Synthesis and antibacterial activity of colloidal silver prepared by electrochemical method

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ABSTRACT

Study was conducted on the electrochemical method of preparing colloidal silver at low DC voltage. A natural stabilizer was used as a carob bean gum from the carob bean. Using a UV-Vis spectrophotometer and a transmission electron microscope (TEM), colloidal silver (silver nanoparticles) was characterized. It is possible to produce colloidal silver of satisfactory quality by means of this electrochemical method. Silver nanoparticles have exhibited surface plasmon resonance as determined by UV-vis analysis. Based on the TEM analysis, the silver nanoparticles appear to be spherical except in sample E1, where they appear rod-shaped and micrometer-sized. Other three samples had particle sizes ranging from 23–52 nm. In a qualitative analysis of colloidal silver solutions, two types of antibacterial activity were observed. At all dilutions, some colloidal solutions showed better antibacterial activity against gram-positive bacteria (S. aureus, S. pyogenes, E. faecalis) while others displayed better antibacterial activity against gram-negative bacteria (S. enteridis, P. aeruginosa, E. coli).

1. Introduction

Nanotechnology is an important area of modern research that deals with the synthesis, design, and manipulation of particle structures in the range of 1–100 nm (Ersöz, Işitan, & Balaban, 2018). Nanoparticles have a wide range of applications in biomedicine (Wu & Li, 2013), cosmetics (Grumezescu, 2019), food industry (Nile et al., 2020), optics, environmental protection (Bhat et al., 2019), and mechanics, chemical industry, etc., due to defined chemical, optical and mechanical properties (Khan, Saeed, & Khan, 2019).

Silver nanoparticles are particularly interesting because of their unique properties that can be used for antimicrobial applications, in biosensor materials, composite fibers, cryogenic superconducting materials, etc. Many studies have investigated the antibacterial efficacy of silver nanoparticles and the possibility of commercial application in the field of health care and medicine (Gurunathan, Choi, & Kim, 2018; Kasitevar, Periakaruppan, Muthupandian, & Mohan, 2017). Currently, the appearance of multidrug-resistant bacteria represents an increasing worldwide problem (Shrivastava, Shrivastava, & Ramasamy, 2018). Therefore, there is an urgent need to find an effective drug to treat infectious diseases (Kramer, Stewardson, & S. Harbarth, 2016). Given this fact, colloidal silver gained renewed interest (Barras, Aussel, & Ezraty, 2018; Tran et al., 2017). Colloidal silver can significantly reduce the duration and severity of many bacterial infections (Said et al., 2014). The main characteristic of colloidal silver is the high microbicidal capacity of silver ions in water at a very low concentration (Baranowski, 1995). Recent studies have found that interactions between silver ions and thiol groups play an important role in the inactivation of bacteria (Gupta & Silver, 1998). Colloidal silver is not yet understood exactly how it acts as a bactericide. Silver nanoparticles are believed to bind to the surface of membranes of cells, causing changes in properties such as permeability and respiration (Yliniemi et al., 2008).

Synthesis of silver nanoparticles can be carried out by various reduction methods such as chemical reduction, photoreduction, electrochemical reduction methods, or thermal decomposition. Factors affecting the synthesis of silver nanoparticles are the temperature of the solution, the concentration of the precursor, type of reducing agent, reaction time, and cleanliness of the glassware. Chemical reduction involves the reduction of a silver salt with a reducing agent in the presence of a stabilizer. The most commonly used methods for the preparation of silver nanoparticles in an aqueous solution are the reduction of silver nitrate with reducing agents such as borohydride (Barbir, Dabić, & Meheș, 2019), citrate (Gakiya-Teruya, Palomino-Marcelo, & Rodriguez-Pallas, 2008).
Reyes, 2018), or ascorbic acid (Chekin & Ghasemi, 2014). Stabilizers are substances used in the synthesis of nanoparticles to prevent their attachment by electrostatic or steric repulsion. In the case of silver nanoparticles, the most commonly used stabilizers are citrate, polyvinylpyrrolidone (PVP), and surfactin.

The electrochemical method is an alternative in the preparation of silver nanoparticles due to lower reaction temperatures, simple equipment, controllable products obtained, and short reaction times. Electrochemical methods have used metallic silver to prepare silver nanoparticles using PVP (Khaydarov, Khaydarov, Gapurova, Estrin, & Scheper, 2009). Among the main advantages of this method are the high purity of the end products, as well as their size and shape, which can be controlled by adjusting the current density without the need for expensive equipment and a vacuum (Rodríguez-Sánchez, Blanco, & López-Quintela, 2000). Additionally, using the electrochemical method is environmentally friendly as it does not require the use of toxic reduction agents. Most of the products produced by this method are sold under the name colloidal silver. Colloidal silver is composed of silver ions and particles. Typical colloidal silver is 85% ionic silver and 15% silver particles. Silver ions obtained by electrolysis are often referred to as dissolved silver. Since most of the silver in colloidal silver is in the form of dissolved silver, it would be more technically correct to call them silver solutions (Haider & Mahdi, 2015).

In this work, the synthesis of colloidal silver was carried out by the low voltage electrochemical method DC. Carob bean gum synthesized from carob bean was used as a stabilizer. Antimicrobial activity of prepared colloidal silver solutions against six microorganisms: Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Enterococcus faecalis, Pseudomonas aeruginosa, and Streptococcus pyogenes has been investigated.

2. Experimental

2.1. Preparation of carob bean gum

Carob beans, which come from the island of Sótla, Croatia, are immersed in boiling water for 2 hours. After the seeds have softened, they are peeled and placed in an oven at 105 °C. After drying, the carob seeds are crushed and brought to heat in high purity water at a ratio of 1:197. Heating is done at a temperature of 70 °C for half an hour, with intense stirring at a speed of 600 rpm. The solution was filtered and the filtrate was treated with isopropanol in the ratio of 2:1 with stirring for about 5 minutes and then the solution was left for 12 hours to precipitate the locust bean gum. The slurry obtained from the solution was taken out and dried at room temperature, after which the carob bean gum was crushed.

2.2. Preparation of colloidal silver

In experimenting, two electrodes (anode and cathode) of high purity silver (99.99% Ag) in the form of a wire with a diameter of 3 mm were used. The electrodes were placed at a distance of 2 cm in a cell containing distilled water with a specific conductivity of 4.82 μS cm⁻¹ and a carob bean gum as a stabilizer. Calculating the current and determining the electrode’s surface area is essential prior to starting the electrolysis. The electrodes are connected with wires to a low voltage generator DC (power supply DC) and the required current is set. The magnetic stirrer is switched on and moderate stirring is set in the cell (300 rpm). Electrolysis was carried out for 100 minutes at room temperature and during the process silver concentration (TDS) was measured every 10 minutes using a conductometer Metler Toledo Seven Compact S230, Switzerland. The resulting colloidal silver solution was then stored in the dark until characterization of the silver nanoparticles.

2.3. Characterization of colloidal silver

The absorbance of the colloids formed was determined using UV-Vis spectrophotometer Analytik Jena SPECORD® 200, Germany, in the wavelength range of 300 to 600 nm. For colloidal silver, the absorbance is in the range of 380 to 420 nm. The size and shape of silver nanoparticles were determined using a transmission electron microscope (TEM) Carl Zeiss EM10A, Germany.

2.4. Determination of antimicrobial activity of colloidal silver

The microorganisms used for the test were Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Salmonella enteritidis ATCC 13076, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 10145, and Streptococcus pyogenes ATCC 13615. During this experiment, microorganisms were subcultured into Muller Hinton Broth (MHB) at 37 °C for 18 h and their concentration was diluted from 10⁸ to 10³ cfu mL⁻¹ in twofold MHB. For each sample of microorganism, 100 μL of colloidal silver solution (4 samples) and 100 μL of MHB were added to microplates (Lkhagvajav, Yaşa, Çelik, Koizhaiganova, & Sari, 2011; Sarkar, Jana, Samanta, & Mostafa, 2007). At 37 °C, the microplates were then incubated for 24 hours. Each microplate was subsequently treated with 40 μL of TTC (2,3,5-Triphenyl tetrazolium chloride). A color change in TTC in a
microplate from colorless to red-pink, an indication that it is positive (+) (Eaton, Clesceri, Greenberg, & Franson, 2005).

3. Results and discussion

Table 1 shows the silver concentration values for four colloidal silver samples obtained by the electrochemical method under different reaction conditions. The designation E1 refers to a sample of colloidal silver obtained using a power supply DC as a current source. The designation E2 refers to a sample also obtained using a power supply DC with 5% by weight of carob gum as stabilizers. E3 denotes a sample of colloidal silver obtained using a low voltage generator DC, and E4 denotes a sample obtained using a generator with the addition of 5% by weight of carob gum.

From Table 1, it can be seen that the silver concentration increases from the beginning to the end of electrolysis, which is expected for all four samples. After 100 minutes of electrolysis, the silver concentration ranged from 19 to 26 mg L\(^{-1}\). A sudden jump in silver concentration occurs at 60 minutes for sample E1 and 40 minutes for sample E2. For the remaining two samples with the use of a generator, the increase in concentration was uniform.

UV-Vis spectroscopy is a very important and simple technique to confirm the formation of silver nanoparticles. The absorption spectrum of silver colloids was obtained in the range of 300–600 nm. The formation of silver nanoparticles during the electrochemical process is evident from the change in color of the solution from colorless to gray, which can be seen in Figure 1.

Metallic nanoparticles have free electrons, which lead to the formation of an absorption band of the surface plasmon resonance due to the mutual oscillations of the electrons in resonance with the light wave. The appearance of peaks in the region of 420 nm shows that silver nanoparticles possess surface plasmon resonance, as shown in Figure 2. From the plots, very low intensities and perturbations on the absorption curves in the region of wavelengths around 300 nm can be seen. This behavior can be attributed to the insufficient time of electrolysis and the formation of colloidal silver.

Previous studies indicate that silver colloids are characterized by UV-Vis spectrogram peaks in the wavelength range from 410 to 450 nm, and it was found that there is a relationship between the width of the absorption peak at 50% intensity and the particle size (Mulfinger et al., 2007). It is evident from the results that both the choice of source DC and the addition of a stabilizer do not affect the absorption maximum, as the values for all four samples are between 410–420 nm.

The shape and size of the obtained silver nanoparticles (Figures 3–5) were determined by TEM analysis. The scale on TEM images is 500 nm. Based on the analysis, it can be seen that all the particles except sample E1 are spherical (Figure 3). Moreover, the particles in sample E1 had a size in the micrometer range, so it can be said that no silver nanoparticles were formed. Silver nanoparticles were formed in the other three samples. The effect of stabilizer on the formation of silver nanoparticles can be observed in sample E2, where silver nanoparticles were formed, unlike sample E1. The average silver particle size in sample E2 was 23 nm, in E3 39 nm, and in sample E4 52 nm. Silver nanoparticles were unaffected by the addition of carob bean gum to sample E4.

Figure 5 and Table 2 show the results of antimicrobial testing with colloidal silver solutions on different microorganisms.

The specific mechanism of antimicrobial activity of silver nanoparticles is still unclear and has not been fully explained. Nanoparticles containing silver can often release silver ions (Ag\(^+\)), which can play a role in their antimicrobial activity. In order for silver to exhibit antimicrobial activities, positively charged Ag\(^+\) should essentially be ionized (Bapat et al., 2018; Klueh, Wagner, Kelly, Johnson, & Bryers, 2000). As Ag\(^+\) ions form complexes with nucleic acids, they
actually interact specifically with nucleotides of these nucleic acids, unlike phosphate groups. Gram-positive and gram-negative bacteria have differences in membrane composition that may have a particular effect on the mechanism of action of prepared colloidal silver solutions (Table 2). The membranes of gram-positive bacteria are composed primarily of anionic phosphatidylglycerol and cardiolipin lipids, whereas the membranes of gram-negative bacteria are composed primarily of zwitterionic lipid
phosphatidylethanolamine (Epand & Epand, 2009; Pedron et al., 2017). Silver nanoparticles were found to be antibacterial against gram-positive and gram-negative bacteria by many scientists (Lara, Ayala-Nunez, Ixtepan Turrent, & Rodriguez Padilla, 2010; Shrivastava et al., 2007; Yogesha, Rabinal, & Ananthamurthy, 2012). Based on their findings, several factors affect antibacterial effectiveness. Generally, silver particles of a certain size are the most important. Silver particles measuring 9 nm in diameter are more effective at killing bacteria than silver particles measuring 64 nm in diameter in colloidal solutions, as reported by Lok et al. (2007). In their study, Sintubin and colleagues found that a different diameter of silver nanoparticle affects its antibacterial activity since the reduction in size results in a higher concentration of active silver (Sintubin et al., 2011).

In the Ostwald-Freundlich equation, silver nanoparticle shape and size play a role in producing Ag⁺ ions. As a result of their large surface areas, finer and spherical silver nanoparticles will promote Ag⁺ ion formation (Shanmuganathan et al., 2018). During our experiments, we found that the sample E1, in which the silver particles are not spherical and are microscopic, exhibited the least antimicrobial activity. By aggregating silver nanoparticles, less Ag⁺ ions are released, meaning this issue could be resolved by using stabilizers (carob gum), which modify the surface of silver nanoparticles.

Table 2. Qualitative analysis of the antimicrobial activity of different colloidal silver solutions.

| Solutions of microorganisms | MHB 1 | MHB 2 | Colloidal silver solutions |
|-----------------------------|-------|-------|---------------------------|
|                             | 10⁸   | 10⁷   | 10⁶   | 10⁵   | 10⁴   | 10³   | 10²   | 10¹   |       |
| S. aureus                   | –     | –     | –     | –     | –     | –     | –     | –     | E1    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E2    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
| E. coli                     | +     | +     | +     | +     | +     | +     | +     | +     | E1    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E2    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
| S. enteritidis              | +     | +     | +     | +     | +     | +     | +     | +     | E1    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E2    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
| E. faecalis                 | +     | +     | +     | +     | +     | +     | +     | +     | E1    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E2    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
| P. aeruginosa               | +     | +     | +     | +     | +     | +     | +     | +     | E1    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E2    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
| S. pyogenes                 | +     | +     | +     | +     | +     | +     | +     | +     | E1    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E2    |
|                             | +     | +     | +     | +     | +     | +     | +     | +     | E3    |
|                             | –     | –     | –     | –     | –     | –     | –     | –     | E4    |
Researchers have found that silver nanoparticles are more likely to kill gram-negative bacteria compared to gram-positive bacteria. Compared to gram-positive bacteria, gram-negative bacteria have thinner cell walls. Silver nanoparticles are less able to diffuse into the cellular environment because of the thick cell wall (Duval, Gouyau, & Lamouroux, 2019). In our study, we were unable to confirm this claim but obtained the opposite results. It follows that the method of obtaining silver nanoparticles is important for antimicrobial activity. The next step of the research will be to determine the Minimum Inhibitory Concentration (MIC) for all tested microorganisms.

4. Conclusions
It can be concluded from the presented results and discussion that electrochemical method was successful in synthesizing colloidal silver solutions. Silver nanoparticle surface plasmon resonance peaks at wavelengths 410–420 nm were detected by UV-Vis analysis. Silver nanoparticles were found to be spherical except in sample E1. The particles in samples E1 are rod-shaped and micrometer-sized. In the three other samples, the particle size ranged from 23 to 52 nm. Antimicrobial activities of colloidal silver solutions were analyzed quantitatively, revealing both positive and negative results. Some colloidal solutions had better antimicrobial activity on gram-positive (S. aureus, S. pyogenes, E. faecalis) and some on gram-negative (S. enteridis, P. aeruginosa, E. coli) bacteria at all dilutions.

Disclosure statement
No potential conflict of interest was reported by the authors.

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