THIN FILMS OF PAH/PAA DECORATED WITH SILVER NANOPARTICLES AS A POTENTIAL BACTERICIDAL AGENTS IN THE MICROBIOLOGICAL TREATMENT OF INDUSTRIAL WASTEWATER

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EXTENDED ABSTRACT

The rapid growth in nanotechnology has spurred significant interest in the environmental applications of nanomaterials. In particular, its potential to revolutionize century-old conventional water treatment processes has been enunciated recently. Nanomaterials are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity. More recently, several natural and engineered nanomaterials have been shown to have strong antimicrobial properties, including chitosan, silver nanoparticles (AgNPs), photocatalytic TiO₂, fullerol, aqueous fullerene nanoparticles, and carbon nanotubes. In view of these facts, many studies have evaluated the bactericidal efficiency of AgNPs, since they show high inhibitory activity against a broad range of microorganisms including Gram-positive and Gram-negative bacteria, fungi and viruses. As nanotechnology continues to grow, it is of prime importance understanding the interactions of nanoparticles with bacteria and viruses in order to protect public health and the environment. In this context, this work presents a novel way to introduce AgNPs in a multilayer polymer produced by the layer-by-layer (LbL) assembling technique. Therefore, the goal of this paper was to prepare and characterize thin films obtained from solutions of weak polyelectrolytes (polyallylamine hydrochloride – PAH, and polyacid acrylic – PAA) decorated with AgNPs and crosslinked with glutaraldehyde, aiming to develop a material of long duration that can be used as a bactericidal agent in the microbiological treatment of industrial wastewater. In general, the results showed that the molecular absorption spectrum of AgNPs in the UV-Vis region presented only a single band centered at 393 nm. Moreover, the transmission electron microscopy (TEM) images allowed observing nanoparticles with spherical shape and an average diameter of 50 nm. The microbiological tests carried out on nutrient agar revealed that the thin films exhibit inhibitory activity against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria. Moreover, the microbiological treatment of an industrial wastewater with (PAH₈,₀/PAA₄,₀)₂₀ thin film reduced around 90% of total coliforms present in an effluent sample. Finally, considering the ease of application, low environmental impact, reduced costs and satisfactory inhibitory action, the thin films show great potential to be used in the microbiological treatment of industrial wastewater.

Keywords: thin films, silver nanoparticles (AgNPs), antibacterial activity, treatment, industrial wastewater, reuse.

1. INTRODUCTION

Nanotechnology, science and technology that focuses on the special properties of materials at the nanoscale, is becoming one of the most promising fields of research today. By virtue of their size, nanomaterials have been shown to possess distinctive chemical, catalytic, electronic, magnetic, mechanical and optical properties (Jortner and
Rao, 2002). In a little over 15 years, the multidisciplinary nano “boom” has led to the development of a wide array of novel technologies for both domestic and industrial applications, ranging from enhanced drug delivery to new methods for the treatment of contaminated water (Crane and Scott, 2012). In this context, self-assembled thin films with polyelectrolytes of opposite charges have gained a considerable scientific interest due to their numerous applications (Cheng et al., 2005). One of the methods used for the manufacture of these materials is based on the self-assembly of multilayer polymeric differentiated by layer-by-layer (LbL) technique.

The LbL is a versatile technique for deposition of thin and ultrathin films and has attracted considerable attention due to their simplicity of preparation and cost-effectiveness. It consists basically of submerging a suitable substrate (a glass coverslip, for example) into a vessel containing the solution of the polycation or polyanion for a certain period of time. Once in contact with the solution of deposit, the charged molecules of poly-ions adsorb on the substrate surface by electrostatic interaction (Jesus, 2009). The substrate is then washed to remove the molecules that were not satisfactorily adsorbed. The system substrate/film is now charged electrostatically which permits the adsorption of a new layer with opposite charge loaded by immersion in another solution polymer. After adsorption of the second layer, the substrate is rinsed again and, at the end of the deposition of the layers, it is air dried.

These self-assembled systems are considered a good medium for nanoparticles. The incorporation of these species that have high surface area can significantly affect the properties of thin films (Dubas et al., 2006). The obtained materials may exhibit improvements in optical, thermal, mechanical, electrical, magnetic properties, among others (Qin et al., 2005). Furthermore, the properties of these materials depend on the way the incorporation of nanoparticles is performed, its size and shape, concentration and type of interaction with the polymer matrix (Sówka et al., 2006).

A class of great interest for the manufacture of nanocomposites of polymeric matrices is the noble metal nanoparticles, synthesized via a simple aqueous solution (Crespilho et al., 2006). Most of the studies relates to silver nanoparticles (AgNPs) since they present activity against a wide range of micro-organisms such as Gram-positive and Gram-negative bacteria, fungi and viruses. Its bactericidal effect was quantified for the first time by Von Naegelis against algae in the form of silver ions. The use of AgNPs (Key and Maass, 2001), however, has been reported in the documents of the late eighteenth century, being its use intensified between 1910 and 1920 (Gibbs, 1999).

In view of these facts, the goal of this work was to prepare and characterize thin films obtained from solutions of weak polyelectrolytes (PAH and PAA) decorated with AgNPs and crosslinked with glutaraldehyde, aiming to develop a material of long duration that can be used as a bactericidal agent in the microbiological treatment of industrial wastewater.

2. MATERIALS AND METHODS

2.1. Preparation of thin films

The self-assembled thin films were prepared with NanoStracto Sequence equipment, according to the flow diagram shown in Figure 1.
Figure 1. Schematic flow diagram of time and polyelectrolytes used in the preparation of thin films

The glass slides were first immersed in a 0.01 mol/L solution of PAH (M₆ = 70,000 g mol⁻¹, Aldrich-Sigma) for 15 min and then removed from the solution and rinsed by immersion in deionized water for 2, 1, and 1 min consecutively. Further, the substrates were immersed in another aqueous solution of the same concentration of PAA (M₆ = 90,000 g mol⁻¹, Polysciences) for 15 min, being rinsed in deionized water in a similar manner to that described previously. The multilayers were obtained with aqueous solutions composed of (PAHₓ/PAAᵧ)ᵢ, where x and y are the pH values of the solutions of polycation and polyanion, respectively; and i is the number of layers. The choice of pH of solutions and the number of layers was carried out based in previous studies (Machado et al., 2011).

2.2. Synthesis of AgNPs

Silver nanoparticles in aqueous medium were prepared by chemical reduction of a silver nitrate solution by sodium borohydride in the presence of sodium citrate (stabilizing agent), at 23 °C an under stirring, and away from light. This procedure was performed based on the method proposed by Jana et al. (2001).

In the preparation, 250 mL of a 0.25 mmol L⁻¹ solution of AgNO₃ were added to 250 mL of 0.25 mmol L⁻¹ solution of sodium citrate for 30 s, under vigorous magnetic stirring. Immediately after mixing these two solutions, an aliquot of 10 mL of 1.00 mmol L⁻¹ solution of NaBH₄ was added to the system (Pinto et al., 2010). After formation of the AgNPs (golden yellow solution), the system was kept under stirring for 1 min and the resulting solution was used immediately in the diffusing of the thin films.

2.3. Diffusion of AgNPs in thin films and crosslinking with glutaraldehyde

After preparation of thin films, they were immersed in a solution of AgNPs for a period of 1, 2 and 4 h, and then rinsed in deionized water for 30 s. The diffusion of AgNPs was performed at room temperature and away from light as well as the drying for a period of 24 h.

To minimize the solubility of thin films in aqueous media, they were crosslinked with the aid of a 2.5% (v/v) aqueous solution of glutaraldehyde for 30 min at room temperature and away from light. Then, the films were rinsed in deionized water for 30 s and dried for 24 h.
2.4. UV-Vis spectroscopy

The AgNPs solution was analyzed in a Thermo Scientific Evolution 60 spectrophotometer in the range of wavelength between 200 and 800 nm using a quartz cuvette of 10 mm path length.

2.5. Transmission electron microscopy (TEM)

The AgNPs have been previously dispersed in deionized water (one drop of colloidal silver solution in 20 mL of this solvent) using ultrasound (Unique USC 1400) for 30 min. Then, one drop of this new solution was deposited on a copper grid with a Formvar film (300 mesh) and allowed to dry for 24 h. Finally, TEM analysis was performed on a Jeol microscope JEM-1200 Ex II, operating at a voltage of 80 kV.

2.6. Microbiological tests

The microbiological tests were performed in Petri dishes with agar nutrient (HIMEDIA). The antimicrobial efficacy was tested on the growth inhibition of *Escherichia coli* (ATCC - 25922) and *Staphylococcus aureus* (ATCC - 25923). Samples of these two bacterial cultures were first diluted in a 0.1 wt% peptonated saline solution. Then, each microorganism was added to a tube containing saline until obtaining a compatible turbidity to 0.5 McFarland scale of bacterial cell density of about $1.5 \times 10^8$ colony forming units (CFU) per milliliter. The suspensions of both bacteria were then dispersed homogeneously in the culture medium of each Petri dish where the glass substrates containing the thin films were also deposited, and subsequently incubated at a temperature of ~ 37 °C for 48 h in a bacteriological incubator (Quimis Q-317B). After the incubation period, the plates were analyzed in order to assess the inhibitory effect of bacterial growth in the samples through formation of an inhibition halo around, under and over the thin films. Preliminary microbiological tests were carried out in stock agar with thin films of 20 layers using only the *E. coli* culture and a time of immersion in silver colloidal solution of 1 h (Table 1). Regarding the discussion of these results, only the PAH$_{8.0}$/PAA$_{4.0}$ film (marked in “green” in Table 1) was discussed by presenting the best result in terms of inhibition.

<table>
<thead>
<tr>
<th>Thin films</th>
<th>AgNPs</th>
<th>pH of AgNPs solution</th>
<th>Crosslinking with glutaraldehyde</th>
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<tr>
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<td>–</td>
<td>No</td>
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<tr>
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<td>No</td>
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<tr>
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<td>3.0</td>
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<td>6.0</td>
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<td>6.0</td>
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2.7. Microbiological treatment of industrial wastewater

The glass slides containing the (PAH$_{8.0}$/PAA$_{4.0}$)$_{20}$ thin films, after immersion in the silver colloidal solution for 4 h and crosslinking with glutaraldehyde, were placed into...
Erlenmeyer flasks containing 250 mL of an industrial wastewater for reuse with a total coliform count of 900 UFC/mL. The substrates were then stirred in an orbital-shaking system at 180 rpm for 5, 10, 15, 30, 45, 60, 120, 240, and 360 min. At the end of this procedure, the total coliform count was performed once again in all flasks.

3. RESULTS

3.1. UV-Vis spectroscopy

The spectrum of the AgNPs colloidal solution (Figure 2) revealed the presence of a single absorption band centered at 393 nm.

![UV-Vis spectrum of AgNPs.](image)

According to the literature, molecular absorption spectra in the UV-Vis region can provide important information concerning the size and geometry of the nanoparticles studied (Pal et al., 2007). Typically, a band around 400 nm indicates the presence of AgNPs of spherical shape (Noguez, 2007). This finding was corroborated by TEM analysis, where it was possible to observe nanoparticles with this format.

3.2. Transmission electron microscopy (TEM)

Figure 3 shows the TEM image of AgNPs. As can be seen, the nanoparticles are dispersed in the solution. Moreover, the nanoparticles showed spherical shape and an average diameter of ~ 50 nm.

![TEM image of synthesized AgNPs.](image)
3.3. Microbiological tests

The microbiological tests showed that the thin films decorated with AgNPs exhibited inhibitory activity against *S. Aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria). In general, the highest activity inhibition was observed in the films tested against the bacteria *E. coli*, since Gram-negative micro-organisms have a peptidoglycan layer much thinner than Gram-positive micro-organisms which facilitate the entry and consequently the diffusion of the AgNPs, increasing the efficiency of inhibition (Durán et al., 2010).

Although the halos in the Petri dishes are small, since the contact area of the glass slides with the agar is reduced due to the thinness of them, it was observed that bacterial growth in and on the slides was very small or not occurred.

Finally, microbiological tests also showed that the diffusion time of AgNPs directly interferes in the bactericidal activity. A longer period of immersion in colloidal silver enhances the bactericidal action due to greater incorporation of nanoparticles in thin films. The test which was performed after the diffusion of AgNPs for 4 h showed a greater transparency in relation to those of 1 and 2 h for both *E. coli* (*Figures 4a, 4b and 4c*) and *S. aureus* (*Figures 4d, 4e and 4f*), showing almost no growth of any bacterial strain under and over the slide.

![Figure 4](image)

**Figure 4.** Microbiological test result for the (PAH$_{8.0}$/PAA$_{4.0}$)$_{20}$ thin film decorated with AgNPs at pH = 6.0 and crosslinked with glutaraldehyde: (a) immersion for 1 h in a solution of AgNPs against *E. coli*; (b) immersion for 2 h in a solution of AgNPs against *E. coli*; (c) immersion for 4 h in a solution of AgNPs against *E. coli*; (d) immersion for 1 h in a solution of AgNPs against *S. aureus*; (e) immersion for 2 h in a solution of AgNPs against *S. aureus*; (f) immersion for 4 h in a solution of AgNPs against *S. aureus*. 
3.4. Microbiological treatment of industrial wastewater

Figure 5 shows the percentage removal of total coliforms as a function of contact time of (PAH<sub>8.0</sub>/PAA<sub>4.0</sub>)<sub>20</sub> thin films with the industrial wastewater sample. As can be observed, in the first 5 min there was a reduction of approximately 27% of total coliforms present in the effluent. After 15 min, over half of the microorganisms have been eliminated, and after 360 min, around 90% of coliforms were removed.

![Figure 5. Influence of contact time in the counting of total coliforms in the effluent sample.](image)

4. CONCLUSIONS

The silver nanoparticles synthesized in this work showed spherical shape and an average diameter of ~ 50 nm. The (PAH<sub>8.0</sub>/PAA<sub>4.0</sub>)<sub>20</sub> thin films, decorated with AgNPs and crosslinked with glutaraldehyde, showed inhibitory activity against Gram-positive (S. aureus) and Gram-negative (E. coli) micro-organisms with higher inhibition activity against the bacteria E. coli. This is due to the fact that Gram-negative have the peptidoglycan layer thinner than the Gram-positive micro-organisms, thus facilitating the entry and diffusion of AgNPs in cells, and consequently increasing the efficiency of inhibition. Moreover, the diffusion time of AgNPs in thin films directly interfered in the bactericidal activity. A longer period of immersion in colloidal silver enhances the bactericidal action due to greater incorporation of nanoparticles in thin films. Concerning the microbiological treatment of the industrial wastewater, there was a reduction of around 90% in the total coliform count. Finally, considering the ease of application, low environmental impact, reduced costs and satisfactory inhibitory action, the thin films presented in this work show great potential to be used in the microbiological treatment of industrial wastewater.
5. ACKNOWLEDGEMENTS

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