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PREPARATION OF SILVER NANOPARTICLES IN REVERSE MICELLES AND ANTIBACTERIAL ACTIVITY OF SILVER MODIFIED-PAINTS

ABSTRACT

Silver particles having fine or ultrafine sizes are one of the fastest growing research interests with wide applications. Here we report the preparation method of silver modified paints which revealed antimicrobial activity against gram-negative bacteria Escherichia coli, gram-positive Staphylococcus aureus, yeast Saccharomyces cerevisiae and pathogenic fungi belonging to Candida family. In this work, we choose heptane and cyclohexane, as the oil phase for preparation nanometer sized metallic particles. We have also studied the effect of different silver precursors – silver nitrate, silver citrate and different surfactants: anionic AOT, non-ionic Triton X100, Span 80 and Tween 85 for stabilization of obtained silver colloids. UV-VIS spectrum contained a strong plasmon band near 410 nm, which confirmed silver ions reduction to Ag° in microemulsion system or aqueous phases. Prepared samples contained from 500 to 2000 ppm of silver. The diameter size of silver nanoparticles was in the range from 16 nm to 82 nm and were stable for 3 months without precipitation.

Key words: *silver, nanoparticle, paint, microemulsion*

INTRODUCTION

Silver has been applied as antibacterial agent for ages. Due to quantum size effects and surface effects colloidal particles present many unique properties like excellent electrical conductivity, catalytical activity, chemical stability and antimicrobial activity. According to the literature data, the antimicrobial activity strongly depends on the silver nanoparticles size and shape [1].

These properties have led to tremendous range of applications of silver nanoparticles, for instance textiles, engineering materials, medical devices [2-5], food preparation surfaces [4] and coated sanitary wares.

Many methods have been developed for the preparation of silver nanoparticles, such as: reverse micelles [6,7], electrochemical [8,9], chemical reduction [10,11], sonochemical [12], photochemical [13,14] and laser ablation-based [15,16].

Microemulsion served as a system of nanoreactors for preparation of ultrafine particles with a narrow size distribution by controlling the growth process of nanoparticles. The interchange

of the reactants takes place during the collisions of the water droplets in the oil phase. The reaction takes place inside the droplets (nucleation and growth), which control the final size of the particles. The properties of silver nanoparticles depend on their size. The smaller silver particles with diameter around 10 nm exhibit higher catalytic and antimicrobial activity. Thus, the control over the shape, size and size distribution is crucial in tuning physical, chemical and optical properties of silver nanoparticles. The most common used surfactant to form reverse micelles is sodium bis (2-ethylhexyl) sulfosuccinate (AOT). AOT microemulsion has extensively been applied to prepare metal nanoparticles. AOT as the anionic surfactant due to its higher solubility in organic phase helps to extract metal cations from the aqueous to reverse micellar phase. Zhang et al. [17] reported that silver nanoparticles prepared in AOT microemulsion have a smaller average size and narrower size distribution compared to the particles prepared with using cationic or nonionic surfactant in microemulsion system. They have also found that the particle size increases as the solvent changes from decane to heptane but decreases for cyclohexane [17].
Here we report the preparation method of silver colloids and silver-modified paints which revealed antimicrobial activity. The effect of silver precursor, stabilizer, reaction medium for antibacterial/antifungal properties was investigated.

EXPERIMENTAL

Materials and instruments

Silver nitrate (pure p.a.) was provided by POCh and used as the starting material for preparation of silver nanoparticles. Ascorbic acid (99%) was purchased from Aldrich and used as the reducing agent. Cyclohexane and heptane were used as the continuous oil phases and were purchased from Aldrich. Sodium bis- (2-ethylhexyl) sulfosuccinate (AOT), Span 80, Tween 85, purchased from Aldrich and Triton X100 purchased from POCh were used as surfactants, 1-hexanol (Aldrich, p.a.) and isopropanol (Eurochem BGD, p.a.) as co-surfactants for stabilization of obtained silver nanoparticles.
The size distribution of samples was measured on Malvern Zetasizer 3000. UV–VIS spectra were recorded with a 1 cm path length quartz cell using an Beckmann spectrophotometer. Deionized water or cyclohexane were used as the reference samples to take the blank spectrum for all measurements. The antimicrobial activity of silver nanoparticles was evaluated through the determination of the minimum inhibitory concentration (MIC) for *Escherichia coli*, *Staphylococcus aureus*, Fungi - *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Saccharomyces cerevisiae* and two recombinant strains of *Saccharomyces cerevisiae*. Disc diffusion sensitivity test was also performed using *Escherichia coli, Staphylococcus aureus* and pathogenic fungi belonging to *Candida* family.

Preparation of silver colloids and silver-modified paints

Silver colloids were prepared by mixing two microemulsion containing silver precursor in water cores with microemulsion containing the reducing agent (ascorbic acid) dispersed in aqueous phase of microemulsion. All experiments were carried out at room temperature**.** Water content was controlled by fixing the molar ratio of water to surfactant (wo) at 2. The molar ratio of silver ions and ascorbic acid was held constant at a value of 1.2. After all the

ascorbic acid microemulsion was added, the vigorous magnetic stirring was maintained for 1 h, the resulting microemulsion mixtures changed to a stable, transparent brown color colloid after the reaction, indicating the formation of Ag nanoparticles.
Silver-modified paints were prepared using three different methods as was shown in Fig. 1. In the first method the commercial phtalic paint (Cieszynka Plus) was diluted with previously obtained silver colloid in aqueous solution. The volume ratio was kept constant at 1:5 (20 cm^3 of microemulsion: 80 cm^3 of paint). In the second method the paint was diluted with microemulsion containing silver ions in water cores. The last method based on reduction of silver ions directly in paint using microemulsion containing ascorbic acid in water cores.

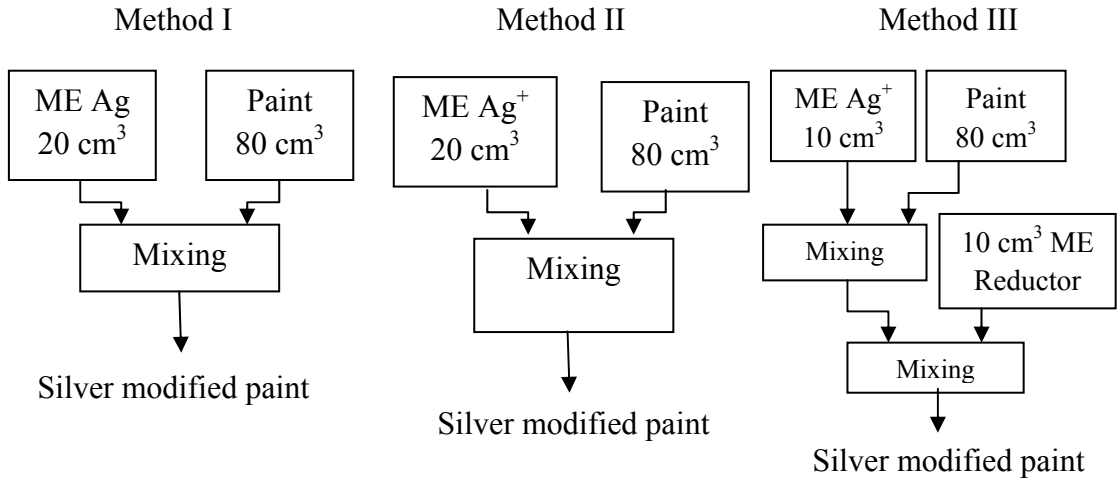

Fig. 1. Schematic illustration of preparation methods of silver-modified paints,(ME - microemulsion)

Antimicrobial activity analysis

The interior modified-paints were coated on paper using K-paint coater at a speed of 1 cm/min and 2 μm. The samples were dried for at least 48 hours before antimicrobial analysis. The coated papers were cut to 15 cm×15 cm squares.
The agar plate method was used for the evaluation of antimicrobial effect of the coatings. Two different bacteria, gram-positive *Staphylococcus aureus* (ATCC 6538) and gram-negative *Escherichia coli* (ATCC 10536) and two different fungi, *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763), were used for testing the antibacterial/antifungal activity of silver-modified paints. The solid YEPG (2 % glucose, 1 % yeast extract, 1 % bacto-peptone, 2 % agar for fungi) or Tryptic Soy Agar (BTL, for bacteria) were liquefied by warming to 100 ^{0}C and then chilled to 50 ^{0}C. For disc diffusion sensitivity tests, the semi-liquid medium was inoculated with 10^5 cells/ml of an overnight culture of microbial cells, poured into Petri plates and left for solidification. The paint samples were placed on the agar medium surface. Plates were incubated for 24 h at 37 °C (antibacterial activity determination) or at 30 °C (antifungal activity determination). The zones of inhibition were measured.
The prepared silver modified-paint was irradiated with UV-Vis lamp (Xenon, 1000 W, Oriel) for 2h in order to evaluate the color changes.

Stability and composition of silver colloids and silver modified-paints

Table 1 shows the characteristic of obtained 0.2M W/O microemulsions. The last column includes the data about stability of the microemulsion. If, after a few days, the microemulsion

stays stable and clear, without precipitation, it is marked as "+". Otherwise, "-", which means it is unstable. The composition of silver-modified paints prepared by method I, II and III are shown in Table 2.

Table 1. *Stability and composition of W/O microemulsions*

Sample label	Ag concentration [ppm]	Ag Precursor	Oil Phase	Surfactant	Co-surfactant	Stability of Microemulsion*
M1	500	$AgNO_3$	cyclohexane	AOT	(0.1M) 1-hexanol + (0.05M) isopropanol	**(+)**
M2	500	$AgNO_3$	cyclohexane	Span 80 + Tween 85	(0.1M) 1-hexanol	(-)
M3	500	$AgNO_3$	dodecane	AOT	-	**(+)**
M4	500	silver citrate	dodecane	AOT	-	(-)
M5	500	$AgNO_3$	dodecane	Triton X-100	(0.1M) 1-hexanol	(-)
M6	2000	$AgNO_3$	cyclohexane	AOT	(0.1M) 1-hexanol + (0.05M) isopropanol	**(+)**
M7	500	$AgNO_3$	heptane	AOT	(0.05M) isopropanol	**(+)**

* visual evaluation: (+) stable for 3 months, (-) unstable, silver precipitates after 1 day

Table 2. *Composition of silver modified-paints (P1-P14 prepared using Method I and P15-P16 prepared using method II, P17 for method III)*

Sample label	Silver colloids sample label	Microemulsion volume added to paint [cm^3]	Cyclohexane volume added to paint [cm^3]	Ag concentration in paint [ppm]
P1	-		20	-
P2	-	20 (ME without Ag)	-	-
P3	M1	2	18	10
P4	M1	4	16	20
P5	M1	10	10	50
P6	M1	20	-	100
P7	M6	0,5	19,5	10
P8	M6	1	19	20
P9	M6	2,5	17,5	50
P10	M6	5	15	100
P11	M6	10	10	200
P12	M6	15	5	300
P13	M6	20	-	400
P14	M7	20	-	100 (in heptane)
P15	Method II			400
P16				800
P17	Method III			400

RESULTS AND DISCUSSION

Particle size distribution of silver nanoparticles

The particle sizes of the silver nanoparticles measured by dynamic light scattering (DLS) are shown in Table 3.

Table 3. *Average Ag participle diameter and dispersity index*

Sample label	Silver concentration [ppm]	Oil Phase	Average Ag particle size [nm]
M1	500	cyclohexane	16
M3	500	Dodecane	6
M6	2000	cyclohexane	82
M7	500	Heptane	21

The particles size for obtained silver colloids was below 100 nm. It was found that the particles size depended on concentration of silver nanoparticles and type of the oil phase of the microemulsion system. The smallest silver nanoparticles were prepared in AOT-dodecane microemulsion (M3) and in AOT-cyclohexane microemulsion (M1). The largest particles were obtained for sample containing 2000 ppm of Ag.

UV-Vis spectra analysis

Fig. 2 shows that the absorption peak at approximately 410 nm corresponding well with that of silver nanoparticles present in microemulsion system. The sample M3 with silver particles of about 6 nm showed lower absorption band and wider peak compared to the sample containing silver nanoparticles with diameter of about 16 nm, as shown in Fig. 2.

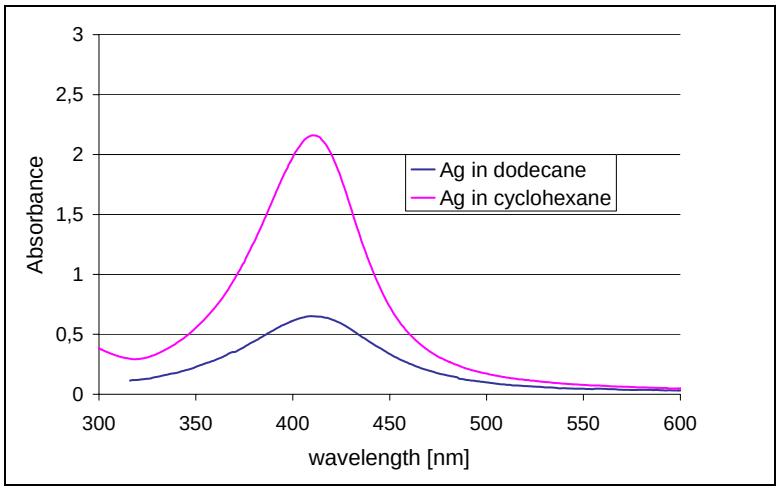

Fig. 2. UV-VIS absorption spectrum of Ag nanoparticles obtained in water-AOT-dodecane microemulsion and water-AOT-cyclohexane microemulsion

Antibacterial activity of silver modified-paint

The diameter of inhibition zone depended on silver particles size and silver concentration in colloids. In Table 4 the diameters of zones of inhibition for silver-modified paints are presented. The labels in the first column are the same as in Tables 1 and 2, and are related to silver modified-paint. The best antimicrobial activity was observed for samples P6, P13 and P16 prepared using silver nanoparticles obtained in microemulsion M1 and M6. The zones of inhibition against *C. albicans* and *S. cerevisiae* were observed for samples of paints P6 and P16.

Table 4. *The inhibition zones against microorganisms for silver modified-paint*

Silver modified-paint			Zone of Inhibition for silver-modified paint [mm]			
Sample label	Ag concentration [ppm]	Ag /oil phase	***Candida albicans*** ATCC 10231	***Saccharomyces cerevisiae*** ATCC 9763	***Escherichia coli*** ATCC 10536	***Staphylococcus aureus*** ATCC 6538
P1	Pure Paint		0	0	0	0
P2	Paint + ME without Ag		**1**	0	0	0
P3	10	Ag cyclohexane	0	0	**2**	**2**
P4	20	Ag cyclohexane	0	0	0	0
P5	50	Ag cyclohexane	**1**	**1**	**3**	**3**
P6	100	Ag cyclohexane	**4**	**2**	**4**	**4**
P7	10	Ag cyclohexane	0	0	0	0
P8	20	Ag cyclohexane	0	0	0	0
P9	50	Ag cyclohexane	0	0	0	0
P10	100	Ag cyclohexane	0	0	0	**1**
P11	200	Ag cyclohexane	0	0	**2**	**3**
P12	300	Ag cyclohexane	**3**	0	**3**	**4**
P13	400	Ag cyclohexane	**4**	**1**	**4**	**6**
P14	100	Ag heptane	0	0	0	0
P15	400	Ag^+ cyclohexane	**3**	**1**	0	0
P16	800	Ag^+ cyclohexane	**5**	**1**	**4**	**7**
P17	400	Ag cyclohexane	**3**	**1**	**3**	**5**

Figure 3 shows an example of zones of inhibition resulting from the disc diffusion test performed using *E. coli*, *S. aureus*. The diameters of zones of inhibition were 5 mm for *C. albicans* and *E. cola* and 7 mm for *S. aureus*. The best bioactivity revealed sample P6 with

100 ppm of silver nanoparticles concentration and sample P16 with 8-times higher Ag^+ amount (800 ppm of silver ions). Our results are in good agreement with others. Parameswari et al. [18] studied the bactericidal potential of silver nanoparticles. They found that silver nanoparticles at concentration of 30 μg/cm^3 showed 100% bacteria growth reduction, whereas in $AgNO_3$ much less effect was observed at the same time. The bactericidal effect of silver nanoparticles is attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes [19]Morones et al. [20] proposed that the antimicrobial mechanism of silver nanoparticles and silver ions are distinctly different. For treatment with silver nitrate, DNA loses its replication ability and expression of ribosomal subunit proteins, as well as other cellular proteins and enzymes essential to ATP production, becomes inactivated (Yamanaka *et al.*, 2005). Silver ions interact with thiol groups in protein, which induce the inactivation of the bacterial proteins.

Silver nanoparticles are incorporated in the cell membrane, which causes leakage of intracellular substances, structural changes and damage to membranes, finally leading to cell death. [12,15]. Some of the silver nanoparticles also penetrate into the cells.

Silver in ionic or nanoparticle forms has a high antimicrobial activity and is therefore widely used for various sterilization purposes including materials and water disinfections.

These results indicate that the antibacterial efficacy of silver modified-paints may depend on the particles size and oxidize form of silver and its concentration in paint. The highest inhibitory efficiency shows silver modified-paint P16 containing 800 ppm of Ag^+ prepared in Method II. On the other hand the paint containing 100 ppm of silver nanoparticles (which was prepared in microemulsion containing 500 ppm of Ag°) showed higher antibacterial efficiency.

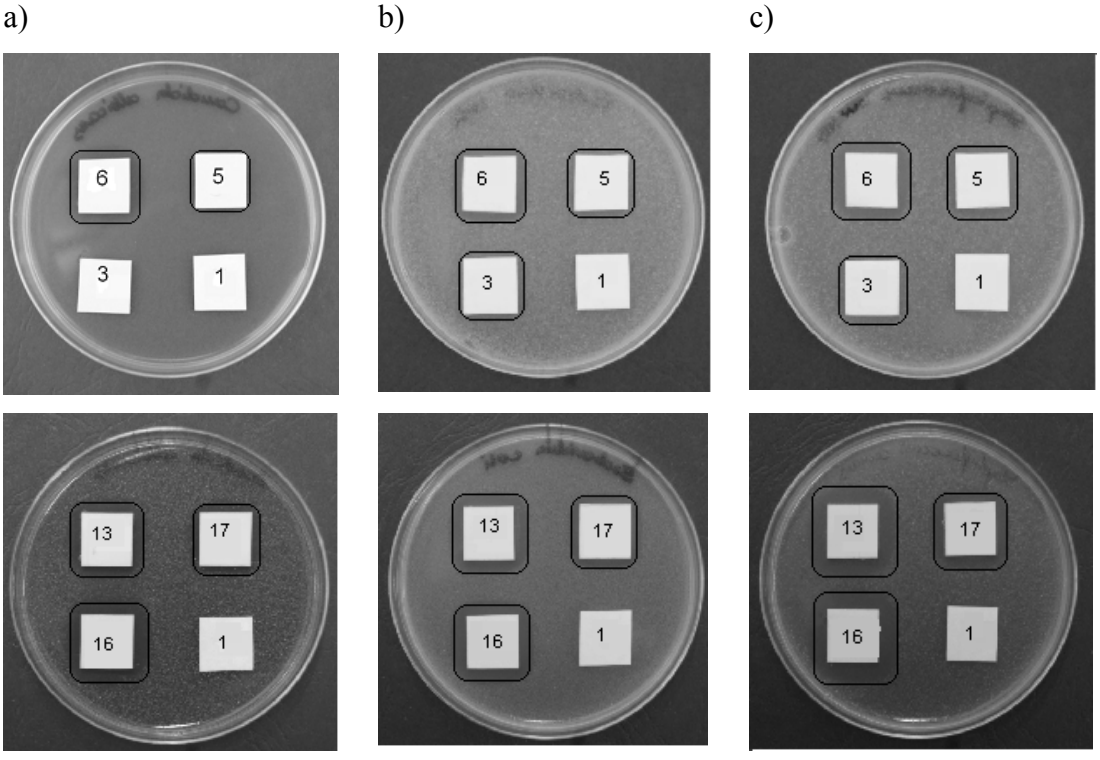

Fig. 3*. The inhibition zones against microorganisms produced by the silver modified-paint a) Candida albicans, b) Escherichia coli, c) Staphylococcus aureus*

Higher antibacterial activity was observed for paint obtained by using Method I. The prepared paints did not alter colors under UV irradiation.

CONCLUSIONS

For all obtained silver-modified paints containing silver nanoparticles prepared in microemulsion system which revealed antimicrobial activity against *E. coli, St. aureus, S. cerevisiae* and pathogenic fungi belonging to *Candida* family, the effect of silver particles size on antimicrobial activity of paint was observed. If the silver nanoparticles were smaller, the paint showed higher antibacterial activity. The obtained paints did not alter colors under UV irradiation. These well-dispersed silver nanoparticles obtained in microemulsion system can be used directly into commercially available paints.

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