

Layer-by-Layer Deposition of Antibacterial Polyelectrolytes on Cotton Fibres

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Published online: 3 August 2012
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Abstract The introduction of molecules with biological properties on textile materials is essential for a number of biotechnological applications. With the purpose of testing new processes applied to textiles, in this study, we present the first results on the feasibility of using the Layer-by-Layer (LbL) deposition process in natural fibers such as cotton, with natural polyelectrolytes like chitosan (CH) and alginic acid sodium salt (ALG), the durability of CH/ALG multilayer on cotton were evaluated. The increase of negative charges to the substrate cotton was made with NaBr and TEMPO, to ensure the success of the process of LbL.

Three characterization methods to assess electrostatic LbL deposition were performed: the contact angle between a liquid (water) and the sample surface, in order to characterize the wettability of the samples with the different layers of CH and ALG; dyeing of the CH/ALG assembled cotton fabric with cationic methylene blue that shows regular changes in terms of color depth (K/S value), which indicate that the surface were alternately deposited with CH and ALG layers and, finally, the analysis by infrared spectroscopy using Fourier Transform with Attenuated Total Reflection (ATR-FTIR), to assess the changes in the interaction between CH and ALG deposited on cotton samples.

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Keywords Layer-by-layer · Contact angle ·
ATR-FTIR · Chitosan · Alginate

Introduction

The challenges facing the textile finishing industry have intensified during the last decade. Current awareness of the negative environmental impact of chemical processing of textiles, combined with increased strict legislation on industrial effluents, has led to the search for advanced, non-polluting processes for coating textiles. Coating on textiles is a new way to give functionalities and properties on textile surfaces without compromising on fabric properties and they open a whole new vista of value-addition possibilities in the textile sector. The coating can be used to give wrinkle resistance, improve color or light fastness, flame retardancy, water or oil repellency and antibacterial properties [1]. Newer methods of coating textiles become possible to improve the functionality and durability of the coating to a higher level compared to the conventional

coating techniques. These techniques include immobilization of enzymes, LbL assemblies, nano coatings and use of plasma for deposition of functional molecules. All these techniques are distinct from conventional finishes in that they impart special functionalities to textile surfaces by bringing about modifications at micro or nano level, without affecting the bulk properties. These processes add functionality with minimum effect on the strength, feel, handle or breathability of textiles [2]. Some of these techniques have been tested and validated at lab scale, but most are still in research stages. Most of the conventional coating techniques either affect the fabric flexibility, comfort and permeability or deteriorate the mechanical properties of the treated fabric [3, 4].

Functional coating methods provide a flexible alternative to conventional finishing methods in that they are independent of fabric type, require low quantities of additives and allow combinations of different functionalities in a simple way [2]. Polyelectrolyte multilayer (PEM) coatings have become a new and general way to functionalize surfaces [5]. This technique has been described as being theoretically able to cover many kinds of surfaces when they are charged [6]. The mechanisms allowing for film coating essentially involve electrostatic interactions, but the assembly of such multilayer structures have also been shown on non-ionic or apolar substrates [7]. The film is constructed by the alternate adsorption of oppositely charged polyelectrolytes at the surface of the material, easily obtained when the material is dipped in polyelectrolyte solutions. The driving force for the film construction is related with the excess charge (alternatively positive and negative) which appears after each new polyelectrolyte adsorption [8, 9]. A deposition cycle creates a bilayer, and these cycles can be repeated as often as needed. The number of deposition cycles and the types of polyelectrolytes used in the construction allow for the control of thickness and roughness of the multilayered film [10]. A broad range of applications for these films has been considered, going from drug delivery to specific bio-applications based on surface modifications. For example, the multilayer film technique has been used to create microcapsules, defined as micro and nano-containers for storage, transport, and release of active macromolecules [11]. Martins et al. analyzed the potential and achievements of LbL technique as a promising approach to functionalize biomaterials surface in a controllable and facile manner. They found that the build-up of CH/ALG system presented a linear growth, meaning that no polymer has the ability to diffuse “in” and “out” of the film after each deposition step [9].

A new method for the modification of textile fibers was introduced by Hyde et al. [12], they demonstrated that a polymer thin film could be deposited directly onto cotton

fabrics by following the widely studied LbL deposition method known as PEM. The LbL method involves essentially electrostatic interactions. Using transmission electron microscopy (TEM) they have shown the uniform coating provided by the LbL onto the cotton fabric [12]. It seems possible that by following the same LbL deposition method, a wide range of molecules, nanoparticles, and other functionalized polyelectrolyte could be deposited on textile fabric leading towards the development of new applications for technical textile applications. So the LbL self assembly method may provide new coatings or films that can be constructed by the alternate adsorption of oppositely charged polyelectrolytes at the surface of the material, easily obtained when the material is dipped in polyelectrolyte solutions. LbL is a simple and inexpensive method for preparation of controlled layered structures and it is applicable to a variety of materials. It has the advantages of simplicity, low cost, ability to incorporate different bio molecules and molecular control. LbL film structures are less densely packed and this is advantageous for diffusion through the films [13]. But certain details of the process are still not clearly understood. The LbL deposition process has not been extensively implemented in textile and natural fibers, as they possess unique challenges including the chemical heterogeneity of their surfaces as well as their irregular shapes.

Numerous studies involving different polymer substrates and several synthetic polyelectrolytes have been published. But, there are very few scientific articles concerning the deposition of alternate polyelectrolyte on natural textile supports. During a deep revision, we found few reports concerning the LbL method involving cotton fibres.

Hyde et al. [14], they evaluated three different levels of cotton cationization. Variations in the cationization degree were achieved by manipulating the ratio of 3-chloro-2-hydroxy propyl trimethyl ammonium to NaOH. The deposition of the polyelectrolytes was monitored using XPS and CHNS elemental analysis. The experimental results they obtained, indicated that the deposition process was not significantly influenced by the degree of cotton cationization. The build-up of further polyelectrolyte layers was found to be less sensitive to variations in the cationic character of the substrate once a critical number of alternating layers was deposited [14].

Wang et al. [15], they have utilized two different methods for the characterization for LbL deposition of two polyelectrolytes poly (sodium styrene sulfonate) (PSS) and poly (dimethyldiammonium chloride) (PDDA) on cotton fabrics, a dyeing method and a UV absorption method. Two types of dyes, anionic Direct Red 80 and cationic Methylene Blue, were utilized to dye the self-assembled cotton in order to reveal the change of surface electric property after LbL deposition of polyelectrolytes on cotton.

The UV absorption method could monitor the growth of polyelectrolytes on cotton substrates in terms of UV absorbance at characteristic absorption wavelengths [15].

Wang et al. [16], they have studied a new approach for UV protection of cotton fabrics based on LbL self-assembly. Three fluorescent brightening agents and polycation PDDA were used on cationized cotton fabrics through direct LbL deposition technique. The assembled cotton fabrics could obtain excellent rating of UV protection when the fluorescent brightening agents and PDDA were built up on the cotton substrates. Good durability to washing revealed the stability of multilayers films on the cationized cotton, which is important for actual application of textiles [16].

Ugur et al. [17], they obtained ZnO nanoparticle-based multilayer nanocomposite films were fabricated on cationized woven cotton fabrics via LbL deposition process. In this study they concluded that the process by LbL provides a novel and simple method for nano-ZnO nanocomposite film deposition on cotton fabrics and their application onto cotton fabrics to gain antibacterial and UV protection functions [17].

Ali et al. [3] developed a study to evaluate the effect of different process parameters on the amount of polyelectrolyte adsorbed on a cotton textile substrate via sequential adsorption of negatively charged PSS and positively charged poly allylamine hydrochloride (PAH) using LbL [3].

Recently Ali et al. [18] deposited CH nanocoating onto a cotton textile substrate using a LbL technique. PSS, was used as the anionic polyelectrolyte, and CH was used as the cationic polyelectrolyte. As a result, they obtained a uniform surface deposition of bilayers, as observed by scanning electron microscopy (SEM) and confirmed that during the LbL deposition, the layers did not block the fabric pores (unlike conventional coatings) [18].

As the commonly used polyelectrolytes such as PSS, poly (acrylic acid) (PAA), PDDA, poly(allylamine hydrochloride) (PAH) and polyethyleneimine (PEI) have no special functions transferred to textiles [12, 15, 16, 19–22] non-polyelectrolytes with negative charges such as nanoparticles and dyestuffs were integrated into the LbL self-assembled multilayers together with polyelectrolytes to modify the surface giving antibacterial properties and dyeability, respectively [16]. It should be noted that this new technique is not limited to polyelectrolytes. Some organic molecules with positive or negative charges have also been integrated into multilayers via LbL deposition. This opens the possibility of developing functional textiles. Therefore, here we report the first results regarding the feasibility of LbL deposition of nanolayers of natural bioactive polyelectrolytes, to give antibacterial properties to natural fibers. The purpose of this experimental work is to determine the feasibility of using the LbL deposition

process in natural fibers with natural polyelectrolytes. To impart higher negative charge to the substrate cotton, CH and ALG were successfully layered over cotton fibers using LbL technique.

The introduction of negative charges onto cotton samples is made with sodium bromide, NaBr and 2,2,6,6-tetramethylpiperidiny-1-oxy free radical (TEMPO) [23–26]. The cotton is composed for the most part of cellulose, cellulose is a natural polymer composed of β -D-glucopyranose units that are linked together by (1 \rightarrow 4)- glycosidic bonds [23]. A cellulose molecular chain, depending on the source of the cellulose, consists of 300–15,000 D-glucose units. The unit has three hydroxyl groups on C2, C3 and C6, respectively, and the hydroxyl group of C6 is much more reactive than that of C2 and C3. TEMPO is a stable nitroxide radical, which can catalytically oxidize primary and secondary alcohols under aqueous condition with high selectivity and efficiency. In this study we follow the oxidation method that uses a mixture of sodium hypochlorite, sodium bromide and TEMPO. With such reagents, the oxidation is selective as it oxidizes exclusively the primary hydroxyl groups while leaving untouched the secondary ones [23, 24, 26, 27]. There are several works where TEMPO-mediated oxidation was applied to cellulose fibers under various conditions with good results [23, 27–29].

The number of biofunctional textiles with antibacterial activity has increased considerably over the last few years [30–32]. Application is now extended to biomedical products, which is perhaps the largest application of antibacterial textiles [33, 34]. There is a wide range of methods available to examine the interaction of microorganisms with textiles. In order to evaluate the activity of antibacterial textiles there are several standard methods available. The most common can be divided into two categories: (1) qualitative methods: AATCC 147:1998 and JIS L 1902:2002—Halo method and (2) quantitative methods: AATCC 100:1999 and JIS L 1902:2002—Absorption method. In the qualitative method, textile samples are placed onto agar plates, which have been inoculated with bacteria and are then incubated under moist conditions at 37 °C for 24–48 h. The intention is that intimate contact between the textile, the bacteria, and the growth medium will result in the inhibition of growth either immediately adjacent to the textile or in an area around the textile. A new approach to evaluate the effectiveness of the antibacterial activity of textile fibers as well as bacteria adhesion on textiles by using SEM is described in a previous paper [35]. The SEM analysis revealed great potential on the evaluation and effectiveness of antibacterial activity of textiles. Also, the bacterial adhesion and the morphology of bacteria after exposure to antibacterial agents, was determined using the same.

Experimental

Layer by Layer Coating of Cotton

Cotton fabrics obtained from James H. Heal & Co. Ltd was used as substrate, cotton samples were used with dimensions of 2 cm × 2 cm. TEMPO, NaBr, Sodium Hypochlorite (NaOCl) 5 %, CH (low molecular weight), Acetic Acid (CH₃COOH), ALG, Sodium Chloride (NaCl), Sodium Hydroxide (NaOH) and Hydrochloric acid (HCl) were purchased from Sigma-Aldrich. All chemicals were of analytical grade and used as received.

Antibacterial polyelectrolyte CH (1 mg/mL) and ALG (1 mg/mL) solutions were prepared by dissolving CH and ALG in 0.1 M CH₃COOH and 0.5 M NaCl solutions, respectively. The pH values were adjusted to 5 using 0.1 M HCl and 1 N NaOH solutions. The pH was selected to 5, to be approximately intermediate between the pK_a of CH (6.3) and ALG, pK_a of 3.38 and 3.65 [36]. ALG and CH are two oppositely charged natural polyelectrolyte materials and very sensitive toward changes in external factor such as pH. At pH 5 the carboxylate group of ALG mainly exists in the form of COO⁻ and the amino group of CH mainly exists in the form of NH₃⁺. In this case the presence of both COO⁻ and NH₃⁺ along polymer backbone could enhance the electrostatic interaction of the network structure [37]. This value of pH 5 was used in several works in the process of LbL with CH and ALG [38–43].

To apply LbL technique, two samples of the substrate (cotton) were charged by immersing cotton samples in (TEMPO + NaBr + NaClO 5 %, pH = 10.5) solution under moderate stirring for 30 and 120 min, respectively, followed by a rinse with deionized water, as described elsewhere [26, 28]. Then, CH and ALG polyelectrolytes multilayer films were deposited over cotton by the LbL assembly whereas CH was used as polycation and ALG as polyanion. For each layer deposition, the cotton substrate was immersed into the corresponding solution at room temperature and for 5 min, followed by rinsing with deionized water to remove excessive polyelectrolyte. Since

cotton samples were charged negatively, the CH was deposited as the first layer. Samples were prepared with five layers (CH/ALG/CH/ALG/CH), six layers (CH/ALG/CH/ALG/CH/ALG), nine layers (CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH) and ten layers (CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH/ALG), respectively in control samples functionalized by LbL (designated by B5, B6, B9 and B10), cotton samples treated during 30 min in TEMPO and then functionalized by LbL (designated by CT5, CT6, CT9 and CT10) and finally cotton samples treated during 120 min in TEMPO and then functionalized by LbL (designated by 2CT5, 2CT6, 2CT9 and 2CT10). After the last deposition, the sample was dried in a desiccator at room temperature overnight. Control samples were also prepared using the same method by LbL, without pretreatment with TEMPO.

The functionalized samples were washed and tested the durability of the CH/ALG multilayers. The durability to washing of samples functionalized by LbL was determined following the NP 1710, textiles—wash fastness test (Portuguese Standard). The Fig. 1 show the SEM images of the cotton (control), the CH/ALG/CH/ALG/CH/ALG (six layers) sample and the sample that were washed after LbL deposition. The functionalized samples show a large and heterogeneous deposition of polyelectrolytes, Fig. 1b. In contrast, the washed samples show a less but more uniform polyelectrolytes deposition Fig. 1c.

The results indicate that the cotton assembled with CH/ALG multilayers had good durability to washing. Good durability to washing revealed the stability of multilayer films on the cotton surface, which is important in various applications of textiles, is not important for application in disposable materials.

Control Tests

Contact Angle

The measurement of the contact angle between water and sample surface is one of the easiest ways to characterize the

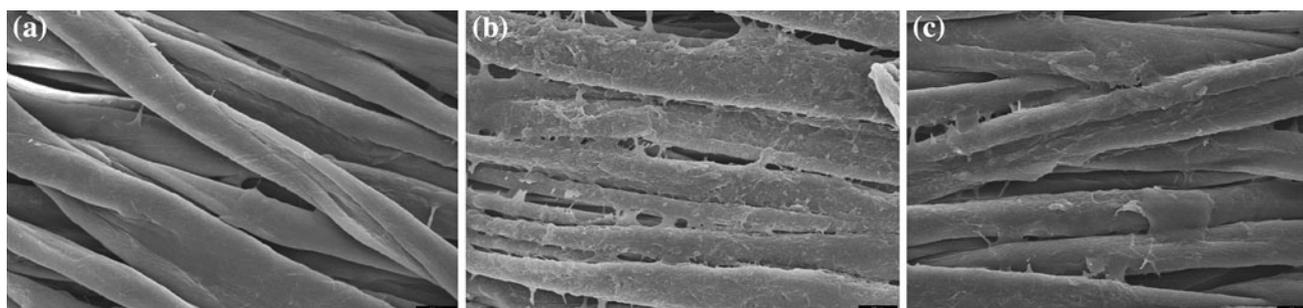


Fig. 1 SEM images. (a) Cotton sample, (b) CT6 and (c) CT6 after washed

wettability of the material [44]. A hydrophilic or hydrophobic surface is defined by these contact angle values. For values higher than 90° the surface is hydrophobic and less than 90° it is hydrophilic [45].

The sessile drop contact angle method [46] was used to measure the contact angles of a water drop on the functionalized cotton samples with a contact angle system Dataphysics model OCAH 200. Contact angles were determined by placing 5 µl drops of deionized water. The tip of the needle was removed, and then an image was recorded. Average values were obtained from multiple contact angle values (at least six) on each sample using an optical system connected to a video display. The measures presented were made at 100, 1,000 and 10,000 ms, according to standard TAPPI T 558 pm-95 “Surface wettability and absorbency of sheeted materials using an automated contact angle tester”.

Control Method for Evaluation of Negatively Charged Layers

The present study attempts to evaluate the effect of different process parameters on the amount of polyelectrolyte adsorbed on a cotton samples via sequential adsorption of positively charged CH and negatively charged ALG using LbL self—assembly process. The amount of polyelectrolyte adsorption on cotton fabric was evaluated by measuring the color value (K/S) of methylene blue absorbed cotton surface [15, 16, 19, 20, 47]. Methylene blue exhibit adsorption is proportional to the amount of anionic groups on fibers [48]. In cotton there is abundance of carbohydrate hydroxyl groups, methods of characterization of these groups are in constant development. Several analytical techniques are being applied and new ones introduced and tested, in this paper we use the adsorption of methylene blue. This dye was extensively studied as an indicator of the amount of anionic groups on fibers [15, 16, 19, 20, 47, 48] A dyeing method in terms of K/S values has been proven a simple and quick means to characterize the change of surface polarities of LbL self—assembled textile substrates based on the attraction or repulsion between the cationic dye and polyelectrolytes. The relative color depth of the dyed fabrics expressed as K/S , was measured by the light reflectance technique using the Kubelka–Munk equation [15].

$$K/S = \frac{(1 - R)^2}{2R}$$

Kubelka–Munk theory describes optical characteristics (e.g. reflectance, transmittance and absorbance) by a variety of light scattering media including paints, textiles and paper [49, 50]. The reflectance (R) of the dyed fabrics was measured at the maximum absorbance wavelength on a

Datacolor Spectraflash SF300. The ratio of the absorption coefficient (K) to the scattering coefficient (S) varies with the total light reflected according to above expression. To determine K , it is necessary to first compute S , which involves measurements either of the transmittance of the thickness, or of the reflectance of the thickness against backgrounds of known reflectance. If it could be assumed that S was reasonably constant under a given set of experimental conditions, then K/S values should be proportional to the amount of dye and could be used in much the same way that absorbance values were used in the analysis of samples by transmitted light, higher dye content leads to an increase in absorbance. Therefore, the K/S values were expected to be proportional to the amount of dye [51] The values obtained of K/S are commonly used to represent the amount of dye fixation or dye content of a given textile fibers [47].

Cotton specimens with different numbers of layers were dyed using 7.5 % owf cationic dye (methylene blue) [16]. The dyeing was performed in petri dishes without stirring at temperature of 40° C for 15 min. After immersion in the dye solution, the samples were soaked in deionized water and air dried.

ATR-FTIR Analysis

The study was made using Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode (ATR-FTIR) with a Vertex 70 spectrophotometer. The transmittance was converted into absorbance for display.

ATR-FTIR reveals information about the molecular structure of chemical compounds and is useful for the characterization of biopolymers. The carbonyl vibrations of a carboxylate and a carboxylic acid group occur at different wave numbers, as does the N–H vibrations of amines and protonated amines [43]. These analyses were made in order to investigate the success of the LbL technique applied to samples of cotton, through the existence or absence of functional groups with specific vibrations.

Results and Discussion

Contact Angle

To identify and distinguish the wettability of each layer in the CH/ALG multilayer film, samples were prepared as described in Table 1. Since the last assembled layer has the most significant effect on the surface property, according to the outermost layer is CH or ALG [52]. The surface wettability is very sensitive to the surface compositions of the outermost layer. The sessile drop contact angle obtained on various surfaces was shown in Table 1. This table presents

Table 1 Contact angle

Sample n°	Description of sample	100 ms	1,000 ms	10,000 ms
1	Cotton	47.52 ± 11.72	–	–
2 (B5)	Control sample—with CH/ALG/CH/ALG/CH	86.13 ± 13.93	55.56 ± 18.33	–
3 (B6)	Control sample—with CH/ALG/CH/ALG/CH/ALG	–	–	–
4 (B9)	Control sample—with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH	42.85 ± 10.14	–	–
5 (B10)	Control sample—with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH/ALG	–	–	–
6 (CT)	Anionic cotton (30 min TEMPO), CT	23.31 ± 4.89	–	–
7 (CT5)	CT—with CH/ALG/CH/ALG/CH	114.6 ± 4.46	106.17 ± 12.93	–
8 (CT6)	CT—with CH/ALG/CH/ALG/CH/ALG	67.67 ± 13.10	–	–
9 (CT9)	CT—with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH	114.63 ± 10.13	101.53 ± 17.27	–
10 (CT10)	CT—with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH/ALG	82.65 ± 12.07	38.6 ± 2.97	–
11 (2CT)	Anionic cotton (120 min TEMPO), 2CT	22.25 ± 14.50	–	–
12 (2CT5)	2CT- with CH/ALG/CH/ALG/CH	122.58 ± 7.76	123.08 ± 3.99	116.95 ± 10.53
13 (2CT6)	2CT- with CH/ALG/CH/ALG/CH/ALG	54.98 ± 6.20	17.35 ± 4.17	–
14 (2CT9)	2CT- with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH	112.57 ± 9.50	115.62 ± 6.97	113.23 ± 7.55
15 (2CT10)	2CT—with CH/ALG/CH/ALG/CH/ALG/CH/ALG/CH/ALG	58.4 ± 8.17	–	–

the contact angle of cotton (sample n° 1), control samples functionalized by LbL (samples n° 2–5, designated by: B5, B6, B9, B10), anionic cotton treated during 30 min in TEMPO (sample n° 6, CT), previous anionic cotton functionalized by LbL (samples n° 7–10, designated by: CT5, CT6, CT9, CT10), anionic cotton treated during 120 min in TEMPO (sample n° 11, 2CT) and previous anionic cotton functionalized by LbL (samples n° 12–15, designated by: 2CT5, 2CT6, 2CT9, 2CT10). Results show that for the control samples (samples B5, B6, B9, B10) untreated with TEMPO, the contact angle values are much lower than those of the other samples. Cotton fibers contain abundant hydroxyl groups, making the fiber surface highly hydrophilic. Although cotton fibers are known to be charged slightly negatively because of the ionization of some hydroxyl groups [53], the TEMPO-oxidation method is selective as it oxidizes exclusively the primary hydroxyl group [24], so the TEMPO-oxidation makes the cotton fibers charge enough for the electrostatic assembly. For cotton, the initial contact angle was around 47.52°, for sample CT and 2CT the contact angle suffered a decrease because of the hydrophilic character of cotton when immersed in TEMPO, due to the formation of a greater number of negative charges on the sample surface. In general, the water contact angle decreases as the extent of surface charges increases and the surface becomes more hydrophilic. For all samples functionalized by LbL, the greatest reduction in contact angle was observed for the samples CT6, CT10, 2CT6 and 2CT10, confirming the presence of permanent negative charges in the polymer chains (ALG) as a generator of a hydrophilic matrix. For the samples CT5, CT9, 2CT5 and 2CT9, where the last layer is CH, higher contact angles were achieved, showing

the hydrophobic character of CH. This is in accordance with previous studies, where the CH layer deposition led to a contact angle increase [52, 54, 55]. The different values of contact angle according to the outermost layer is CH or ALG which are similar to those found in literature, pure CH membranes were more hydrophobic than ALG membranes [54–60].

Figure 2 shows the contact angle (measured at 100 ms) of the multilayer films with layer number, from 0 (cotton sample), 5, 6, 9 and 10, for samples treated 30 min and 120 min in TEMPO. For all the conditions, the contact angle exhibits the zigzag feature with the layer number, indicating the alternate assembly of CH and ALG on the surface. These observations are due to the surface

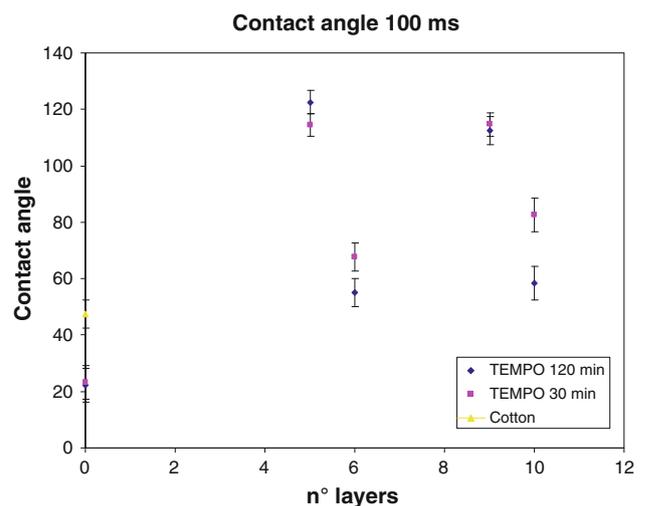


Fig. 2 Contact angle of CH/ALG multilayer films with 5, 6, 9 and 10 numbers of layers. The sample without LbL functionalized is layer zero

composition as a result of the contact angle of CH that is bigger than that of the ALG. Typically, the ALG layer induced an decrease of contact angle whereas CH layer deposition led to a contact angle increase. The wettability of a surface depends on the nature of the outermost layer and not on the initial substrate film [61].

Control Method for Evaluation of Negatively Charged Layers

The electrical properties of the cotton surface would change alternately between positively charged and negatively charged after LbL electrostatic assembly of polyelectrolytes. So, it is valuable to determine the change in surface polarity of assembled cotton to demonstrate the stepwise buildup of CH/ALG multilayers. The cationic methylene blue was used to dye the self-assembled cotton in order to reveal the change of cotton surface. The increase in absorbance at 600 nm of the samples was monitored using a reflectance spectrophotometer. We can use the increase of the K/S value as a characteristic of the LbL deposition of the dye on fibers. The growth of multilayers on the cotton surface based on LbL self-assembly was assessed in terms of the UV change absorbance of assembled substrates.

As shown in Fig. 3 the regular changes in color depth of assembled cotton samples indicate that the surfaces were alternately deposited with every CH and ALG layers. As the outermost layer alternated between CH and ALG, the K/S values present a regular oscillations, revealing that the surface is covered by CH or by ALG which is enough to change the surface polarities. The high K/S values shown in Fig. 3 indicate a high level of adhesion of the ALG on the

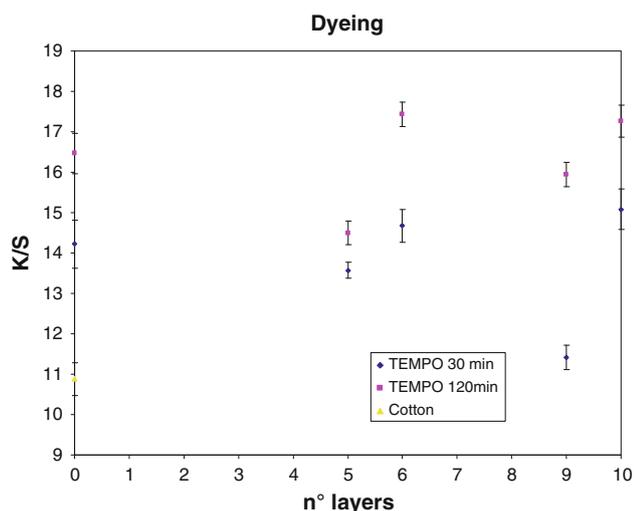


Fig. 3 Tracing of the color depth (K/S) versus the number of monolayers in CH/ALG assembled multilayers on cotton dyed with methylene blue. The first layer is CH and the surface layer alternates between CH and ALG

sample. Moreover, the fact that the data measured at four different locations, including both sides of the fabric, was nearly the same demonstrates that the surface is uniformly coated. When CH first layer is deposited on activated cotton, a part of the NH_3^+ groups in CH were bound with anionic groups of cotton through ionic bonds, the remained would make the cotton surface present net positive charges. The newly formed positively charged surface rejects the absorption of cationic direct dye because of the repulsion between the same charges, resulting in a decrease of color depth. However, when ALG was subsequently assembled on the CH coated cotton surface, the electric properties of cotton surface were reversed. The newly formed cotton surface with net negative charges would attract the cationic dye, causing the increase of K/S values. The composition of the polyelectrolytes deposited on the surface of assembled cotton directly relates to the linear increases in UV absorbance. Therefore, the growth of these multilayers could be recorded by monitoring the UV spectra of assembled cotton specimens. The behavior observed in Fig. 3, where the K/S value exhibits a zigzag feature through even/odd layer numbers, indicating the alternate assembly of CH and ALG on the surface, is consistent with other results found in the literature [15, 16].

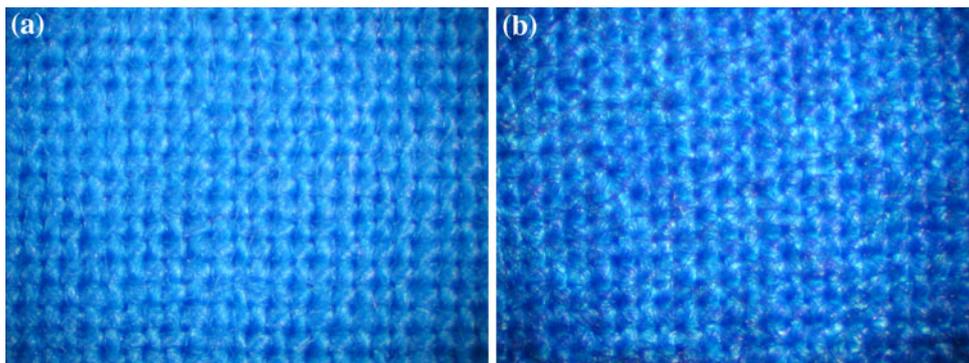
The electrical properties of the cotton surface changed alternately between positively charged and negatively charged after LbL electrostatic assembly of polyelectrolytes. Therefore, the change in surface polarity of assembled cotton demonstrates the stepwise fabrication of CH/ALG multilayers. As the outermost layer alternated between CH and ALG the K/S values presented regular oscillations, revealing that the surface coverage of CH by ALG and vice versa is enough to change the surface polarities.

We initially used the method for evaluation of negatively charged in samples of cotton and cotton pre-treated with TEMPO. In Fig. 4b we found that the sample has a more intense color than in Fig. 4a. This is due to the cotton sample pre-treated with TEMPO get negative charges, so there is a greater absorption to the surface of the cationic dye methylene blue. The complexity of textile surface makes the monitoring of the multilayers become difficult, because the textile substrates in fabric form have a non-planar surface. Considering the change of surface electric property of the cotton specimen during self-assembly procedure, which had been demonstrated by determining the color depth of cotton surface, it can be concluded that CH were produced on the cotton substrate via bonding with oppositely charged ALG.

ATR-FTIR

In the current study, an LbL assembly was produced on a cotton fabric to explore the interaction between CH and

Fig. 4 Images for evaluation of negatively charged layers. **a** Cotton sample with methylene blue; **b** cotton sample pre-treated with TEMPO



ALG. In order to evaluate the ability of the functionalization process with TEMPO to produce a surface capable of supporting polyelectrolyte films via LbL deposition, ATR-FTIR spectrum of the received cotton, Fig. 5a, functionalized cotton samples when immersed 30 min in TEMPO (CT sample), Fig. 5b and functionalized cotton samples when immersed 120 min in TEMPO (2CT sample), Fig. 5c, were assessed. When cotton was immersed into TEMPO, Fig. 5b, c and took new spectrum of the functionalized cotton, the spectrum was almost identical to the original. Figure 5, shows characteristic cellulose peaks around $1,000\text{--}1,200\text{ cm}^{-1}$, which are the main components of cotton [62–64]. As shown in Fig. 5, in the current study, the absorbance intensity of the characteristic peak at around $1,600\text{ cm}^{-1}$ varied. The band is absent in the cotton sample (Fig. 5a), but can be detected in CT and 2CT samples (Fig. 5b, c). A maximum, but not significant value for this band is reached in the spectrum (Fig. 5c) for the 2CT sample, corresponding to the maximum oxidation conditions. Thus, we can conclude that the TEMPO-mediated oxidation conditions were selectively converted to carboxylate ion ionized form (COO^-), imparting a negative surface charge to the cotton, as discussed previously [62]. The TEMPO-mediated oxidation treatment was

proven to be able of modifying a surface of samples cotton to produce a surface capable of supporting multilayer films.

Figure 6 shows the ATR-FTIR spectra of cotton and cotton assembled with CH/ALG multilayers. In this figure six regions can be distinguished: first at $770\text{--}830\text{ cm}^{-1}$, $1,180\text{--}1,300\text{ cm}^{-1}$, $1,330\text{--}1,450\text{ cm}^{-1}$, $1,600\text{--}1,800\text{ cm}^{-1}$, $2,800\text{--}2,980\text{ cm}^{-1}$ and finally at $3,100\text{--}3,550\text{ cm}^{-1}$. Figure 6 showed characteristic cellulose peaks around $1,000\text{--}1,200\text{ cm}^{-1}$ [64]. Other characteristic bands related to the chemical structure of cellulose were hydrogen-bonded OH stretching around $3,100\text{--}3,550\text{ cm}^{-1}$, the C–H stretching around $2,800\text{ cm}^{-1}$ and the asymmetrical COO^- stretching around $1,600\text{ cm}^{-1}$ [53, 62–64]. If the carboxylate existed in ionized form (COO^-), it would show two peaks at $1,600$ and $1,400\text{ cm}^{-1}$ for the asymmetric and the symmetric stretching of COO^- ion, respectively [62]. In carboxylate ion, if it is protonated, it would become COOH in which double bond (C=O) and single bond (C–OH) would exist. The C=O stretching would show at around $1,750\text{ cm}^{-1}$ and C–OH stretching at $1,200\text{ cm}^{-1}$ [62, 64]. This was confirmed by the spectrum of samples shown in Fig. 6. Based on several studies [41–43, 65] the characteristic peaks of CH were detected in a region around $1,700\text{--}1,500\text{ cm}^{-1}$ corresponding to amino group. The

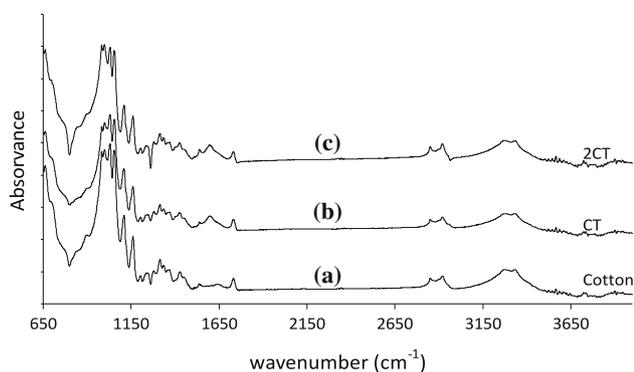


Fig. 5 ATR-FTIR spectra of cotton and of cotton TEMPO- oxidized with different immersion times. (a) Cotton sample, (b) immersed 30 min in TEMPO, (c) immersed 120 min in TEMPO

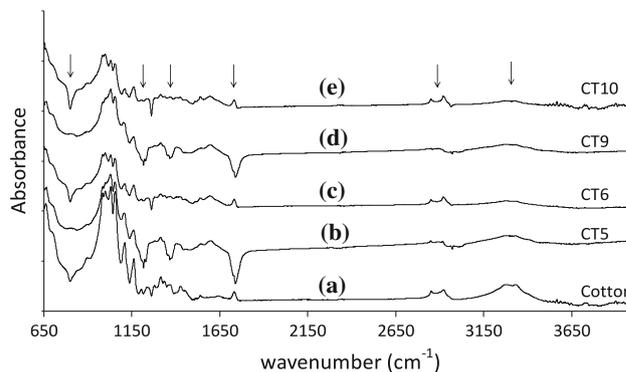


Fig. 6 ATR-FTIR spectra of cotton (a) and samples CT5 (b), CT6 (c), CT9 (d) and CT10 (e). Arrows indicates the six regions can be distinguished

ALG spectrum shows characteristic band of carboxylate (COO^-) band at $1,600$ and $1,400\text{ cm}^{-1}$. The observation of these peaks in Fig. 6, indicated that the degree of ionic interaction between the negatively charged carboxylic ion group of ALG and the positively charged amino group of CH. The study of Alves et al. and Lawrie et al. [41, 43] present values of the characteristic stretching bands in ATR-FTIR spectrum which correspond to CH and ALG related to the chemical structure. When CH is the outermost layer in an LbL assembly (Fig. 6b, d), CT5 and CT9 samples, the amine groups extending into solution during fabrication will become deprotonated upon washing and drying (around $1,400\text{ cm}^{-1}$).

However, when ALG is the outermost layer (Fig. 6c, e), CT6 and CT10 samples, the amine groups of the underlying CH layer will be protonated to a larger degree due to interaction with the deprotonated carboxylate groups of ALG. It was therefore expected that a higher amount of protonated amines will be present when ALG is the outmost layer [41, 43]. This behavior is observed in the peak around $1,400$ and $1,200\text{ cm}^{-1}$ in Fig. 6. It can be seen in Fig. 6 that after the assembly of 5 bilayers, the absorption band around $3,300\text{ cm}^{-1}$, corresponding to the hydroxyl groups of cellulose, became less evident, this result also confirms the presence of CH/ALG multilayers on cotton. The same study was done for samples 2CT5, 2CT6, 2CT9 and 2CT10, and it was found that the analysis of spectra was identical to that described previously. The fourth region in the FTIR spectrum at $1,680\text{--}1,800\text{ cm}^{-1}$, Fig. 7b, d the spectrum for samples B5 and B9 have the same behavior that samples CT5, 2CT5, CT9 and 2CT9, but for samples B6 and B10 the behavior is the inverse of the samples CT6, 2CT6, CT10 and 2CT10. On the other hand, for this last group of samples, on the second region ($1,180\text{--}1,300\text{ cm}^{-1}$) and third region ($1,330\text{--}1,450\text{ cm}^{-1}$) the band disappeared. The behavior of ATR-FTIR spectrum of control samples is exactly the same in the various layers, this does not happen in samples CT5, CT6, CT9 and CT10, comparatively to spectra of Fig. 6. This indicates that the LbL process was

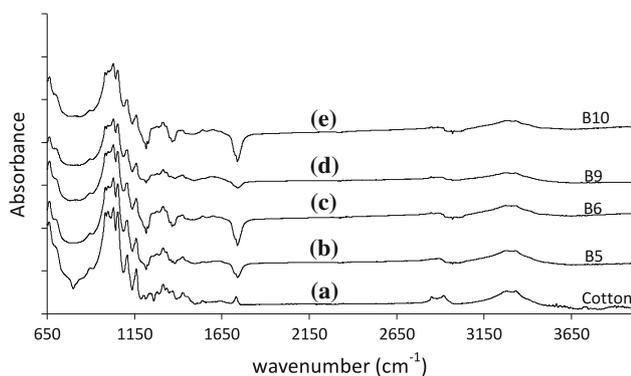


Fig. 7 ATR-FTIR spectra of cotton (a) and control samples (b–e)

not successful in these samples, because these samples were not activated with TEMPO-oxidation. Two different levels of TEMPO-oxidation (30 and 120 min) were used to functionalize cotton samples substrate in order to investigate the role of the supporting surface in the buildup of the multilayer. According to the results, we found that the activation of the substrate with TEMPO-oxidation is necessary to ensure the success of the LbL technique applied to cotton. Concerning the level of oxidation, there are no relevant differences in results for of the immersion times of 30 and 120 min in TEMPO-oxidation. Be noted that some characteristic peaks of absorption could not almost be identified from assembled cotton, even after a (CH/ALG) multilayer was fabricated on cotton. This might be due to the fact that ATR-FTIR is not sensitive enough to the small amount of the deposited materials. In addition, partial overlapping of characteristic absorption peaks between the functionalized cotton and the polyelectrolytes might also result in the above phenomenon [15].

The samples used in this study have been tested on the antibacterial properties on a previous study [35]. Antibacterial activity of functionalized cotton samples was determined in terms of inhibition zone formed around the sample and analyzed by SEM. Control samples showed low antibacterial activity and bacterial growth on the surface of control samples analyzed by SEM indicated the presence of colonies. LbL functionalized cotton sample presented a clear area around it with no bacterial growth (zone of inhibition). Analyzing these samples by SEM, we observed damaged bacteria under and around them.

Conclusions

The LbL deposition process was used to deposit alternate layers of CH and ALG on cotton substrates. Treatment of the cotton samples with TEMPO was proven to be an effective procedure to create a substrate able to support multilayer films. This result was confirmed by various methods of analysis used in this work. The activation of the substrate with TEMPO-oxidation is necessary to ensure the success of the LbL technique applied to cotton.

The surface wettability is very sensitive to the surface compositions of the outermost layer. Using the contact angle method it was found that differently functionalized samples presented different values. Samples where the last layer was CH had a higher value of contact angle compared to samples having ALG on the last layer. This result is consistent with the considerations found in the literature, classifying CH as having hydrophobic character and ALG as having hydrophilic character. This fact demonstrated the formation of alternating layers of CH and ALG, indicating that the process of LbL was successfully applied in cotton samples.

Dyeing of the CH/ALG assembled cotton samples with cationic methylene blue shows regular and observable “odd–even” changes in terms of color depth (K/S value), indicating the variation of surface composition of the cotton substrates due to the alternate deposition of CH and ALG on them.

ATR-FTIR provided direct and indirect evidence of the efficacy of the deposition process. It was possible to follow the formation of the multilayers of CH/ALG on cotton samples, analyzing the chemical changes in each layer. These experimental results validate the feasibility of using the LbL self-assembled deposition of natural polyelectrolytes on cotton substrates as a novel processing method for textile functionalization.

The LbL of CH/ALG on cotton textile has potent antibacterial activity toward both Gram-positive and Gram-negative bacteria. These coating cotton textiles are potentially useful as antibacterial fabrics in a wide variety of biomedical and general use applications.

Overall, the results showed a promising eco-friendly and simple technique to give functionalized textiles with antibacterial properties using natural polyelectrolytes with antibacterial agents. This method can open new avenues towards the development of non-toxic and safe biomedical textiles.

Excellent durability to washing of the CH/ALG multilayers was obtained, which indicates good adhesion between the multilayer coatings and the cotton surfaces.

Acknowledgments The authors wish to acknowledge the Fundação para a Ciência e Tecnologia and Compete for the financial support of the project: PTDC/EBB-BIO/113671/2009 Skin2Tex.

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