



Green Synthesis, Characterization and Antimicrobial Evaluation of Silver Nanoparticles for an Intracanal Dressing

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Green chemistry has been applied in different areas due to the growing demands for renewable processes and one of them is nanotechnology. The aim of this study was to characterize a formulation containing silver nanoparticles (AgNPs) produced by a green synthesis and to evaluate its antimicrobial activity. The formulation will be used as an intracanal dressing exploiting the AgNPs' antimicrobial properties, which are crucial to prevent infections and bacterial reinfections that can compromise endodontic treatments. In the green synthesis, silver nitrate was employed as the precursor salt, maltose as a reducing agent, and gelatin as a stabilizing agent. The formulation was prepared mixing 50 % of a liquid containing the AgNPs and 50 % of hydroxyethylcellulose gel at 1.5 % with proper evaluation of the process inherent parameters. Techniques such as molecular absorption spectrometry and dynamic light scattering were used in characterization step. The antimicrobial activity of the AgNPs against *Escherichia coli* ATCC 25922, *Enterococcus faecalis* NCTC 775, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175 was verified according to National Committee for Clinical Laboratory Standards (NCCLS) by determining minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The obtained results indicated the formulation containing AgNPs produced by a green synthesis was properly characterized by the selected techniques. Furthermore, the formulation assessment proved that it is suitable for the proposal as well as it has potential to be used as an intracanal dressing since presented antimicrobial activity against all bacterial strains evaluated.

Key Words: green synthesis, silver nanoparticles, antimicrobial activity, intracanal formulation.

Introduction

In endodontic therapy, some substances are traditionally used as irrigation solutions and intracanal medication, helping to disinfect the root canal system. Among them, it is possible to mention calcium hydroxide, sodium hypochlorite and chlorhexidine (CHX). Calcium hydroxide [Ca(OH)₂] has good acceptance due to its alkalinizing and enzymatic action, in addition to antimicrobial and mineralizing properties (1).

When it comes to disinfection procedures, the use of silver nanoparticles is an important alternative, due to their dimensions that would allow penetration into the dental canaliculi. In addition, nanoparticles have antimicrobial action on a wide spectrum of microorganisms, including Gram-positive and Gram-negative bacteria (2,3), fungi (4) and viruses (5). Therefore, these nanomaterials have great potential to be used as an irrigating solution and/or intracanal medication during endodontic therapy (2).

Most of the methods described in the literature for nanoparticles production are based on hazardous organic solvents. This is a consequence of the desirable hydrophobicity of the capping agents employed. In addition, the proposed methods massively use highly toxic reducing

agents such as hydrazine, sodium borohydride (NaBH₄), and dimethyl formamide (DMF). All of them are extremely reactive chemicals and present serious potential to cause environmental and biological damage (6). Accordingly, in order to prevent environmental and biological risks and to guarantee economic viability and method renewability, there is an increasing trend to pursue the development of the nanoparticles synthesis based on green chemistry principles employing low cost, sustainable and natural resources (7).

Thus, a renewable and low cost synthesis to produce silver nanoparticles to be added to an intracanal dressing formulation for use in endodontic therapy is proposed. The green synthesis employed water as solvent, silver nitrate as precursor salt, maltose as reducing agent and gelatin as stabilizer agent, allowing classified it as a mild and eco-friendly system. In order to make feasible this proposal, the characterization and antimicrobial assessment of the formulation were carefully carried out.

Material and Methods

Synthesis of the Silver Nanoparticles

The AgNPs were initially synthesized by the reduction

of silver nitrate 0.500 mol/L by maltose 1.000 mol/L, and solubilized using 1.0 g gelatin as the stabilizing agent. The silver nitrate and maltose solutions were sequentially added to the gelatin solution, at a temperature of 75 °C. This temperature was also used to evaluate the reaction time interval, varying from 30 min to 3 h reaction, during which the medium was kept under vigorous stirring. The longest time interval was selected to evaluate the added volumes of the precursor salt and the reducing agent. Different volume proportions were established between the precursor salt and the reducing agent, and the ratio of 5 mL of silver nitrate for each 10 mL of maltose was defined. In the subsequent step, the concentrations of silver nitrate and maltose solutions from 0.500 mol/L and 1.000 mol/L respectively were evaluated, which were successively reduced using a dilution factor of 2 until concentrations of 0.031 mol/L of silver nitrate and 0.062 mol/L of maltose were reached, which were the lowest concentrations evaluated. The amount of gelatin was also proportionally reduced from 1.0 g until a mass of 0.062 g was achieved. During these studies, the temperature was maintained at 75 °C. The lowest concentrations of the reagent solutions and the lowest mass of gelatin were then selected and used for the evaluation of the reaction temperature. Temperatures of 75, 85, 90 and 95 °C were investigated. The colloidal solutions obtained in this study were analyzed by flame atomic absorption spectrometry.

Tables 1 and 2 show the total silver concentrations of the colloidal solutions obtained through analysis by flame atomic absorption spectrometry in studies related to the concentrations of reagents and temperature respectively.

Formulation Evaluation

Once obtained, the AgNPs were incorporated into the formulation, which was prepared using gel with 1.5 % hydroxyethylcellulose as a vehicle containing 50 % AgNP. During the evaluation of the formulation, the gel containing the nanoparticles was stored away from light at room temperature and also in a refrigerator. The color, pH and

effect of the centrifugation were observed in relation to changes in phase and/or precipitation, considering periods of 24 h and 7 days. In evaluating the effect of the centrifugation, 2.0 g of gel were subjected to centrifugation at 3000 rpm for two cycles of 15 min each. At the end of this study a centrifugation step was subsequently included, with an interval of 1 h at 15,000 rpm and 4 °C to separate any products generated by heating, as well as the remaining ionic silver.

Characterization

The nanoparticles were evaluated by means of the color produced, which is concentration dependent, and a simple test was used to check their presence based on the Tyndall effect. In the next step, on a molecular absorption spectrophotometer (CARY 1E, Varian, Melbourne, Australia), a wavelength scan was carried out in the range of 350 to 650 nm, to obtain the absorption spectrum of the nanoparticles. To determine their dimensions the four groups shown in Table 3 were defined, using the dynamic light scattering technique (Zetasizer Nano ZS, Malvern Instruments Ltd. Worcestershire, England). For the AgNP D group, an alteration related to the reducing agent concentration was introduced, changing it from 0.062 to 0.12 mol/L. After this analysis, the groups AgNP A and AgNP D were selected for subsequent tests. The quantification of silver before and after centrifugation was performed by flame atomic absorption spectrometry (SpectrumA-200, Varian, Melbourne, Australia).

Microbiological Analysis

Bacterial strains

To verify the antibacterial properties of AgNPs, the groups AgNP A and AgNP D were selected, using five bacterial strains representing the species frequently associated with endodontic infections: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* NCTC 775, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175.

Antimicrobial Activity Tests

The evaluation of the AgNPs antimicrobial activity was

Table 1. Silver concentrations of colloidal solutions depending on the variation of reagent concentrations (3 h, 75 °C)

Colloidal solution	Reagent concentration (mol/L)		Mean±sd (µg/mL)
	AgNO ₃	Maltose	
1	0.500	1.000	1.888.00±22.85
2	0.250	0.500	877.24±1.99
3	0.125	0.250	624.31±5.52
4	0.062	0.125	351.58±12.64
5	0.031	0.062	146.65±2.99

Table 2. Silver concentrations of colloidal solutions as a function of temperature variation

Colloidal Solution	Mean Concentration±sd (µg/mL)	Temperature (°C)
1	156.80±2.99	75
2	185.09±4.25	85
3	345.15±2.76	90
4	507.73±5.52	95

preliminarily performed by the agar diffusion method, using sterile filter paper discs with a diameter of 6 mm (LB, Laborclin Ltda, Pinhais, PR, Brazil), as described by the Clinical and Laboratory Standards Institute - CLSI (8). Aliquots of 10 μ L containing the AgNPs were used to impregnate previously sterilized paper discs.

Bacterial suspensions were prepared in saline solution until the turbidity referring to the McFarland 0.5 scale was achieved (approximately 1.5×10^8 CFU/mL) and inoculated on Petri dishes containing Agar Muller Hinton (Difco, Detroit, MI, USA), as recommended by the CLSI (8). The disks impregnated with the AgNPs were placed on the bacterial cultures and incubated at 37 °C for 18 h. After this period, whether or not there was the formation of bacterial growth inhibition halos around the disks, it was verified the antimicrobial activity proportional to the size of the inhibition halo formed. For all tests, 2 % CHX solution was used as a positive control and saline solution (NaCl 0.9 % m/v) as a negative control.

Determination of Minimum Inhibitory and Bactericidal Concentrations

After the tests of antimicrobial activity, determination of the minimum inhibitory concentration (MIC) was carried out for the five bacterial strains tested using the groups AgNP A and AgNP D.

For MIC assessment, the 96-well microplate dilution method was used with a method adaptation related to the used bacterial growth indicator, which was resazurin, as described by Pitondo-Silva et al. (9). The MIC value that is related to bacteriostatic activity was determined in the well, in which the lowest concentration of AgNPs prevented the visible growth of bacteria.

For determination of the minimum bactericidal concentration (MBC) values related to bactericidal activity for each strain, the readings referring to the MIC values of the different groups evaluated were taken into account. Thus for the MBC tests, all wells where there was no visible bacterial growth were selected. A 10 μ L aliquot from each selected well was transferred to Agar Müller Hinton culture medium (Difco, Detroit, MI, USA) by using a micropipette.

Table 3. Groups used in the evaluation of the antimicrobial activity of AgNPs (reaction time = 3 h).

Group	Reaction temperature (°C)	Reagent concentration	
		AgNO ₃ (mol/L)	Maltose (mol/L)
AgNP A	85	0.031	0.062
AgNP B	90	0.031	0.062
AgNP C	95	0.031	0.062
AgNP D	85	0.031	0.120

The plates were incubated for 24 h at 37 °C, and after this period a visual assessment was made to determine MBC by the presence or absence of bacterial colonies. MBC was determined as the lowest concentration for which there was no bacterial growth in the inoculation region.

Results

Synthesis of Silver Nanoparticles

At the end of the studies the following conditions were adopted: 5 mL of silver nitrate 0.031 mol/L, 10 mL of maltose 0.062 mol/L, gelatin mass of 0.062 g, reaction time of 3 h and temperature of 85 °C. These parameters allowed the obtaining of silver nanoparticles through green synthesis, which were properly characterized.

Formulation Evaluation

There were no visible changes in the gels after 24 h, and after seven days a slight darkening was observed. Regarding the pH, a small variation from 5.64 for the value observed in the first 24 h, to 5.91 for the value verified after 7 days was observed, after keeping the gel sheltered from light at room temperature and also in a refrigerator.

After 24 h at room temperature and also in a refrigerator, no visible changes were observed in the gels, such as phase separation and precipitation. However, after seven days at room temperature and also in a refrigerator, no visible change in consistency or phase separation was observed, but a slight dark color precipitation could be observed.

The centrifugation step included after obtaining the nanoparticles allowed the separation of the supernatant liquid containing the AgNPs, from the silver ions and potential by-products derived from the heating of the unreacted maltose, deposited as a dark precipitate. This allowed the elimination of the dark colored precipitated material that had been observed in the tests involving the formulation.

Characterization of the Nanoparticles

After 3 h of reaction, nanoparticles with yellow coloring, characteristic of silver nanoparticles, were obtained using the parameters previously described. The wavelength scan in the 350 to 650 nm range using molecular absorption spectrophotometry allowed the obtainment of an absorption spectrum, whose maximum was close to the 400 nm wavelength (Fig. 1).

According to the results obtained in the light scattering analysis, the silver nanoparticles obtained in the groups in which the temperature of 85 °C, 0.031 mol/L of silver nitrate and 0.062 mol/L of maltose (AgNP A), and 85 °C, 0.031 mol/L of silver nitrate and 0.120 mol/L of maltose (AgNP D) presented the smallest dimensions. For this reason, they were selected to be used in the subsequent

studies. The AgNP A group presented nanoparticles with dimensions that varied in the range from 7.5 to 50 nm, with the size distribution percentage corresponding to this interval of 3.67%. In the AgNP D group, the dimensions of the nanoparticles ranged from 21.04 to 50.75 nm,

corresponding to a size distribution percentage of 15.87%, as it can be seen in Figure 2. The Polydispersity Index (Pdl), which represents nanoparticle size distribution, was calculated and a value of 0,277 was obtained.

Once the parameters that affect the formation process

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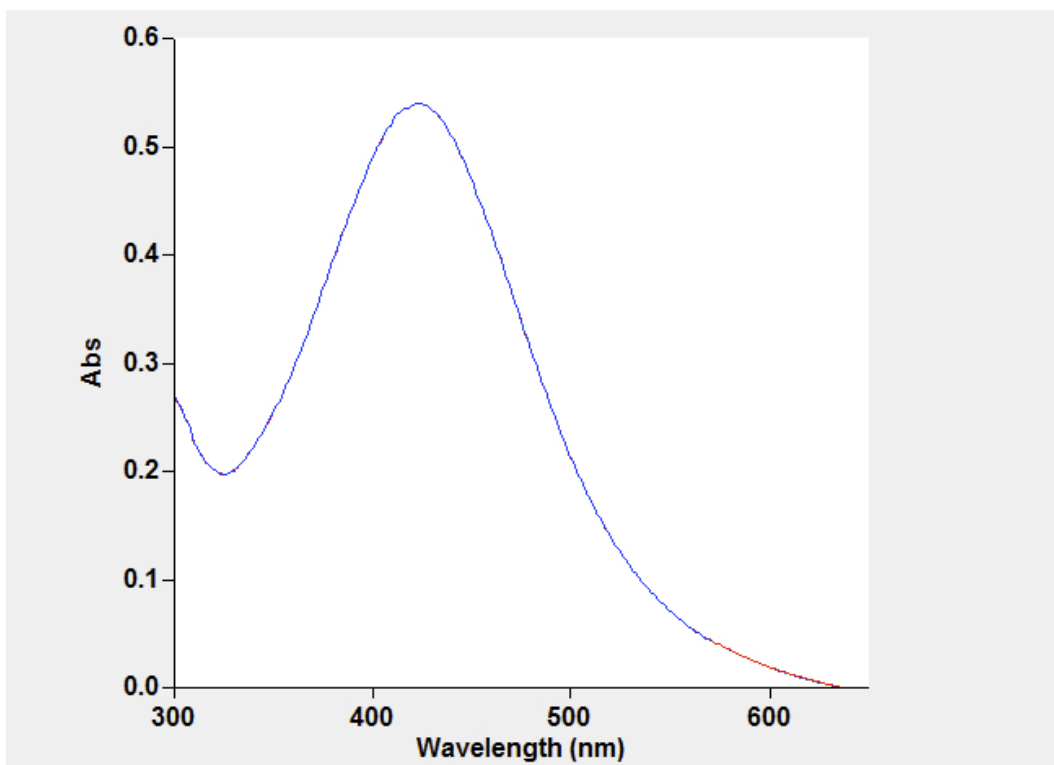


Figure 1. Absorption spectrum of the AgNP D group nanoparticles.

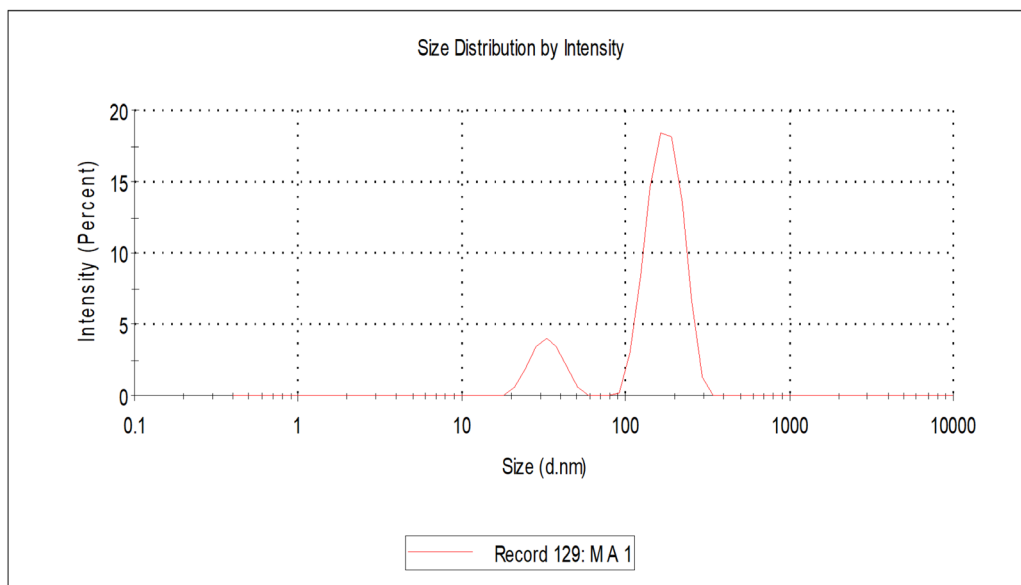


Figure 2. Distribution of the dimensions of the nanoparticles according to the percentage of intensity of the AgNP D group.

of the nanoparticles were defined, an analysis of the AgNP A and AgNP D groups was carried out by atomic absorption, having obtained the total silver concentrations of 194.82 $\mu\text{g/mL}$ and 194.34 $\mu\text{g/mL}$, respectively.

Antimicrobial Activity

The results showed that all the evaluated AgNPs showed antimicrobial activity for all studied bacterial species, since they formed bacterial growth inhibition halos ranging from 6 to 9 mm for all of them. For all bacterial strains, the 2 % CHX solution (positive control) showed halos of inhibition ranging between 14 and 18 mm; as expected, sodium chloride 0.9 % (negative control) did not inhibit bacterial growth.

The minimum inhibitory concentrations were established as well as the minimum bactericidal concentrations for the groups AgNP A and AgNP D and both showed bactericidal and bacteriostatic activities at different values, as shown in Table 4. As expected, no minimum inhibitory concentration was observed for sodium chloride, and for CHX a value of 9.77 $\mu\text{g mL}^{-1}$ was obtained for both the minimum inhibitory and minimum bactericidal concentrations.

Discussion

In the health area, nanoparticles are particularly interesting due to their antimicrobial properties (10). Although there is a large variety of biological, physical and chemical methods to produce nanoparticles, lately there has been a trend towards development of green chemistry based methods (6,7,11) aiming at the establishment of renewable and sustainable processes. Following this trend, carbohydrates such as starch and different sugars have been used in several methods due to their chemical simplicity and low cost (7).

In this work, the synthesis of silver nanoparticles (AgNP) based on the redox reaction, in which the hydrolysis of maltose results in two glucose molecules and an aldehyde,

reducing the Ag^+ ions to Ag^0 and producing glyconic acid reported by Oluwafemi et al. (12) was employed. However, considering the planned application, *i.e.*, the use of AgNPs as an intracanal dressing in endodontic treatments, changes were made to the original synthesis parameters related to temperature and reaction time, as well as reagent concentrations. Such parameters were modified as a function of the application and also because they can influence the size and morphology of the nanoparticles (13) and therefore, their properties.

The evaluation of the concentrations of the reagents aimed not only to ensure the formation of nanoparticles with dimensions that would guarantee the antimicrobial action, but also that would not cause staining of the dental element. In this sense, the selected concentrations of silver nitrate and maltose used have been reduced by approximately 32 times compared to those employed by Oluwafemi et al. (12). Even so, it was possible to obtain AgNPs with the characteristics and properties suitable for maintaining the antimicrobial activity. The lower concentrations of the reagents concomitantly led to the generation of nanoparticles with a pale yellow color that potentially would not offer a risk of causing tooth staining. However, with the reduction in the concentration of reagents, the reaction time interval could not be shorter than three hours for AgNP formation. It is also important to emphasize that in the present study, the use of a green chemistry based synthesis to obtain AgNPs, in addition to ensuring the desirable antimicrobial activity, virtually contributed to the reduction of cytotoxicity by the significant decrease in the concentration of the precursor silver salt, known to be cytotoxic, when compared to the concentration previously used (12).

The increase in the reducing agent/precursor salt ratio (AgNP D group) favored the formation of silver nanoparticles (14), which was evidenced by the decrease in the ratio between the concentrations of total and metallic silver. The greater conversion of ionic silver to metallic silver was verified through the analysis of the colloidal solutions carried out using flame atomic absorption spectrometry, before and after the centrifugation step, since most of the remaining Ag^+ ions precipitated. The silver concentrations of 194.82 $\mu\text{g/mL}$ and 194.34 $\mu\text{g/mL}$ of the AgNP A and AgNP D groups respectively, obtained by atomic absorption spectrometry after the centrifugation step, are consistent with the used concentration of the precursor salt, considering both precipitation of ionic silver and loss of part of the liquid by evaporation during the three hours of reaction.

Among the temperatures evaluated, 75, 85, 90 and 95 °C, the two highest led to the achievement of a greater number of nanoparticles, indicated by the darker color of the

Table 4. Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) for the AgNP A and AgNP D groups

Microorganisms	Groups			
	AgNP A		AgNP D	
	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
<i>E. coli</i>	1.52	1.52	1.52	1.52
<i>E. faecalis</i>	6.09	0.19	6.09	0.19
<i>P. aeruginosa</i>	1.52	1.52	1.52	3.04
<i>S. aureus</i>	1.52	3.04	24.35	24.29
<i>S. mutans</i>	12.18	12.15	12.18	12.15

medium. According to Darroudi et al. (15), the dimensions of the nanoparticles are inversely proportional to the increase in temperature, therefore the higher the temperature, the smaller the dimensions of the nanoparticles. However, it should be noted that the increase in temperature, as well as in the concentrations of reagents, would lead to the formation of a greater amount of nanoparticles, and consequently, to the generation of a very dark color. It is necessary therefore to consider the relationship among the quantity, characteristics of the nanoparticles formed and the corresponding coloring. Thus, the temperature of 85 °C was selected, which allowed the formation of AgNPs with adequate characteristics and in such quantity, whose corresponding color did not reveal to be a potential source of staining of the dental element.

In the characterization of AgNPs, the dispersion of light through the liquid in the test based on the Tyndall effect was observed, indicating the path of the laser beam due to presence of the colloidal solution. Considering nanoparticle morphology, an absorbance maximum at 400 nm is expected for spherical AgNPs (16). This characteristic absorption spectrum is due to the surface plasmon resonance band, whose response can be observed in the range from 350 to 700 nm, depending on parameters such as shape, size and type of nanoparticle agglomeration (17).

In this work, however, a slight red shift absorption spectrum with maximum absorbance at 424 nm was obtained by using molecular absorption spectrometry. This technique was very useful for the characterization of the AgNPs, since the profiles of the spectra obtained indicated the respective stage of nanoparticle formation over the period of time considered. The steps could, to a certain extension, be accompanied by the variation in the color of the formed colloidal solution, which at the beginning was colorless, passing through gold yellow to dark brown, indicating the formation of nanoparticles (17). This has been verified as the reagent concentration, and time and temperature reaction were increased during evaluation of such parameters. During the studies, several spectra were obtained by analyzing the aliquots collected time to time in order to observe AgNP formation process step by step when the parameters were being evaluated. These spectra played an important role in revealing the characteristics of the nanoparticles formed so that it was possible to observe changes in the profiles of the absorption spectra over time. It must be highlighted AgNPs have a more intense plasmon resonance than, for example, copper and gold nanoparticles in the visible region. This is due to the different dielectric properties resulting from the small overlap between the surface plasmon resonance and the interband transitions of silver that start at 320 nm (18).

AgNP dimensions, on its turn, were determined by

dynamic light scattering, which revealed the population of nanoparticles was divided into two sets in terms of size for both AgNP A and AgNP D groups. For this last one, the average dimensions were 33.60 nm (15.9% of size distribution percentage) and 177.9 nm (84.1% of size distribution percentage). The red shift earlier mentioned is probably due to the increase in the diameter of the nanoparticles, since the set with larger nanoparticles has greater signal intensity contributing more to the definition of the spectra profiles (16). However, the results observed with respect to these absorption spectra profiles and the proximity to the maximum absorption of 400 nm corroborate which is described in the literature (19).

Particle size uniformity, expressed by Pdl, was estimate using dynamic light scattering (DLS), which is able to determine this index in a range of 0.05 to 0.7. Acceptable values of Pdl depend on the application area, e.g., 0.221 or below for silver nanoparticles (20). The authors observed an increase in Pdl value, attributed to the higher reagent concentration, revealing the instability of silver nanoparticles due to the agglomeration process. Therefore, lower Pdl values are strongly desirable and, in this work, the Pdl presented a value of 0.277, with good agreement to the mentioned work, so that it could be considered quite suitable.

Durán et al. (10) presented several proposed mechanisms which involve multiple aspects of bacterial cell/AgNPs interaction in an extensive revision on mechanistic aspects related to antimicrobial activity. According to the authors, the role of reminiscent silver ions adsorbed on nanoparticle surface and the silver nanoparticles dimensions are usually highlighted by different researchers as the main mechanisms related to AgNPs antimicrobial activity, although this is not a consensus. The reduced dimensions of the nanoparticles are supposedly related to their antimicrobial activity (10), which justifies the need to determine them by using dynamic light scattering. Smaller nanoparticles have a larger surface area improving antibacterial efficiency due to increased release of Ag⁺ ions that remain naturally adsorbed on the nanoparticle surface, a fundamental step for antibacterial action (21). In addition, particles smaller than 10 nm can pass through the nuclear pores, interact with chromosomes and DNA (10) and interrupt the production of ATP molecules increasing its bactericidal potential.

In this work, however, such dimensions were not reached. Actually, it was observed that the nanoparticles of the AgNP A group, which had a smaller dimension, whose average was 28.75 nm, had a reduced intensity percentage, being only 3.67 %. The AgNP D group, on the other hand, presented a dimension average of 33.60 nm with intensity percentage correspondent to 15.87 %. Such dimensions did

not preclude the respective bacteriostatic and bactericidal actions of the AgNPs as indicated by the obtained results. Nevertheless, it should be emphasized that in the case of the AgNP A group, nanoparticles were found as small as 7.5 nm, and in the case of the AgNP D group, 21 nm.

The formulation was based on a previous work (2) and it was evaluated by their macroscopic aspect, considering its homogeneity and consistency. In the formulation assessment, studies involving pH, temperature and centrifugation effect, and the product color and consistence revealed the formulation presented suitable characteristics that allow it to be used as intracanal dressing containing AgNPs. However, during the evaluation of the formulation a slight darkening of the formulation was observed, indicating a precipitation process which could probably be attributed to the reasonable amount of silver ions that had not been reduced. To overcome this problem, the maltose concentration was doubled in order to increase the efficiency of the conversion process from ionic silver to metallic silver (14), maintaining the amount of gelatin used as a stabilizer. As this did not completely solve the problem, after obtaining the nanoparticles a centrifugation step was introduced. At the end of this process, a dark precipitate was observed at the bottom of the tube, possibly indicating the presence of silver ions and/or by-products resulting from the heating of the maltose. The release of the remaining silver ions, adsorbed on the surface of the nanoparticles, can lead to the interaction of these ions with the cell wall proteins that contain the thiol groups, affecting cell functions (10). At the same time, the excess of these ions in the medium could cause the dental elements to darken, as well as their precipitation could affect the stability and quality of the formulation produced. To avoid such shortcoming, gelatin was used as a stabilizing agent due to its biocompatibility and biodegradability. In addition, the amine groups linked to the gelatin structure stabilize the nanoparticles preventing their aggregation, which eventually helps in the silver-gelatin complex formation (22), which is produced in the reaction beginning.

The studied AgNPs showed antimicrobial effectiveness against *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, and *S. mutans*, even though particles larger than 10 nm were obtained. The results obtained are in agreement with other studies that proved the effectiveness of AgNPs against planktonic bacteria (2,5,23).

The efficacy of AgNPs on *E. faecalis* is especially relevant, as it is a specie present in the etiology of persistent endodontic infections (5) and most of the contamination of endodontically treated canals is due to the presence of this bacterium and its ability to survive in adverse conditions inside the root canal (24). In addition, this specie is less sensitive to changes in pH when compared to other bacteria

(5) due to defense intrinsic mechanisms such as the proton pump (25), which makes it more resistant to the action of calcium hydroxide, the usual choice as intracanal dressing.

The AgNPs were properly characterized by different analytical techniques, proving the adequacy of the reaction parameters selected for their production. The reaction based on green chemistry, using maltose as a reducing agent, in addition to ensuring the biocompatibility of the material, was efficient for the generation of silver nanoparticles with antimicrobial potential against *S. mutans*, *S. aureus*, *P. aeruginosa*, *E. faecalis*, and *E. coli*, with bacteriostatic and bactericidal activity. The formulation obtained showed color and consistency characteristics considered satisfactory for the proposed application in endodontics.

The results obtained are very promising and indicate that formulations containing AgNPs have the potential to compose an intracanal dressing formulation, especially when considering the studied bacteria. Complementation of the studies carried out with the evaluation of the effects of the action of AgNPs on other microorganisms, which cause periapical lesions, and mature and polymicrobial biofilms normally found in the root canal is already in progress.

Resumo

A química verde tem sido aplicada em diferentes áreas devido à crescente demanda por processos renováveis e uma delas é a nanotecnologia. O objetivo deste estudo foi caracterizar uma formulação contendo nanopartículas de prata (AgNPs) produzidas por meio de síntese verde e avaliar sua atividade antimicrobiana. A formulação será usada como curativo intracanal explorando as propriedades antimicrobianas das AgNPs que são cruciais para prevenir infecções e reinfecções bacterianas que podem comprometer os tratamentos endodônticos. Na síntese verde, nitrato de prata foi empregado como sal precursor, maltose como agente redutor e gelatina como agente estabilizador. A formulação foi preparada misturando-se 50% do líquido contendo as AgNPs e 50% de gel de hidroxietilcelulose a 1,5% com avaliação adequada dos parâmetros inerentes ao processo. Técnicas como espectrometria de absorção molecular e espalhamento dinâmico de luz foram usadas na etapa de caracterização. A atividade antimicrobiana das AgNPs contra *Escherichia coli* ATCC 25922, *Enterococcus faecalis* NCTC 775, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 e *Streptococcus mutans* ATCC 25175 foi verificada de acordo com o National Committee for Clinical Laboratory Standards (NCCLS), determinando-se a concentração inibitória mínima (MIC) e a concentração bactericida mínima (MBC). Os resultados obtidos indicaram que a formulação contendo AgNPs produzidas por meio de síntese verde foi devidamente caracterizada pelas técnicas selecionadas. Além disso, a avaliação da formulação provou que ela é adequada para a proposta, bem como tem potencial para ser utilizada como curativo intracanal já que apresentou atividade antimicrobiana contra todas as cepas bacterianas avaliadas.

Acknowledgements

The authors are grateful to the University of Ribeirão Preto (UNAERP) for financial support, to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the doctorate scholarship to João Felipe Bonatto Bruniera, to the pharmacist José Orestes Del Ciampo, from the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRP/USP), for the analysis by dynamic light scattering, and to John Carpenter, for language assistance.

References

- Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in Endodontics and dental traumatology. *Int Endod J* 2011;44:697-730.
- Bruniera JF, Silva-Sousa YTC, Lara MG, Pitondo-Silva A, Marcaccini AF, Miranda, CES. Development of intracanal formulation containing silver nanoparticles. *Braz Dent J* 2014;25:302-306.
- Abbaszadegan A, Ghahramani Y, Gholami A, Hemmateenejad B, Dorostkar S, Nabavizadeh M, et al. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study. *J Nanomater* 2015;16:53-60.
- Alananbeh KM, Al-Refaei WJ, Al-Qodah Z. Antifungal effect of silver nanoparticles on selected fungi isolated from raw and waste water. *Indian J Pharm Sci* 2017;79:559-567.
- Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Fard MJ. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. *J Dent* 2015;43:1573-1579.
- Raveendran P, Fu J, Wallen SL. Completely "green" synthesis and stabilization of metal nanoparticles, *J Am Chem Soc* 2003;125:13940-1394.
- Meshram SM, Bonde SR, Gupta IR, Gade AK, Rai MK. Green synthesis of silver nanoparticles using white sugar. *IET Nanobiotechnol* 2013;7:28-32.
- CLSI (Clinical and Laboratory Standard Institute). Performance standards for antimicrobial disk susceptibility tests; Approved standard 15th Edition 2015;M02-A12.
- Pitondo-Silva A, Gonçalves GB, Stehling EG. Heavy metal resistance and virulence profile in *Pseudomonas aeruginosa* isolated from Brazilian soils. *APMIS* 2016;124:681-688.
- Durán N, Durán M, De Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine* 2016;12:789-799.
- Lakkappa B A, Jasmith S C, Prabhuodeyara M G. Effect of concentration and ph on the size of silver nanoparticles synthesized by green chemistry. *Organic & Medicinal Chem IJ* 2017;3:555622.
- Oluwafemi OS, Lucwaba Y, Gura A, Masabeya M, Ncapayi V, Olujimi OO, et al. A facile completely 'green' size tunable synthesis of maltose-reduced silver nanoparticles without the use of any accelerator. *Colloids Surf B Biointerfaces* 2012;102:718-723.
- Rout A, Jena PK, Sahoo D, Bindhani BK. Green synthesis of silver nanoparticles of different shapes and its antibacterial activity against *Escherichia coli*. *Int J Curr Microbiol App Sci* 2014;3:374-383.
- Liu H, Zhang H, Wang J, Wei J. Effect of temperature on the size of biosynthesized silver nanoparticle: Deep insight into microscopic kinetics analysis, *Arab J of Chem* 2020;13:1011-1019.
- Darroudi M, Ahmad MB, Abdullah AH, Ibrahim NA. Green synthesis and characterization of gelatin-based and sugar-reduced silver nanoparticles. *Int J Nanomedicine* 2011;6:569-574.
- Paramelle D, Sadovoy A, Gorelik S, Free P, Hobbey J, Fernig DG. A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectrum. *Analyst* 2014;139:4855-4961.
- Amendola V, Bakr OM, Stellacci F. A study of the surface plasmon resonance of silver nanoparticles by the discrete dipole approximation method: effect of shape, size, structure, and assembly. *Plasmonics* 2010;5:85-97.
- Noguez C. Surface plasmons on metal nanoparticles: the influence of shape and physical environment, *J Phys Chem C* 2007;111:3806-3819.
- Mehmood A, Murtaza G, Bhatti TM, Kausar R, Ahmed MJ. Biosynthesis, characterization and antimicrobial action of silver nanoparticles from root bark extract of *Berberis lycium* Royle. *Pak. J Pharm Sci* 2016;29:131-137.
- Lestari TP, Tahlil FA, Sukweenadhi J, Kartini K, Avanti C. Physical characteristic and antibacterial activity of silver nanoparticles from green synthesis using ethanol extracts of *Phaleria macrocarpa* (Scheff.) Boerl Leaves, *Trad Med J* 2019;24:16-21.
- Li P, Li S, Wang Y, Zhang Y, Han G. Green synthesis of β -CD-functionalized monodispersed silver nanoparticles with enhanced catalytic activity. *Colloid Surf. A - Physicochem Eng Asp* 2017;520:26-31.
- Zhang J, Gu M, Zheng T, Zhu JJ. Synthesis of gelatin-stabilized gold nanoparticles and assembly of carboxylic single-walled carbon nanotubes/Au composites for cytosensing and drug uptake. *Anal Chem* 2009;81:6641-6648.
- Corrêa JM, Mori M, Sanches HL, Da Cruz AD, Poiate Jr E, Poiate IA. Silver nanoparticles in dental biomaterials. *Int J Biomater* 2015;2015:485275.
- Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006;32:96-98.
- Siqueira Jr JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J* 1999; 32:361-369.

Received June 16, 2020
Accepted September 29, 2020