

Antibacterial Action and Physicochemical Properties of Stabilized Silver and Gold Nanostructures on the Surface of Disperse Silica

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Abstract

This work is devoted to the synthesis and stabilization of nanosized Ag/SiO₂ and Au/SiO₂ disperse materials and investigation their morphology, optical and antimicrobial properties. First, Ag and Au nanoparticles (NPs) were produced in colloids via chemical (Ag) or photochemical (Au) reduction of appropriate ions. To prevent the oxidation of Ag NPs in colloid solution, external binary stabilizing agents PVP and SDS were used. Then, Ag and Au NPs (0.01–0.05% wt) were adsorbed from their colloid solutions on high disperse silica surface ($S_{sp}=260\text{m}^2/\text{g}$) and samples prepared were dried. Materials obtained were studied by UV-vis, XRD, and TEM methods. Ag and Au NPs adsorbed on silica demonstrated a fair crystallinity in XRD. The surface plasmon resonance (SPR) band positions inherent to Ag and Au NPs on silica surface as well as the intensities of optical spectra were stable during 7 month and more. Obtained Ag NPs in colloids and Ag/SiO₂ composites demonstrated excellent antimicrobial activity against a series of the microorganisms (*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*). Au/SiO₂ samples did not reveal any bactericide properties relative to the test microorganisms grown. The mechanisms of Ag(Au) NPs interaction with silica surface were analyzed.

Keywords: Silver, Gold Nanoparticles, Binary Stabilizer PVP and SDS, Adsorption, High Disperse Silica, Antimicrobial Activity

1. Introduction

Water related infections belong to the one of the main health problems. The most dangerous water pathogenic pollutions such as *Escherichia coli*, *Staphylococcus aureus* are resistant to biodegradation. Silver nanoparticles (NPs) or silver ions have long been known to have strong inhibitory and bactericidal effect in solutions and composites with silica films and particles (Ag/SiO₂) with developed surface area [1,2]. In case of Ag/SiO₂ application, the specific surface area of silica is of great importance for effective adsorption of the species desirable. That is why developed silica surface covered with OH groups offers unique environment for the fixation and stabilization of nanosized silver and gold particles (Ag and Au NPs) for applications in optics, sensing, catalysis and as bactericides. However, Ag NPs are unstable on silica surface under ambient conditions due to fast oxida-

tion [3]. It is important to prevent or inhibit the aggregation of NPs and oxidation process at the nanocluster formation stage, especially in case of silver. There are some methods to prevent the contact of Ag NPs with environment such as the protection with the inverse micelles, polymers, or their introduction into the solid materials, metal oxides or polymer films. Thus, stabilizing agents used for NPs surface modification control the particles formation, growth and aggregation rate [4,5].

Various methods are available for the NPs preparation. The most widely used techniques are photo- or radiation method in presence of stabilizers, and chemical reduction using sodium tetrahydroborate, hydrazine, glucose, sodium citrate, and other reactants [6–10].

Previously we synthesized stable Ag and Au NPs within silica films prepared using sol-gel method via thermal reduction of appropriate metal ions [11]. These films were bright colored, transparent, stable and had

characteristic SPR bands belonging to Ag or Au NPs. However, Ag clusters formed on disperse silica surface were found to be stable only for a few days [3]. The self-agglomeration and oxidation of Ag NPs were detected also within the porous silica films [12]. Photochemically generated Ag and Au NPs prepared in colloid solutions aggregated during 4-5 weeks, and Ag NPs were covered with oxide layer [9,10,13,14].

We suppose that the combination of the surfactant and polymer as the metal NPs stabilizers proposed for solution in [15,16] can be successfully used to prevent the oxidation and aggregation of Ag NPs in adsorbed state on high disperse silica surface in air.

The aim of this work was the synthesis of stable nano-sized Ag/SiO₂ and Au/SiO₂ disperse materials using binary stabilizer SDS/PVP and investigation of their morphology, optical and antimicrobial properties.

2. Experimental Section

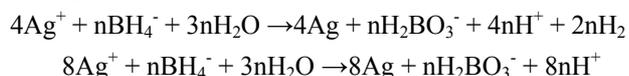
2.1. Initial Reagents

HAuCl₄, AgNO₃ (Merck); Sodium dodecyl sulfate (SDS, Sigma Aldrich), Polyvinylpyrrolidone (PVP, grade: VFS 42-1491-85(Russia), FW=12000); Sodium tetrahydroborate (NaBH₄, Fluka), Benzophenone (BP, Aldrich), *i*-propanol Sigma Aldrich); high-dispersed silica (HDS, brand A-300 with S_{sp}=260m²/g, "Khlorvinil").

2.2. Ag and Au NPs Preparation

Ag/SiO₂ and Au/SiO₂ powders were obtained by adsorption of previously synthesized Me NPs colloids (total metal concentration 1.5·10⁻⁴ mol/l) on the surface of thermal treated HDS under stirring at room temperature. All Me colloids (0.01-0.05 wt % Me) were adsorbed on silica surface.

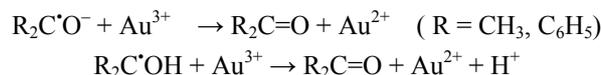
Nanosized Ag particles in colloid solution were prepared via chemical reduction of AgNO₃ with NaBH₄ analogously to [9] in presence of SDS/PVP mixture previously dissolved in water. The reaction scheme is:



Synthesized Ag NPs were adsorbed on the surface of HDS pretreated at 500 C. The Ag/SiO₂ powders were dried at 85-95°C during 4 hours.

Au colloids were synthesized via photochemical reduction of HAuCl₄ [10] under UV-irradiation in water-*i*-propanol solution with mercury lamp irradiation (λ=254 nm) in presence of mesoporous SiO₂ powder with adsorbed benzophenone as the photocatalyst of Au³⁺ ions reduction and SDS as a stabilizer. Total reaction scheme including formation of ketyl-radicals from benzophenone

and *i*-propanol is:



disproportionation: $2\text{Au}^{2+} \rightarrow \text{Au}^{3+} + \text{Au}^+$

reduction of Au(I): $\text{R}_2\text{C}^{\cdot}\text{O}^- + \text{Au}^+ \rightarrow \text{R}_2\text{C}=\text{O} + \text{Au}^0$

Synthesized Au NPs were adsorbed on the surface of HDS pretreated at 500°C. The Au/SiO₂ powders were dried and treated at 500°C during 5 hours.

2.3. Characterization

UV-VIS Absorption Spectroscopy studies were performed with a Perkin-Elmer Lambda 35 spectrophotometer in the wavelength range of 200–1000 nm. Measurements were made in 1 cm quartz cuvettes. Diffuse reflection spectra of Me/SiO₂ powders were recorded with Perkin-Elmer Lambda 35 spectrophotometer with a Labsphere RSA-PR-20 integrating sphere and handled using a Kubelka-Munk equation. The crystal structures of powders were determined with X-ray diffractometer (DRON-4-07, CuK_α). The size and morphology of the Ag and Au nanoparticles in colloids and on silica surface were characterized with a JOEL JEM-100C transmission electron microscope (accelerating potential 100kV).

2.4. Antimicrobial Properties Study

Antimicrobial actions of NPs in colloids and on silica surface were determined using suspension tests according to the European standards EN 13727:2004 [17] and EN 13624:2002 [18]. Test microorganisms Staphylococcus aureus ATC C 6538, Escherichia coli K12 NCTC 10538 were used for evaluation of bactericidal activity and Candida albicans ATCC 10231 was used for evaluation of fungicidal activity of disinfectants.

Test microorganisms were grown in agar medium:

Tryptic soy agar for bacteria and Saburo agar for Candida albicans (HIMEDIA, India). Their specific quantities in test suspension (bacteria – up to 1.5·10⁸ – 5.0·10⁸ colony-forming units CFU/ml or 8lg, yeast-like fungi – up to 1.5·10⁷ – 5.0·10⁷ CFU/ml or 7lg) were monitored with a KFK-3 photoelectric colorimeter (λ = 620 nm, cuvette length – 10 mm).

Suspension method involved mixing of 1 ml of the test microorganisms with 1 ml of diluent and then adding 8 ml of the test disinfectant. The experimental mixture was maintained at (20.0±1.0)°C for the chosen contact times of 1; 2; 4; 24 h. After exposing for the required contact time, 1 ml was transferred to 9 ml of neutralizer (8.0 ml 0.1% Na₂S + 1.0 ml H₂O). After neutralizing for 5 min 1.0 ml of the test mixture was taken in duplicate and plated to detect survived test microorganisms. After incubation the numbers of survived bacteria (at 37.0±1.0°C for 24 to

48 h) or fungi (at $30.0 \pm 1.0^\circ\text{C}$ for 48 h) in each sample were found.

The reduction values $R = N_0 - N_a$ were expressed in decimal logarithms: $\lg R = \lg N_0 - \lg N_a$, where N_0 – number of cells per ml in the test mixtures at the beginning and N_a – that at the end of the contact time t . An excellent antibacterial activity was in agreement with 99.9 percent reduction of microorganisms ($R > 99.9\%$). This related to 51g for bacteria and 41g for fungi.

3. Results and Discussion

SPR absorption band positions of the Ag and Au colloids obtained were at 417 and 530 nm respectively, and the average particle diameters were of 10-12 nm for Ag and 20-30 nm for Au (Figures 1(a), 1(b), 2(a), 2(b)).

When NPs from prepared colloids were adsorbed on the HDS surface adsorption equilibrium occurred in a few minutes. An irreversible binding of both types of NPs with silica was found. It should be noted, that a homogeneous distribution of the NPs was observed also after thermal treatment of bright-colored Ag/SiO₂ at 85-90°C and Au/SiO₂ at 500°C.

In the X-ray spectra of Me/SiO₂ powders two clear reflections were noticed at $2\theta = 38^\circ$ and 44° , more evident in case of Au/SiO₂. These peaks were assigned to (111) and (200) planes of cubic Me respectively.

Sample color and characteristic optical spectra of Me NPs in colloids were stable for 7 month and more. Ag/SiO₂ powders treated at 85-90°C were stable and

oxidation-resistant on the developed HDS surface. Au/SiO₂ powders were stable after treatment at 500°C after destruction of protective shell.

The oxidation of Ag NPs on silica run after the thermal treatment of Ag/SiO₂ powders in air at app. 200°C due to destruction of organic protective shell and further interaction of Ag NPs with oxygen: $4\text{Ag} + \text{O}_2 \leftrightarrow 2\text{Ag}_2\text{O}$.

Repeated treatment of Ag₂O/SiO₂ at 250 - 300°C resulted in the reduction of yellow color of Ag NPs which disappeared again when the samples were cooled to the room temperature. Obviously the presence of stabilizing shell was necessary to protect Ag NPs on HDS surface against oxidation.

Separately used PVP and SDS stabilizers provided the stability of Ag on the SiO₂ during several days after drying. After that the oxidation of Ag on silica surface occurred and the color disappeared. The protection mechanism of Ag NPs in solution by binary PVP/SDS stabilizer consisted in capping of Ag NPs by SDS chains and absorption by PVP functional groups in accordance with [19]. Double shell of SDS micelles and polymer probably prevented Ag NPs against contact with oxygen. Binding of the metal nanoclusters by the surface of HDS is strong due to interaction between the functional groups of binary stabilizer of Ag NPs and OH-groups of silica surface [9] TEM image of Ag/SiO₂ is shown in the Figure 1(d). According to [20], PVP in specified concentration range was adsorbed irreversibly. At the same time the binary shell did not prevent biocide action of Ag NPs as it is shown later.

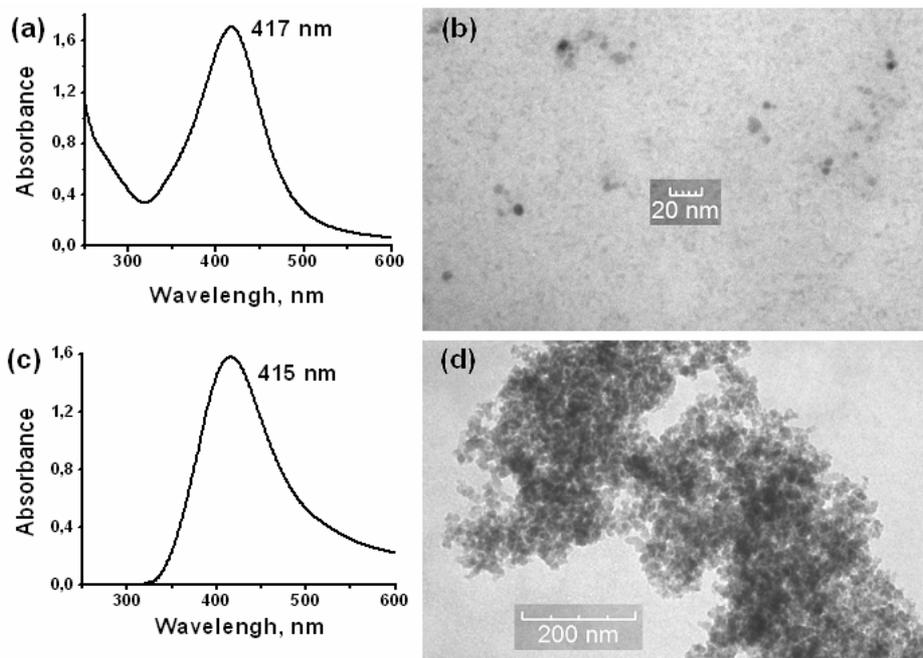


Figure 1. Optical spectra (a,c) and TEM images (b,d) of Ag NPs colloid and Ag/SiO₂ powder respectively.

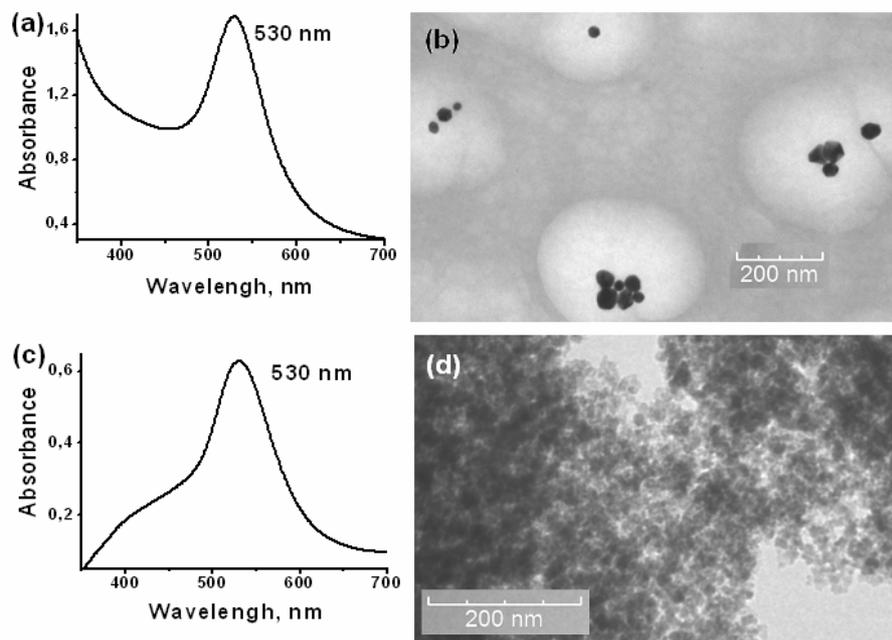


Figure 2. Optical spectra (a,c) and TEM images (b,d) of Au NPs colloid and Au/SiO₂ powder respectively.

In accordance with [21,22], colloid silica particles in presence of SDS micelles after drying formed 3D structures with developed porosity. We suggested that Au NPs produced within colloid using only SDS stabilizer were localized within these “secondary” pores of HDS. TEM image of Au/SiO₂ is shown in the Figure 2(d). Such location can provide an explanation for strong binding of Au NPs by silica after 500 C treatment.

Absorption maxima in the DRS of Ag/SiO₂ and Au/SiO₂ powders calculated via Kubelka-Munk equation were equal to those in colloid solutions. This is an evidence of nanoscale dimensionality of metal particles after absorption (Figure 1(c), 2(c)).

3.1. Me/SiO₂ Composites Antimicrobial Activity

To explain the inhibitor effects of silver on bacteria it was suggested that silver reacted with proteins by combining the thiol (-SH) groups leading to the bacteria inactivation [23]. In this work we examined the antibacterial activity of Ag/SiO₂ and Au/SiO₂ suspensions and of Ag (Au) colloids. Ag/SiO₂ powders were tested after treating at 85-90°C, Au/SiO₂ – after 500°C. The probes were diluted in distilled water with the concentration of 0.0016% wt. Me and of 3.13% wt. HDS in suspension. Colloids were tested with the concentration of 0.0016% wt. Me.

AgNO₃ solution was used in the control experiments in the equal concentration as Ag NPs. Binary stabilizer PVP/SDS and NaBH₄ were studied in the control with the same amount as in the colloids. Initial HDS was tested also at the concentration 3.13 % wt. in presence of

the stabilizers and NaBH₄.

The results of antimicrobial activity of Ag NPs in colloid and suspension against *E. coli*, *S. aureus* и *C. albicans* are presented in the Table 1.

Essential reduction values for bacteria *E. coli* (5 lg) and fungi *C. albicans* (4 lg) in colloids were achieved after 1 hour of exposure of microbial cells with Ag NPs. Staphylococcus were more Ag NPs-resistant, particularly 4.35 lg reduction was achieved only after 4 hour of exposure. Decrease in Ag concentration allowed to reveal the phenomenon mentioned. *C. albicans* bacteria were the most sensitive among of objects studied.

The control AgNO₃ solution showed no antimicrobial action. The same result was also revealed for PVP/SDS and NaBH₄ mixture. Thus experimental data presented indicated a high antimicrobial activity of silver colloids to all microorganisms.

Ag NPs embedding into SiO₂ surface slightly decreased activity of Ag/SiO₂ suspension. The exposure time increased and changes in interaction character of Ag NPs with the microbial cells appeared.

The contact time for 4 lg reduction reached for *C. albicans* remained the same as that in colloid (1 h). At the same time, Ag/SiO₂ resistance of *E. coli* rose. The reduction value was only 3.58 lg after 4 hour contact time. On the contrary, *S. aureus* was more sensitive to Ag/SiO₂ then to Ag in colloid.

Nevertheless the antimicrobial activity of Ag NPs/SiO₂ complex remained high in general.

Nanosized gold at the same concentration range showed no antibacterial action on the indicated microorganisms. As it was shown in [24], Au NPs suppressed bacteria

Table 1. The antimicrobial activity of Ag NPs in colloids and suspensions.

Samples and concentrations	Exposure time, h	test-strains, lgR*		
		<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Ag NPs (0.0016 % wt) colloid	1	> 5.22	< 1.57	> 4.27
	2	> 5.22	2.07	> 4.27
	4	> 5.22	4.35	> 4.27
	24	> 5.22	> 5.24	> 4.27
initial microorganisms amount, lgN ₀		7.37	7.39	6.42
Ag NPs (0.0016 % wt)/SiO ₂ (3.13 % wt)	1	< 1.39	3.89	> 4.54
	2	1.48	5.14	> 4.54
	4	3.58	> 5.17	> 4.54
	24	> 5.06	> 5.17	> 4.54
SiO ₂ (3.13 % wt) (control)	1	< 1.39	< 1.5	< 0.87
	2	< 1.39	< 1.5	< 0.87
	4	< 1.39	< 1.5	< 0.87
	24	< 1.39	< 1.5	< 0.87
initial microorganisms amount, lgN ₀		7.21	7.32	6.69

*According to European standards if the counts of CFU/ml sample are higher or lower than usual limits for counting microorganisms it must be reported the number as “> value” or “< value” for further calculation of number of survivors at the end of the contact-time t (Na) and reduction (R) next.

growth for the first stage of their reproduction.

Experimental data presented in Table 1 indicated almost total growth inhibition of all studied microbes when the extremely low concentrations of nanosized Ag were used. It should be noted that antimicrobial rates were over 1–2 orders of magnitude than those given in literature.

4. Conclusions

Stable Au and Ag nanoparticles on SiO₂ surface were obtained by adsorption of previously synthesized Me NPs colloids in presence of SDS and binary SDS/PVP stabilizing agents.

The fixation of the metal nanoclusters on silica surface was a result of: 1) interaction between the functional groups of stabilizer shell of NPs and OH-groups of silica; 2) location of Me NPs within secondary pores of HDS globules.

Composite systems obtained containing strongly bound and homogeneously distributed Ag NPs on silica surface served as potential wide-spectrum effective antimicrobial materials for water cleaning as well as for medical and pharmaceutical application.

5. References

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