, usually written as drug/micelle binding constant, $K_b$ and micelle water partition coefficient, $K_x$. The elucidation of these parameters help to understand the interactions of drugs with biomembranes, quantitative structure-activity relationship of drugs, micellar HPLC or micellar electrokinetic capillary chromatography (MEKC) used in drug delivery control [2]. The amphiphilic drugs can show surface activity effects on structural and dynamical properties of membranes through interaction [4].

Many drugs, particularly those with local anesthetic, tranquilizer, antidepressant and antibiotic actions, put forth their action by interaction with biological membranes. This membrane affinity is due to the hydrophilic–hydrophobic interactions in a molecule and associated with the surface activity of drugs at air-solution interface [15]. Formation of colloidal sized huddles, also called micelles, is one of the important properties of surfactants having particular significance in pharmacy as they have ability to enhance solubility of sparingly soluble substances. Because of anisotropic distribution of water within structure of micelles, spatial position of solublized drug molecules in micelle depends on their polarity: non polar molecules being solublized in micellar core and those with intermediate polarity will be distributed in certain intermediate positions [8].

1.8.6. Micelles as a simple model of Biomembranes

Surface activity is of ubiquitous presence in living system. Surface tension of any body fluid or cell soup is always less than that of water because the most of biomolecules, proteins, lipids etc. are surface active in nature. Surface active nature of these molecules is very important for living matter and its organization. Formation of biological cell membranes as well as location of receptor proteins in the lipid bilayer are the result of surface activity. It is, therefore, logical to expect that the drugs acting by
altering the permeability of cell membranes after interacting with them may also be surface active in nature. A number of circumstantial evidences indicate that there may exist some similarities in mechanism of action of all surface active drugs. Such drugs are likely to adsorb at interfaces and form a layer at the site of action and modify the access of relevant molecules to the action site [16].

A layer of material serving as a selective barrier between two phases and remains impermeable to specific substances under the action of a driving force is called membrane. Some components are allowed to pass through it whereas others are retained [17]. A biomembrane is an enclosing amphiphilic layer that performs function as a barrier within or around a cell. The presence of such membranes gives rise to enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment different from the outside. Having selectively-permeable structure is probably the most important feature of a biomembrane. The size, charge and other chemical properties of various species decide their ability to cross such membranes. The effective separation of a cell or organelle from its surroundings largely depends on selective permeability of its membranes. [18].

The understanding of cellular function largely depends on the study of cell membrane and the role it plays in living cells. Biological membranes are built of lipids in association with proteins and glycoproteins. According to present accepted model bio membrane has lipid bilayer structure. Many biological processes take place at surface of membranes or within its hydrophobic moiety. The surface of biological membrane have, frequently, a net charge owing to ionic head groups of lipid and the same is responsible for binding of charged and uncharged drug molecules with it. It is, therefore, necessary to pay attention toward relationship between binding properties of drugs and its location in membrane.

Surfactants have variety of applications while carrying out membrane studies. Surfactants, being amphiphilic molecules, like lipids, follow same rules, to some extent, governing lipid behaviour. Micelles, being relatively simple, provide an interesting alternative to study interactions of membranes with a number of compounds [8].

1.9. Protein
Proteins are condensation, linear biopolymers of amino acids joined by peptide linkage between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acid molecules in polypeptide chain is due to the sequence of a gene, which is encoded in the genetic code [19]. Proteins can exhibit joint action to execute a particular function and they, in this way, have ability to form stable complexes [20].

The ability of proteins to bind other molecules specifically and tightly is the chief characteristic responsible for their diverse set of functions. The region of the protein where binding takes place is known as the binding site and is in the form of a depression or "pocket" on the molecular surface. This binding ability of protein is due to its tertiary structure and the chemical properties of the surrounding amino acid’s side chains. Binding with protein can be extraordinarily tight and specific; for example, the ribonuclease inhibitor protein binds to human angiogenin with a dissociation constant (<10-15 M) but does not bind at all to its amphibian homolog onconase (>1 M). The binding may be prevented even by extremely minor chemical changes in binding partner such as the addition of a single methyl group; for example, the aminoacyl tRNA synthetase specific to the amino acid valine discriminates against the very similar side chain of the amino acid isoleucine [21].

1.9.1. Serum albumins

Serum albumins, because of being easily available, less expensive, stable and having unusual ligand binding properties that is being applied and studied on the large scale. Owing to these reasons, a large number of research papers have been published so far. It is, without question, the protein with which blood is rich to maximum extent and it acts for storage and transportation of many endogenous and exogenous compounds. The colloid osmotic pressure of the blood is mostly controlled by albumin and it is main possible source of amino acids for various tissues [22].

Albumin is a chemical of extraordinary importance having wide range of functions and applications. Its most marvelous property is to bind reversibly a copious range of ligands. Its physiological significance is due to transportation of ligands and relative ease of isolation and purification that has resulted into large number of binding studies. Most ligands undergo reversible binding and typical association constant (K_a)
range from $10^4$ to $10^6$ M$^{-1}$. As large variety of ligands bind to albumin, researchers, in past, underwent a misapprehension that albumin-ligand binding was as non-specific process and they fail to recognize the discrete sites on protein surface and, instead, envisioned that ligands are attached to surface of protein in random manner. This point of view has been rejected over the past years, and now theory of distinct binding sites has got strength [22].

Human Serum Albumin (HSA) is a globular protein having single polypeptide chain of 583 amino acids. Its molar mass of 66500 g mol$^{-1}$. It is used as a model protein to study interaction with various ligands. X-ray crystallography shows its structure to be asymmetric heart-shaped molecule with sides of 8.0 nm and thickness of 3.0 nm. It can be, more or less, taken as an equilateral triangle. Two hydrophobic binding sites are present at the two heart shaped lobes while polar groups are present at outside. Its secondary structure has high content of $\alpha$-helices (67%). It has high value of diffusion coefficient and is the first protein that acts as a carrier of fatty acids and several amphiphiles from blood stream to tissues, after being adsorbed on the foreign. It is, therefore, a very good choice to study of interaction of proteins with amphiphilic molecules [23].

1.9.2. Interaction of drugs with proteins

Drug protein interactions are important because the large number of drugs travel through the blood because of being reversibly bound to serum albumin forming a drug-protein complex. The biological activity of drug is strongly influenced by the nature and magnitude of drug protein interaction. The pharmacological response and dosage drug depend on the binding parameters [24].

Proteins being multifaceted macromolecules can exist in large number of conformations, slightly different from each other. Functional parameters of protein undergoes big change due to small structural differences between these substrates. The native conformation is slightly stable due to delicate balance of various interactions in proteins (van der Waals, electrostatic, hydrogen bonds, hydrophobic and disulphide bridges). This balance is affected by pH, temperature or addition of small molecules such as substrate, coenzymes, inhibitors and activators that bind to native state and disturbing
this equilibrium. The drugs bind to protein (as HSA) at various sites with different affinities and is carried to target. Adverse effects are faced by body if drug gets accumulated at certain places and drug medical therapy can be complicated due to ligand induced protein structure conformational changes. Therefore, it is very interesting to study the effect of various parameters such as such as pH, temperature, salinity, etc. on conformational changes in protein and the analysis of binding mechanism between protein and amphiphilic drug as well as structure of the drug-protein complex formed. Such research helps us to elaborate regulation of ligand affinity and the alteration of the protein conformation upon complexations being very important in many biochemical phenomena [25].

The concentration of free drug in plasma decreases due to its strong binding with protein, whereas low circulation time or poor distribution is result of weak binding. The fluctuation in different conformation of native proteins governs the pharmacokinetic function of HSA in adsorption, distribution metabolism and excretion of drugs and other ligands. It is believed that N-B transitions control the transport function of HSA. In addition, binding of ligand to protein also induces conformational alterations in its native structure, altering this fragile equilibrium. Therefore, the parameters that are of particular interest to explain the regulation of ligand affinity and alteration of protein conformations are the analysis of binding capacity and structure of complexes between HSA and amphiphilic drugs because these factors are of key importance in a variety of biochemical phenomenon [26].

Protein folding has resulted into the variety of structures that are highly specific. It brings key functional groups close to each other. The development of diversity and selectivity, by living systems, in their underlying chemical processes, including the flow of molecule to specific cellular locations and the regulation of cellular growth and differentiation is also outcome of protein folding. Thus, only those proteins have long term stability in crowded biological environment which are correctly folded and the same are able to interact selectively with their natural partners.

In fact the malfunction of protein to fold correctly or its ability to remain correctly folded is the origin of a wide diversity of pathological conditions. A perfect familiarity
of mechanism underlying protein folding –unfolding pathways is, therefore, obligatory in order to elaborate adequate strategies to prevent and/or treat these pathologies [27].
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Chapter – 2

THEORETICAL BACKGROUND

The following experimental techniques have been applied;

❖ Surface Tension
❖ Electrical conductivity
❖ UV/Visible Spectroscopy
❖ Florescence Spectroscopy
❖ Laser light Scattering(LLS)

2.1. Surface Tension

Surface tension of a liquid stands for a contractile force, which tends to shrivel the surface and operates about outskirts of the surface.

Model 2.1. Adsorption of surfactant at the interface

It is property of the interface between two phases. Surface tension is a force that operates at surface and acts perpendicular and inward from boundaries of surface tending to decrease the area of interface. Let us consider the case of water; the molecules in the
interior have ability to form up to four hydrogen bonds. At the surface of water, however, the molecule has fewer neighbors, and water tends to minimize broken bonds by minimizing surface area [1].

Model 2.2. Surface tension in liquids

The measurement of surface tension enables us to surmise, indirectly, the amount of material adsorbed per unit area of interface. The adsorption at these interfaces is described by the plot of surface or interfacial tension as a function of surfactant concentration. Such plot helps to calculate the amount of surfactant adsorbed per unit area of interface by employing Gibbs adsorption equation

\[ d\gamma = -\sum \Gamma_i d\mu_i \]  

(2.1)

Where \(d\gamma\) is the change in surface or interfacial tension, \(\Gamma_i\) is the surface excess concentration of any component of the system and \(d\mu_i\) is the change in chemical potential of any component of the system

The surface excess concentration is excess amount of any component, per unit area of interface, actually present in the system than that in the reference system of same volume. At equilibrium between interface and bulk phase concentration,
\[ d\mu_i = RT d\ln a_i \] where \( a_i \) is activity of any component in the bulk, \( R \) is gas constant and \( T \) is absolute temperature. Thus

\[ d\gamma = -RT \Sigma_i d\ln a_i \] (2.2)

\[ d\gamma = -RT \Sigma_i d\ln x_i f_i \] (2.3)

\[ d\gamma = -RT \Sigma_i d(\ln x_i + \ln f_i) \] (2.4)

Where \( x_i \) is mole fraction of any component in bulk phase and \( f_i \) is activity coefficient.

For solution consisting of solvent and one solute only,

\[ d\gamma = -RT (\Gamma_1 d\ln a_1 + \Gamma_2 d\ln a_2) \] (2.5)

Where subscript 1 and 2 refers to the solvent and solute respectively. For dilute solutions (10-2M or less) having only one non dissociating surface active solute, the activity of solvent and activity coefficient of solute may be considered as constant and mole fraction of solute \( x_2 \) may be replaced by its molar concentration \( C_2 \). Thus

\[ d\gamma = -RT \Gamma_2 d\ln C_2 \] (2.6)

\[ d\gamma = -2.303RT \Gamma_2 d\log C_2 \] (2.7)

\[ \Gamma_2 = -\frac{1}{2.303RT} \left( \frac{d\gamma}{d\log C_2} \right) \] (2.8)

Taking concentration in term of molality

\[ \Gamma_2 = -\frac{1}{2.303RT} \left( \frac{d\gamma}{d\log m} \right) \] (2.9)

The factor \( \frac{d\gamma}{d\log m} \) is obtained from slope of surface tension-molality plot in pre micellar region [1].
From the surface tension measurement, the following parameters have been calculated.

(i) Surface excess concentration ($\Gamma$)
(ii) Area per molecule at interface ($A$)
(iii) Free energy of adsorption ($\Delta G_{oad}$)
(iv) Free energy of micellization ($\Delta G_{om}$)

2.1.1. Surface excess concentration

For surface-active solute, the surface excess concentration can be considered to be actual surface concentration without significant error. For ionic surfactant $\Gamma_2$, can be determined by the application of Gibbs Adsorption Equation [2].

\[
\Gamma_2 = -\frac{1}{2.303RT_x} \left( \frac{d\gamma}{d\log m} \right)_T
\]  

(2.10)

Where $R$ is the gas constant, $T$ the temperature in Kelvin. The variable $x$ is introduced to allow for the simultaneous adsorption of cations and anions.

\[
x = 1 + \left[ \frac{m}{m + m_s} \right]
\]

(2.11)

The expressions used in calculation of $x$ were proposed by Mateejavic and Patheca [2], where $m$ and $m_s$ are concentrations of drug and added electrolyte, if any. The value of $x$ is 2 in water and approaches to 1 in the presence of excess inert electrolyte.

2.1.2. Area per molecule at interface

The area per molecule at interface ($A$) gives information about the degree of packing and orientation of adsorbed surfactant. Where $A = 1/NA\Gamma_2$

A typical $\gamma$-log $C$ plot for dilute solution of surfactants is shown below,
Figure 2.1. Typical plot of surface tension as a function of logarithmic concentration

The break in the curve occurs at critical micelle concentration (CMC), the concentration at which surfactant molecules start forming aggregates known as micelles. Above CMC surface tension remains almost constant because only monomeric form of surfactant is responsible for reduction in surface tension. At concentration below but near CMC, the slope of surface tension curve is constant because surface excess concentration has acquired constant maximum value. In this range interface is considered to be saturated with surfactant and further decrease in surface tension is due to increased activity of surfactant in bulk phase rather than at interface. In case of ionic surfactant having constant concentration of counter ion, this region of saturated adsorption may extend down to one third of the CMC [3].

2.1.3. Free energy of Micellization

The free energy of micellization ($\Delta G_{\text{mic}}$) can be calculated by using the equation;

$$\Delta G_{\text{mic}} = (1 + \alpha)RT \ln X_{\text{CMC}}$$

(2.12)
Where $\alpha$ is the counter ion binding, $R$ is the gas constant having value 8.314 J mol$^{-1}$K$^{-1}$, $T$ is the absolute temperature, $X_{\text{CMC}}$ is CMC in term of mole fraction.

### 2.1.4. Free energy of adsorption

The standard free energy of adsorption, $\Delta G_{\text{ads}}$ for pure surfactant solutions as well as mixed system can be calculated by using equation below.

$$
\Delta G_{\text{ads}}^o = \Delta G_m^o - \frac{\pi_{\text{CMC}}}{\Gamma_m}
$$

Where $\pi_{\text{CMC}}$ is the surface pressure at critical concentration and is given by

$$
\pi_{\text{CMC}} = \gamma_o - \gamma_{\text{cmc}}
$$

Here $\gamma_o$ is surface tension of pure solvent and $\gamma_{\text{CMC}}$ is that of surfactant solution at CMC.

### 2.2. Electrical conductivity:

The conductivity (or specific conductance) of an electrolyte solution is a measure of its ability to conduct electricity.

The reciprocal of electric resistance is called electric conductance and that of specific resistance is called specific conductivity ($\kappa$).

$$
\kappa = \frac{1}{\rho} = \frac{1}{R} \times \frac{l}{A}
$$

Where $l/A$ is called cell constant.

Specific conductance = observed conductance $\times$ cell constant

The SI unit of conductivity is Siemens per meter (S/m) [4]. The experimental determination of electrical conductivities of amphiphilic solution helps to calculate its critical micellar concentration (CMC). At each temperature concentration dependence of the electrical conductivity shows a gradual increase of slope [5]. The following parameters have been calculated from electrical conductivity measurement.

### 2.2.1. Critical micelle concentration (CMC)
The measurement of specific conductivities provides more scientific technique to detect CMC. The dependence of electrical conductivities on concentration shows a gradual increase of slope at each temperature. According to William et al. [6] the CMC can be determined by intersection of two straight lines of concentration-conductivity plot, in premicellel and post micellel region. The exactitude of measurement depends on the concentration range over which the change in physical properties are observed [7].

2.2.2. Degree of Ionization ($\beta$)

The degree of ionization ($\beta$) can be calculated from the ratios of the slopes of post-micellar and pre-micellar regions of conductivity-concentration plot using following equation [8].

$$\beta = \frac{S_2}{S_1}$$  \hspace{1cm} (2.16)

Where $S_1$ and $S_2$ represent the slopes of the straight lines in the pre micellar and post micellar region respectively.

2.2.3. Degree of counter ion binding ($\alpha$)

The degree of ionization ($\beta$) is related to the degree of counter- ion binding ($\alpha$) as $\alpha = 1-\beta$ [8].

2.2.4. Enthalpy of micellization

The following equation can be used to calculate enthalpy of micellization [1].

$$\Delta H_m^o = -2.3(1 + \alpha)RT^2 \left[ \frac{\partial (\log X_{cmc})}{\partial T} \right]_p$$  \hspace{1cm} (2.17)

2.2.5. Entropy of micellization

We have calculated entropy of micellization by the following equation [1].

$$T\Delta S_m = \Delta H_m - \Delta G_m$$  \hspace{1cm} (2.18)

2.3. Ultraviolet visible (UV- Vis) spectroscopy
When organic compounds absorb electromagnetic radiations in either ultraviolet (10-400 nm) or visible region (400-800 nm), they undergo same type of molecular excitation and follow same basic principles. Absorption in both regions is, thus, discussed together as UV/Visible spectroscopy. It is also termed as electronic spectroscopy because it involves transition among the electronic energy levels of molecule. The wavelengths of UV/Visible radiations are usually expressed in nanometers (1nm = 10^{-9}m). The UV region below 200 nm cannot be studied by the conventional UV-visible spectrophotometer because oxygen (in air) absorbs strongly in this region, however if oxygen is expelled by flushing the instrument with nitrogen, then the range of spectrophotometer can be extended down to 150 nm below which nitrogen also absorbs strongly. The region below 200 nm is thus called vacuum ultra violet region. For routine practical purpose we are mainly interested in ordinary (quartz) UV region extending from 200 nm to 400 nm [9].

2.3.1. Absorption of radiations and electronic transitions

When molecules absorb UV/Visible radiations, they undergo excitation of valency electrons from ground state to higher energy state. In fact, the transition of electron takes place between highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). HOMO may be bonding or nonbonding molecular orbital while LUMO is antibonding molecular orbital. The wavelength of absorbed radiation depends on energy difference between HOMO and LUMO. When a molecule absorbs UV/Visible radiation of a particular wavelength, only one photon is absorbed and it is assumed that only one electron is promoted while all other electrons remain unaffected. Furthermore, during electronic transitions, the atoms of molecule undergo no motion. According to Frank-Condon principle “the electronic transition occurs so rapidly that vibrating atoms do not change their internuclear distance appreciably, during this period”. The electronic transition that are associated with the absorption of UV/Visible radiations are of four types i.e.,

(i) \( \sigma \) to \( \sigma^* \)  
(ii) \( n \) to \( \sigma^* \)  
(iii) \( \pi \) to \( \pi^* \)  
(iv) \( n \) to \( \pi^* \)
The σ to σ* transition occur in saturated hydrocarbons, such as ethane, which contain only strongly bound sigma electrons. Such excitation require a large amount of energy thus it falls in vacuum UV region below 150nm. The C-C single bond generally absorbs at 135 nm while a C-H bond absorbs at 125 nm. Since vacuum UV region is not accessible in most of spectrophotometer, these transitions are generally of no use for routine analysis [9].

The n to σ* transitions occur in saturated hydrocarbons containing heteroatoms such as oxygen, nitrogen, sulphur, and halogens, and involve the excitation of an electron from a nonbonding orbital of the heteroatoms to an antibonding sigma orbital of the molecule. Such transition involve less energy than in case of σ to σ* transitions and consequently results in absorption at higher wavelength end of vacuum UV region and shorter wavelength end of ordinary UV region i.e., between 150 nm to 250 nm of electromagnetic spectrum. Methyl alcohol has n to σ transition band at 183 nm and trimethylamine at 227 nm. Trimethylamine shows no n to σ* transition in acidic media because the protonated amine contains no nonbonding electrons. Many of n to σ* transitions are observable with ordinary UV/Visible spectrophotometers [9].

The π to π* transitions occur in molecules having double or triple bonds or aromatic rings. These transitions generally absorbs at 160-190nm, e.g. Ethylene absorbs at 171 nm. However conjugated system of unsaturated bond absorbs at much longer wavelength, e.g., butadiene absorbs at 217 nm.

The n to π* transition occur in compounds having double or triple bonds involving heteroatoms e.g., >C = O, -C ≡ N, etc. In these transitions an electron in nonbonding atomic orbital associated with the heteroatoms is excited to an antibonding π* orbital associated with double or triple bond. These transitions require less energy than π to π* transition and occur at longer wavelength, usually well within the range of ordinary UV/Visible spectrophotometer, for example, saturated aldehydes and ketones absorb at 275-295 nm. These transitions are, therefore, the most useful for analysis [9].

2.3.2. Positions and Intensities of absorption band
Two main characteristics of an absorption band are:

- Position
- Intensity

The position of absorption band corresponds to the wavelength of radiation whose energy is equal to that required for transition. While intensity of transition depends on probability of transition which, in turn, depends on relative symmetries of orbital in ground and excited states. For example, $\pi$ to $\pi^*$ transition have high intensity because both $\pi$ and $\pi^*$ orbitals have same symmetry while $n$ to $\pi^*$ transition although involve lower energy but has lower intensity too because both $n$ and $\pi^*$ orbital have different symmetry [9].

2.3.3. Chromophore

A group responsible for absorption of electromagnetic radiations, and hence for giving colour to chemical compound is known as chromophore e.g. azo group (-N=N-) is a chromophore in azo dyes. Initially this term was limited to groups that impart visible colours to compounds but now it has been extended to describe “Any functional group that enables a compound to absorb electromagnetic radiations in either UV or visible region”. The chromophores are generally unsaturated groups [9].

2.3.4. Auxochromes

The functional groups that do not act as chromophores themselves but shift absorption of chromophore to higher wavelength are called auxochromes. For example, -OH, -NH2, -SH, Halogens etc.

2.3.5. Bathochromic shift or red shift

A shift of absorption maximum to higher wavelength is called red shift or bathochromatic shift.

2.3.6. Hypsochromic shift or blue shift
A shift of absorption maximum to lower wavelength is called blue shift or hypsochromic shift.

2.3.7. Hyperchromic effect

An increase in intensity of absorption caused by a substituent is called hyperchromic effect.

2.3.8. Hypochromic effect

A decrease in intensity of absorption caused by a substituent is called hypochromic effect.

2.3.9. Absorption and intensity

The intensity of absorption may be measured either as Transmittance (T) or as absorbance (A) [9].

2.3.10. Parameters calculated

The data obtained from UV/Visible spectroscopy helps to calculate following parameters

- Drug/micelle Partition coefficient
- Free energy of partition
- Drug/surfactant binding constant
- Free energy of binding
- Number of drug molecules incorporated per micelle

2.3.10.1. Drug/micelle Partition coefficient

Partition coefficient gives us idea to which extent drug is partitioned from aqueous to micellar phase. It is calculated from Kawamura model [10].
\[
\frac{1}{\Delta A} = \frac{1}{K_a \Delta A_x \left(C_a + C_s^{mc}ight)} + \frac{1}{\Delta A_x}
\]  
(2.19)

Where \(C_a\) is concentration of additive (drug) in mol/dm³, \(C_{smo}\) represents \(C_s-CMCo\), in the same units. Here, CMCo is CMC of SDS in water and \(C_s\) is total surfactant concentration in mol/dm³. \(\Delta A\) is differential absorbance and \(\Delta A_\infty\) represents its value at infinity. \(K_c\) is partition constant having value in dm³/mol. The dimensionless partition coefficient \(K_x\) is obtained as \(K_x = Kcnw\), where \(nw\) is number of moles of water per dm³.

2.3.10.2. Free energy of partition

Free energy of partition is measure of ease with which partition occurs. The more negative is its value, the more spontaneous is partition of drug from bulk water to micelle. It is calculated as [11]

\[
\Delta G_p = -RT \ln K_x
\]

(2.20)

Where \(R\) is general gas constant and \(T\) is absolute temperature.

2.3.10.3. Drug/surfactant binding constant

An ion-pair complex is formed between surfactant and drug having opposite charge. Following equation provides quantitative approach to calculate binding constant [12].

\[
\frac{C_s C_a}{\Delta A} = \frac{C_s}{\Delta \varepsilon} + \frac{1}{K_b \Delta \varepsilon l}
\]

(2.21)

Here \(C_a\) is concentration of additive (drug), \(C_s\) is that of surfactant, \(\Delta A\) is difference of absorbance between complex formed and drug. \(\Delta \varepsilon\) is difference of absorption coefficient; \(l\) is path length while \(K_b\) stands for binding constant.

2.3.10.4. Number of drug molecules per micelle (n)
The approximate number of drug molecules incorporated per micelle is given as

\[ n = \frac{C_m}{M} \]  \hspace{1cm} (2.22)

Where \( C_m \) is concentration of solubilized drug and \( M \) is micelle concentration. They are calculated from equation (2.23) and (2.24).

\[ M = \frac{C_s - CMC}{N} \]  \hspace{1cm} (2.23)

\[ C_m = \frac{A_o - A}{\varepsilon_o - \varepsilon_m} \]  \hspace{1cm} (2.24)

Where \( C_s \) is total surfactant concentration, \( N \) is mean aggregation number, \( A_o \) is absorbance of drug in absence of surfactant, \( A \) is absorbance at concentration after which no change in absorbance takes place, \( \varepsilon_o \) and \( \varepsilon_m \) are calculated from Beer-Lambert law.

### 2.4. Fluorescence spectroscopy

Fluorescence spectroscopy (also called Luminescence spectroscopy, Fluorometry or spectrofluorometry) is one of the most extensively used spectroscopic techniques used in chemistry, biochemistry as well as biophysics. Although it does provide detailed structural information, it is popular because of being acute sensitive to changes in structural and dynamic properties of biomolecules and biomolecular complexes [13].

#### 2.4.1. Basic rules

The rules given under provide base for fluorescence.

1. The Franck-Condon principle: the excitations take place to excited vibrational level of excited electronic state while nucleus remains stationary.

2. Emission takes place from lowest vibrational level of the lowest excited
singlet state because relaxation from excited vibrational level is much faster than emission.

3. The Stokes shift: emission is always of lower energy than absorption due to nuclear relaxation in the excited state.

4. The mirror image rule: the emission spectra are mirror image of the lowest energy absorption band [13].

2.4.2. Jablonski Diagram

Jablonski diagram illustrates the processes occurring between absorption and emission of light named after Alexander Jablonski [14]. A typical Jablonski diagram is shown in figure 2.3.

The singlet ground, first and second excited electronic states are depicted by So, S1 and S2 respectively. The fluorophore, at each of electronic level, can exist in a number of vibrational energy levels, denoted by the horizontal lines. This diagram excludes a number of interactions, such as quenching and solvent interactions.

2.4.3. Internal Conversion

A fluorophore is usually excited from ground electronic state to some higher vibrational level of either S1, or S2 (excited electronic states). The molecules in condensed phases rapidly relax to the lowest vibrational level of S1, with few rare exceptions. This process is called internal conversion with life time of 10-12 second or less. Since life time of fluorescence is typically near 10-8 seconds, internal conversion is generally prior to emission. Hence, fluorescence emission generally occurs from the lowest–energy vibrational level of excited electronic state to higher vibrational level of ground electronic state. The emission spectrum is, thus, typically a mirror image of the absorption spectrum of the So→S1 transition because electronic excitation does not greatly alter the nuclear geometry. Hence, the spacing of the vibrational energy levels of the excited state is similar to that of the ground state [14].
Figure 2.2. Sketch of excitation followed by Fluorescence
If the energy levels of the singlet state overlaps those of the triplet state then vibrational coupling may take place between the two states giving rise to a phenomenon called intersystem crossing in which molecules in the singlet excited state can switch over to the triplet excited state. Such cross over generally being forbidden has high life time (one nano second) [14].

2.4.5. Fluorescence Quenching

Any process that decreases the fluorescence intensity of a sample is called fluorescence quenching. Quenching processes are of wide variety and include excited state reactions, molecular rearrangements, ground state complex formation, and energy transfer. Quenching occurs either via static or dynamic (collisional) mechanism. Both types require an interaction between the fluorophore and quencher. In the case of dynamic quenching, the quencher must diffuse to the fluorophore during the lifetime of the excited state causing fluorophore to return to the ground state without emission of a
photon. Static quenching involves formation of non fluorescent complex between the fluorophore and quencher.

### 2.4.6. Dynamic or Collisional Quenching

Collisional quenching occurs when excited state fluorophore is deactivated by colliding with some other molecule (quencher) in solution. Consequently fluorophore returns to ground state due to diffusive encounter with the quencher, however, no chemical alteration occurs. For collisional quenching decrease in intensity is described by well known Stern Volmer equation [15]:

\[
\frac{F_0}{F} = 1 + K_{sv} [Q] = 1 + K_q \tau_0 [Q]
\]

(2.25)

Where \(F_0\) and \(F\) are the observed fluorescence intensity in the absence and presence of quencher, \(K_{sv}\) is the Stern-Volmer quenching constant, \(K_q\) is the bimolecular quenching constant, \(\tau_0\) is the lifetime of protein in the absence of quencher, and \([Q]\) is the quencher concentration.

A wide variety of molecules act as collisional quenchers e.g. oxygen, halogens, amines, and electro deficient molecules like acrylamide [15].

### 2.4.7. Static Quenching

Besides dynamic mechanism, fluorescence quenching may also take place by a variety of other processes. Fluorophore may form non fluorescent complex with quencher. Such processes may be referred as static quenching since it occurs in the ground state and does not depend on diffusion or molecular collision. It follows modified Stern Volmer equation.

\[
\log \left( \frac{F_0 - F}{F} \right) = \log K_b + n \log [Q]
\]

(2.26)
It is a straight line equation giving value of binding constant from intercept and that of number of binding sites from slope [15].

2.4.8. Important Parameters calculated from Fluorescence Emission Spectroscopy

Fluorescence emission spectroscopy is helpful to calculate a number of very important parameters. We have applied this technique to study drug-protein system and have calculated a number of parameters such as binding constant (Kb), free energy of binding ($\Delta G_b$), number of binding sites (n) and association constant (Ksv).

2.4.8.1. Quenching Constant

The value of quenching constant is calculated from Stern Volmer equation (2.25).

2.5.8.2. Binding Constant or Association Constant (Kb)

The values of binding constant (Kb) is calculated from intercept of modified Stern Volmer equation (2.26).

2.4.8.3. Number of binding sites

The number of binding sites (n) are calculated from slope of modified Stern Volmer equation (2.26).

2.4.8.4. Free Energy of Binding ($\Delta G_b$)

The binding energy is calculated by the following equation

$$\Delta G_b = -RT \ln Kb$$

Where Kb is the binding constant, R is the gas constant and T is the absolute temperature.

In a typical fluorescence experiment, the different frequencies of fluorescent light emitted are measured, while wavelength of excitation light is kept constant.

2.5. Laser Light Scattering
When light strikes an object, its direction as well as intensity suffers from change, this phenomenon is called light scattering. This change is due to the cumulative effects of reflection, refraction and diffraction (in the absence of absorption).

Light scattering, being an excellent method for the study of colloidal suspensions, has been in use for at least three decades [16]. Maxwell, Raleigh, Lorenz, Einstein and Debye have made remarkable contribution in this field to develop the theoretical basis of the subject. Nowadays the conventional light sources have been replaced by lasers. The use of intense, coherent laser light, efficient spectrum analyzers and autocorrelators, have brought a revolution in this field. This technique is, no doubt, one the most extensively used methods for determinations of molar mass, molecular size of the macromolecules and to study the molecular motions e.g. diffusion and flow, and other dynamic processes as well as equilibrium properties of solutions. The fine filtration is of utmost importance and filtration technology for clarifying samples has also been significantly improved [17]. It also helps to study the quantitative characterization of solute-solvent interactions and aggregation phenomenon in solutions of amphiphiles [18].

On being directed at a solution, some of the light may be absorbed, some is scattered and the rest of it is transmitted uninterrupted through the sample. Scattering of light by independent particles is divided into three classes:

i) **Rayleigh Scattering:** In this case scattering particles are small enough to act as point sources of scattered light.

ii) **Debye Scattering:** In this case particles are relatively large but difference between their refractive index and that of the dispersion medium is small.

iii) **Mie Scattering:** In this case particles are relatively large and have a refractive index significantly different from that of dispersion medium [19].

### 2.5.1. Dynamic laser light scattering

Dynamic light scattering is also known as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering. In this technique variation in the light intensity due to the Brownian movements of the particles helps to extract information. Fast photon counter is used to measure time dependent fluctuations in the scattered light signals. Since
diffusion coefficient determines particle motion, the quantity actually measured by DLS is the translational diffusion coefficient, D which can be converted to hydrodynamic radius through Stoke-Einstein equation. At present the dynamic light scattering experiment is a routine laboratory technique for measuring diffusion coefficients, particle size and particle size distributions in colloidal suspension. In a typical scattering experiment, a detector measures the intensity of the scattered radiation over a period of time t. In contrast to static light scattering (SLS) experiments, in DLS the photons arriving at the detector are correlated instead of being accumulated and averaged. As Brownian motion of particles results in fluctuation of light intensity. These fluctuations give information about particles motions.

2.5.2. Mechanism of Dynamic Light Scattering

When light strikes small particles, it becomes scattered in all directions (Raleigh scattering) provided the particles are small compared to the wavelength of light (below 250 nm). If laser, being monochromatic and coherent, is used as light source then a time-dependent fluctuation in the scattering intensity is observed. These fluctuations are due to Brownian motion due to which the distance between the particles in the solution is constantly changing with time. The light from neighbouring particles undergoes either constructive or destructive interference and within this intensity fluctuation, information is contained about the time scale of movement of the scatterers. Quasi-elastic laser light scattering is one of the methods to derive information in this technique.

2.5.3 Data Analysis Method from DLS

The data from DLS can be analyzed by several methods. The cumulants method, introduced by Koppel, is the simplest one and gives useful information on the average value of decay constant and its variance and is useful for narrow size distribution [20]. For the wide distribution of particle size, however, the distribution of decay time is also wide and the above simple method remains no more useful. Provencher developed a computational program known as CONTIN used for the smoothest and most optimal solutions of entire distributions. We have analyzed the system by the constrained
regularized CONTIN method, thus gaining information of the distribution of decay rates \( \Gamma \) [21].

### 2.5.4. Intensity Correlation Function

In DLS intensity-intensity time correlation function, \( G^{(2)}(t, q) \) in self beating mode was measured. It has following form [22, 23]

\[
G^{(2)}(t) = A \left[ 1 + \beta |g^{(1)}(t)|^2 \right] \tag{2.28}
\]

Where \( q \) is scattering vector, \( \beta \) is a parameter depending on coherence of detection. \( T \) is delay time, \( g^{(1)}(t) \) is a normalized first order electric field time correlation function and \( A \) is the measured base line. For a polydisperse sample \( g^{(1)}(t) \) is related to line width distribution \( G(\Gamma) \) by

\[
c(\tau) = g^{(1)}(t) = \int_0^{\infty} G(\Gamma) e^{-\Gamma \tau} d\Gamma \tag{2.29}
\]

Where \( \Gamma \) is delay time rate. The relaxation time, \( \tau \), is inversely proportional to delay time rate.

A Laplace inversion of \( g^{(1)}(t) \) can lead to \( G(\Gamma) \). For diffusive relaxation, \( \Gamma \) can be written as [24]

\[
\frac{\Gamma}{q^2} = D \tag{2.30}
\]

Where \( D \) is the translational diffusion coefficient and \( q \) is the scattering angle.

Stokes law [17] gives relationship between diffusion coefficient and hydrodynamic radius (\( R_h \)),

\[
R_h = \frac{K_b T}{6\pi \eta D} \tag{2.31}
\]
REFERENCES


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18 Kratchvil, P. Classical Light Scattering from Polymer Solutions; Ed.; Jenkins, A; D., Elsevier 1987.


3.1 Material used

Following materials were used in this study,

- A number of antibiotic, antidepressant and antiallergic drugs with amphiphilic properties were selected for this study. The antibiotic drugs selected were Clindamycin Phosphate (CLN), Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ), Dexamethasone Sodium Phosphate (DSP) and Pefloxacin Mesylate (PFL) while the antidepressant ones are Citalopram HBr (CIT), Fluphenazine (FLP) and Trifluperazine HCl (TRF). The anti allergic drug studied is Certizine 2HCl (CRT). These drugs were purchased from Sigma Aldrich and were of analytical grade and were used as such.

- Anionic surfactant Sodium dodecyl Sulphate (SDS), cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB) and Human Serum Albumin (HSA) were also obtained from Sigma Aldrich and used as received.

- Water was distilled using Water Still Apparatus Model IM-100 and then deionized by passing though Elga B114 deionizer.

- Buffer solutions of pH 7.4 and 3.0 were used. Structural formulas of drugs used are given on the next page.

3.2 Preparation of solution

For measurement of surface tension and conductivity aqueous solution of each drug was prepared, in term of molality, in deionized water ranging from pre micellar to post micelle concentration. However, for spectroscopic study the stock solution of each additive (drug) was prepared in distilled deionized water and diluted further so that maximum absorbance may not exceed 1 and Lambert-Beer Law may be obeyed. The
drug solution was used as solvent to prepare surfactant solutions of varying concentration, keeping concentration of drug constant.

Quinacrine 2HCl (QUN)  
Chloroquine diphosphate (CLQ)  
Citalopram HBr (CIT)  
Pefloxacin Mesylate (PFL)  
Clindamycin phosphate (CLN)  
Fluphenazine (FLP), X=CH2-CH2-OH  
Trifluperazine (TRF), X=CH3
Figure 3.1. Chemical structures of different amphiphilic drugs.
3.3 Surface Tension

Surface Tension of aqueous solutions of drugs was determined using Torsion balance (White Elect.Inst. Co.Ltd) equipped with Platinum ring (4.0 cm circumference) along with water circulator (Irmeco I-1800) to control temperature at 30°C (303K). A homemade glass cell with a special hollow space as well as an inlet and outlet for water circulation was used to ensure the constant temperature. The sample was taken in the cell around which water at constant temperature was circulated in the hollow portion of the cell in order to achieve the desired temperature. The torsion balance was placed on a fixed smooth surface to minimize chances of disturbance. Two leveling screws in the tripod base were used to adjust the position of the bubble in the spirit level. Platinum ring was attached to the extension hook. The instrument was kept free from vibrations. The torsion balance was checked for zero and calibrated with water.

The solution was taken in the measuring cell and placed on the platform below the ring after having calibrated the instrument. The platinum ring was dipped in sample surface. The index pointer was gradually moved along to maintain zero at vernier, s scale. After having reached the corresponding value of surface tension the ring got detached from the liquid surface and outer main scale gives value of surface tension (\( \gamma \)) in N/m. The experiment was repeated thrice to ensure accuracy.

Molal solutions were prepared and carefully diluted to avoid any chances of error. It is well known that critical concentration derived by surface tension is particularly sensitive to impurities. No minima were evidenced in this region of critical concentration which was proof of absence of surface active impurities [1].

3.4 Specific conductivities

Besides a number of techniques available, measurement of electrical conductivity provides an efficient and sensitive way to find CMC of amphiphilic compounds and study of their thermodynamic properties. It even helps to detect 2nd or 3rd critical concentration, if any. Electrical conductivities of drug aqueous solutions are carried out to study the physicochemical properties of amphiphilic drugs (antibiotic, antiallergic and antidepressant) to have knowledge about changes in aggregation process of these drugs in aqueous solution. Specific conductivities were measured with Jenway 4310. This
The instrument has auto ranging from 0.01 μS to 199.9 mS, conductivity control with accuracy of ±0.5 % ± 2 digits and temperature control accuracy of 0.5°C. The electrode used has cell constant of 0.98 cm⁻¹ and was coated with platinum black in order to avoid the polarization effect. The conductivities were measured at temperature range of 293-323K with increment of 10K. The temperature was controlled using water circulator (IRMÉCO I-2400 GmbH Germany). The electrode was calibrated using KCl over the appropriate concentration range.

The critical micelle concentration (CMC) of drugs and surfactant was determined from plot of specific conductivity versus concentration. The effect of additives (drugs) on CMC of surfactant, SDS, during solublizization was also studied. For this purpose the stock solution of drug was prepared and a portion of it was used to prepare stock solution of surfactant ranging from pre-micellar to post micellar region. The concentration of additives was kept constant during each experimental run.

3.5 Ultra violet visible Spectroscopy

The UV-Visible spectroscopic data was used to study interaction of drugs with anionic surfactant Sodium dodecyl Sulphate (SDS), cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB) and protein i.e. Human Serum Albumin (HSA). All absorption spectra of the sample in UV-Visible range were measured on a computer interfaced Perkin Elmer Double beam lambda 20 UV-Visible spectrophotometer equipped with a water jacketed cell compartment to control the temperature. The instrument has two light paths, one for sample and the other for blank or reference. The detector alternatively perceives the reference and the sample beam and the outputs of the detector is proportional to the ratios of intensities of two beams It/Jo. The cells used were square cuvettes of quartz, 1.0cm thick and slit width used is 1.0nm. Different spectra of sample are automatically noted by computer under given conditions. This technique may also be used to detect CMC [2] but its applications are limited because presence of additives may affect CMC of surfactants [3, 4]

3.5.1 Measurement of simple absorption spectra

Aqueous solution of drugs were prepared and divided into two portions. A part of this solution is used as reference and the other one is used to prepare surfactant solution
ranging from pre to post micellar region. Initially deionized water was kept in both light paths to run auto zero (to make base line correction). Then spectra of drug solution were obtained keeping drug solution at sample side. Finally a series of surfactant solutions having concentration range from pre micellar to micellar region was run to find effect of surfactant on UV/Visible spectra of drug. All spectra of a particular drug were taken as close together as possible using same stock solution.

3.5.2 Measurement of differential absorption spectra

In double beam spectrophotometer, a difference spectrum between sample and reference is obtained. Differential UV/Visible spectroscopy is useful tool to demonstrate, qualitatively, whether interaction between two components occurs or not. We have employed this technique to study surfactant solublization. First we prepared aqueous solution of each drug and divided it into two portions. A part of this solution is used as reference and the other one is used to prepare surfactant solution. The base line correction was made by keeping drug solution at reference as well as sample side. Then a series of surfactant solutions was run to get differential UV/Visible spectra. For this purpose surfactant solution in presence of constant concentration of drug was kept at sample side while solution of pure drug was kept at reference side.

3.6 Fluorescence Spectroscopy

Fluorescence intensity of HSA in the absence and presence of drug was recorded at 298 K at blood (pH 7.4) using Perkin Elmer Luminescence spectrophotometer (model LS55, made in Germany). The cell used was clear in all dimensions and of path length 1.0 cm. The instrument was equipped with external water circulator to avoid temperature fluctuations. The concentration of HSA was kept constant at 2×10-6M (2.0μM) while that of drug was changed regularly. The pH was maintained using phosphate buffer. Fluorescence quenching spectra was recorded from 300 to 450nm at an excitation wavelength of 278nm. Emission slit, exit slit, scan speed, start wavelength and end wavelength was adjusted according to requirement of each drug.

3.7 Dynamic Light Scattering Measurements
Dynamic light scattering experiment was carried out by a commercial LLS spectrometer BI-200SM motor-driven goniometer equipped with BI-9000AT digital autocorrelator or the BI-9025AT photon counter and a cylindrical 22mW uniphase He–Ne laser ($\lambda = 637$ nm) and BI-ISTW software was used.

The instrument is very sensitive to dust particle so as to avoid discrepancy, all the glassware were washed with acetone before use and dried carefully in oven. Solutions analyzed contain fixed amount of protein (HSA) and varying the amount of drug. The solutions were filtered into quartz LLS cell (10 mm in diameters) to remove dust by using 0.22 μm millipore filter. The experiment duration was 3.0 min. Scattering intensities were measured at 298K for various concentrations. Other solutions were prepared by diluting the stock solutions for each drug-protein system.
Figure: 3.2: Laser light scattering spectrophotometer.
# REFERENCES


In this chapter micellar behavior of some amphiphilic drugs and their interactions with ionic surfactants and Human Serum Albumin (HSA) has been discussed in detail. We have selected some antibiotic, antidepressant and antiallergic drugs with amphiphilic properties for this study. The antibiotic drugs are Clindamycine Phosphate (CLN), Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ), Dexamethasone Sodium Phosphate (DSP) and Pefloxacin Mesylate (PFL) while the antidepressants are Citalopram HBr (CIT), Fluphenazine (FLP) and Trifluperazine HCl (TRF). The only antiallergic studied is Certizine 2HCl (CRT).

4.1. Surface Behaviour of Drugs

Differences between energies of molecules located at surface and in the bulk phase manifest themselves as surface tension. This concept, historically, has been a key factor in understanding molecular behaviour of liquids [1] and we have applied it to study the surface properties of different drugs in aqueous solution. As amphiphiles (surfactants, block copolymers or amphiphilic drugs) are added in water, their molecules get accumulated at surface, thus, decreasing surface tension of water. As we go on increasing concentration of amphiphile in solution surface tension goes on decreasing. But after a certain value of concentration, known as critical micelle concentration (CMC), surface tension becomes almost constant. After CMC, in most of cases, no more surface adsorption takes place so no change in surface tension is observed. At this point amphiphilic molecules start forming micelles. However sometimes slow adsorption, at surface continues even after having reached at CMC, so surface tension keeps on decreasing slowly after CMC. This phenomenon indicates that adsorption and micellization may take place at the same time but rate of micellization is much faster than that of adsorption. It is generally accepted that values of CMC vary to a certain extent according to what physical properties are considered for its determination.

4.1.1. Surface activity in aqueous solution
Two processes have important influence on surface activity of amphiphiles in aqueous solution. One concerns the effect of solute on water structure and the other concerns degree of freedom for movement of hydrocarbon groups. According to modern theories of water structure, water is composed of both structured as well as unstructured region. There is tetrahedral arrangement of hydrogen bounded water molecules in structured region similar to that in ice while unstructured region consists of free unbound molecules. This particular model is known as “flickering cluster model of water structure” which describes continuous destruction and reconstruction of ordered region. When amphiphiles are added in water, initially there is disruption of the hydrogen bonds between water molecules so that surfactant molecules may be accommodated in highly structured network. No hydrogen bonding is possible between hydrocarbon groups of amphiphiles and water molecules to compensate this bond disruption. Water molecules in immediate neighborhood of hydrocarbon groups are restructured into even more ordered arrangement than they were in pure water. This phenomenon is called hydrophobic hydration and is responsible for entropy decrease making dissolution of amphiphiles an unfavorable process. The hydrophobic parts of amphiphiles are brought into water by virtue of their attachment with hydrophilic groups. The hydrophilic groups have strong tendency to form hydrogen bonding with water molecules, which compensate, energetically, for initial disruption processes.

The amphiphiles in solution is, thus, bordered by a cage of highly structured water molecules. Consequently internal torsional vibration of hydrophobic portion is restricted in solution. Several authors have suggested that decrease in entropy is due to this process, rather than hydrophobic hydration. The removal of hydrophobic part from the aqueous environment leads to disruption of highly ordered water structure and relaxation of hydrophobic part from restriction on mobility and is, therefore, an entropically favourable process. Amphiphilic molecules, due to this reason, accumulate at air/water interface in such a way that their hydrophilic parts remain attached with water surface and hydrophobic parts are expelled out of water bulk. Hydrophilic head groups, being in contact with water, are responsible to anchor the molecule at the surface. Surfactant molecules, thus, replace some of water molecules. The attractive force between water
and amphiphilic molecules is weaker than that between water molecules which causes reduction in surface tension [2].

Beyond the CMC surface tension undergoes little or no change because monomeric form of amphiphile no more exists. The slope of surface tension-concentration curve is almost constant at concentration below but near CMC because surface excess concentration has acquired constant value [3]. The surface behaviour of various drugs studied at 303K is explained in detail one by one.

4.1.2. Clindamycin Phosphate (CLN)

A plot of surface Tension, $\gamma$, verses molal concentration ($m$) for CLN in water at 303K (Figure 4.1) shows that surface tension remains constant below inflection in plot signifying the formation of full Gibbs monolayer at air/solution interface. The inflection in surface tension curve at 0.045 molkg-1 is in reasonable agreement with that detected by conductivity at 303K (0.0377 molkg-1). It seems, in first glance, that both values are different but in actual practice it is accepted, by and large, that values of CMC vary to a certain extent according to what physical properties are considered to find the CMC. The slope of plot of $\gamma$ against molal concentration below CMC concentration was used to compute an approximate value of minimum area per molecule in full surface monolayer, $A$, from the surface excess concentration, $\Gamma$. A value of minimum area of 2.24nm2/molecule was calculated in this manner. Area per molecule at interface gives information about degree of packing and orientation of adsorbed amphiphilic molecule, when compared with the dimension of molecules obtained by molecular models. Table 4.1 shows the various parameters calculated from surface tension.
Figure 4.1: Typical plot of Surface tension as a function of molality for aqueous solution of CLN at 303K.

Table 4.1. Different parameters calculated from Surface Tension measurement of CLN at T=303K.

<table>
<thead>
<tr>
<th>Surface Excess Concentration (Γ) ×107 mol/m2</th>
<th>Minimum area per molecule (A) nm²</th>
<th>Free Energy of Adsorption (ΔGads) kJ/mol</th>
<th>Free Energy of Micellization (ΔGm) kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.43</td>
<td>2.24</td>
<td>-54.21</td>
<td>-22.52</td>
</tr>
</tbody>
</table>

The standard Gibbs free energy of adsorption (ΔGads) at 303K was calculated by equation (2.13) giving a value of -54.2 kJ/mol while value of free energy of micellization (ΔGm) as calculated from equation (2.12) is -22.5 kJ/mol. The value of ΔGads is more negative than that of ΔGm which indicates that process of adsorption is more
spontaneous than micellization. Therefore surface adsorption takes place earlier than micellization [1-10].

4.1.3. Pefloxacin Mesylate (PFL)

A plot showing variation in surface tension of PFL versus molal concentration (m) in water at 303K (Fig.4.2) shows that surface tension remains constant after turning point in plot indicating that full Gibbs monolayer is formed at air/solution interface. The value of CMC obtained from surface tension (0.093mol/kg) and conductivity (0.11mol/kg) are in acceptable agreement with each other. The slight difference between two CMC values is due to the fact that CMC does not represent a sharp value of concentration but a range of concentration and its value depends on which technique is being used to find it. The value of minimum area per molecule at interface gives idea about how molecules are packed and oriented at interface during adsorption. The standard Gibbs free energy of adsorption (ΔGads) at 303K gives value of –38.0 kJ/mol while value of free energy of micellization (ΔGm) is –19.8 kJ/mol. The more negative value of ΔGads than that of ΔGm is indicative of more spontaneous nature of adsorption than that of micellization [1-10].
Figure 4.2: Typical plot Surface tension as a function of molality for aqueous solution of PFL at 303K.

Table 4.2. Different parameters calculated from Surface Tension measurement of PFL at $T=303K$

<table>
<thead>
<tr>
<th>Surface Excess Concentration ((\Gamma) ×106 mol/m2)</th>
<th>Minimum area per molecule (A) nm²</th>
<th>Free Energy of Adsorption ((\Delta G_{ads})) kJ/mol</th>
<th>Free Energy of Micellization ((\Delta G_{m})) kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.40</td>
<td>1.19</td>
<td>-38.20</td>
<td>-19.83</td>
</tr>
</tbody>
</table>
4.1.4. Quinacrine 2HCl (QUN)

The surface tension of various dilutions of QUN was determined and the plot of surface tension versus molal concentration of drug were plotted, from which inference can be made about CMC by intersection of lines showing surface tension in pre and post micellar region. The value of CMC determined from Surface tension is 0.07mol/kg, which is in level headed concurrence with that determined from electrical conductivity at same temperature (0.078mol/kg).

Table 4.3 shows the adsorption parameters at air/solution interface. These values are obtained on the basis of adsorption isotherm using Gibbs equation for ionic amphiphiles. Here again standard Gibbs free energy of adsorption ($\Delta G_{ads}$) has more negative value at 303K than that of free energy of micellization ($\Delta G_m$). The more negative value of $\Delta G_{ads}$ than that of $\Delta G_m$ is showing more spontaneity of adsorption over micellization [1-10].
Figure 4.3: Typical plot of Surface tension as a function of molality for aqueous solution of QUN at 303K.

**Table 4.3. Different parameters calculated from Surface Tension measurement of QUN at T=303K.**

<table>
<thead>
<tr>
<th>Surface Excess Concentration (Γ) ×10⁶ mol/m²</th>
<th>Minimum area per molecule (A) nm²</th>
<th>Free Energy of Adsorption (ΔGads) kJ/mol</th>
<th>Free Energy of Micellization (ΔGm) kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.34</td>
<td>1.24</td>
<td>-28.81</td>
<td>-22.02</td>
</tr>
</tbody>
</table>

4.1.5. Chloroquine di phosphate (CLQ)

Plot of surface tension against molality, m, for CLQ at 303K are shown in Figure 4.4. The CMC was detected at the point of intersection from γ-log m plot before and after micellization.
Figure 4.4: Typical plot of Surface tension as a function of molality for aqueous solution of CLQ at 303K.

There is no minimum observed in this plot which means no impurity is present in solution. The values of surface parameters obtained for this drug are similar to those for other ones. The free energy of adsorption $\Delta G_{ads}$ and that of micellization $\Delta G_{m}$ have negative values, signifying that voyage of drug molecules from bulk to air-water interface before micellization as well as their back journey toward bulk after micellization is spontaneous in nature. However $\Delta G_{ads}$ has more negative value than $\Delta G_{m}$ showing greater degree of spontaneity of adsorption over micellization. The value of CMC at 303K found from surface tension is 0.1mol/kg, which is comparable with that determined electrical conductivity at same temperature (0.128mol/kg) [1-10]. The results calculated from CMC are given in table 4.4.

4.1.6. Comparison between surface behaviour of QUN and CLQ

Both QUN and CLQ are two structurally related amphiphilic drugs so we can compare their surface parameters as exposed in Table 4.4. QUN being more hydrophobic undergo micellization easily and, thus, having lesser value of CMC. The value of free energy of micellization is more negative for QUN that is clear indication for its micellization to be more spontaneous. As QUN consist of larger molecules it, therefore, has greater value for area per molecule at interface than CLQ. The presence of larger molecules causes QUN to have less value of surface excess concentration than CLQ [1-10].

Table 4.4. Comparison of parameters calculated from surface tension measurement of QUN and CLQ at $T=303K$.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>CMC (mol/kg)</th>
<th>Free Energy of Adsorption (kJ/mol)</th>
<th>Free Energy of Micellization (kJ/mol)</th>
<th>Surface Excess concentration (mol/m²)$\times$106</th>
<th>Area per molecule (nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>m</td>
<td>γ (m)</td>
<td>cmc (m)</td>
<td>ΔG_m</td>
<td>ΔG_ads</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----</td>
<td>-----------</td>
<td>---------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Quinacrine 2HCl</td>
<td>0.075</td>
<td>-40.40</td>
<td>-33.67</td>
<td>1.34</td>
<td>1.24</td>
</tr>
<tr>
<td>Chloroquine Diphosphate</td>
<td>0.10</td>
<td>-51.90</td>
<td>-32.70</td>
<td>1.67</td>
<td>0.99</td>
</tr>
</tbody>
</table>

4.1.7. Citalopram HBr (CIT)

A plot of surface tension as a function of m is given in Figure 4.5; it is clear that surface tension becomes constant in post CMC region. The inflection in surface tension curve is observed at 0.0277 mol kg\(^{-1}\) and is taken as CMC. It is in reasonable agreement with that detected by conductivity (0.0283 mol kg\(^{-1}\)). No additional critical concentration was detected: surface tension is related to amount of amphiphile at surface. At CMC surface becomes saturated and surface tension becomes almost constant. Surface tension has nothing to do with structural changes taking place in the bulk.

The slope of $\gamma$-log m below critical concentration was used to calculate the amount of drug adsorbed at air-water interface (surface excess concentration, $\Gamma$) and area per molecule at interface. The distance between drug molecules at surface monolayer is because their aromatic ring link with each other and cause drug molecules to be at reasonable distance. The values of free energy of micellization $\Delta G_m$ and free energy of adsorption $\Delta G_{ads}$ have been calculated using equations 2.12 and 2.13 and found negative because both adsorption and micellization are spontaneous process. However $\Delta G_{ads}$ has more negative value than $\Delta G_m$ because adsorption is more spontaneous than micellization. The value of CMC, $\Delta G_{ads}$ and $\Delta G_m$ are given in Table 4.5 [1-10]. The standard Gibbs free energy of micellization, $\Delta G_m^\circ$, at 303K has a value of -22.4 kJ/mol while the value of standard Gibbs free energy of adsorption at the same temperature was -32.2 kJ/mol.
Figure 4.5: Typical plot of Surface tension as a function of molality for aqueous solution of CIT at 303K.

Table 4.5. Different parameters calculated from Surface Tension measurement for CIT

at $T=303K$.

<table>
<thead>
<tr>
<th>Surface Excess Concentration ($\Gamma \times 10^6$ mol/m²)</th>
<th>Minimum area per molecule (Å²)</th>
<th>Free Energy of Adsorption ($\Delta G_{ads}$) (kJ/mol)</th>
<th>Free Energy of Micellization ($\Delta G_{m}$) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.78</td>
<td>0.93</td>
<td>-32.21</td>
<td>-22.40</td>
</tr>
</tbody>
</table>

4.1.8. Certizine 2HCl (CRT)
A plot of surface Tension, $\gamma$, versus molality for Certizine 2HCl in water at 293K (Figure 4.6) shows that surface tension remains constant above clear inflection in data, indicating the formation of full Gibbs monolayer at air /solution interface. The inflection in surface tension curve at 0.075molkg-1 is in reasonable agreement with that detected by conductivity (0.0712molkg-1). Surface parameters are calculated, at 293 K, by using equations 2.10, 2.12 and 2.13 and tabulated under (Table 4.6).

![Figure 4.6 Typical plot of Surface tension as a function of molality for aqueous solution of CRT at 293K.](image)

**Table 4.6** Different parameters calculated from Surface Tension measurement of CRT at $T=293K$.

<table>
<thead>
<tr>
<th>Surface Excess Concentration $(\Gamma)\times10^6$</th>
<th>Minimum area per molecule (Å)</th>
<th>Free Energy of Adsorption $(\Delta G_{ads})$</th>
<th>Free Energy of Micellization $(\Delta G_m)$</th>
</tr>
</thead>
</table>


4.1.9. Biological consequence of Drug Surface Activity

The drugs we have selected can form aggregates at concentration, which they do not usually manage in vivo even then their surface active behaviour is more important biologically because surface-active drugs may bind hydrophobically to proteins and other biological macromolecules, such as dyes, other drugs, bile salts and with receptors[2].

4.2. Micellar behaviour of Drugs

The amphiphilic molecules have penchant to either adsorb at interfaces or to form self-aggregates within bulk of solution depending upon their solution concentration. The aggregates formed by self-assembly of amphiphiles are called micelles and their aqueous solutions behave as association colloids. Either in surface adsorption or self association, head to head and tail to tail ordering takes place as a result of physical interaction rather than by covalent bonding. The only difference between two is that the former phenomenon takes place at surface while the latter in the bulk.

A large number of drugs behave as amphiphiles and form association colloids in solution. Although pharmacological activities of these drugs are evident at very low concentration even then the study of their colloidal behaviour is attention grabbing. It is because their molecules, if administered in large amount, may get accrued at certain sites of organism and may lead to formation of aggregates. These large sized aggregates are unable to pass through membranes, decreasing transport rates and, thus, cause adverse effects on health. Physico-chemical properties of such drugs are important to be studied from chemical, physical, biological and pharmaceutical point of view [1-3].

4.2.1. Biological relevance of micelle formation by drugs

A large number of drugs, being amphiphilic in nature, associate, usually at very high non-physiological concentration, to form aggregates of various sizes. These drugs are likely to accumulate at certain sites in body and lead to aggregation and several
biologically significant changes occur in system of which they constitute a part: the monomeric concentration of drug either decreases or increases very slowly with increase in total concentration of drug in solution and the transport and colligative properties of system are changed as well as biological activity of drug may suffer from alteration. The change in biological activity produced by micellization may be due to change in transport rate, ability to pass through biological barrier or change in ability of aggregated specie to interact with other biological specie [2]. On the basis of aggregation behaviour drugs have been divided into three categories

- Drugs showing two critical concentrations.
- Drugs showing one critical concentration.
- Drugs showing aggregation other rather than micellization.

4.2.2. Drugs showing two critical concentrations

Drugs showing two inflection points in conductivity-concentration plot are Quinacrine 2HCl (QUN), Chloroquine diphosphate, Fluphenazine (FLP) and Trifluperazine HCl (TRF). The hydrophobic parts of such drugs have fused aromatic rings.

Such drugs are again of two types

- Drugs showing premicellar aggregation e.g. QUN and CLQ.
- Drugs do not showing premicellar aggregation e.g. FLP and TRF.

4.2.3. Specific Conductivity and CMC

The conductivity is one of the best, reliable, sensitive and very simple modus operandi to identify CMC. Specific conductivity is plotted against concentration of amphiphiles and CMC was determined from the point of intersection of premicellar and post micellar region of this plot. The conductivity of amphiphilic solution undergoes abrupt change at CMC. The slope of conductivity-concentration plot decreases after CMC due to formation of less mobile micelle and decrease in concentration of free ions as large number of ions get attached with charged micelle surface forming stern layer while diffused layer of counter ions exist around micelle. The CMC value is low for the
drugs having high hydrophobicity and may increase or decrease with temperature depending upon nature of hydrophilic and hydrophobic groups as explained in case of each drug.

4.2.4. Electrical Conductivity of aqueous solutions of some amphiphilic drugs

4.2.4.1 Quinacrine 2HCl (QUN)

Since micellization is very complex process hence it is very difficult to pinpoint the concentration where it actually does start. That’s why people are still trying to make an easy way accessible to determine this concentration, which is very important in many biological phenomena and of great industrial importance [11].

The experimental determination of specific conductivities provides more scientific technique to detect CMC. Figure 4.7 shows plots of electrical conductivity of aqueous solution of QUN as a function of molality at various temperatures while Figure 4.8 represents typical plot at 293K showing two turning points symbolized by cc1 and cc2. The first critical point cc1 is due to premicellar aggregation while and next one cc2 stands for CMC. The value of cc2 calculated from electrical conductivity and that of CMC obtained from surface tension are very close to each other. At each temperature concentration dependence of electrical conductivity shows a gradual increase of slope.
Figure 4.7. Plots of Electrical Conductivity versus molality for aqueous solution of Quinacrine 2HCl at 

\[293\text{K (■), 303K (●), 313K (▲) and 323K (▼).}\]

Figure 4.8. Typical plot of Electrical Conductivity versus molality for aqueous solution of Quinacrine 2HCl at 293K.

Different thermodynamic and micellar parameters calculated from CMC are given in Table 4.7.

Table 4.7: Thermodynamic and micellar parameters calculated from Electrical conductivity measurements for Quinacrine 2HCl at different temperatures.

<table>
<thead>
<tr>
<th>(T/K)</th>
<th>CC1/(mol/kg)</th>
<th>CC2=CMC (mol/kg)</th>
<th>(\Delta H_m/\text{kJmol}^{-1})</th>
<th>(\Delta G_m/\text{kJmol}^{-1})</th>
<th>(\Delta S_m/\text{JK}^{-1}\text{mol}^{-1})</th>
<th>(\alpha)</th>
<th>(\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>0.028</td>
<td>0.075</td>
<td>-12.50</td>
<td>-32.16</td>
<td>67.15</td>
<td>0.34</td>
<td>0.66</td>
</tr>
<tr>
<td>303</td>
<td>0.030</td>
<td>0.078</td>
<td>-13.36</td>
<td>-33.11</td>
<td>65.13</td>
<td>0.31</td>
<td>0.69</td>
</tr>
</tbody>
</table>
The large negative value of $\Delta G_m^\circ$ shows that micellization is thermodynamically favorable process. The values of $\Delta G_m^\circ$ become more negative with increase in temperature which means that micellization become more spontaneous at high temperature. The values of $\Delta S_m^\circ$ are positive while that of $\Delta H_m^\circ$ are negative at each temperature. These values indicate that the process of micellization is both entropy and enthalpy driven [12]. These values also indicate that in addition to hydrophobic, electrostatic interaction also plays a vital role in aggregation.

The positive value of $\Delta S_m^\circ$ decreases while the negative values of $\Delta H_m^\circ$ increase with increase in temperature. It is because hydrophobic interactions become weaker while electrostatic become stronger as temperature increases. The $\Delta H_m^\circ$ is the sum of change in enthalpies arising from hydrophobic interactions, electrostatic interactions, hydration of polar head groups and counter ion binding to micelles. A negative value of $\Delta H_m^\circ$ occur when hydration of water molecules around hydrophilic heads groups become more important than destruction of water structure around hydrophobic groups of monomers.

The positive values of $\Delta S_m^\circ$ are due to transfer of hydrophobic chains of drugs from aqueous environment to micelle core [12-17]. It is accepted that in the immediate vicinity of hydrophobic groups there is strengthening of hydrogen bonding between water molecules. This hydration of hydrophobic groups is quite different than the usual solvent-solute interaction and is termed as hydrophobic hydration. The water molecules in neighborhood of hydrophobic groups are more attracted by nearby water molecules. This corresponds to tightening of water structure around hydrophobic groups.

A consequence of this situation is that internal torsional vibrations of chains are restricted in solution. The more ordered structure of water molecules around hydrophobic chains and restriction in vibrations of hydrophobic groups leads to decrease in entropy of system. The removal of hydrophobic groups from aqueous environment is
entropically favourable leading to disruption of highly organized water structure and removal of mobility constraints on hydrocarbon chain [2]. The decrease in $\Delta S^o_m$ values with temperature is due to decrease in the degree of hydration of hydrophobic parts at high temperature.

4.2.4.2. Chloroquine diphosphate (CLQ)

The CLQ belongs to family of drugs that exhibit premicellar association. The main driving force behind its micellization is expulsion of hydrocarbon portion (aromatic rings) out of aqueous environment. The counter ions are bound with hydrophilic groups to form stern layer around it. The hydrocarbon core is, thus, surrounded by concentrated electrolytic solution. Water is also present here both as free molecules as well as water of hydration. Besides the stern layer counter ion are also present in solution. These ions are under influence of two types of forces: an electrostatic attraction drawing them toward micelle and thermal jostling, which tends to disperse them. The equilibrium resultant of two competing forces is a diffused ion atmosphere. Drug molecules within micelle are in dynamic equilibrium with unimers in solution and perform to and fro motion within micelle.

![Figure 4.9](image_url)  

**Figure 4.9**. Plots of Electrical Conductivity versus molality for aqueous solution of CLQ at 293K (■), 303K (●), 313K (▲) and 323K (▼).
The surface of micelle is quite chaotic at molecular level due to comings and goings of ions and water molecules. It is, therefore, not wrong to say that regions of double charged layer are not sharply defined on a molecular level [12-17].

The change in electrical conductivity of aqueous solution of CLQ with respect to molality at various temperatures is evidenced in Figure 4.9 and typical conductivity-concentration plot of same drug is given in figure 4.10 which indicated two critical concentrations. The first critical change occurs at cc1 due to premicellar aggregation while cc2 represents CMC. The variation in values of critical concentrations and thermodynamic parameters are given in Table 4.8.

**Table 4.8.** Micellar and thermodynamic parameters calculated from Electrical conductivity measurements for aqueous solution of CLQ at different temperature.

<table>
<thead>
<tr>
<th>$T/K$</th>
<th>CC1/(mol/kg)</th>
<th>CC2=CMC (mol/kg)</th>
<th>$\Delta H_m$ $kJmol^{-1}$</th>
<th>$\Delta G_m$ $kJmol^{-1}$</th>
<th>$\Delta S_m$ $JK^{-1}mol^{-1}$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>0.066</td>
<td>0.10</td>
<td>-12.85</td>
<td>-30.80</td>
<td>62.40</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>303</td>
<td>0.056</td>
<td>0.125</td>
<td>-13.74</td>
<td>-30.91</td>
<td>56.70</td>
<td>0.43</td>
<td>0.57</td>
</tr>
<tr>
<td>Temperature</td>
<td>CMC Value</td>
<td>ΔHm</td>
<td>ΔSm</td>
<td>ΔGm</td>
<td>ΔHm</td>
<td>ΔSm</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>0.050</td>
<td>-14.66</td>
<td>-31.53</td>
<td>53.80</td>
<td>0.46</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>0.041</td>
<td>-15.60</td>
<td>-32.53</td>
<td>52.40</td>
<td>0.38</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>

The CMC value of CLQ increases with increase in temperature. It is due to greater degree of hydrophilic dehydration than hydrophobic dehydration which disfavors micellization. The variation in behaviour of ΔGm, ΔHm, and ΔSm is same for both QUN and CLQ.

4.2.4.3. Fluphenazine (FLP) and Trifluperazine (TRF)

As shown in Figure 4.11-4.14, the conductivity plots for both drugs show two turning points, cc1 and cc2. For both drugs cc1 is in reasonable agreement with the value obtained by surface tension data of these drugs and is taken as CMC. There is again a change in slope in conductivity-concentration plot at cc2 which is related with structural rearrangement of aggregates previously formed [5].

![Figure 4.11](image-url) 

**Figure 4.11.** Plots of Electrical Conductivity versus molality for aqueous solution of Fluphenazine 2HCl at

293K (■), 303K (●), 313K (▲) and 323K (▼).
Figure 4.12. Typical plot of Electrical Conductivity versus molality, \( m \), (mol/kg), for aqueous solution of Fluphenazine 2HCl at 293K.

Figure 4.13. Plots of Electrical Conductivity versus molality for aqueous solution of Trifluperazine 2HCl at 293K (■), 303K (●), 313K (▲) and 323K (▼).
Figure 4.14 Typical plot of Electrical Conductivity versus molality, m, (mol/kg), for aqueous solution of

Trifluperazine 2HCl at 293K.

The value of CMC increases with increase in temperature because at high temperature, the degree of hydrophilic dehydration is greater than that of hydrophobic dehydration, which enhances the repulsion among hydrophilic groups, thus, making micellization difficult so increasing the CMC value [12]. The CMC of Trifluperazine 2HCl is less than that of Fluphenazine 2HCl because the former is more hydrophobic. The OH group present in Fluphenazine 2HCl reduces its hydrophobicity. Table 4.9 shows the values of thermodynamic parameters along with the values of cc1, cc2, degree of counter ion binding (α) and degree of ionization (β) for both Phenothiazine drugs Fluphenazine 2HCl and Trifluperazine 2HCl respectively.

**Table 4.9:** Micellar and thermodynamic parameters calculated from Electrical conductivity measurements for Fluphenazine 2HCl and Trifluperazine 2HCl at different temperatures.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>T</th>
<th>CMC</th>
<th>cc2</th>
<th>ΔHm</th>
<th>ΔGm</th>
<th>ΔSm</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>molkg⁻¹</td>
<td>molkg⁻¹</td>
<td>kJmol⁻¹</td>
<td>kJmol⁻¹</td>
<td>Jk⁻¹mol⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>Concentration</td>
<td>ΔG°m</td>
<td>ΔH°m</td>
<td>ΔS°m</td>
<td>Value</td>
<td>ΔG°m</td>
<td>ΔH°m</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>---------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Fluphenazine 2HCl</td>
<td>293</td>
<td>0.0170</td>
<td>-5.60</td>
<td>-26.8</td>
<td>72.15</td>
<td>0.59</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>303</td>
<td>0.0175</td>
<td>-5.98</td>
<td>-27.9</td>
<td>72.44</td>
<td>0.60</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>313</td>
<td>0.0193</td>
<td>-6.38</td>
<td>-28.75</td>
<td>71.47</td>
<td>0.61</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>323</td>
<td>0.0205</td>
<td>-6.80</td>
<td>-29.7</td>
<td>70.87</td>
<td>0.61</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Trifluperazine 2HCl</td>
<td>293</td>
<td>0.0135</td>
<td>-20.70</td>
<td>-28.24</td>
<td>25.70</td>
<td>0.62</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>303</td>
<td>0.0149</td>
<td>-22.15</td>
<td>-29.50</td>
<td>24.29</td>
<td>0.62</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>313</td>
<td>0.0175</td>
<td>-23.64</td>
<td>-30.18</td>
<td>20.88</td>
<td>0.63</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>323</td>
<td>0.0184</td>
<td>-25.17</td>
<td>-31.33</td>
<td>19.07</td>
<td>0.64</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

The large negative value of $\Delta G^o_m$ indicates thermodynamically favorable nature of micellization process. The values of $\Delta G^o_m$ become more and more negative with increase in temperature which means that micellization become more spontaneous at high temperature. The values of $\Delta S^o_m$ are positive while that of $\Delta H^o_m$ are negative at each temperature. These values indicate that both entropy and enthalpy favour the process of micellization [12-17]. The negative values of $\Delta H^o_m$ at each temperature show that micellization is exothermic.

The value of $\Delta S^o_m$ is positive and that of $\Delta H^o_m$ is negative which is a sign of the imperative role played by electrostatic interactions for aggregation besides the hydrophobic ones. Furthermore hydration of hydrophilic groups becomes more significant rather than destruction of water structure around hydrophobic groups of monomers. The decrease in positive value of $\Delta S^o_m$ and increase in the negative value of $\Delta H^o_m$ with temperature signifies that temperature favors the electrostatic interactions but disfavors the hydrophobic ones.

Both FLP and TRF show two critical concentrations but without premicellar aggregation.
The hydrophobic hydration gives rise to stronger hydrogen bonding among water molecules in the close neighborhood of hydrophobic groups and causes the internal torsional vibrations of hydrophobic groups to be constrained in solution. The entropy of system thus decreases. The exclusion of hydrophobic groups from aqueous environment, when micellization starts, enhances the value of entropy due to disruption of highly ordered water structure and removal of mobility constriction on hydrocarbon chain [2, 12-14].

The $\Delta G_m^\circ$ value for Trifluperazine 2HCl is more negative than for Fluphenazine 2HCl because the former is more hydrophobic so its micellization is more spontaneous.

Molecules of both drugs produce dication on being dissolved in water. Let the general formula of each drug is DCl2. When they are dissolved in water they become ionized and in premicellar region cation and neutral molecules are in equilibrium with each other as given as $\text{DCl}_2 \leftrightarrow \text{D}^2+ + 2\text{Cl}^-$. 

As we go on increasing concentration of drug molecules in solution, while being not beyond pre CMC region equilibrium gets disturbed but according to Le Chatlier principle system soon reinstates its state of dynamic equilibrium by increasing rate of ionization of drug molecules, a forward process. However, as we enter in post micellar region dynamic equilibrium gets established between micelle and counter ions. There is positive charge at surface of micelle and negative counter ions at solution side are attached to it forming an electrical double layer around micelle. Due to unequal distribution of charges between micellar phase and aqueous phase, the micellar surface gets overall positive charge. According to Stern model, electrical double layer may be divided into two parts: (1) a layer of strappingly held ions adsorbed very close to micellar surface at fixed sites forming Stern layer being formed according to Langmuir Adsorption isotherm and (2) a diffused layer of counter ions as per Gouy-Chapman model. Electrical potential drops rapidly in portion of Stern layer and gradually in that of diffused layer [3].

4.2.5. The drugs showing one critical concentration
Citalopram HBr (CIT), Pefloxacin Mesylate (PFL), Clindamycin Phosphate (CLN) and Certizine 2HCl (CRT) are the drugs that show only one inflection point in conductivity concentration plot and hence one critical concentration. All of these drugs have flexible structure because benzene rings in these compounds are not fused.

4.2.5.1. Citalopram HBr (CIT)

CIT is an antidepressant drug having flexible structure consisting of two non fused benzene rings. It has flexible structures so it is very convenient for it to be micellized thus it has the low value of CMC. Plots of conductivity for aqueous solution of CIT versus molality are shown in Figure 4.15 and typical conductivity-molality plot at 293K is represented in Figure 4.16.

![Figure 4.15](image)

**Figure 4.15.** Plots of Electrical Conductivity versus molality for aqueous solution of CIT at 293K (■), 303K (●), 313K (▲) and 323K (▼).
Figure 4.16. Typical plot of Electrical Conductivity versus molality for aqueous solution of CIT at 293K.

The conductivity-concentration plot undergoes change in slope at CMC. The value of CMC calculated from conductivity and surface tension are very close to each other. The micellar and thermodynamic parameters calculated from CMC are given in Table 4.10.

Table 4.10. Micellar and thermodynamic parameters calculated from electrical conductivity for CIT at different temperatures.

<table>
<thead>
<tr>
<th>$T/K$</th>
<th>$CMC/molg^{-1}$</th>
<th>$\Delta H_m/kJmol^{-1}$</th>
<th>$\Delta G_m/kJmol^{-1}$</th>
<th>$\Delta S_m/ JK^{-1}mol^{-1}$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>0.029</td>
<td>-18.57</td>
<td>-21.88</td>
<td>11.31</td>
<td>0.19</td>
<td>0.81</td>
</tr>
<tr>
<td>303</td>
<td>0.0283</td>
<td>-19.86</td>
<td>-22.35</td>
<td>8.20</td>
<td>0.17</td>
<td>0.83</td>
</tr>
<tr>
<td>313</td>
<td>0.027</td>
<td>-21.2</td>
<td>-22.71</td>
<td>4.76</td>
<td>0.15</td>
<td>0.86</td>
</tr>
<tr>
<td>323</td>
<td>0.026</td>
<td>-22.57</td>
<td>-23.24</td>
<td>2.08</td>
<td>0.13</td>
<td>0.87</td>
</tr>
</tbody>
</table>
The large negative value of $\Delta G_m^-$ is an evidence for spontaneity of micellization. The more negative values of $\Delta G_m^-$ with temperature means that micellization become more spontaneous at higher temperature. The values of $\Delta S_m^+$ are positive while that of $\Delta H_m^+$ are negative at each temperature. These values indicate that the process of micellization is both entropy and enthalpy driven [12-17]. The decrease in positive value of $\Delta S_m^+$ and increase in negative value of $\Delta H_m^+$ with increase in temperature gives us an idea that hydrophobic interactions become weaker while electrostatic ones become stronger with increase in temperature. The degree of hydration of hydrophobic parts decreases at high temperature, which results in reduction of $\Delta S_m^+$ values. A negative value of $\Delta H_m^+$ may occur when hydration of water molecules around hydrophilic heads groups become more important than destruction of water structure around hydrophobic groups of monomers. The positive values of $\Delta S_m^+$ are due to transfer of hydrophobic chains of drugs from aqueous environment to micelle core [12, 14]. Figure 4.16 shows a typical plot of Electrical Conductivity of CIT versus molality at 303K showing CMC at 0.0283mol/kg. The decrease in CMC of CIT with temperature is due to the fact that hydrophobic ends are dehydrated more readily than hydrophilic heads.

4.2.5.2. Clindamycin Phosphate (CLN)

Figure 4.17 shows plots of electrical conductivity versus molality of CLN at different temperatures while typical plot of conductivity as a function of molality is visible in Figure 4.18. The CMC values were determined from the intersection points.
**Figure 4.17.** Plots of Electrical Conductivity versus molality for aqueous solution of CLN at 293K (■), 303K (●), 313K (▲) and 323K (▼).

**Figure 4.18.** Typical plot of Electrical Conductivity versus molality for aqueous solution of CLN at 303K.

Various parameters obtained from electrical conductivity of aqueous solution of CLN are tabulated as well as discussed under.
Table 4.11. Micellar and thermodynamic parameters calculated from Electrical conductivity of CLN at different temperatures.

<table>
<thead>
<tr>
<th>$T$</th>
<th>$CMC$</th>
<th>$\Delta H_m$</th>
<th>$\Delta G_m$</th>
<th>$\Delta S_m$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>molkg$^{-1}$</td>
<td>kJmol$^{-1}$</td>
<td>kJmol$^{-1}$</td>
<td>JK$^{-1}$mol$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>0.0352</td>
<td>-34.52</td>
<td>-23.65</td>
<td>6.93</td>
<td>0.29</td>
<td>0.71</td>
</tr>
<tr>
<td>303</td>
<td>0.0377</td>
<td>-35.68</td>
<td>-23.66</td>
<td>3.55</td>
<td>0.29</td>
<td>0.71</td>
</tr>
<tr>
<td>308</td>
<td>0.0383</td>
<td>-36.87</td>
<td>-23.84</td>
<td>1.38</td>
<td>0.28</td>
<td>0.72</td>
</tr>
<tr>
<td>313</td>
<td>0.0411</td>
<td>-38.08</td>
<td>-23.92</td>
<td>-1.7</td>
<td>0.27</td>
<td>0.73</td>
</tr>
</tbody>
</table>

The values of CMC increase with temperature, which is due to greater degree of hydrophilic dehydration rather than hydrophobic dehydration. Hydrophilic ends, thus, get exposed to each other and repulsion between them increases which increases CMC. The negative value of $\Delta G_m$ and positive value of $\Delta S_m$ point toward the spontaneous nature of micellization. The negative value of $\Delta S_m$ at 323K show more organized behaviour of molecules in micellar form than in free state [15].

Positive value of $\Delta S_m$ is primarily responsible for spontaneous nature of micellization. The process of micellization is shown by the equilibrium $nS \leftrightarrow Sn$. At first glance positive value of entropy looks unforeseen because above equilibrium shows that there is decrease in number of independent kinetic units during micellization. So value of $\Delta S_m$ should be negative. The problem is that actually we have ignored what happens to water structure during micelle formation. The reason behind this entropy increase is the extensive hydrogen bonding in water.

Water molecules have tetrahedral structure with oxygen atom at center while two hydrogen atoms and two lone electron pairs are at corners of tetrahedron. A loose network is formed between water molecules due to hydrogen bonding between hydrogen atom of one molecule and lone pair of other. This network keeps on breaking and reforming at various points due to thermal fluctuation but at equilibrium a high average
level of hydrogen bond prevails. When amphiphilic molecules are added in water no hydrogen bond develops between water molecules and hydrophobic part of amphiphile so it behaves as if it is embedded in water merely occupying a hole in water structure. During formation of holes or cavities hydrogen bonding between water molecules is broken and the molecules at the surface of cavities regenerate hydrogen bonding and, as a result, become more ordered around hydrophobic groups with decrease in entropy. On micelle formation hydrophobic groups are removed from water into micellar environment and cavities revert to the structure of pure water. The highly ordered water molecules at surface of so called cavity become disordered with an increase in entropy[13].

4.2.5.3. Pefloxacin Mesylate (PFL)

Figure 4.19 shows plots of electrical conductivity as a function of molality for PFL at different temperatures. The CMC values are determined from the intersection point as shown in Figure 4.20.

![Graph showing conductivity vs molality](image)

**Figure 4.19.** Plots of Electrical Conductivity ($\mu$S) versus molality, $m$, (mol/Kg), for aqueous solution of PFL at 293K (■), 303K (●), 313K (▲) and 323K (▼).
Figure 4.20. Typical plot of Electrical Conductivity versus molality for aqueous solution of PFL at 303K.

Various parameters calculated from electrical conductivity are given in Table 4.12.

Table 4.12. Micellar and thermodynamic parameters calculated from Electrical conductivity of aqueous solutions of PFL at different temperatures.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>CMC (mol/kg)</th>
<th>$\Delta H_m$ (kJ/mol)</th>
<th>$\Delta G_m$ (kJ/mol)</th>
<th>$\Delta S_m$ (JK$^{-1}$mol$^{-1}$)</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>0.120</td>
<td>-18.57</td>
<td>-21.88</td>
<td>11.31</td>
<td>0.40</td>
<td>0.68</td>
</tr>
<tr>
<td>303</td>
<td>0.114</td>
<td>-19.86</td>
<td>-22.35</td>
<td>8.20</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>313</td>
<td>0.102</td>
<td>-21.22</td>
<td>-22.71</td>
<td>4.76</td>
<td>0.21</td>
<td>0.80</td>
</tr>
<tr>
<td>323</td>
<td>0.089</td>
<td>-22.57</td>
<td>-23.24</td>
<td>2.08</td>
<td>0.18</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The hydrophobic portion of this diphillic drug gets banished from the aqueous medium to minimize free energy of system. The negative value of $\Delta G_m$ is indicative of spontaneous nature of micellization and is mainly due to large positive value of $\Delta S_m$. Thus both enthalpy and entropy favour the micellization [11, 16].
In case of PFL, CMC decreases with increase in temperature. This is because degree of hydrophilic dehydration is greater than that of hydrophobic dehydration. The increase in negative value of free energy of micellization points toward increase in spontaneity of process at higher temperature. Association of PFL molecules is favoured by both enthalpy and entropy due to negative value of enthalpy and positive value of entropy.

4.2.5.4. Certizine 2HCl (CRT)

Figure 4.21 shows the concentration dependence of electrical conductivity of CRT at various temperatures while typical plot of concentration dependence of electrical conductivity of Certizine 2HCl at 293K is shown in Figure 4.22. Only one inflection point was detected at all temperatures.

![Figure 4.21](image)

**Figure 4.21.** Plots of Electrical Conductivity versus molality for aqueous solution of CRT at 298K (■), 303K (●), 308K (▲) and 313K (▼).
Figure 4.22. Typical plot of Electrical Conductivity versus molality for aqueous solution of CRT at 298K.

The electrical conductivity increases rapidly before CMC but in post CMC region it raises slowly due to low mobility of larger micelles. The CMC value goes up as temperature increases due to greater degree of hydrophilic dehydration than hydrophobic dehydration. This situation does not support micellization thus cause increase in CMC.

Table 4.13 shows CMC values and other parameters at different temperature.

**Table 4.13** Micellar and thermodynamic parameters calculated from conductivity data of CRT at different temperatures.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>CMC (mol/kg)</th>
<th>$\Delta H_m$ (kJ/mol)</th>
<th>$\Delta G_m$ (kJ/mol)</th>
<th>$\Delta S_m$ (J/K mol)</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>0.0758</td>
<td>-20.20</td>
<td>-25.47</td>
<td>17.9</td>
<td>0.585</td>
<td>0.415</td>
</tr>
<tr>
<td>303</td>
<td>0.0761</td>
<td>-19.86</td>
<td>-26.29</td>
<td>15.4</td>
<td>0.583</td>
<td>0.417</td>
</tr>
<tr>
<td>308</td>
<td>0.0763</td>
<td>-21.2</td>
<td>-26.84</td>
<td>12.02</td>
<td>0.570</td>
<td>0.430</td>
</tr>
</tbody>
</table>
The value of CMC obtained from surface tension is in conformity with that we get from conductivity. The large negative value of $\Delta G^\circ_m$ is an indication for impetuosity of micellization. The more negative values of $\Delta G^\circ_m$ with temperature means micellization become more spur-of-the-moment at higher temperature. The values of $\Delta S^\circ_m$ are positive while that of $\Delta H^\circ_m$ are negative at each temperature. These values point out that the process of micellization is supported by entropy as well as enthalpy and in addition to hydrophobic interactions, electrostatic ones are no less behind to play a vital role in aggregation. The decrease in positive value of $\Delta S^\circ_m$ and increase in negative value of $\Delta H^\circ_m$ with increase in temperature gives us an idea that hydrophobic interactions become weaker while electrostatic ones become stronger with increase in temperature. The degree of hydration of hydrophobic parts decreases at high temperature, which results in reduction of $\Delta S^\circ_m$ values.

A negative value of $\Delta H^\circ_m$ may occur when hydration of water molecules around hydrophilic heads groups become more important than destruction of water structure around hydrophobic groups of monomers. The positive values of $\Delta S^\circ_m$ are due to transfer of hydrophobic chains of drugs from aqueous environment to micelle core [12, 14, 16, 17].

### 4.2.5.5. Dexamethasone Sodium Phosphate (DSP)

|  |  |
|---|---|---|---|---|---|---|
| 313 | 0.0766 | -22.57 | -26.95 | 7.36 | 0.560 | 0.440 |
Drugs represent an interesting variety of amphiphilic structures ranging at one extreme from cationic quaternary ammonium germicides, which are easily recognized as typical surfactants, to more complex aromatic or heterocyclic molecules such as the phenanthrene narcotic analgesics. It is important to recognize that micellization is not the only way of association; amphiphilic molecules may also exhibit open or non-micellar association in solution. Typical surfactants have hydrocarbon groups, which can intertwine during micellization process to form approximately spheroidal aggregates. Replacement of their flexible hydrophobic moiety with a rigid aromatic or heterocyclic ring system can have very pronounced effect on the way in which molecules are disposed within aggregates to such an extent that process of aggregation can no longer be regarded as micellization. A well-known illustration of this effect is association of cationic dyes and purines and pyrimidines bases of nucleotides, which associate by stacking process. This self-association process is generally continuous having no equivalent to CMC in it and there is wide range of aggregate sizes in solution. Many of drug molecules lie in between these two extremes. Although the hydrophobic groups of most of drugs are aromatic but they resemble typical surfactants because of having high degree of flexibility. On the other hands the rigid aromatic ring system, for example, phenothiazine, differ from cationic dye in that their charges are generally localized at a terminal group of a relatively long side chain rather than delocalized in the ring system, as is common with dyes molecules. Drugs thus provide an opportunity to investigate those factors, which are responsible for type of association exhibited by particular amphiphilic molecules in solution [3]. It is this aspect of studies on colloidal properties of drug, rather than any pharmaceutical consequences of colloidal behaviour, which have been emphasized in this work [2].

Dexamethasone Sodium Phoshate (DSP) is among drugs having rigid non flexible structure that undergoes open or continuous aggregation not to be regarded as micellization and inflection point may be called critical aggregation concentration (CAC) rather than CMC. The experimental determination of specific conductivities is a promising technique for the detection of CAC.

DSP is one of the widely used freely water-soluble drugs with potent anti-inflammatory and antineoplastic activities. Figure 4.23 shows the concentration
dependence of electrical conductivity of DSP at various temperatures. As drug has rigid cyclic structure so critical aggregation concentration (CAC) doesn’t have a sharp value and was determined using linear regression of the two data series. The value of CAC increases with temperature due to increase in solubility and dehydration of hydrophilic groups, which enhances repulsion between them, making aggregation difficult, and thus increasing CAC value.

Figure 4.23. Plots of Conductivity versus molality for aqueous solution of DSP at 298K (■), 303K (●), 308K (▲) and 313K (▼).
Figure 4.24. Typical plot of electrical conductivity ($\Delta S$) versus molality for aqueous solution of DSP at 298 K.

The summary of variation of aggregation and thermodynamic parameters is given in Table 4.14.

Table 4.14. Aggregation and thermodynamic parameters for aqueous solution of DSP at different temperature.

<table>
<thead>
<tr>
<th>$T/K$</th>
<th>$CAC/molkg^{-1}\times10^3$</th>
<th>$\Delta H_{agg}/kJmol^{-1}$</th>
<th>$\Delta G_{agg}/kJmol^{-1}$</th>
<th>$\Delta S_{agg}/JK^{-1}mol^{-1}$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>3.4</td>
<td>-46.31</td>
<td>-26.69</td>
<td>-0.066</td>
<td>0.11</td>
</tr>
<tr>
<td>303</td>
<td>4.8</td>
<td>-48.70</td>
<td>-25.84</td>
<td>-0.076</td>
<td>0.10</td>
</tr>
<tr>
<td>308</td>
<td>6.9</td>
<td>-51.13</td>
<td>-25.08</td>
<td>-0.085</td>
<td>0.09</td>
</tr>
<tr>
<td>313</td>
<td>7.9</td>
<td>-53.62</td>
<td>-24.85</td>
<td>-0.092</td>
<td>0.08</td>
</tr>
</tbody>
</table>
The increasing trend of CAC with temperature shows aggregation becomes difficult as temperature increases. Gibbs free energy of aggregation becomes less negative with temperature, showing that the process becomes less spontaneous as temperature increases. Similarly lower magnitude of negative value of enthalpy of aggregation is an evidence for lower degree of spontaneity of aggregation at high temperature and that process is enthalpy driven. The negative value of entropy may be due to more pronounced ordering effect of randomly oriented monomers from solvated form into highly organized core of aggregate than disordering effect caused by destruction of water structure around hydrophobic groups of monomer molecules. Moreover, negative value of $\Delta H$ is indicative of greater contribution of electrostatic interaction during aggregation [15-19].

4.3. Drug Surfactant interaction

During the last few decades, one important insight has triggered a tremendous upsurge of interest in surfactant structures. This is the recognition that these structures may mimic biological structures in some ways. Enzymes, for example, are proteins into which a reactant somehow fits to form a reactive intermediate. Likewise cell membranes not only compartmentalize biological system but also play a variety of functions in life. Surfactant systems can be used as a model system to impersonate both enzymes and membranes. This concept has given birth to a whole new field of mimetic chemistry and the colloidal structures produced by the surfactants are at the center of whole subject. Self assembled structure such as micelles and reverse micelles also play increasingly important role in separation process in engineering and environmental sciences and technology. The nature and mechanism of interactions of surfactants with additives are not still clearly understood. Electrostatic and/or hydrophobic interactions may take place leading to formation of complexes of specific physico-chemical characteristics [20-25].

4.3.1. Solublization

Surfactants are known to play a vital role in many processes of interest in both fundamental and applied science. One of important property of surfactant is formation of colloidal sized clusters in solution, known as micelle, which has particular significance in pharmacy because of their ability to increase solubility of sparingly soluble substance in
water. Micelles are known to have anisotropic water distribution within their structures. In other words water concentration decrease from surface to core of micelle. Consequently, spatial position of solublized drug in a micelle will depend on its polarity: nonpolar substance will be solublized in micelle core and those with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions [20].

This process of formation of thermodynamically stable, isotropic solution is called solublization. The substance dissolved is called solublizate and surfactant is called solublizer. Since enhancement of solubility begins at CMC, this phenomenon provides a method to determine CMC of surfactant. It must, however, be used cautiously as solublizate may change CMC of surfactant.

Drug interaction with heterogeneous media typically induce changes in physico-chemical properties of drug, and by monitoring these changes we can quantify the degree of drug/membrane interaction, which is normally expressed by related quantities drug/micelle binding constant and partition coefficient [21]. Here it is assumed that the mother micelle retains its original size and shape when solublization amount is relatively small [22]. Solublization can be considered as a process in which hydrophobic substance is incorporated into micelle accompanied by enthalpic and entropic changes in the system. It includes partition of solublizate between micellar phase and bulk phase [23, 24]. The partitioning behaviour of solublizate between micellar and aqueous phase is an indication of hydrophilic lipophilic balance of molecules, change in this balance cause to change entropy of system. Interaction of micelle with solublizate and water plays an important role in partitioning system which results in thermodynamically stable system. It also depends on structure of surfactant and solublizate that constituted the system. In addition to solublization, micro viscosity of micellar interior and location of solublizate also play an important in thermodynamics of system [25].

4.3.2. Effect of additive on CMC of surfactant

The drug molecule may penetrate in micelle in such a way that its hydrophobic part may reside in palisade layer while hydrophilic part may remain at outer portion of micelle close to micelle water interface. The repulsions among head groups is, thus, weakened because penetrated drug molecules may lower surface charge density of
micelle. The shorter chain compounds have greater possibility to be adsorbed on interface while longer chain ones inside the micelle core. This fashion of adsorption of drug molecules reduces magnitude of work for micellization thus decreasing CMC [26].

4.3.3. UV/Visible Spectroscopic study of Drug Surfactant interaction

Spectroscopic behavior of amphiphilic drugs in ordered media of ionic surfactant provides an insight into process that leads to partition of drugs between surfactant micelles and aqueous media.

Some antibiotic, antidepressant and anti allergic drugs have been selected for this study. The antibiotic drugs chosen are Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ), and Pefloxacin Mesylate (PFL) while the antidepressant ones are Citalopram HBr (CIT) and Trifluperazine HCl (TRF). The simple and differential UV/Visible spectroscopic technique was employed to study how these drugs interact with micellar structure of surfactant (an artificial membranes), and how they are partitioned between aqueous and micellar phase.

4.3.4. Polynuclear aromatic hydrocarbon

Spectral shifts arise due to change in environment around chromophore of additive molecules. Shifts in electronic transition may be due to its hydrophobicity and its stereo chemical factors. In case of aromatic hydrocarbon the change in microenvironment, in term of polarity, has a little effect on \( \pi \)-electron system due to resonance energy. Below CMC, surfactant monomers form cage like structure and drug molecule get entrapped in this cage. The increase in absorbance with increases in SDS concentration shows that these cage like structures get stronger and leads to micellization and more additives get entrapped in cage. However, after CMC no further increase in absorbance is observed which refers to maximum incorporation of additive molecule by micelle.

We have, in this work, studied, interaction of various drugs with cationic and anionic surfactants. The unimers of surfactant form micelles after a certain value of concentration. Micelles have potential to mimic membrane system and to interact with various neutral and charged molecules. Generally, for hydrocarbon and polar
compounds, the order of solubilization capacity of micelle occurs as cationic > anionic for surfactants with same carbon chain length [27].

4.3.5. Quinacrine dihydrochloride (QUN)

QUN, a tricyclic drug having IUPAC name \(N^-(6\text{-chloro-2-methoxy-acridin-9-yl})-N, N\text{-diethyl-pentane-1, 4-diamine}\) was initially approved in the 1930s as an antimalarial drug as well as being used against tapeworm infections.

4.3.5.1. UV/Visible spectroscopy

Interaction of QUN with SDS is studied by using UV/Visible spectroscopy. The effect of different concentration region of SDS on UV/Visible spectrum of QUN has been checked. The data obtained from UV/Visible spectra is utilized for determining partition characteristics of cationic drug QUN with anionogenic tenside SDS.

The partition coefficient and binding constant of QUN to micelles of SDS has never been carried out so far and no literature is available about such interaction of drug with SDS. The determination of QUN binding to SDS will help to understand the mode of action of QUN. The information obtained from this study may be supportive to develop a new drug with increased activity and fewer side effects.

4.3.5.1.1. Simple Absorption Spectra

Figure 4.25 shows simple UV/Visible spectrum of Quinacrine in water in the absence of SDS. The peak at 278nm shows maximum absorbance having a shoulder at 263nm which indicates that formation of dimer in aqueous solution has taken place. The drug molecules in dimer are held by two types of forces, the dispersion of \(\pi\)-system of drug molecules and forces emerging from hydrophobic effects. The cumulative effect of these forces should be greater than repulsion between the positive charges of drug molecules [28]. The simple absorption spectrum at different concentration of SDS gives interaction of QUN with surfactant.
Figure 4.25. Simple absorbance spectra of Quinacrine in aqueous solution.

Figure 4.26. Simple absorption spectra of QUN in presence of pre micellar and post micellar amount of SDS.
The UV/visible spectrum of Quinacrine dihydrochloride (8.76 x10^-6M) with varying concentration of surfactants were obtained. The data obtained in aqueous surfactant solution is compared to those obtained in water. The UV/visible spectra represented in Figure 4.26 clearly shows that drug surfactant interaction causes bathochromic shift (red shift) which indicates strong host guest relationship between cationic drug and anionic surfactant. It is because Quinacrine is mainly hydrophobic in nature having cationic hydrophilic group. It is, therefore, expected to have strong interaction with anionic surfactant. Figure 4.27 shows the UV / Visible spectrum of QUN in the presence of pre micellar and post micellar concentration of SDS. The bathochromic shift takes place from 278nm to 281nm. However, hypochromic effect is also observed in premicellar region. As we add SDS to drug solution monomeric form of drug interacts with hydrophilic head group of surfactant resulting in shift of the dimer↔monomer equilibrium toward monomer. While increasing SDS concentration hyperchromic effect takes place due to increasing drug surfactant interaction. The absorbance increases rapidly till CMC and then increases very slowly. It appears that before CMC structural environment of drug molecule changes with SDS concentration till CMC however at post micellar concentration drug molecules do not experience any change in environment [27-29].

It may be concluded that the structure of additive molecule (in this case QUN molecule) and the charge present on surfactant molecules contributes largely towards the phenomenon of solublization. Since the drug is cationic thus have an attraction for anionic surfactant SDS and, consequently, incorporation of drug molecules into micelles is facilitated. Figure 4.28 shows that absorbance increases with SDS concentration. This indicates that a larger number of drug molecules are taken into SDS micelle. However, after CMC absorbance becomes almost constant due to maximum incorporation of drug molecules into micelle or in some cases absorbance increases very slowly even after CMC because the greater the amount of surfactant added in solution, above CMC, give birth to large number micelles and increase in absorbance is due to incorporation of drug molecules in newly formed micelle [29].
Figure 4.27. Simple absorption spectra of QUN in presence of various amounts of SDS.

Figure 4.28. Plot of simple Absorbance of QUN versus molar concentration of SDS.

While going from the hydrated micelle surface to non-polar core we face a continuum of environment. The solubilizate may stay anywhere from surface of micelle
to its core. The dynamic nature of solublization process let the solublizate to spend different time at different places between core and the surface. The hydrophilic and hydrophobic forces being not in balance keep the solublizate somewhat dynamic in micelle, thus giving random values of absorbance [30].

The orientation of additive molecules at the interface of micelle depends on hydrophilic-hydrophobic forces. The less hydrophobic additives resides in core while hydrophilic ones near the surface region of micelle. The hydrophobic interactions cause the additive molecules to be buried deep in the core of micelle [31].

The CMC of surfactant increases in presence of QUN which is due to structure breaking effect of QUN which causes destruction of water structure and causes less increase in entropy thus making micellization entropically less favourable and increasing CMC. Another factor is the balance between hydrophilic and hydrophobic forces. For additive having shorter chain, there are greater chances of hydrogen bonding between hydrophilic parts of additive and water, so orientation of their molecules is more likely in outer portion of micelle close to micelle water interface. Such fashion of adsorption of drug molecules increases work of micellization by producing less increase in entropy thus making micellization less convenient and increasing CMC [26].

The partitioning behavior of solublizate between bulk phase and micellar phase also depends on the hydrophilic-lipophilic balance. It has been discussed that affinity of water for solublizate is important in partitioning due to water dragging effect where the water is carried as a shell around solublizate into organic (micellar) phase. It follows that interaction between the solublizate and water, between the solublizate and micelle and between the water and micellar phase play a vital role in partitioning process. The partition coefficient is dependent on the structure of solublizate and the surfactant that constitute the micelle [32].

However, the red shift in pre micelle to micellar region is because the micellar phase is less polar than aqueous phase, a phenomenon known as solvatochromism. It is a phenomenon in which absorption spectra of additives shift to higher wavelength due to decrease in solvent polarity.

4.3.5.1.2. The differential spectroscopy
To find whether interaction and binding of drug with surfactant occurs or not differential (or difference) spectroscopy is the best tool to be used which helps to have a look into partition behaviour of drug between aqueous and micellar phase and get values of partition constant as well as binding constant. The value of partition coefficient is calculated from Kawamura equation [2.19].

The increase in value of differential absorption with increasing surfactant concentration suggests stronger interaction between drug molecules and SDS micelles. The partition of drug molecules between aqueous bulk phase and micellar phase depends on their hydrophobicity as well as their polarity [30]. The continuous increase in differential absorbance with SDS concentration is indicative of incessant inclusion of additive within micelle. The spectral properties of drugs have been investigated through a series of absorption experiment under varying amounts of the surfactant concentration.

Figure 4.29 shows the differential absorption spectra of aqueous solution of Quinacrine in the presence of SDS at a certain concentration of drug. The differential absorbance is zero at very low concentration of SDS and increases with increasing SDS concentration. The increase in differential absorbance, \( \Delta A \), with SDS concentration may be attributed to the increase in amount of solublized drug in micelles. The following description for analysis of amount of solublized drug is made at the wavelength, \( \lambda_{\text{max}} \), where the highest peak appears in the spectra [25, 33].
Figure 4.29. Differential UV/Visible spectrum of QUN at different concentration of SDS.

Fig 4.30. Plot of differential absorbance of QUN at different concentration of SDS.

Table 4.15. Calculation of parameters indicating partitioning of QUN in SDS micellar media

<table>
<thead>
<tr>
<th>Cs×103 (Mol/dm3)</th>
<th>ΔA×102</th>
<th>1/ΔA</th>
<th>CMCo×103</th>
<th>Csmo×103</th>
<th>Ca×106</th>
<th>Csmo+Ca×104 [1/Cs + Ca] ×10^{-3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>10.76</td>
<td>9.29</td>
<td>8.20</td>
<td>0.80</td>
<td>8.75</td>
<td>8.09</td>
</tr>
<tr>
<td>10</td>
<td>11.20</td>
<td>8.93</td>
<td>8.20</td>
<td>1.80</td>
<td>8.75</td>
<td>18.10</td>
</tr>
<tr>
<td>15</td>
<td>12.08</td>
<td>8.28</td>
<td>8.20</td>
<td>6.80</td>
<td>8.75</td>
<td>68.10</td>
</tr>
<tr>
<td>20</td>
<td>12.60</td>
<td>7.94</td>
<td>8.20</td>
<td>11.80</td>
<td>8.75</td>
<td>118.00</td>
</tr>
</tbody>
</table>
The CMC of SDS in presence of drug can be determined from plot of absorbance of drug in micellar system against SDS concentration but it doesn’t let us detect the precise value of CMC. It is, therefore, preferable to determine CMC of surfactant in the presence and absence of drug by specific conductivity method. The value of CMC of surfactant increases with an increase in concentration of drug. QUN being more hydrophobic drug has negative value of free energy of partition which is an indicator for ease of penetration of additive drug into micelles [31].

4.3.5.1.3. Partition Coefficient (Kx)

The partition coefficient of solublizate between micellar and aqueous phase is imperative not only to expound mechanism of solublization but also to comprehend biological phenomenon e.g. interaction between drugs and biological membranes. Kx is ratio of concentration (in mole fraction) of drug molecules in micelle to that in bulk aqueous solution. Higher value of partition coefficient displays higher concentration of drug in micelle than in surrounding water. Partitioning of drug molecules between two phases is governed by partition Law. Partition coefficient is determined by differential absorbance method reported by Kawamura et al. suggesting that Beer-Lambert law holds good for solublized additive.
Model 4.1. Locus of QUN molecule in micelle.
Figure 4.31. Relationship between $1/\Delta A$ and $(1/ (C_s + C_{smo})$ for calculation of $K_x$ for QUN/SDS system.

Table 4.16. Various parameters calculated from UV/Visible spectroscopy for QUN/SDS system.

<table>
<thead>
<tr>
<th>$K_x \times 10^{-4}$</th>
<th>$\Delta G_x/kJmol^{-1}$</th>
<th>$K_b$</th>
<th>$\Delta G_b/kJmol^{-1}$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.68</td>
<td>-30.55</td>
<td>400</td>
<td>-14.84</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Water-micelle partition coefficient is determined by relationship given in Kawamura equation. The extent of solublization depends on structure of additive molecule that may penetrate deeply into hydrocarbon core or remain adsorbed at polar surface of micelle. Polar additives are solublized by the virtue of strong attractive interaction between their polar groups and ionic head groups of surfactant. In the present work, value of $K_x$ points toward the adsorption of QUN in such a way that its polar group are attached to surface whereas, their aliphatic and aromatic moieties extend partially into hydrophobic core of micelle. There is electrostatic interaction between polar ends of
drug additives and ionic surfactant as there is a strong ion-dipole interaction due to large surface charge density of micelle [34, 35].

4.3.5.1.4. Standard free energy change (ΔGp)

Equation 2.20 gives value of standard free energy change of transfer of additive from aqueous to micellar phase. The value of Kx increases and that of ΔGp decreases with hydrophobicity of drug molecules. The negative value of ΔGp indicates spontaneous nature of partitioning. The stabilization of systems directly depends on negative value of Kx.

4.3.5.1.5. Relative solubility (St/So)

Relative solubility may be defined as relative increase in solubility after adding surfactant. It gives amount of drug portioning into micelles. It is denoted as St/So where St is total solubility while So is the intrinsic medium solubility [36]. The value of relative solubility increases with surfactant concentration and hydrophobic interaction of additives with micelles.

![Figure 4.32](image_url)

**Figure 4.32.** Change in relative solubility of QUN in micelles of SDS while changing concentration of surfactant.
4.3.5.1.6. Interaction of QUN with CTAB

The cationic surfactant CTAB produces no perturbation in visible spectra of drug. This behaviour is indicative of no interaction between Quinacrine and CTAB because the repulsion between the cationic drug molecules and positively charged head group of cationic surfactant CTAB keeps drug molecules away from micelles in the aqueous phase [37].

4.3.5.2. Conductometric study QUN/SDS interactions

The presence of colloidal particles in solution is indicated by bulk properties. Both attractive and repulsive forces equally contribute toward aggregation phenomenon. Electrostatic repulsion, in case of ionic surfactants, is responsible for repulsive interactions while hydrophobic interaction between non polar ends for attractive interactions [38, 39]. Electrical conductivity measurement has been found to be highly useful for studying association behaviour of drugs and probe the structural changes occurring in system [40]. The conductivity is linearly related to surfactant concentration in both premicellar as well as post micellar concentration. The CMC is determined from intersection of conductivity-concentration plot in pre and post micellar region.

In order to gain an insight into the surfactant/ additive/water (S/A/W) system the conductivity was measured as a function of surfactant concentration at constant additive concentration in temperature ranges of 293-323K. The graphical representation for variation of conductivity of SDS in presence of QUN at varying concentration of SDS is depicted in figure 4.33.

Each plot shows single break point. According to William’s method [14], the critical micelle concentration is obtained from intersection of fitting lines of conductivity-concentration plot above and below the break point. The precision of the method depends upon the width of concentration range over which the change in physical properties is observed.
**Figure 4.33.** Plot of specific Conductivity versus SDS concentration in the presence of QUN at 293K (■), 303K (●), 313K (▲) and 323K (▼).

**Figure 4.34.** Typical plot of specific Conductivity versus SDS concentration in the presence of QUN at 293K.
The Conductivity shows a gradual increase with temperature, which may be due to an increase in thermal energy of molecular entities [40].

4.3.5.2.1. Thermodynamic Parameters

The stability of micellization can be judged by having knowledge about thermodynamic parameters being calculated from CMC i.e. \( \Delta G_m \), \( \Delta H_m \), and \( \Delta S_m \). The \( \Delta G_m \) can be split into entropic and enthalpic contribution. The enthalpy change represents net change in intermolecular forces upon micelle formation. The entropy change measures the change in degree of freedom of solvent and surfactant molecules. The presence of additive (drug molecules) can change these parameters. The physical behaviour of surfactant micelle has been visualized as the construction of model membrane to imitate biological system. This experimental model is being used to study the effects of solubilization on micellization of surfactant and thermodynamics of system. The major driving force for micellization is hydrophobic interaction and value of \( \Delta G_m \) is more negative as hydrophobicity of amphiphile increases. \( \Delta G_m \) becomes more negative with temperature, because temperature tends to drive equilibrium toward hydrophobic bonding and, hence, micellization is favoured. It is clear from results that the value of \( \Delta H_m \) is positive at 293K and negative at higher temperature indicating micellization to be exothermic process at higher temperature. The positive value of \( \Delta S_m \) at 293K and 303K can be attributed to the fact that solubilization of drug causes distraction of more structured water molecules around hydrophobic parts of drug molecules as the latter move from bulk phase to non-aqueous micelle interior. Thus as inferred from decrease in \( \Delta S_m \) and \( \Delta H_m \), the increase in CMC on addition of surfactant should be seen in term of establishment of additional hydrophobic interaction between hydrophobic part of surfactant and that of drug molecules [41-43]. The large positive value of \( \Delta S_m \) is clear indicative of the fact that the system becomes more haphazard after micellization and governing force of micellization is hydrophobic interaction between surfactant monomers resulting in break down of structured water surrounding hydrophobic heads. The entropy gets decreased at higher temperature due to less degree of freedom of hydrophobes.

Table 4.17. Micellar and Thermodynamic parameters calculated for SDS/QUN system
The value of CMC first decreases and then increases with temperature. The effect of temperature on CMC of surfactant in aqueous solution is usually analyzed in term of two opposing factors. First, as the temperature increases the degree of dehydration of hydrophobic heads increases which favours micellization; however, an increase in temperature also causes dehydration of hydrophilic groups and this is not favorable for micellization. It seems from the data that hydrophobic dehydration is predominant till 303K after which hydrophilic dehydration becomes the leading one.

4.3.6. CHLOROQUINE DIPHOSPHATE (CLQ)

The extent of solublization and the site occupied by the solublizate depend upon structural and chemical nature of both the surfactant and solublizate as well as concentration of the specie in solution and temperature. In present work efforts have been made to solublize antimalarial drug Chloroquine \( N' - (7\text{-chloroquinolin}-4\text{-yl})-N, N\text{-diethyl-pentane-1, 4-diamine} \) (CLQ) in the aqueous SDS micellar media in term of concentration variation. It has rigid planar Chloroquinoline ring to which is attached an \( N, N\text{-diethylpentane-1, 4, diamine} \) group. The solublization of drug in SDS micelle is monitored spectrophotometerically by using UV/Visible spectrophotometer as a function of surfactant concentration [40].

4.3.6.1. Simple UV/Visible absorption spectra

A systematic UV/Visible spectroscopic investigation of the samples was carried out to dig out all-possible information about interaction of surfactant with CLQ. These
informations can be utilized to thrash out partition characteristics of drugs with surfactants. The absorption spectrum of pure CLQ is shown in figure 4.35.

![Absorption spectrum of CLQ](image)

**Figure 4.35.** The simple UV/Visible absorption spectra of CLQ in aqueous media.

The peak at 222 nm is selected for study as it shows the maximum absorption. The concentration of CLQ used is $2.0 \times 10^{-5}$. The absorbance increases with [SDS] till micellar domain gets start.
Figure 4.36. Simple absorption spectra of CLQ in the presence of various amounts of SDS.

Figure 4.37. Plot of simple absorbance of CLQ versus molar concentration of SDS.
The Figure 4.36 shows UV/Visible Spectrum of aqueous solution of CLQ as a function of concentration of SDS concentration. It is observed that on addition of SDS in CLQ solution a significant hypochromic shift is produced along with red shift in wavelength. After that absorbance starts increasing till 9.13 mmol/dm3, which is CMC of SDS in presence of CLQ. Beyond the CMC there is very little increase in absorbance [43, 44]. The significant shift in characteristic peak of additive (CLQ) corresponds to association of additive with surfactant molecules.

### 4.3.6.2. Differential UV/Visible Absorption Spectra

Differential spectroscopy helps to study partition of drugs into micelles. Figure 4.38 shows differential UV/Visible spectra of CLQ. The value of Kc, the partition coefficient was calculated using Kawamura model.

![Differential UV/Visible absorbance spectra of CLQ in different SDS concentration.](image)

**Figure 4.38.** Differential UV/Visible absorbance spectra of CLQ in different SDS concentration.

**Table 4.18.** Calculation of parameters indicating partitioning of CLQ in SDS micellar media

<table>
<thead>
<tr>
<th>Cs×103 (Mol/dm3)</th>
<th>ΔA×102</th>
<th>1/ΔA</th>
<th>CMCo×103</th>
<th>Csmo×103</th>
<th>Ca×105</th>
<th>(Csmo+Ca)×103</th>
<th>[ \frac{1}{C_s^{mo} + C_a} ]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS Concentration (moldm⁻³)</td>
<td>∆A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.010</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.015</td>
<td>0.16</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.020</td>
<td>0.20</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
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<td>0.030</td>
<td>0.28</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.39.** Plot of differential absorbance of CLQ as a function of SDS concentration.
Figure 4.40. Relationship between $1/\Delta A$ and $(1/ (Cs +Csmo)$ for the calculation of $K_x$ for CLQ/SDS system.

Model 4.2. Micellization and Solublization.
Model 4.3. Locus of CLQ molecule in micelle.

4.3.6.2.1. Partition Coefficient (Kx)

Kawamura equation helps to calculate partition coefficient using data of differential absorbance. The structure of additive will decide to which extent the solublization may takes place. Kx value for CLQ, in the present case, shows that polar heads of drug molecules are attached with surface of micelle by virtue of electrostatic force of attraction while the non polar ones get penetrated into the hydrophobic core of micelle[44].

4.3.6.3. Comparison between solublization behaviour of Chloroquine and Quinacrine

The Table 4.19 gives a view of comparative study of solublization parameters of both amphiphilic drugs QUN and CLQ. The QUN being more hydrophobic has higher value of partition coefficient that indicates QUN is partitioned between aqueous and micellar phase to much greater extent than CLQ. Similarly free energy of partition is more negative for QUN than in case of CLQ being a sign of more spontaneous solublization in the former than the latter.
Table 4.19. Comparison of solubilization parameters calculated for QUN and CLQ.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Kx $\times$ 10^{-4}</th>
<th>$\Delta G_x$ (kJ/mol)</th>
<th>Kb (dm$^3$/mol)</th>
<th>$\Delta G_b$ (kJ/mol)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUN</td>
<td>22.68</td>
<td>-30.55</td>
<td>400</td>
<td>-14.80</td>
<td>0.44</td>
</tr>
<tr>
<td>CLQ</td>
<td>6.48</td>
<td>-27.50</td>
<td>500</td>
<td>-15.20</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Binding constant is, however, higher and free energy of binding is more negative for CLQ than that of QUN. As molecule of CLQ is relatively smaller than that of QUN so it binds more effectively with surfactants. Molecules of QUN are large in size so less number of this drug are accommodated in the micelle.

4.3.7. Citalopram HBr (CIT)

4.3.7.1. Simple Absorption Spectra

Figure 4.41 shows simple UV/Visible spectrum of CIT in the presence and absence of SDS. The peak at 238 nm shows maximum absorbance. The initial decrease in absorbance suggests that drug forms aggregates of insoluble or less soluble salts or less absorbing specie with SDS. Further addition of SDS resulted in increase of absorbance showing bathochromic shift due to dissolution of dye-surfactant aggregates [45].
The variation in simple absorbance with respect to change in SDS concentration is evident from Figure 4.42. Since CIT is cationic drug and thus have an attraction for anionic surfactant SDS and as a result it is easily incorporated into micelle. The increase in absorbance of CIT indicates that larger number of drug molecules are taken into SDS micelle. After having reached CMC, absorbance becomes almost constant as maximum incorporation of drug molecules into micelle has taken place. Sometimes absorbance increases very slowly even after CMC which is due to incorporation of drug molecules in newly formed micelles [30].

Drug molecules are oriented in the micelle under influence of hydrophilic-hydrophobic forces. For less hydrophobic additive like CIT this balance is prevalent for hydrophilic forces, and thus additive molecules are solublized near the surface region of micelle while for hydrophobic additives, hydrophobic interactions are favored, and additive is solublized deep in palisade layer [46].
Figure 4.42. Plot of simple UV/Visible absorbance as a function of SDS concentration.

CIT being structure breaker has caused CMC to increase due to destruction of water structure, a phenomenon responsible for less increase in entropy. The process of micellization, thus, becomes entropically less favourable. The hydrogen bonding between hydrophilic parts of additive and water molecules do not let the drug molecules to be incorporated deep in micelle and force it to be oriented near micelle water interface. The work of micellization is thus, increased making micellization less opportune and increasing CMC [28]. The red shift in simple UV/Visible absorption spectra is because the micellar phase is less polar than aqueous phase. It is phenomenon in which absorption spectra of additives shift to higher wavelength due to decrease in solvent polarity.

4.3.7.2. The differential spectroscopy

The interaction and binding of drug with surfactant is studied with the help of differential spectroscopy. The increase in value of differential absorption with increasing surfactant concentration suggests stronger interaction between drug molecules and SDS
micelles. The partition of drug molecules between aqueous bulk and micellar phase depends on their hydrophobicity as well as their polarity [31].

Figure 4.43 shows the differential absorption spectra of aqueous solution of CIT in the presence of SDS at a certain concentration of drug. The continuous increase in differential absorbance of CIT with SDS concentration is a sign of persistent inclusion of additive within micelle [47]. The spectral properties of drug have been investigated through a series of absorption experiment under varying amounts of surfactant concentration.

![Figure 4.43. Change in differential absorbance of CIT in the presence of different concentration of SDS.](image)

Figure 4.43. Change in differential absorbance of CIT in the presence of different concentration of SDS.
Figure 4.44. Plots of change in differential absorbance of CIT as a function of SDS concentration.

Table 4.20. Calculation of parameters indicating partitioning of CIT in SDS micellar media

<table>
<thead>
<tr>
<th>Cs×10^3 (Mol/dm^3)</th>
<th>ΔA×10^2</th>
<th>1/ΔA</th>
<th>CMCo×10^3</th>
<th>104×Csmo×10^5</th>
<th>Ca×10^5</th>
<th>Csmo+Ca×10^4</th>
<th>( \left[ \frac{1}{C_{s}^{mo} + C_{a}} \right] \times 10^{-3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.75</td>
<td>3.91</td>
<td>25.57</td>
<td>8.20</td>
<td>0.55</td>
<td>3.75</td>
<td>0.93</td>
<td>10.80</td>
</tr>
<tr>
<td>9.55</td>
<td>4.90</td>
<td>20.41</td>
<td>8.20</td>
<td>1.35</td>
<td>3.75</td>
<td>1.73</td>
<td>5.80</td>
</tr>
<tr>
<td>11.67</td>
<td>5.68</td>
<td>17.61</td>
<td>8.20</td>
<td>3.47</td>
<td>3.75</td>
<td>3.85</td>
<td>2.60</td>
</tr>
</tbody>
</table>
Figure 4.45. Relationship between $1/\Delta A$ and (1/ ($C_s + C_{smo}$) for CIT/SDS system.

Various parameters obtained are given in table 4.21. The value of free energy of partition becomes more negative for more hydrophobic drugs and, thus, is an indicator for ease of penetration of additives into micelles [48-49].

Table 4.21. Various parameters obtained from UV/visible absorption data for CIT/SDS system

<table>
<thead>
<tr>
<th>Kx $\times 10^{-4}$</th>
<th>$\Delta G_p$/kJmol$^{-1}$</th>
<th>Kb</th>
<th>$\Delta G_b$/kJmol$^{-1}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.43</td>
<td>-28.38</td>
<td>375</td>
<td>-14.70</td>
<td>1.85</td>
</tr>
</tbody>
</table>
4.3.8. Pefloxacin Mesylate (PFL)

4.3.8.1. Simple UV/Visible Absorption Spectra

In order to have a deep insight into micellar system, spectroscopic and conductometric measurements were carried out. The UV/Visible spectra of aqueous solution of PFL are shown in Figure 4.46. In the experiment with drug/surfactant/water ternary system, the concentration of drug was kept constant at 1.65x10^{-5}M while that of surfactant was varied from 0.006M to 0.015M. The absorption maxima of PFL shifted toward higher wavelength in presence of SDS. This change is by virtue of penetration of drug molecules into SDS micelles. The low concentration of drug was used so as to avoid its micellization. The spectrum of pure PFL in deionized water shows characteristic peak at 276nm. The Figure 4.47 portrays absorbance spectra of drug/surfactant/water ternary system. It shows a significant shift in the characteristic peaks of drug on addition of surfactants.

Absorbance of PFL-SDS system increases rapidly till CMC while in post micellar region value of absorbance increases very slowly showing a saturation plateau for the reason that maximum incorporation of drug molecules has taken place in micelles as evident in figure 4.48. The drug PFL has increased the CMC of surfactant because drug has structure breaking effect and hydrogen bonding between hydrophilic parts of drug and water cause drug molecules to be oriented near micelle surface inside palisade layer thus increasing work of micellization making process less favourable entropically [28].

Monomer absorbance becomes even more pronounced in micelle than that in absence of surfactant for same drug concentration. The drug (D)-surfactant (S) aggregate formation occurs in following manner. At very low concentration, far below the CMC, there is formation of drug surfactant salt starting with ion pair (D+S-) and continuing with drug surfactant aggregates represented as (D+S-)n. Near and just below the CMC, the progress of reorganization of (D+S-)n aggregates into premicelles with a monomeric D+ content results in an increase in the absorbance in this premicellar region (just below the CMC), showing that presence of premicelles provides drug with micelle-like environment. On further increase in SDS concentration, the absorbance reaches its limiting value and all drug molecules are compartmentalized into normal micelle [30].
The presence of drug PFL increases the CMC of surfactant SDS as also confirmed by conductivity measurement.

**Figure 4.46.** Simple UV/Visible absorption spectra of PFL in aqueous media.
Figure 4.47. Simple UV/Visible absorption spectra of PFL in the presence of different SDS concentration.
Figure 4.48. Plot of absorbance as a function of SDS concentration for PFL.
4.3.8.2. Differential UV/Visible Absorption Spectra

Figure 4.49 demonstrates that the differential absorption increases with increase in surfactant concentration implying stronger interaction between drug molecules and SDS micelles. The increasing value of differential absorption with growing value of SDS concentration indicates that more amount of drug is preferentially being taken into micelle. Hydrophobicity and polarity of drug govern partition of drug within micelle [47-49].

**FIGURE 4.49.** Differential absorbance of aqueous solution of PFL in the presence of different SDS concentration.
FIGURE 4.50. Plot of change in differential absorbance of PFL with SDS concentration.

Table 4.22. Calculation of parameters indicating partitioning of PFL in SDS micellar media

<table>
<thead>
<tr>
<th>Cs×10^3 (Mol/dm^3)</th>
<th>ΔA×10^2</th>
<th>1/ΔA</th>
<th>CMCo×10^3</th>
<th>Csmo×10^4</th>
<th>Ca×10^5</th>
<th>Csmo+Ca×10^4</th>
<th>( \frac{1}{C_s^{mo} + C_a} )×10^-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.82</td>
<td>10.22</td>
<td>9.79</td>
<td>8.40</td>
<td>4.20</td>
<td>1.65</td>
<td>4.37</td>
<td>2.29</td>
</tr>
<tr>
<td>9.37</td>
<td>10.46</td>
<td>9.56</td>
<td>8.40</td>
<td>9.70</td>
<td>1.65</td>
<td>9.87</td>
<td>1.01</td>
</tr>
<tr>
<td>10.00</td>
<td>10.56</td>
<td>9.47</td>
<td>8.40</td>
<td>16.0</td>
<td>1.65</td>
<td>16.20</td>
<td>0.62</td>
</tr>
<tr>
<td>10.71</td>
<td>10.64</td>
<td>9.40</td>
<td>8.40</td>
<td>23.10</td>
<td>1.65</td>
<td>23.30</td>
<td>0.43</td>
</tr>
<tr>
<td>11.54</td>
<td>10.51</td>
<td>9.52</td>
<td>8.40</td>
<td>31.40</td>
<td>1.65</td>
<td>31.60</td>
<td>0.32</td>
</tr>
<tr>
<td>12.50</td>
<td>10.55</td>
<td>9.48</td>
<td>8.40</td>
<td>41.00</td>
<td>1.65</td>
<td>41.20</td>
<td>0.24</td>
</tr>
<tr>
<td>13.64</td>
<td>10.49</td>
<td>9.53</td>
<td>8.40</td>
<td>52.40</td>
<td>1.65</td>
<td>52.60</td>
<td>0.19</td>
</tr>
<tr>
<td>15.00</td>
<td>11.08</td>
<td>9.03</td>
<td>8.40</td>
<td>66.00</td>
<td>1.65</td>
<td>66.20</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Figure 4.51. Relationship between 1/ΔA and (1/ (Cs +Csmo) for the calculation of Kx for PFL/SDS system.
Table 4.23. Different parameters obtained from UV/visible absorption data for PFL/SDS system

<table>
<thead>
<tr>
<th></th>
<th>Kx×10⁻⁴</th>
<th>ΔGp/kJmol⁻¹</th>
<th>Kb</th>
<th>ΔGb/kJmol⁻¹</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>76.11</td>
<td>-33.5</td>
<td>50</td>
<td>-9.70</td>
<td>1.30</td>
</tr>
</tbody>
</table>

4.3.8.3. The conductometric study of partition of PFL in SDS micelle

Interaction between PFL and SDS is studied by conductivity measurement also. The technique has been widely used to obtain the CMC of pure and mixed ionic surfactants in water and thus is an important tool to study the thermodynamics of system. Figure 4.52 shows the behaviour of conductivity for aqueous solution of surfactant in presence of drug. The critical concentration was determined by the intersection of two straight lines of conductivity-concentration plot before and after CMC as shown in figure 4.53. The value of CMC and thermodynamic parameters are shown in Table 4.24.

The CMC of ternary system is greater than that of binary system showing that micellization is delayed due to presence of drug. In accordance with pseudo-phase separation model, the standard free energy of micellization (ΔGmo), enthalpy of micellization (ΔHmo) and entropy of micellization (ΔSmo) for ternary system has been calculated and tabulated under.
FIGURE 4.52. Plot of variation in conductivity of SDS as a function of concentration in the presence of
PFL at 293K (■), 303K (●), 313K (▲) and 323K (▼).

FIGURE 4.53. Typical plot of variation in conductivity of SDS as a function of concentration in the
presence of PFL at 303K.
Table 4.24. Micellar and thermodynamic parameters calculated at different temperatures for PFL/SDS system.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$cmc \times 10^3$ (mol/kg$^{-1}$)</th>
<th>$\Delta H_m$ (kJ/mol$^{-1}$)</th>
<th>$\Delta G_m$ (kJ/mol$^{-1}$)</th>
<th>$\Delta S_m$ (mol J/K mol$^{-1}$)</th>
<th>$\alpha$</th>
<th>$\beta$</th>
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<tr>
<td>293</td>
<td>8.60</td>
<td>94.85</td>
<td>-29.88</td>
<td>425.80</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>303</td>
<td>8.79</td>
<td>66.27</td>
<td>-31.30</td>
<td>322.10</td>
<td>0.42</td>
<td>0.58</td>
</tr>
<tr>
<td>313</td>
<td>8.95</td>
<td>33.20</td>
<td>-33.41</td>
<td>212.81</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>323</td>
<td>8.79</td>
<td>-4.61</td>
<td>-33.82</td>
<td>90.33</td>
<td>0.44</td>
<td>0.56</td>
</tr>
</tbody>
</table>

The large value of entropy shows that micellization is highly entropy supported. However, entropy contribution decreases with temperature and becomes less significant than enthalpic contribution at 323K. As temperature increases, the hydrogen bonding between water molecules diminishes and, therefore, less energy is required to break up the water cluster [12, 48-49].
4.3.9. TRIFLUPERAZINE (TRF)

4.3.9.1. Simple UV/Visible Absorption Spectra

Figure 4.54 shows the simple UV/Visible spectrum of TRF in aqueous media with maximum absorbance at 257nm while Figure 4.55 and 4.56 represent the same in the presence of SDS. The red shift is undoubtedly the consequence of interaction taking place between TRF and SDS. The cations of TRF are being transferred from highly polar phase (water) to less polar site (within micelle).

![UV/Visible absorbance spectra of TRF in aqueous solution.](image)

**Figure 4.54.** UV/Visible absorbance spectra of TRF in aqueous solution.

For TRF, as in case of other drugs, absorbance first increases significantly with increasing concentration of surfactant. However, beyond the CMC this increase is not so noteworthy and curve levels off due to maximum incorporation of drug molecules within
micelles (Figure 4.57). The enhancement in absorbance is attributed to complex formation between SDS and TRF. Similarly TRF being structure breaker causes SDS to micellize at higher concentration. Unfortunately absorbance is not a method sensitive enough to obtain value of CMC; hence the same was determined from the other techniques like conductivity [47-49].

Figure 4.55. Change in absorbance of TRF in the absence and presence of SDS.
**Figure 4.56.** Change in absorbance of TRF in the presence of various concentration of SDS.
**Figure 4.57.** Plot of change in simple absorbance of TRF as a function of SDS concentration.

### 4.3.9.2. Differential Absorption spectra of TRF

Figure 4.58 displays the relationship between differential absorbance of TRF and increasing concentration of SDS. The differential absorbance rises due to greater interaction between two at higher concentration. It is evident that the differential absorbance rises sharply and then becomes constant at higher concentration of surfactant. The increasing value of $\Delta A$ signifies that drug is preferentially taken into micelle with increasing SDS concentration and the leveling off curve indicates the maximum uptake of drug molecules in the micelles [47, 50].

### 4.3.9.3. Determination of partition constant and Binding Constant

Absorption spectroscopy was used to calculate partition coefficient ($K_x$), for TRF cation between micelle and aqueous pseudo phase and binding constant $K_b$, of TRF ion to SDS micelle. The value of $\Delta A$ (differential absorbance) can be used for calculation of
partition coefficient, $K_x$, a thermodynamic parameter that represents the affinity of a given solubilizate to the micellar phase relative to aqueous one [50].

**Figure 4.58.** Differential absorbance of aqueous solution of TRF in the presence of different concentration of SDS.
Figure 4.59. Plot of differential absorbance of TRF with SDS concentration.
Table 4.25. Different parameters indicating partitioning of TRF in SDS micellar media

<table>
<thead>
<tr>
<th>Cs $\times 10^3$ (Mol/dm³)</th>
<th>$\Delta A \times 10^3$</th>
<th>$\frac{1}{\Delta A}$</th>
<th>CMCo $\times 10^3$</th>
<th>Csmo $\times 10^4$</th>
<th>Ca $\times 10^6$</th>
<th>Csmo+Ca $\frac{1}{C_{sm} + C_a}$ $\times 10^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.30</td>
<td>71.54</td>
<td>13.97</td>
<td>8.20</td>
<td>1.30</td>
<td>9.07</td>
<td>1.39</td>
</tr>
<tr>
<td>8.80</td>
<td>90.93</td>
<td>10.99</td>
<td>8.20</td>
<td>5.50</td>
<td>9.07</td>
<td>5.59</td>
</tr>
<tr>
<td>9.60</td>
<td>75.90</td>
<td>13.17</td>
<td>8.20</td>
<td>13.50</td>
<td>9.07</td>
<td>13.60</td>
</tr>
<tr>
<td>1.05</td>
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<td>9.42</td>
<td>8.20</td>
<td>93.00</td>
<td>9.07</td>
<td>93.10</td>
</tr>
</tbody>
</table>
Figure 4.60. Relationship between $1/\Delta A$ and $1/(C_s + C_{smo})$ for the calculation of $K_x$ for TRF/SDS system.
Table 4.26. Different solublization parameters calculated for TRF/SDS system.

<table>
<thead>
<tr>
<th>Kx×10^-4</th>
<th>ΔGp/KJmol^-1</th>
<th>Kb</th>
<th>ΔGb/KJmol^-1</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.70</td>
<td>-27.80</td>
<td>875</td>
<td>-16.80</td>
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</tr>
</tbody>
</table>

4.4. Drug Protein interaction

Protein is a compound of prime importance for our body and life and the main target of medicine in body. The major transport of unesterified fatty acids is carried out by it and it can bind with large number of metabolites, drugs and organic compounds. The most abundant protein in our circulatory system is Human Serum and is, therefore, most studied one [51].

HSA accounts for 60% of total human protein and corresponds to a concentration of 42g/L and provides about 80% of osmotic pressure. Its ionizable group contains 116 acidic groups and 100 basic groups. At pH 7.4 its shape in solution is thought to be a prolate ellipsoid of revolution with major and minor axis with thickness of 12.0 and 2.7 or 14.1 and 4.1nm, respectively, linking three homologues, globular domain in series [52].

Recently three-dimensional structure of HSA has been determined by X-Ray crystallographic measurement. It consists of three structurally homologues domains which assemble to form heart shaped molecule each domain consists of two sub domains. Albumin Serums have ability to bind and transport many ligands, including fatty acids, amino acids, hormones, cations and anions and a variety of pharmaceuticals. It is suggested that principal regions of ligands binding to HSA are present in hydrophobic cavities located in sub domain IIA and IIIA and single tryptophan residue of HSA is in sub domain IIA [53].

HSA undergoes pH dependent conformational transition: the N-F transition between pH 5.0 and 3.5, the F-E transitions between pH 3.5 and 1.2 and the N-B transition between pH 7.0 and 9.0. The N-F isomerization involves unfolding and
separation of domain III from rest of molecule without significantly affecting the latter [54].

Serum albumins are the most extensively studied and applied because of their availability, low cost, stability, and unusual ligand binding proteins. The most outstanding property of albumin, perhaps, is its capacity to bind reversibly a numerous variety of ligands. The physiological importance of albumin as transporting proteins and relative ease with which it can be isolated and purified on a large scale have resulted in a great number of binding studies. Most of ligands are bound reversibly and typical association constant is in range of $10^4$ to $10^6$ M$^{-1}$. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cell in vivo and in vitro. Consequently the mechanism of interaction between bioactive compounds and proteins is important to understand. Many drugs are transported in blood while being bound to albumin mainly as a complex with proteins. The biological activity (efficacy and rate of delivery) of drug is influenced by the nature and magnitude of drug-protein interaction. It is, therefore, important to study the binding parameters in order to know and try to control pharmacological response of drugs and designs of dosage formed. This kind of studies is helpful to provide the salient information on structural feature that determine curative effectiveness of drugs and hence become important research field in chemistry, life sciences and clinical medicines. Serum albumin is, thus, taken as a model for studying drug protein interaction in vitro since it is a major binding protein for drugs and other physiological substance [55].

HSA is produced in liver, exported as nonglycylated protein and present in blood. The proteins containing tryptophan residues have intrinsic fluorescence. Information about these proteins can be obtained by measuring the intrinsic fluorescence intensity of tryptophan unit before and after addition of drugs [56].

Drug protein binding plays a very important role in drug pharmacology and pharmacokinetics and greatly influences the absorption, distribution, metabolism and excretion properties of typical drugs. Moreover, the therapeutic drug effects are related
to the balance between the bound and unbound fraction of drugs. Therefore, studies on this aspect can provide information about the structural feature that determine therapeutic activity of drug and have become an interesting research field [57].

In recent years, several research groups devoted to the study of the interaction of proteins with drugs. We have also tried to contribute in this field while bringing under study the interaction of Human Serum Albumin (HSA) with different drugs i.e. Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ), Pefloxacin Mesylate (PFL), Citalopram HBr (CIT) and Trifluperazine HCl (TRF) using UV/Visible spectroscopy, Florescence spectroscopy and Laser Light Scattering (LLS) technique.

4.4.1. Interaction of Quinacrine Dihydrochloride (QUN) with HSA

4.4.1.1. UV/Visible spectroscopy

The UV/Visible spectra is a very simple method and applicable to explore structural changes and to know complex formation. For HSA a strong absorption peak occurs at 277nm and peak intensity increases with addition of QUN. Figure 4.61 and 4.62 depict absorbance spectra of pure QUN and pure HSA at pH 7.4 respectively. The absorbance of QUN/HSA system increases with increasing drug concentration as evident in figures 4.63. A reasonable explanation of this phenomenon may come from complex formation [58].
Figure 4.61. UV/Visible Absorbance spectra of 0.028mM of QUN at pH 7.4.

Figure 4.62. Absorbance spectra of 2μM of HSA at pH 7.4

Figure 4.63. Absorbance of different concentration of QUN in the presence of 2μM of HSA at pH 7.4
4.4.1.2. Florescence spectroscopy

Florescence technique, being highly sensitive, rapid and to be implemented easily, provides a great aid in study of interaction between drug and plasma protein in general and serum albumin in particular. For macromolecules, this technique is helpful to obtain some information about binding of small substance to protein, such as binding mechanism, binding mode, binding constants and intermolecular distances etc [57].

The intensity of fluorescence decreases by a wide variety of processes called quenching [59]. Fluorescence quenching is decrease of quantum yield of fluorescence from a fluorophore induced by a variety of molecular interaction with quencher molecules, including exciting state reaction, molecular rearrangement, energy transfer, ground state complex formation and collisional quenching process [58].

In principal, both native protein florescence and ligand florescence (if any) can be exploited to study the interactions. However, most of the studies rely on quenching of protein florescence. In order to study the interaction of drug with protein, the concentration of protein is kept constant and increasing concentration of ligand is added. Florescence spectra were recorded at wavelength range of 300-450 nm upon excitation at 278 nm. This excitation selectively excite s tryptophan without letting the tyrosine to excite. Monitoring the quenching of tryptophan florescence yields much better signal to noise ratio than monitoring the increase in ligand florescence. On increasing QUN concentration there is a decrease in HSA florescence but emission maxima doesn’t move toward shorter or longer wavelength. This result shows the interaction between QUN and HSA [55].

4.4.1.2.1. The study of quenching mechanism

Quenching can be classified as either dynamic or static depending upon its mechanism. In dynamic quenching, collision takes place between fluorophore and quencher, and static quenching results from formation of ground state complex between fluorophore and quencher. In general, static and dynamic quenching are distinguished by how they depend on temperature [58]. The quenching constant decrease with temperature for static quenching while for dynamic quenching the case is reverse.
However, possible quenching mechanism may also be interpreted by fluorescence quenching spectra of protein using Stern Volmer equation [56].

Figure 4.65 shows fluorescence emission spectra of HSA in the presence of different concentrations of QUN. The pure HSA shows strong emission band at 368nm. Increasing concentration of QUN caused an altering of microenvironment around tryptophan residue and reducing fluorescence [60]. The results provide evidence about interaction between HSA and QUN

![Figure 4.64. Fluorescence Intensity of 2μM HSA in pure form.](image-url)
Figure 4.65. Fluorescence intensity of HSA (2μM) in presence of different concentration of QUN (from “a” to “i”) 0, 0.028 mM, 0.04 mM, 0.048 mM, 0.056 mM, 0.06 mM, 0.064 mM, 0.072 mM, and 0.076 mM.
There is no fluorescence emission from QUN in range of 300-450nm thus contribution from QUN can be neglected when measuring protein fluorescence intensity at different concentration of QUN. In order to confirm quenching mechanism of HSA with QUN, we have employed Stern Volmer equation and the process is supposed to be dynamic. Within certain concentration, Stern Volmer curve would be linear if the quenching type is dynamic or static. If both static and dynamic mechanisms were involved in quenching, the Stern Volmer plot shows an upward curvature, being concave toward y-axis [61]. Alternatively Stern Volmer plot can deviate from linearity toward x-axis when two fluorophore populations are present but one class is not accessible to quencher. This result is frequently found for quenching of tryptophan fluorescence in protein by polar or charged quencher. These molecules do not readily penetrate the hydrophobic interior of protein and tryptophan residues on the surface of protein are quenched only [55].

Diffusion controlled quenching typically results in the value of $K_q$ near $10^{10}$dm$^3$/mol.s. The value of $K_q$ smaller than this value results from steric shielding of fluorophore or a low quenching efficiency. Apparent value of $K_q$ larger than diffusion controlled limit usually indicates some type of binding interaction. The dynamic quenching parameters of QUN and HSA could be obtained from experimental data using Stern Volmer Equation. The linear fit of experimental data gives value of $K_{sv}$ from slope. Figure 4.66 shows such plot for quenching of HSA fluorescence by QUN. The plot shows that the results agree with Stern Volmer equation within investigated range of concentration [55, 60].

The fluorescence time for biopolymers is 10-8s. The value of quenching constant ($K_q$) in our case, is $2.73 \times 10^{12}$dm$^3$/mol-s-1. Compared with maximum scatter collision quenching constant of various quenchers with biopolymers, $2.0 \times 10^{10}$dm$^3$/mol/s, the rate constant of HSA quenching procedure initiated by QUN is much greater. It may be concluded that the quenching is not initiated by dynamic collision but formation of a new compound. A new complex might have been formed between HSA and QUN, which are responsible for quenching of HSA. In order to verify formation of this complex the UV/Visible Spectra of HSA in presence and absence of QUN has been recorded. The
absorption peak of HSA is recorded at 277nm. The increase in intensity of HSA in presence of QUN is indicative of formation of HSA-QUN complex [56].
4.4.1.2.2. Evaluation of binding constant and binding sites

Figure 4.66. Plots of Fo/F for HSA against [QUN], where [HSA] = 2μM, λex = 280nm and pH =7.4.

When small molecules are bound independently to a set of equivalent sites on macromolecules, the binding constant, Kb and the number of binding sites, n, can be derived as given in Table 4.27. The value of “n” shows that there is about one independent class of binding site on HSA for ligand. The result also shows that there is strong binding interaction between HSA and QUN [56].
Figure 4.67. Plot of log (Fo-F/F) vs log [QUN] for QUN/HSA system at pH = 7.4
Table 4.27. Different parameters calculated from fluorescence of QUN/HSA system at pH = 7.4.

<table>
<thead>
<tr>
<th>Ksv ×10^-3 (dm3mol⁻¹)</th>
<th>Kb ×10⁻³ (M⁻¹)</th>
<th>ΔGb (kJ/mol)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.76</td>
<td>82.00</td>
<td>-28.03</td>
<td>1.28</td>
</tr>
</tbody>
</table>

4.4.2. CHLOROQUINE (CLQ)

4.4.2.1. Interaction of CLQ with Human Serum Albumin (HSA)

After being absorbed in cell, distribution of drug present in plasma is governed by several factors. A proportion of it is bound by plasma protein, thus, forming drug protein complex, a part of it passes to extra cellular space and hence into tissues; another portion of it reaches to site of action. The rest of drug remains unbound in plasma and is in dynamic equilibrium with all these fractions. This unbound drug may, however, suffer from loss by excretion or metabolism, thus, disturbing the balance. It is the only pharmacologically active fraction of drug passing across the membranes separating the compartments. The bound form of protein is regarded as inert, except when bound to specific receptors. The binding of drug to serum albumin may influence the bioavailability and level of response to certain pharmaceuticals.

Physicochemical forces concerned in protein drug interactions include covalent and ionic bonding between polar or nonpolar and ionized groups of drug with a protein molecules; vander Waals forces are probably also involved. Serum proteins have several binding sites for basic drugs but only one or two for acidic drugs. Different drugs compete for same binding site on a protein molecule. The level of unbound drug in plasma will increase if a drug is displaced by the other having greater affinity for that particular site.
Model 4.4. Adsorption of amphiphilic molecules on protein.
The binding of drug to specific site is the result of a number of possible types of interactions, e.g. electrostatic, hydrogen bonding and hydrophobic interactions [62]. Several pharmaceutical drugs including certain antibiotics bind reversibly with serum albumins to different extents, depending upon their side chains functional groups [63].

The florescence probe methods have emerged as an important tool for biomembrane research. Its advantages are high degree of sensitivity and low degree of membrane perturbation.

UV/Visible absorption measurement is a way to explore the structure change and complex formation between HSA and CLQ. The UV/Visible absorption spectra of the HSA-CLQ were measured under simulative physiological conditions. The absorbance of HSA increases with the increase in CLQ concentration as shown in Figure 4.68 [64].

![Figure 4.68](image)

**Figure 4.68.** Absorbance of different concentration of CLQ in the presence of 2.0μM of HSA at pH 7.4.
4.4.2.2. Analysis of Fluorescence quenching of HSA by CLQ

The fluorescence of HSA comes from tryptophan, tyrosine and phenylalanine residues. Actually, the intrinsic fluorescence of HSA is almost contributed to tryptophan alone, because phenylalanine has a very low quantum yield and fluorescence of tyrosine is almost totally quenched if it is ionized or near amino group or carboxylic group or tryptophan. When small molecule binds to HSA, the changes in intrinsic fluorescence intensity of HSA are induced by the microenvironment of tryptophan residue [57]. Figure 4.69 shows fluorescence spectra of 2μM of HSA. The maximum fluorescence intensity is visible at 369nm. Figure 4.70 displays that fluorescence intensity shows gradual decline with increasing concentration of CLQ which behaves as quencher for HSA.

Although the fluorescence of HSA decreases with CLQ concentration but maximum wavelength of HSA does not undergo significant change. Under condition of fixed pH, temperature and ionic strength, fluorescence quenching may result from ground complex formation, energy transfer and dynamic quenching process [65].

Dynamic quenching refers to process in which the fluorophore and quencher come into contact with each other during lifetime of excited state, where static quenching refers to fluorophore-quencher complex formation. To shed light on fluorescence quenching mechanism, the quenching data was analyzed by the modified Stern Volmer Equation.

The binding strength of drug to HSA is a main factor for its availability to diffuse from circulatory system to target. Most of ligands bound reversibly and exhibit moderate affinity for proteins (its binding constant is in the range of 1-15×104M-1). So the value of Kb shows that binding between HSA and CLQ is moderate, which indicates that a reversible drug-HSA complex formation takes place and drug can be stored and carried in body by HSA [59].
Figure 4.69 Florescence spectra of 2µM of HSA at T=298K.

Figure 4.70. Plot of fluorescence intensity for HSA as a function of CLQ concentration where [HSA] = 2µM, λex = 280nm and pH = 7.4.
**Figure 4.71.** Plots of Fo/F for HSA against [CLQ] at [HSA] = 2μM, λex = 280nm and pH 7.4.

**Figure 4.72.** Plot of log (Fo-F/F) vs log [CLQ] for CLQ/HSA system at pH 7.4

### 4.4.2.3. Comparison between binding parameters of CLQ and QUN

The binding parameters of CLQ and QUN for HSA have been made clear in the table 4.28. The greater value of Quenching constant as well as binding constant for QUN
than for CLQ is indicative of greater binding ability of QUN. Binding energy for QUN is more negative pointing toward more spontaneous binding of QUN. Number of binding sites is 1 for both drugs that hints that only one binding site is available for binding of each of two aforesaid drugs.

Table 4.28. Comparison between binding parameters of CLQ and QUN with HSA.

<table>
<thead>
<tr>
<th>Drug/HAS System</th>
<th>$K_q \times 10^{-11}$ (dm$^3$/mol.s)</th>
<th>$K_b \times 10^{-4}$ (M$^{-1}$)</th>
<th>$\Delta G_b$ (kJ/mol)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUN/HSA</td>
<td>19.80</td>
<td>82.00</td>
<td>-28</td>
<td>1.28</td>
</tr>
<tr>
<td>CLQ/HSA</td>
<td>6.50</td>
<td>16.60</td>
<td>-24</td>
<td>1.00</td>
</tr>
</tbody>
</table>

4.4.3. Interaction of PFL and TRF with Human Serum Albumin (HSA)

Although we have carried out interaction of QUN, CLQ, PFL, and TRF with HSA utilizing florescence and UV/Visible spectroscopy. QUN and CLQ have been proved to be efficient quenchers for HSA while PFL and TRF exhibited no such activity. UV/Visible spectroscopy, however, has provided evidence for interaction of said drugs with HSA. The increase in UV/Visible absorbance of PFL and TRF is depicted in Figure 4.73 and 4.74 respectively. Ascending behaviour in absorbance provides a clue for interaction between drugs and protein and, thus, supports conclusion drawn from fluorescence data.
Figure 4.73. Absorbance spectra of different concentration of PFL in presence of 2×10-6M of HSA at pH 7.4

Figure 4.74. Absorbance spectra of different concentration of TRF in presence of 2×10-6M of HSA at pH 7.4
4.4.4. CYCLIC VOLTALMETTRY (CV)

Trifluperazine (TRF) is an electrochemically active compound and in present work our interest is devoted to the study of its interaction with HSA. Figure 4.75 shows cyclic voltamograms of pure TRF and that in the presence of various concentration of HSA. On addition of HSA, a decrease in cathodic current without significant shift was observed. Two factors may be considered for lessening in reductive peak current. First, is competitive adsorption of TRF and HSA on GCE (Glassy carbon electrode) and the second one is formation of electro inactive complex.

The CV of antidepressant drug Trifluperazine 2HCl indicates the irreversible, one step, oxidation to an electrochemically inactive product. In the presence of increasing amount of HSA, the peak current of TRF decreases which can be attributed to the interaction between HSA and TRF [66-69].

![Figure 4.75. CV spectra of TRF in the presence of different concentration of HSA at pH 7.4.](image)

4.4.5. Laser Light Scattering Study (LLS)

Reversible binding of physiological active compounds (PAC) to serum albumins has grabbed attention of wide circle of research workers. In this work the effect of pH on binding of some of PACs like Clindamycine Phosphate (CLN), Quinacrine 2HCl (QUN), Chloroquine diphosphate (CLQ) and Citalopram HBr (CIT) on the size and sub molecular structure of globular anionic protein HSA has been studied using Dynamic
Laser light scattering (DLS). The aim of study was to elucidate the peculiarities of PAC’s influence on albumin conformation.
4.4.5.1.1. Study of QUN/HSA system by DLS

DLS has been performed to determine size of drug/protein complexes expressed as hydrodynamic radius. The gradual increase in Rh shows strong electrostatic interaction between drug and protein. This phenomenon suggests saturation rather than denaturation process. Magnitude of this change is not sufficient to account for any appreciable unfolding or extension [70].

Many studies have revealed that HSA has lot of binding sites for many drugs and interaction between them will cause conformational changes. The interaction between Quinacrine 2HCl and HSA in different buffer solution was investigated by DLS. Analysis of QUN/HSA system as a function of QUN concentration has provided information on Hydrodynamic radius (Rh).

Figures 4.76 (A-D) shows some representative plots of distribution of hydrodynamic radius of QUN/HSA complex at pH 7.4 in the presence of increasing concentration of QUN while HSA concentration is 0.1%. There are two peaks evident at 1.48mM; the larger one is for protein. The second smaller peak corresponds to a very limited association of HSA or due to formation of very small fraction of clusters produced by complex association. Intensity of this peak increases with increase in drug concentration. Pure HSA gives only one peak showing Rh value at 3.23nm. This peak broadens in presence of drug. At low drug concentration there is no appreciable change in Rh upon binding. At higher drug concentration, however, due to increase in size of drug/protein complex, broadening in peak is observed.

Figures 4.77 (A-D) shows some selected plots of Particle size distribution of Quinacrine 2HCl in presence of 0.1% HSA at pH 3.0 in aqueous solution of different concentration of Quinacrine 2HCl below CMC. The peak broadens as drug concentration increases. At 1.48mM small proportion of large aggregates having average size in range of 40-50nm was detected whose origin may be related to partial association of HSA or due to formation of a small fraction of clusters produced by complex association [72-73].

Figure 4.78 displays that in QUN/HSA system, non cooperative binding takes place till 6.2mM of Quinacrine concentration at pH 7.4 while cooperative binding occurs till 3.08 mM after this concentration of drug saturation region gets started. However at
pH 3.0, for the same system non cooperative binding takes place till 1.4mM while cooperative binding occurs till 14.0 mM after this concentration we enter in saturation region. At pH 3.0 Rh values is greater than at pH 7.4 and there is rapid increase in Rh within cooperative binding region at pH 7.4 as compared to pH 3.0. This is because at pH 3.0, protein is already in expanded form.

**Figure 4.76.** Representative plots of particulate size distribution in aqueous solution of different concentration of Quinacrine 2HCl where pH is 7.4 and [HSA] = 0.1%.
Figure 4.77. Representative plots of particle size distribution in aqueous solution of different concentration of Quinacrine 2HCl where pH is 3.0 and [HSA] = 0.1%.
Figure 4.78. Plot of Hydrodynamic radii of HSA/QUN complexes versus concentration of QUN at
pH=3.0 and pH=7.4.

Table 4.29. Hydrodynamic radii of HSA/QUN complexes at two different pH values.

<table>
<thead>
<tr>
<th>[QUN]/mM</th>
<th>Rh(nm) at pH 7.4</th>
<th>Rh(nm) at pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure HSA</td>
<td>3.23</td>
<td>3.89</td>
</tr>
<tr>
<td>1.48</td>
<td>3.32</td>
<td>3.97</td>
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<td>2.83</td>
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</tr>
<tr>
<td>19.30</td>
<td>5.60</td>
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</tr>
</tbody>
</table>
4.4.5.1.2. CLQ/HSA system

Studies of interaction of complexes formed between protein and amphiphilic molecules in aqueous solution has been center of attention for scientists since few years and great progress has been made in this field. The understanding of such system is of great importance in many biological and clinical uses of drugs.

Figures 4.79 (A-D) shows selected plots for particle size distribution of CLQ/HSA complex in pre micellar region of drug at pH 7.4. Pure HSA gives only one peak showing Rh value at 3.63nm. Presence of drug molecules makes this peak broad owing increase in size of drug/protein complex. At 10mM another peak appears due to formation of associated drug/protein complex present as a small fraction but providing a significant scattering intensity due to large size. The increasing value of Rh owe to strong electrostatic interaction between drug and protein [70-73]. Intensity of second peak increases with the increase in drug concentration.

Selected plots of Particle size distribution of CLQ and 0.1% HSA at pH 3.0 in aqueous solution of different concentration drug below CMC are shown in Figures 4.80 (A-D). The peak widens as we increase drug concentration. At 27mM very small proportion of large aggregates of average size 40-50nm was detected whose origin is related to a very limited association of HSA or due to formation of very small fraction of clusters produced by complex association [72-73].

Figure 4.81 shows the plot of hydrodynamic radius as a function of CLQ concentration at pH 3.0 and 7.4. It is clear that in CLQ/HSA system, non cooperative binding happens till 27mM of CLQ concentration at pH 7.4, while cooperative binding occurs till 53.8mM. After this concentration of drug, there is beginning of saturation region. However, at pH 3.0, for the this system non cooperative binding occurs till 20mM while cooperative binding happens till 44.8mM after this saturation region is reached. In CLQ/HSA system, as in QUN/HSA, system value of Rh is greater at pH 3.0 than at pH 7.4. The increase in value of Rh is greater at pH 3.0 than at pH 7.4 within domains of cooperative binding region due to pre expanded form of protein at acidic pH.
Figure 4.79. Representative plots of particle size distribution in aqueous solution of different concentration of Chloroquine diphosphate where pH is 7.4 and [HSA] = 0.1%.
Figure 4.80. Representative plots of partial size distribution in aqueous solution of different concentration of Chloroquine diphosphate where pH is 3.0 and [HSA] = 0.1%.
Figure 4.81. The plot of Hydrodynamic radii of HSA/CLQ complexes versus concentration of CLQ at pH=3.0 and pH=7.4.

Table 4.30: Hydrodynamic radii of HSA/CLQ complexes at two different pH values.

<table>
<thead>
<tr>
<th>[CLQ]/mM</th>
<th>Rh(nm) at pH 7.4</th>
<th>Rh(nm) at pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (pure HSA)</td>
<td>3.23</td>
<td>3.89</td>
</tr>
<tr>
<td>8.00</td>
<td>3.34</td>
<td>3.97</td>
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<td>10.00</td>
<td>3.42</td>
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<tr>
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<td>5.96</td>
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<td>6.04</td>
</tr>
<tr>
<td>100.00</td>
<td>5.89</td>
<td>6.15</td>
</tr>
</tbody>
</table>
4.4.5.1.3. CIT/HSA system

The variation in size of drug/protein complexes has been studied by using dynamic laser light scattering. The increase in value of hydrodynamic radius (Rh) with increase in number of drug molecules, at pH 7.4, being adsorbed on protein surface as shown in figures 4.82 (A-D), which offers a clue about the distribution of Rh value of CIT/HSA complex in presence of increasing concentration of CIT. The strong electrostatic interaction between drug and protein is evident from gradual increase in Rh value of drug. Thus we are able to conclude, unmistakably, that adsorption of drug on protein surface is saturation process rather than denaturation and no palpable unfolding or extension may be possible [72]. Pure HSA produces a single peak with Rh value at 3.63nm. The broadening in peak is, however, observed at higher drug concentration, owing to polydispersity and increase in size of drug/protein complex.

Figures 4.83 (A-D) show some selected plots of Particle size distribution of Citalopram HBr (CIT) in presence of 0.1% HSA at pH 3.0 in aqueous solution of different concentration of aforementioned drug in premicellar region. The peak goes on broadening with the increase in concentration. At 1.6 mM concentration of CIT very small proportion of large aggregates of average size 40-50 nm was detected. The limited association of HSA is responsible for formation of this peak [70-73].

Figure 4.84 shows that non cooperative binding happens till 1.6mM of CIT concentration at pH 7.4 while cooperative binding occurs till 5.0mM after this concentration of drug there is beginning of saturation region. However at pH 3.0, for the this system non cooperative binding occurs till 1.6mM while cooperative binding happens till 4.5mM after which we have saturation region. At pH 3.0, Rh values is greater than at pH 7.4 and there is rapid increase in Rh within cooperative binding region at pH 7.4 than at pH 3.0 due to already in expanded form of HSA at acidic pH.
Figure 4.82. Representative plots of particle size distribution in aqueous solution of different concentration of Citalopram 2HBr where pH is 7.4 and [HSA] = 0.1%.
Figure 4.83. Representative plots of particle size distribution in aqueous solution of different concentration of Citalopram 2HBr where pH is 3.0 and [HSA] = 0.1%.
**Figure 4.84.** Plots of Hydrodynamic radii of HSA/CIT complexes versus concentration of CIT at pH=3.0

and pH=7.4.

**Table 4.31.** Hydrodynamic radii of HSA/CIT complexes at two different pH values.

<table>
<thead>
<tr>
<th>[CIT]/mM</th>
<th>Rh(nm) at pH 7.4</th>
<th>Rh(nm) at pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure HSA</td>
<td>3.23</td>
<td>3.89</td>
</tr>
<tr>
<td>0.64</td>
<td>3.30</td>
<td>3.96</td>
</tr>
<tr>
<td>0.82</td>
<td>3.41</td>
<td>4.01</td>
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<tr>
<td>1.15</td>
<td>3.50</td>
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<td>1.334</td>
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<td>4.68</td>
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</tr>
<tr>
<td>3.70</td>
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<td>4.94</td>
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<td>4.50</td>
<td>4.63</td>
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</tr>
<tr>
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<td>4.79</td>
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<td>7.14</td>
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<td>5.38</td>
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<tr>
<td>10.00</td>
<td>5.00</td>
<td>5.41</td>
</tr>
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</table>
4.4.5.1.4. CLN/HSA system

Figures 4.85 (A-D) show selected plots for Particle size distribution in aqueous solution of different concentration of CLN in the presence of 0.1% HSA at pH 7.4. The electrostatic force of attraction between drug and protein causes Rh to rise gradually. This phenomenon implies neither denaturation nor unfolding or extension but a saturation process [70].

Two peaks were observed at 1.4mM CLN concentration. The first peak was assigned to the pure protein and the smaller one to aggregates having size in the range of 40-50nm. At lower concentration of drug there is little change in Rh of drug/protein complex upon binding. However, size of said complex increases with the increase in drug concentration [70-73].

Figures 4.86 (A-D) show some selected plots of Particle size distribution of Clindamycin phosphate (CLN) and 0.1% HSA at pH 3.0 in aqueous solution of different concentration of CLN. The broadening is produced in this peak as we go on increasing drug concentration. At 0.52mM very small proportion of large aggregates of average size 40-50nm were observed as limited association among molecules of HSA takes place [70-73].

Figure 4.87 indicates that in case of CLN/HSA system, non cooperative binding happens till 1.115mM of CLN concentration at pH 7.4 while cooperative binding occurs till 3.08mM after this concentration of drug there is beginning of saturation region. However, at pH 3.0, for the this system non cooperative binding occurs till 1.4mM while cooperative binding happens till 4.0mM after which we have saturation region.
Figure 4.85. Representative plots of particle size distribution in aqueous solution of different concentration of CLN where pH is 7.4 and [HSA] = 0.1%.
Figure 4.86. Representative plots of particle size distribution in aqueous solution of different concentration of CLN where pH is 3.0 and [HSA] = 0.1%.
Figure 4.87. Plot of Hydrodynamic radii of HSA/CLN complexes as a function of CLN concentration at pH=3.0 and pH=7.4.

Table 4.32.: Hydrodynamic radii of HSA/CLN complexes at two different pH values.

<table>
<thead>
<tr>
<th>[CLN]/mM</th>
<th>Rh(nm) at pH 7.4</th>
<th>Rh(nm) at pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>3.23</td>
<td>3.89</td>
</tr>
<tr>
<td>0.52</td>
<td>3.39</td>
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<td>5.41</td>
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<tr>
<td>10.0</td>
<td>5.20</td>
<td>5.44</td>
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Surface tension of aqueous solutions of aforementioned amphiphilic drugs was measured at 303K and specific conductivity in the temperature range of 293-323K. The results obtained from these techniques were successfully employed for the determination of surface and thermodynamic parameters. The surface tension enabled us to conclude that distance between drug monomers at the surface is due to linking of hydrocarbon rings in the structure of drugs that causes molecules to be separated at interface. Conductivity data makes possible for us to detect even two critical concentrations for trifluperazine, fluphenazine, Quinacrine and Chloroquine indicating that this technique is more sensitive toward structural rearrangements taking place in bulk of solution. The surface tension data, on contrary, gives one value of critical concentration due to formation of drug monolayer at air-water border. Free energy of micellization became more negative with increase in temperature showing more spontaneity of micellization at higher temperature.

Partition behaviour of some amphiphilic drugs were studied with the help of UV/Visible spectroscopy. The transfer of drug molecules from aqueous phase to organic micellar phase provides a model to foretell passage of drug molecules across the biological membranes. Shifts monitored in UV/Visible spectra of drugs in the presence of surfactant indicated that interactions were taking place between drugs and surfactants. The structure and chemical nature of drugs decide whether drug molecules are adsorbed at the micellar surface or get penetrated inside micelle and to which extent. Orientation of drug molecules in micelle is governed by hydrophilic-hydrophobic balance. The partition coefficient not only provides an idea about mechanism of solubilization but also helps to understand how drug is partitioned through biological membranes within living body. The value of partition coefficient of Quinacrine (being more hydrophobic) was found greater than that of Chloroquine.

The complexation of amphiphilic drugs, Quinacrine and Chloroquine, with HSA at physiological conditions showed that only one binding site of fluorescent protein HSA was available for these drugs. Both drugs reduced the fluorescence intensity of HSA and a complex was formed between drug molecules and HSA by static quenching procedure.
The results from UV/Visible spectroscopy also supported the interaction taking place between drugs and protein.

The results obtained from dynamic laser light scattering showed that hydrodynamic radius increases with increase in drug concentration, which was attributed to the binding of drugs with protein.
Recommendations for Future Study

This project is interdisciplinary; it coordinates chemistry, biology and pharmacology. The results obtained are helpful to have knowledge about interactions of various drugs with human blood protein and body membranes. For researchers who want to extend this work in future, following suggestions are recommended.

- This study may be extended to other drugs, surfactants and proteins and then decision should be made about usefulness of a drug and duration to which it can produce the desired result.
- Interaction of various drugs with real bio membranes e.g. Kidney membranes, liver membranes etc. may be worked out under physiological conditions.
- Efficiency of drugs should be related to its thermodynamic properties.
- Human friendly surfactants should be searched out and their micelles should be used as drug carrier in body.
- Computer aided drug designing should be applied to design more drugs that may be more effective and more efficient for binding with proteins and membranes.