5.1 Introduction

In pharmacy, the interaction of small molecules with drugs has been most extensively studied. In this respect, many drugs, particularly those with local anesthetic, antidepressant, and antibiotic actions, exert their activity by interaction with biological membranes, which can be considered as complex form of amphiphilic bi-layers. Thus, a full knowledge of the mechanism of the interactions of drugs with other foreign material is required before the actual application in human body. The drug-membrane interactions provide a full insight into more complex biological processes such as the passage of drugs through cell membranes**.** Due to the much complex structure of biomembranes, surfactant micelles (less complex structure) have been used as model to mimic the biomembrane environment [1-9]**.**

A most common challenge is to design and develop drugs and drug carriers with good aqueous solubility while simultaneously retaining potency and selectivity. Use of aqueous micellar system as a drug carrier [10] presents advantages in comparison to other alternatives as it has more drug solubility [10-13], low viscosity, small aggregate size, narrow size distribution, simple preparation, long shelf life, bioactivity [14,15] and the enhanced bioavailability/ stability of the drug [16,17]. However, conventional surfactants are toxic in context to their use for physiological applications.

Bile salts are yet another class of surfactants which can be used as drug carrier. It has been reported that the bile salts form mixed micelles with the drugs [18]. Mixed systems containing phospholipids and a bile salt (NaDC) have also been used for the preparation of amphotericin B (antifungal agent) formulation for oral use or intravenous injections [19].

Emulsions based on bile salt and lecithin for the formulation of paclitaxel, so called bilosomes (bile salt stabilized vesicles) has been developed as a drug delivery system for the oral applied vaccines. For example, Valium MM and Konakion MM are two bile acid and phospholipid based formulations currently available in the pharmaceutical market. Various toxicological, safety and in vivo studies have shown that bile salts, especially formulated with phosphatidylcholine, can be used for the development of drug delivery systems especially for the solubilization of hydrophobic drugs. NaDC is known to enhance the permeation of various drugs across biological membranes [20].

Phase behavior of bile salts in presence of drugs has not been studied much. Before their use in combination with a drug, it is desirable to understand the solution behavior under various conditions (temperature, additives or pH) of applications. From the previous studies (chapters 3 and 4) it is known that surfactant solutions undergo clouding/ phase separation on heating (*vide supra*). As the surfactant rich phase is small in volume and contains almost all the surfactant (and drug, if present), the concentration of drug may increase many fold in a small volume. Such a high concentration may change the drug's biological activity due to decreased ability to pass through biological barriers. Therefore, it may be of genuine interest to have a good CP data based on biosurfactant + drug systems with and without additives. Keeping all these points in mind, a study on CP behavior of NaDC in presence AMT has been undertaken in this chapter. AMT (scheme 1 of chapter 2) is a widely prescribed tricyclic antidepressant drug. This study would provide a composition of NaDC + AMT for the study of the effect of various additives (inorganic salt, organic salt, protein denaturant, sugar, conventional surfactant).

5.2 Results and Discussion

5.2.1. Clouding in NaDC solution

Though clouding in ionic surfactants has been reported since last two decades [21-29], it is not observed with anionic bile salts. To check clouding in NaDC solution, we have tried various combinations using simple salts (like NaCl, KCl), organic salts (NaSal, PTHC, TBAB, TBPB) and AMT (an antidepressant drug). Clouding was observed only with NaDC-AMT combination. Various compositions of NaDC and AMT were tried to get the idea about the NaDC concentration to be used for the study of effect of additives.

Figure 1 show the variation of CP of NaDC with different concentration of AMT. From the perusal of data it is clear that CP decreases with the increase of AMT concentration. The ternary amine becomes protonated (cationic) at low pHs, and deprotonated (or neutral) at high pHs. Since we made solution in water, both charged and uncharged fraction of AMT would be available to interact with NaDC aggregates (which bear a negative charge).The micellar surface is characterized by the presence water of hydration. The AMT molecule consists of an almost planar tricyclic ring system and a short hydrocarbon chain carrying a terminal nitrogen atom. [30, 31]. Hence, AMT can interact with anionic NaDC micellar surface hydrophobically and electrostatically or both. As the temperature is raised, the hydration of ionic species of the mixed micelles decreases with concomitant increased interactions between the oppositely charged groups. In a similar type of work, it has been reported that the proximity of oppositely charged species at the micellar surface assists in removing the water from the micellar surface [32-34]. Here also, the removal of water is expected from the mixed micelle water - interface due to the presence of AMT.CP data in Figure 1 also show the interplay between [NaDC] and [AMT]. At higher [NaDC], more amount of AMT was needed to get clouding. This may be due to the presence of more number of anionic micelles which require higher amount of AMT in order to show clouding.

Figure 2 shows the variation of [AMT] with respect to NaDC to get clouding at a fixed temperature (*e.g.* ~ 41˚C). It seems that there exist some sort of compensation between temperature and AMT concentration. On the basis of the studies of variation of CP with the addition of AMT, 30 mM NaDC + 7 mM AMT system has been chosen to study the effect of various additives. The chosen composition has enough temperature window for making variation above and below the CP (39 ˚C).

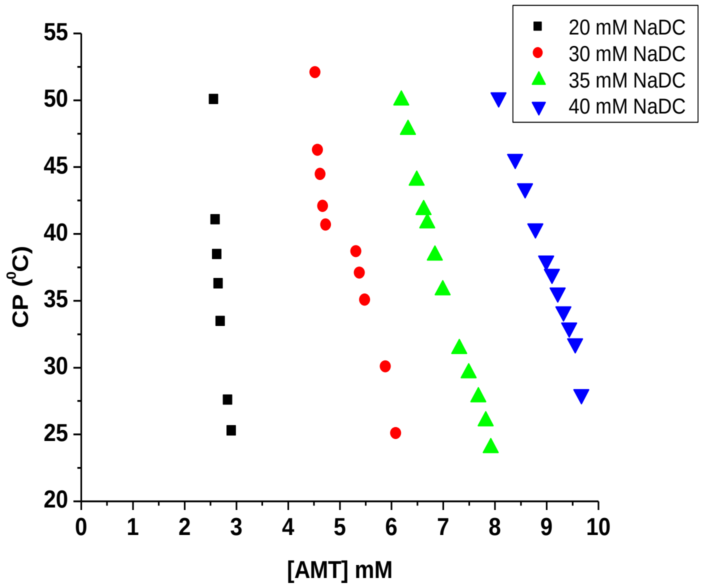

Figure 1.Variation of CP with [AMT] at different fixed [NaDC].

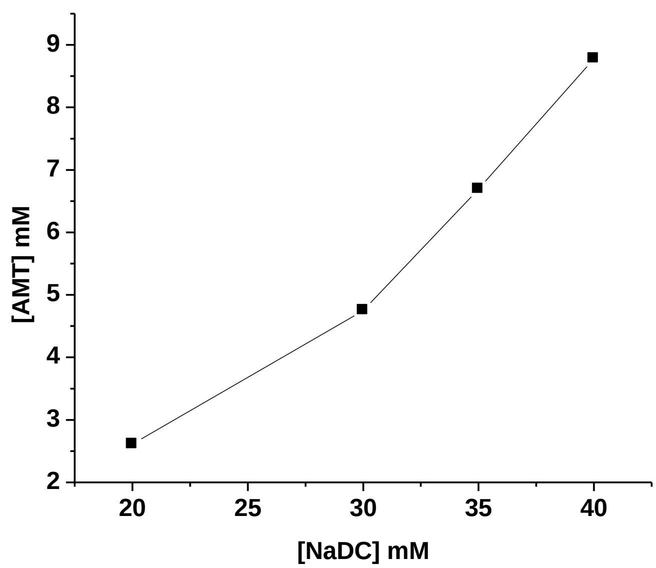

Figure 2. Plot between [NaDC] and minimum [AMT] needed for the appearance of CP (40.6˚C).

5.2.2. Effect of salts

In ionic micellar solutions, presence of counterion near the micellar surface decides effective charge on the micelle and their mutual interactions. The presence of salts may influence these factors as well as solvation/desolvation equilibria. Therefore, salts can affect clouding phenomenon of the NaDC- AMT system by virtue of variations in the factors mentioned above. Figure 3 shows the CP variation on addition of various inorganic salts. The salt addition causes an increase in CP. The presence of salt provides additional counterions (Na^+ or K^+) which can compete with AMT moiety to acquire micellar surface. In doing so, above counterions would carry additional water to the micellar surface and may be the reason of CP increase. On comparing the CP behavior of NaCl and NaSal, it has been found that co-ion has little influence on overall CP phenomenon. However, Na^+ is more effective in increasing CP than K^+. This may be due to the more hydration of Na^+. However, addition of PTHC caused immediate precipitation which hampered further CP study with such type of salts (binding salts).

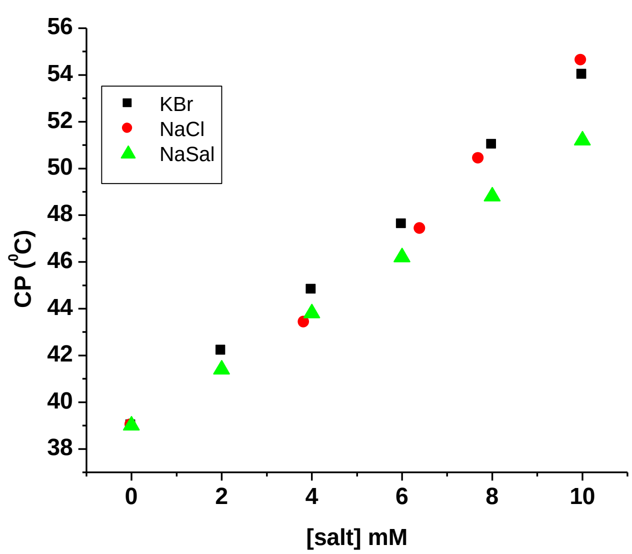

Figure 3. Variation of CP of 35 mM NaDC + 7 mM AMT solutions with added salts.

In contrast to inorganic cations, quaternary ammonium counterions are less hydrated [35]. Figure 4 shows the variation of CP with the addition of quaternary ammonium salts. Here again one can expect exchange of counterions with the micellar surface. Due to hydrophobic nature of these counterions, both hydrophobic and electrostatic interactions can take place with the anionic micellar surface. However, due to bulky nature of AMT, TBA^+ would remain at the micellar surface forming water mediated ion pairs which is responsible for CP increase. However, when one of the butyl chain in the counterion has been replaced by a phenyl ring [PTA^+], a decrease in CP was observed. This may be due to the additional hydrophobic interaction between the phenyl rings of AMT and PTA^+. Due to these interactions, the resultant aggregates are expected less hydrated and responsible for CP decrease. Further, addition of TPAB also causes a decrease in CP which is again due to the additional hydrophobicity provided by $TePA^+$ to the resultant aggregates. These hydrophobic interactions are responsible for the effective decrease in CP on the addition of relatively more hydrophobic counterions.

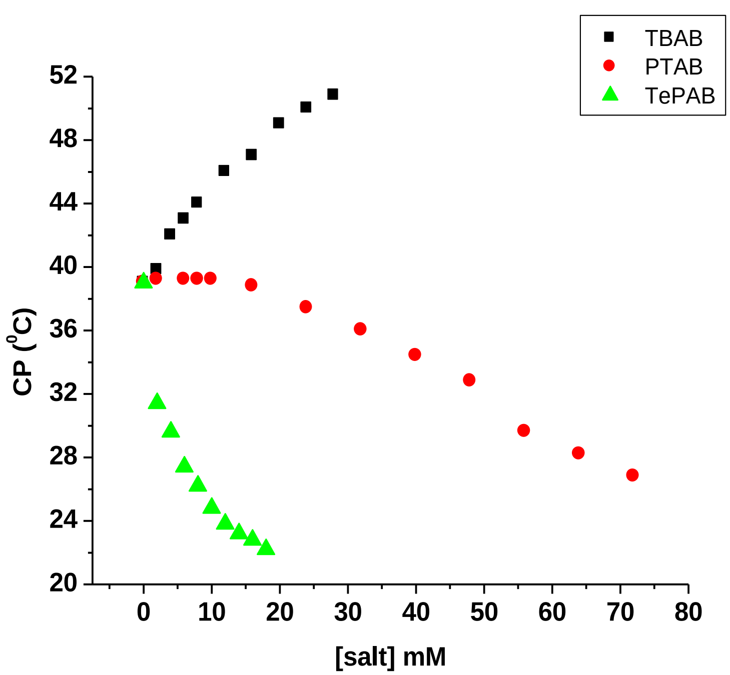

Figure 4. Variation of CP of 35 mM NaDC + 7 mM AMT solutions with added quaternary salts.

5.2.3.Effect of urea and thiourea

The variation of CP with added urea and thiourea is shown in Figure 5. Both additives show an increase in CP of NaDC-AMT system. To understand the microscopic basis of urea action two different mechanisms has been proposed [36-38]: I) urea alters the water structure which facilitates solvation of hydrophobic chains; II) urea replaces some water molecules that solvate the alkyl tails and the headgroup. In another study it was reported that urea partitions relatively less near the micellar surface than the background aqueous solution and destabilizes ion-pair formation with a concomitant effect on micellar properties [39]. The weak dependence of CP on [urea], are in similar lines as observed in above study. The effect of thiourea can also be understood in the light of its water structure breaking nature. The CP data in presence of urea and thiourea are similar as observed in the case of nonionic surfactant [36].

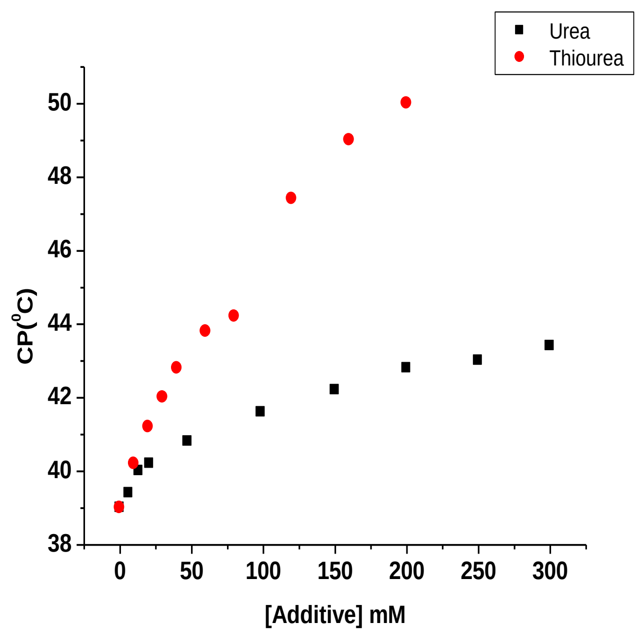

Figure 5. Variation of CP of 35 mM NaDC + 7 mM AMT solutions with added urea/thiourea.

5.2.4. Effect of sugars

Effect of addition of sugars (glucose and sucrose) on the CP variation of NaDC-AMT system is shown in Figure 6. These compounds produce lowering in the CP. These sugars may interact with the NaDC-AMT mixed aggregates via H_2O mediated interaction between the hydroxyl groups of sugars and NaDC. Due to these interactions the formation of more hydrated aggregates is expected. If this is correct, higher temperature is required to remove this water and observance of clouding. Data of figure 6 can be understood in the light of these facts.

5.2.5. Effect of conventional surfactants

The effect of varying [surfactant] on the CP of NaDC-AMT system is shown in Figure 7. CTAB causes precipitation of the system and could not be studied. Addition of SDS shows a drastic increase in CP at low [SDS] (at 2 mM CP observed at 59.6 ˚C while at 4 mM it was not observed even at 80 ˚C). This drastic change in CP hampered the study at further high [SDS]. The CP increase seems due to the adsorption of SDS monomers to the nearly uncharged NaDC-AMT micelles (*pseudo-nonionic micelles*). This adsorption may impart negative charge with a concomitant increase in electrostatic repulsions between the micelles with a simultaneous increase in CP. Similar behavior was reported in the case of nonionic micellar solution in which a ionic surfactant was added [40]. Data on CP variation (Figure 7) of NaDC-AMT system in presence of tween-20 (nonionic surfactant) clearly illustrate that CP increases initially followed by a continuous decrease as the concentration of tween-20 is increases in the system. The initial increase may be due to the partitioning of all the tween-20 near headgroup region which hinders evaporation of water molecules. However, increase in [tween-20] behaves oppositely to that with SDS. This may be due to increased hydrophobic interactions between tween-20 and NaDC-AMT mixed micelles at higher [tween-20]. Probably dehydration of the micelles seems responsible for the decrease in CP. This indeed was observed in Figure 7.

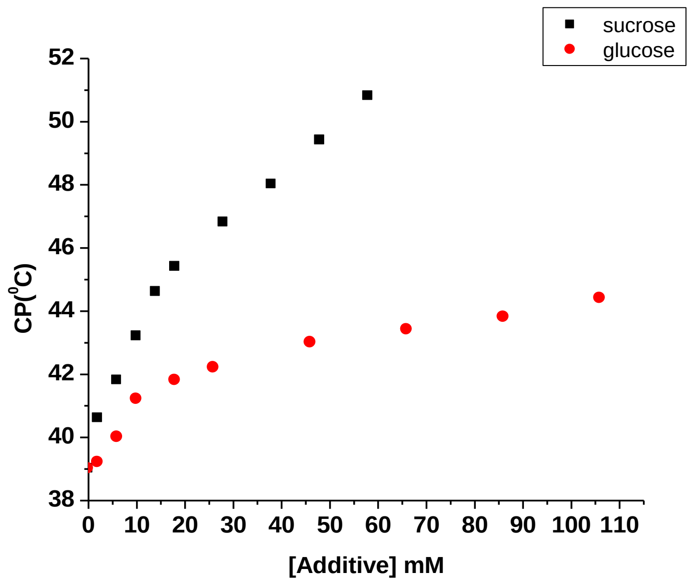

Figure 6. Variation of CP of 35 mM NaDC + 7 mM AMT solutions with added sugars.

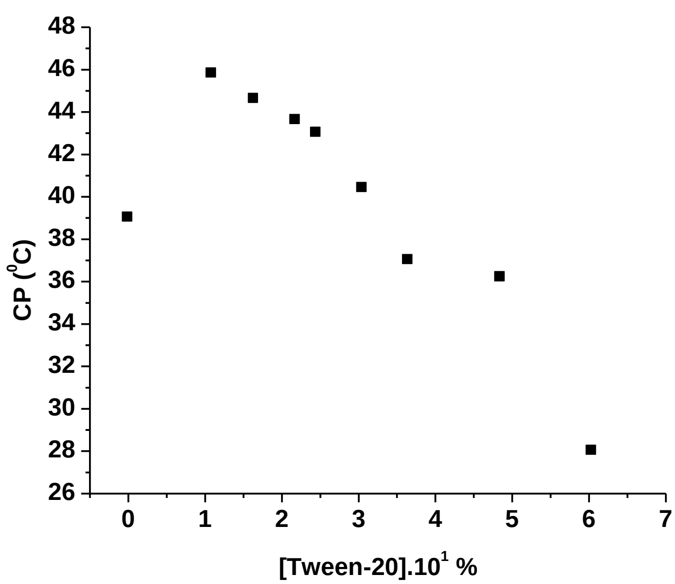

Figure 7. Variation of CP of 35 mM NaDC + 7 mM AMT solutions with added Tween-20.

5.3 Conclusions

Clouding phenomenon has also been observed in aqueous NaDC system in presence of AMT. CP decreases with increase in [AMT]. It can be tuned in either direction (increase or decrease) by the presence of an appropriate additive. NaDC and AMT are biocompatible material and a optimum composition can potentially be used for the extraction of thermally labile material (*e.g.*, proteins, vitamins, amino acids etc.).

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