

## Stability of *Vicugna Pacos* Fibers Functionalized with Silver Nps and Their Antibacterial Effect over Time

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This research aimed to evaluate the stability and antibacterial effect of *Vicugna pacos* “alpaca” white fibers functionalized with silver Nps on *Escherichia coli* ATCC 25922 over time. An experimental design in completely randomized blocks (DEBCA) was used, considering as blocks the 1st, 2nd, and 3rd months of ageing time and treatments the variations of the concentration of trisodium citrate (TSC) of 2, 6, and 10 mg. The results showed the presence of significant differences in the concentration and ageing time. A comparison of means showed that the antibacterial effect remained almost intact for the highest concentrations of trisodium citrate, while the concentration of 2 mg showed gradual decay. Furthermore, the Raman spectroscopy study indicates that the VPA fibers have not deteriorated due to functionalization with Nps-Ag. Therefore, it is concluded that the fibers functionalized using a high concentration of trisodium citrate are stable and keep their antibacterial effect over time.

Keywords: functionalization, alpaca fibers, antibacterial, stability

### 1. Introduction

*Vicugna pacos* is a native species of the Andean highlands, with an estimated global population of about 3.5 million individuals (Quispe et al., 2013). Its fiber is a high-quality natural material known for its softness, strength, elasticity, and thermal insulation. It is highly valued in the industry due to its softness, hygroscopicity, breathability, and its ability to reduce skin abrasion and support thermoregulation (Naffa et al., 2020). Additionally, the fiber shows good wash stability and appropriate mechanical properties, making it a suitable substrate for functionalization with bioactive agents to develop materials with enhanced or novel properties (Vaca-Cardenas et al., 2021).

With the support of nanotechnology and nanoscience, these characteristics can be improved through the design of innovative materials that enhance the lives of people affected by weather and certain enteropathogenic bacteria (Ruiz et al., 2018). Over the years, this bacterium has developed an unprecedented ability to evade antibacterial action through mutations and the acquisition of new transfer mechanisms.

The nanomaterials with antibacterial properties can be made into fabrics with a composition of natural polymers such as animal fibers or synthetics, including cotton, linen, or polyester, which have a coating of Nps and are widely used in multiple areas, such as medicine, chemistry, pharmaceutical industries, etc (Anwar and Alghamdi, 2020).

Yujia et al. (2019) reported the use of melanin extracted from alpaca fiber in combination with TiO<sub>2</sub> Nps to develop textiles with high protection against ultraviolet radiation and greater light stability. Unlike that study, the present work focuses on obtaining a material with antibacterial effect, using alpaca fibers functionalized with Nps-Ag. Rehan et al. (2018) synthesized Ag/AgX (X=Cl, I) Nps *in situ* on viscous fibers to impart brilliant colors, UV protection, antimicrobial, self-cleaning, and photocatalytic properties. The colored fibers showed excellent photocatalytic activity and remarkable antimicrobial activity against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans*. In turn, Messaoud et al. (2010) corroborated that active agents in the form of Nps can be considered as new antibacterial materials. Likewise, Betancur et al. (2016) highlight that these materials, through different mechanisms, can reduce or eliminate pathogenic microorganisms. Panáček et al. (2018) refer that Nps-Ag have high bactericidal activity at concentrations that are not toxic to human cells and interact synergistically with conventional antibiotics to improve antibacterial activity, even against multi-resistant bacteria.

This study aims to evaluate the stability and antibacterial effect of *V. pacos* fibers functionalized with Nps-Ag on *Escherichia coli* ATCC 25922 over time. To improve this natural fiber for multifunctional use and to add value for the benefit of the communities that produce this native fiber in Peru.

## 2. Materials and methods

### 2.1 Functionalization of *Vicugna pacos* “alpaca” fibers with Nps-Ag.

Nps-Ag were synthesized *in situ* on ultrasonically cleaned alpaca fibers using silver nitrate and trisodium citrate (TSC) as the reducing agent. The reaction was carried out at 95 °C with TSC concentrations of 2, 6, and 10 mg to study their effect on nanoparticle formation. After 25 minutes of stirring, the fibers were cleaned and dried. A progressive color change from yellow to dark yellow indicated a higher density of Nps-Ag with increasing TSC concentration.

### 2.2 Characterization of *Vicugna pacos* “alpaca” fibers functionalized with NPs-Ag.

UV reflectance spectroscopy (Lambda 750 - Perkin Elmer spectrophotometer with an integration sphere of Spectralon) was used to confirm the presence of Nps-Ag in the functionalized white *V. pacos* fiber samples (Ansari et al., 2023). Raman spectra (Wintec - Alpha 300R, 532nm) show vibrational modes corresponding to the formation of characteristic bonds, which provide clues to the functionalization mechanism when compared with natural samples of white *V. pacos* fibers (Anwar and Alghamdi, 2020). The micrograph SEM was collected with Vega 3 TESCAN.

### 2.3 Evaluation of antibacterial activity

After the reactivation of the *Escherichia coli* ATCC 25922 strains, the evaluation of the antibacterial activity was carried out with the modified AATCC-100 standard method to work with individual fibers, which were incubated in a shaker (200 rpm) at 37°C for 24 hours, obtaining results of the first, second and third month for white *V. pacos* fibers functionalized with Nps-Ag produced from 6 mg of silver nitrate and three different concentrations of trisodium citrate of 10 mg (A), 6 mg (B), 2 mg (C) (American Association of Textile Chemists and Colorists, 2010). After the bacterial growth period, the plate count technique was applied. The reduction of bacteria was calculated from the equation:

$$R (\%) = (C_i - C_f / C_i) \times 100.$$

R is the percentage reduction, C<sub>f</sub> is the counted colonies exposed to the functionalized fibers after 24 hours of incubation, and C<sub>i</sub> are the counted colonies of the bacteria at 24 hours, excluding the fibers (Noorian et al., 2019).

### 2.4 Data processing

The plate count method was applied after 24 hours of incubation at 37°C, determining the colony-forming units (CFU) and subsequently the reduction percentage of the first, second, and third month for white *V. pacos* fibers functionalized with Nps-Ag produced from 6 mg of AgNO<sub>3</sub> and three different concentrations of trisodium citrate of 10 mg (A), 6 mg (B), 2 (C) (Wu et al., 2020).

A completely randomized block experimental design (DEBCA) was carried out using Analysis of Variance and Tukey range test with the obtained data. Software R (free) was used to determine the logarithmic growth curve, with a PE = 0.05.

### 3. Results and discussion

Several authors suggest that there are different methods of action of Nps-Ag on bacteria, such as the formation of reactive oxygen species (ROS), capable of affecting the cell membrane, increasing cell permeability, and filtering the intercellular content through cell disruption; these can be superoxide anions, hydrogen peroxide, hydroxyl radicals, among other things. Another procedure for attacking bacteria is the condensation of DNA and the dehydration of microbial cells. The methods of action on bacteria depend on the concentration, stability, size, and shape of the Nps (Anwar and Alghamdi, 2020). For the standardization of the synthesis (*in situ*) of Nps-Ag on *V. pacos* fibers. One methodology section used by Kelly and Johnston (2011) was used in this work. They indicate that the synthesis of Nps in the fibers of *V. pacos* occurred *in situ* by the reduction of silver ions in solution by trisodium citrate at high temperature. The Nps bind primarily to the sulphur-containing amino acids of the keratin fiber protein due to their affinity for silver, using trisodium citrate as a binding agent. This suggests that the role of TSC as a binding agent for the Nps is fundamental during the synthesis. The results observed during the process show a slight color change, which may indicate the formation of Nps; this process improves with increasing concentration. TSC has three functions in nanoparticle synthesis: it is a reducing agent, a production agent, and a pH mediator (Huang *et al.*, 2018). In addition, this could explain the results obtained in Figure 1, where it is observed that the reduction of silver nitrate in concentration C (2 mg of TSC) is not as large as in concentrations A and B, which contained 6 mg and 10 mg of TSC, respectively. In this sense, the stability of the antibacterial effect over time of the functionalized fibers was evaluated. When TSC is at a high concentration, there is stability, and at a low concentration, a progressive degradation is observed over time.

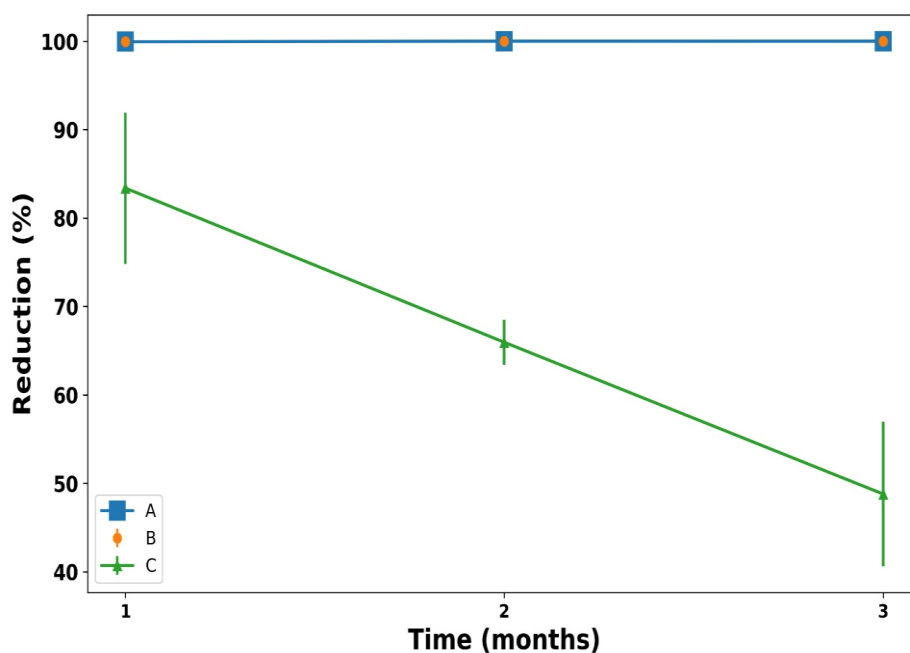


Figure 1: Average percentage reduction of the antibacterial effect against *E. coli* ATCC 25922 of *V. pacos* "alpaca" white fibers functionalized with Nps-Ag produced from 6 mg of  $\text{AgNO}_3$  and different concentrations of TSC of 10 mg (A), 6 mg (B), and 2 mg (C) with error bars.

Figure 2 (a) shows what is mentioned above; the greater the amount of TSC in the functionalization of the fibers, the flatter the curve becomes, so there is a greater reduction of  $\text{AgNO}_3$ . This explains that the percentage of reduction is better in those fibers that have a greater amount of TSC in their functionalization.

In this research, TSC is a protection agent in addition to being a reducing agent, as stated by Bhardwaj and Jha (2020), as well as polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and so on, producing modifications in the morphology of the nanomaterial. In this case, the TSC allows the Nps not to deteriorate due to the action of different environmental factors that can end their useful life. One of the main problems that occurs with Nps-Ag is the sulfation that they may suffer. Over time, its properties diminish considerably, but TSC forms a protective layer.

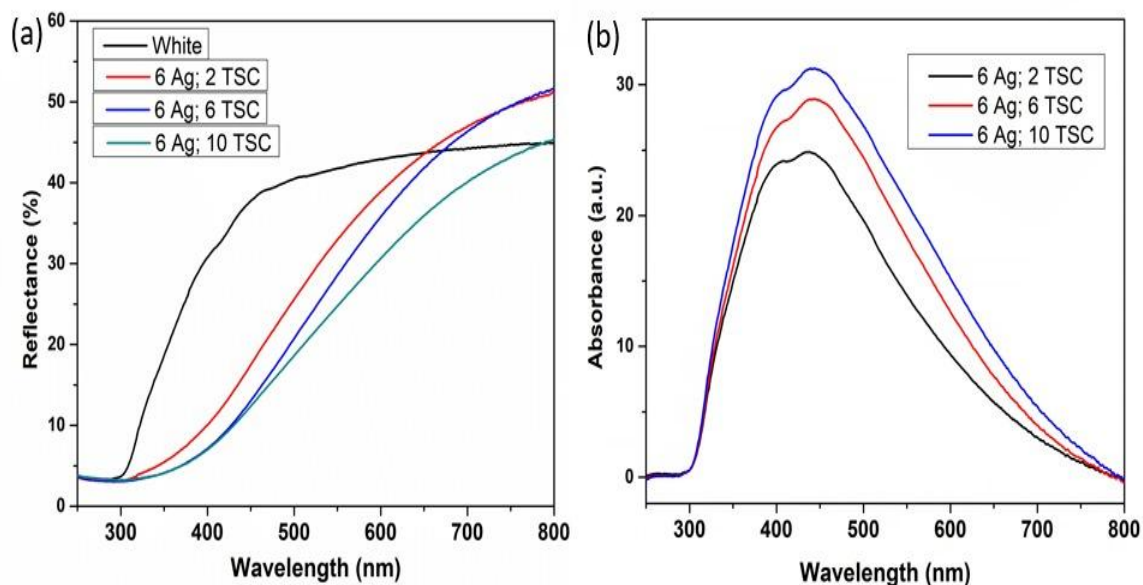


Figure 2: (a) The reflectance of non-functionalized and functionalized *V. pacos* “alpaca” white fibers with silver Nps produced from 6 mg of  $\text{AgNO}_3$  and different concentrations of TSC; (b) The absorbance of silver Nps in white *V. pacos* “alpaca” fibers functionalized with silver Nps.

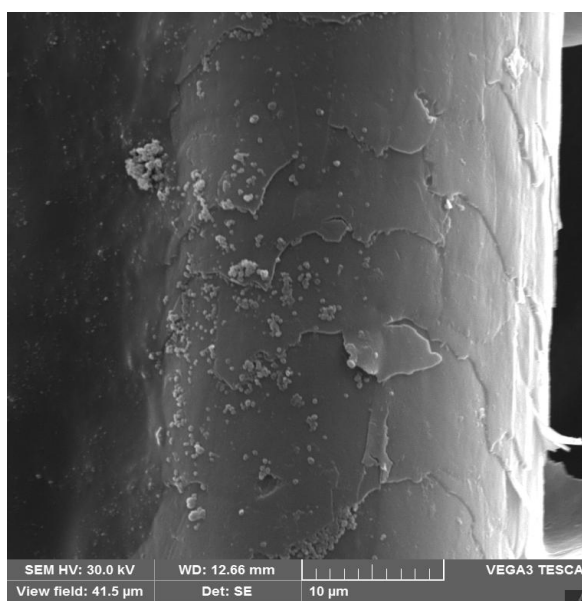


Figure 3: Photomicrograph of *V. pacos* fibers with Nps-Ag.

The effective functionalization of white fibers of *V. pacos* with Nps-Ag was verified through their reflectance spectra (Figure 2a). Subsequently, according to Baba *et al.* (2014), a direct subtraction of the same was carried out to find the plasmon band centred around 450 nm, typical of Nps-Ag. This is shown in Figure 2(b), which allows us to affirm that the functionalization has been carried out successfully during the synthesis of the Nps. Regarding the stability of the fiber, a Raman spectroscopy analysis was carried out, which provides information on the chemical and structural composition of the fibers (Cabrales *et al.*, 2014). Clearly, bands assigned as the CH stretching ( $\text{CH}_2$ ,  $\text{CH}_3$ ) at 2935 ( $\text{cm}^{-1}$ ); CH stretching at 3738 ( $\text{cm}^{-1}$ ); C=O Amide I at 1656 ( $\text{cm}^{-1}$ );  $\text{CH}_3$ , CCH olefinic,  $\text{CH}_2$  rock at 958 ( $\text{cm}^{-1}$ ); Amide III (unordered) at 1245 ( $\text{cm}^{-1}$ );  $\text{CH}_2$  bending mode at 1449 ( $\text{cm}^{-1}$ );  $\text{CH}_2$  bend, Trp at 1339 ( $\text{cm}^{-1}$ ); Ring breathing mode of Tyr at 851 ( $\text{cm}^{-1}$ ) have been identified (Essendoubi *et al.*,

2019). The Raman shift is uniform in all samples, without significant variation in the pattern obtained (unfunctionalized fiber). The *V. pacos* fibers remain stable even after being functionalized with Nps-Ag (Figure 3, 4). This result is desirable because if their properties are altered, functionalization could not be considered as a good treatment option for them.

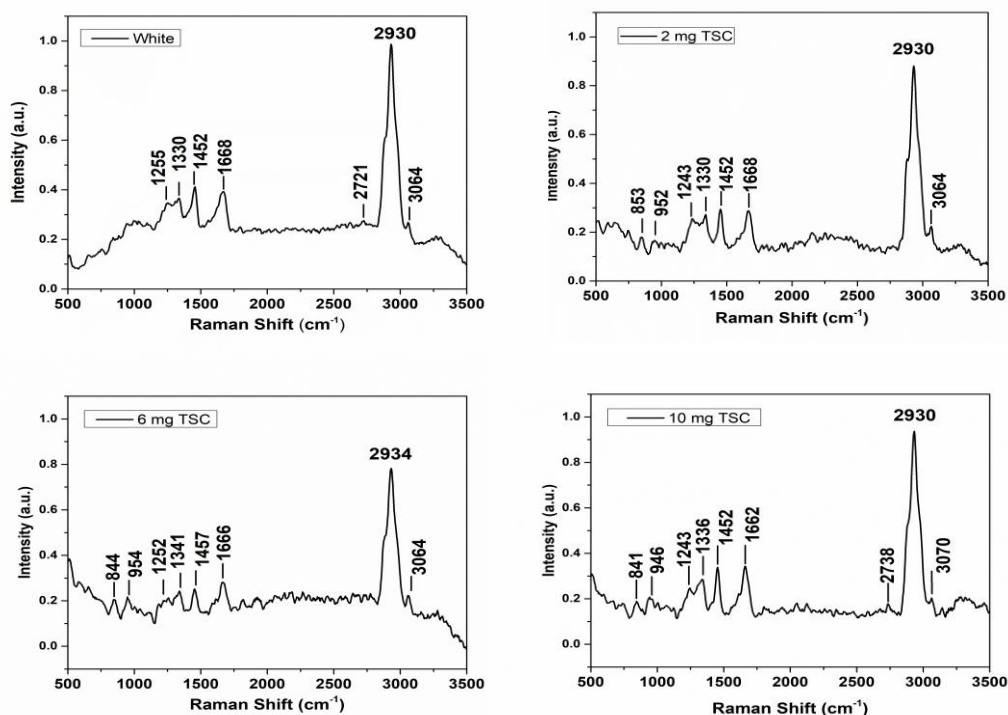


Figure 4: Raman spectra of white fibers of *V. pacos* “alpaca” non-functionalized and functionalized with Nps-Ag formed from 6 mg of  $\text{AgNO}_3$  and variations in the concentration of TSC. (Right) Photomicrograph of *V. pacos* fibers with silver Nps.

#### 4. Conclusions

White *V. pacos* fibers functionalized with Nps-Ag using trisodium citrate concentrations of 6 and 10 mg presented expected stability in the percentage of growth reduction on *E. coli* ATCC 25922 over time. *V. pacos* white fibers functionalized with Nps-Ag using a concentration of 2 mg of trisodium citrate show a gradual decay in the percentage of growth reduction on *E. coli* ATCC 25922 over time. The white *V. pacos* fibers maintained their stability after the functionalization process, as shown in the Raman spectroscopy analysis.

#### Acknowledgments

The authors acknowledge the economic support of Proyecto Concytec – Banco Mundial “Mejoramiento y Ampliación de los Servicios del Sistema Nacional de Ciencia, Tecnología e Innovación Tecnológica” 8682-PE, by the Fondecyt. [Contract number 048-2019- FONDECYT-BM-INC.INV]. Special thanks to Dr. Luis Angelats Silva for his support in part of characterizing the fibers.

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