Colloid Polym Sci (2007) 285:1573–1579
DOI 10.1007/s00396-007-1727-4

ORIGINAL CONTRIBUTION

Influence of organic additives on the clouding phenomena of promethazine hydrochloride solutions

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Received: 12 October 2006 / Revised: 19 June 2007 / Accepted: 19 June 2007 / Published online: 25 July 2007

Abstract This paper reports the influence of organic additives (alcohols, amino acids, sugars) on the micellization and cloud point (CP) of a phenothiazine drug, promethazine hydrochloride (PMT). The critical micelle concentration (CMC) of the drug, determined by surface tension measurements in the presence of a representative of each additive class (i.e., butanol, leucine, arabinose), are used to evaluate the maximum surface excess concentration (Γ_{max}) and the minimum area per surfactant molecule (A_{min}) at the air/water interface. Γ_{max} increases and CMC/A_{min} decreases with increasing concentration of the additives, which indicate mixed micelle formation. The intermicellar interaction coefficients in the mixed micelles (β^m and β^σ) are also calculated, and their negative values imply attractive interactions. Effect of pH revealed CP decrease with increasing pH due to deprotonation of PMT molecules. Effect of amino acids depended upon their nature and polarity, whereas sugars caused a CP decreasing effect. Aliphatic alcohols as well as cycloalkanols and diols decreased the CP. In the presence of arabinose, increase in drug concentration resulted in the CP increase, while increase in pH showed an opposite trend. Results are interpreted on the basis of mixed micelle formation, hydrophobic interactions, and change in solvent structure.

Keywords Cloud point · Phenothiazine drug · Promethazine hydrochloride · Mixed micelles · Aliphatic alcohols · Cycloalkanols · Amino acids · Sugars

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Introduction

Phenothiazine drugs are amphiphilic in nature and associate in water in a surfactant-like manner [1]. Association and thermodynamic properties of these drugs have earlier been examined [1–5]. Promethazine hydrochloride (PMT), a tranquilizer, although it possesses a rigid planar hydrophobic ring system (Fig. 1), associates in water to form micelles [5]. Like surfactants, phase transitions (dependent upon concentration, temperature, and pH) occur in this drug also. The temperature of phase transition is called the cloud point (CP) [6–8]. Above the CP, solutions spontaneously separate into two distinct phases; one phase is surfactant-rich and the other is surfactant-lean. The CP phenomenon is reversible, and when the temperature falls below the CP, a single phase appears again. After the phase separation above the CP, the chemical potential of a surfactant molecule should be equal between surfactant-rich and surfactant-lean phases. Presence of additives has been found to affect the CP, but the mechanism of phase transition has not yet been fully delineated.

As computer simulations have indicated that partitioned drugs accumulate heterogeneously in the membrane, local concentrations may be much higher than in the bulk aqueous phase or even in the other regions in the lipid bilayer [9, 10]. Because clouding is concentration-dependent, it is essential to have a knowledge of clouding behavior of drugs under varying conditions of concentration, pH, and presence of additives. We, therefore, thought it worthwhile to study the effect of various additives (viz. alcohols, amino acids, sugars) on the clouding in promethazine hydrochloride in the presence of sodium phosphate buffer. Under special circumstances, these additives may be found in blood (e.g., alcohols in alcoholics and excess sugar in diabetic patients) and can interact with the drug, inducing CP in the drug solution. This clouding would accumulate the drug at certain sites, which may prove harmful.

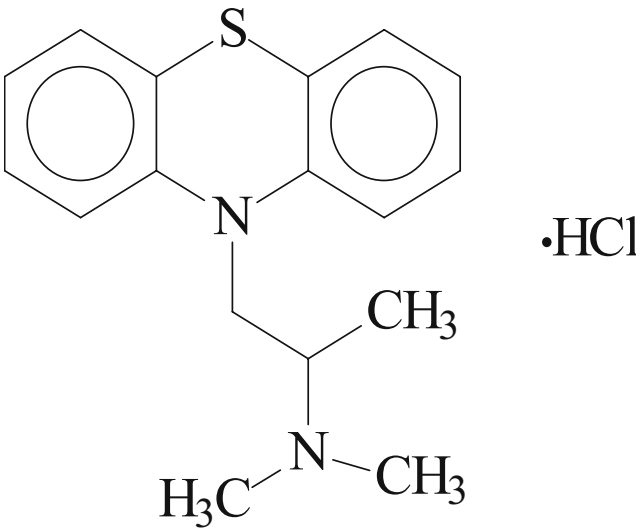

Fig. 1 Molecular structure of promethazine hydrochloride (PMT)

Experimental

Materials

PMT, ≥98%, supplied by Sigma, was used as received. DL-Asparatic acid, ≥99%; glutamic acid, ≥99%; glycine, ≥99.5%; L-phenylalanine, ≥99%; β-alanine, ≥99% (all SISCO, India); L-leucine, ≥99% (E. Merck, Germany); asparagine, ≥99% (Reanal, Hungary); DL-threonine, ≥98.5% (BDH, England) L-lysine, ≥98%; L-arginine, ≥99.5% (all Fluka, Switzerland); L-histidine, ≥99% (LOBA Chemie, India), and their hydrochloride salts, L-histidine monohydrochloride (BDH, England); L-histidine methylester dihydrochloride (Fluka, Switzerland); L-(+)-lysinium monohydrochloride, ≥99% (E. Merck, Germany); L-arginine monohydrochloride, ≥99% (LOBA Chemie, India) were used as received. Dextrose, ≥99%; D-(−)-fructose, ≥99% (all Merck, India); D-(+)-mannose, ≥99%; D-(+)-xylose (all s. d. fine, India); L (−) sorbose, ≥99% (Fluka, Switzerland) and L-(+)-arabinose, ≥98% (Sigma, USA) were used as received. Trisodium phosphate dodecahydrate (TSP) and sodium dihydrogen phosphate monohydrate (SDP) were of reagent grade obtained from Merck, India. Methanol, C_1OH, >99.8% (Ranbaxy, India); ethanol, C_2OH, ≥99.8% (Merck); 1-propanol, C_3OH, ≥99.9%; 1-hexanol, C_6OH, >99%; 1-heptanol, C_7OH, >99% (all BDH); *n*-butanol, C_4OH, ≥99.9% (Sarabhai, India); 1-pentanol, C_5OH, >99%; 1-octanol, C_8OH, >97%; cyclopentanol, ≥98% (all Fluka); ethane-di-ol, ≥99%; propane-1, 2-di-ol; cyclohexanol, >98% (all BDH, India) and allyl alcohol, >95% (Duchem Lab, India) were used as received. Steam distilled water (first time over $KMnO_4$) was used (specific conductivity 1–2× 10^{-6} Ω^{-1} cm^{-1}).

CMC and CP determinations

The CMC of PMT in pure water and in presence of additives was determined by measuring the surface tension of drug solutions of various concentrations at 27±0.5 °C. The surface tension (ST) was plotted against log [PMT] (not shown), and the constancy in ST was taken as the CMC of the drug. Ten millimolar SP buffer (prepared from SP monobasic monohydrate, 5.5 mM and SP tribasic dodecahydrate, 4.5 mM) was used throughout as solvent. To determine the CP, the sample solution was taken in a Pyrex glass tube, which was then stoppered and placed in a controlled heating device. The temperature was slowly raised, taking special care near the CP with the rate 0.1 °C/min. The onset of sudden clouding in the solution was taken as CP [11–13]. The solution was then cooled until the sample became clear again. The temperature was cycled (at least twice) in this way to obtain the CP temperature (reproducibility ±0.5 °C). The pH and drug concentration of the solution were fixed, respectively, at 6.7 and 50 mM (unless mentioned otherwise). An ELICO pH meter (model LI 120, India) was used for pH and a du Noüy type tensiometer (H. D. Hardson, Kolkata, India) for surface tension measurements.

Results and discussion

The pK_a value for promethazine hydrochloride, whose molecular structure is shown in Fig. 1, is 9.1 [14]. The critical micelle concentration (CMC) of aqueous PMT solution was determined by ring method and was found to be 45.14 mM (Table 1), which is close to the reported value (44.0 mM) [1]. As is well known, CMC of amphiphiles changes in the presence of additives. We have determined the CMC of PMT in the presence of electrolytes and observed that it decreases with the increasing concentration of additives [15]. The CMC of binary amphiphile systems (i.e., drug + additive) were determined by surface tension measurements, and the other relevant parameters (maximum surface excess concentration at the air/water interface, Γ_{max}, minimum area per surfactant molecule at the air/water interface, A_{min}, mole fraction of component 1, X_1^m, interaction parameter for mixed micelle formation in an aqueous medium, β^m, interaction parameter for mixed monolayer formation at the aqueous solution/air interface, β^σ, and the activity coefficients, f_1 and f_2, see Tables 1 and 2) were evaluated using appropriate equations [15–27]. Physicochemical properties of mixed micelles are known to be different from those of pure micelles of the individual components. Although CMCs of some binary mixtures are found above or below that of the individual components, most systems have values in between [16, 17]. Our CMC results (Table 1) agree well with the expected behavior, and

Table 1 Effect of additive concentrations on the CMC (determined by surface tension measurements) and A_{min} as well as Γ_{max} values of PMT in aqueous solutions at ~300 K

	Butanol			Leucine			Arabinose		
[Additive] (/mM)	CMC (/mM)	$10^{10}\times\Gamma_{max}$ (/mol m^{-2})	A_{min} (/Å^2)	CMC (/mM)	$10^{10}\times\Gamma_{max}$ (/mol m^{-2})	A_{min} (/Å^2)	CMC (/mM)	$10^{10}\times\Gamma_{max}$ (/mol m^{-2})	A_{min} (/Å^2)
0	45.14 (44.0)[a]	2.0985	79.12	45.14 (44.0)[a]	2.0985	79.12	45.14 (44.0)[a]	2.0985	79.12
50	31.99	1.934	85.86	32.84	1.564	106.18	31.37	1.636	100.14
100	27.57	2.040	81.79	26.81	1.615	102.79	25.59	1.665	99.69
150	25.59	2.095	79.23	22.08	1.672	99.27	21.88	1.774	93.59
200	23.34	2.238	74.17	17.85	1.848	89.83	17.53	1.876	88.51

[a] [1]

thus, indicate mixed micelle formation. Notwithstanding the above (i.e., decrease of CMC), other relevant parameters (i.e., decrease of A_{min}, increase of Γ_{max}, the negative β values—Tables 1 and 2) also support the conclusion [15]. Further, negative β values at all mole fractions of the mixed systems suggest that the interactions between the two components are more attractive in the mixed micelles.

As shown in Fig. 2, the CP of drug solution (50 mM) decreases with the increase in pH from 6.15 to 6.75 due to deprotonation of nitrogen atom of tertiary amine portion of the PMT molecule. The deprotonation increases micellar compactness due to a decrease in head group repulsion, which leads to decrease in CP.

Variation of CP with the addition of alcohols, as depicted in Figs. 3 and 4, shows that the added alcohols decrease the CP of PMT solutions over entire concentration range (although the behavior, just as for CMC, depends upon the chain length of the alcohol [28]). The CP remains nearly constant with the addition of C_1OH. With C_2OH–C_4OH, the decrease is slow (in fact, the CP remains almost constant up to certain concentration, and then slight decrease takes place). With C_5OH, the change is much pronounced. With C_6OH–C_8OH, the CP decrease becomes progressively steeper: with C_8OH, for example, it takes only 15–20 mM alcohol to cause a decrease of 23 °C. This behavior can be explained by taking cognizance of the nature of alcohols. As alcohols contain a hydrophilic –OH group and a hydrocarbon alkyl part, their distribution between aqueous and micellar phases depends upon these two factors. It is well known that short chain length alcohols (C_1OH–C_3OH) prefer aqueous phase, medium chain length alcohols (C_4OH and C_5OH) distribute between both the phases, and longer chain length ones (C_7OH and above) prefer micellar phase [28–32].

While considering micelle formation in ionic amphiphiles, two factors should be kept in mind. First is the electrostatic repulsion among charged head groups which favors small micelles with high surface area per head group and second is the hydrophobic interaction among alkyl chains which favors large, tightly packed structures with small surface area per head group [33]. C_1OH, being hydrophilic in nature, dissolves in water in all proportions and would not affect the micelles in any way; therefore, CP remains nearly constant on addition of C_1OH. C_2OH–C_4OH, at high concentrations, start solubilizing in the micelles, resulting in micellar growth and the resultant slight decrease in CP. However, longer chain length alcohols are

Table 2 Micellar compositions (X_1^m, X_1^σ), interaction parameters (β^m, β^σ), and activity coefficients (f_1^m, f_2^m, f_1^σ, f_2^σ) of binary mixtures of PMT and additive at different mole fractions of additive (α_1)

α_1	X_1^m	β^m	f_1^m	f_2^m	X_1^σ	β^σ	f_1^σ	f_2^σ
Butanol								
0.091	0.443	−1.600	0.609	0.730	0.287	−0.268	0.873	0.978
0.167	0.521	−3.448	0.453	0.392	0.469	−0.257	0.930	0.945
0.231	0.622	−6.044	0.422	0.096	0.596	−0.462	0.927	0.849
Leucine								
0.091	0.339	−0.224	0.907	0.975	0.194	−0.190	0.884	0.992
0.167	0.527	−0.259	0.944	0.931	0.349	−0.737	0.732	0.914
0.231	0.629	−0.296	0.960	0.889	0.422	−0.822	0.760	0.864
0.286	0.666	−0.388	0.956	0.842	0.479	−1.820	0.611	0.658
Arabinose								
0.091	0.340	−0.160	0.933	0.982	0.190	−0.068	0.956	0.997
0.167	0.495	−0.184	0.954	0.956	0.352	−0.641	0.764	0.923
0.231	0.585	−0.239	0.959	0.921	0.431	−0.850	0.759	0.854

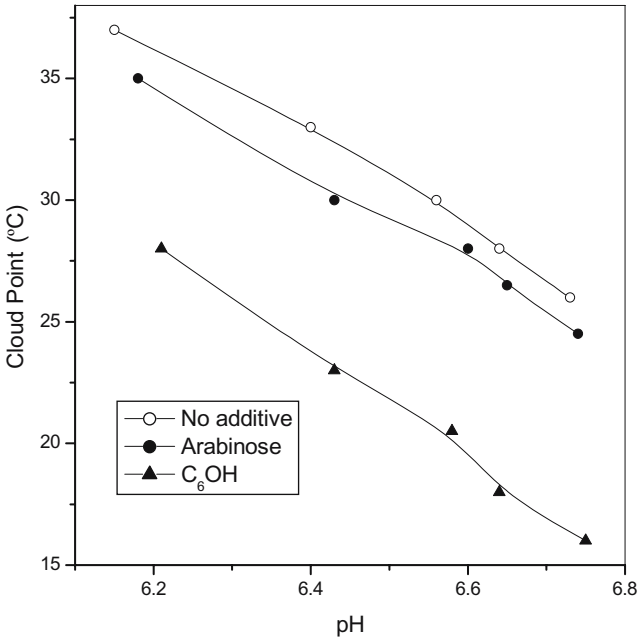

Fig. 2 Effect of pH on the cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution containing no or a fixed concentration of additives

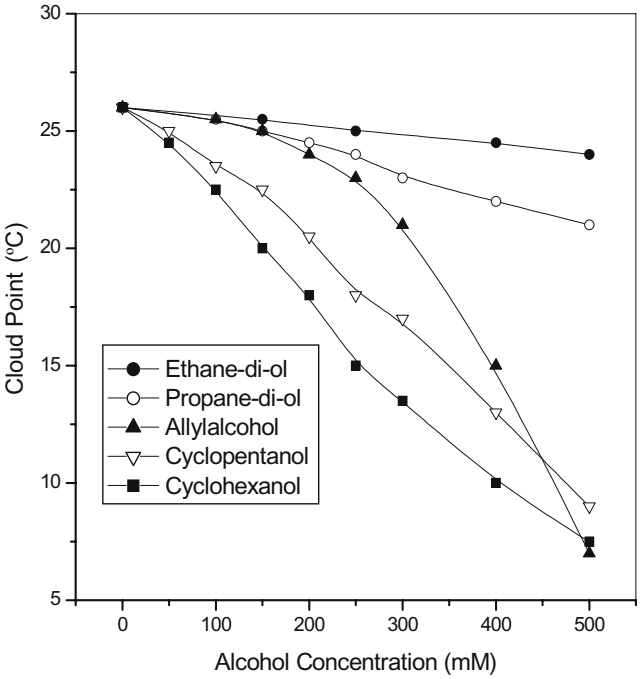

Fig. 4 Variation of cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution (pH=6.7) with addition of alcohols

hydrophobic in nature (hydrophobicity increases with the increase in chain length) but, due to the presence of hydrophilic –OH group, solubilize in head group region with the alkyl chain penetrating into the micellar core. This penetration increases the volume of the hydrocarbon part of the monomer (V_o) [34–36]. It also reduces the intermicellar repulsion between monomeric head groups by holding the –OH group between charged head groups of the drug molecule, decreasing surface area per molecule (a_o) and water content of the micelles [34–36]. As a result, the Mitchell–Ninham parameter R_p ($=V_o/a_o l_c$) [34] increases. Hence, larger aggregates would form with the longer chain length alcohols. It has been found on the basis of SANS studies [32] that the volume of SDS micelle increases with the increase of longer chain length alcohols, which is due to the increase in aggregation number as well as the increase in alcohol molecules in the micelles.

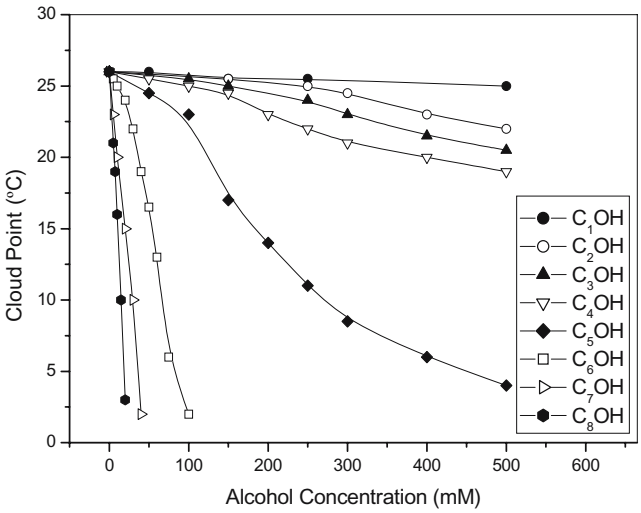

Fig. 3 Effect of addition of alcohols on the cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution (pH=6.7)

All the factors discussed above help in decreasing the CP of the drug system in the presence of C_6OH–C_8OH. Because of its large hydrophobic volume, only small amount of C_8OH is required to remove more water and, hence, it shows the steepest decrease in CP. C_5OH shows intermediate results. Gu and Galera-Gomez [37] also observed similar CP decreasing effect in the TX-100 (a non-ionic surfactant system) when the alcohol chain length was longer than C_3.

In view of the above discussion, the results of Fig. 4 are self-explanatory. The added alcohols penetrate the palisade layer and replace water from that region, decreasing the CP. With ethane-di-ol and propane-di-ol, insignificant decrease in CP is seen. Diols possess two –OH groups and are highly miscible with water due to their ability to form hydrogen bonds at both –OH groups with water. Therefore, they would not affect the micelle hydration and CP decreases are only marginal.

Figure 5 shows the effect of added amino acids on the CP of PMT. All the amino acids have similar functional groups, but different side chains (Table 3). Therefore, their effect on CP can be explained by taking into account the acidic/basic as well as polar/non-polar characteristics of the side chains.

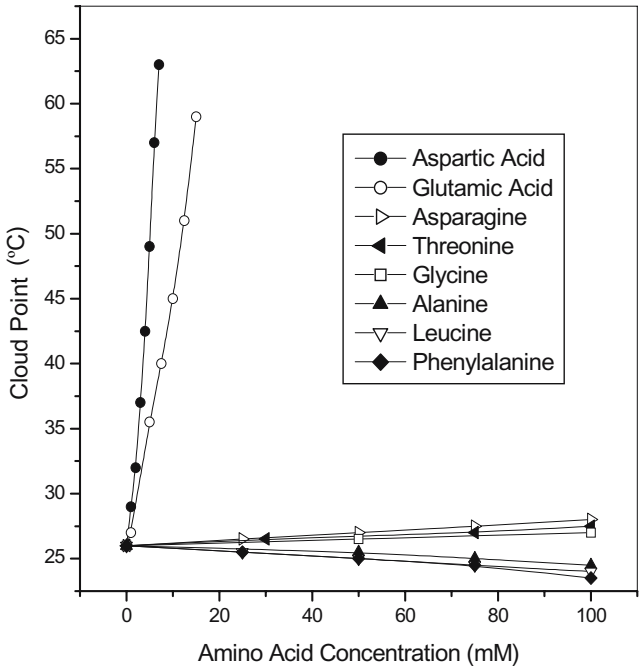

Fig. 5 Variation of cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution with addition of amino acids. The initial pH of PMT solution was 6.7

Acidic amino acids increase the CP sharply, while addition of non-polar and uncharged polar amino acids cause only slight changes in CP. As per their pK values (Table 3), acidic amino acids possess negatively charged side chain at pH 6.7, which would interact with the amine group of the PMT molecule. This interaction could make the micelle more hydrophilic, leading to an increase in CP. However, non-polar and uncharged polar amino acids would partition in micellar interior or bulk water and would not affect the micelles or CP of the system. This indeed is observed in Fig. 5.

Basic amino acids behave in a manner opposite to that of acidic amino acids. They prefer polar environment and would intercalate between monomer head groups. This would replace water from the head group region, resulting in dehydration of micelles. Hydrochloride salts have a positive charge on them, and their interaction with the PMT micelles would increase the repulsion among the head groups. Our results of CP decrease with basic amino acids, and its increase with their hydrochloride salts support the explanation (Fig. 6).

Figure 7 shows the variation of CP of PMT solutions with the addition of sugars. All the added sugars were found to decrease, although slightly, the CP. Sugars are well known water-structure makers [38]; therefore, hydrophobic interactions increase with their addition. Sugars remove water molecules surrounding the micelles; this affects the system in two ways: (1) micelles approach each other easily and (2) micelle hydration is decreased. Both the effects favor larger micelle formation and, therefore, CP appears at lower temperatures on addition of sugars.

Table 3 pK values for the ionizing groups of the amino acids at 25 °C[a]

Amino acid	pK_1 (–COOH)	pK_2 ($-NH_3^+$)	pK_R (R group)
Acidic			
Aspartic acid	2.09	9.82	3.86 (R= $-CH_2-COOH$)
Glutamic acid	2.19	9.67	4.25 (R= $-(CH_2)_2-COOH$)
Basic			
Lysine	2.18	8.95	10.53 (R= $-(CH_2)_4-NH_2$)
Arginine	2.17	9.04	12.48 (R = $-(CH_2)_3NH-C(=NH)-NH_2$)
Histidine	1.82	9.17	6.0 (R = $-CH_2-C=CH-$ imidazole)
Uncharged polar			
Glycine	2.34	9.60	
Asparagine	2.02	8.80	
Non-polar			
Alanine	2.34	9.69	
Leucine	2.36	9.60	
Threonine	2.63	10.43	
Phenylalanine	2.58	9.24	

[a] [39]

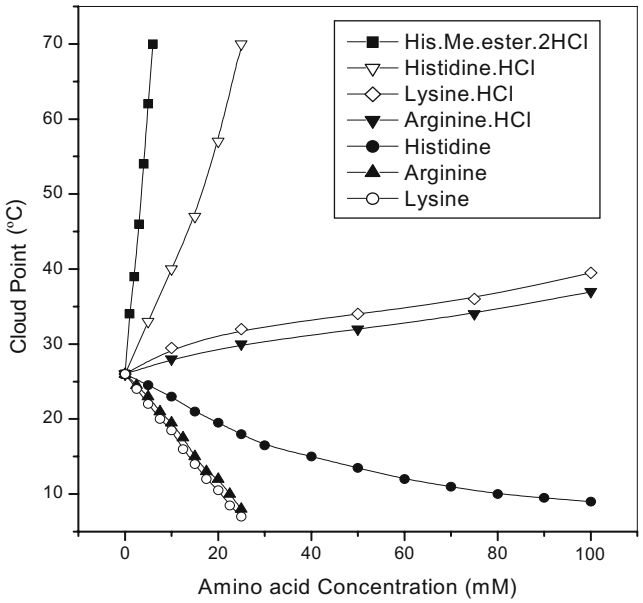

Fig. 6 Variation of cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution with addition of basic amino acids and their salts. The initial pH of PMT solution was 6.7

Figure 8 shows the effect of arabinose addition on the CP of PMT solutions of different fixed concentrations (50, 75, 100 mM). The CP decreasing effect of the sugar is again evidenced. Although the values of CP are higher for higher drug concentration, CP behavior is similar for all the PMT concentrations. At a constant arabinose concentration, increase in drug concentration increases the number, size and charge of micelles. This increases both intermicellar and intramicellar repulsions, causing an increase in CP.

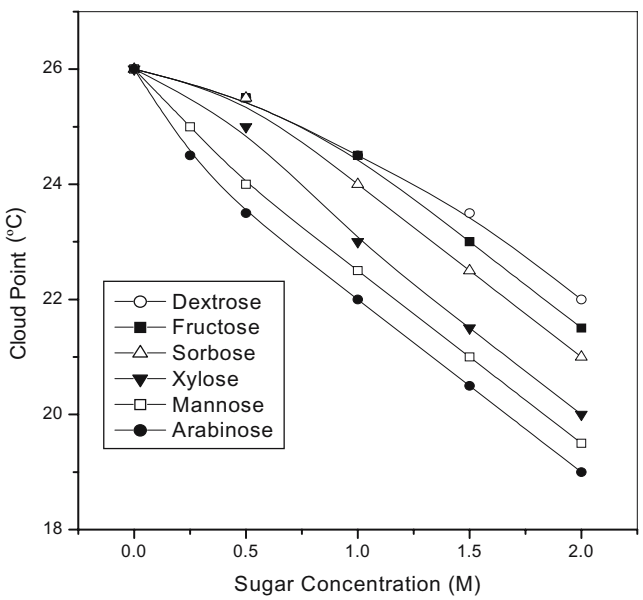

Fig. 7 Variation of cloud point of 50 mM PMT in a 10 mM sodium phosphate buffer solution (pH=6.7) with addition of sugars

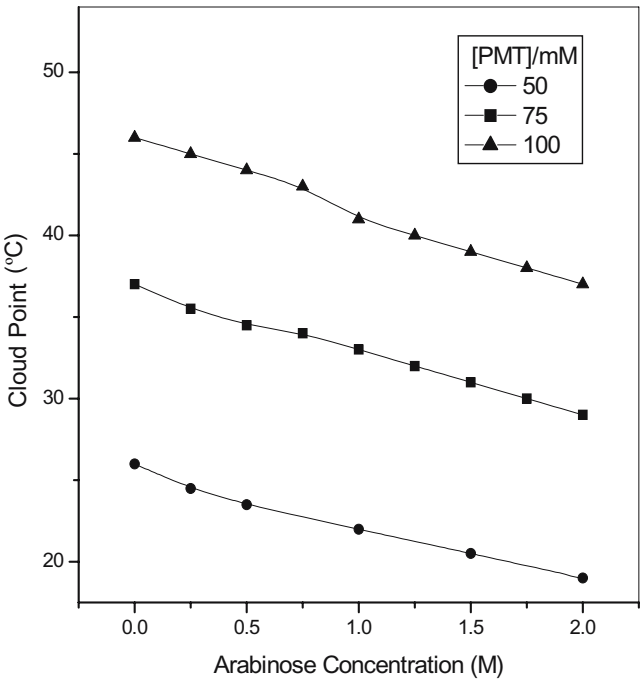

Fig. 8 Effect of arabinose concentration on the CP of different fixed concentrations (50 to 100 mM) of PMT solution, prepared in 10 mM sodium phosphate buffer (pH=6.7)

Effect of arabinose addition on the CP of 50 mM PMT micellar solutions at different fixed pHs was also seen (Fig. 9). At any fixed [arabinose], decrease in CP is observed with the pH increase which, once again, shows that the higher the deprotonation (with increase in pH), the lower is the electrostatic repulsion (among micelles) with a concomitant higher decrease in CP.

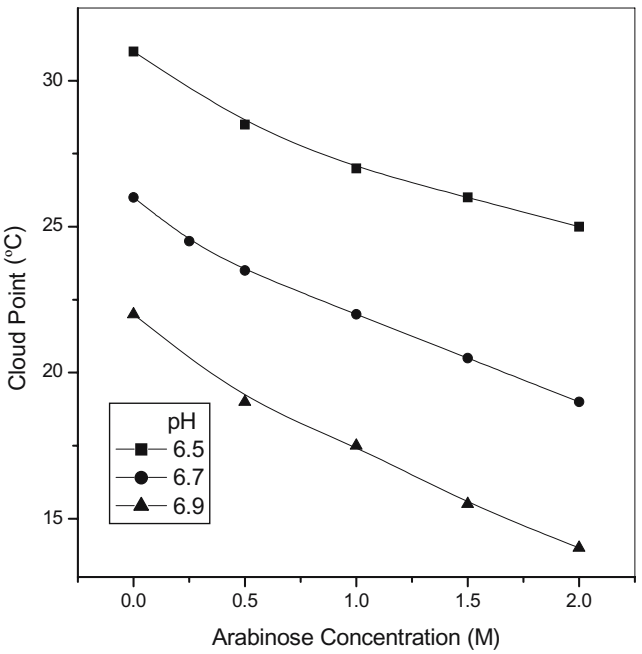

Fig. 9 Effect of arabinose concentration on the CP of 50 mM PMT solution, prepared in 10 mM sodium phosphate buffer at different pHs

Conclusion

CP phenomenon has been studied in PMT solutions in the presence of organic additives. CP decreases with the increase in pH. Alcohols decrease the CP by their screening effect and palisade layer penetration. Behavior of amino acids depends upon the nature of their side chain. Acidic amino acids and hydrochloride salts of basic amino acids increase the CP, while basic amino acids decrease the CP and non-polar and polar uncharged ones are less effective. Sugars promote water structure and decrease the hydration of micelles, leading to a decrease in CP. The CMC and other relevant surface parameters evaluated for the binary amphiphilic systems (drug + additive) indicate mixed micelle formation.

Acknowledgment The authors are thankful to Council of Scientific and Industrial Research, New Delhi, India, for providing a research grant (Project No. 01(1873)/03/EMR-II).

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