J. Pharm. Pharmacol. 1986, 38: 494–498
Received December 19, 1985

Self-association of local anaesthetic drugs in aqueous solution

D. ATTWOOD* AND P. FLETCHER

Pharmacy Department, University of Manchester, Manchester M13 9PL, UK

The aggregation characteristics of a series of local anaesthetic drugs in water and electrolyte solution have been examined using total intensity light scattering, photon correlation spectroscopy and vapour pressure osmometry. The association of cinchocaine hydrochloride was micellar. An appreciable increase in the effective diffusion coefficient as solutions of cinchocaine were diluted close to the critical micelle concentration was observed and has been discussed. The association of amethocaine hydrochloride could be described using a co-operative stepwise association model. No association could be detected in water or 0·1 mol dm^{-3} electrolyte for butacaine hemisulphate and the hydrochlorides of procaine, proparacaine, mepivacaine, lignocaine, bupivacaine and prilocaine at drug concentrations of less than 0·2 mol dm^{-3}.

Several workers have studied the association characteristics of local anaesthetic drugs (see Attwood & Florence 1983). Jaenicke (1966) reported a micellar mode of association for the hydrochlorides of amethocaine (tetracaine), stadacain and cinchocaine (dibucaine) and determined aggregation numbers by an ultracentrifugation technique. Johnson & Ludlum (1969) also concluded that cinchocaine formed micelles in aqueous solution and reported a critical micelle concentration (CMC) from total intensity light scattering methods which was in good agreement with that of Jaenicke. These workers also noted limited association of amethocaine and procaine hydrochlorides in water and electrolyte solution. The light scattering plots for these drugs were not of a typically micellar form and it was suggested that there may be complex multiple equilibria between aggregates of different sizes. A similar conclusion was reached by Faradieh et al (1967) who also failed to detect CMCs for these two drugs from vapour pressure osmometry and conductivity techniques.

In this present investigation we have studied in more detail the association of cinchocaine and amethocaine in water and electrolyte solutions using total intensity light scattering, photon correlation spectroscopy (PCS) and vapour pressure techniques. Other amphiphilic local anaesthetic drugs have been examined for evidence of association.

MATERIALS AND METHODS

Materials

The following drugs were sufficiently well characterized and purified to be used as received: procaine hydrochloride BP, butacaine hemisulphate, cinchocaine hydrochloride BP and lignocaine hydrochloride (Sigma); prilocaine hydrochloride BP bupivacaine hydrochloride (Astra Pharmaceuticals), mepivacaine hydrochloride (Leo), proparacaine hydrochloride (Squibb) and amethocaine hydrochloride BP (Smith and Nephew Pharmaceuticals). Sodium chloride (BDH) was of Analar grade and sodium sulphate (BDH) was laboratory grade.

Total intensity light scattering

Measurements were made at 303 ± 0·1 K with a Fica 42000 photogonio-diffusometer using a wavelength of 546 nm. Solutions were clarified by ultrafiltration through 0·1 μm filters until the ratio of light scattering at angles of 30° and 150° did not exceed 1·10. The refractive index increments were measured at 546 nm by differential refractometry.

Photon correlation spectroscopy

Measurements were made at 303 ± 0·1 K using a Malvern 7027 digital autocorrelator equipped with a 2W Argon ion laser (Coherent Innova 90) operating at 488 nm on the single clipped homodyne mode and using 60 linearly spaced channels with a far point delay of 1024 sample times. Solutions were clarified as described above.

Vapour pressure osmometry

A Knauer vapour pressure osmometer type 11·15 calibrated with sodium chloride solutions of known activity was used at 303 K.

* Correspondence.

RESULTS AND DISCUSSION

Plots of the light scattering intensity from drug solutions relative to that of a scattering standard, S_{90}, as a function of solution molality (Fig. 1) clearly show the micellar nature of the association of cinchocaine hydrochloride in water and in the presence of added electrolyte. There is no evidence of micellar growth in solutions of high ionic strength such as has been reported for the phenothiazine drugs (Attwood 1983; Attwood & Natarajan 1983; Atherton & Barry 1985). CMC values as determined from inflection points in these plots are given in Table 1 together with aggregation numbers, N, and degrees of ionization, α, as determined using the theory of Anacker & Westwell (1964). Vapour pressure plots (Fig. 2) also confirm that this drug forms micelles and CMC values determined from inflections in these plots are in reasonable agreement with those from light scattering (Table 1). The CMCs in water are within the range quoted by previous workers (6·0–6·6 × 10^{-2} mol dm^{-3}), although the aggregation number in water is lower than the value (N = 15) reported by Johnson & Ludlum (1969) at room temperature.

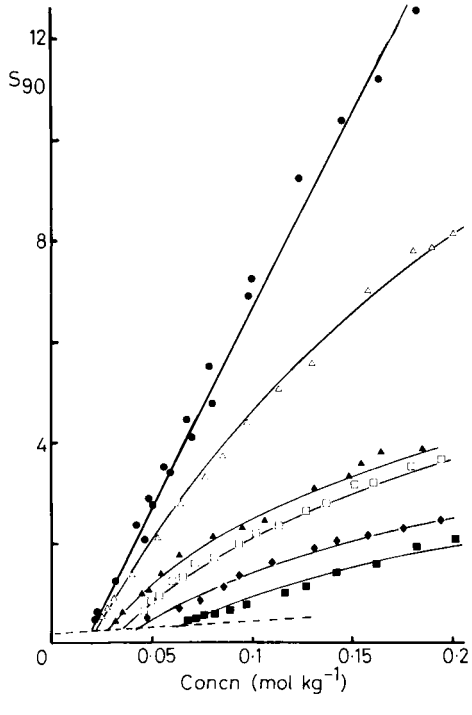

FIG. 1. Variation of the scattering ratio, S_{90}, with concentration for cinchocaine hydrochloride in ■, H_2O and ◆, 0·05; □, 0·1; ▲, 0·2; △, 0·3 and ●, 0·4 mol dm^{-3} sodium chloride solutions at 303 K. (– –) monomer line.

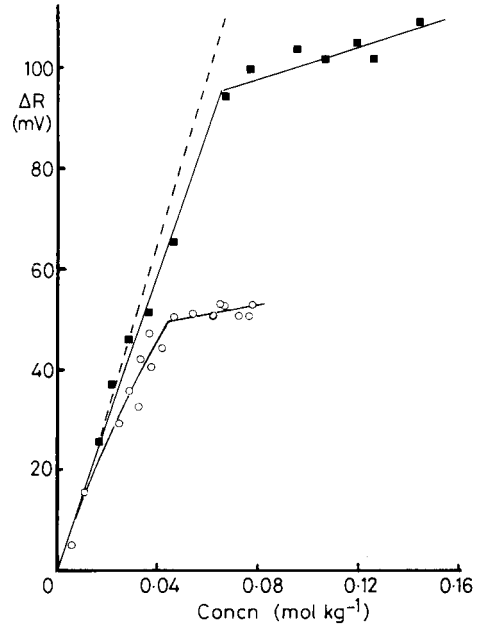

FIG. 2. Vapour pressure osmometer bridge resistance, ΔR, against concentration for cinchocaine hydrochloride in ■, H_2O and ○, 0·1 mol dm^{-3} sodium chloride solution at 303 K. (– – –) ideal 1 : 1 electrolyte.

A plot of log CMC against log counterion concentration, X^-, was linear in accordance with

$$\log \text{CMC} = -(1 - \alpha)\log X^- + \Delta G_m^{\circ}/2{\cdot}303RT + 1/N \log F\,(m^{+P}) \quad (1)$$

where m^{+P} is the mole fraction of micelles and F is a term involving the activity coefficients of all species in solution. A value of α of 0·42 was calculated from the slope assuming solution ideality. The standard free energy of micellization (per mole of monomeric drug ion), ΔG_m°, as calculated from the intercept of the plot was 27·4 kJ mol^{-1}.

The concentration dependence of the effective diffusion coefficient, D_{eff}, of cinchocaine hydrochloride is shown in Fig. 3. The very pronounced increase in D_{eff} as the concentration approaches the CMC is a characteristic feature of PCS plots of

Table 1. Micellar properties of cinchocaine hydrochloride in water and electrolyte solutions.

NaCl concn (mol dm^{-3})	CMC (mol kg^{-1} × 10^2) Light scatt.	vap. press.	N	α	r_h (nm)
0·000	6·0	6·6	9	0·23	—
0·050	4·3	—	10	0·26	2·62
0·100	3·6	4·3	15	0·27	2·11
0·200	2·8	—	18	0·32	2·01
0·300	2·3	—	31	0·25	2·20
0·400	1·8	—	35	—	2·25

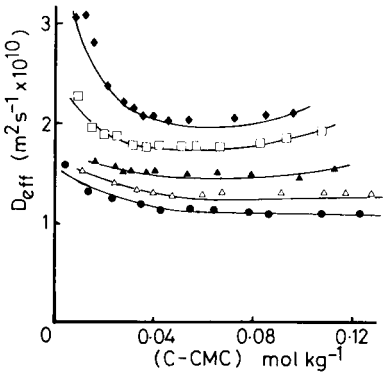

FIG. 3. Variation of effective diffusion coefficient, D_{eff}, with micellar concentration for cinchocaine hydrochloride in ◆, 0·05; □, 0·1; ▲, 0·2; △, 0·3; and ●, 0·4 mol dm^{-3} sodium chloride solutions at 303 K. (——) from eqn (4).

micellar systems with low aggregation numbers and high CMCs (Turq et al 1983). In solutions of low electrolyte concentration the curves exhibit a shallow minimum before becoming linear with a positive slope at higher drug concentration due to intermicellar repulsion. In the presence of higher concentrations of added electrolyte the intermicellar electrostatic forces are partially screened and attractive forces predominate giving rise to a negative slope and, consequently, an absence of a minimum in the curve. The reason for the upswing in the curve in the vicinity of the CMC has been attributed by Degiorgio & Corti (1984) to the increasing importance of the monomer contribution to D_{eff} as the solution is diluted in the vicinity of the CMC. In cases where the micellar lifetime is much greater than the measured correlation time, the effective diffusion coefficient may be represented by

$$D_{eff} = \frac{c_1 D_1 + N c_m D_m}{c_1 + N c_m} \qquad (2)$$

where c_1, c_m, D_1 and D_m are the monomer and micelle concentrations and diffusion coefficients, respectively. The true micellar diffusion coefficient corresponds to the value determined by extrapolation to the CMC of D_{eff} against concentration graphs, which for typical surfactants (N = 100), are linear at concentrations exceeding approximately 3 CMC. For the much lower aggregation numbers of the drugs under investigation it would be necessary to go to even higher concentrations than this to ensure linearity. Measurements have not been made over this concentration range for solutions of cinchocaine in low electrolyte and it is evident from Fig. 3 that, in the case of these solutions, linearity is not established over the concentration range of the experiment.

A method of avoiding the high concentrations of drug required to determine the micellar diffusion coefficient by the extrapolation technique is to simulate the diffusion curves in the following manner. From the phase separation model of micellization it may be shown that

$$D_m = D_m^0 (1 + K\emptyset_m) \qquad (3)$$

where D_m^0 is the true micellar diffusion coefficient, $\emptyset_m$ is the volume fraction and K is a constant. Substituting for D_m in equation (2) and expressing concentration as volume fraction yields (Turq et al 1983).

$$D_{eff} = \frac{D_m^2 N^2 \emptyset_m (1 + K\emptyset_m) + D_1 \bar{V}_m CMC}{\bar{V}_m CMC + N^2 \emptyset_m} \qquad (4)$$

$\emptyset_m$ and the partial molal volume of the micelles, $\bar{V}_m$, were calculated from

$$\emptyset_m = \frac{4}{3}\pi r_h^3 N_A c_m/N = \bar{V}_m c_m/N \qquad (5)$$

where r_h is the micellar radius as calculated from the diffusion coefficient using the Stokes-Einstein equation. The experimental data were fitted to equation (4) using a least squares routine in which D_1, D_m and K were optimized simultaneously. A good fit of the experimental data was obtained (see Fig. 3) with the best fit values of these parameters given in Table 2.

Table 2. Micellar D_m and monomeric D_1 diffusion coefficients for cinchocaine hydrochloride as determined from equation (4).

NaCl concn (mol dm^{-3})	D_1 ($m^2 s^{-1} \times 10^{10}$)	D_m ($m^2 s^{-1} \times 10^{10}$)	K
0·050	10·59	1·06	1·4 ± 0·03
0·100	6·51	1·30	2·0 ± 0·10
0·200	3·64	1·36	0·6 ± 0·92
0·300	4·77	1·24	0·3 ± 0·95
0·400	4·01	1·20	−1·1 ± 1·48

The results in 0·05 mol dm^{-3} NaCl are anomalous, giving a very much higher estimate of the monomer diffusion coefficient than was determined in higher electrolyte solutions, and a micellar diffusion coefficient which does not conform to the general trend of decreasing D_m with increase of electrolyte concentration. Whether this apparent anomaly is due to insufficient data or is a consequence of the inapplicability of this treatment, which is based on the phase separation theory, for a system of such a low aggregation number, is not clear. Inspection of Fig. 3 shows that at high salt concentration where the experimental concentration range extends suffi-

ciently beyond the CMC to permit meaningful extrapolation of data, a good agreement is obtained between extrapolated and calculated values of D_m. The hydrodynamic micellar radii calculated from the D_m values are included in Table 1.

The light scattering plots (Fig. 4) for amethocaine hydrochloride in water and in the presence of added electrolyte show evidence of continuous association, in agreement with the conclusions of other workers (Johnson & Ludlum 1969; Faradieh et al 1967). The vapour pressure curves (Fig. 5) similarly show no distinct inflection points, with the exception of the amethocaine—0·4 mol dm^{-3} NaCl system in which there is an abrupt inflection over the concentration range 0·06–0·07 mol kg^{-1}. Although there is a significant increase of light scattering intensity in this same concentration region, the S_{90} values at lower concentrations are well in excess of the monomer line and consequently this inflection cannot be interpreted as a CMC. It is clear from Fig. 5 that this is a very non-ideal system. At low drug concentrations the measured vapour pressure exceeds that predicted for a monomeric drug indicating an osmotic coefficient greater than unity.

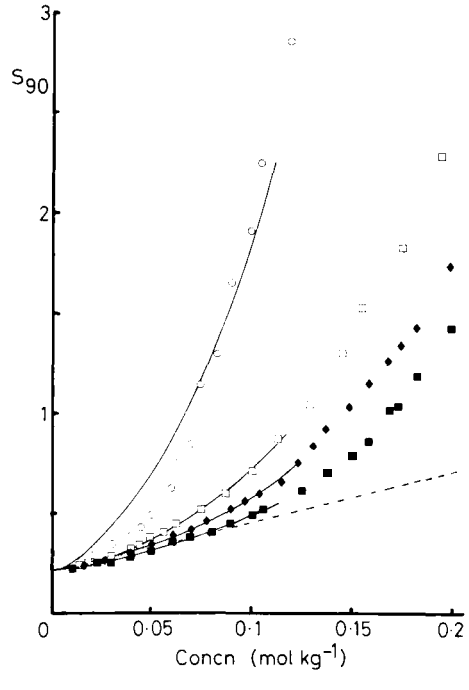

FIG. 4. Variation of the scattering ratio, S_{90}, with concentration for amethocaine hydrochloride in ■, H_2O; ◆, 0·05; □, 0·1 and ○, 0·4 mol dm^{-3} sodium chloride solutions at 303 K. (– – –) monomer line. (——) from eqn (6).

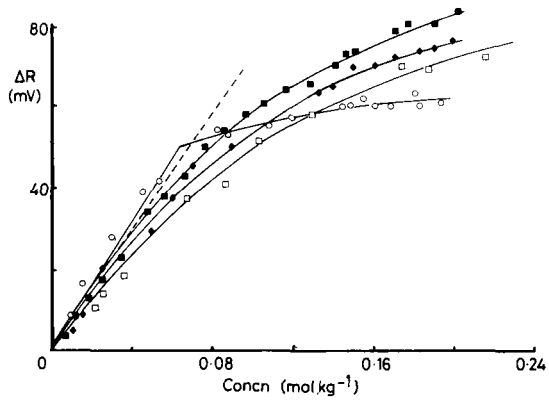

FIG. 5. Vapour pressure osmometer bridge resistance, ΔR, against concentration for amethocaine hydrochloride in ■, H_2O; ◆, 0·05; □, 0·1 and ○, 0·4 mol dm^{-3} sodium chloride solutions at 303 K. (– – –) ideal 1 : 1 electrolyte.

The total intensity light scattering data have been analysed using models which assume that association proceeds by the stepwise addition of monomers. Several association models covering a range of co-operative and antico-operative association have been described (Attwood & Florence 1983) in which the variation of the equilibrium constant K_N for the formation of an aggregate containing N monomers is expressed in terms of a generalized equilibrium constant K. The method of selection of the most suitable model using computer simulation of the data has been previously described (Gormally et al 1984). The association in water and in all concentrations of added electrolyte, except 0·4 mol dm^{-3} NaCl, was most accurately described by the co-operative model.

$$K_N = K\,(N - 1)/N \qquad (6)$$

with K values of 6·48, 13·4 and 21·3 kg mol^{-1} in water, 0·05 and 0·10 mol dm^{-3} NaCl, respectively. In the simulation procedure, only data for drug concentrations below 0·10 mol kg^{-1} were considered, to minimize the error arising from solution non-ideality at higher drug concentrations. The continuous lines of Fig. 4 are theoretical lines calculated in this way and it is clear that a good fit of data is achieved over this concentration range for all systems except cinchocaine—0·4 mol dm^{-3} NaCl. However in view of the high non-ideality of this system as indicated by the vapour pressure results, we do not feel justified in analysing the data using association models which are derived on the assumption of ideality.

The concentration dependence of the effective diffusion coefficient in the amethocaine solutions is shown in Fig. 6. The curves show a similar pronoun-

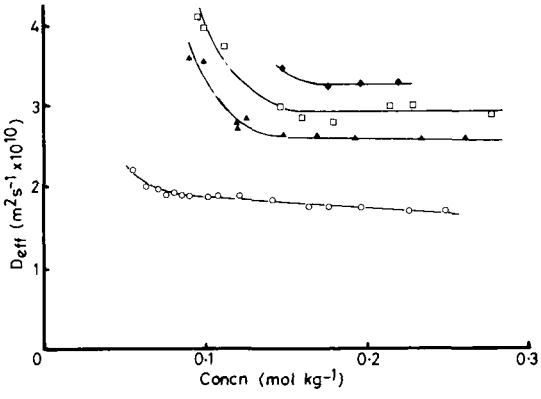

FIG. 6. Variation of effective diffusion coefficient, D_{eff}, with concentration for amethocaine hydrochloride in ◆, H_2O and □, 0·05; ▲, 0·1 and ○, 0·4 mol dm^{-3} sodium chloride solution at 303 K.

ced upswing due to monomer contribution to D_{eff} as the solution was diluted at low concentrations as was noted in the cinchocaine systems. However, unlike these systems, the diffusion curves for amethocaine, in low salt solutions do not show evidence of minima due to a change of slope on increasing concentration. The tendency for increase of D_{eff} arising from repulsive interaction between amethocaine aggregates is partially counteracted by a concomitant tendency for D_{eff} to decrease as a result of aggregate growth, such that D_{eff} becomes independent of concentration and no minimum is observed.

No evidence of association of procaine hydrochloride was apparent in water or in 0·1 mol dm^{-3} NaCl. The total intensity light scattering from solutions of this drug with concentrations up to 0·7 mol dm^{-3} conformed to that calculated for unassociated monomers in both solvents. Previous workers (Johnson & Ludlum 1969) have reported limited association of procaine within this concentration range in water and 0·4 mol dm^{-3} NaCl. Procaine is clearly a less hydrophobic compound than amethocaine (the hydrophobic moiety is $H_2N.C_6H_4$—as compared with $CH_3.(CH_2)_3\ NHC_6H_4$—for amethocaine) and it would be anticipated that any association would be of a more limited nature than that observed for amethocaine. None of the other local anaesthetic drugs examined, including butacaine, lignocaine, bupivacaine, mepivacaine, proparacaine and prilocaine showed evidence of association from total intensity light scattering and vapour pressure measurements in the presence of 0·1 mol dm^{-3} NaCl (or 0·1 mol dm^{-3} Na_2SO_4 in the case of butacaine hemisulphate) for drug concentrations up to 0·2 mol dm^{-3}.

Acknowledgements

The authors thank S.E.R.C. for financial support and Astra Pharmaceuticals, Warner-Lambert, Leo, Squibb, and Smith and Nephew Pharmaceuticals for generous gifts of drugs.

REFERENCES

Anacker, E. W., Westwell, A. E. (1964) J. Phys. Chem. Ithaca 68: 3490–3494

Atherton, D., Barry, B. W. (1985) J. Coll. Interface Sci. 106: 479–489

Attwood, D. (1983) J. Chem. Soc. Faraday Trans. I, 79: 2669–2677

Attwood, D., Florence, A. T. (1983) Surfactant Systems, their Chemistry, Pharmacy and Biology, Chapman & Hall, London, Chap. 4, p. 124.

Attwood, D., Natarajan, R. (1983) J. Pharm. Pharmacol. 35: 317–319

Degiorgio, V., Corti, M. (1984) J. Coll. Interface Sci. 101: 289–291

Faradieh, B., Hall, N. A., Hammarlund, E. R. (1967) J. Pharm. Sci. 56: 18–23

Gormally, J., Natarajan, R., Wyn-Jones, E. W., Attwood, D., Gibson, J., Hall, D. G. (1984) J. Chem. Soc., Faraday 2, 80: 243–259

Jaenicke, R. (1966) Kolloid. Z. 212: 36–45

Johnson, E. M., Ludlum, D. B. (1969) Biochem. Pharmacol. 18: 2675–2677

Turq, P., Drifford, M., Hayoun, M., Perara, A., Tabony, J. (1983) J. Phys. Lett. 4: L471–L479