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Modification of Cellulose Films by Adsorption of CMC and Chitosan for Controlled Attachment of Biomolecules

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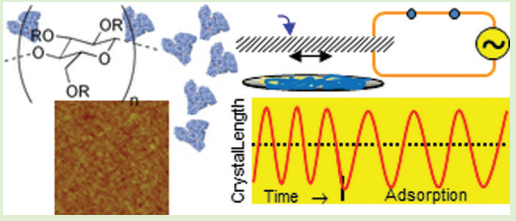
ABSTRACT: The adsorption of human immunoglobulin G (hIgG) and bovine serum albumin (BSA) on cellulose supports were investigated. The dynamics and extent of related adsorption processes were monitored by surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation monitoring (QCM-D). Amine groups were installed on the cellulose substrate by adsorption of chitosan from aqueous solution, which allowed for hIgG to physisorb from acid media and produced a functionalized substrate with high surface density (10 mg/m^2). hIgG adsorption from neutral and alkaline conditions was found to yield lower adsorbed amounts. The installation of the carboxyl groups on cellulose substrate via carboxymethylated cellulose (CMC) adsorption from aqueous solution enhanced the physisorption of hIgG at acidic (adsorbed amount of 5.6 mg/m^2) and neutral conditions. hIgG adsorption from alkaline conditions reduced the surface density. BSA was used to examine protein attachment on cellulose after modification with chitosan or carboxymethyl cellulose. At the isoelectric point of BSA (pI 5), both of the surface modifications enhanced the adsorption of this protein when compared to that on unmodified cellulose (a 2-fold increase from 1.7 to 3.5 mg/m^2). At pH 4, the electrostatic interactions favored the adsorption of BSA on the CMC-modified cellulose, revealing the affinity of the system and the possibility of tailoring biomolecule binding by choice of the surface modifier and pH of the medium.



INTRODUCTION

Development of functional materials from renewable, nontoxic plant-derived biopolymers for diagnostic and medical applications has lately sparked attention in academia and industry. Among these materials cellulose has been qualified by Pelton as "particularly protein and biomolecule friendly".[1] Cellulose is a high molecular weight linear homopolymer of D-anhydroglucopyranose units connected through β-(1−4) linkages. Sharing same level of interest is chitosan, another widely available linear polysaccharide which is composed of *N*-acetyl-D-glucosamine randomly linked via β-(1−4) bonds to D-glucosamine units.[2] Chitosan is obtained after deacetylation of chitin which is the structural element in the exoskeleton of crustaceans and cell walls of fungi. Degrees of deacetylation in chitosan are usually in the range of 70−80%, making it hydrosoluble due to the free amines with a pK_a value of ∼6.5, which leads to a protonation in acidic to neutral solutions.[3] However, the deprotonation of the amine groups in neutral and alkaline media reduces the solubility of chitosan and thus limits processing in such conditions. Carboxymethyl cellulose (CMC), a derivative of cellulose, is negatively charged in aqueous solutions due to its anionic carboxyl groups (pK_a of ∼4.5). In the presence of salt CMC adsorbs irreversibly on cellulose and can therefore be used to increase the negative charges of cellulosic fibers.[4]

Chitosan has shown antibacterial properties,[5−8] and its cationic charge has rendered it useful in the manufacture of hydrogels for drug delivery,[9,10] and bioadhesives to bind to negatively charged surfaces such as mucosal membranes and nanocapsules.[11] Moreover, it is ideally suited to complex with anionic CMC to enable a number of applications[12,13] and can be conjugated with functional molecules,[14] antibodies,[15] biotin,[16] enzymes,[17] and heparin.[18] Of relevance to the present work is the fact that both of the studied polysaccharides adsorb irreversibly from aqueous solution on cellulose,[19−21] most likely by virtue of their opposite charges (chitosan) and molecular/structural affinity (chitosan and CMC).[22] Such adsorptions are known to influence the swelling of cellulosic fibers.[23,24] Recently, the conjugation of chitosan with cellulose fibers after sodium-metaperiodate oxidation has been reported.[25]

Surface plasmon resonance (SPR) has been used to study biopolymer adsorption.[26−28] It is based on the principles of total internal reflection, whereby light creates surface plasmons along a thin metal layer adjacent to materials possessing different refractivity.[29] Molecular adsorption on a SPR wafer changes the refractivity of the metal film and leads to a minimum in reflected light intensity at a given angle (SPR angle). In principle, the change in the SPR angle is monitored as a function of time, and further analyzed for calculating the properties of the adsorbed layers. A common substrate employed in SPR is carboxymethylated dextran (CMD) since it facilitates information on adsorption and conjugation of biomolecules. For example, the interactions of bacteria,[30]

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globular protein (BSA),[31] and antibodies (rbST)[32] on CDM surfaces have been documented. Recently, SPR has been used to investigate the adsorption of polymers on cellulosic materials, including nanofibrillated cellulose (NFC)[33] and thin films of regenerated cellulose.[34] The adsorption of cellulose derivatives such as ethyl(hydroxyethyl) cellulose onto hydrophilically- and hydrophobically-modified gold has been followed with SPR.[35] In the case of chitosan surfaces, SPR has also been utilized to quantify binding of ferric ions[36] and BSA[37] and to study the conjugation of coiled peptides via enzymatic oxidation.[38] Moreover, the adsorption of chitosan on lipid bilayers, and its effects on layer properties have been reported.[39]

While SPR is a powerful tool for detecting adsorption events at the molecular level and allows determination of the adsorbed mass, in situ quartz crystal microbalance with dissipation monitoring (QCM-D) can provide complementary information. QCM-D is sensitive to the adsorbed mass and also to the state of hydration (coupled water), which can be useful to monitor changes in viscoelasticity at the interface. QCM-D technique is based on the acoustic oscillation of quartz crystal which can be conveniently measured to monitor adsorption events.[40,41] The combination of SPR and QCM-D allows for better understanding of hydration and (de)swelling phenomena, both of which are very relevant to the present study involving chitosan and cellulose.[42]

In this paper we describe a generic method for attaching biomolecules on cellulose primed with preadsorbed CMC and chitosan. CMC was used to control protein binding because nonspecific protein interactions are prevented in the presence of its hydrogel-like layers. Preadsorbed chitosan provides cationic charges on cellulose surfaces, which in turn can be used to control and enhance protein adsorption. Therefore, the overall goal of this study is to develop simple platforms for controlling protein adsorption on cellulose as part of recent efforts in the area of bioactive-paper research.

MATERIALS AND METHODS

Chitosan (50−190 kDa molecular weight, deacetylation degree of 75−85%, #448869), carboxymethyl cellulose, CMC (250 kDa molecular weight, degree of substitution of 0.7, #419311), polystyrene (280 kDa molecular weight, #182427), and bovine serum albumin, BSA (#A7906) were all obtained from Sigma-Aldrich (U.S.A.). Trimethylsilyl cellulose (TMSC) was synthetized as described elsewhere.[43,44] Purified human immunoglobulin G (#SLH661) was purchased from Equitec-Bio (Kerrville, TX, U.S.A.). Bare gold SPR wafers were obtained from KSV Ltd. (Espoo, Finland). QCM-D bare gold sensors were obtained from Q-Sense (Västra Frölunda, Sweden). The water used in all solutions was deionized and further purified with a Millipore Synergy UV unit. All used laboratory chemicals were analytic grade.

Preparation of Langmuir-Schaeffer (LS) Cellulose Films. The base substrates for deposition of cellulose consisted of gold-coated wafers and quartz resonators for SPR and QCM-D measurements, respectively. The gold wafers were first cleaned with UV/ozone treatment followed by spin coating with 0.1 wt % polystyrene in toluene (4000 rpm, 30 s). The obtained polystyrene-coated wafers were then heat-treated in an oven at 60 °C for 10 min to ensure a uniform hydrophobic layer suitable for trimethylsilyl cellulose (TMSC) deposition. TMSC was deposited on the polystyrene-coated substrates by using the horizontal LS-deposition technique as described by Tammelin et al.[45] The TMSC layer was then converted to cellulose via desilylation with hydrochloric acid vapor as described elsewhere.[46] The crystallinity degree, thickness, and roughness of the LS-cellulose films prepared in the same manner have previously been observed to be 53.5%, 17.8 nm, and 0.5 nm, respectively.[47] Before QCM-D or SPR experiments the cellulose substrates were allowed to stabilize overnight in the respective buffer solution.

Surface Plasmon Resonance (SPR). The SPR experiments were performed using a SPR Model Navi 200 (Oy BioNavis Ltd., Tampere, Finland). Adsorbing molecules at the interface between the solid and the surrounding medium produce a shift in optical resonance properties causing a change in the SPR angle where the reflected light intensity is minimum.[48] The thickness of adsorbed layers was determined by using eq 1:[49]

$$d = \frac{l_d}{2}\frac{\Delta_{\text{angle}}}{m(n_a - n_0)} \quad (1)$$

where the Δ_{angle} is a change in the SPR angle, the l_d is a characteristic evanescent electromagnetic field decay length (120 nm) and estimated as 0.37 of the light wavelength; m is a sensitivity factor for the sensor (109.94°/RIU) obtained after calibration of the SPR, n_0 is the refractive index of the bulk solution (1.334 RIU), and n_a is the refractive index of the adsorbed species. The refractive indexes used in this work were assumed to be 1.57 for BSA,[49] 1.5 for chitosan,[50] 1.4 for CMC,[51] and 1.352 for PEG.[52] The surface excess concentration was calculated according to eq 2:

$$\Gamma = d \times \rho \quad (2)$$

where d is the calculated thickness of the adsorbed layer and ρ is the specific volume of an adsorbate. The specific volumes (g/cm^3) were assumed to be 1.36 for BSA,[53] 1.20 for PEG,[54] 1.77 for chitosan,[55] and 1.61 for CMC.[56] All experiments were performed at a constant flow rate of 100 μL/min and the temperature was maintained at 25 °C.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D). In situ QCM-D experiments were performed using a Q-Sense E4 instrument (Västra Frölunda, Sweden). The adsorbed mass can be determined by using the Sauerbrey's equation provided the adsorbed layer is rigid, uniformly distributed on the surface, and small compared to the crystal's mass:

$$\Delta m_{\text{Sauerbrey}} = C_{\text{QCM-D}} \Delta f n^{-1} \quad (3)$$

where $C_{\text{QCM-D}}$ is 17.7 ng/cm^2 for 5 MHz crystal, Δf is change in frequency, and n is an overtone number.[57,58] However, if the adsorbed layer does not meet Sauerbrey conditions, the equation underestimates the adsorbed mass. Under these conditions the Voigt viscoelastic model (Q-Tools software, version 2.1, Q-Sense, Västra Frölunda, Sweden) is more indicated. We estimated the adsorbed masses (CMC and chitosan) on cellulose by using the Voigt approximation and assuming 1200 g/m^3 for the density of the adsorbed layer.[59] The QCM-D measurements were performed under continuous flow rate of 100 μL/min at 25 °C.

Surface Modification of Cellulose with Chitosan and CMC. Thin films of cellulose were modified by adsorption of chitosan and CMC from aqueous solutions of 0.5 mg/mL concentration and the process was monitored by using QCM-D and SPR. Before use, CMC was dissolved in Milli-Q water and dialyzed with 12−14 kDa Spectrapor dialysis membrane tubes (Spectrumlabs) to remove any impurities. The dialyzed CMC was dried to solid form and stored in a desiccator until use.

Before adsorption on cellulose, the dialyzed CMC was dissolved in 50 mM NaOAc buffer at pH 5.0 and 50 mM ionic strength adjusted with NaCl. Chitosan, used as received, was dissolved and adsorbed on cellulose from 50 mM NaOAc buffer at pH 5.0 and 50 mM ionic strength, also adjusted with NaCl. Both solutions were allowed to dissolve and stabilize overnight before measurements and filtered using 0.2 μm filters. Both CMC and chitosan were allowed to adsorb on the cellulose surface until signals from the QCM-D or SPR indicated adsorption plateau. Thereafter, rinsing with polymer-free buffer solution was applied. The signals recorded in QCM-D and SPR after rinsing were used to ascertain the irreversibility of the binding of the given molecule to the cellulose substrate. The resulting films of cellulose carrying the respective preadsorbed layer (chitosan or CMC)

were used in additional experiments; these substrates are referred thereafter as chitosan- or CMC-modified cellulose.

X-ray Photoelectron Spectroscopy (XPS) Analysis of Cellulose Surfaces. Chemical composition of the surfaces was investigated via XPS. Prior to measurement the samples were evacuated in a prechamber overnight. An in situ reference consisting of standard filter paper (S&S 5893 Blue ribbon ashless, 100% cellulose)[60] was measured along with each sample in order to verify satisfactory experimental conditions during the XPS analysis. The measurements were conducted using a Kratos Analytical AXIS 165 electron spectrometer with monochromatic Al Kα X-ray irradiation at 100 W. All spectra were collected at an electron takeoff angle of 90°. Both elemental wide-region data and high resolution spectra of carbon (C 1s) and oxygen (O 1s) were collected. All spectra were recorded at three different locations on each sample; the area and depth of analysis was 1 mm^2 and less than 10 nm, respectively. No sample degradation due to ultrahigh vacuum or X-ray radiation was observed during the XPS measurements.

Atomic Force Microscopy (AFM). Topographical changes on the cellulose surfaces after CMC and chitosan modifications were analyzed by AFM equipped with Nanoscope IIIa Multimode scanning probe from Digital Instruments Inc., Santa Barbara, CA, U.S.A. The images were scanned using tapping mode in air with silicon cantilevers. Scan sizes of 5 × 5 and 1 × 1 μm^2 were employed. At least three different regions on each sample were measured; no image processing except flattening was performed.

Adsorption of BSA on Cellulose, Chitosan-Modified, and CMC-Modified Cellulose. SPR was used to measure BSA adsorption from 10 mM NaOAc buffer at pH 4, and 5 and also in 10 mM phosphate buffer at pH 6.2, and 7.4. Ionic strength of the buffer solutions was adjusted to 50 mM with NaCl. BSA (0.1 mg/mL) was allowed to adsorb on pure cellulose or on cellulose with preadsorbed layers of chitosan and CMC. The adsorption process was monitored until an adsorption plateau was observed and then the system was rinsed with the respective buffer solution to remove loosely bound BSA molecules.

hIgG Adsorption on Cellulose, CMC-Modified Cellulose, and Chitosan-Modified Cellulose. hIgG adsorption on cellulose, CMC-modified, and chitosan-modified cellulose was investigated by using SPR. The experiments were conducted in 10 mM NaOAc buffer at pH 5, and in 10 mM phosphate buffer at pH 6.2, 7.4, and 8. The ionic strength of the buffer solutions was adjusted to 50 mM by adding NaCl. hIgG (0.1 mg/mL) was allowed to adsorb on cellulose, CMC-modified, and chitosan-modified cellulose until the adsorption plateau was observed. The respective buffer solution was injected to rinse out any free molecules.

RESULTS AND DISCUSSION

Adsorption of Chitosan and CMC on Cellulose. The backbones of chitosan and CMC polymers are similar to that of cellulose, which has been postulated as a reason for their affinity with cellulose.[22] In acidic solution, chitosan's amine groups are positively charged, while in alkaline conditions, chitosan is uncharged and its solubility in water is diminished. It has been observed that chitosan adsorbs on cellulose in both conditions.[23] CMC is negatively charged in aqueous solutions due to its anionic carboxyl groups (pK_a of ~4.5).[61] It adsorbs irreversibly on cellulose in the presence of salt and can therefore be used to increase the density of negative charges on cellulosic fibers.[4] While the utility of Langmuir−Schaefer (LS) films as a model substrate for cellulose has been widely reported in relation to fundamental inquires, mainly because its chemical similarity with cellulose in fibers, the results from adsorption experiments with LS needs to be put into context. The lower surface roughness and lack of voids in LS films are in contrast with those of actual fibers. Therefore, it should be noted that albeit the adsorption data collected from regenerated LS cellulose model surfaces is very useful in understanding interfacial phenomena, the added complexity of fiber systems has to be factored in, depending on their source and processing details.

Figure 1a shows the SPR sensogram upon adsorption of chitosan and CMC on cellulose, under the same experimental

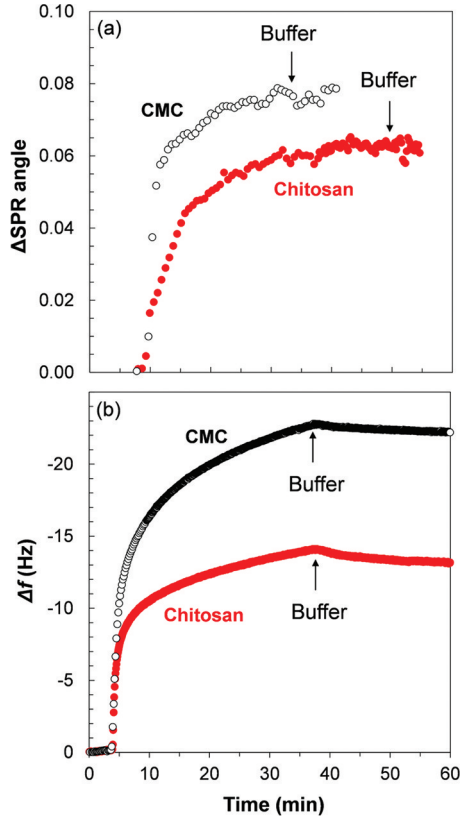

Figure 1. Dynamics of adsorption of chitosan and CMC on LS cellulose, as monitored by SPR angle shift (a) and changes in QCM-D's fifth overtone frequency Δf (b).

conditions as those used in similar QCM-D experiments, Figure 1b. The adsorption kinetics of both polysaccharides was observed to be Langmuir-type and revealed similar adsorption mechanisms. This is reasonable since the high ionic strength used screened the electrostatic interactions and the structural differences between the polymers, both of which lead to similar dynamics. From SPR data, it can be observed that the adsorption leveled off after 30 min equilibration time and no desorption was evident after rinsing with buffer solution. In contrast, QCM-D experiment indicated a small change in the frequency signal (Figure 1b). These observations indicate the important role of water coupled to the adsorbed layer and the changes in hydration/swelling that occurred after replacing the background solution. Such effects are easily detected with the QCM-D but not with SPR.

The changes in QCM-D energy dissipation were plotted against the shift in frequency in the so-called $\Delta D-\Delta f$ profiles (Figure 2). These profiles are useful to elucidate the adsorption process: It can be clearly observed that the extent of adsorption (as determined by the maximum Δf) was more limited for chitosan than for CMC (ca. −12 Hz compared to ca. −22 Hz, respectively). Interestingly, the slopes of the $\Delta D-\Delta f$ profiles were comparable in both cases, indicating that a similar adsorption mechanism took place. Only a small degree of

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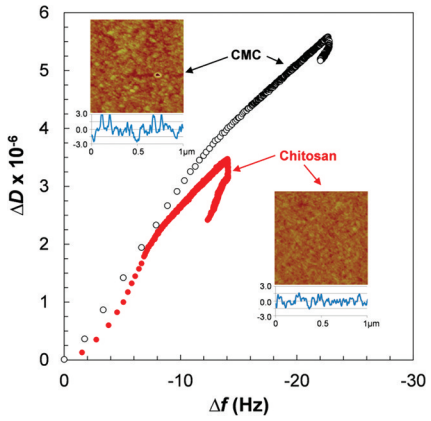

Figure 2. QCM-D $\Delta D-\Delta f$ fifth normalized overtone profiles upon adsorption from 0.5 mg/mL aqueous solution of chitosan or carboxymethyl cellulose (CMC) on LS cellulose (50 mM ionic strength). Insets: 1 × 1 μm^2 AFM height images and corresponding roughness profiles.

polymer desorption was observed after rinsing with the buffer solution, as can be determined by the small loop in $\Delta D-\Delta f$ at the end of the experiment. This is indicative of the highly irreversible adsorption that occurred even when electrostatic repulsion was favored, that is, when the charges for CMC and cellulose were of the same sign. Furthermore, negligible desorption was observed for both of the polysaccharides when rinsing was conducted under more stringent conditions (Milli-Q water, pH of ca. 8.5, data not included). AFM images of the modified cellulose showed only minor topographical changes thus indicating rather thin and uniform adsorption layers (Figure 2, insets).

The difference in adsorbed mass measured by QCM and SPR is related to water coupling, as indicated before. Such comparison of adsorption data was conducted by fitting the data (see Material and Methods), and the main results are summarized in Table 1. The 51 nm thickness of the adsorbed CMC layer determined by QCM-D (Voigt fitting parameters used: ρ = 1.2 kg/m^3, $\eta = 0.95 \times 10^3$ N·S·m^2, and $\mu = 2.3 \times 10^3$ N·m^{-2}) was found to be significantly higher than that obtained from SPR data (1.3 nm), confirming the hydrogel-like nature of CMC in aqueous solution: 97% water was calculated to be present in the adsorbed CMC layer. The thicknesses of the adsorbed chitosan layers measured by QCM-D (Voigt fitting parameters used: ρ = 1.2 kg/m^3, $\eta = 1.35 \times 10^3$ N·S·m^2, and $\mu = 110 \times 10^3$ N·m^{-2}) and SPR were found to be 3.6 and 0.4 nm, respectively. The hydration % of the adsorbed chitosan layer was considerable, 83, but lower compared to that calculated in the case of the thicker CMC adsorbed layer. Overall, it is likely that carboxyl groups in CMC facilitate strong hydrogen bonding, water coupling, and swelling.

Adsorption of BSA on Cellulose Surfaces. Preventing nonspecific adsorption of biomolecules is a requirement in affinity adsorption and in biosensing as it defines the detection limits of the system as well as its suitability for deployment in complex matrices. As such, the adsorption of bovine serum albumin (BSA) on cellulose, CMC-, and chitosan-modified cellulose was studied by using the SPR technique. Relevant to this discussion is the fact that the surface was enriched with carboxyl and amine groups after priming the cellulose surface with CMC or chitosan, respectively. X-ray photoelectron spectroscopy indicated an elevated amount of nitrogen in the case of the chitosan-modified cellulose and a slight change in the carbon/oxygen ratio for the CMC-modified cellulose (see Table 2). More importantly, the treatment of cellulose with the respective polymer is expected to affect the adsorption of probing proteins, such as BSA, depending on the solution pH: BSA is negatively charged above its isoelectric pH of 5 and positively charged below it.[62]

Table 2. XPS Atomic Surface Concentration of Oxygen, Carbon, and Nitrogen on Cellulose, CMC-, and Chitosan-Modified Cellulose

	XPS surface concentration (atomic %)			O/C ratio
	O	C	N	
cellulose	42.4 ± 0.2	57.6 ± 0.2	0.1 ± 0.1	0.74
chitosan-modified cellulose	41.2 ± 0.1	57.4 ± 0.1	1.4 ± 0.1	0.72
CMC-modified cellulose	43.7 ± 0.1	55.9 ± 0.1	0.3 ± 0.2	0.78

Figure 3 illustrates the adsorption from BSA solutions of given pH onto cellulose (Figure 3a), CMC-modified cellulose (Figure 3b), and chitosan-modified cellulose (Figure 3c). The respective maximum areal BSA adsorbed mass was calculated from the SPR data by using eqs 1 and 2 and values at the different pH reported in the same figure (insets).

It is observed that at the isoelectric point of BSA (ca. pH of 5) a rather large amount of this protein was adsorbed on each of the surfaces investigated. In the case of cellulose and chitosan-modified cellulose, a maximum adsorption can be clearly observed close to the isoelectric pH. In general, similar observations for the adsorption of BSA on noncellulosic surfaces have been noted elsewhere as a result of reduced solvency at the pI.[63] In contrast, CMC-modified cellulose did not display a maximum at the isoelectric pH; adsorption of BSA was reduced monotonically with solution pH. In this case, the strongest (electrostatic) affinity was observed at pH 4.

BSA adsorption on cellulose at pH 4 and 7.4 was very limited. At pH 6.2, the adsorbed amount was twice that measured at pH 4 and 7.4. The higher BSA adsorbed mass at pH 6.2 might be explained by a minimum hydrodynamic radius close to this pH,[62] which facilitates better packing of the adsorbed molecules and thus higher surface coverage.

The presence CMC in CMC-modified cellulose was found to influence dramatically the adsorption of BSA. As expected, the adsorption of BSA was significantly elevated at pH 4 due to the increased anionic charge. On the other hand, at pH 7.4, the adsorbed mass was much less. At the isoelectric point of BSA,

Table 1. Calculated (QCM-D and SPR Data) Mass (Δm, mg/m^2), Layer Thickness (nm), and % Water Content of Layer of Chitosan and CMC Adsorbed on LS Cellulose in Aqueous Solution

	thickness$_{QCM-D}$, nm	thickness$_{SPR}$, nm	Δm_{QCM-D}, mg/m^2	Δm_{SPR}, mg/m^2	% coupled water
CMC	51.0 ± 3.0	1.3 ± 0.1	53 ± 3	2 ± 0.2	97
chitosan	3.6 ± 0.2	0.4 ± 0.05	4.4 ± 0.2	0.7 ± 0.1	83

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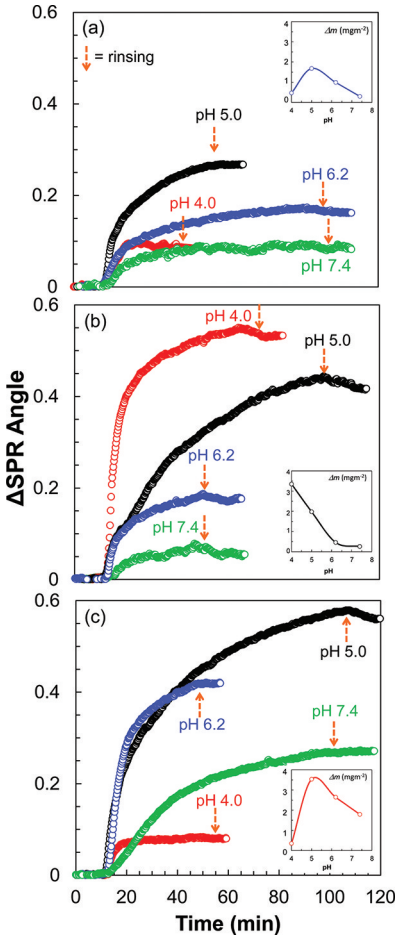

Figure 3. SPR sensograms for the adsorption of bovine serum albumin (BSA) from 0.1 mg/mL aqueous solution on cellulose (a), CMC-modified cellulose (b), and chitosan-modified cellulose (c). The isotherms were obtained at pH 4, 5, 6.2, and 7.4. The corresponding adsorbed amounts (calculated from SPR) as a function of solution pH are added as inset in the respective figure.

the adsorption was found to be approximately 30% higher than that on the unmodified cellulose.

The chitosan layer deposited onto cellulose in chitosan-modified cellulose enhanced BSA adsorption, depending on the pH: while the adsorption of positively charged BSA (pH 4) was rather similar to that on the unmodified cellulose, the adsorption significantly increased at pH 6.2, likely by virtue of the electrostatic attraction of the negatively charged BSA and the cationic chitosan layer. At pH 7.4, chitosan is unprotonated and water insoluble, resulting in a neutral thin adsorbed layer, with lower hydrophilicity. Under this condition, adsorption was also higher than at pH 4 and the contribution of hydrophobic interactions dominate.

To the best of our knowledge, no reports are available describing the adsorption of BSA onto cellulose surfaces. Therefore, the results presented here can only be compared to those related to metals, for example, hydroxyl-modified gold at pH 7.4. In this case, adsorption of BSA was limited relative to the amount measured on cellulose, 0.10 versus 0.30 mg/m^2.[64] Adsorption of BSA onto the CMC-modified cellulose was found to be over 60% lower (0.26 mg/m^2) when compared to the adsorption on carboxyl-functionalized gold at pH 7.4 (6.8 mg/m^2).[64] Finally, the adsorption of BSA on chitosan-modified cellulose was over 70% lower (1.8 mg/m^2) compared to amino-functionalized gold at pH 7.4 (6.3 mg/m^2).[64] These differences in adsorption highlight important features of cellulose as support for biomolecule attachment, and therefore, further exploration on how its functionalization affects the adsorption of proteins is warranted.

Some differences were observed in the initial rate of BSA adsorption. At pH of 5, the rate of adsorption was significantly slower for all tested surfaces, which may be an indication of the role of hydrophobic/hydrophilic interactions compared to the rapid protein adsorption process when electrostatic interactions are dominant. The slowest adsorption rate was observed at pH 7.4 for all substrates considered. This is likely due to the influence of electrostatic repulsion between BSA and the surface (cellulose and CMC). Whereas in the case of chitosan-modified cellulose, no electrostatic interactions were present at pH 7.4, and the adsorption was driven mainly by hydrophobic effects. The dynamics of adsorption on chitosan-modified cellulose was found to be significantly slower relative to cellulose and CMC-modified cellulose.

Electrostatic interactions have been postulated to influence the binding of proteins onto cellulose in ion-exchange devices.[65] Preadsorbed CMC increases the number density of negative charges on the surface and forms highly hydrated layers, increasing the adsorption of positively charged proteins while preventing adsorption of either negatively charged and

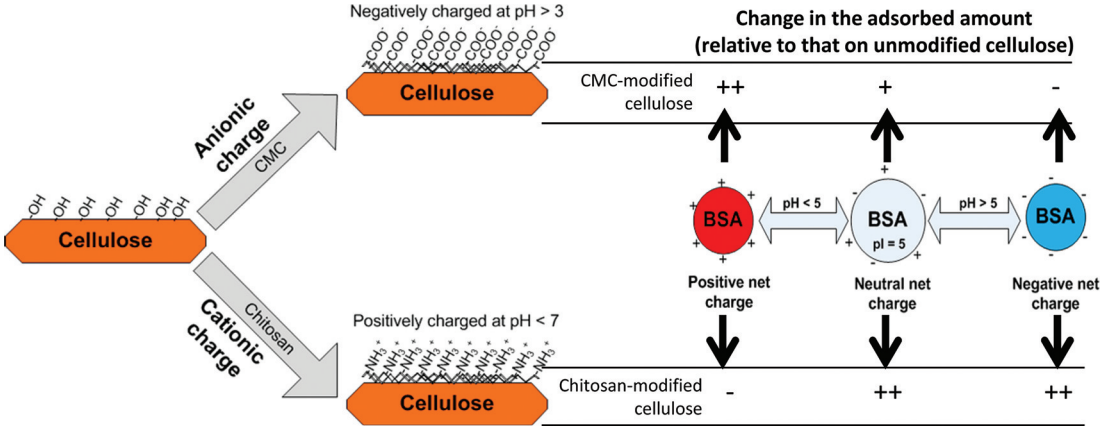

Figure 4. Illustrative schematics summarizing main observations regarding the adsorption of BSA on cellulose-based substrates under different conditions of solution pH and respective charge balance.

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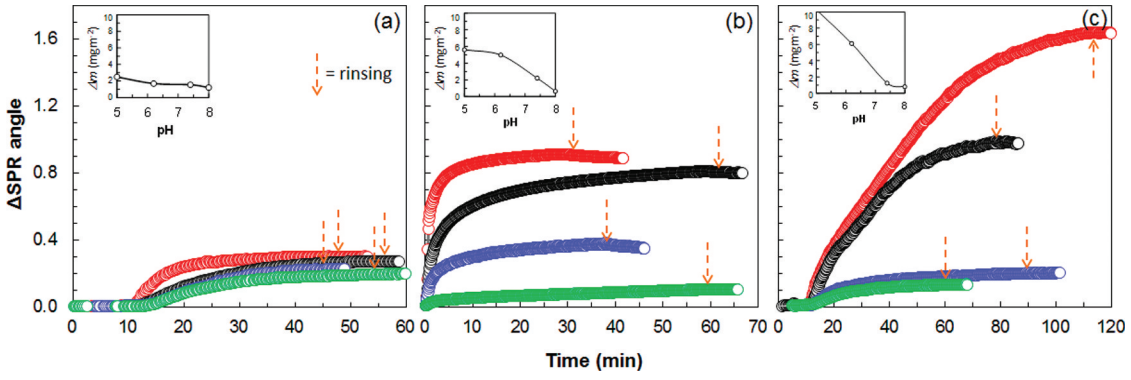

Figure 5. SPR sensograms on the adsorption of 0.1 mg/mL human immunoglobulin G (hIgG) on cellulose (a), CMC-modified cellulose (b), and chitosan-modified cellulose (c) from aqueous solutions of pH 5.0, 6.2, 7.4, and 8.0 (the respective curves follow the same order from top down). Corresponding calculated adsorbed mass is indicated as a function of pH in the insets (calculated from SPR data by using eq 1).

hydrophobic proteins. On the other hand, a preadsorbed chitosan layer increases cationic charges; this enhances the adsorption of negatively charged proteins. However, a preadsorbed chitosan layer is prone to enhance the adsorption of the proteins even in the deprotonated (insoluble) state. This is likely due to the increased hydrophobicity of the adsorbed layer. It is also worth mentioning here that the adsorption capacity may be enhanced by increasing the positive charge, hydrophobicity, or negative charge of the absorbent.[66] Overall, the results discussed so far underscore the prospects of surface modification of cellulose with CMC and chitosan to control the adsorption of globular proteins. Figure 4 provides an illustration with a summary of main results regarding the adsorption of BSA on cellulose-based surfaces under different conditions of solution pH and respective charge balance.

Adsorption of hIgG on Cellulose, CMC-Modified, and Chitosan-Modified Cellulose. hIgG is the largest antibody group in the human immune system: it binds pathogens such as viruses, bacteria, and fungus. In fact, the specific interaction of hIgG with protein A has already been utilized in immunoassay applications.[14] The ability to detect certain pathogens allows for the manufacture of substrates with specific binding affinities. Therefore, the functionalization of cellulosic substrates with hIgG could render a platform for detection and removal based on these biopolymers.

Figure 5 shows typical SPR sensograms and calculated adsorbed mass for hIgG on cellulose (a), CMC-modified cellulose (b), and chitosan-modified cellulose (c). It can be observed that hIgG adsorbed on unmodified cellulose mostly at pH 5. At this pH, hIgG is positively charged (the isoelectric pH of hIgG is between 6.8 and 7.4) and therefore the acidic condition is expected to favor attractive electrostatic interactions on unmodified cellulose. The presence of the preadsorbed CMC layer significantly increased the adsorbed mass of hIgG at acidic conditions. This is likely due to the attractive electrostatic forces between the negatively charged CMC layer and positively charged hIgG. However, at pH 8, hIgG adsorbed mass was found to be lower than that on unmodified cellulose, which is indicative of repulsive (electrostatic) forces. In addition, it is observed that the initial adsorption of hIgG occurred faster on CMC-modified than on unmodified cellulose. The presence of the preadsorbed chitosan layer was also found to dramatically increase the extent of adsorption of hIgG at low pH (pH 5 and 6.2). However, at higher pH (pH 7.4 and 8.0), the preadsorbed chitosan layer reduced the adsorption of hIgG when compared to the unmodified cellulose. Chitosan is positively charged in acidic conditions (pI of 6.5) and it becomes uncharged above the isoelectric point. At pH 5, the adsorbed amount was found to be more than five times higher when compared to that on unmodified cellulose. This indicates that the adsorption of hIgG on chitosan-modified cellulose is likely governed by nonelectrostatic interactions. On the other hand, the adsorbed amounts of hIgG were reduced at higher pH (pH 7.4 and 8), indicating that the uncharged and more hydrophobic preadsorbed chitosan layer prevented adsorption of hIgG.

Figure 6 summarizes the results of adsorption from solutions at different pH. It is observed that in acidic conditions the mass

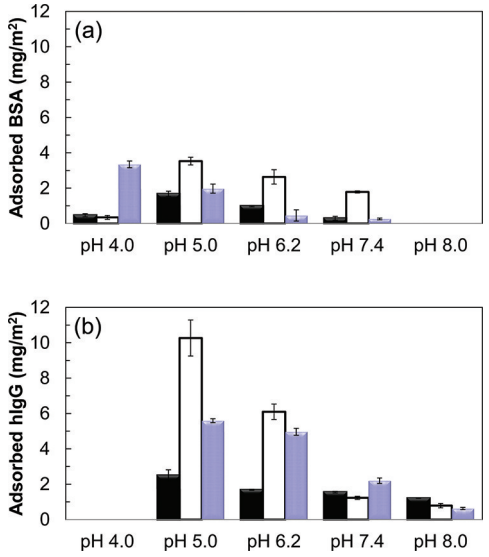

Figure 6. Adsorbed mass of (a) bovine serum albumin (BSA) and (b) human IgG (hIgG) on cellulose (filled black bars), chitosan-modified cellulose (open bars), and CMC-modified cellulose (gray bars). The adsorbed amount was calculated by using SPR data. Adsorption of BSA and hIgG was negligible at pH 8 and 4, respectively.

of hIgG adsorbed is higher compared to BSA (see Figure 6). This fact indicates that preadsorbed chitosan or CMC layers can be used to increase affinity with the surface in such conditions. Yet, in neutral and alkaline conditions, the adsorption of hIgG was limited on the chitosan-modified cellulose. This observation supports our hypothesis that other interactions are relevant in this condition. In fact, it has been

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reported that hIgG adsorbed to a larger extent on hydrophilic surfaces compared to hydrophobic ones: adsorption of hIgG on chitosan was favored at lower pH.[67] Moreover, it was shown that the adsorption of hIgG increased as a function of protonation degree of the chitosan,[67] in agreement with our observations.

The adsorption of hIgG on cellulose, CMC-modified cellulose, and chitosan-modified cellulose was found to be irreversible because only a negligible desorption was observed after rinsing the system with the respective background buffer solution. A long adsorption time was required until reaching saturation or plateau adsorption, which has been shown to induce irreversible adsorption due to the time allowed for slow conformational changes of the proteins binding to the substrate.[68] Moreover, it has been demonstrated that low adsorption concentrations lead to a stronger binding because of a larger protein footprint.[69]

CONCLUSIONS

Substrates consisting of cellulose and cellulose carrying preadsorbed layers of chitosan or CMC were investigated for protein adsorption. It was found that the type of the cellulose modification and the solution pH played an important role in controlling BSA binding. The highest adsorption on the chitosan-modified cellulose was observed at pH 5, whereas on the CMC-modified substrate it occurred at pH 4. Moreover, the presence of preadsorbed layers of chitosan or CMC significantly increased the binding of hIgG on cellulose at acidic pH. Overall, the proposed platforms based on cellulose-based model films and physical adsorption of BSA or hIgG proteins is expected to open new venues for deployment of this support in biodetection or filtration of pathogens.

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