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SILVER NANOPARTICLES SYNTHESIZED BY DECOMPOSITION OF A SILVER ORGANIC COMPLEX WITH VALENCE TAUTOMERISM AND THEIR PROPERTIES

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Features of the formation of silver nanoparticles (Ag-NPs) via intramolecular redox transformation of the Ag(I) complex of 4,6-di-tert-butyl-2,3-dihydroxybenzaldehyde isonicotinoylhydrazone in organic solvents with donor numbers DN > 19 were studied. The stability of the organic sols depended on the nature of the dispersion medium and the presence of oxygen and water in it. The physical chemistry and morphology of the Ag-NP in the organic sol were investigated using molecular absorption spectroscopy, transmission electron microscopy, and atomic force microscopy. The silver sol consisted of spherical Ag-NPs 5–20 nm in size with a characteristic absorption band near 440 nm. It was found that the silver complex with valence tautomerism was a promising precursor for Ag-NPs. The synthesized Ag-NPs showed high antimicrobial activity compared with standard antibiotics and Ag-containing agents (MIC = 0.007 μmol/mL).

Keywords: *silver nanoparticles, silver complex with valence tautomerism, molecular absorption spectroscopy, transmission electron microscopy, atomic force microscopy, antimicrobial activity.*

Introduction. The preparation of homogeneous nanoparticles (NPs) with given sizes, shapes, and physicochemical properties is of great interest for fabricating new drugs and medical materials [1–3]. Silver compounds are known to be toxic to a broad spectrum of microorganisms [4–6] and are used as antimicrobial drugs against antibiotic-resistant strains [7, 8]. They act simultaneously as wound-healing and anti-inflammatory agents [9]. NPs have large specific surface areas that increase the probability of contact with bacterial cells [10]. Therefore, silver nanoparticles (Ag-NPs) and their nanocomposites (1–100 nm) are extremely promising as antimicrobial agents [11]. However, known pharmacopoeial colloidal silver preparations, i.e., Protargol and Collargol, are characteristically unstable as aqueous solutions. Therefore, they are produced only as powders that are used to compound drugs on demand. These powders also change during storage because of hygroscopicity and the ability to react with CO_2. Thus, it seemed interesting to develop methods for preparing silver salts and isolating solid NPs that could be subsequently dispersed.

The preparation method is known to determine the ultimate size and morphology of NPs and; therefore, their physicochemical and biological properties. The principal known methods for preparing Ag-NPs are reduction of Ag(I) salts [12], electrochemical synthesis [13], chemical precipitation from the vapor phase [14], laser ablation [15], microwave synthesis [16], and decomposition of organometallic compounds [17, 18]. Generation of Ag–NPs by decomposition of metal complexes in solution deserves special attention [19–21]. This method is advantageous because of the fact that the metal complex contains the oxidant, reductant, and sol stabilizer. Previously, Ag(I) complexes of 4,6-di-*tert*-butyl-2,3-dihydroxybenzaldehyde isonicotinoylhydrazone (L^I) and 4,6-di-*tert*-butyl-2,3-dihydroxybenzaldehyde thiosemicarbazone (L^{II}) were synthesized and characterized physicochemically [20]:

The complexes were shown to have partial charge transfer and belonged to a special class of transition-metal compounds with redox-active ligands [22]. They included metal complexes with *o*-dioxolenes in which intramolecular

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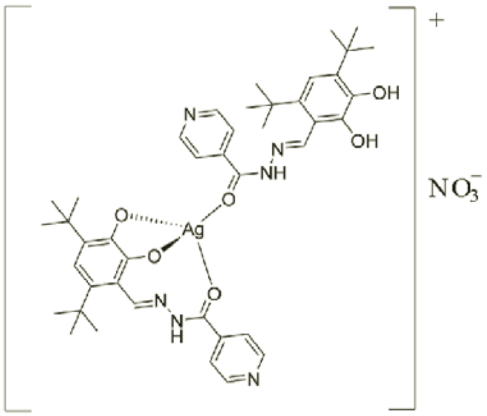

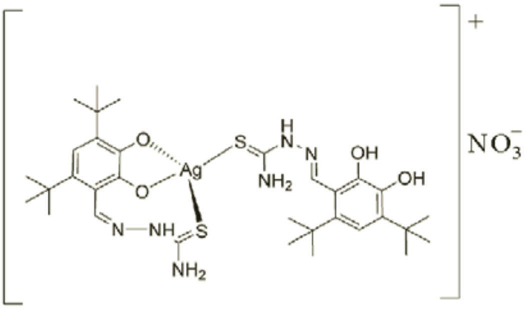

electron transfer between the ligand and metal ion (valence tautomerism or partial charge transfer) was observed. The ligand in these complexes existed partially in the semiquinone form. An intramolecular redox reaction occurred in solvents with high solvating capability for the $Ag(L^I)_2NO_3$ complex. As a result, Ag was reduced although the aforementioned complex of this type $Ag(L^{II})_2NO_3$ remained stable. Based on these results, a new method for generating Ag-NPs using an Ag(I) complex with valence tautomerism as their precursor was proposed.

The goals of the present work were to establish that the Ag(I) complex of 4,6-di-*tert*-butyl-2,3-dihydroxybenzaldehyde isonicotinoylhydrazone $[Ag(L^I)_2NO_3]$ could be used as a new precursor for preparing stable Ag-NPs and to assess the antibacterial and antifungal activity of the synthesized Ag-NPs as compared with standard antibiotics and Ag-containing agents.

Experimental. Reagents for synthesizing L^I and L^{II} and their Ag(I) complexes were commercially available and were used without further purification. Organic solvents (EtOH, PrOH-2, 2-methylpropanol-1, octanol-1, DMF, DMSO, MeCN) were purified by distillation and dehydration. Methods for synthesizing the ligands and their Ag(I) complexes in addition to their analytical data and physicochemical characteristics were published before [20, 22, 23]. The purity of the compounds was checked by TLC. Their structures were elucidated by PMR, ^{13}C NMR, IR, and mass spectroscopy. UV/Vis absorption spectra (300–700 nm) were recorded on a Specord M 400 UV-Vis dual-beam spectrophotometer. Sols were stored in a dark place in tightly closed containers with preliminary de-aeration (Ar) in order to study temporal changes in the absorption spectra. The sizes and shapes of sol particles were determined by transmission electron microscopy (TEM) using an EM-125 K instrument. The particle sizes and shapes were also found using atomic force microscopy (AFM). Ag-NPs were deposited from the organic sols onto silicon substrates modified by several layers of polyelectrolytes such as polydiallylmethylammonium chloride (PDDA, M_w = 200,000–350,000, Aldrich) and sodium polystyrenesulfonate (PSS, M_w = 70,000, Aldrich). The surface acquired a negative charge after depositing four polyelectrolyte layers (PDDA/PSS/PDDA/PSS) and a positive charge after depositing three layers (PDDA/PSS/PDDA). Modified plates were immersed in organic sols for 12–20 min, rinsed in deionized H_2O for 1 min to reduce the concentration of surface impurities, and then dried in air. AFM images of the surface were taken on a FemtoScan 001 instrument in contact mode using Si_3N_4 (silicon nitride) tips with force constant 0.32 N/m. The scan rate varied in the range 1–5 Hz; force of action, 1–10 nN. Statistical analysis used the Student *t*-test. A value of $P < 0.05$ was considered statistically significant. X-ray diffraction studies of solid Ag-NPs were performed on an HZG 4A diffractometer (Co K_α-radiation, MnO_2 filter).

Silver organic sols were prepared via chemical decomposition of $Ag(L^I)_2NO_3$ in solvents with high donor numbers $DN > 19$ (DMF, DMSO, EtOH, PrOH-2, 2-methylpropanol-1) with constant stirring. Sols were prepared by dissolving a weighed portion of complex corresponding to a sol silver concentration of $1\cdot10^{-4}$ M and were stored in closed containers in a dark place. EtOH containing H_2O (10 vol.%) was used to study the effect of H_2O on silver sol formation during complex decomposition. Dissolved oxygen was removed if necessary by passing Ar through the solutions. Solid Ag-NPs were prepared by evaporating solvent in an RV 8 V rotary evaporator (IKA). The silver content in the solid was determined by inductively coupled plasma atomic emission spectrometry (Spectroflame Modula).

Antimicrobial activity was determined as the minimum compound concentration that inhibited visible microorganism growth (minimum inhibitory concentration, MIC, μmol/mL). Compound activity was studied *in vitro* using double dilutions in liquid growth medium [24] and microorganisms from the collection of the Department of Microbiology, Belarusian State University (Gram-negative: *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella typhimurium*, *Escherichia coli*; Gram-positive: *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Mycobacterium smegmatis*; yeast-like *Cryptococcus laurentii*, *Lipomyces lipofer*, *Candida albicans*, *Candida boidinii*, *Candida utilis*,

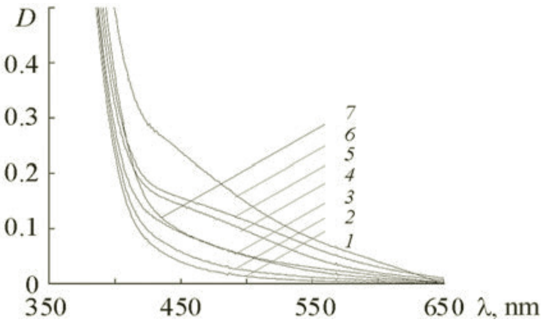

Fig. 1. Absorption spectra of Ag-NPs in EtOH: 30 s (1), 20 min (2), 50 min (3), 2 h (4), 3.5 h (5), 24 h (6), 48 h (7).

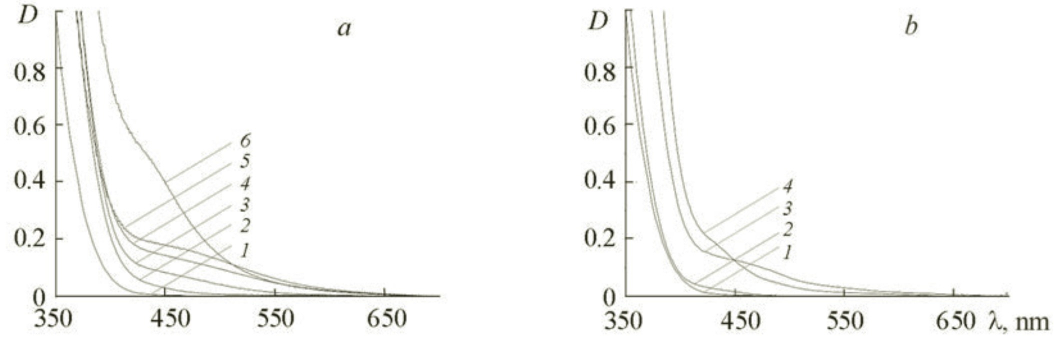

Fig. 2. Absorption spectra of the compounds in alcohols [oxidized ligand (1), Ag(I) complex in octanol-1 (2), Ag-NPs in propanol-2 (3), Ag-NPs in EtOH (4), Ag-NPs in EtOH:H_2O (9:1) (5), Ag-NPs in 2-methylpropanol-1 (6)] (a) and in other solvents [oxidized ligand (1), Ag(I) complex in MeCN (2), Ag-NPs in DMF (3), Ag-NPs in DMSO (4)] (b).

Saccharomyces cerevisiae; and mold fungi *Aspergillus niger*, *Fusarium* spp., *Mucor* spp., *Penicillium lividum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria alternata*). The antimicrobial activity of the Ag-NPs was compared with those of standard antibacterial antibiotics (tetracycline, chloramphenicol, ampicillin, streptomycin), Ag-containing preparations (silver nitrate, silver sulfadiazine), antituberculosis agents (isoniazid), and antifungal antibiotics (terbinafine, nystatin, fluconazole, amphotericin B).

Results and Discussion. The absorption spectrum of the complex $Ag(L^I)_2NO_3$ dissolved in EtOH displayed a maximum at 440 nm that belonged to Ag-NPs (Fig. 1) [25]. The characteristic absorption maximum in the spectrum appeared as a shoulder because it partially overlapped a ligand intrinsic absorption band at 270–420 nm. The absorption spectrum of the ligand in EtOH did not show noticeable changes due to oxidation during storage in air for 3 d.

Plasmon absorption bands were clearly visible in spectra in solvents (EtOH, PrOH-2, 2-methylpropanol-1, DMF, DMSO) with $DN > 19$ and dielectric permittivity $\varepsilon > 17$ (Fig. 2). On the other hand, the complex did not decompose in octanol-1 and MeCN to form Ag-NPs. Alcohol sols containing H_2O were unstable. Coarsely disperse silver separated from these systems only several hours after dissolving the complex. The complex in de-aerated solutions decomposed faster to form Ag-NPs in higher yields than with oxygen (Fig. 3a). Thus, dissolution of the complexes in solvents with high donor numbers that actively solvate metal cations created conditions favorable for redox decomposition of the complexes and formation of Ag-NPs.

The changes of spectral maximum intensities and positions were due to changes in the silver sol state during storage. The optical density increased over time in the plasmon-absorption region and reached the greatest value 3.5 h after dissolution. Furthermore, the band broadened and underwent a bathochromic shift. According to the literature [25], this was related to an increase in the sol-particle size and surface adsorption of organic compounds. The maximum gradually subsided upon further storage because the NPs aggregated and then settled. Ultrasonication of the destabilized system redispersed the solid and formed alcohol silver sols, spectra of which exhibited a characteristic absorption band (430–450 nm).

Sols were most stable in EtOH and 2-methylpropanol-1. The synthesized disperse systems could be stabilized by the ligand, its oxidation products, and the organic solvent. However, water could affect the structure of the dispersion medium and; therefore,

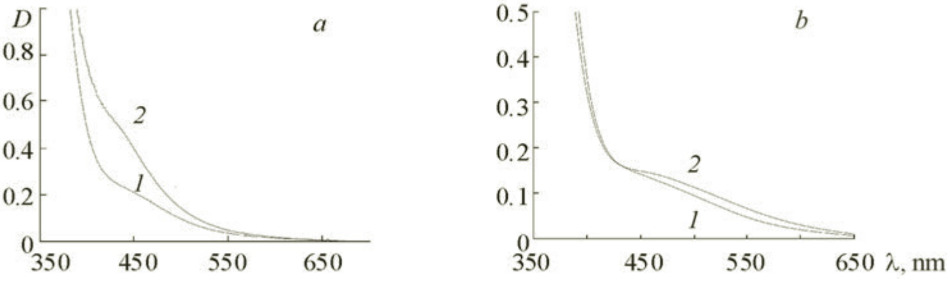

Fig. 3. Absorption spectra of Ag-NPs in aerated (1) and de-aerated (2) 2-methylpropanol-1 (a) and in EtOH before (1) and after (2) redispersion (b).

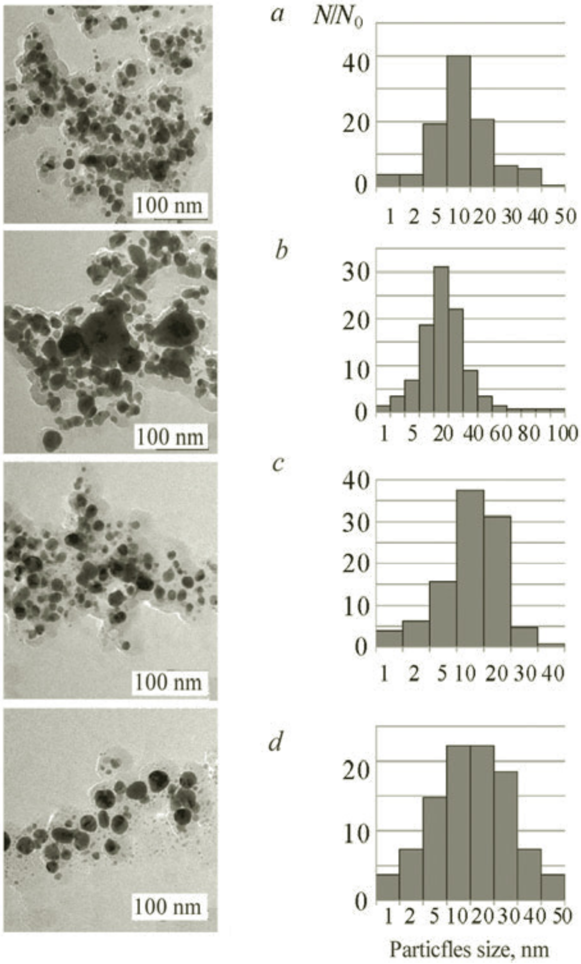

Fig. 4. TEM images and size-distribution diagrams of Ag-NPs in 2-methylpropanol-1 after 30 min (a) and 5 h (b) and in EtOH after 5 h (c) and after redispersion (d).

was a destabilizing factor for the silver alkaline sols because of the possible involvement of redox reactions. Obviously, oxygen could interfere with the redox reaction between the ligand and Ag(I) during decomposition of the complex in solution (Fig. 3a).

TEM was used to study organic sols containing 2-methylpropanol-1 and EtOH as the dispersion media. Most of the Ag-NPs in freshly prepared sols obtained by decomposing the complex in 2-methylpropanol-1 and EtOH were 5–20 nm in size (Fig. 3b) although a few (~7%) of them were smaller (1–2 nm) according to TEM data. The particles grew (10–40 nm)during storage of the sol for 5 h as a consequence of their aggregation and gradual precipitation (Fig. 4). The size of the primary silver particles in the organic sols was determined by AFM. Features of the Ag-NP structure were studied using AFM by precipitating them onto positively charged substrates (three polyelectrolyte layers PDDA/PSS/PDDA).

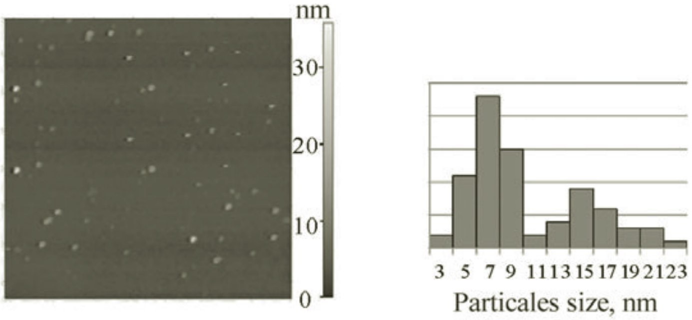

Fig. 5. AFM images and size-distribution diagrams of Ag-NPs in 2-methylpropanol-1.

According to AFM data, the size of the primary silver particles in the organic sols was 5–10 nm (Fig. 5). The sizes of the Ag-NPs that were found using TEM and AFM (Figs. 4 and 5) agreed well with data obtained from absorption spectra of the sols [26].

Ag-NPs formed via decomposition of $Ag(L^I)_2NO_3$ in EtOH were isolated from alcohol sols as solids by evaporating the solvent. A sol with particles 10–30 nm in size was again formed after they were redispersed in EtOH (Figs. 3b and 4). Solid Ag-NPs were identified by x-ray phase analysis [27]. The silver concentration in the organic sols was determined by measuring the mass of the solid residue (88.9–90.0 μg/mL), which had a silver content of 12.1 ± 0.1%.

Pharmacological screening of the synthesized Ag-NPs showed high activities (MIC = 0.007 μmol/mL) against test cultures of microorganisms that were comparable with those of standard antibiotics and Ag-containing preparations [20, 21]. A result with practical significance was the fact that Ag-NPs were equally effective against the tested Gram-positive and Gram-negative bacteria and yeast-like and mold fungi. It is noteworthy that the MIC values of Ag-containing preparations Collargol and Protargol against Gram-positive (0.008–0.009 μmol/mL) and Gram-negative bacteria (0.022–0.231 μmol/mL) were poorer [28, 29].

Conclusions. Redox decomposition of an Ag(I) complex with valence tautomerism in organic solvents could be used to produce Ag-NPs. A method was proposed for preparing Ag-NPs via an intramolecular redox reaction of the Ag(I) complex with 4,6-di-*tert*-butyl-2,3-dihydroxybenzaldehyde isonicotinoylhydrazone in solvents with high solvating capability (*DN* > 19, DMF, DMSO, EtOH, PrOH-2, 2-methylpropanol-1). Absorption spectroscopy, TEM, and AFM found that the size of the primary Ag-NPs in the organic sol was 5–20 nm. The synthesized Ag-NPs suppressed the growth of bacteria and yeast-like and mold fungi at very low concentrations (MIC = 0.007 μmol/mL). The results were interesting for designing new drugs and medical materials because the number of anti-infectious agents with high levels of antibacterial and antifungal activity is extremely limited [29, 30].

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