

# ZELENA SINTEZA NANOČESTICA SREBRA POMOĆU ZELENOG ČAJA: KARAKTERIZACIJA I ANTIBAKTERIJSKA SVOJSTVA

## GREEN SYNTHESIS OF SILVER NANOPARTICLES USING GREEN TEA: CHARACTERIZATION AND ANTIBACTERIAL PROPERTIES

Damir Barbir<sup>1</sup>, Pero Dabić<sup>1</sup>, Ivana Weber<sup>2</sup>, Anita Rakić<sup>3</sup>

<sup>1</sup> University of Split, Faculty of Chemistry and Technology, R. Boškovića 35, 21 000 Split, Croatia;

<sup>2</sup> University of Split, Faculty of Science, R. Boškovića 33, 21 000 Split, Croatia;

<sup>3</sup> Teaching Institute for Public Health, Split - Dalmatia County, Vukovarska 46, 21 000 Split, Croatia

### ABSTRACT

A relatively new branch of nanotechnology is the green synthesis of silver nanoparticles, which has many advantages over traditional chemical and physical methods. Safety, simplicity, environmental friendliness, lower energy requirements, are all advantages of this process. Recently, the trend has been to synthesize various nanoparticles using plants and plant parts due to their high biodiversity and secondary metabolites. In this paper, silver nanoparticles were synthesized in an ecological way and without using organic or toxic solvents. The synthesis was carried out with green tea as reducing and stabilizing agent using 1 mmol dm<sup>-3</sup> silver nitrate solution and distilled water as solvent. A considerable amount of catechins in green tea is involved in silver ion reduction. Catechins are flavanols related to catechins. The characterization of colloidal silver was performed by UV-Vis spectrophotometer, FTIR, DLS (Dynamic Light Scattering) and SEM (Scanning Electron Microscopy). UV-Vis spectrophotometer proved the presence of silver nanoparticles by the occurrence of surface plasmon resonance, while DLS and SEM analysis was used to evaluate the size and shape of the resulting silver nanoparticles and the uniformity of the resulting nanoparticle sample. The antibacterial efficacy of the silver nanoparticles was tested on the bacteria *Escherichia coli* NCTC 13216 and *Staphylococcus aureus* ATCC 25923. The antimicrobial effect of silver nanoparticles obtained with green tea was demonstrated for these two bacteria up to certain concentrations.

### BACKGROUND

Due to the great biological diversity of plants and their potential secondary metabolites, plants and plant parts have recently been frequently used for the synthesis of various nanoparticles. Plant extracts can serve as both reducing and stabilizing agents for the formation of nanoparticles, thus avoiding the use of chemical reducing agents and stabilizers. Plant extracts contain compounds such as flavonoids and alkaloids that are soluble in water. These compounds act as reducing and stabilizing agents. The polyphenolic functional group is responsible for the reduction of silver ions and the stabilization of silver nanoparticles. The synthesis is very simple and requires a precursor such as AgNO<sub>3</sub> and a plant extract in the form of a water-soluble powder (Fig 1). The size of the nanoparticles obtained depends on the ratio of precursor and reducing agent, i.e. the concentration, synthesis time, mixing speed, temperature and pH at which the synthesis is carried out.

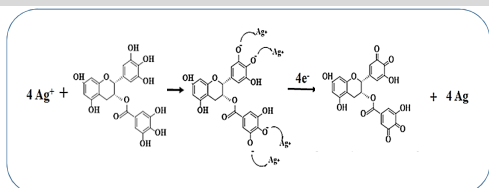


Fig. 1. Proposed reaction mechanism between silver ions and (-)-epigallocatechin-3-gallate (ECGC)

### EXPERIMENTAL

After the solutions of silver nitrate with a concentration of 0.001 mol dm<sup>-3</sup>, sodium carbonate with a concentration of 0.1 mol dm<sup>-3</sup> and double-filtered green tea were prepared, the synthesis is carried out by adding a solution of silver nitrate to the green tea solution at a rate of 1 drop/sec with mixing on a magnetic stirrer at a speed of 700 rpm (Fig 2). The pH of the solution was then adjusted with sodium carbonate solution (Fig 3). 45 mL of AgNO<sub>3</sub> solution and 15 mL of tea solution are required for one sample, i.e. the synthesis was performed at a ratio of 3:1. 4 colloidal silver samples (pH values 6, 7, 8 and 9) were synthesized.

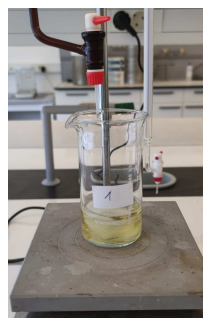


Fig. 2. Preparation of silver nanoparticles

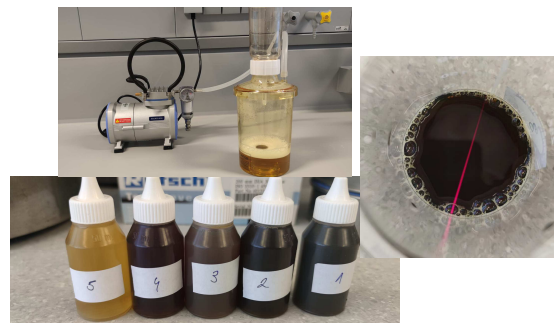


Fig. 3. Samples of silver nanoparticles with adjusted pH value

### RESULTS

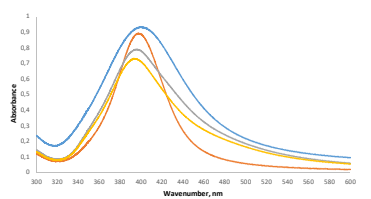


Fig. 4. Absorption spectra of colloidal silver obtained at pH=6, 7, 8, and 9

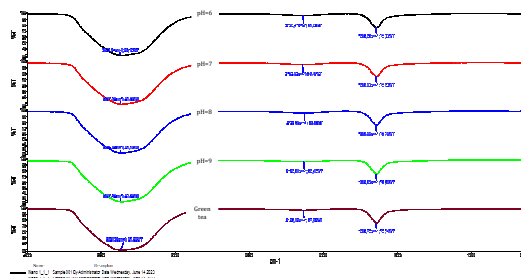


Fig. 5. FTIR absorption spectra of colloidal silver synthesized at pH values of 6, 7, 8, 9 and green tea solution

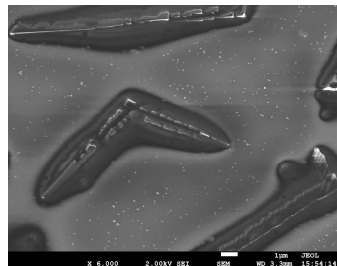
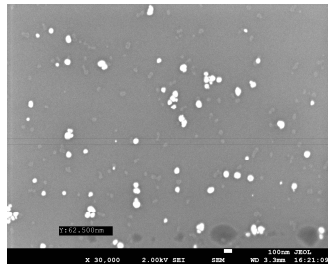


Fig. 6. SEM images of silver nanoparticles



Tab. 1. Polydispersity index (PDI) and hydrodynamic diameter of colloidal silver samples synthesized at different pH values

Sample	PDI	Hydrodynamic radius, nm
pH=6	0.243	50
pH=7	0.277	32
pH=8	0.177	95
pH=9	0.108	52

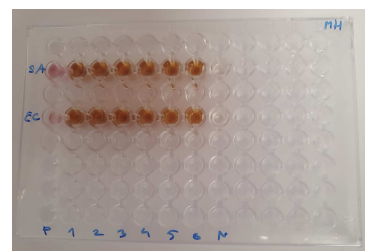


Fig. 7. Determination of antibacterial activity of colloidal silver - Microplate after addition of TTC

Tab. 2. Antibacterial activity of colloidal silver

Suspensions of microorganisms	+ kontrola	MHB						- Control	
		10 <sup>8</sup> 1	10 <sup>7</sup> 2	10 <sup>6</sup> 3	10 <sup>5</sup> 4	10 <sup>4</sup> 5	10 <sup>3</sup> 6		
Colloidal silver	+	+	+	-	-	-	-	-	<i>S. aureus</i> ATCC 25922
	+	+	+	+	-	-	-	-	<i>E. coli</i> NCTC 13216

### CONCLUSIONS

The green synthesis of silver nanoparticles can be successfully carried out with green tea, which is a natural reducing and stabilizing agent. The appearance of absorption spectra at wavelengths around 400 nm was observed in all samples, which is evidence for the synthesis of silver nanoparticles. DLS analysis showed a particle distribution in colloidal silver with nanoparticle sizes ranging from 32 nm to 95 nm. A high uniformity of the sample is characteristic of samples in an alkaline environment, while the smallest particles were obtained at pH 7 with a wider distribution of nanoparticle sizes in the sample. All colloidal silver samples obtained are monodisperse. FT-IR analysis confirmed the theoretical mechanism of reduction of silver ions, and neutral pH proved to be the best for synthesizing the smallest nanoparticles with a diameter of 32 nm. Colloidal silver has an antimicrobial effect, i.e. it kills bacteria *Escherichia coli* up to a concentration of 10<sup>7</sup> and *Staphylococcus aureus* up to a concentration of 10<sup>6</sup>.