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Self-Association of Amphiphilic Penicillins in Aqueous Electrolyte Solution: A Light-Scattering and NMR Study

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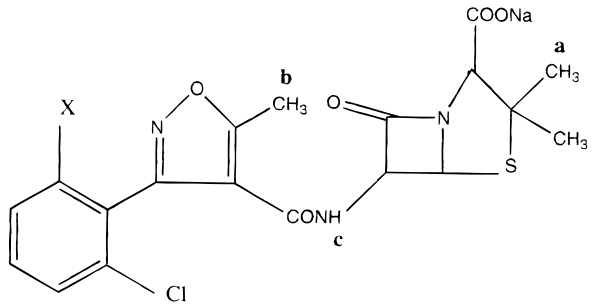
The self-association of the penicillins cloxacillin, dicloxacillin, and flucloxacillin in water and in the presence of added electrolyte (0.025−0.40 mol kg^{-1} NaCl) at 30 °C has been examined by light-scattering and NMR techniques. Inflections in the data from both techniques were observed at a single critical concentration for solutions of cloxacillin and at two critical concentrations for dicloxacillin and flucloxacillin. Aggregation numbers and effective micellar charges were calculated from the static light-scattering data for the stable aggregates formed at the first critical concentration. Application of the valance-generalized light-scattering theory for multicomponent systems to data at concentrations above the second critical concentration provided an estimate of the aggregate size of the associated species present at high solution concentration. The interaction between aggregates was interpreted from diffusion data from dynamic light-scattering using DLVO theory. Micellar properties have been determined by the application of mass action theory to the concentration dependence of ^{1}H NMR chemical shifts, confirming the results obtained by the light-scattering technique.

Introduction

The study of the properties of surface active drugs in solution provides an opportunity to investigate the influence of the molecular structure of the hydrophobe on the association characteristics of amphiphilic molecules.[1,2] The penicillin drugs selected for study form an interesting series of molecules in which the only variation in the molecular structure is the number and nature of the substituents on the aromatic ring of the hydrophobe (see Chart 1). The penicillins under investigation are cloxacillin (X = H), dicloxacillin (X = Cl) and flucloxacillin (X = F). Interest in the colloidal properties of penicillins extends back to the late 1940s and includes studies by McBain and co-workers,[3] Hauser et al.,[4] and Few and Schulman.[5] These early investigations, mainly on penicillin G, were adversely affected by surface active impurities. A more recent study[6] has reported the micellar properties of several synthetic penicillins (including flucloxacillin and cloxacillin) both in water and in the presence of 0.15 M NaCl.

The present study extends this work and considers the influence of electrolyte on the mode of association, the micellar properties, and the intermicellar interactions of the selected series of penicillins using static and dynamic light-scattering techniques and NMR. The measurements were carried out at higher penicillin concentration than previously examined in order to detect any second critical concentration, which is a characteristic feature of some drugs.[2,7,8] To quantify the interaction between the aggregates, the data have been interpreted using the Corti and Degiorgio[9] treatment of diffusion data based on the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory of colloid stability.[10]

Chart 1



Experimental Section

Materials. Sodium cloxacillin monohydrate ([5-methyl-3-(*o*-chlorophenyl)-4-isoxazolyl]penicillin) and sodium dicloxacillin monohydrate ([3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]-penicillin) were obtained from Sigma Chemical Co. Sodium flucloxacillin monohydrate ([3-(2-chloro-6-fluorophenyl)-5-methyl-4-isoxazolyl]penicillin) was a generous gift from Smithkline Beecham Pharmaceuticals. Sodium chloride was of Analar grade. Water was double-distilled, deionized, and deaerated before use.

Light-Scattering Measurements. Static light-scattering measurements were performed at 30 ± 0.1 °C using a Malvern 7027 laser light-scattering instrument equipped with a 2-W argon

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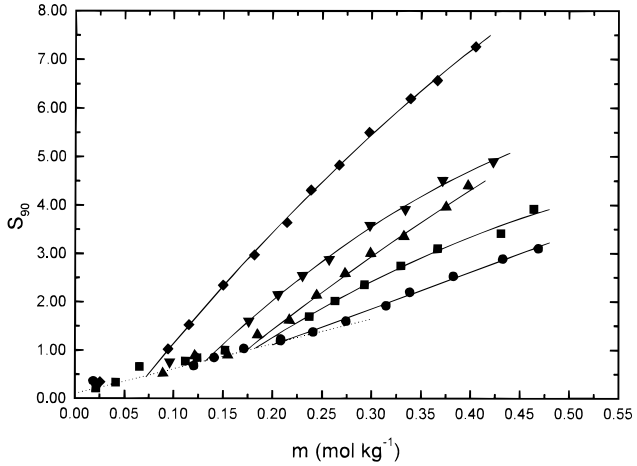

Figure 1. Variation of the scattering ratio S_{90} with molality m for sodium cloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentration (■) 0.05, (▲) 0.1, (▼) 0.2, and (◆) 0.4 mol kg^{-1} at 30 °C. (···) Monomer line.

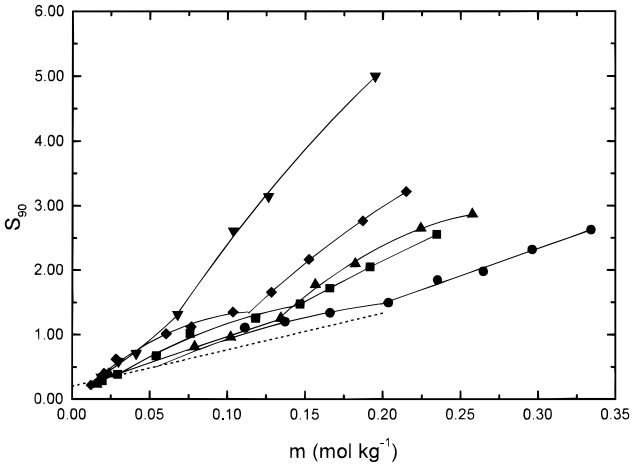

Figure 2. Variation of the scattering ratio S_{90} with molality m for sodium dicloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentrations (■) 0.025, (▲) 0.05, (▼) 0.1, and (◆) 0.2 mol kg^{-1} at 30 °C. (···) Monomer line.

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.

The refractive index increments of the penicillin aggregates were measured at 30 ± 0.1 °C using an Abbé 60/ED precision refractometer (Bellingham and Stanley Ltd.), giving values of 0.080 ± 0.001, 0.083 ± 0.002, and 0.079 ± 0.002 kg mol^{-1} for cloxacillin, dicloxacillin, and flucloxacillin, respectively. Measurements in water and in the most concentrated electrolyte solutions showed no effect of electrolyte on the value of the refractive increment within the limits of error of measurement. Similarly, no inflections in the refractive index data were detectable at the second critical concentration, and consequently the same values of refractive index increments were used for the aggregates present in solutions above and below this concentration. The refractive index increment of NaCl was taken from the literature.[11]

Dynamic Light Scattering. Measurements were made at 30 ± 0.1 °C and a scattering angle of 90° with the Malvern instrument described above combined with a Brookhaven BI 9000AT 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. Hydrodynamic radii were calculated from measured diffusion coefficients by means of the Stokes−Einstein equation.

Nuclear Magnetic Resonance. ^{1}H NMR spectra were recorded on a JEOL EX270 270 MHz spectrometer at 25 ± 1 °C. The chemical shifts of selected peaks were accumulated using a "peak pick" facility. All spectra were compared with sodium 3-(trimethylsilyl)propionate (TSP), which acted as an internal standard.

Results and Discussion

The concentration dependence of the light-scattering ratio S_{90} (intensity of light scattered by the solution relative to that obtained from benzene) shows abrupt discontinuities at well-defined critical concentrations (one in the case of cloxacillin and two for both dicloxacillin and flucloxacillin) at all electrolyte concentrations (Figures 1−3).

The first critical concentrations (cc_1's) were determined from the intersection of the scattering curves and the theoretical line (represented by a dashed line in Figures 1−3) representing ideal scattering from monomers. The second critical concentrations (cc_2's) were taken as the inflection points of the scattering curves at higher

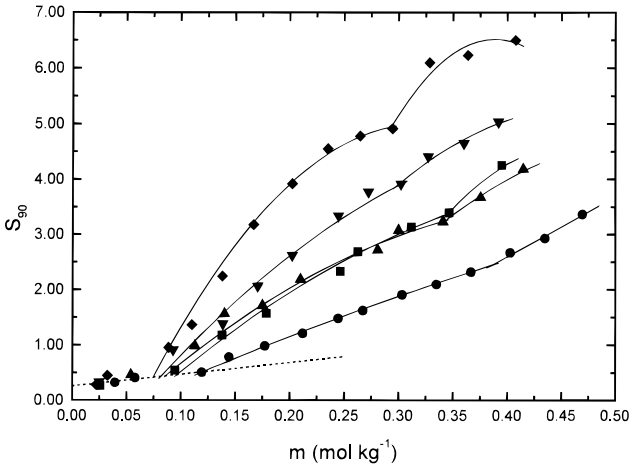

Figure 3. Variation of the scattering ratio S_{90} with molality m for sodium flucloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentrations (■) 0.05, (▲) 0.1, (▼) 0.2, and (◆) 0.4 mol kg^{-1} at 30 °C. (···) Monomer line.

concentrations. A second inflection point has been reported for a wide range of ionic surfactants and has generally been interpreted in terms of a transition from spherical to cylindrical micelles.[1] Other amphiphilic drugs, notably those based on a phenothiazine ring system, also have two or three critical concentrations,[2,7,8] although with such drugs the additional critical concentrations are thought to denote a restructuring of the stacked aggregates rather than a sphere-to-rod transition.

Inspection of the cc_1 values of Table 1 shows an increase of hydrophobicity in the order cloxacillin < flucloxacillin < dicloxacillin; that is, the second Cl substituent in a meta position to the first has a greater effect on the hydrophobicity than a F substituent in this position. It is interesting to note a similar trend with the values of cc_2, the least hydrophobic drug cloxacillin showing no evidence of a second inflection in the concentration range examined. Comparison of the cc_1 values of cloxacillin and flucloxacillin with previously measured values in water[6] shows good agreement in the case of flucloxacillin (lit. value = 0.112 mol kg^{-1}); the poorer agreement of values for cloxacillin (lit value = 0.135 mol kg^{-1}) is possibly a consequence of the difficulty of locating an inflection point in systems of such low aggregation number. The range of concentration over which scattering measurements were previously

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Table 1. First (cc_1) and Second (cc_2) Critical Concentrations, Aggregation Numbers (N_1), and Charges (z_1) Corresponding to the First Critical Concentration, and Global Aggregation Numbers ($N = N_1N_2$) of Sodium Cloxacillin, Dicloxacillin, and Flucloxacillin Monohydrates in Aqueous Electrolyte Solution at 30 °C from Static Light-Scattering Measurements

[NaCl] (mol kg^{-1})	cc_1 (mol kg^{-1})	cc_2 (mol kg^{-1})	N_1	z_1	$N(N_1N_2)$
		Cloxacillin			
0.00	0.20		3		
0.05	0.18		3	0.6	
0.10	0.17		4	1.3	
0.20	0.13		5	1.8	
0.40	0.07		6	2.2	
		Dicloxacillin			
0.000	0.05	0.20	2		4
0.025	0.03	0.14	3	1.0	9
0.050	0.02	0.13	3	2.1	12
0.100	0.02	0.11	5	3.7	20
0.200	0.01	0.06	6	4.9	36
		Flucloxacillin			
0.00	0.12	0.39	2		6
0.05	0.10	0.35	3	0.7	12
0.10	0.09	0.34	4	1.9	16
0.20	0.08	0.30	6	2.8	30
0.40	0.08	0.29	8	4.2	80

made for flucloxacillin was more restricted than that in the present study and did not extend to the cc_2 value.

The micellar aggregation number N_1 and the effective micellar charge z_1 corresponding to the first critical concentration (Table 1) were calculated according to the Anacker and Westwell[12] treatment, in which the light scattering from solutions of ionic aggregates is represented by

$$\frac{K'm_2}{\Delta R_{90}} = \frac{2m_3 + N_1^{-1}(z_1 + z_1^2)m_2}{[2N_1 + (2N_1)^{-1}(z_1 + z_1^2)f^2 - 2fz_1]m_3 + z_1m_2} \quad (1)$$

where ΔR_{90} is the Rayleigh ratio of the solution in excess of that of a solution at the critical concentration, m_2 is the molality of the micellar species in terms of monomer, m_3 is the molality of the supporting electrolyte, and $f = (\mathrm{d}n/\mathrm{d}m_3)_{m_2}/(\mathrm{d}n/\mathrm{d}m_2)_{m_3}$. K' for vertically polarized incident light is defined by

$$K' = 4\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}m_2)_{m_3}^2 V^\circ/L\lambda^4 \quad (2)$$

with n_0 being the refractive index of the solvent, V° the volume of solution containing 1 kg of water, L Avogadro's number, and λ the wavelength of the incident light (488 nm). Expansion of eq 1 in powers of m_2 leads to

$$\frac{K'm_2}{\Delta R_{90}} = A + Bm_2 + \ldots \quad (3)$$

where

$$A = 4N_1[(2N_1 - fz_1)^2 + z_1f^2]^{-1} \quad (4)$$

and

$$B = z_1A(2m_3)^{-1}[(1 + z_1)N_1^{-1} - A] \quad (5)$$

Table 1 shows very limited association of these drugs, values in water for cloxacillin and flucloxacillin being of similar magnitude to those reported previously.[6]

Estimation of the size of the aggregates formed at the second critical concentration is more speculative. We assume that these aggregates are formed by the single-step association of the aggregates present at the first critical concentration and are in equilibrium with these primary aggregates. Following Anacker and Jacobs,[13] we have applied the general fluctuation theory of light scattering by multicomponent systems[14] in the presence of added salt to obtain an approximate value for the size of the aggregates at cc_2. The excess turbidity due to a micellar component of a three-component system with regard to the solvent is given by

$$\tau = \frac{C_3 + C_4m_2'}{C_1 + C_2m_2'}(Hn_2'^2V^\circ m_2') \quad (6)$$

where $H = 16\pi K'/3$ and m_2' is the molality of the micellar species at concentrations above cc_2,

$$n_2' = (\partial n/\partial m_2')_{T,P,w_1,w_3},\ C_1 = \theta(\theta + \phi)m_3,\ C_2 = z_2(z_2 + 1)\phi m_2'/N_2,\ C_3 = [\theta(\theta + \phi)N_2 + z_2(z_2 + 1)(f_1^2/N_2)(\theta/(\theta + \phi)) - 2f_1z_2\theta]m_3,\ C_4 = z_2\theta$$

T is the absolute temperature, P is the pressure, w_1 is the number of water molecules, w_2 is the number of micelles, $(zw_2 + \theta w_3)$ is the number of counterions, θw_3 is the number of surfactant ions and co-ions, $f_1 = N_2n_3/n_2$, N_2 is the aggregation number, and z_2 is the charge of the aggregate. Expression 6 can be expanded in powers of m_2', leading to

$$\frac{Hn_2'^2V^\circ m_2'}{\tau} = A + Bm_2' + \ldots \quad (7)$$

where

$$A = \frac{C_1}{C_3} = \frac{\theta(\theta + \phi)^2N_2}{\theta(\theta + \phi)^2N^2 + \theta(z_2 + z_2^2)f_1^2 - 2\theta(\theta + \phi)z_2f_1N_2} \quad (8)$$

$$B = \frac{(C_2 - C_4A)A}{C_1} = \frac{\phi A(z_2 + z_2^2 - z_2AN_2)}{\theta(\theta + \phi)m_3N_2} \quad (9)$$

A and B can be determined experimentally as the intercept and limiting slope, respectively, of the $Hn_2'^2V^\circ m_2'/\tau$ versus m_2' plot. By solving eqs 8 and 9 simultaneously, the following expressions for z_2 and N_2 are obtained:

$$z_2 = \frac{[\theta\phi(\theta + \phi)^3m_3B]^{1/2} + \theta(\theta + \phi)f_1m_3B}{(\theta + \phi - f_1A)\phi A} \quad (10)$$

$$N_2 = \frac{(z_2 + z_2^2)\phi A}{\theta(\theta + \phi)m_3B + z_2\phi A^2} \quad (11)$$

Table 1 shows the values obtained for the global aggregation numbers ($N = N_1N_2$) for dicloxacillin and flucloxacillin, respectively. The global aggregation numbers increase with electrolyte concentration, the increase

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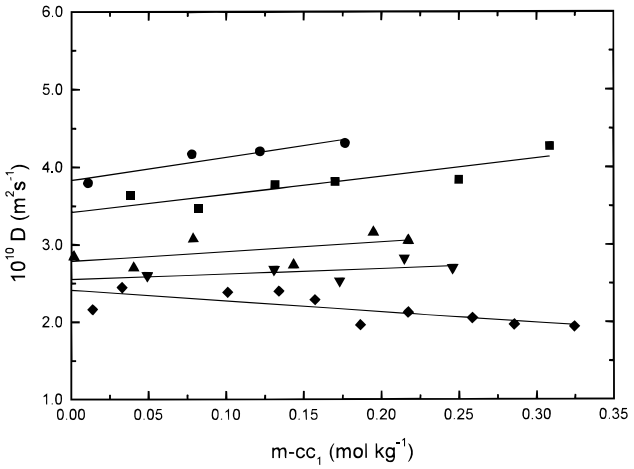

Figure 4. Diffusion coefficient D as a function of the micellar concentration for sodium cloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentration (■) 0.05, (▲) 0.1, (▼) 0.2, and (◆) 0.4 mol kg^{-1} at 30 °C.

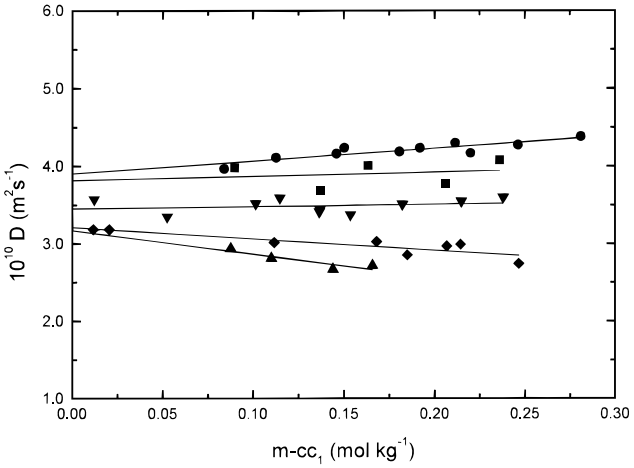

Figure 5. Diffusion coefficient D as a function of the micellar concentration for sodium dicloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentration (■) 0.025, (▼) 0.05, (◆) 0.1, and (▲) 0.2 mol kg^{-1} at 30 °C.

being a consequence of increases of both N_1 and N_2 with increase in added electrolyte concentration.

The correlation functions from dynamic light scattering were single exponentials (even at concentrations above cc_2) and were analyzed by the cumulants method. Polydispersity indices generated by this analytical method were less than 0.1 indicative of a reasonable degree of monodispersity of sizes. Apparent diffusion coefficients D are plotted as a function of micellar concentration ($m - cc_1$, where m = molality of the solution) in Figures 4−6. Although the concentration range of the measurements exceeded the second critical concentration, no significant inflection in the data could be detected at cc_2. This lack of sensitivity of the diffusion measurements to the changes in aggregate structure at the critical concentration is surprising in view of the significant changes noted in the static light-scattering data. It should be noted that the apparent diffusion coefficients measured at such high concentrations are influenced by interparticle interactions, changes of which may counteract changes arising from the presence of larger aggregates. The contribution of monomers to the effective value of D in the vicinity of the first critical concentration may cause considerable curvature of the data.[15] For this reason, measurements of D were restricted to a concentration region in which D was a linear function of molality. Extrapolation of the data to

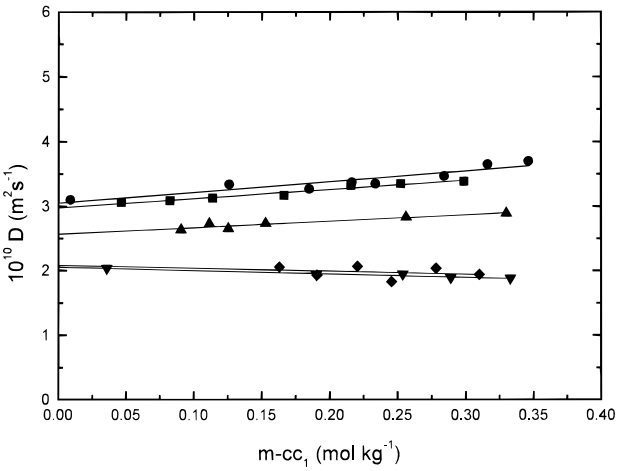

Figure 6. Diffusion coefficient D as a function of the micellar concentration for sodium flucloxacillin monohydrate in water (●) and in aqueous NaCl solutions of concentration (■) 0.05, (▲) 0.1, (◆) 0.2, and (▼) 0.4 mol kg^{-1} at 30 °C.

Table 2. Limiting Diffusion Coefficients D_0, and Hydrodynamic Radii r_h, of Sodium Cloxacillin, Dicloxacillin, and Flucloxacillin Monohydrates in Aqueous Electrolyte Solution at 30 °C

[NaCl] (mol kg^{-1})	$10^{10}D_0$ (m^2 s^{-1})	r_h (nm)
	Cloxacillin	
0.00	3.83	2.3
0.05	3.42	2.6
0.10	2.79	3.1
0.20	2.55	3.4
0.40	2.41	3.6
	Dicloxacillin	
0.000	3.91	2.2
0.025	3.82	2.3
0.050	3.42	2.6
0.100	3.21	2.7
0.200	3.17	2.8
	Flucloxacillin	
0.00	3.05	2.9
0.05	2.98	2.9
0.10	2.57	3.4
0.20	2.08	4.2
0.40	2.05	4.3

cc_1 yielded the limiting diffusion coefficient D_0 of the aggregates formed at this critical concentration. Hydrodynamic radii r_h, derived assuming sphericity of the aggregates from D_0, using the Stokes−Einstein equation,

$$r_h = \frac{k_B T}{6\pi\eta D_0} \tag{12}$$

where k_B is the Boltzmann constant and η the solvent viscosity, are given in Table 2 and show the expected increase of aggregate size with increase of the electrolyte concentration.

To relate the changes of the gradient of the diffusion−concentration plots to changes in the interactive forces between aggregates, the data were analyzed according to the Corti and Degiorgio treatment.[9] For interacting particles, the concentration dependence of D may be described as

$$D = D_0[1 + k_D(m - cc_1)] \tag{13}$$

(15) Attwood, D.; Fletcher, P. *J. Colloid Interface Sci.* **1987**, *115*, 104.

Expressed in terms of the volume fraction ϕ of the particles

$$D = D_0(1 + k'_D\phi) \tag{14}$$

where $k'_D = k_D/\bar{\nu}$ and $\bar{\nu}$ is the apparent molar 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 the expression proposed by Felderhof[16]

$$k_D = 1.56 + \int_0^\infty [24(1 + x)^2 - F(x)][1 - \exp(-V(x)/k_BT)]dx \tag{15}$$

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

$$F(x) = 12(1 + x) - \frac{15}{8}(1 + x)^{-2} + \frac{27}{64}(1 + x)^{-4} + \frac{75}{64}(1 + x)^{-5} \tag{16}$$

The interaction potential $V(x)$ as it is usually written in DLVO theory[10] is the sum of an attractive London−van der Waals interaction $V_A(x)$ and a repulsive force $V_R(x)$. The expression for $V_A(x)$ derived by Hamaker[17] for the case of two spheres is

$$V_A(x) = -\frac{A}{12}\left[(x + 2x)^{-1} + (x^2 + 2x + 1)^{-1} + \frac{2\ln(x^2 + 2x)}{(x^2 + 2x + 1)}\right] \tag{17}$$

where A is the 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

$$V_R(x) = \frac{\epsilon a\psi_0^2}{2}\ln[1 + \exp(-2\kappa a x)] \tag{18}$$

which is appropiate for the aggregates investigated here. In eq 18 ψ_0 is the surface potential and κ is the Debye−Hückel reciprocal length parameter, expressed by the equation

$$\kappa^2 = \frac{8\pi c_s e^2 z^2}{\epsilon k_B T} \tag{19}$$

where ϵ is the relative dielectric constant of the suspending medium, z is the valence of the ionic species in solution, c_s the concentration of these species, and e is the electronic 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 electrolyte concentration for each penicillin. This procedure assumes that both parameters are independent of NaCl concentration. Agreement between computed and experimental values of k_D (Table 3) is similar to that reported by other workers[9] and is reasonable in view of the assumptions inherent in these calculations.

Table 3. Experimental and Theoretical Slopes k_D, and Reduced Potential at Shear Surface $e\psi_0/k_BT$ of Sodium Cloxacillin, Dicloxacillin, and Flucloxacillin Monohydrates at 30 °C as a Function of Electrolyte Concentration

[NaCl] (mol kg^{-1})	k_D exp	k_D theor	$e\psi_0/k_BT$
	Cloxacillin		
0.05	2.5	2.7	1.22
0.10	1.6	0.3	0.55
0.20	1.0	0.0	0.32
0.40	−2.10	−0.1	0.20
	Dicloxacillin		
0.025	0.5	0.8	0.69
0.050	0.3	−1.2	0.39
0.100	−1.7	−1.5	0.24
0.200	−3.5	−1.6	0.17
	Flucloxacillin		
0.05	1.8	2.0	1.16
0.10	1.4	0.1	0.59
0.20	−0.8	−0.3	0.27
0.40	−0.9	−0.4	0.19

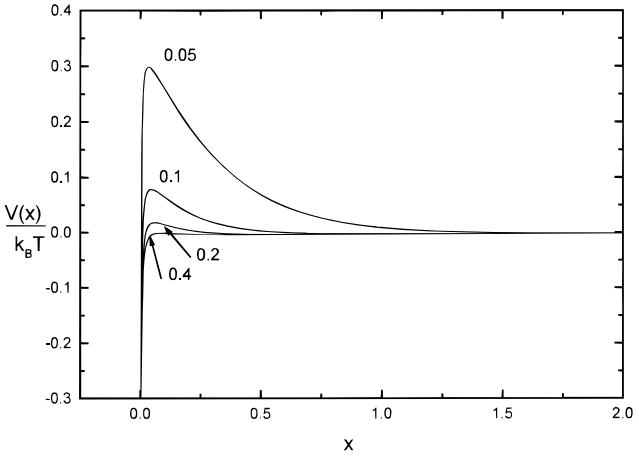

Figure 7. Pair-interaction potential $V(x)$ for sodium cloxacillin monohydrate at the electrolyte concentrations (mol kg^{-1}) indicated. Values of the parameters are given in the text.

The micellar charge q is related to the surface potential, ψ_0, by the expression[18]

$$\psi_0 = \frac{2k_BT}{e}\sinh^{-1}\left(\frac{2\pi\epsilon\kappa^{-1}qe}{4\pi a^2\epsilon k_BT}\right) \tag{20}$$

The values of q derived from eq 20 were 1.2, 0.4, and 1.5 units of electron charge for cloxacillin, dicloxacillin, and flucloxacillin, respectively, and the corresponding Hamaker constants were 1.2×10^{-22}, 2.2×10^{-22}, and 1.4×10^{-22} J. The similarity of these values is in agreement with the small differences in the aggregation numbers at cc_1 of Table 1. In Table 3 we can see the reduced potential, $e\psi_0/k_BT$, as determined from eq 20, and in Figures 7−9 we can see the effects of electrolyte on the potential $V(x)/k_BT$ for cloxacillin, dicloxacillin, and flucloxacillin, respectively. It is clear from these figures that at low electrolyte concentrations the electrostatic repulsion is predominant. The electrostatic potential becomes progressively more screened with increase of electrolyte concentration, and the London−van der Waals attraction becomes more important, resulting in precipitation of drug at high electrolyte concentration. In the case of dicloxacillin, such

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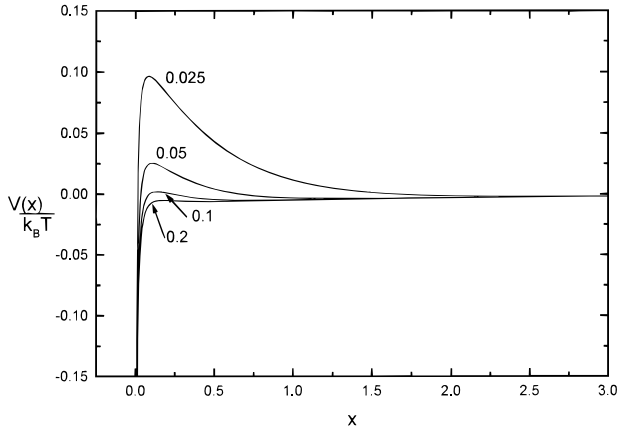

Figure 8. Pair-interaction potential $V(x)$ for sodium dicloxacillin monohydrate at the electrolyte concentrations (mol kg^{-1}) indicated. Values of the parameters are given in the text.

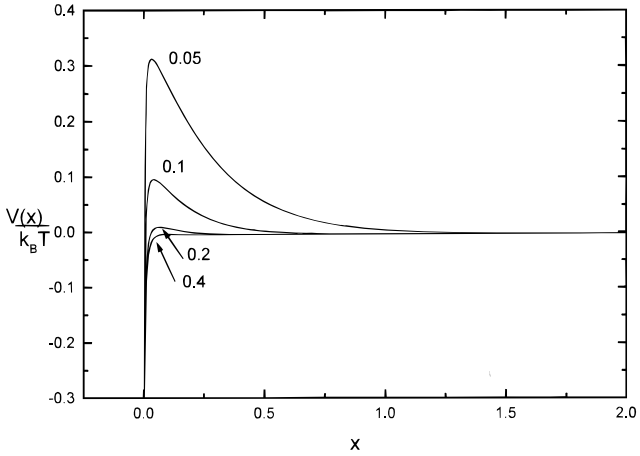

Figure 9. Pair-interaction potential $V(x)$ for sodium flucloxacillin monohydrate at the electrolyte concentrations (mol kg^{-1}) indicated. Values of the parameters are given in the text.

precipitation occurs in the presence of lower electrolyte concentrations (>0.2 mol kg^{-1}) than those with the other two drugs (>0.4 mol kg^{-1}), in agreement with the higher Hamaker constant for dicloxacillin.

The aggregation behavior of the penicillins was investigated by high-resolution ^{1}H NMR spectroscopy. Figure 10 shows the chemical shift of the protons of the aromatic ring as a function of reciprocal concentration. Curves for all three penicillins show upfield shifts on increase of concentration above the first critical concentration. Values obtained for cc_1 from the intersection of the linear portions of the plots of Figure 10 at concentrations well above and below the inflection region (see Table 4) are lower than equivalent values from light scattering for all three penicillin drugs but are in the same rank order. In view of the error involved in the determination of the inflection points from the NMR data due to curvature at cc_1, greater reliance should be placed on the values from light scattering. An interesting feature of the curves for dicloxacillin and flucloxacillin is the change of shift from upfield to downfield with concentration increase in the region of the second critical concentration. No such changes were observed for cloxacillin for concentrations up to 0.38 mol kg^{-1}.

The direction of the chemical shift provides information about the changes in the environment of the aromatic protons during the two association stages. For surfactants containing phenyl rings, the large changes in proton shifts upon micellization are generally attributed to an inter-

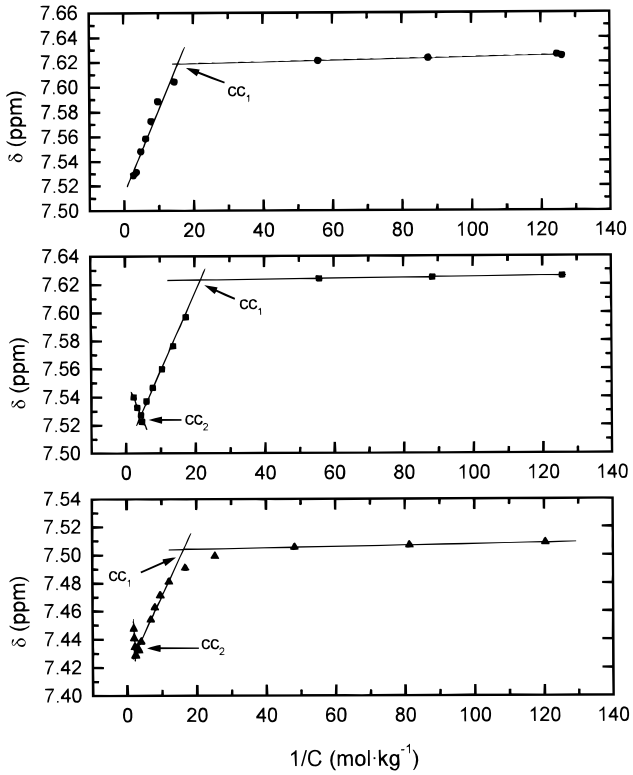

Figure 10. Variation of the ^{1}H chemical shift of the aromatic protons of sodium cloxacillin monohydrate (top), sodium dicloxacillin monohydrate (middle), and sodium flucloxacillin monohydrate (bottom), as a function of the reciprocal concentration.

Table 4. Aggregation Properties of Sodium Cloxacillin, Dicloxacillin, and Flucloxacillin Monohydrates in Aqueous Solution at 25 °C from Nuclear Magnetic Resonance Measurement

compound	cc_1 (mol kg^{-1})	N_1	cc_2
cloxacillin	0.06	3	
dicloxacillin	0.05	2	0.24
flucloxacillin	0.06	4	0.44

molecular aromatic ring current effect.[19,20] An upfield shift of aromatic protons as the concentration is increased above the cmc has been noted, for example, for the micellization of ω-phenylalkyltrimethylammonium bromides[21,22] and sodium ω-phenyldecanoate.[23] Similar shifts are also characteristic of surfactants which exhibit a stacking mode of association and have been reported in studies of the association of nucleotides,[24] dyes,[25,26] and phenothiazine drugs.[27] The change to a downfield shift at cc_2 suggests a decrease of packing density in the larger aggregates. It is interesting to note that a change of the direction of the shift has also been observed at the transition from globular to cylindrical micelles for nonylammonium bromide.[28]

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Measurement of the chemical shifts of protons at positions a, b, and c (see Chart 1) of the three penicillins shows clear upfield shifts at cc_1 (but of lower magnitude than the corresponding shifts of the aromatic protons) in the case of cloxacillin and dicloxacillin. For flucloxacillin, a small downfield shift of all three protons was observed at cc_1, suggesting the possibility of a different aggregate structure for this penicillin.

If we assume that the mass action law may be applied to the formation of the aggregates at cc_1, then the chemical shift accompanying aggregation may be written as[28]

$$\delta_{\mathrm{obsd}} = \frac{C_{\mathrm{m}}}{C_{\mathrm{t}}}\delta_{\mathrm{m}} \tag{21}$$

where C_{m} and C_{t} are the concentration of micellized amphiphile and the total amphiphile concentration, respectively. δ_{obsd} is the observed chemical shift, and δ_{m} is the shift of micellized amphiphile both taken relative to the chemical shift of the monomer as determined from measurements at high dilution. From the mass action equation we may express the concentration of monomer as

$$[A] = C_{\mathrm{t}}\frac{\delta_{\mathrm{m}} - \delta_{\mathrm{obsd}}}{\delta_{\mathrm{m}}} \tag{22}$$

and the aggregate concentration as

$$N_1[A_n] = C_{\mathrm{t}}\frac{\delta_{\mathrm{obsd}}}{\delta_{\mathrm{m}}} \tag{23}$$

The expression for the equilibrium constant K may be rewritten as

$$\ln(C_{\mathrm{t}}\delta_{\mathrm{obsd}}) = N_1 \ln[C_{\mathrm{t}}(\delta_{\mathrm{m}} - \delta_{\mathrm{obsd}})] + \ln K + \ln N_1 - (N_1 - 1)\ln \delta_{\mathrm{m}} \tag{24}$$

Plots of $\ln(C_{\mathrm{t}}\delta_{\mathrm{obsd}})$ against $\ln[C_{\mathrm{t}}(\delta_{\mathrm{m}} - \delta_{\mathrm{obsd}})]$ may in principle give the aggregation number N_1 and the equilibrium constant. Figure 11 shows chemical shift data plotted according to eq 24 for solutions of cloxacillin. Similar plots were obtained for the other penicillins. In general, aggregation numbers obtained from NMR shifts tend to be lower than those obtained using other techniques,[29,30] but Table 4 shows reasonable agreement with aggregation numbers from light scattering for the very small aggregates of this study.

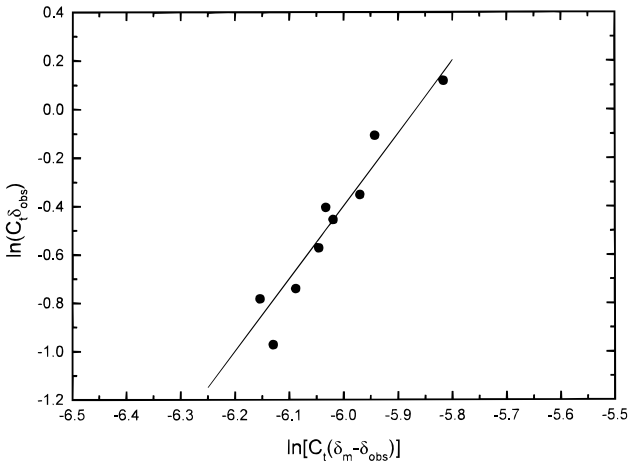

Figure 11. NMR chemical shift data plotted according to eq 24 for sodium cloxacillin monohydrate in aqueous solution.

Summary

This study of the aggregation of three structurally related penicillins in water and aqueous electrolyte has shown the influence of different substituents on the phenyl ring on the micellar properties. Using the critical concentration for aggregation as an indicator of hydrophobicity, it is clear that the introduction of a second Cl substituent meta to the first in this ring increases the hydrophobicity to a greater extent than a F substituent in this position. Aggregation numbers are low in solutions of all three penicillins and, although increasing with added electrolyte concentration, do not exceed 8 in 0.4 mol kg^{-1} NaCl. Despite this, clear inflections were observed at critical concentrations in both static light-scattering and NMR data, and application of mass action theory in the interpretation of data from both techniques has yielded aggregation numbers in reasonable agreement. There is evidence from both techniques of a second critical concentration in solutions of dicloxacillin and flucloxacillin, the NMR technique suggesting a structural rearrangement of the aggregate and light scattering showing an increase of aggregate size at this concentration. Application of DLVO theory in an analysis of the concentration dependence of the apparent diffusion coefficient in electrolyte solutions of increasing molality has quantified the intermicellar interactions in solutions of these penicillins.

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