

Effects of Antibiotics on the Surface Properties of Bovine Serum Albumin Solutions

Chang Tan *

School of Education, University of Barcelona, Barcelona 08035, Spain

* Corresponding Author Email: tanchanges@163.com

Abstract. Antibiotics, as widely used antibacterial agents, are commonly delivered through the bloodstream, where they bind to plasma proteins and may alter the surface and rheological properties of blood, thereby influencing drug transport and absorption. Serum albumin, the most abundant carrier protein in plasma, is a well-established model for studying drug–protein interactions. Taking bovine serum albumin (BSA) as the research subject, this study measured the surface tension and contact angle on a silicon nitride substrate of mixed solutions of BSA with three classes of antibiotics: quinolones (sparfloxacin, ofloxacin, gatifloxacin), tetracyclines (oxytetracycline, doxycycline, chlortetracycline hydrochloride), and macrolides (erythromycin, erythromycin ethylsuccinate, azithromycin) at two concentrations. The solid surface free energy of silicon nitride was calculated using the Fowkes, OWRK, and Wu methods, and the solid–liquid interfacial tension was obtained via the Young equation. Results show that quinolones exert little influence on the surface properties of BSA solutions; tetracyclines mainly increase surface tension through competitive adsorption and electrostatic effects but do not alter the contact angle; macrolides, owing to the strong hydrophobicity of their 14-membered lactone ring, enhance BSA hydrophobicity, reduce surface tension at high concentration, and lower the solid–liquid interfacial tension on silicon nitride. The findings indicate that hydrophilic–hydrophobic interactions play a decisive role in shaping the surface behavior of antibiotic–BSA systems.

Keywords: Bovine serum albumin, antibiotics, surface tension, contact angle, interfacial tension.

1. Introduction

Proteins are amphiphilic biomacromolecules carrying both polar and non-polar groups, and therefore exhibit pronounced surface activity. When dissolved in water, proteins spontaneously adsorb at interfaces because such adsorption lowers the total free energy of the system: hydrophobic residues are driven to the air side while hydrophilic residues face the aqueous phase. Miller et al. described this process with a three-step model in which protein molecules first move from the bulk solvent to a sub-interface region, then flip onto the interface, and finally undergo conformational rearrangement in response to the altered molecular environment [1].

Once adsorbed, proteins can unfold, aggregate, and even gel, which often reduces their biological activity, alters their pharmacokinetic profile, decreases target-binding affinity, and may trigger immunogenic reactions. Surface tension is therefore a sensitive probe of the structural state of a protein at an interface. McClellan and Franses showed that the interfacial density of BSA rises with concentration, while changes in surface tension remain modest, indicating reorientation rather than further packing of molecules at the air/water interface [2]. Xiao et al. further demonstrated, using exogenous fluorescent probes and steady-state fluorescence spectroscopy, that the surface tension of a protein solution correlates negatively with surface hydrophobicity ($r \approx -0.70$, $P < 0.05$), so that additives lowering surface tension also tend to promote aggregation [3].

In the human body, drugs are transported through the bloodstream, where serum albumin is the principal binding partner. Most drugs bind reversibly and extensively to albumin, and the nature of this interaction strongly modulates their pharmacological activity. When drugs perturb the interfacial behavior of plasma proteins, they may alter blood surface properties, induce protein aggregation, and ultimately interfere with drug transport and absorption.

Antibiotics are widely used in clinical practice and daily life owing to their bacteriostatic or bactericidal activity through inhibition of cell-wall synthesis, disruption of membrane permeability,

or interference with protein and nucleic acid synthesis. Kamat and Seetharamappa, for example, studied the in vitro interaction between tricyclic compounds and BSA and showed that drug binding can significantly modify the conformation and microenvironment of the protein [4]. Although a substantial body of work has examined drug–protein binding from a spectroscopic or thermodynamic perspective, the interfacial response of BSA to small antibiotic molecules has received comparatively little attention.

To address this gap, the present study takes BSA as a model protein and systematically investigates how three classes of antibiotics—quinolones, tetracyclines, and macrolides—affect the surface properties of BSA solutions. By measuring the surface tension of BSA–antibiotic mixtures and their contact angles on silicon nitride substrates, and by calculating the solid surface free energy and the solid–liquid interfacial tension, the work aims to clarify the role of molecular structure, hydrophobicity, and charge in mediating antibiotic–protein interfacial behavior, and to provide a reference for understanding how antibiotics may influence drug transport in vivo.

2. Materials and Methods

2.1 Experimental Design

This study adopted a comparative experimental design to examine how antibiotics from three different chemical classes affect the surface properties of BSA solutions. The design was structured around three measurable indicators: surface tension of the mixed solution, contact angle on a silicon nitride substrate, and the derived solid–liquid interfacial tension obtained at both a low antibiotic concentration (0.005 mg/mL) and a high concentration corresponding to a drug-to-BSA molar ratio of 1:1. This dual-concentration scheme was chosen because the low value is comparable to the plasma concentration observed about ten minutes after intravenous administration, while the high value allows the saturation behavior of drug–BSA binding to be probed.

2.2 Reagents and Instruments

Reagents: bovine serum albumin (BSA); phosphate-buffered saline (PBS, pH $\approx$ 7.4); silicon nitride wafers; ultrapure water; diiodomethane; ethylene glycol; quinolones (ofloxacin, gatifloxacin, sparfloxacin); tetracyclines (chlortetracycline hydrochloride, doxycycline, oxytetracycline); and macrolides (erythromycin, erythromycin ethylsuccinate, azithromycin). Instruments: an ultrasonic cleaner; a drying oven; an SDC-200S contact-angle measuring apparatus; an electronic analytical balance; 1 mL syringes equipped with stainless-steel needles of 1.6 mm and 0.5 mm outer diameter for surface-tension and contact-angle measurements, respectively; Petri dishes; tweezers; and test tubes.

2.3 Preparation of Solutions

Because the serum-albumin concentration in human blood lies in the range 35–50 mg/mL, a BSA concentration of 40 mg/mL was used throughout. A stock BSA solution was prepared by dissolving 40 mg of BSA in 1 mL of PBS. Stock solutions of each antibiotic (10 mg/mL) were prepared by sonicating 10 mg of drug in 10 mL of PBS until fully dissolved. For low-concentration mixtures, 5 μ L of each antibiotic stock was added to 1 mL of BSA solution to obtain 0.005 mg/mL antibiotic–BSA mixtures. For high-concentration mixtures, drug stock volumes were adjusted so that the antibiotic-to-BSA molar ratio was 1:1, with PBS added to bring the total volume to 1 mL.

2.4 Surface-Tension Measurement

Surface tension was determined by the pendant-drop method using the SDC-200S apparatus and its accompanying software. A syringe filled with the sample solution was fitted with a 1.6 mm needle and mounted on the holder. Air was first expelled by continuous dripping, after which a droplet was formed by manual control until a white spot appeared at the top of the drop, indicating equilibrium.

The software then computed the surface tension from the drop profile. Each sample was measured several times to reduce random error; humidified ambient air was maintained to prevent evaporation, and droplets were generated 1 cm above the liquid surface. All measurements were performed at 293 K and standard atmospheric pressure.

2.5 Contact-Angle Measurement

Contact angles were measured with the same instrument operated in sessile-drop mode using a 0.5 mm needle. A cleaned silicon nitride wafer was placed on the stage; a 2 μ L droplet of the test solution was dispensed and brought into contact with the substrate by raising the stage. After baseline selection and drop fitting in the software, the contact angle on each side of the droplet was recorded. Between samples, the substrate was rinsed with ultrapure water and dried; needles and syringes were replaced. Each measurement was repeated to limit random error. Measurements were carried out at 293 K and standard atmospheric pressure.

2.6 Calculation of Solid–Liquid Interfacial Tension

When a liquid drop reaches equilibrium on a solid surface, the three interfacial tensions satisfy the Young equation:

$$\gamma_{lv}\cos\theta = \gamma_{sv} - \gamma_{sl} \quad (1)$$

Where γ denotes interfacial tension and the subscripts l, v, and s refer to the liquid, vapor, and solid phases respectively, and θ is the contact angle. Because γ_{sv} cannot be measured directly, it was estimated using three approaches based on the dispersion and polar components of surface tension,

$$\gamma = \gamma^d + \gamma^p \quad (2)$$

$$\gamma_{lv}(1 + \cos\theta) = 2\sqrt{\gamma_{sv}^d\gamma_{lv}^d} \quad (3)$$

$$\gamma_{lv}(1 + \cos\theta) = 2\sqrt{\gamma_{sv}^d\gamma_{lv}^d} + 2\sqrt{\gamma_{sv}^p\gamma_{lv}^p} \quad (4)$$

$$\gamma_{lv}(1 + \cos\theta) = 4\left(\frac{\gamma_{sv}^d\gamma_{lv}^d}{\gamma_{sv}^d + \gamma_{lv}^d} + \frac{\gamma_{sv}^p\gamma_{lv}^p}{\gamma_{sv}^p + \gamma_{lv}^p}\right) \quad (5)$$

Water and diiodomethane were used as reference liquids. Their measured surface tensions and contact angles on silicon nitride were substituted into Eqs. (2)–(4) to obtain γ_{sv}^d , γ_{sv}^p and γ_{sv} . Once γ_{sv} was known, γ_{sl} for each antibiotic–BSA mixture on silicon nitride was obtained from Eq. (1).

3. Results

The measured surface tensions and contact angles on silicon nitride for BSA, low-concentration mixtures, and 1:1 molar mixtures are summarized in Tables 1 and 2. The solid surface free energy of silicon nitride calculated from the reference liquids is given in Table 3, and the corresponding solid–liquid interfacial tensions are compiled in Table 4.

Table 1. Surface properties of antibiotic–BSA mixtures at 0.005 mg/mL (low concentration)

Group	BSA	Sparfloxacin	Ofloxacin	Gatifloxacin	Erythromycin
Surface tension (mN/m)	51.44 (± 1.01)	53.65 (± 0.96)	52.77 (± 1.19)	52.14 (± 1.77)	53.81 (± 0.79)
Contact angle ($^\circ$)	80.02 (± 0.49)	79.59 (± 1.01)	79.78 (± 0.86)	80.39 (± 1.11)	79.59 (± 0.99)
Surface tension (mN/m)	52.72 (± 1.19)	52.83 (± 0.76)	53.77 (± 1.33)	54.33 (± 1.35)	51.75 (± 1.70)
Contact angle ($^\circ$)	81.03 (± 1.16)	79.76 (± 1.03)	80.42 (± 0.41)	79.66 (± 1.13)	79.71 (± 1.49)

Table 2. Surface properties of antibiotic–BSA mixtures at a 1:1 molar ratio (high concentration)

Group	Eryth. ethylsucc.	Azithromycin	Oxytetracycline	Doxycycline	Chlortetracycline
Surface tension (mN/m)	53.65 (±0.96)	53.21 (±1.19)	52.68 (±1.07)	52.61 (±0.79)	Surface tension (mN/m)
Contact angle (°)	79.87 (±1.59)	79.33 (±1.15)	79.38 (±1.09)	75.87 (±0.82)	Contact angle (°)
Surface tension (mN/m)	49.70 (±0.32)	51.40 (±1.55)	53.83 (±1.54)	53.70 (±1.08)	52.80 (±1.01)
Contact angle (°)	75.82 (±1.17)	75.39 (±1.06)	80.50 (±1.18)	80.36 (±1.84)	79.53 (±1.35)

3.1 Surface Tension

The surface tension of the 40 mg/mL BSA solution was 51.44 mN/m, slightly below the value of about 52.7 mN/m reported in the literature for 10 mg/mL BSA, consistent with the higher concentration used here. The measured values for PBS and water were 71.19 mN/m and 72.04 mN/m, respectively, confirming that BSA significantly lowers the surface tension of the aqueous medium owing to its amphiphilic character.

At 0.005 mg/mL, all nine antibiotic–BSA mixtures exhibited surface tensions higher than that of pure BSA, with increments of 1–4 mN/m. The doxycycline mixture showed the highest value (54.33 mN/m) and the chlortetracycline mixture the lowest (51.75 mN/m). When the concentration was increased to a 1:1 molar ratio, the three quinolones and the tetracyclines oxytetracycline and chlortetracycline remained essentially unchanged. By contrast, all three macrolides showed lower surface tension at the higher concentration, with erythromycin ethylsuccinate decreasing markedly to 49.70 mN/m below that of pure BSA.

3.2 Contact Angle on Silicon Nitride

At low concentration, the contact angles of all nine antibiotic–BSA mixtures on silicon nitride were within about 1° of that of pure BSA (80.02°), with differences within the range of experimental error. At the 1:1 molar ratio, the quinolones and tetracyclines again showed negligible variation. The three macrolides, however, produced a decrease of approximately 4–5° in contact angle (erythromycin 75.87°, erythromycin ethylsuccinate 75.82°, azithromycin 75.39°), suggesting an enhanced affinity between the BSA solution and the silicon nitride substrate when these drugs are present at higher concentrations.

3.3 Solid Surface Free Energy of Silicon Nitride

The reference-liquid measurements yielded the following values: surface tension of water 72.04 mN/m (contact angle 88.81°) and diiodomethane 14.41 mN/m (contact angle 59.94°). Substituting these values into Eqs. (2)–(4) gave the surface free energies summarized in Table 3.

Table 3. Solid surface free energy of silicon nitride calculated by three methods (mN/m)

Method	Polar component	Dispersion component	Solid surface energy
Fowkes		31.589	31.589
OWRK	23.752	0.558	24.309
Wu	21.575	4.565	26.140

Because the Fowkes method neglects polar interactions between solid and liquid, its result deviates considerably from those of the OWRK and Wu methods. The Wu method, which combines dispersion and polar contributions through a harmonic mean, yielded $\gamma_{sv} = 26.140$ mN/m and was adopted in subsequent calculations.

3.4 Solid–Liquid Interfacial Tension

Substituting the surface tensions, contact angles, and Wu-method γ_{sv} into Eq. (1) yielded the solid–liquid interfacial tensions of all mixtures on silicon nitride, listed in Table 4.

Table 4. Solid–liquid interfacial tension γ_{sl} on silicon nitride (mN/m). Low = 0.005 mg/mL; high = 1:1 molar ratio with BSA

Drug	Concentration	γ_{sl} (mN/m)
BSA	40 mg/mL	17.221
Sparfloxacin	low	16.445
	high	16.700
Ofloxacin	low	16.779
	high	16.290
Gatifloxacin	low	17.436
	high	16.526
Erythromycin	low	16.414
	high	13.300
Eryth. ethylsuccinate	low	17.922
	high	13.968
Azithromycin	low	16.748
	high	13.179
Oxytetracycline	low	17.194
	high	17.250
Doxycycline	low	16.388
	high	17.149
Chlortetracycline	low	16.894
	high	16.546

At low concentration, γ_{sl} for all mixtures lay close to that of pure BSA (17.221 mN/m). At the 1:1 molar ratio, only the three macrolides erythromycin (13.300), erythromycin ethylsuccinate (13.968), and azithromycin (13.179) produced a substantial decrease in γ_{sl} . The other six antibiotics left γ_{sl} essentially unchanged.

4. Discussion

4.1 Origin of the Surface-Tension Increase: Competitive Adsorption

Interfacial stabilization of proteins by excipients generally arises through two mechanisms: direct interaction with the protein and competitive adsorption at the air/water interface [3]. Antibiotics used here have molecular weights about two orders of magnitude smaller than BSA and therefore diffuse much faster to the interface. At 0.005 mg/mL, drug–BSA binding is sparse, so the increase in surface tension is best attributed to competitive adsorption: drug molecules occupy interfacial sites that would otherwise be filled by surface-active BSA, lowering the BSA fraction at the interface and raising the surface tension. This interpretation is consistent with the three-step adsorption model of Miller et al. [1], in which interfacial coverage is controlled by the kinetics of transfer from the sub-interface region.

Within the tetracycline series, oxytetracycline and doxycycline gave the highest surface tensions at low concentration. Both molecules contain amino groups that are protonated in PBS at pH $\approx$ 7.4 and therefore carry a positive charge. Because the isoelectric point of BSA is around 4.7, BSA is also positively charged under these conditions, so electrostatic repulsion drives more drug molecules from the bulk solution onto the air/water interface, further lowering the BSA share of the interface. Chlortetracycline hydrochloride dissociates into H^+ and Cl^- in PBS; electrostatic attraction then favors its binding to BSA over interfacial adsorption, which is why the surface tension of the chlortetracycline mixture remains closest to that of pure BSA.

4.2 Macrolides: Hydrophobic Enhancement and Reduced Interfacial Tension

BSA consists of three structurally homologous domains, each containing several hydrophobic regions. The three macrolides studied here possess a common 14-membered lactone ring together with additional six-membered rings, conferring pronounced hydrophobicity. At a 1:1 molar ratio, drug molecules bind efficiently to the hydrophobic pockets of BSA, increasing the overall hydrophobicity of the protein surface. Because protein surface tension is negatively correlated with hydrophobicity [3], the surface tension of the high-concentration macrolide mixtures decreases relative to the low-concentration case. This shift is particularly large for erythromycin ethylsuccinate, whose extra ester-containing carbon chain further enhances hydrophobicity and drives γ_{lv} below that of pure BSA. Such concentration-dependent modulation is also reminiscent of the orientation changes reported for BSA at the air/water interface by McClellan and Franes [2].

The enhanced hydrophobicity of BSA also explains the contact-angle behavior on silicon nitride. The silicon nitride substrate is itself moderately hydrophobic; water and diiodomethane form contact angles of 88.81° and 59.94° , respectively. When macrolides increase the hydrophobicity of the BSA solution, the hydrophobic affinity between the solution and the substrate strengthens, the contact angle decreases by roughly 5° , and γ_{sl} drops from ≈ 17 mN/m for pure BSA to 13–14 mN/m for the three macrolide mixtures. Hence the reduced contact angle and the reduced solid–liquid interfacial tension are two manifestations of the same underlying hydrophobic interaction.

4.3 Quinolones and Tetracyclines: Limited Effect on Interfacial Properties

Sparfloxacin, ofloxacin, and gatifloxacin produced only minor and largely concentration-independent changes in surface tension, while leaving the contact angle and γ_{sl} essentially unchanged. Two factors plausibly account for this behavior. First, the interfacial fraction occupied by these molecules may already approach saturation at 0.005 mg/mL, so further increases in bulk concentration cannot meaningfully raise interfacial coverage. Second, although quinolones are hydrophobic, their binding to the hydrophobic regions of BSA appears not to produce a conformational rearrangement sufficient to alter the measurable surface response. The three tetracyclines likewise leave the contact angle and γ_{sl} close to those of pure BSA: the increase in surface tension arises predominantly from competitive interfacial adsorption rather than from a structural perturbation of BSA that would propagate to the solid–liquid interface.

4.4 Drug–Protein Interaction and Implications for Drug Transport

Drug–albumin interactions modulate not only thermodynamic binding parameters but also the conformation and microenvironment of the protein, as demonstrated for tricyclic compounds by Kamat and Seetharamappa [4]. The present results complement that view from an interfacial standpoint: at concentrations comparable to those reached in plasma shortly after intravenous administration, antibiotics measurably perturb the surface tension of BSA solutions mostly through competitive adsorption, but for macrolides also through enhancement of BSA hydrophobicity that lowers γ_{sl} on a hydrophobic substrate. Because the surface and rheological properties of blood depend strongly on plasma–protein behavior, such interfacial perturbations may, in turn, influence drug transport and absorption. Macrolides, in particular, deserve attention given their concentration-dependent capacity to alter both bulk surface tension and the solid–liquid interfacial tension of protein solutions on hydrophobic surfaces.

5. Conclusion and Research Prospects

This study examined the influence of three classes of antibiotics—quinolones, tetracyclines, and macrolides—on the surface properties of 40 mg/mL BSA solutions. Surface tension and contact angle on silicon nitride were measured at two concentrations, the solid surface free energy was determined by the Wu method (26.140 mN/m), and the solid–liquid interfacial tension was derived from the Young equation. Quinolones (sparfloxacin, ofloxacin, gatifloxacin) had little effect on the surface

properties of BSA solutions across both concentrations. Tetracyclines (oxytetracycline, doxycycline, chlortetracycline hydrochloride) increased surface tension mainly through competitive interfacial adsorption modulated by electrostatic interactions, but left the contact angle and γ_{sl} on silicon nitride essentially unchanged. Macrolides (erythromycin, erythromycin ethylsuccinate, azithromycin) exhibited the most pronounced effects: through their hydrophobic 14-membered lactone rings they enhanced the hydrophobicity of BSA, reduced the surface tension of the mixed solution at high concentration, and substantially lowered γ_{sl} , in turn decreasing the contact angle by about 5°. The work thus demonstrates that hydrophilic–hydrophobic interactions dominate the interfacial response of antibiotic–BSA systems and offers an experimental basis for relating drug structure to plasma–protein interfacial behavior.

Several limitations should be acknowledged. Only a single BSA concentration (40 mg/mL) and a single substrate (silicon nitride) were considered, and the analysis relied on static rather than dynamic surface-tension data. Future studies could extend the work in several directions: incorporating dynamic surface-tension and dilatational rheology to capture the kinetics of antibiotic–BSA co-adsorption; exploring a wider range of substrates with different polarities to test the generality of the macrolide effect; combining interfacial measurements with spectroscopic or calorimetric methods to correlate conformational changes with surface behavior; and extending the model system to human serum albumin and physiological blood-like fluids in order to draw closer connections with drug transport in vivo.

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