Protein Adsorption onto Zirconia Modified with Terminally Grafted Polyvinylpyrrolidone

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The potential effectiveness of poly(vinyl pyrrolidone) (PVP) as a zirconia surface modifier for protein adsorption reduction was investigated using lysozyme (LYS). The relatively small size of LYS (45 × 30 × 30 Å) allowed for testing the adequacy of the graft polymerization method for producing a dense surface chain coverage to exclude LYS from direct interaction with the zirconia surface. The study demonstrated that a PVP brush layer is capable of reducing lysozyme adsorption. Overall, the maximum adsorption capacity decreased (by up to about 76%) due to surface modification with increasing polymer/silane surface coverage ratio (mol/mol). Adsorption reduction, due to protein exclusion from the surface by the tethered polymer layer, increased significantly when the distance between surface chains was less than the large axis of LYS (i.e., 45 Å). The present results are encouraging and suggest further consideration of polymer-modified ceramic surfaces for reducing fouling of ceramic membranes during protein ultrafiltration and producing ceramic biocompatible surfaces for biomedical applications.

Key Words: graft polymerization; protein adsorption; ceramic; polymer brush.

INTRODUCTION

Adsorption of proteins occurs on a variety of solid–liquid interfaces. In many instances, protein adsorption results in undesirable effects such as surface-induced thrombosis due to adsorption of plasma proteins (1) or fouling of membranes used in food and beverage processing (2–4) and performance degradation in analytical protein liquid chromatography (5). Protein adsorption is affected by numerous factors including protein size, charge, shape, hydrophobicity, pH, surface charge, surface topology, coadsorption of low-molecular-weight ions, intermolecular forces between adsorbed molecules, strength of functional groups bonds, composition of the protein solution, and chemistry of the solid surface (6, 7). Although various factors affect protein adsorption, the surface chemistry of the substrate has been studied most intensively with the goal of affecting protein adsorption via a variety of surface modification techniques. Of the various surface modification methods, the use of polymers to alter the surface chemistry is of particular interest in the present work.

Polymer coatings designed to reduce protein adsorption have been used to modify a variety of polymeric substrates such as polycarbonate (8), polysulfone (8, 9), polypropylene (10), low-density polyethylene (11), polystyrene latex (12), as well as glass (13) and ceramic surfaces (14–21). Over the last decade, various researchers have also investigated the potential use of adsorbed diblock and triblock copolymers for reduction of protein adsorption (22–27). In the latter approach, the hydrophilic part of the polymers is first adsorbed onto the substrate, with the hydrophilic end serving as the barrier for protein adsorption from aqueous solutions. PEO and di-/triblock copolymers of PPO and PEO (PPO–PEO and PEO–PPO–PEO) have been particularly popular and demonstrated reasonable levels of protein adsorption reduction (10–12, 16–19). Coatings of other nonionic polymers such as poly(vinyl pyrrolidone) (PVP), poly(vinyl alcohol) (PVA), poly(vinyl methyl ether) (PVME), dextran, and methylcellulose have also proven effective in reducing adsorption of certain proteins (β-lactoglobulin and bovine serum albumin) onto polysulfone surfaces (26). It has been suggested that the degree of protein adsorption reduction is determined largely by the effectiveness of protein exclusion from the native surface due to the polymer surface chains that provide a steric barrier (10).

Reduction of protein adsorption onto polymeric substrates can be also achieved through chemical binding of preformed polymer chains (29), i.e., polymer grafting, or in situ polymerization onto the surface (30), i.e., graft polymerization. Graft polymerization involves the growth of polymer chains from surface active sites by sequential monomer polymerization, while polymer grafting involves chemical bonding of live polymer chains to the support surface. In both cases, and in contrast to coated polymer phases, the resulting polymer phase is highly stable since the polymer chains are covalently bonded to the surface. With polymer grafting it is possible to graft monodisperse polymers onto the surface; however, since polymer molecules must diffuse to the solid surface, diffusional limitations and steric hindrance effects can severely reduce the degree of surface chain density and overall polymer graft yield. In contrast, in graft polymerization diffusion limitations and steric hindrance effects are

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minimized owing to the much smaller size of the monomeric units that diffuse to react with surface chains or active surface sites. Therefore, with graft polymerization it is possible to achieve a higher degree of surface coverage (especially for porous supports) than via polymer grafting (5). It is important to note that, with all of the above approaches, it is possible to create a polymer surface with terminally anchored chains that are completely soluble in aqueous systems. Terminally anchored (tethered) polymer chains can be effective in reducing protein adsorption provided that polymer chains completely cover the surface to effectively exclude proteins from the native surface. At sufficiently high surface densities, the grafted polymer chains are forced to extend away from the surface to minimize their free energy, in a conformation analogous to the bristles of a brush. In this so-called “brush regime,” a high degree of protein rejection is generally observed for a variety of proteins, irrespective of whether covalent grafting or physical adsorption of diblock copolymers is used (13, 31–33).

Given the interest in producing highly stable polymer surface layers, polymer grafting and graft polymerization modification techniques have become popular, especially for the modification of polymeric ultrafiltration membranes (34–38). In contrast, less effort has been devoted to evaluating the effectiveness of terminally and covalently anchored chains for reduction of protein adsorption onto ceramic surfaces. In particular, it appears that the effectiveness of PVP, a known water-soluble and biocompatible polymer (39) which is of interest in this work, as a surface modifier of ceramic surfaces has not been fully explored. The recent review of Cohen et al. (5) suggests that PVP brush layers can be effective in reducing surface adsorption of water-soluble macromolecules and thus suitable for protein adsorption reduction. Other recent studies (37, 38) have reported that low-temperature plasma graft polymerization of N-vinyl-2-pyrrolidone (NVP) onto poly(ether sulfone) membranes reduced membrane fouling during the filtration of bovine serum albumin, confirming the relevance of PVP for protein adsorption reduction.

In the present study, the potential effectiveness of PVP as a modifier for reducing protein adsorption onto zirconia surfaces was investigated using lysozyme as the model protein. Lysozyme is a well-characterized protein commonly used as a model protein in adsorption (40–42) and membrane ultrafiltration (43–45) studies. Moreover, its relatively small size demands a high surface polymer chain density to effectively exclude the protein from the native surface, thereby testing the adequacy of the graft polymerization method used in the present study.

**EXPERIMENTAL**

**Materials**

Zirconia powder with a particle size of 5 μm was obtained from Aldrich (Milwaukee, WI). The specific surface area was determined by BET nitrogen adsorption analysis (Autosorb-1, Quantachrome Inc., Syosset, NY) to be 6.13 m²/g. The characteristics of the zirconia particles are provided in Table 1. Surface silylation was carried out with vinyltrimethoxysilane (VTMS) obtained from Petrach Systems Inc. (Bristol, PA). Reagent-grade xylene purchased from Fisher Scientific (Tustin, CA) was used as a solvent. Vinylpyrrolidone (1-vinyl-2-pyrrolidone) monomer, containing 0.1% potassium hydroxide as inhibitor, was supplied by Kodak Chemical Company (Rochester, NY). A 50% aqueous solution of ammonium hydroxide (Mallinckrodt Inc., Paris, KY) was the source of ammonium ions used in the graft polymerization. Ammonium hydroxide acts as a buffer for the reaction mixture and prevents the formation of acetaldehyde, a by-product that promotes chain transfer (5, 46). The initiator for the PVP grafting reaction was reagent-grade hydrogen peroxide available as a 30% solution (Aldrich).

Lysozyme (LYS) grade I (L6876), obtained from Sigma (St. Louis, MO), was the model protein selected for the protein adsorption studies. The properties of LYS are summarized in Table 2. Reagent-grade potassium dihydrogen phosphate and disodium hydrogen phosphate of reagent quality, used as a buffer for the aqueous protein solutions, were obtained from Baker Chemical Company (Phillipsburg, NJ). The ionic strength of the solutions was adjusted with NaCl (S640-500) obtained from Fisher Scientific. Finally, protein concentration was determined by UV analysis (HP 8452A Diode Array Spectrophotometer, Hewlett-Packard, Palo Alto, CA), at λ = 280 nm.

**Surface Modification**

PVP-grafted zirconia particles were prepared by a graft polymerization process consisting of two main sequential steps: (i) silylation, and (ii) surface graft polymerization. The graft polymerization process followed the method of Cohen and co-workers (5, 46) and a brief description of the synthesis methods is provided below.

The initial surface treatment step involves modification of –OH groups, present on the ceramic surface, by silylation with vinyltrimethoxysilane, H₂C=CH-Si–(OCH₃)₃, to generate
vinyl surface sites for subsequent surface polymerization. Prior to silylation, the zirconia particles were washed with 1% HCl solution and dried at 110°C overnight under vacuum. Vinylsilylation was subsequently carried out for a period of 5 h in a 3-L batch slurry reactor filled with a 10% (v/v) solution of VTMS in xylene. The condenser temperature was kept at 70°C, above the boiling point of methanol, but below the boiling of the xylene solution, to remove alcohol produced by the reaction. At reaction termination, the particles were washed several times with xylene and allowed to cure at 140°C in a vacuum oven for 24 h. The surface methoxy groups were subsequently hydrolyzed by immersion in an aqueous NaOH solution (pH 9.5) for 3 days. This latter step partially restored the hydrophilicity of the particles, thereby enabling dispersion in the aqueous polymerization mixture. The treated particles were then rinsed, dried, and reserved for the graft polymerization reaction.

PVP graft polymerization of a batch of 10 g of silylated particles was performed in a 1-L jacketed reaction flask under nitrogen atmosphere. Nitrogen atmosphere was necessary to eliminate atmospheric oxygen, which is known to increase the latent period of polymerization and reduce the rate of polymerization. The silylated particles were dispersed in an aqueous vinylpyrrolidone solution (30% by volume) and then heated to the desired reaction temperature (typically 70°C). The grafting reaction was initiated with 2 mL of hydrogen peroxide solution (30%) and 0.8 mL of ammonium hydroxide solution (50%). Ammonium hydroxide acts as a buffer for the reaction mixture and prevents the formation of the undesirable acetaldehyde by-product under acidic conditions. In addition, ammonium hydroxide has a strong activating effect on the polymerization reaction, shortening the latent period and increasing the rate of polymerization. At the termination of the polymerization reaction, the grafted particles were filtered, rinsed with water, and oven dried at 100°C overnight.

**Analytical Methods**

Titration of the zirconia particles was conducted to determine the –OH surface concentration on the native and modified surfaces and to evaluate the surface charge density of the particles. The particles were titrated at constant temperature (30°C) and N2 blanket to avoid acidification of the solution due to dissolution of atmospheric CO2. A carefully weighted particle charge (about 0.2 g) was dispersed in a 50-mL solution of 0.1 M NaCl. Separate acid and base titrations were conducted using 0.1 M HCl and 0.1 M NaOH, respectively, with the pH continuously monitored with a pH-meter (Accumet Model 805A, Allied Fisher Scientific, Pittsburgh, PA). Blank titrations were also conducted for salt solution without particles.

The effectiveness of surface hydroxyl replacement and masking by graft polymerized PVP surface phase was also evaluated by both determination of the surface charge density and ζ-potential measurements. Surface charge density, \( \sigma_0 \) (C/m²), was determined by a potentiometric acid–base titration

\[
[(V_{H}C_{H} - V_{OH}C_{OH}) - V_0([H^+] - [OH^-])]F/S = \sigma_0, \tag{1}
\]

where \( V_{H} \), \( V_{OH} \), \( C_{H} \), and \( C_{OH} \) are the volumes (L) and initial concentrations (M) of acid and base added, respectively, \( V_0 \) is the final volume of the solution, \([H^+]\) and \([OH^-]\) are the ionic concentrations (M) in the solution calculated from the pH, \( F \) is the Faraday constant (C/mol), and \( S \) is the specific area (m²). ζ-potential measurements were performed with a Laser Zee Meter 501 (Pen Kem Inc., Bedford Hills, NY). The measurements were conducted by dispersing about 0.5 g of particles (native or modified) in a 1-L solution of 0.1 M NaCl solution with pH adjustment using aqueous NaOH or HCl solutions.

Identification of chemical groups on the surface of the unmodified, silylated, and PVP-grafted particles was made by diffuse reflectance infrared-Fourier transform (DRIFT) spectroscopy using a Bio-Rad FTS-40 FTIR (Digilab Division, Cambridge, MA) with a diffuse reflectance accessory. Quantitative measurements of the yield (µmol/m² or mg/m²) of VTMS and PVP grafted onto the ceramic substrate were performed by thermogravimetric analysis (TGA). References were taken with respect to the cleaned unmodified substrate and hydrolyzed particles. Finally, the silylation and polymer graft yields were determined by TGA conducted with a Perkin–Elmer TGS-2 thermogravimetric analyzer, for a range of temperatures between 100 and 900°C.

Direct observation of the topology of the grafted PVP surface phase was accomplished by atomic force microscopy (AFM) imaging of a PVP-modified silicon (100) prime-grade wafer (Wafernet, San Jose, CA). The wafer was soaked first in acetone and subsequently in methanol to remove soluble organic contaminants. The wafer was cleaned (47) using a 7:3 (by volume) solution of sulfuric acid (certified ACS grade, Fisher Scientific) and 30% hydrogen peroxide in water (certified ACS grade, Fisher Scientific). Drying of the wafer was accomplished by oven drying, under vacuum at 110°C. Subsequently, the wafer was silylated and graft polymerized under the same reaction conditions as for the particles. AFM images were obtained using a Digital Instruments (Santa Barbara, CA) multimode atomic force microscope with a Nanoscope IIIa SPM controller, operating in tapping mode.

**Adsorption**

Adsorption experiments were carried out by standard batch equilibrium adsorption studies at 25°C. Carefully weighed quantities of unmodified, silylated, or grafted ceramic particles were added to 10-mL glass vials with Teflon-lined screw-cap tops. The vials were filled with previously prepared aqueous protein solutions to a level that kept the headspace negligible. Protein adsorption vials and blanks containing water and water with grafted-ceramic particles were prepared in triplicates. The vials were agitated using a 3-ft-long rack (capacity of 66 adsorption vials) that rotated the tubes end-over-end at 12 rpm. An equilibrium time of about 30 h was determined to be sufficient
for the adsorption study. At the end of the adsorption period the particles were removed from the solution by filtration using a 0.8-μm cellulose acetate membrane. The supernatant solutions were then analyzed in triplicate by UV spectrophotometry ($\lambda = 280$ nm).

Protein adsorption capacity was determined from a mass balance on the adsorption vials

$$q = \frac{V}{m}(C_0 - C_{eq}),$$

in which $C_0$ and $C_{eq}$ are the initial and final (equilibrium) protein concentrations (mg/L), respectively, $V$ is the solution volume (L), $m$ is the mass of particles in the vial (g), and $q$ is the amount of adsorbed protein (mg/g).

**RESULTS AND DISCUSSION**

**Surface Characterization**

The presence of the silylated vinyl silane and grafted poly(vinylpyrrolidone) on the ceramic surface was verified by DRIFT spectroscopy as illustrated in Fig. 1 for silylated and PVP-grafted zirconia. As shown in Fig. 1, a clear identification

**FIG. 1.** DRIFT spectrographs of the surface of unmodified (bottom), silylated (middle), and PVP-grafted (top) zirconia particles.
of the peak corresponding to the isolated hydroxyl groups at 3770 cm\(^{-1}\) or corresponding to neighboring hydroxyl groups at 3670 cm\(^{-1}\) (46) on the unmodified ceramic surface could not be made due to the broad spectrum associated with surface water. However, for the silylated surfaces, the presence of vinyl groups on the silylated surface was confirmed by the bands at 1600 and 1410 cm\(^{-1}\), corresponding to the stretch of the \(-\text{C}==\text{C}–\) double bond, and by the bands between 2900 and 3100 cm\(^{-1}\), corresponding to the C–H bond next to alkene. The above peaks remain visible in the spectrum of the PVP-grafted particles since there is a residual fraction of unreacted vinyl groups (belonging to the surface anchored VTMS) and \(-\text{C}==\text{C}–\) double bond-terminated surface PVP chains (46). The presence of PVP on the grafted surface was confirmed by the peaks in the region 1705–1680 cm\(^{-1}\), which correspond to the C–O groups in the PVP chain.

Previous studies on vinyl pyrrolidone graft polymerization have shown that the molecular weight distribution of the grafted polymer chains is typical of the expected distribution for free radical polymerization (5, 49). The example AFM image of a graft-polymerized PVP surface topology, shown in Fig. 2, is clearly consistent with the expected polydispersity of the surface chains. It is important to note that the AFM images were acquired under dry conditions and thus do not reflect the actual conformation of the surface layer when exposed to the protein solution. Nonetheless, the images clearly demonstrate that the surface is fully covered with PVP chains, with regions between the larger chains (or collection of large chains) populated with shorter PVP chains. Surface topology indicates layer features that rise in excess of 20 nm (relative to the native wafer surface) which is consistent with recent hydrodynamic thickness measurements for grafted PVP layers, prepared under the identical grafting conditions (51). The root-mean-square (RMS) surface roughness for the depicted graft-polymerized PVP surface, prepared with initial monomer concentrations of 2.81 M, was determined to be about 0.8 nm, relative to 0.1-nm RMS surface roughness for the unmodified wafer. Average RMS surface roughness deviation, based on analysis of five different 5 × 5-μm areas on the wafer, was less than about 10%.

The level of polymer graft yield is affected by the surface concentration of vinyl groups as shown in Fig. 3 for grafting onto zirconia particles. It is expected that the polymer graft yield will increase as more chains are initiated with increasing vinyl silane surface coverage (49, 50). However, in the present study a maximum graft yield was obtained at a silylation coverage of about 16.5 μmol/m\(^2\). Although the number of surface vinyl groups increases with increasing silylation coverage, at sufficiently high silylation coverage the progressive formation of a polysilane network can screen underlying vinyl groups, especially inbetween surface protrusions on the rough zirconia surface. As a consequence, the degree of inaccessibility of surface vinyl groups also increases, leading to the observed decrease in the polymer graft yield. A limiting polymer graft yield should be expected at sufficiently high surface vinyl silane concentration; however, this limit was not reached in the present set of experiments. We note that silylation coverage above a monolayer on zirconia (about 9.4 μmol/m\(^2\)) is indicative of the formation of polysilanes. Irrespective of the silylation coverage, the molecular weight of the grafted polymer should be similar for different batches of PVP-modified particles since the graft polymerization reaction conditions were kept identical.

The number-average molecular weight of the PVP surface chains, \(\bar{M}_n\), was estimated as \(\bar{M}_n = 1000 \cdot G/f S_a\), where \(G\) is the polymer graft yield (mg/m\(^2\)), \(f\) is the polymer grafting efficiency (49), and \(S_a\) is the vinyl silane surface concentration (μmol/m\(^2\)). An estimate of the molecular weight is best achieved using the zirconia-2 batch for which the silylation coverage of 10.6 μmol/m\(^2\) is near the theoretical monolayer value of 9.8 μmol/m\(^2\). Accordingly, using the above approach and a grafting efficiency of 0.0047 under the present reaction conditions (51) results in \(\bar{M}_n = 14,600\). The molar density of polymer chains (\(n_{\text{chains}}\)) on the surface is given as \(n_{\text{chains}} = (G/1000)/\bar{M}_n\), and the distance between grafted polymer
TABLE 3
Arrangement of Graft-Polymerized PVP Chains on Modified Zirconia Surface and LYS Adsorption Parameters

<table>
<thead>
<tr>
<th>Modified zirconia</th>
<th>Silylation coverage (µmol/m²)</th>
<th>PVP graft yield (mg/m²)</th>
<th>D (Å)</th>
<th>q_{max}^c (mg/m²)</th>
<th>K_d (L/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zirconia-1</td>
<td>21.93</td>
<td>0.45</td>
<td>73.2</td>
<td>3.0</td>
<td>0.42</td>
</tr>
<tr>
<td>Zirconia-2</td>
<td>10.60</td>
<td>0.73</td>
<td>57.6</td>
<td>4.8</td>
<td>0.45</td>
</tr>
<tr>
<td>Zirconia-3</td>
<td>21.93</td>
<td>0.8</td>
<td>54.9</td>
<td>5.3</td>
<td>0.42</td>
</tr>
<tr>
<td>Zirconia-4</td>
<td>21.80</td>
<td>1.03</td>
<td>48.4</td>
<td>6.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Zirconia-5</td>
<td>20.01</td>
<td>1.69</td>
<td>37.8</td>
<td>11.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Zirconia-6</td>
<td>13.33</td>
<td>1.72</td>
<td>37.5</td>
<td>11.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Zirconia-7</td>
<td>17.40</td>
<td>2.31</td>
<td>32.4</td>
<td>15.2</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*a pH 7; buffer: phosphate, ionic strength: 0.1 M.
*b Calculated from Eq. [3].
*c Maximum adsorption capacity: \( q_{max} \approx 0.94 \text{ mg/m}^2 \) for unmodified zirconia.

 chains, \( D \), can be estimated from

\[
D = (n_{\text{chain}} N_A)^{-1/2},
\]

where \( N_A \) is Avogadro's number. This distance can be used to evaluate surface graft density, \( \sigma \), defined as

\[
\sigma = (a/D)^2,
\]

where \( a \) is the monomer size estimated to be 4 Å for PVP repeating units. The properties of the grafted chains (\( D \) and \( \sigma \)), estimated as described above, are provided in Table 3. The regime of surface chain density can be determined following the scaling criterion of de Gennes (52), which specifies that terminally anchored chains, exposed to a good solvent, are in the “brush” (stretched) regime when \( \sigma > N^{-6/5} \), where \( N \) is the degree of polymerization of the grafted polymer chains. For PVP of \( M_n = 14,600 \), \( N^{-6/5} = 2.87 \times 10^{-3} \), and thus the above inequality is satisfied for all the PVP-grafted particles (Table 3); i.e., the polymer layer on the modified particles is in the brush regime.

Protein adsorption onto inorganic oxide surfaces is known to be significantly impacted by the presence of surface hydroxyls (–OH) on these substrates (5, 18, 53). Therefore, the success of screening or eliminating surface –OH groups, by a surface modification method, is best quantified by determining the presence of hydroxyls on the surface before and after the modification procedure. The concentration of hydroxyls on the surface of the unmodified zirconia particles was determined to be about 5.06 –OH/m² using the acid–base titration method described earlier. Following Rigney et al. (54), hydroxyl concentration can be also approximated from the TGA curve of the unmodified particles assuming that up to 350°C the weight lost is due to loss of strongly hydrogen bonded water, while surface hydroxyls condense at 350–800°C (Fig. 4). This analysis method results in an estimated surface –OH concentration of 5.65 –OH/m², in good agreement with Rigney et al. (54), who reported a concentration of 5.9 –OH/m². It is important to note that, after PVP modification of the ceramic particles, the concentration of surface hydroxyls as determined by the titration method was only 0.39 –OH/m² (about 7% residual surface hydroxyls). In contrast, it is interesting to note that studies with adsorbed PEO have reported to leave about 15–70% of the initial –OH groups unmasked (18).

The effectiveness of PVP graft polymerization was also quantified by surface charge density and \( \xi \) potential measurements of the unmodified and modified particles. The surface charge density for the PVP-grafted zirconia was found to be near zero over a wide pH range (Fig. 5). In contrast, the unmodified zirconia particles display a strongly positive surface charge as the pH decreases below about 6.5 and strongly negative surface charge...
density at pH above about 8. The surface charge density for the unmodified zirconia particles approaches zero at pH ≈ 7.5, in agreement with previously reported values (55, 56). The above results suggest that indeed any surface –OH groups that may be present on the modified zirconia are efficiently masked by the PVP-grafted chains. These results are also supported by the near-zero ζ potential for the PVP-grafted zirconia, in the range of studied pH (Fig. 6). In contrast, the ζ potential for the unmodified zirconia particles is 35 at pH = 2 and strongly decreases with increasing pH. At pH ≈ 6.8, the unmodified particles did not show any significant mobility (isoelectric point, pI), in agreement with other studies (44, 57, 58) that reported zero mobility for unmodified zirconia particles at 6.5 < pH < 7. It is plausible that the ζ potential could vary with the length of the grafted polymer chains; such an effect, however, was not observed in the present study since all grafted PVP surfaces were prepared under identical reaction polymerization conditions.

Protein Adsorption

Lysozyme adsorption isotherms were obtained at pH 7 and ionic strength 0.1 M. At pH 7, both the unmodified and PVP-grafted surfaces reveal a similar point of zero charge and ζ potential (Figs. 5 and 6). As a result, pH 7 LYS protein–surface electrostatic interactions should be negligible; therefore, LYS adsorption at this pH level should be indicative of the impact of the polymer brush on LYS adsorption.

The adsorption isotherms all appeared to be of the Langmuir-type isotherm, as displayed in the representative isotherms for zirconia-7 (Figs. 7a–7c). Accordingly, the experimental adsorption data were fitted with the Langmuir isotherm

\[ q = q_{\text{max}} \frac{K_d C}{1 + K_d C}, \]

where

\[ q = \frac{\text{adsorbed mass}}{\text{surface area}}, \]

\[ q_{\text{max}} = \frac{\text{maximum amount adsorbed}}{\text{surface area}}, \]

\[ K_d = \frac{\text{association constant}}{\text{surface area}}. \]
where \( q \) is the adsorption capacity (g protein/m\(^2\) adsorbent), \( C \) is the liquid phase concentration (g/L of solution), \( q_{\text{max}} \) is the maximum binding capacity (g protein/m\(^2\) of adsorbent), and \( K_d \) (L/g) is the apparent dissociation constant. Although the theoretical basis of the Langmuir isotherm is not strictly applicable to protein adsorption, it is a convenient and popular model for estimating the maximum adsorption capacity, \( q_{\text{max}} \), and assessing adsorption affinity from the magnitude of the initial isotherm slope. Scatter in the reported values of \( K_d \), for zirconia-1 through zirconia-4 batches is due to the fact that few data points could be obtained at low concentration for these high-affinity adsorption isotherms. Nonetheless, it is apparent that lysozyme has lower affinity for PVP-grafted zirconia surfaces, relative to the unmodified or silylated, as deduced from the lower initial adsorption isotherm slope (Table 3). Also, the maximum adsorption capacity (\( q_{\text{max}} \)) is lower for the modified surfaces, being lowest for the PVP-grafted surface. Overall, maximum adsorption capacity reductions of about 76 and 52% were achieved, relative to the native and silylated surfaces, respectively, upon the introduction of the PVP chains onto the surface (zirconia-7; polymer chain density of 0.158 mol/m\(^2\), graft yield of 2.31 mg/m\(^2\)).

LYS at pH 7 is positively charged and interacts with inorganic oxide surfaces mainly through surface –OH groups. Increased hydrophobicity of the surface upon silylation results in reduction of the maximum adsorption capacity as illustrated in Fig. 8. The decrease in the maximum adsorption capacity for the silylated surface, \( q_{\text{max, silyl}} \), appears to be linear with silane coverage, for the range of conditions in the present study. It is reasonable to expect that hydrophobic interactions between the protein and the silylated surface would increase with increased silylation coverage. However, given the reduction in adsorption upon silylation, it is likely that hydrophobic interactions between LYS and the silylated surface are less significant relative to reduction of protein interactions with surface –OH groups.

The adsorption capacities for the PVP-grafted zirconia particles decreased with increasing polymer graft yield (Fig. 9), in a manner quantitatively similar to results reported in the literature for adsorption of LYS onto PEO-grafted layers (33) and in the same range of fibrinogen adsorption onto PEO-grafted layers (31). The above behavior suggests that LYS adsorption onto the tethered PVP layer, for the high-polymer-surface-density samples (Table 3), is determined largely by the surface density of the chains. The effect of polymer surface chain density can be more effectively illustrated by displaying the maximum adsorption capacity of lysozyme, \( q_{\text{max}} \), as a function of the distance, \( D \), between polymer chain anchoring points (Fig. 10). Clearly, the adsorption capacity increases as the distance between chains increases, a result that is consistent with previous experimental and theoretical studies (13, 31, 32, 42, 59, 60). For example, Du et al. (13) demonstrated that, when polymer chains are sparsely positioned on the surface, in the so-called “mushroom regime,” the adsorption capacity of BSA, laminin, and fibronectin, onto a PEO tethered surface decreased with increasing polymer surface density as less of the surface was available for protein adsorption. For the above tethered PEO surface, a lower rate of adsorption reduction was observed at higher polymer surface densities in the brush regime. The decrease in protein adsorption capacity was most pronounced at low polymer surface coverage. At surface coverage higher than about 0.1 polymer chain/nm\(^2\) an adsorption plateau was reported for LYS and fibrinogen adsorption onto PEO layers (33). It is also interesting to note that the theoretical study of Szleifer (60), in which a single-chain mean-field (SCMF) theory was employed, also concluded that surface chain density is a paramount parameter in determining protein interactions.
adsorption reduction, although the size of the grafted chains may influence the kinetics of adsorption.

Above chain spacing of about 45 Å (the large axis of LYS), it is expected that lysozyme molecules could penetrate the polymer surface layer to some degree. On the other hand, when the distance between grafted points is lower than about 45 Å, chain spacing is sufficiently small that LYS molecules should be excluded from the dense brush layer; indeed, such behavior is noticeable in Fig. 10. It is also noted that, at approximately $D < 45$ Å, $q_{\text{max}}$ decreases faster with decreasing $D$ since it becomes energetically more difficult for the protein to penetrate the brush layer at this range of chain densities. Malmsten et al. (31) argued, based on studies with grafted PEO onto a silicon surface, that once a sufficiently high surface density is achieved ($\sigma/\sigma_0 = 3$, where $\sigma_0$ corresponds to the interfacial polymer chain overlap concentration, which is estimated in the studied system to be about 0.033 chain/nm$^2$), it is the protein size, relative to the average distance between grafted chains, that determines the rejection capacity of the grafted polymer phase. Sofia et al. (32) concluded, based on protein adsorption studies onto adsorbed PEO surface, that effective adsorption reduction requires polymer surface chains that are roughly half-overlapping ($D \approx R_G$, where $R_G$ is the radius of gyration of the chain). In the Sofia et al. (32) study chain spacing was greater than the size of the protein. The present study confirms, consistent with previous studies, that adsorption reduction is most effective when chain spacing is smaller than the protein size. A number of recent studies (61, 62) have suggested that lysozyme aggregation may occur at protein concentrations greater than about 0.3 mM which is significantly above the highest concentration of 0.05 mM in the present study. Therefore, lysozyme aggregation was not expected for the present study but is worth investigating. Finally, we note that the present results suggest that total exclusion of a protein the size of lysozyme may require grafted chain spacing lower than achievable even with the present method of free radical graft polymerization.

**CONCLUSIONS**

The present study demonstrated that adsorption of lysozyme onto zirconia can be reduced by a covalently bonded PVP brush layer. The adsorption capacity decreased with increasing polymer surface coverage, due to the increasing barriers for accommodating the protein molecule in the spacing between grafted polymer chains. In principle, total exclusion should occur when chain spacing is smaller than the protein size. However, residual adsorption will depend on specific protein–polymer interactions. The present results are encouraging and suggest further exploration of the potential application of polymer-modified ceramic surfaces for producing nonfouling ceramic ultrafiltration membranes and also for producing biocompatible ceramic surfaces for biomedical applications. Future work would need to address the optimization of surface chain density and length in relation to the specific selected polymer chemistry to reduce adsorption over wide ranges of pH and ionic strength.

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