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Chemical and Biochemical Modification of Surfaces for Control of Wettability, Adsorption, and Drug Delivery

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Chemical and Biochemical Modification of Surfaces for Control of Wettability, Adsorption, and Drug Delivery

Part I: Surface Modification of Calcium hydroxyapatite and Human Enamel to Control its Wetting and Adhesion Properties

Part II: Surfaces with Reversible Wettability Using Biochemical Surface Reactions

Part III: The Use of Nanoporous Silicas for Controlled Release

Ph.D. Thesis
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Abstract

Covalent modification of surfaces has proven to be an efficient strategy in the development of materials with controllable surface properties. This work investigates surface modification of calcium hydroxyapatite, and human dental enamel using self-assembled monolayers of organophosphonic acids with the goal of preparing calcium hydroxyl apatite surfaces with desired adsorption and adhesion properties. We also investigated biospecific surface reactions for the engineering of self-assembled monolayers supported on silica. We report the reactions of enzymes with lipid- and protein-rich surfaces for the preparation of surfaces with reversible polarity, e.g. capable of switching from hydrophobic to hydrophilic and back. The application of these biospecific self-assembled monolayers in the capillary system with gating properties has been demonstrated. This concept of surface chemistry control through the implementation of different self-assembled monolayers was also used to control the release of model drug molecules from porous silicas for oral drug delivery.
Covalent modification of surfaces has proven to be an efficient strategy in the development of materials with controllable surface properties. Different selective surfaces and modification techniques have been the focus of research and technology for a variety of applications including sorption and separation, adsorption and adhesion, waterproofing, lubricants, fillers, sensors, and optical and electronic devices.\(^1\)

Most surface modification work has been done on metals and metal oxides (primarily silica), the reactions of which have been extensively characterized and are well understood. In contrast, very little is known about surface modification of non-oxide surfaces, such as that of inorganic phosphates like calcium hydroxyapatite, CaHAP, \(\text{Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2\), the major mineral component of hard tissue (teeth and bones). Surface modification of CaHAP biological and biomimetic surfaces is of great interest in the development of materials with controllable adhesion and adsorption properties.

The use of organosilanes, mostly trialkoxysilanes, for surface modification of CaHAP and calcium phosphate ceramics has
been described. Organosilanes were used to control adhesion in polymer-CaHAP composites and to improve the mechanical properties of dental materials. The use of fluorinated silane coupling agents was shown to decrease the adhesion of bacterial plaque on the tooth. Reactions with organophosphates (ROPh(O)(OH)2) have been described in the works of Ishikawa and colleagues. According to refs 10-12, reaction mechanisms differ with solution concentration of alkyl phosphate: (1) surface modification was reported for low concentration solutions; (2) bulk modification of CaHAP and the formation of layered alkyl phosphate structures were reported for reactions of high concentration solutions. Recently, the use of isocyanates (RNCO) for surface coupling of polymers on CaHAP has been described.

The use of organosilanes for the modification of the surface of human dental enamel is of great importance for antifouling applications and the prevention of cariious formation. It is commonly accepted that salivary proteins adsorb to the enamel surface, aggregate, and form the acquired pellicle. Though the exact composition of the pellicle is unknown, it has been proposed that oral bacteria have receptors for portions of the adsorbed proteins, adhere to them, and form the biofilm known as plaque. After biofilm formation, the metabolic byproducts of the oral bacteria lower the pH of the
oral cavity which leads to carious formation. SAM formation on the surface of human enamel can not only be used to decrease the adhesions of salivary proteins and oral bacteria; a closely packed monolayer present on the surface could prevent exposure of enamel to a decreased pH in the oral cavity. Before one can examine the effects of a SAM supported on human enamel, some surface properties which will define the effectiveness of these surfaces must be explained.

**Dynamic Contact Angle and Surface Wettability**

The wettability of a surface can be demonstrated by how a liquid spreads on it. Figure 1.1 show examples of both wetting and non-wetting surfaces. On a wetting surface, the probe fluid, water, spreads over the surface while forming a drop on the non-wetting one. The most common method of evaluating the wetting phenomenon is through contact angle measurements.
Figure 1.1: Wetting and non-wetting surfaces. (A) example of a probe fluid on a wettable surface (B) example of probe fluid on a non-wettable surface.
It is known that an equilibrium contact angle of liquid on a solid surface results from an energy balance between three interfaces: the solid-liquid, the solid-vapor, and the liquid-vapor (see Figure 1.2). The famous Young equation\(^{28}\) describes this balance in interfacial energies:

\[ r_s = r_d + \gamma_v \cos \theta_e \]

where \( \gamma_s \) is the solid surface free energy, \( \gamma_d \) is the solid-liquid interfacial free energy, \( \gamma_v \) is liquid-vapor interfacial free energy, and \( \theta_e \) is the equilibrium contact angle. This equation, in conjunction with others, can be used to calculate the surface energy of a solid. In this work, our main focus is the wettability of the given surface. By definition, a wettable surface has a contact angle \( \theta < 90^\circ \), while a non-wettable surface has a contact angle \( \theta > 90^\circ \). Surfaces with high surface energies tend to be wettable, where liquid is spread over the surface to reduce energy. In contrast, liquids ball up on low surface energy surfaces to decrease the size of the interface. Since the contact angle is formed from an equilibrium between three interfaces, it would be expected that only one contact angle exists for a given surface. In reality, two contact angles, advancing and receding, are obtained from the same surface. The difference between these two is called contact angle hysteresis.
Figure 1.2: The equilibrium contact angle results from an energy balance between three interfaces: solid-vapor, solid-liquid, and liquid vapor.
Historically, hysteresis has been attributed to surface roughness and heterogeneity, but it has also been reported for molecularly smooth surfaces\textsuperscript{21-23}. Given its ubiquitous nature, contact angle hysteresis is now viewed as a characteristic of the surface instead of a flaw and can be used to calculate the free energy of the surface\textsuperscript{10}. Through out this work, contact angle measurements using both polar and non-polar probe fluids (water and hexadecane respectively) are reported along with contact angle hysteresis. These measurements demonstrate how, through the use of surface chemistry, the properties of a surface can be controlled e.g. wettability, adhesion, and adsorption.
1.1 Experimental Section

**General Information:** Infrared spectra were obtained with a Perkin Elmer Spectrum One FTIR instrument using a narrow band MCT detector. Spectra were collected in the reflectance mode using a Harrick Seagull accessory (Harrick, Ossining, NY). Spectra were taken at a 50° angle of incidence, with 124 scans and resolution 4cm⁻¹. X-ray photoelectron spectra (XPS) were recorded at the University of Massachusetts, Amherst, with a Perkin-Elmer Physical Electronics 5100 with Mg Kα excitation (400 W). Spectra were obtained at two different takeoff angles, 15° and 75° (between the plane of the surface and the entrance lens of the detector optics). Contact angle measurements were made with a Kame-Hart telescopic goniometer and a Gilmont syringe. The probe fluids used were reverse osmosis purified water and hexadecane. Dynamic advancing (θ_a) and receding angles (θ_r) were recorded while the probe fluid was added to and withdrawn from the drop, respectively. Chemical analysis was performed by Schwarzkopf Microanalytical Lab (Woodside, NY) using the ASTM method. Grafting densities (ρ, groups/ nm²) of the monolayers supported on CaHAP powders were calculated using the formula²⁴
\[
\rho = \frac{6 \pi \theta^{\circ}(\%C)}{[1200 \varepsilon_e - MW(\%C)]} \times \frac{1}{S(BET)}
\]  
(1)

where \( MW \) is the molecular weight of the grafted group, \( n_c \) is the number of carbon atoms in the grafted molecule, \( \%C \) is the weight carbon percentage in the sample, and \( S(BET) \) is the \( N_2 \) surface area of the bare CaHAP (m²/g). Low-temperature \( N_2 \) adsorption measurements were performed using a Coulter 100CX instrument.

The BET surface area for bare CaHAP was calculated assuming the cross section for nitrogen \( a(N_2) = 13.5 \AA^2 \), the value recommended by Jelinek and Kovats for metal oxides.\textsuperscript{25} The surface area for CaHAP modified with BP(O)\((CH)\_2\) was calculated using the cross section for nitrogen \( a(N_2) = 16.5 \AA^2 \), the value recommended for hydrophobic surfaces.\textsuperscript{25,26}

**Organophosphonic Acids:** \( n \)-Octadecyl- and \( n \)-octylphosphonic acids were purchased from Gryza Labs (Chelmsford, MA) and used as received. 1,1,2,2-Tetrahydrofluorodecylphosphonic acid was synthesized using the procedure described in reference 27 via the following reactions:

\[
\text{C}_8\text{F}_{17}\text{CH}_2\text{OH} + \text{NaP(OXOC)}\text{CH}_2\text{Cl} \rightarrow \text{C}_8\text{F}_{17}\text{CH}_2\text{P(OXOC)}\text{CH}_2\text{Cl} \rightarrow \text{C}_8\text{F}_{17}\text{CH}_2\text{P(OXOC)}\text{CH}_2\text{OH}
\]

The product was purified by crystallization from heptane. The structure was confirmed by chemical analysis and IR spectroscopy.
Calcium Hydroxyapatite: CaHAP powder was obtained from Aldrich and used as received. Thin film samples of CaHAP supported on Ti-coated Si wafers were prepared via a solution deposition process, following the method described in reference 28. The wafers were placed in the solution prepared by mixing equal volumes (5 mL) of sodium hydroxophosphate (1.86 mM) and calcium chloride (3.1 mM) for one week at 40°C.
Chemisorption of organophosphonic acids on CaHAP: A 0.1g sample of CaHAP was placed in a GC vial and dried at 100°C in an oven overnight. A 20 mL volume of RP(O)(OH)_2 solution was injected into the vial using a syringe. The reaction in four different solvents was investigated using toluene, ethyl alcohol, 1-butyl alcohol, and tetrahydrofuran (THF). All solvents (HPLC grade) were purchased from either Aldrich or Fisher. Upon addition of the solution, the reaction vessels were either placed in a controlled water bath or left at room temperature. After reacting for a given time (0.01 - 24h for kinetic measurements), the reactions were quenched by filtration on a Buchner funnel with a fritted disk. The samples were then washed with 3 x 20mL of the reaction solvent followed by 2 x 20 mL THF. The samples remained on the filter until dry and were dried in the oven overnight at 100°C. Reactions with CaHAP thin films were carried out in a similar fashion using smaller volumes of solvents. For measurements of the adsorption isotherms, solutions of organophosphonic acids in THF were allowed to contact with CaHAP for 5 days. Adsorbed amounts (Γ, mol/ g) were determined from carbon analysis. The equilibrium concentrations of the solutions were calculated using the following formula:

\[ C_n = C_0 - \Gamma \frac{m}{V} \]  
(2)
where \( C_0 \) is the initial concentration of the solution (M), \( V \) is the volume of the solution (0.1L) and \( m \) is the mass of CaHAP (0.1g). Adsorption isotherms of \( C_{14}H_{27}PO_{3}H_{2} \) were measured at three different temperatures (253, 298, and 323 K). The enthalpy of adsorption was calculated using the following equation:

\[
\ln \left( \frac{C_1}{C_2} \right) = \frac{\Delta H}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)
\]

where \( C \) is the equilibrium concentration of \( C_{14}H_{27}PO_{3}H_{2} \) that corresponds to the adsorption \( \Gamma \) at temperature \( T \). \( R \) is the universal gas constant.

1.2 Results

Characteristics of CaHAP samples: The characteristics of the CaHAP substrates are given in Table 1.1. An infrared spectra of bare CaHAP substrates is shown in Figure 1.3. The spectra are consistent with the spectra of CaHAP published in the literature.\(^{29,10}\) The sharp peak at \(-3570 \text{ cm}^{-1}\) is due to the lattice OH groups of CaHAP. Broad bands centered at \(-3300\) and \(-1650 \text{ cm}^{-1}\) are due to molecularly adsorbed water and hydrogen bonded OH groups. The peaks at \(-1450 \text{ cm}^{-1}\) are due to the chemisorption of carbon dioxide to the surface and, perhaps, due to contamination with calcium carbonate. The strong absorbance at \(-1160-960 \text{ cm}^{-1}\) is attributed to the stretching of the
phosphate group in CaHAP. The chemical composition of the powders was determined by chemical analysis, while XPS was used for thin films (Table 1.1). According to the Ca/P ratio, the materials were calcium deficient hydroxyapatite. The high carbon content in the CaHAP thin film is explained due to carbon contamination from ambient.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Chemical composition</th>
<th>Ca/P (mol)</th>
<th>$S_{\text{bet}}$ (m$^2$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>P</td>
<td>O</td>
</tr>
<tr>
<td>CaHAP powder</td>
<td>35.66$^a$</td>
<td>17.83$^a$</td>
<td>-</td>
</tr>
<tr>
<td>CaHAP film</td>
<td>16.41$^b$</td>
<td>9.23$^b$</td>
<td>5.52$^b$</td>
</tr>
<tr>
<td>Supported on Ti/Si</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ determined from chemical analysis; $^b$ determined from XPS.
Figure 1.3. IR spectra of CaHAP powder as is (d) and dehydrated at 600°C (a) 300°C (b) and 100°C (c).
Grafting Density of the Monolayers of RP(O)(OH)₂: Analysis of the molecular models (ACD Software) shows that the packing density in the monolayers of n-alkyl-phosphonic acids is limited by the cross section (σ) of the phosphonic group and not by the cross section of the alky chain. The cross section of the phosphonic group is ~0.23 nm² as estimated from the molar volume of phosphorous acid (H₃PO₃). A similar number (0.24 nm²) was used by the authors of reference 31 for the cross section of octadecylphosphonic acid adsorbed on titania and zirconia.

Assuming a vertical orientation of alkyl chains, and using σ = 0.23 nm², the grafting density of closely packed monolayers of alkylphosphonic acids can be obtained as follows: \( \rho = \frac{1}{\sigma} = \frac{1}{0.23} \approx 4.35 \) groups/nm². This agrees well with the grafting density obtained for self-assembled monolayers (SAMs) of C₁₈P(O)(OH)₂ supported on titania.²² It is noted that this number is smaller than ~5 groups/nm², the value reported for closely packed SAMs of long-chain alkyls, e.g. alkyltrichlorosilanes on silica.²³ From the mismatch of the cross sections it follows that molecules in the SAMs of alkylphosphonic acids must be tilted from vertical to achieve maximum contact between alkyl chains.
Figure 1.4. Adsorption isotherms (RT) of \(\text{C}_{12}\text{H}_{27}\text{P(O)(OH)}_2\) (solid symbols) and \(\text{C}_{6}\text{H}_{5}\text{P(O)(OH)}_2\) (open symbols) from the THF solutions on CaHAP powder. Dotted line corresponds to the monolayer capacity of CaHAP assuming cross-sectional area \(\sigma(\text{P(O)(OH)}_2)=0.23\, \text{nm}^2\).
Thermodynamics and Kinetics of the Chemisorption of RP(O)(OH) 

CaHAP. Adsorption isotherms of the alkylphosphonic acids on CaHAP from solutions in THF are shown in Figure 1.4. Isotherms have two steps. The first step corresponds to the adsorbed amount (Γ) ~0.24 mM/g and is practically independent of the length of the alkyl group in alkylphosphonic acid. Using the surface area of CaHAP (S_{BET}=60 m^2/g), it can be determined that Γ = 0.24 mM/g corresponds to the grafting density of ~2.4 group/nm^2 or ~0.42 nm^2 per one molecule of the alkylphosphonic acid. It is noted that this grafting density is only ~0.55 of the maximal grafting density of alkylphosphonic acids on the surface (4.35 group/nm^2). The low grafting density of alkylphosphonic acids suggests a disordered structure of the surface, which is also supported by nitrogen adsorption and wettabiliy data (see below). Molar enthalpy for the adsorption of octadecylphosphonic acid was determined from the adsorption isotherms measured at different temperatures (Figure 1.3) using eq.3. The value of ΔH obtained (~5615 kJ/mol) is quite large, demonstrating the strong interactions of the phosphonic group with P-OH and, perhaps, with Ca-OH groups on the surface of CaHAP. FTIR data provides more insight into the surface bonding of the monolayers and suggest the formation of covalent bonding at the interface. This data will be discussed in the following section.
According to Figure 1.4, the adsorption rapidly increases with the equilibrium concentration of RP(O)(OH)₂ and levels off at ~14-16 mM/g. Considering that this value of $\Gamma$ exceeds the monolayer capacity by a factor of ~35, it implies the aggregation of alkylphosphonic acids on the surface. We note that "two-step" adsorption isotherms similar to those shown in Figure 1.4 are typically reported in the literature for the adsorption of surfactants on different surfaces. The initial increase in the isotherm (the first step) is described as a monolayer adsorption, which is primarily driven by ionic interactions or hydrogen bonding between surfactant and the surface. Further increase in the adsorption is described as the growth of surface aggregates of surfactant in the direction perpendicular to the surface. We point out, however, that formation of micellar aggregates of RP(O)(OH)₂ on the surface is unlikely for the following reasons. The chemisorption of RP(O)(OH)₂ was carried out in organic solvent, which provide poor environments for the formation of micelles from long chain, amphiphilic molecules. Further, the surfaces are rather stable for non-covalent, micellar aggregates. For example, no desorption of the surfactant was observed after rigorous washing and ultrasound treatment, which would remove surface micelles. Adsorption studies using various octadecyl surfactants (C₁₈H₃₇NH₂, C₁₈H₃₇COOH, C₁₈H₃₇SCNNa, C₁₈H₃₇OH) in THF solutions failed to show a rapid increase in adsorption seen for RP(O)(OH)₂ (Figure 1.4). This phenomenon must, therefore, be unique to the interactions of phosphonic acid and CaHAP.
The closest work reported in the literature to the work presented here was done by Ishikawa e.a.,\textsuperscript{10-12} who studied the reactions of alkylphosphates (ROP(O)(OH))\textsubscript{2}, R=ethyl, hexyl, octyl, decyl) with CaHAP. Based on the XRD studies, two reaction mechanisms were proposed. (1) The surface modification of CaHAP was proposed for the reaction of low concentration solutions of ROP(O)(OH))\textsubscript{2} (less than 0.1M). (2) The bulk modification of CaHAP and the formation of layered alkylphosphate structures was proposed for the reactions of high concentration solutions (>0.1M and greater). Based on the similarity of the structure of alkylphosphates and alkylphosphonic acids, one can expect similarity in their reactions with CaHAP. Investigation of the structure of CaHAP-ROP(O)(OH))\textsubscript{2} composites is currently underway in our lab.
Figure 1.5. Initial regions of the adsorption isotherms of $\text{C}_2\text{H}_3\text{P(O)}(\text{OH})_2$ on CaHAP from THF at different temperatures. Adsorption at 253K (open symbols), adsorption at 298K (black symbols), adsorption at 323K (gray symbols).
The reaction kinetics of $\text{C}_{18}\text{H}_{35}\text{P}(\text{O})(\text{OH})_2$ with CaHAP was studied in different solvents. Chemical analysis data (C, H) was used to follow the surface concentrations of grafted molecules. The uptake plots (Figure 1.6) were adequately described as a first-order process:

$$\frac{P}{\rho_0} = 1 - e^{-kt}$$

(4)

$\rho$ is the grafting density at time $t$, $\rho_0$ is the maximum grafting density, and $C$ is the concentration of the solution. Table 1.2 presents the rate constants ($k$) obtained through the curve fitting procedure using MathCad 8.0 software.

The data in Table 1.2 and Figure 1.6 shows that solvent has a profound effect on the rate constant. The values of $k$ change more than one thousand times. According to the rate constant, the solvents range as follows: toluene ≥ ethanol > 1-butanol >> tetrahydrofuran. It is noted that the solubility of $\text{C}_{18}\text{H}_{35}\text{P}(\text{O})(\text{OH})_2$ in these solvents ranges in the reverse order. Toluene and ethanol are rather poor solvents for $\text{C}_{18}\text{H}_{35}\text{P}(\text{O})(\text{OH})_2$ with the solubility ~2.5 and ~3 g/L respectively. Solubility in 1-butanol is ~10 g/L. The highest solubility of $\text{C}_{18}\text{H}_{35}\text{P}(\text{O})(\text{OH})_2$ is observed for THF (> 40g/L). The effect of the solvent can be explained in terms of the solvent-solute and the solute-surface interactions. In a good solvent, strong solvent-solute interactions are favored, diminishing solute adsorption and
Figure 1.6. Kinetics of the reaction of \( \text{C}_4\text{H}_7\text{P(O)(OH)}_2 \) with CaHAP in different solvents: toluene (squares), 1-butanol (diamonds), tetrahydrofuran (circles). Reaction conditions: 25°C, concentration of \( \text{C}_4\text{H}_7\text{P(O)(OH)}_2 \) 9.4 mM.
reaction with the surface. Conversely, in a poor solvent, solute-surface interactions are favored. We note that careful analysis of the interactions in the system should also account for the possible specific interactions between the solvent and the surface, which may include π-bonding, hydrogen bonding etc. However, since no irreversible adsorption of pure solvents on CaHAP was observed, we assumed that these interactions are not significant. The activation energy of the reaction ($E_a$) was determined from the temperature dependence of the rate constants (Arrhenius plots). For all solvents, the same value, $E_a = 20 \pm 3$ kJ/mol, was obtained.
Table 1.2

Kinetic parameters for the reactions of C$_6$H$_5$F(O)(OH)$_2$ with CaHAP

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Reaction temp., °C</th>
<th>Rate constant, 1/mol·h</th>
<th>$E_a$, kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>25</td>
<td>1620</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>7012</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>25</td>
<td>1005</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5020</td>
<td></td>
</tr>
<tr>
<td>1-Butanol</td>
<td>25</td>
<td>170</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1191</td>
<td></td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>25</td>
<td>1.2</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>
Reaction of Organophosphonic Acids with CaHAP According to FTIR.

Since the spectra obtained for both CaHAP powder and CaHAP thin films were similar, only the spectra for CaHAP powder will be discussed. Figure 1.7 presents the reflectance spectra of CaHAP that reacted with solutions of CmHnPO(OH)2 of different concentrations. According to Figure 1.7, spectra substantially differ depending on the adsorbed amount of octadecylphosphonic acids. Samples with grafting density -2.4 group/nm2 and less (corresponds to the adsorbed amount -0.24 mM/g and less, i.e. the first step of the isotherm, Figure 1.4) show the following characteristic features. First, compared to bare CaHAP there is a decrease in the absorbance at ~3400-3300 and 1650 cm⁻¹ (ν(stretching and deformation of adsorbed molecular water)).

Removal of the adsorbed water is explained by the formation of hydrophobic surfaces through the reaction of CaHAP with RP(O)(OH)2. Inspection of the high frequency region of the spectra (OH stretches) reveals a decrease in the absorption at ~3690 cm⁻¹ (surface PO-H groups32,33), while the sharp peak at ~3570 cm⁻¹ (OH from the lattice of CaHAP32,33) remains unchanged. Bands at ~2920, 2850 cm⁻¹ (ν(CH₃)) and ~1469 cm⁻¹ (δ(CH₃)), 782 and 716 cm⁻¹ (ν(PO)) are consistent with the appearance on the surface of alkyl groups from the alkylphosphonic acids. Analysis of the P-O stretching region (Figure 1.8) is complicated due to strong background absorbance of the CaHAP matrix (ν(P=O)). It is noted from Figure 1.6 that the bands from P-OH (~1074 and 928 cm⁻¹, ν₁ and ν₂ P(OH)₂) and the P-O group
(-1229 cm⁻¹, ν(P=O)), present in the spectra of pure octadecylphosphonic acid, are not seen in the spectra of CaHAP with low grafting density of octadecylphosphonic acid. This indicates a strong interaction between the phosphonic acid and the surface P-OH groups, probably, through the formation of P-O-P bonds:

![Chemical structure diagram](image)
Figure 1.7. IR spectra of CaHAP with different amounts of adsorbed $\text{C}_{12}\text{H}_{27}\text{P(O)}(\text{OH})_2$: 0.2 mM/g (b); 1.2 mM/g (c); 14 mM/g (d) and bare CaHAP (a).
Figure 1.8. Spectra in the P-O stretching region for CaHAP with different amounts of adsorbed $\text{CaH}_{3}P(OH)_{2}$ (0.2 mM/g (a); 1.2 mM/g (b); 14 mM/g (c) and the spectrum of pure $\text{CaH}_{3}P(OH)_{2}$ (d)).
In previous works on the reaction of alkylphosphonic acids with TiO$_2$, ZrO$_2$, and Al$_2$O$_3$, the absence of the P-O band in the spectra has been interpreted as evidence for the formation of phosphonate species (RPO$_3^-$) attached to the surface via three P-O bonds. We disagree with this explanation and point out that this mechanism would require a change in the oxidation state of phosphorus (from plus five to plus four), which is not acknowledged in prior works. We believe that the disappearance of the P=O stretches from the spectra can be explained due to hydrogen bonding (P$_2$-OH...O=P). We also offer that the disappearance of this band from the spectra may be due to a decrease in the extinction coefficient, which it is not uncommon in the spectroscopy of adsorbed species. Obviously, more work is needed to elucidate the exact mechanism of surface bonding in this system.

For samples with high loading of RP(O)(OH)$_2$, the following additional features are present in the spectra. Bands at -2340 cm$^{-1}$ (v(PO-H)), -1080 and -930 cm$^{-1}$ (v(P=OH)) appear and grow as the concentration of adsorbed RP(O)(OH)$_2$ exceeds 5 group/nm$^2$ (corresponds to the adsorbed amount of 0.5 mM/g). These bands indicate the presence of non-coupled P-OH groups of alkylphosphonic acid in the samples. The vanishing of the 3570 cm$^{-1}$ band (lattice OH groups) allows us to propose that the reaction of CaHAP with C$_{14}$H$_{27}$P(O)(OH)$_2$ at high concentrations
results in changes of the crystalline structure and bulk modification of the CaHAP matrix. The mechanism of this process is yet unclear and is the subject of further investigation.17

Perhaps, the most notable feature of Figures 1.7 and 1.8 is a series of bands in the ~1100-1400 cm\(^{-1}\) range that appear only in the spectra of samples with high loadings of Ca\(_{12}H_{7}P(O)(OH)\(_{2}\) (effective grafting density greater than ~5 group/nm\(^{2}\)). These peaks are assigned to the CH\(_{2}\) wag-twist modes, whose appearance indicates the presence of all-trans sequences of the alkyl chains.\(^{39,40}\) The average spacing between these bands (\(\Delta \nu = 1841\) cm\(^{-1}\)) agrees with the value of 18 cm\(^{-1}\) predicted for fully extended C\(_{12}\) chains,\(^{39,40}\) indicating a high degree of ordering in the system.

Further details about the ordering of alkyl chains in the modified CaHAP can be assessed from the position of the CH\(_{2}\) stretching. For a completely disordered structure, the frequency is close to that of a liquid alkane (\(\nu_{s} = 2928\) and \(\nu_{s} = 2856\) cm\(^{-1}\)). For well-ordered monolayers, e.g. SAMs of alkylthiols on gold or alkyltrichlorosilanes on silica,\(^{41-45}\) the frequency is close to that of crystalline alkane\(^{46}\) (\(\nu_{s} = 2915-18\) and \(\nu_{s} = 2846-50\) cm\(^{-1}\)). For a series of CaHAP samples that reacted with octadecylphosphonic acid over different periods of time, the frequency of CH\(_{2}\)-stretching gradually shifted to lower wave numbers, indicating a transition from the completely disordered to ordered structures. For surfaces with grafting density ~2.4 group/nm\(^{2}\) (the first plateau in the adsorption isotherm, Figure
the stretching frequency $v_1$ is around 2920-21 cm$^{-1}$ and $v_3$ -2851-52 cm$^{-1}$ respectively. These numbers indicate a certain degree of order in the monolayers, which is, however, lower than for the closely-packed octadecyl SAMs. For samples with high loadings of C$_{18}H_{37}P(O)(OH)$, (effective grafting density greater than -5 group/nm$^2$, the raise and the final plateau in the adsorption isotherms, Figure 2) the stretching frequency $v_1$ is around 2916 cm$^{-1}$ and $v_4$ ~2848 cm$^{-1}$ respectively, indicating a high degree of ordering of alkyl groups.

**Adsorption and Wettability Studies of Organophosphonic Modified CaHAP Surfaces.** The main focus of this study was to demonstrate that the adsorption and wettability properties of CaHAP surfaces can be effectively modified using organophosphonic acids. For adsorption studies surfaces were prepared by reacting RP(O)(OH)$_2$,

$$(R = C_6H_{13}, C_{18}H_{37}, (CH_2)_2CF_{17})$$

with CaHAP powder. For wettability studies CaHAP thin films were used.

Table 1.3 shows water and hexadecane contact angle data for a series of CaHAP supported surfaces. Bare CaHAP shows low contact angles, demonstrating the presence of a high energy, polar surface covered with OH groups. After the reaction with alkylphosphonic acids, the water contact angles increased to ~100-105°, indicating the formation of hydrophobic surfaces. Although the obtained values of the water contact angles are
Table 1.3
Wettability Data for CaHAP Surfaces Modified with RP(O)(OH),

<table>
<thead>
<tr>
<th>Surface</th>
<th>Water CA (adv/rec), °</th>
<th>Hexadecane CA (adv/rec), °</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaHAP bare</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CaHAP-O,PC₄H₁₁</td>
<td>105/80</td>
<td>15/0</td>
</tr>
<tr>
<td>CaHAP-O,PC₆H₁₁</td>
<td>100/75</td>
<td>20/0</td>
</tr>
<tr>
<td>CaHAP-O,PC₈H₁₁</td>
<td>115/95</td>
<td>45/15</td>
</tr>
</tbody>
</table>
quite high, they are notably smaller than 110-115°, the numbers typically reported for the CH₃-terminated SAMs of long chained alkylsilanes on silica or alkylthiols on gold. The hexadecane contact angles are also lower than 45°, the number reported for CH₃-terminated SAMs. Low water and hexadecane contact angles suggest disordered surfaces of alkyl groups enriched with methylene (CH₂) rather than with methyl (CH₃) functionalities. For fluorinated alkylphosphonic acid, the contact angles obtained are also notably lower than the values typically reported for closely packed CF₃-surfaces, −125° for water and −75° for hexadecane. All surfaces show large values of contact angle hysteresis, 20-25° for water and 15-30° for hexadecane. High contact angle hysteresis indicates the presence of different chemical functionalities interacting with the probe fluids and is consistent with the disordered structure of the surfaces.

While wettability data characterizes the surfaces at the solid-liquid interface, the vapor phase adsorption provides insight into the interactions at solid-vapor interface. Typical adsorption isotherms for bare CaHAP and alkylphosphonic-modified CaHAP are shown in Figure 1.9. The adsorption isotherms are Type II,¹⁰ indicating physical adsorption of N₂ on the surface. It is noted that the isotherm for bare CaHAP goes higher and is substantially more convex than those for modified CaHAP. This
indicates weakening of the adsorption interactions for the modified surfaces, which is consistent with the hydrophobic nature of the monolayers of RP(O)(CH)₂. The intensity of the adsorption interactions can be characterised by the constant $C$ of the BET equation$^{51}$:

$$\frac{P}{v(P_s-P)} = \frac{1}{v_mC} + \frac{C-1}{v_mC} \frac{P}{P_s}$$

where $v$ is the adsorption at pressure $P$, $v_m$ is the monolayer capacity, $C$ is a constant, and $P_s$ is the saturation vapor pressure of the adsorbate. According to the authors of the BET model,$^{51}$ the average heat of adsorption can be estimated as follows:

$$\Delta H = RT \ln C$$

where $R$ is the gas constant, and $T$ is absolute temperature. The high value of $C$ constant (220) obtained for bare CaHAP demonstrates a high energy, polar surface. Modification of CaHAP with RP(O)(OH)₂ results in a drop of the $C$ constants, indicating a decrease in energy of the adsorption interactions. $C$ constants for the modified CaHAP range as follows: 33 (CaH₇PO₄-CaHAP) and 35 (C₁₀H₁₇PO₄-CaHAP). The typical value of the BET $C$ constant reported for the surfaces of disordered alkyl chains is around 20-24, while for closely-packed CH₃ surfaces the $C$ constant is around 11-12.$^{51,53}$ The values of $C$ constants observed for alkyl-modified CaHAP surfaces suggest that adsorption takes
place primarily on disordered alkyl groups rather than on the bare surface of CaHAP. Alkyl groups of alkylphosphonic acids effectively shield CaHAP, acting as a structural barrier, preventing nitrogen molecules from interacting with the polar centers of CaHAP.

By preparing surfaces with intermediate grafting densities (e.g. by controlling the reaction time) one can control the energy of adsorption interactions over a wide range. As shown in Figure 1.10, the C constant decreases as the monolayer grafting density increases. It is noted that the BET surface area of the modified CaHAP varies only insignificantly and remains close to that of bare CaHAP (Figure 1.10). To conclude this section, wettability and adsorption data agree well to each other and indicate that the intensity of the interactions at the CaHAP surfaces can be effectively modified through the reaction with PF(O)OH₂.
Figure 1.9. Nitrogen adsorption isotherms (77K) for bare CaHAP and $\text{C}_{18}\text{H}_{37}\text{PO}_{3}$-CaHAP (grafting density 3.4 group/nm$^2$).
Figure 1.10. BET surface areas (open symbols) and C constants of the BET equation (closed symbols) for CaHAP treated with C$_4$H$_8$P(O)(OH)$_2$ vs. grafting density.
Bacterial Adhesion to Surface Modified CaHAP

In an effort to assess the effect the formation of SAMs on the surface of CaHAP have on bacterial adhesion; collaboration was set up with Dr. Frank Scannapieco of the Department of Oral Microbiology at SUNY Buffalo Dental School. Yi-Ping Liu, a dental school graduate student, performed the following experiment which is the accepted way to assess bacterial adhesion: Samples of bare and organophosphonic acid modified CaHAP was placed in a chamber and a stream of oral bacteria was passed over the samples. The number of bacteria was counted before and after passing through the sample. The difference was assumed to be the number of bacteria adhered to the surface. Figure 1.11 depicts the findings of bacterial adhesion to the modified CaHAP samples. The bare CaHAP sample showed the highest level of bacterial adhesion, while the carboxy ethyl modified CaHAP showed the least. It is noted that there is only a small difference between all samples tested. These results do not show an optimal decrease in bacterial adhesion but do show the potential of altering surface chemistry to control bacterial adhesion.
Figure 1.11: Bacterial Adhesion to the Surface of Modified CaHAP

Adhesion of oral bacteria to bare and modified CaHAP (carboxy ethyl, trishydroxymethyl, ODPA, hexamethyl diisocynate, and phosphoryl choline)

Graph done in collaboration with Dr. Frank Scannapieco, SUNY Buffalo Dental School
1.3. Conclusions

In this work, we investigated the surface modification of calcium hydroxyapatite (CaHAP) with organophosphonic acids (RP(O)(OH))₄. The solution-phase reactions of RP(O)(OH)₄ (R = n-C₃H₇, n-C₄H₉, and n-C₅F₃(CH₃)₃) were studied with two types of CaHAP substrates: high surface area powder (S₆₈, BET=60 m²/g) and thin films supported on Ti/Si wafers. It was found that the concentration of the RP(O)(OH)₄ solution has a dramatic effect on the structure of modified surfaces. Solutions of low-concentration (<5-10mM or less) yielded covalently-attached monolayers supported on CaHAP. According to FTIR, the major products of the reaction were organophosphonic groups bound to the surface via P₄-O-P bonds. Grafting densities of alkyl groups in these monolayers were ~2.4 group/µm², which is only ~0.55 of the maximum values typically observed for closely-packed self-assembled monolayers, e.g. alkyltrichlorosilanes on silica, indicating a disordered structure of the surfaces. Loose packing of alkyl groups was also supported by adsorption and wettability studies. Solutions of high-concentration (~10-20mM or higher) yielded the formation of composites with a high loading of organics believed to be due to the bulk modification of CaHAP. The kinetics of the reactions followed the first-order equation with rate constants ranging from ~1 L/mol·h using a good solvent (THF) to ~1000 L/mol·h using poor solvents (toluene, ethanol). Modified surfaces showed good hydrolytic and thermal stability. No loss of the grafted material was observed after rigorous washing with solvents and
ultrasound, which makes this surface modification technique useful for tailoring the CaHAP surfaces and modifying the adsorption and wettability properties of the CaHAP adsorbents, fillers, and biomaterials. It was also shown that the grafted layer did reduce the ability of oral bacteria to adhere to CaHAP.
Reactions of Organophosphonic Acids with Human Enamel surfaces

Introduction

Self-assembled monolayers (SAMs) have proven to be an efficient biomimetic strategy in the development of materials with controllable surface properties such as adsorption and adhesion. Surface modification of biological CaHAP surfaces, e.g. surface of teeth, is of great interest in the development of biomaterials with controllable adhesion and adsorption properties.

The presence of a SAM on the surface of the tooth has the potential to modify the development of the acquired pellicle via changes in surface free energy to alter the adsorption and adhesion properties of the tooth surface. SAMs may also protect the surface of the tooth against demineralization. The use of organosilanes for surface modification of calcium phosphate ceramics and dental materials has been described. The use of fluorinated silane coupling agent was shown to decrease the adhesion of bacterial plaque on the tooth. However, the toxicity of the reagents used is a concern. SAMs of organophosphonic acids have been studied in various metal and metal oxide surfaces. These monolayers are robust, easily tailored for adsorption, wettability, and adhesion properties of the interfaces. Organophosphonic acids, in general, are known for low toxicity in mammals. They are routinely used as
treatments for metabolic disorders of the bone (Paget's disease) and therapy of tumor induced hypercalcemia.\textsuperscript{44} Recently, we described the use of organophosphonic acids for the surface modification of CaHAP powders.\textsuperscript{69} In this work we present the results on synthesis and characterization of SAMs of octadecylphosphonic acid (C\textsubscript{18}H\textsubscript{37}P(O)(OH)\textsubscript{2}) supported on human teeth. The SAMs demonstrate good hydrolytic stability and are efficient for the modification of surface energy of enamel as assessed by wetting experiments.

2.1. Experimental

**General Information:** Solvents (HPLC grade) were purchased from Aldrich. Infrared spectra were obtained with a Perkin Elmer Spectrum One FTIR instrument using a narrow band MCT detector. Spectra were collected in the ATR mode with a germanium crystal using a Harrick Seagull accessory (Harrick, Ossining, NY). Spectra were taken at 124 scans, resolution 4 cm\textsuperscript{-1}. Contact angle measurements were made with a Ramé-Hart telescopic goniometer and a Gilmont syringe. The probe fluids used were RO purified water and hexadecane. Dynamic advancing ($\theta_a$) and receding angles ($\theta_r$) were recorded while the probe fluid was added to and withdrawn from the drop, respectively. All contact angle measurements were made on flat pieces of modified enamel. On average, six advancing angle and two receding angle
measurements were made per sample. The average data is presented below. The standard deviations for these measurements were in the range of 4-8 degrees.

**Human Tooth Specimens.** Extracted human teeth were used in this work. Human teeth were obtained from Kernan Dental Associates after autoclave sterilization treatment in accordance with the OSHA procedure. Samples were cut into an average of six to eight enamel chips per tooth. Enamel chips were cleaned via sonication with Alconox detergent followed by deionized water.

**Reaction of Octadecylphosphonic Acid with Enamel.** Octadecylphosphonic acid (ODPA) was purchased from Oryza Labs (Chelmsford, MA) and used as received. Prior to the reaction, the enamel chips were soaked in THF for 10-12 h. 20 ml of the solution of ODPA in THF (0.03M) was added to the vial. Upon addition of the solution, the reaction vessels were sealed and left at room temperature. The reactions were quenched by rinsing the samples with an excess of THF (4x20 ml) followed by ethanol (20 ml) and dried at room temperature.

**Atomic Force Microscopy.** AFM studies were performed at Clemson University on a Dimension 3100 (Digital Instruments, Inc.) microscope. We used the tapping and phase modes to study the surface morphology of the bare enamel surface and the surface covered with SAM. Silicon tips with spring constants of 50 N/m were used. Imaging was done at scan rates in the range 1 - 2 Hz. The scanning was conducted at the highest set-point value.
that permitted a reproducible imaging. Power spectra density plots (PDS) representing Fourier transform of the SPM images were evaluated using commercial software (Digital Instruments and Origin). Picks on the PDS plots were associated with dominant lateral dimensions of the surface located structural elements. The root-mean-square roughness (RMSR) of the samples was evaluated from topographical SPM images recorded in the tapping mode. RMSR is the standard deviation of feature height \( Z \) values within a given area:

\[
Roughness = \sqrt{\frac{\sum (Z_i - Z_{av})^2}{N}}
\]

(1)

where \( Z_{av} \) is the average \( Z \) value within the given area, \( Z_i \) is the current \( Z \) value, and \( N \) is the number of points within a given area.

2.2: Results

Reaction of Octadecylphosphonic Acid with Enamel by ATR. The solution-phase reactions of \( \text{C}_{18}\text{H}_{37}\text{P(O)(OH)}_2 \) with enamel surface have been investigated. Surface reactions were monitored by ATR. Figure 2.1 presents the ATR spectra of bare enamel and enamel samples that were reacted with solutions of \( \text{C}_{18}\text{H}_{37}\text{P(O)(OH)}_2 \) over different periods of time. The spectrum of bare enamel agrees with those reported in the literature\(^6\) and shows the
Figure 2.1. ATR spectra of enamel after soaking in THF (a); enamel reacted with C_{12}H_{25}PO(OH)_{2} (1% solution in ethyl alcohol) for 1h.
following peaks: \(-3570\text{cm}^{-1}\) (bulk OH from hydroxyapatite matrix), \(-3250\text{cm}^{-1}\) and \(1640\text{cm}^{-1}\) (stretching and deformation of adsorbed water), \(-1100-1050\text{cm}^{-1}\) (stretching of \(\text{PO}_4\) from hydroxyapatite matrix), and \(-630\text{cm}^{-1}\) (OH liberation). The spectra of SAMs supported on enamel showed the following additional peaks: \(2916\text{cm}^{-1}\) and \(2849\text{cm}^{-1}\) (CH\(_2\) stretching from alkyl groups), \(781\text{cm}^{-1}\) and \(719\text{cm}^{-1}\) (stretching of P-O-C). Careful analysis of the P-O region \((-1200-900\text{cm}^{-1}\)) of the spectra is complicated due to strong background absorbance of the hydroxyapatite \((\nu(\text{PO}_4))\). It is noted, however, that the bands from P-OH \((-1030\text{ and } 940\text{ cm}^{-1}\)), and \(\nu_\text{c} \text{ P(OH)}_2\)) and the P=O group \((-1230\text{ cm}^{-1}\), \(\nu(\text{P=O})\), present in the spectra of pure octadecylphosphonic acid, are not seen in the spectra of enamel-supported SAMs. This is arguing for the strong interaction between the phasmonic group and the surface with the formation of a tridentate species \((\text{RPO}_3)\) grafted to the surface through P-O-P bonds. A similar mechanism of surface bonding was proposed for the reactions of alklyphosphonic acids with CaHAP powder\(^{11}\) and with different metal oxides.\(^{48,49}\)

The ordering of alkyl chains in the SAMs can be assessed from the position of the CH\(_2\) stretching (Figure 2.2). For a completely disordered structure, the frequency is close to that of a liquid alkane (\(\nu_\text{c} = 2928 \text{ and } \nu_\text{c} = 2856 \text{ cm}^{-1}\)). For well-ordered structures, e.g. crystalline alkane\(^{10}\) the frequency is \(\nu_\text{c} = 2915-18\)
and ν₂-2846-50 cm⁻¹. For a series of enamel-supported SAMs the position of CH₂ stretches changes over time from ~2919 cm⁻¹ to ~2916 and 2848 cm⁻¹ (> 1 h of reaction time), indicating the formation of a highly ordered surfaces similar to the closely-packed SAMs of long chained alkyls.⁷¹-⁷⁴
Figure 2.2. The CH\textsubscript{2} stretching region of ATR spectra of enamel reacted with 1% of solutions of C\textsubscript{10}H\textsubscript{25}P(O)(OH)\textsubscript{2} in THF: (a-e) 10 min, 1 h, 10 h, 24 h and 100 h of the reaction time respectively.
Contact Angles of Enamel-Supported SAMs. The focus of this study was to demonstrate that wettability and, therefore, the surface energy of tooth can be effectively modified using SAMs of organophosphonic acids. Water and hexadecane contact angle data for a series of enamel-supported surfaces are shown in Table 2.1. Advancing/receding water contact angles of bare enamel are 60°/35°, which indicates the presence of organics (most likely protein) contamination on the surface. Rinsing with organic solvent (THF) gives surfaces with water contact angles of 40°/21°, which agrees with those previously reported for bare enamel.\textsuperscript{12} We note, however, that these angles are still higher than those reported for clean CaHAP surfaces (<10°).\textsuperscript{12} This may indicate the presence of strongly adsorbed organics or structural proteins (hydrophobic) incorporated in the enamel matrix. According to reference\textsuperscript{12}, the surface of enamel consists of ~90% of CaHAP and ~10% of matrix proteins.
<table>
<thead>
<tr>
<th>Surface</th>
<th>Water CA (adv/rec), deg.</th>
<th>Hexadecane CA (adv/rec), deg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel-Untreated</td>
<td>60/35</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Enamel-Cleaned (detergent, THF)</td>
<td>40/21</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Enamel-O,PC&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;, reaction time 100h</td>
<td>98/68</td>
<td>20/&lt;10</td>
</tr>
</tbody>
</table>
Figure 2.3 shows the evolution of contact angles as a function of enamel exposure to solutions of octadecyl phosphonic acid. The water contact angles for SAMs increase over time and level off after ~24 h of reaction time at ~100°, indicating the formation of low energy, hydrophobic surfaces. Although the obtained values of the water contact angles are quite high, they are notably smaller than 110°-115°, the numbers typically reported for the CH₃-terminated SAMs of long chained alkyls. The hexadecane contact angles are also lower than 45°, the number reported for CH₃ terminated SAMs. Low water and hexadecane contact angles may be interpreted as either the presence of a disordered SAM structure or incomplete surface coverage. Since IR data shows high degree of order of alkyl groups in SAMs, we believe incomplete surface coverage is responsible for contact angle data lower than expected. In order to assess the different group contributions to wettability, we used the Israelachvili-Gee model for the wetting of heterogeneous surfaces:

\[ (1+\cos\theta)^2 = f_1(1+\cos\theta_1)^2 + f_2(1+\cos\theta_2)^2 \]  
\[ f_1 + f_2 = 1 \]  

We treat the enamel-supported SAM as a mixture of methyl groups (θ₁ = 110°) and bare enamel (θ₂ = 40°). Equation (2) gives an angle of 100° for mixed surface with the following composition: \( f_1 = 0.9 \) and \( f_2 = 0.1 \) respectively. Thus, the contact angle data suggest ~10% of uncovered enamel present on the surface, even in the SAMs prepared after 24-100 h of reaction. Incomplete surface
coverage with SAMs can be explained by the heterogeneity of enamel, primarily due to the presence of matrix proteins. According to a previously published work, \( -10\% \) of the surface of enamel is presented by structural proteins incorporated into the matrix.
Figure 2.3. Advancing (diamonds) and receding (circles) water contact angles and contact angle hysteresis (triangles) for enamel reacted with 1% of solutions of $C_{12}H_{25}PO(OH)_2$ in THF over different periods of time.
Analysis of contact angle hysteresis, the difference between advancing and receding contact angles, deserves additional comment. All enamel supported SAMs demonstrate high contact angle hysteresis, ~20-50°. The data in Figure 2.3 also show that contact angle hysteresis increases for the early reaction times and then decreases as the reaction is allowed to progress. In the literature, contact angle hysteresis is most often attributed to surface roughness and/or chemical heterogeneity. According to AFM imaging (see Figure 2.6 and the next section below), the root-mean-square roughness of the enamel supported surfaces is of the order of 5-10 nm, which is comparable with the thickness of the SAMs (length of all-trans ODPA molecule is ~2.5 nm). We note that on this scale, the effects from roughness and chemical heterogeneity are almost indistinguishable. The chemical heterogeneity of SAMs, e.g. incomplete surface coverage, gives rise to surface roughness and vice versa. We, therefore, propose that the large wetting hysteresis observed for enamel supported SAMs is attributed to the molecular sized heterogeneity of the enamel supported surfaces. Higher hysteresis at shorter reaction time points (Figure 2.3) can be explained by incomplete surface coverage. The decrease in hysteresis for longer reaction times is consistent with increased surface coverage over time, exposing the probe liquid to a more uniform SAM. This picture agrees well with the results obtained via AFM imaging, which gives further
Insight into the microstructure of the SAM-enamel surface and is discussed in the section below.

**Morphology of Enamel-Supported SAMs.** Surface morphology of the enamel specimens was studied at Clemson University with AFM in the tapping mode. To ensure the obtained images were not influenced by sample to sample heterogeneity, enamel chips taken from the same tooth were used in this investigation. Enamel chips were mounted to a support prior to use. Surface feature heights, determined from topographical AFM were used to calculate surface roughness, while phase images gave insight into the mechanical properties of the surface. Topographical and phase SPM images of virgin enamel surface and the enamel washed with THF (solvent used for SAM deposition) at room temperature are presented in Figure 2.4. These images reflect the morphology of bare tooth surfaces prior to SAM deposition. The structure of hydroxyapatite crystallites was observed all over the specimens. Single apatite crystals of about 60-90 nm width were found. The outcome of the imaging was close to the results reported in the previous studies of the enamel surface.80,81 It was noted that the structures depicted on the AFM images represented the projections of the apatite crystals from the prismatic layer known to be present in most teeth. The comparison between the pristine enamel sample (Figures 2.4a and 2.4b) and the sample washed with organic solvent (THF) (Figures 2.4c and 2.4d) showed that the surface morphology of the samples was rather close. The same structure of
hydroxyapatite crystallites was detected. However, the roughness of the virgin surface (0.8x0.8 μm RMSR = 9 nm) was sufficiently higher than the roughness of the solvent treated substrate (0.8x0.8 μm RMSR = 5 nm). This agrees well with the contact angle data and is explained due to the removal of adsorbed protein material as a result of washing with solvent.
Figure 2.4. AFM topographical (a, c) and phase (b, d) images (3.8x0.8 μm) of virgin enamel surface (a, b) and the enamel treated with THF at room temperature (c, d). Vertical scale 30 nm and 50 degrees for topography and phase images, respectively.
Figure 2.5 shows the AFM topographical images of the enamel supported SAMs at different reaction times. The RMSR values go through the maximum in the course of SAM growth (Figure 2.6). For an early reaction time (10 min) patchy structure of SAM can be readily detected (compare Figures 2.4c and 2.5a). The RMSR undergoes a notable increase as compared to bare enamel (from -5 to 9 nm, Figure 2.6). From the wettability studies the SAM after 10 min of reaction has an advancing water contact angle of -70°, which corresponds to ~50% of the surface coverage (from equation 2). We believe that the patchy structure of SAM explains the increase of the RMSR for this sample. For longer reaction times, the patchy structure of the surface is still present (Figures 2.5b-d), but the RMSR values decrease and the surface become smoother than bare enamel (Figure 2.6). This suggests that during the course of SAM growth ODPA molecules were placed in between the primary islands until full surface coverage is achieved.
Figure 2.5. APM topographical images (0.8x0.8 μm) of the enamel surface after the SAM deposition at different times. (a) 10 min, (b) 1 h, (c) 24 h, and (d) 100 h. Vertical scale 30 nm.
Figure 2.6. Roughness (RMSR 0.8x0.8 μm) of the enamel surface after the organophosphonic acid deposition versus time of the reaction (size of symbols denotes standard deviation, 0.2 nm).
Figure 2.7 displays the AFM phase image changes during the course of SAM formation. The phase images reflect tip-sample interactions and can be used to identify areas with different adhesive or mechanical properties on the surface.\(^{82,83}\) In general, the phase imaging corroborates the conclusions drawn from the topography of the SAMs. After 10 min of the reaction (Figure 2.7a) a somewhat complex situation was observed. There were regions on the surface, which interacted differently with an AFM tip. First, the structure of hydroxyapatite crystallites was still clearly detected on the surface. Also, the areas covered with the SAM could be observed as brighter spots on the image. This result is consistent with the island-like deposition mechanism of the SAM formation. After 1 h of the reaction, the islands merged yielding larger domains, which cover almost the entire surface (Figure 2.7b). The average lateral dimensions of the grains were about 60-50 nm and were close to the size of the single apatite crystals observed on bare enamel surface. As the reaction time increases, the grainy structure is still present. The size of the grains, however, increased to 80-120 nm and exceeds the dimensions of apatite crystals. As the reaction time increases further (130 h of the reaction time), bigger grains transform into smaller ones with the lateral size of 40-60 nm (Figure 2.7d). The average grain size in this case is significantly smaller than the average size of apatite crystals.
Figure 2.7. AFM phase images (0.8x0.8 μm) of the enamel surface after the organophosphonic acid deposition at different times. (a) 10 minutes, (b) 1 h, (c) 24 h, and (d) 100 h. Vertical scale 50 degrees.
It should be mentioned that the maximal RMS value, as well as the maximal heterogeneity of the surfaces as assessed from the phase imaging and wettability data (Israelevitch-Gee model), are observed for the SAMs with incomplete surface coverage. Given the fact that AFM and contact angles are quite different, such correlation between these two techniques is quite encouraging. On the other hand, the maximum value of roughness probed by the AFM tip, though, is observed for the SAM after 10 min of reaction, while the maximum heterogeneity obtained from hysteresis probed by advancing/receding contact angles is observed for the SAM after 1-24 h. This difference is probably due to the difference in "the probe size" used in the AFM and contact angles. The size of the AFM tip is ~10 nm, while in contact angles "the probe size" is of molecular size, i.e. an order of magnitude smaller. Thus, surfaces that appear smooth for an AFM tip may be heterogeneous for a wetting liquid.

2.3 Conclusions

This work investigated self-assembled monolayers (SAMs) of organophosphonic acids supported on the surface of human teeth. The SAMs were prepared via a solution-phase reaction of C₉H₄PO(OH), with enamel chips. AFM suggests the formation of SAMs with highly ordered alkyl groups (based on the CH₃ stretching). Water contact angles of the enamel-supported SAMs are in the range ~100°/70° (advancing/receding), which is consistent with the formation of hydrophobic surfaces. SAMs with
incomplete surface coverage and intermediate hydrophobicity were prepared via controlling the reaction time. According to AFM imaging, the SAM growing process appears as the formation of islands on the surface of the tooth. At the late stages of the reaction, islands merge yielding larger domains covering almost the entire surface of the tooth. The size of these domains undergoes a complex change during the course of the deposition and after 100 h of reaction it becomes ~40-60 nm in lateral dimension. The surfaces prepared demonstrate good hydrolytic stability showing the potential uses of SAMs of organophosphonic acid for permanent modification of the surface properties of the tooth.
PART II: Surfaces with Reversible Wettability Mediated by Biochemical Reactions and Capillary Gates

Introduction

The high specificity and mild reaction conditions of enzymatic reactions are attractive features for use in the chemistry and engineering of surfaces. Through the use of enzymes, new surfaces can be developed whose synthesis may not be possible using conventional chemical methods. The reactions of enzymes with surfaces have been reported for the preparation of chromatographic stationary phases, biosensors, polymer fibers with improved adhesion, and drug delivery systems. This chapter reports the reactions of enzymes with lipid- and protein-rich surfaces that are employed for the preparation of surfaces with reversible polarity, i.e., surfaces capable of switching from hydrophobic to hydrophilic and back. Figures 3.1 and 3.2 show a schematic representation of this biochemically controlled reversible wettability. The two systems' reversible hydrophilic-hydrophobic behavior is described. The first system employs adsorption of phospholipids on C₁₈ surfaces followed by enzymatic cleavage with lipases. The second system employs adsorption of bovine serum albumin on C₁₈ surfaces followed by enzymatic digestion of adsorbed protein with trypsin.
Figure 3.1: Schematic representation of protein controlled reversible wettability. CAMs of alkylsilanes (top) produce a hydrophobic surface. Deposition of protein (bottom right) changes the surface from hydrophobic to hydrophilic. A hydrophobic surface can be restored (bottom left) through removal of adsorbed protein by enzyme proteolysis.
Figure 3.2: Schematic representation of lipid controlled wettability. CAMs of alkylsilanes (top) produce a hydrophobic surface. Deposition of lipid (bottom right) changes the surface from hydrophobic to hydrophilic. A hydrophobic surface can be restored (bottom left) through removal of adsorbed lipid by digestion with lipase.
7.1 Experimental

General Information. Solvents (HPLC grades) were purchased from Aldrich and Fisher. Infrared reflectance spectra were obtained with a Perkin Elmer Spectrum One FTIR instrument using a Harrick Seagull accessory (Harrick, Ossining, NY). Spectra were taken at a 50° angle of incidence, 124 scans, resolution 4 cm⁻¹. The ellipsometric measurements were performed with Inomtech automatic ellipsometer (West Hartford, CT) at 70° angle of incidence. The following refractive indices were used for the thickness calculations: n₀(Si substrate) = 1.74, n₁(silicon dioxide) = 1.462, n₂(octadecylsilane) = 1.45, n₃ = 1.46 (adsorbed BSA) and 1.45 (adsorbed phospholipids). Contact angles were measured with a Ramé-Hart telescopic goniometer. The probe fluids used were H₂O purified water and hexadecane. Dynamic advancing (θa) and receding angles (θr) were recorded while the probe fluid was added to and withdrawn from the drop (Gilmont syringe), respectively. Ellipsometric measurements were performed with Inomtech automatic ellipsometer, West Hartford, CT, at 70° angle of incidence.
Capillary rise measurements

All capillary rise measurements were taken on a home made catatometer system. Capillary rise and depression measurements were made for each sample as follows: The height of the probe fluid ($H_p$) was subtracted from the height of the fluid in the capillary ($H_c$) to give the capillary rise (capillary rise = $H_c - H_p$) (see Figure 3.3).
Figure 3.3 Capillary Rise Measurements: Representation of device used for capillary rise measurements (A) Capillary rise (B) Capillary depression.

(A)

(B)
Contact angles using capillary rise data were calculated from the Laplace equation:

$$\cos \theta = \frac{h(\rho_1 - \rho_2)g \cdot r}{\gamma}$$

Where $\rho_1$ is the density of the probe fluid ($\rho_{H_2O} = 0.997 \text{ g/cm}^3$, $\rho_{methanol} = 0.76 \text{ g/cm}^3$), $g$ is the gravitational constant 980.66 cm/s², $\gamma$ is the surface tension of the probe fluid ($\gamma_{H_2O} = 72$ dyn/cm, $\gamma_{methanol} = 27.4$ dyn/cm) and $h$ is the capillary rise.

**Silica Substrates.** Si wafers (100, P/B dopant, thickness 435-575 μm, growth method CZ, polished/etched semi standard flats) were purchased from International Wafer Service (Santa Clara, CA). Filter frits were cut from coarse glass filters (Fisher). Prior to the reaction with alkylsilanes all the silica substrates were cleaned via soaking in a saturated solution of K₂Cr₂O₇ in concentrated H₂SO₄ for 1 h, rinsed with excess of water and dried in an oven at 100°C for at least 1 h. Water contact angles of cleaned silicas were <10°. Polydimethylsiloxane/phenylmethyl siloxane (DMDPDM) capillaries, I.D. 0.53 mm, were purchased from J&W Scientific, Pansom, CA, cut into 3 inch segments, and used without further modification.
Biological Detergents: Zwitterionic detergents CHAPS (C₁₂H₂₅N₂SO₃, CMC ~8 mM), Zwittergent 3-10 (C₁₃H₂₅NO₅S, CMC ~25-40 mM), and the non-ionic detergents MEGA 9 (C₁₆H₃₃NO₆, CMC ~19-25 mM) and Big CHAP (C₁₆H₃₃N₂O₄S, CMC ~3-4 mM) were purchased from Fisher and diluted to their CMC in distilled water.

Lipids, Proteins, Proteases, and Lipases: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18:1 PC) (Avanti Polar Lipids, Alabaster, AL) were used without further purification. Bovine Serum Albumin (BSA), Trypsin (from bovine pancreas, sequencing grade), Phosphodiesterase I from Crotalus atrox (PDEase I), Phospholipase C from Bacillus cereus (PLC) (all from Sigma) were used without further purification.

Deposition and Removal of BSA: Adsorption of BSA on Si wafers and filters was performed by incubation of the substrates in a 10-50 mg/mL solution of protein in phosphate buffered saline (PBS) at room temperature for ~2 hours. After incubation, samples were washed three times with PBS and dried. The enzymatic digestion of the adsorbed BSA was performed using Trypsin. The substrates were incubated in a solution of 12.5 ng/μL Trypsin in 25 mM NH₄HCO₃, pH 8.5, for 3 hours at 37°C. After the reactions, the substrates were washed three times with DI water and dried under N₂.
Deposition and Removal of Lipid: For the deposition of lipids, the best results were obtained using the vesicle unrolling technique.35-37 The vesicles were prepared via the injection of ethanol solution of lipids in water. The silica substrates were incubated in the vesicle solution at room temperature for 16-24 hours. Then the samples were washed three times with water and dried under vacuum at room temperature. We also used the deposition of lipids by incubation of the substrates in 25 mg/mL chloroform solution of lipids for five minutes. The samples were taken out of chloroform, placed in DI-water and sonicated for two minutes, then removed from the solution and dried. The lipid surfaces obtained by this technique appeared cloudy and showed significant variation in thickness and contact angles throughout the surface. The enzymatic cleavage of adsorbed lipids was performed using phospholipases PLA2e I and PLC. The silica substrates were incubated in the enzyme solutions with pH 8.5, for 3 hours at 37°C. After the reactions, the substrates were washed three times with DI water and dried under nitrogen.
Hydrophobization of Silica Substrates: The surface reactions reported here were studied on three silica substrates: Si wafers, glass capillaries, and porous glass filters. Si wafers were mainly used for the ellipsometric and spectral characterisation of the surfaces; filters were used for demonstration of gating properties of the systems. Given the similarity of surfaces of glass and oxidized Si wafer, we assumed that the structure of the surfaces supported on two silica substrates were very close.

\[
(SiO_2)Si-OH + (CH_3)3NSi(CH_3)2C_{18}H_{37} \rightarrow (SiO_2)Si-O-Si(CH_3)2C_{18}H_{37} + (CH_3)2NH
\]

Hydrophobization of the silicas was done through reactions with octadecyl(dimethyl-N,N-dimethylamino)silane (Gelest), which produced covalently-attached monolayers (CAM) of the C18 groups.88,98

For C18 surfaces supported on Si wafers, the contact angles (adv/rec) were -105°/95° (water) and -34°/31° (hexadecane). The thickness of the monolayer assessed by ellipsometry was 1.4±0.1 nm (~50% of the length of fully-stretched C18 chains) indicating hydrophobic surfaces of disordered alkyls, in a good agreement with the literature on CAMs of alkyl(dimethyl)silanies.89,96 For polydimethyl siloxane and DMPOMS capillary surfaces, the contact angles (adv/rec) were -105°/101° and 98°/95° respectively, while
hexadecane contact angles were -46°/42°. A summary of contact angles and monolayer thickness can be found in Table 3.1.
<table>
<thead>
<tr>
<th>Modification</th>
<th>$\theta_{\text{w}}$ water</th>
<th>$\theta_{\text{h}}$ hexadecane</th>
<th>Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{6}$H$</em>{5}$Si(CH$<em>{3}$)$</em>{3}$NH$_{2}$</td>
<td>105/95</td>
<td>34/31</td>
<td>1.13</td>
</tr>
<tr>
<td>(CH$<em>{3}$)$</em>{3}$SiCl$_{3}$</td>
<td>105/101</td>
<td>46/42</td>
<td>1.16</td>
</tr>
<tr>
<td>DMPG/OMG</td>
<td>98/95</td>
<td>X</td>
<td>$-200$-$300$</td>
</tr>
</tbody>
</table>
Lipid Adsorption and Removal: Lipid-Lipase System. Adsorption of phospholipids on the C18 filters (through the fusion of vesicles) produced hydrophilic surfaces through which water runs freely (Figure 3.4). Two lipids were investigated: 1,2-dioleoyl-sn-glycero-3-phosphoethanol-amine (DOPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC). The results for both lipids were very close. For the C18 surfaces on Si wafers, water contact angles dropped to -36°/25° (adv/rec) and the thickness of the lipid layers was in the range ~1.7-2.2 nm. The value of thickness and low water contact angles suggested that the lipid molecules formed a monomolecular layer with their polar head groups facing away from the C18 surfaces. Tables 3.2 and 3.3 show contact angle and thickness data for both lipids.
Figure 3.4. Adsorption of amphiphilic compounds (lipids or proteins) on hydrophobic porous filters (top) produced hydrophilic surfaces (bottom), through which water runs freely. Enzymatic cleavage of the adsorbed layers restores original hydrophobic surfaces (top).
The infrared study suggests that the alkyl chains in adsorbed lipids were ordered. The position of the CH₂ stretchings was νa-2918 and νs-2848 cm⁻¹ which is indicative of the high degree of order of alkyls similar to that reported for crystals and closely-packed self-assembled monolayers of long chained alkyls. The other peaks in the infrared spectra were consistent with the presence of phospholipids on the surface (Figure 3.5). The peak at ~1465 cm⁻¹ was attributed to the deformation of the C-H bonds. A small peak ~3010 cm⁻¹ was due to carbon-carbon double bond from the unsaturated alkyl chains of the lipid. The peak at ~1720 cm⁻¹ was due to carbonyl stretching and peaks ~1200-1000 cm⁻¹ were assigned to stretches of P=O, C-O and P-O bonds. A broad peak at ~3200-3300 cm⁻¹ was O-H stretching from the surface water. It is noted that lipids adsorbed rather strongly to C₁₄ surfaces; no removal of lipids (by ellipsometry and contact angles) was observed after washing the surfaces with pure water or Tris-buffer solutions.

After incubation (37°C, 3 h) of the lipid-coated surfaces with PDase I, hydrophobic surfaces were obtained. The water contact angles were ~100°-105°/90°-95° (adv/rec), i.e. close to those of the original C₁₄ surfaces (Tables 3.2 and 3.3).
Lipid-coated filters, after the incubation with PDase I, also turned hydrophobic, so water did not go through the pores. Figure 3.4 shows that such surfaces can act as a capillary gating system. A filter with hydrophobic surface of pores (water CA > 90°) does not let water to run through; the gate is closed. Water runs freely through filters with hydrophilic coatings; the gate is open. Through use of PDase I, adsorbed lipids are destroyed and, the hydrophobicity of the original surface can be restored. PDase I is the enzyme that cleaves the P-O-C bonds from the phosphate head group to the hydrocarbon portion of the molecule. It has been reported that this enzyme is active for the lipids adsorbed and even covalently grafted to the surfaces. We believe that enzymatic cleavage of the polar head groups from the hydrophobic tails destroys the adsorbed lipid layer and causes desorption of fragments off the surface. The detergent MEGA 9 was also shown to successful remove adsorbed lipid from modified C₁₈ surfaces and restore their hydrophobic nature. Figure 3.6 shows the effects of lipid adsorption and removal with detergent on the wettability of a C₁₈ modified filter.
Figure 3.5. IR spectra of phospholipid (DOPE) adsorbed on C18 surfaces.
<table>
<thead>
<tr>
<th>CAM Surface</th>
<th>( \theta_a/r ) After Adsorbed Lipid</th>
<th>Removal Technique</th>
<th>( \theta_a/r ) After Lipid Resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_9H_{16}-(CH_2)_4 ), 106/82</td>
<td>33/26</td>
<td>PDase I 37°C 3h</td>
<td>105/94</td>
</tr>
<tr>
<td>( C_9H_{16}-(CH_2)_4 ), 105/95</td>
<td>36/22</td>
<td>PDase I 37°C 3h</td>
<td>103/96</td>
</tr>
<tr>
<td>( C_9H_{16}-(CH_2)_4 ), 110/92</td>
<td>41/30</td>
<td>Tris pH 8.0 3h, 37°C</td>
<td>52/20</td>
</tr>
<tr>
<td>( C_9H_{16}-(CH_2)_4 ), 109/96</td>
<td>41/22</td>
<td>Tris pH 8.0 3h, RT</td>
<td>40/30</td>
</tr>
</tbody>
</table>
### Table 3.3: Ellipsometry and contact angle data for DOPS adsorption and removal

<table>
<thead>
<tr>
<th>CAM Surface 0a/r°</th>
<th>Thickness (nm)</th>
<th>0a/r° After Lipid Adsorption</th>
<th>Thickness (nm) After Lipid Adsorption</th>
<th>Removal Technique</th>
<th>0a/r° After Remov</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₉H₁₈-(CH₃)₂</td>
<td>1.30</td>
<td>33/26</td>
<td>2.22</td>
<td>PDEase I</td>
<td>105/5</td>
</tr>
<tr>
<td>106/02</td>
<td></td>
<td></td>
<td></td>
<td>3 h, 37°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₉H₁₈-(CH₃)₂</td>
<td>1.17</td>
<td>36/22</td>
<td>PDEase I</td>
<td>103/5</td>
</tr>
<tr>
<td>105/95</td>
<td></td>
<td></td>
<td></td>
<td>3 h, 37°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₉H₁₈-(CH₃)₂</td>
<td>1.14</td>
<td>41/22</td>
<td>Tris</td>
<td>40/3</td>
</tr>
<tr>
<td>109/96</td>
<td></td>
<td></td>
<td></td>
<td>3 h, 37°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₉H₁₈-(CH₃)₂</td>
<td>-</td>
<td>44/21</td>
<td>H₂O</td>
<td>39/1</td>
</tr>
<tr>
<td>105/98</td>
<td></td>
<td></td>
<td></td>
<td>3 h, 37°C</td>
<td></td>
</tr>
</tbody>
</table>

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Figure 3.6: Wettability study of lipid adsorption and removal on C<sub>18</sub> filter using water as a probe fluid. (A) C18 filter with adsorbed DOPE (hydrophilic surface). (B) Second wash with 20mM MEGA 9. (C) Fifth wash with MEGA 9. (D) Sixth wash with MEGA 9 (restoration of hydrophobic surface).
**BSA adsorption onto capillary surfaces:** For both capillary CAM surfaces, \((\text{CH}_3)_3\text{SiCl}_3\), and DMEM, BSA deposition at either 10 or 50 mg/mL increases the wettability of the surface (Table 3.4). This can be seen in the decrease in contact angles for CAM surfaces from \(\Theta > 90^\circ\) to an average of \(\Theta = 72/44^\circ\) and \(\Theta = 54/39^\circ\) for \((\text{CH}_3)_3\text{SiCl}_3\), and DMEM respectively. Though the contact angle of the surface does decrease after protein adsorption, it is also noted that BSA does not have the ability to make the surface completely hydrophilic. Contact angles in the fifties and forties are expected from the deposition of the very hydrophobic protein BSA. As expected, higher concentrations of BSA (50mg/mL) had a greater effect on contact angle than incubation in solutions of lower concentration (10mg/mL). It is also noted that, though this data would seem to suggest a trend in protein concentration and wettability decrease, these concentrations have not been optimized. Wettability change as a function of concentration on modified silica wafers to optimize this protein system will be discussed in the next section.

After incubation with either non-ionic or zwitterionic detergents or the protease trypsin, contact angles were restored to close to their original values. Since the non-ionic detergent MEGA 9 showed the greatest restorative properties, all subsequent biomolecule removals were done with non-ionic detergents. Restoration with the protease trypsin also proved to be successful in the restoration of a hydrophobic surface. Table 3.5 shows a summary of ellipsometric data for BSA removal with
both trypsin and MEGA 9. Trypsin cuts C-terminal side of hydrophilic, basic residues located on the surface of adsorbed molecule. Larsericsdotter et al. found that upon adsorption, no additional cleavage sites were exposed on BSA even though the protein had lost much of its native structure”. They also report that upon albumin adsorption to a hydrophobic surface, trypsin preferentially cuts over chymotrypsin, which cuts hydrophobic residues. This confirms that BSA is deposited onto the surface with it hydrophobic portion towards the surface and hydrophilic portion exposed”, inferring some hydrophobic effect or van der walls forces between the hydrophobic portion of the molecule and the alkyl chains of the CAM surface.
<table>
<thead>
<tr>
<th>CAM Surface</th>
<th>%a/r</th>
<th>Deposition</th>
<th>%a/r</th>
<th>Removal</th>
<th>%a/r</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CH₃)₂SiCl₂</td>
<td>101/96</td>
<td>10mg/ml BSA</td>
<td>64/47</td>
<td>8mM CHAPS 2h, RT</td>
<td>94/65</td>
</tr>
<tr>
<td>(CH₃)₂SiCl₂</td>
<td>105/99</td>
<td>10mg/ml BSA</td>
<td>81/52</td>
<td>20mM MEGA 9 2h, RT</td>
<td>100/90</td>
</tr>
<tr>
<td>DMDMP</td>
<td>115/78</td>
<td>50mg/ml BSA</td>
<td>54/39</td>
<td>20mM MEGA 9 2h, RT</td>
<td>112/83</td>
</tr>
<tr>
<td>DMDMP</td>
<td>115/78</td>
<td>50mg/ml BSA</td>
<td>54/39</td>
<td>3.4mM Big CHAP 2h, RT</td>
<td>103/74</td>
</tr>
<tr>
<td>DMDMP</td>
<td>115/78</td>
<td>50mg/ml BSA</td>
<td>54/39</td>
<td>12.5mM Trypsin 3h, 37°C</td>
<td>108/75</td>
</tr>
</tbody>
</table>
**Table 3.5**: Thickness data for the removal of 25mg/mL BSA adsorbed to C18 Si-wafers using trypsin and MEGA 9

<table>
<thead>
<tr>
<th>BSA Thickness (nm)</th>
<th>Removal Technique</th>
<th>Final Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0213</td>
<td>Trypsin</td>
<td>3.50515</td>
</tr>
<tr>
<td>4.392</td>
<td>Trypsin</td>
<td>2.9158</td>
</tr>
<tr>
<td>4.526</td>
<td>MEGA 9</td>
<td>3.748</td>
</tr>
<tr>
<td>4.392</td>
<td>MEGA 9</td>
<td>2.748</td>
</tr>
<tr>
<td>2.743</td>
<td>PBS Control</td>
<td>2.84</td>
</tr>
</tbody>
</table>
BSA Adsorption and Removal: BSA-Trypsin System. The adsorption of BSA onto C₈ surfaces resulted in a substantial drop of the water contact angles. We found, however, that the concentration of the BSA solution and the adsorption time had a profound effect on the wettability of the adsorbed protein layers. Figure 3.7 and 3.8, show that the change in advancing and receding contact angle is dependent on the concentration of BSA adsorbed. Surfaces exposed to high concentrations of BSA (50mg/mL) showed a sharp, initial decrease in both advancing and receding contact angles which slowly increased and leveled after ~2-3 h. This behavior is believed due to the tendency of the protein to aggregate at higher concentrations. If the protein adsorbed to the hydrophobic surface and formed a monolayer, the hydrophobic portions of the molecule would interact with the surface. If the protein aggregates during adsorption, the hydrophobic portions of some molecules would interact with the substrate while others would be free to interact with the contact angle probe fluid. This would cause an initial decrease in contact angle which would then increase as BSA in solution aggregated to those molecules already adsorbed. If the concentration of BSA is too low (10mg/mL), there are not enough molecules to cover the hydrophobic surface and decrease the contact angle. For these solutions, the adsorption was low and
the water contact angles decreased insignificantly (to ~90°).

The surfaces with the lowest water contact angles (~50/40°
(adv/rec)) that did not change over time (up to 2 h) were
obtained using BSA solutions with a concentration of 25 mg/mL.

The thickness of the adsorbed layer was ~2 nm, which suggested
formation of a monomolecular layer of BSA on the surface.44,95

Figure 3.9 shows the decrease in contact angle and thickness of
the protein monolayer for a 25 mg/mL solution of BSA.
Figure 3.7: Advancing contact angle vs. time for three different SSA concentrations
Figure 3.8: Receding contact angle vs. time for several different BSA concentrations
Figure 3.9: Contact angle and thickness vs. time for 25mg/mL BSA adsorbed onto a C18 Si-wafer
C₁₈ filters after adsorption of BSA (25 mg/mL) were hydrophilic enough for water to run freely through them. Figure 3.10 shows the wettability change (hydrophobic to hydrophilic) for a modified C₁₈ filter. When a drop of BSA solution (25 mg/mL) is initially placed on the hydrophobic filter, it does not pass through. Over time, protein adsorbes to the modified surface, increasing the wettability, and allowing the solution to pass through the filter. After removal of the protein with detergent, the hydrophobic nature of the filter’s surface is restored.

The infrared spectra of the BSA-coated surfaces were consistent with the presence of adsorbed protein (data not shown). No removal of BSA was observed after rinsing the surfaces with water or buffer solution used for the adsorption (ammonium-bicarbonate, pH 8).

After incubation with trypsin solutions (37°C, 3 h), the hydrophilic BSA coatings were destroyed and the surfaces became hydrophobic. For surfaces supported on Si wafers, water contact angles increased up to ~100°/90° (adv/rec). Porous filters, after the incubation with trypsin, turned hydrophobic and water beaded off their surface. The observed changes in wettability are explained due to proteolytic digest of the adsorbed BSA. We believe that trypsin cuts protein into smaller pieces that do
not adsorb strongly to the $C_{18}$ surface and can be washed away by water. The activity of trypsin towards adsorbed BSA allows for some speculations regarding the orientation of protein molecules on the surface. It is known that trypsin cleaves the C-terminal side of hydrophilic, basic residues (lysine and arginine) located on the surface of a protein molecule. Larsenicsdotter et al. reported that upon adsorption, no additional cleavages sites were exposed on BSA even though the protein had lost much of its native structure. They also report that upon albumin adsorption to a hydrophobic surface, trypsin preferentially cuts over chymotrypsin, which is known to cleave hydrophobic residues. Our data further supports the conclusions of work that BSA adsorbed onto the $C_{18}$ surfaces with it hydrophobic portion towards the surface and hydrophilic portion exposed to the solution.
Figure 3.10: The effects of BSA adsorption onto a C18 filter.
(A) Drop of 25mg/mL BSA on the surface of C18-filter. (B) Drop of BSA solution after 0.5h. (C) Drop of BSA solution after 3h. (D) Drop of water on filter after removal of adsorbed BSA with MEGA 9.
3.3 Conclusion

To conclude the chapter, we have demonstrated that the adsorption of a monolayer of phospholipids or protein onto a hydrophobic surface of C18 CAM produced hydrophilic surfaces. The hydrophobicity of the surface can be restored by using the enzymes that cut the adsorbed hydrophilic layers: lipase for lipids and protease for proteins. These reactions supported on porous surfaces were used to prepare a biospecific capillary system with gating properties.

Protein adsorption onto hydrophobized capillaries showed the ability to increase wettability as demonstrated by a decrease in contact angle as measured by the capillary rise technique and use of the Laplace equation. Though BSA does not have the ability to make the surface completely wettable, the observed decreases were expected with the use of such a hydrophobic protein. Ellipsometric for the protein adsorption studies done at 25 mg/mL BSA are indicative of the formation of a single polymeric layer adsorbed to the surface. The non-ionic detergent MEGA 9 at its CMC proved the best detergent for the removal of BSA and showed the best restorative property of the detergents used. The protease trypsin also showed good restorative properties. The enzyme was able to selectively digest the BSA protein adsorbed the CAM surface, suggesting that the orientation of the molecule on the CAM surface must be so the hydrophilic portions of the molecule are facing away from the CAM
surface. This would suggest that the hydrophobic portion of the molecule is attached to the surface through van der Waals interactions.

In the case of CAM adsorbed lipids, both 16:0-18:1 PC and DOPC showed the ability to switch the surface from hydrophobic to hydrophilic. This would indicate that the lipid molecules were oriented on the surface with their polar phosphate head groups facing away from the hydrophobic CAM surface. The presence of lipid on the surface is also confirmed from the FTIR data which, after lipid adsorption, shows an increase in C-H stretching from lipid alkyl chains, and the appearance of other strong peaks from the presence of the lipid on the surface. Once again, the non-ionic detergent MEGA 9 proved to be the most effective detergent in restoring wettability. Phases I was shown to have the ability to both cleave the adsorbed molecule and restore the hydrophobicity of the surface. Given that the enzyme cleaves the ester linkage between the phosphate head and the aliphatic portion of the molecule, it is believed that the lipid molecules are also "flopped over" (data also suggested by the ellipsometric data). Adsorbed lipid molecules must also be spaced far enough apart to allow the enzyme access to its cleavage site. It is noted that no orientation study of lipid adsorption and removal has not been done and will be examined in the future.

From the presented data it has been demonstrated that protein and lipid molecules adsorbed onto a hydrophobic CAM surface have the ability to switch the wettability of the surface. Detergents and enzymes with specific active sites for
portions of the adsorbed molecules have the ability to restore the hydrophobic nature of the surface. These systems were also shown to be effective in the creation of a biochemically controlled gate as evidenced by the modified filters.
Part III: THE USE OF NANOPOROUS SILICAS FOR CONTROLLED RELEASE

Introduction

The oral cavity is an attractive site for drug delivery given the ease of accessibility, increasing the level of patient compliance, elimination of the “first pass effect” through direct drug absorption into the internal jugular vein. Protease inhibitors and tissue permeability adjuvants can be locally administered with the drug to increase tissue permeability.97 Potential disadvantages of an oral based delivery system are the relatively short residence time due to salivary wash,98 the relatively small surface area and lower permeability of the oral cavity as compared to other delivery sites e.g. intestine, and the necessity to design “user friendly” palatable doses.97 The formulation of drug delivery systems which could increase drug residency time, target specific body sites, and have controllable drug delivery properties would have a great impact on the health care system.

Bioadhesion was introduced by Park and Robinson98 in 1984 and can be described as attachment of a synthetic or biological molecule to biological tissue.98 Carrier molecules such as nanoparticles, microparticles, biodegradable and non-biodegradable polymers, and liposomes are all used to modulate the release and
adsorption characteristics of a drug. Microspheres show both small size and act as efficient carrier molecules, but their short residence times at the site of adsorption limit their effectiveness. Production of microspheres with increased residence time and controllable drug diffusion properties is necessary. Hombeiro-Perez et al. have shown success with non-degradable microparticles of amoxicillin methacrylate copolymers containing both hydrophilic (propranolol HCl) and lipophilic (nifedipine) model drugs. Both drugs were shown to be successfully incorporated into the microparticles and release rates were shown to be controlled over periods of at least eight hours.

It has been proposed that diffusion of a drug from a polymer can be controlled by varying the degree of cross linking for cross-linked polymers, for semicrystalline polymers, varying the polymer molecular weight and degree of crystallinity and for uncross linked polymers, by varying the polymer type and molecular weight. Porous silica particles with uniform, uninterrupted pores can be synthesized using the method developed by Stucky et al. Porous silica particles, having a pore diameter from 50-70 Å and surface areas ranging from 500 to 1000 m²/g can further be tailored through the covalent modification of organosiloxane to increase pore compatibility for both hydrophobic and hydrophilic drugs. Through the
modification of porous silicas with organosilanes and changes in
the pore diameter, it is possible to control the release of drug
molecules. CAM modified, drug "loaded," SBA's are of interest
for controlled release in the oral cavity. Figure 4.1 shows a
diagram of the loading and release of drug from modified SBA
particles. The particles can be adsorbed to the dental enamel
in the oral cavity where drug release can begin.
Figure 4.1: Schematic representation of drug loading and release from SBA particles. (A) Synthesized porous silica particles with uniform pores can be modified with SAMs of organosilanes. (B) Modified SBAs can be filled with drug molecules. (C) Drug molecules will slowly release from the pores into solution. (D) Release of drug molecules can be monitored by UV.

(A) Porous Silica Particle  (B) Porous Silica Particle Loaded with Drug Molecule

(C) Drug Slowly Released from Pores  (D) Fraction Released

Time
4.1 Experimental

General Information: All solvents (HPLC grade) were purchased from Fisher and used as received. The following silanes were purchased from Aldrich and used as received: 3-aminopropyl trimethoxy silane, triethyl silanol, 3-glycidopropyl trimethoxy silane, and bromopropyl trichlorosilane. The porous silica Prodigy (with large, non-uniform, interconnected pores) purchased from Phenomenex, with surface area = 1m²/g, and a pore volume 0.7-1cc/g. Ibuprofen and pseudoephedrine were purchased from Sigma. Ibuprofen was used with out further modification while pseudoephedrine was titrated with HCl to increase its solubility in water. Eugenol and thymol, USP grade, were received as a donation from Andrea Aromatics Inc. Trenton, NJ, and were used with out further purification. Chemical analysis was performed by Schwarzkopf Microanalytical Lab (Woodside, NY) using the ASTM method. Grafting densities (p, groups/ nm²) of the monolayers supported on SBAs were calculated using Formula 1 from Part I. UV spectra were taken using a Per Spectrophotometer scanning from λ=190-300 nm using a quartz cuvette with at 1cm path length. Surface area measurements were obtained by the BET method described earlier.

Microscopy images: All TEM images were provided in collaboration with Dr. Chaoying Ni and the University of
Delaware. AFM images were provided in collaboration with Dr. Igor Luzinov, Department of Materials Science at Clemson University. All SEM images were provided in collaboration with Dr. Brian Kirkmeyer from IFF Inc.

Synthesis of Porous Silicas: SBA silicas were synthesized from TEOS, HCl, and a detergent (BRIJ 30, Pluronic 123) following the protocol by Stucky et al\textsuperscript{134}. Changes in pore diameter were made by varying the detergent or reaction temperature\textsuperscript{4}. TEOS was used to create medium sized pores while BRIJ 30 was used for small pores. Table 1 shows data for the BET characterization of the SBAs used.

Human Tooth Specimens. Extracted human teeth were used in this work. Human teeth were obtained from Kernan Dental Associates, Cherry Hill, NJ, after autoclave sterilization treatment in accordance with the OSHA procedure. Samples were cut into an average of six to eight enamel chips per tooth. Enamel chips were cleaned via sonication with Alconox detergent followed by deionized water.

Chemisorption of Organosilanes onto SBAs: For the modification of SBAs, a 300-500mg sample was dried in the oven overnight at 100°C prior to the reaction. A 1% solution of organosilane in toluene (-20mL) was added to a GC vial along with the dried SBA
and capped. The vials were kept at 80°C overnight. The reaction was quenched on a filter followed by 3 x 20mL toluene and 3 x 20mL acetone. During each wash step, the sample was sonicated in the wash solution to remove any un-reacted silane from the pores. After washing, samples were dried overnight at 80°C.

**SBA Loading and Release:** To load SBAs, solutions of drug molecules were made. For pseudoephedrine and ibuprofen, a 25mg/mL and a 50mg/mL solution in water was made respectively. For eugenol and thymol, non-polar drug molecules, a 106.7mg/mL and 3.97mg/mL solution in ethanol were made respectively. Since the solubility of both non-polar drugs is < 1mg/mL in water, all loadings were done in ethanol. Figure 4.2 shows the structures of the drug molecules investigated. Prior to loading, ~100mg of SBA, modified or bare, was dried in the oven at 100°C for minimum 1 h. For pseudoephedrine and ibuprofen, the maximum pore volume for an SBA sample was calculated, and the calculated volume of drug solution was added while mixing. If the volume of drug solution added was enough to fill the pore volume, the sample remained dry, as all liquid was quickly absorbed into the pores. For the loading of non-polar drugs dissolved in ethanol, pore volume was once again calculated and that volume added. Ethanol loaded samples, however, were dried in the oven at ~80°C until the ethanol carrier was evaporated.
Release for all drug molecules was carried out as follows: A 25-100mg sample of drug loaded SBA was added to a scintillation vial. A 6mL aliquot of 18.2mΩ water was added to the vial and the sample was incubated for 1 h. 4mL of the incubated liquid was the removed to a fresh scintillation vial. 4mL of fresh water was then added to replace the removed aliquot. This process was repeated for 24h.

Prior to spectrophotometer measurements, the removed aliquots were filtered through a 2µm PTFE syringe filter to remove any SBA particles. Spectrophotometric measurements were taken at the following λ_{max}: Pseudoephedrine λ_{max} = 257nm, Ibuprofen λ_{max} = 263nm, Eugenol λ_{max} = 260nm, and Thymol λ_{max} = 273nm.

To calculate the release of drug from the SBAs, the obtained absorbencies were compared to those of a drug-specific standard curve. For each drug, standard curves in water were constructed with concentrations ranging from minimal to maximal detection levels for a given drug. All curves showed a linear relationship between concentration and absorbance.

Given our release measuring technique, release into 6mL where only 4mL are removed and replaced, corrections were made to the total amounts released which were calculated from the standard curves. The following is an explanation of the correction calculation. The table shows hypothetical data from
a release experiment and the application of the correction factor:

<table>
<thead>
<tr>
<th>Release Time (h)</th>
<th>Total Release (mg) From STD Curve</th>
<th>Total Release Corrected (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3.45</td>
<td>3.45</td>
</tr>
<tr>
<td>2</td>
<td>2.56</td>
<td>1.38</td>
</tr>
<tr>
<td>3</td>
<td>0.82</td>
<td>0.379</td>
</tr>
</tbody>
</table>

The values reported from hour one are a representation of the total mass of drug released into the 6mL aliquot, therefore, its value is the same in the corrected column. For the release for the second hour, the release calculated from standard curve was multiplied by a correction factor to account for the incomplete removal of liquid from the first measurement. This is reflected in the decrease of drug weight released in the corrected column.

Adsorbed SBA on enamel and Si-wafers for microscopy: Human enamel chips were incubated at room temperature for ~2h in a scintillation vial containing 2~3mL of human saliva. After incubation, samples were blown dry under nitrogen and placed in a fresh scintillation vial containing ~50mg SBA. The enamel was shaken with the SBA for a minute, removed and blown with nitrogen to remove excess SBA on the surface. These samples were then used for microscopy. For microscopy of SBAs on Si-
wafers, wafers (described earlier) were washed as previously
described and dried in the oven. Adsorption of SBA to the
surface was carried out as described for enamel.
<table>
<thead>
<tr>
<th>SBA</th>
<th>Pore Diameter (Å)</th>
<th>Description</th>
<th>Pore Volume (cc/g)</th>
<th>Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA 24/80</td>
<td>500-1000</td>
<td>Uniform uninterrupted</td>
<td>0.7</td>
<td>567</td>
</tr>
<tr>
<td>Prodigy</td>
<td></td>
<td>Non-uniform interconnected</td>
<td>0.7-1</td>
<td>350</td>
</tr>
<tr>
<td>SRIJ 30</td>
<td>21</td>
<td>Small, uniform, uninterrupted</td>
<td>0.3</td>
<td>547</td>
</tr>
</tbody>
</table>
Figure 4.2: Model drug molecules used in SBA release. (A) Pseudoephedrine HCl, (B) Ibuprofen, (C) Eugenol, (D) Thymol.
4.2 Results

Porous Silicas: Figures 4.3 and 4.4 show TEM images of synthesized SBA particles. The pores present in the silica particles can be described as uniform, non-interconnected pores in a hexagonal array. It can be seen that these pores are uninterrupted and run the length of the silica particle. Table 4.2 shows grafting densities calculated from the elemental analysis results for modified SBAs.
Figure 4.3: TEM image of synthesized SBA particles. Uniform pores in a hexagonal array are present.
Figure 4.4: TEM image of synthesized SBA particles. Non-interconnected pores running the length of the particle can be seen.
<table>
<thead>
<tr>
<th>SBA Surface</th>
<th>Properties</th>
<th>Grafting Density (groups/nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromopropyl</td>
<td>Hydrophobic w/ dipole</td>
<td>1.61</td>
</tr>
<tr>
<td>Aminopropyl</td>
<td>Ion exchange, part</td>
<td>1.646</td>
</tr>
<tr>
<td></td>
<td>hydrophobic</td>
<td></td>
</tr>
<tr>
<td>Triethyl</td>
<td>Hydrophobic, floppy</td>
<td>0.838</td>
</tr>
<tr>
<td>Epoxy</td>
<td>Hydrophobic, reactive</td>
<td>0.750</td>
</tr>
</tbody>
</table>
Drug release from SBA particles: Though the release of several model drug molecules was examined, we will concentrate mostly on the release of Eugenol because it best illustrated the effects of pore diameter, pore uniformity, and surface grafted molecules. The release of ibuprofen, pseudoephedrine, and thymol will be shown mainly as examples for the release of drugs having differing solubilities in water.

Figure 4.5 shows pseudoephedrine release from bare and modified SBAs. All SBAs, except for the SBA modified with triethyl all show a constant release of pseudoephedrine over the first five hours. The profiles of aminopropyl, bare SBA, and epoxy release almost the same quantity of drug, while bromopropyl releases much less. Very little release was seen from the triethyl modified SBA. It is noted that complete release was not seen for any of the SBA samples tested. Though elemental analysis was preformed on the same samples after release, we were unable to determine if the remainder of the pseudoephedrine was still present in the pores. There was, however, a difference in the release of drug from the bare and modified SBAs with in the first five hours. Since surface modification did seem to play a role here, the experiments were repeated with the more readily water soluble drug ibuprofen.

Figure 4.6 shows the release profile for ibuprofen from the same SBA substrates. The release profiles for all samples show
the same, continued release of drug over the first five hours and are all identical except for the profile of triethyl SBA. Like pseudoephedrine, none of the samples showed complete release of drug, though triethyl SBA did show the best whit -85% release. Again, elemental analysis was unable to determine if the remainder of the drug was still in the pores.
Figure 4.5: Pseudoephedrine release from SBAs. (diamonds) bare SBA, (squares) aminopropyl SBA, (triangles) epoxy SBA, (circles) bromopropyl SBA, and (stars) triethyl SBA.
Figure 4.6: Ibuprofen release from SBA. (diamonds) bare SBA, (circles) epoxy SBA, (triangles) bromopropyl SBA, (squares) aminopropyl SBA, and (stars) triethyl SBA.
Figure 4.7 shows the release of thymol from bare SBA. We believe that the release of thymol is governed by its solubility in water. Unlike the previous two drugs, a constant release over the first five hours is not present for thymol. Since thymol is a solid and must be dissolved in ethanol for loading, it may have precipitated inside the pores when the sample was dried prior to release. This would mean that release was not controlled by chemical potential but by dissolution of the solid in solution. Given that the desired controlled release was not seen for the bare SBA, further release studies involving thymol were abandoned.
Figure 4.7: Release of Thymol from bare SBA
Figure 4.8 show the release of eugenol from modified SBA. Through the first five hours of release, all SBAs tested showed identical, controlled release, after which, almost all (~80-85%) of the eugenol was released. We can describe the first five hours of release as bulk eugenol being released from the pores. This bulk eugenol does not have contact with the modified surfaces of the SBA pores and its release appears to be controlled by its dissolution in solution. When looking at the second half of the release profile (6-24 hours) the release profiles for different SBAs begin to change. It appears that surface chemistry begins to play a larger role in the release. Once the bulk molecules have left the pores, the remaining drug molecules are those who have direct interaction with the modified pore walls. It is noted that the supported monolayers may not be the optimal ones for the release of this given molecule, but the data as a whole shows much promise for the use of surface chemistry and the controlled release of model drugs.
Figure 4.8: Release of Eugenol from modified SBAs. (diamonds) bare SBA, (squares) aminopropyl SBA, (triangles) triethyl SBA, (circles) epoxy SBA, and (open triangles) bromopropyl SBA.
It has been shown that surface chemistry does play a role in the controlled release of drugs, but that is only one component of the controlled drug release system. Pore size and uniformity is another approach to control the release of drug molecules. Figure 4.9 shows the release of eugenol from three bare porous silicas having different pore characteristics. The release of eugenol from prodigy shows a typical burst effect in which all the drug is released within the first half an hour. This phenomenon is attributed the interconnected network of large pores (diameter 10nm) present in the commercially available prodigy. Both synthesized SBAs, SBA 24/80 (diameter 4.5nm) and BRAl 30 (diameter 2nm) show sustained release of eugenol over the first five hours. As expected, release of eugenol from the smaller diameter pores of the BRAl 30 SBA takes longer and is less than that of SBA 24/80. It is noted, once again, that not all the drug is released from the synthesized SBA particles.
Figure 4.9: Release of eugenol from SBA's having different pore diameters. (circles) prodyg, interconnected pores with 19nm diameter, (triangles) SBA 24/80 uniform pores with 4.5nm diameter (diamonds) Brij 30 small uniform pores with 2nm diameter.
Adhesion of SBA particles to human dental enamel: It has been demonstrated that the release of model drugs can be controlled through the use of surface modification and pore diameter, we will now examine the adhesion of these particles to the surfaces of human dental enamel. Figure 4.10 shows an AFM image of bare SBA 24/80 on the surface of human dental enamel. From the 2-D image, it is seen that the heights of the particles adhered to the surface are about 1 micron. This is consistent with the size of the SBA particles obtained by QELS analysis (data not shown). From the 3-D image it is clear that the bare SBA particles adhere to the surface rather spread apart. This would imply that there is little interaction between particles either before or after adhesion.
Figure 4.10: AFM images of bare SBA 24/80 adhered to the surface of human dental enamel. (A) 2D image of the enamel surface (B) 3D image of the enamel surface.

(A) 2-D image of the enamel-1 surface (4X4 micron)  
(B) 3-D image of the enamel-1 surface
Figures 4.11 and 4.12 show APM images of modified SBA on the surface of human enamel. Unlike the images of bare SBA, where particles adhered independently of one another, modified SBA molecules seem to aggregate prior to or after adhesion. This would imply that there is some attraction due to the presence of SAMs on the surface. It also shows that SAMs may also be used to increase the adhesion of SBA particles to the tooth.

The presence of bare SBA on enamel was also confirmed by SEM images. Figure 4.13 shows bare SBA on the surface of a Si-wafer. The particles appear cylindrical in shape and do not seem to aggregate upon adhesion. Figure 4.14 shows an SEM image of bare enamel while Figure 4.15 shows bare SBA on the surface of enamel. The cylindrical SBA particles can clearly be seen on the surface of the enamel.
Figure 4.11: AFM image of aminopropyl modified SBA on the surface of human enamel. (A) 2D image (B) 3D image both show aggregation of the modified SBA on the surface.

(A) 2-D image of the enamel-2 surface (4X4 micron)  
(B) 3-D image of the enamel-2
Figure 4.12: AFM image of epoxy modified SBA on the surface of human enamel (A) 2D image (B) 3D image both showing aggregation of modified SBA on the surface.

2-D image of the enamel-3 surface (4X4 micron)

3-D image of the enamel-3
Figure 4.13: SEM images of bare SBA on the surface of a silica wafer (A-D) different zoom scale views of the same wafer.

(C) In collaboration with Dr. Brian Kirkmeyer, JFF
Figure 4.14 SEM images of bare human dental enamel (A-B)
different views of the same tooth 10μm scale

(A)

(B)
Figure 4.15: Bare SBA adhered to the surface of human dental enamel (A) 50μm scale (B) 10μm scale
4.3 Conclusion

In this chapter it has been shown that controlling the release of drug molecules in the oral cavity is of great interest. The oral cavity is a desirable site for drug delivery but is complicated by constant salivary wash, eating, and other factors. Through the use of porous silica particles having uniform, uninterrupted pores, the burst effect for model drug molecules is avoided and a sustained drug release is observed over five hours. As expected, SBA particles having smaller pores showed slower drug release. Release can further be controlled through the use of SAMs of organosilanes grafted to the surface. These SAMs can be optimized not only for release but also for drug compatibility. It is noted that in all release studies, except for those using silica with large, non-uniform pore networks, not all model drug was released. Future work will include optimization of release.

We have also shown by both AFM and SEM that the biocompatibility of these SBAs can be increased by changing the nature of the grafted molecules. Rare SBA particles were shown to adhere to enamel independently of one another while those supporting SAMs showed aggregation. Future work will include the release of drug from adhered SBA and characterization of SBA adhesion to the enamel surface.
Part IV. Thesis Conclusion

This work aimed to demonstrate the importance of surface chemistry and interfacial interactions to control surface properties, e.g. wettability and adhesion. For calcium phosphate surfaces, CaRAP and human dental enamel, organophosphonic acid molecules were covalently grafted to the surface through the formation of P-O-F bonds. Once formed, these stable monolayers were shown to decrease the wettability of the surface and alter bacterial adhesion.

For silica surfaces, Si-wafers, capillarier, and SBA porous particles, modification was done using organosilane molecules which formed covalent Si-O-Si bonds to the surface. After the formation of these monolayers on the surface, the wettability of the Si-substrates changed from wettable to very hydrophobic. We have also demonstrated that this change in wettability can be further modified through the use of biomolecules. Specific interactions between enzymes and their substrates (proteases/protein or lipases/lipid) were used to reversibly alter the wettability of the supported SAM surface. These reactions were used to create the wettability controlled gates demonstrated on Si-filters.

Using information learned from both the silica substrate and calcium phosphate substrate investigations, the use of SAMs
supported on porous silica particles was employed for the
controlled release of model drug molecules. Synthesis of SBAs
with different pore diameters in conjunction with SAM
modification showed very effective in the continuous, controlled
release of several model drugs over a five hour period, thus
eliminating the "burst effect" of drug release. After this
initial five hour period, release seems to be more affected by
the SAM attached to the surface. Bulk drug molecules have been
released and those remaining in the pores have direct
interaction with the modified pore walls. SAMs are not only
useful for increasing the compatibility of the SBA pores with
both hydrophobic and hydrophilic drugs, but can also improve
adhesion to the enamel surface. In AFM images of modified SBAs
on the enamel surface, particles were seen to aggregate, and had
visibly more adhered particles as compared with bare SBA.

For all investigations mentioned in this work, SAMs proved
to be an efficient, robust chemical method for altering the
surface properties of a given surface.
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