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A Study of the Interaction of the Amphiphilic Penicillins Cloxacillin and Dicloxacillin with Human Serum Albumin in Aqueous Solution

J. M. Ruso,[†] D. Attwood,[‡] M. García,[†] P. Taboada,[†] L. M. Varela,[†] and V. Mosquera*,[†]

Grupo de Física de Coloides y Polímeros, Departamento de Física Aplicada y Departamento de Física de la Materia Condensada, Facultad de Física, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain, and School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, U.K.

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The complex formed by the interaction of the structurally similar amphiphilic penicillin drugs cloxacillin and dicloxacillin and human serum albumin (HSA) in water at 25 °C has been investigated using a range of physicochemical techniques. The colloidal dispersion has been considered as a binary system in which water and drug molecules are regarded as the solvent for the HSA/drug complex. Measurements of the solution conductivity and the electrophoretic mobility of the complexes have shown hydrophobic adsorption of the drug on the protein surface, leading to surface saturation. Measurements of the size of the complex and the thickness of the adsorbed layer by dynamic light scattering have shown a gradual increase in hydrodynamic radius of the complex with increasing drug concentration typical of a saturation rather than a denaturation process. A larger hydrodynamic radius of the HSA/dicloxacillin complex has been attributed to a more pronounced extension or unfolding of the HSA molecule than in complexes formed with cloxacillin. The interaction potential between the HSA/drug complexes and their stability were determined from the dependence of diffusion coefficients on protein concentration by application of the DLVO colloidal stability theory. The results indicate decreasing stability of the colloidal dispersion of the protein/drug complexes with increase in the concentration of added drug.

1. Introduction

Studies on the structures and interactions of the complexes formed between proteins and surfactants in aqueous solutions have been extensively reviewed.[1,2] Protein−surfactant complexes can be conveniently divided into three main categories:[3] (i) polyelectrolyte and charged ionic amphiphilic systems, where the protein and the amphiphilic molecule can have opposite charge, giving rise to Coulombic and hydrophobic interactions, or the same charge (as in the case of the present work), where the interaction is primarily hydrophobic; (ii) neutral polymer and ionic amphiphilic systems; and (iii) less common kinds of systems, containing either a polyelectrolyte and a nonionic amphiphile, or two neutral species.

The globular protein human serum albumin (HSA), which consists of 583 amino acids in a single polypeptide chain with a molar mass of 66,411 g mol^{-1}, is widely used as a model protein in the study of such interactions.[4] X-ray crystallography[5] has shown an asymmetric heart-shaped molecule with sides of 8 nm and thickness of 3 nm that can be roughly approximated as an equilateral triangle with a height of 6.9 nm. The two heart lobes contain the molecule's hydrophobic binding sites while the outside of the molecule contains most of the polar groups. HSA constitutes approximately half of the total blood protein, acting as a carrier for fatty acids and several amphiphiles from bloodstream to tissues, and hence is an appropriate choice of protein for a study of interaction with amphiphilic drugs. The binding of the drugs to albumin has important effects in their transport to tissues and to the kidneys, where they are cleared from the blood, and on their effective therapeutic concentration. In the present study we have examined the protein binding of two penicillins with amphiphilic structures, cloxacillin and dicloxacillin. Our previous studies of the solution properties of a large number of penicillins[6−11] have characterized their self-assembly in aqueous solution as a function of electrolyte content and temperature. Cloxacillin and dicloxacillin are structurally similar, differing only in an additional chlorine atom on the phenyl ring of dicloxacillin (see below, Chart 1). They are anionic with pK_a's of 2.7 and 2.8, respectively, and will be fully ionized at the pH of the present study. Static light scattering and NMR studies[6] have shown that both drugs form small aggregates (dimers and trimers) at a well-defined critical aggregation concentration (cac) in aqueous solution, but there is evidence from both techniques of a second critical concentration in solutions of dicloxacillin (but not cloxacillin), the NMR technique suggesting a structural rearrangement and light

[†] Universidad de Santiago de Compostela.
[‡] University of Manchester.
* To whom correspondence should be addressed. Tel +34 981 563-100; Fax +34 981 520 676; e-mail fmvictor@usc.es.

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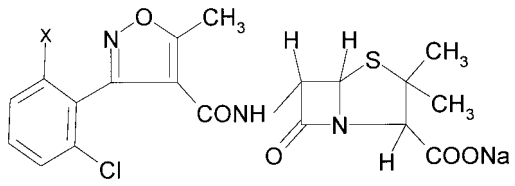
Chart 1



X= H CLOXACILLIN
X= Cl DICLOXACILLIN

scattering showing an increase of aggregate size at this concentration.

Since HSA also carries a net negative charge in aqueous solution (the isoelectric point is in the range 4.7−4.9), the interaction between these penicillins and protein will be expected to be primarily hydrophobic and nonspecific,[12] although as with interactions between sodium dodecyl sulfate (SDS) and HSA[13] it is likely that some negatively charged drug molecules may bind specifically to cationic amino acid side chains. We have recently reported[12,14−16] the interactions between proteins and amphiphilic molecules with a view to understanding the mechanism for the adsorption of these molecules to such biopolymers. Equilibrium dialysis in aqueous phosphate buffered saline solution (ionic strength 0.188 M, pH 7.4 at 25 °C) has shown that the numbers of cloxacillin and dicloxacillin molecules bound per HSA molecule approach ∼1200 (dicloxacillin) and ∼3000 (cloxacillin) as the free drug concentration approaches the cac. Cloxacillin shows a maximum in the binding isotherm possibly related to maxima in drug activity in the vicinity of the critical concentration. We have recently presented a theoretical formalism based on the combination of the Brunauer−Emmett−Teller (BET) multilayer adsorption model with an electrolytic adsorbate to predict binding isotherms of the amphiphilic penicillins onto HSA.[17] Adsorption maxima in these binding processes were seen to be due to the ionic character of the adsorbate, and the increase of the number of bound molecules was related to the hydrophobic nature of the drugs. The dicloxacillin binding isotherm shows a slight plateau above the first cac and a steep rise before the second cac. All these results indicate that the second chlorine atom of dicloxacillin has a marked effect on the binding characteristics compared to cloxacillin.

In the present work we have studied the nature and the stability of the complexes formed by the two anionic amphiphilic penicillins and human serum albumin in water. Measurements of the electrophoretic mobility of the HSA−penicillin complex in a wide range of penicillin concentrations have provided information on the adsorbed layer, the zeta potential of the complex, and the energies of adsorption. Zeta potential and conductivity measurements have been used to monitor the influence of protein on the onset of self-association of both penicillins. Dynamic light scattering techniques have been used in the determination of the size and charge interactions of the complexes. Finally, the interaction potential between complexes has been quantified by application of the DLVO theory of colloidal stability to diffusion data using the Corti−Degiorgio model,[18] assuming that the interpretation of the protein−surfactant interactions can be understood by a comparison with the self-assembly of free surfactant. In this respect, there are many similarities between the binding of surfactant to polyelectrolytes and micelle formation, both involving a cooperative process over a narrow concentration range.[19]

2. Experimental Section

2.1. Materials. Human serum albumin (70024-90-7, 98% purity), 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. Experiments were carried out using double distilled, deionized, and deaerated water.

2.2. Adsorption of Cloxacillin and Dicloxacillin onto Albumin. Aliquots of 2.5 cm^3 of a 0.125% (w/v) solution of HSA were added to equal volumes of solutions of the penicillins to give solutions in which the final concentration of albumin was 0.0625% (w/v) and that of the drugs covered the required range. The solutions were maintained at 25 °C until equilibrium adsorption onto the protein was achieved.

2.3. Specific Conductivities. Conductivities were measured with a HP 4285A Precision LCR meter equipped with a HP E5050A colloid dielectric probe. The probe is especially designed to measure inductances and to avoid the polarization that occurs when the probe is constructed from plain condenser plates. Specific conductivities were measured at 25.0 °C. The measuring cell was immersed in a thermostat bath, keeping the temperature control to within ±0.01 °C.

2.4. Zeta Potential Measurements. ζ-potentials of the HSA−cloxacillin and HSA−dicloxacillin complexes were measured using a Zetamaster 5002 (Malvern Instruments Ltd.) by taking the average of five measurements at a stationary level. The cell used was a 5 mm × 2 mm rectangular quartz capillary. The temperature of the experiments was 25.0 ± 0.1 °C controlled by a HETO proportional temperature controller.

2.5. Dynamic Light Scattering. Measurements were made at 25.0 ± 0.1 °C and at a scattering angle of 90° 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 in combination with a Brookhaven BI 9000AT digital correlator with a sampling time range of 25 ns to 40 ms. Solutions were clarified by ultrafiltration through 0.1 μm Millipore filters.

3. Results and Discussion

3.1. Conductivity. Figure 1 shows plots of the specific conductivity, κ, of solutions of cloxacillin in water and in the presence of 0.0625% (w/v) HSA as a function of cloxacillin concentration m (mol kg^{-1}). Similar plots (not shown) were obtained for solutions of dicloxacillin. The curvature of the plots of the drug in water in the region of the cac is a consequence of the low aggregation number of the drug aggregates. Precise values of cac were derived from these plots by an analytical procedure[20] based on the Phillips definition of the critical micelle concentration[21]

$$\left(\frac{d^3\kappa}{dm^3}\right)_{m=\mathrm{cmc}} = 0 \qquad (1)$$

The procedure involves a Gaussian approximation of the second derivative of the conductivity/concentration data, followed by two consecutive numerical integrations

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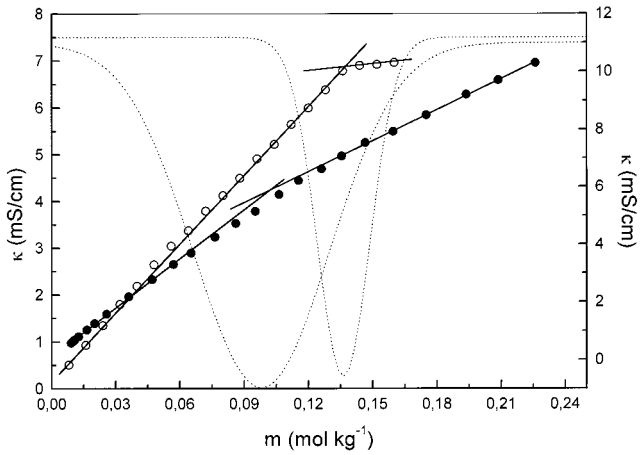

Figure 1. Specific conductivity, κ, of cloxacillin (●) (left side of the figure) and HSA (0.0625% w/v)/cloxacillin (○) (right side of the figure) vs concentration of cloxacillin. The dashed line represents the second derivative of the conductivity−concentration curve.

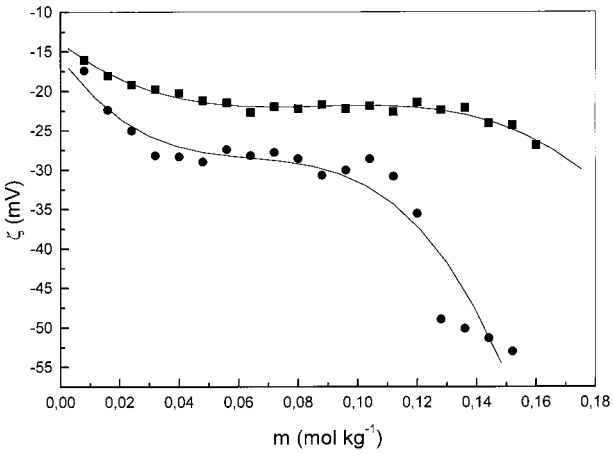

Figure 2. ζ potential of HAS (0.0625% w/v)/penicillin complexes as a function of cloxacillin (■) and dicloxacillin (●) concentration.

by the Runge−Kutta method and the Levenberg−Marquardt least-squares fitting algorithm. Figure 1 shows the minimum of the second derivative and therefore the cac of the drug in the presence and absence of albumin. The increase of the cac results from a decrease in the number of free drug molecules in solution as a consequence of their adsorption onto the HSA, micelles being formed only after saturation of the protein surface.[22] Other authors using a range of different techniques[23−25] have detected similar changes in the critical concentration of amphiphiles following adsorption on to proteins. Critical aggregation concentrations (mol kg^{-1}) of 0.098 and 0.136 were obtained for cloxacillin and HSA/cloxacillin and 0.093 and 0.114 for dicloxacillin and HSA/dicloxacillin, respectively. No inflections corresponding to the onset of complex formation could be detected for either system, indicating that interaction between penicillin molecules and protein starts at very low drug concentration.

The average numbers, N_0, of drug molecules adsorbed onto the protein in the proximity of the cac as estimated from the difference between the cac values in the absence and presence of protein were ∼2500 and ∼1050 for cloxacillin and dicloxacillin, respectively. These values, which show a much greater adsorption of cloxacillin, are in reasonable agreement with those from equilibrium dialysis[12] (∼3000 for cloxacillin and ∼1200 for dicloxacillin). This notable difference in the number of adsorbed molecules is due to the greater hydrophobicity of cloxacillin (lower cmc) that induces a greater extent of adsorption on cloxacillin molecules onto HSA.

3.2. Electrokinetic Behavior. The ζ potential was calculated from the electrophoretic mobility, u, assuming a protein radius, a, of approximately 3 nm[26] using the relationship[27]

$$\zeta = \frac{3\eta u}{2\epsilon_0\epsilon_r} \frac{1}{f(\kappa a)} \qquad (2)$$

where the permittivity of vacuum, ϵ_0, the relative permittivity of the medium, ϵ_r, and the viscosity of water, η, were taken as 8.854×10^{-4} J^{-1} C^2 m^{-1}, 78.5, and 8.904×10^{-4} N m^{-2} s, respectively. The factor $f(\kappa a)$ depends on the particle shape; for a sphere with $\kappa a > 1$ it is given by

$$f(\kappa a) = \frac{3}{2} - \frac{9}{2\kappa a} + \frac{75}{2\kappa^2 a^2} - \frac{330}{\kappa^3 a^3} \qquad (3)$$

κ being the reciprocal Debye length.

Figure 2 shows the ζ potential of the two HSA/penicillin systems as a function of the concentration of each drug. The plots show an initial decrease of the potential at approximate drug concentrations (mol kg^{-1}) $m \leq 0.03$ for dicloxacillin and $m \leq 0.05$ for cloxacillin as a consequence of adsorption of the negatively charged penicillin monomers within the hydrophobic cavities of the protein molecule.[28] The plateau region, at concentrations (mol kg^{-1}) ($0.03 \leq m \leq 0.11$ for dicloxacillin and $0.05 \leq m \leq 0.13$ for cloxacillin), may be attributed to the saturation of the protein sites, leading to a decrease of ξ potential in the third region, which commences at similar concentrations to those determined by conductivity for the formation of aggregates of the penicillins in solutions containing protein. There are therefore contributions to the zeta potential in this third region from both the micelles and the protein complexes. The more abrupt the change in the case of dicloxacillin may also possibly indicate a loosening of the protein structure.

The surface charge density enclosed by the shear plane, σ_ζ, was obtained from the corresponding ζ potentials below the critical concentration using the following equation for a z:z electrolyte[29]

$$\sigma_\zeta = \frac{\epsilon_r\epsilon_0 k_B T\kappa}{ze}\left[2\sinh\left(\frac{ez\zeta}{2k_BT}\right) + \frac{4}{\kappa a}\tanh\left(\frac{ez\zeta}{4k_BT}\right)\right] \qquad (4)$$

where e is the elemental charge, z is the valence of the ion, a is the particle radius, κ is the reciprocal Debye length, k_B is the Boltzmann constant, and T is the thermodynamic temperature. Equation 4 takes into account the particle curvature and gives σ_ζ to within 5% for $\kappa a > 0.5$ for any ζ potential. The values of the calculated charge vary from −2.1 to −3.5 and −2.3 to −7.1 μC cm^{-2}, corresponding to the lowest and highest concentrations of cloxacillin and dicloxacillin, respectively.

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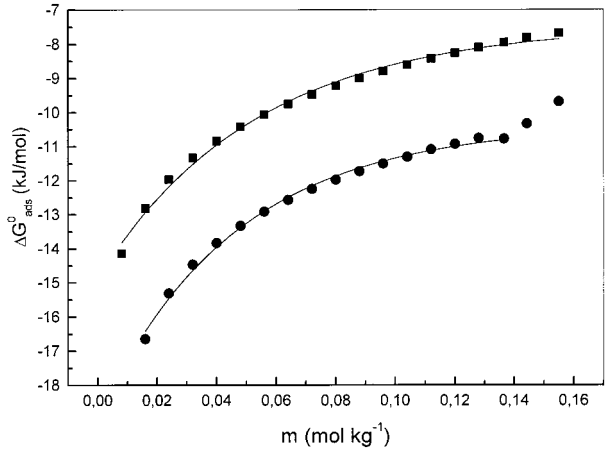

Figure 3. Gibbs energies of adsorption on human serum albumin (0.0625% w/v) as a function of cloxacillin (■) and dicloxacillin (●) concentration.

The standard free energy of adsorption, ΔG^0_{ads}, was calculated from

$$k_2 = \exp(-\Delta G^0_{ads}/k_B T) \tag{5}$$

where k_2 is the adsorption constant estimated from the relationship

$$\frac{1}{m} = k_2\left(\frac{zeN_1}{\sqrt{8n_0\epsilon k_B T}[\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)]} - 1\right) \tag{6}$$

and m is chosen as the concentration at the ζ potential midpoint between selected values ζ_1 and ζ_2.

The standard Gibbs energies of adsorption evaluated in this manner (Figure 3) are large and negative at low penicillin concentrations where binding to the high-energy sites takes place and become less negative as more penicillin molecules bind, suggesting a saturation process. Similar behavior was found for the system sodium *n*-dodecyl sulfate/histone.[30] The higher (more negative) adsorption free energy of dicloxacillin is a consequence of its greater hydrophobicity compared to that of the monochloro-substituted drug.

Results obtained for the free energies of adsorption by this method are of the same order but generally slightly lower than those obtained from equilibrium dialysis.[12] The discrepancies in values from the two techniques arise from differences in the experimental conditions and the assumptions inherent in each method. In equilibrium dialysis the chemical potential of unbound ligand is identical in the donor and receptor compartments, and therefore the activities of unbound ligand are the same since we reasonably choose the same standard state for the ligand in each compartment. However, it does not necessarily follow that the concentrations of ligand are identical, because it is conceivable that the ligand activity coefficients are different in the two compartments, whose contents obviously have different compositions.[31] In the calculation of free energies from the zeta potential the assumption is made that only hydrophobic interactions are present, which is unlikely to be the case in many systems.

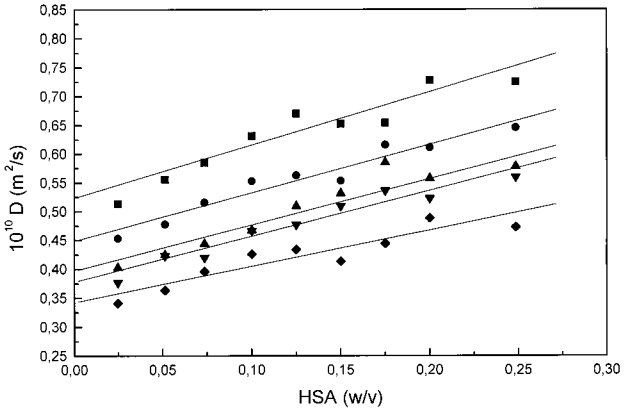

Figure 4. Diffusion coefficient, *D*, as a function of the human serum albumin concentration in aqueous solutions of cloxacillin: (■) 0.03, (●) 0.06, (▲) 0.09, (▼) 0.12, and (◆) 0.15 mol kg^{-1}.

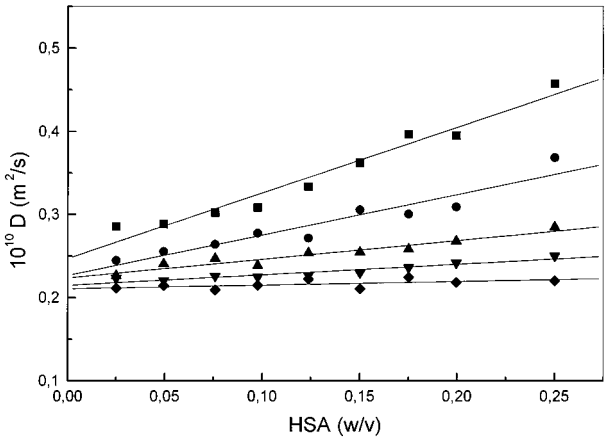

Figure 5. Diffusion coefficient, *D*, as a function of the human serum albumin concentration in aqueous solutions of dicloxacillin: (■) 0.03, (●) 0.06, (▲) 0.09, (▼) 0.12, and (◆) 0.15 mol kg^{-1}.

3.3. Dynamic Light Scattering (DLS). Apparent diffusion coefficients, *D*, of human serum albumin in water and aqueous solutions of cloxacillin and dicloxacillin are presented in Figures 4 and 5 as a function of protein concentration, *c*. The concentration dependence of *D* is due to interactions between complexes and may be fitted to the linear function:

$$D = D_0[1 + k'_D c] \tag{7}$$

where D_0 is the limiting diffusion coefficient at zero protein concentration. Hydrodynamic radii, R_h, were calculated from D_0, using the Stokes−Einstein equation

$$R_h = \frac{k_B T}{6\pi\eta D_0} \tag{8}$$

where η is the solvent viscosity. It should be noted that the radii determined from dynamic light scattering measurements at 90° using eq 9 assume spherical aggregates. Consequently, values determined here should be regarded as approximate, although any deviation from sphericity in these systems is not thought to be significant.

Table 1 shows an increase of the globular size with penicillin concentration as a consequence of adsorption on the protein surface. A feature of the diffusion results

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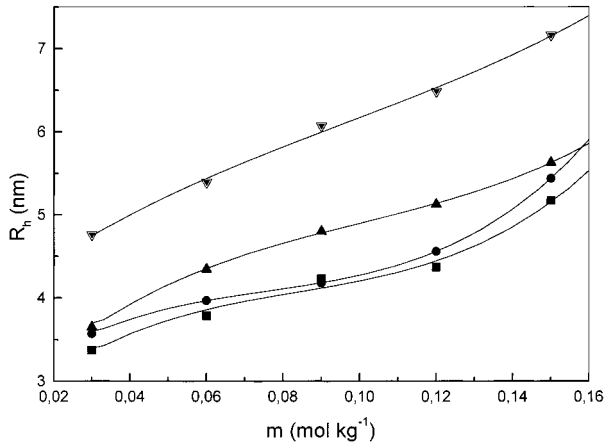

Figure 6. Hydrodynamic radius of the HSA/cloxacillin complex vs cloxacillin concentration for HSA concentrations of (■) 0.25, (●) 0.125, (▲) 0.075, and (▼) 0.025% (w/v).

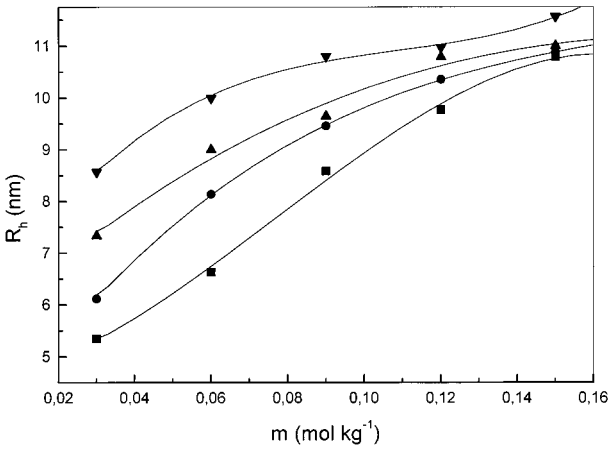

Figure 7. Hydrodynamic radius of the HSA/dicloxacillin complex vs dicloxacillin concentration for HSA concentrations of (■) 0.25, (●) 0.125, (▲) 0.075, and (▼) 0.025% (w/v).

Table 1. Limiting Diffusion Coefficients, D_0, and Hydrodynamic Radii, R_h, from DLS for HSA/Penicillin Complexes as a Function of the Penicillin Concentration

penicillin conc/mol kg^{-1}	$10^{10}D_0$/m^2 s^{-1}		R_h/nm	
	HSA/clox	HSA/diclox	HSA/clox	HSA/diclox
0.03	0.515	0.247	4.75	9.90
0.06	0.446	0.227	5.48	10.77
0.09	0.387	0.223	6.32	10.96
0.12	0.374	0.214	6.50	11.42
0.15	0.343	0.210	7.13	11.64

is the marked difference in the size of the complexes formed by the two drugs, the apparent radius of the HSA/dicloxacillin complex being twice that of the complex formed with cloxacillin, suggesting the possibility of a more unfolded or extended structure.

Figures 6 and 7 show plots of the hydrodynamic radius of the drug/protein complexes against drug concentration for different protein concentrations. These plots can be compared with typical binding isotherms, which show the average number of surfactant molecules bound per protein molecule as a function of the free surfactant concentration. Two general types of behavior may be identified from such plots. Systems that exhibit cooperative interactions show a marked response to a small change in amphiphile concentration, attributable to the denaturation of the protein and the subsequent appearance of new binding sites. Relative insensitivity of the plots to changes in amphiphile concentration, as noted in the present study for both drug/protein systems, is indicative of an anticooperative process and a folding of the protein as the adsorption of drug occurs.

Figure 8 shows decay time distributions of free cloxacillin and HSA/cloxacillin complex in solutions containing a range of protein concentrations and a fixed cloxacillin concentration of 0.15 mol kg^{-1} (well above the cac). Similar plots were obtained for the HSA/dicloxacillin system. The plots show two different relaxation modes, indicating two distinct diffusional species in the solution: the slow mode corresponding to the HSA/penicillin complex and the fast mode to the penicillin aggregate. Similar behavior was also reported in the system lysozyme/sodium dodecyl sulfate.[32] The sequence of plots shows an increase of the relative height of the peak of the complex to that of the aggregate as the protein concentration increases that is most pronounced at low protein concentrations. The protein is behaving in a manner similar to an adsorption interface, adsorption onto which is energetically more favorable than the formation of aggregates, in agreement with the observed higher value of the critical concentration of the penicillin in the presence of HSA.

3.4. Stability HSA/Penicillin Complexes. To correlate experimental results of diffusion with the interactive forces between complexes, the data were analyzed according to the treatment proposed by Corti and Degiorgio.[33] For the purpose of data interpretation, the concentration dependence of the diffusion coefficient (eq 7) was expressed in terms of the volume fraction, ϕ, of the particles rather than their molality,

$$D = D_0(1 + k_D\phi) \tag{9}$$

with $k_D = k'_D/\bar{v}$, where $\bar{v}$ is the specific volume of the solute particles as determined from density measurements. k_D may be related to the pair-interaction potential, $V(x)$, between spherical particles of radius a using the expression proposed by Felderhof:[34]

$$k_D = 1.56 + \int_0^\infty [24(1+x)^2 - F(x)][1 - \exp(-V(x)/k_BT)]\,dx \tag{10}$$

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

$$F(x) = 12(1+x) - \frac{15}{8}(1+x)^{-2} - \frac{27}{64}(1+x)^{-4} + \frac{75}{64}(1+x)^{-5} \tag{11}$$

The interaction potential $V(x)$ as it is usually written in the DLVO theory 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, $V_R(x)$. The expression for $V_A(x)$ derived by Hamaker for the case of two spheres is

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

where A is the Hamaker constant. The approximate

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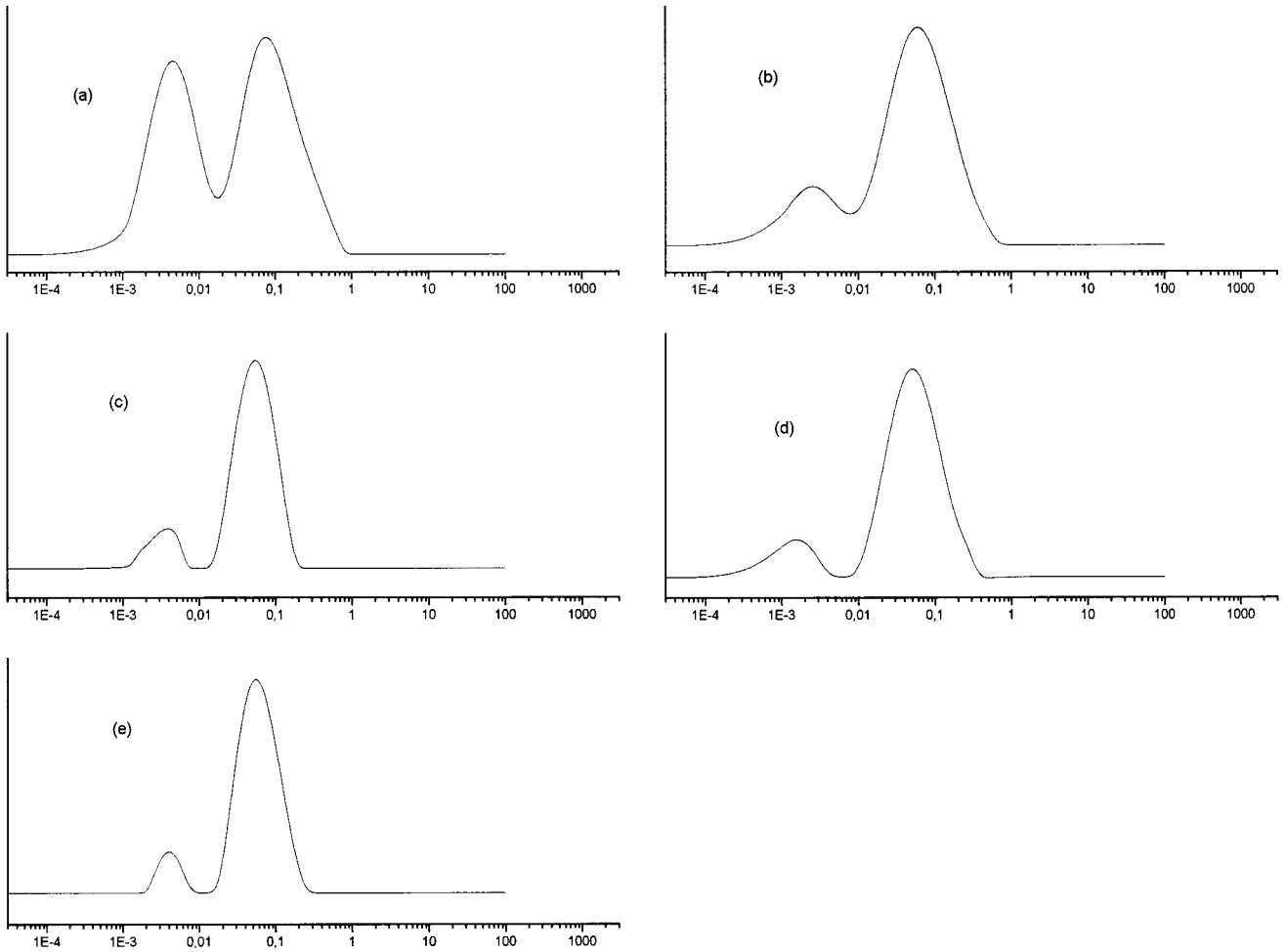

Figure 8. Decay time distribution (ms) in aqueous solutions of cloxacillin (0.15 mol kg^{-1}) and HSA concentrations of (a) 0.025, (b) 0.075, (c) 0.125, (d) 0.175, and (e) 0.25% (w/v).

expression for the repulsive interaction, $V_R(x)$, appropriate for values of $\kappa a > 1$ is

$$V_R(x) = \frac{\epsilon a \Psi_0^2}{2} \ln[1 + \exp(-2\kappa a x)] \qquad (13)$$

The aggregate charge, q, is related to the potential corresponding to the net charge of the particle, Ψ_0, by the expression[35]

$$\psi_0 = \frac{2k_B T}{e} \sinh^{-1}\left(\frac{\pi e^2 \kappa^{-1} q}{2\pi a^2 \epsilon k_B T}\right) \qquad (14)$$

The computational procedure involved the iterations of values of A and ψ_0 to give the best fit of computed and experimental values of k_D over the range of surfactant concentration. Table 2 shows reasonable agreement between these values. Our calculations indicate that complexes of similar charge and interaction are formed by both drugs: the values of q and A and obtained for cloxacillin and dicloxacillin were 1.13 and 1.05 electrostatic units of charge and 4.1 × 10^{-23} and 2.5 × 10^{-23} J, respectively.

Figure 9 shows the reduced potential, $V(x)/k_BT$, as a function of distance for a set of dicloxacillin concentrations. On increasing penicillin concentration, the double-layer thickness is reduced; hence, the electrostatic potential is screened, and London−van der Waals attraction becomes increasingly important. However, the curves indicate the predominance of electrostatic repulsion, leading to stable systems over the drug concentration range studied. The corresponding curves for cloxacillin show similar behavior.

Table 2. Experimental and Theoretical Slopes, k_D, and Reduced Potential, $e\Psi_0/k_BT$, as a Function of the Penicillin Concentration

penicillin conc/mol kg^{-1}	k_D exptl	k_D theor	$e\Psi_0/k_BT$
HSA/Cloxacillin			
0.03	3.41	4.12	1.54
0.06	3.23	2.32	0.85
0.09	3.10	1.92	0.64
0.12	2.94	1.76	0.32
0.15	2.20	1.68	0.15
HSA/Dicloxacillin			
0.03	2.87	3.05	1.11
0.06	1.77	1.24	0.57
0.09	0.81	0.77	0.26
0.12	0.46	0.55	0.19
0.15	0.17	0.44	0.14

Combining the surface potential, ψ_0, and the ζ potential it is possible to estimate the thickness of the adsorbed layer[36] (Δ) by the following equation:

$$\mathrm{tgh}\left(\frac{ze\zeta}{4k_BT}\right) = \mathrm{tgh}\left(\frac{ze\Psi_0}{4k_BT}\right) \exp(-\kappa\Delta) \qquad (15)$$

where z is the valence of the counterion. The estimated

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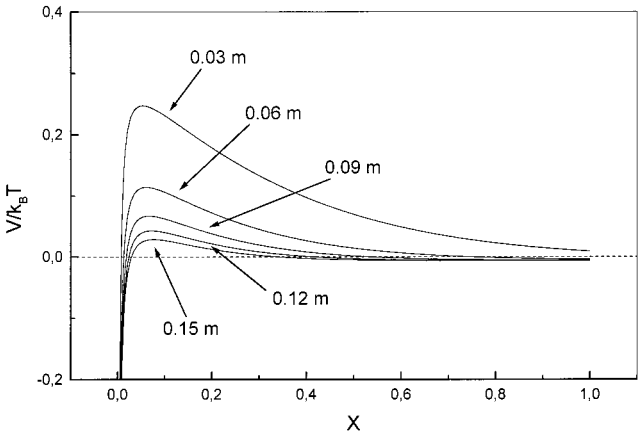

Figure 9. Plots of the reduced pair interaction potential $V(x)/k_BT$ as a function of the reduced and normalized distance x at the dicloxacillin concentrations (mol kg^{-1}) indicated.

thickness varies from 1.3 to 1.8 nm and 2.9 to 4.5 nm for the lowest and highest concentrations of cloxacillin and dicloxacillin, respectively. The apparent difference in adsorbed layer thickness for complexes formed by the two drugs may be a consequence of the more bulky dicloxacillin molecule, although assumptions of sphericity, particularly in the case of the dicloxacillin complex, will introduce significant errors in these calculations.

The interaction potential can be related to the stability ratio W by the relationship[37]

$$W = 2\int_0^\infty \frac{\exp(V/k_BT)}{(x+2)^2}\,dx \qquad (16)$$

It can be seen from Figure 9 that the position of the maximum (x_m) is not dependent on penicillin concentration, and hence W is determined almost entirely by the value of the maximum of the reduced potential, so eq 16 can be written

$$W = \frac{2}{(x_m+2)^2}\int_0^\infty \exp(V/k_BT)\,dx \qquad (17)$$

Expanding V in a Taylor series around V_m, neglecting terms higher than two, eq 17 becomes

$$W = \frac{2\pi^{1/2}}{x_m+2}\exp\frac{V_m}{k_BT} \qquad (18)$$

Table 3 shows the values of V_m/k_BT and W obtained from eq 19 using values of $x_m = 0.01$ and 0.025 for cloxacillin and dicloxacillin, respectively. The decrease in

Table 3. Maximum Value of the Reduced Interaction Potential (V_m/k_BT) and Stability Ratio (W) as a Function of the Penicillin Concentration

penicillin conc/mol kg^{-1}	V_m/k_BT		W	
	HSA/clox	HSA/diclox	HSA/clox	HSA/diclox
0.03	0.264	0.603	0.99	0.99
0.06	0.140	0.283	0.88	0.73
0.09	0.095	0.119	0.84	0.62
0.12	0.072	0.035	0.82	0.57
0.15	0.056	0.000	0.81	0.54

the stability of the solutions of the albumin complex as penicillins are adsorbed onto the protein is apparent from these values, the effect being more marked in the case of dicloxacillin.

4. Summary

This study has compared the adsorption characteristics of two structurally similar penicillin drugs on the surface of human serum albumin. With both drugs the adsorption was accompanied by a gradual change in hydrodynamic radius of the complex with increasing drug concentration indicative of a saturation rather than a denaturation process, saturation of the protein surface occurring at drug concentrations of approximately 0.135 and 0.110 mol kg^{-1} for cloxacillin and dicloxacillin, respectively. The number of available adsorption sites per unit area of protein was appreciably larger for the cloxacillin molecule, a larger number of which were adsorbed (approximately 2500 compared to 1050 for dicloxacillin), the difference reflecting the larger area occupied on the protein surface by the dicloxacillin molecules as a consequence of the additional Cl atom. Similarly, the adsorption free energy of dicloxacillin was higher (more negative) as a consequence of its greater hydrophobicity compared to the monochloro-substituted drug. HSA/dicloxacillin complexes were of larger size, suggesting a more appreciable extension or unfolding of the HSA molecule than in the HSA/cloxacillin systems. Application of the DLVO theory of colloidal stability to the diffusion data has indicated decreasing stability of the colloidal dispersion of the drug/protein complexes with increase in the concentration of added drug.

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