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Influence of Electrolytes on the Micellar Growth of Amphiphilic Drug Chlorpromazine Hydrochloride

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Abstract: The effect of electrolytes on the micellar behavior of an amphiphilic drug, chlorpromazine (CPZ) hydrochloride, was studied using cloud point (CP) and dye solubilization techniques. In the presence of KBr, increase in pH led to decrease in the CP of 50 mmol·L^{-1} drug solution (prepared in 10 mmol·L^{-1} sodium phosphate (SP) buffer) because of deprotonation of drug molecules at high pH. The visible absorbance increased (due to dye solubilization) with the increase in pH from 6.5 to 6.9, which indicated micellar growth. At fixed pH (6.7), addition of inorganic salts (KF, KCl, and KBr) to drug solutions (50 mmol·L^{-1}) caused an increase in the CP as well as in the visible absorbance, with effectiveness being in the order: $F^-<Cl^-<Br^-$. The results were discussed on the basis of counter-ion binding and their effect toward micellar growth. Cations (co-ions) also led to an increase in the CP (and also the visible absorbance), with their effectiveness order being $Li^+>Na^+>K^+$, which was explained by considering cognizance of their hydrated radii. Compared with anions, their effect was small. Increase in [CPZ] caused micellar growth and hence the CP as well as the visible absorbance increased. The overall behavior was discussed in terms of electrostatic interactions and micellar growth.

Key Words: Phenothiazine drug; Chlorpromazine hydrochloride; Cloud point; Dye solubilization; Hofmeister series; Micelles

Many pharmacologically active compounds are amphiphilic and hence undergo different kinds of association in a surfactant-like manner[1–5]. Despite the investigation of the micellar properties of these drugs by different workers, there is lack of data on the effect of temperature on their micellar solutions. When the temperature is increased to a particular value, the clouding phenomenon is generally observed in nonionic surfactant solutions[6]. When the nonionic surfactant solution has a temperature higher than the cloud point (CP), phase separation occurs, resulting in the formation of the micellar-rich phase or coacervate and the micellar dilute phase. The CP has been found to be highly dependent on the presence of additives [7–11]. Encouraged by our own findings of the dependence of the CP in anionic surfactant solutions on the nature and concentration of additives[12–15], and hence its possi ble tuning, we have started exploring clouding behavior in solutions of amphiphilic drugs.

The dye solubilization is a well-known technique for studying micellar behavior of surfactants, amphiphilic block copolymers, and amphiphilic drugs[16–22]. The underlying principle is the dependence of solubilization of otherwise water-insoluble dye on the state of aggregation of the amphiphile. As a result, the absorbance value is nearly negligible prior to micellization, which then rises steeply due to micelle formation and solubilization of the dye therein.

Chlorpromazine (CPZ) hydrochloride, a phenothiazine with neuroleptic activity, has shown a high capacity to interact with biological membranes and is often regarded as model drug for the investigation of interactions between drug and membranes (both biological and model)[1,23–37]. As the electrolyte concentration in membranes may vary, the CP of a drug may be affected. With this viewpoint, CPZ (which shows clouding at 37.5 ℃ in 50 mmol·L^{-1} CPZ at pH 6.7 in 10 mmol·L^{-1} phosphate buffer[38,39]) was employed herein as a model to study the CP phenomenon in the drug solutions with additives. Furthermore, as the effect of Br^- was significant[39], most of the systems of the present study contained KBr. The objective of the current study was to extend the scope of our previous work by studying dye solubilization in order to get insight into the morphological aspects involved when a drug solution approaches the CP. The results of this study provide support to the mechanism of increase in CP due to the growth of CPZ micelles.

1 Experimental

CPZ hydrochloride (≥95.0%, Fluka, Switzerland) was used

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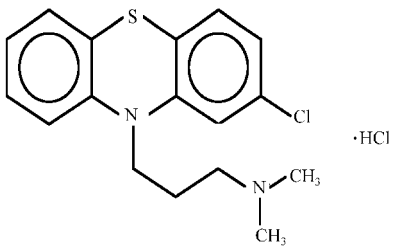
as received. The electrolytes, lithium bromide (LiBr, ≥99%, E. Merck, Germany), sodium bromide (NaBr, ≥99.9%, BDH, England), potassium fluoride (KF, ≥99%, BDH, England), potassium chloride (KCl, ≥99.8%, BDH, England), and potassium bromide (KBr, ≥99%, Merck, Germany) were of analytical grade. The components of buffer, trisodium phosphate dodecahydrate (TSP), and sodium dihydrogen phosphate (SDP) monohydrate were also purchased from Merck.

The water used was doubly distilled and deionized (sp. cond.= 1–2 μS·cm^{-1}). SP buffer solution of 10 mmol·L^{-1} was prepared from TSP (6.1 mmol·L^{-1}) and SDP (3.9 mmol·L^{-1}) and subsequently used throughout as solvent. The pH of the CPZ solutions was measured with an ELICO pH meter (model LI 120). All CPs were obtained by placing Pyrex glass tubes (containing the sample drug solutions) in a temperature-controlled bath, the temperature was ramped at the rate of 0.1 ℃·min^{-1} near the CP, and onset of clouding was noted by visual inspection. The temperature at the commencement of clouding was taken as CP. However, the temperature was oscillated slowly through the CP until the results were reproducible (±0.5 ℃)[12–15]. Dye solubilization experiments for the aqueous drug solutions (with or without electrolytes) were performed at room temperature. The sample solutions with Sudan III dye (equilibrated for 24 h) were filtered and then the spectra were recorded using a UV-visible Spectronic-20D+ spectrophotometer (Thermo Electron Corp., Madison, WI, USA). The surface tensions were measured using the ring detachment method using a S.D. Hardson tensiometer (Kolkata, India).

2 Results and discussion

The critical micelle concentration (cmc) of CPZ in pure water, determined by surface tension method, was found to be approximately 17 mmol·L^{-1}. The literature values, reported by different authors[34,39] using variety of techniques and experimental conditions, are scattered over a range of two orders of magnitude (10^{-5} up to 10^{-3} mol·L^{-1}). CPZ has an amino group and, because of its pK_α=9.4[40], exists essentially in its charged form (cationic) at the physiological pH (with a small fraction in neutral form). At low pH values, the tertiary amine portion of the CPZ molecule becomes positively charged, whereas at high pH values, it becomes neutral (see the structure, Scheme 1). Another point worth mentioning is that no minima were obtained in the surface tension *vs* lg[drug] plots[22]: this indicates that despite 95% purity, the drug sample is free of surface-active impurities.



Scheme 1 Molecular structure of chlorpromazine (CPZ) hydrochloride

Previously we found that the CP decreased with the increase in pH (ranging from 5.5 to 6.9)[39]. As the pH increases, the drug molecules become progressively deprotonated and hence repulsion, due to positive charge on the headgroups, decreases. This increases the compactness of micelles and decreases the CP (whether or not an electrolyte is present)[39].

Fig.1 shows the effect of KBr concentration on the CP of CPZ solutions at different fixed pHs (6.5, 6.7, 6.9——all below the pK_α value of the drug). At all the three pHs, CP follows the same trend (at any fixed [KBr], increase in pH causes a decrease in the CP). As the pH is increased more and more, drug molecules become unionized, reducing the inter- and intra-micellar repulsions. These reduced repulsions may give rise to more compact micelles and cause a decrease in the CP. Presence of Br^- (as KBr) causes a decrease in the surface area occupied per CPZ head group (A_o), with a simultaneous increase in the Mitchell-Ninham parameter R_p (=V_c/l_cA_o, where V_c is the volume of the hydrophobic part of the CPZ monomer and l_c is its length)[41] of the CPZ monomer. Therefore, a decrease in A_o results in an increase in R_p and in micellar growth. The results of Fig.1 favorably support this interpretation. As the Br^- ions are hydrated, their presence (binding!) near the micellar head is expected to make the micelles relatively wet compared with grown and compact micelles formed with the increase in pH. The increase in the CP with micellar growth is in accordance with Kim and Shah′s earlier findings[21], where large increases were recorded in amitriptyline solutions with the addition of KBr.

Fig.2 shows the visible spectra of Sudan III in 50 mmol·L^{-1} CPZ in 10 mmol·L^{-1} sodium phosphate solutions at different pHs in the wavelength range of 400–600 nm. The visible absorbance increases with increase in pH from 6.5 to 6.9, which indicates an increase in the dye (Sudan III) solubility, resulting mainly from the enlarged micellar size[21]. Thus, the results clearly suggest that an increase in the visible absorbance with increasing pH mainly results from the enlarged micellar size. Fig.3 illustrates the visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ in water containing different fixed KBr concentrations. In this case also, the visible absorbance increases with

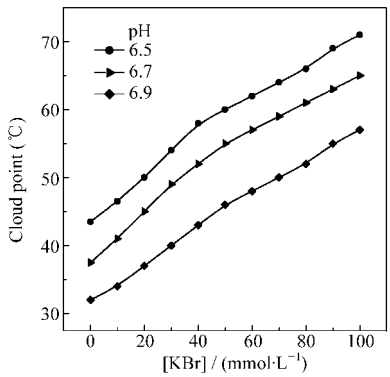


Fig.1 Effect of KBr concentration on the CP of 50 mmol·L^{-1} CPZ solutions prepared in 10 mmol·L^{-1} sodium phosphate buffer solutions at different pHs

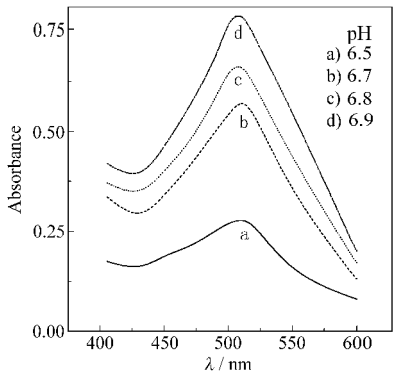


Fig.2 Visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ prepared in 10 mmol·L^{-1} sodium phosphate buffer solutions at different pHs

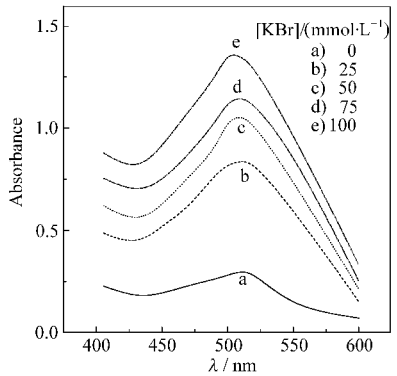


Fig.3 Visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ in water containing different fixed amounts of KBr concentrations

increasing KBr concentration, indicating an increase in the dye solubility. The addition of the electrolyte raises the aggregation number of ionic micelles due to electrostatic effects.

Addition of electrolytes (KF, KCl, and KBr) at constant pH (6.7) causes an increase in the CP and the order being $Br^- > Cl^- > F^-$ (Fig.4). The order correlates well with their position in Hofmeister series. As halide ions carry a charge opposite to the drug micelles, they interact electrostatically with the micelles. The degree of counter-ion binding is known to affect the size and shape of the micelles[1,42]. F^- binding to cationic headgroups is weak (as F^- is highly hydrated; crystal radius: 0.136 nm and hydrated radius: 0.352 nm) and therefore, with NaF addition, slow changes occur in the micelle shape/size, as a consequence of which the CP also increases slowly. Br^- ions bind strongly to the micelles and hence are more effective in increasing the size of micelles. However, as Br^- is also hydrated, although less than F^- (crystal radius: 0.195 nm and hydrated radius: 0.330 nm), it increases micelle hydration as well as the CP. The trend (Figs.4, 5, and see Fig.3 also for additional support to the effectiveness of KBr addition) is similar to earlier findings[21] of large increase in the CP as well as the absorbance intensity with the electrolyte addition.

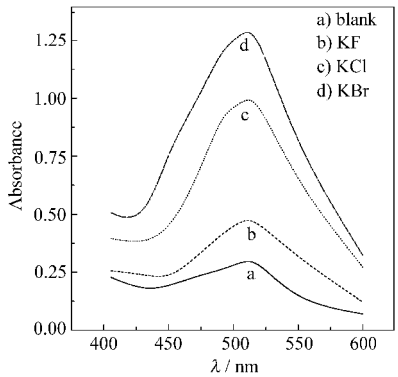


Fig.5 Visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ in water containing 100 mmol·L^{-1} KX

The influence of cationic co-ions (Li^+, Na^+, K^+) on the CP of 50 mmol·L^{-1} CPZ solutions (Fig.6) shows the increase in effectiveness order of CP as: $Li^+ > Na^+ > K^+$. Bearing similar charge as the micelles, these ions would remain in the aqueous solution and would not affect the micelles directly. Being the smallest in size, Li^+ is the most hydrated. Therefore, to remove water from micelles, it needs much energy, which leads to the highest CP (Fig.6). K^+ being the largest in size and least hydrated, less energy would be required to remove water from micelles, which is

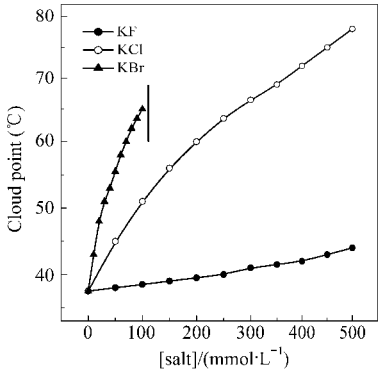


Fig.4 Effect of anionic counter-ions on the CP of 50 mmol·L^{-1} CPZ solutions prepared in 10 mmol·L^{-1} sodium phosphate buffer (pH 6.7)

"|" indicates precipitation occurring beyond [KBr]>100 mmol·L^{-1} at room temperature (which could be due to formation of nonmicellar phases).

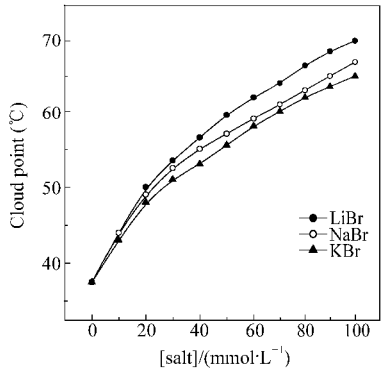


Fig.6 Effect of cationic co-ions on the CP of 50 mmol·L^{-1} CPZ solutions prepared in 10 mmol·L^{-1} sodium phosphate buffer (pH 6.7)

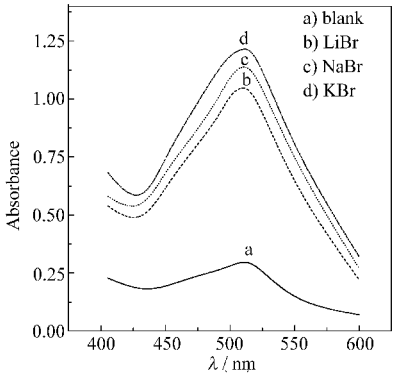


Fig.7 Visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ in water containing 100 mmol·L^{-1} MBr

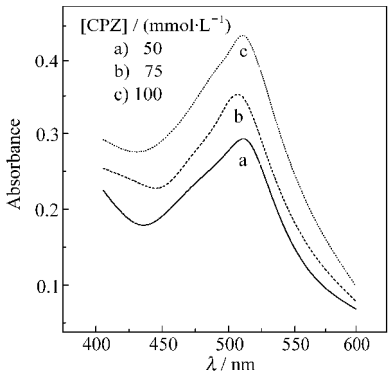


Fig.9 Visible spectra of Sudan III solubilized in different fixed amounts of CPZ concentrations in water

demonstrated by the lowest CP observed with KCl (Fig.6). Obviously, Na^+ imparts an intermediate effect.

The visible spectra of Sudan III solubilized in 50 mmol·L^{-1} CPZ in water at 100 mmol·L^{-1} bromide salt are shown in Fig.7. The absorbance changes with the size of cations and fully endorses the above-discussed order of the cations toward the CP increase of CPZ micellar solutions. The above results also imply that the effect of cations (co-ions) is small compared with anions (counter-ions).

In view of the results described above, the Br^- ion has been found to be an effective cloud-point booster for CPZ solutions. Its effect, therefore, was further explored for the drug solutions containing different fixed CPZ concentrations (50, 75, and 100 mmol·L^{-1}) (Figs.8, 9). The increasing trend observed for CP is the same for all the CPZ concentrations (Fig.8). Increase in [CPZ], in the presence of KBr, increases the number of micelles (which are charged). For a fixed [KBr], increase in drug concentration, increases the number, size, and charge of micelles, which increase both inter- and intra-micellar repulsions, causing an increase in the CP. The corresponding visible spectra of Sudan III solubilized in different fixed amounts of CPZ concentration in water are shown in Fig.9. The absorbance increases with increasing [CPZ], indicating increase in the dye solubility as well as micellar growth, which supports the above explanation (see Fig.8).

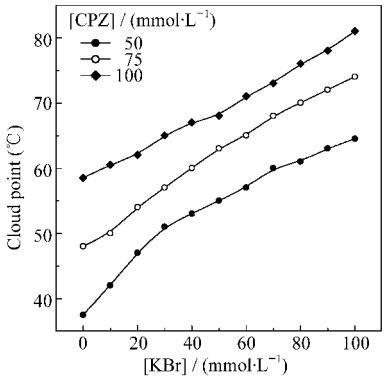


Fig.8 Effect of KBr concentration on the CP of CPZ solutions containing different fixed amounts of the drug prepared in 10 mmol·L^{-1} sodium phosphate buffer (pH 6.7)

3 Conclusions

We have performed the CP and dye solubilization measurements to investigate the influence of electrolytes on the micellar behavior of CPZ. The CP of CPZ micellar solutions decreased with the increase in pH because of deprotonation of the drug molecules. Dye (Sudan III) solubilization experiments performed with increasing pH indicated micellar growth. Addition of KX (X=F, Cl, Br) decreased the electrical repulsion, resulting in micellar growth and increase in the CP as well as visible absorbance of the dye, with the order being: $Br^- > Cl^- > F^-$. The binding effect of co-ions is in the order: $Li^+ > Na^+ > K^+$. The effect of co-ions (cations) is small compared with the counter-ions (anions).

References

1 Attwood, D.; Florence, A. T. Surfactant systems. London: Chapman & Hall, 1983
2 Attwood, D.; Mosquera, V.; Villar, V. P. *J. Chem. Soc., Faraday Trans 1,* **1989, 85**: 3011
3 Ruso, J. M.; Attwood, D.; Taboada, P.; Suarez, M. J.; Sarmiento, F.; Mosquera V. *J. Chem. Eng. Data,* **1999, 44**: 941
4 Taboada, P.; Attwood, D.; Garcia, M.; Jones, M. N.; Ruso, J. M.; Samiento, V. *J. Colloid Interface Sci.,* **2000, 221**: 242
5 Attwood, D.; Boitard, E.; Dubes, J. P.; Tachoire, H. *J. Colloid Interface Sci.,* **2000, 227**: 356
6 Gu, T.; Galera-Gomez, P. A. *Colloids Surf. A,* **1995, 104**: 307
7 Schott, M. *Colloids Surf.,* **1984, 11**: 51
8 Karlstrom, G. *J. Phys. Chem.,* **1985, 89**: 4962
9 Myers, D. Surfactant science and technology. 2nd ed. New York: VCH, 1992
10 Gu, T.; Galera-Gomez, P. A. *Colloids Surf. A,* **1999, 147**: 365
11 Shigeto, K.; Olsson, U.; Kuneida, H. *Langmuir,* **2001, 17**: 4717
12 Kumar, S.; Sharma, D.; Kabir-ud-Din. *Langmuir,* **2000, 16**: 6821
13 Kumar, S.; Sharma, D.; Khan, Z. A.; Kabir-ud-Din. *Langmuir,* **2001, 17**: 5813
14 Kumar, S.; Sharma, D.; Khan, Z. A.; Kabir-ud-Din. *Langmuir,* **2002, 18**: 4205
15 Kumar, S.; Sharma, D.; Kabir-ud-Din. *Langmuir,* **2003, 19**: 3539

16 Mitra, S.; Dungan, S. R. *Colloids Surf. B,* **2000, 17**: 117
17 Mata, J.; Varade, D.; Ghosh, G.; Bahadur, P. *Colloids Surf. A,* **2004, 245**: 69
18 Mata, J.; Varade, D.; Bahadur, P. *Thermochim. Acta,* **2005, 428**: 147
19 Bhattarai, N.; Bhattarai, S. R.; Khil, M. S.; Lee, D. R.; Kim, H. Y. *Eur. Polym. J.,* **2003, 39**: 1603
20 Kelarakis, A.; Mai, S. M.; Havredaki, V.; Brett, A.; Booth, C. *J. Colloid Interface Sci.,* **2004, 275**: 439
21 Kim, E. J.; Shah, D. O. *Langmuir,* **2002, 18**: 10105
22 Alam, M. S.; Naqvi, A. Z.; Kabir-ud-Din. *J. Chem. Eng. Data,* **2007, 52**: 1326
23 Seeman, P.; Weinstein, J. *Biochem. Pharmacol.,* **1966, 15**: 1737
24 Kwant, W. O.; Seeman, P. *Biochim. Biophys. Acta,* **1969, 183**: 530
25 Seeman, P. *Pharmacol. Rev.,* **1972, 24**: 583
26 Cater, B. R.; Chapman, D.; Hawes, S. M.; Saville, J. *Biochim. Biophys. Acta,* **1974, 363**: 54
27 Leterrier, F.; Mendyk, A.; Viret, J. *Biochem. Pharmacol.,* **1976, 25**: 2469
28 Lee, A. G. *Mol. Pharmacol.,* **1977, 13**: 474
29 Frenzel, F.; Arnold, K.; Nuhn, P. *Biochim. Biophys. Acta,* **1978, 507**: 185
30 Romer, J.; Bickel, M. H. *Biochem. Pharmacol.,* **1979, 28**: 799
31 Yamagushi, T.; Watanabe, S.; Kimoto, E. *Biochim. Biophys. Acta,* **1985, 820**: 157
32 Guth, P. S.; Spirtes, M. A. *Int. Rev. Neurobiol.,* **1964, 7**: 231
33 Sheetz, M.; Singer, S. J. *Proc. Natl. Acad. Sci. USA,* **1974, 71**: 4457
34 Conrad, M. J.; Singer, S. J. *Proc. Natl. Acad. Sci. USA,* **1979, 76**: 5202
35 Paiement, J. *J. Pharm. Pharmacol.,* **1984, 36**: 614
36 Luxnat, M.; Galla, H. J. *Biochim. Biophys. Acta,* **1986, 856**: 274
37 Bindford, J. S.; Rao, M. S.; Pollock, V.; Malloy, R. C. *J. Phys. Chem.,* **1988, 92**: 3522
38 Wajnberg, E.; Tabak, M.; Nussenzveig, P. A.; Lopes, C. M. B.; Louro, S. R. W. *Biochim. Biophys. Acta,* **1988, 944**: 185
39 Alam, M. S.; Kumar, S.; Naqvi, A. Z.; Kabir-ud-Din. *Colloids Surf. B,* **2006, 53**: 60
40 Kartzung, B. G. Basic and clinical pharmacology. 9th ed. New York: McGraw Hill, 2004
41 Mitchell, D. J.; Ninham, B. W. *Faraday Trans. 2,* **1981, 77**: 601
42 Rosen, M. J. Surfactants and interfacial phenomena. New York: Wiley, 1989