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Static and dynamic light scattering study on the association of some antidepressants in aqueous electrolyte solutions

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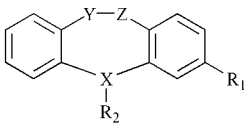
The self-association of the antidepressant drugs amitriptyline, nortriptyline, doxepin and desipramine hydrochlorides in aqueous solution has been studied by static and dynamic light scattering at 298.15 K in the presence of added electrolyte (0.0–0.4 mol kg^{-1} NaCl). Self-assembly was by a closed association process, commencing at a well-defined critical concentration for all systems. Micelle properties determined from static and dynamic light scattering techniques as a function of electrolyte concentration were related to differences in the structure of the hydrophobe. The pair interaction potential between aggregates has been discussed in terms of the DLVO theory of colloidal stability.

1. Introduction

A large number of drug molecules are amphiphilic and self-associate in aqueous environments to form small aggregates. The motivation for study of the solution properties of such compounds is two-fold. Although the pharmacological effects of amphiphilic drugs are usually manifest at concentrations well below the critical micelle concentration (c.m.c.), it is likely that accumulation of drug molecules in certain sites in the body may cause a localised high concentration resulting in aggregation and consequent changes in biological activity due to decreased transport rates or decreased ability to pass through biological barriers.[1] In addition, amphiphilic drug molecules also provide an opportunity for an examination of the influence of the structure of the hydrophobe on the association characteristics of surface active molecules. The tricyclic antidepressant drugs provide an interesting series of compounds with which to investigate the influence of hydrophobic substituents on association properties. These substances possess an almost planar tricyclic ring system with a short hydrocarbon chain carrying a terminal, charged nitrogen atom (see Scheme 1 for structures). It has been established from earlier studies on these compounds[2] that aggregates of approximately 8–10 monomers are formed in water by a closed association process at a well-defined critical concentration. More detailed examinations of the physicochemical properties of two of this series of compounds, imipramine and clomipramine, have been reported.[3–5]

We now report a study of the self-association in aqueous electrolyte of four antidepressant drugs, amitriptyline, nortriptyline, doxepin and desipramine hydrochlorides (see Scheme 1) and discuss structural features of the molecules that influence their association properties using static and dynamic light scattering techniques. To quantify the interaction between the aggregates the data have been interpreted using the Corti and Degiorgio[6] treatment of diffusion data, based on the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability.[7]



	X	Y–Z	R_1	R_2
Desipramine HCl	N	CH_2–CH_2	H	–$[CH_2]_3NH[CH_3]$
Doxepin HCl	C	O–CH_2	H	=$CH[CH_2]_2N[CH_3]_2$
Amitriptyline HCl	C	CH_2–CH_2	H	=$CH[CH_2]_2N(CH_3)_2$
Nortriptyline HCl	C	CH_2–CH_2	H	=$CH[CH_2]_2NH(CH_3)$

Scheme 1

2. Experimental

A. Materials

Amitriptyline, nortriptyline, doxepin and desipramine hydrochlorides of at least 98.5% purity were purchased from Sigma Chemical Co. and used without further purification. Solutions of the antidepressants were prepared with double-distilled and degassed water. Sodium chloride was of Analar grade.

B. Static light scattering

Static light-scattering measurements were made at 298.15 ± 0.1 K using a BI-200SM Brookhaven laser light-scattering instrument equipped with a 4 W argon ion laser (Coherent Innova 90) operating at 488 nm with vertically polarized light. Solutions were clarified by ultrafiltration through 0.1 μm filters with the ratio of light scattering at angles of 45° and 135° not exceeding 1.10. Toluene was used as a reference standard for which a Rayleigh ratio of 31.6×10^{-6} cm^{-1} was assumed.[8] The refractive index increments of the four drugs were measured at 298.15 ± 0.1 K using an Abbé 60/DE precision refractometer (Bellingham and Stanley Ltd.). Measurements over the range of electrolyte concentration showed no effect of electrolyte on the value of the refractive index increment within the limits of error of measurement. Values of

0.0736, 0.0715, 0.0727 and 0.071 kg mol^{-1} were obtained for amitriptyline, nortriptyline, doxepin and desipramine in water, respectively.

C. Dynamic light scattering

Measurements were made at 298.15 ± 0.1 K and at a scattering angle of 90° with the Brookhaven instrument described above combined with a Brookhaven BI 9000 AT digital correlator with a sampling time range of 25 ns to 40 ms. Solutions were clarified as described above. Diffusion coefficients were determined from a single exponential fit to the correlation curve. The hydrodynamic radius was calculated from measured diffusion coefficients by means of the Stokes–Einstein equation.

3. Results and discussion

A. Static light scattering

Fig. 1 shows the concentration dependence of the static light scattering intensity, S_{90}, (intensity of light scattered by the solution relative to that of toluene) for doxepin hydrochloride in the presence of added electrolyte at 298.15 K. Plots for amitriptyline, nortriptyline, and desipramine hydrochlorides were similar. Measurements on nortriptyline were restricted to a maximum electrolyte concentration of 0.05 mol kg^{-1} because of the onset of precipitation of the drug at higher NaCl concentrations. Critical concentrations (cc) determined from the inflections in the light scattering curves are given in Table 1; values in the absence of electrolyte are in good agreement with those previously obtained from density and ultrasound measurements[9] and those of an earlier study[2] from light scattering, conductivity and pH measurements.

Comparison of the cc values indicates that the hydrophobicity follows the sequence nortriptyline > amitriptyline > desipramine > doxepin. The differences in the cc values arise solely from the different substituents in the molecular structure of these drugs; all the drugs have Cl^- counterions and all are fully ionised at the pH of the solutions. The lone pair of electrons on the ring N of desipramine can undergo resonance with the aromatic rings giving rise to tautomers with zwitterionic character, so decreasing the hydrophobicity of the ring system. A similar situation exists with the ring O of doxepin and may account for the higher hydrophilicity of these two drugs compared with amitriptyline and nortriptyline which are not ring substituted.

Micelle characteristics were determined from the concentration dependence of the static light scattering intensity, S_{90},

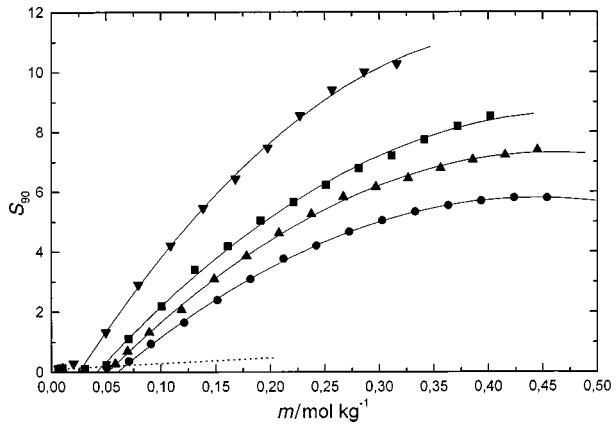


Fig. 1 Variation of the scattering ratio, S_{90}, with molality, m, for doxepin hydrochloride in (●) water and in aqueous NaCl solutions of concentrations (▲) 0.05, (■) 0.1, (▼) 0.2 mol kg^{-1} at 298.15 K. (– – –) Monomer line.

Table 1 Critical concentrations (cc), aggregation numbers (N_1) and charges (z_1) of amitriptyline, nortriptyline, doxepin and desipramine hydrochlorides in aqueous electrolyte solution at 298.15 K from static light scattering measurements

[NaCl]/mol kg^{-1}	cc/mol kg^{-1}	N_1	z_1
Amitriptyline			
0.00	0.033	6	—
0.05	0.023	13	2.4
0.10	0.019	17	3.1
0.20	0.016	32	5.7
0.40	0.014	49	8.0
Nortriptyline			
0.00	0.024	5	—
0.05	0.010	15	5.5
Doxepin			
0.00	0.067	9	—
0.05	0.059	11	1.6
0.10	0.043	12	1.9
0.20	0.029	18	3.7
Desipramine			
0.000	0.042	6	—
0.025	0.039	9	1.3
0.050	0.037	11	2.2
0.075	0.034	13	2.3
0.100	0.031	15	2.5

using the Anacker and Westwell[10] treatment in which the light scattering from the solutions of ionic aggregates is represented by

$$\frac{Km_2}{\Delta R_{90}} = \frac{2m_3 + N_1^{-1}(z_1 + z_1^2)m_2}{[2N_1 + (2N_1)^{-1}(z_1 + z_1^2)f^2 - 2fz_1]m_3 + z_1 m_2} \quad (1)$$

where m_2 is the molality of the micellar species in terms of monomer, m_3 is the molality of supporting electrolyte, z_1 the charge of the aggregate and $f = (\mathrm{d}n/\mathrm{d}m_3)_{m_2}/(\mathrm{d}n/\mathrm{d}m_2)_{m_1}$ with n the refractive index of the solution. The optical constant K for vertically polarized incident light is defined by

$$K = \frac{4\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}m_2)_{m_3}^2 V^0}{N_A \lambda^4} \quad (2)$$

n_0 being the refractive index of the solvent, V^0 the volume of solution containing 1 kg of water, N_A the Avogadro number and λ the wavelength of the incident light (488 nm). ΔR_{90} is the Rayleigh ratio of the solution in excess of that at the cc.

Eqn. (1), which is derived using the general fluctuation theory of light scattering by multicomponent systems, is applicable in the correction of the aggregation numbers of ionic micelles for charge effects when both the surfactant and added electrolyte are uni-univalent and have a common ion. A simplifying assumption is that the monomer concentration is equal to the c.m.c. and can be treated as part of the supporting electrolyte. Such an assumption is not thought to introduce significant error.[10]

Expansion of eqn. (1) in powers of m_2 leads to

$$\frac{Km_2}{\Delta R_{90}} = A + Bm_2 + \cdots \quad (3)$$

where

$$A = 4N_1[(2N_1 - fz_1)^2 + z_1 f^2]^{-1} \quad (4)$$

and

$$B = z_1 A(2m_3)^{-1}[(1 + z_1)N_1^{-1} - A] \quad (5)$$

Table 1 shows the aggregation numbers, N_1, and the effective micelle thermodynamic charge, z_1, so obtained; N_1 values in water are in good agreement with light scattering values from an earlier study.[2] It is of interest to compare the associ-

ation properties of the tricyclic molecules of this study with those of the tranquillising drugs based on the tricyclic phenothiazine ring system, which have been extensively studied.[11] In the presence of low concentrations of added electrolyte (<0.1 mol kg^{-1}) the phenothiazine drugs show discontinuities in solution properties (including light scattering intensity) at two and sometimes three ccs. Moreover, in the presence of higher electrolyte concentration the association pattern changes from closed to open association above the cc indicative of the growth of the primary micelle with drug concentration. Only one cc could be detected in the scattering curves of the four antidepressant drugs in water and added electrolyte over a drug concentration range which, except in the case of nortriptyline, is similar to that used in the light scattering studies of the phenothiazine drugs. Similarly, Debye plots of all systems were characteristic of those for closed association. The striking difference in behaviour between these two series of drugs in the presence of electrolyte suggests differences in the stacking of molecules in the aggregates that might arise from the partial flexibility of the hydrophobic core of the antidepressants[12] in contrast to the rigid phenothiazine ring system.

B. Dynamic light scattering

Dynamic light scattering measures a time profile of the normalized autocorrelation function of the light intensity, $g^{(2)}(t)$, which is related to the electric field normalized correlation function, $g^{(1)}(t)$, through the Siegert relation:

$$g^{(2)}(t) = 1 + \beta\,|g^{(1)}(t)|^2 \tag{6}$$

where β is the correlation factor ($0 < \beta \leqslant 1$).

$g^{(1)}(t)$ can be written as the Laplace transform of the distribution of the relaxation rates, $G(\Gamma)$:

$$g^{(1)}(t) = \int_0^\infty G(\Gamma)\exp(-\Gamma t)\,\mathrm{d}\Gamma \tag{7}$$

where Γ is the relaxation rate. For relaxation times, τ, $g^{(1)}(t)$ will be expressed as

$$g^{(1)}(t) = \int_0^\infty A(\tau)\exp(-t/\tau)\,\mathrm{d}\tau \tag{8}$$

where $\tau A(\tau) \equiv \Gamma G(\Gamma)$. The translational mutual diffusion coefficient, D, was calculated from the average relaxation rate, $\bar{\Gamma}$, according to the equation

$$D = \frac{\bar{\Gamma}}{q^2}; \quad (q \to 0) \tag{9}$$

where $q = 4\pi n_s \sin(\theta/2)/\lambda_0$ is the scattering vector. At $\theta = 90°$, the condition $q \to 0$ is fulfilled due to the small size of the particles in the solution.

For interacting particles, the concentration dependence of D may be described as:

$$D = D_0[1 + k_D(m - \mathrm{cc})] \tag{10}$$

where D_0 is the translational diffusion coefficient at zero concentration, k_D a constant and m the molality of the solution.

Fig. 2 shows the apparent diffusion coefficients, D, of amitriptyline plotted as a function of micellar concentration (m − cc) in water and aqueous electrolyte solutions. The other antidepressant drugs gave similar plots. The contribution of monomers to the effective value of D in the proximity of the critical concentration may cause considerable curvature of the data.[13] For this reason, measurements of D were restricted to a concentration region in which D was a linear function of molality. The limiting diffusion coefficients, D_0, were obtained by extrapolation of the data to the critical concentration.

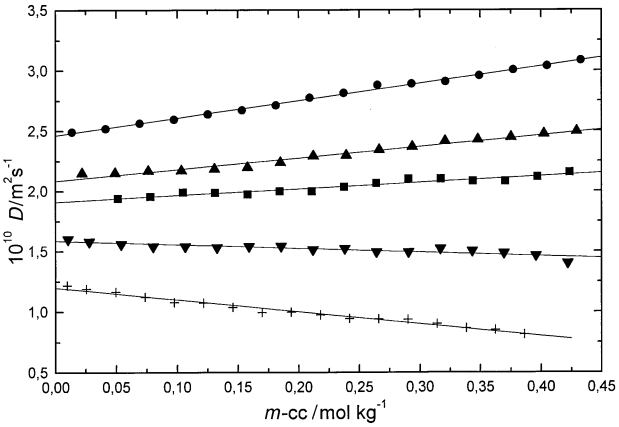


Fig. 2 Diffusion coefficient, D, as a function of the micellar concentration for amitriptyline hydrochloride in (●) water and in aqueous NaCl solutions of concentrations (▲) 0.05, (■) 0.1, (▼) 0.2 and (+) 0.4 mol kg^{-1} at 298.15 K.

Hydrodynamic radii, r_h, were derived assuming spherical aggregates from D_0, using the Stokes–Einstein equation,

$$D_0 = \frac{k_B T}{6\pi\eta r_h} \tag{11}$$

where k_B is the Boltzmann constant, T the absolute temperature and η the viscosity of the solvent. Table 2 shows the expected increase of aggregate size with increasing electrolyte concentration.

To relate the changes of gradient of the diffusion–concentration plots with changes in the interacting forces between aggregates, the data were analyzed according to the Corti and Degiorgio treatment.[6] Eqn. (10) may be expressed in terms of the volume fraction ϕ of the particles:

$$D = D_0(1 + k'_D\,\phi) \tag{12}$$

where $k'_D = k_D/\bar{v}$ and $\bar{v}$ is the specific volume of the solute particles. k_D may be related to the pair-interaction potential, $V(x)$, between spherical particles of radius a (equated to r_h) using

Table 2 Limiting diffusion coefficients, D_0, and hydrodynamic radii, r_h, of amitriptyline, nortriptyline, doxepin and desipramine in aqueous electrolyte solution at 298.15 K

[NaCl]/mol kg^{-1}	$D_0/10^{-10}$ m^2 s^{-1}	r_h/nm
Amitriptyline		
0.00	2.46	0.99
0.05	2.08	1.16
0.10	1.91	1.27
0.20	1.59	1.53
0.40	1.20	2.03
Nortriptyline		
0.00	3.36	0.72
0.05	2.59	0.94
Doxepin		
0.00	2.33	1.04
0.05	2.16	1.13
0.10	1.95	1.24
0.20	1.73	1.40
Desipramine		
0.000	2.59	0.94
0.025	2.37	1.02
0.050	2.26	1.07
0.075	2.12	1.14
0.100	2.00	1.21

Table 3 Experimental and theoretical slopes, k_D, and reduced potential at shear surface, $e\psi_0/k_BT$, of amitriptyline, doxepin and desipramine at 298.15 K as a function of electrolyte concentration

[NaCl] /mol kg^{-1}	k_D		$e\psi_0/k_BT$
	Experimental	Theoretical	
Amitriptyline			
0.05	1.7	2.0	0.75
0.10	0.9	0.6	0.40
0.20	−0.7	−1.2	0.16
0.40	−3.0	−1.5	0.02
Doxepin			
0.05	3.7	2.4	0.45
0.10	1.4	1.2	0.24
0.20	−0.1	0.1	0.12
Desipramine			
0.025	3.1	3.4	1.15
0.050	2.7	1.5	0.70
0.075	2.1	1.1	0.46
0.100	0.5	0.9	0.33

the expression proposed by Felderhof[14]

$$k_D = 1.56 + \int_0^\infty [24(1+x)^2 - F(x)][1 - \exp(V(x)/k_BT)]\,dx \tag{13}$$

where $x = (R - 2a)/2a$, R is the distance between the centers of two particles and $F(x)$ is given as:

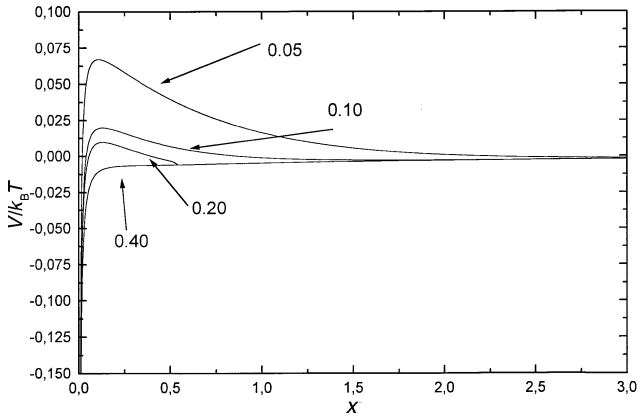


Fig. 3 Pair interaction potential, $V(x)$, for amitriptyline at different electrolyte concentrations (mol kg^{-1}). Values of the parameters are given in the text.

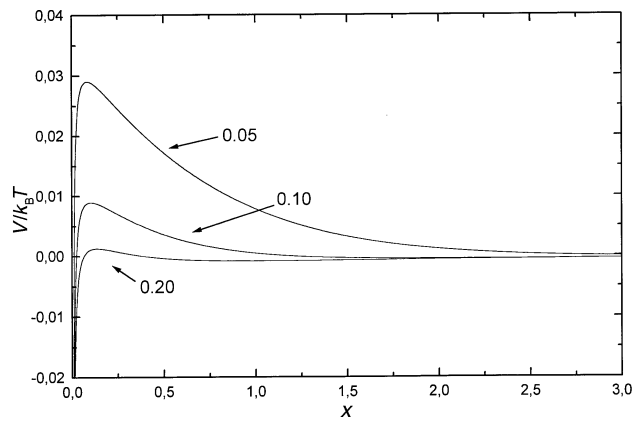


Fig. 4 Pair interaction potential, $V(x)$, for doxepin at different electrolyte concentrations (mol kg^{-1}). Values of the parameters are given in the text.

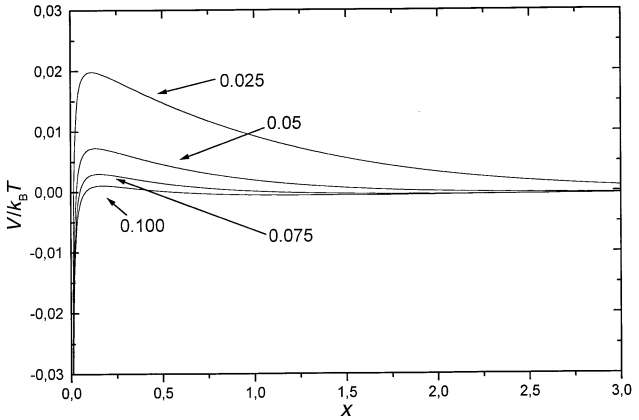


Fig. 5 Pair interaction potential, $V(x)$, for desipramine at different electrolyte concentrations (mol kg^{-1}). Values of the parameters are given in the text.

$$F(x) = 12(1+x) - \tfrac{15}{8}(1+x)^{-2} + \tfrac{27}{64}(1+x)^{-4} + \tfrac{75}{64}(1+x)^{-5} \tag{14}$$

The interaction potential $V(x)$ as it is usually written in DLVO theory[7] is the sum of an attractive London–van der Waals interaction $V_A(x)$ and a repulsive interaction due to the electric charge of the spheres, $V_R(x)$. $V_A(x)$ derived by Hamaker[15] for the case of two spheres is given by the expression:

$$V_A(x) = -\frac{A}{12}\left[(x^2+2x)^{-1} + (x^2+2x+1)^{-1} + \frac{2\ln(x^2+2x)}{(x^2+2x+1)}\right] \tag{15}$$

where A is the attractive Hamaker constant. Two approximate expressions have been proposed for the repulsive interaction, $V_R(x)$, for the limiting cases of $\kappa a < 1$ and $\kappa a > 1$. We have used the expression:[16]

$$V_R(x) = \frac{\varepsilon a \Psi_0^2}{2} \ln[1 + \exp(-2\kappa a x)] \tag{16}$$

which is appropriate for the aggregates investigated here. In eqn. (16) Ψ_0 is the surface potential and κ the Debye–Hückel reciprocal length parameter, expressed by the equation:

$$\kappa^2 = \frac{8\pi c_s e^2 z^2}{\varepsilon k_B T} \tag{17}$$

where ε is the relative permittivity of the suspending medium, z the valence of the ionic species in solution, c_s the concentration of the same species and e the proton charge.

The computational procedure involved the iteration of values of A and Ψ_0 to give the best fit of computed and experimental values of k_D over the range of electrolye concentration for each drug. Agreement between computed and experimental values of k_D, shown in Table 3, is reasonable in view of the assumptions inherent in these calculations.

The charge of the agregate including the Stern layer, p, is related to the surface potential, Ψ_0, by the expression[17]

$$\Psi_0 = \frac{2k_BT}{e} \sinh^{-1}\left(\frac{2\pi e\kappa^{-1}pe}{4\pi a^2 \varepsilon k_B T}\right) \tag{18}$$

The values of micellar charge number, p, derived in this manner were 0.18, 0.10 and 0.13. Although there is a fundamental difference between the effective charge measured from light scattering (z_1), which is a thermodynamic charge, and that derived from transport properties such as diffusion (p),[18]

the values of the number of charges from the two techniques should be of similar magnitude. The large discrepancy between the two sets of values presented here may be a consequence of the assumptions inherent in the theoretical treatment of the data. The most probable source of error is that neither of the two expressions available for the evaluation of the repulsion interaction, $V_R(x)$, are strictly applicable to the systems under investigation where $\kappa a \approx 1$. Consequently, of the two estimations of number of charges per micelle, those from static light scattering are considered to be the more reliable. The values of the Hamaker constants were 2.1×10^{-22}, 1.5×10^{-22} and 1.3×10^{-22} J for amitriptyline, doxepin and desipramine, respectively. These values of the Hamaker constant are very similar to those values previously reported for other drugs including the penicillins[19] and the phenothiazine tranquillizers.[20,21] In order to evaluate the unknown parameters appearing in the expression for the interaction potential $V = V_A(x) + V_R(x)$ we have made the simplifying assumption that A and p are both independent of the salt concentration. With regard to A, there are measurements of forces between two surfaces in aqueous electrolyte solutions[22] which clearly show that the attractive London–van der Waals forces are largely independent of the type and concentration of the aqueous electrolyte solution. Nevertheless, the results obtained from the analysis of the dynamic light scattering data serve mainly to provide a qualitative description of the changes in the interaction between the aggregates as the electrolyte concentration is increased, rather than to give precise values of the interaction parameters.

Increase of electrolyte concentration caused a progressive screening of the electrostatic potential and hence increased importance of the London–van der Waals attraction as seen from the changes in the reduced potential $e\Psi_0/k_BT$ and $V(x)/k_BT$ shown in Table 3 and Fig. 3–5.

4. Summary

Static light scattering measurements on four tricyclic antidepressant drugs have shown the influence of the molecular structure of the hydrophobe on the association characteristics in aqueous solution. The presence of a ring N (desipramine) or O (doxepin) decreased the hydrophobicity, the lone pairs of electrons undergoing resonance with the aromatic rings and producing tautomers with zwitterionic character. Comparison of the association characteristics with those of tranquillising drugs based on the tricyclic phenothiazine ring system showed differences in response to added electrolyte. Self-assembly of all four antidepressant drugs was by closed association. Measurements on one (amitriptyline) in the presence of added electrolyte of concentration up to 0.4 mol kg^{-1} showed no conversion to an open association model at high electrolyte concentration as was noted for the phenothiazine drugs. Moreover, there was no evidence of the presence of more than one critical concentration, in contrast to the phenothiazines in water and dilute electrolyte. Such differences in association behavior suggest possible differences in the stacking of molecules in the aggregates which merits further study.

Estimation of the reduced potential at the shear surface by application of the DLVO theory to dynamic light scattering data has shown a progressive screening of the electrostatic potential with increase of electrolyte concentration leading to the eventual instability of the dispersed systems.

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