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SHORT COMMUNICATION

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Influence of additives on the clouding behavior of amphiphilic drug solutions

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Abstract The present work focuses on the clouding phenomenon in an amphiphilic drug [amitriptyline (AMT), which is a tricyclic antidepressant] solution. A 50-mM AMT solution prepared in 10 mM of sodium phosphate (SP) buffer was taken where the cloud point (CP) was found to decrease with increasing pH. The same CP decreasing trend (with pH increase) followed in the presence of a fixed concentration (50 mM) of added salts [NaBr, and tetra-*n*-butylammonium bromide (TBuAB)]. The addition of increasing amounts of quaternary bromides (tetramethylammonium bromide, tetraethylammonium bromide, tetra-*n*-propylammonium bromide, TBuAB, and tetra-*n*-pentylammonium bromide) to 50 mM of AMT solution (prepared in 10 mM of SP buffer) caused continuous increase in CP, which was found to be dependent upon the alkyl chain length of that particular salt. The similar type of CP increase was also observed in the presence of conventional (cetyltrimethylammonium bromide and tetradecyltrimethylammonium bromide) and gemini surfactants [bis(hexadecyldimethylammonium)hexane, bis(hexadecyldimethylammonium) pentane, and bis(hexadecyldimethylammonium)butane]. The overall behavior was discussed in terms of electrostatic interactions, micellar growth, and mixed micelle formation.

Keywords Clouding phenomenon · Amphiphilic drugs · Amitriptyline · Gemini surfactants · Quaternary bromides

Introduction

Molecules or ions, which are amphiphilic, in aqueous solution frequently assemble at interfaces and self-associate in an attempt to sequester their apolar regions from contact with the aqueous phase. Surface-active behavior among many diverse classes of drugs was reported and attempts were made to correlate surface activity and biological activity [1–4]. The aggregation of such drugs follows the same principles as of conventional surfactants [5]. Drug self-association depends on the molecular structure of the drug, its concentration, and the experimental conditions such as temperature, pH, and salt concentration [2, 6]. Amphiphilic drugs solubilize in body fluids and interact with membranes in the organism before reaching their final targets.

Over the years, several of the phase structures produced by surfactants were of interest to the pharmaceutical scientist, either as drug vehicles/carriers or, recently, as targeting systems [7]. Nonionic surfactants were widely investigated as a means of producing a clear stable solution of a poor water-soluble drug suitable for intravenous or oral administration [8, 9]. The cloud point (CP) phenomenon is generally observed in nonionic surfactant micellar solutions when the temperature of the solution is raised to a certain value [10–12]. It was reported that the CP of a nonionic surfactant increased when small amounts of ionic surfactants were added, while the addition of inorganic

salts caused a decrease in CP of the nonionic–ionic surfactant mixtures [13]. Moreover, an increase in CP of TX-100 solutions was observed when quaternary ammonium bromides were added [14, 15].

After introducing a drug (solubilized in surfactant solution) into the body, the system may, depending upon its route of administration, undergo a large dilution. If the surfactant is diluted below its critical micelle concentration (CMC), precipitation of transported drug may occur. Because nonionic surfactants generally exhibit lower CMC than ionic surfactants, they are preferred for the purpose of drug delivery. When considering a nonionic system as a drug delivery vehicle, it should also be borne in mind that clouding can be induced by a change in temperature (as the normal human body temperature is usually ~37 °C), which can put using nonionic surfactant micelle as a drug vehicle in question.

A new class of surfactants has recently appeared in the scientific literature. These surfactants are made up of two amphiphilic moieties connected at the level of the head groups (so-called gemini surfactants) [16–19]. CMCs of such surfactants are one to two orders of magnitude lower than for the corresponding conventional surfactants. Therefore, such surfactants may be used as a substituent of nonionic surfactants, which are usually used for drug delivery [7]. A knowledge of the interaction with various biological surface active agents (lipids, bile salts, enzymes, etc.), which the drug molecule may encounter in vivo, is also essential as these may alter or even destroy the efficacy of the drug itself. It was reported that drug solutions undergo a concentration, temperature, and pH-dependent transition leading to the clouding phenomenon [20]. Human blood plasma normally has a pH close to 7.40. Should the pH-regulating mechanisms fail, as may happen in severe uncontrolled diabetes because of the acidosis caused by over production of metabolic acids, the pH of the blood can fall to 6.8 or below and can lead to irreparable damage and death [21]. And in other diseases, the pH may rise to the point of no return. These observations indicate that pH effect should be taken into account when dealing with a drug solution. Investigations on the effect of additives and experimental pH on the clouding behavior of amphiphilic drugs were started fairly recently [22, 23]. Therefore, to get insight of morphological aspects involved when a drug solution approaches the CP, studies embodied in the present paper should be continued.

Hence, clouding behavior of an antidepressant drug, amitriptyline (AMT), in aqueous buffer solution [10 mM sodium phosphate (SP) buffer] was examined by investigating the following factors: (1) pH, (2) nature and concentration of externally added salts, and (3) addition of cationic surfactants (gemini and conventional).

Experimental

Materials

AMT hydrochloride (≥98%; Sigma, USA), cetyltrimethylammonium bromide (CTAB, ≥99%; BDH, England), tetradecyltrimethylammonium bromide (TTAB, 99%; Sigma), tetramethylammonium bromide (TMeAB, ≥97%; Fluka, Switzerland), tetraethylammonium bromide (TEtAB, ≥98%; Fluka), tetra-*n*-propylammonium bromide (TPrAB, >98%; Fluka), tetra-*n*-butylammonium bromide (TBuAB, 99%; Fluka), tetra-*n*-pentylammonium bromide (TPeAB, 99%; Fluka), and NaBr (≥99%; Loba Chemie, India) were used as received. The gemini surfactants, namely, bis(hexadecyldimethylammonium) hexane (16-6-16), bis(hexadecyldimethylammonium) pentane (16-5-16), and bis(hexadecyldimethylammonium)butane (16-4-16) were prepared and characterized by the method reported elsewhere [24]. Trisodiumphosphate dodecahydrate and sodium dihydrogen phosphate monohydrate were of reagent grade obtained from Merck, India. All the solutions were prepared in double distilled deionized water.

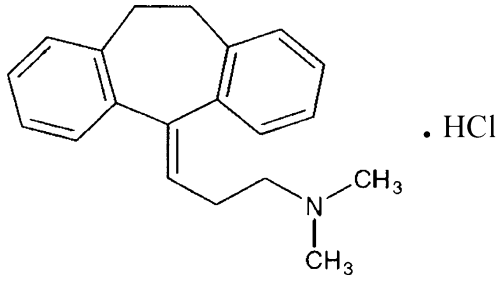
CP and CMC determinations

The CMC of AMT in pure water was determined by measuring the surface tension (ST) of pure drug solutions of various concentrations at 30±0.5 °C. CMC of the drug was obtained by plotting ST against log [AMT] (data not shown). The constancy in ST vs log [AMT] plot was taken as the CMC of AMT. Ten millimoles of SP buffer solution was prepared by the method reported by Kim and Shah [23] and subsequently used for preparing AMT samples for CP determinations. CPs were obtained by placing Pyrex glass tubes (containing the drug solutions) into a temperature-controlled bath, the temperature of which was ramped at the rate of 0.1 °C/min near the CP and onset of clouding was noted (CP) by visual inspection. However, the temperature was oscillated slowly through the CP until reproducible (±0.1 °C) [25, 26].

Results and discussion

General considerations

The CMC of AMT (a tricyclic antidepressant drug, Fig. 1) in pure aqueous solution is found to be about 38 mM, which is in close agreement with the values reported in literature [1, 22]. The concentration (50 mM) considered in this work is above the CMC. The tertiary amine portion of the drug AMT is hydrophilic, and the tricyclic portion is hydrophobic. The hydrophilic portion gets protonated (cationic) in acidic range (low pH) and deprotonated



Amitriptyline hydrochloride (AMT)

Fig. 1 Structure of amphiphilic drug (AMT)

(neutral) in basic range. The ionization constant pK_a of AMT in the free molecular state is 9.4 [27]. Just like various nonionic surfactants in aqueous solution [10, 28], it was found out that AMT micellar solution undergo a phase transition characterized by a change from a clear to turbid solution (at CP) [22]. The phenomenon was explained by a decrease of the hydrophilic group hydration so that the intermicellar hydration repulsion decreases. Due to hydration decrease with increasing temperature, micelles undergo secondary aggregation at the CP and separate into two isotropic phases, one of which is rich in water and the other in surfactant [10].

Clouding in the presence of salts and surfactants

As we have mentioned above that AMT can behave as a cationic surfactant at pH<7, intermolecular and intermicellar interactions depend on the polar head charge and counterion concentration. Therefore, the clouding behavior of AMT solution should be influenced both by pH and ionic strength. Indeed, maintaining the [AMT] fixed (50 mM) and increasing the pH permitted us to observe the clouding phenomenon. Figure 2 shows that CP decreases as the pH value of the AMT solution increases. A considerable decrease in CP with increasing pH from 5.7 to 6.7 implies a significant change in the micellar surface charge in this pH range. Such type of behavior was explained in terms of reduction of electrical repulsion due to increased fraction of deprotonated AMT with the concomitant enhancement in micellar aggregation number leading to a decrease in CP [22]. Figure 2 also shows the influence of cationic coions (50 mM; Na^+, and $TBuA^+$) on the CP of the AMT solution as a function of pH. Here one can see that depending upon the pH of the AMT solution, both decrease and increase in CP takes place. At lower pH due to protonation of tertiary amine group, both charged and uncharged fractions of AMT molecules would be available for aggregate (so-called AMT micelle) formation. Therefore, each micelle would bear a cationic charge and hence Br^- would cause a micellar growth with the concomitant decrease in CP. This understanding of micellar growth in decreasing the CP was recently developed by Kim and Shah [22]. Marszall [29] reported that the addition of NaCl to sodium dodecyl sulfate + Triton X-100 solution (mixed micelle with a negative charge) causes a decrease in CP, which is in line with the present results (Fig. 2). However, after attaining a certain pH (~6.2) of the solution, the addition of salt shows an increase in CP. This may be due to the fact that at higher pH the fraction of deprotonated AMT in the overall aggregate would be higher and the aggregate would behave like a nonionic one. Therefore, both kinds of ions (cations and anions) would influence the clouding behavior of the AMT solution. All reports on the effect of inorganic salts on the

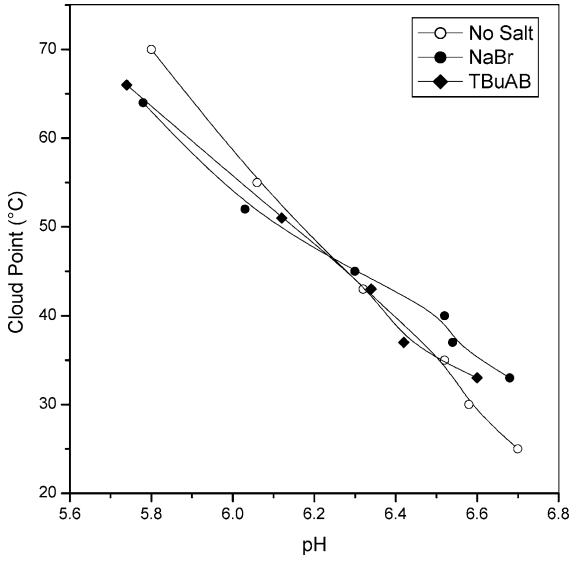


Fig. 2 Influence of pH on the CP of 50 mM of AMT in a 10-mM SP buffer solution containing no salt or 50 mM of salt

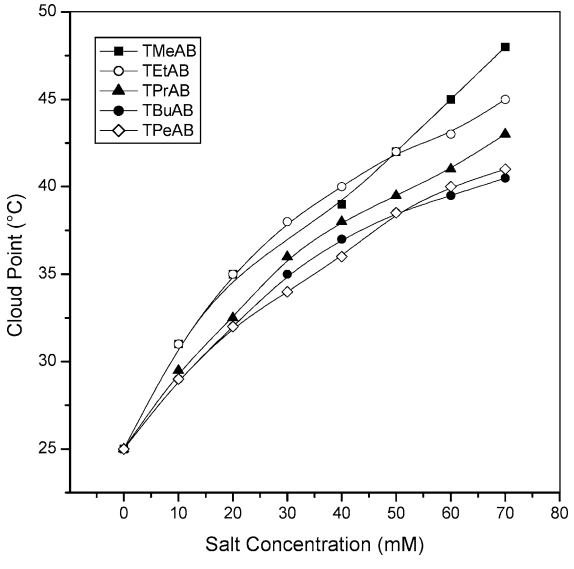


Fig. 3 Influence of the addition of QABs on the CP of 50 mM of AMT in a 10-mM SP buffer solution (pH=6.7)

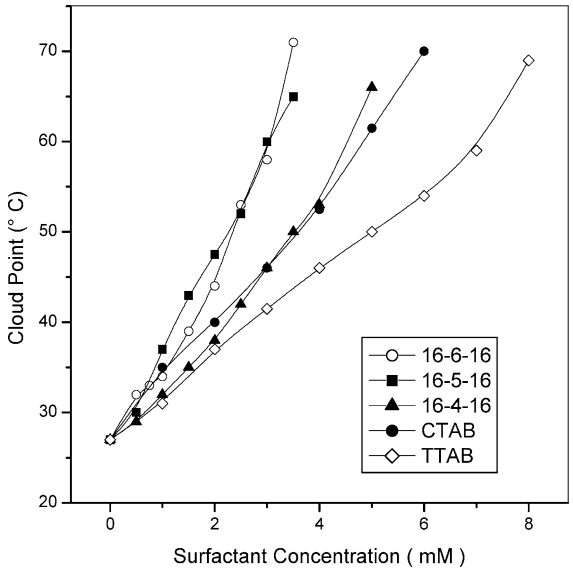


Fig. 4 Influence of the addition of various cationic surfactants on the CP of 50 mM of AMT in a 10-mM SP buffer solution (pH=6.7)

lowering of CP have considered the competition for water of hydration between oxygen of the ethylene oxide group and cation and anion of the inorganic salt. Unfortunately, AMT has no such ether oxygen and the literature is not of much help in explaining the increase in CP in AMT at higher pH in the presence of salts. Probably, water structure breaking nature of the cation in some way is playing its role in increasing the CP at higher pH. Although Kim and Shah [22] have not offered any explanation for such a fact, the effect of salts on raising the CP is understandable by following the scheme of Schott and Han [30].

Figure 3 shows the variation of CP of 50 mM of AMT solution prepared in 10 mM SP buffer (pH=6.7) as a function of the concentration of added quaternary ammonium bromides (QABs). All the QAB salts cause an increase in CP and the increase is in the order of TMeAB>TEtAB>TPrAB>TBuAB>TPeAB. The QABs are less hydrated than the inorganic salts. It was reported that the tetraalkylammonium ions are water structure formers and this effect increases with increase in the length of the alkyl group. The CP rising in the case of QAB cations is ascribed to adsorption/mixed micelle formation predominating over water structure formation [31]. In such cases, micelles would experience greater intermicellar repulsions and consequently higher CPs. This indeed is observed in Fig. 3

Figure 4 depicts the effect of the addition of conventional and gemini cationic surfactants on the 50 mM of AMT solutions prepared in 10 mM of SP buffer. It can be seen that the CP increases upon the addition of each surfactant and the magnitude of CP increase is found to be dependent upon the length of the alkyl chain present in the conventional surfactant (CTAB or TTAB). This behavior is similar to the findings of an earlier work [23]. The addition

Table 1 Values of CMC for various cationic surfactants at 30 °C

Surfactant	CMC (M)
16-6-16[a]	4.37×10^{-5}
16-5-16[a]	3.63×10^{-5}
16-4-16[a]	2.83×10^{-5}
CTAB[a]	1.00×10^{-3}
TTAB[b]	3.93×10^{-3}

[a][34]
[b][32]

of gemini surfactants provides higher CPs than the conventional cationic surfactants. The added surfactants would exist in the AMT solution as either monomers or micelles depending upon their CMC (Table 1). Because the surfactants are all around the AMT micelle in the bulk solution, their presence would hinder AMT micellar association responsible for the CP phenomenon. This hindering effect increases with the chain length of the surfactant monomer (TTAB to CTAB case). This result indicates that hydrophobicity of the surfactant monomer has a role to play toward the overall CP phenomenon. The CP data shown in Fig. 4 illustrate that the gemini surfactants show a stronger effect on CP increase in comparison to the conventional ones. One outcome of the covalent spacer insertion between two N^+ centers at the level of head groups is the imposition of additional geometric constraint on the surfactant intramolecular packing. This, in turn, influences the micellar morphology. Because spheroids and ellipsoids differ in terms of curvature [16], a larger effective charge would be expected

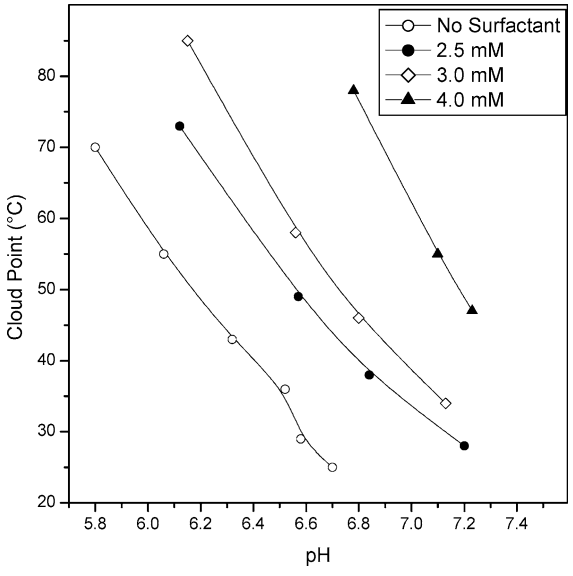


Fig. 5 Influence of pH on the CP of 50 mM of AMT in a 10-mM SP buffer solution containing different fixed concentrations of 16-6-16

for a spherical micelle compared to an ellipsoidal morphology. It was reported that micellar charge increases with increasing spacer chain length, although not monotonically [16, 33]. In our CP results on AMT solution, one can see that the role of spacer length is quite distinct. Therefore, both micellar morphology and charge play important roles in deciding the CP of the drug solution. As mentioned earlier that if the surfactant is in monomeric form, it would form mixed micelles while cationic micelles of the surfactant would exist if the [surfactant] is above CMC. Both cases increase the interaggregate repulsion (as discussed for QAB) with an increase in the CP of the AMT solution (Fig. 4).

Variation of CP with pH in the presence of different fixed concentrations of 16-6-16 (Fig. 5) shows that the CP of the AMT solution increases with the concentration of the gemini surfactant and clouding region appears at a comparatively much higher temperature. This allows us to say that by judicious selection of the surfactant and its concentration, one can safely produce a 16-6-16–AMT system, which can check the phenomenon of clouding and phase separation at body temperature and pH. Hence, for transporting AMT into human body, we need a surfactant system with the above two characteristics in addition to its body compatibility.

Conclusion

In conclusion, it can be pointed out that it is possible to increase the CP of AMT drug solutions near the physiological pH (6.7) by the presence of a suitable additive. Gemini surfactants seem to be ideal candidates for increasing the CP of the drug solution and their presence may check the phenomenon of clouding in real applications where AMT is in use and delivered through a surfactant system.

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