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OPTIMIZATION OF THE ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES USING GOJI BERRIES' BIOACTIVE COMPOUNDS

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ABSTRACT. The numerous applications of metallic nanoparticles in different fields such as materials science, medicine, biology, led to the rapid development of various synthesis methods of these nanomaterials, among which the biogenic approach in obtaining nanoparticles has been proved to be an efficient alternative compared to other methods. The present study aims to investigate the potential of goji berries extract as source of bioactive compounds able to reduce the silver ions and to stabilize the resulting nanoparticles. Reaction parameters such as pH and $AgNO_3$ concentration were analyzed and optimized in order to obtain spherical, well dispersed and high yield silver nanoparticles. Five different pH values (6; 7; 8; 9; 10) and five ratios fruit extract: silver nitrate solution (1:1; 1:3; 1:7; 1:10; 1:15) were investigated and it was found that the silver nanoparticles obtained at pH=9 and 1:10 ratio demonstrated the highest monodispersity and were obtained in the highest yield. The obtained nanoparticles were characterized in terms of their size and shape using transmission electron microscopy (TEM) and UV-vis spectroscopy.

Keywords: *silver nanoparticles, goji berries*

INTRODUCTION

The wide range of applications of nanosized materials led in the last decades to an explosion of nanotechnology research. Metallic nanoparticles are of a great interest due to their important uses in medicine, materials science, environment remediation, catalysis, biology, pharmacy [1-3]. There is a strong relationship between the size and shape of the nanoparticles and their properties and, consequently, their applications. These parameters are

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strongly influenced by the synthesis methods used to obtain the nanoparticles. Physical and chemical approaches such as thermal decomposition, chemical/ electrochemical reduction, laser ablation, attrition, mechanical milling, sol-gel techniques, have been developed but usually these methods involve either the use of hazardous reagents or expensive instruments, high temperature or pressure and can lead to low fabrication rate, significant energy consumption, poor size control and low stability of the obtained nanoparticles. Compared to these methods, the green synthetic approaches offer solutions to all these disadvantages, being fast, simple, non-toxic, low cost, and environmentally friendly [4-6]. Various biological resources can be used in the green synthesis methods of metallic nanoparticles, such as microorganisms, enzymes and plants. There are numerous studies which report the biosynthesis of metal nanoparticles by exploiting the reductive properties of phenolics, proteins, flavonoids and carbohydrates from various plant extracts, such as: *Camellia sinensis* [7], *Cornus sanguinea* [8], *Cornus mas* [9], *Citrus limon* [10], *Solanum licopersicum* [11].

Among metallic nanoparticles, silver nanoparticles are essential materials, especially because of their medical uses, possessing strong antibacterial, antifungal and antiviral properties [12] and also because of their high catalytic activity.

The plant extracts, especially those with pharmacological properties, have been widely used for the green synthesis of the AgNPs. Goji berries have been intensively used for medicinal purposes, especially in Asia as they contain a wide range of compounds with health promoting properties being a good source of phenolic compounds, proteins, carotenoids, fibres and minerals. Goji berries are the fruits of *Licium barbarum* L., a perennial shrub from Solanaceae family originating from South-West Asia and nowadays naturalized in America and Europe [13].

Goji berries extract has been reported to possess various biological properties, such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anti-obesity effects. Their potent antioxidant activity originates in the presence of polyphenols in the goji berries, among which caffeic acid is the main compound [14]. Kaempferol, myricetin, catechin, apigenin, rutin, chlorogenic, coumaric, ferulic, ellagic and gallic acids are also identified in these fruits [15].

The presence of these compounds and their high reductive properties enabled us to choose goji berries as source of reducing and capping agents for the green synthesis of AgNPs. The aim of the present study was to exploit goji phytocompounds in the reduction reaction of the silver ions and to optimize the reaction conditions (pH and reactants' ratio) to obtain low dimensions, monodispersed, spherical in shape silver nanoparticles.

RESULTS AND DISCUSSION

The goji berries are well known for their remarkable antioxidant capacity, due to the presence of inherent metabolites, of which flavonoids and phenolic acids confer them this property. The presence of high amounts of chlorogenic acid, scopoletin, coumaric acid, rutin, caffeic acid, N-feruloyl tyramine [16] recommend them as suitable fruits for the plant assisted mediated green synthesis of metallic nanoparticles, as all these compounds can successfully act as reducing agents of metallic ions as well as capping and stabilizing agents of the obtained metallic nanoparticles.

The total phenolic content of the goji extract used in this study, assessed by the widely applied Folin-Ciocalteu method, was 7125±216.42 mg GAE/L.

The antioxidant activity of the extract was evaluated using the ABTS free radical scavenging assay and was found to be 1038.46±32.48 µM Trolox.

The optimal synthesis conditions of the silver nanoparticles using goji berries extract were determined by evaluating two synthesis parameters: pH value and fruit extract: $AgNO_3$ mixing ratio (v/v). These factors are known as key parameters in obtaining AgNPs using natural compounds. The impact of the pH value on the size, morphology and yield of the obtained AgNPs was investigated at 6; 7; 8; 9 and 10. The formation of silver nanoparticles was first visually observed by a colour change of the reaction mixture from faint orange to yellowish brown or deep brown, depending on pH of the reaction medium. The synthesis of AgNPs was validated using UV-vis spectroscopy. Silver nanoparticles possess unique optical properties due to their surface plasmon resonance (SPR). The surface plasmon vibration excitation of colloidal silver results in absorption peaks between 400-500 nm [17]. The UV-Vis spectra of the obtained silver nanoparticles are depicted in Figure 1.

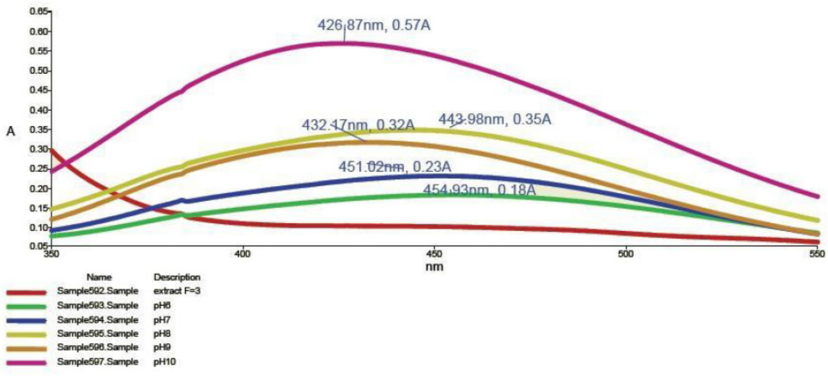

Figure 1. UV-Vis spectra of AgNPs obtained at different pH values

The synthesized AgNPs at all the investigated pH values presented the characteristic SPR peaks of silver nanoparticles between 426 and 454 nm. It can be observed that the intensity of the absorption bands decreases with the value of pH. As higher intensity of these bands can be attributed to higher concentration of nanoparticles, one can conclude that pH=10 conducted to the highest reaction yield. As predicted, acidic pH values could not stabilize the silver nuclei for subsequent growth of silver nanoparticle. By increasing the reaction medium pH, a gradually red shifted absorption band, until 426 nm for pH=10 was observed, indicating the obtain of smaller sized AgNPs [18]. At alkaline pH, the bioactive metabolites from the goji extract act as capping agents of the obtained nanoparticles, stabilizing these by preventing agglomeration.

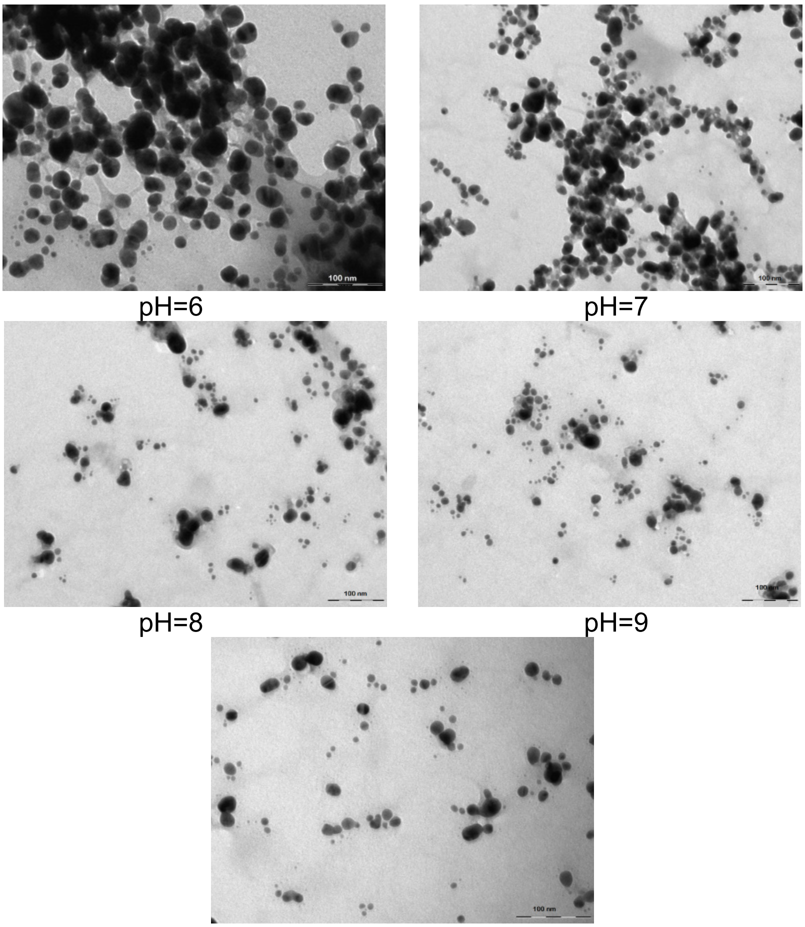

Figure 2. TEM images of AgNPs obtained at different pH values

The size and morphology of the goji berries mediated synthesized silver nanoparticles was analysed using the Transmission electron Microscopy (TEM) (Figure 2).

At pH = 6 and 7 the TEM images show the formation of mostly spherical nanoparticles with two broad size distributions, some presenting a diameter in the range of 20-30 nm and others being larger and of irregular shapes. There was also noticed an agglomeration tendency of the obtained nanoparticles, especially at pH=6. For the pH values in the range 8-10, smaller sized, dispersed nanoparticles, with more regular shapes were obtained. The alkaline conditions proved to be more appropriate for the synthesis of the AgNPs using goji extract, resulting uniform size and shape and small diameter nanoparticles, the influence of the pH on their properties being less important in this pH range, result in accordance with other studies reporting the phytomediated synthesis of silver nanoparticles [19]. The size of the nanoparticles obtained in alkaline conditions was in the range of 10-20 nm, with an average size of 17 nm at pH=10. All the obtained results indicated that the synthesis of AgNPs with the goji berry phytocompounds occurred with the highest yield at pH=10, resulting regular in shape, monodispersed and small sized nanoparticles.

The concentration of the reducing phytocompounds from the fruit extract has a strong influence on the size, shape and stability of the metallic nanoparticles [20]. Thus, establishing the optimal ratio between the fruit extract and silver nitrate solution could be of a great importance for obtaining small, uniform and stable AgNPs.

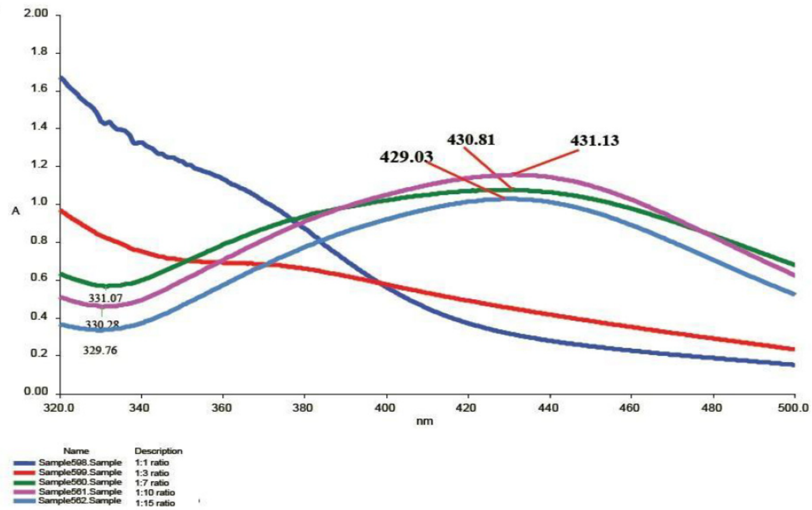

Figure 3. UV-Vis spectra of AgNPs obtained at different fruit extract: $AgNO_3$ ratios

Five different ratios: 1:1; 1:3; 1:7; 1:10 and 1:15 were analysed, the reaction being conducted at the optimum determined pH value, pH=10. The UV-Vis spectra of the silver nanoparticles obtained at these ratios (Figure 3) indicate that no reaction occurred at 1:1 and 1:3 ratio as no characteristic absorption band of the silver nanoparticles was noticed. The AgNPs obtained at 1:7; 1:10 and 1:15 displayed similar position of the SPR absorption band with a maximum in a very narrow range of wavelengths between 429 and 431 nm. This fact indicates that for ratios between 1:7 to 1:15, the size of the obtained nanoparticles was not influenced by this parameter. The highest yield was obtained for the 1:10 ratio.

CONCLUSIONS

The present study reports the green synthesis of silver nanoparticles using goji berries as source of phytocompounds which act as reducing and stabilizing agents. By optimizing the reaction conditions, among which pH and reactants ratio is very important, our protocol confers an easy way to obtain in high yield small sized uniform nanoparticles, spherical in shape, using a green method which involves non-toxic chemicals. The optimum determined pH value was 10, while the optimum fruit extract: metal precursor ratio was 1:10.

EXPERIMENTAL SECTION

Chemicals and reagents

All chemicals and reagents were purchased from Merck (Darmstadt, Germany), were of analytical grade and were used without further purification. A TYPDP1500 Water distiller (Techosklo LTD, Držkov, Czech Republic) was used to obtain the distilled water.

Fruit extract preparation

Commercially available dried goji berries were used to obtain the fruit extract, by mixing 5 grams of milled fruits with 100 mL distilled water. After 1 hour of stirring at room temperature, the mixture was vacuum filtered and the filtrate was further used to obtain the desired silver nanoparticles.

Determination of total phenolic content

The method developed by Singleton [21] using the Folin-Ciocalteu reagent was applied to determine the total phenolic content (TPC).

A 0.2 N Folin-Ciocalteu solution (1.5 mL) was mixed with goji berries extract (0.3 mL) and incubated in the dark for 5 min. After that, 1.2 mL of 0.7N Na_2CO_3 solution were added. After 2 hours incubation at room temperature, the absorbance of the resulting solution was measured at 765 nm, using an UV-VIS Perkin Elmer Lambda 25 double beam spectrophotometer. A calibration curve of gallic acid was used for quantitative determinations. The results were expressed as mg gallic acid equivalents (GAE)/L fruit extract.

Determination of antioxidant capacity

The antioxidant activity of the extract was evaluated using the $ABTS^{\cdot +}$ assay of Re et al. [22]. 360 mg of 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) were dissolved in 100 mL distilled water. To this solution, 100 mL potassium persulfate solution (2.45 mM) were added, in order to generate the stable ABTS radical cation. The mixture was allowed to react for 24 hours in the dark at room temperature. The resulting radical solution was diluted to an absorbance of 0.8 with distilled water. To 6 mL diluted ABTS solution, 0.1 mL goji extract were added and the absorbance was measured at 734 nm after 15 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard to determine the antioxidant capacity of the investigated fruit extract, which was expressed in μM Trolox equivalents.

Synthesis and characterization of silver nanoparticles

Silver nitrate solution (1mM) was used as silver ions source for the phytomediated synthesis of the silver nanoparticles. 2 mL goji fruit extract was mixed with 20 mL $AgNO_3$ solution at room temperature. The pH values of the resulting reaction mixtures were adjusted in the range 6 to 10 by dropping 0.1 M NaOH solution. After 2 hours of stirring, the obtained nanoparticles were purified by centrifugation at 10.000 rpm for 20 min and washing with double distilled water. In order to investigate the influence of reactants' ratio on the reaction yield and on the size and shape of the obtained AgNPs, different volumes of silver nitrate solution were mixed with 2 mL fruit extract in order to achieve the desired ratios.

The obtained silver nanoparticles were characterized using consecrated methods, such as UV-Vis spectroscopy (using a Perkin Elmer Lambda 25 double beam spectrophotometer) and transmission electron microscopy (using a H-7650 120 kV Automatic transmission electron microscope, Hitachi, Tokyo, Japan).

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