Slobodan, G., Momčilo, C., & Miodrag, Š. (2023). Microbiological activity of silver nanoparticles stabilized with dextran derivatives, 2(1), 1-12. doi: 10.35120/medisij020101g
UDK: 579.67:[546.57:620.3

MICROBIOLOGICAL ACTIVITY OF SILVER NANOPARTICLES STABILIZED WITH DEXTRAN DERIVATIVES

Slobodan Glišić[1], Momčilo Conić[1], Miodrag Šmelcerović[1]*

[1]Academy of professional studies South Serbia, Leskovac, Serbia
e-mail: glisictfl@gmail.com, momcilo.conic@gmail.com, msmelcerovic@yahoo.com

Abstract: The paper shows the microbiological activity of silver nanoparticles (AgNPs) stabilized with dextran derivatives with carbox-ymethyl dextran (CMD) and dextran sulfate (DS). Non-toxic, green procedures for the synthesis of AgNPs with CMD, DS were developed. The increasing application of silver nanoparticles in many areas, especially favors the so-called non-toxic, i.e. "green" synthesis procedures. Unlike physical and chemical synthetic methods, green synthesis has a number of advantages: lower energy consumption, simple execution, costs are reduced, use of non-toxic chemicals as reducing and stabilizing agents. Nanoparticles AgNPs-DS and AgNPs-CMD showed microbiological activity against the analyzed test microorgan-isms. Silver nanoparticles obtained in this way, due to their stability and preserved antimicrobial activity, can be widely used in various branches of industry. The results of testing the antimicrobial activity of synthesized nanoparticles from the technologi-cal aspects are important; these compounds have potential application in the biomedical field, and simple procedures have many advantages, such as low costs, compatibility for medical and pharmaceutical applications, as well as the production of other commercial products in the food industry.

Keywords: *microbiological activity, silver nanoparticles, green synthesis, food industry.*

Introduction

Nanotechnology is a relatively young, multidisciplinary field in which the knowledge of natural sciences and engineering principles are applied with the aim of obtaining, characterizing and applying nanometer-sized materials (Ramsten, 2014). Materials of nano dimensions have specific characteris-tics. When the number of atoms that make up a material is significantly reduced, the atoms occupy a different arrangement and the distance between the surface atoms changes, which leads to a change in physical and chemical characteristics in relation to materials of the same composition, but with mac-roscopic dimensions (Trajković and Marković, 2010). In the last few decades, metal nanoparticles (gold, silver, platinum, palladium and many metal oxides, and composite materials based on them) have been the focus of many research groups (Krstić, 2013, Nadagouda and Varma, 2007, Liu et al. 2009, Shevchenko et al. 1985, Tolmachev et al.1985).

The size of metal nanoparticles ranges from 1 to 100 nm. Nanoparticles, thanks to their large specific surface area in relation to their volume, as well as their high surface energy, have unique catalytic, electrical, magnetic, optical and mechanical properties. (Horikoshi and Sorpone 2013). Silver nanoparticles are due to their catalytic properties (Jiang et al., 2005) and optical (McFarland and Duy-ne, 2003) properties, as well as antimicrobial activities (Bhattacharya and Mukherjee 2008), attracted the attention of many research groups. They can be synthesized by different procedures - methods: chemical reduction of silver ions (in the presence of a reducing and stabilizing agent) in aqueous solutions (Leopold and Lendl, 2003), Caswell et al., 2003, Chaki et al., 2004) as in non-aqueous solu-tions (Chen and Huang 2002, Wang et al., 2003, Faure et al., 2003), electro chemical method (Johans et al., 2004), reduction of silver ions using ultrasound (Zhang et al. 2004), by photo-induced or cata-lyzed reduction (Shchukin et al., 2003, Zhang et al., 2003, Cozzoli et al., 2004), by microwave-induced synthesis (Komarneni et al., 2002, Yamamoto et al., 2004), by radiation-chemical reduction (Hornebecq et al., 2003, Lihui et al., 2004), micro emulsion method (Zheng et al., 2003, Zheng et al., 2004, Maillard et al., 2002, Maillard et al., 2003) and biochemical reduction of silver ions (Naik et al., 2002, Bhainsa and D'Souza 2006). In all AgNPs synthesis procedures, the basic reaction is the reduc-tion of silver ions to elemental silver. In doing so, one should bear in mind the great tendency towards agglomeration, which reduces the ratio of surface area to volume, and thus changes the properties of nanoparticles. Therefore, when choosing a reducing agent, one must take into account its properties as a protective agent that prevents agglomeration. For this

*Corresponding author: glisictfl@gmail.com

purpose, various substances can be used, most often polymers of synthetic or natural origin, especially biopolymers of the polysaccharide type and their derivatives (Krstić, 2013, Nadagouda and Varma, 2007, Liu et al. 2009, Shevchenko et al. 1985, Tolmachev et al.1985, Quelemes et al., 2013,Hamedi et al., 2012, Chudobova et al., 2013b, Garza-Navarro et al., 2013, Prinz et al.,2010). The increasing application of silver nanoparticles in many areas, especially favors the so-called non-toxic, relatively "green" synthesis procedures. In con-trast to physical and chemical synthetic methods, green synthesis has a number of advantages: lower energy consumption, simple execution, costs are reduced, use of non-toxic chemicals as reducing and stabilizing agents, which enables biocompatible and in vivo application of the formed particles (Amooaghaie et al., 2015, Sathishkumar et al., 2009, Bar et al., 2009, MubarakAli et al., 2011). There is an ever-greater interest in green synthetic procedures where plant or other natural products are used as reducing and protective agents. One of the ways to switch to non-toxic syntheses is to use natural products, i.e. water extracts of biomass, as well as polymers of natural or synthetic origin, produced by various microorganisms, as a reducing and stabilizing agent. For biomedical applications, honey has proven to be an attractive green agent, which simultaneously reduces silver and stabilizes silver na-noparticles. Honey has been used for medicinal purposes since ancient times due to its composition and specific characteristics. Honey consists of various carbohydrates (>80%), with fructose and glu-cose being the most abundant, accounting for about 70%, while sucrose (table sugar) represents ~5%. The rest is water, about 20%, while proteins are represented in a small percentage, about 0.3%. During the synthesis of silver nanoparticles in honey, glucose serves to reduce silver, and honey proteins to stabilize nanoparticles. In order for silver reduction to begin, the presence of sodium hydroxide is nec-essary, which facilitates the opening of the glucose ring, which allows the silver ions to oxidize glucose to gluconic acid. During this chemical reaction, silver nanoparticles and gluconic acid are formed, and all other components of honey remain unchanged. The most commonly used biopolymers are poly-saccharides and their derivatives: cellulose and carboxymethyl cellulose, dextran (Berry et al., 2003), CMD and DS, pullulan, alginate, agar, starch (Chairam and Somsook 2008, Chairam et al., 2009), chi-tosan (Park et al., 2005) and glycogen. From synthetic polymers, due to biocompatibility, PVP, PVA, poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(methyl methacrylate) (PMMA) are used. (Stasica et al., 2000, Darwis et al.,2002). The mechanism of reduction of silver ions and stabilization of formed AgNPs by polysaccharides is well explained in the paper (Bankura et al., 2012) on the example of the synthesis of silver nanoparticles in water, using dextran as a reducing and protective agent. Silver nanoparticles cannot be synthesized using a solution of dextran in purified water, because purified water has a weak acidity (pH = 6) that can prevent the reduction of Ag+ to Ag0 in the nanometer range by forming R-O+H2. The presence of a very low concentration of NaOH can liberate the hydroxyl groups of dextran by removing protons and help the formation of silver nanoparticle which is observed by the color change of the solution (Bankura et al., 2012).

Microbiological properties of silver nanoparticles

The microbiological properties of silver have been known for centuries. Thus silverware was used to store water, while compounds containing silver were used in traditional medicine (Armentano et al., 2010, Slawson et al.,1992). In the last few decades, there has been a significant increase in mi-croorganisms that are resistant to existing antibiotics, which has brought silver back into medical use. (Monteiro et al., 2009). Nanoparticles have been shown to have greater microbiological activity than silver ions (Lok et al., 2006). Nanoparticles act at the level of the cell (cytoplasmic) membrane by in-teracting with structural proteins, changing the permeability of the membrane, and then with membrane enzymes, inhibiting their activity. Nanoparticles also penetrate the cell by interacting with DNA, which affects the cell's ability to replicate. In doing so, the nanoparticles also dissolve, releasing silver ions that also have an anti-microbial effect (Morones et al., 2005). Silver nanoparticles have been shown to act on various viruses (Xiang et al., 2011), bacteria (Guzman et al., 2012, Radzig et al., 2013) and fun-gi (Panáček et al., 2009). Chemically synthesized silver nanoparticles in solutions of different saccha-rides showed antibacterial activity against 10 different types of bacteria, whereby the smallest nanopar-ticles in this study with a diameter of 25 nm were the most effective, while the largest nanoparticles with a diameter of 50 nm had the weakest microbiological activity (Panáček et al.,2006). It has also been shown that the shape of nanoparticles significantly affects microbiological activity (Pal et al.,2007). When examining the microbiological activity of silver nanoparticles of different shapes (rod-shaped, spherical and triangular nanoparticles) against E. coli ATCC 10536, it was shown that the most effective nanoparticles were triangular-shaped, while the rod-shaped nanoparticles had the weakest microbio-logical efficiency. Silver nanoparticles are already commercially used for wound dressings (Acticoat, Smith&Nephew, USA), but have potential applications for antimicrobial creams and gels (Jain et al., 2009), as well as for coverings for catheters, drains and

implants (Knetsch and Koole, 2011). Silver nanoparticles are also, due to their strong antimicrobial activity, very attractive components for improv-ing the functionality of wastewater treatment membranes and water filters (Jain and Pradeep 2005). However, the main problem with the application of silver nanoparticles is their rapid release from the surface of the polymer from which the membranes are made (Tseng et al.,2006), therefore, the con-centration of silver in the filtered water is above the permitted concentration of 0.1 mg/l according to the EPA standard (Environmental Protection Agency-EPA). Therefore, the functionality of the membranes is improved by immobilizing the silver nanoparticle inside the polymer fibers from which the membrane is made. In this way, a small percentage of nanoparticles is released from the polymer into the filtered water, and the antimicrobial activity remains unchanged.

Carbohydrates as ligands

Carbohydrates represent a very widespread group of natural products that are synthesized by living organisms and that occupy an important place in life cycles. They make up about 80% of the mass of the dry substance of plants and about 2% in animals, as energy reserves (eg as starch) or as building materials (cellulose). Polysaccharides have different and, in many cases, complex chemical structures, numerous physiological functions and a wide range of potential applications. The basis of the carbohydrate structure of polysaccharides is represented by monosaccharide units that can be connected to each other through different positions and orientations, which makes the structural chemistry of polysaccharides very complex. They can be covalently bound to proteins or lipids and in the form of complex glycoconjugates they form biologically important compounds that have different functions. According to the sources from which they are obtained, they are divided into plant, animal and microorganism polysaccharides. Polysaccharides are used in industry as thickeners, stabilizers and gelling agents. Also, they are used as agents for removing pollutants from the environment, and due to their biological effects, such as antioxidant, probiotic or antitumor, the interest in polysaccharide chemistry is growing every day (Đaković,1985, Liu et al., 2010a). They can be isolated from different sources (bacteria, fungi, algae and plants), although polysaccharides from algae and higher plants are dominant on the world market. These biopolymers are isolated by direct extraction from biomass and can be used in their native form, while in chemical form they are used in various fields. Thus, for example, dextran, which is a polysac-charide obtained by microbiological synthesis, is a molecular chain of anhydro-D-glucopyranose units connected by α-1,6 glycosidic bonds (in addition to these α-1,6 there may be others, α-1,2, α-1 ,3 and α-1,4). It is used as the most effective substitute for blood plasma, it is used in postoperative therapy to prevent venous thrombosis, as well as for the production of granulated gels in gel chromatography (Nikolić, 2001).

Exopolysaccharide dextran

Dextran is a branched exopolysaccharide consisting of D-glucopyranose residues interconnected by α-1,6 glycosidic bonds in the main sequence, with different content of α-1,2), α-1,3 and α-1,4 branches, and different conformation of the glucopyranose unit (Sarwat et al., 2008) (see Fig. 1). Dextran is a natural product, but it can be synthesized from sucrose by means of certain lactic acid bacteria, the most famous of which are Leuconostoc mesenteroides and Streptococcus mutans. It is used medically as an antithrombotic, to reduce blood viscosity, and as a means of increasing volume in anemia. It is used as a substitute for blood plasma in the treatment of shock caused by loss of body fluids (Rehm, 2010). Polysaccharide dextran has an extraordinary ability to form water-soluble complexes with various biometals (Fe, Cu, Co, Zn, Ca, Mg), as well as other metals (Tb, Al, Cd, Pb, Ni, Mn). (Cakić et al.,2008, Nikolić et al.,2009, Mitić et al., 2009, Nikolić et al., 2008, Mitić et al.,2007, Mitić et al., 2008, Vasko et al., 1971, Barker et al.,1953). Raw dextran cannot be used for clinical purposes, because it has toxic and antigenic properties. For the above reasons, raw dextran is depolymerized and repeatedly fractionated with hydrophilic solvents in order to obtain fractions with the narrowest distribution of molar masses. Due to their toxicity, dextran molecules with a high molar mass cause side reactions in the body, while molecules with a low molar mass are quickly excreted from the body. For clinical purposes, only dextrans with a low degree of branching are used, i.e. those with a content of α-1,6 bonds over 90%. Partially hydrolyzed dextran dissolves in water, formamide, dimethylformamide, dimethylsulfoxide, ethylene glycol, glycerol and in alkali solution. Various alcohols and acetones do not dissolve dextran, but rather precipitate it out of solution.

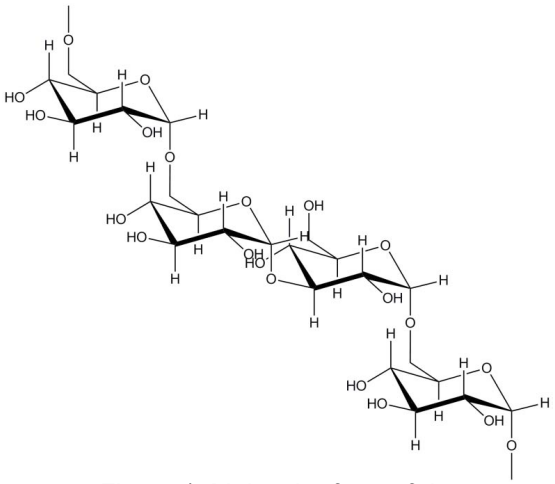

Figure 1. Molecular form of dextran

The reactivity of dextran (but also of other polysaccharides, for example pullulan, inulin, starch, cellulose, agar, agaroid, chondroitin sulfate, etc.) primarily depends on the reactivity of the secondary, equatorially oriented hydroxyl groups of the glucopyranose unit OH at C-2, C-3 and C-4 . Therefore, dextran can build various compounds, among which, due to its biological function and pharmacological activity, esters with inorganic or organic acids, but also complex compounds with biometals are important (Ricketts,1952b, Kentaro and Kotaro 1974). From the group of esters and ethers, the most famous are dextran and cellulose, i.e. and carboxymethyl dextran and carboxymethyl cellulose, as well as dextran sulfate. These compounds are used in pharmacy and cosmetics for the production of many preparations in which they have the function of fillers or, due to their hydrophilicity, for the production of moisturizing creams. Given the presence of carboxyl and sulfo functional groups, they can build complexes with biometals or other derivatives, mostly with Schiff's bases (amino acids), which can be further complexed, say with platinum, and used in cancer therapy, as reducing and stabilizing agents for the synthesis of MNPs or as their carriers (Tao et al., 2011). Certainly the most famous compound of dextran (but also of other polysaccharides, pullulan and inulin) is its polynuclear complex with iron (more correctly with β_2-FeOOH form of iron hydroxide), bearing in mind its wide application in human medicine and veterinary medicine in the treatment of iron deficiency anemia (Cakić et al., 2007).

Dextran derivatives

Carboxymethyl dextran (CMD) is a derivative of dextran, which is obtained by etherification of dextran with halogen acetic acid, (most often chlorine- or bromine-chlorine) in a mixture of alcohol and aqueous sodium hydroxide solution. The synthesis of carboxymethyl dextran is carried out in two stages, the first represents alkalization of dextran, and the second represents carboxymethylation of dextran with chloroacetic acid. CMD dissolves in distilled water and can be precipitated with ethanol.

Dextran sulfate (DS) is an ester of sulfuric acid and dextran. Given that there are three OH groups per glucopyranose unit of dextran, the degree of esterification. In addition to dextran sulfate, inulin sulfates, pullulan sulfates, and others (agar and agaroid, chondroitin sulfate, etc.) are also known (Ricketts 1952a).

Materials and methods

Synthesis of silver nanoparticles (AgNPs) with CMD. In a reactor with a volume of 100 ml, at room temperature, 20 ml of freshly prepared CMD (see Fig. 2) (0.002 M) was first added, and after 10 minutes, with constant stirring (300 rpm), and 10 ml of $AgNO_3$ solution (0.001 M). The suspension was stirred for 2 hours, and then the pH value of the suspension was adjusted to 7 by adding 0.4 ml of NaOH solution (0.001M). The mixture was kept under reflux for 24 hours. The change of white to yellow color of the reaction mixture indicates the formation of AgNPs-CMD nanoparticles. The reaction mixture was then cooled to room temperature, and the AgNPs-CMD nanoparticles were precipitated with 96% ethanol. After standing overnight, the ethanol was decanted, and the precipitate was re-dissolved with redistilled water. Finally, the nanoparticles were precipitated from the solution with 96% ethanol and, after decanting the alcohol, dried for 3 hours at 105°C in a vacuum oven.

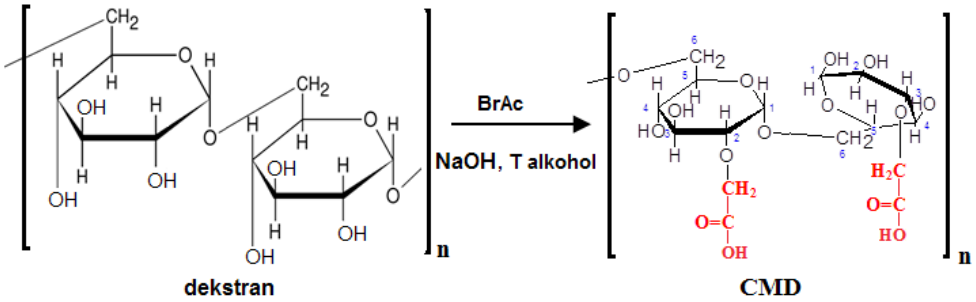

Figure 2. Schematic representation of CMD synthesis

Synthesis of silver nanoparticles (AgNPs) with DS. In a reactor with a volume of 100 ml, at room temperature, 20 ml of freshly prepared DS (see Fig. 3) (0.002 M) was first added, and after 10 minutes, with constant stirring (300 rpm) and 10 ml of AgNO3 solution (0.001 M). The suspension was stirred for 2 hours, and then the pH value of the suspension was adjusted to 7 by adding 0.4 ml of NaOH solution (0.001 M). The mixture was kept under reflux for 24 hours. The change from white to yellow in the reaction mixture indicates the formation of AgNPs-DS nanoparticles shown in Figure 3. The reaction mixture was then cooled to room temperature, and the AgNPs-DS nanoparticles were precipitated with 96% ethanol. After standing overnight, the ethanol was decanted, and the precipitate was re-dissolved with redistilled water. Finally, the nanoparticles were precipitated from the solution with 96% ethanol and, after decanting the alcohol, dried for 3 hours at 105oC in a vacuum oven.

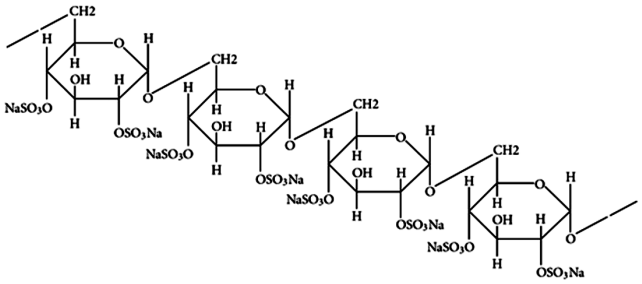

Figure 3. Structural formula of the sodium salt of dextran sulfate DS

Microbiological activity of silver nanoparticles. For testing, standard inoculums of microorganisms were used, which were prepared by sowing the appropriate microorganism culture (100 µl) on a liquid TSB medium (3 ml). After incubation of the inoculum at 37 ºC overnight, the microorganisms were taken from their exponential growth phase (16-18 hours after seeding) and used as such further in the experiment. The method itself is based on adding sterile Erlenmeyer flasks in the sterile atmosphere of the burner in the following order: physiological solution (50 ml), inoculum of the appropriate microorganism (500 µl) and tested silver nanoparticles (1 mg). In order to maintain a sterile environment, the Erlen-meyer flasks were capped and kept at a temperature of 37 ºC in a water bath for 2 h, with agitation. After that, an aliquot of suspension of 1 ml was taken from each Erlenmeyer which was further diluted deci-mally with physiological solution 0.9% NaCl (sterilization for 20 minutes at 121°C). After homogenization of the suspension on a vortex, the density of the suspension was adjusted by comparison with the 0.5 McFarland standard, which corresponds to the number of microorganisms of 1-2x108 CFU/ml (eng. Colony Forming Units) (Andrews, 2005). A 0.1 ml aliquot of the appropriate dilution was transferred to sterile Petri dishes, after which it was covered with melted TSA medium (~20 ml), with mixing. Sterile discs with a diameter of 9 mm were placed on the surface of the seeded substrate and impregnated with 60 µl of AgNPs-CMD, AgNPs-DS nanoparticle solution in concentrations of 0.25 mgcm-3, 0.5 mgcm-3, 1 mgcm-3. Petri dishes were incubated in a thermostat at a temperature of 37 ºC for the next 24 hours (Hamblin and Jori 2011). After incubation, the diameter of the zone of inhibition of the growth of micro-organisms was measured. The following microorganisms were used for the microbiological activity of silver nanoparticles as an indicator of strains: Gram-positive bacteria: Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 11778, Bacillus luteus in haus strain, Listeria monocytogenes ATCC 15313, Gram-negative bacteria: Escherichia coli ATTC 25922, Pseudomonas aeruginosa ATTC 27853, Klebsiella pneumoniae ATTC

700603, Proteus vulgaris ATTC 8427, and fungus: Candida albicans ATTC 2091.

Results and Discussion

Microbiological activity of AgNPs silver nanoparticles with CMD. A large amount of literature data shows that AgNPs possess significant microbiological activity, which is explained by the accumulation of silver ions from the solution in bacterial membranes, which can lead to cell death (Delić, 2011). Namely, the silver cation can react with thiol groups and proteins in cells, and in addition, it can inactivate en-zymes that are required for normal cellular metabolism (Sondi and Salopek, 2004). The microbiological activity of stabilized AgNPs-CMD was performed using the disk-diffusion method on agar. Clinical iso-lates of bacterial pathogens were used to test the antimicrobial activity of synthesized AgNPs-CMD (B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa, Klebsiella) and fungal strains (C. albicans) as indicators of strains for analysis. The results of testing the antimicrobial activity of the AgNPs-CMD na-noparticles solution are shown in Table 1.

Table 1.
Radial diameter of inhibition zones of tested bacterial and fungal strains

Radial diameter of inhibition (mm)				
AgNPs-CMD				
		G_3 = 0,25 mg/ml	G_4= 0,5 mg/ml	G_5=1 mg/ml
Bacterial strains	*Bacillus lutea*	11	13	20
	Bacillus aureus	12	18	21
	Bacillus cereus	11	12	14
	Enterococcus faecalis	-	-	11
	Pseudomonas aeruginosa	-	-	12
	Klebsiella	13	14	15
Fungal strains	*Candida albicans*	-	-	11

The radial growth of the zone of inhibition increases with increasing concentration of AgNPs-CMD from 0.25 to 1 mg/ml except for strains Enterococcus faecalis in which the zone of inhibition is reached only at a AgNPs-CMD concentration of 1 mg/ml. Fungus Candida albicans is more sensitive to AgNPs-CMD, while an inhibition zone of about 11 mm is achieved at a AgNPs-CMD concentration of 1 mg/ml. AgNPs-CMD nanoparticles showed the highest activity against bacteria B. lutea and B. aureus. The antifungal activity of AgNPs-CMD was analyzed according to the strain C. albicans. Zones of inhibition according to C. albicans are very small. Zone of inhibition B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa and K. pneumoniae were 20, 21, 14, 11, 12 and 15 mm, respectively. AgNPs-CMD at a concentration of 1 mg/ml showed a number of specificities according to their antimicrobial activity (see Fig. 4).

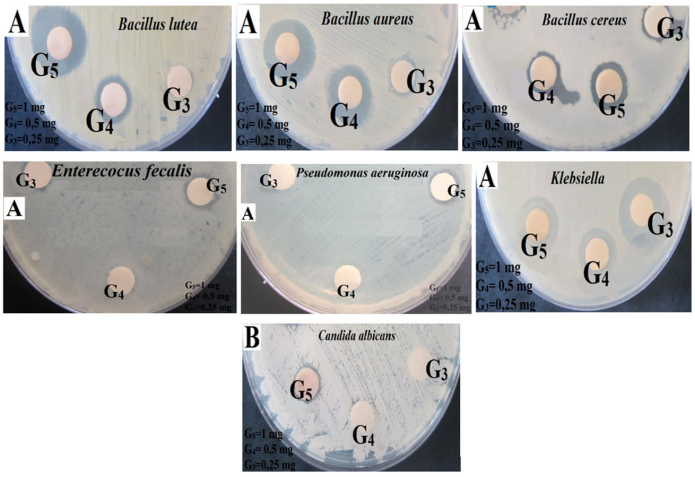

Figure 4. Microbiological activity of AgNPs-CMD nanoparticles of different concentrations against bacterial strains (B. lutea, B. aureus, B. cereus, E. faecalis, P. aeruginosa and Klebsiella) (A) and fungal strain (C. albicans) (B)

Microbiological activity of AgNPs silver nanoparticles with DS. AgNPs-DS solution showed micro-biological activity against bacteria, Staphylococcus aureus, Bacillus cereus, Bacillus luteus in haus strain, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris which proves the presence of clear zones of bacterial growth inhibition around the disks. The radial diameters of inhibition zones are shown in Table 2.

Table 2.
Radial diameter of inhibition zones for tested bacterial and fungal strains

Radial diameter of inhibition (mm)					
AgNPs-DS					
			G_1 = 0,25mg/ml	**G_2= 0,5mg/ml**	**G_3= 1mg/ml**
Bacterial strains	Gram +	*Staphylococcus aureus*	17	18	19
		Bacillus cereus	16	18	19
		Bacillus luteus in haus strain	20	21	24
		Bacillus subtilis	16	17	19
		Listeria monocytogenes	16	17	18
	Gram -	*Escherichia coli*	17	18	21
		Pseudomonas aeruginosa	23	24	26
		Klebsiella pneumoniae	16	18	19
		Proteus vulgaris	13	14	15
Fungal strain		*Candida albicans*	-	16	-

Inhibition was observed in all analyzed bacterial strains at a concentration of 0.25 mg/ml AgNPs-DS, indicating that the minimum inhibitory concentration for these microorganisms is low. For example, in literature (Dhand et al.,2016) it was stated that the minimum inhibitory concentrations for Escherichia coli and Staphylococcus aureus were about 0.26 mg/l. The highest zones of inhibition were recorded against Pseudomonas aeruginosa and Bacillus luteus in haus strain. The zone of inhibition against these microorganisms for a concentration of 1 mg/ml AgNPs-DS was 26 and 24 mm, respec-tively. Among the bacterial strains, Proteus vulgaris it was the least sensitive to the activity of AgNPs-DS with an inhibition zone of 15 mm, when a concentration of 1 mg/ml AgNPs-DS was used. Testing the activity of different concentrations of AgNPs-DS in relation to all other used bacterial strains showed similar results with inhibition zones in the range of 16-17 mm, 18-19 mm and 18-21 mm for concentra-tions of 0.25, 0.5 and 1 mg/ml AgNPs - DS. Results for Klebsiella pneumoniae, Bacillus luteus in haus strain and Pseudomonas aeruginosa are higher than previously reported for AgNPs-CMD. Antimicrobial activity against Candida albicans was observed only at a concentration of 0.5 mg/ml AgNPs-DS. Low antimicrobial activity of AgNPs against Candida albicans has already been reported for AgNPs stabi-lized by CMD. The mechanism of antimicrobial activity of AgNPs can be explained by the accumulation of silver in bacterial membranes that can lead to cell death. Dissolved Ag cation can react with thiol groups and proteins in cells. Additionally, it can inactivate enzymes that are required for normal cellular metabolism (Dhand et al.,2016). AgNPs-DS at a concentration of 1 mg/ml showed a number of speci-ficities according to antimicrobial activity. But a higher concentration of silver can also be harmful to microbes. Therefore, lower concentrations are much more applicable for this purpose. The lower effec-tive concentrations of AgNPs, which cause an effect on organisms different from the control, range from a few ng/l to tens of mg/l depending on the organism and many other factors. This silver nanoparticle synthesis design has great potential due to its antimicrobial activity.

Conclusions

Synthesized AgNPs-DS and AgNPs-CMD nanoparticles showed microbiological activity against the analyzed test microorganisms. AgNPs-DS and AgNPs-CMD showed no activity on the fungal strain C. albicans, and AgNPs-CMD and on P. aeruginosa in a concentration of 0.5 mg/ml. Mutual comparison of the size of the radial zones of inhibition shows that they are slightly smaller with AgNPs-DS, and the smallest with AgNPs-CMD. Also, the zones of inhibition in all analyzed bacterial strains are larger when

the concentration of AgNPs is 1 mg/ml than for 0.5 mg/ml. The results of testing the antimicrobial activity of the synthesized AgNPs show that they can be used in the biomedical field for the production of var-ious preparations in human medicine, cosmetics, veterinary medicine and the food indus-try.Nanoparticles have been shown to have greater microbiological activity than silver ions. Nanoparti-cles act at the level of the cell (cytoplasmic) membrane by interacting with structural proteins, changing the permeability of the membrane, and then with membrane enzymes, inhibiting their activity. Nanopar-ticles also penetrate the cell by interacting with DNA, which affects the cell's ability to replicate. In doing so, the nanoparticles also dissolve, releasing silver ions that also have an anti-microbial effect. Silver nanoparticles have been shown to act on various viruses, bacteria and fungi. Chemically synthesized silver nanoparticles in solutions of different saccharides showed antibacterial activity against 10 different types of bacteria, whereby the smallest nanoparticles in this study with a diameter of 25 nm were the most effective, while the largest nanoparticles with a diameter of 50 nm had the weakest microbiological activity. It has also been shown that the shape of nanoparticles significantly affects microbiological ac-tivity. When examining the microbiological activity of silver nanoparticles of different shapes (rod-shaped, spherical and triangular nanoparticles) against E. coli ATCC 10536, it was shown that the most effective nanoparticles were triangular-shaped, while the rod-shaped nanoparticles had the weakest microbiological efficiency. Silver nanoparticles are already commercially used for wound dressings (Acticoat, Smith&Nephew, USA), but have potential applications for antimicrobial creams and gels, as well as for coverings for catheters, drains and implants. Silver nanoparticles are also, due to their strong antimicrobial activity, very attractive components for improving the functionality of wastewater treatment membranes and water filters.

Conflict of interests

The authors declare no conflict of interest.

References

Amooaghaie, R., Saeri, M.R., & Azizi, M., (2015), Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from Nigella sativa leaf extract in comparison with chemical silver nanoparticles. Ecotoxicology and environmental safety, 120, 400-408.

Andrews, J.M., (2005), BSAC standardized disc susceptibility testing method. Journal of Antimicrobial Chemotherapy 56 pp. 60.

Armentano, I., Dottori M., Fortunati E., Mattioli S., Kenny J. M., (2010), Biodegradable polymer matrix nanocomposites for tissue engineering: a review. Polymer Degradation and Stability 95: 2126–46.

Bankura, K. P., Maity, D., Mollick, M. M., Mondal, D., Bhowmick, B., Bain, M. K., ... & Chattopadhyay, D., (2012), Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. Carbohydrate polymers, 89(4), 1159-1165.

Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P., & Misra, A., (2009), Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and surfaces A: Physicochemical and engineering aspects, 339(1), 134-139.

Barker, S.A., Bourne, E.J., Stacey, M., & Whiffen, D.H., (1953), Infra-red absorption spectra of dextran and other polyglucosans. Chemistry & Industry, (9), 196-197.

Berry, C.C., Wells, S., Charles, S., Curtis, A.S.G., (2003), Dextran and albumin derivatised iron oxide nanoparticles: influence on fibroblasts in vitro. Biomaterials 24: 4551-7.

Bhainsa, K.C., D'Souza S.F., (2006), Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigates. Colloids and Surface B: Biointerfaces 47: 160-4.

Bhattacharya, R., Mukherjee, P., (2008), Biological properties of "naked" metal nanoparticles. Advanced Drug Delivery Reviews 60: 1289–306.

Cakić, M., Mitić, Ž., Nikolić, G. S., Ilić, L., & Nikolić, G. M., (2008), The investigations of bioactive copper(II) complexes with reduced low-molar dextran. Journal of Spectroscopy, 22(2-3), 177-185.

Cakić, M., Nikolić G., Cvetković D., Ilić Lj., Kompleksi Fe(III) sa oligosaharidima – antianemici. Naučna monografija, (Tehnološki fakultet, Leskovac, 2007).

Caswell, K.K., Bender, C.M., Murphy, C.J., (2003), Seedless, surfactantless wet chemical synthesis of silver nanowires. Nano Letters 3: 667-9.

Chairam, S., Poolperm, C., Somsook E., (2009), Starch vermicelli template assisted synthesis of size/shape-controlled nanoparticles. Carbohydrate Polymers 75: 694–704.

Chairam, S., Somsook, E., (2008), Starch vermicelli template for synthesis of magnetic iron oxide nanoclusters. Journal of Magnetism and Magnetic Materials 320(15): 2039–43.

Chaki, N.K., Sharma J., Mandle A. B.,. Mulla I. S, Pasricha R., Vijayamohanan K., (2004), Size dependent redox behavior of monolayer protected silver nanoparticles (2–7 nm) in aqueous medium. Physical Chemistry Chemical Physics 6: 1304-9.

Chen, D.H., Huang, Y.W., (2002), Spontaneous Formation of Ag Nanoparticles in Dimethylacetamide Solution of Poly(ethylene glycol). Journal of Colloid Interface Science, 255, 299-302.

Chudobova, D., Nejdl, L., Gumulec, J., Krystofova, O., Rodrigo, M. A. M., Kynicky, J., ... & Kizek, R. (2013b). Complexes of silver (I) ions and silver phosphate nanoparticles with hyaluronic acid and/or chitosan as promising antimicrobial agents for vascular grafts. International journal of molecular sciences, 14(7), 13592-13614.

Cozzoli, P.D., Comparelli R., Fanizza E., Curri M.L., Agostiano A., Laub D., (2004), Photocatalytic synthesis of silver nanoparticles stabilized by tio2 nanorods: a semiconductor/metal nanocomposite in homogeneous nonpolar solution. Journal of the American Chemical Society. 126(12):3868-79.
Đaković, Lj., (1985), Koloidna hemija, Tehnološki fakultet, Zavod za izdavanje udžbenika, Novi Sad.
Darwis, D., Stasica P., Razzak M.T., Rosiak J.M., (2002), Characterization of poly(vinyl alcohol) hydrogel for prosthetic intervertebral disc nucleus. Radiation Physics and Chemistry, 63: 539-42.
Delić, D., (2011), Toxicity of silver nanoparticles in duckweed (Lemna minor L.), Sveučilište u Zagrebu, 43.
Dhand, V., Soumya L., Bharadwaj S., Chakra S., Deepika B., Sreedhar B., (2016), Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity." Materials science and engineering. C, 58, 36-43.
Faure, C., Derre A., Neri W., (2003), Spontaneous Formation of Silver Nanoparticles in Multilamellar Vesicles. Journal of Physical Chemistry B, 107: 4738-46.
Garza-Navarro, M.A., Aguirre-Rosales, J.A., Llanas-Vázquez, E.E., Moreno-Cortez, I.E., Torres-Castro, A., & González-González, V., (2013), Totally ecofriendly synthesis of silver nanoparticles from aqueous dissolutions of polysaccharides. International Journal of Polymer Science, 2013.
Guzman, M., Dille J., Godet S., (2012), Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology, and Medicine 8: 37–45.
Hamblin, MR & Jori G., (2011), Photodynamic inactivation of microbial pathogens: Medical and environmental applications, Hamblin MR, Jori G (Eds.), From series: Comprehensive Series in Photochemistry and Photobiology No. 11, The Royal Society of Chemistry, European Society for Photobiology, UK, ISBNs: 978-1-84973-144-7 (Hardback) & 978-1-84973-308-3 (Electronic).
Hamedi, S., Ghaseminezhad, S. M., Shojaosadati, S. A., & Shokrollahzadeh, S., (2012), Comparative study on silver nanoparticles properties produced by green methods, Iranian Journal of Biotechnology, 10(3), 191-197.
Horikoshi, S., Sorpone N., (2013), Microwaves in Nanoparticle Synthesis: Fundamentals and Applications 1. Introduction to Nanoparticles Published Online: 26 APR DOI: 10.1002/9783527648122.ch1
Hornebecq, V., Antonietti, M., Cardinal, T., Treguer-Delapierre, M., (2003), Stable silver nanoparticles immobilized in mesoporous silica. Chemistry of Materials, 15: 1993-99.
Jain, J., Arora, S., Rajwade, J. M., Omray, P., Khandelwal, S., Paknikar, K. M., (2009), Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Molecular Pharmacology, 6(5):1388-401
Jain, P., Pradeep T., (2005), Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnology and Bioengineering, 90(1): 59-63.
Jiang, Z.J., Liu C.Y., Sun L.-W., (2005), Catalytic properties of silver nanoparticles supported on silica spheres. Journal of Physical Chemistry B, 109(5), 1730-5.
Johans, C., Clohessy, J., Fantini, S., Kontturi, K., Cunnane, V.J., (2004), Electrosynthesis of polyphenylpyrrole coated silver particles at a liquid– liquid interface. Electrochemistry Communications, 4: 227-30.
Kentaro, M., Kotaro, K., (1974), Flange cover mounting structure; Int C1 Fi6B9/02, Appl. No 287 67; Jap. Pat 120 62A2 (2000) dop. JpP120 626
Knetsch, M.L.W, Koole, L.H., (2011), New strategies in the development of antimicrobial coatings: The example of increasing usage of silver and silver nanoparticles. Polymers 3(1): 340-66.
Komarneni, S., Li, D.S., Newalkar, B., Katsuki, H., Bhalla, A.S., (2002), Microwave-Polyol Process for Pt and Ag Nanoparticles. Langmuir 18: 5959-62.
Krstić, N.S., (2013), Study of the interaction of M(II) biometal ions in model systems with pharmaceuticals and supplements type acids as potential ligands. PhD Thesis. Faculty of Sciences and Mathematics Nish, Serbia.
Leopold, N., Lendl, B., (2003), A new method for fast preparation of highly surface-enhanced raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride. Journal of Physical Chemistry B., 107: 5723-27.
Lihui, X., Ruimin, Z., Gracien, E.B., Francis, A.O., (2004), Synthesis of silver nano-particles by EB irradiation. Journal of Radiation Research and Radiation Processing 22(2): 69-72.
Liu, C., Lu, J., Lu, L., Liu, Y., Wang, F., Xiao, M., (2010a), Isolation, structural characterization and immunological activity of an exopolysaccharide produced by Bacillus licheniformis 8-37-0-1, Bioresource Technology, 101, 5528–5533.
Liu, M., Yue, X. L., Dai, Z.F., Ma, Y., Xing, L., Zha, Z., Liu, S., Li, Y., (2009), Novel thrombo-resistant coating based on iron-polysaccharide complex multilayers, ACS Applied Materials & Interfaces, 1(1), 113-123.
Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun H., Tam, P.K.H., Chiu, J. F., Che C.M., (2006), Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research 5: 916-24.
Maillard, M., Giorgio, S., Pileni, M.P., (2002), Silver nanodisks. Advanced Materials 14(15): 1084-86.
Maillard, M., Giorgio, S., Pileni, M.P., (2003), Tuning the size of silver nanodisks with similar aspect ratios: Synthesis and optical properties. Journal of Physical Chemistry B, 107(11): 2466-70.
McFarland, A.D., Duyne, R.P.V., (2003), Single silver nanoparticles as real-time optical sensors with zeptomole sensitivity. Nanoletters, 3, 1057- 62.
Mitić, Ž., Nikolić, G. S., Cakić, M., Nikolić, R., & Ilić, L., (2007), Synthesis and spectroscopic characterization of copper (II)-dextran complexes. Russian Journal of Physical Chemistry A, 81(9), 1433-1437.
Mitić, Ž., Nikolić, G. S., Cakić, M., Premović, P., Ilić, Lj., (2009), FTIR spectroscopic characterization of Cu(II) coordination compounds with exopolysaccharide pullulan and its derivatives. Journal of Molecular Structure, 924, 264-273.
Mitić, Ž., Nikolić, G.S., Cakić, M., Development of ATR-FTIR Microspectroscopyc for Analysis of Bioactive Cooper(II)-Polysaccharide Complexes „Shedding Light of Diseanse" Optical Diagnosis for the New Millennium, Conference SPEC 2008, (Sao Jose dos Campos, Sao Paulo, Brazil, 86-88, 2008).
Monteiro, D.R., Gorup, L.F., Takamiya, A.S., Ruvollo-Filho, A.C., Camargo, E.R., Barbos, D.B., (2009), The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. International Journal of Antimicrobial Agents, 34: 103-10.
Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J., (2005), The bactericidal effect of silver nanoparticles. Nanotechnology 16: 2346–53.

MubarakAli, D., Thajuddin, N., Jeganathan, K., & Gunasekaran, M., (2011), Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces, 85(2), 360-365.
Nadagouda, M.N., & Varma, R.S., (2007), Synthesis of thermally stable carboxymethyl cellulose/metal biodegradable nanocomposites for potential biological applications. Biomacromolecules, 8(9), 2762-2767.
Naik, R.R., Stringer, S.J., Agarwal, G., Jones, S.E., Stone, M.O., (2002), Biomimetic synthesis and patterning of silver nanoparticles. Nature materials 1(3): 169-72.
Nikolić, G., (2001), Sinteza, fizičkohemijska svojstva i struktura kompleksa gvožđa (III) sa oligosaharidima, Doktorska disertacija, Tehnološki fakultet, Leskovac.
Nikolić, G.S., Cakić, M., Mitić, Ž., Ilić, B., & Premović, P., (2009), Attenuated total reflectance-fourier transform infrared microspectroscopy of copper (II) complexes with reduced dextran derivatives. Russian Journal of Physical Chemistry A, Focus on Chemistry, 83(9), 1520-1525.
Nikolić,G.S., Cakić, M., Mitić, Ž., & Ilić, Lj., (2008), Deconvoluted Fourier-transform LNT-IR study of coordination copper (II) compounds with dextran derivatives. Russian Journal of Coordination Chemistry, 34(5), 322-328.
Pal, S., Tak K.Y., Song M.J., (2007), Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Applied and Environmental Microbiology, 73: 1712–20.
Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., Hamal, P., Zbořil, R., Kvítek, L., (2009), Antifun-gal activity of silver nanoparticles against Candida spp. Biomaterials 30: 6333–40.
Panáček, A., Kvítek, L., Prucek, R., Kolář, M., Večeřová, R., Pizúrová, N., Sharma, V.K., Nevěčná, T., Zbořil R., (2006), Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry B, 110: 16248-53.
Park, J. H., Im, K.H., Lee, S.H., Kim, D.H., Lee, D.Y., Lee, Y.K., Kim, K.M., Kim, K.N., (2005), Preparation and characteriza-tion of magnetic chitosan particles for hyperthermia application. Journal of Magnetism and Magnetic Materials 293: 328-33.
Prinz, E. M., Eggers, R., Lee, H.H., Steinfeld, U., & Hempelmann, R., (2010), Synthesis of drug loaded magnetic nanoparticles and their uptake into immune cells. In Journal of Physics: Conference Series 200(12) p. 122009). IOP Publishing.
Quelemes, P. V., Araruna, F.B., de Faria, B.E., Kuckelhaus, S.A., da Silva, D.A., Mendonça, R.Z., ... & Leite, J.R.S., (2013), Development and antibacterial activity of cashew gum-based silver nanoparticles. International journal of molecular sciences, 14(3), 4969-4981.
Radzig, M.A., Nadtochenko, V.A., Koksharova, O.A., Kiwi, J., Lipasova, V.A., Khmela, I.A., (2013), Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. Colloids and Surfaces B: Biointerfaces 102: 300–6.
Ramsten, J.J., (2014), What is Nanotechnology? Applied Nanotechnology, Second Edition. http://dx.doi.org/10.1016/B978-1-4557-3189-3.00001-4
Rehm, B.H.A., (2010), Bacterial polymers: biosynthesis, modifications and applications, applied and industrial microbiology, Nature Reviews Microbiology, 8, 578– 592.
Ricketts, C.R., (1952a), Interaction of dextran and fibrinogen. Nature, 169(4310), 970-970.
Ricketts, C.R., (1952b), Dextran sulphate-a synthetic analogue of heparin. Biochemical Journal, 51(1), 129-133
Sarwat, F., Qader, S.A.U., Aman, A., Ahmed, N., (2008), Production & characterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713, International Journal of Biological Sciences, 4(6), 379–386.
Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S., & Yun, Y.S. (2009), Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids and Surfaces B: Biointerfaces, 73(2), 332-338.
Shchukin, D.G., Radtchenko, I.L., Sukhorukov, G.B., (2003), Photoinduced reduction of silver inside microscale polyelectrolyte capsules. ChemPhysChem, 4: 1101-3.
Shevchenko, L.I., Lugovaya, Z.A., & Tolmachev, V.N., (1985), Study of the complexing properties of the dextran carboxymethyl ether with transition metal ions in solutions. Polymer Science USSR, 27(9), 2242-2247.
Slawson, R.M.; Van Dyke, M.I.; Lee, H., Trevors J.T., (1992), Germanium and silver resistance, accumulation, and toxicity in microorganisms. Plasmid, 27: 72-9.
Sondi, I., Salopek, B., (2004), Uticaj srebra na E.coli, Journal of Colloid and Interface Science, 27(5), 17-18.
Stasica, P., Rosiak, J.M., Ciach, M., Radek, M., (2000), Approach to construct hydrogel intervertebral discs implants – experimental and numerical investigations. Engineering of Biomaterials 3: 9-14.
Tao, K., Song, S., Ding, J., Dou, H., & Sun, K., (2011), Carbonyl groups anchoring for the water dispersibility of magnetite nanoparticles. Colloid and Polymer Science, 289(4), 361-369.
Tolmachev, V.N., Polovinkina, L.I., & Lugovaya, Z.A., (1985), Study of the acid properties of carboxymethyl dextran. Polymer Science USSR, 27(2), 273-278.
Trajković, V., Marković, Z., (2010), Nanomedicina: Stanje i perspektiva. In book: Biomaterijali. Rakovic D., Uskoković D. (eds), Institut tehničkih nauka Srpske akademije nauke i umetnosti, Beograd, 762-75.
Tseng, C.H., Wang, C.C., Chen, C.Y., (2006), Polypropylene fibers modified by plasma treatment for preparation of Ag nanoparticles. Journal of Physical Chemistry B 110: 4020–9.
Vasko, P.D., Blackwell, J., & Koenig, J.L., (1971), Infrared and raman spectroscopy of carbohydrates: Part I: Identification of O-H and C-H related vibrational modes for D-glucose, maltose, cellobiose, and dextran by deuterium-substitution methods. Carbohydrate Research, 19(3), 297-310.
Wang, X.Q., Itoh, H., Naka, K., Chujo, Y., (2003), Tetrathiafulvaleneassisted formation of silver dendritic nanostructures in acetonitrile. Langmuir, 19: 6242-46.
Xiang, D.X., Chen, Q., Pang L., Zhenga, C.L., (2011), Inhibitory effects of silver nanoparticles on H1N1 influenza A virus in vitro. Journal of Virological Methods, 178, 137–142.
Yamamoto, T., Yin, H.B., Wada, Y., Kitamura, T., Sakata, T., Mori, H., Yanagida S., (2004), Morphology-control in micro-wave-assisted synthesis of silver particles in aqueous solutions. Bulletin of the Chemical Society of Japan 77: 757-61.
Zhang, J., Chen, P., Sun, C., Hu, X., (2004), Sonochemical synthesis of colloidal silver catalysts for reduction of complexing

silver in DTR system. Applied Catalysis A: General 266: 49–54.
Zhang, L.Z,. Yu, J.C, Yip, H.Y., Li, Q., Kwong K.W., Xu A.W., Wong P.K., (2003), Ambient Light Reduction Strategy to Synthesize Silver 316.
Zheng, X.W., Zhu, L.Y., Wang, X.J., Yan, A.H., Xie, Y., (2004), A simple mixed surfactant route for the preparation of noble metal dendrites. Journal of Crystal Growth 260: 255-62.
Zheng, X.W., Zhu, L.Y., Yan, A.H., Wang, X.J., Xie, Y., (2003), Controlling synthesis of silver nanowires and dendrites in mixed surfactant solutions. Journal of Colloid Interface Science 268: 357-61.