

# Novel in-situ synthesis of cellulose AGNPS characterization and antibacterial properties

## Abstract

The present study reported a simple and a fast method for in-situ antibacterial Ag NPs loading on cellulosic white plain weave (bandage) through chemical reduction technique using sodium borohydride ( $\text{NaBH}_4$ ). The effect of adding a stabilizing agent, such as polyvinyl pyrrolidone (PVP), was evaluated. The prepared cellulosic samples were characterized by Fourier Transform Infrared spectroscopy (FTIR) and DSC analysis. The treated supports displayed obvious antibacterial properties for both gram-negative and gram-positive bacteria. The obtained results revealed that the addition of the protective colloid (PVP) generated better antibacterial activity than the fabric treated without PVP.

**Keywords:** cotton, antibacterial activity, silver nano particles, stabilizing agent

Volume 4 Issue 1 - 2018

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**Received:** December 30, 2017 | **Published:** January 12, 2018

**Abbreviations:** PVP, polyvinyl pyrrolidone; FTIR, Fourier transform infrared spectroscopy;  $\text{NaBH}_4$ , sodium borohydride;  $\text{AgNO}_3$ , silver nitrate; DSC, differential scanning calorimetry

## Introduction

Many bacteria are harmless and even highly helpful but a lot of them can cause hundreds of illnesses.<sup>1</sup> Therefore, there exists a need to develop antibacterial supports which exhibit satisfactory antibacterial properties allowing the protection of the human body. Cellulose is one of the most abundant biopolymers in the nature. It is a renewable material which exhibited hygroscopicity, non-toxicity, biocompatibility, biodegradability and chemical reactivity. It is a carbohydrate polymer composed of repeating D-glucopyranose units and consists of three hydroxyl groups per a hydroglucose unit conferring the cellulose molecule a high degree of functionality. The materials based on cellulose and its derivatives have been employed in a large variety of applications such as textile, food, paper production, biomaterials and pharmaceuticals.<sup>2-4</sup> Functionalization of cellulose with nanoparticles has attracted great attentions of researchers in recent years. A number of studies have been investigated to improve functions of this material such as antibacterial treatment.<sup>5</sup> Among different metals, silver is by far the most widely used antibacterial agent because of its efficiency and its strong toxicity against many forms of both gram-positive and gram-negative bacteria.<sup>6</sup> The structural differences between the two types of bacteria lie in the organization of a key constituent of the membrane, peptidoglycan. Gram negative bacteria display only a thin peptidoglycan layer ( $\approx 2-3\text{nm}$ ) between the cytoplasmic membrane and the outer membrane.<sup>7</sup> However; Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer of about 30nm thick.<sup>7</sup>

The Ag metal has the capacity to attach to bacterial cell membranes, to enter and attack the respiratory chain of the cell, hence inducing the death of the bacteria.<sup>7</sup> Ag NPs have been added to a variety of polymeric fibers and have shown successful antibacterial activity.<sup>8-11</sup> In this investigation, nano silver particles were introduced on the structure of a cellulosic material (a bandage) via a chemical reduction method using sodium borohydride ( $\text{NaBH}_4$ ) in the presence or not

of polyvinyl pyrrolidone (PVP) as stabilizer agent. The in situ direct metallization method was applied in the attempt to obtain a high loading amount and strong bonding force of Ag NPs on the cellulosic surface, thus evading the  $\text{Ag}^+$  contamination problem. The presence of Ag NPs on the surface of the used support was verified by Fourier transform infrared spectroscopy and DSC characterizations. The impact of the adopted treatment on some mechanical properties of the tested fabric was then evaluated. Finally, the survey of the bacterial activity of loaded Ag NPs cellulosic support was evaluated against gram positive and gram negative bacteria.

## Experimental

### Materials

The cellulosic support used in this study is a bandage that was supplied by the central pharmacy of Sousse, Tunisia. The used fabric has the following specification: Plain weave, surface mass  $25\text{g/m}^2$ , thickness 0.3 mm, weft density=warp density=18 thread/cm, weft yarn finesses=warp yarn finesses=15tex. Silver nitrate ( $\text{AgNO}_3$ ), sodium borohydride ( $\text{NaBH}_4$ ) and polyvinyl pyrrolidone (PVP) were purchased from Sigma-Aldrich Chemical Company and used as received without further purification.

### Preparation of cell/Ag<sup>o</sup> material

In the present work, cellulose fibers were functionalized with Ag nanoparticles by in-situ synthesis. Firstly, the cellulosic support was treated with an aqueous solution of  $\text{AgNO}_3$  ( $0.06\text{ mol.L}^{-1}$ ) at room temperature for 24hours. Then, it was washed with distilled and deionized water several times until no more silver ions could be identified in the washing water by the chloride test. Subsequently, the  $\text{Ag}^+$  incorporated in cellulose membrane (cell/Ag<sup>+</sup>) were then treated with a reducing agent, namely  $\text{NaBH}_4$ . In order to evaluate the role of PVP, as a protective colloid, we have prepared another solution (the same that the earlier) and we have gradually added an aqueous solution containing 0.2% wt/v of PVP at pH 13-14. This addition was carried out in an ice ultrasound bath (temperature around  $5^\circ\text{C}$ ) for 15minutes. The mixture was then maintained for a further 1hour at room temperature. At the end of reduction process, the Cell/Ag<sup>o</sup>

became dark brown. The obtained materials were finally dried at 100°C.

### Characterization of silver treated fabrics

**FTIR analysis:** The chemical changes to cellulosic fabric treated with AgNPs were analyzed on FTIR spectroscopy. Infrared spectra were obtained from KBr pellets using a Fourier Transform Infrared Spectrometer (Nicolet® 510M) in a transmittance mode in the range of 500–4000cm<sup>-1</sup>.

**DSC analysis:** Heat-flow analysis differential scanning calorimetry (DSC) analysis was conducted using a Setaram instrument by heating the sample from room temperature to 300°C at a rate of 10°C.min<sup>-1</sup>.

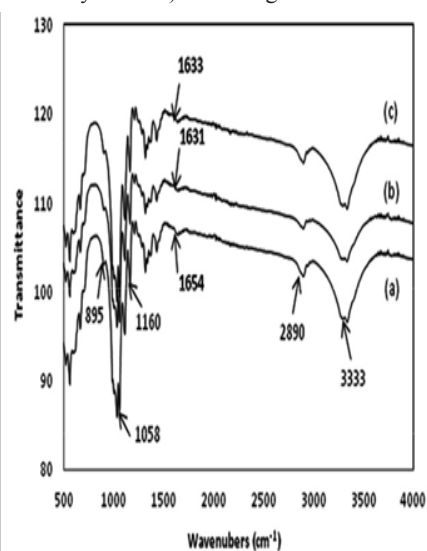
**Mechanical properties:** The bandage fabric was tested for the tensile properties<sup>12</sup> before and after nano Ag treatment. Mechanical properties (breaking load, % elongation-at-break, Young modulus and rigidity) were determined using LLOYD LR5 Instrument. The tested fabric possess identical warp and weft yarns characteristics. Therefore the mechanical properties were studied only in one direction of the bandage.

**Antibacterial activities:** Antibacterial activity of untreated and treated samples was tested on the basis of the agar diffusion standard test method.<sup>13</sup> Four type of bacterial pathogen strains were used: *Staphylococcus aureus* (ATCC 25923) and *Vibrio parahaemolyticus* (CECT 511) (gram- positive bacteria). *Escherchia Coli* (ATCC 25922) and *Salmonela typhimurium* (ATCC 1408) (gram-negative bacteria).

## Results and discussion

### FTIR analysis

The FTIR spectra of untreated and treated supports have been shown in Figure 1. The spectrums of the three supports exhibited the main characteristic adsorption peaks of cellulose.<sup>6</sup> Indeed, the wide peak at around 3333cm<sup>-1</sup> is assigned to the hydrogen-bonded OH stretching. The peak at 2890cm<sup>-1</sup> is associated to the CH stretching and the one at 895cm<sup>-1</sup> belonged to the CH<sub>2</sub>-OH bending groups of cellobiose. The band at 1160cm<sup>-1</sup> is related to C-O-C stretching at the β-(1→4)-glycosidic linkage. The peak at 1058cm<sup>-1</sup> corresponded to the C-OH (secondary alcohol) stretching vibration.



**Figure 1** FTIR spectra of, Untreated support, (b) Cell/Ag prepared with NaBH<sub>4</sub> without PVP and (c) Cell/Ag prepared with NaBH<sub>4</sub> using PVP.

The small absorption at around 1654 cm<sup>-1</sup> (Figure 1(a)), which confirmed the existence of the double bond C=C, was shifted to 1631cm<sup>-1</sup> for Cell/Ag prepared with NaBH<sub>4</sub> without PVP (Figure 1(b)) and to 1633cm<sup>-1</sup> for Cell/Ag prepared with NaBH<sub>4</sub> using PVP (Figure 1(c)). These peaks are assigned to the vibration of the inter-cellobiose bonds.

The shift of the peak might be explained by the fact that the nanoparticles of Ag are fixed 77 between the electron pair carried by two local oxygen's (two oxygen's carried by the same 78 carbon). For the rest of the spectra, we noted a perfect juxtaposition between the bands of 79 vibrations in the plan and outside the plan of the different bonds constitutive of the cellulosic 80 structures. The FTIR characterization confirmed that the deposit of Ag NPs is exclusively 81 done at the point of meeting between the two pyranose cycles.

### Differential scanning calorimetry (DSC) analysis

The heat treatment DSC curves of the cellulosic material before and after Ag NPs functionalization are given in Figure 2. As can be observed, there is a general stabilization of the cellulose structure that is characterized by:

- A small shift in the temperature of water loss which passes from 66°C for the untreated support to 69°C for the treated supports with Ag NPs.
- A thermal stabilization characterized by a weight loss of the nano Ag treated supports relatively reduced compared to that of untreated one.
- The temperature of the cellulose decomposition passes from around 235°C for the untreated cellulosic material to approximately 270°C for the treated ones accompanied by a relatively negligible weight loss compared to that of the untreated sample.

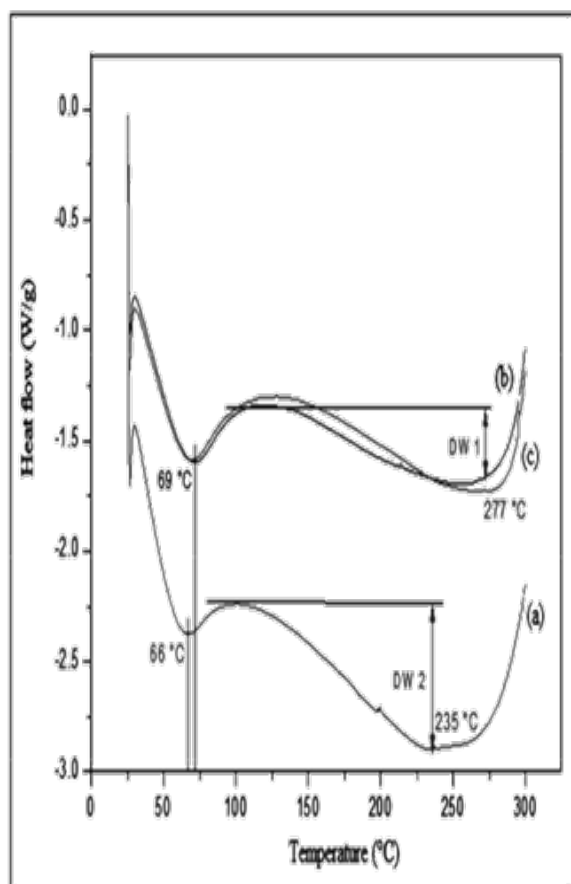
The ratio (*R*) of the weight loss between the treated sample (DW<sub>1</sub>) and the untreated one (DW<sub>2</sub>) is represented as follows:

$$R = \frac{DW_2}{DW_1} \quad (1)$$

Based on the results presented in Figure 2, *R* is equal to 1.49. This means that the Ag NPs treatment led to a sample's weight loss of 1.49 times than that of the weight loss of the untreated cellulosic sample.

### Mechanical properties studies

The effect of silver nano treatment on breaking load, maximal strain, Young modulus and rigidity has been evaluated in this study. As it can be seen from Table 1, the introduction of silver nano particles into the structure of the fibre causes an improvement in the mechanical properties of the samples. This could be attributed to the small size of nano Ag particles which could penetrate in between the polymer molecules and may be act as filler or cross linking agent which also contribute to the load sharing phenomenon during load application to the material.<sup>14</sup> In this context, Ag NPs perform as binding components which joins the fibrils with the "bodies" of the fibre, thus strengthening their structure.<sup>15</sup> Results also revealed that there is a little improvement in the mechanical properties of the Cell/Ag support prepared with NaBH<sub>4</sub> using PVP in comparison the Cell/Ag support prepared with NaBH<sub>4</sub> without PVP. This suggested that the addition of the protective colloid agent allows more insertion of the silver nanoparticles inside the textile structure inducing an increase in the mechanical performances of the tested materials.



**Figure 2** DSC analysis of, (a) Untreated cellulose, (b) Cell/Ag prepared with NaBH<sub>4</sub> without PVP and (c) Cell/Ag prepared with NaBH<sub>4</sub> using PVP.

**Antimicrobial activity studies**

The untreated and treated cellulosic substrates (Cell/Ag prepared with NaBH<sub>4</sub> without PVP and Cell/Ag prepared with NaBH<sub>4</sub> using PVP) were taken for antimicrobial effect against five well-known bacteria by agar diffusion test. To evaluate the antibacterial activity of the treated cellulosic materials, we have measured the surface of the bandage sample noted by  $S_p$ , and the surface of the inhibition

**Table 1** Mechanical properties of treated and untreated supports

Sample	Breaking load (N)	Elongation at break (%)	Young modulus (MPa)	Rigidity (N/m)
Untreated support	40	5.6	50	10 521
Cell/Ag prepared with NaBH <sub>4</sub> without PVP	44	8.8	61.88	19 425
Cell/Ag prepared with NaBH <sub>4</sub> using PVP	45	9.23	62	21 098

**Table 2** Antimicrobial activities of treated cellulosic samples

Sample	<i>E. Coli</i>	<i>S. typhimurium</i>	<i>V. parahaemolyticus</i>	<i>S. aureus</i>
Cell/Ag prepared with NaBH <sub>4</sub> without PVP	< 1	1	< 1	1
Cell/Ag prepared with NaBH <sub>4</sub> using PVP	1.05	1.15	1.17	1.16

zone (if it exists), noted by  $S_{finish}$ . Then, we calculate the ratio  $S_{finish}/S_0$ . The presence of a clear zone around the sample was recorded as an inhibition against the bacterial strains. As currently envisioned, the reference cellulosic sample, without the Ag addition, has not produce any antimicrobial activities since no inhibition zone was observed. So, the cellulose material was only used as matrix for the dispersion of Ag NPs.

The antibacterial activities results (the ratio  $S_{finish}/S_0$ ) of loaded supports with Ag nanoparticles are given in Table 2. As can be seen, the cellulosic supports loaded with Ag NPs exhibited good inhibitory activities against the tested bacterial strains. Indeed, the nano silver particles have an extremely large relative surface area, thus increasing their contact with bacteria or fungi, and vastly improving their bactericidal and fungicidal effectiveness. Nano silver is very reactive with proteins. When contacting bacteria and fungus, it adversely affects cellular metabolism and inhibits cell growth. It also suppresses respiration, the basal metabolism of the electron transfer system, and the transport of the substrate into the microbial cell membrane. Furthermore, it inhibits the multiplication and growth of those bacteria and fungi which cause infection, odor, itchiness and sores.

Results also showed that the Cell/Ag prepared with NaBH<sub>4</sub> using PVP displayed higher antimicrobial efficiency than that of Cell/Ag prepared with NaBH<sub>4</sub> without PVP, independently of the microbe type. Indeed, this colloid provides overall increase of silver active surface due to high surface to volume ratio of each nanoparticle. In this case, silver nanoparticles were entrapped physically on the cellulose structure and it might be firmer in comparison with the fabric without PVP. In addition to that, when the bandage with Ag ions are immersed in the reducing agent solution in the presence of PVP (as a protective colloid), the Ag ions are reduced to Ag<sup>0</sup> particles, which are stabilized at the ion selective sites of the cellulosic surface without becoming grown or agglomerated.<sup>16</sup> The obtained results showed the importance of the addition of the stabilizing agent (PVP) in the reduction's reaction of Ag<sup>+</sup> ions.

As can be shown in Table 1, the *E. Coli* has lower resistance against antimicrobial activity of Cell/Ag prepared with NaBH<sub>4</sub> using PVP in comparison with the other ones. This could be attributed to the thicker cell wall of *S. aureus*, *V. parahaemolyticus* and *S. typhimurium* compared to that of *E. Coli*. For this, the nano silver particles cannot destroy these bacteria easily.

## Conclusion

In this survey, a cellulosic support (a bandage) has been antimicrobially finished through in situ preparation of silver functionality by applying chemical reduction method using  $\text{NaBH}_4$  as reducer. The use of a colloid protector, such as PVP, has also been evaluated. Afterwards, the antibacterial activity of the functionalized supports against some bacterial stumps has been tested. This activity was measured by calculating the ration of the inhibition zone surface and bandage sample surface placed in the culture medium. Results showed that the Cell/Ag prepared with  $\text{NaBH}_4$  using PVP exhibited higher antimicrobial effectiveness than that of Cell/Ag prepared with  $\text{NaBH}_4$  without PVP. The mechanical properties of the untreated and treated supports has also been examined and it was found that the introduction of AgNPs into the structure of the cellulosic fibres deals with an improvement in terms of breaking load, elongation at break, young modulus and rigidity.

## Acknowledgements

None.

## Conflict of interest

Author declares there is no conflict of interest in publishing the article.

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