# Surface Behavior of Aqueous Protein + Penicillin Solutions

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The interactions between human serum albumin (HSA) and two anionic amphiphilic penicillins, sodium cloxacillin and dicloxacillin, in aqueous solution of pH 4.5 (ionic strength 0.05 M) and 7.4 (ionic strength 0.17 M) at 298.15 K have been determined by surface tension measurements. The surface behavior for both penicillins in such buffered solutions was also analyzed for comparison. Critical micelle concentrations (cmc's) for both drugs were obtained. Measurements in the presence of a mass of HSA per unit volume of 0.125% were carry out under conditions where the protein molecule was positively or negatively charged. In the absence of HSA and for both pHs, lower values of the cmc for dicloxacillin reflect its greater hydrophobicity. The presence of the protein in solution resulted in a decrease of the surface tension and in an increase of the cmc's of the penicillins, suggesting the formation of surface-active complexes. Their adsorptions at the air/solution interface have been calculated and compared.

## 1. Introduction

Adsorption of proteins onto interfaces plays a fundamental role in many biological and technological phenomena.[1−3] Human serum albumin (HSA) is the most abundant protein in the circulatory system, accounting for 60% of the total serum protein,[4,5] and it has been one of the most studied proteins for over 40 years. Its primary structure is well-known, and its tertiary structure was determined a few years ago by X-ray crystallography.[6] HSA is a globular protein, which consists of 585 amino acids in a single polypeptide chain with a molar mass of 66 411 g·mol$^{-1}$ and an isoelectric point of 4.9,[8] being widely used as a model protein to study the interactions between proteins and surfactants.

The anionic penicillins cloxacillin and dicloxacillin were found to bind extensively to human serum albumin in aqueous solution.[8,9] Our previous studies on the physicochemical properties of these penicillins[10−12] have characterized the self-aggregation process in aqueous solution as a function of electrolyte concentration and temperature. The cloxacillin and dicloxacillin structures differ only in an additional chlorine atom on the phenyl ring of dicloxacillin (see Chart 1).

In the present work, the adsorption of cloxacillin and dicloxacillin in the absence and in the presence of a mass of HSA per unit volume of 0.125% at pH 4.5 ($I$ = 0.05 M) and 7.4 ($I$ = 0.17 M), that is, below and above the isoelectric point of the protein (4.9), has been examined by surface tension measurements. The change in pH alters the charge distribution within protein molecules and, hence, also the electrostatic interaction within the adsorbed layer and the degree of hydration of the protein molecules at the interface.

## 2. Experimental Section

**Materials.** Human serum albumin, [HSA (70024-90-7)], sodium cloxacillin monohydrate ([5-methyl-3-(*o*-chlorophenyl)-4-isoxazolyl]penicillin), and sodium dicloxacillin monohydrate ([3-(2,6-dichlorophenyl)-5-methyl-α-isoxazolyl]penicillin) were obtained from Sigma Chemical Company.

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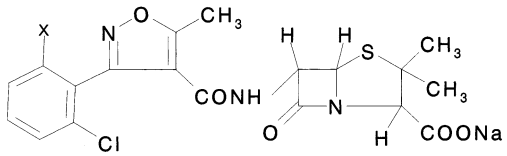
**Chart 1**




CLOXACILINA X=H
DICLOXACILINA X=Cl

Experiments were carried out using buffered solutions of sodium acetate (20 mmol·dm$^{-3}$) + acetic acid (20 mmol·dm$^{-3}$) for pH 4.5 ($I$ = 0.05 M) and phosphate buffered saline tablets for pH 7.4 ($I$ = 0.17 M). Water was double-distilled and deionized before use.

**Surface Tension.** Measurements were made by the Wilhelmy plate method using a Kruss K-12 surface tension equipment, equipped with a processor to acquire the data automatically. The equipment was connected to a HETO circulating water bath with a proportional temperature controller to keep the temperature constant at (25.0 ± 0.1) °C. Penicillin or penicillin−HSA solutions of known molality were progressively diluted with buffer or buffer−HSA solutions using an automatic pump (Dosimat 665 Metrohm). The usual precautions were taken to ensure cleanliness. The surface tension of the buffers was regularly measured in order to check the experimental technique. To ensure thermodynamic equilibrium at the air/solution interface, penicillin solutions in the presence of HSA were left for 90 min prior to measurements. In the absence of protein, 10 to 15 min was enough to reach equilibrium. Measurements of both drugs were very limited at pH 4.5 in the presence of HSA due to precipitation.

Surface tension values of the buffers were (72.70 and 73.10 ± 0.05) mN·m$^{-1}$ for pH values 4.5 and 7.4, respectively, and those for a mass of HSA per unit volume of 0.125% in such buffered solutions were (53.50 and 56.00 ± 0.05) mN·m$^{-1}$ at pH 4.5 and 7.4, respectively.

## 3. Results and Discussion

**3.1. Penicillins in Buffered Solution.** Although the surface activities of both drugs in aqueous solution had

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**Table 1. Surface Tension Values, $\gamma$,[a] for Cloxacillin at pH 4.5 and pH 7.4 at 25 °C**

| pH 4.5 | | pH 7.4 | |
|---|---|---|---|
| $\gamma$ / mN·m$^{-1}$ | $m$ / mol·kg$^{-1}$ | $\gamma$ / mN·m$^{-1}$ | $m$ / mol·kg$^{-1}$ |
| 68.20 | 0.0003 | 66.78 | 0.0003 |
| 68.00 | 0.0005 | 66.87 | 0.0005 |
| 67.86 | 0.0007 | 67.14 | 0.0006 |
| 67.63 | 0.0008 | 66.31 | 0.0010 |
| 67.37 | 0.0010 | 65.22 | 0.0024 |
| 66.10 | 0.0015 | 62.73 | 0.0045 |
| 64.75 | 0.0020 | 60.98 | 0.0060 |
| 63.97 | 0.0024 | 58.41 | 0.0092 |
| 60.76 | 0.0045 | 57.19 | 0.0111 |
| 59.04 | 0.0060 | 55.85 | 0.0158 |
| 58.20 | 0.0071 | 53.68 | 0.0240 |
| 57.00 | 0.0085 | 50.32 | 0.0400 |
| 54.30 | 0.0120 | 49.11 | 0.0570 |
| 49.42 | 0.0250 | 48.00 | 0.0752 |
| 46.38 | 0.0455 | 48.01 | 0.1220 |
| 45.20 | 0.0550 | 47.79 | 0.1440 |
| 43.86 | 0.0920 | 47.94 | 0.1670 |
| 43.36 | 0.1300 | 47.73 | 0.2010 |
| 43.40 | 0.1530 | 47.92 | 0.2610 |
| 43.44 | 0.1600 | | |
| 43.50 | 0.1800 | | |
| 43.46 | 0.2010 | | |

[a] Uncertainties in $\gamma$ are ±0.05 mN·m$^{-1}$.

**Table 2. Surface Tension Values, $\gamma$,[a] for Dicloxacillin at pH 4.5 and 7.4 and 25 °C**

| pH 4.5 | | pH 7.4 | |
|---|---|---|---|
| $\gamma$ / mN·m$^{-1}$ | $m$ / mol·kg$^{-1}$ | $\gamma$ / mN·m$^{-1}$ | $m$ / mol·kg$^{-1}$ |
| 68.64 | 0.0003 | 64.00 | 0.0009 |
| 68.17 | 0.0004 | 63.31 | 0.0015 |
| 66.38 | 0.0006 | 61.60 | 0.0025 |
| 64.32 | 0.0008 | 57.94 | 0.0045 |
| 62.13 | 0.0009 | 55.75 | 0.0060 |
| 58.73 | 0.0015 | 52.37 | 0.0076 |
| 54.51 | 0.0030 | 51.59 | 0.0091 |
| 52.07 | 0.0045 | 48.89 | 0.0151 |
| 50.49 | 0.0060 | 48.41 | 0.0240 |
| 48.42 | 0.0096 | 49.36 | 0.0300 |
| 48.38 | 0.0152 | 48.61 | 0.0414 |
| 48.18 | 0.0252 | 48.81 | 0.0710 |
| 48.09 | 0.0302 | 48.75 | 0.0890 |
| | | 49.03 | 0.1120 |
| | | 49.03 | 0.1310 |
| | | 49.09 | 0.1570 |
| | | 49.30 | 0.1800 |

[a] Uncertainties in $\gamma$ are ±0.05 mN·m$^{-1}$.

been previously studied,[11] no data were available in buffered solutions at the ionic strength used in this study, which is required for the determination of the influence of albumin on the surface activity. Surface tension data for cloxacillin and dicloxacillin penicillins at pH 4.5 and 7.4 at 298.15 K are displayed in Tables 1 and 2. To obtain the critical micelle concentrations, the surface pressure, $\pi$ ($\pi = \gamma_0 - \gamma$, where $\gamma_0$ is the surface tension of the solvent and $\gamma$ is that of the solution), was plotted against logarithm of molality for both drugs at each pH (see Figures 1 and 2 for cloxacillin and dicloxacillin, respectively). The critical micelle concentrations were calculated as the intersection points of the linear fits corresponding at each branch of the $\pi$–log $m$ curves. Lower values of critical micelle concentrations (cmc's) and surface pressure at the cmc, $\pi_{cmc}$ (see Table 3), for dicloxacillin reflect the greater hydrophobicity of this drug compared with cloxacillin due to the

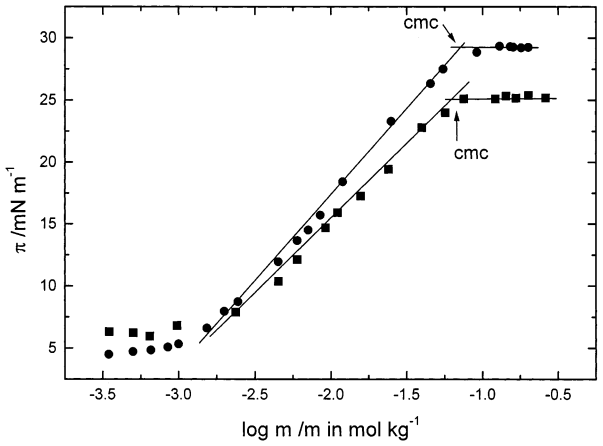


**Figure 1.** Surface pressure, $\pi$, versus logarithm of molality, $m$, for cloxacillin at (●) pH 4.5 and (■) pH 7.4. The arrow denotes the critical micelle concentration (cmc).

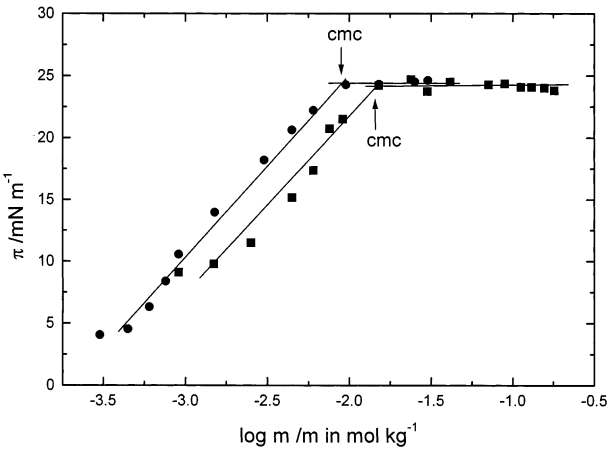


**Figure 2.** Surface pressure, $\pi$, versus logarithm of molality, $m$, for dicloxacillin at (●) pH 4.5 and (■) pH 7.4. The arrow denotes the critical micelle concentration (cmc).

**Table 3. Critical Micelle Concentrations, cmc, and Surface Tension at the cmc, $\gamma_{cmc}$, of Cloxacillin and Dicloxacillin at pH 4.5 and 7.4 and 25 °C**

| | cloxacillin | | dicloxacillin | |
|---|---|---|---|---|
| | cmc[a] / mol·kg$^{-1}$ | $\gamma_{cmc}$[b] / mN·m$^{-1}$ | cmc[a] / mol·kg$^{-1}$ | $\gamma_{cmc}$[b] / mN·m$^{-1}$ |
| pH 4.5 | 0.073 | 44.9 | 0.010 | 48.5 |
| pH 7.4 | 0.060 | 47.5 | 0.012 | 48.2 |

[a] Estimated uncertainties ±10%. [b] Estimated uncertainties ±0.05 mN·m$^{-1}$.

presence of an extra Cl atom in the hydrophobic benzene ring of its molecular structure.

The Gibbs surface excess concentrations, $\Gamma_2$, of the penicillins at the air/buffer solution interface (see Table 4) were calculated, assuming ideality, using the Gibbs equation, which in the presence of excess electrolyte may be written as

$$\Gamma_2 = -\frac{1}{2.303xRT}\frac{d\gamma}{d\log m} \quad (1)$$

where $R$ is the gas constant, $T$ is the absolute temperature, and the variable $x$ is introduced to allow the simultaneous adsorption of cations and anions. The expression used in the calculation of $x$ was that proposed by Matijevic and Pethica,[13] $x = 1 + m/(m + m_s)$, where $m_s$ is the concentra-

**Table 4. Gibbs Surface Excess Concentration, $\Gamma_2$, and Minimun Area Per Molecule, *A*, of Cloxacillin and Dicloxacillin at pH 4.5 and 7.4 and 25 °C[a]**

| | cloxacillin | | dicloxacillin | |
|---|---|---|---|---|
| | $10^6\Gamma_2$ | $A$ | $10^6\Gamma_2$ | $A$ |
| | mol·m$^{-2}$ | nm$^2$ | mol·m$^{-2}$ | nm$^2$ |
| pH 4.5 | 2.42 | 0.69 | 2.61 | 0.64 |
| pH 7.4 | 1.90 | 0.87 | 2.98 | 0.56 |

[a] Estimated uncertainties ±15%.

**Table 5. Surface Tension Values, $\gamma$,[a] for Cloxacillin in the Presence of a Mass of HSA per Unit Volume of 0.125% at pH 4.5 and 7.4 and 25 °C**

| pH 4.5 | | pH 7.4 | |
|---|---|---|---|
| $\gamma$ | $m$ | $\gamma$ | $m$ |
| mN·m$^{-1}$ | mol·kg$^{-1}$ | mN·m$^{-1}$ | mol·kg$^{-1}$ |
| 51.3 | 0.0001 | 56.0 | 0.0008 |
| 51.0 | 0.0002 | 55.7 | 0.0015 |
| 50.4 | 0.0003 | 55.3 | 0.0020 |
| 50.1 | 0.0004 | 54.7 | 0.0027 |
| 49.7 | 0.0005 | 53.3 | 0.0035 |
| 49.1 | 0.0006 | 52.5 | 0.0045 |
| 48.9 | 0.0008 | 52.7 | 0.0060 |
| 49.0 | 0.0009 | 52.8 | 0.0076 |
| 49.1 | 0.0012 | 53.9 | 0.0082 |
| 49.3 | 0.0014 | 54.5 | 0.0100 |
| 49.5 | 0.0020 | 53.2 | 0.0160 |
| 49.7 | 0.0025 | 52.1 | 0.0200 |
| 49.8 | 0.0030 | 50.7 | 0.0300 |
| 49.8 | 0.0035 | 49.5 | 0.0390 |
| 49.3 | 0.0041 | 47.9 | 0.0500 |
| 48.4 | 0.0045 | 46.4 | 0.0580 |
| 47.6 | 0.0055 | 45.5 | 0.0710 |
| 47.0 | 0.0066 | 44.7 | 0.0790 |
| 46.5 | 0.0076 | 44.2 | 0.0910 |
| 45.7 | 0.0081 | 43.8 | 0.1450 |
| | | 43.7 | 0.2130 |

[a] Uncertainties in $\gamma$ are ±0.05 mN·m$^{-1}$.

tion of the added electrolyte. Thus, $x$ has a value of 2 in water and approaches 1 in the presence of excess inert electrolyte. The values of $\Gamma_2$ so obtained are shown in Table 4. Changes in the minimum surface area per molecular headgroup at the air/buffer solution interface, $A$, were evaluated from

$$A = \frac{1}{N_A \Gamma_2} \qquad (2)$$

where $N_A$ is Avogadro's number. The data (see Table 4) show the expected decrease of $A$ with the increase of buffer ionic strength due to a progressive charge shielding and a closer packing of the drug ions on the surface. The values of $A$ obtained are similar to those reported for other drug molecules: the phenothiazine drugs, for example, have areas per molecule of between 0.66 and 0.77 nm,[2,14] and antidepressant drugs have values between 0.77 and 0.96 nm$^2$.[15]

**3.2. Penicillin−HSA Solutions.** Surface tension data of cloxacillin and dicloxacillin in the presence of a mass of HSA per unit volume of 0.125% at pH 4.5 and 7.4 at 25 °C are shown in Tables 5 and 6, respectively. Figure 3 shows $\gamma$−log $m$ plots for cloxacillin in the presence of HSA at pHs 4.5 and 7.4. Similar plots were obtained for dicloxacillin (not shown). For pH 7.4, the plot presents four distinct regions, named as A, B, C, and D. In zone A, the lowering of surface tension at low drug concentration is related to the adsorption of penicillin−protein ions onto the air/buffer solution interface. Comparison with Figure 1 shows a much lower surface tension in this region compared to that of cloxacillin at pH 7.4 at similar concentrations, supporting the formation of surface-active complexes, as mentioned previously. The sharp inflection at the beginning of region B is referred to as the critical aggregation concentration (cac), which is taken as the concentration at which cooperative binding of ions to the protein starts. Values of cac for cloxacillin and dicloxacillin are given in Table 7. The plateau observed in Figure 3 may be attributed to the preferential adsorption of drug at the protein rather than at the air/solution interface. The increase of surface tension at higher concentrations may be a consequence of dissolution of complexes in the bulk solution from the air/solution interface. The decrease of surface tension in region C represents the saturation of the protein surface and adsorption of excess drug ions at the air/buffer solution interface. Finally, inflection in region D is a consequence

**Table 6. Surface Tension Values, $\gamma$,[a] for Dicloxacillin in the Presence of a Mass of HSA per Unit Volume of 0.125% at pH 4.5 and 7.4 and 25 °C**

| pH 4.5 | | pH 7.4 | |
|---|---|---|---|
| $\gamma$ | $m$ | $\gamma$ | $m$ |
| mN·m$^{-1}$ | mol·kg$^{-1}$ | mN·m$^{-1}$ | mol·kg$^{-1}$ |
| 50.3 | 0.0003 | 53.6 | 0.0015 |
| 49.6 | 0.0004 | 52.2 | 0.0020 |
| 49.4 | 0.0005 | 51.2 | 0.0025 |
| 49.1 | 0.0006 | 49.9 | 0.0030 |
| 48.9 | 0.0007 | 49.9 | 0.0040 |
| 48.7 | 0.0008 | 49.8 | 0.0050 |
| 48.5 | 0.0009 | 49.8 | 0.0060 |
| 48.2 | 0.0010 | 50.2 | 0.0080 |
| 48.4 | 0.0012 | 50.8 | 0.0090 |
| 48.2 | 0.0013 | 51.2 | 0.0100 |
| 48.2 | 0.0015 | 51.1 | 0.0120 |
| 48.3 | 0.0017 | 49.9 | 0.0150 |
| 48.3 | 0.0020 | 49.0 | 0.0200 |
| 48.3 | 0.0025 | 46.9 | 0.0280 |
| 48.3 | 0.0031 | 46.1 | 0.0355 |
| 48.2 | 0.0041 | 45.9 | 0.0450 |
| 48.2 | 0.0050 | 45.6 | 0.0600 |
| 48.2 | 0.0060 | 45.6 | 0.0795 |
| | | 45.6 | 0.0890 |
| | | 45.5 | 0.1000 |
| | | 45.4 | 0.1200 |
| | | 45.6 | 0.1440 |

[a] Uncertainties in $\gamma$ are ±0.05 mN·m$^{-1}$.

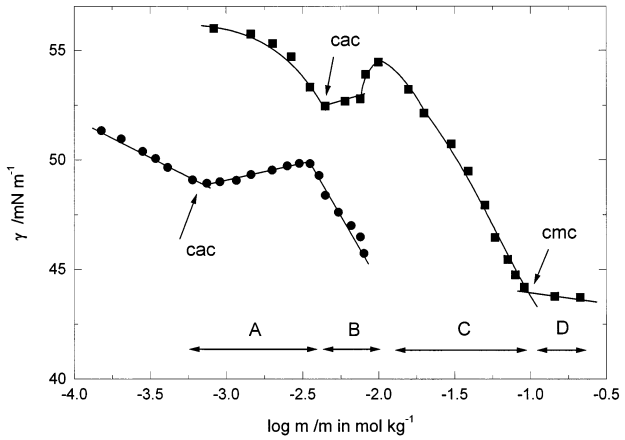


**Figure 3.** Surface tension, $\gamma$, versus logarithm of molality, $m$, for cloxacillin in the presence of a mass of HSA per unit volume of 0.125% at (●) pH 4.5 and (■) pH 7.4. The arrow denotes the critical micelle concentration (cmc) and the critical aggregation concentration (cac).

**Table 7. Critical Micelle Concentration, cmc, and Critical Aggregation Concentration, cac, of Cloxacillin and Dicloxacillin in the Presence of a Mass of HSA per Unit Volume of 0.125% at pH 4.5 and 7.4 and 25 °C[a]**

| | cloxacillin | | dicloxacillin | |
|---|---|---|---|---|
| | $10^3$(cac) | cmc | $10^3$(cac) | cmc |
| | mol·kg$^{-1}$ | mol·kg$^{-1}$ | mol·kg$^{-1}$ | mol·kg$^{-1}$ |
| pH 4.5 | 0.7 | precipitate | 1.0 | precipítate |
| pH 7.4 | 4.0 | 0.092 | 3.1 | 0.039 |

[a] Estimated uncertainties ±10%.

of the formation of free penicillin micelles in solution as a consequence of completion of the Gibbs monolayer at the solution surface. At pH 4.5, a distinct behavior is observed as a consequence of the positive charge of the protein at this pH. Therefore, electrostatic interactions are established between penicillin and protein molecules, leading to a higher number of penicillin molecules bound to the protein and, thus, giving rise to precipitation of the complexes at concentrations below the critical micelle concentration. The critical micelle concentrations and critical aggregation concentrations for cloxacillin and dicloxacillin in the presence of a mass of HSA per unit volume of 0.125% are shown in Table 7.

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