Surface and Bulk Properties of Two Anionic Amphiphilic Penicillins in a Selective Solvent

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The surface physicochemical properties of two anionic penicillins—cloxacillin and dicloxacillin—in mixed ethanol−water solvent were investigated by surface tension and dynamic light scattering (DLS). The data were analyzed according to the treatment of the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory to study the stability of the systems. The aim of the study is to obtain information about the effects of ethanol on the surface activity, bulk properties, and aggregate stability of these amphiphilic drugs, keeping in mind that both penicillins have the same counterion, and the difference in their structures is only a Cl atom in the phenyl ring that makes dicloxacillin more hydrophobic. The surface tension data show a minimum area per molecule increment with ethanol concentration that is related to the variation of the dielectric constant with the alcohol. Dicloxacillin has lower values of the standard Gibbs energies of adsorption than does cloxacillin, which gives this drug a more marked escaping tendency from the aqueous environment to the air−water monolayer. DLS data was fitted to an exponential function for cloxacillin at any drug or alcohol concentration in the range of concentrations studied that indicates that the system can be modeled as an ergodic system of dilute diffusing monodisperse particles. Dicloxacillin DLS data at an ethanol concentration of 5% (v/v) had to be fitted at a sum of an exponential and a stretched exponential function, which indicates that, besides the drug aggregates, a small population of penicillin clusters with longer relaxation times is present. The stability curves predicted by the DLVO theory, for both penicillins, indicate the predominance of electrostatic repulsion, leading to a stable system over the drug−ethanol concentration range studied, but the height of the reduced pair interaction potential energy barrier decreases with ethanol concentration, thus it is expected to undergo a transition from a stable dispersion to a coagulated one.

1. Introduction

The effect of short and medium chain-length alcohols on the properties of classical surfactants has been extensively studied with the objective of determining the role of these alcohols as cosurfactants in microemulsion systems.[1−3] The alcohols are partitioned between the micellar and aqueous phases[4−7] and are often present in amphiphilic drug delivery formulations since they affect the dielectric constant of the aqueous medium, the interionic attraction, and the solute−solvent interaction.[8] The immobilization of the amphiphilic drug on a biodegradable polymer carrier is one of the effective ways of transport. The addition of alcohol to the aqueous drug solution can modify adsorption provided that polymer swelling and the solvation of ionic groups are altered in the water/alcohol solution. One of the key factors of drug immobilization on a biodegradable polymer is the achievement of a sufficient therapeutic concentration of drug in the polymer phase, and drug adsorption from a water/alcohol solution can increase drug uptake. This requires profound investigation of the characterization of drug behavior in water/alcohol solutions and elucidation of factors affecting the adsorption. Recently, Zimnitsky et al.[9] showed that the adsorption process of some β-lactam antibiotics on samples of oxidized cellulose from water/ethanol solutions increases with an increase in the alcohol mole fraction. The effects of alcohol on the aggregation behavior of amphiphiles with a hydrophobic aromatic ring have not been investigated in detail.

The study of the properties of surface-active drugs in an aqueous solution provides an opportunity to investigate the influence of the molecular structure of the hydrophobe on the association characteristics of the amphiphilic molecules.[8,10−14] Cloxacillin and dicloxacillin are two β-lactam amphiphilic penicillins that can be considered hydrotropes or hydrotropic agents if we considered that the term comprises hydrophilic and hydrophobic moieties that form aggregates by a stacking mechanism of the planar aromatic ring present in their chemical structure, as is the case for both penicillins.[15] These two molecules are structurally similar, differing only in an additional chlorine atom on the phenyl ring of dicloxacillin (see Figure 1). They are anionic amphiphiles with pK_a's of 2.7 and 2.8, respectively, and are fully ionized at the pH of the present study. There is evidence from static light scattering and NMR studies that the two penicillins exhibit a stacking model of association and have a second critical concentration in aqueous solution.[10,16] Since the penicillins ionize in an aqueous−ethanol medium, variation of the dielectric constant effects the stability of the penicillin aggregates.

The aim of this study was to obtain information about the surface and bulk properties of the two ionic amphiphilic penicillins cloxacillin and dicloxacillin in aqueous solution and in the presence of ethanol. As it has been seen before, both penicillins have the same counterion, so their different behavior

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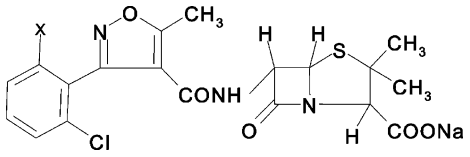

Figure 1. Structure of cloxacillin (X = H) and dicloxacillin (X = Cl) penicillins.

in solution will be due to the molecular structure differences. Surface tension and dynamic light scattering (DLS) techniques were used. This study is complementary to a recent thermodynamic study that was carried out for the same systems,[17] where the partition coefficients were determined using a proposed indirect method based on the pseudophase model and with the help of apparent molar data. The partition coefficients showed that dicloxacillin, the more hydrophobic of the two penicillins, solubilizes a larger quantity of ethanol. The dependence of the apparent molar volume of both penicillins on concentration was that of typical surfactants, being that the critical concentrations decreased because of a reduction in the headgroup repulsion due to the presence of the alcohol.

2. Experimental Section

2.1. 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) with molecular masses of 475.9 and 510.3 g mol^{-1}, respectively, were obtained from Sigma Chemical Co. and were well characterized by surface tension to be used as received. Ethanol (Sigma) was of AnalaR grade and stored over molecular sieves. The alcohol was treated as a cosolvent in the usual way; that is, only the amphiphile concentration was progressively changed. Solutions were made up by weight at room temperature, using a METTLER AT20 balance with a precision of 0.001 mg, with water treated with a Millipore Q system. The experiments were carried out at 25 °C.

2.2. Surface Tension. The surface tensions (γ) of dilute aqueous ethanol solutions of cloxacillin and dicloxacillin penicillins were measured by the Whilhelmy plate method on a Krüss K-12 instrument equipped with a processor to acquire the data automatically. The instrument was connected to a circulating bath with a proportional temperature controller to keep the temperature constant to ±0.1 °C. Techniques were followed to ensure that the plate and glassware used in the measurements and preparation of the solutions were scrupulously clean. The plate was cleaned by washing with doubly distilled water followed by heating in an alcohol flame. Penicillin solutions of known molality were progressively diluted in ethanol/water solutions using an automatic pump (Dosimat 665 Metrhom). Ethanol concentrations were kept constant at 2, 7.5, 10, 12.5, 14, and 15% (v/v). Triply distilled water of low conductivity was used to prepare the solutions. Equilibrium was considered to be obtained when successive values taken at 5 min intervals agreed within ±0.1 mN m^{-1}. It is well-known that critical concentrations derived from surface tension techniques are particularly sensitive to traces of impurities.[18] The accuracy of measurement was checked by frequent determination of the surface tension of pure water, and there was no evidence of minima in the region of the critical concentrations that generally arise when surface active impurities are present.

2.3. Light Scattering. All glassware was washed with condensing acetone vapor before use. Ethanol concentrations were kept constant at 0.15, 0.5, 2, and 5% (v/v), and solutions were clarified by filtering through Millipore Millex filters (Triton free, 0.22 or 0.10 μm porosity) directly into the cleaned scattering cell. DLS intensity was measured for solutions at temperature of 25.0 ± 0.1 °C by means of an ALV-5000F instrument with a vertically polarized incident light of wavelength $\lambda = 532$ nm supplied by a CW diode-pumped Nd:YAG solid-state laser (Coherent Inc.) operated at 400 mW. The intensity scale was calibrated against scattering from toluene. Measurements were made at a scattering angle of $\theta = 90°$ to the incident beam, but some measurements were made at other angles to ensure that the angular dependence of intensity was unimportant. DLS correlation data were analyzed by the constraint regularized CONTIN method to obtain distributions of decay rates.[19] Solutions were equilibrated at 25 °C for 30 min before a measurement was made. Experiment duration was in the range 3−5 min, and each experiment was repeated two or more times.

Viscosities were measured with an Anton Paar automated microviscometer (AMVn) based on the "rolling ball" principle. The samples of solvent were introduced into a glass capillary in which the steel ball rolls and the viscous properties of the text fluid can be determined by measuring the rolling time of the steel ball. The temperature was maintained by the Peltier effect with a resolution of ±0.01 °C. The viscosities for the solutions of water and ethanol at concentrations of 0, 0.15, 0.5, 2, and 5% v/v were 0.8904, 0.8946, 0.9072, 0.9499, and 1.0448 cp, respectively.

3. Theory and Data Evaluation

3.1. Surface Tension. On the basis of a plot of the surface tension, γ, as a function of the equilibrium concentration of the penicillins in water/ethanol, the amount of amphiphilic drug adsorbed at the air−water interface, Γ_2, can be determined by application of the Gibbs equation: $\Gamma_2 = -1/2.3RTx(d\gamma/d \log m)$, where R is the gas constant, T is the temperature in Kelvin, and the variable x is introduced to allow for the simultaneous adsorption of cations and anions, taking the value of 2 in water, as suggested by Matijevic and Pethica.[20] The minimum area per molecule, A, at the air−water interface was calculated from Γ_2: $A = 1/N_A\Gamma_2$, where N_A is the Avogadro constant.

Values (per mol of monomer) of the standard Gibbs energy change upon aggregation were calculated from the expression[21] $\Delta G^0_m = 2RT \ln x_{cc}$, where x_{cc} is the critical concentration expressed in mole fraction units. The standard Gibbs energy of adsorption, ΔG^0_{ads}, in water for these penicillins was calculated from the standard Gibbs energy and the surface tension data through the equation[21]

$$\Delta G^0_{ads} = \Delta G^0_m - \frac{\pi_{cc}}{\Gamma_2} \tag{1}$$

where π_{cc} is the surface pressure at the critical concentration.

3.2. DLS. In general, the fluctuation of scattered light in a colloidal dispersion is due to local changes in the particle concentration, which gives rise to fluctuations in the refractive index of the scattering medium. Therefore, the scattered electric field time autocorrelation function measured in a DLS experiment is proportional to the time autocorrelation function of the fluctuations in the refractive index:

$$g_1(t) = \langle \delta n(q,0)\delta n(q,t)\rangle \tag{2}$$

where $\delta n(q,t)$ is a Fourier transform of the refractive index fluctuation $\delta n(\vec{r},t)$ at position $\vec{r}$ in time t. The term q is the scattering vector:

$$q = |k_f - k_i| = \frac{4\pi n}{\lambda_0} \operatorname{sen} \frac{\theta}{2} \quad (3)$$

with k_i and k_f being the wave vectors of the initial and final scattered beam, respectively, n being the refractive index of the medium, λ_0 being the light wavelength in the vacuum (532 nm), and θ being the scattering angle (90°).

DLS 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:

$$g^{(2)}(t) = 1 + \beta|g^{(1)}(t)|^2 \quad (4)$$

where β is a nonideality factor ($0 < \beta \leq 1$). The term $g^{(1)}(t)$ can be written as the Laplace transform of the distribution of relaxation rates, $G(\Gamma)$:

$$g^{(1)}(t) = \int_0^\infty G(\Gamma) \exp(-\Gamma t) d\Gamma \quad (5)$$

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

$$g^{(1)}(t) = \int_0^\infty A(\tau) \exp(-t/\tau) d\tau \quad (6)$$

where $\tau A(t) \equiv \Gamma G(\Gamma)$. The total scattering intensity is given as

$$I = \int_0^\infty A(\tau) d\tau \quad (7)$$

The apparent translational diffusion coefficient, D, was calculated from the average relaxation rate, $\bar{\Gamma}$, according to the equation

$$D = \bar{\Gamma}/q^2 \ (q \rightarrow 0) \quad (8)$$

At $\theta = 90°$, the condition $q \rightarrow 0$ is fulfilled because of the small size of the particles in the solution. The diffusion coefficient is affected by the concentration of the scattering particles. For interacting particles, the concentration dependence of D may be described as[22]

$$D = D_0[1 + k_D(c - cc)] \quad (9)$$

where D_0 is the translational diffusion coefficient at zero concentration (free particle diffusion coefficient), c is the concentration of the solution, cc is the critical concentration, and k_D is a constant. D_0 is related to the hydrodynamic radius, r_h, through the Stokes−Einstein equation:

$$r_h = \frac{k_B T}{6\pi\eta D_0} \quad (10)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

3.3. Colloidal Stability. To relate the changes in gradient of the diffusion concentration plots with changes in the interacting forces between aggregates, the data were analyzed according to the treatment proposed by Corti and Degiorgio[23] of the Derjaguin, Landau, Verwey, and Overbeek theory (DLVO theory). For the purpose of data interpretation, the concentration dependence of the apparent diffusion coefficient (eq 9) was expressed in terms of the volume fraction, ϕ, of the particles:

$$D = D_0(1 + k'_D\phi) \quad (11)$$

where $k'_D = k_D/\bar{v}$ and $\bar{v}$ is the specific volume of the solute particles as determined from density measurements. The term k'_D may be related to the pair-interaction potential, $V(x)$, between spherical particles of radius a using the expression proposed by Felderhof:[24]

$$k'_D = 1.56 + \int_0^\infty [24(1+x)^2 - F(x)][1 - \exp(-V(x)/k_B T)] dx \quad (12)$$

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

$$F(x) = 12(1+x) - \frac{15}{8}(1+x)^{-2} + \frac{27}{64}(1+x)^{-4} + \frac{75}{64}(1+x)^{-5} \quad (13)$$

The interaction potential $V(x)$ 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, $V_R(x)$.[25] The expression for $V_A(x)$ derived by Hamaker[26] for the case of two hard spheres is given by the expression

$$V_A = -\frac{A}{12}\left[(x^2+2x)^{-1} + (x^2+2x+1)^{-1} + \frac{2\ln(x^2+2x)}{(x^2+2x+1)}\right] \quad (14)$$

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 $\kappa a > 1$. In our study, the values of κa were in the range 0.55−0.66, so we used the expression[27]

$$V_R(x) = \frac{\epsilon a \Psi_0^{\ 2}}{2} \ln[1 + \exp(-2\kappa a x)] \quad (15)$$

In eq 15, ψ_0 is the potential at the inner limit of the diffuse part of the double layer, and κ is the Debye−Hückel reciprocal length parameter, expressed by the equation $\kappa^2 = 8\pi c_s e^2 z^2/\epsilon k_B T$, where ϵ is the dielectric constant of the suspending medium, z is the valence of the ionic species in solution (1:1 in our case), c_s is the concentration of the same species, and e the proton charge. The charge of the aggregate including the Stern layer, p, is related to the surface potential, ψ_0, by the expression[28]

$$\psi_0 = \frac{2k_B T}{e} \sinh^{-1}\left(\frac{pe^2}{2a^2\kappa\epsilon k_B T}\right) \quad (16)$$

The computational procedure involved the interaction of values of A and p to give the best fit of computed and experimental values of k'_D over the range of electrolyte concentration for each drug. The values of p and A were calculated by solving the following optimization problem: $\min J = \sum (k'_{D(ex)} - k'_{D(th)})^2$, where J is the objective function to be minimized and corresponds to a least-squares sum of the experimental ($k'_{D(ex)}$) and theoretical ($k'_{D(th)}$) values over the different values of concentration considered. The optimized values of p and A are those that provide $k'_{D(th)}$, which makes J minimum. To solve this problem, a MATLAB code was developed, and FMINCON was established for the optimization.

4. Results and Discussion

Surface tension and DLS techniques have been applied to study the surface and bulk properties of two amphiphilic ionic

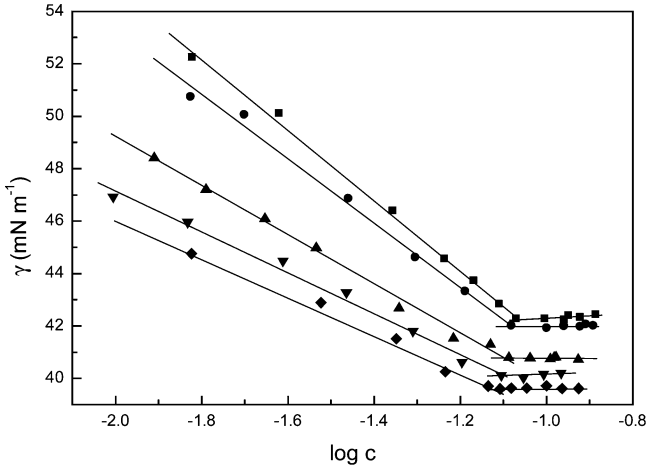

Figure 2. Surface tension, γ, as a function of the logarithm of concentration, c (mol dm^{-3}), for aqueous solutions of cloxacillin containing (■) 2, (●) 7.5, (▲) 10, (▼) 12.5, and (◆) 15% added ethanol at 25 °C.

TABLE 1: Interfacial Properties of Cloxacillin and Dicloxacillin Penicillins in Water−Ethanol Solutions

Cloxacillin			
ethanol % (v/v)	*cc* (mol dm^{-3})	*A* (nm^2/molecule)	ΔG^0_{ads} (kJ mol^{-1})
2	0.085	1.41	−37.78
7.5	0.081	1.56	−37.60
10	0.080	1.78	−38.10
12.5	0.079	2.44	−38.55
15	0.070	2.94	−38.71
Dicloxacillin			
ethanol % (v/v)	*cc* (mol dm^{-3})	*A* (nm^2)	ΔG^0_{ads} (kJ mol^{-1})
2	0.066	1.53	−39.31
7.5	0.062	2.28	−39.25
10	0.051	2.52	−39.78
12.5	0.0506	4.32	−40.83
14	0.049	5.52	−40.93
15	0.049	7.60	−41.59

penicillins—cloxacillin and dicloxacillin—in an aqueous-ethanol medium. Experimental data were evaluated using the methods described in the preceding section. This study is complementary to the recent thermodynamic study that was carried out for the same water/ethanol/penicillin system.[17]

Figure 2 shows a representative plot of surface tension, γ, against the logarithm of concentration for cloxacillin in different water−ethanol concentrations, at 25 °C. Similar plots were obtained for dicloxacillin. The critical concentration (*cc*) was determined by the intersection of the two straight lines of the γ-log c curves above and below the *cc*. The values obtained are shown in Table 1. A comparison of the critical concentration values indicates that dicloxacillin is more hydrophobic than cloxacillin for all the ethanol concentrations. The differences in the critical concentrations of both penicillins arise solely from the different substituent in the molecular structure of these drugs. Both penicillins have the same counterions; therefore, the extra Cl atom in the molecular structure of dicloxacillin, when compared with cloxacillin, makes this substance more hydrophobic. The same behavior has been reported on the study of several structurally related antidepressants.[29−32] It can also be observed in Table 1 that lower values of the *cc* are obtained as ethanol increases its concentration. In general, an increase in the alcohol concentration of an aqueous solution makes the solvent more hydrophobic with lower values of the dielectric constant; therefore, in the presence of surfactants, the electrostatic interaction between the ionic groups of ionic surfactants increases and so does the critical micelle concentration (cmc). However, in our case, the opposite effect occurs: the alcohol leads to a diluted surface charge density in the palisade layer of the penicillins that causes a better shielding of the negative headgroups of the monomers and thereby reduces the electrostatic repulsive interaction (as can be observed in the analysis of the aggregate stability; see Figure 6), a process that promotes lower values of the *cc*.

TABLE 2: Hydrodynamic Radius of Cloxacillin and Dicloxacillin Aggregates, r_h, Slope, k_D', Charge, p, and Hamaker Constant, A, in Water−Ethanol Solutions

Cloxacillin				
ethanol/% (v/v)	r_h (nm)	$\sqrt{k_D'}$	p (u.e.)	10^{-22} A (J)
0.15	0.66	−0.17	0.24	1.16
0.5	0.67	−0.28	0.23	0.92
2	0.67	−0.34	0.23	0.91
5	0.64	−0.745	0.23	0.90
Dicloxacillin				
ethanol/% (v/v)	r_h (nm)	$\sqrt{k_D'}$	p (u.e.)	10^{-22} A (J)
0.15	0.68	−0.27	0.29	1.51
0.5	0.69	−0.4	0.29	1.30
2	0.7	−0.55	0.27	0.84
5	0.69	−1.01	0.26	0.74

On the basis of the plot of the surface tension as a function of the equilibrium concentration, changes in the minimum area per molecule, A, of the monomers at the air/water−ethanol interface were evaluated by application of the Gibbs equation (see the preceding section and Table 1). The values of A in the presence of a low ethanol concentration (2% (v/v)) are similar to those reported for cloxacillin and dicloxacillin in water[33] (1.48 and 1.58 nm^2 for cloxacillin and dicloxacillin, respectively). The data show an area increment with ethanol concentration that can be related to two processes: (1) a progressive increase in the alcohol molecules in the surrounding polar groups as part of the drug hydration layer and thus an effective increase in the occupied volume of the polar heads,[17] and (2) the incorporation of the alcohol molecules into the interfacial films. It is also worth mentioning the higher values of the minimum area per molecule of dicloxacillin compared with those of cloxacillin, since similarly charged ionic heads repel the monomers at the interface. The greater distance of dicloxacillin monomers means a higher repulsion between them due to the substitution of a Cl atom by a hydrogen atom in the phenyl group (as can be observed in Figure 1), which gives rise to a higher net electrical charge of dicloxacillin, as demonstrated by the DLVO theory (see Table 2), and causes the molecules of this drug to be more separated at the interface.

The standard Gibbs energies of adsorption, ΔG^0_{ads}, were calculated through eq 1. The values obtained are shown in Table 1. ΔG^0_{ads} is lower for dicloxacillin, which is the most surface-active drug. Therefore, its escaping tendency from the aqueous environment to the air−water monolayer is more marked because of its greater hydrophobicity.

The bulk properties (association) of both penicillins and their aggregate stability in aqueous-ethanol solutions are manifested in dynamic light experiments and data analysis (DLVO theory). Cloxacillin and dicloxacillin were studied at ethanol concentrations of 0.15, 0.5, 2, and 5% (v/v).

In the DLS experiments, the fluctuations of the scattering intensity with time are monitored, yielding a time-dependent intensity autocorrelation function, $g^{(2)}(t)$, where t is the time on the relaxation time scale. An example of the selected intensity

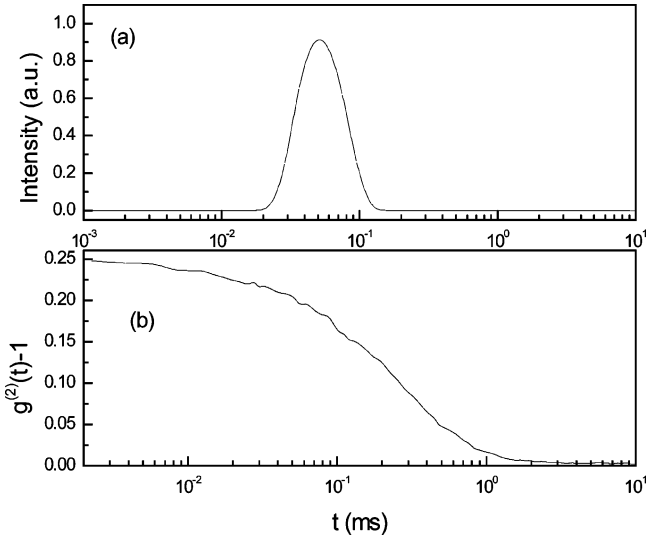

Figure 3. (a) Intensity−relaxation time distribution for cloxacillin in an aqueous concentration of 0.3 mol dm^{-3} and 5% (v/v) ethanol. (b) Normalized intensity autocorrelation function for the same concentrations.

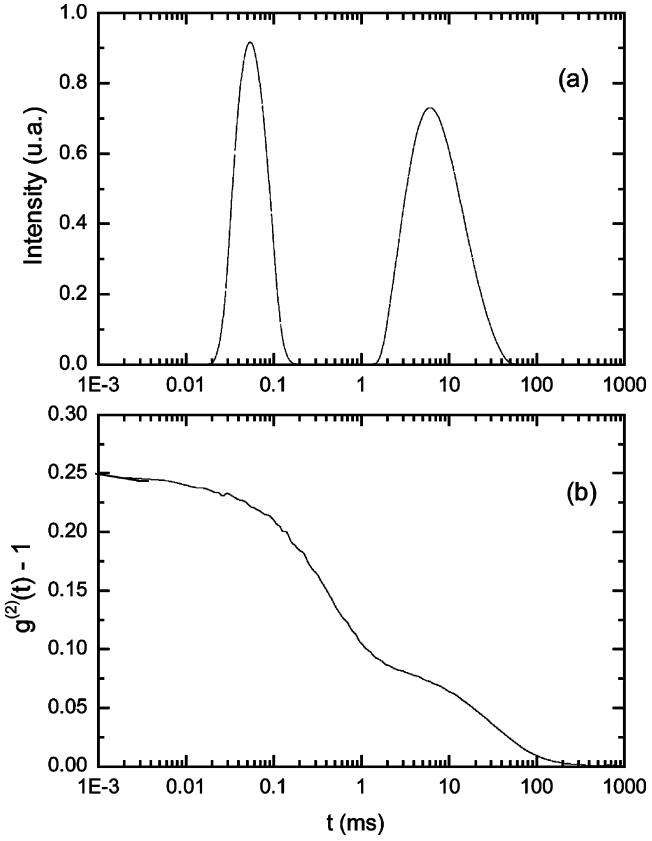

Figure 4. (a) Intensity−relaxation time distribution of dicloxacillin in an aqueous concentration of 0.3 mol dm^{-3} and 5% (v/v) ethanol. (b) Normalized intensity autocorrelation function for the same concentrations.

autocorrelation and intensity−relaxation time of an aqueous concentration of 0.3 mol dm^{-3} cloxacillin and 5% (v/v) ethanol are shown in panels a and b of Figure 3, respectively, in the form of logarithmic plots. Similar plots were obtained for other concentrations of cloxacillin and ethanol. The relaxation time distribution, Figure 3a, shows one single and quite narrow peak that indicates the penicillin aggregate as a diffusional monodisperse species in the solution. Therefore, $g^{(1)}(q,t)$ can be modeled as an ergodic system of dilute diffusing monodisperse

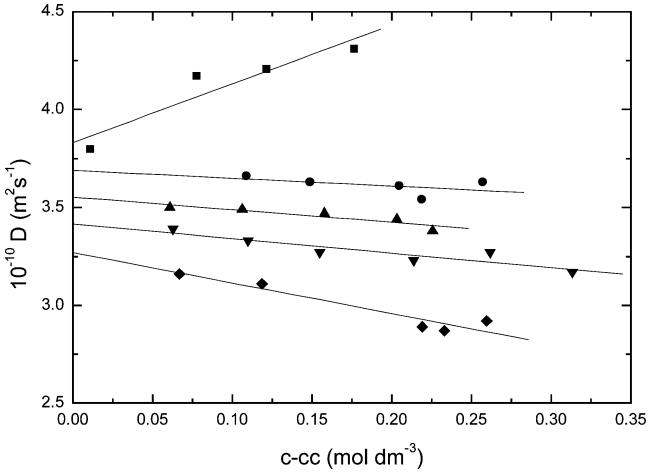

Figure 5. Self-diffusion coefficient as a function of the concentration of aqueous solutions of cloxacillin containing (■) 0, (●) 0.15, (▲) 0.5, (▼) 2, and (◆) 5% (v/v) ethanol at 25 °C.

particles by eq 6. The same results were obtained for the other concentrations of cloxacillin and ethanol. The autocorrelation function (Figure 3b) shows a single-exponential decay. Therefore, the autocorrelation functions were fitted to eq 6. The exponential decay may be assumed to correspond to the cooperative motions of the penicillin aggregate since it has a relaxation frequency similar to that of the cooperative mode in solutions of surfactants.

Figure 4 shows selected intensity−relaxation time distributions and normalized autocorrelation functions for a dicloxacillin concentration of 0.3 mol dm^{-3} and 5% (v/v) ethanol. The intensity−relaxation time distribution (Figure 4a) shows two different relaxation modes, indicating two distinct diffusional species in solution: the slower mode represents the diffusion of the drug aggregates, while the faster one can be related to a small proportion of clusters formed by the random association of drug aggregates. Thus, we have a mixture of monodisperse aggregates and clusters ∼21 nm in size. These two modes appeared in the entire range of dicloxacillin concentrations studied at 5% (v/v) ethanol. Figure 4b shows the normalized intensity autocorrelation function. The autocorrelation function exhibits a bimodal shape that indicates the presence of two different populations in the sample: besides the drug aggregates, a population of larger particles with longer relaxation times is present. From the scattering theory, we learn that the scattering intensity increases with the sixth power of the particle radius, meaning that only a small number of larger particles in the scattering volume could completely hide the whole population of smaller structures.[34] So, it can be said that the population of large particles is much smaller than that of the drug aggregates because the much weaker scattering intensity of the smaller particles would be hidden in the overall signal, which is dominated by the contribution of the larger structures. The CONTIN method was unable to provide an exact "two-peak" solution corresponding to these two different species in solution since the analysis has a tendency to split broad distributions into two or more contributions (overfitting); therefore, the electric field autocorrelation functions were fitted to a sum of stretched exponential functions with exponent β:[35−37]

$$g^{(1)}(t) = A_c \exp(-[t/\tau_c]^{\beta_C}) + A_s \exp(-[t/\tau_s]^{\beta_S}) \quad (17)$$

where the values τ_i ($i = s$ or c) are the characteristic decay times, and A_i values are the relative scattering amplitudes. The exponents β_i are related to the width of the corresponding

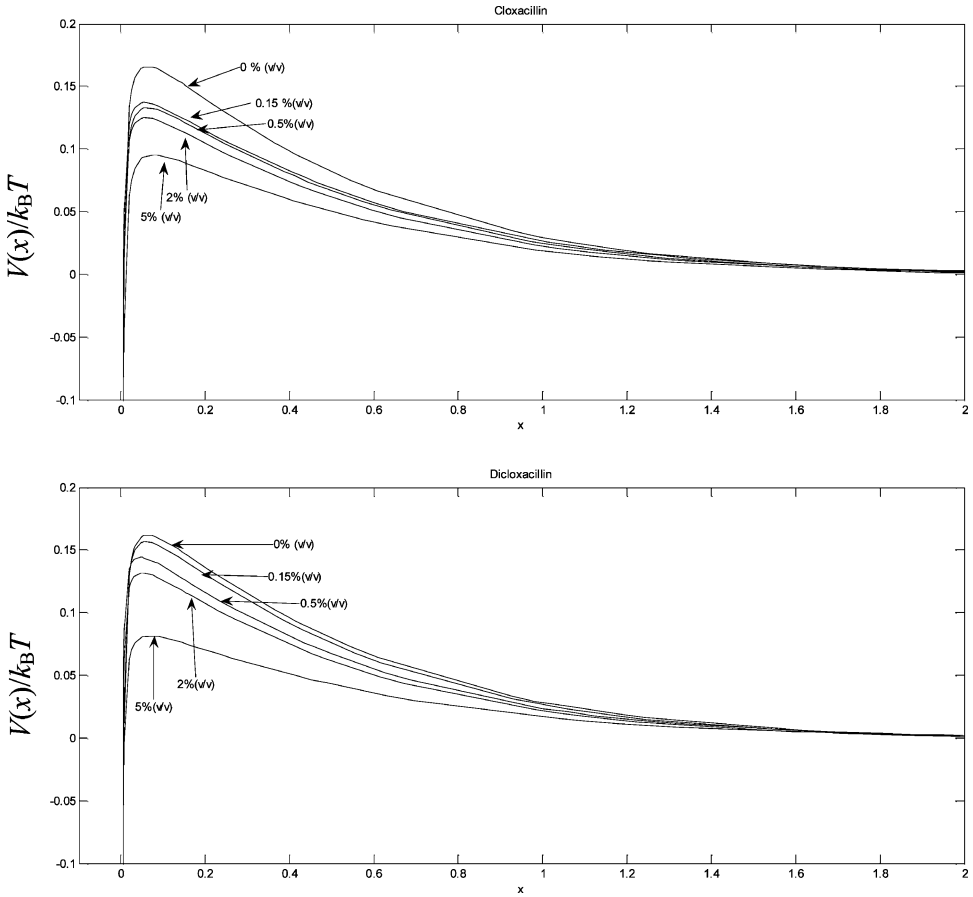

Figure 6. Plots of the reduced pair interaction potential $V(x)/k_BT$ of cloxacillin and dicloxacillin as a function of the reduced and normalized distance x at ethanol concentrations (% (v/v)) indicated.

distributions of the relaxation times τ_i: the smaller the value of β_i, the broader the distribution. The values obtained for β_i were $\beta_c \approx 1$ and $\beta_s \approx 0.8$ and were practically constant with ethanol concentration. In stretched autocorrelation functions, a decrease in β with concentration reflects an increase in the polydispersity of the distribution of characteristic relaxation times, $A(\tau)$, with increasing concentration.[35] The behavior of β_i observed for a water−ethanol−dicloxacillin system led us to conclude that the system can be considered nearly monodisperse for each of the species in solution, at least for the drug and ethanol concentrations that were studied in this work.

Figure 5 shows the self-diffusion coefficient as a function of the concentration of aqueous solutions of cloxacillin in the presence of different ethanol concentrations. Similar plots were obtained for dicloxacillin in the presence of the same concentrations of ethanol.

Hydrodynamic radii of the penicillin aggregates in the presence of ethanol were calculated using the Stokes−Einstein equation (eq 10). It should be noted that the radii determined from DLS measurements using eq 10 assume spherical aggregates. Consequently, values determined here should be regarded as approximate. These compounds exhibit a stacking mode of association[10] comparable to those that have been reported in studies of the association of nucleotides,[38] dyes,[39,40] and phenothiazine drugs.[41] Table 2 shows a slight increase in the hydrodynamic radius of the aggregates with ethanol concentrations. In some ways it can be thought that the penicillin aggregate is behaving in a manner similar to an adsorption interface. A small amount of clusters of dicloxacillin at an ethanol concentration of 5% (v/v) with a hydrodynamic radius of 20.96 nm was also detected.

To correlate the experimental results of diffusion with the interactive forces between aggregates, the data were analyzed according to the treatment proposed by Corti and Degiorgio[23] (see section 3.3). The results are shown in Table 2. There were no great differences between the experimental and predicted values of k'_D, so only the experimental k'_D values are shown. Comparisons of the variations in the net charge of the aggregate, p, with ethanol concentration indicate that, for cloxacillin, its value is independent of alcohol concentration and dicloxacillin lowers its value. The Hamaker constant lowers its value with ethanol concentration for both penicillins.

Figure 6 shows the reduced potential, $V(x)/k_BT$, of cloxacillin and dicloxacillin as a function of distance, x, for a set of alcohol concentrations. It can be observed that, upon increasing ethanol concentration, the double-layer thickness is reduced; hence, the electrical potential is screened, and the London−van der Waals attraction becomes increasingly important. The alcohol leads to a diluted surface charge density in the palisade layer of the penicillins that causes a better shielding of the ionic headgroups of the monomers and thereby reduces the electrostatic repulsive interaction and the electrical double layer, a process that promotes lower values of the *cc*, as has been shown by surface tension. In some aspects the ethanol behavior can be considered as that of an electrolyte (reduces the critical concentration and the double layer). The height of the reduced pair interaction potential energy barrier decreases with ethanol concentration and does so faster for dicloxacillin, as can be observed in Figure 6. These two amphiphilic drugs are thus expected to undergo a transition from a stable dispersion to a coagulated one with additions of ethanol. However, the curves in Figure 6 indicate

the predominance of electrostatic repulsion, leading to a stable system over the drug−ethanol concentration range studied.

5. Conclusions

The relationship between the structures of the penicillins cloxacillin and dicloxacillin and interfacial tension was studied in aqueous solutions in the presence of ethanol. It was found that the critical concentrations indicate that dicloxacillin is more hydrophobic than cloxacillin for all the ethanol concentrations because of a structural difference. Changes in the minimum area per molecule of the monomers at the air/water−ethanol interface were evaluated by surface tension. The data show an area increment with ethanol concentration that can be related to a progressive decrement of the dielectric constant and, thereby, an increment of the interaction between the ionic groups that gives place to a loose packing of the drug ions in the surface and to the incorporation of alcohol molecules into the interfacial films. The greater distance between dicloxacillin monomers in the surface, when compared with those of cloxacillin, means higher repulsion among them due to the substitution of a Cl atom by a hydrogen atom in the phenyl group, which gives rise to an increase in its net electrical charge, as has been calculated by DLVO theory, and causes the molecules of dicloxacillin to be more separated at the interface. The standard Gibbs energies of adsorption calculated gave lower values for dicloxacillin. Therefore, its "escaping tendency" from the aqueous environment to the air−water monolayer is more marked. DLS data had to be fitted to a sum of an exponential and a stretched exponential function at concentrations of ethanol of 5% (v/v) because, besides the drug aggregates, a small population of nearly monodisperse clusters of penicillin with longer relaxation times is present. For cloxacillin, the relaxation time distribution shows a single peak, which indicates that the system can be modeled as an ergodic system of dilute diffusing monodisperse particles. The analysis of the aggregate stability showed that alcohol leads to a diluted surface charge density in the palisade layer of the penicillins that causes a better shielding of the positive headgroups of the monomers and thereby reduces the electrostatic repulsive interaction, a process that promotes lower values of the *cc*, as has been shown by surface tension. Comparisons of the variations of the net charge of the aggregate with ethanol concentration indicate that, for cloxacillin, its value is independent of the alcohol concentration and dicloxacillin lowers its value. The Hamaker constant lowers its value with ethanol concentration for both penicillins. The stability curves over the drug−ethanol concentration range studied indicate the predominance of electrostatic repulsion, leading to a stable system, but the height of the reduced pair interaction potential energy barrier decreases with ethanol concentration; so, as the ethanol concentration increases, it is expected to undergo a transition from a stable dispersion to a coagulated one.

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