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Effect of pH on the micellar properties of amphiphilic drugs in aqueous solution

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The influence of pH on the micellar properties of several amphiphilic drugs under conditions of constant ionic strength has been investigated. No significant effect of pH on the critical micelle concentration or micellar size of chlorpromazine hydrochloride was noted over a pH range well below the pK_a. The micellar properties of opipramol, thiopropazate, flupenthixol, clopenthixol, and trifluoperazine, which contain a piperazine moiety showed considerable pH dependence. The concentration dependence of the pK_a in these micellar systems was taken into consideration in the selection of pH values representative of complete protonation of either one or both of the piperazine N atoms. A lower aggregation number and higher critical micelle concentration was observed at a low pH corresponding to complete protonation of both charge centres. Mepyramine maleate exhibited a non-micellar mode of association at pH 5·5 which could be described by a stepwise association model in which association constants, K_N, increased sequentially with aggregation number, N, according to the relationship, $K_N = K(N - 1)/N$ where $K = 31{\cdot}3$ dm^3 mol^{-1}. No significant association could be detected at pH 2 when the pyridine ring N was fully protonated.

It is well known that as the pH of an aqueous solution of a cationic, micelle-forming drug is increased in the region of its pK_a, a dramatic increase of micellar size occurs due to solubilization of the non-ionized base which is usually only sparingly water soluble. Further increase in pH usually leads to precipitation of free base. Very few studies have been reported of the effect of pH on micellar properties at pHs well below the pK_a. One of the problems associated with such studies is the marked effect which electrolyte, added in the form of buffer components, has on the micellar properties. Not only will variation in the concentration of the buffer components have an effect but also, as demonstrated by Zografi & Zarenda (1966), various buffers may have widely differing effects on surface activity of drug molecules and by inference will probably likewise affect the micellar properties. Failure to recognize these potential sources of error may give rise to misleading conclusions regarding the effect of pH.

In this study the association of several drugs containing piperazine ring systems has been studied under conditions of complete protonation of either one or both of the piperazine N atoms. The influence of pH on the critical micelle concentration (cmc) of one of the drugs, clopenthixol, has recently been reported by Thoma & Albert (1980). Increase of pH from 2 to 6·5 was reported to decrease the cmc from $2{\cdot}03 \times 10^{-3}$ mol dm^{-3} to $4{\cdot}4 \times 10^{-4}$ mol dm^{-3}.

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Also investigated is the effect of protonation of the pyridine ring N atom on the association of the antihistamine, mepyramine maleate. Previous studies on this compound by Attwood & Udeala (1975, 1976) have revealed a non-micellar mode of association in water and electrolyte solution.

MATERIALS AND METHODS

Materials. The following drugs were sufficiently well characterized and purified to be used without further purification; chlorpromazine hydrochloride (M & B) the dihydrochlorides of opipramol (Geigy), thiopropazate (Searle) trifluoperazine (SKF), flupenthixol and clopenthixol (Lundbeck). Mepyramine maleate was from Cambrian Chemicals. Boric acid was Analar, other buffer components and inorganic compounds were reagent grade.

Preparation of solutions. A phosphoric acid–acetic acid acid–boric acid buffer containing equimolar proportions (0·04 mol dm^{-3}) of each component was used throughout. pH adjustment was achieved by the dropwise addition of either HCl (1 mol dm^{-3} solution) or KOH (5 mol dm^{-3} solution) and the final ionic strength was adjusted to a predetermined value using solid KCl.

Light scattering measurements. Measurements were made at 303K with a Fica 42000 photogoniodiffusometer (A.R.L. Ltd) using a wavelength of 546 nm. The solutions were clarified by ultrafiltration through 0·1 μm Millipore filters until the ratio of light scattering at angles of 30° and 150° did

not exceed 1·10. Refractive index increments of the drugs in the presence of buffer components, $(dn/dm_2)_{m_3}$ were determined using a differential refractometer at 546 nm. The following values (kg mol^{-1}) were obtained; opipramol 0·0899 (pH 1) and 0·0862 (pH 5·5); thiopropazate 0·1001 (pH 1) and 0·0951 (pH 4·8); flupenthixol 0·0909 (pH 1) and 0·0891 (pH 4·8); clopenthixol 0·1002 (pH 1) and 0·0973 (pH 4·8); trifluoperazine, 0·0989 (pH 0·5 and pH 5·5); chlorpromazine 0·0710 (pH 2) and 0·0700 (pH 5·5); mepyramine maleate 0·0735 (pH 2) and 0·0711 (pH 5·5).

pK_a determination. pK_a values were determined by potentiometric titration (Albert & Serjeant 1971) using a Pye Model 290 pH meter fitted with a combined glass–silver chloride electrode.

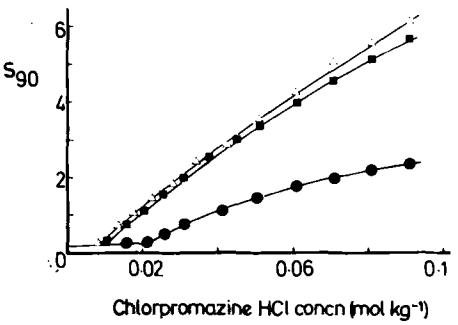

FIG. 1. Variation of the scattering ratio, S_{90}, with concentration for chlorpromazine hydrochloride ● at pH 2 (buffer only); △ at pH 2 (buffer + 0·1 mol kg^{-1} KCl); ■ at pH 5·5 (buffer + 0·1 mol kg^{-1} KOH).

RESULTS AND DISCUSSION

The marked effect of added electrolyte on micellar properties presents problems in the determination of the effect of pH on aggregation. In this study, all solutions were prepared in the same buffer solution and brought to the same ionic strength by the addition of KCl. Consequently at low pH the solutions contain, in addition to the buffer components, Cl^- counterions from the added HCl and KCl. At higher pH, solutions contain Cl^- counterions from the KCl and also OH^- counterions from the added KOH. The comparison of micellar properties at low and high pH is thus subject to possible errors arising from the differing effects of Cl^- and OH^- counterions. An indication of the possible magnitude of such error was ascertained by determinations on chlorpromazine hydrochloride, which has a single ionizable group, at pH values well below the pK_a of 9·2 (Sorby et al 1966). The light scattering results for this drug are presented in Fig. 1 as graphs of the scattering intensity at an angle of 90°, S_{90}, as a function of molal concentration, m. The effective micellar charge, p, and the aggregation number, N, were evaluated using equations proposed by Anacker & Westwell (1964).

$$p = [2fm_3 B \pm (8m_3B)^{\frac{1}{2}}]A^{-1}(2 - fA)^{-1} \quad .. \quad (1)$$

$$N = p(p + 1) A (2m_3B + pA^2)^{-1} \quad .. \quad (2)$$

A and B are the intercept and slope respectively of plots $K'm_2/\Delta R_{90}$ against the molal concentration of micelles, m_2. ΔR_{90} is the Rayleigh ratio of the solution in excess of that of a solution at the cmc; $K' = 2\pi^2 n_0^2 (dn/dm_2)^2_{m_3} V^0/L\lambda^4$; n_0 is the refractive index of the solvent, V^0 is the volume of solution containing 1 kg of water; L is the Avogadro number, λ is the wavelength of incident light, m_3 is the molality of added electrolyte and $f = (dn/dm_3)_{m_2}/(dn/dm_2)_{m_3}$. The determination of the refractive index increment is complicated by the presence of mixed counterions. Since the systems contained identical concentrations of the same buffer and since the drug counterions were Cl^- ions, account was taken only of the possible error arising from the additional presence of OH^- ions at high pH. In systems containing mixed counterions the refractive index increment of the solute, dn/dm_2 is given by (Anacker & Ghose 1968):

$$dn/dm_2 = (a + b)^{-1} a\,(dn/dm_{DOH}) + b\,(dn/dm_{DHCl}) \quad (3)$$

where a and b are the molalities of the potassium hydroxide and the hydrochloride salt of the drug, respectively and dn/dm_{DOH} and dn/dm_{DHCl} are the refractive index increments of the hydroxide and hydrochloride salts of the drug respectively. Theoretical values were calculated for dn/dm_{DOH} since the hydroxide salts of the drugs were not available. The method of calculation was that outlined by Anacker & Ghose (1968). A solution that is a molal in KOH and b molal in drug hydrochloride was regarded as being b molal in drug hydroxide, b molal in KCl and (a–b) molal in KOH. The measured refractive index difference between a b molal solution of drug hydrochloride and the KOH solution was corrected by adding the quantity $b(dn/dm_{KOH} - dn/dm_{KCl})$ to give the corresponding refractive index for drug hydroxide. The correction term was however found to be insignificant since refractive index increments for KOH and KCl were very similar. Consequently dn/dm_{DOH} could be equated with dn/dm_{DHCl} and

no correction of the measured refractive index of the drug ion due to the mixed counterions was necessary. Values of N and $\alpha(= p/N)$ for chlorpromazine are given in Table 1 together with cmc values determined from the discontinuities in the light scattering plots. No significant differences were noted in the micellar properties at pH 2 in the presence of 0·1 mol kg^{-1} KCl and at pH 5·5 in the presence of 0·1 mol kg^{-1} KOH. However, failure to add to the buffer at pH 2 an amount of KCl equivalent to the KOH added to bring the pH to 5·5, leads to large discrepancies in the micellar parameters from which erroneous conclusions regarding the importance of pH might be drawn.

Table 1. Effect of pH on micellar properties of chlorpromazine hydrochloride.

pH	Solvent	cmc (mol kg^{-1})	N	α
2·0	Buffer only	0·020	20	0·42
2·0	Buffer + 0·1 mol kg^{-1} KCl	0·0085	34	0·20
5·5	Buffer + 0·1 mol kg^{-1} KOH	0·0095	37	0·28

Piperazine derivatives. Light scattering graphs for trifluoperazine at a series of pHs are given in Fig. 2. The generalized valency theory of light-scattering of Anacker & Jacobs (1974) was used to calculate the micellar charge and aggregation number at low pH where the drug molecules possess two charge centres. According to this theory,

$$p = \frac{[\theta\phi\,(\theta + \phi)^3\, m_3\, B]^{\frac{1}{2}} + \theta\,(\theta + \phi) f m_3 B}{(\theta + \phi - fA)\phi A} \quad (4)$$

$$N = \frac{(p + p^2)\,\phi\, A}{\theta\,(\theta + \phi) m_3 B + p\phi A^2} \quad (5)$$

$\theta = \rho + r\sigma$ and $\phi = \nu + r\nu$ where r is the ratio of the molality of monomeric surfactant to the molality of added salt, ρ, σ and ν are the valencies of the coion, drug ion and counterion respectively. Application of equations 4 and 5 at low and high pH is straightforward since the drug ions are doubly and singly charged respectively at these pHs. At intermediate pHs, ionization of one of the charge centres of the trifluoperazine molecule is incomplete. Because of the uncertainty in the pK_a (see below) it was not possible to calculate the fractional charge on this centre and the N and α values are subject to error. Calculations using σ values of between 1 and 2 showed that although α was dependent on the value of σ, the variation of N was insignificant.

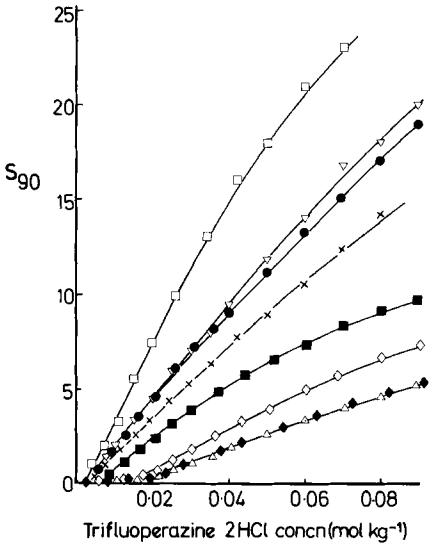

FIG. 2. Variation of the scattering ratio, S_{90}, with concentration for trifluoperazine dihydrochloride at pH △ 0·5, ◆ 1·0, ◇ 2·0, ■ 2·9, × 3·4, ● 4·3, ▽ 5·0, and □ 5·5. Ionic strength = 0·37.

The effect of pH on the micellar properties of trifluoperazine is shown in Table 2. The decrease of N and increase in cmc with decrease of pH below pH 5 is a consequence of the gradual increase in charge on the molecule as the second N atom of the piperazine ring becomes fully protonated. The pK_{a1} and pK_{a2} values of this drug are 3·8 and 8·4 respectively (Chatten & Harris 1962). A pH of 5·5 should be sufficiently lower than pK_{a2} for virtually complete ionization of one of the N atoms and thus the dramatic increase of micellar size as the pH is raised to 5·5 which suggests a significant decrease in ionization, is unexpected. Similarly at a pH of 2 the second N atom should also be almost completely ionized. However the results shown in Table 2 suggest that at this pH the micellar properties have not yet reached the constant values representa-

Table 2. Effect of pH on micellar properties of trifluoperazine dihydrochloride at ionic strength = 0·37.

pH	cmc (mol kg^{-1})	N	α
0·5	0·020	17	0·46
1·0	0·019	17	0·46
2·0	0·016	24	0·41
2·9	0·007	35	0·42
3·4	0·005	40	0·23
4·3	0·003	48	0·20
5·0	0·003	49	0·18
5·5	0·002	82	0·20

tive of this state. Determination of the pK_a of trifluoperazine as a function of concentration revealed that the cause of these apparently anomalous results was a decrease in pK_a with increase in solution concentration (see Fig. 3). A change in the

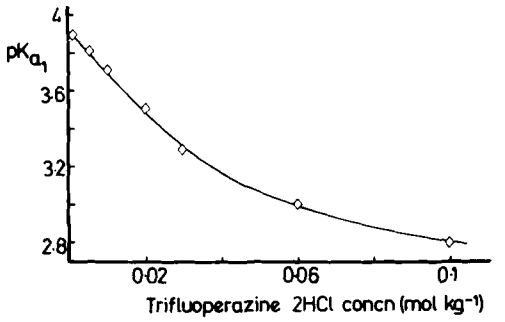

FIG. 3. Concentration dependence of the apparent pK_{a_1} of trifluoperazine dihydrochloride.

apparent pK_a in the region of a cmc is well known and is attributable to the changing ratio of monomeric to micellar surfactant in this region. In other surfactants (Yalkowsky & Zografi 1970) a constant pK_a is observed below the cmc and the pK_a reaches a limiting value at concentrations well in excess of the cmc when the monomer/micelle ratio is reasonably constant. The failure to observe a constant value at low concentrations of the drugs investigated here presumably arises because of the variation of the cmc with pH (see Table 2). The extrapolated value of 3·9 for pK_{a_1} at zero concentration is identical with that quoted by Chatten & Harris (1962).

The micellar properties of the other drugs containing piperazine moieties were measured at two pHs which were approximately 3 pH units below the literature values of pK_{a_1} and pK_{a_2}. These selected pH values thus represent conditions of complete ionization of either both or only one of the charge centres respectively. The results, summarized in Table 3, show as expected a higher aggregation number and a lower cmc when the drugs are present as singly charged compounds. The energy of micellization, ΔG_m^θ, can be arbitrarily divided into a hydrocarbon contribution, ΔG_h^θ and an electrical contribution, ΔG_e^θ. For any particular drug, ΔG_h^θ per mole of monomer will not be significantly affected by the number of charges on the molecule. Hence the decrease in N and increase of cmc associated with the protonation of the second N atom is a direct consequence of changes in the electrical contribution to the overall ΔG_m^θ.

Table 3. Effect of pH on micellar properties of drugs containing a piperazine moiety. Ionic strength = 0·37.

Compound	pH	cmc (mol kg^{-1})	N	α
Clopenthixol	1·0	0·005	36	0·46
	4·8	0·0005	87	0·38
Flupenthixol	1·0	0·005	41	0·44
	4·8	0·001	62	0·28
Opipramol	1·0	0·034	8	0·49
	5·5	0·011	23	0·36
Thiopropazate	1·0	0·016	23	0·44
	4·8	0·003	47	0·33

Pyridine derivatives. Previous studies (Attwood & Udeala 1975, 1976) both in the presence and absence of added electrolyte have indicated a non-micellar mode of association of several antihistamine drugs which contain pyridine rings such as pheniramine, brompheniramine, chlorpheniramine and mepyramine when these drugs are associated with a maleate counterion. Mepyramine maleate was selected for an examination of the effect of pH on this type of drug. Fig. 4 shows a curved light scattering plot at pH 5·5 with no inflection point. Such plots are typical of a non-micellar mode of association. The data were fitted to a stepwise association as described previously (Attwood & Agarwal 1980). The monomer concentration $[b_1]$ was determined as a function of the total solution concentration c (g dm^{-3}) by integration of the light scattering data according to Steiner (1952):

$$\ln x = \int_0^c [(M/M_{app}) - 1]\, d\ln c \quad .. \quad (6)$$

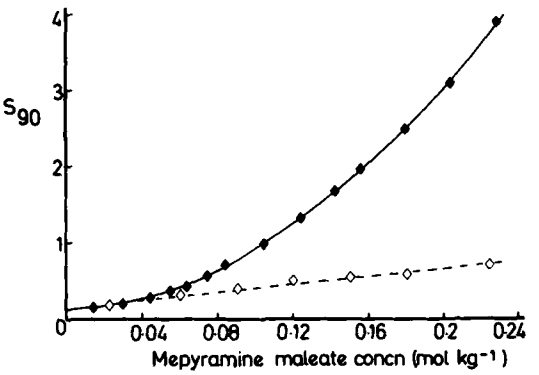

FIG. 4. Variation of the scattering ratio, S_{90}, with concentration for mepyramine maleate in buffer at ◆ pH 5·5 and ◇ pH 2. Ionic strength = 0·255. (——) theoretical scattering calculated using stepwise association Model 2 (eqn 10) with K = 31·3 dm^3 mol^{-1}. (– – –) theoretical scattering for unassociated monomers.

when x is the weight fraction of monomers and M is the monomer molecular weight. The apparent weight-average molecular weight, M_{app}, was calculated from the light scattering intensity assuming ideality. Because of the presence of mixed counterions (OH^- and maleate ions) it was necessary to correct the measured refractive index increment of mepyramine using the treatment described above. A value of 0·0511 kg mol^{-1} was calculated for the refractive index increment of mepyramine hydroxide. Substitution into an equation of similar form to equation 3 yielded a mean $(dn/dm_2)_{m3}$ value of 0·060 kg mol^{-1} which was subsequently used in the calculation of M_{app}.

Several models of self-association were considered in which explicit relationships between all stepwise association constants were assumed, the relationships being expressed using a generalized parameter K. Both cooperative and anticooperative models were considered.

Model 1. This model assumes the equality of all K values i.e. $K_2 = K_3 = K_N = K$, leading to the relation (Ghosh & Mukerjee 1970)

$$([b_1]/m)^{\frac{1}{2}} = 1 - K[b_1] \quad .. \quad (7)$$

Model 2. Stepwise association constants increase sequentially with N according to $K_N = K(N - 1)/N$ i.e. $K_2 = K/2$, $K_3 = 2K/3$ etc. This model leads to

$$(m/[b_1]) = 1 + Km \quad .. \quad (8)$$

Model 3. Stepwise association constants decrease sequentially with N according to $K_N = K/N$ i.e. $K_2 = K/2$, $K_3 = K/3$ etc. This model leads to the relation (Attwood & Tolley 1980)

$$N_w = 1 + K[b_1] \quad .. \quad (9)$$

where N_w is the weight-average aggregation number as determined from light scattering results.

Fig. 5 shows a linear plot when data were plotted in accordance with equation (8) indicating that model 2 was applicable to the association process. As a check on the value of K obtained from the slope of Fig. 5, values of S_{80} were recalculated from N_w values generated using equation 10 which has been derived for this model (Attwood et al 1980).

$$N_w = 1/(1 - K[b_1]) \quad .. \quad (10)$$

A satisfactory fit of the experimental data was obtained with K = 31·3 dm^3 mol^{-1} (see Fig. 4).

At pH 2 the experimental results could be described by a line calculated for monomeric scattering (Fig. 4) indicating that the additional protonation of the pyridine N atom renders the molecule sufficiently hydrophilic to prevent significant association over the concentration range studied.

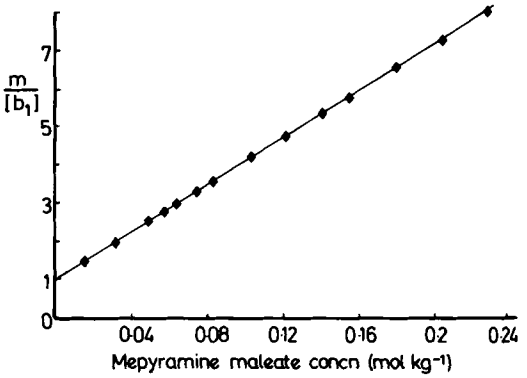

FIG. 5. Mepyramine maleate data at pH 5·5 plotted according to eqn 8.

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