CHEMICAL PREPARATION AND PROPERTIES OF SOME HIGH CONCENTRATED COLLOIDAL SILVER SOLUTIONS FOR ANTIMICROBIAL APPLICATIONS

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Some stable colloidal silver solutions of 600-1700 ppm silver nanoparticles (AgNps) concentration by an original and efficient chemical synthesis method were prepared. This method is based on the reduction of an aqueous silver nitrate solution with a sodium borohydride solution in the presence of a stabilizer agent of anionic polyelectrolyte type namely poly(acrylic acid sodium salt). The synthesis was carried out in proper conditions relating to concentration, temperature, pH, dropping rate and stirring. The synthesized AgNps were characterized by various techniques like: UV-Vis spectrophotometry, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and antimicrobial analyses. AgNps had a spherical shape and a maximum diameter of 32 nm indicated by TEM and DLS analyses. Moreover, the DLS analyses revealed a narrow grain size distribution of AgNps and a high stability of the solutions, showed by the zeta potential values. The antibacterial and antifungal tests proved that the prepared colloidal silver solutions have high antimicrobial properties.

INTRODUCTION

Silver (Ag) provides a good example of developing nanoparticle technology because it has a long history of use and relatively well described modes of action.[1] Comparatively with antibiotics and organic biocides that are known for certain health risks, Ag is non-toxic, skin friendly and proved efficiency against a broad range of bacteria, fungi, protozoa and mutant pathogens, including antibiotic-resistant strains causing serious illness. Ag has also antistatic, electromagnetic shielding and electrical and thermal conductive properties, which represent an advantage for its use in many applications. Silver as bioactive silver nanoparticles (AgNps) with sizes up to 10 nm presents high antibacterial and antifungal activity even at very low concentrations (0.001-0.2 wt. %) due to their stability, high surface to volume ratio and high surface energy.[2-4] Also, AgNps have anti-inflammatory and regenerative effects.[5]

Up to now, many methods for AgNps preparation were carried out. The classical ones are physical, chemical, electrochemical or photochemical reduction of metals in different media.[6-8] However, the most of these methods have limitations in controlling particle size, colloidal stabilities, industrial scale production and dispersion ability, especially. To overcome these limitations some organic surfactants or functional polymers were used to protect small particles from agglomeration or losing their surface properties and to achieve a stabilization of colloidal solutions.[9] Aggregation can be inhibited by the thick electrical double layers that form around AgNps in low-ionic-strength suspensions. For high ionic strengths or organic-phase suspensions, capping agents such as

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self-assembled monolayers, surfactants, polymers, and dendrimers can be employed to protect the particles from aggregation.[7] Moreover, the surfactants presence can also affect the growth process of nanocrystallites and allow controlling AgNps shapes and sizes.

This paper presents the research results on the obtaining of high concentrated and stable colloidal silver solutions (CSSs) by an original and efficient chemical method for antimicrobial applications.

EXPERIMENTAL

In the experiments, the following starting materials were used: silver nitrate aqueous solutions of 0.2-0.5 wt. % ($AgNO_3$, p.a., crystallized, $\geq$ 99.9 %, Fluka), poly(acrylic acid sodium salt) (PAAS) solutions of 1-4 wt. % (M_w = 5,100, Fluka), sodium borohydride solutions of 0.1 wt. % ($NaBH_4 \geq$ 96 %, M_w = 37.83, Merck) and bi-distilled water with pH of 5.5 and electrical conductivity of 5 μS at 25 °C.

The CSSs were synthesized by reduction of Ag^+ ions from an $AgNO_3$ aqueous solution with a $NaBH_4$ solution in the presence of the PAAS polyelectrolyte used as a capping agent as well as a dispersant in proper experimental conditions relating to concentration, temperature, pH, dropping rate and stirring. The PAAS solution was added drop wise to the $AgNO_3$ aqueous solution, which was previously heated at 70-90 °C and vigorously stirred. These conditions were kept up to a light yellow color appeared, that reveals the Ag nuclei formation. Then in the suspension that was rapidly cooled at 4 °C to protect AgNps against uncontrolled growing and flocculation, the $NaBH_4$ solution was slowly added drop wise and kept for 0.5 h under vigorously stirring. The $NaBH_4$:$AgNO_3$ molar ratio was of 2:1.

For the AgNps characterization, some tests such as UV-Vis spectroscopy using a V570 (Able & Jasco, Japan) apparatus, transmission electron microscopy (TEM) using a CM 100 (Philips, Holland) microscope and dynamic light scattering (DLS) technique using a 90 Plus apparatus with BI-Zeta option (Brookhaven, USA) were carried out. For TEM observation, the samples were prepared using a drop of CSS on a carbon-coated copper grid and allowing the drop to dry completely in a vacuum desiccator. For the UV-Vis absorbance measurements, a quartz cuvette filled with 3 mL of concentrated CSS and a quartz cuvette filled with 3 mL of bi-distilled water as reference were used. For the DLS measurements, a plastic cuvette filled with 3 mL of CSS sample was used. The 90 Plus apparatus is equipped with a high power diode laser of 35 mW that provided the light source to illuminate the CSS sample contained in the cuvette. A detector whose position was of 90° was used to measure the scattered light to record the grain size distribution, average diameter of AgNps and polydispersity index. The zeta potential was measured with BI-Zeta option.

For the antifungal assays of the CSSs carried out according to the standards SR EN 60068-2-10:2006 and SR EN 1275:2006, the antibiogram method was used upon the following germs: Aspergillus niger, Aspergillus flavus, Penicillium glaucum, Penicillium funiculosum, Paecilomyces variotii, Chaetomium globosum, Myrothecium verrucaria, Scopulariopsis brevicaulis, Stachybotrys atra and Trichoderma viride. A sterile filter paper disc with diameter of 10 mm was immersed in the CSSs. As inoculum, a suspension of the above-mentioned spores in the complete Czapek - Dox mineral salts solution (with sucrose as an additional source of C) was used. The samples were incubated in a thermostat with humidification at the temperature of 30 ± 2 °C and air relative humidity of 90-100 %. The moulds growth was monitored 28 days using a stereomicroscope with magnification of 50x. The antifungal activity was evaluated by measuring (in mm) the size of the zone of no fungal growth called inhibition zone (I.Z.) around each sample of filter paper disc impregnated with CSSs.

For the antibacterial assays of the CSSs carried out according to the standard SR EN 1040:2006, the agar microdilution method was used upon the following bacterial strains: Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Serratia marcescens, Enterobacter cloacae, Acinetobacter baumannii, Escherichia Coli ESBL and Klebsiella pneumonia, to find out the Minimum Inhibitory Concentration (MIC), which is the lowest concentration of AgNps that inhibits the visible growth of the test strains.

RESULTS AND DISCUSSION

It is well known that the absorbance of AgNps depends mainly upon particle size and shape, dielectric medium and chemical surroundings.

The physical origin of the strong light absorption by noble metal nanoparticles is the coherent oscillation of the conduction band electrons induced by interaction with an electromagnetic field. These resonances are known as surface plasmons and are associated with both a kind of quasiparticle and a surface effect because they are absent in the individual atoms as well as in the bulk.[10]

Ag is the only material whose surface plasmon resonance (SPR) can be tuned to any wavelength in the visible spectrum. The SPR peaks number decreases as the symmetry of the AgNps increases.[11]

The UV-Vis spectra of the AgNps showed a well defined surface plasmon band centered at around 422-424 nm (Fig. 1), which is the characteristic of AgNps and clearly indicated the formation of nanoparticles in CSSs. Also, the absorption band in visible region and plasmon peak at 452-453 nm is typical for AgNps. The absorbance peaks from 344-349 nm correspond to the wavelength at which the real and imaginary parts of the dielectric function of Ag almost vanish.[12] The plasmon bands are broad with an absorption tail in the longer wavelengths, which could be in practice due to the AgNps size distribution.
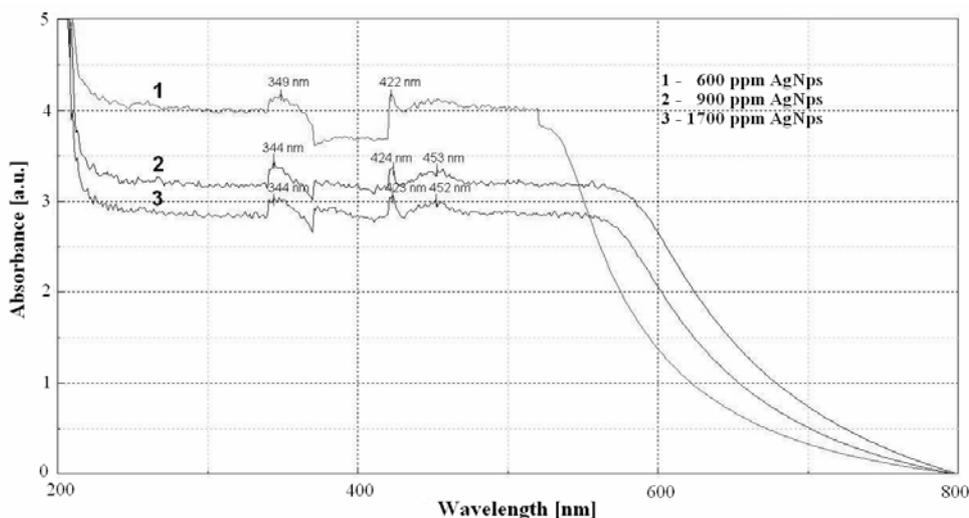
Fig. 1 – UV-Vis absorbance spectra of the CSS
of 600 ppm AgNps (1), 900 ppm AgNps (2) and 1700 ppm AgNps (3).

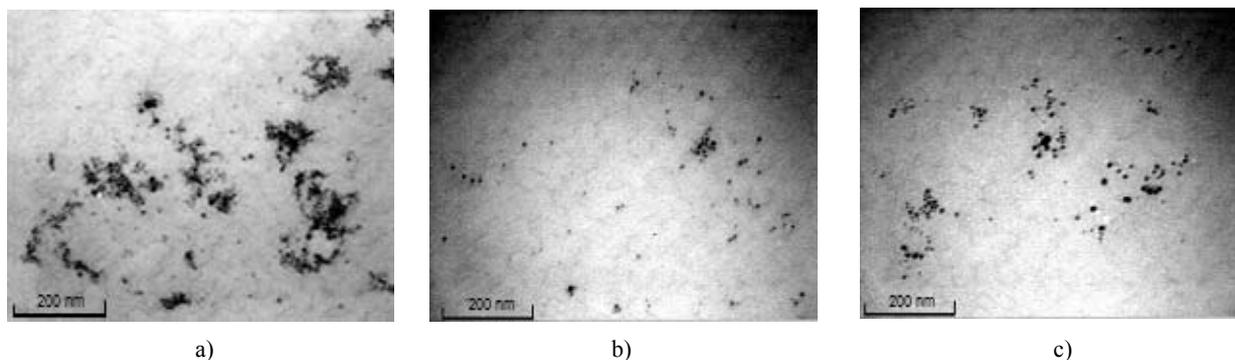a)   b)   c)

Fig. 2 – TEM images of the CSS of:
a) 600 ppm AgNps, b) 900 ppm AgNps, c) 1700 ppm AgNps.

The color of the all CSSs was of dark reddish brown. The origin of this color is the result of the occurrence of the strong absorption at the SPR peaks when the frequency of the electromagnetic field becomes resonant with the coherent electron motion.

Absorbance is directly proportional to concentration of particles and it is decreasing with the increasing concentration of AgNps. The wavelengths of the peaks are very little differentiated for the CSSs while the absorption intensities are higher in the case of the CSS of 600 ppm AgNps.

Fig. 2 presents typical bright-field TEM images of the AgNps from the CSSs.

The TEM analysis revealed polydispersed AgNps with a spherical shape and a maximum diameter of the AgNps of 32 nm, and an average diameter of 4 ± 2 nm for the CSS of 600 ppm AgNps, of 9 ± 2 nm for the CSS of 900 ppm AgNps and of 20 ± 2 nm for the CSS of 1700 ppm AgNps.

In Fig. 3 the grain size distributions of the AgNps from the CSSs are presented.

The DLS measurements revealed a grain size distribution of AgNps in a narrow range for the all CSSs.

The polydispersity index that is a measure of the size ranges present in the CSS was of of 0.259 for the CSS of 600 ppm AgNps, of 0.291 for the CSS of 900 ppm AgNps and of 0.328 for the CSS of 1700 ppm AgNps.

The AgNps diameter that is measured in DLS, which is also called the hydrodynamic diameter is that of a sphere that has the same translational diffusion coefficient as the particle being measured. This coefficient depends not only on the size of the particle "core", but also on any surface structure, as well as the concentration and type of ions in the medium.[13]
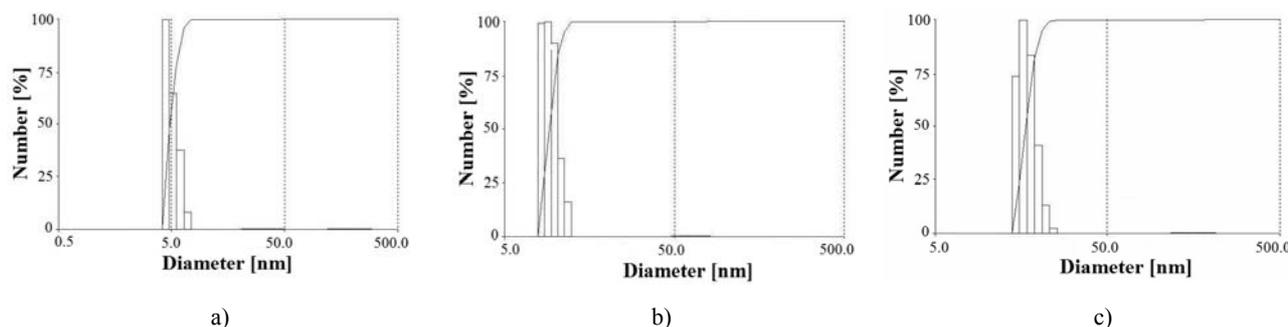
Fig. 3 – Grain size distribution of the AgNps from the CSS of:
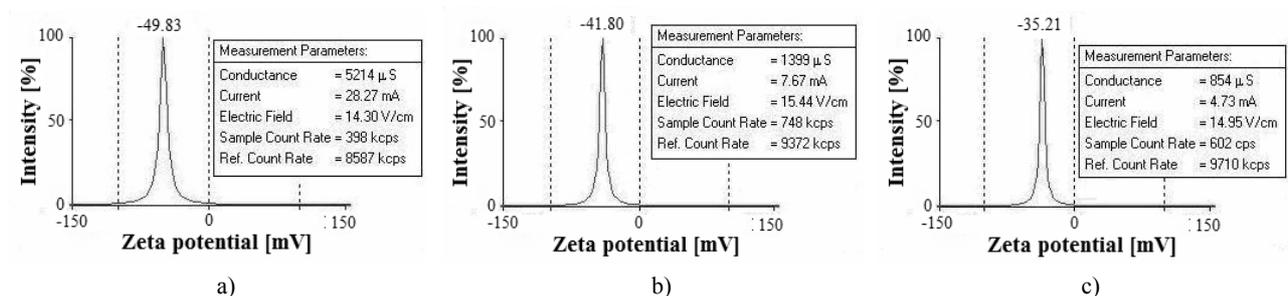a) 600 ppm AgNps, b) 900 ppm AgNps, c) 1700 ppm AgNps.

Fig. 4 – Zeta potential of the the CSS of:
a) 600 ppm AgNps, b) 900 ppm AgNps, c) 1700 ppm AgNps.

The AgNps average hydrodynamic diameter is of 6.3 ± 1.2 nm for the CSS of 600 ppm AgNps, of 14.3 ± 1.5 nm for the CSS of 900 ppm AgNps and of 25.5 ± 1.8 nm for the CSS of 1700 ppm AgNps. It is observed the tendency for growing AgNps with the increase of concentration.

The AgNps hydrodynamic diameter is a little bit higher than the one measured by TEM, where the AgNps are removed from their native environment.

The difference between the AgNps size data obtained by TEM and the ones obtained by DLS can be explained by a tendency of the DLS measurements toward larger particles.

In the first stage of synthesis, by heating, in the aqueous solution, the polyacrylate anion couples easily the silver cations, Ag^+, by electrostatic attraction forces, catching them like in a cage with small volume. In these nanoreactors reduction reactions of Ag^+ cations in AgNps germs or nanoclusters can be driven, which remain highly protected by the polymeric chain.

Since the PAAS polyelectrolyte is of a weak type, the first stage of synthesis was a soft one. Therefore, a second stage of reduction was performed with a strong reductive agent such as $NaBH_4$ in order to obtain uniform and size controllable AgNps.

The zeta potential is determined by the overall charge a particle acquires in a specific medium. It is a measure of the repulsive forces in electrostatically-stabilized systems, being an excellent indicator of relative stability. The sign and magnitude of zeta potential, as a function of pH, salt, or dispersing agent concentration are indicators of stability against flocculation or coagulation. The colloidal suspensions with zeta potentials more negative than -30 mV and more positive than +30 mV are normally considered stable.[14]

Fig. 4 presents the zeta potential curves of the CSSs, where the pH of the CSSs was of 8.7-9.3.

The zeta potential values were of -49.83 mV for the CSS of 600 ppm AgNps, of -41.80 mV for the CSS of 900 ppm AgNps and of -35.21 mV for the CSS of 1700 ppm AgNps. Since these values are more negative than -30 mV, it means that the all CSSs are stable.

The high stability of the CSSs is the result of the chemical method used for the AgNps synthesis that controls the size and the dispersion of the AgNps by reducing the surface energy through the stabilization with electrosteric repulsion that is assured by the PAAS.

The results from the antifungal assays of the CSSs are presented in Table 1. The image of a blank filter paper disc with diameter of 10 mm used for immersion in the CSSs in the antifungal assays is shown in Fig. 5. In Figs. 6-8 are shown the images of the samples of filter paper discs immersed in the CSSs after 3 days, 7 days and 28 days from the moulds inoculation. It was observed that after 3 days, respectively 7 days from the moulds inoculation the all CSSs have a high fungistatic effect (no growth apparent under the microscope) and an inhibition zone of 2-7 mm (after 3 days) and 1-4 mm (after 7 days), with the highest inhibition zone of 2-7 mm (after 3 days) and 4 mm (after 7 days) for the CSS of 600 ppm AgNps. After 14 days from the moulds inoculation, the all CSSs still have a high fungistatic effect (no growth visible without magnifying apparatus, but clearly visible under the microscope), but a growth of Trichoderma viride was observed under the microscope for the all CSSs. Additionally the CSS of 600 ppm AgNps presented a growth of Aspergillus species and

Myrothecium verrucaria and the CSS of 1700 ppm AgNps presented a growth of Penicillium species. The inhibition zone of the moulds growth is slightly decreased with 0.5-2 mm, proving in this way the decrease of the fungistatic effect on time. After 28 days from the moulds inoculation, the all CSSs maintained the high fungistatic effect, the best antifungal property being achieved for the CSS of 600 ppm AgNps, which has the smallest average diameter of AgNps.

The results of the antibacterial assays carried out on the CSSs showed that the AgNps formed in all CSSs exhibited good antibiotic activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Enterobacter cloacae, Acinetobacter baumannii, Klebsiella pneumonia) pathogens, proved by the obtained MIC values of up to 18.8 ppm AgNps against the majority of the bacterial strains and of up to 75 ppm AgNps against the Pseudomonas aeruginosa ATCC 9027.

Table 1

Antifungal properties of the CSSs

Sample	Behavior at moulds action							
	Growth rating*				Inhibition zone [mm]			
	3 days	7 days	14 days	28 days	3 days	7 days	14 days	28 days
CSS of 600 ppm AgNps	0	0	1	1	2-7	4	2	2
CSS of 900 ppm AgNps	0	0	1	1	2-4	3	2	2
CSS of 1700 ppm AgNps	0	0	1	1	2	1.5	1	1

* according to the standard SR EN 60068-2-10:2006 the growth rating of 0 means no fungal growth apparent under the microscope (magnification 50x) and of 1 means no fungal growth visible without magnifying apparatus, but clearly visible under the microscope.
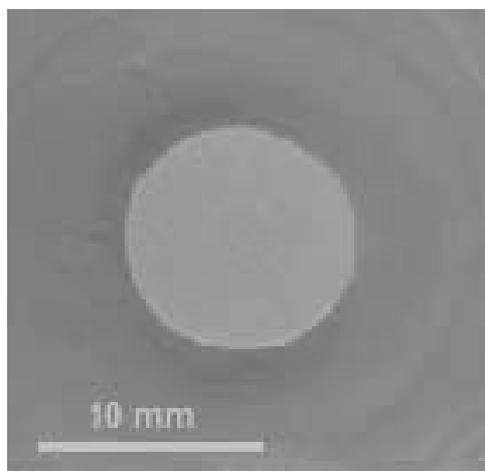

Fig. 5 – Image of a blank filter paper disc with diameter of 10 mm
used for immersion in the CSSs in the antifungal assays.

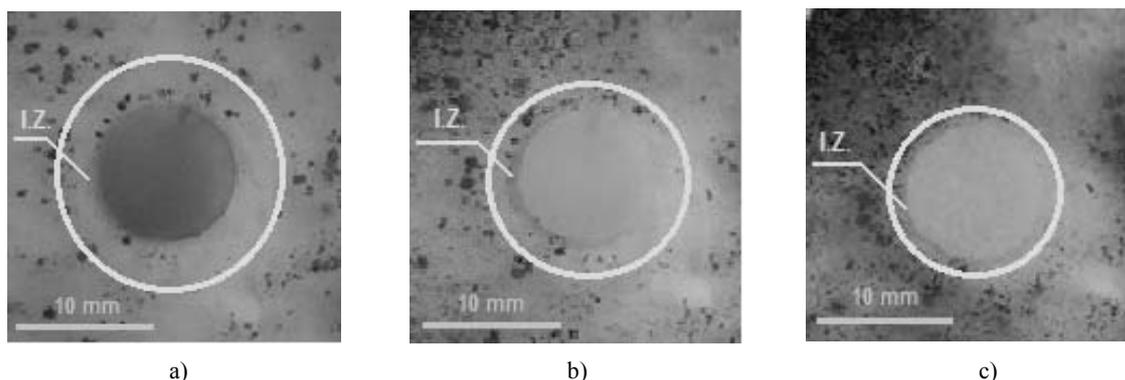a)   b)   c)

Fig. 6 – Images of the sample of filter paper disc immersed in the CSS of 600 ppm AgNps after: a) 3 days,
b) 7 days and c) 28 days from the moulds inoculation, where I.Z. is the inhibition zone around the sample.

a)   b)   c)
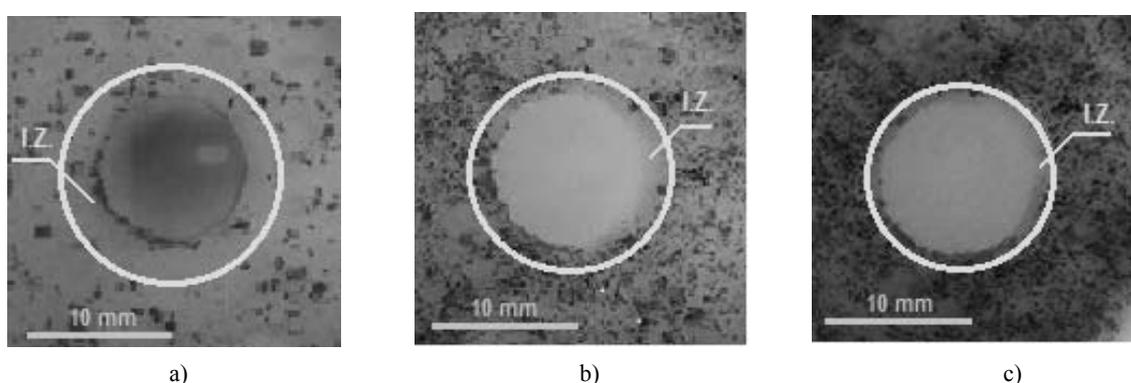
Fig. 7 – Images of the sample of filter paper disc immersed in the CSS of 900 ppm AgNps after: a) 3 days,
b) 7 days and c) 28 days from the moulds inoculation, where I.Z. is the inhibition zone around the sample.

a)   b)   c)
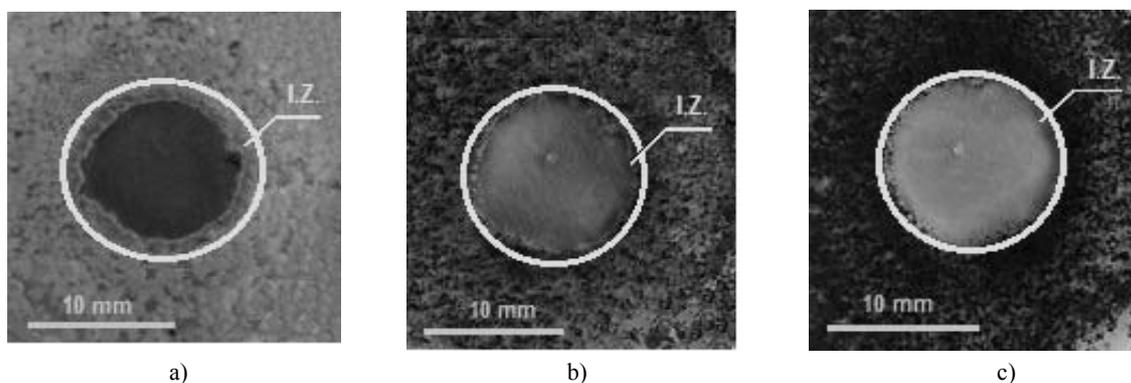
Fig. 8 – Images of the sample of filter paper disc immersed in the CSS of 1700 ppm AgNps after: a) 3 days,
b) 7 days and c) 28 days from the moulds inoculation, where I.Z. is the inhibition zone around the sample.

CONCLUSIONS

An efficient chemical synthesis method for high concentrated CSSs obtained using the PAAS as a stabilizer agent was carried out. Owing to the electrosteric repulsion characteristic of PAAS, the prepared CSSs exhibited a remarkable dispersion stability, which was confirmed by zeta potential measurements.

The synthetized CSSs were characterized in terms of UV-Vis absorbance, stability, shapes, grain size distribution and average diameter of AgNps. Also some antimicrobial tests were performed.

The UV-Vis absorbance measurements showed characteristic SPR peaks of AgNps at wavelengths of 422-424 nm and 452-453 nm. The TEM analysis revealed polydisperse AgNps with

spherical shape and size of 4-20 ± 2 nm. The DLS measurements showed AgNps with a narrow grain size distribution (polydispersity index of 0.259-0.328), a small average diameter (from 6.3 ± 1.2 nm to 25.5 ± 1.8 nm) and a negative charged surface with zeta potential ranges between -49.83 mV and -35.21 mV.

The biocidal AgNps, due to their small particle size obtained by synthesis, gave rise to a high surface area of AgNps and consequently to a high antimicrobial activity of the CSSs. The results of the antibacterial and antifungal tests proved the high biocidal activity of AgNps against a broad spectrum of moulds and strains.

These excellent properties recommend AgNps for using in antimicrobial applications from different fields such as medicine, biology, consumer goods, cosmetics and health care.

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