Silver Nanoparticles: Synthesis, Characterization and Antibacterial Activity

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Silver nanoparticles have attracted attention because of their unique chemical and physical properties and pronounced antibacterial activity, which provide one of the most cost effective alternatives in developing new antibacterial agents. Preparation of silver nanoparticles by reduction of silver nitrate using polyvinyl alcohol and tannic acid as reducing agents is presented. Colloidal silver nanoparticles obtained have been characterized by Dynamic Light Scattering and UV-Vis analysis indicating the formation of nanoparticles. The antibacterial activity of synthesized nanoparticles against etalon strains of gram-negative bacteria (Escherichia coli) has been studied. The silver nanoparticles showed a strong bactericidal effect against E. coli. The minimum inhibitory concentration (MIC) was also determined. The lowest value for MIC parameter was for nanoparticles having radius below 5 nm, for larger nanoparticles the MIC value increases.

Keywords: silver nanoparticles, antibacterial activity, tannic acid, polyvinyl alcohol

Synthesis of noble metal nanoparticles by reducing metal salts have been the subject of interest in different areas such as: material science, biotechnology and organic chemistry [1–3]. The metal nanoparticles, especially noble metal nanoparticles, show new physico-chemical properties that were not observed in the individual molecules [4]. Silver nanoparticles (AgNPs) were extensively studied as they exhibit strong absorption of electromagnetic waves in the visible range due to surface plasmon resonance (SPR), highly stable dispersions, chemical inertness and biocompatibility [5, 6]. A small change in the AgNPs size, shape, surface nature or the distance between particles leads to major changes in their optical properties [7].

AgNPs are the subject of many recent researches especially due to their antimicrobial activity [8-23]. Having good antimicrobial properties AgNPs can be used in a wide range of applications: water filtration [24], catalysis [25], optics [26], medicine [26] and food packaging [27].

This paper aim is the preparation of some silver nanoparticles colloidal dispersions from an aqueous solution of $AgNO_3$ and using PVA and tannic acid as reducing agents. Silver nanoparticles were characterized by UV-Vis spectroscopy and Dynamic Light Scattering (DLS) analyses in terms of stability and influence of reactants on the formation and size of nanoparticles.

Antibacterial susceptibility of silver nanoparticles was tested against *Escherichia coli* using the Kirby-Bauer disk diffusion method and minimal inhibitory concentration (MIC) analysis.

Experimental part

Materials

The reagents were of analytical purity and they were used as received: $AgNO_3$ (Chimopar), NaOH (Chimopar), tannic acid ($C_{76}H_{52}O_{46}$) M_W=1701.2 g/mol (Merck), ultrapure water (conductivity 6 MΩ). PVA used has M_W = 27000 g/mol and hydrolysis degree 98 - 99%.

Test organisms

For evaluating the antibacterial activity were used pure references strains of pathogen Gram-negative bacteria (*Escherichia coli* ATCC 25922), purchased from USA.

Silver nanoparticles synthesis

For obtaining silver nanoparticles were used solutions of $AgNO_3$ (5 and 10 mM), PVA (5 wt%), tannic acid (15 mM), NaOH (10 mM) and ultrapure water as presented in table 1. In the reaction vial were added: PVA solution, tannic acid solution, NaOH solution and distilled water. The vial was heated to 70 Celsius degrees, after which was added $AgNO_3$ solution. The reaction mixture was maintained at this temperature for 5 minand then the reaction vial was cooled to 20 Celsius degrees under stirring.

Nanoparticle dispersions thus obtained are kept in a refrigerator in brown vials, for further analysis.

Table 1
EXPERIMENTAL CONDITIONS, TOTAL VOLUME 10 mL

Sample no.	Reactants volume (mL)					[Ag] (mg/mL)	[Ag] (mM)	molar ratio Tannic acid: $AgNO_3$	molar ratio PVA : $AgNO_3$
	$AgNO_3$	Tannic acid	PVA	NaOH	Water				
1	1.00	0.83	0.05	0.5	7.61	1.7	10	0.5	0.001
2	1.00	0.83	0.54	0.5	7.13	1.7	10	0.5	0.01
3	1.00	0.83	5.40	0.5	2.27	1.7	10	0.5	0.1
4	0.50	0.42	0.27	0.5	8.31	0.85	5	0.5	0.01
5	1.00	0.83	0.54	0.25	7.38	1.7	10	0.5	0.01

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Characterization

In order to verify formation and stability of obtained silver nanoparticle dispersions UV-Vis spectra (from 200 to 600 nm) were recorded using a Jasco V550 Spectrometer. UV-Vis spectra were recorded using a blank sample containing the same concentrations of reactants except silver nitrate. For UV-Vis analysis all samples were diluted by a dilution factor of 0.1.

The diameter size of obtained nanoparticles was studied by DLS analysis, using a Zetasizer Nano ZS device from Malvern Instruments. The samples were analyzed undiluted. At least 3 measurements were made up to obtain consistent results.

Although the whole range of diameter is shown in the intensity-weighted distribution (I-distribution), the proportionality to the sixth power of diameter of particle underestimates the small particles, which are only very weakly weighed [28]. The corresponding number-weighed distribution converted using Mie theory, is proportional to the first power of diameter and determines the real number of particles that yield the observed intensity of each size class [29]. The number distribution was validated by TEM analysis (fig. 9).

Particle size was measured also using high resolution transmission electron microscopy (HRTEM) associated with microanalyses techniques (EELS electron energy loss spectroscopy) which enabled real optical images of the nanoparticles and allowed their characterization through the assessment of the chemical bond type. The apparatus used was TECNAI F30 G2 transmission electron microscope with linear resolution of 1Å and dots resolution of 1.4 Å.

Preparation of test organisms

The cultures of test organisms were maintained in agar slants (PCA – Plate Count Agar; Biokar Diagnostics, France). From reference stock culture inoculated in TSB (Tryptic Soy Broth) at 37°C for 18-24 h were obtained bacterial inoculums. From the freshly grown cultures were made decimal dilutions in sterile Peptone Physiological Serum, up to the concentration of 10^6 cfu/mL used for further testing of the silver nanoparticle dispersions.

Antibacterial testing

Antibacterial susceptibility of silver nanoparticles was evaluated using Kirby-Bauer disk diffusion method. Inhibition zones were measured after 24 h s of incubation at 37°C. Antibacterial activity was determined using standard micro-dilution method by determining minimum inhibitory concentration (MIC). MIC is the largest dilution concentration at which no bacterial growth occurs.

Results and discussions

There were synthesized various dispersions of silver nanoparticles starting from different concentrations of silver nitrate and varying all other reagents used in order to follow their influence on the obtained nanoparticles.

Stability of the silver nanoparticles dispersions

UV-Vis spectra of each sample were recorded in the same day of the synthesis and after a week for analyzing the stability of the obtained dispersions. The presence of an absorption peak at a wavelength between 400 and 420 nm indicates formation of silver nanoparticles. As shown in table 2, spectra recorded for each of the silver nanoparticles dispersion showed no significant differences within one week (variation of wavelength, peak width and absorbance). This indicates a good stability of silver nanoparticles dispersions. The dispersion with the highest stability is the dispersion with the lowest concentration of silver (sample no. 4).

Peak width at half length is a good measure of particles dispersion [30]. As expected, at low silver concentrations are obtained less dispersed populations of nanoparticles.

The influence of PVA: $AgNO_3$ molar ratio on obtained silver nanoparticles

Analyzing the results presented in figure 1 and table 2 it can be noticed the increasing of nanoparticles dispersions absorbance with increasing of molar ratio PVA / $AgNO_3$.

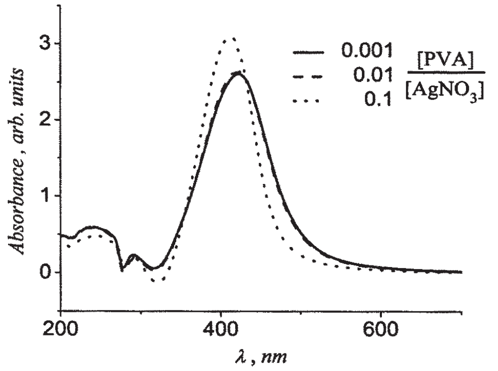

Fig. 1. Influence of PVA: $AgNO_3$ molar ratio on obtained silver nanoparticles (UV-VIS spectra)

Absorbance of sample no.3 (molar ratio PVA / $AgNO_3$ 0.1) is higher than for sample no.1 (molar ratio PVA / $AgNO_3$ 0.001) and sample no.2 (molar ratio PVA / $AgNO_3$ 0.01).

The samples no. 1, 2, and 3 which differ by molar ratio PVA / $AgNO_3$ were analyzed by DLS technique. It can be observed a slight increasing of nanoparticles diameter by increasing of PVA amount (fig. 2). Both tannic acid and polyvinyl alcohol are reductants for silver precursor and stabilizers for nanoparticles formed [31, 32]. Tannic acid plays a more pronounced reduction role and polyvinyl alcohol a stabilizing role.

The combined use better highlight their properties. The narrow distributions and the smallest particles are obtained when tannic acid is used in sufficient quantity to reduce

Table 2

UV-Vis SPECTRAL ANALYSIS OF SILVER NANOPARTICLES

Sample no.	$[AgNO_3]$ (mM)	molar ratio PVA:$AgNO_3$	[NaOH] (mM)	λ (nm)	Abs (a.u)	FWHM * (a.u.)	Peak area (a.u)
1	10	0.001	10	422.5	2.609	94.38	**261.87**
1w **	10	0.001	10	420	2.697	88.55	**208.81**
2	10	0.01	10	420	2.649	93.24	**264.97**
2w **	10	0.01	10	419.5	2.712	86.80	**208.54**
3	10	0.1	10	412	3.095	78.03	**278.17**
3w **	10	0.1	10	410.5	3.107	72.84	**221.55**
4	5	0.01	10	402.0	3.020	63.92	**213.43**
4w **	5	0.01	10	403.5	2.999	63.81	**190.62**
5	10	0.01	5	420.5	1.858	127.99	**228.25**
5w **	10	0.01	5	418.5	1.876	126.90	**150.58**

* FWHM = peak width at half height; ** Sample analyzed after a week

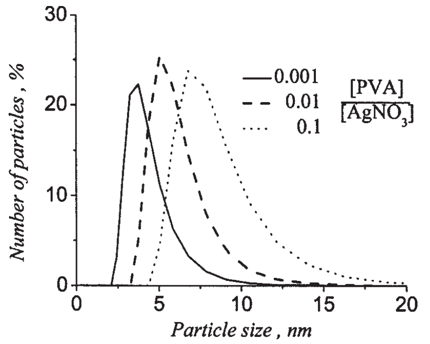

Fig. 2. Influence of PVA: $AgNO_3$ molar ratio on obtained silver nanoparticles (DLS analysis)

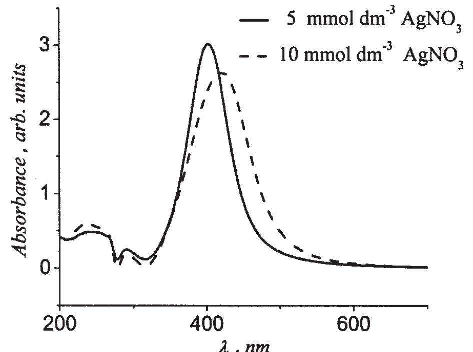

Fig. 3. Influence of $AgNO_3$ concentration on obtained silver nanoparticles (UV-Vis spectra)

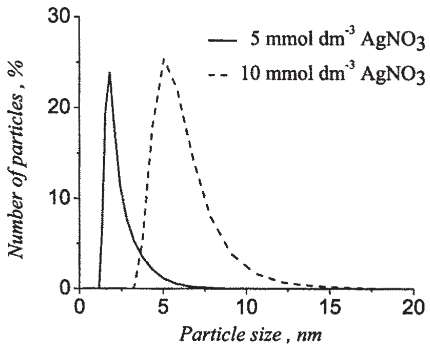

Fig. 4. Influence of $AgNO_3$ concentration on obtained silver nanoparticles (DLS analysis)

the silver precursor and polyvinyl alcohol is especially designed as stabilizer (sample 1).

The influence of $AgNO_3$ concentration on obtained silver nanoparticles

As can be seen in figure 3 and table 2, at lower silver concentrations the conversion of silver ions in silver nanoparticles with low size is higher; that can be deduced from highest value of relative absorbance (absorbance divided by $AgNO_3$ conc.).

At higher silver concentrations (20 mM) the agglomeration phenomena is observed and for this concentration the sample is colorless.

Only at this silver concentration dispersions were instable and the nanoparticles were agglomerated; in all other cases the dispersions were stable during more weeks. Influence of silver concentration on nanoparticles size is highlighted by DLS analysis of 2 dispersions with silver concentration of 5 and 10 mM (fig. 4).

At the lowest silver concentration nanoparticles have the smallest radius, at highest concentration (20 mM) the

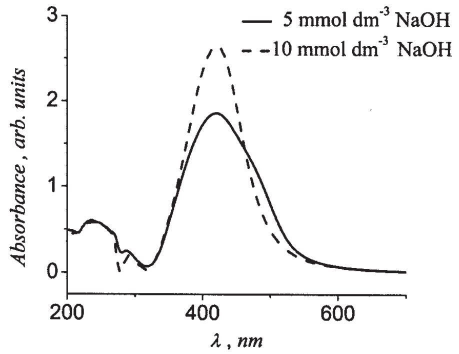

Fig. 5. Influence of NaOH concentrations on obtained silver nanoparticles (UV-Vis spectra)

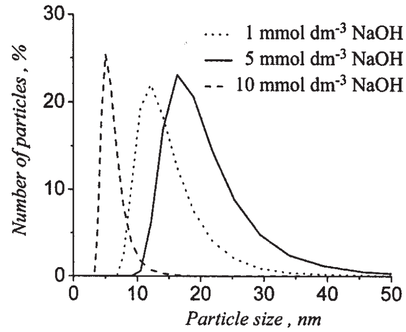

Fig. 6. Ifluence of NaOH concentrations on obtained silver nanoparticles (DLS analysis)

aggregation of nanoparticle is developed and DLS analysis cannot be obtained.

Influence of NaOH concentration on obtained silver nanoparticles

The influence of NaOH concentration is significant, as we can see analyzing figure 5 and table 2. The amount of produced silver nanoparticles is higher if the NaOH concentration is higher.

The sample containing the lowest NaOH concentration has the lowest absorbance. It was also found that in absence of NaOH the silver nanoparticles formation is not possible.

In figure 6 is shown the influence of NaOH concentration on particle size. The smaller silver nanoparticles are obtained at relatively high concentration of NaOH (10 mM). Sample containing 1 mM NaOH was used only for this test in order to evaluate the influence of NaOH concentration. At low NaOH concentration (1 and 5 mM) the size distribution is worst, many large nanoparticles are obtained.

Antibacterial assays

All obtained silver nanoparticles dispersions were tested in terms of antibacterial susceptibility. The antibacterial activity evaluation against a pure culture of Escherichia coli was followed. Antibacterial tests presented in Figure 7 were made for each sample (table 3). Antibacterial activity was determined for silver nanoparticles samples obtained but also for samples containing $AgNO_3$, Tannic acid, PVA and NaOH in the same concentrations as used for obtaining silver nanoparticles. From the pictures of figure 7 it can be noticed that Tannic acid, PVA and NaOH solutions don't have antimicrobial effect and silver nanoparticles dispersions have inhibition zones comparable with $AgNO_3$ solutions. Measured inhibition zones diameters of all nanoparticles dispersions were higher than 10 cm; this indicates a very strong antibacterial activity against *E. coli*.

Sample no.	[Ag] (mg/mL)	UV-VIS analysis		DLS analysis	Antimicrobial properties	
		Abs (a.u)	Abs / $[AgNO_3]$	r (nm)	MIC μg/mL	D_i (mm)
1	1.7	2.609	0.261	3.5	54	13.0
2	1.7	2.649	0.265	5.1	54	11.3
3	1.7	3.095	0.31	7	107	11.0
4	0.85	3.020	0.604	2	54	12.0
5	1.7	1.858	0.186	20	213	12.3

Table 3
ANALYSIS AND ANTIMICROBIAL PROPERTIES OF SILVER NANOPARTICLES

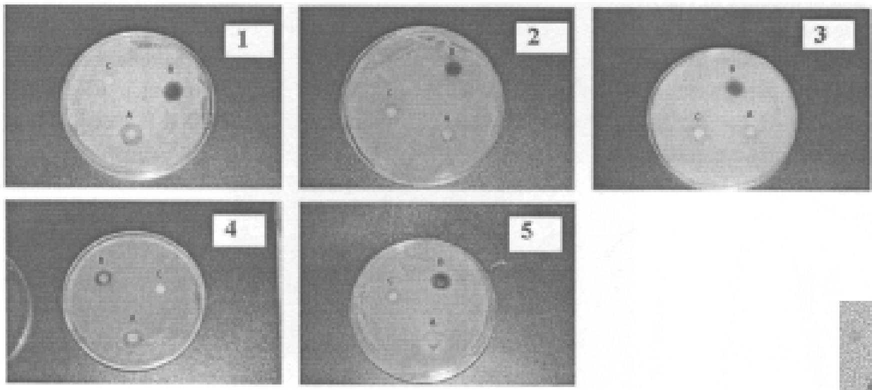

Fig. 7 The antibacterial activity: A- silver nanoparticle dispersions (samples from table 2); B - $AgNO_3$ solutions with the same concentration as silver nanoparticle samples; C - blank samples (TA + PVA + NaOH without $AgNO_3$)

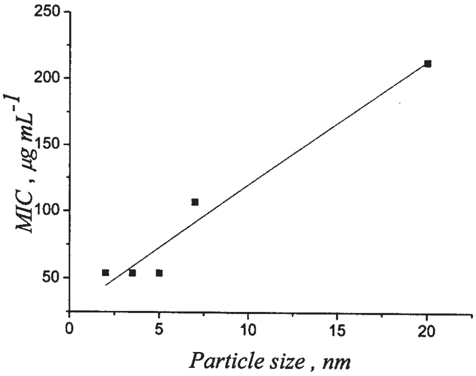

Fig. 8 The influence of silver nanoparticle size on the MIC

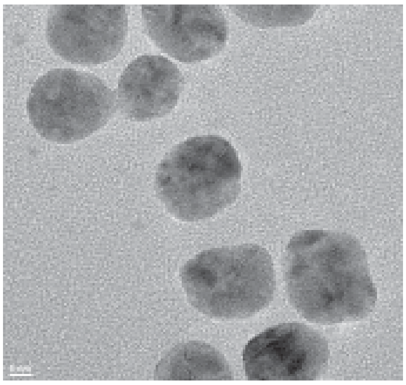

Fig. 9. TEM image of obtained nanoparticles in sample no. 2

Samples 1, 2 and 3 have the same silver concentration, the same tannic acid / $AgNO_3$ molar ratio, but different PVA / $AgNO_3$ molar ratio. The largest diameter of inhibition zone is given by the sample no. 1 which contains a sufficiently large number of nanoparticles with small diameter. To determine the minimum inhibitory concentration (MIC) was used the classical method of successive dilutions. The influence of nanoparticle size is presented in figure 8. It can be noticed that below 5 nm the MIC value is minimum and increasing the nanoparticle size the MIC is increasing also.

The diameter of inhibition zones (D_i) was determined for all dispersions using the Kirby-Bauer diffusion method. All samples have a value of inhibition zone diameter above 10 mm and they present a very good activity.

Conclusions

This paper aim is the synthesis of silver nanoparticles from $AgNO_3$ as precursor and using tannic acid and PVA as reducing and stabilizer agents. Obtained dispersions were analyzed by UV-Vis spectroscopy to reveal the formation of nanoparticles and their stability. The presence of an absorption peak at wavelength between 400 and 420 nm indicate the formation of silver nanoparticles. All obtained dispersions are stable over time. The dispersion with the lowest silver concentration (5 mM) proved to be the most stable. UV-Vis study reinforced the fact that at low silver concentration are obtained the less disperse nanoparticles populations. At lower silver concentration, ionic silver transformation in silver nanoparticles is higher.

DLS analysis showed that at lower silver concentrations the diameter of obtained nanoparticles is smaller. Also, increasing the amount of PVA, the nanoparticles diameter increase slightly, forming even particles with diameter size larger than 100 nm.

The use of tannic acid and polyvinyl alcohol in proper concentrations make it possible to obtain stable nanoparticle dispersion with a narrow distribution.

By determining the minimum inhibitory concentration it was pointed out that the particles having radius less than 5 nm are most active.

Diameters of the inhibition zones measured for all silver nanoparticles dispersions were higher than 10 cm, signifying a very good antibacterial activity against *Escherichia coli*.

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