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# Aggregation of antidepressant drugs in aqueous solution

D. ATTWOOD* AND J. GIBSON

*Pharmacy Department, University of Manchester, Manchester M13 9PL, U.K.*

Light scattering, conductivity and pH methods have been used to examine the aggregation in aqueous solution of a series of antidepressant drugs. The drugs investigated included the hydrochlorides of amitriptyline, butriptyline, protriptyline, nortriptyline, imipramine, desipramine, clomipramine, dothiepin, dibenzepin, opipramol, iprindole, doxepin, mianserin and maprotiline. No significant association of dibenzepin, mianserin or maprotiline hydrochlorides could be detected up to their respective solubility limits. A micellar pattern of association was established for all other compounds. Critical micelle concentrations and micellar properties are reported.

Typical colloidal behaviour is exhibited by a large number of drugs from many pharmacological groups of compounds (see reviews by Florence, 1968; Felmeister, 1972, and more recent work on antihistamines; Attwood, 1972; Attwood & Udeala, 1974; 1975a,b; Thoma & Siemer, 1976; and antiacetylcholine drugs, Attwood, 1976a,b1). Thoma & Siemer (1976) have determined the critical micelle concentration, (cmc), of the tricyclic antidepressant, amitriptyline hydrochloride, in 0·9% NaCl. Other workers (Seeman & Bialy, 1963, Kitler & Lamy, 1971; Nambu, Sakurai & Nagai, 1975) have reported on the surface activity of several tricyclic antidepressants, although none have worked at sufficiently high concentrations to detect any onset of aggregation.

This paper examines the solution properties of a series of antidepressants. Light scattering, conductivity and pH methods have been used to establish the type of association (i.e. whether micellar or non-micellar) and to determine the properties of any aggregates formed.

## MATERIALS AND METHODS

*Materials.* The following drugs were sufficiently well characterized and purified by the manufacturers to be used without further purification; the hydrochlorides of clomipramine, desipramine B.P., imipramine B.P. and opipramol (Geigy Pharmaceuticals); amitriptyline B.P. and protriptyline B.P. (Merck, Sharp and Dohme); nortriptyline B.P. (Eli Lilly); dibenzepin (Wander Pharmaceuticals); doxepin B.P. (Pfizer); iprindole (Wyeth); dothiepin B.P. (Boots); butriptyline (Ayerst); mianserin (Beecham Pharmaceuticals) and maprotiline (Ciba).

* Correspondence.

*Light scattering measurements.* Measurements were made at 303 K with a Fica 42000 photogoniodiffusometer (A.R.L. Ltd.), using a wavelength of 546 nm. The aqueous solutions were clarified by ultrafiltration through 0·1 $\mu$m Millipore filters. The refractive index increments of the micellar species were measured at 546 nm using a differential refractometer.

*Conductivity measurements.* Measurements were made at 303 $\pm$ 0·01 K using a Wayne Kerr autobalance universal bridge (Model B642). The conductivity cell (Mullard E 7591/B) was calibrated with potassium and sodium chloride (Analar) solutions.

*pH measurements.* A Pye Model 290 pH meter, fitted with a combined glass-silver chloride electrode, was used in the determination of pH. Carbon dioxide-free water was used in the preparation of the solutions and an atmosphere of nitrogen was maintained during measurement. Measurements were made at 303 $\pm$ 0·01 K. Cmc values were determined from inflections in the curves of pH vs log concentration, in the usual way.

## RESULTS

Light scattering results are presented in Figs 1 and 2 as plots of the scattering at an angle of 90°, $S_{90}$, as a function of molal concentration, m. Scattering graphs for maprotiline, mianserin and dibenzepin hydrochlorides followed closely the theoretical lines derived for scattering from unassociated monomers, up to the solubility limit for each compound. The scattering from opipramol hydrochloride (Fig. 1) increased continuously with increasing concentration, with no apparent discontinuity which could be attributed to a cmc. The remaining compounds

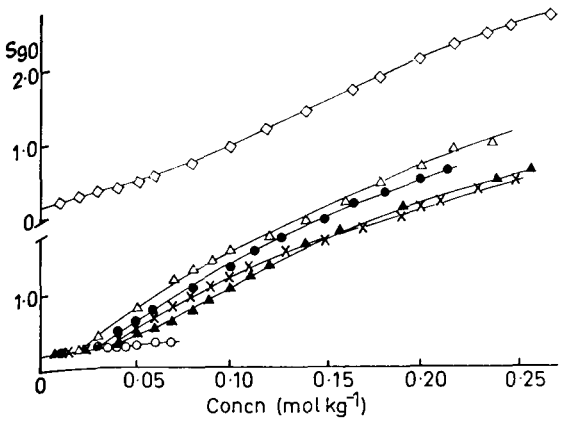



FIG. 1. Variation of the scattering ratio, $S_{90}$, with concentration (mol kg$^{-1}$) for aqueous solutions of ◇ opipramol 2 HCl; △ clomipramine HCl; ● amitriptyline HCl; × butyriptyline HCl; ▲ imipramine HCl; ○ mianserin HCl. Continuous line for opipramol 2 HCl was calculated from eqns 1–5 (see text).

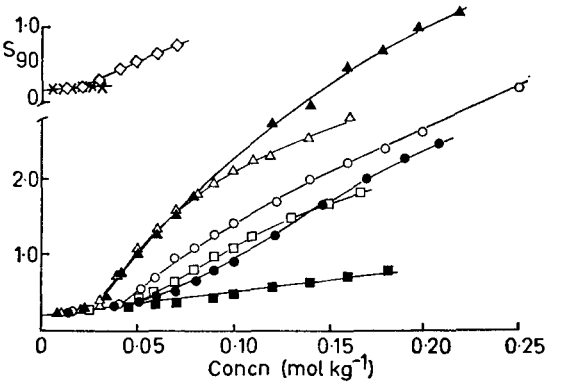



FIG. 2. Variation of the scattering ratio, $S_{90}$, with concentration (mol kg$^{-1}$) for aqueous solutions of ◇ nortriptyline HCl; × maprotiline HCl; ▲ dothiepin HCl; △ iprindole HCl; ○ protriptyline HCl; □ desipramine HCl; ● doxepin HCl; ■ dibenzepin HCl.

exhibited the scattering behaviour normally associated with surfactant solutions. Cmc values for these compounds were determined from the clearly defined inflections in the $S_{90}$ *vs* m plots. The scattering in the pre-cmc region did not deviate significantly from that calculated for unassociated monomers. For such compounds the micellar aggregation number, N, and the effective thermodynamic micellar charge, p, were evaluated using equations proposed by Anacker & Westwell (1964). In a solution containing no added electrolyte

$$K^1 m_{mic} / R_{90mic} = A + Bm_{mic} \quad .. \quad .. \quad (1)$$

where

$$K^1 = 2\pi^2 n_0^2 (dn/dm)^2 V^\circ / L\lambda^4 \quad .. \quad .. \quad (2)$$

$$A = 4N ((2N - p)^2 + p)^{-1} \quad .. \quad .. \quad (3)$$

$$B = pA ((1 + p)N^{-1} - A) (2m_{mon})^{-1} \quad .. \quad (4)$$

In these equations, $m_{mic}$ is the molal concentration of micelles; $R_{90mic}$ is the Rayleigh ratio of the solution in excess of that of a solution at the cmc, $n_0$ is the refractive index of the solvent; $V^\circ$ is the volume of solution containing 1 kg of water; L is the Avogadro constant and $\lambda$ is the wavelength of the incident light. The intensity of scattering from nortriptyline hydrochloride was not sufficiently high to allow the calculation of a reliable value of p and in this case N was equated with $A^{-1}$. The light scattering results are summarized in Table 1.

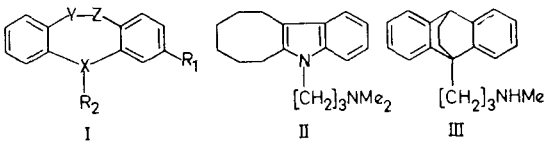
The scattering behaviour exhibited by opipramol illustrates a problem which is often encountered when low aggregation numbers are involved (Attwood, 1976b). The absence of discontinuities in the scattering curves may be indicative of non-micellar association or may result from micellar association processes involving a combination of low values of both N and micellar equilibration constant $K_m$. A similar absence of a cmc was noted from conductivity and pH graphs for opipramol. According to the mass action theory of micellization, $K_m$, may be approximated to

$$K_m = \frac{(M^{p+})}{((X^-) - p(M^{p+}))^N (X^-)^{N-p}} \quad (5)$$

where $(M^{p+})$ and $(X^-)$ represent the mole fraction of micellar species and counterions respectively. The scattering intensity arising from the micellar species, $S_{90mic}$, was calculated from equations 1–4 using values of $m_{mic}$ generated by eqn 5 for selected combinations of the variables N, $K_m$ and p. The monomeric scattering, $S_{90mon}$, was estimated in a similar manner using values of $m_{mon}$ generated from equation 5 for the same combination of variables. The total scatter was then a summation of $S_{90mic}$, $S_{90mon}$ and the intensity of light scattered by the solvent. An iterative method was developed for the solution of equations 1–5, details of which are to be published. A good fit of the scattering data of opipramol was achieved for N = 4·9, p = 2·4 and $K_m = 1 \times 10^{16}$ (see Fig. 1) suggesting that the aggregation process was micellar.

In general, a reasonable agreement between cmc values from light scattering, conductivity and pH

Table 1. *Micellar properties of antidepressant drugs at 303 K.*




I II III

| Compound | Structure | X | Y–Z | $R_1$ | $R_2$ | dn/dm kg mol$^{-1}$ | N | P | α |
|---|---|---|---|---|---|---|---|---|---|
| Imipramine HCl | I | N | $CH_2$–$CH_2$ | H | $[CH_2]_3NMe_2$ | 0·0710 | 7·5 | 1·7 | 0·23 |
| Clomipramine HCl | I | N | $CH_2$–$CH_2$ | Cl | $[CH_2]_3NMe_2$ | 0·0777 | 6·2 | 0·8 | 0·13 |
| Desipramine HCl | I | N | $CH_2$–$CH_2$ | H | $[CH_2]_3NHMe$ | 0·0711 | 7·2 | 1·5 | 0·21 |
| Opipramol 2HCl | I | N | CH=CH | H | $[CH_2]_3$·N N·$[CH_2]_2OH$ | 0·104 | 74·9 | 2·4 | 0·49 |
| Amitriptyline HCl | I | C | $CH_2$–$CH_2$ | H | $=CH[CH_2]_2NMe_2$ | 0·0738 | 6·7 | 1·0 | 0·15 |
| Nortriptyline HCl | I | C | $CH_2$–$CH_2$ | H | $=CH[CH_2]_2NHMe$ | 0·0718 | 4·0 | — | — |
| Butriptyline HCl | I | C | $CH_2$–$CH_2$ | H | $CH_2$.CH.$CH_2$.$NMe_2$ (Me) | 0·0662 | 8·7 | 1·6 | 0·18 |
| Protriptyline HCl | I | C | CH=CH | H | $[CH_2]_3NHMe$ | 0·0771 | 8·6 | 1·8 | 0·21 |
| Doxepin HCl | I | C | O–$CH_2$ | H | $=CH[CH_2]_2NMe_2$ | 0·0728 | 6·6 | 1·1 | 0·17 |
| Dothiepin HCl | I | C | S–$CH_2$ | H | " | 0·0836 | 9·9 | 1·4 | 0·14 |
| Dibenzepin HCl | I | N | N–C=O | H | Me | 0·0803 | 1 | — | — |
| Mianserin HCl | | $CH_2$ | $[CH_2]_2NMe_2$ CH–N N Me | H | — | 0·0715 | 1 | — | — |
| Iprindole HCl | II | — | — | — | — | 0·0733 | 18·8 | 3·6 | 0·19 |
| Maprotiline HCl | III | — | — | — | — | 0·0744 | 1 | — | — |

determinations was noted (Table 2) confirming the micellar nature of the association process. The lack of any clearly defined inflection in the pH and conductivity curves (Figs 3, 4) for nortriptyline and opipramol illustrates the lack of sensitivity of these techniques when compounds with low aggregation numbers are involved.

## DISCUSSION

One of the requirements for micellization is a co-operativity of aggregation, which is most easily achieved by surfactants with flexible hydrocarbon chains and terminal polar groups. It has been shown (Attwood & Udeala, 1974; Attwood, 1976a)

Table 2. *Critical micelle concentrations of antidepressant drugs at 303K.*

| Compound | cmc (mol kg$^{-1}$) Light scattering | Conductivity | pH |
|---|---|---|---|
| Imipramine HCl | 0·050 | 0·048 | 0·042 |
| Clomipramine HCl | 0·020 | 0·023 | 0·024 |
| Desipramine HCl | 0·051 | 0·057 | 0·041 |
| Amitriptyline HCl | 0·030 | 0·044 | 0·035 |
| Nortriptyline HCl | 0·023 | — | — |
| Butriptyline HCl | 0·037 | 0·046 | 0·044 |
| Protriptyline HCl | 0·044 | 0·044 | 0·035 |
| Doxepin HCl | 0·062 | 0·068 | 0·050 |
| Dothiepin HCl | 0·026 | 0·029 | 0·031 |
| Iprindole HCl | 0·029 | 0·031 | 0·032 |

No significant inflections could be detected in data for opipramol 2 HCl, dibenzepin HCl, mianserin HCl or maprotiline HCl.

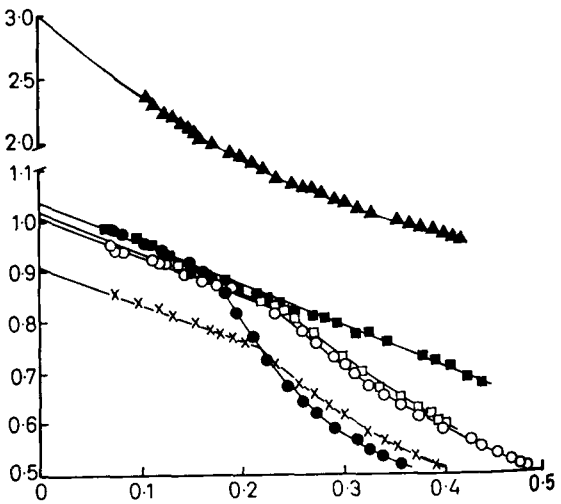



Fig. 3. Equivalent conductivity, $\Lambda[(\Omega^{-1}m^2\,mol^{-1}) \times 10^2]$, of aqueous solutions of ▲ opipramol 2HCl; ■ dibenzepin HCl □ desipramine HCl; ○ imipramine HCl; ● iprindole HCl; × butriptyline HCl. Abscissa; $\sqrt{m}$ (mol$^{\frac{1}{2}}$ dm$^{-3/2}$).

that drug molecules with hydrophobic groups constructed around a diphenylmethane skeleton are also sufficiently flexible to allow micellization. In contrast, rigid, planar aromatic or heteroaromatic ring structures with short flexible chain substituents,

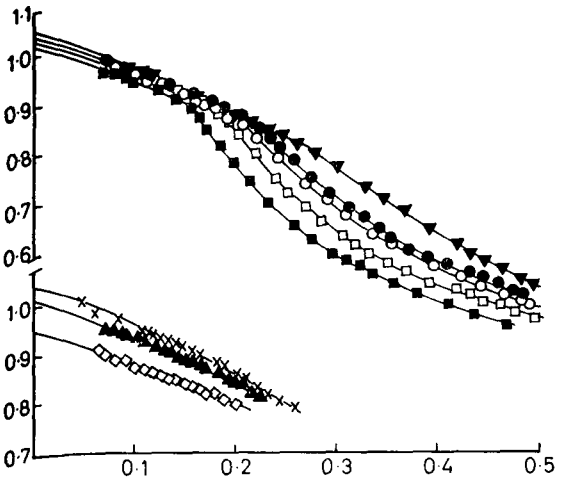



FIG. 4. Equivalent conductivity, $\Lambda[(\Omega^{-1}\,m^2\,mol^{-1}) \times 10^2]$ of aqueous solutions of ▼ doxepin HCl; ● amitriptyline HCl; ○ protriptyline HCl; □ dothiepin HCl; ■ clomipramine HCl; × mianserin HCl; ▲ nortriptyline HCl; ◇ maprotiline HCl. Abscissa: $\sqrt{m}$ ($mol^{\frac{1}{2}}\,dm^{-3/2}$).

aggregate by a stacking-type association in which each associating monomer can lie flat on top of a stack containing one or more monomers (Mukerjee, 1974). Such non-micellar association produces a polydisperse system characterized by the absence of a cmc. The methylene blue systems, extensively investigated by Mukerjee & Ghosh (1970), have been interpreted assuming an association of this type. The tricyclic ring systems of the antidepressants studied here are skewed and bent (Wilhelm & Kuhn, 1970) in contrast to the planar tricyclic ring systems of methylene blue. More significantly, the antidepressant drug molecules exhibit a certain flexibility as evidenced by the variable temperature nmr spectroscopic studies of Abraham, Kricka & Ledwith (1975). The results presented here suggest that the flexibility of the hydrophobic groups of the antidepressants may be sufficient to enable a micellar pattern of association to be established. An exception to this generalization is the rigid analogue, maprotoline hydrochloride. Unfortunately the $-CH_2-CH_2-$ 'bridge' which confers rigidity to this molecule is also likely to act as a structural barrier to face-to-face stacking beyond the dimer stage. A lack of any significant association was noted over the limited concentration range which could be studied.

The antidepressant drugs provide an interesting series of compounds with which to illustrate the effect of substituents on solution properties. In the following examples, pairs of compounds have been selected in which each member of the pair has an identical side chain ($R_2$ group) so that any changes in hydrophobicity may be attributed entirely to the structural changes of the hydrophobic moiety. As all of these drugs are hydrochloride salts, the effect of the counterion is also eliminated. A comparison of imipramine with clomipramine shows the increased hydrophobicity (as evidenced by a decrease in the cmc) which is conferred by a -Cl substituent on one of the phenyl rings. This is a well known effect and is noted, for example, in a comparison of the phenothiazine drugs, promazine and chlorpromazine (Attwood, Florence & Gillan, 1974) and the antihistamines, diphenhydramine and bromodiphenhydramine (Attwood & Udeala, 1975b). A comparison of amitriptyline, doxepin and dothiepin demonstrates the effect on solution properties of a heteroatom in position 10 of the dihydrodibenzocycloheptene ring system. Table 1 shows that hydrophobicity increases according to $0 < CH_2 < S$. The hydrophobic nature of the S atom is well established and it is this heteroatom which is responsible, for example, for the increased hydrophobicity of the tricyclic antiacetylcholine drug, methixene, compared with the structurally similar drugs, methantheline and propantheline in which the S is replaced by an O atom.

It is interesting to compare the antidepressants with the phenothiazine tranquillizers, the micellar properties of some of which have been reported by Scholtan (1955) and Attwood & others (1974). A direct comparison of compounds with identical side chains is possible in the case of the pairs, imipramine and promazine and also clomipramine and chlorpromazine. In both cases, the S atom of the phenothiazines confers a greater hydrophobicity than the 2 $CH_2$ groups which replace it in the antidepressant drugs. Consequently, the antidepressants have, in general, higher cmcs and slightly lower aggregation numbers than the phenothiazine drugs.

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