Cloud Point and Dye Solubilization Studies of Amphiphilic Drug Solutions: The Effect of Electrolytes and Nonelectrolytes

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The effect of electrolytes and nonelectrolytes on the micellar behavior of amitriptyline (AMT), an amphiphilic drug, has been investigated using cloud point (CP) and dye solubilization techniques. The critical micelle concentration (cmc) of AMT determined by the Wilhelmy plate method was about 40 mM in the presence of 10 mM sodium phosphate, and it reduced to about 20 mM in the presence of 500 mM sodium phosphate. Interestingly, the CP phenomenon was observed even below the cmc in cationic AMT solutions. Increasing AMT concentration reduced the CP below the cmc, while it had the opposite effect above the cmc. An increase in the pH of the AMT solutions from 6.2 to 6.8 at a given AMT concentration decreased the CP and increased the AMT micellar size. Addition of an electrolyte such as sodium chloride to 100 mM AMT in water increased both the CP and the AMT micellar size. On the other hand, added nonelectrolytes such as dextrose, sucrose, and urea decreased both the CP and the micellar size. Experimental results have been interpreted in terms of electrostatic effect and interactions between additives and AMT head groups.

Keywords Cloud point; Micelle; Amphiphilic drug; Amitriptyline; Electrolyte; Nonelectrolyte

Introduction

Amphiphilic drugs tend to self-associate as micelles in aqueous solution in a surfactant-like manner. The self-association of amphiphilic drugs is governed by hydrophobic interactions among the nonpolar alkyl chains and the electrostatic interactions of the polar or ionic head groups with themselves (Tanford, 1980; Baar et al., 2001). Drug self-association depends on the molecular structure of the drug, the drug concentration, and physicochemical conditions such as temperature, pH, and additive concentration (Attwood and Natarajan, 1981; Atherton and Barry, 1985; Attwood, 1995; Schreier et al., 2000; Taboada et al., 2000). Attwood and Natarajan (1981) reported that the critical micelle concentration (cmc) of

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piperazine-containing drugs increased with decreasing pH, due to protonation of the second nitrogen atom of the drugs' piperazine ring. Taboada et al. (2000) studied the self-association of antidepressant drugs such as amitriptyline (AMT), nortriptyline, doxepin, and desipramine hydrochlorides in aqueous solution in the presence of added electrolyte (0.0–0.4 mol kg^{-1} NaCl) using static and dynamic light scattering techniques. They found that the aggregation number of amphiphilc drug micelles increased on addition of NaCl to the aqueous amphiphilic drug solutions.

The cloud point (CP) phenomenon has been generally observed in nonionic surfactant micellar solutions at elevated temperatures (Schott, 1984; Xie et al., 1989; Gu and Galera-Gómez, 1995; Al-Ghamdi and Nasr-El-Din, 1997). The increase in turbidity of the surfactant solution at the CP is generally considered to be due to the formation of large aggregates (Paradies, 1980; Glatter et al., 2000). Corti et al. (1984) interpreted the increase in turbidity as being due to critical fluctuations of micellar concentrations and not to a temperature dependence of the micellar size. On the other hand, Laughlin (1994) suggested that micellar interaction and micellar growth have no need to relate to the CP because the CP phenomena are also observed in non-surfactant systems. Recently, the CP has been also observed in the anionic surfactant sodium dodecyl sulfate solution in combination with symmetrical quaternary bromides (Kabir-ud-Din et al., 1996; Kumar et al., 2000a, 2000b). The CP appearance in this system is explained in terms of increased hydrophobic interactions due to the alkyl chains of the quaternary bromides. Most recently, we have observed the CP phenomenon in amphiphilic drug AMT solutions. In our earlier studies, we explored the effect of addition of alcohols and surfactants on the CP of the cationic AMT micellar solutions (Kim and Shah, 2003a, 2000b).

The dye solubilization technique has been widely used to study the micellar behavior of surfactants (Mitra and Dungan, 2000; Mata et al., 2004, 2005), amphiphilic block copolymers (Bhattarai et al., 2003; Kelarakis et al., 2004), and amphiphilic drugs (Kim and Shah, 2002). The solubilization of a water-insoluble dye depends on the state of aggregation of surfactants. The amount of the dye solubilized is negligible up to the cmc of a surfactant, and thereafter a sudden and steep rise is observed with the formation of surfactant micelles.

Amphiphilic drugs solubilize in body fluids and interact with membranes in the organism before they reach their final targets. Mixed micelles and polymeric micelles have been extensively used as drug solubilizing agents as well as drug delivery vehicles. An understanding of the self-aggregation mechanism of amphiphilic drugs at the molecular level and their solubilization by the surfactants is crucial in the rational design of more effective drug delivery systems (Schreier et al., 2000). Few studies have been reported on the role of additives in ionic drug micellar behavior. In this work, AMT, a tricyclic antidepressant agent, was employed as a model amphiphilic drug to study the effect of electrolytes and nonelectrolytes on the micellar behavior of amphiphilic drugs using CP and dye solubilization techniques. The molecular structure of AMT is shown in Figure 1 (Taboada et al., 2000, 2001). The tricyclic portion of the AMT molecule is hydrophobic, and the tertiary amine portion is hydrophilic. The tertiary amine becomes protonated (cationic) at low pHs and deprotonated (neutral) at high pHs. Sodium chloride and sodium phosphate were used as electrolytes. Dextrose, sucrose, and urea were employed as nonelectrolytes. The effect of additives on the CP and the AMT micelle size has been discussed in terms of electrostatic effect and additive-AMT head group interactions.
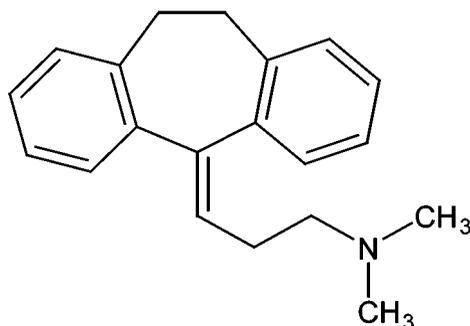
Figure 1. Molecular structure of AMT.

Experimental Section

Materials

AMT hydrochloride ($\geq$98%) supplied by Sigma Chemical Company was used as received. Sodium chloride, sodium phosphate, and urea were of ACS reagent grade and were obtained from Fisher Scientific. Dextrose and sucrose obtained from Sigma Chemical Company were of ACS reagent grade. Sudan III (90%) supplied by Eastman Kodak Company was used as a dye in the solubilization experiments. Distilled and deionized water with a resistivity of 18.3 M$\Omega\cdot$cm was used to prepare AMT solutions. The 500 mM sodium phosphate buffer solutions used in the CP experiments were prepared from sodium phosphate monobasic monohydrate (356 mM) and sodium phosphate tribasic dodecahydrate (144 mM). The 10 mM sodium phosphate buffer solutions consisted of 6.1 mM sodium phosphate mono-basic monohydrate and 3.9 mM sodium phosphate tribasic dodecahydrate. The pH of the AMT solutions was adjusted using 1 N NaOH obtained from Fisher Scientific.

Surface Tension and pH Measurements

Surface tensions of the AMT solutions were measured at room temperature using a Rosano surface tensiometer made by Biolar Corp. Surface tension measurements were made by the Wilhelmy plate method with a platinum plate to determine the cmc of AMT. Before each measurement, the plate was cleaned thoroughly with acet-one and doubly distilled water and was carefully flame-dried. The accuracy of measurement was checked by frequent determination of the surface tension of pure water. The pH of the AMT solutions was measured with an ATI Orion 720 A pH meter.

Cloud Point Measurements

The CP of the AMT solutions was determined visually by noting the temperature at which the continuously heated solution suddenly became cloudy. The AMT solution was heated at about 0.5°C/min starting from room temperature. CP measurements were made three times for one data point. The repeatability of the CP measurements was $\pm$0.2°C. The AMT solution became clear on cooling below the CP.

Dye Solubilization Measurements

Dye solubilization experiments for the AMT solutions were performed at room temperature by vigorously stirring 20 mL of AMT solution with 20 mg of Sudan III dye for 5 min, separating the insoluble dye by filtering the mixture through a 0.2 μm syringe filter (Millipore, Millex-FG) and measuring the UV-visible absorbance in the wavelength range of 400–800 nm on a Hewlett Packard 8453 UV-visible spectrophotometer.

Results and Discussion

Figure 2 illustrates the effect of AMT concentration on the CP of AMT solutions in the presence of small and large amounts of sodium phosphate. Here the concentration of AMT varies from 5 to 200 mM and the pH of the AMT solutions is fixed at 6.2. As shown in Figure 2, the CP becomes a minimum at an intermediate AMT concentration at a fixed sodium phosphate concentration. Similar CP behavior was observed in an ethylene oxide/propylene oxide random copolymer/water system (Johansson et al., 1993; Cunha et al., 1998).

In the case of the 10 mM sodium phosphate solution, the CP decreases with increasing AMT concentration up to 50 mM, and then it increases with further increase in AMT concentration above 50 mM. The cmc of AMT determined by the Wilhelmy plate method was about 40 mM in the presence of 10 mM sodium phosphate, and it reduced to about 20 mM in the presence of 500 mM sodium
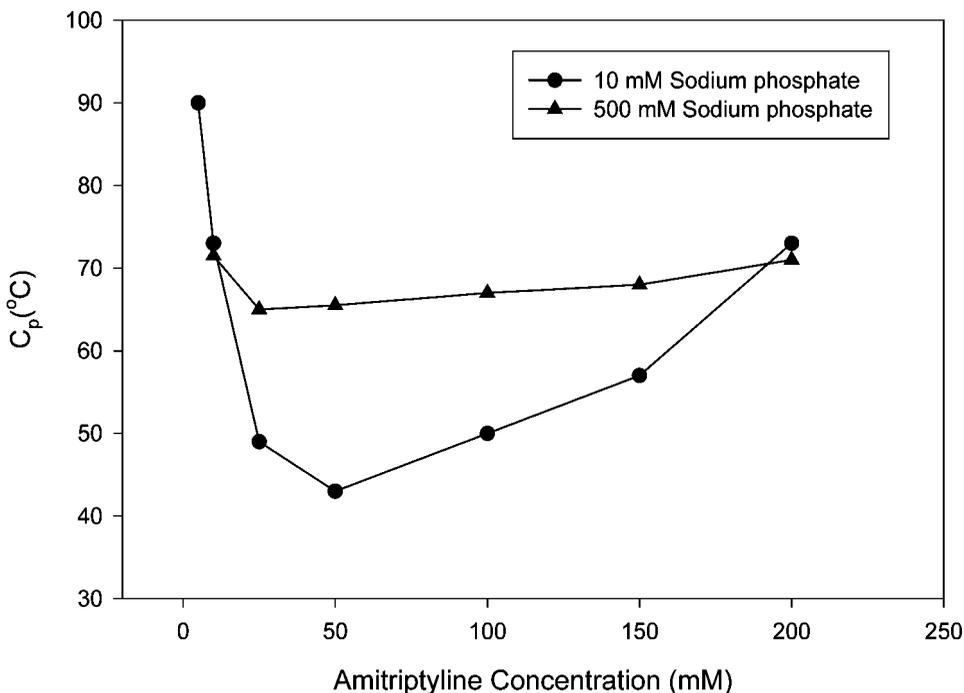

Figure 2. Variation of cloud point of AMT solutions with AMT concentration in the presence of small and large amounts of sodium phosphate. The pH of the AMT solutions is 6.2.

phosphate. Accordingly, the AMT concentration with the minimum CP in Figure 2 approximately corresponds to the cmc at a given sodium phosphate concentration. The minimum CP shifts toward lower AMT concentration as the sodium phosphate concentration increases from 10 to 500 mM. This implies that the cmc is lowered with increasing sodium phosphate concentration. In general, the cmc of ionic micelles decreases with increasing electrolyte concentration (Tanford, 1980; Taboada et al., 2001). As shown in Figure 2, the CP decreases with AMT concentration below the cmc. Apparently, an occurrence of the CP below the cmc is not related to micellar interaction and micellar growth. Below the cmc, attractive interactions between AMT monomers appear to trigger phase separation as a result of an aggregation of AMT monomers. In this case, higher AMT concentration would favor molecular aggregation, resulting in a decrease in CP with increasing AMT concentration. On the other hand, the CP increases with AMT concentration above the cmc. The size of AMT micelles increases with increasing AMT concentration. Since the ionization constant, pKa, of AMT is 9.4 (Hansch, 1990), ionized AMT molecules prevail at a pH of 6.2. As a result, electrical repulsion between cationic AMT micelles would become greater for larger micelles, leading to an increase in CP.

It is worth noting in Figure 2 that the CP is less sensitive to AMT concentration in the presence of 500 mM sodium phosphate. Large amounts of phosphate counterions will screen out the surface charge of the micelles, considerably reducing intermicellar electrostatic repulsion. In this case, the CP would be less sensitive to the micellar size. In the present system, van der Waals attraction, electrical repulsion, and the solvation effect play a role in the occurrence of the CP. As the temperature of the AMT solution is raised, progressive dehydration of the polar heads of the AMT monomers takes place. This process of dehydration increases interaction between the cationic heads and anions. At the CP, an aggregation of the monomers or micelles seems to occur (Kumar et al., 2000a, 2000b).

Figure 3 depicts the effect of AMT concentration on the CP of AMT solutions in the presence of 500 mM sodium phosphate at different pHs. The pH of the AMT solutions ranges from 6.2 to 6.8. One can see that increasing the value of pH lowers the CP at a fixed AMT concentration. However, the trend in CP behavior does not change with pH. As mentioned above, the ionization constant, pKa, of AMT in the free molecular state is 9.4. However, a considerable decrease in the CP with increasing pH from 6.2 to 6.8 implies significant changes in the micellar surface charge in this pH range. This pH-dependent CP behavior of AMT molecules in the micellar state is likely to be different than their behavior in the free molecular state. Electrostatic effects in AMT micelles would shift the pH equilibrium of AMT toward the deprotonated form. AMT molecules in micelles become unionized as the value of pH increases. Thus, electrical repulsion between micelles reduces with increasing pH. This may enhance micellar aggregation leading to a decrease in CP. In addition, the aggregation number, i.e., the micellar size, increases at higher pHs, due to a decrease in head group repulsion (Wajnberg et al., 1988). Note also that the minimum CP shifts toward lower AMT concentration as the value of pH increases, implying that the cmc decreases with increasing pH. This result is consistent with the observation of Attwood and Natarajan (1981).

Figure 4 shows the UV-visible spectra of Sudan III in the presence of 50 mM AMT in a 10 mM sodium phosphate solution at different pHs in the wavelength range of 400–800 nm. One can see that the UV-visible absorbance increases with increasing pH from 5.3 to 6.6. An increase in the UV-visible absorbance indicates
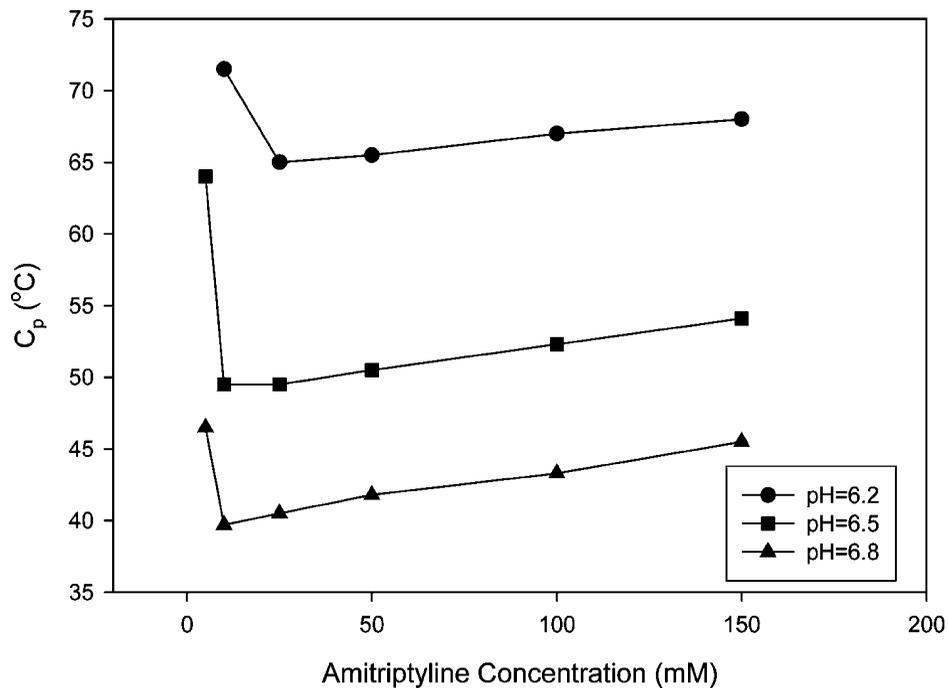
Figure 3. Variation of cloud point of AMT solutions with AMT concentration in the presence of 500 mM sodium phosphate at different pHs. The pH of the AMT solutions ranges from 6.2 to 6.8.
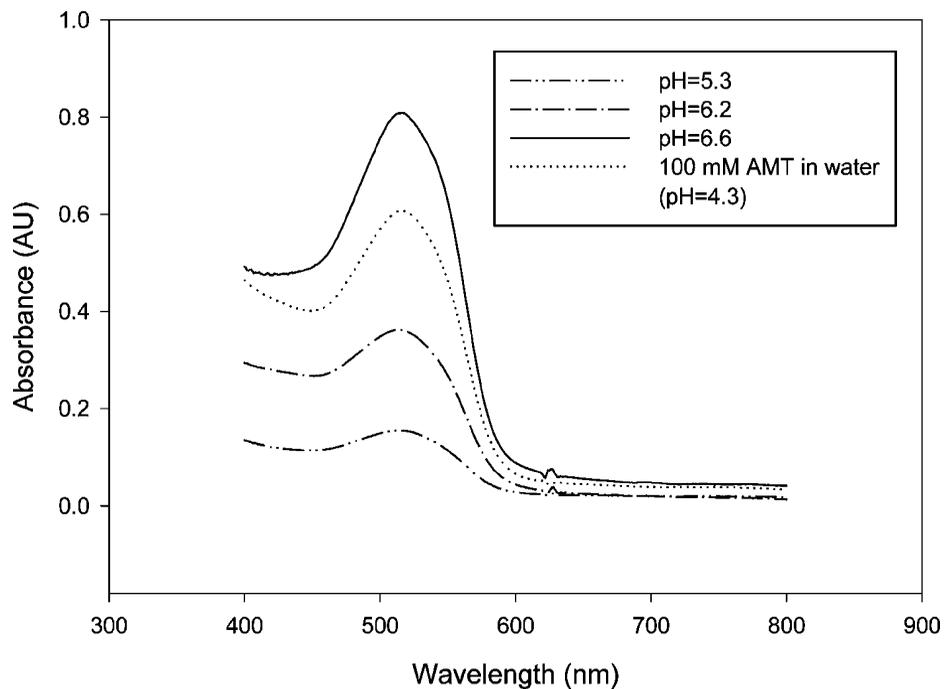

Figure 4. UV-visible spectra of Sudan III in the presence of 50 mM AMT in a 10 mM sodium phosphate solution at different pHs. The pH of the AMT solutions ranges from 5.3 to 6.6.

an increase in the dye (Sudan III) solubility, resulting mainly from the enlarged micellar size (Kim and Shah, 2002). As discussed above, the micellar size increases and the cmc is lowered as the value of pH increases. It is important to note in Figure 4 that the intensity of the absorbance peak of 50 mM AMT at a pH of 6.6 is greater than that of 100 mM AMT in water at a pH of 4.3 where the micellar concentration is about 60 mM. This result suggests that an increase in the UV-visible absorbance with increasing pH results mainly from the enlarged micellar size. An increase in pH leads to a decrease in surface area occupied per AMT head group (A_o), with a simultaneous increase in the Mitchell–Ninham parameter R_p ($=V_c/l_cA_o$, V_c being the volume of the hydrophobic part of the AMT monomer and l_c its length) (Kabir-ud-Din et al., 1996; Mitchell and Ninham, 1981) of the AMT monomer. Thus, a decrease in A_o seems to result in an increase in R_p and micellar growth. The results of Figure 4 favorably support this interpretation.

Figure 5 illustrates the UV-visible spectra of Sudan III in the presence of 100 mM AMT in water at different additive concentrations in the wavelength range of 400–800 nm. One can see that the UV-visible absorbance increases on addition of an electrolyte such as NaCl, while it decreases on addition of nonelectrolytes such as sucrose and urea. Addition of electrolytes raises the aggregation number of ionic micelles due to electrostatic effects (Evans and Wennerström, 1999). Taboada et al. (2000) also observed from their static light scattering studies that the aggregation number of AMT in NaCl solution considerably increased from 6 to 49 with increasing NaCl concentration from 0 to 400 mM. On the other hand, a decrease in the
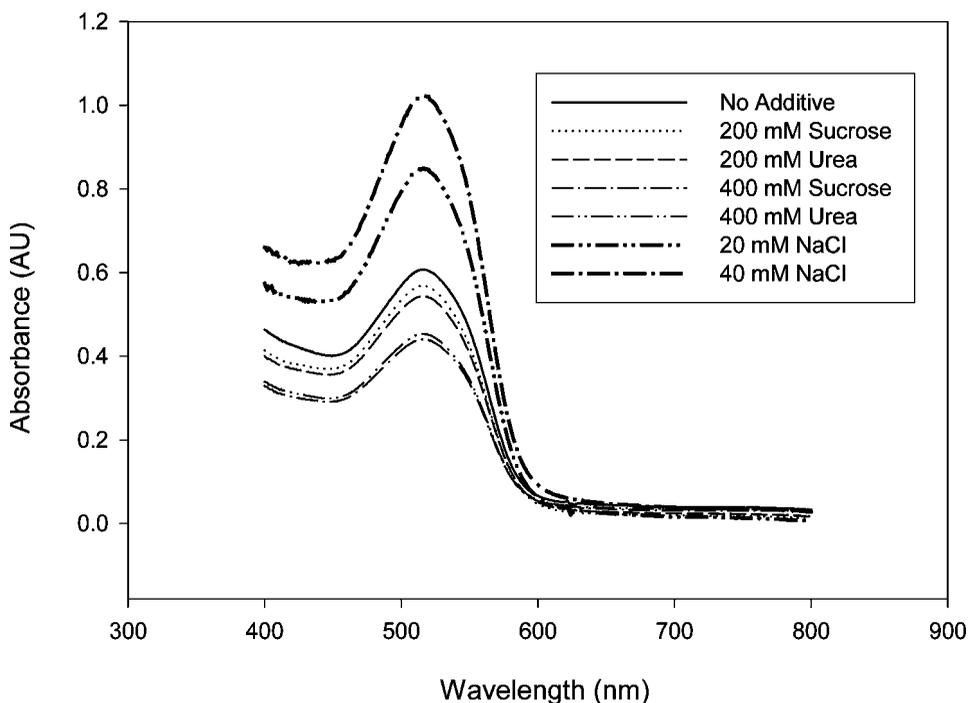

Figure 5. UV-visible spectra of Sudan III in the presence of 100 mM AMT in water at different additive concentrations.
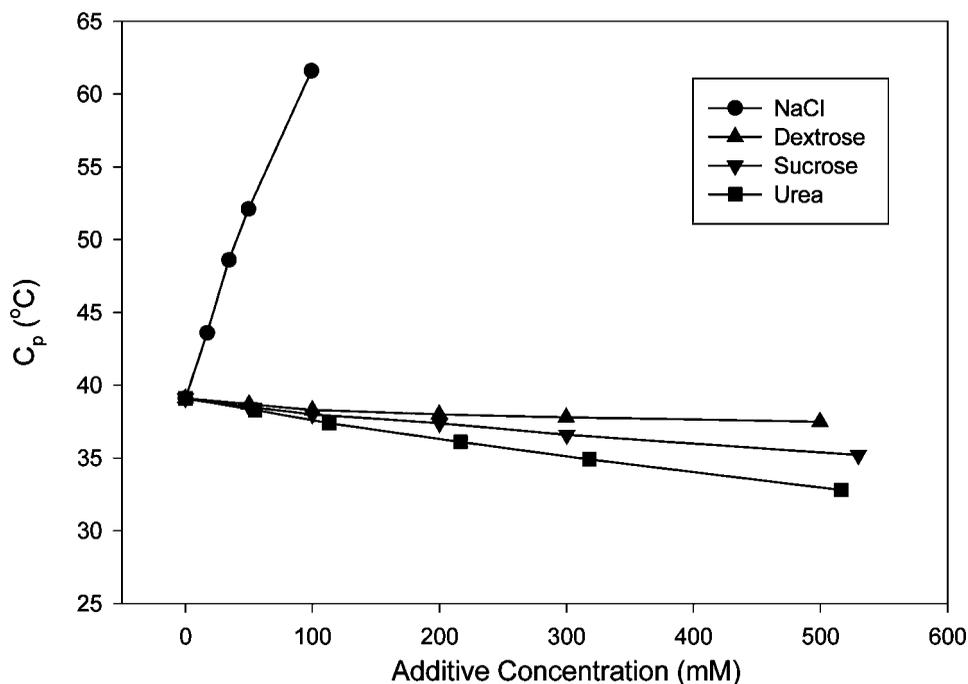
Figure 6. Variation of cloud point of 100 mM AMT in water with additive concentration. The pH of the AMT solutions is 6.5.

UV-visible absorbance on addition of sucrose and urea results from a decrease in the size of micelles.

Figure 6 shows the effect of additive concentration on the CP of 100 mM AMT in water. The pH of the AMT solutions is here fixed at 6.5. It can be seen that addition of NaCl to the AMT solution increases the CP, whereas addition of dextrose, sucrose, and urea decreases the CP. Al-Ghamdi and Nasr-El-Din (1997) observed in their experiments that addition of NaCl to the Triton-X nonionic surfactant solutions reduces the CP, while addition of urea increases the CP. In this case, Cl^- ions are water structure makers and decrease solute solubility, lowering the CP of nonionic surfactants due to the salting-out effect. Addition of urea increases the CP of nonionic surfactants because urea breaks the water structure and enhances solubility of nonionic surfactants through hydrogen bonding. On the other hand, addition of dextrose and sucrose is known to decrease the CP of nonionic surfactants because they make the water structure and reduce solubility of nonionic surfactants.

We see the opposite trend in the CP behavior on addition of NaCl and urea to the cationic AMT solution. Combined results of Figures 5 and 6 suggest that the micellar size increases with increasing NaCl concentration due to both the electrostatic effect and the salting-out effect. An increase in the cationic AMT micellar size would enhance electrical repulsion between the cationic AMT micelles even though Cl^- ions partly screen out the surface charge of the AMT micelles. This would result in an increase in CP. In contrast, addition of nonelectrolytes including dextrose, sucrose, and urea decreases the micellar size and depresses the CP due to reduced electrical repulsion between the AMT micelles resulting from the reduced micellar

size. Note that addition of sucrose, a disaccharide, lowers the CP greater than dextrose, a monosaccharide. In our system, the variations of CP and micellar size with added nonelectrolytes may not be attributed to the water solvent effect. It appears that the adsorption of nonelectrolytes such as sucrose and urea at the micelle-water interface originates a restriction of micellization and is responsible for a decrease in the UV-visible absorbance. It would be plausible to assume that oxygen atoms in added nonelectrolytes interact with AMT head groups, which may reduce the micellar size and in turn lower the CP.

Mechanisms for AMT micellar growth and interactions between AMT micelles and additives discussed above are a preliminary explanation. Further studies employing light scattering and fluorescence measurements are needed to make a thorough interpretation of the data.

Conclusions

CP and dye solubilization measurements were performed to investigate the effect of electrolytes and nonelectrolytes on the micellar behavior of AMT. The CP decreased with increasing AMT concentration below the cmc, while it increased with increasing AMT concentration above the cmc. The CP of AMT micelles decreased with increasing pH due to reduced electrical repulsion between AMT micelles. Furthermore, the aggregation number, i.e., the micellar size, increases at higher pHs, due to a decrease in head group repulsion. Addition of electrolytes increased the CP due to an increase in electrical repulsion between the cationic AMT micelles as a result of AMT micelle growth. Addition of a nonelectrolyte decreased the CP and the micellar size as a result of interactions between nonelectrolytes and AMT head groups. The effect of addition of electrolytes was considerable compared with nonelectrolytes. The CP and dye solubilization results presented in this article are consistent with the micellar behavior of ionic surfactants reported in the literature.

Acknowledgment

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