A Neutron Reflectometry Study of Human Serum Albumin Adsorption in Situ

Andrea Liebmann-Vinson, Lorraine M. Lander, M. D. Foster, and W. J. Brittain
Institute of Polymer Science, The University of Akron, Akron, Ohio 44325-3909

Erwin A. Vogler
Polymer Science and Technology Department, Becton Dickinson Research Center, Research Triangle Park, North Carolina 27709-2016

C. F. Majkrzak and S. Satija
Reactor Radiation Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

Reprinted from
LANGMUIR, Volume 12, Number 9, Pages 2256–2262
A Neutron Reflectometry Study of Human Serum Albumin Adsorption in Situ

Andrea Liebmann-Vinson,*† Lorraine M. Lander,‡ M. D. Foster,* and W. J. Brittain

Institute of Polymer Science, The University of Akron, Akron, Ohio 44325-3909

Erwin A. Vogler

Polymer Science and Technology Department, Becton Dickinson Research Center, Research Triangle Park, North Carolina 27709-2016

C. F. Majkrzak and S. Satija

Reactor Radiation Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20859

Received July 31, 1995. In Final Form: January 2, 1996

Reflectometry is used to characterize silicon-wafer-supported NH₃⁺-terminated self-assembled silane monolayers (SAMs) and silicon oxide (SiO₂) surfaces against air, water, and human serum albumin (HSA) solutions. X-ray reflectometry (XR) of the NH₃⁺-terminated SAM in air revealed a 14 Å thick, close-packed silane monolayer consistent with an all-trans extended hydrocarbon chain packing tilted about 30° with respect to the surface normal. Neutron reflectometry (NR) of the NH₃⁺ surface against D₂O was consistent with (XR) results if it was assumed that labile protons exchange with deuterium resulting in an interface comprised largely of NH₂⁺. NR results obtained with the SiO₂ surface against D₂O were interpreted in terms of a porous, hydrated oxide layer about 20 Å thick. NR of HSA adsorbed from D₂O buffer onto NH₃⁺ terminated SAM surfaces revealed a two-layer adsorption regime. The first layer directly adjacent to the solid surface was about 40 Å thick and was essentially independent of bulk liquid concentration whereas the second layer was more strongly dependent on liquid phase concentration studied, extending an additional 40 Å into solution at the maximum solubility concentration (0.1% wt/v). NR results are thus consistent with multilayer or mixed-layer protein adsorption mechanism and formation of an interphase region about 80 Å in thickness that separates bulk solid and bulk liquid phases. By contrast, no such interphase could be resolved for an HSA solution in contact with a water-wettable SiO₂ surface, suggesting that HSA does not adsorb to fully-water-wettable surfaces from water. Implications of these results in biomaterials science, especially blood contact phenomena, are briefly discussed in the summary.

Introduction

We report herein use of neutron reflectometry (NR) in the study of protein adsorption. Reflection of neutrons from a solid–liquid interface is sensitive to any solute density gradient at that interface and can thus be used to measure the thickness of a compositionally distinct intervening interphase that separates bulk solid and bulk liquid phases. This technique has been used to study adsorption of surfactants and synthetic polymers at the solid–liquid interface in situ and application to protein adsorption has been reported recently as well.

We selected human serum albumin (HSA) for this study because it is an important blood protein for which the molecular dimensions are well-known. HSA adsorption from aqueous (D₂O) buffer was studied on two types of surfaces with very different surface chemistry, water wettability, and blood-contact properties. One of these types was NH₃⁺-terminated self-assembled silane monolayers (SAMs) supported on electronic-grade silicon wafers. NH₃⁺-terminated SAMs are poorly-water-wettable surfaces (θₐдв = 46° ± 6°, θₑₑ = 40° ± 10°) that are found experimentally not to activate the blood coagulation cascade. The second type of surface studied was fully-water-wettable (θₐдв = θₑₑ = 0°) silicon oxide (SiO₂) on

* Authors to whom correspondence should be addressed.
† Current address: Polymer Science and Technology Department, Becton Dickinson Research Center, Research Triangle Park, NC 27709-2016.
‡ Current address: IBM Almaden Research Center, San Jose, CA 95120.
silicon wafers. In contrast to the NH$_3^+$-terminated SAM, water-wettable SiO$_2$ surface is found to be highly efficient activators of the blood coagulation cascade. Thus, comparison of HSA adsorption onto NH$_3^+$ and SiO$_2$ surfaces provides interesting insights into the behavior of blood proteins at surfaces with widely different chemistry (cationic vs anionic) and energies (water-wettable vs non-water-wettable).

We report direct observation by NR of an HSA concentration gradient emanating from the surface of NH$_3^+$-terminated SAMs in contact with aqueous solutions of HSA. A two-layer interphase concentration was revealed. The first layer directly adjacent to the solid surface was about 40 Å thick and essentially independent of bulk liquid concentration. The second layer was more strongly dependent on liquid phase concentration, extending an additional 40 Å into solution at the maximum solution concentration studied (0.1% wt/v). NR results are thus consistent with a multilayered, mixed-layer adsorption mechanism and formation of an interphase region about 80 Å thick that varies in solute composition and separates bulk liquid and bulk solid phases. By contrast, no such interphase could be resolved for an HSA solution in contact with the water-wettable silicon wafer. This observation corroborates contact angle studies indicating that water-wettable surfaces do not support protein adsorption.\textsuperscript{15}

**Experimental Section**

**Reagents and Solutions.** All reagents were obtained from Aldrich or Fisher, unless otherwise noted. (11-Bromodecaneyl)-trichlorosilane was synthesized by the platinum-catalyzed hydroisolation of undecl-10-enyl bromide (Lancaster, distilled prior to use). Hexadecane was purified by percolation twice through neutral alumina and dried over 4 Å molecular sieves. N$_2$-Dimethyformamide was dried over 4 Å molecular sieves. D$_2$O was obtained from Cambridge Isotopes. Distilled water was deionized using a Millipore System PF with disposable cartridges. All other reagents were used as received.

A 0.1% HSA stock solution was made by dissolving 100 mg of HSA powder (Sigma, 99%, virtually globulin free, amino acid content <0.05%) in a solution buffered to a pH of 7.0 and diluting to 100 mL in a volumetric flask. Dilution of 20 mL of this stock solution with 20 mL of buffer resulted in a 0.05% HSA solution. The phosphate buffer was prepared by dissolving 0.2959 g of KH$_2$PO$_4$ and 1,060 g of Na$_2$HPO$_4$ in 250 mL of D$_2$O. No extraordinary measures were taken to avoid the possibility of HSA dimerization, and the possibility of dimer adsorption can not be rigorously excluded on the bases of the HR results.

D$_2$O was used in the NR experiments for the improved contrast associated with the increased atomic scattering lengths of the isotope D over H (6.674 compared to −3.74 × 10$^{-12}$ cm, respectively). D$_2$O also provides much lower background scattering than H$_2$O.

**Surface Preparation and Characterization.** The substrates were silicon wafers (Semiconductor Processing Inc.) polished on one side and having a diameter of 4 in. and thickness of 0.5 in. In order to remove all organic residues from the surface, the silicon wafers were cleaned using "piranha solution", a mixture of 70% concentrated sulfuric acid and 30% hydrogen peroxide (30%). Wafers were immersed in the piranha solution and heated to 60-90 °C for a period of 30 min. Piranha treated wafers were immersed in distilled water and subsequently rinsed a minimum of five times with distilled water. The influence of this surface treatment on the silicon surface was studied by multiple treatment of a wafer with piranha solution and measurement of its surface roughness after each treatment. No change in surface roughness after multiple treatments within the sensitivity range of XR was found. Cleaned wafers were stored under distilled water until use, at which time any residual water was removed with a stream of nitrogen or argon. This treatment yielded substrates with fully-water-wettable silicon oxide (SiO$_2$) surfaces.\textsuperscript{16}

NH$_4^+$-terminated SAM surfaces were prepared by a three-step process, beginning with the formation of a bromide-terminated SAM of the form O$_2$Si(CH$_2$)$_n$Br. Clean silicon wafers were immersed in a 0.3% solution of (11-bromodecaneyl)-trichlorosilane in hexadecane and heated to 60-90 °C. After 6 h, the wafers were removed from the solution, rinsed with anhydrous methylene chloride and chloroform to adsorb an argon stream. Bromide-terminated SAMs were further converted by an in situ reaction to an azide-terminated SAM by immersion in a 10% NaN$_3$/N,N-dimethyformamide solution for 24 h. Surfaces were rinsed with distilled water, acetone, and chloroform and dried with argon. The third step involved the reduction of the azide functionality to ammonia using lithium–aluminum hydride. Azide-terminated wafers were immersed in lithium aluminum hydride (1.0 M in THF) for 24 h. Upon removal, the wafers were rinsed with 20% HCl, large amounts of distilled water, acetone, and chloroform. The resulting substrate were washed with ethanol.

Contact angles ($\theta_{\text{D}\theta_{\text{D}}}$ and $\theta_{\text{D}_2\text{O}}$) were measured using the tilting-plate method\textsuperscript{17} implemented on a Ramé-Hart goniometer with 10 μL of 0.9% saline solution droplets. Contact angles were found to be consistent with an NH$_3^+$ surface functionality with only a small portion of free amines NH$_2$ groups, $\theta_{\text{D}_2\text{O}}$ observed for this surface was 46°±6°. Taking 42° as the expected value for a surface completely covered with NH$_2$ groups and 62° for a surface with only NH$_2$ functionality,\textsuperscript{17} bounds on the surface coverage of 62 and 87% can be estimated using either the Cassie equation or a modified Cassie equation proposed by Israelachvili and Gee.\textsuperscript{19}

**Reflectometry.** XR of SAMs was performed in air using Cu $K\alpha$ radiation ($\lambda = 1.54 \AA$) generated by a rotating anode (Rigaku). Constant-wavelength resolution of $\Delta \lambda / \lambda = 0.022$ and incidence angle resolution $\Delta \theta / \theta = 0.002$ were used. X-ray data were corrected for background before analysis. NR measurements were performed on the BT7 reflectometer at the National Institute of Standards and Technology (NIST) NBSR research reactor. A fixed-wavelength ($\lambda = 2.35 \AA$) with a constant resolution of about $\Delta \lambda / \lambda = 0.015$ was used, while the relative resolution of incidence angle, $\Delta \theta / \theta$, improved somewhat with 6, even though the collimating and deflection slits sizes were increased gradually through the course of the measurement to maximize intensity. The data were corrected for background and the effect of varying slit size.

The influence of diffuse scattering on background correction has not been rigorously taken into account. We assumed that the entire measured background was uncoupled, and thus it is possible that the background is therefore somewhat overestimated. More precise treatment of the background could alter the qualitative fit of the data. However, we do not anticipate that the essential qualitative features of the layer model would change.

Figure 1 illustrates the different geometries used for XR (Figure 1A) and NR (Figure 1B) reflectometry measurements. In the XR experiment, the X-ray beam strikes the sample—air interface from the air. A sample cell especially designed to investigate adsorption phenomena at the solid–liquid interface was utilized in the NR measurements. The fact that researchers can use macroscopic distances in single crystals, such as silicon, without any measurable loss was exploited. As shown in Figure 2, the neutron beam enters the thick silicon wafer edge-on and penetrates through the silicon to the interface under investigation, where it is reflected back through the silicon into the detector. The 4 mm deep Teflon trough was filled with protein solution through a hole using disposable pipets. Before each measurement, the trough was cleaned with HCl followed by intense rinsing with deionized water.

\textcopyright 1993 Marcel Dekker, Inc. All Rights Reserved.
Results and Discussion

This section is divided into three main parts. The first section briefly introduces general aspects of reflectometry and provides a background for readers unfamiliar with reflectometry techniques. The second section details computational aspects of fitting reflectometry curves. Structures of surfaces and interfaces deduced from reflectometry are discussed in the third and final section. Conclusions are drawn together in the Summary section, along with brief discussion of the implications of HSA adsorption in blood coagulation and biomaterials surface science in general.

General Aspects of Reflectometry. The principles of XR and NR are similar, with the primary difference being the radiation, X-rays or neutrons, used to probe the samples. An incoming beam strikes a flat sample surface under an incident angle \( \theta_i \), as sketched in Figure 3. Below a sample-dependent critical angle, \( \theta_c \), total reflection occurs (in the absence of absorption). Above \( \theta_c \), the beam penetrates into the sample, and reflection occurs from both the surface and any interior interfaces. Reflectivity 
\[
R = \frac{I_{\text{reflected}}}{I_{\text{incoming}}},
\]
which is the ratio of reflected to incoming intensity, is unity in case of total reflection and less than unity for \( \theta > \theta_c \). Variation of \( R \) with incident angle, \( \theta \), or equivalently \( q \), the momentum transfer (\( q = (4\pi \lambda \sin \theta) \)) where \( \lambda \) is the radiation wavelength), is sensitive to features in the sample structure in the direction perpendicular to the surface. For example, for ideal atomically smooth surfaces with no internal interfaces within the radiation penetration depth, the measured reflectivity follows Porod's law (\( R \sim q^{-4} \)) for \( q \gg q_c \). When interfaces between materials of distinct contrast for either X-ray radiation or neutrons are present, interference of radiation reflected from these interfaces modulates the form of the measured reflectivity curve producing “Kissig fringes”. Reflectivity curves for real samples with measurable surface rugosity and roughness at interior interfaces are generally more complex.

Computational Aspects. Reflectivity curves of rough surfaces or interfaces comprised of multiple layers with differing X-ray or neutron contrast are complex, and structural information can be obtained only through detailed mathematical modeling. We use herein an optical matrix formalism model as described in ref 20. Briefly, the modeling process involves dividing a hypothetical construction of the surface or interface into a number of parallel regions of constant scattering length densities, \((b/v)\), propagating along the surface normal from the sample surface into the sample depth \((d)\). Sharp step transitions between two regions of different \((b/v)\) are convolved with an error function to account for the non-ideality of rough interfaces. The result of the model is a \((b/v) vs d\) profile, as illustrated in Figure 4B. A reflectivity curve \((\log R vs q)\) is then computed from the \((b/v)\) profile and compared to the experimental data as shown in Figure 4A. The parameters \((b/v), d,\) and roughness \((d\) as well as the number of constant \((b/v)\) regions are varied until the model adequately simulates the experimental reflectivity. As a consequence, the fitted model is not necessarily unique and, in principle, a number of alternative models may be possible. However, the number of \((b/v)\) profiles that simulate experimental data are limited by physical constraints on all adjustable parameters, and ultimately, the model must make physicochemical sense.

The method of Tidwell and co-workers\(^{21}\) was used in modeling XR curves of the NH\(_3\)-terminated SAMs in air. This model decomposes the film into three regions of different \((b/v)\) layers representing the hydrocarbon chain.

---


Figure 4. XR curve of SAM₃ together with model fit (A) and (b/u) profile (B) compared to NR curve of SAM₂ against D₂O with model fit (C) and (b/u) profile (D).

(A), an interfacial layer (B) containing the SiO₃ head groups, and a native silicon oxide layer (C) on top of the bulk silicon substrate (D), as illustrated in Figure 4B. A similar model construction was used to interpret NR of the SAM against either D₂O or HSA solutions in D₂O, except that the SiO₃ head group interface layer was replaced with a rough SAM/SiO₂ interface.

NR (b/u) values for the hydrocarbon region were computed from XR measurements using eq 1:

\[
\frac{b}{u} = \frac{\sum b_i}{r_0 \sum z_i u_i}
\]  

(1)

where \( b_i \) are the atomic scattering lengths, \( z_i \) the atomic numbers, and \( r_0 \) is the classical electron radius. The scattering length density of the D₂O was corrected for the addition of the buffer salts, resulting in a (b/u) value slightly higher than that of pure D₂O.

It is estimated from contact angle measurement that the SAMs have a composition of 82–87% NH₃⁺ and 13–18% NH₂ (see the Experimental Section). Calculated (b/u) values taking this surface composition into account were between −0.002 198 and −0.003 109 × 10⁻¹² cm²Å⁻³ respectively, depending on the ammonium surface composition.

Surfaces, Interfaces, and Protein Adsorption

Surfaces. XR was used to characterize SAM surfaces in air. XR curves of the two NH₃⁺-SAM surfaces selected for study herein (designated SAM₁ and SAM₂) are compared in Figure 5. Both reflectivity curves exhibit one well defined minimum that results from destructive interference between reflection at air—hydrocarbon and hydrocarbon—substrate interfaces. The model fit for the reflectivity curve of SAM₂ is shown in Figure 4A, with the corresponding (b/u) profile shown in Figure 4B. Final parameters obtained from model fits for both SAMs are summarized in Table 1 and are comparable to values given in the literature.²¹ Some differences between SAM₁ and SAM₂ could be resolved. First, it was apparent that the SiO₂ layer thicknesses were not equivalent for both samples. This is not surprising since the thickness of the SiO₂ layer is known to vary between 10 and 20 Å.²² Secondly, electron densities of both the interface and the hydrocarbon region for SAM₂ was higher than that of SAM₁, suggesting that that SAM₂ had a more densely packed silane monolayer than SAM₁. Roughnesses for all interfaces comprising the (b/u) model were very similar for both SAM samples.

SAM thicknesses represent 86% of the expected value for a fully extended hydrocarbon chain in the all-trans configuration (calculated for a $C_{12}H_{25}$ hydrocarbon chain according to the well-known formula $L = 1.26n + 1.5 \text{ Å}$). This implies a tilting of the molecules with respect to the surface normal by about 30°–31°. For comparison, tilt angles reported in the literature vary between 14 and 21°. While this work does not specifically address the origin of hydrocarbon chain tilting, it is interesting to speculate that the observed variability in tilt angle may be due to structural variability in the SiO$_2$ head group region. Taking the bond lengths of 1.35 and 1.52 Å for Si–O and Si–OCH$_3$ linkages, respectively, the calculated thickness of the SiO$_2$ head group interface is 2.85 Å. This value is about one-third of the 8 Å experimental value, suggesting that each SiO$_3$ head group may not be covalently attached to the SiO$_2$ surface through three rigid Si–O linkages, but by a more flexible network comprised of intermolecular cross-links between SiO$_3$ head groups with commensurately fewer covalent attachments to the SiO$_2$ surface.

**Aqueous Interfaces.** NR was used to characterize NH$_3$–SAMs in contact with pure D$_2$O phosphate buffer solutions. The NR curve and model fit corresponding to SAM$_1$ against D$_2$O buffer is shown in Figure 4C along with the (b/v) profile in Figure 4D. The SAM–liquid interface is well defined, with a roughness only slightly higher than that of the same SAM in air. However, experimental data could not be fit using calculated (b/v) values (see Computational Aspects section). Instead, it was determined by iterative fitting that (b/v) had to be increased to 0.003 $52 \times 10^{-12}$ cm$^2$/Å$^2$. We can advance two possibilities to explain the increased scattering length density. The first possibility is that D$_2$O penetrates into the hydrocarbon layer, increasing NR contrast. The second possibility is that terminal protons rapidly exchange with deuterium in the presence of D$_2$O buffer. While we cannot rigorously evaluate the relative contributions of these two possibilities, the second option seems much less likely than the second because NH$_3^+$-SAMs are shown to be closely packed by XR and D$_2$O penetration into a dense hydrocarbon region seems unlikely. On the other hand, exchange of terminal hydrogens with deuterium from buffer solution introducing NH$_3^+$–D$_2^-$ and NH$_2$–D$_3$ functionalities with commensurate increase in NR contrast sensibly agrees with chemical intuition. As an example, (b/v) = 0.003 $52 \times 10^{-12}$ cm$^2$/Å$^2$ is calculated for a 100% NH$_3^+$ surface, which, when compared to the fitted value, suggests that about 65% of the available protons have exchanged with deuterium.

**Protein Adsorption.** The NR curve of SAM$_1$ in contact with pure D$_2$O is compared to that of SAM$_2$ against 0.05 and 0.1% HSA in D$_2$O buffer solution in Figure 6C. In the region around the critical edge, all three curves were almost identical. However, as scattering vector values between 0.02 and 0.15 Å$^{-1}$ reflectivity was observed to drop off significantly faster with increasing HSA concentration relative to reflectivity from the pure D$_2$O buffer/NH$_3^+$ interface.

The experimental data could be rationalized using a two-layer model for the region of adsorbed protein incorporating the crystallographic dimensions for the HSA molecule. This idealized model does not include dimensional changes that may occur upon HSA adsorption. However, other work suggests that albumin retains its secondary structure in the adsorbed state. The first layer (E) directly adjacent to the NH$_3^+$ surface had a greater HSA concentration than the second layer (F) with a concentration profile that decayed with depth to the bulk liquid concentration (G). An example of a fit of this model to experimental data (SAM$_3$ 0.1% HSA in D$_2$O solution) is shown in Figure 6A and the corresponding (b/v) profile is shown in Figure 6B. Table 2 collects fitted parameters of all three interfaces for this model. The resulting HSA composition profiles within the interphase separating bulk liquid and solid phases are shown in Figure 6D.

Thicknesses of both protein-rich layers were about 40 Å. Whereas the thickness of the HSA layer directly adjacent to the NH$_3^+$ surface varied only 6 Å between 0.05 and 0.1% HSA, the thickness of the second layer increased 15 Å over the same concentration interval. Most recent crystallographic data on HSA’s quaternary structure suggests that it is “heart-shaped” with a width of 82 Å, a maximum dimension from the apex of the heart to the end of the domains on each side of 83 and 70 Å, respectively, and a depth of about 30 Å, as sketched in Figure 7A. Thus it seems reasonable to suggest a side-on “Langmuir-like” adsorption regime dominated by charge–charge interactions in which negatively charged moieties on HSA$_{12}$tetrionate cationic NH$_3^+$ groups comprising the SAM surface. The second layer may likewise be comprised of HSA molecules in a side-on orientation resting on the first layer as suggested in Figure 7B. The second possibility is the combination of side-on and tilted end-on orientations, as shown in Figure 7C, with mainly side-on adsorbed molecules and a few tilted end-on adsorbed molecules sticking out into the bulk solution.

An HSA concentration profile, as shown in Figure 6D, can be estimated from the model (b/v) profile using an arithmetic proportion of (b/v) values of the interphase constituents HSA and D$_2$O. The empirical structure formula $C_{5288}H_{8091}O_{1473}N_{1530}S_{41}$ for HSA leads to a calculated (b/v) = 0.1723 $52 \times 10^{-12}$ cm$^2$/Å$^2$ (ignoring D$_2$O within the HSA molecule and any exchange of labile hydrogens on the protein). The (b/v) value for the pure D$_2$O buffer is 0.0634 $52 \times 10^{-12}$ cm$^2$/Å$^2$. Thus, the interphase separating bulk liquid and solid phase is composed of 16 and 26% HSA for the first layer adjacent to the NH$_3^+$ surface at

Table 1. Fitting Parameters for NH₃⁺-Terminated SAMs in Air and Comparison to Literature Values

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>ρₑ (g/cm³)</th>
<th>d (Å)</th>
<th>σ (Å)</th>
<th>air/A</th>
<th>A/B</th>
<th>B/C</th>
<th>C/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM₁</td>
<td>2.79</td>
<td>5.25</td>
<td>6.91</td>
<td>7.00</td>
<td>0.81</td>
<td>13.3</td>
<td>6.2</td>
<td>15.5</td>
<td>4.55</td>
<td>1.92</td>
<td>1.00</td>
</tr>
<tr>
<td>SAM₂</td>
<td>2.92</td>
<td>5.42</td>
<td>6.91</td>
<td>7.00</td>
<td>0.85</td>
<td>13.2</td>
<td>6.1</td>
<td>11.8</td>
<td>4.03</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>literature</td>
<td>3.01</td>
<td>5.74</td>
<td>6.72</td>
<td>7.04</td>
<td>0.85</td>
<td>7.0</td>
<td>10-20</td>
<td>2.4-2.9</td>
<td>2.40</td>
<td>1-2</td>
<td>1-2</td>
</tr>
</tbody>
</table>

* See Figure 4B for definition of layers.

Figure 6. NR curve of SAM₂/0.1% HSA in D₂O solution interface with model fit (A) and (b/v) profile (B). (C) Comparison of NR from interface between SAM₂ and D₂O—buffer (open squares), the interface between SAM₁ and 0.05% HSA/D₂O—buffer solution (open squares), and the interface between SAM₂ and 0.1% HSA/D₂O—buffer solution (open diamonds). (D) Composition profile of the interphase separating bulk solid and bulk liquid phase derived from NR data for 0.05% (solid line) and 0.1% (broken line) HSA solutions.

Table 2. Fitting Parameters for HSA Adsorbed from D₂O-Buffer Solutions onto NH₃⁺-Terminated SAMs

<table>
<thead>
<tr>
<th></th>
<th>HSA concentration</th>
<th>E</th>
<th>E*</th>
<th>F</th>
<th>F*</th>
<th>A/E</th>
<th>E/F</th>
<th>F/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>scattering length density (Å⁻¹)</td>
<td>5 × 10⁻¹² cm²/Å³</td>
<td>35.5</td>
<td>35.2</td>
<td>11</td>
<td>18</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>layer thickness, d (Å)</td>
<td>±5 Å</td>
<td>41.5</td>
<td>50.2</td>
<td>13</td>
<td>22</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rms roughness, σ (Å)</td>
<td>±2 Å</td>
<td>0.0555</td>
<td>0.0630</td>
<td>0.0626</td>
<td>0.0626</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See Figures 4B and 6B for definition of layers.

bulk solution compositions of 0.05 and 0.1% HSA, respectively. At the same bulk solution concentrations, the second layer compositions are only 1% and 2%, respectively. Thus, the picture of HSA at NH₃⁺-SAM surfaces emerging from NR is that of a protein-rich interphase separating bulk solid and bulk liquid phases dominated by a Langmuir-like lattice site adsorption regime.

It is of interest to compare the above results to those obtained with fully-water-wettable SiO₂ surfaces because, as mentioned in the Introduction, these surfaces exhibit blood contact properties very different from that of the NH₃⁺-SAMs, in addition to the pronounced differences in surface chemistry and energetics. Figure 8 compares reflectivity from the SiO₂/D₂O buffer interface to that from SiO₂ against a 0.5% HSA solution in D₂O. The two reflectivity curves are identical up to a scattering vector of about 0.15 Å⁻¹. At higher scattering vectors, the two curves deviate with the SiO₂—D₂O interface having a
Figure 7. (A) Shape of HSA molecule. Comparison of the suggested two different protein layer models, one being a double layer of side-on Langmuir-like adsorbed molecules (B) and the second representing a mixed layer of side-on and end-on adsorbed HSA molecules (C).

reflectivity lower than the one where protein is present in solution.

Features in the reflectivity from the case where an HSA solution is next to the wafer can best be rationalized as arising from the existence of two separate layers of silicon oxide having different amounts of water trapped in the oxide's porosity. The oxide layer closest to the silicon has a thickness of 15.3 Å thick with a surface roughness of 1.7 Å. An additional layer of thickness 5.2 Å and 2.3 Å surface roughness is required as well. While one might argue that this second layer is not oxide, but rather some sort of protein rich layer, we find such a model highly improbable, as such a thin layer suggests that the protein in that layer had become entirely denatured, losing all vestiges of a folded, tertiary structure. An overall oxide layer thickness of 20.5 Å is well within the range of values found in the literature. Thus we conclude that HSA is not adsorbed to the SiO2 surface.

Summary

Reflectometry has been used to characterize NH3+-terminated self-assembled monolayer (SAM) and silicon oxide (SiO2) surfaces in contact with air, water (D2O), and an aqueous-buffered solution of human serum albumin (HSA). X-ray reflectometry (XR) of the NH3+-terminated SAM in air revealed a 14 Å thick, close-packed silane monolayer consistent with an all-trans extended chain hydrocarbon packing tilted about 30° with respect to the surface normal. Neutron reflectometry (NR) of the NH3+ surface against D2O was consistent with (XR) results if it was assumed that labile protons exchange with deuterium resulting in an interface comprised largely of ND3+. NR of the SiO2 surface against D2O was consistent with a porous, hydrated oxide layer about 20 Å thick.

NR of HSA adsorbed from D2O buffer onto NH3+-terminated SAM surfaces revealed a two-layer adsorption regime. The first layer directly adjacent to the solid surface was about 40 Å thick and essentially independent of bulk liquid concentration whereas the second layer depended more strongly on liquid phase concentration and extended an additional 40 Å into solution at the maximum solution concentration (0.1% wt/v). NR results are thus consistent with an adsorption mechanism which confines either multilayer or mixed-layer adsorption and formation of an interphase region of about 80 Å in thickness that separates bulk solid and bulk liquid phases. By contrast, no such interphases could be resolved for HSA solutions in contact with a water-wettable SiO2 surfaces, implying that HSA does not adsorb to fully-water-wettable surfaces from water.

The NH3+-terminated SAM and SiO2 surfaces were selected for study here because each exhibits very different blood contact properties. The poorly-water-wettable NH3+-terminated SAM is found experimentally to be a very inefficient activator of the blood coagulation cascade. In contrast, water-wettable SiO2 surfaces are found experimentally to be very efficient activators of blood coagulation. In this connection, it is of interest that efficient coagulation activator (SiO2) does not adsorb HSA, an important blood protein, whereas HSA readily adsorbs to an inefficient activator (NH3+-terminated SAM). Thus, NR corroborates contact angle studies indicating that water-wettable surfaces do not support protein adsorption as well as results of blood coagulation studies indicating that efficient adsorbents of blood serum proteins are inefficient blood coagulation activators and that, conversely, efficient activators do not support adsorption of serum proteins.

This work demonstrates utility of NR as a new tool in the arsenal of techniques for studying protein adsorption. Techniques in this arsenal range from contact angles to ellipsometry, and from microcalorimetry to radiometry, and include a host of different spectroscopies. The principle advantage of NR in this pursuit is that it provides a nondestructive in situ view of protein adsorption gradients and can measure thickness of this compositionally distinct intervening interphase that separates bulk solid and bulk liquid phases.

Acknowledgment. The authors would like to acknowledge the assistance of S. Petrash, T. Vierbeller, H. Wu, and C. Li with the neutron measurements and financial support from the Army Research Fund DAAL03-92-G-0402 and support from the Deutsche Forschungsgemeinschaft for Andrea Liebmann-Vinson.

LA950642D