

Cloud Point Phenomenon in Amphiphilic Drug Solutions

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We report here the cloud point (CP) phenomenon in amphiphilic drug amitriptyline (AMT) solutions. We have investigated the influence of electrolytes on the micellar behavior of AMT by using CP and dye solubilization techniques. The CP was found to considerably decrease with increasing pH due to a reduction in electrical repulsion between AMT micelles. It increased on addition of electrolyte due to an increase in electrical repulsion resulting from an increase in the cationic AMT micelle size. The binding effect of anionic counterions was in the order: $\text{Br}^- > \text{Cl}^- > \text{F}^-$. The effect of cations was insignificant compared to anions.

Introduction

Amphiphilic drugs bear an ionic or nonionic polar headgroup and a hydrophobic portion. They tend to self-associate as micelles in aqueous solution in a surfactant-like manner. Micelle formation is a highly dynamic process, the equilibrium between monomer and aggregate being established within a few milliseconds. When micelles are formed, their aggregation number (N) is usually small. Drug self-association depends strongly on the pH and the ionic strength of added electrolytes.¹ For example, the critical micelle concentration (CMC) of piperazine-containing drugs increases and N decreases with decreasing pH, as a result of protonation of the second nitrogen atom of the drugs' piperazine ring, thus resulting in an increase in electrical repulsion.² The CMC of phenothiazine micelles also decreases and N increases with increasing electrolyte concentration. The CMC decreases and N increases in the order $\text{F}^- < \text{Cl}^- < \text{Br}^- < \text{I}^-$.³

Cloud point (CP) phenomenon is generally observed in nonionic surfactant micellar solutions when the temperature of the system is raised to a certain value. Gu and Galera-Gómez⁴ reported that the CP of TX-114 increased on adding small amounts of either cationic surfactant cetyltrimethylammonium bromide (CTAB) or anionic surfactant sodium dodecyl sulfate (SDS). Moreover, the CP of a mixed solution of TX-114 and ionic surfactants decreased when small amounts of inorganic salts were added. The decreasing effect depended on the nature and concentration of the salt and, particularly, on the valency of the salt ion with charges opposite in sign to the charges of the nonionic–ionic mixed micelles. Recently, Kumar et al.⁵ observed the CP phenomena in the anionic surfactant SDS in combination with symmetrical quaternary bromides. They found that the CP depends on the nature and concentration of salt and the surfactant concentration.

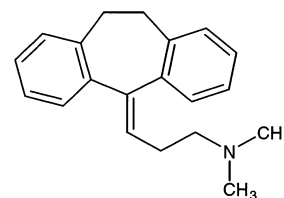


Figure 1. Molecular structure of AMT.

To the best of our knowledge, the CP phenomenon of amphiphilic drugs has not been reported yet in the literature.

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.¹ In this article, amitriptyline (AMT), a tricyclic antidepressant agent, was employed as a model amphiphilic drug to study the CP phenomenon in the drug solutions. The molecular structure of AMT is shown in Figure 1. 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. The effect of pH and electrolyte on the CP of AMT solution has been discussed in terms of electrical repulsion between cationic AMT micelles.

Experimental Section

AMT hydrochloride ($\geq 98\%$) supplied by Sigma Chemical Company was used without further purification. All electrolytes including sodium fluoride, sodium chloride, sodium bromide, sodium phosphate (SP), lithium chloride, and potassium chloride were of the reagent grade and were obtained from Fisher Scientific. 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 Ω cm was used to prepare AMT solutions. 500 mM SP buffer solutions used in the CP experiments were prepared from SP monobasic monohydrate (356 mM) and SP tribasic dodecahydrate (144 mM). 10 mM SP buffer solutions consisted of 6.1 mM SP monobasic monohydrate and 3.9 mM SP tribasic dodecahydrate.

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[†] University of Ulsan.

(1) Schreier, S.; Malheiros, S. V. P.; de Paula, E. *Biochim. Biophys. Acta* **2000**, *1508*, 210.

(2) Attwood, D.; Natarajan, R. *J. Pharm. Pharmacol.* **1981**, *33*, 136.

(3) Atherton, A. D.; Barry, B. W. *J. Colloid Interface Sci.* **1985**, *106*, 479.

(4) Gu, T.; Galera-Gómez, P. A. *Colloids Surf., A* **1995**, *104*, 307.

(5) Kumar, S.; Sharma, D.; Kabir-ud-Din *Langmuir* **2000**, *16*, 6821.

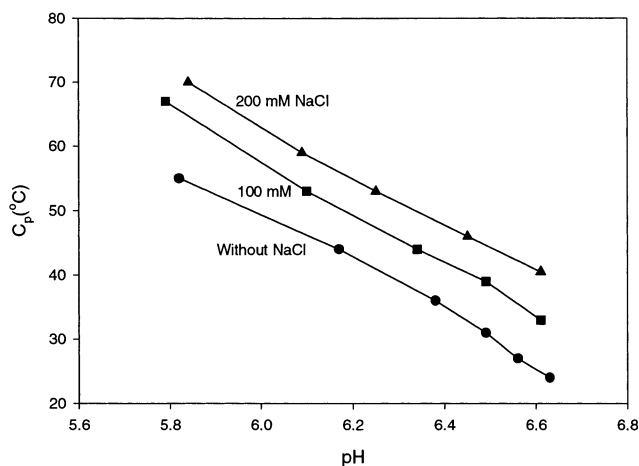


Figure 2. Effect of pH on the CP of 50 mM AMT in a 10 mM sodium phosphate buffer solution at different NaCl concentrations.

Surface tensions of AMT in water were measured at room temperature by the Wilhelmy plate method to determine the CMC of AMT. The pH of the AMT solutions was adjusted using 1 N NaOH obtained from Fisher Scientific and was measured with an ATI Orion 720A pH meter. Dye solubilization experiments for the AMT solutions in the absence of SP buffer 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. 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 starting from room temperature. Heating was regulated to about 0.5 $^{\circ}\text{C}/\text{min}$ around the CP. The repeatability of the CP measurements was ± 0.2 $^{\circ}\text{C}$.

Results and Discussion

The CMC of AMT in pure water determined by both the Wilhelmy plate and the dye solubilization methods is found to be about 40 mM, which is close to the value (36 mM) reported in the literature.⁶ The CMC of AMT decreases with increasing pH due to deprotonation of the nitrogen atom in the tertiary amine portion of the AMT molecule. It also decreases with increasing electrolyte concentration. The concentrations of AMT considered in this work are above the CMC of AMT in pure water where AMT micelles start to form in aqueous solutions.

Figure 2 depicts the effect of pH on the CP of AMT in a 10 mM SP buffer solution at different NaCl concentrations. Here the concentration of AMT is fixed at 50 mM. As shown in Figure 2, the CP decreases as the value of pH increases at a given NaCl concentration. The ionization constant, pK_a , of AMT in the free molecular state is 9.4.⁷ However, a considerable decrease in the CP with increasing pH from 5.8 to 6.6 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 from that in the free molecular state. Electrostatic effects in AMT micelles would shift the pH equilibrium of AMT toward the deprotonated form. As the value of pH increases, more AMT molecules in micelles become un-ionized. Thus, electrical repulsion

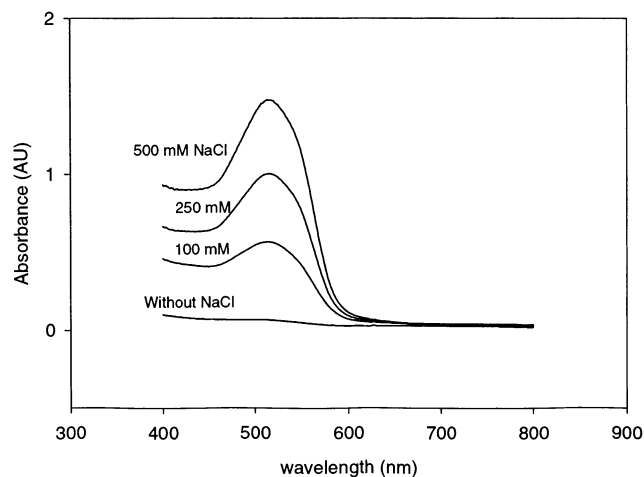


Figure 3. UV-visible spectra of 50 mM AMT in water at various NaCl concentrations. The pH of the AMT solutions is 5.8.

between micelles reduces with increasing pH. This may enhance micellar aggregation leading to a decrease in CP. In addition, intramicellar compactness increases at higher pH, due to a decrease in headgroup repulsion.⁸ As a result, the aggregation number, i.e., the micellar size, may increase with increasing pH. It is interesting to note in Figure 2 that the CP increases with increasing NaCl concentration at a given pH. It is well-known that simple inorganic salts such as NaCl lower the CP of nonionic surfactants.^{9,10} Accordingly, we see the opposite trend in the CP behavior on addition of NaCl in our system. Schott¹¹ mentioned that polyoxyethylated nonionic surfactants such as Triton X-100 owe their solubility to hydration of their ether linkages by hydrogen bonding. Ions which make the structure of water and increase the hydrogen bonding among water molecules decrease the hydration of the ether groups of the surfactant, reducing its solubility, salting it out, thus lowering its CP. Marszall¹² observed in his experiments that when NaCl is added to the SDS-Triton X-100 surfactant solution where SDS is present in small amounts, the CP is lowered. The author speculated that the original charge distribution is swamped and the corresponding repulsions are screened, resulting in a dramatic cloud point lowering. Figure 3 shows the UV-visible spectra of 50 mM AMT in water at various NaCl concentrations in the wavelength range of 400–800 nm. One can see that the UV-visible absorbance increases with increasing NaCl concentration. An increase in the UV-visible absorbance indicates an increase in the dye (Sudan III) solubility. Addition of electrolyte raises the aggregation number of ionic micelles due to electrostatic effects.¹³ It also lowers the CMC, thus creating more micelles. We found that the intensity of the absorbance peak of 50 mM AMT in the presence of 100 mM NaCl is comparable to that of 100 mM AMT in the absence of NaCl, where the micellar concentration is about 60 mM. Furthermore, the peak intensity of 50 mM AMT in the

(8) Wajnberg, E.; Tabak, M.; Nussenzeig, P. A.; Lopes, C. M.; Louro, S. R. *Biochim. Biophys. Acta* **1988**, *944*, 185.

(9) Doscher, T. M.; Myers, G. E.; Atkins, D. C. *J. Colloid Sci.* **1951**, *6*, 223.

(10) Travalloni-Louvisse, A. M.; Gonzalez, G.; Smith, D. H., Eds.; ACS Symp. Ser. 373; American Chemical Society: Washington, DC, 1988; Chapter 11.

(11) Schott, H. *Colloids Surf.* **1984**, *11*, 51.

(12) Marszall, L. *Colloids Surf.* **1987**, *25*, 279.

(13) Evans, D. F.; Wennerström, H. *The Colloidal Domain: where physics, chemistry, and biology meet*, 2nd ed.; Wiley-VCH: New York, 1999.

(6) Attwood, D.; Florence, A. T. *Surfactant systems: their chemistry, pharmacy and biology*; Chapman and Hall: New York, 1983.

(7) Hansch, C. *Comprehensive medicinal chemistry: the rational design, mechanistic study & therapeutic application of chemical compounds*; Pergamon Press: New York, 1990.

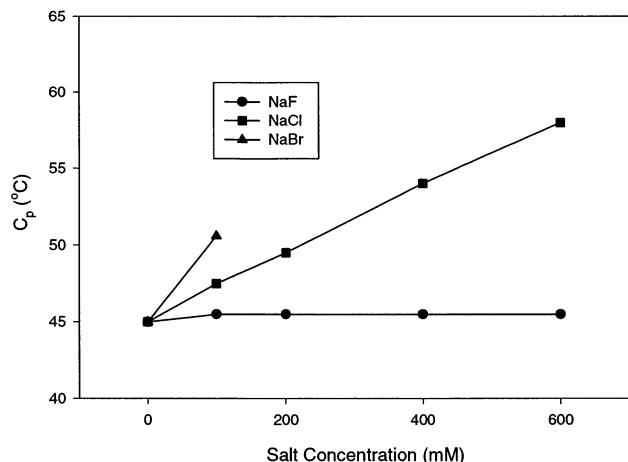


Figure 4. Effect of anionic counterions on the CP of 100 mM AMT in a 500 mM sodium phosphate buffer solution. The pH of the AMT solutions is 6.7.

presence of 500 mM NaCl was even slightly greater than that of 150 mM AMT in the absence of NaCl. These results suggest that an increase in the UV-visible absorbance results mainly from the enlarged micellar size. The presence of a counterion (Cl^-) is responsible for the decrease in surface area occupied per AMT headgroup (A_0), with a simultaneous increase in the Mitchell-Ninham parameter R_p ($= V/l_c A_0$, V_c being the volume of the hydrophobic part of the AMT monomer, and l_c its length)^{14,15} of the AMT monomer. Accordingly, a decrease in A_0 seems to result in an increase in R_p and micellar growth. The results of Figure 3 favorably support this interpretation. Combined results of Figures 2 and 3 suggest that the micellar size increases with increasing NaCl concentration due to the salting-out effect. This would enhance electrical repulsion between the micelles, resulting in an increase in CP as well as an increase in the dye solubility. 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 ionic heads of the AMT monomers take place. This process of dehydration increases interaction between the cationic heads and Cl^- . At the CP, a collapse of the micelles seems to occur.⁵

Figure 4 illustrates the influence of anionic counterions (F^- , Cl^- , Br^-) on the CP of 100 mM AMT in a 500 mM SP buffer solution. In this case, there is a copious amount of phosphate ions in the AMT solution, thus the electrostatic effects being already considerably reduced before other salts are added. Since the CP of the AMT solution is found to be highly sensitive to pH as shown in Figure 2, 500 mM SP buffer solution is used in the CP experiments to ensure that the pH of the AMT solution remains constant when other electrolytes are added. On the other hand, we observed that a slight change in the pH of the AMT solution upon addition of salt in the dye solubilization measurements does not have a significant effect on the UV-visible absorbance. The pH of the 500 mM SP buffer solution is 6.7. It is found that the CP remains almost constant at around 45 °C with NaF concentration up to 600 mM, implying that NaF has a negligible effect on the CP. In the case of NaCl, however, the CP significantly increases

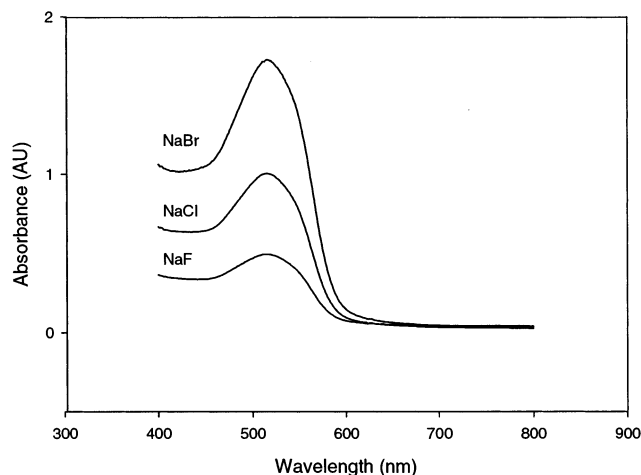


Figure 5. UV-visible spectra of 50 mM AMT in water at a 250 mM salt concentration. The pH of the AMT solutions is 5.8.

with salt concentration. The CP difference between NaCl and NaF becomes larger at higher sodium salt concentrations. At a 100 mM salt concentration, NaBr yields the highest CP and NaF the lowest CP. At higher NaBr concentrations above 100 mM in the presence of phosphate ions, the AMT solution became turbid at room temperature. This may result from the formation of nonmicellar phases such as lamellar or other liquid crystalline phases leading to phase separation. Figure 5 shows the UV-visible spectra of 50 mM AMT in water at a 250 mM salt concentration in the wavelength range of 400–800 nm. As seen in Figure 5, the UV-visible absorbance increases as one goes from NaF to NaBr. From the results of Figures 4 and 5, the effect of counterion binding is found to be in the order $\text{Br}^- > \text{Cl}^- > \text{F}^-$. The anions can be classified into the so-called Hofmeister or lyotropic series according to their salting-out strengths at a given molar concentration.¹⁶ Two mechanisms have been proposed to explain the Hofmeister series behavior. In one mechanism, inorganic salts affect the solvent property of water; the salts on the left-hand side of the Hofmeister series (kosmotropes) are considered to be structure makers decreasing solute solubility while those on the right-hand side (chaotropes) are structure breakers increasing solute solubility.¹⁷ In an alternative mechanism, the salting-in and salting-out phenomena are directly related to adsorption and desorption of ions to the hydrophilic parts of the organic compounds.¹⁸ The controversy between two mechanisms has not been solved yet.¹⁹ Lu et al.²⁰ found that the properties of ionic surfactants are affected by the counterions because the substitution of one counterion by another alters the interaction between counterion and surfactant, which may change the extent of binding or ionization. The effect of counterion binding on the structure of anionic surfactants in the aqueous system has been widely investigated with interest largely focused on micellization.²¹ The degree of counterion binding has an effect on the size and shape of micelles.^{6,22} The order of

(16) Hofmeister, F. *Arch. Exp. Pathol. Pharmacol.* **1888**, 24, 247.

(17) Franks, F. *Water: A comprehensive treatise*; Plenum Press: New York, 1972.

(18) Hall, D. G. *J. Chem. Soc., Faraday Trans. 2* **1974**, 70, 1526.

(19) Iwanaga, T.; Kunieda, H. *J. Colloid Interface Sci.* **2000**, 227, 349.

(20) Lu, J. R.; Marrocco, A.; Su, T. J.; Thomas, R. K.; Penfold, J. *J. Colloid Interface Sci.* **1993**, 158, 303.

(21) Berr, S. S.; Coleman, M. J.; Marriot, J.; Johnson, J. S., Jr. *J. Phys. Chem.* **1986**, 90, 6492.

(22) Rosen, M. J. *Surfactants and Interfacial Phenomena*, 2nd ed.; Wiley: New York, 1989.

(14) Kabir-ud-Din; Kumar, S.; Kirti; Goyal, P. S. *Langmuir* **1996**, 12, 1490.

(15) Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2* **1981**, 77, 601.

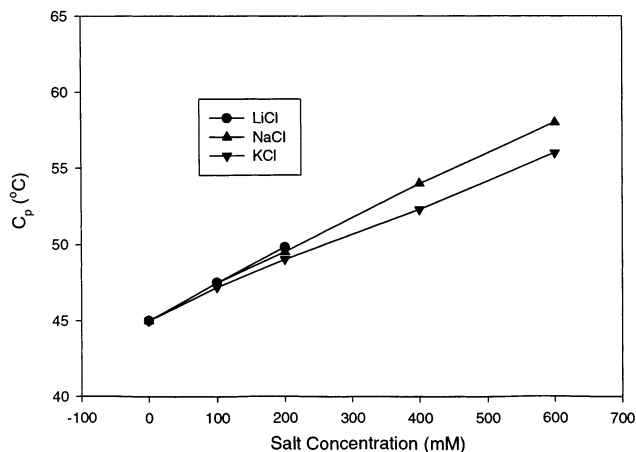


Figure 6. Effect of cationic co-ions on the CP of 100 mM AMT in a 500 mM sodium phosphate buffer solution. The pH of the AMT solutions is 6.7.

the counterion effect shown in Figures 4 and 5 correlates with the position of the ions in the Hofmeister series.

Figure 6 shows the influence of cationic co-ions (Li^+ , Na^+ , K^+) on the CP of 100 mM AMT in a 500 mM SP buffer solution. The CP increases linearly with chloride salt concentration. At higher LiCl concentrations above 200 mM, the AMT solution became turbid at room temperature as observed in NaBr. Note that the CP difference between NaCl and KCl is not significant even at a higher chloride salt concentration of 600 mM (compare with Figure 4). Figure 7 shows the UV-visible spectra of 50 mM AMT in water at a 250 mM chloride salt concentration in the wavelength range of 400–800 nm. The UV-visible absorbance changes slightly with the size of cations. The above results imply that the effect of cations is small compared to anions. Our observations are consistent with the Schulze-Hardy rule,¹³ indicating that the valence of the ion of opposite charge to the colloid has a principal effect on the stability of the colloid.

Conclusions

We have observed the CP phenomenon in amphiphilic drug AMT solutions. CP and dye solubilization measure-

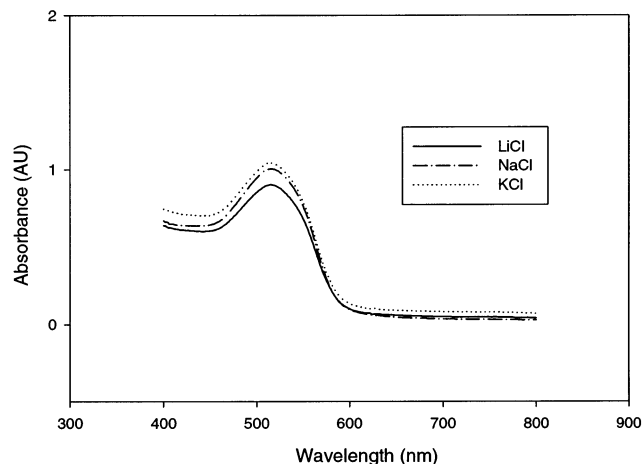


Figure 7. UV-visible spectra of 50 mM AMT in water at a 250 mM chloride salt concentration. The pH of the AMT solutions is 5.8.

ments were performed to investigate the effect of electrolytes on the micellar behavior of AMT. The CP remarkably decreased with increasing pH due to a reduction in electrical repulsion between AMT micelles. Also, intramicellar compactness may increase at higher pH, due to a decrease in headgroup repulsion. Addition of electrolyte increased the CP due to an increase in electrical repulsion as a result of the cationic AMT micelle growth. The binding effect of anionic counterions was in the order $\text{Br}^- > \text{Cl}^- > \text{F}^-$. The order of the effect correlates with the position of the ions in the Hofmeister series. The effect of cations was insignificant compared to anions. The CP and dye solubilization results presented in this paper are consistent with the micellar behavior of ionic surfactants reported in the literature.

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