BINDING OF FERROCYPHEN BY SDS, CTAB AND TRITON X-100 IN WATER-ETHANOL CO-SOLVENT

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Abstract

The binding of Ferrocyphen $[Fe(phen.)_2(CN)_2]^{2+}$ to sodium dodecylsulphate (SDS), cetyltrimethylammonium bromide (CTAB) and Triton X-100 surfactants was studied spectrophotometrically in water-ethanol medium. The equilibrium binding constant (K_b) and the number of binding sites (n) per surfactant monomer were obtained from Scatchard plots. The change in free energy of binding ΔG_b, and the change in free energy of dissociation of bound complex per binding site $\left[-\Delta G_b/n\right]$ were calculated from K_b. It is proposed that ethanol stabilizes the surfactant monomers when compared with hydrophobic hydration and hence enhances hydrophobic binding. K_b values suggest that the binding is driven largely by favourable entropic contribution moderated primarily by hydrophobic and electrostatic interactions. The entropic, hydrophobic and hydrogen bond interaction effects are suggested to go through multiple equilibria steps. For CTAB the results indicate preferential electrostatic interaction between π – electron rich phenanthroline ligands of the metal complex and the cationic ammonium head group over hydrophobic interaction.

1. Introduction

The bulky ferrocyphen [Dicyano-bis-(1,10-Phenanthroline) Iron II] complex, with significant hydrophobic character and the three surfactants, SDS (sodium dodecylsulphate), CTAB (cetyltrimethylammonium bromide) and Triton X-100 were especially selected for their overall varying hydrophobicity. Since binding in some biological systems is predominantly hydrophobic in origin (Tanford, 1978) the present study may give at least some qualitative insight into such interactions, where ethanol is a moderating substrate. Hydrophobicity is significant not only in micelle formation but also in binding or surface adsorption (Shigeyosh *et al.*, 1989)

Surfactants are used extensively in drugs, food industry and environmental clean-ups. In most of these applications, surfactants are used with a number of additives to improve their effectiveness as binders, solubilizers and emulsifying agents, alcohol being frequently used (Griffith, 2002). Substrate-surfactant binding studies, in addition to yielding information on the effectiveness of substrate additives as binders, are important for biological reasons and attempts have been made to use such binding studies to mimick important biological processes (Linda *et al.*, 1981). Recently, reverse micelle environment has been proposed to provide the best conditions for mimicking reactions in cells. One of the most common biological processes is the reversible combination of ligand with known sites on the surface of biomacromolecules (Saboury, 2002). For example Solubilization of membrane proteins like serotonin (Shanti and Amitabha, 2005) is known to depend on ligand (small ion or molecules) binding affinity while the presence of additives affect the hydrophobic bonds in enzyme protein binding (Marilyn and Alfred, 1967). In fact most biological functions have binding as a primary process. For example, a protein complex like RNA polymerase has to bind to DNA to be able to perform a DNA template directed RNA synthesis (Karstern, 1997). Many of these bindings have strong hydrophobic contributions which vary significantly in the presence of a co-solvent or additive like ethanol.

Ethanol used as co-solvent in the present study, is known to affect the onset of micellization in ionic and neutral surfactant media (Porter, 1991), an interaction that is likely to affect binding sites and the energetics of the binding process. In the present work, the free energy of binding and the corresponding changes in hydrophobic interactions and number of binding sites are examined as a function of ethanol composition. Ferrocyphen is known to have a high affinity for ethanol in which it is quite soluble (Mounir, 1999). The role of ethanol in these interactions is also of interest. Alcohol transferrase, a protein which metabolises alcohol in the liver, must first bind the alcohol prior to oxidation. Ethanol which has been investigated as a hydrotrope, is proposed to undergo self association in aqueous solution with the ability to modify the amphiphilic properties of surfactants (Zana, 1995) by modifying the properties of water and hence the chemical potential of the free surfactant.

2. Experimental

SDS and CTAB (BDH) were used as supplied without further purification. Their degree of purity

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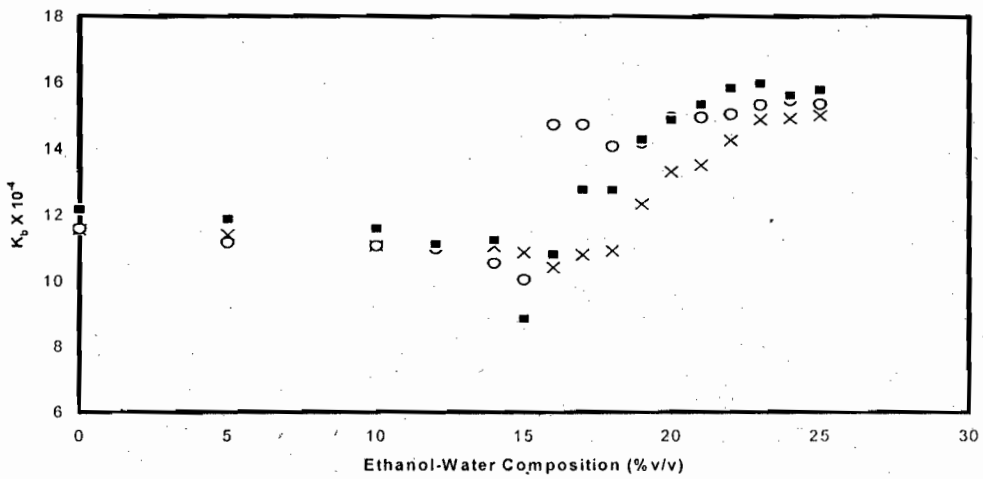


Fig. 3: The variation of K_b versus ethanol-water composition (%v/v) at 25 °C. SDS (×), CTAB (o), TX-100 (●)

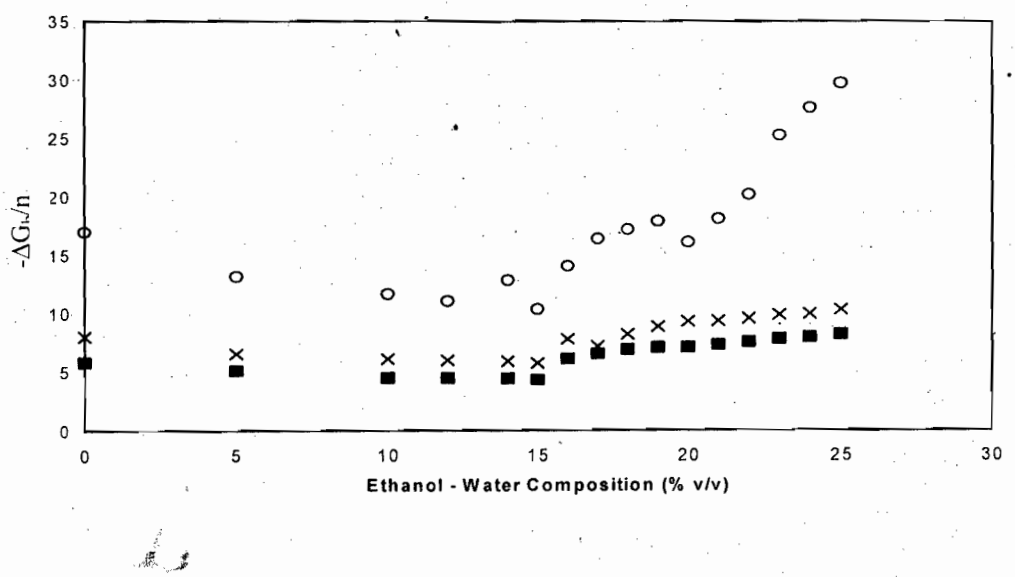


Fig. 4: The variation of $-\Delta G_b/n$ versus ethanol-water composition (%v/v) at 25 °C. SDS (×), CTAB (o), TX-100 (●)

factors responsible for the observed structures [maximum, minimum] in the binding profiles [Fig. 3 and 4].

The lowering of the Gibb's free energy is entropy driven by equilibria [9] and [13], that is ethanol-dehydration of the surfactant monomer and hydrophobic interaction of the bound complex and ethanol molecule respectively.

$$Ferrocyphen + S.\beta H_2O \overset{K_1}{\rightleftharpoons} [Ferrocyphen.S + \beta H_2O] \quad (8)$$

As the concentration of ethanol increases, ethanol being comparatively more hydrophobic than water, more water molecules in the immediate hydration shell of the surfactant monomer [equilibrium 9] are replaced.

$$EtOH + S.\beta H_2O \overset{K_2}{\rightleftharpoons} [EtOH.S] + \beta H_2O \quad (9)$$

The physical origin of hydrophobic hydration is well discussed in the literature (Finney and Soper, 1994). Equilibrium [9] is represented approximately in more detail by scheme 1 below.

Scheme 1 is supported by the fact that alcohol-water mixtures are better solvents for surfactants. The affinity of mixed water-alcohol for surfactants is so strong that at greater than 25 % ethanol, the monomers are preferentially stabilized over micelle formation.

The transition from configuration I to II must be accompanied with a less positive entropy change since EtOH which is removed from the water to the surfactant environment is more bulky than H_2O molecules, resulting in less distruption of the water structure. This coupled with the comparatively less hydrophobic monomer in configuration I accounts for the initial decrease in K_b at low alcohol composition [Fig. 3]. *Ferrocyphen* is bound by the surfactant in configuration I or II. Configuration II obviously provides a stronger hydrophobic interaction. This is compatible with what is found in the solubilisation of alcohols where CH_3CH_2 is directed in the hydrophobic region and the -OH can remain on the surface where it is largely hydrated (Stilb, 1981). At high alcohol composition, configuration II is predominant, so that the stronger hydrophobic binding provided by configuration II leads to an increase in K_b. Increase in binding site hydrophobicity normally increases binding ability between a neutral hydrophobe and the surfactant.

Binding of the *Ferrocyphen* hydrophobe in configuration I and II is cooperative, and the number of available binding sites per monomer will therefore initially increase with the addition of alcohol and will decrease at high alcohol composition when only configuration II is predominant. Competitive binding to configurations I and II adequately explains the variation in $[-\Delta G_b/n]$ and n as functions of alcohol composition [Fig. 2 and 4].

As explained by scheme 1, the entropy change accompanying *Ferrocyphen* binding to the surfactant monomer in its initial transition from configuration I to II for which some of the hydration water molecules have been displaced by EtOH, is a lower positive value. This process contributes less to the lowering of the binding Gibbs free energy and is reflected as a decrease in K_b. Simultaneously the established affinity of the metal complex for ethanol via H-bonding between N of the cyano ligands and -OH of the alcohol, implies that the metal complex will readily bind ethanol molecules. Further evidence for strong H-bonding tendency of cyano ligands of transition metal complexes was reported by present authors (Ojo et al., 2006). The ethanol bound complex [equilibrium 10]

$$EtOH + Ferrocyphen \overset{K_3}{\rightleftharpoons} EtOH.Ferrocyphen \quad (10)$$

is entropically more favourably bound to the surfactant monomer because it is more bulky and will displace more water molecules in the immediate hydration shell of the surfactant monomer [equilibrium 11], with the ethyl group enhancing hydrophobic interaction.

$$EtOH.Ferrocyphen + S.\beta H_2O \overset{K_4}{\rightleftharpoons} EtOH.Ferrocyphen.S + \beta H_2O \quad (11)$$

This effect predominates at high ethanol concentrations, and explains the observed increase in K_b as ethanol composition is increased [Fig. 3].

There are three other possible equlibria [12 to 13] which must also be taken into consideration.

$$EtOH + Ferrocyphen \overset{K_5}{\rightleftharpoons} EtOH.Ferrocyphen.S \quad (12)$$

$$Ferrocyphen.S + EtOH \overset{K_6}{\rightleftharpoons} EtOH.Ferrocyphen.S \quad (13)$$

Addition of equilibria (8) to (13) gives

$$Ferrocyphen + EtOH + S.\beta H_2O \overset{K_b}{\rightleftharpoons} EtOH.Ferrcyphen.S + \beta H_2O$$

the overall binding equilibrium.

The binding constant K_b is hence composite and is given by

$$K_b = K_1 K_2 K_3 K_4 K_5 = \prod_{i}^{6} K_i \qquad (14)$$

At high ethanol concentrations, the affinity of the metal complex for the alcohol will enhance equilibria (10) and (11) and will contribute to increased K_b values. This affinity predominates over the hydrotropic ability of ethanol. This can only further enhance the surfactant binding of the metal complex. Large positive K_b values [Fig. 3] show that binding is spontaneous for all the three surfactants. In figure 4, we have plotted [$-\Delta G_b/n$] against ethanol-water composition so that obtained values reflect the tendency of the bound complex to dissociation from the surfactant. Hence relatively high positive values reflect comparatively less strongly bound complex. The strength of binding or stability of bound complex is hence CTAB<SDS<Triton X-100.

Triton X-100 the most hydrophobic of the surfactants is most stabilized by ethanol and most strongly binds the Ferrocyphen. In the study (Guiseppe *et al.*, 1991) of the solubilisation of doubly charged Iron [II] complexes in aqueous neutral micelles, the degree of binding of the iron [II] complexes to the neutral micelles increases as the co-ordinated ligand to the metal becomes increasingly hydrophobic. Our results are in excellent agreement with these observations. In addition, Triton X-100 has more sites for hydrogen bonding via the –OH end group and oxygen atoms of the oxy-ethylene units. This coupled with its tertiary octly group para to the phenoxy group accounts for high n values relative to the two other surfactants.

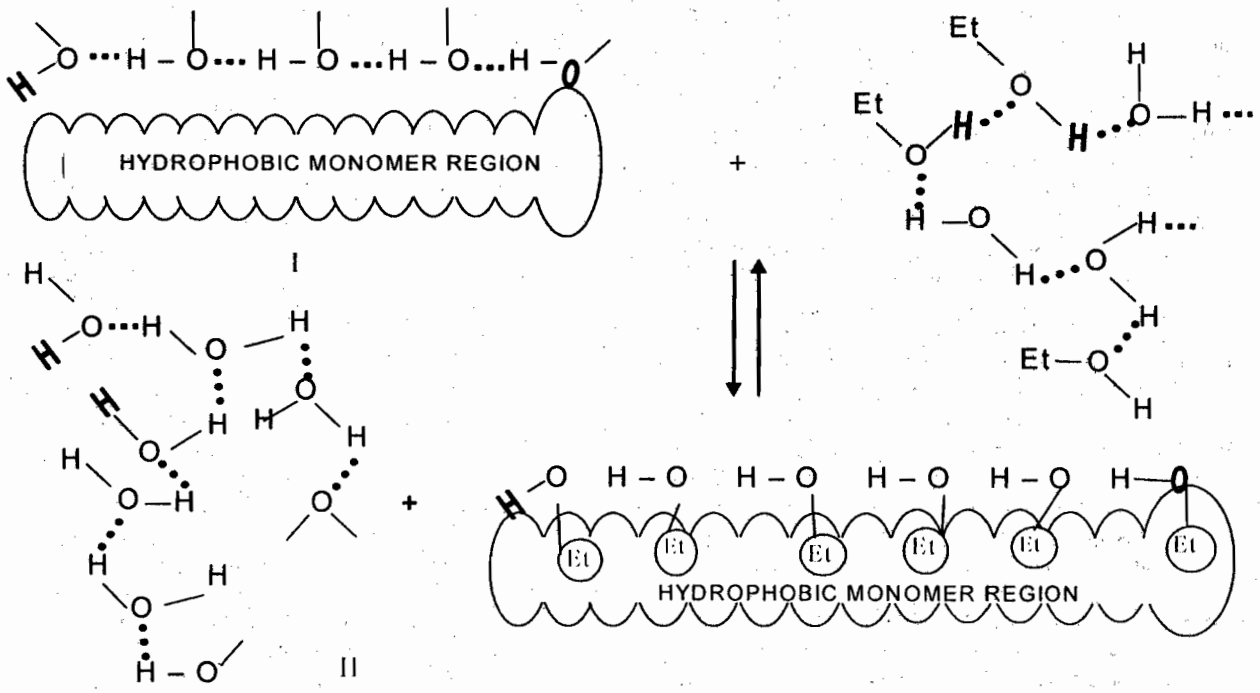
There is deeper penetration of aromatic compounds into SDS micelles relative to CTAB micelles (Fornasiero *et al.*, 1988). Multi-parameter analysis (Quina *et al.*, 1995) of non-ionic solubilisates into micelles shows that hydrophobic interactions are more efficient for the solubilisation process with the binding of the solubilisates in the hydrophobic region. The bulky ferrocyphen hydrophobe fits into this category. The same analysis suggests that micellar microenvironment is less efficient hydrogen-bond donor than bulk water. This does not in anyway completely exclude the hydrogen-bond interactions between Triton X-100 and ethanol via the –OH head groups and the oxygen of the oxy-ethylene units of the surfactant. In the same way simultaneous covalent and hydrogen bonding interactions cannot be ruled out (Kurtz, 1962). The hydrogen bonding or electrostatic interactions possible in Triton X-100 and SDS binding of the Ferrocyphen hydrophobe is absent in the case of CTAB.

The contribution of the various hydrogen bonding interactions to the overall energetics of the binding process is not as significant as the hydrophobic interactions. For example, enzyme-bound alcohols have been reported (Anderson *et al.*, 1984) to participate in hydrogen bonding interactions without providing any pronounced contribution to the net energetics of alcohol binding. However, it is expected that directional position of CH_3CH_2-hydrogen bonded ethanol molecules will enhance the hydrophobicity of the surfactant methylene groups and the resulting hydrophobic bonding between the Ferrocyphen hydrophobe and the surfactant.

CTAB has more methylene groups than SDS. A *priori*, one expects CTAB to exhibit a stronger hydrophobic interaction with the Ferrocyphen than SDS. It is significant therefore that K_b values for CTAB are lower. Experimental data from the literature (Motsadage and Kostenbauder, 1963) suggest strong ion-dipole interaction between the ammonium cationic head group of CTAB and the electron rich π – system of the phenanthroline ligands in the metal complex hydrophobe. This is the case in the hydrophobic binding of water soluble organic molecules by chiral hosts (Micheal *et al.*, 1988). On this basis, we expect relatively higher concentration of the complex between the head-group region and only a few carbon atoms below the stern layer with less concentration of the complex in the lower hydrophobic region of CTAB. The net effect is a relatively weaker hydrophobic binding interaction. This coupled with the sterically located methyl groups on the ammonium cation and the bulky metal complex results in the observed lowest K_b values for CTAB.

The simple hydrophobic nature of the metal complex predisposes it as a suitable probe of the relative hydrophobicity of a homologous series of surfactants in a suitable medium. All other conditions being equal, the variation in K_b values will be indicative of the relative degree of hydrophobicity in a family of surfactants.

Comparative agreement of our data and the binding in biological systems can be viewed as mainly qualitative in the sense that the same electrostatic and hydrophobic interactions are at play. Hydrophobic hydration and subsequent dehydration of the surfactant monomers by ethanol molecules may be compared to the expulsion of water molecules from the hydrated cavities of enzymes by ethanol with the difference that some of the ethanol is coordinated to ions in these cavities, which are the binding sites. While it is true that ethanol binding is not directly studied in the present work, it moderates the final binding of the Ferrocyphen hydrophobe through stabilization of the surfactant monomer, and enhancing the hydrophobic interaction between the hydrophobe and the surfactant. Small molecules like ethanol have small binding energies and are effective only at high concentrations (Trudel and Harris, 2004). This effect is in agreement with the observed



increase in K_b values at high ethanol compositions where surfactant monomer stabilization is most significant and predominant. We propose that this simple hydrophobic stabilization of surfactant monomers may be similar to the way ethanol interacts with proteins in the nervous system (Kruse *et al.*, 2003).

4. Conclusion

In the presence of ethanol, hydrophobic and electrostatic interactions are factors that do not only affect the number of available of binding sites but also the strength of the binding interaction between the metal complex hydrophobe and the surfactants SDS, TritonX-100 and CTAB. K_b, n, $[-\Delta G_b/n]$ - ethanol composition profiles exhibit minima and maxima. These structures are due to a combination of the dehydration of the surfactant monomers, hydrogen-bonding and hydrophobic interactions. Electrostatic interaction between the positively charged head group of CTAB and the aromatic phenanthroline ligands of the metal complex predominates over expected stronger hydrophobic interactions with the methylene group much down the surfactant chain, below the ammonium cationic head group, yielding the lowest K_b values for CTAB compared with SDS and Triton X-100. The strength of binding is CTAB < SDS < Triton X-100. The binding takes place in a series of equilibrium steps.

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References

Alfred, A.S., 1960. Mixed Ligand Complexes of Iron II and III with cyanide and Aromatic Di-Imines., *J. Am. Chem. Soc.*, 82, 3000-3005.

Anderson, P.J., Kvaisman, B. Olden and Pettersson, G., 1984. Synergism between Coenzyme and alcohol binding to liver alcohol dehydrogenase. *Eur. J. Biochem.*, 144, 317-324.

Fendler, J.H. and Fendler E.J., 1975. Catalysis in Micellar and Macromolecular Systems. Academy Press, New York and London.

Finney, J.L. and Soper; A.K., 1994. Structure of High and Low Density Amorphous Ice by Structural Determination of the Hydrophobic Hydration. *Chem. Soc. Rev.* 23, 1-10.

Fornasiero, D., Griesir, F. and Sawyer, W.H., 1988. NMR Study of the location of the anthroyloxy moiety on some n-(9-anthroyloxy) stearic acid probes solubilized in surfactant micelles. *J. Phys. Chem.* 92, 2301-2305.

Griffith, P.C., 2002. Solvency effect on surfactant micelle size and shape. *ISIS experimental Report RB Number 12969.*

Guiseppe, C.F., Paolo, F.C. and Carmelo, S., 1991. Solubilization of doubly charged Iron II complexes in aqueous neutral micellar aggregate: A kinetics investigation. *J. Chem. Soc. Faraday Trans.* 87, 3033-3036.

Karstern, R., 1997. Analysis of Protein-DNA binding at equilibrium *B.I.F. Futura*; 12, 20-26.

Kruse, S.W., Zhao, R., Smith, S.P. and Jones, D.N.M., 2003. Structure of a specific Alcohol-Binding Site Defined by the Odorant Binding Protein LUSH from Drosophila Melanogaster. *Life Sciences.*, 9, 2-76.

Kurtz, J.L., 1962. Effects of Micellization on the kinetics the Hydrolysis of Monoalkylsulfate. *J. Phys. Chem.*, 66, 2230-2246.

Linda, J., Kijiro, K.and Cralg, A.M., 1981. Binding of phenols to inverted micelles and microemulsion aggregate. *J. Phys. Chem;* 85, 1434-1439.

Marilyn, E.F. and Alfred, H., 1967. The Hydrophobic Bond in Micellar systems: Effects of various Additives on the stability of micelles sodium Diodecyl sulfate and of n-Dodecytrimetyl aminonium bromide. *J. Phys. Chem;* 71, 3320-3330.

Melo, E.C.C. and Costa S.M.B., 1987. A Critical Evaluation of Partition Constants in Non-ionic Micelles. *J. Phys Chem.*, 99, 5635-5640.

Michael, A.P., Timothy, J.S., Richard, E.B. and Dougherty, D.A., 1988. "Hydrophobic" binding of water-soluble guests by high symmetry. Chiral hosts. An electron-rich receptor site with a general affinity for quaternary ammonium compounds and electron in deficient system. *J. Am. Chem. Soc.*, 110, 6825-684.

Motsavage, V.A. and Kostenbauder, H.B., 1963. The influence of the state of aggregation on the specific acid-catalysed hydrolysis of sodium dodecylsulfate. *J. Colloid Sci.* 18, 603-615.

Mounir, A.M., 1999. *Experimental Inorganic/Physical Chemistry Oscar Publication, New Delhi, India.* pp. 34-36.

Ojo, J.F., Ige, J., Ogunlusi, G.O., Owoyomi, O. and Olaseni, E.S., 2006. Protonation of Chloro-, bromo-and Iodo-pentacyno cobaltate (III) complexes. *Trans. Met. Chem.* 31, 337-339.

Porter, M.R., 1991. *Handbook of Surfactant. Blackie Academic and Professional U.K.*, pp. 10-60.

Quina, F.H., Alonso, E.O. and Farah, J.P.S., 1995. Incorporation of Nonionic Solute into Aqueous micelles: A linear solvation free Energy Relationship Analysis. *J. Phys. Chem.*, 99, 11708-11714.

Saboury, A.A., 2002. A new form of scatchard plot to study binding of fluoride ion urease by isothermal titration calorimetry. *J. Biochem. Biophys. (Indian)*, 37, 347-350.

Scatchard, G., 1949. The Interaction of Proteins for small molecules and ligands. *Ann. N. Y. Acad. Sci.*, 660-672.

Shanti, K. and Amitabha, A.C., 2005. Membrance protein solubilization: Recent Advances and Challenges in Solubilization of Serotonin Reciptors$_{1A}$ *Life*, 57, 505-512.

Shigeyosh, M., Tsuyoshi, A. and Moric, N., 1989. Hydrophobicity and surface activities of sodium salt of N-dodecanoyl amino acids. *J. Colloid Interface Sci.* 131, 68-73.

Stilbs, P., 1981. Solubilization equilibria determined through Fourier transform NMR Self-diffusion measurement. *J. Colloid. Interface. Sci.*, 80, 608-610.

Tanford, C., 1978. The hydrophobic effect and the organization of living matter. *Science;* 200, 1012-1018.

Trudel, J.R. and R.A. Harris, 2004. Are Sobricty and Consciousness Determined by water in protein Cavities? Alcohol. *Clin. Exp. Res.*, 28, 1-3.

Zana, R, 1995. Aqueous Surfactant-Alcohol Systems: *A Review Advances in Collid and Interface Science*, 57, 1-64.