J. Pharm. Pharmac., 1970, **22**, *Suppl.*, 121 S–125 S *Received February*, 11 1970

Nuclear magnetic resonance studies on micelle formation by promethazine hydrochloride

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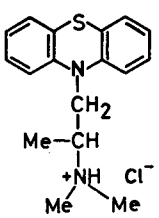
The pmr spectra of solutions of promethazine hydrochloride in D_2O have been obtained as a function of concentration above and below the critical micellar concentration of the drug. Chemical shifts were obtained relative to an external standard of a 20% promethazine solution (micellar). The shifts of the spectrum of the hydrophilic $\overset{+}{N}H(CH_3)_2$ group to higher fields on increasing concentration were explained by the increased dissociation of the group at the micelle surface, an effect illustrated by the decrease in bulk pH at the critical micelle concentration. Diamagnetic shifts of the aromatic ring protons are compatible with the non-polar environment and a parallel stacking of the phenothiazine rings in the interior of the micelle.

Micelle formation by drugs in solutions is a widespread phenomenon (Florence, 1968). With ionic compounds in aqueous solution micellization results in the removal of hydrophobic portions of the monomer from contact with water and concentration of the hydrophilic head groups at the surface of the micelle. The changes in the environment of these groups leads to changes in their proton magnetic resonance absorption. Nuclear magnetic resonance investigations on surfactant solutions have, since Eriksson's (1963) study, led to a more detailed understanding of the process of micelle formation and of the structure of micelles at the molecular level (Inoue & Nakagawa, 1966; Müller & Birkhahn, 1967; Clifford & Pethica, 1964).

The low critical micellar concentration (CMC) of the cetyl pyridinium bromide with which he worked prevented Eriksson (1963) from making measurements on the monomeric state before micellization. To obviate this difficulty we have chosen a phenothiazine derivative, promethazine hydrochloride (I),



(I)

which has a CMC of approximately 4×10^{-2}M (1·33%). This compound has the additional advantage that the proton resonance of *three* portions of the molecule,

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namely the aromatic, the CH–C$\underline{H}_3$ and N(C$\underline{H}_3$)$_2$ groups, can be examined in monomeric and micellar form. Promethazine HCl forms micelles containing 27 monomers in 0·9% sodium chloride (Scholtan, 1955).

The purpose of the present paper is to discuss the chemical shifts which occur on micellization, in particular those caused by the proximity of the groups at the micellar surface.

EXPERIMENTAL

Promethazine hydrochloride, used without further purification, was obtained from May & Baker Ltd. Solutions of the compound in deuterium oxide (D_2O) were prepared immediately before use to minimize breakdown of the promethazine.

Proton magnetic resonance spectra (60 MHz) were obtained at 34° on a Perkin-Elmer R-12 spectrometer with a 20% solution of promethazine hydrochloride in D_2O as external reference. Chemical shifts of the proton absorptions of $\overset{+}{N}H(CH_3)_2$ (singlet), CH–C$\underline{H}_3$ (doublet) and the aromatic ring (multiplet) were measured relative to the corresponding reference resonance line at a sweep width of 100 Hz. Results were not corrected for bulk magnetic susceptibility effects which are expected to be small (cf. Bailey & Cady, 1969; Arrington, Clouse & others, 1970).

Coaxial sample tubes were prepared by the accurate concentric drilling of nylon bushes and the insertion of a sealed capillary containing the external reference into the drill holes: this unit was then fitted into a standard 5 mm pmr sample tube.

pH measurements were made with a Pye Model 78 pH meter by titration of the drug solution into distilled water at room temperature (about 22°).

RESULTS AND DISCUSSION

The pmr spectrum of a 20% solution of promethazine HCl in D_2O with sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) as internal reference, is not well resolved. At this solution strength the environment of most promethazine hydrochloride molecules is micellar and the broadening of the signal may, therefore, be attributed to the more facile spin-lattice relaxation of the micellar species.

Pmr data on promethazine hydrochloride in D_2O and of the base in deuterochloroform and benzene-D_6 are summarized in Table 1. Fig. 1 shows the scale-expanded $\overset{+}{N}H(CH_3)_2$ resonance signal of solutions of promethazine hydrochloride. The $\overset{+}{N}H(CH_3)_2$ signal of a 5·2% solution is almost indistinguishable in shift from the 20% reference solution line and can be detected only in the form of line broadening at the base of the absorption band. As solution concentration is lowered, two

Table 1. *Pmr data on promethazine*

Compound	Solvent	$\overset{+}{N}(C\underline{H}_3)_2$	CH–C$\underline{H}_3$	Aromatic
Promethazine HCl 20% solution ..	D_2O	7·17 s	8·71 d *J* 6Hz	2·8 m
Promethazine HCl 5% solution ..	D_2O	7·17 s	8·66 d	2·85 m
Promethazine base	$CDCl_3$	7·68 s	8·97 d	2·97 m
Promethazine base	Benzene-D6	7·87 s	9·10 d	3·07 m

s = singlet d = doublet m = multiplet

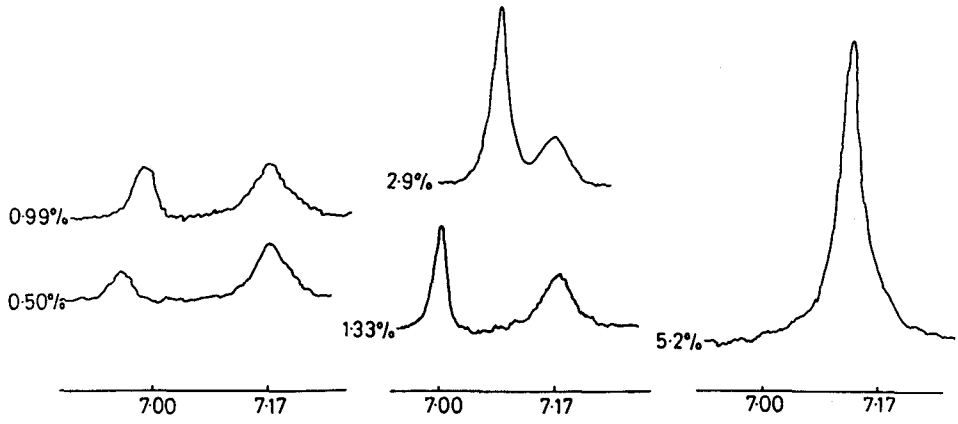

FIG. 1. Pmr signal of the $\overset{+}{N}(CH_3)_2$ protons of promethazine hydrochloride in D_2O. The high-field line of constant intensity is due to the external reference signal of 20% promethazine HCl in D_2O. The concentrations of the solution are marked.

distinct lines appear, the separation increasing with decrease in concentration. The broad high-field line in Fig. 1 does not change in intensity whereas the sharper low-field signal decreases in intensity as the solution concentration decreases. We therefore assign the broad high-field line to the reference (micellar) form and the low-field signal to the solution containing an increasing concentration of monomer.

The diamagnetic shift (13·1 Hz maximum) observed on changing from a solution composed mainly of promethazine HCl monomers (0·51%) to the solution consisting largely of micellized drug may be attributed to an increased dissociation of the polar head groups at the micelle surface. Koch & Doyle (1967) have shown that the chemical shift of the NCH_3 group in a number of amines was linearly dependent on the fractional number of free base and salt molecules present. That increased dissociation is occurring above the CMC is confirmed by the dramatic fall in pH which

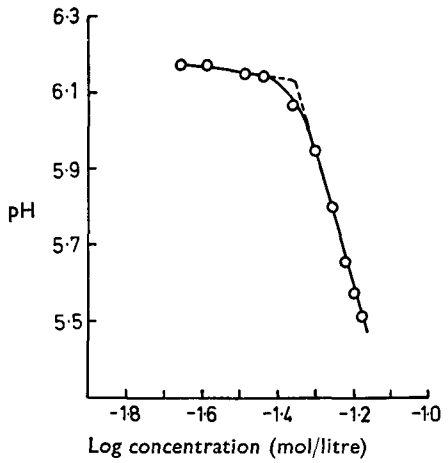

FIG. 2. The variation of pH of promethazine hydrochloride, at room temperature, in H_2O as a function of the concentration of drug.

occurs at the CMC (Fig. 2). Titration of promethazine HCl solutions with NaOH above and below the CMC shows that the change in apparent pK_a on micellization is −0·48. Similar pK_a changes on micellization of decylamine and dodecylamine hydrochlorides have been reported (−1·1 and −1·63 units respectively) (Veis & Hoerr, 1960). Since $\Delta pK_a = \Delta G_e/2{\cdot}303kT$ (Veis & Hoerr, 1960) this allows the calculation of the electrostatic free energy of micellization ($\Delta G\hat{e}$) for promethazine hydrochloride as +1·1 kT. This is much lower than the 3·75 kT obtained for dodecylamine hydrochloride probably because the packing of the phenothiazine molecules into a micelle is more difficult than with n-aliphatic amines and, as a result, the polar head groups do not approach each other as closely in the smaller micelle.

The $CH–C\underline{H}_3$ doublet which is proximate to the polar head group also experiences a change in magnetic environment (23·4 Hz maximum to high-field values) (Table 2) being more shielded in the micelle. This shift, greater than that observed for the $N(CH_3)_2$ signals, may be explained by the alkyl chains of adjacent molecules in the micelle providing extra shielding.

Table 2. *Chemical shifts* of promethazine HCl as function of concentration in D_2O at 34°*

Concentration† (%)	Shifts (Hz) $\overset{+}{N}(CH_3)_2$	$-CH-C\underline{H}_3$	Aromatics
5·23	—	5·8	5·9
4·161	2·4	7·5	7·0
2·89	4·6	9·9	9·4
1·585	9·4	18·0	11·7
1·355	10·3	19·9	13·0
1·027	11·1	21·1	15·0
0·986	11·4	21·6	15·8
0·724	12·1	22·1	18·2
0·509	13·1	23·4	19·6

* Shifts measured relative to the corresponding resonance lines of the external standard (20% promethazine HCl in D_2O).

† % w/w.

A diamagnetic shift also occurs in the major peak of the phenothiazine ring protons. This is compatible with the aromatic groups residing in a non-polar environment and being influenced by the diamagnetic anisotropy of adjacent aromatic moieties. The shift to a lower field on dilution suggests that the rings are stacked parallel to each other in the micelle. Rings packed in a coplanar configuration would produce a high-field shift on being removed from the micelle, according to Blears & Danyluk (1966).

Fig. 3 shows the observed shifts of the three parts of the molecule as a function of concentration. All plots show breaks of varying abruptness at the CMC. The mean CMC value obtained at 34° in D_2O is 1·3% w/w ($4{\cdot}06 \times 10^{-2}$M). The changes in proton magnetic absorption below the CMC might be explained for the $\overset{+}{N}(CH_3)_2$ peak by the changes in bulk pH (see Fig. 2), but the changes in the $CH–CH_3$ and aromatic absorptions cannot. It is surprising that the aromatic protons show such large shifts below the CMC. This contrasts with the behaviour of ω-phenylalkyltrimethylammonium bromides (Inoue & Nakagawa, 1966), in which the signal of the

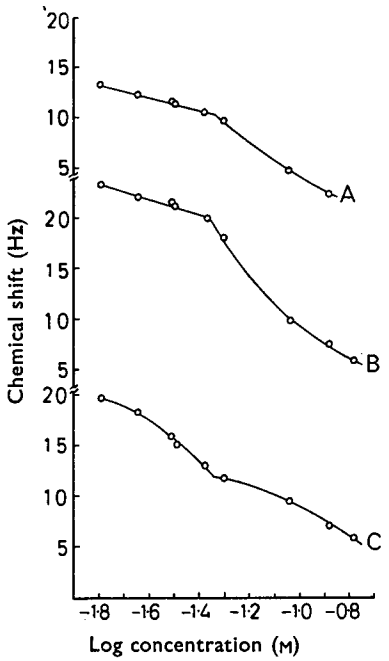

FIG. 3. Chemical shifts (Hz) of signal of A: $\overset{+}{N}(C\underline{H}_3)_2$, B: CH–C$\underline{H}_3$ and C: aromatic ring protons, as a function of the concentration of solution, relative to position of 20% promethazine HCl signals from the respective groups.

phenyl protons remained at the same position at concentrations below the CMC. It is possible that dimers of promethazine are forming below the critical micellar concentration constituted in such a way that the $-\overset{+}{N}(C\underline{H}_3)_2$ groups are unaffected.

Acknowledgements

We thank May & Baker Ltd. for the gifts of drug.

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