

# Aggregation of antiacetylcholine drugs in aqueous solution: monomer concentrations in non-micellar drug systems

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The self-association of the antiacetylcholine drugs, propantheline bromide, methantheline bromide and methixene hydrochloride in aqueous solution, has been examined by surface tension, light scattering and conductimetric methods. Surface tension graphs were similar to those of conventional surfactants, showing apparent critical micelle concentrations (cmc) at distinct inflection points. Surface tension measurements in the presence of increasing amounts of electrolyte indicated a decrease in the apparent cmc with increase in electrolyte concentration for propantheline bromide. Light scattering curves for propantheline bromide in electrolyte solution showed no significant discontinuity attributable to a cmc. A mode of self association involving aggregate growth by the stepwise addition of monomers was assumed and equilibrium constants for the initial stages of the association were evaluated. An increase in the magnitude of the stepwise association constants with increase in electrolyte concentration was noted. Integration of the light scattering data according to  $\ln x = \int_0^c [(M/M_{app}) - 1] \ln c$  (where  $M$  and  $M_{app}$  are the monomer and apparent aggregate weights respectively and  $x$  is the weight fraction of monomers) showed an asymptotic increase in monomer concentration towards a limiting concentration, as the solution concentration,  $c$ , was increased. Limiting monomer concentrations determined by this method were in reasonable agreement with the apparent cmcs from surface tension studies. It was not possible to detect a cmc for methantheline or propantheline bromide from conductivity measurements.

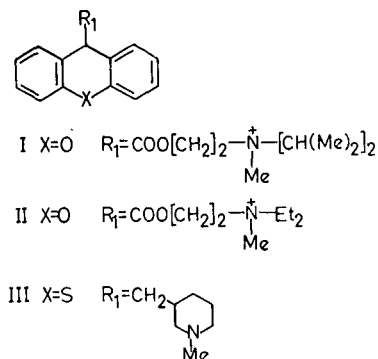
Previous treatments (Attwood 1976a) of the light scattering data for propantheline bromide, methantheline bromide and methixene hydrochloride in water have shown that the association is non-micellar. It may be described by a stepwise association process, possibly involving the vertical stacking of the almost planar hydrophobic rings. Other antiacetylcholine drugs, with hydrophobic regions based on the diphenylmethane nucleus, exhibited typical micellar behaviour (Attwood 1976b). An essential distinction between the two types of association is in the size distribution of the products of association. In micellar association there is a predominance of micelles of an energetically preferred size, whilst in the stepwise association model, the products of association are generally multimers with a broad size distribution. Since the active form of the drug in solution is usually the monomer rather than the aggregate, it is of interest to study the variation of monomer concentration with total solution concentration. A knowledge of monomer concentration is important in the interpretation of the binding of drugs to proteins or receptors and their diffusional transport through membranes. In micellar solutions the drug is predominantly in the monomeric form

below the critical micelle concentration (cmc). Above the cmc, the monomer concentration decreases according to the mass action theory of micellization. In non-micellar drug systems, there is no equivalent to a cmc and the monomer concentration can not readily be calculated. The variation of surface tension with solution concentration is primarily dependent on monomer concentration and hence this is a useful technique in the study of this problem.

In this investigation, surface tension measurements have been made on solutions of propantheline bromide, methantheline bromide and methixene hydrochloride in water and aqueous electrolyte. The results are compared with those from light scattering and conductivity techniques.

## MATERIALS AND METHODS

*Materials.* The compounds I-III were used as received: propantheline bromide B.P. (I) (Searle); methantheline bromide (II) (Searle) and methixene hydrochloride (III) (Wander). Water was distilled twice from alkaline permanganate in a glass apparatus and its surface tension checked against the literature value before use. Sodium bromide was of Analar grade.



**Surface tension measurement.** Measurements were made at 303K by the Wilhelmy plate method using a Cahn Electrobalance Model RG. Solutions were aged for 3–24 h until an equilibrium surface tension was attained.

**Light scattering measurements.** Measurements were made at 303K with a Fica 42 000 photogoniometer (A.R.L. Ltd) using a wavelength of 546 nm. Aqueous solutions were clarified by ultrafiltration through 0.1  $\mu\text{m}$  Millipore filters until the ratio of the light scattering at angles of 30 and 150° did not exceed 1.10. The refractive index increments were measured at 546 nm using a differential refractometer.

**Conductivity measurements.** Measurements were made at  $303 \pm 0.01\text{K}$  using a Wayne Kerr auto-balance universal bridge (Model B641).

### RESULTS

The variation of surface tension,  $\sigma$ , with log molal concentration for propantheline, methantheline and methixene in aqueous solution is shown in Fig. 1A. The curves are similar to those obtained with conventional micellar solutions showing apparent cmcs

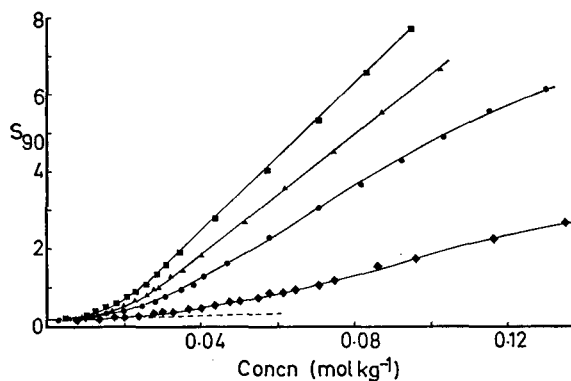


FIG. 2. Variation of the scattering ratio,  $S_{90}$ , with concentration for propantheline bromide in aqueous solutions containing  $\blacklozenge$  0.00;  $\bullet$  0.15;  $\blacktriangle$  0.30 and  $\blacksquare$  0.45 mol  $\text{kg}^{-1}$  NaBr. Monomer scattering (---).

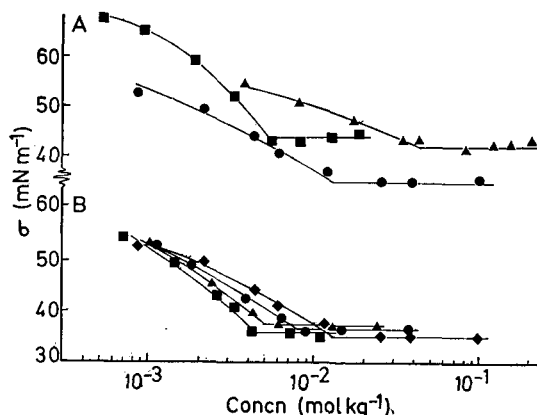


FIG. 1. Surface tension,  $\sigma$  ( $\text{mN m}^{-1}$ ), as a function of log molal concentration for A aqueous solutions of  $\bullet$  propantheline bromide;  $\blacktriangle$  methantheline bromide; and  $\blacksquare$  methixene hydrochloride; and B propantheline bromide in aqueous solutions containing  $\blacklozenge$  0.00;  $\bullet$  0.15;  $\blacktriangle$  0.30 and  $\blacksquare$  0.45 mol  $\text{kg}^{-1}$  NaBr.

at distinct inflection points. Fig. 1B shows a progressive decrease in the apparent cmc of propantheline with increase in the sodium bromide concentration over the range 0.15 to 0.45 mol  $\text{kg}^{-1}$ . A linear relation exists between log cmc and log counterion concentration.

Light scattering plots for propantheline bromide in aqueous solutions of sodium bromide (0.15 to 0.45 mol  $\text{kg}^{-1}$ ) are presented in Fig. 2. The curves were of the form previously noted for propantheline in the absence of electrolyte (Attwood 1976a) and showed a continuous increase in the scattering at 90°,  $S_{90}$ , with increase in solution concentration,  $m$ . No discontinuities in the concentration dependence of  $S_{90}$ , attributable to a cmc, were observed, although

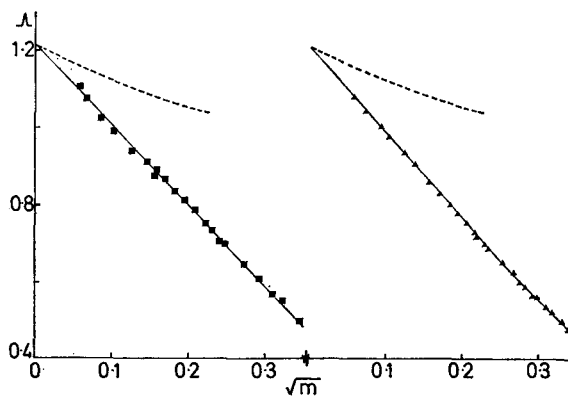
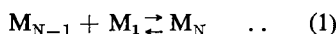


FIG. 3. Equivalent conductivity of aqueous solutions of  $\blacktriangle$  propantheline bromide and  $\blacksquare$  methantheline bromide. eqn 4 (---). Abscissa  $-\Lambda^{-1}(\Omega^{-1}\text{m}^2\text{mol}^{-1}) \times 10^2$ . Ordinate  $\sqrt{m}$  ( $\text{mol}^{1/2}\text{dm}^{-3/2}$ ).

the scattering intensities were much higher than those calculated for unassociated monomers. The scattering data were analysed as described previously (Attwood & Udeala, 1975a). Aggregate growth was assumed to occur by the stepwise addition of monomers,  $M_1$ ,



where  $N$  is the aggregate number. Stepwise equilibrium constants,  $K_N$ , were estimated from equation 2 using an analytical treatment proposed by Steiner (1952).

$$\begin{aligned} [(M_w/xM)-1]/(xc/M) &= 4K_2 + 9K_2K_3(xc/M) \quad \dots \\ &+ N^2(\pi K_N)(xc/M)^{N-1} \quad \dots \quad (2) \end{aligned}$$

$M$  and  $M_w$  are the monomer molecular weights and weight-average aggregate weights respectively, and  $c$  is the weight concentration ( $\text{g dm}^{-3}$ ). The weight fraction,  $x$ , of compound existing as the monomer was determined by integration of the light scattering data according to,

$$\ln x = \int_0^c [(M/M_w)-1] d \ln c \quad (3)$$

This treatment does not take into account non-ideality effects arising from interactions between the charged aggregates. Such interactions are reduced in the presence of excess electrolyte. Errors in the calculation of  $K_N$  are cumulative and only the lower values have been calculated. Table 1 shows a general increase in the magnitude of  $K_N$  with increasing electrolyte concentration. A similar effect was noted following the addition of electrolyte to aqueous solutions of several antihistamines (Attwood & Udeala, 1976).

Graphs of the equivalent conductivity,  $\Lambda$ , against  $m^{\frac{1}{2}}$ , (Fig. 3), for propantheline, methantheline and methixene, did not show any significant inflection attributable to a cmc. Both compounds exhibited

slopes greater than those predicted for 1 : 1 electrolytes by equation 4 (Robinson & Stokes, 1954) which is reportedly valid for  $m \leq 0.05 \text{ mol dm}^{-3}$

$$\Lambda = \Lambda^\circ - [(B_1\Lambda^\circ + B_2)/(1 + ka)] m^{\frac{1}{2}} \quad (4)$$

$B_1$  and  $B_2$  are constants with values of  $0.2321 \text{ mol}^{-\frac{1}{2}} \text{ dm}^{3/2}$  and  $6.791 \times 10^{-3} \Omega^{-1} \text{ m}^2 \text{ mol}^{-1} (\text{mol dm}^{-3})^{-\frac{1}{2}}$  respectively at 303K and  $k = 1.04 \times 10^8 \text{ m}^{\frac{1}{2}}$  at this temperature. Values of the ionic radius,  $a$ , were estimated from molecular models.

#### DISCUSSION

It is clear from the light scattering data presented here and in previous reports, that the self association of these drugs does not occur by the normal micellization process. The failure to detect a cmc by conductimetric techniques provides further supporting evidence. Since both monomers and aggregates contribute to the conductance of the solution, a gradual rather than abrupt change in conductivity might be expected for a stepwise mode of association. A similar failure to detect an inflection point was noted in conductivity studies on solutions of several antihistamines which are thought to associate in this manner (Attwood & Udeala, 1975a). The surface tension graphs (Fig. 1A and B) are, however, similar to those of conventional micellar systems. Similar apparent discrepancies between light scattering and interfacial behaviour have been reported by other authors. Corkill, Goodman & Ottewill (1961) and Balmbra, Clunie & others (1962, 1964) noted curvature of the light scattering plots of several polyoxyethylene non-ionic detergents at concentrations considerably greater than the cmc determined from conventional surface tension plots. Light scattering measurements (Attwood, 1968) on aqueous solutions of the non-ionic detergent, heptaoxyethylene glycol monohexadecyl ether showed a pronounced concentration-dependence of micellar size at concentrations considerably in excess of the cmc determined from the typically micellar surface tension graphs of Elworthy & Macfarlane (1962). More recently, Attwood & Udeala (1975b) noted similar discrepancies for several antihistamines in aqueous solution.

If the usual assumption is made that surface tension depends entirely on the monomer concentration,  $m_{\text{mon}}$ , then Fig. 1A and B suggest that  $m_{\text{mon}}$  reaches a limiting value and remains approximately constant over a wide range of solution concentration,  $m$ . It is therefore of interest to consider the concentration-dependence of  $m_{\text{mon}}$  as indicated by the application of equation 3 to the light scattering data.

Table 1. Stepwise association constants for propantheline bromide in aqueous sodium bromide solutions.

Concn of sodium bromide (mol kg <sup>-1</sup> )	Stepwise association constants (dm <sup>3</sup> mol <sup>-1</sup> )		
	$K_2$	$K_3$	$K_4$
0.00*	12.5	76	25
0.15	25	98	105
0.30	41	117	257
0.45	69	190	345

\* Values from Attwood (1976a).

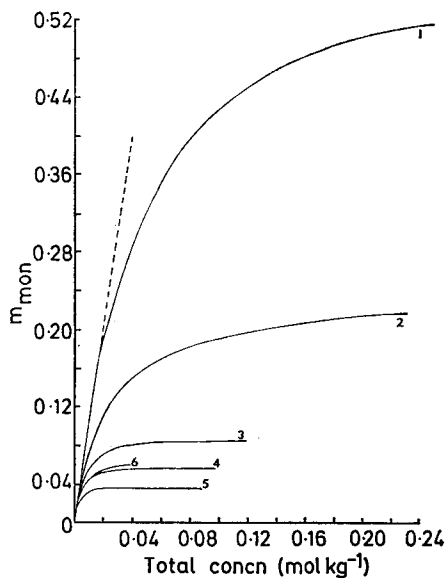


FIG. 4. Variation of monomer concentration,  $m_{\text{mon}}$ , ( $\text{mol kg}^{-1}$ ) with solution concentration ( $\text{mol kg}^{-1}$ ), calculated from light scattering data using eqn 3. 100% monomers (---). 1, methantheline bromide in  $\text{H}_2\text{O}$ ; 2, proprantheline bromide in  $\text{H}_2\text{O}$ ; 3,  $0.15 \text{ mol kg}^{-1}$  NaBr; 4,  $0.30 \text{ mol kg}^{-1}$  NaBr; and 5,  $0.45 \text{ mol kg}^{-1}$  NaBr; 6, methixene hydrochloride in  $\text{H}_2\text{O}$ .

Fig. 4 shows the results of such calculations for proprantheline in water and electrolyte solutions and also for methantheline and methixene in water (calculated using light scattering data reported previously). The curves differ, from those calculated by the mass action theory for micellar solutions (Elworthy, Florence & Macfarlane, 1968).  $m_{\text{mon}}$  is appreciably less than  $m$  in dilute solutions and

approaches a limiting value asymptotically as concentration is increased. In micellar solutions, the monomer concentrations may generally be equated with solution concentration below the cmc and decreases above the cmc. Table 2 shows reasonable

Table 2. Limiting monomer concentrations from surface tension I [apparent cmc from surface tension ( $\text{mol kg}^{-1}$ )  $\times 10^3$ ] and light scattering data II [limiting monomer concn from light scattering\* ( $\text{mol kg}^{-1}$ )  $\times 10^3$ ].

Compound	Solvent	I	II
Proprantheline Br	$\text{H}_2\text{O}$	13.5	$\sim 22^\dagger$
	$0.15 \text{ mol kg}^{-1} \text{ NaBr}$	8.2	8.3
	0.30 "	5.1	5.7
	0.45 "	4.6	3.7
Methantheline Br	$\text{H}_2\text{O}$	44	$\sim 52^\dagger$
Methixene HCl	$\text{H}_2\text{O}$	5.5	6.0 $^\dagger$

\* Calculated using eqn 3

$^\dagger$  Evaluated from light scattering data of Attwood (1976a).

agreement between the limiting value of  $m_{\text{mon}}$  from the light scattering data and the apparent cmcs indicated by the surface tension graphs. Integration of the light scattering data of systems undergoing stepwise association would therefore seem to be an acceptable method of determining the limiting monomer concentration in such systems.

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