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Formation, antimicrobial activity, and controlled release from cotton fibers with deposited functional polymers

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ABSTRACT: Chitosan and alginate are biopolymers with interesting bioactivity that can be transferred to cotton fibers for medical and health care applications. These polymers in solution can be attached onto cotton fibers by a layer-by-layer technique. Confirmation of polymer deposition onto fibers was verified by morphology analysis, coomassie blue dye coloration, and contact angle of water on fibers. Also, weight gain and level of whiteness after each layer deposition were determined. Antimicrobial activity on treated cotton samples against *E. coli* and *S. aureus* was evaluated after each layer deposition and high inhibition rate of bacteria growth was observed in samples with chitosan outer layer ($\sim$ 100%). Polyelectrolyte layers on cotton fibers not only provide interesting bioactivity by themselves, but can also serve as a matrix for small bioactive molecules. In this regard, a model molecule was added during sample preparation to study its release behaviors in a buffer solution by monitoring with UV–vis spectroscopy. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43054.

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INTRODUCTION

Textile materials have been used in medicine with long tradition and their main application fields are wound care and infection prevention.[1] According to the application purpose, textile materials in the medical field and health services are classified into two basic groups, for internal and implantable uses (inside tissues), and for external and nonimplantable uses (on the surface). The textile materials for internal and implantable uses are like vascular grafts, meshes, stents, tendons and ligament implants, surgical threads, etc and for external uses are gauzes, bandages, surgical covers, nappies, tampons, and so on.[2] Fibers used in medical applications, especially in wound dressing, can be divided into biodegradable and nonbiodegradable, in this respect, natural cellulose fibers are preferred due to their shorter time of degradation compared to synthetic fibers, such as polyester and nylon.[3] However, cellulosic fibers have a large active surface area that retains moisture which creates an undesirable environment that favors microorganism growth on the fabric surface,[4] therefore, antimicrobial activity is beneficial in medical textiles. In this regard, various bioactive finishing processes for fabrics have been attempted using synthetic or natural polymers.[5,6]

Among the natural polymers used for surface modification of cellulose fibers, chitosan has been studied broadly. Chitosan is a biopolymer composed of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units linked through β-(1→4) bonds and it is derived from alkaline deacetylation of chitin, one of the most abundant natural polysaccharides.[3] Many advantageous properties, such as biodegradability, antibacterial activity, noninflammatory property, nontoxicity, and high charge density, can be transferred to traditional textiles by attachment of chitosan on their surfaces.[6] Chitosan-based materials have been successfully used as wound dressings for burns and chronic wounds like leg ulcers, and chitosan fibers or fibers covered with chitosan can also help local blood coagulation.[1] In the functionalization of cellulose fibers with chitosan, the active

amino groups are attached to the surface of cellulose fibers. The similarity of chemical structures and functional groups of cellulose and chitosan promotes a high affinity between both polymers.[2]

Currently, chitosan is the most used biopolymer agent for antimicrobial treatment of textiles and several authors have showed its excellent antimicrobial activity onto fibers.[6–11] The actual mechanism for inhibition activity of chitosan against microbes is not yet fully understood however, the most accepted hypothesis is that the active amino groups present in chitosan provide positive charges on the fibers which react with the negatively charged surface of microbes.[5]

Chitosan, being a cationic polyelectrolyte, binds strongly to negatively charged species. In the process of chitosan attachment onto cellulose substrates, alginate was chemically integrated by ionic interaction to improve mechanical properties of the dressing.[12] Alginate is an anionic polysaccharide also used with chitosan in modification of textiles for medical applications. As wound dressing material, alginates provide many advantages like optimal moist environment by retaining large amounts of water and absorbing wound exudates by swelling and diffusion mechanisms which speed up wound healing and prevent a secondary trauma caused by sticking to the wound.[11] Alginate dressing has a function as an antimicrobial agent by absorbing microorganism-infected exudates which are removed in the process of changing dressings.[13] In calcium alginate wound dressings, a reverse ion exchange process takes place, sodium ions in blood and wound exudates are exchanged with the calcium ions in the alginate.[11]

Polyelectrolytes of opposite charges can be deposited successively onto fibers by the layer-by-layer technique. This treatment can also serve as a matrix to incorporate a bioactive compound that can be released when applied over an open wound. This work presents the study of polycationic and polyanionic layer formation on cotton fibers, the antimicrobial activity and the release of a model compound into a buffer solution.

EXPERIMENTAL

Materials

High molecular weight chitosan (670 kDa and degree of deacetylation 95%) was obtained from chitin kindly provided by Idebio (Chile). Bleached white woven 100% cotton fabrics (80/65 warp/weft cm^{-1}) and commercial cotton gauze were used as cellulose substrates for functionalization. The cationic polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA) with low molecular weight, 100–200 kDa, in aqueous solution (20% w/w), the sodium salt of alginic acid (viscosity 200,000–400,000 cps) and other chemicals were purchased from Sigma–Aldrich. The 2,4-dichlorophenoxyacetic acid (2,4-D) was isolated and purified from commercial herbicide Hedonal-6® purchased from Bayer.

Polyelectrolyte Assembling onto Cotton Fibers

For polyelectrolyte assembling, three cotton fiber samples of 15 × 20 cm^2 were prepared for each treatment. A solution to fiber ratio of 30:1 was used for all treatments. Fiber samples were washed with distilled water after each treatment (10 cycles × 150 mL) and air dried.

PDDA Deposition. Charge modification of cotton fibers surface was assessed by cationic layer deposition using PDDA. A 1 g L^{-1} PDDA solution was prepared dissolving the polymer in a 5 g L^{-1} sodium hydroxide aqueous solution. Cotton fibers, prewashed with nonionic surfactant Tween 80, were immersed in PDDA solution and incubated at 60°C for 30 min under stirring.

Alginate Deposition. A 5 g L^{-1} of alginate solution was prepared in distilled water. Prewashed cotton fibers were incubated in alginate solution for 1 h at 60°C under stirring.

Chitosan Deposition. A 5 g L^{-1} chitosan solution was prepared in 1% (v/v) acetic acid aqueous solution under strong agitation. Prewashed and previously treated with PDDA-AL cotton fibers were incubated in the chitosan solution for 1 h at 60°C.

Characterization of Layer by Layer Deposition of Polyelectrolyte on Cotton Fibers

Scanning Electron Microscopy (SEM) Analysis. After depositions of different types of polyelctrolytes, the longitudinal view of cotton fibers was observed with a scanning electron microscope, model Tabletop Microscope TM-3000, from Hitachi at 1000× magnification.

Anchoring Efficiency of Polymers onto Cotton Fibers. The anchoring of polymers onto fibers was evaluated by measuring the weight gain of the cotton fibers after each deposition step. The weight gain is proportional to the amount of polyelectrolyte attached to the fibers (PDDA, chitosan and alginate). After each incubation process, the samples were washed with distilled water and air-dried overnight. Before weighing, samples were conditioned in a controlled humidity chamber at 65–68% relative humidity at room temperature for 24 h. The anchoring efficiency (AE) was calculated according to the following equation [eq. (1)]. All measurements were run in triplicate.

$$\mathbf{AE}\ \% = 100 \times \frac{[BC]b - [BC]a}{[BC]a} \quad (1)$$

where AE is anchoring efficiency, [BC]a is the weight of cotton fibers previous to layer deposition and [BC]b is the weight of cotton fibers after layer deposition.

Visualization for Polyelectrolyte Layers. The formation of a cationic layer of PDDA and chitosan on the surface of cotton fibers were visually verified by coomassie brilliant blue dye G-250 attachment. A 0.1 g L^{-1} of the dye solution was prepared in distilled water. Each fiber sample with different polyelectrolyte layers was cut to 5 × 5 cm^2 and immersed in the same dyeing solution. Three samples were tested for each treatment. The dyeing process was carried out at room temperature for 10 min followed by washing with water for 30 min at room temperature to remove unabsorbed dye from the fibers surface. Chemical structures of PDDA, coomassie brilliant blue dye G-250, and 2,4-D are shown in Figure 1.

Whiteness Index. The effect of depositions of polyelectrolyte layers on whiteness was studied. The whiteness index was

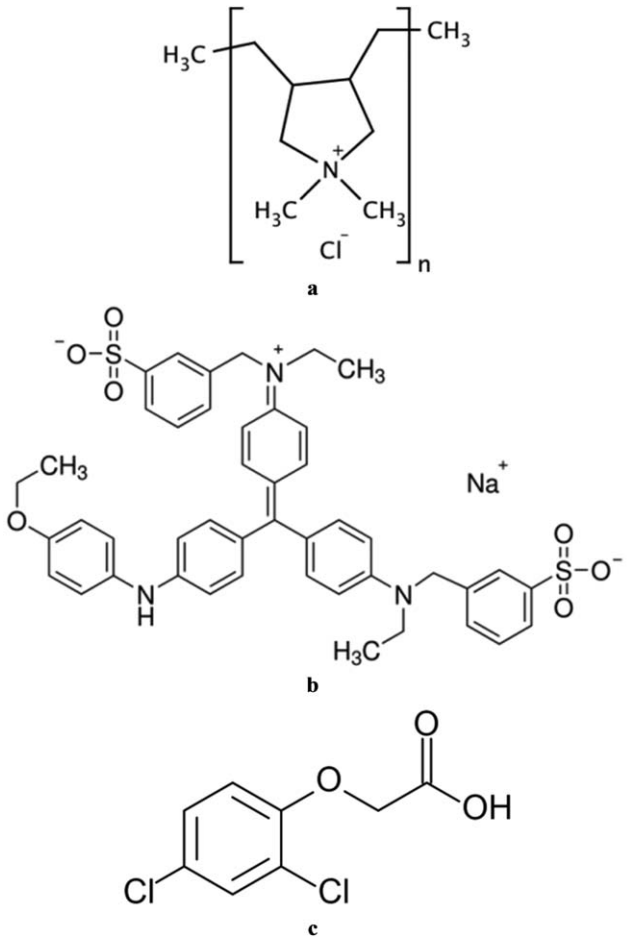

Figure 1. Chemical structures of (a) PDDA, (b) Coomassie brilliant blue dye G-250 and (c) 2,4-D.

spectrophotometically obtained by Datacolor apparatus at standard illuminant D65 and observer 10° combination.

Wettability. The surface hydrophilicity of the modified cotton fibers was evaluated by placing a drop of water. To visualize the absorption of water onto the fiber's surface, a reactive red dye generally used in cotton dying was used. For contact angle measurements, a 10-μL drop of distilled water was deposited on the sample surface with a microsyringe. The measurement was carried out at three different points on each fiber sample.

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis. FT-IR spectra were collected with a Perkin Elmer FT-IR 100 spectrophotometer. Cotton fiber was cut very fine to obtain powder sample and mixed with potassium bromide (KBr) which was used as matrix.

Antimicrobial Test. The antimicrobial activity of chitosan, PDDA, and alginate on cotton fabrics was evaluated as growth inhibitor using a turbidimetric method following AATCC Test Method 100 (quantitative test). The gram-negative bacteria *E. coli* (ATCC 25922) and gram-positive bacteria *S. aureus* (ATCC 25923) were tested. All the inocula were grown overnight and diluted to 1.0×10^8 UFC using 0.5 of McFarland standard solution.

The reduction rate of bacteria was obtained following equation [eq. (2)]:

$$\boldsymbol{R}\ \%=100\ \text{x}\ \frac{[N^\circ]\text{bc}-[N^\circ]\text{mc}}{[N^\circ]\text{bc}} \quad (2)$$

where $[N^\circ]$bc is the number of detected bacteria in bleached white (unmodified) cotton and $[N^\circ]$mc is the number of detected bacteria in modified cotton by chitosan, alginate, and cationic layer deposition.

***In Vitro* Release of 2,4-D from Modified Cotton and Cotton Gauzes.** Release profiles of 2,4-D from modified cotton and cotton gauzes from assembled chitosan/alginate layers were studied in 10 m*M* acetate buffer at pH 5.5 (normal skin pH) at 37°C. A 30 g of treated cotton fibers (5×5 cm^2) and gauze fibers (8×16 cm^2) were immersed in 25 mL acetate buffer and incubated for 10 days. To measure the released 2,4-D, 1 mL of solution was taken at different time intervals and replaced with the same amount of fresh buffer solution. The release rate of 2,4-D was measured using a Perkin Elmer Lambda 2 UV–vis spectrophotometer at 290 nm where 2,4-D has its maximum absorbance. The cumulated amount of released 2,4-D in buffer medium was presented. A calibration curve was obtained with 1, 2, 4, 10, and 20 m*M* of 2,4-D in acetate buffer (pH 5.5). The measurement was performed in once from each sample bath for 10 days.

RESULTS AND DISCUSSION

Cellulose fibers are not charged when dry, in aqueous medium they become slightly negatively charged showing a zeta-potential of −11 mV because of their characteristic carbonyl and hydroxyl groups.[14] Therefore, the attachment of positively charged polymers, such as chitosan, on the surface is very convenient for surface modification of cellulose fibers in terms of polymer coating.[3] The positively charged modified surfaces of fibers offer now a great affinity for polymers with negative charges due to electrostatic interactions, making it feasible to assemble multiple layers on the fibers. Moreover, the deposition of these layers is easy and simple and their thickness can be finely controlled, even at nano-scale.[13,15,16]

Chitosan with a high degree of deacetylation (95%) was attached to cotton fibers in an acidic medium (pH 5). At this pH chitosan becomes a polycation due to protonation of its free amino groups (pK_a 6.5), which favors its dissolution.[17] On the other hand, PDDA is a pH-independent polycation due to its quaternary ammonium groups. In this case, chitosan and PDDA were successfully deposited on cotton fibers to produce a positively charged surface.

On the contrary, alginate is an anionic polyelectrolyte because of the carboxylate groups in each repeat unit, and can interact strongly with both chitosan and PDDA (as shown in Figure 2). This electrostatic attraction favors the deposition of the polyelectrolytes layer by layer.

Deposition of multiple layers of alginate (AL) and chitosan (CS) over PDDA or chitosan covered cotton fibers was accomplished and the layer deposition sequences are shown in Figure 3. First, the polycation (PDDA or CS) was deposited on the

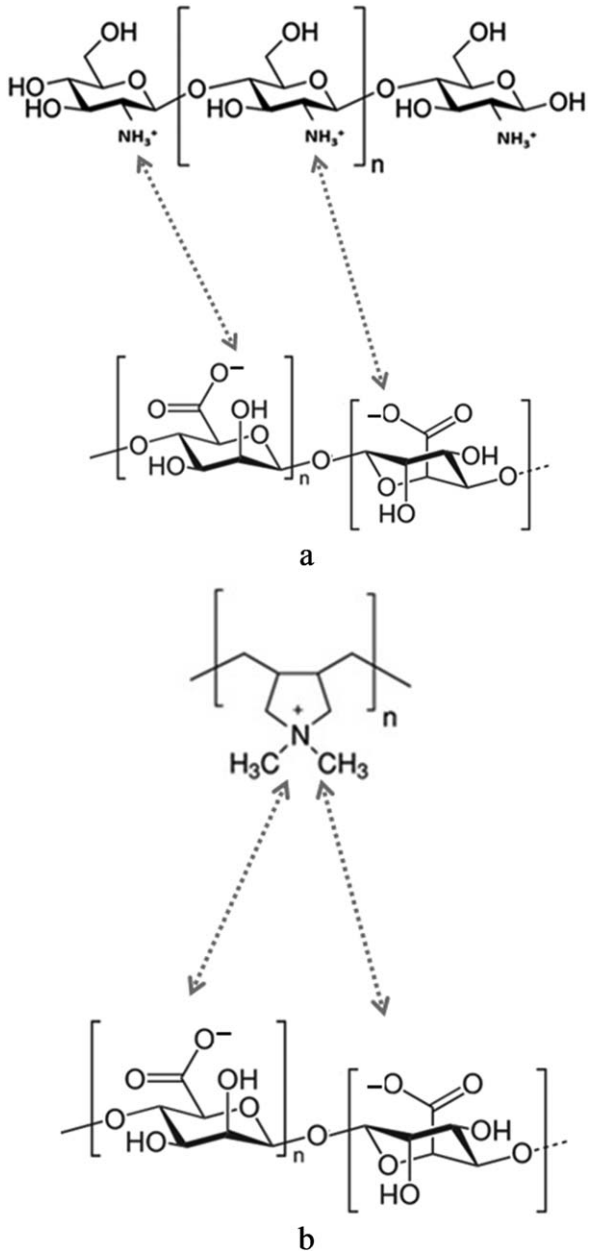

Figure 2. Ionic interactions of (a) chitosan and alginate (CS-AL) and (b) PDDA and alginate (PDDA-AL).

cotton surface. In the next step, the polyanion (AL) attached to the CS or PDDA positively charged surface. This was followed by a new layer of chitosan and then alginate.

The morphology of cotton fibers before and after depositions of different types of polyelectrolytes was analyzed by SEM and the obtained images are presented in Figure 4. No remarkable changes were detected in the sample with layer of CS, the sample with layer of CS-AL, and the sample with layer of PDDA comparing to the control sample (BC). The morphology of cotton fibers became irregular after the third layer deposition, like CS-AL-CS and CS-AL-CS-AL, shown in Figure 4(B,C), respectively. When alginate was deposited after PDDA layer, small particles were detected over the fibers. This can be explained by the aggregation of the two polyelectrolytes that occurred by the strong electronic interaction between PDDA attached onto the fibers and alginate in the solution [Figure 4(D)]. Dissimilarly to sample A (control), samples E and F showed irregularities of the surface of fibers which is generally detected in images of fibers covered with polymers.[5]

The weight gain was measured to verify the anchoring efficiency (AE) of the polyeletrolytes onto the fibers after each deposition, and is presented in Figure 5. The amount of polymer attached onto the fiber's surface was evidenced by an increase of weight. Among the four different compositions of layers deposited onto cotton fibers, chitosan only and PDDA only showed a similar behavior in attaching to bleached white cotton fibers, resulting in the highest amount of polymer attachment onto the fibers (anchoring efficiency of 1.6–2.1%, respectively). This may be due to the high available surface area on the fibers where the polymers can attach. For the second step (alginate deposition), the smallest amount of polymer was attached but the anchoring efficiency of alginate is higher when deposited on PDDA only covered fibers than on chitosan only covered fibers. This could be explained by the higher positive charge density of PDDA considering not only the number of charges per weight but also the degree of deacetylation of chitosan and pK_b of its amino groups.

The rate of weight gain slightly increased in the third layer of CS deposition onto CS-AL and PDDA-AL layers, and finally, the last layer of AL deposition presented a higher rate of weight gain compared to the second and third layers. This can be explained as the enhancement of the ionic interactions between opposite charged polymers.

The validation of polyelectrolyte deposition onto cotton fibers was performed by coloration using coomassie brilliant blue dye. Commassie brilliant blue is broadly used for protein staining and detection due to its anionic character and its affinity with protonated amino groups.[18] Therefore, chitosan and PDDA layers on cotton fibers have a high affinity to this dye. Color strength on the fibers is related to the quantity of cationic polyelectrolyte attached onto fibers, the stronger color the more affinity to the fiber surface. Color appearance of dyed fibers after each layer deposition is shown in Figure 6.

As it was expected, when cationic polymers, PDDA and chitosan, were on the outer layer (fiber surface), a strong blue color adsorption was observed (CS, PDDA, CS-AL-CS and PDDA-AL-CS). On the contrary, a much lighter color (less dye attachment) was observed on fibers with anionic polyelectrolyte (alginate) deposited on the outer layer (CS-AL, PDDA-AL, CS-AL-CS-AL, and PDDA-AL-CS-AL) due to the reduced dye affinity (electrostatic repulsion). These results confirmed the successful layer by layer polymer deposition of the polyelectrolyets onto the cotton fibers.

The whiteness index (WI) of cotton fabrics after anchoring polyelctrolytes was studied and compared with white cotton as control. In the obtained results (Figure 7), most of cotton samples with polymer layers showed the reduction of WI comparing to white bleached cotton (WI = 76) and the reduction rate is

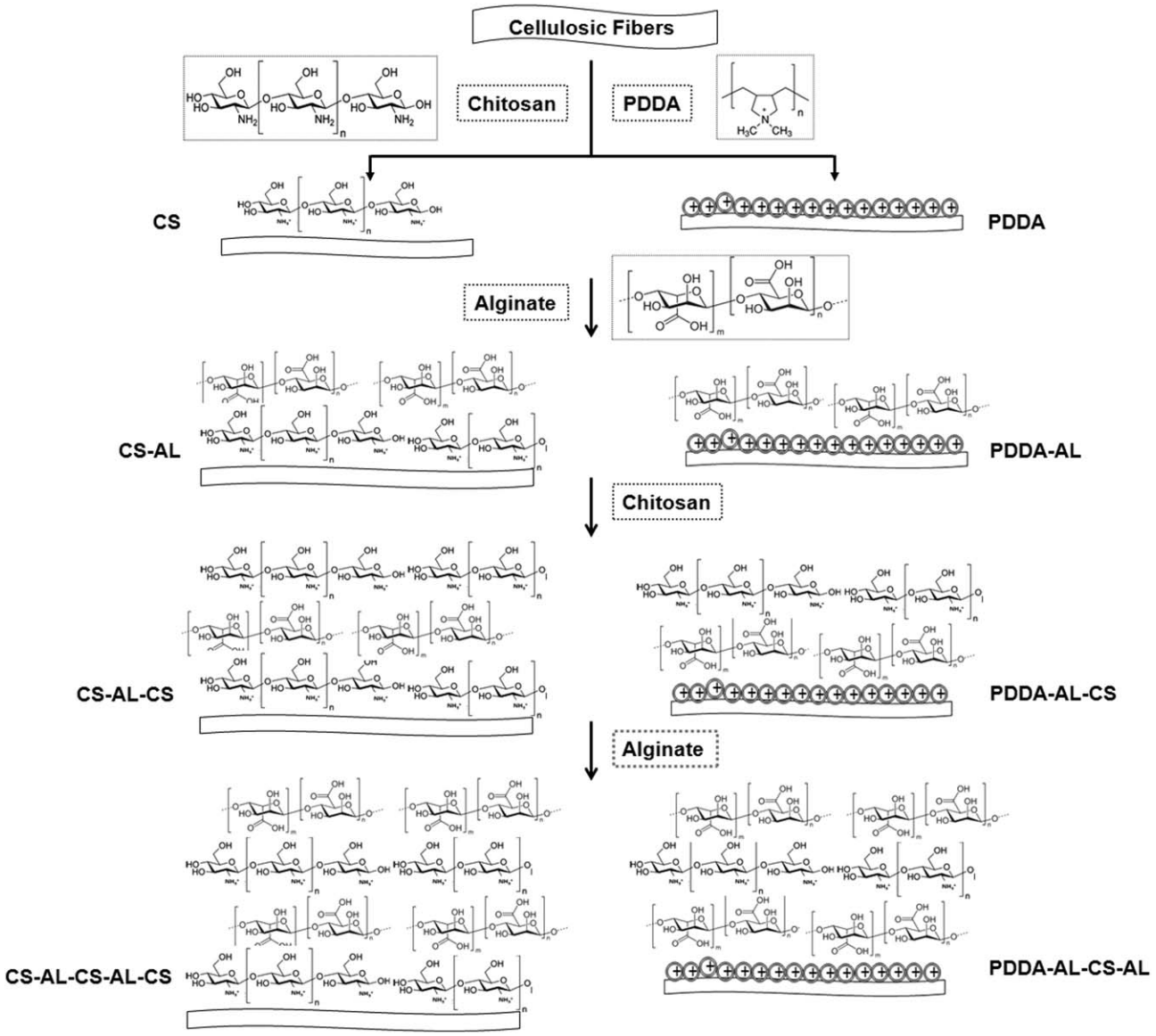

Figure 3. Schematic representation of cellulose fibers with layers of chitosan (CS), alginate (AL) and PDDA.

increased with the numbers of layers except the cases of PDDA-AL and PDDA-AL-CS. The WI values of CS and PDDA single layer were similar with white bleached cotton and the compound affecting the most on the whiteness was alginate layer. As alginate solution has yellowish color, the adsorption of alginate onto fabrics might reduce the whiteness of cotton fabrics. Comparing to CS-AL and CS-AL-CS-AL layers, PDDA-AL and PDDA-AL-CS-AL layers showed higher rate of WI reduction which may be occurred due to higher positive charge density of PDDA as same reason of results presented in Figure 5.

Polymer deposition on fibers can change dramatically its natural properties, for example, water absorption and surface wettability. Bleached white cotton fibers are very hydrophilic materials, which manifests as high water absorption.[4] When the surface is modified with multi layers, the wettability depends mainly on the property of the outermost layer,[19] chitosan covered substrates show an hydrophobic character, while external alginate layers are hydrophilic.[20] This agrees with the literature[21] chitosan films are hydrophobic depending on its degree of deacetylation and crystallinity, and, on the other hand, surfaces of alginate are highly hydrophilic and absorb water readily.[22] However, the conditions during the assembling of the layers can cause different results in the surface wettability.[19,20]

Figure 8 shows colored water absorption on cotton fibers with different layers of polymers. As it is apparent, CS-AL-CS and PDDA-AL-CS surfaces are the most hydrophobic, they show no water absorption and high water contact angles ($121.5° \pm 2.1°$ and $120° \pm 1°$, respectively). However, even though its surface is covered with chitosan, sample CS is hydrophilic. This might be due to the small amount of chitosan deposited onto fibers (as evidenced by its anchoring efficiency of 1.6%, Figure 5), its water permeability or the fiber's surface roughness.

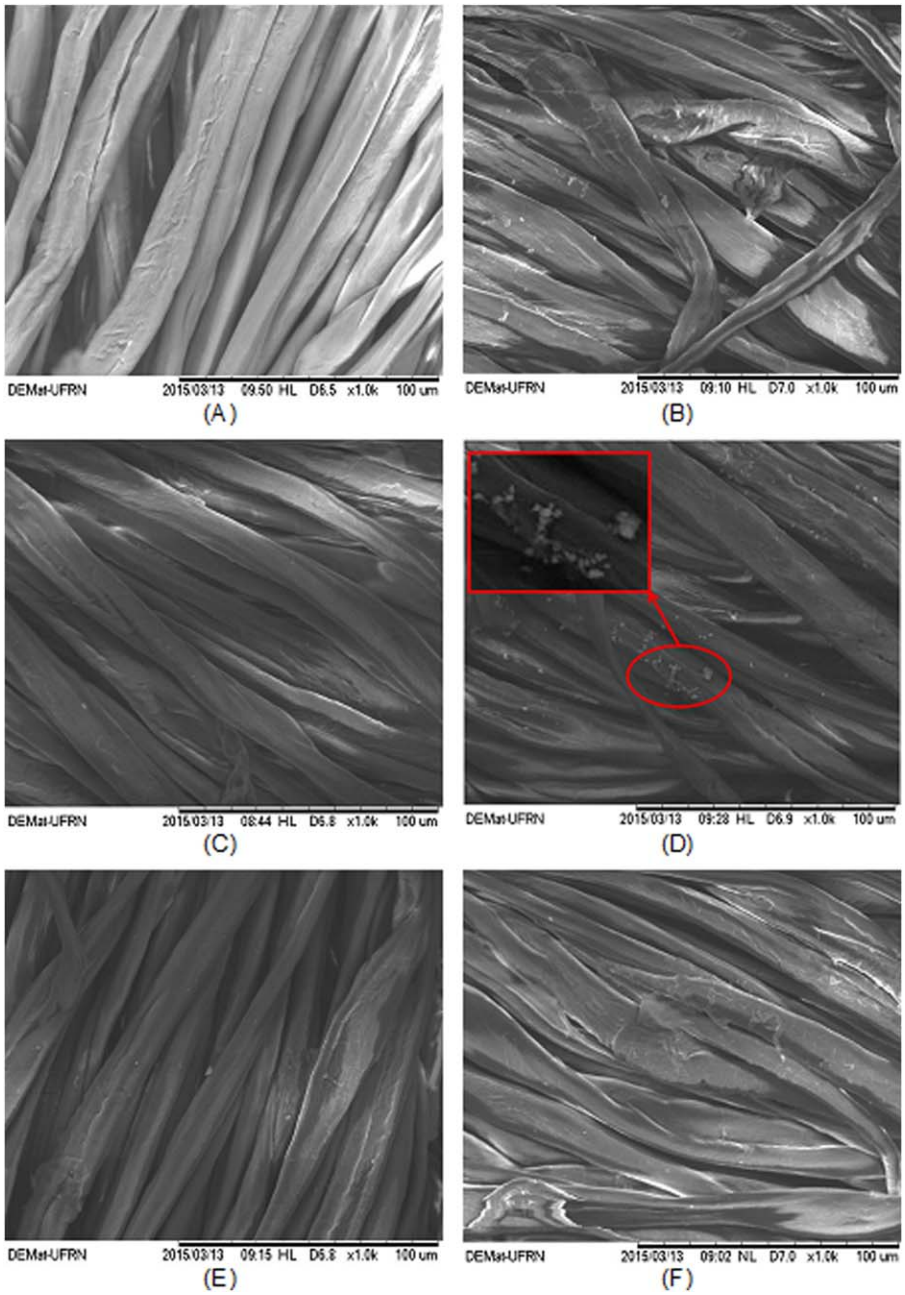

Figure 4. Scanning electron microscopy images of cellulose fibers before and after depositions of polyelectrolytes: (a) BC: bleached white cotton, (b) sample with layers of CS-AL, (c) sample with layers of CS-AL-CS-AL, (d) sample with layers of PDDA-AL, (e) sample with layers of PDDA-AL-CS and (f) sample with layers of PDDA-AL-CS-AL; all the images were amplified ×1000. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

As expected, samples having alginate as the outermost layer showed hydrophilic behavior similar to what has been previously reported[20] however, the measured contact angles varied depending on the number of alginate layers. CS-AL and PDDA-AL behaved similarly, they absorbed water immediately as expected for an alginate covered surface. It is interesting to see that samples CS-AL-CS-AL and PDDA-AL-CS-AL, in spite of having alginate on its surface, became less hydrophilic, water was absorbed slowly and the measured contact angles were $39° \pm 1.4°$ and $45° \pm 2.8°$, respectively. This may be due to the interaction with cationic polymers like chitosan previously deposited onto fibers. As it is mentioned before, the outermost layer is a decisive factor on wettability; however, the interpenetration of layers can cause changes of their natural properties.[19] In this case, a high amount of chitosan was deposited previously onto the substrate and its hydrophobicity can interact with the other layers (as AL) and result in less hydrophilicity than fibers with less layers, such as CS-AL or PDDA-AL layers.

Modification of cotton fibers by layer-by-layer deposition of added polyelectrolytes was also verified by FT-IR spectroscopy and presented in Figure 9. The characteristic absorption bands,

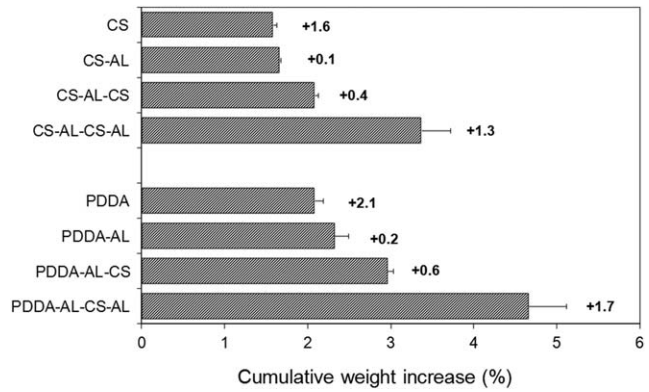

Figure 5. Weight increase rate of cotton fibers by each layer deposition presented. Number values of anchoring efficiency (%) of each layer added in the graph. The data is statistically obtained by mean value of three samples and the error bar represents the value of standard deviation. *CS: sample with layers of CS, CS-AL: sample with layers of CS-AL, CS-AL-CS: sample with layers of CS-AL-CS, CS-AL-CS-AL: sample with layers of CS-AL-CS-AL, PDDA: sample with layers of PDDA, PDDA-AL: sample with layers of PDDA-AL, PDDA-AL-CS: sample with layers of PDDA-AL-CS, PDDA-AL-CS-AL: sample with layers of PDDA-AL-CS-AL.

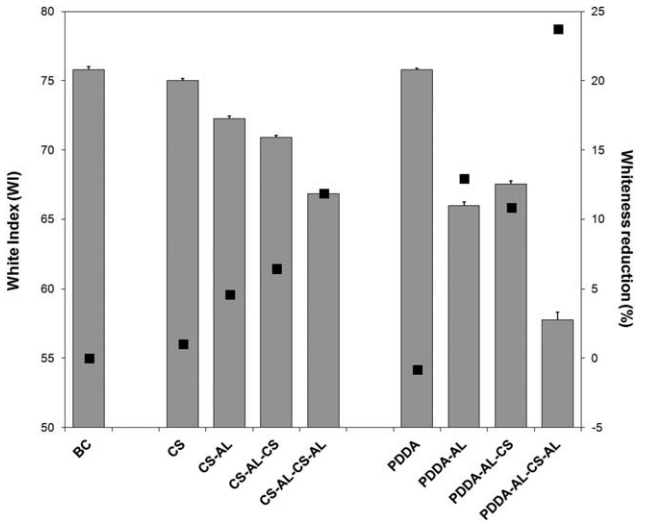

Figure 7. Whiteness Index (WI) data and their reduction rate are presented. The data is statistically obtained by mean value of three different points of each cotton fibers and the error bar represents the value of standard deviation.

corresponding to chitosan, can be seen in the cotton samples treated with both CS and CS-AL such as O—H and N—H stretch bonds at 3350 cm^{-1}, C—H stretch bond at near 2870 cm^{-1}, amide I at 1645 cm^{-1}, and stretching vibrations of aliphatic C—H bonds at 1324 cm^{-1} The absorption bands of saccharide structure of chitosan can be found at 1120 cm^{-1} attributed to antisymmetric stretch C—O—C and C—N stretch and at near 1070 cm^{-1} attributed to skeletal vibration involving the C—O stretching.[23,24] In sample of CS-AL, the peaks corresponding to alginate, as its broad range O—H vibration band at 3000–3500 cm^{-1}, and antisymmetric CO_2^- stretch at near 1600 cm^{-1} and symmetric CO_2^- stretch bond at near 1420 cm^{-1} were also identified.[23]

A general problem in hospitals and healthcare institutions is microbial contamination of surfaces of wound treating materials, including textiles (sheets, gowns, etc.), their contamination can cause infections in open wounds and consequently result in cross-infections.[4,25] Thus, antimicrobial activity in textiles for these applications is strongly required. Figure 10 shows the results as percentage of growth inhibition rate obtained for gram negative bacteria *E. coli* and gram-positive bacteria *S. aureus* cultivated on the surface of bleached white cotton (control) and layer-by-layer modified cotton fibers. Chitosan has been reported as an antimicrobial agent for textile functionalization.[5,7,8,12] It is believed that the interaction of the positively charged chitosan with the negatively charged residues at the cell walls of bacteria further causes extensive cell surface alteration and modifies cell permeability.[5] When a single chitosan layer was deposited on cotton fibers, a similar antimicrobial activity was observed with both, gram negative and gram positive bacteria, showing 58 and 52% inhibition rate, respectively. In the

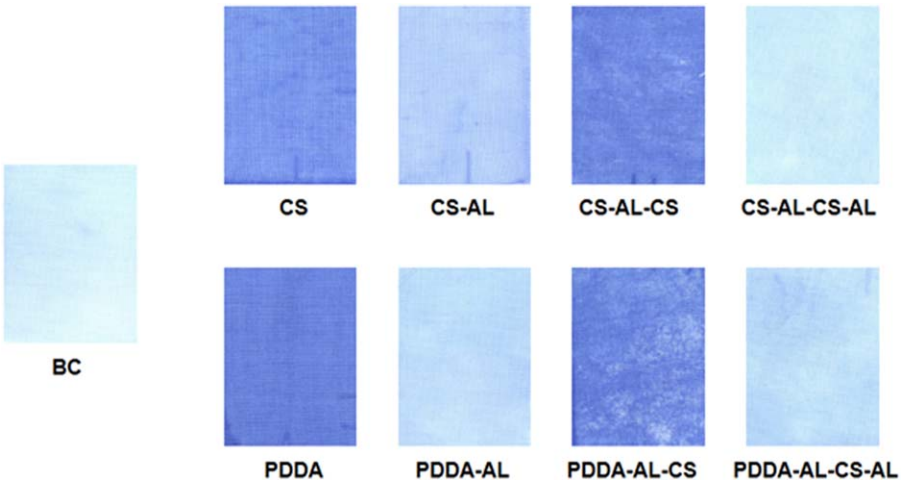

Figure 6. Dye adsorption of deposited layers onto cellulose fibers with coomassie brilliant blue dye. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

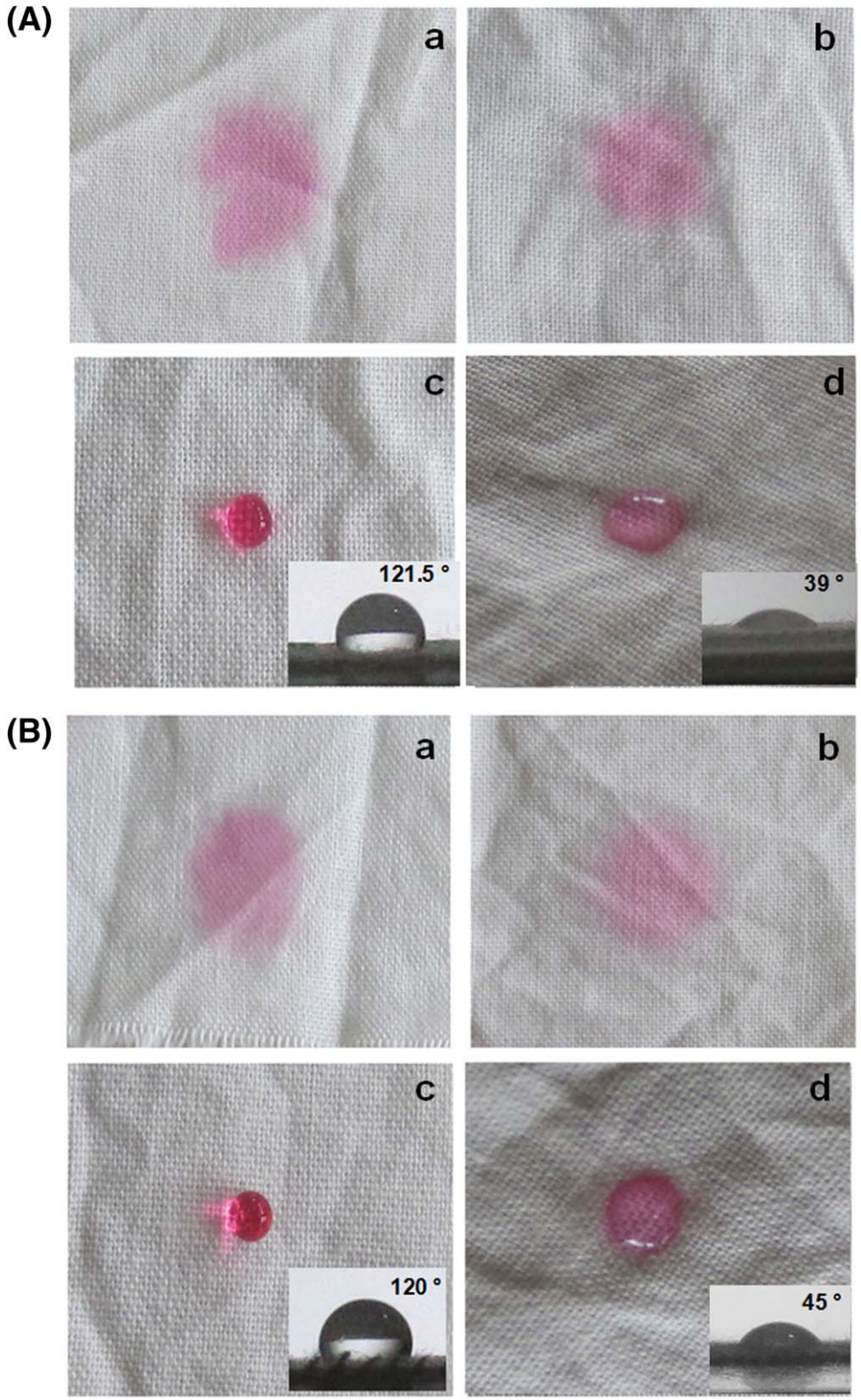

Figure 8. Water absorption and contact angle on cellulose fibers obtained by water drop test: (A)- (a) CS, (b) CS-AL, (c) CS-AL-CS, (d) CS-AL-CS-AL, and (B)- a: PDDA, b: PDDA -AL, c: PDDA -AL-CS, d: PDDA -AL-CS-AL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

case of CS-AL-CS layers, the sample showed a higher inhibition rate of microorganism growth in gram positive bacteria (74% compared to 52%) but a lower inhibition rate for the gram negative bacteria than only one chitosan layer (38% compared to 58%). This might be due to the specific inhibitory behavior of chitosan, as reported by Badawy *et al.*, who reported that the

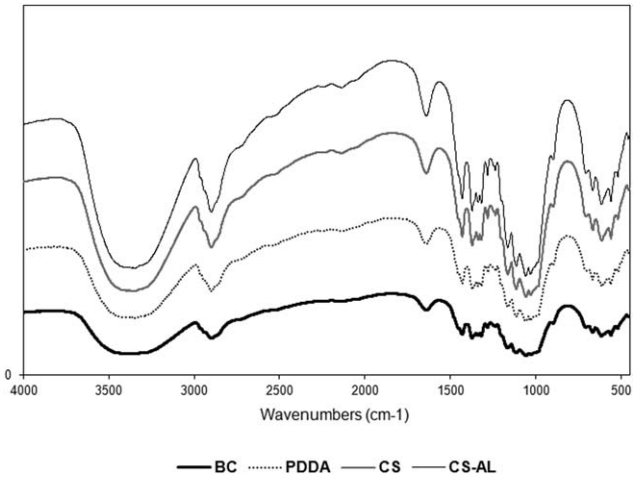

Figure 9. FT-IR spectra of BC: bleached white cotton, PDDA: cotton fabric with PDDA layer, CS: cotton fabric with chitosan layer, and CS-AL: cotton fabric with chitosan and alginate layers.

antimicrobial activity of chitosan is higher against gram-positive compared to gram-negative bacteria.[10] Also Silva *et al.* presented a work of chitosan grafting onto linen fibers showing better microbial inhibition of *S. aureus* than *E. coli*.[5] The decrease of inhibition rate on gram-negative bacteria can be due to the interaction with the alginate layer previously deposited which functioned as a negative factor on microorganism inhibition of chitosan.

PDDA layer on cotton fibers presented an excellent value of inhibition rate of microorganism growing (close to 100%). The quaternary ammonium compounds like PDDA have been broadly studied and evidence a great antibacterial and antimicrobial activity.[26,27] It has been shown that they bind strongly to microbial membrane surfaces through ionic and hydrophobic interactions which cause cell leakage and permanent membrane damage.[28]

It is interesting to observe that samples CS-AL and PDDA-AL showed almost no antimicrobial activity (close to 0%) or even higher bacteria rate growth despite there was chitosan or PDDA underneath the alginate outer layer. Although alginate is used as an effective wound dressing agent and it acts as an antimicrobial substance, its mechanism of action is different. The alginate in the dressing material does not affect the development of bacteria but absorbs the exudates infected with them and, in this way, removes the pathogens and cleans the wound when the material is changed.[11] Therefore, the alginate in the outer layer of treated samples does not inhibit bacteria growth, but acts as an absorbent of bacteria in the growing media.

As results show, chitosan and PDDA are very good at inhibiting bacteria growth and alginate can remove exhudates from wounds, therefore, dressing materials with combinations of these polyelectrolytes on textiles could show enhanced efficiency for open wound treatment.[4,9]

Because of its specific properties like nontoxicity, antimicrobial activity, and biocompatibility, chitosan is one of the most used natural polymers for medical applications, not only for applications such as dressing materials for burns and chronic wounds, but also as a vehicle for drug delivery systems.[17] As a consequence of the results obtained from polymer anchoring efficiency and antimicrobial activity, the treated fibers became an interesting matrix for controlled release of drugs. This added capacity to wound dressing materials will improve their performance and create new potential applications.[29] In this respect, the release of a model molecule, 2,4-D, was studied.

Because of its carboxylic group, 2,4-D becomes negatively charged at pH above 2.6, so it is an interesting model molecule than can interact with the positive charges introduced in the cationized cotton fibers with CS or PDDA. The 2,4-D was incorporated to the fibers together with alginate, since both species are negatively charged and compete to interact electrostatically with chitosan or PDDA on the fiber's surface.

Release of 2,4-D from treated cotton fibers in acetate buffer, at pH 5.5 was determined by UV–vis spectroscopy and is shown in Figure 11. Both cotton woven fibers and cotton gauze fibers presented a similar behavior, the initial release of 2,4-D from fibers is initially high up to a certain period of time, after which the release rate increases gently until the end (240 h), which is a general release behavior of drug loaded fibers.[30,31] The amount of 2,4-D released into the medium is related to the amount of 2,4-D attached onto the fibers. Taking into account that 2,4-D interacts with the fibers during alginate layer deposition (2,4-D is dissolved in alginate solution), it is evident that samples with two alginate layers have a higher amount of 2,4-D and therefore show a higher released quantity of 2,4-D, than samples with only one alginate layer. Results show that the amount of 2,4-D in the matrix and its release can be controlled by forming several layers.[30]

However, the profile of the initial (high release) period and the amount of 2,4-D released from cotton woven fibers was substantially higher (up to seven times) than that of cotton gauze, except for the case of samples with CS-AL layers [Figure 10(a)]. Another difference is that the high release initial period was

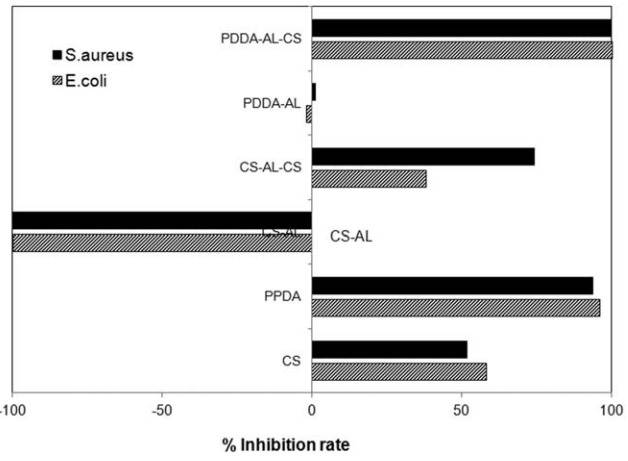

Figure 10. Antimicrobial activities of treated cotton with chitosan, PDDA, and alginates. The number of bacteria growing on white cotton (untreated cotton) was used as control.

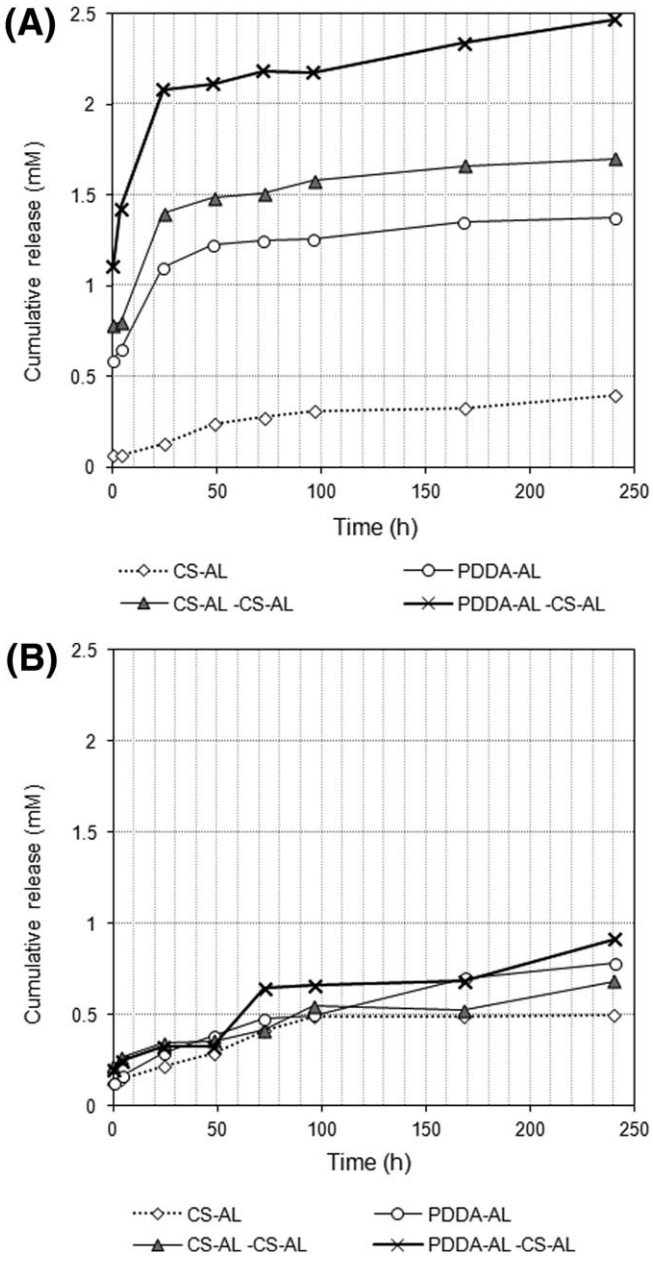

Figure 11. *In vitro* release profile of 2,4-D from a. cotton woven fibers and b. cotton gauze. Incubation was performed at 37°C in acetate buffer pH 5.5.

much shorter for cotton woven (~24 h) than for cotton gauze (~72 h).

For cotton woven fibers [Figure 10(a)], sample PDDA-AL-CS-AL, which contains multiple layers, presented the greatest initial release as well as cumulative release of 2,4-D. Following this, fibers with CS-AL-CS-AL layers showed the next highest amount of 2,4-D release. The main difference between these two samples (PDDA-AL-CS-AL and CS-AL-CS-AL), relies on their first layers, PDDA and chitosan, respectively. This could be explained considering the higher density of positive charges on PDDA than on chitosan, as explained earlier. The higher number of cations allow the fiber to interact with more anions (2,4-D and carboxylate groups from alginate). This is also correlated to the higher anchoring efficiency for alginate on PDDA than on chitosan (as was explained from Figure 5). The same can explain the release behavior of samples CS-AL and PDDA-AL as Figure 10 shows.

Contrary to cotton woven fibers, it was difficult to differentiate the release behavior in cotton gauze samples. Comparing to woven fibers, cotton gauze showed a lower amount of 2,4-D released to the medium, which indicates also less amount of 2,4-D attached onto cotton fibers (note the difference in the *y* xis units).

CONCLUSIONS

Functional polymers like chitosan, alginate, and PDDA were successively attached onto cotton fibers using the layer-by-layer deposition technique and their formation was confirmed by coloration with coomassie brilliant blue dye, weight gain (anchoring efficiency), FT-IR and SEM analysis. Difference in water contact angles and water absorption behavior of the different layers also proved formation of polymer layers. PDDA's greater functionality (cationic density) results in a higher affinity to alginate than chitosan. The number of layers and the type of polymer determine the substrate antimicrobial activity. The combination of PDDA and chitosan layers presented very interesting antimicrobial activity showing a synergistic effect. It has been shown that the layer-by-layer deposition technique could also be useful to insert molecules to be released from the treated cotton woven and cotton gauze fibers. In this case, more 2,4-D (an anionic model molecule) was released from samples with higher number of layers. Therefore, deposition of chitosan and alginate biopolymers on cotton fibers, cannot only change its wettability and antimicrobial activity but also incorporate drugs for medical and health care applications.

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