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ABSTRACT

Bactericidal water filters were developed. For this purpose, nitrocellulose membrane filters were impregnated with different biosynthesized silver nanoparticles. Silver nanoparticles (AgNPs) from *Aspergillus niger* (AgNPs-Asp), *Cryptococcus laurentii* (AgNPs-Cry) and *Rhodotorula glutinis* (AgNPs-Rho) were used for impregnating nitrocellulose filters. The bactericidal properties of these nanoparticles against *Escherichia coli*, *Enterococcus faecalis* and *Pseudomona aeruginosa* were successfully demonstrated. The higher antimicrobial effect was observed for AgNPs-Rho. This fact would be related not only to the smallest particles, but also to polysaccharides groups that surrounding these particles. Moreover, in this study, complete inhibition of bacterial growth was observed on nitrocellulose membrane filters impregnated with 1 mg L^{-1} of biosynthesized AgNPs. This concentration was able to reduce the bacteria colony count by over 5 orders of magnitude, doing suitable for a water purification device.

Keywords: Silver nanoparticles; Nitrocellulose membrane filter; Water purification; Bactericidal filters

1. Introduction

Globally, eighty percent of gastrointestinal infectious and parasitic diseases are due to the use of non-potable water. Poor hygiene and lack or malfunctions of health services are some of the reasons for which diarrhea remains a major health problem in developing countries. Contaminated water and food are regarded as the main vehicles involved in the transmission of bacteria, viruses or parasites. Most pathogenic microorganisms contained in the water are removed in the early stages of treatment for water purification. However, water disinfection is necessary as one of the last steps to prevent drinking water is harmful to our health. In special situations, silver salts are used to maintain the bacteriological quality of drinking-water [1]. However, high levels of silver content in water could cause possible human health effects [2]; so it must be taken with special care.

In the last years, the biosynthesis of metallic nanoparticles has been proposed as an environmental friendly alternative to chemical and physical methods [3]. These last methods, usually, employ hazardous and toxic chemicals such as reducing agents, organic solvents, or non-biodegradable stabilizing agents which are potentially dangerous to the environment and biological systems [4].

Recently, microbiological methods for synthesis of metallic nanoparticles have attracted an ever-growing research interest [5-9]. These biological methods are a green chemistry approach that interconnects nanotechnology and microbial biotechnology. On the other hand, among the metallic nanoparticles, silver nanoparticles (AgNPs) have received much attention in various fields, such as antimicrobial activity [10], therapeutics [11], bio-molecular detection [12], silver nanocoated medical devices [13] and optical receptor [14].

Hence, biogenic approach, in particular the usage of microorganisms has offered a reliable, simple, nontoxic and environmental friendly method [15-17].

According to the World Health Organization [18], many people worldwide do not have access to clean and potable water sources. The greatest water borne threat to human health is bacterial contamination of drinking water sources, leading to outbreaks of several diseases [19]. Recently, considerable interest has arisen in the use of nanotechnology for water purification [20]. In particular, nanomaterials can be used for small-scale or point-of-use systems for water systems [2, 21] that are not connected to a central network and for emergency response following disasters. Such systems should be cheap, highly portable, non toxic, easy to use and distribute and require low energy input.

In the present study, we have incorporated different kind of biosynthesized AgNPs obtained from *Aspergillus niger*, *Cryptococcus laurentii* and *Rhodotorula glutinis* into nitrocellulose membrane filter. The antibacterial effectiveness of developed filters was evaluated in contaminated water samples with *Escherichia coli*, *Enterococcus faecalis* and *Pseudomona aeruginosa*. To our best knowledge, until now, has not been found bibliography to report the use of biosynthesized AgNPs incorporated to nitrocellulose membrane filter used to purify drinking water.

2. Materials and methods

2.1. Silver nanoparticles used and their characteristics

In the present work were used commercial AgNPs (Sigma Chemical, St. Louis, MO, USA) obtained by chemical synthesis and different extracellular biosynthesized AgNPs obtained from *A. niger* (NRRL 1419), *C. laurentii* (BNM 0525) and *R. glutinis* (BNM 0524). The nanoparticles were synthesized and characterized in Industrial Microbiology Laboratory of Universidad Nacional de San Luis (Data unpublished). Characteristics are shown in Table 1.

Table 1

2.2. Microorganisms and culture

Water borne pathogens such as *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027) and *E. faecalis* (ATCC 29212) were used as testing organisms. *E. coli* measures approximately 0.5 µm in width by 2 µm in length, while *E. faecalis* measures 0.6 µm in width by 2 µm in length and *P. aeruginosa* measures 0.5 µm in width by 1.5 µm in length. To prepare bacterial culture, bacteria were added to 100 mL of sterilized nutrient broth and agitated on a shaker (120 rpm) at 37 ºC during 24 h. The bacteria were separated from the nutrient broth by centrifuging for 10 min at 2800 rpm and then suspended in sterilized tap water and diluted to approximately 10^5 colony-forming units (CFU) mL^{-1}.

2.3. Impregnation of nitrocellulose membrane filters with AgNPs

The characteristics of nitrocellulose membrane filters employed (Sartorius Stedim Biotech) were: pore size 0.45 µm, thickness 130 µm, bubble point 2.4 bar and diameter 47 mm. Prior to application, AgNPs were diluted with deionized water to achieve the appropriate concentrations. Solutions of the following concentrations were prepared: 1 mg L^{-1}, 0.5 mg L^{-1} and 0.1 mg L^{-1}. The nitrocellulose membrane filters were sprayed with 250 µL of the different solutions of AgNPs. Then, the filters were dry at 60 ºC for 30 min and cooled at room temperature by 4 hour (Figure 1). The filters were characterized by scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM–EDX) using a LEO 1450 VP. This instrument equipped with an energy dispersive X-ray microanalyzer (EDAX Genesis 2000) and a Si(Li) detector allowed the analytical electron microscopy measurements. The samples were sputter coated with gold.

Figure 1

2.4. Antimicrobial activity of nitrocellulose filter impregnated with Ag NPs

The antimicrobial activity of the nitrocellulose membrane filters impregnated with AgNPs was measured by membrane filter method using APHA procedures [22]. Different solution with microorganisms were filtered through nitrocellulose membrane filter with the different AgNPs and subsequently incubated at 37 ºC for 48 h. Three replicates were performed for each sample. The retention capacity of the filter was also analyzed in the filtered liquid using the plate count method [22]. The culture of *E. coli*, *E. faecalis* and *P. aeruginosa* were performed in Chromocult Coliform Agar (Merck KGaA), Azide Agar (Merck KGaA) and Pseudomonas Agar P (Britania) respectively. In addition, control growth of different microorganisms was performed using filters without AgNPs.

2.5. Silver content in effluent

The silver loss from nitrocellulose membrane filter impregnated with AgNPs was determinate in the filtrate by ICP-OES [BAIRD (Bedford, MA, USA) ICP 2070]. The 1 m-Czerny Turner monochromator had a holographic grating with 1800 grooves mm^{-1}. The 328.068 silver wavelength was used and systems were expressed as peak height, which was corrected against the reagents blank.

3. Results and Discussion

3.1. Characterization on nitrocellulose paper impregnated with Ag NPs

Nitrocellulose filter impregnated with different AgNPs were characterized. The aggregation of AgNPs in the nitrocellulose paper was studied by SEM. Figure 2 shows SEM characterization of the nitrocellulose filters with different AgNPs and water (negative control). There, it can be seen that the adhesion of the chemical nanoparticles (Sigma Chemical, St. Louis, MO, USA) in filter fibers was less successful than the nanoparticles biosynthesized. Compounds surrounding the biosynthesized nanoparticles would be responsible of this phenomenon. Functional groups would interact with functional groups of nitrocellulose. It is possible that an interaction would occur between the carbonyl groups of proteins coated to the biosynthesized AgNPs and the nitrate groups of nitrocellulose filters (Figure 1). The macromolecules bind to nitrocellulose is not well understood, but both electrostatic and hydrophobic interactions have been suggested as possible binding mechanisms. According to van Oss et al. [23], the mechanism of binding of macromolecules to nitrocellulose by hydrophobic interaction is demonstrated by a minimum contact area of 100 Å and minimum value of free energy of adhesion (ΔG) of -1.5 kT being required for stable fixation to the matrix. An example of this is SDS-protein complexes, which are negatively charged, bind to negatively charged nitrocellulose membranes [24]. Additional support for the hypothesis that hydrophobic interactions play a dominant role in the binding of proteins to nitrocellulose membranes comes from a study that compared the binding of antibodies and other proteins to nitrocellulose membranes in acidic, neutral and basic buffers [25].

In Figure 3, the different deposited AgNPs on the nitrocellulose filters was also evidenced using EDS (energy dispersive spectrometry). The presences of Ag peaks were observed in greater proportion in the nitrocellulose filters with biosynthesized AgNPs (Figure 3b, 3c and 3d) than commercial AgNPs (Figure 3a). These results confirmed micrographs obtained by SEM.

In order to analyze the dispersion of the nanoparticles on the nitrocellulose filters, an Ag mapping was performed (Figure 4). As can be observed, the higher silver loading retained is observed in Figure 4b, 4c and 4d; in agreement with that seen in Figures 2 and 3.

Figure 2

Figure 3

Figure 4

3.2. Antibacterial effect of AgNPs in nitrocellulose filter

E. faecalis and *E. coli* are considered to be indicator organisms of fecal contamination. Moreover, in costal marine waters, enterococci are the preferred fecal indicator [26]. On other hand, *P. aeruginosa* is an opportunistic pathogen and is capable of growing in water and colonizing various surfaces including water pipes [27].Thus, these bacteria were selected to evaluate the effectiveness of the system.

After filtration, the filtered liquids were analyzed by plate count method [28]. In all cases the bacterial growth was negative, since the bacteria were retained on the filter also in the case of nitrocellulose filter without nanoparticles. But the control nitrocellulose membrane filter (without silver particles) did not show any inhibitory activity against the pathogen bacteria when the count of pathogens was performed with the retained by the filters. Table 2 show the degree of inhibition of filters impregnated with the different AgNPs. The bacterial growth is represented with the plus sign (+), and growth inhibition is shown with a minus sign (-). It is noted that the inhibition of *E. coli*, *P. aeruginosa* and *E. faecalis* was depended on the concentration and the origin of AgNPs.

Filters with nanoparticles from *R. glutinis* and *C. laurentii* were more effectives than filters with nanoparticles from *A. niger* or chemicals (Sigma Chemical, St. Louis, MO, USA), against all bacteria. But, filters with nanopaticles from *A. niger* were more effectives against *P. aeruginosa*, than filters with chemical nanoparticles.

The increased antimicrobial activity by biosynthesized AgNPs incorporated to filters, could be given by the polysaccharides arabinogalactan, arabinan and galactoglucomannan confirmed by Fourier transform infrared spectroscopy (FTIR) analysis (data not shown). These polysaccharides could contribute to the enhancement of the antibacterial activity of the biosynthesized AgNPs due to its ability to anchor the microbial cell wall and then penetrate it, thereby causing structural changes that affect the permeability of the cell membrane and cause cell death [29, 30]. In addition, the size and surface area of the nanoparticles are closely related since with decreasing size increases the surface area of biosynthesized AgNPs leaving a greater number of atoms exposed on the surface, which will be available interactions with cells [31, 32]. For example, the smaller particle size of AgNP-Rho (see Table 1) contributes to greater bactericidal activity. Moreover, this also justifies the best antimicrobial activity of AgNP-Asp regarding AgNP-Com. Although both have similar particle sizes, the polymers associated with the biosynthesized AgNPs allowed a better bactericidal activity.

According to US EPA standards, for a water purification device to meet, it must reduce the bacteria colony count by over 5 orders of magnitude [33-37]. In this context, biosynthesized AgNPs inhibited bacterial growth at level of concentration equal 1 mg L^{-1}. Moreover, AgNPs-Rho obtained the same results with a concentration of 0.5 mg L^{-1}.

It is necessary to emphasize that the tested AgNPs have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria. This irreversible inhibition of bacterial growth is desirable to prevent bacterial colonization of drinking water sources. In addition, all the impregnated filters preparations were perfectly stable for at least 8 month.

Table 2

3.3. Leaching of silver ion released from AgNPs

The content of silver was analyzed in the effluent water by ICP-OES. The maximum average concentration of Ag^+ leached from Ag NPs in the liquid filtrate were 16.10^{-5} mg L^{-1}, $2\ .10^{-5}$ mg L^{-1}, 7.10^{-5} mg L^{-1} and 1.10^{-5} mg L^{-1} for AgNPs-Com, AgNPs-Asp, AgNPs-Cry and AgNPs-Rho respectively. According to World Health Organization (WHO) [1], higher levels of silver, up to 0.1 mg L^{-1} (a concentration that gives a total dose over 70 years of half the human with no observed adverse effect level of 10 g), could then be tolerated without risk to health. In all case; levels of silver were below the limit recommended by WHO in relation to human health.

Furthermore, the average concentration of Ag^+ in the liquid filtrate of commercial AgNPs was an order of magnitude higher than biosynthesized AgNPs. Probably the major adherence to the biosynthesized AgNPs could be due to the protein and carbohydrate groups, encouraging a greater interaction with nitrocellulose membrane filter.

3.4. Stability of AgNPs and porosity of the nitrocellulose membrane filter

In order to establishing the stability of filters impregnated with AgNPs, a tap water solution was prepared with *E. feacalis* at a concentration of 10^5 CFU mL^{-1}. Different levels of the solution (0.5, 1, 3, 5 and 7 L) were filtered at a flow of 25 mL min^{-1}. The filters used were impregnated with AgNPs-Asp. Subsequently, the filters were incubated for 48 hours. No growth was observed on any of the filters. These results showed a significant stability of impregnated filters, making them cheap and reliable for purifying water.

Another test was conducted to evaluate if AgNPs will affect the porosity of the nitrocellulose membrane filter. In this context, the different membrane filters were analyzed by “bubble point method”, to detect the largest pores in a membrane filter. The analysis was performed using Bubble Point Tester "it-BP-HP” (measure range 200-7500 mbar), wetted with water. According to the results observed in Table 3, the pores sizes were slightly reduced by applying of different nanoparticles, especially by biosynthesized nanoparticles. However, using ANOVA test, there were no significant differences ($p < 0.05$) between the groups with respect to commercial nitrocellulose membrane filter without nanocomposites.

Table 3

4. Conclusions

In general, biosynthesized AgNPs had a higher antimicrobial activity than commercial AgNPs. The coated of polysaccharides on the surface of the biosynthesized AgNPs contributed to a better antimicrobial activity. The content of silver particles in the effluents water was below the limit recommended by WHO. The higher antimicrobial effect was observed for AgNPs- Rho, which is related not only to the smallest particles, but also to polysaccharides groups that surrounding the particles. Moreover, in our study, complete inhibition of bacterial growth was observed on nitrocellulose membrane filters impregnated with 1 mg L^{-1} of biosynthesized AgNPs. This concentration was able to reduce the bacteria colony count by over 5 orders of magnitude, doing suitable for a water purification device.

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Highlights

A new method including nanotechnologies was used for water treatment.

An impregnated filter with commercial and biosynthesized AgNPs was used.

Antibacterial effectiveness was evaluated in water samples according to WHO.

Biosynthesized AgNPs reduced the bacteria colony count.

Antimicrobial activity could be given by polysaccharides over silver nanoparticles.

*Graphical Abstract (for review)

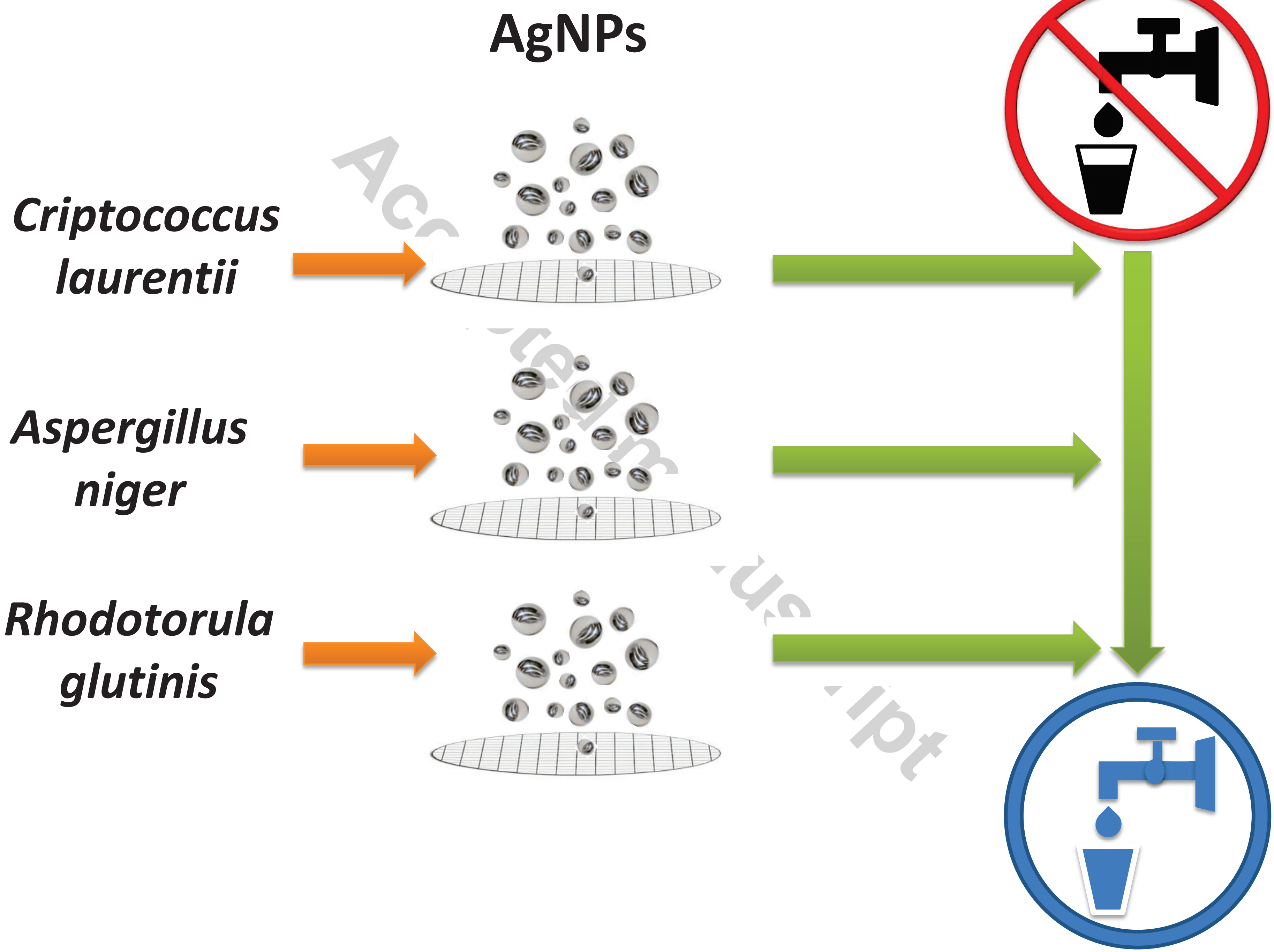


Table 1. Principal characteristics of AgNPs used

Origin	Size (nm)	Compounds surrounding nanoparticles
Chemical	40 ± 4	None
Aspergillus niger	40 ± 5	High concentration of proteins and low concentration of carbohydrates
Cryptococcus laurentii	35 ±10	High concentration of carbohydrates type arabinogalactan. Low concentration of proteins.
Rhodotorula glutinis	15 ± 8	High concentration of carbohydrates type arabinan and galactoglucomannan

Table 2. Bacterial growth exposed to different concentration and type of AgNPs.

	Ag NPs-Asp[a]			**Ag NPs-Rho**[a]			**Ag NPs-Cry**[a]			**Ag NPs-Com**[a]		
	1	0.5	0.1	1	0.5	0.1	1	0.5	0.1	1	0.5	0.1
E. coli	-	-	+	-	-	+	-	-	+	-	+	+
E. faecalis	-	+	+	-	-	+	-	-	+	-	+	+
P. aeruginosa	-	+	+	-	-	+	-	-	+	+	+	+

[a] Concentration expressed as mg L^{-1}

Table 3. Study of pore size change of the nitrocellulose membrane filters by AgNPs, using the bubble point method.

Type of membrane filter	Bubble point (bar)
Without nanoparticles	2.48±0.01
+ AgNPs-Com	2.55±0.01
+ AgNPs-Asp	2.67±0.03
+ AgNPs-Cry	2.73±0.02
+ AgNPs-Rho	2.74±0.01

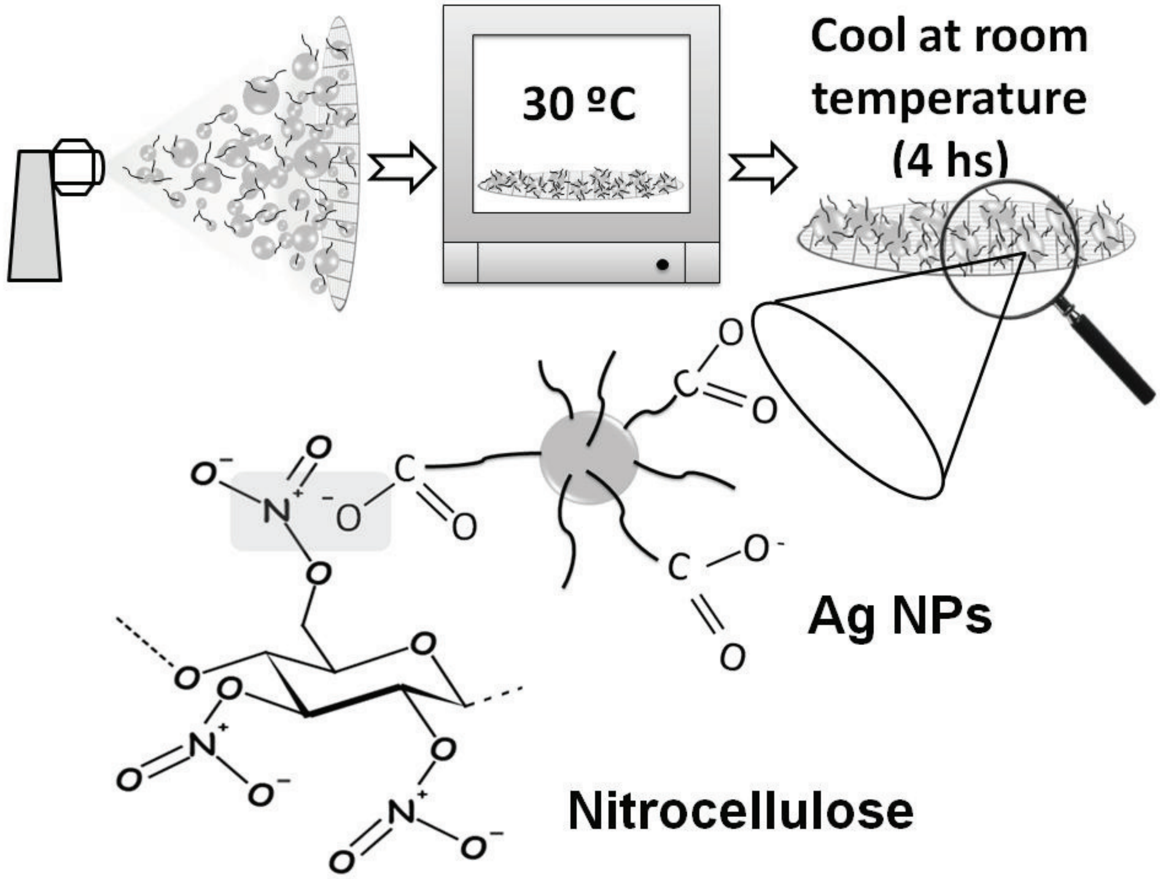
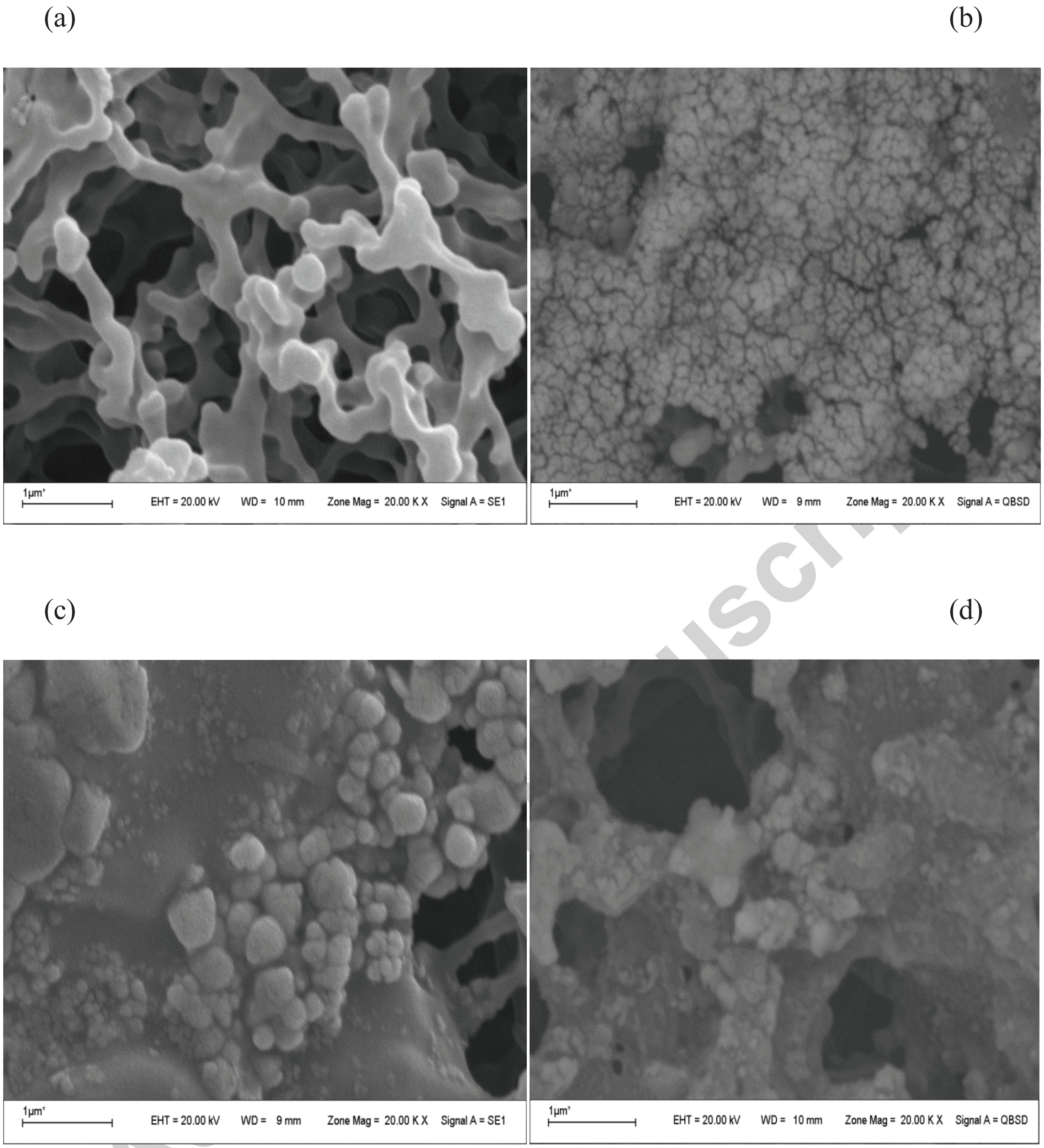


Figure 1. Scheme for possible interaction mechanism between the carbonyl groups of proteins coated to the biosynthesized AgNPs with the nitrate groups of nitrocellulose filters.

Figure 2


(a)
(b)
1µm
EHT = 20.00 kV
WD = 10 mm
Zone Mag = 20.00 K X
Signal A = SE1
1µm
EHT = 20.00 kV
WD = 9 mm
Zone Mag = 20.00 K X
Signal A = QBSD
(c)
(d)
1µm
EHT = 20.00 kV
WD = 9 mm
Zone Mag = 20.00 K X
Signal A = SE1
1µm
EHT = 20.00 kV
WD = 10 mm
Zone Mag = 20.00 K X
Signal A = QBSD

(e)

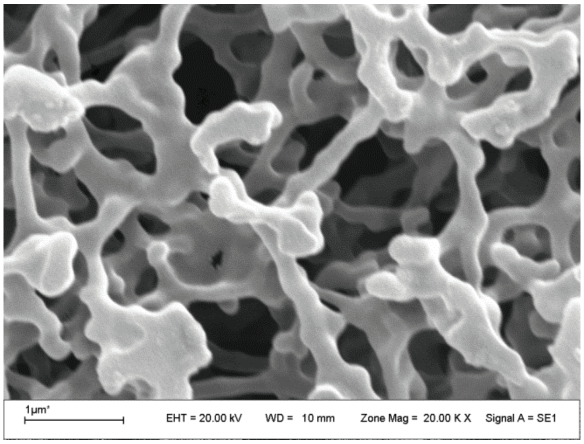
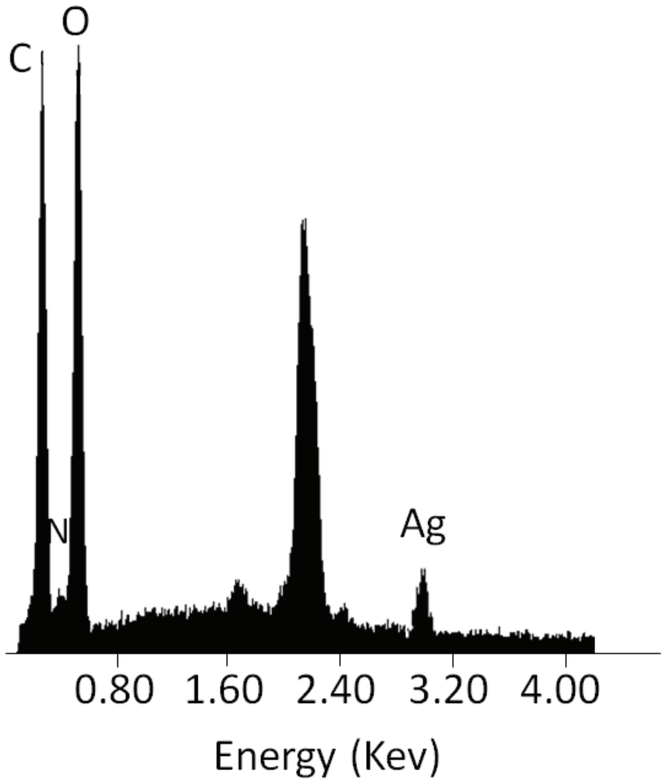


Figure 2. SEM characterization of the nitrocellulose filters with different AgNPs and water (negative control). (a) AgNP-Com, (b) AgNP-Asp, (c) AgNP-Cry, (d) AgNP-Rho and (e) negative control.

Figure 3

(a)



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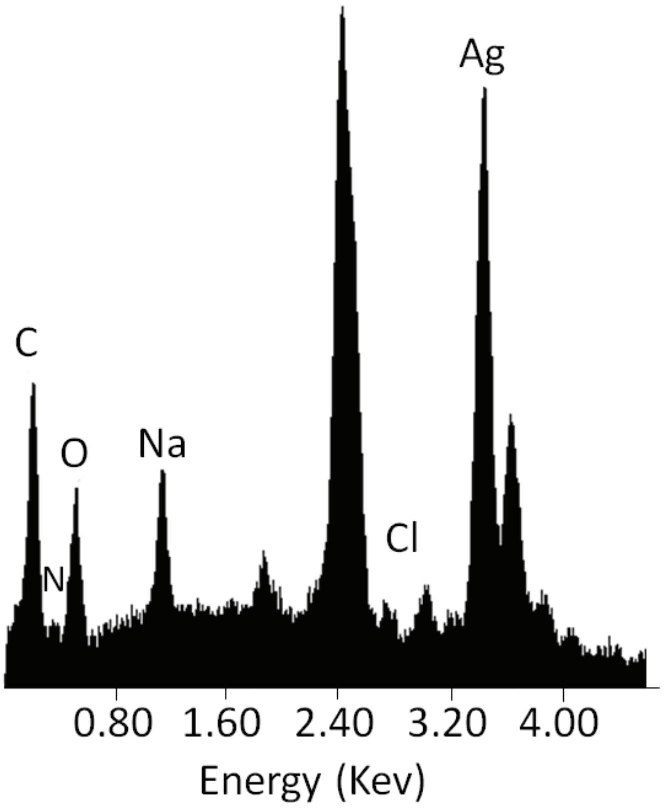

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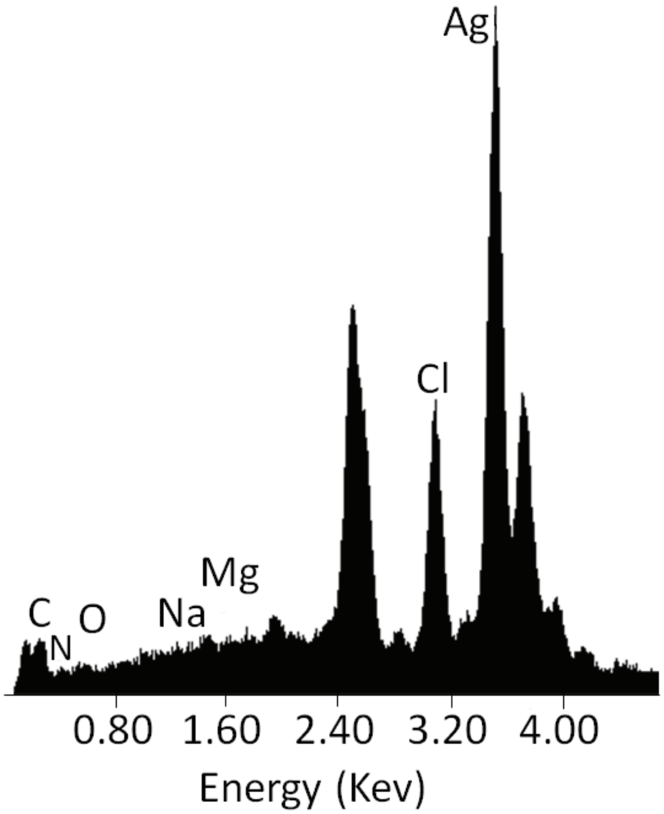
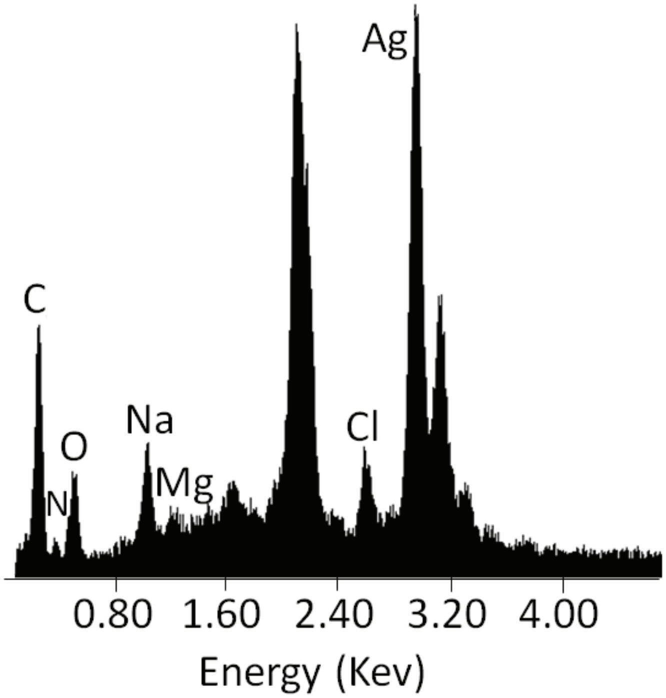


(d)



(e)

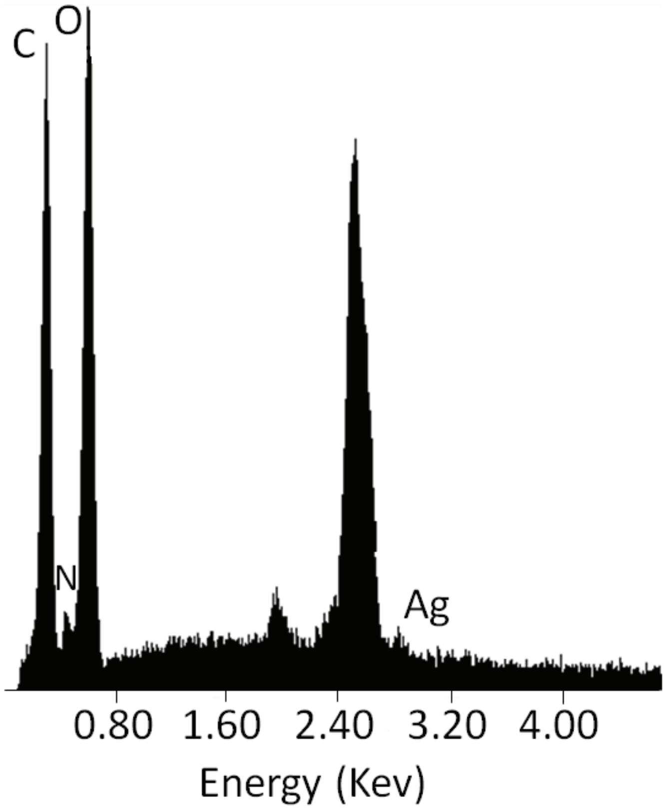
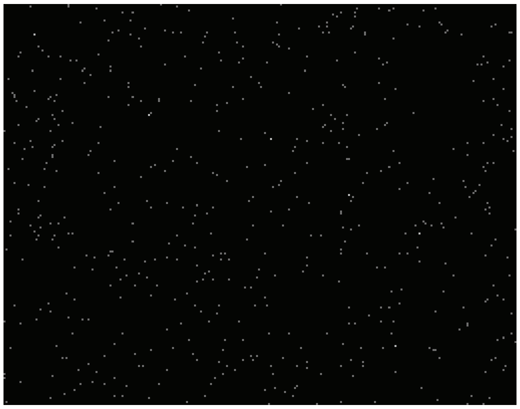


Figure 3. EDS spectra of the nitrocellulose filters with different AgNPs. (a) AgNP-Com, (b) AgNP-Asp, (c) AgNP-Cry, (d) AgNP-Rho, and (e) a negative control.

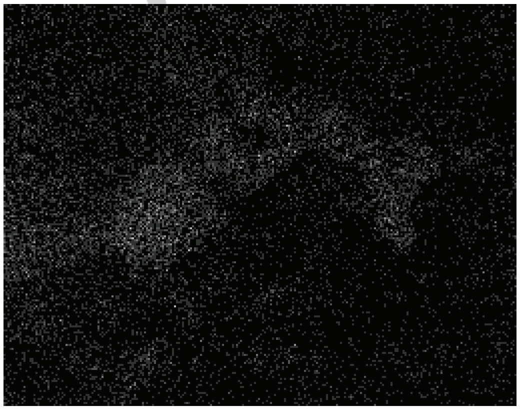
Figure 4

(a)



(b)

(c)



(d)

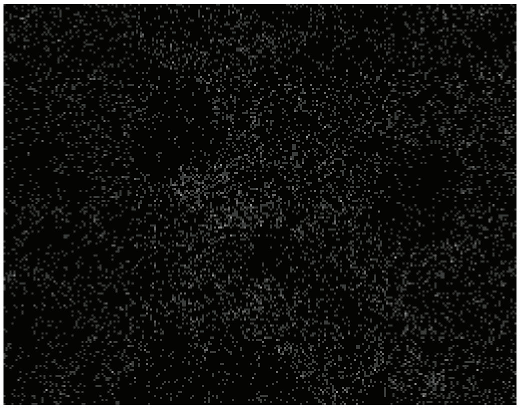


Figure 4. Silver mapping on nitrocellulose filters with different AgNPs. (a) AgNP-Com, (b) AgNP-Asp, (c) AgNP-Cry and (d) AgNP-Rho. The bright areas correspond to AgNPs.