

Antimicrobial colloidal silver solutions. Preparation and characterization

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Colloidal silver solutions involving electrochemical technique, through "sacrificial" Ag electrodes method have been prepared, using different stabilizers and co-stabilizers. A constant current pulse generator, model CS1-12 with stirrer has been used at different working times. The effects of various surfactants content on the Ag concentration (ppm) and UV-Vis spectra for silver nanoparticles were determined with a spectrophotometer UV-Vis Jasco V500; the nanoparticles sizes have been analyzed through DLS (Dynamic Light Scattering) technique involving a Zetasizer Nano equipment and their morphology has been evidenced by TEM. To evaluate antimicrobial effect antibiogram method has been used, involving various test fungi containing different micotoxines. Using this method a stable nano-Ag based colloidal solutions until 35 ppm Ag were obtained and colloidal solutions have good antimicrobial properties.

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1. Introduction

In recent years, antibacterial agents that have been used on large scale have included quaternary ammonium salts, metal salt solutions and antibiotics. Recently, the photoinduced bactericidal activity of TiO₂ thin films has been demonstrated [1]. Unfortunately, some of these agents are toxic or poorly effective, which makes them not suitable for applications in human and veterinary medicine, pharmacology, food industry, water purification and so on. In contrast, silver is a non-toxic, tolerant disinfectant that can reduce significantly many bacterial infections [2,3]. Polymeric or fibrous nano-composites of polymers with silver nanoparticles have been reported to have special properties [4,5]. Antibacterial properties of padded PP/PE nonwovens incorporating nano-sized silver were reported [6]. Beside silver nanoparticles with very small sizes (< 20 nm) and high dispersion, used in composite materials, colloidal silver solutions can be used like antibacterial agent. Recently, various antibacterial finishes and disinfecting techniques have been developed for all types of textiles, cellulosic and synthetic [7]. Authors reported antibacterial efficacy against *S.aureus* and *K.pneumoniae* by a padding process with silver colloidal solution on textile fabrics.

The present paper presents some aspects regarding the preparation and characterization of colloidal silver solutions and their antimicrobial effect. Colloidal silver solutions involving an electrochemical method have been prepared, the technique offering the advantage of a high purity of final formed solution, especially for bio-medical applications. In order to decrease dispersion in particle size during electroformation, two important principles should be taken into account. Firstly, the crystal seed formation has to occur spontaneously, thus preventing progressive nucleation. Secondly, the crystal growth has to be conducted at a slow rate, i.e. at low current density [8].

There are two electrochemical ways to obtain silver colloidal solutions, respectively: (i) by chemical reduction of metal salts, usually AgNO₃ and (ii) by using silver electrodes in deionised water, the so-called "sacrificial anode method".

2. Experimental

2.1. Synthesis of colloidal silver solutions

The synthesis of colloidal silver solutions was performed involving a constant current pulse generator, model CS1-12 with stirring and alternating polarity. To ensure small particle size, pulsed high frequency waveform was used. Electrodes are made of Ag of 99.999 purity, with sizes of 155 / 27 mm and they are introduced in ultra pure deionised water with C < 1µS. The working parameters of the silver colloidal solution in the electroforming process have been varied in the range of 1-5 mA for the applied direct current and between 3-24 hours for the process time. To prepare stable colloidal solutions with an optimum content of silver nanoparticles, different stabilizers and co-stabilizers agents have been tested, respectively PVP [poly(N-vinylpyrrolidone)], Naphthalene sulphonate, Na-lauryl sulfate.

A schematically representation of the device used for nano-colloidal silver solutions preparations is shown in Fig. 1.

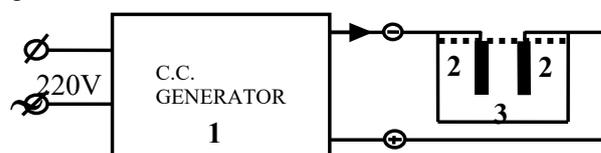


Fig. 1. Scheme of the electrochemical device for colloidal silver obtention. 1 Constant current pulse generator CS1-12; 2 Silver electrodes of 99,999% purity; 3 Deionized water ultra pure

2.2. Characterization of silver colloidal solutions

The Ag concentration of the obtained colloidal solutions has been determined through UV-VIS spectra recording, involving a JASCO V 500 spectrophotometer. The role of stabilizers (PVP) and co-stabilizers (N-naphtalenesulfonate, Na-lauryl sulfate) was also studied by means of spectrophotometer. The nanoparticles sizes have been analyzed through DLS (Dynamic Light Scattering) technique involving a Zetasizer Nano equipment and their morphology has been evidenced by TEM micrographs using an electronic microscope Philips CM 100. The specimens for TEM investigations have been prepared through deposition on 400 mesh grids with formvar film. To evaluate antimicrobial effect, antibiogram method has been used, involving various test fungi containing different micotoxines (e.g. aflatoxine). According to this method, Petri vessels with agarized Czapek Dox medium were inoculated, by spraying, with fungi mix from the following species: *Aspergillus*, *Penicillium*, *Trichoderma*.

3. Results and discussion

3.1. Role of stabilizers

In order to obtain stable colloidal silver solutions, different types of surfactant agents have been used, namely: PVP[poly(N-vinylpyrrolidone)] with molecular weight of 10000, 25000 and 55000 as stabilizers and N-naphtalenesulphonate (Na-NS), Na-lauryl sulfate (Na-LS), as co-stabilizers. Applying concentrations of 3 – 20 g/l PVP and 0.25 – 1g/l Na-naphtalene sulphonate or Na-lauryl sulfate, stable colloidal nanosilver solutions were obtained with concentrations up to 35 ppm, having a light yellow towards dark orange color as a function of the total Ag particles content. Many experiments were performed with various concentrations and electroformation durations. Table 1 shows briefly the dependence of nanosilver concentration against the type and content of PVP and electroformation period.

According to Table 1, the use of solutions with no stabilizer agent lead to colloidal Ag solutions with a low Ag content, regardless the processing period. The addition of PVP stabilizer facilitates an increase of nano Ag concentration, more evidenced for higher molecular weights of PVP. Thus the use of PVP 55 practically doubled the Ag content, as compared with blank solution, for the same duration of the process. The increase of PVP stabilizer concentration over 6 g/L, worsened the quality of colloidal solution regardless PVP molecular weight, materialized by formation of grayish appearance suggesting a growth of metallic particle size that exceeds nanometer domain.

The role of co-stabilizer nature and content was proved to have a significant influence on the nano Ag particles content in the final colloidal solution, even for relatively short electroformation periods, of 3 hours. Both additions of Na-NS and Na-LS lead to great contents of nano Ag particles in the formed colloidal solutions that attain values more than 30 ppm for 6 hours processing

time. As it is exemplified in Fig. 2 for a solution of 3 g/L PVP 10, the addition of a co-stabilizer facilitates formation of relatively high concentrated colloidal solutions. In the case of Na-NS the doubling of concentration determined an increase of Ag content from about 14 ppm to 34 ppm; the use of Na-LS also determined high contents of nano Ag particles, but with no significant influence of its concentration within the solution. It should be also mentioned that the best results from nano Ag particles concentration in the colloidal solution view point, have been obtained using PVP 10 with any of the two investigated co-stabilizers.

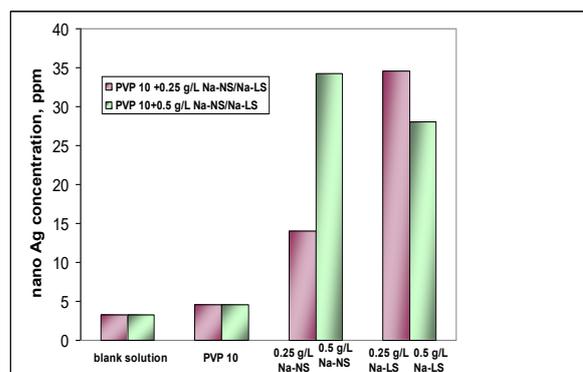


Fig. 2. Dependence of nano Ag concentration on co-stabilizer type and content, for a solution containing 3 g/l PVP 10 and 6 hours processing duration.

The protective mechanism of PVP [9,10] in the electrochemical synthesis of silver particles is generally proposed on the basis of its structural features. PVP has a structure of a polyvinyl skeleton with polar groups. The donated lone pairs of both nitrogen and oxygen atoms in the polar groups of one PVP unit may occupy two sp orbitals of silver ions in the presence of PVP [11,12]. The first stage is the formation of the Ag_m^0 -PVP complex (m is the number of silver adatoms anchored at a PVP molecule). Then PVP accelerated formation of a large amount of silver nuclei when the color becomes yellow. On the basis of Zhang and coworkers' results [13], it is inferred that the scattering of sunlight by nuclei with a diameter smaller than 10 nm is responsible for the light yellow color. The coalescence of the silver clusters during the growth process is prevented since the PVP has the role to promote silver nucleation and to prohibit grain growth and particle aggregation [13]. The steric effect arising from long polyvinyl chain of PVP on the surface of silver particles can contribute to anti agglomeration, whereas the chemical bond between the PVP and silver particles may prohibit aggregation of silver particles. Co-stabilizers are common surfactants which have amphiphilic properties due to C12 chain (lipophilic) attached to a sulfate / sulfonate group (hydrophilic). It can be concluded that stabilizers together with co-stabilizers make possible a high concentration of nano-Ag to be obtained; colloidal silver solutions are stable and had colors varying at yellow to dark – orange or red.

The influence of presence and concentration of PVP with molecular weights of 10000, 25000 and 55000 and of co-stabilizers over the concentrations of silver particle was studied through UV-Vis spectroscopy as well as the influence of electroformation time. It should be mentioned that according to the literature [9], the absorption peak at 420 nm may be assigned to Ag nanoparticles presence in colloidal solution.

Fig. 3 presents the influence of co-stabilizers concentration on nano Ag content at the same PVP 10 addition. The maximum of absorption was evidenced in the presence of 0.25 g/l Na-LS and 0.5 g/l Na-NS, in close agreement with the results presented in Fig. 2. A longer electroformation time also leads to a growth of nano Ag particles, as exemplified in Fig. 4 for solutions containing 6 g/l PVP 25 and 0.5 g/l Na-NS.

Table 1. Dependence of nano-Ag concentration in the colloidal solution against PVP molecular weight and co-stabilizer nature and content for 3 g/L PVP different electroformation durations.

No. crt	Composition	Working time (h)	Ag concentration ppm
1	Without stabilizer	3	0.87
		6	3.30
2	3g/l PVP 10	6	4.52
3	3g/l PVP 10 + 0,25 g/l Na- naphtalenesulphonate	3	8.95
		6	14.02
4	3g/l PVP 10 + 0,25 g/l Na-lauryl sulfate	3	20.72
		6	34.28
5	3g/l PVP 10 + 0,5 g/l Na- naphtalenesulphonate	3	17.50
		6	34.57
6	3g/l PVP 10 + 0,5 g/l Na-lauryl sulfate	3	17.70
		6	28.08
7	3g/l PVP 25	6	5.80
8	3g/l PVP25 + 0,25 g/l Na- naphtalenesulphonate	3	7.65
		6	15.10
9	3g/l PVP25 + 0,5 g/l Na- naphtalenesulphonate	3	11.20
		6	14.80
10	3g/l PVP 25 + 0.25g/l Na-lauryl sulfate	3	18.10
		6	19.72
11	3g/l PVP25 + 0,5g/l Na-lauryl sulfate	6	14.74
12	3g/l PVP 55	6	6.48
13	3g/l PVP55 + 0,25 g/l Na- naphtalenesulphonate	6	6.76
		16	17.90
		3	13.74
14	3g/l PVP55 + 0,5 g/l Na- naphtalenesulphonate	3	16.65
		6	19.50
15	3g/l PVP55 + 0,5 g/l Na-lauryl sulfate	6	21.5

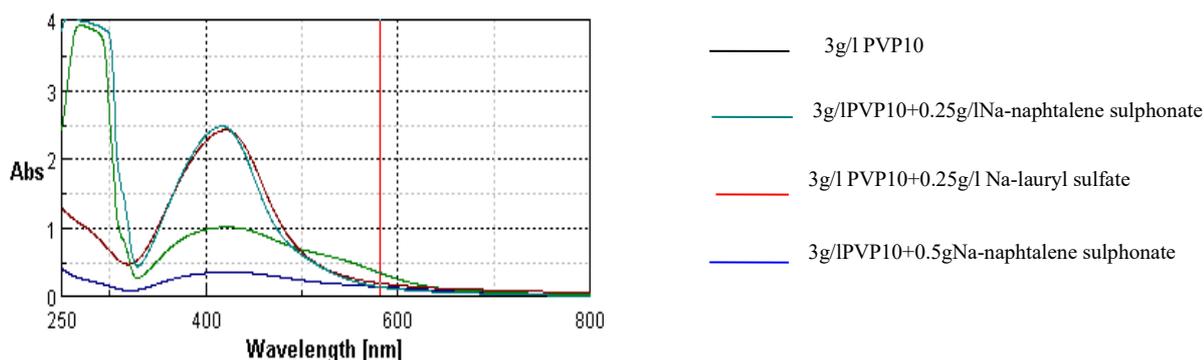


Fig. 3. UV-Vis spectra for silver nanoparticles obtained with different co-stabilizers contents at a concentration of PVP 10 of 3g/l.

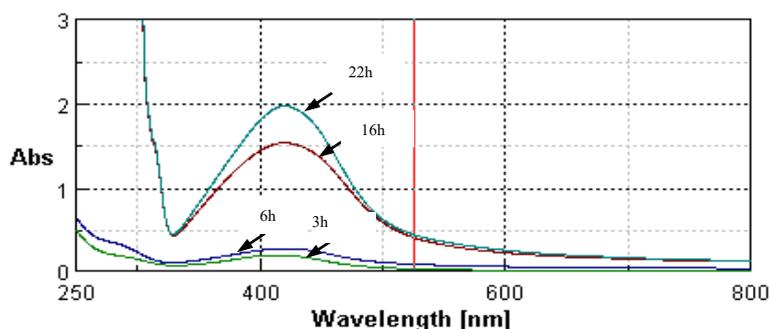


Fig. 4. UV-Vis spectra for silver nanoparticles obtained in solutions containing 6g/l PVP 25 + 0.5g/l sodium naphthalene sulphonate for various processing durations.

3.2. Size and stability of nanosilver solutions

To analyze the nanoparticles sizes their granulometric distribution has been recorded, expressed against the scattered light intensity, the particles number and their volume. The stability of obtained colloidal solutions has been determined by Zeta potential measurement that indicates if the particles present in a liquid tend to associate. For the obtained nano Ag particles based colloidal solutions Zeta potentials has not been affected either by the consequent modification of particles' sizes in time or by the slight sedimentation tendency that appears during measurement performing. According to Figs. 5, 6, 7 the obtained solutions are stable and don't exhibit sedimentation tendency (materialized by peaks displacing to the left) or agglomeration (peaks displacement to the right). This behavior evidence again the role of the used stabilizers. Particles sizes are found to be between 10-30 nm. Zeta potential distribution (see Fig. 8) is a monomodal one, with values between $-18 \div -30$ mV that suggest the particles are entirely covered by stabilizer agents and thus the solution is stable.

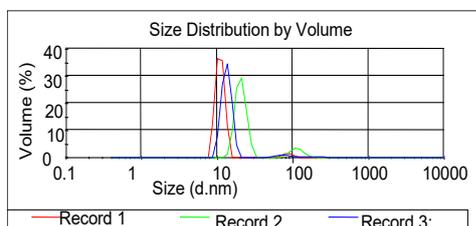


Fig. 5. Granulometric distribution of particles against their occupied volume for a solution containing 5.6 ppm Ag formed in the presence of 3 g/l PVP 25, 6 hours processing time.

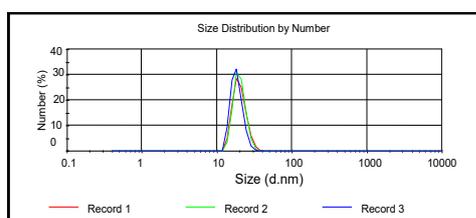


Fig. 6. Granulometric distribution of particles against their number for a solution containing 21.5 ppm Ag formed in the presence of 3g/l PVP55+ 0.5g/l Na-LS, for 6h.

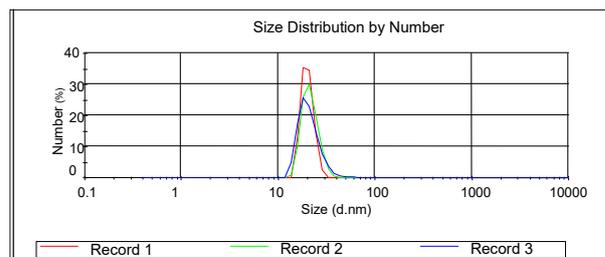


Fig. 7. Granulometric distribution of particles against their number for a solution containing 12.5 ppm Ag, formed in the presence of 6g/l PVP25+0.25 g/l Na-NS, for 6 h.

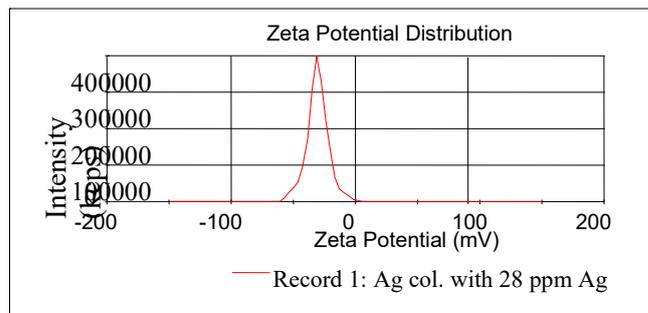


Fig. 8. Zeta potential distribution for 28 ppm Ag colloidal solution (max.: -30.5 mV).

3.3. Nanosilver particles morphology

As TEM micrographs show (see Fig. 9) the obtained colloidal solutions contain Ag nanoparticles less than 20 nm, having a spherical shape, uniformly distributed.

3.4. Antimicrobial effect

The obtained nano Ag based colloidal solutions have been tested to evidence their antifungal and antiseptic properties. According to antibiogram method, fungistatic properties are expressed by the presence and magnitude of inhibition area for mould growth around filter paper padded with Ag colloidal solution. Test fungi used contained various micro-toxines, as aflatoxine. The best results after 7 and 14 days of exposure have been evidenced for solutions with more than 15 ppm Ag that have been obtained in the presence of PVP and Na-NS/Na-LS stabilizers. Figs. 10 and 11 present photographic images of inhibition area (evidenced by the arrow) produced by a 15 ppm and 18 ppm nano Ag colloidal

solution on a mould colony containing *Aspergillus*, *Penicillium*, *Trichoderma*, after 14 days of exposure.

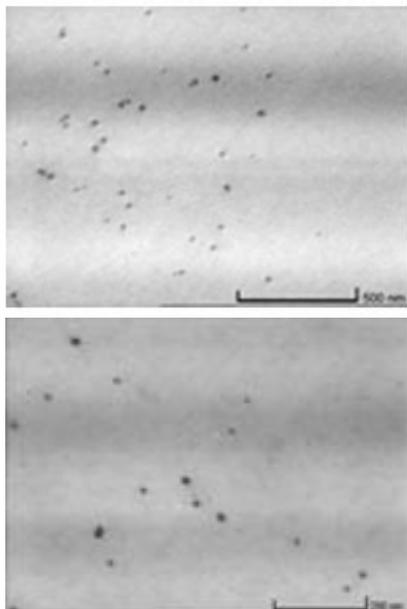


Fig. 9 TEM micrography of a colloidal solution containing 15.1 ppm Ag, obtained in the presence of 3g/lPVP25+0,25g/l Na-NS, 6h.



Fig. 10 Photographic image of an inhibition zone produced by a silver colloidal solution with 15 ppm Ag (made with PVP and Na 1- naphthalene sulphonate) against a mouldiness cologne, containing *Aspergillus*, *Penicillium*, *Trichoderma*, after 14 days.

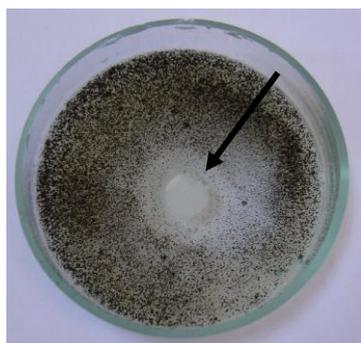


Fig. 11 Photographic image of an inhibition zone produced by a silver colloidal solution with 18 ppm Ag (made with PVP and Na lauryl-sulphate) against a mouldiness cologne, containing *Aspergillus*, *Penicillium*, *Trichoderma*, after 14 days.

4. Conclusions

As a result of the performed experiments it has been concluded that:

- ❖ the electrochemical method allows formation of stable and of high purity nano-Ag based colloidal solutions containing up to 35 ppm Ag;
- ❖ PVP stabilizer and co-stabilizers as N-naphthalenesulphonate, Na-lauryl sulfate, facilitate a suitable dispersion of nanoparticles and hinders their agglomeration;
- ❖ a good antimicrobial effect has been evidenced evaluated as 7-14 days of exposure at test fungi with no appearance of mould in the area treated with the prepared nano-Ag colloidal solution;
- ❖ the results are promising for the use in antimicrobial applications and for composite materials obtention

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