Adsorption of Alginic Acid and Chondroitin Sulfate-A to Amine Functionality Introduced on Polychlorotrifluoroethylene and Glass Surfaces

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ABSTRACT: The amine functionality is introduced onto a glass surface by using 3-(aminopropyl)-triethoxysilane (3-APTS)—a silane coupling agent—as well as by the adsorption of poly(L-lysine). The amine functionality is introduced onto the hydroxyl-functionalized polychlorotrifluoroethylene surface (PCTFE-OH) using 3-APTS as well. The amine-functionalized surfaces were thoroughly characterized by X-ray photoelectron spectroscopy (XPS). Selected polysaccharides, involved in fouling processes, such as alginic acid and chondroitin sulfate-A were allowed to adsorb onto amine-functionalized surfaces. It is shown that alginic acid adsorbs from calcium-free artificial seawater onto amine-functionalized glass surface prepared by the adsorption of poly(L-lysine) and onto amine-functionalized polychlorotrifluoroethylene surfaces (PCTFE-NH_2). The adsorption of alginic acid to PCTFE-NH_2 is very rapid, resulting in high-affinity isotherms and ∼5 Å thick dry overlayer of the polysaccharide. In addition, we observe that the adsorbed alginic acid protects the PCTFE-NH_2 surface from hydrolysis by phosphate buffer 2 and from hydrolysis/oxidation by copper(II) acetate monohydrate solution. It is also shown that chondroitin sulfate-A adsorbs from potassium hydrogen phthalate buffer to the amine-functionalized glass surface prepared by the reaction between silanol groups on glass and 3-APTS. It is observed that alginic acid and chondroitin sulfate-A do not adsorb to the unmodified glass surface, which is polar and negatively charged, and the PCTFE surface, which is nonpolar, from aqueous solutions under various solution conditions. This study clearly indicates the importance of surface functional groups on the adsorption of polysaccharides, an important preliminary step in the biofouling of surfaces.

Introduction

The study of the adsorption of polysaccharides containing uronic acid and sulfated sugar residues is critical toward the fundamental understanding of marine and biofouling processes.[1] The first layer of slime formed during the fouling process has been identified to contain polysaccharides and peptidoglycans (polysaccharides cross-linked by small peptides).[1] Fouling is the process in which marine plant and animals adhere and grow on objects submerged in water. Fouling impacts the functioning of underwater devices such as hydrophone and reduces fuel efficiency of aircraft carriers by increasing frictional resistance.[2] In addition, the loss of time and money in fixing the problem is an important factor in itself. Fouling also affects buoyancy of installations in contact with water as growth of organisms causes plugging of orifices that should remain open and interferes with the functioning of mechanically moving devices.[2] Fouling is also the cause for paint destruction and corrosion in some cases.[2]

A typical biotic succession in the fouling of a clean surface involves the formation of a slime layer (secreted extracellular polysaccharides) that is produced by diatoms and bacteria containing uronic acid and sulfated sugar residues. This is followed by attachment and growth of bacteria (up to 48 h), protozoa (up to 3 weeks), barnacles (4−10 weeks), tunicates (10−16 weeks), and bryozoa (within 26 weeks the surface is totally covered).[1] Currently fouling is being minimized by application of toxic paints and by the use of metals that produce toxic ions when they corrode. Though these methods work well, environmental concern has forced researchers to look for nontoxic alternatives. Recent patent literature is replete with claims involving the use of functionalized polymers, nontoxic inorganics, immobilized bacteria, and metal tubules with biocidal agents.[3,4] The development of nontoxic fouling protection agents requires that the mechanisms of fouling be understood.

In effort to better understand the physicochemical basis of fouling, we choose to study the adsorption of some marine polysaccharides to "clean" unmodified substrate surfaces as well as to those with tailored surface structure. Our choice of polysaccharides was limited by their solubility in aqueous solution, being limited to a narrow pH range. We selected two polysaccharides: alginic acid containing uronic acid residues soluble in the pH range 6−9 and chondroitin sulfate-A containing sulfated sugar residues soluble in the pH range 4−6. Having selected polysaccharides with acidic functionality, our natural choice of surface functionality was the amine group. Well-characterized amine-functionalized semiconductor and glass surfaces are known in the literature.[5,6] The use of the 3-APTS coupling agent to modify semiconductor surfaces and glass fibers has been reported as well.[7] Literature work on the incorporation of primary amine functionality on polymer surfaces does not exist although several other functionalities such as carboxylic acid, hydroxyl, ester, and many others have been introduced to desired depths on several polymer surfaces such as polyethylene, polypropylene, PCTFE, PTFE, and ethylene−propylene copolymer.[8−18]

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Our speculation was that acid−base chemistry between surface functional groups and natural macromolecules in solution probably initiates fouling. To test this hypothesis, we carried out a detailed investigation of the adsorption of alginic acid and chondroitin sulfate-A to unmodified and amine-functionalized glass and PCTFE surfaces.

Experimental Section

Materials. Alginic acid ($M_w \sim 240\,000$ Da), chondroitin sulfate-A, and poly(L-lysine) hydrobromide (PLL) ($M_w \sim 502\,500$, PDI-1.10) were purchased from Sigma and were used as received. Glass microscope slides (Fisher) were cut to a dimension of ∼2 cm × 1.5 cm and were used after chemical etching of surface impurities using nochromix and sulfuric acid mixture.[30] PCTFE film (5 mil Allied Aclar 33C) was extracted in refluxing dichloromethane for 2 h and dried (0.05 mm, room temperature, >24 h). Benzene, heptane, and toluene (Fisher) were distilled under nitrogen from calcium hydride. Tetrahydrofuran (THF) was distilled under high-purity nitrogen from sodium benzophenone dianion as and when required. 3-Bromo-1-propanol and ethyl vinyl ether (Aldrich) were purified as reported earlier by us.[16] Acetaldehyde 3-bromopropyl ethyl acetal was also prepared and purified according to published procedures.[16] *tert*-Butyllithium (Aldrich) was standardized by titration with 4-biphenylmethanol in THF from time to time. 3-(Aminopropyl)triethoxysilane (3-APTS; Huls America) was diluted with equal volume of dry solvent (benzene/THF/toluene as the case may be) and used. Methanol, dichloromethane, and house distilled water were used after they were sparged with nitrogen, as wash solvents. HCl (0.1 N), NaCl, KCl, $MgCl_2{\cdot}6H_2O$, $Mg_2SO_4{\cdot}7H_2O$, Na_2HPO_4, K_2HPO_4, Na_2CO_3, $NaHCO_3$, $Ca(OH)_2$, $Na_2B_4O_7{\cdot}10H_2O$, and potassium hydrogen phthalate (all from Fisher) were used as received. Potassium hydrogen phthalate (pH = 3.9), monobasic phosphate (pH = 7.2; phosphate buffer 1), dibasic phosphate (pH = 7.4; $\mu = 0.13$ mol/L phosphate buffer 2), and Borax (pH = 9.1) buffer solutions and calcium-free artificial seawater were prepared as reported in refs 19 and 20.

Methods. X-ray photoelectron spectra (XPS) were obtained with a Perkin-Elmer-Physical Electronics 5100 spectrometer. Spectra were routinely recorded at two takeoff angles, 15° and 75°, using reported procedures.[12] Angle-resolved XPS was performed at several different takeoff angles (from 15° to 75° at intervals of 5°; 300 W X-ray power was used to minimize sample damage), if the thickness of the modified film were to be determined. The thickness values were obtained from the slope of ln[element$^{\theta}$/element^{75}] vs [$(1/\sin\theta) - 1$] plot for a particular photoelectron emission of an element, as reported before.[21] The slope of such a plot would be t/λ where t is the thickness of the film on the substrate and λ is the mean free path of the particular photoelectron under consideration through the overlayer and the substrate. For example, if we were interested in monitoring the thickness of glass surface upon surface modification, we determine the area under the Si_{2p} photoelectron peak at a given takeoff angle and divide it by the area at a takeoff angle of 75, giving us the [element$^{\theta}$/element^{75}] ratio. Dynamic advancing and receding contact angles were measured with a Rame-Hart telescopic goniometer and a Gilmont syringe with a flat-tipped 24 gauge needle, as water was added (θ_A) or withdrawn (θ_R) from the surface. pH measurements were performed using a Fisher 825MP pH meter standardized using several commercial buffer solutions (Fisher).

Preparation of Amine-Functionalized Surfaces By the Coupling Reaction of Glass with 3-APTS. Microscope glass slides were left in freshly prepared nochromix (100 g) + sulfuric acid (36 N, 2 L) bath, overnight. Subsequently, they were rinsed several times with distilled water, sonicated in distilled water once (15 min), rinsed several times with spectroscopic grade methanol, sonicated in methanol once (15 min), and dried in a Schlenk tube under nitrogen purge (2 h minimum). Dry benzene (20 mL) was added to the Schlenk tube followed by the addition of 2 mL of 3-APTS, all transfers being performed via a cannula under nitrogen. The coupling reaction was allowed to proceed overnight (minimum reaction time allowed was ∼15 h) at 25 °C. After the desired reaction time the reaction mixture was removed via a cannula (under nitrogen), and the slide was rinsed thrice with benzene and dried (0.05 mm, room temperature, >24 h). The reactions were performed using dry THF or toluene as the solvent as well. In each case the reaction was performed at least thrice with three independent glass slides to verify the reproducibility of the reaction through XPS atomic composition and the overlayer thickness.

Preparation of Amine-Functionalized Surfaces by the Adsorption of PLL to Glass. A 0.5 mg/mL solution of PLL in calcium-free artificial seawater was transferred to a clean glass slide in a Schlenk tube, and adsorption was allowed to proceed at 25 °C in a Haake constant-temperature bath for a day. After the desired time the slides were rinsed thrice with the medium used for adsorption and thrice with distilled water and dried (0.05 mm, room temperature, >24 h). The dry slides were analyzed by ARXPS and by water contact angle analysis, as before. All reactions were performed at least thrice, independently, on three different glass slides, and the analyses were performed at least twice, independently, to check their reproducibility. Amine-functionalized glass slides can also be prepared by the adsorption of poly(L-lysine) from Borax buffer.

Preparation of Amine-Functionalized Surfaces by the Chemical Modification of PCTFE Surface Followed by Coupling Reaction with 3-APTS. PCTFE-OH surfaces were prepared at −78 °C, in dry THF, as reported before. This methodology enables the surface selective incorporation of −OH functionality to a depth of ∼50 Å, and the density of −OH groups is four for every five PCTFE repeat units. The amine functionality was introduced using 3-APTS, following the procedure reported in the earlier section for the glass surface. The dry films were analyzed by ARXPS and water contact angle, as also before.

Adsorption Experiments. A typical adsorption of a polysaccharide to a functionalized or an unfunctionalized surface was performed at 25 °C under ambient conditions as follows: The substrate of interest was placed in a glass tube and was allowed to equilibrate to 25 °C in a constant-temperature bath for 2 h. The polysaccharide solution of desired concentration in the appropriate buffer was also equilibrated in a glass tube to 25 °C for 2 h. It was then transferred quickly to the tube containing the substrate, in air. After the desired time the solution was removed using a Pasteur pipet and was rinsed several times (at least thrice) with the buffer solution at the adsorption temperature followed by several distilled water rinses. The substrate was dried under vacuum and was analyzed by XPS and water contact angle, as before.

Labeling of Adsorbed Alginic Acid Using Cupric Acetate Monohydrate. Alginic acid adsorbed onto the surface of PCTFE-NH_2 after adsorption was labeled using copper(II) acetate monohydrate. Divalent cations are known to chelate to alginic acid and other polysaccharides to different extents.[22] We choose Cu^{2+} as it is an effective XPS label (has reasonable cross section for electron emission). Typically, the dry polymer film after the adsorption was immersed in a 20% solution of copper(II) acetate monohydrate in distilled water, for 48 h. After this time the film was rinsed repeatedly with distilled water (at least five times), dried under vacuum, and analyzed by XPS.

Results and Discussion

The initial adsorption experiments were performed on unmodified glass and PCTFE substrates. Several solution conditions were tried. These include the variation of the pH of the solution, use of calcium-free artificial seawater (alginic acid forms a chelate in the presence of calcium ions which precipitates out of the solution), variation of the concentration of the polysaccharide in solution, and the time of adsorption. Follow-

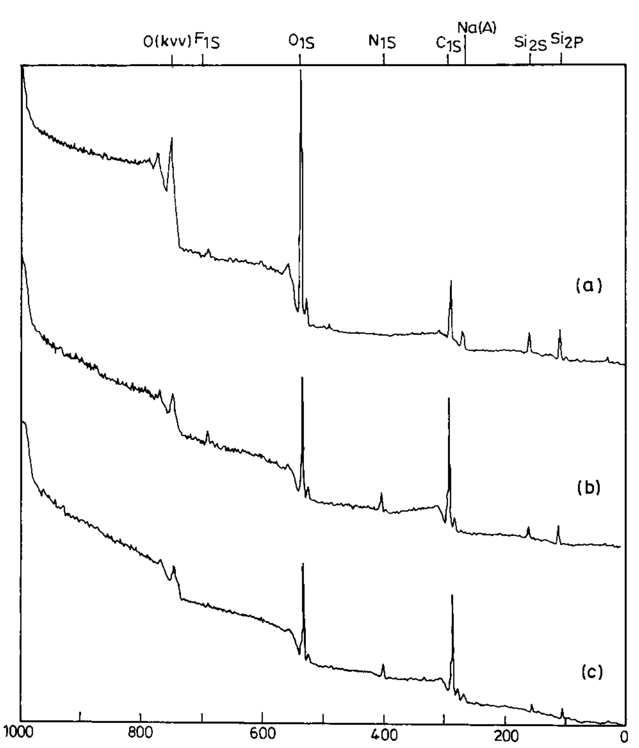


Figure 1. X-ray photoelectron spectra of glass surfaces following (a) nochromix cleaning, (b) after reaction with 3-APTS in benzene, and (c) after the adsorption of poly(L-lysine) from calcium-free artificial seawater. Takeoff angle is 15°.

ing the adsorption experiments the substrates were dried and analyzed by XPS for the presence of polysaccharide and by water contact angle for interfacial energy change. XPS analysis following the adsorption of alginic acid to glass surface indicated the presence of a small quantity of nitrogen (~2%), an element not present in alginic acid. Nitrogen was not detected on the surface when the pH of the solution was increased above 8.0. We attribute this to the adsorption of a proteinous impurity present in alginic acid, below a pH of 8.[23] The water contact angle (θ_A/θ_R) following the adsorption changed from 15/0 to 41/12−14 below a solution pH of 8 and did not change above a solution pH of 8, confirming the XPS finding that the impurity adsorbs onto the glass surface below a pH of 8. Similar results were observed on PCTFE surfaces as well. XPS analysis of glass and PCTFE surfaces following the adsorption of chondroitin sulfate-A did not reveal the presence of sulfur, confirming that this polysaccharide does not adsorb onto the unfunctionalized surfaces studied.

The amine functionality was incorporated onto the glass surfaces by using 3-APTS as described in the Experimental Section. The dry glass slides following the coupling reaction were analyzed by ARXPS for the thickness of the organic thin film and its composition. The XPS survey spectrum of glass at a takeoff angle of 15°, before and after the coupling reaction, is shown in Figure 1 (traces a and b). The appearance of the nitrogen (N_{1s} or N) electron peak at a binding energy of 400 eV, following the coupling, confirms the incorporation of nitrogen on the glass surface. The atomic composition data under various conditions are given in Table 1 to emphasize an important point that these coupling reactions are reproducible within the limits of XPS atomic composition determination. An important point of observation is that the nitrogen atomic composition decreases monotonically with increase in takeoff angle when the coupling is performed in THF, suggesting that the amine functionality is present very much at or near the surface.

Table 1. ARXPS Compositions of Glass Surface Following Amine Functionalization by 3-APTS Coupling Reaction in Benzene and THF

	benzene				THF			
angle	N	C	Si	O	N	C	Si	O
15	6.74	64.06	9.01	20.20	7.21	63.62	8.02	21.15
20	6.86	62.48	9.72	20.94	6.95	61.15	9.16	22.75
30	6.68	56.24	12.06	25.02	7.15	54.94	11.22	26.69
40	6.89	50.04	13.53	29.54	6.62	50.68	13.44	29.27
50	7.18	45.44	15.48	33.37	6.48	46.87	14.07	32.58
60	5.90	43.26	16.24	34.61	5.25	44.73	19.56	30.46
75	5.59	40.22	16.89	37.30	6.39	43.89	16.08	33.65
Following Repetition of the Reaction with Different Slides								
15	7.08	64.57	9.07	19.28	6.60	62.55	7.95	22.89
20	7.46	62.25	9.36	20.93	6.30	59.29	9.22	25.19
30	7.19	55.48	11.84	25.48	5.86	53.20	10.83	30.10
40	7.05	50.07	13.17	29.11	5.30	48.27	11.95	34.48
50	6.77	46.12	15.10	32.01	5.29	45.24	12.69	36.77
60	6.21	43.55	15.93	34.31	4.65	42.81	13.67	38.87
75	5.87	41.04	16.58	36.51	4.75	39.93	14.45	41.47

The overall atomic composition of the monolayer at a takeoff angle of 15° is $Si_{1.28}C_{9.12}N_1O_{2.72}$ and $Si_{2.82}C_{6.99}N_1O_{6.24}$ at a takeoff angle of 75°. The chemical formula of the coupling agent used is $Si_1C_9N_1O_3H_{23}$, and the small deviation of the overlayer composition from that expected of a monolayer is due to the contribution from the glass substrate to the Si and O peaks. Several independently prepared amine-functionalized glass slides were analyzed by XPS independently (those prepared in the same solvent as well as those prepared in different solvents), and the data are reproducible within the limits of experimental error. For example, the composition of N observed is in the range 6.6−7.2%, and this variation is observed from slide to slide in the same solvent as well as in different solvents.

The thickness of the overlayer formed in benzene was determined using the area under Si_{2p} electrons peak, as described in the Experimental Section. The data are best fitted by the straight line $y = 0.0134 + 0.2872x$ with an R^2 value of 0.997. We estimated thickness values in the range of 6.3 ± 0.4 Å, assuming a mean free path of 22 ± 1.5 Å for the Si_{2p} electrons through the glass.[21] Similar results were obtained from the Si_{2s} and O_{1s} photoelectrons emission data as well. The thickness value obtained for coupling reactions in THF was 6.2 ± 0.4 Å and is within the limits of experimental error to those obtained in benzene. The water contact angles (θ_A/θ_R) observed are 78/52 when benzene is used for the coupling reaction and 78/55 when THF is used, suggesting that the surface formed is less polar than glass.

The glass surface was also modified by the physisorption of PLL from calcium-free artificial seawater and Borax buffer solution. The XPS survey spectrum following adsorption from calcium-free artificial seawater is shown in Figure 1, trace c. The ARXPS atomic composition data from these experiments are shown in Table 2. The N atomic composition decreases monotonically with increasing takeoff angle when adsorbed from artificial seawater, suggesting that the amine functionality is present at or very close to the surface, and goes through a maximum when adsorbed from Borax buffer, suggesting that the amine functionality is buried under

Table 2. ARXPS Compositions of Glass Surface Following Amine Functionalization by the Adsorption of Poly(L-lysine) from Calcium-Free Artificial Seawater and Borax Buffer[a]

	calcium-free artificial seawater, pH = 7.2				borax buffer, pH = 8.3			
angle	N	C	Si	O	N	C	Si	O
15	6.79	63.56	7.53	22.12	4.32	28.60	2.49	64.59
20	6.66	56.58	10.16	26.61	6.42	46.35	5.54	41.69
30	5.70	45.67	14.12	34.51	5.25	47.89	12.36	34.50
40	5.34	36.60	16.60	41.46	4.93	40.98	14.90	39.19
50	4.43	30.47	18.57	46.53	4.71	34.42	17.03	43.84
60	3.87	26.15	19.53	50.45	4.48	31.73	17.58	46.21
75	3.74	23.25	20.10	52.90	4.61	34.84	21.66	38.99

[a] The adsorptions were carried out from a 0.48 mg/mL solution of PLL for 10 days at room temperature. The water contact angles following the adsorption are 70/32 (artificial seawater) and 78/21 (Borax buffer).

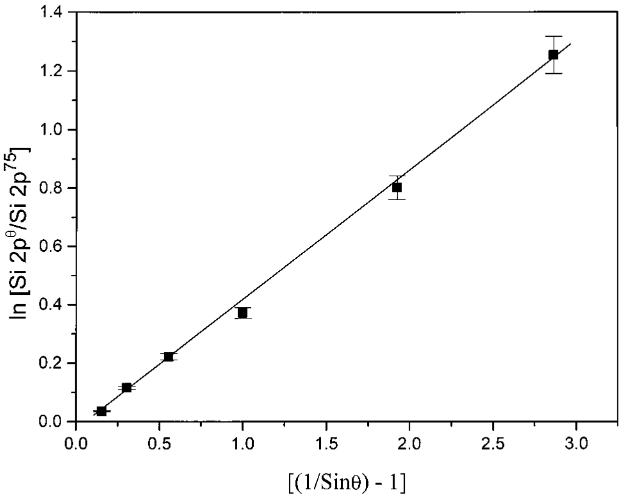


Figure 2. Natural logarithm of Si_{2p} peak area at a given takeoff angle (θ) normalized to that at 75° versus ($1 - \sin\theta$) from glass surface, following the adsorption of poly(L-lysine) from calcium-free artificial seawater.

the surface. The thickness of the adsorbed dry film was determined from the ARXPS data plotted in Figure 2 to be 9.8 ± 0.2 Å. The water contact angles (θ_A/θ_R) following adsorption from artificial seawater are found to be 70/32 and those from Borax buffer to be 78/21.

PCTFE surfaces with the amine functionality were prepared from the PCTFE-OH surfaces,[24] as described in the Experimental Section. The XPS survey spectra of PCTFE, PCTFE-OH, and PCTFE-NH_2 surfaces are shown as traces a, b, and c in Figure 3. Examination of the survey spectra of PCTFE-NH_2 surface reveals the presence of the N_{1s} peak at 400 eV and the disappearance of the fluorine (F_{1s} or F) peak at ~700 eV due to the fluoropolymer, suggesting that the coupling reaction takes place with equal versatility on the PCTFE-OH surface. The peaks seen at around 100 and 150 eV are due to the silicon (Si 2s and 2p electrons) present in 3-APTS. The atomic compositions are Si, 12.55; Cl, 1.95; C, 51.40; N, 12.01; O, 21.18; F, 0.91 at a takeoff angle of 15° and Si, 12.08; Cl, 3.46; C, 47.84; N, 11.91; O, 23.46; F, 1.25 at a takeoff angle of 75°. The composition of oxygen (O) is ~12% in PCTFE-OH at a takeoff angle of 15°, and after the reaction with 3-APTS the composition falls in the range ~21–24%.[24] With lateral reaction among the ethoxy silane groups (two oxygens for every silicon), it is reasonable to assume the structure shown in Scheme 1 for the PCTFE-NH_2 surface. The water contact angles (θ_A/θ_R) following amine functionalization are 71/22. The advancing angle is comparable to those observed with other amine surfaces. The receding angle of 22 is much lower than that observed for other amine surfaces (say 3-APTS modified glass surface) and suggests that the monolayer of amine formed using PCTFE-OH is quite uniform in the plane of the film. This could be due to the greater number of −OH groups per unit area on the PCTFE surface (it is four −OH groups per five repeat units in the case of PCTFE-OH while it is less in the case of glass[16,17]).

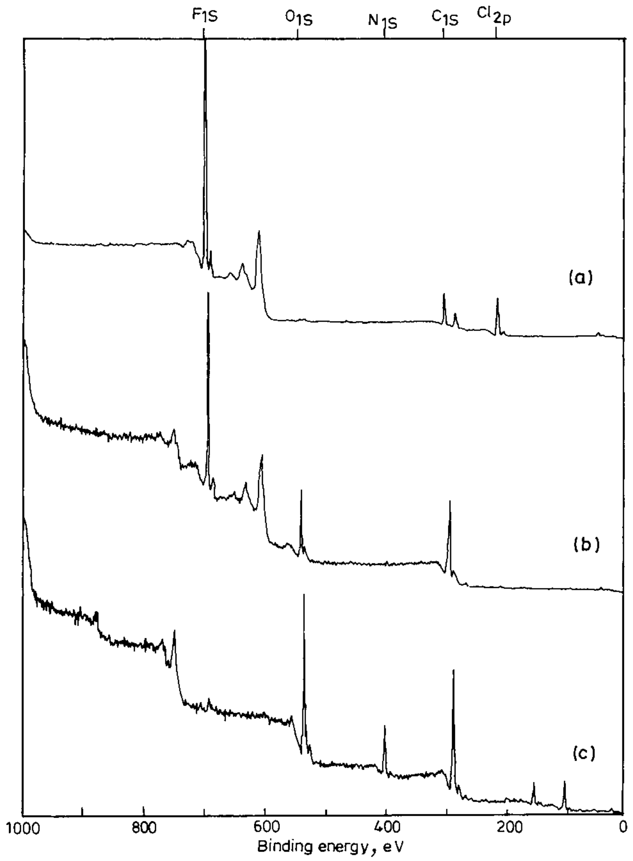
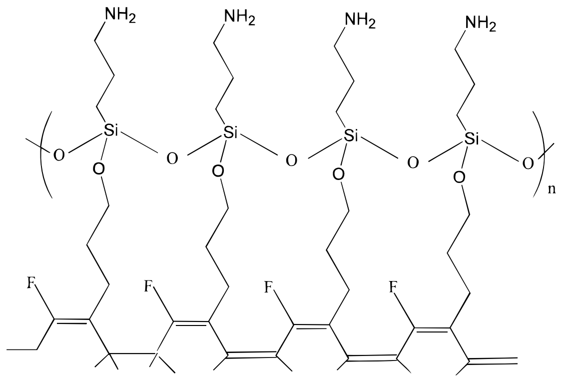


Figure 3. X-ray photoelectron spectra of (a) PCTFE, (b) PCTFE-OH, and (c) PCTFE-NH_2 surfaces at a takeoff angle of 15°.

Scheme 1. Proposed Structure of PCTFE-NH_2 Surface



The adsorption of alginic acid to amine-functionalized glass and PCTFE was carried out under various solution conditions. Since we were able to modify the PCTFE surface (of well-defined surface chemistry as established before[13−18]) with amine groups of high surface density and characterize it unambiguously, we selected it for

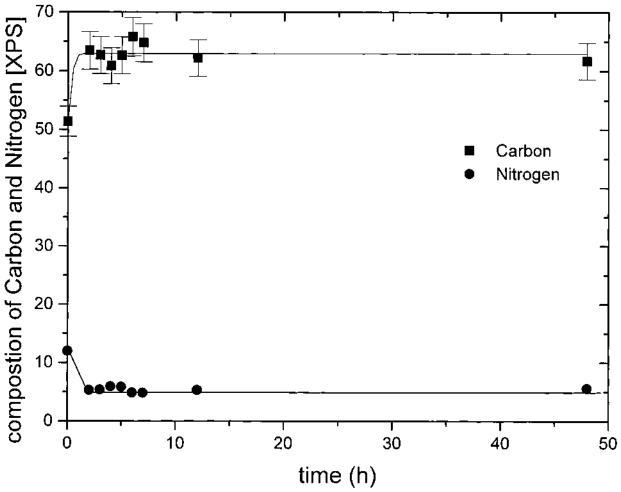


Figure 4. XPS atomic composition of carbon (●) and nitrogen (○) versus the time of adsorption following the adsorption of alginic acid from a 2 mg/mL solution in phosphate buffer 2 and PCTFE-NH_2 surfaces at a takeoff angle of 15°.

detailed investigations. Adsorption of alginic acid was carried out from phosphate buffer 2 (pH = 7.4) from solutions of various concentrations for various desired times. The kinetics of adsorption (25 °C) was followed by the XPS atomic composition (of the dry overlayer) of carbon (C), expected to increase following adsorption, and the decrease in atomic composition of N, expected due to the formation of an adsorbed overlayer. The results at a solution concentration of 2 mg/mL are shown in Figure 4. The C atomic composition increases from 51.4% at zero time and reaches a plateau value around 65.8% after 6 h while the N composition decreases from ~12% to ~5%. It can also be seen from the figure that the increase or decrease in atomic composition is very rapid with time, and it reaches the plateau value within an hour at a solution concentration of 2 mg/mL. Independent experiments with a solution of 0.1 mg/mL indicated that plateau adsorbance was reached within 5 h (data not shown).[25] The control for these experiments was the exposure of the PCTFE-NH_2 surface to the buffer for 6 and 24 h. This resulted in partial hydrolysis of the amine surface (i.e., conversion to the PCTFE-OH surface) as evident from the decrease in XPS atomic composition of Si and N from the PCTFE-NH_2 surface.[26] The XPS survey spectra for the control experiments are shown in Figure 5 as traces b and c. Comparison of these traces (in particular the N, Si, and F peaks) with the XPS survey spectra of PCTFE-NH_2 surface shown in Figure 3, trace c, clearly confirms the finding that in the absence of the polysaccharide the buffer hydrolyzes the 3-APTS-based overlayer.

The adsorption isotherm was determined by the change in the XPS atomic composition of the dry films after 48 h of adsorption at 25 °C as a function of several solution concentrations. The results of these experiments are presented as an adsorption isotherm in Figure 6. This indicates the formation of a high-affinity adsorption isotherm. The XPS survey spectrum following the adsorption from a solution concentration of 2 mg/mL is shown in Figure 5, trace a. Comparing this with that of the PCTFE-NH_2 surface shown in Figure 3, trace c clearly suggests the formation of an adsorbed overlayer as evident from the large decrease in peak intensities due to N (400 eV) and Si (100 and 150 eV) and the absence of F (~699 eV) peak. Thickness calculation following ARXPS analysis suggests the formation of a dry polysaccharide layer of ~5 Å thickness. The contact angle following adsorption changes with changing solution concentration. It is 67/15 at 2 mg/mL, 66/15 at 1 mg/mL, 64/17 at 0.5 mg/mL, 78/25 at 0.2 mg/mL, 79/36 at 0.1 mg/mL, and 79/38 at 0.01 mg/mL. This confirms the XPS finding that at lower polysaccharide solution concentration (≤0.2 mg/mL) hydrolysis of the 3-APTS layer to generate the PCTFE-OH surface competes with adsorption.

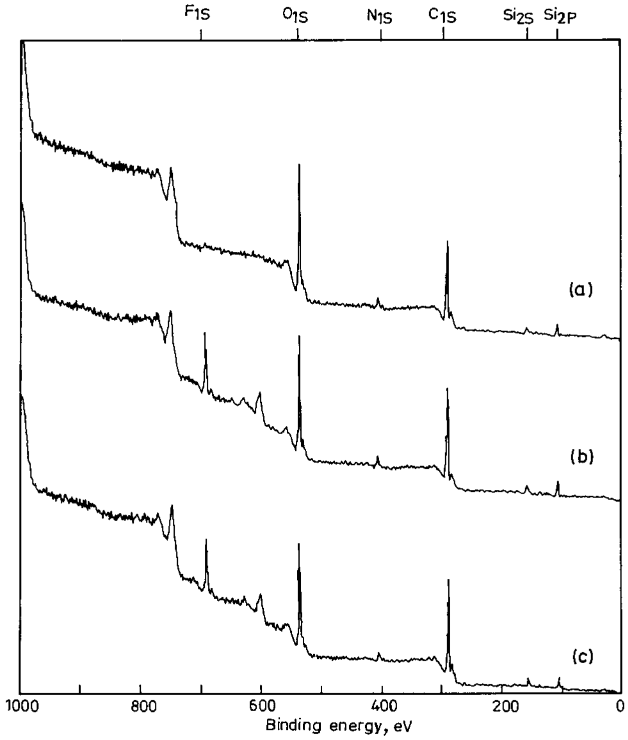


Figure 5. X-ray photoelectron spectra of the PCTFE-NH_2 surface: (a) following the adsorption of alginic acid from phosphate buffer 2; following exposure to the buffer alone for 6 h (b) and 24 h (c). Takeoff angle is 15°.

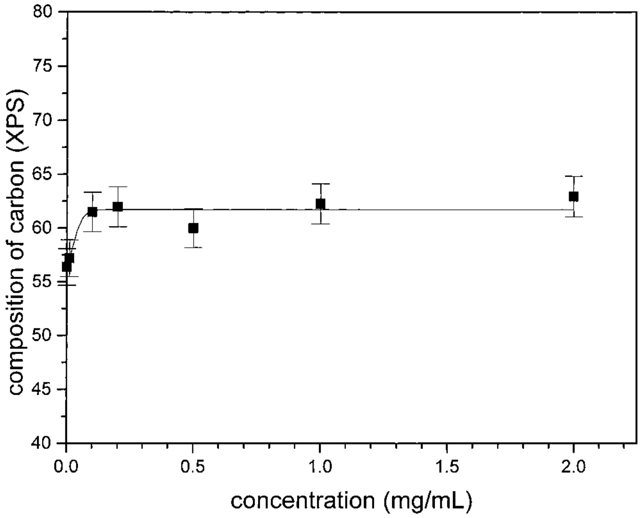


Figure 6. XPS atomic composition of carbon (●) versus the concentration of alginic acid in solution following its adsorption from phosphate buffer 2 to the PCTFE-NH_2 surface. Takeoff angle is 15°.

In an effort to confirm the presence of alginic acid following adsorption, surface-labeling experiments were performed. The XPS survey spectrum of the labeled surface is shown in Figure 7, trace a. The appearance of the peak due to Cu at ~935 eV confirms that alginic

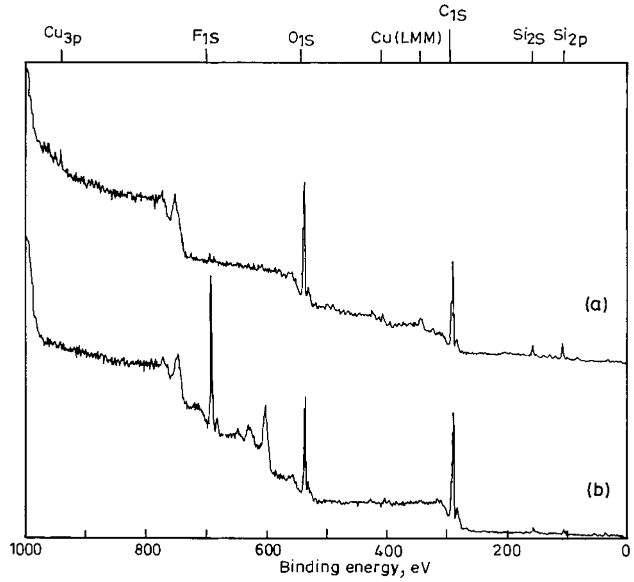


Figure 7. X-ray photoelectron spectra of PCTFE-NH_2 surface: (a) following the adsorption of alginic acid from phosphate buffer 2 and Cu(II) labeling; following exposure to the Cu(II) solution alone. Takeoff angle is 15°.

Table 3. ARXPS Composition Data Following the Adsorption of Alginic Acid from Calcium-Free Artificial Seawater to Glass Modified Earlier by PLL Adsorption[a]

takeoff angle	N	C	Si	O
15	2.85	78.38	5.32	13.45
20	2.89	74.59	6.49	16.03
30	3.54	64.81	9.41	22.24
40	3.10	57.84	11.83	27.22
50	3.02	50.35	14.02	32.61
60	2.89	45.14	16.02	35.94
75	2.98	40.84	16.49	39.69

[a] The adsorptions were carried out from a 0.5 mg/mL solution for 24 h at room temperature.

acid adsorbs to the PCTFE-NH_2 surface. In a control experiment, performed with the labeling solution in contact with the amine surface, it was quite evident that the amine surface was almost completely converted to the PCTFE-OH surface as evident from the XPS survey spectrum shown as Figure 7, trace b. Atomic compositions determined by XPS also confirm the same conclusion.[27] These results also indicate conclusively that the adsorbed alginic acid protects the amine surface from hydrolysis/oxidation by copper(II) acetate solution.

Alginic acid adsorbs from calcium-free artificial seawater (pH ~ 7) to the PLL-modified glass surface as well as to the 3-APTS-modified glass surface from phosphate buffer 2. A representative example following the adsorption from a 0.5 mg/mL solution in calcium-free artificial seawater to PLL-modified glass surface is given in Table 3 in the form of XPS atomic composition. Comparison of these data with that presented in Table 2 reveals very clearly that alginic acid adsorbs to this surface as well (for example, the C composition increases from ~64% to 78% and the N composition decreases from ~7% to 3%).

Table 4. ARXPS Composition Data Following the Adsorption of Chondroitin Sulfate-A to Amine-Functionalized Glass Surface[a]

takeoff angle	N	C	Si	O	S
15	5.55	66.64	5.31	21.59	0.90
20	6.10	62.73	6.27	23.86	1.03
30	6.35	55.80	8.13	28.24	1.48
40	6.11	51.73	9.96	30.95	1.25
50	6.14	46.62	12.13	33.91	1.20
60	5.63	43.01	13.58	36.64	1.13
75	5.34	39.82	14.49	39.42	0.93

[a] The XPS atomic compositions for the control are Si = 16.02, S = 0, N = 0.4, and O = 43.89 at a takeoff angle of 15° and Si = 24.27, S = 0, N = 0.22, and O = 64.87 at a takeoff angle of 75°.

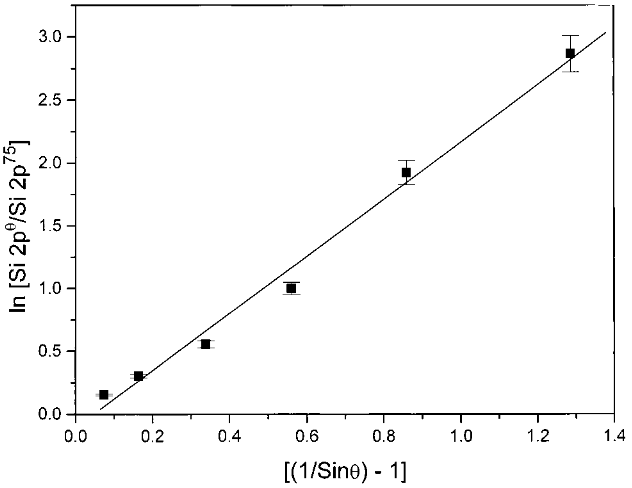


Figure 8. Natural logarithm of Si_{2p} peak area at a given takeoff angle (θ) normalized to that at 75° versus (1 − sin θ) from glass surface, following the adsorption of chondroitin sulfate-A to 3-APTS-modified glass surface.

We also carried out a detailed investigation of the adsorption of Chondroitin sulfate-A to amine-functionalized surfaces. Chondroitin sulfate-A adsorbs from a 0.1 mg/mL solution in potassium hydrogen phthalate buffer (pH = 3.9) to glass surface amine-functionalized using 3-APTS in benzene. The atomic composition data corresponding to this are shown in Table 4. The presence of sulfur (S)—a element unique to the polysaccharide—confirms its adsorption. The atomic composition of S increases with takeoff angle from 0.9 at 15° through a maximum of 1.48 at 30° to 0.93 at 75°. This suggests that the polysaccharide adsorbs with the sulfur functionality pointing toward the amine surface. The thickness of the adsorbed overlayer and the 3-APTS underlayer was calculated using the area under the Si_{2p} peak (ARXPS) as described before. The results of these experiments are shown in Figure 8. The overall thickness is estimated to be 9.7 Å. Since the thickness of the 3-APTS coupled glass surface is 6.7 Å, we infer that the thickness of the dry adsorbed polysaccharide layer is 3 Å. The control for this experiment was the exposure of a similar amine surface to the same buffer for the same period of time. This resulted in partial hydrolysis of the amine surface as evidenced by the XPS atomic composition change. [28]

Conclusions

The primary amine functionality is incorporated on glass and the PCTFE-OH surfaces using 3-APTS, a silane coupling agent. The modified layer's thickness is shown to be ~6.7 Å in the case of the glass−3-APTS reaction. The amine functionality is also incorporated on glass by the adsorption of PLL. In this case the layer thickness is shown to be ~9.8 Å. The systematic decrease in nitrogen atomic composition with increase in takeoff angle in most cases suggests that the amine group is present at or very close to the surface, a result shown conclusively for the first time. Alginic acid adsorbs to amine-functionalized surfaces while it does not to unfunctionalized surfaces. The adsorption of alginic acid is rapid, and a high-affinity isotherm results. It is shown that a thin layer of $\sim$5 Å of the adsorbed polysaccharide is present which protects the amine surface from hydrolysis/oxidation. Chondroitin sulfate-A adsorbs to amine-functionalized glass surface and results in the formations of $\sim$3 Å layer of the polysaccharide which again is shown to protect the surface from hydrolysis. Our experiments clearly suggest that surface functional groups play an important role in the adsorption of polysaccharides containing uronic acid and sulfated sugar residues.

We hypothesize that the marine/biofouling process could be initiated by any one of the two physicochemical processes or both: (1) a high local concentration of polysaccharide(s) (insoluble?) at the bacterial cell wall that could displace water and bind to the surfaces; (2) an initial adsorption of protein(s) (from the cell debris present in water) could lead to the adsorption of polysaccharide(s) by the specific chemical interaction between the acidic functional groups (charged or otherwise) with the amino groups (charged or otherwise). An XPS spectrum of a PCTFE film fouled by immersion in a fish tank for a month (figure not shown) indicates the presence of nitrogen whose concentration increases with increasing takeoff angle, suggesting that hypothesis 2 is not unreasonable.[29]

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(23) It was found that the alginic acid as received contained $\sim$2% of proteinous impurity, a fact which was later confirmed with the supplier, Sigma Chemical Co.

(24) The atomic compositions for PCTFE-OH are Cl, 1.17; C, 65.39; O, 11.74; F, 21.71 at a takeoff angle of 15° and Cl, 2.66; C, 62.08; O, 12.31; F, 22.96 at a takeoff angle of 75°. The water contact angles are 74/24.

(25) At alginic acid concentrations of 0.01 mg/mL and below the hydrolysis of the O−Si bond of the aminosilane competes with the adsorption of alginic acid, as evidenced by XPS results.

(26) The XPS atomic compositions (takeoff angle 15°) are Si, 6.78; Cl, 1.08; C, 61.06; N, 4.55; O, 19.67; F, 6.85 for the 6 h control and Si, 5.59; Cl, 0.66; C, 62.63; N, 5.12; O, 20.37; F, 5.63 for the 24 h control and Si, 12.55; Cl, 1.95; C, 51.40; N, 12.01; O, 21.18; F, 0.91 for the PCTFE-NH_2 surface.

(27) The XPS atomic compositions (takeoff angle 15°) following treatment with Cu(II) for 48 h: Si, 8.19; Cl, 0.72; C, 58.83; N, 4.71; O, 25.24; F, 0.55; Cu, 1.67 following adsorption from a 2 mg/mL solution and Si, 8.41; Cl, 1.78; C, 60.39; N, 5.92; O, 19.98; F, 1.93; Cu, 1.11 following adsorption from a 0.1 mg/mL solution. For the control PCTFE-NH_2 surface it is Si, 2.46; Cl, 0.0; C, 67.0; N, 2.10; O, 14.21; F, 14.23; and Cu, 0.0.

(28) The XPS atomic composition ratios for the aminated glass slide used in this experiment were C/Si = 9.43, O/Si = 2.1, and N/Si = 0.99. The ratios after chondroitin sulfate-A adsorption were C/Si = 12.55, O/Si = 4.07, N/Si = 1.05, and S/Si = 0.17. The ratios after the exposure of the aminated glass to the buffer for 72 h are C/Si = 3.89, O/Si = 2.28, and N/Si = 0.20 and after the adsorption of chondroitin sulfate-A to unfunctionalized glass for 72 h are C/Si = 2.48, O/Si = 2.74, N/Si = 0.0, and S/Si = 0.0. These ratios did not change with increasing adsorption time up to 2 weeks.

(29) A clean film of PCTFE (Aclar 33C) was immersed in a fish tank for $\sim$1 month. After this time the film was thoroughly rinsed with distilled water several times. The resulting film was dried under vacuum and was analyzed by XPS. The composition of the resulting film at two different takeoff angles are at 15° Cl = 2.26, C = 73.66, N = 4.56, O = 15.69, and F = 3.84 and at 75° Cl = 5.23, C = 62.34, N = 4.92, O = 18.34, and F = 9.17. The composition of PCTFE as received is the same at both takeoff angles, and it is Cl = 16.7, C = 33.3, and F = 50.0.

(30) Glass slides following nochromix cleaning through water rinse and workup in benzene exhibit XPS atomic compositions of C = 30.28, O = 52.19, and Si = 17.53, and those worked up in THF exhibit C = 5.86, O = 68.29, and Si = 25.89 at a takeoff angle of 15°.

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