https://doi.org/10.24867/GRID-2024-p52 Original scientific paper

STUDY OF EFFECT OF SILVER NANOPARTICLES SYNTHESIZED USING EXTRACT FROM SIDERITIS SCARDICA, INCORPORATED INTO WATER-BASED AND UV COATINGS

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Abstract: *In this work, we present an efficient and rapid green method for silver nanoparticles synthesis by using extract from Sideritis Scardica and direct sunlight irradiation. The synthesized nanoparticles were characterized by UV-Vis spectroscopy and Transmission Electron Microscopy (TEM). Then, the as obtained silver nanoparticles were mixed with water-based varnish, which is widely used for paperboard packaging in the food industry, pharmaceutical industry and others. Evaluation of the results of a microbiological study showed that water-based varnish with incorporated silver nanoparticles exhibited antibacterial activity against Escherichia coli K12 NBIMCC 407 bacteria and Bacillus subtilis NBIMCC 3562 bacteria. From the results obtained it is clear that these water-based and UV coatings with incorporated silver nanoparticles could find industrial applications in medicine and food industry as packaging with antibacterial properties.*

Key words: coatings, antibacterial coatings, packaging, silver nanoparticles, green synthesis, antibacterial activity

1. INTRODUCTION

Silver nanoparticles are increasingly used across various fields due to their unique physical and chemical properties. Because of these specific properties, they find applications in a number of areas, including catalysts, in electronics, optics, and especially in medicine as a bactericidal and therapeutic agent (Zhang et al., 2016). They also exhibit strong antimicrobial properties, which are believed to be due to the action of the Ag^+ ions released by these particles (Yin, 2020). The biological, also known as 'green,' synthesis of nanoparticles is attracting great attention as a reliable, sustainable, and environmentally friendly approach for synthesizing a wide range of nanomaterials, including metal and metal oxide nanomaterials, hybrid, and biomaterials. In this method, biomolecules replace traditional stabilizers and reducing agents. In biological techniques, silver nanoparticles are typically obtained using various plant raw materials, molds, or bacteria (Alaqad et al., 2016; Sharma et al., 2009). Different plants contain varying amounts of phytochemicals. The main phytochemicals present in plants are flavones, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides, which are responsible for the reduction of silver ions (Prathna et al., 2010). The synthesis of metallic nanoparticles using plant extracts generally occurs in three stages. The first one involves the reduction of metal ions to metal atoms. During the second stage, coalescence of small adjacent nanoparticles into larger particles takes place, and during the third stage, the growth process is halted while the final shape of the nanoparticles is imparted (Dikshit et al., 2021). The size and shape of the silver nanoparticles synthesized using plant extracts vary depending on the concentration of the plant extract, reaction time, temperature, pH, and the ratio of the metal ions to the reducing agents available in the extracts (Jose et al., 2014). Extracts obtained from Sideritis scardica are rich in flavonoids and phenols—substances with numerous pharmacological effects, such as antioxidant, anticancer, and antiviral properties (Ververis, 2023). The active components (flavonoids, terpenoids, phenols) present in S. Scardica play an important role due to their anti-inflammatory, antioxidant, and antimicrobial properties. The application of coatings to printed and packaging production is used to improve various properties such as: optical properties, physical and mechanical properties, barrier and antibacterial properties (Bozhkova et al., 2017; Bozhkova et al., 2015). In the present study, the two types of coatings were chosen. Both two types of coatings are built up to be applied in-line in printing presses using anilox cylinders with different transfer, depending on the specific conditions, used materials and quality requirements. The aim of the present experiment is to improve the antibacterial properties of the coatings, along with the optical and barrier ones.

2. METHODS

2.1 Silver Nanoparticles Synthesis

To prepare the extracts from *Sideritis scardica*, 5 grams of the dried herb were combined with 100 mL of distilled water. The mixture was stirred using a magnetic stirrer with heating, set at 500 rpm. Once the solution reached temperature around 80°C, stirring was continued for an additional 10 minutes. Afterward, the extracts were cooled to room temperature and filtered using a vacuum filtration system. An accurate concentration of silver nitrate was prepared by dissolving $AgNO_3$ (> 99.8 %, Sigma-Aldrich) in distilled water under vigorous magnetic stirring (Boeco MMS-3000, Germany) at room temperature. Preliminary experiments indicated that the most reliable results were achieved with $AgNO_3$ concentrations of 5 and 7 mmol/L. To the aqueous $AgNO_3$ solution, 60 µL of the prepared plant extract was added dropwise under vigorous stirring. The resulting mixtures were then exposed to direct sunlight, with continuous stirring, for 10 minutes. The synthetized silver nanoparticles were analyzed by UV-Vis spectrophotometer (T60, PG Instruments Ltd., U.K.). The samples morphology was observed by Transition Electron Microscopy (TEM) on a JEOL JEM 2100, 80-200 kV (Jeol Ltd. Japan).

2.2 Mixing Silver Nanoparticles with two types of coatings

In this study, two types of coatings were utilized: water-based (Actega Terrawet G9 Food Safe) and UV-curable (Actega Terragloss). Silver nanoparticles, synthesized using a 7 mmol/L $AgNO_3$ solution, were incorporated into each varnish at two volume percentages: 20% and 30%. The total volume of each mixture was 50 mL. After adding the nanoparticles, the dispersions were thoroughly homogenized by stirring.

2.3 Antibacterial experiments

Liquid and solid (agar) medium, Luria Bertani (LB) for E. coli K12 and Nutriant Broth (NB) for B. subtilis 3562, both from HiMedia Laboratories were prepared for the bacteria. The strains were obtained from the Bulgarian National Bank of Industrial Microorganisms and Cell Culture. The cultures were incubated in Shaker ES-20/60. Sterile filter paper discs (6 mm in diameter) by HiMedia Laboratories were used for the antibacterial experiments. The antibacterial activity of the two types of Varnish was evaluated against facultative anaerobic Gram-negative E. coli K12 and aerobic Gram-positive B. subtilis 3562 by using the agar disk diffusion test. The cultures were grown, sub-cultured and maintained in LB and NB solid medium and stored in the fridge at 4°C. For the experiments a single colony of each organism was inoculated into 50 ml LB and NB broth and incubated overnight (24 h) at 37°C and 30°C for E. coli K12 and B. subtilis 3562 respectively with shaking at 220 rpm. A 100 µl of bacterial suspensions with concentration of 0.5 McFarland (comparable to a bacterial suspension of 1.5 x 108 CFU/ml) were seeded on agar plates with solid medium-LB or NB respectively by the pour plate technique. Sterile paper discs were impregnated with 5 µl of the varnish samples, and placed on the surface of the agar plate. A disc with the equal amount of distilled water was used as a control. The presence of a clear zone (restricted bacterial growth) is an indication of antibacterial activity for the obtained materials. Inhibition zones were measured edge to edge across the zone of inhibition over the center of the disk according to the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol after incubation overnight at the appropriate temperature for each strain (Hudzicki, 2009). Mean values of the inhibition zones were determined by performing the experiments in triplicate.

3. RESULTS

3.1 Silver nanoparticles

When exposed to sunlight, the initially colorless aqueous solutions of silver nitrate and 60 µL of plant extract undergo a visible color change to yellow or yellow-brown within approximately 10 minutes. Figure 1 shows the UV-Vis absorption spectra obtained during the characterization of the solutions synthesized using *Sideritis scardica* extract after 10 minutes of direct sunlight exposure. The observed peaks, with maxima around 448 nm, correspond to the characteristic surface plasmon resonance of spherical silver nanoparticles.

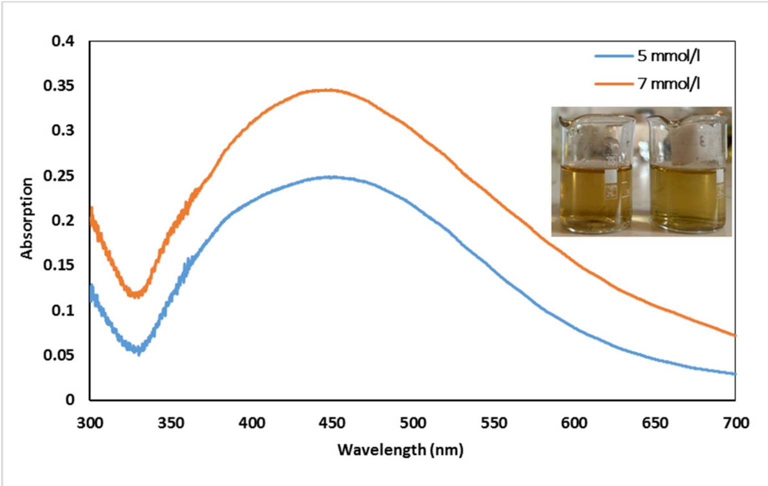

Figure 1: UV-Vis spectra of silver nanoparticles synthesized with 5 mmol/l and 7 mmol/l aqueous solution of silver nitrate and extract from Sideritis scardica *for 10 minutes under direct sunlight*

In Figure 2, TEM images at different magnifications revealed spherical particles ranging from 10 nm to 100 nm. The electron diffraction patterns showed concentric rings, confirming the crystalline structure of the nanoparticles.

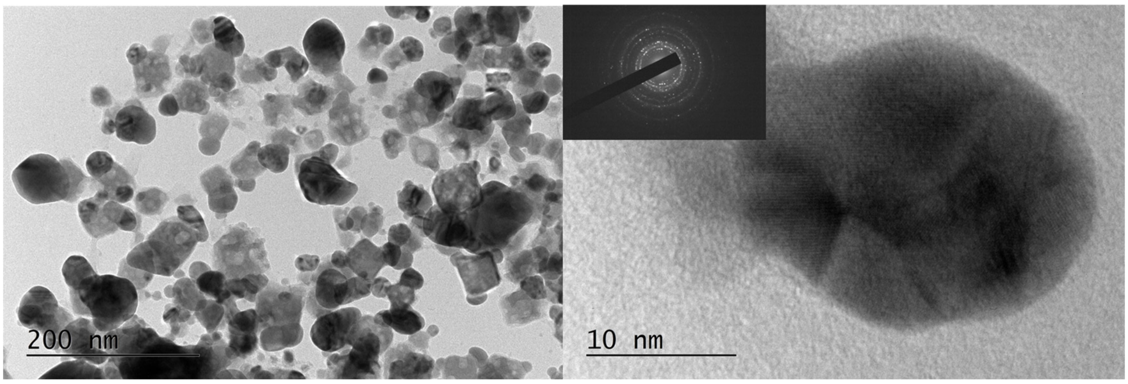

Figure 2: TEM images and the corresponding electron diffraction patterns of silver nanoparticles obtained with Sideritis scardica extract

3.2 Antibacterial activity

In Table 1 are presented the results from the agar disk diffusion test. Coating 1 (water-based varnish) and Coating 2 (UV varnish) were tested at two volume concentrations of the antibacterial agent: 20 vol% and 30 vol%. The inhibition zones are measured in mm.

Table 1: Antibacterial test results of Varnish 1 and 2 samples against B. subtilis 3562 and E. coli K12

Bacterial strain	Coating 1 (water based)		Coating 2 (UV)	
	20 vol%	30 vol%	20 vol%	30 vol%
	Inhibition zone (mm)			
B. subtilis 3562	18.3	18.0	14.5	0
E. coli K12	0	9.5	8.5	9.33

Both samples showed antibacterial activity which was more pronounced against the Gram- positive strain. AgNPs have exhibited significant antibacterial effect against multiple bacteria. However, the exact mechanism of growth inhibition has not yet been fully explained (Bruna T, 2021). Many studies have demonstrated that NPs have greater effectiveness against Gram-positive bacteria than Gram-negative bacteria. This is due to the cell wall of Gram-negative bacteria, which is composed of lipopolysaccharides, lipoproteins, and phospholipids, which form a penetration barrier that allows the entrance of only macromolecules. In contrast, the cell wall of Gram-positive bacteria includes a thin layer of peptidoglycan as well as teichoic acid and abundant pores that allow foreign molecules to penetrate, resulting in cell membrane damage and cell death (Wang L, 2017). A maximum inhibition zone of 18.3 mm was observed for the Varnish 1 sample (20%). According to our results, the last mentioned is a stronger antibacterial agent than Varnish 2. No inhibition was observed against the *E. coli K12* strain when a smaller amount of the antibacterial agent was added.

4. CONCLUSIONS

Experiments were conducted to explore the "green" synthesis of silver nanoparticles using Sideritis scardica extracts, utilizing direct sunlight and continuous stirring with a magnetic stirrer. This approach is energy-efficient, stable, and straightforward, requiring no advanced technology, and results in the formation of well-defined nanoparticles. Sunlight facilitates a cost-effective and rapid synthesis process, minimizing the risk of particle agglomeration. UV-Vis spectra revealed distinct peaks characteristic of well-formed nanoparticles. Transmission electron microscopy (TEM) analysis confirmed the presence of spherical silver nanoparticles with sizes ranging from 10 nm to 100 nm.

The integration of silver nanoparticles into two types of coatings (water based and UV) was investigated to assess whether the resultant products exhibit antibacterial activity. Microbiological tests were conducted using two bacterial strains. The results demonstrated that both types of varnish exhibited antibacterial activity, with a more pronounced effect against the gram-positive strain. The water-based varnish sample showed a maximum inhibition zone of 18.3 mm.

The results obtained from the conducted experiment shows that the addition of silver nanoparticles obtained by the “green” synthesis to different types of coatings increases their antibacterial activity. Improving the antibacterial properties of coatings is important from a scientific and applied point of view, due to the great possibilities for practical application in the field of packaging of foods, medicines, etc. Refinement of the concentrations of silver nanoparticles, expansion of the types of researched coatings in the field of packaging is pending. A number of experiments will be conducted with applying of variety of quantities and different anilox rollers, on papers and cardboards in order to study their antibacterial properties and practical application of this method.

5. ACKNOWLEDGMENTS

This study is funded by the European Union - NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0002, "BiOrgaMCT".

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