Evaluation of the Antimicrobial Activity of Chitosan Biofilms Modified with Silver Nanoparticles

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Abstract: Antibiotic resistance is recognized as a severe global health issue, with increasing morbidity and mortality rates each year. Consequently, innovative biomaterials have been developed over the years to combat bacterial proliferation, particularly focusing on biofilms modified with metallic nanoparticles. For this work in the initial phase, silver nanoparticles (AgNPs) were synthesized at pH levels of 10.9, 11.5, and 12 using the coprecipitation method. Subsequently, chitosan biofilms were chemically synthesized via the sol-gel method, which allowed for the modification of these biofilms through the incorporation of AgNPs at concentrations of 0, 0.25, 0.5, and 0.75 %. Antimicrobial evaluation was performed against *E. coli*, revealing that the addition of AgNPs to chitosan biofilms enhanced antimicrobial activity, with the greatest inhibition diameters observed in biofilms containing 0.75 % AgNPs at pH 10.9, achieving a maximum inhibition halo of 11.93 mm. The synthesized materials were characterized using FTIR, XRD, and SEM.

Keywords: Chitosan, Silver Nanoparticles, Biofilms, Antimicrobial Activity

Evaluación de la Actividad Antimicrobiana de Biopelículas de Quitosano Modificadas con Nanopartículas de Plata

Resumen: La resistencia a los antibióticos es reconocida como un grave problema de salud global, con tasas crecientes de morbilidad y mortalidad cada año. Como consecuencia, a lo largo de los años se han desarrollado biomateriales innovadores para combatir la proliferación bacteriana, centrándose especialmente en biopelículas modificadas con nanopartículas metálicas. Para este trabajo, en la fase inicial, se sintetizaron nanopartículas de plata (AgNPs) a niveles de pH de 10.9, 11.5 y 12 mediante el método de coprecipitación. Posteriormente, las biopelículas de quitosano fueron sintetizadas químicamente mediante el método sol-gel, lo que permitió su modificación mediante la incorporación de AgNPs en concentraciones de 0, 0.25, 0.5 y 0.75 %. La evaluación antimicrobiana se realizó contra *E. coli*, revelando que la adición de AgNPs a las biopelículas de quitosano mejoró la actividad antimicrobiana. Se observaron los mayores diámetros de inhibición en las biopelículas que contenían 0.75 % de AgNPs a pH 10.9, alcanzando un halo máximo de inhibición de 11.93 mm. Los materiales sintetizados fueron caracterizados mediante FTIR, XRD y SEM.

Palabras clave: Quitosano, Nanopartículas de Plata, Biopelículas, Actividad Antimicrobiana

1. INTRODUCTION

In a global context where the constant evolution of various species is an indisputable fact, innovation and the search for viable solutions to combat the spread of pathogens have become crucial, driving the development of new antimicrobial materials. Currently, one of the greatest challenges of modern medicine is the prevention and/or treatment of bacterial infections, especially those caused by pathogens that have developed resistance. This resistance reduces the chances of achieving complete healing and the total elimination of bacteria, leading to increased treatment costs as well as morbidity and mortality rates (Hughes, 2011, cited in Noriega et al., 2014). Every year, more than 700,000 deaths worldwide are recorded due to infections caused by bacteria resistant to antimicrobials, which constitutes a serious global public health problem (OPS, 2021).

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In response, the creation of new materials with antimicrobial properties has captured the attention of the scientific community, with chitosan biofilms standing out, mainly used in the food industry and biomedicine (Khubiev et al., 2023; Alvarado, 2023). These chitosan biofilms have been modified with other materials, such as essential oils, plasticizers, and metal nanoparticles. In particular, silver nanoparticles have shown great effectiveness against various microorganisms, suggesting that their integration into chitosan biofilms could enhance the antimicrobial properties of this material.

Among these modifications, the incorporation of silver nanoparticles (AgNPs) has demonstrated remarkable antimicrobial activity, which has been extensively demonstrated in various studies. Badawy et al. (2019) evaluated the antibacterial activity of chitosan-AgNPs films against *E. coli* and *S. typhimurium*, reporting inhibition zones of up to 25 mm for AgNPs concentrations of 150 and 200 mg/mL, respectively. Ribeiro et al. (2009) developed chitosan-AgNPs hydrogels that exhibited 99.9% growth inhibition of *P. aeruginosa* and *S. epidermidis* within 24 hours. Zhang et al. (2020) investigated chitosan-AgNPs coatings on urinary catheters, observing a 95% reduction in *E. coli* biofilm formation. Furthermore, Trotta et al. (2021) applied these nanocomposites in food packaging, achieving a 7-day extension of chicken fillet shelf life by reducing microbial load by 90%. These results highlight the efficacy of chitosan-AgNPs nanocomposites in diverse antimicrobial applications, with significant inhibition outcomes against a broad spectrum of pathogens. Therefore, the integration of silver nanoparticles into chitosan biofilms could enhance the antimicrobial properties of this material.

In this regard, the present study arises with the objective of evaluating the antimicrobial activity of chitosan biofilms modified with silver nanoparticles, synthesized using the sol-gel method. The study aims to provide valuable information that contributes to the development of new applications of these modified biofilms in sectors such as medicine, dentistry, the food industry, agriculture, and the manufacturing of antimicrobial materials, among others. Additionally, the use of this compound will be promoted, encouraging more sustainable and environmentally friendly practices, as chitosan is a biodegradable polymer derived from natural sources. Therefore, the objective of this work is to synthesize chitosan biofilms modified with silver nanoparticles using the sol-gel technique to determine their antimicrobial activity.

2. METHODOLOGY

2.1 Reagents

Chitosan medium molecular weight (M_w = 300 kDa and 82% of degree of deacetylation) was purchased from Sigma–Aldrich (St. Louis, MO). Silver nitrate (≥ 99%: Merck) acetic acid (glacial, 99–100%: Merck), sodium citrate (≥ 99%: Merck), sodium hydroxide (≥ 99%: Merck), sodium citrate (≥ 99%) were also purchased from Sigma Aldrich.

2.2 Synthesis of Silver Nanoparticles by Coprecipitation

For this specific synthesis, the methodology described by Martínez et al. (2013) was followed with some modifications, carrying out the following steps:

Firstly, aqueous solutions of ascorbic acid ($C_6H_8O_6$) at 0.6 mM, sodium citrate ($Na_3C_6H_5O_7$) at 3 mM, sodium hydroxide (NaOH) at 1.5 M, and silver nitrate ($AgNO_3$) at 0.1 M were prepared. Next, 750 mL of the ascorbic acid and sodium citrate solutions were mixed in a beaker. To adjust the pH to 10.9, 11.5, and 12, this mixture was repeated three times. Then, the pH of each mixture was measured and adjusted using an OAKTON potentiometer and the 1.5 M NaOH solution. Afterward, 187.5 mL of the $AgNO_3$ solution were gradually added, allowing for complete precipitate decantation. Following decantation, the supernatant was removed with a 20 mL automatic pipette. Subsequently, the precipitate was dried in a REBELK oven at 60 °C for 72 hours, resulting in a dark powder. Finally, the obtained nanoparticles were stored in light-protected containers, ensuring their stability.

2.3 Synthesis of Chitosan Biofilms Modified with Silver Nanoparticles

For the synthesis of the biofilms, the method proposed by Herrera et al. (2018) was followed with some adaptations, carrying out the following steps:

Initially, a chitosan solution was prepared by dissolving 0.5 g of chitosan in 50 mL of a 2% v/v acetic acid solution. Consequently, the mixture was stirred at 50°C for 1 hour using a CIMAREC magnetic stirrer, until a homogeneous solution was achieved. To ensure reproducibility, this procedure was repeated three times for each studied pH value, including a control. In parallel, three silver nanoparticle solutions were prepared at concentrations of 0.25, 0.5, and 0.75 % w/v in distilled water, for each pH. Subsequently, 50 mL of each silver nanoparticle solution was added to the chitosan solutions, and vigorously stirred for 10 minutes at room temperature. Following this, the resulting solutions were transferred to crystallizers, and 1.5 mL of glycerin was added to each, followed by an additional 10-minute stirring on the magnetic stirrer. After homogenization, the solutions were allowed to rest for 15 minutes. Finally, the solutions were dried at room temperature for 72 hours.

2.4 Material Characterization

To determine the properties and composition of the synthesized materials, the following techniques were used*:*

2.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

A Thermo Scientific - Nicolet iS10 equipment was used to identify the functional groups present in the synthesized samples, using a frequency range of 4000-500 cm^{-1}.

2.4.2 X-ray Diffraction

An Empyrean PANalytical diffractometer was used, operating in a θ-2θ configuration (Bragg-Brentano geometry) with a Cu X-ray tube (Kα radiation λ = 1.54056 Å) working at 40 kV and 40 mA. This technique allowed confirming the composition

and correct synthesis of the materials through the obtained diffractograms.

2.4.3 Scanning Electron Microscopy (SEM)

Elemental analysis was carried out by scanning electron microscopy (SEM) combined with energy-dispersive X-ray spectroscopy (EDX), using a FEG-SEM camera equipped with a Bruker X-Flash 6|30 detector, which has a resolution of 123 eV in Mn Kα.

2.5 Evaluation of the Antimicrobial Activity of Chitosan Biofilms Modified with Silver Nanoparticles

To evaluate the antibacterial capacity of chitosan biofilms modified with silver nanoparticles, the agar diffusion technique for bacterial sensitivity was used, based on the methods described by Varón et al. (2023), Fontalvo (2020), and Qin et al. (2019). This procedure was carried out using agar culture media in Petri dishes, uniformly inoculated with *Escherichia coli* ATCC 25922, through the massive or film seeding technique, and consisted of the following steps:
A pre-inoculum of *E. coli* was prepared in lysogeny broth (LB) (TM MEDIA) 24 hours before the experiment.
After 24 hours, the microorganism was massively seeded in Petri dishes containing Mueller Hinton (MH) agar.
Chitosan biofilms modified with silver nanoparticles at different pH levels and concentrations (0.25, 0.5, and 0.75 % w/v), along with a control, were cut into discs of 6 mm in diameter.
The discs were placed on the agar, gently pressed to ensure adherence. This process was repeated with discs of different concentrations and pH levels, performed in triplicate, and two additional discs were placed for the control. Subsequently, the plates were incubated at 37°C for 24 hours. After incubation, inhibition halos around the discs were observed and measured with a stereomicroscope (ZEISS).

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles by Coprecipitation

For this synthesis, silver nitrate ($AgNO_3$) was used as the metal precursor agent, allowing the reduction of silver from its ionic state (Ag^+) to its metallic state (Ag^0). Ascorbic acid ($C_6H_8O_6$) was used as the reducing agent, while NaOH was employed to regulate the pH of the solution. Additionally, sodium citrate ($Na_3C_6H_5O_7$) was added as a stabilizing agent, which prevents the formation of large particles by inhibiting coalescence (Martínez et al., 2013).
After preparing the solution, as shown in Figure 1, the solvent was removed, yielding silver nanoparticles (AgNPs), as observed in Figure 2. The final net weight of the obtained nanoparticles was 0.907 g.
Previous studies, such as those by Cuervo et al. (2020) and Martínez et al. (2013), have observed that during AgNPs synthesis, an increase in $AgNO_3$ concentration results in a darker brown hue of the solution. This study confirms this relationship using a 0.1 M concentration of $AgNO_3$, as depicted in Figure 1. Furthermore, it was found that pH influences both the hue and the antibacterial properties of the nanoparticles, utilizing pH levels of 10.9, 11.5, and 12.

Figure 1. Disolution of AgNPs

The synthesis of nanoparticles by reduction was performed due to its simplicity, low cost, and high effectiveness. This coincides with Polinarski et al. (2021), who argue that this method is one of the most widely used due to its practicality and efficiency. Martínez et al. (2013) also note that this procedure uses readily accessible reagents with low toxicity and easily manageable waste, such as ascorbic acid, which is completely biodegradable. For these reasons, this technique was considered highly viable for the synthesis of silver nanoparticles.

Figure 2. AgNPs obtained by coprecipitation method

3.2 Synthesis of Chitosan Biofilms Modified with Silver Nanoparticles

In this phase of the study, the sol-gel method was selected as the most suitable, as proposed by Rojas (2015), due to its ability to control and adjust each stage of the process. This occurs through the creation of a colloidal dispersion followed by its gelation, generating a network immersed in a continuous liquid phase (Arreche, 2016).
According to Martínez (2009), when water was removed, the concentration of the biopolymer increases, which facilitates the alignment and compaction of the molecules, forming a gel and, consequently, a biofilm. Additionally, Arreche (2016)

explains that if the solvent evaporates under room temperature and atmospheric pressure, the result is a xerogel, as was the case in this study. When supercritical temperatures or specific pressure conditions are applied, an aerogel is the obtained product. These conditions could alter the structure of the biomaterial.

Using the sol-gel method, nine chitosan biofilms were successfully synthesized and modified with different concentrations of silver nanoparticles at pH levels of 10.9, 11.5, and 12, along with a control sample without AgNPs but containing glycerin as a plasticizer. The results are shown in Figure 3. According to Martínez (2009), chitosan biofilms are generally fragile, so glycerol was added to make them more flexible and prevent breakage during handling.

Espinoza et al. (2020) highlight that the interest in nanoparticles as reinforcements in polymer matrices lies in their compatibility with the functional groups of polar polymers, allowing electrostatics or coordination bonds formation. These bonds improve the properties of the NPs when supported by the polymers, thus optimizing the performance of the final material.

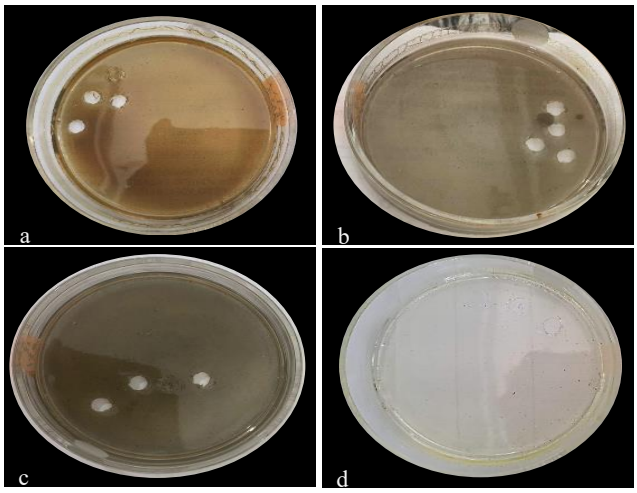

Figure 3. Chitosan biofilms modified with AgNPs (a: pH 10.9; b pH 11.5; c: pH 12; d: Control)

To achieve high-quality biofilms, the "casting" or box casting method was used, which proved to be highly effective. According to Sayyar et al. (2017), this method offers advantages such as obtaining biofilms with considerable optical purity and homogeneous volume distribution. Additionally, Escobar (2020) highlights the dimensional stability and flatness of biofilms generated under these conditions.

3.3 Material Characterization

3.3.1 Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was utilized to elucidate potential intermolecular interactions within the composite materials, specifically between modified and unmodified chitosan biofilms. This was accomplished through comparative analysis of the FTIR spectra depicted in Figure 4. Both spectra exhibited a high degree of similarity in terms of characteristic chitosan functional groups. The broad absorption band observed within the 3300-3500 cm^{-1} spectral region, present in both spectra, is attributed to the stretching vibrations of O-H and N-H bonds, corresponding to the hydroxyl groups of saccharides and amino/amide groups within the chitosan structure, respectively. The presence of glycerol, a component of the formulation, also contributes to this band due to its inherent hydroxyl groups. The C-H stretching vibrations of CH_2 and CH_3 groups were evidenced by distinct peaks within the 2850-3000 cm^{-1} range, common to both spectra. Furthermore, absorption bands within the 900-1200 cm^{-1} region, consistent with the vibrational modes of C-O bonds in chitosan (Suárez et al., 2014), were observed across both spectra. The absorption band at approximately 1640 cm^{-1}, indicative of C=O stretching vibrations in amides, was present in both spectra, albeit with a notable reduction in intensity within the AgNPs-modified biofilm.

Detailed spectral analysis revealed a shift in the C-N stretching band from 1555 cm^{-1} in pure chitosan to 1560 cm^{-1} in the AgNPs-modified chitosan, consistent with observations reported by Barraza et al. (2013), suggesting an interaction between AgNPs and chitosan amino/amide groups. The absorption bands associated with CH_2 and CH_3 alkyl bending (1450-1375 cm^{-1}) and O-H and N-H bending (922 and 850 cm^{-1}) remained consistent across both spectra. However, the carbonyl region exhibited the most significant change, with a marked decrease in the 1640 cm^{-1} band intensity in the presence of AgNPs. This observation suggests a specific interaction between AgNPs and the C=O groups of chitosan, possibly through coordination or electrostatic interactions. Such behavior aligns with the reported affinity of AgNPs for functional groups including O-H, N-H, C-H, and C=O.

Furthermore, a subtle difference in the intensity and shape of the O-H stretching band (3300-3500 cm^{-1}) was observed between the two spectra. The AgNPs-modified chitosan spectrum exhibited a slight decrease in peak intensity and a broadening of the O-H band compared to the pure chitosan spectrum. This variation suggests that AgNPs interact with chitosan hydroxyl groups, potentially through hydrogen bonding or coordination, which may influence biofilm properties. The observed decrease in C=O band intensity, the shift in the C-N band, and the variations in the O-H band collectively provide compelling evidence for the interaction between AgNPs and chitosan, specifically through the modification of amide, amino, and hydroxyl groups within the polymer.

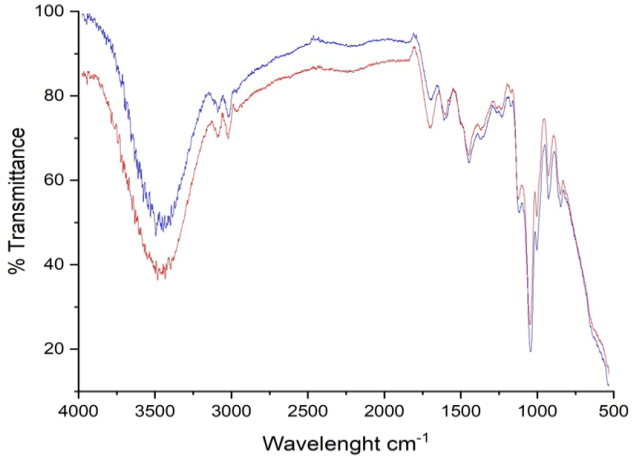

Figure 4. Comparative FTIR spectra of modified and unmodified biofilms (Red: Chitosan; Blue: Chitosan with AgNPs)

3.3.2 X-ray Diffraction (XRD)

The technique provides valuable data on the atomic structure and particle size, which has been crucial for analyzing the synthesized materials.

Data was collected over a 2θ range from 10 to 90 degrees. The diffractogram in Figure 5 obtained using the HighScore plus software was compared with the Crystallography Open Database, No. 96-900-8460. Four peaks at 2θ values of 38.17, 44.31, 64.50, and 77.47 degrees in the experimental diffractogram were identified as being due to silver metal and corresponding to the (hkl) values - (111), (200), (220), and (311) planes of silver. The XRD study thus confirmed that the resulting particles in the prepared sample are silver nanoparticles with a face-centered cubic crystal structure (Pal et al., 2007; Hernández, 2013). There are three additional peaks in the diffractogram at 29.35, 32.52, 47.38. These peaks have been identified as being due to $AgNO_3$, which may not have been fully reduced and therefore remained in the sample. According to the full width at half-maximum (FWHM) of the diffraction peaks, the average sizes of AgNPs were estimated from the Scherrer equation (formula 1) to be about 23.29 nm

$$D_{hkl} = k \times \lambda / (\beta_{hkl} \times \cos\theta_{hkl}) \quad (1)$$

Where D_{hkl} is the particle size perpendicular to the normal line of (*hkl*) plane, *k* is a constant (it is 0.9), β_{hkl} is the full width at half maximum of the (*hkl*) diffraction peak (it is 0.3515), θ_{hkl} is the Bragg angle of (*hkl*) peak (it is 13.97°) and λ is the wavelength of X-ray (it is 0.15406 nm).

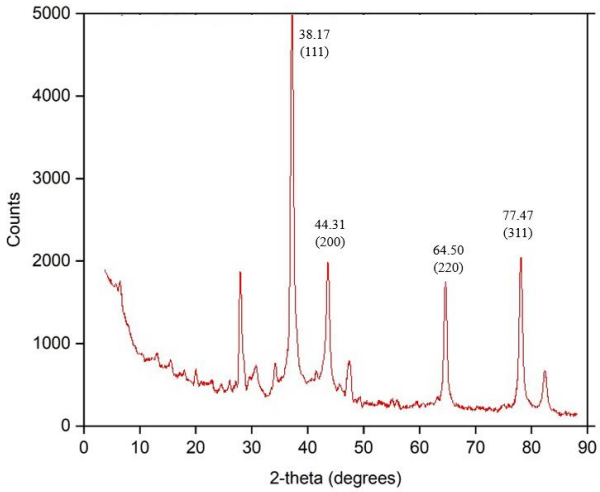

Figure 5. Diffractogram of AgNPs at pH 10.9

In Figure 6, the characteristic peaks of Chitosan and AgNPs in the obtained material can be observed. Chitosan exhibits a semi-crystalline nature, and indeed, according to the literature (Corazzari et al., 2015; Souza et al., 2010; Paul et al., 2018), two diffraction peaks are attributable to it. In particular, the first peak at 2θ = 11.17° (020) is related to the hydrated structure, and the second at 2θ equal to 20.21° (110) is due to the α-chitin chain segments. Furthermore, the characteristic peaks of AgNPs corresponding to (111), (200), (220), and (311) are observed, coinciding with those found in the Crystallography Open Database 96-901-1608 (Hernández, et al., 2013). The chitosan used in the synthesis process did not cause the formation of silver oxides. The shape of each peak was broadened due to the presence of the chitosan polymer (Souza, et al., 2010).

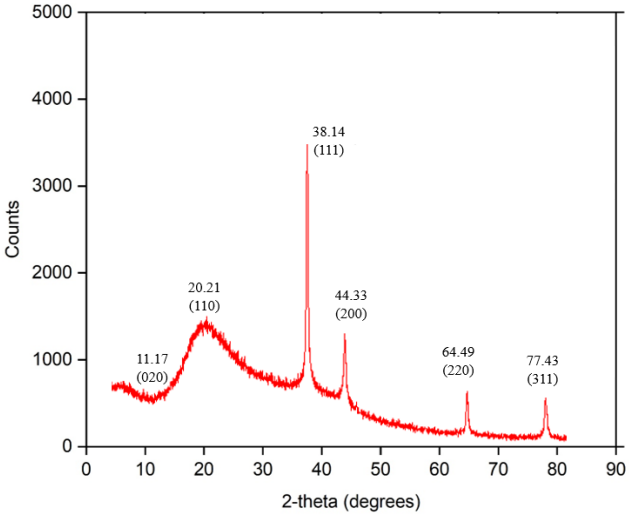

Figure 6. Diffractogram of chitosan modified with AgNPs at pH 10.9

3.3.3 Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) technique allowed the visualization and analysis of the microstructural morphology of the chitosan biofilm at pH 10.9, with a 0.75 % concentration of AgNPs. In Figure 6, the surface of the biofilm is shown, where various structures with variability in shapes and sizes, associated with chitosan, can be distinguished. Additionally, small white flashes are identified, indicating the presence of silver nanoparticles. These details are more evident in Figure 7, thanks to the higher resolution and scale used.

It is important to note that the surface of the modified biofilm does not present pores or cracks, unlike other studies where fibrillar formations and porous structures have been observed in similar biofilms (Cadinoiu et al., 2022; Popescu et al., 2022).

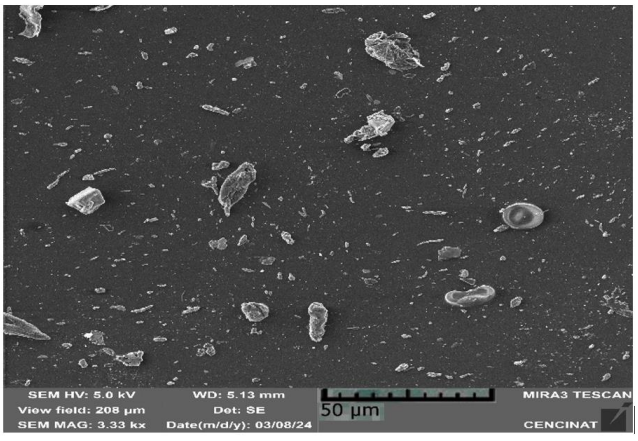

Figure 7. SEM micrograph of modified biofilms at 50 µm

The presence of each element is denoted by the normalized weight percentage, which is the weight percentage assuming the selected elements represent the total composition of the sample. The EDX spectrum of AgNPs presented in Figure 8 shows the three characteristic silver peaks below 4 keV due to surface plasmon resonance. The abundance of silver is

approximately 49 % normalized by mass. The other elements are Na (22 %), N (26 %), and O (22.5 %).

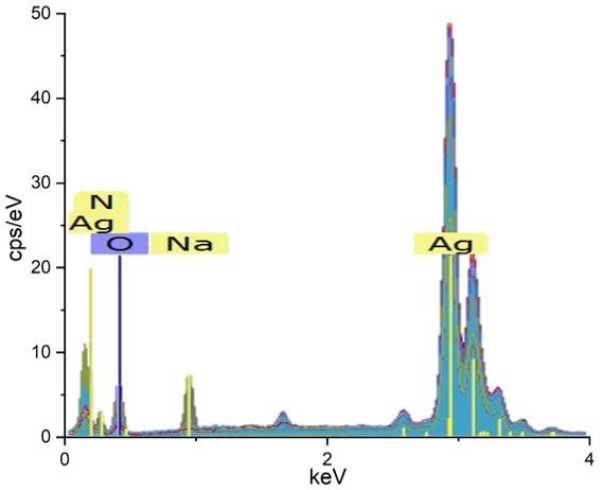

Figure 8. EDX spectrum AgNPs

3.4 Evaluation of the Antimicrobial Activity of Chitosan Biofilms Modified with Silver Nanoparticles

Regarding the evaluation of antimicrobial activity, the Kirby Bauer antibiogram is one of the most feasible methods to perform, as it has been widely implemented and recommended by organizations such as the National Committee for Clinical Laboratory Standards (NCCLS). This technique allowed the determination of *E. coli* sensitivity to chitosan biofilms modified with silver nanoparticles at various concentrations, as shown in Figure 8.

After 24 hours of incubation, inhibition halos were observed around the discs, confirming the antimicrobial action of the modified biofilms. As shown in Figure 9, the inhibition halos formed by the antimicrobial activity of the modified biofilms differ significantly from the control, which contains unmodified chitosan.

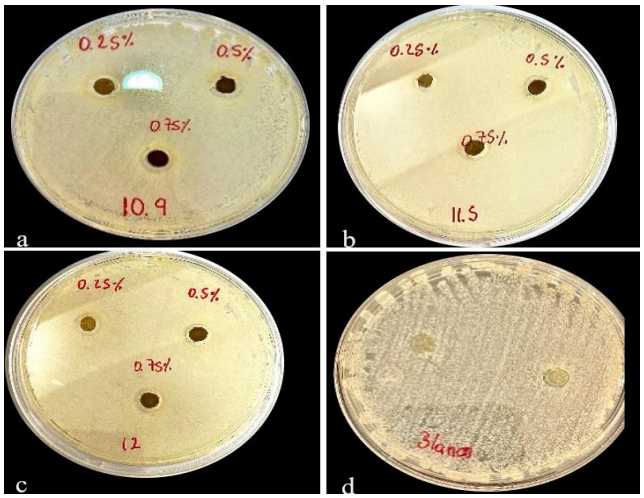

Figure 9. Antibiograms of chitosan biofilms modified with AgNPs (a: pH 10.9; b: pH 11.5; c: pH 12; d: Control) against a *E. Coli.*

As shown in Figure 10, biofilms modified with AgNPs at a concentration of 0.75 % achieved the largest inhibition diameters, indicating that these biofilms exhibit higher antimicrobial effects against *E. coli*. Additionally, better results were obtained with pH 10.9 compared to the others. Biofilms with AgNPs concentrations of 0.25 and 0.5 % also showed greater antimicrobial activity than the unmodified biofilms. Suggesting that the addition of AgNPs enhanced the inherent antimicrobial properties of chitosan.

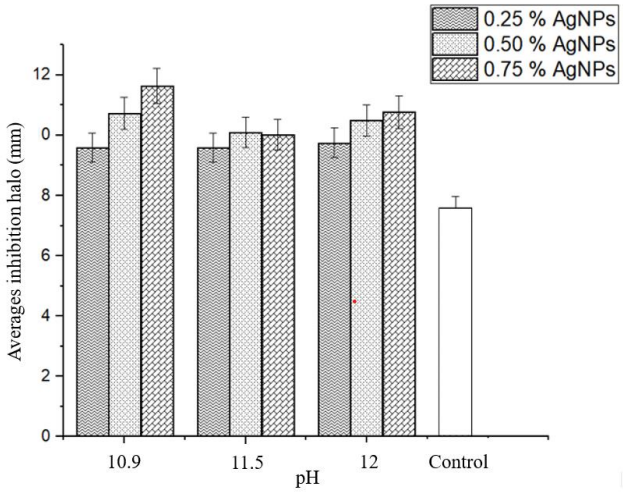

Figure 10. Averages of inhibition diameters against *E. Coli*

The low antimicrobial activity of the control compared to the other biofilms can be justified by the fact that chitosan possesses various factors that directly influence its inhibitory capacity. Ming Kong (2010) stated that the antimicrobial effect of chitosan is conditioned by several factors:

Intrinsic factors: molecular weight, solubility, degree of deacetylation, positive charge density, and chelating capacity.

Environmental factors: pH, temperature, and time.

Microorganism under study.

Physical state of chitosan: liquid (colloid) or solid (membrane).

Ayala (2015) pointed out that the aggregation of NPs enhances the antimicrobial capacity of chitosan. It is suggested that the optimization of antimicrobial activity is due to the increased positive charge density, resulting in competition between -NH_3^+ groups and the positive charges of the NPs for the same negative charge groups present in the microorganisms.

Herrera et al. (2018) evaluated the antimicrobial activity of chitosan-PVA biofilms modified with silver nanoparticles, concluding that the greatest effect was obtained with the highest nanoparticle concentration, which was 0.5 %. The authors attribute this result to the antibacterial properties of the silver ions present in the biomaterial, as they exhibit bacteriostatic and bactericidal characteristics upon release due to interactions with cellular enzymes.

Qin et al. (2019) investigated the antimicrobial activity of chitosan biofilms containing 0.064 g AgNPs per gram of chitosan against *E. coli*, reporting inhibition zones with a diameter of 8.42 ± 0.14 mm. In this study, larger inhibition halos were observed compared to Qin et al. (2019), despite the biofilms having a lower AgNPs content (0.025 g and 0.05 g). As expected, biofilms with a higher AgNPs content (0.075 g) demonstrated a more significant increase in the inhibition halo.

4. CONCLUSIONS

The instrumental methods used for the characterization of the synthesized materials provided valuable information about their structure, arrangement, and interactions. The use of

Fourier-transform infrared spectroscopy (FTIR) allowed the identification of the interaction sites of the AgNPs with chitosan, revealing changes in their representative bands. Similarly, X-Ray Diffraction (XRD) characterization provided valuable data on the particle size, which was estimated to be approximately 23.29 nm, and identified four peaks at 2θ values of 38.17, 44.31, 64.50, and 77.47 degrees, corresponding to the (111), (200), (220), and (311) planes of silver metal, confirming the face-centered cubic crystal structure of the silver nanoparticles. Scanning electron microscopy (SEM) enabled the observation of the morphological structures of the biofilms, identifying the distribution and shape of their components. Moreover, the chitosan biofilms modified with AgNPs exhibited enhanced antimicrobial effects compared to the unmodified biofilm against the *E. coli* pathogen, with the highest inhibitory effect observed in the biofilms with the highest concentration of AgNPs at a pH of 10.9, achieving an inhibition halo of 11.9 mm.

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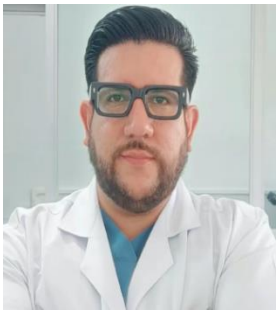
BIOGRAPHIES



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