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Adsorption of an amphiphilic penicillin onto human serum albumin: characterisation of the complex

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Abstract

The complex formed by the interaction of the amphiphilic penicillin drug nafcillin and human serum albumin (HSA) in water at 25°C has been characterised using a range of physicochemical techniques. Measurements of the solution conductivity and the electrophoretic mobility of the complexes have shown an ionic adsorption of the drug on the protein surface leading to a surface saturation at a nafcillin concentration of 0.012 mmol kg^{-1} and subsequent formation of drug micelles in solutions of higher nafcillin concentration. Measurements of the size of the complex and the thickness of the adsorbed layer by static and dynamic light scattering have shown a gradual change in hydrodynamic radius of the complex with increasing drug concentration typical of a saturation rather than a denaturation process, the magnitude of the change being insufficient to account for any appreciable extension or unfolding of the HSA molecule. The interaction potential between the HSA/nafcillin complexes, and the stability of the complexes 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 drug/protein complexes with an increase in the concentration of added drug.

Keywords: Human serum albumin; Penicillins; Drug micelles; Drug–protein interaction

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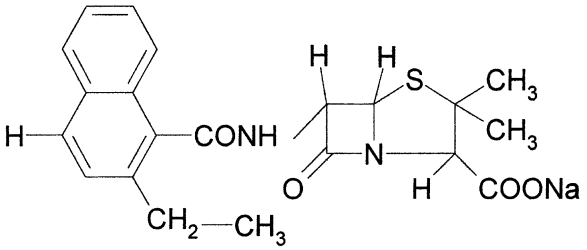

Scheme 1. Nafcillin.

1. Introduction

In the present study we have examined the interaction between the globular anionic protein human serum albumin (HSA) and the amphiphilic drug nafcillin hydrochloride (see Scheme 1) with a view to understanding the adsorption process and the properties of the resulting complex. 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 interaction of penicillins with albumin is known to be the basis for the allergic reaction of humans against penicillin and hence the study of the interaction between HSA and the penicillins, of which nafcillin is selected as the representative, is of particular importance.

The properties of several penicillins including nafcillin, cloxacillin, dicloxacillin, and penicillin V, in aqueous solution and in the presence of electrolyte, have been previously reported [1–4] and the self-assembly of these molecules at a well-defined critical concentration to form small aggregates (typically 8–10 molecules) under different conditions has been well characterised. Nafcillin, in common with the other penicillins, is anionic with a pK_a of 2.7 and will be fully ionised at the pH values used in the present study. Since HSA also carries a net negative charge in aqueous solution, the interaction between the drug and protein will be primarily hydrophobic.

In previous studies [5,6] we have used equilibrium dialysis in a study of changes in the binding of nafcillin to HSA in aqueous phosphate buffered saline solution (ionic strength 0.188 M, pH 7.4 at 25°C) as the free drug concentration approaches the critical concentration. Maxima in the binding isotherms were found which, it was suggested, possibly relate to maxima in drug activity in the vicinity of the critical concentration. In the present work, the electrophoretic mobility of the serum albumin–nafcillin complex was measured over a wide range of nafcillin concentrations providing information on the adsorbed layer, the zeta potential of the complex and the energies of adsorption. A comparison of zeta potential and conductivity measurements is used to determine the commencement of nafcillin aggregate formation in the solution and how this is affected by the presence of the protein. Static and dynamic light scattering have been used in the determination of the molar mass 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 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 co-operative process over a narrow concentration range [7].

2. Experimental

2.1. Materials

Human serum albumin (70024-90-7, 98% purity) and nafcillin (6-[2-ethoxy-1-naphthamidol]-penicillin), sodium salt (product no. N-3269) were obtained from Sigma Chemical Co. It should be noted that the sample of human serum albumin was not guaranteed to be fatty acid free and the extent of drug binding determined from this study may be reduced by the presence of any fatty acids in this sample. Experiments were carried out using double distilled, deionized and degased water.

2.2. Zeta potential measurements

ζ-potentials of the HSA–nafcillin complexes were measured using a Malvern Instruments Ltd Zetamaster 5002 by taking the average of five measurements at stationary level. The cell used was a 5×2-mm rectangular quartz capillary. The temperature of the experiments was 25.0 ± 0.1°C controlled by a HETO proportional temperature controller.

2.3. Adsorption of nafcillin onto albumin

A series of solutions were prepared by the addition of 2.5-cm^3 aliquots of a stock solution of HSA to an equal volume of solutions of nafcillin of concentrations covering the required range, such that the final concentration of albumin was 0.0625% w/v. The solutions were equilibrated at 25°C for 24 h to obtain complete adsorption onto the protein.

2.4. Specific conductivities

Conductivities were measured at 25.0 ± 0.01°C 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 polarisation that occurs when the probe is constructed from plain condenser plates.

2.5. Light scattering measurements

Static light-scattering measurements were made at 25 ± 0.1°C 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 polarised light. Solutions were clarified by ultrafiltration through 0.1-μm filters with the ratio of light scattering at angles of 45° and 135° not exceeding 1.10. Toluene was used as a reference with a value of 3.1×10^{-5} cm^{-1} for the Rayleigh ratio [8]. Refractive index increments were measured at 25 ± 0.1°C using an

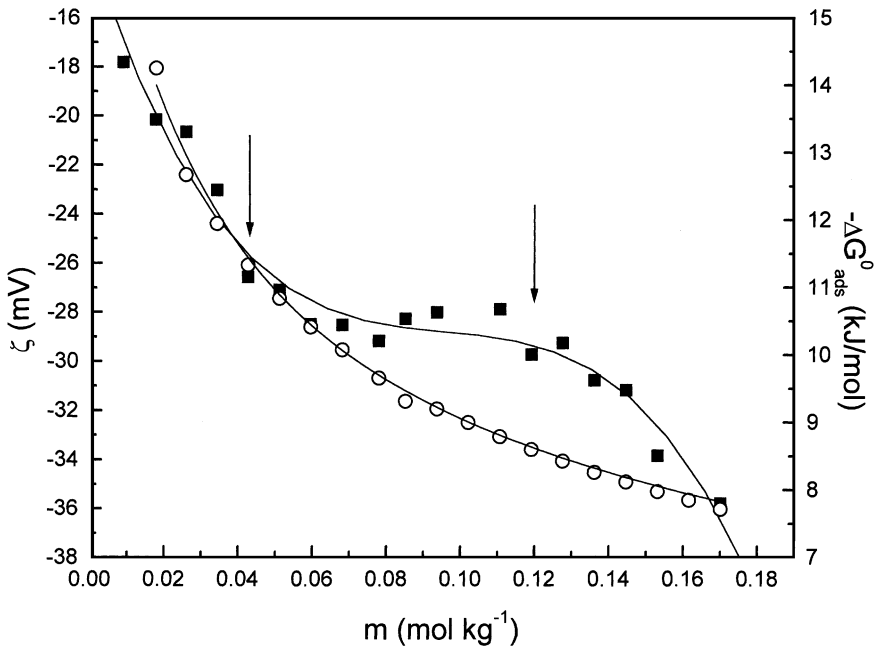

Fig. 1. ζ-Potential of human serum albumin (0.0625% w/v) (■) and Gibbs energies of adsorption (○) as a function of nafcillin concentration at 25°C

Abbé 60/DE precision refractometer (Bellingham and Stanley Ltd).

Dynamic light scattering measurements were made at 25 ± 0.1°C and at a scattering angle of 90° using the equipment described above in combination with a Brookhaven BI 9000AT digital correlator with a sampling time range from 25 ns to 40 ms. Solutions were clarified by ultrafiltration through 0.1-μm filters. Hydrodynamic radii were calculated from measured diffusion coefficients by means of the Stokes–Einstein equation.

3. Results and discussion

3.1. Electrokinetic behaviour

The zeta potential, ζ, was calculated from the electrophoretic mobility, u, assuming a protein radius [10], a, of approximately 3 nm, using [9,11]

$$\zeta = \frac{3\eta u}{2\varepsilon_0 \varepsilon_r} \frac{1}{f(\kappa a)} \tag{1}$$

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^{-12} N m^{-2} s, respectively. A value of 1.14 was calculated for the Henry factor, $f(\kappa a)$, using

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

where κ is the reciprocal Debye length. Eq. 2 is applicable to spheres with $\kappa a > 1$ and hence may be applied to the HSA/drug complexes in which the product κa is approximately 4.

Fig. 1 shows the ζ-potentials of the system HSA/nafcillin as a function of nafcillin concentration. The adsorption process may be divided into three steps. The initial decrease of zeta potential ($m \leq 0.045$) is a consequence of adsorption of the negatively charged nafcillin ions within the hydrophobic cavities of the protein molecule [12]. The plateau region ($0.045 \leq m \leq 0.12$) may be attributed to the saturation of the protein sites, leading to a decrease of zeta potential in the third region at high nafcillin concentration ($m \geq 0.12$) that is associated with the formation of aggregates of nafcillin in the solution.

The surface charge density enclosed by the shear plane, σ_ζ, was obtained at concentrations of nafcillin below the critical concentration from the corresponding ζ-potentials using the following relation for a $z{:}z$ electrolyte [13]

$$\sigma_\zeta = \frac{\varepsilon_r \varepsilon_0 k_B T \kappa}{ze} \times \left[2\sinh\left(\frac{ez\zeta}{2k_B T} \right) + \frac{4}{\kappa a} \tanh\left(\frac{ez\zeta}{4k_B T} \right) \right] \tag{3}$$

e is the elemental charge, z the valence of the ion, k_B the Boltzmann constant and T the thermodynamic temperature. Eq. 3 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 -1.7 to -3.5 μC cm^{-2}, corresponding to the lowest and highest concentrations of nafcillin, respectively.

It may be reasonably assumed that the decrease of the ζ-potential as a function of drug concentration is a consequence of adsorption due to the hydrophobic effect, since the protein and the drug ion have charges of the same sign. Consequently, the following equation may be used to calculate the number of adsorption sites N_1 [14,15]:

$$\left(\frac{\mathrm{d}\zeta}{\mathrm{d}\log m} \right) = \frac{4.606 k_B T}{ze} \times \left(\frac{\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)}{\cosh(ze\zeta_2/2k_B T)} \right) \times \left(\frac{\sqrt{8 n_0 \varepsilon k_B T}\,[\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)]}{zeN_1} - 1 \right) \tag{4}$$

ζ_1 and ζ_2 are selected zeta potentials on the curve, m the nafcillin concentration and n_0 the ionic concentration. The number of available hy-

drophobic adsorption sites per unit area of protein calculated from Eq. 4 was 1.09×10^{16} m^{-2}.

The adsorption constant k_2 may be calculated from the equation:

$$\frac{1}{m} = k_2 \left(\frac{zeN_1}{\sqrt{8n_0\varepsilon k_B T}\,[\sinh(ze\zeta_1/2k_BT) - \sinh(ze\zeta_2/2k_BT)]} - 1 \right) \quad (5)$$

Here, m is chosen as the concentration at the ζ-potential midpoint between ζ_1 and ζ_2.

The standard Gibbs energies of adsorption, ΔG^0_{ads}, evaluated from Eq. (6) are plotted in Fig. 1 as a function of the drug concentration.

$$k_2 = \exp(-\Delta G^0_{ads}/k_BT) \quad (6)$$

The results show that Gibbs energies are large and negative at low drug concentration where binding to the high energy sites takes place, and become less negative as more nafcillin molecules bind, suggesting a saturation process. Similar behaviour was found for the system sodium *n*-dodecyl sulfate/histone [16].

3.2. Specific conductivities

Fig. 2 shows plots of specific conductivity, κ, for nafcillin and nafcillin/HSA (0.0625% w/v) as a function of the concentration of nafcillin. The curvature of the plot for nafcillin in the region of the critical concentration (cc) is a consequence of the low aggregation number of the drug aggregates. Since there was no clear inflection point in this plot, the results were analysed to detect a precise value of cc, using the Phillips definition of the critical micelle concentration (cmc) [17], in which the cmc is defined as the concentration corresponding to the maximum change in gradient in plots of the solution conductivity vs. concentration:

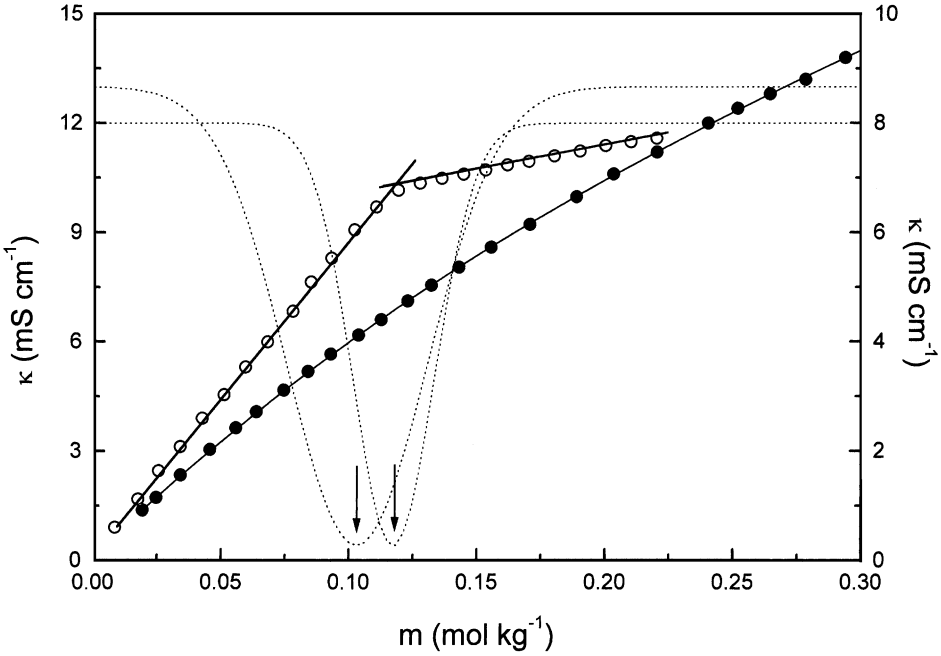

Fig. 2. Specific conductivity, κ, of nafcillin (●) (left side of the figure) and of HSA (0.0625% w/v)/nafcillin (○) (right side of the figure) vs. concentration of nafcillin. The dashed line represents the second derivative of the conductivity–concentration curve.

$$\left(\frac{\partial^3 \kappa}{\partial m^3}\right)_{m=\mathrm{cmc}} = 0 \quad (7)$$

An algorithm was applied in the numerical analysis of the data that was recently developed for the determination of precise values of the critical concentrations of drugs and surfactants of low aggregation number [18]. The method consists of a Gaussian approximation of the second derivative of the conductivity/concentration data, followed by two consecutive numerical integrations by the Runge–Kutta method and the Levenberg–Marquardt least-squares fitting algorithm. Fig. 2 shows minima of the second derivatives and therefore critical concentrations at 0.103 and 0.118 mol kg^{-1} for nafcillin and the HSA/nafcillin systems, respectively. The apparent increase of the critical concentration results from a decrease in the number of free nafcillin molecules in solution as a consequence of their adsorption onto the HSA. This mechanism was confirmed by other authors using different techniques [19–21] and is expected when one considers that adsorption on the protein surface effectively removes nafcillin from solution and thus an apparently higher drug concentration is required before aggregation is observed.

The average number of surfactant molecules adsorbed onto the polymer molecule, N_0, as calculated from the difference between the critical concentration of nafcillin in water and the value obtained for the nafcillin-HSA system was 739.

3.3. Static light scattering

The static light scattering data were analysed using the Debye equation

$$\frac{Kc}{R_{90}} = \frac{1}{M_W} + 2A_2c \quad (8)$$

c is the concentration of albumin, M_W is the molar mass, A_2 is the second virial coefficient, K is an optical constant, and R_{90} is the Rayleigh ratio at 90°.

As written, Eq. (8) assumes small particles relative to the wavelength of the incident radiation. In this respect, the dissymmetry ratio (scattering

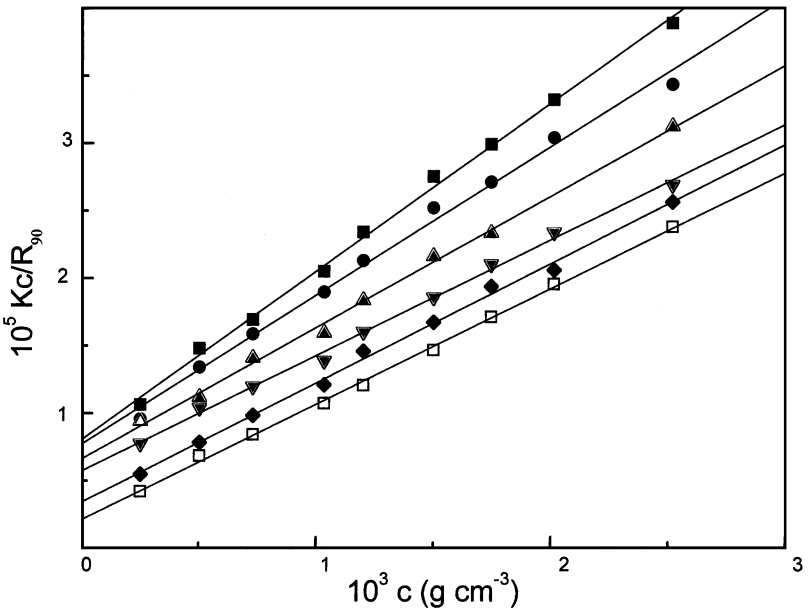

Fig. 3. Kc/R_{90}, as a function of the human serum albumin concentration in aqueous solutions of nafcillin (■) 0.015, (●) 0.03, (▲) 0.045, (▼) 0.7, (♦) 0.1 and (□) 0.15 mol kg^{-1} at 25°C.

intensity at a scattering angle of 45° relative to that at 135°) was consistently near to unity to validate its use.

Fig. 3 shows static light scattering data for HSA in aqueous solutions containing between 0.015 and 0.15 mol kg^{-1} nafcillin. In our treatment of the data we have considered nafcillin as an electrolyte, so our system is acting as a binary system formed by the protein complex as solute, and the nafcillin/water as solvent. Molar masses, M_W, derived from the intercepts of Fig. 3 increased linearly with concentration of added drug (Fig. 4). The molar mass obtained using the Debye equation for the HSA in pure water was $7.9 \pm 0.1 \times 10^4$ g mol^{-1}, which corresponds to an association number $N' = M_W/M_0$ (where M_0 is the molecular weight of the HSA, 6.641×10^4 g mol^{-1}) of 1.19 indicating only limited association.

Table 1 shows the average number of nafcillin molecules adsorbed onto the protein as calculated from the difference between the molar mass of HSA (6.6×10^4 g mol^{-1}) and that of the complex, divided by the molar mass of nafcillin (454.5 g mol^{-1}). There is good agreement between the N_0 value derived by this method for the system containing 0.1 mol kg^{-1} nafcillin (735) and the equivalent value estimated from an analysis of conductivity data (739).

Second virial coefficients, A_2, which reflect deviations from ideality due to the existence of intermolecular interactions, were positive indicating that the effective interaction between complexes was repulsive. Table 1 gives values of the radius of the sphere, R_A, having the same excluded volume, calculated assuming the hard sphere model, from

$$R_A^3 = \frac{3A_2M_W^2}{16\pi N_A} \tag{9}$$

Decreases in R_A with increasing concentration of added drug (and increasing size of the complex) are a consequence of the decreasing charge of the drug/protein complex due to drug adsorption.

In this analysis of light scattering data it was assumed that the increase of scattering was attributable to an increase of complex size due entirely to the adsorption of nafcillin, any aggre-

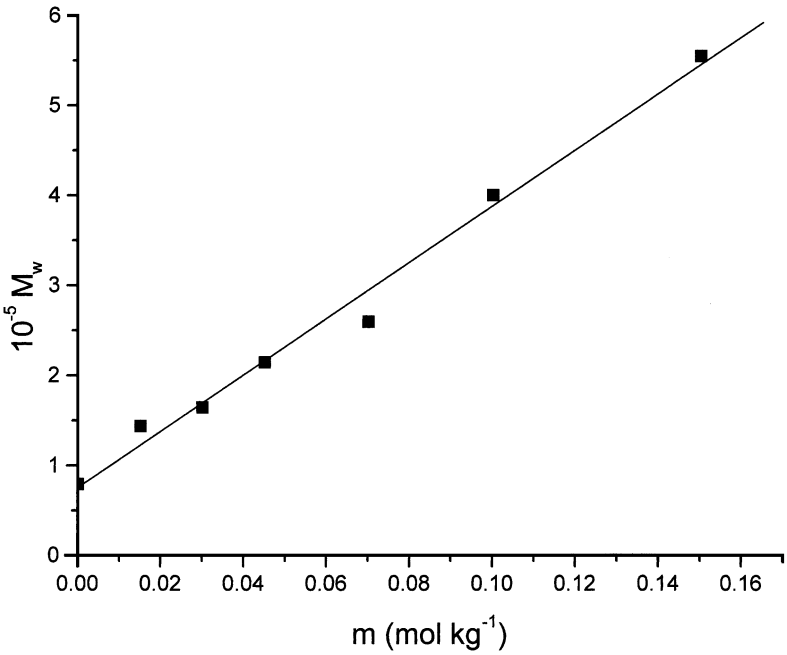

Fig. 4. Apparent molar mass, M_W, of the HSA (0.0625% w/v)/nafcillin complex as a function of nafcillin concentration at 25°C.

Table 1
Number of molecules, N_0, of nafcillin adsorbed onto HSA, and the hard sphere radii, R_A, of the HSA (0.0625%)/nafcillin complex from static light scattering data as a function of nafcillin concentration

[Nafcillin] (mol kg^{-1})	N_0	R_A (nm)
0.015	171	13.96
0.030	216	13.92
0.045	326	13.89
0.070	425	13.34
0.100	735 (739)[a]	12.62
0.150	1075	12.29

[a] Value in parentheses was obtained by conductivity.

gation of the complexes was assumed to be negligible. This seems a reasonable assumption since the complexes are highly negatively charged. Moreover, the sample polydispersity indexes (the variance divided by the square of the average) from dynamic light scattering measurements (below) for this system were very low, indicative of a reasonable degree of monodispersity of size. It is known that charged proteins pose additional problems when interpreting the light scattering data from aqueous systems composed of electrolyte and a diffusible species interacting with it. Such problems arise from the contribution of charged fluctuations to the intensity of the scattered light and the preferential interaction to the refractive index term $(\partial n/\partial m)$ in the optical constant of Eq. (8). The first problem is usually bypassed by operating at constant ionic strength, the second problem may be solved by operating at constant chemical potential, μ, of all diffusible species [22,23], including in this case nafcillin. In this study this was achieved by carrying out a dialysis equilibrium prior to the scattering experiment and $(\partial n/\partial m)_\mu$ measurements.

3.4. Dynamic light scattering

Apparent diffusion coefficients, D, of human serum albumin in aqueous solutions of nafcillin are presented in Fig. 5 as a function of protein

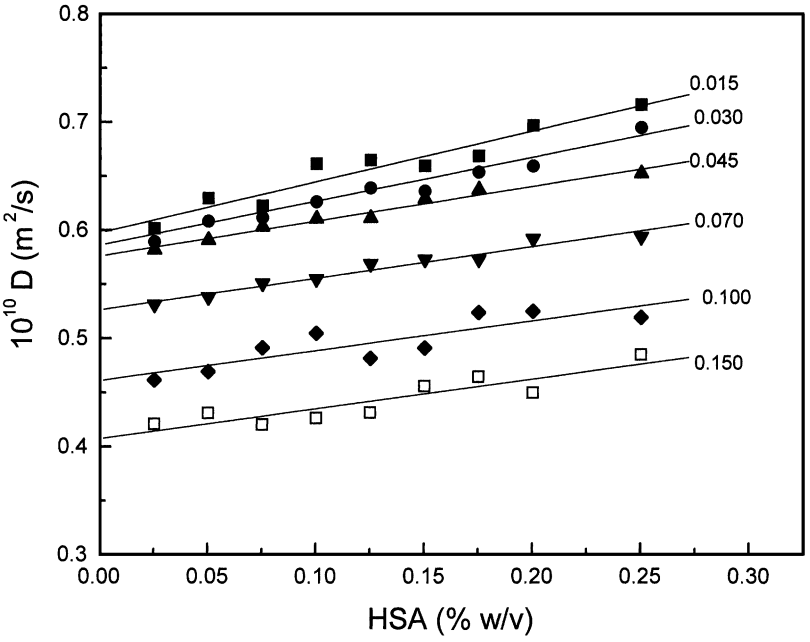

Fig. 5. Diffusion coefficient, D, as a function of the human serum albumin concentration in aqueous solutions of nafcillin at 25°C at the nafcillin concentrations (mol kg^{-1}) indicated.

Table 2
Limiting diffusion coefficient, D_0, hydrodynamic radius, R_h, experimental and theoretical k_D, and reduced potential, $e\Psi_0/k_BT$, of the HSA (0.0625%)/nafcillin complex as a function of nafcillin concentration

[Nafcillin] (mol kg^{-1})	10^{10} D_0 (m^2 s^{-1})	R_h (nm)	k_D		$e\Psi_0/k_BT$
			Exptl	Theor	
0.015	0.600	4.14	2.17	2.56	0.46
0.030	0.580	4.21	2.11	1.42	0.32
0.045	0.576	4.24	2.09	1.20	0.26
0.070	0.526	4.65	1.91	1.09	0.21
0.100	0.461	5.30	1.67	1.04	0.18
0.150	0.407	6.00	1.45	1.02	0.14

concentration. Experimental data were fitted with the linear function:

$$D = D_0[1 + k'_D c] \tag{10}$$

where D_0 is the limiting diffusion coefficient at zero HSA concentration and k'_D is an interaction coefficient. Hydrodynamic radii, R_h, were calculated from D_0, assuming sphericity, using the Stokes–Einstein equation

$$R_h = \frac{k_BT}{6\pi\eta D_0} \tag{11}$$

Table 2 shows the expected increase of the globular size with added nafcillin concentration

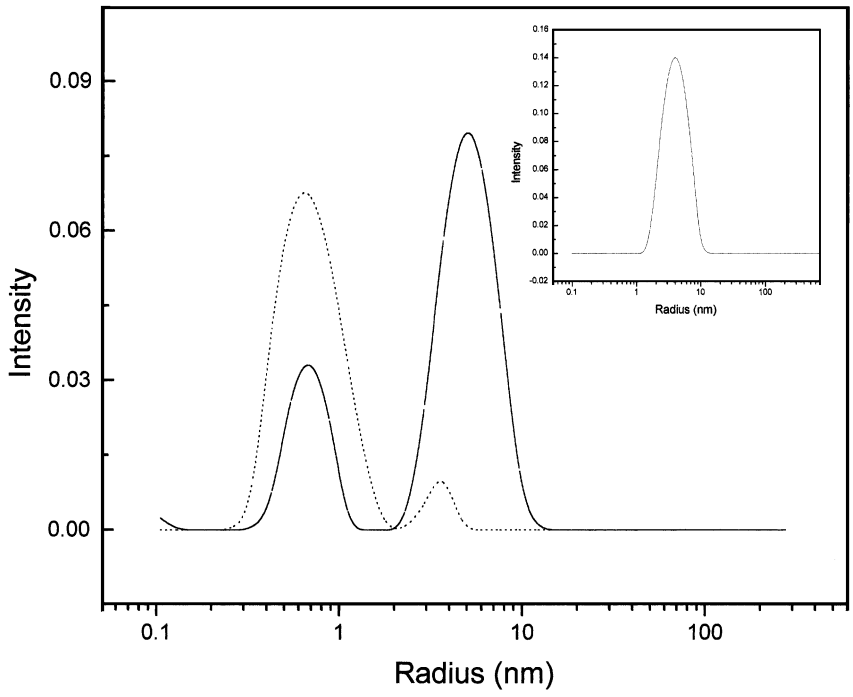

Fig. 6. Particle size distribution in aqueous solutions of nafcillin (0.15 mol kg^{-1}) and HSA. The solid line corresponds to a HSA concentration of 0.25% w/v, the dotted line to 0.025% w/v. The inset shows the corresponding plot for HSA in the absence of added nafcillin.

as the number of drug molecules adsorbed on the protein surface increases. Comparing these values of the radius with those calculated from the second virial coefficient, R_A, suggests strong electrostatic interactions between complexes. Similar behaviour was found with the system sodium dodecyl sulfate-lysozyme [24].

Table 2 shows that the thickness of the adsorbed layer of the HSA/nafcillin complex calculated from the difference between the particle hydrodynamic radius before and after adsorption (the hydrodynamic radius for the bare protein is approximately 3 nm) increased by between 1 and 3 nm with an increase of nafcillin concentration, in line with the observed increase in the number of adsorbed drug molecules.

The gradual change in hydrodynamic radius shown in Table 2 suggests a saturation process rather than a denaturation process, the binding isotherms for which are typically characterised by a sudden response to a small change in ligand concentration due to the creation of new binding sites. Moreover, the magnitude of this change is not sufficient to account for any appreciable extension or unfolding of the HSA molecule. The complex may resemble to some extent the 'necklace model' in which micelle-like clusters are envisaged to bind to a protein molecule [25]. In the present case, a complex consisting predominantly of one HSA molecule and micelle-like clusters around the hydrophobic patches of the protein backbone can be imagined, with little resultant expansion of the protein molecule.

In Fig. 6, we show the size distribution of the species present in solutions of nafcillin and HSA at HSA concentrations of 0.025 and 0.25% as derived by application of CONTIN analysis to the dynamic light scattering data. The nafcillin concentration was 0.15 mol kg^{-1} in both, a concentration well above the critical micelle concentration. The smaller sized aggregates of Fig. 6 correspond to micelles of nafcillin (radius 0.65 nm); the larger to the nafcillin/HSA complex. The figure shows both a decrease in the intensity of the micelle peak and an increase of size of the complex (compare also with the inset that shows HSA in the absence of nafcillin) as a consequence of the adsorption of nafcillin onto the protein. These results are in agreement with the higher value of the critical concentration of the penicillin in the presence of HSA, and suggest as expected, that adsorption onto the protein is energetically more favourable than the formation of aggregates.

3.5. Complex stability

To correlate experimental results of diffusion with the interactive forces between complexes, the data were analysed according to the treatment proposed by Corti and Degiorgio [26]. For interacting particles, the concentration dependence of the diffusion coefficient, D, can be expressed in terms of the volume fraction, ϕ, of the particles:

$$D = D_0(1 + k_D\phi) \tag{12}$$

where $k_D = k'_D/\bar{v}$ and $\bar{v}$ is the specific volume of the solute particles 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 [27]:

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

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

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

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:

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

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

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

which is appropriate for values of $\kappa a > 1$.

The aggregate charge, q, is related to the potential corresponding to the net charge of the particle, ψ_0, by the expression [28]

$$\psi_0 = \frac{2k_BT}{e}\sinh^{-1}\left(\frac{\pi e^2 \kappa^{-1} q}{2\pi a^2 \varepsilon k_B T}\right) \quad (17)$$

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. The value of q derived from Eq. (17) was 0.35 and the Hamaker constant was 4.11×10^{-23} J. Agreement between computed and experimental k_D values was reasonable in view of the assumptions inherent in these calculations (Table 2). Fig. 7 shows the reduced potential of the complex, $V(x)/k_BT$, for a series of nafcillin concentrations. Although electrostatic repulsion predominates at all nafcillin concentrations examined, the size of the primary maximum decreases with an increase of nafcillin concentration indicating a screening of the electrostatic potential and the increasing importance of London–Van der Waals attraction. The results show that the drug is acting as an electrolyte, compressing the electrical double layer and eventually causing instability of the dispersion.

Combining the surface potential, ψ_0, and the ζ potential it is possible to estimate the thickness of the adsorbed layer [29,30] (Δ) from:

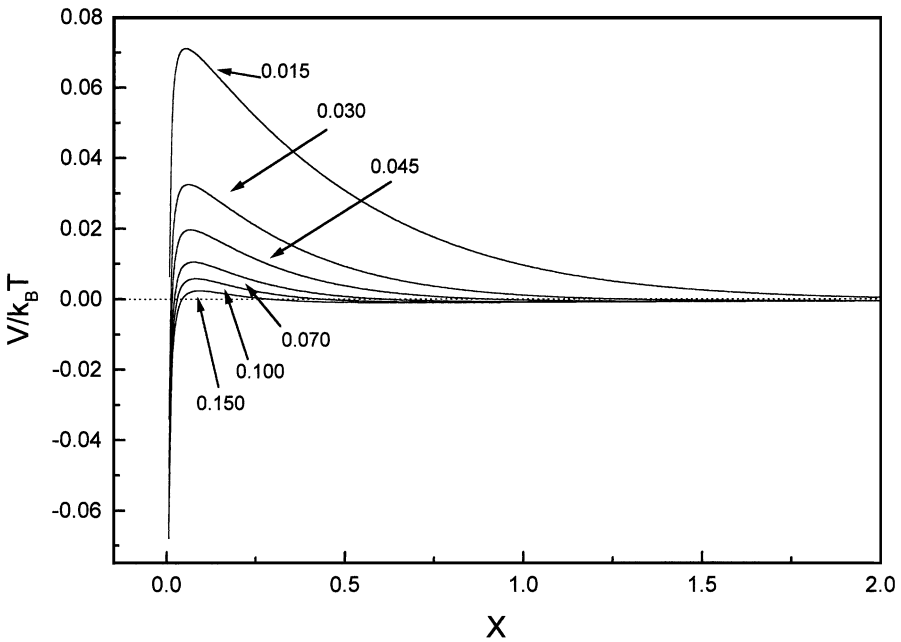

Fig. 7. Plots of the reduced pair interaction potential $V(x)/k_BT$ as a function of the reduced and normalised distance x at the nafcillin concentrations (mol kg^{-1}) indicated.

$$\mathrm{tgh}\left(\frac{ze\zeta}{4k_BT}\right) = \mathrm{tgh}\left(\frac{ze\Psi_0}{4k_BT}\right)\exp(-\kappa\Delta) \quad (18)$$

where z is the valence of the counterion. The estimated thickness varied from 1.3 to 2.0 nm for the lowest and highest concentrations of nafcillin, respectively. These values are in reasonable agreement with those calculated from dynamic scattering (1–3 nm).

The interaction potential can be related to the stability ratio W by the relationship [31]:

$$W = 2\int_0^{\infty} \frac{\exp(V/k_BT)}{(x+2)^2}\,\mathrm{d}x \quad (19)$$

It can be seen from Fig. 7 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 this maximum (V_m/k_BT), so Eq. (19) can be written as

$$W = \frac{2}{(x_m+2)^2}\int_0^{\infty} \exp(V/k_BT)\,\mathrm{d}x \quad (20)$$

Expanding V in a Taylor series around V_m, neglecting terms higher than two, Eq. (20) becomes

$$W = \frac{2\pi^{1/2}}{(x_m+2)}\exp\frac{V_m}{k_BT} \quad (21)$$

Values of V_m/k_BT and W obtained from Eq. (21) using a value of $x_m = 0.055$ (see Table 3) decrease with the increase of nafcillin concentration indicating a decreased stability of the albumin complex as nafcillin is adsorbed.

Table 3
Maximum values of the reduced interaction potential, V_m/k_BT, and stability ratio, W, of the HSA (0.0625%)/nafcillin complex in aqueous electrolyte solution as a function of nafcillin concentration

[Nafcillin] (mol kg^{-1})	V_m/k_BT	W
0.015	0.071	0.95
0.030	0.033	0.92
0.045	0.020	0.90
0.070	0.010	0.89
0.100	0.006	0.89
0.150	0.002	0.88

4. Summary

The results presented in this study have characterised the nature of the interaction between the globular anionic protein human serum albumin (HSA) and the anionic drug nafcillin and have relevance for the transport of this surface active drug in the blood stream. Changes in the zeta potential and conductivity of nafcillin/HSA solutions as a function of drug concentration have indicated hydrophobic adsorption of this anionic amphiphilic drug on to the surface of HSA. Saturation of the protein surface occurred at drug concentrations of approximately 12 mmol kg^{-1} with a further increase of nafcillin concentration leading to the formation of drug aggregates in solution. The gradual change in hydrodynamic radius of the complex with increasing drug concentration is indicative of a saturation rather than a denaturation process, the magnitude of this change being insufficient to account for any appreciable extension or unfolding of the HSA molecule. 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 an increase in the concentration of the added drug.

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