ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2014, *59*(3-4), 213-218

CHARACTERIZATION OF SILVER NANOPARTICLES OBTAINED BY USING *ROSMARINUS OFFICINALIS* EXTRACT AND THEIR ANTIOXIDANT ACTIVITY

Radu Claudiu FIERASCU, Ioana Raluca BUNGHEZ,* Raluca SOMOGHI, Irina FIERASCU and Rodica Mariana ION

National R&D Institute for Chemistry and Petrochemistry – ICECHIM, 202 Spl. Independentei, 060021, sect. 6, Bucharest, Roumania

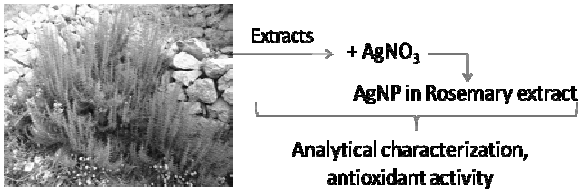
Received September 12, 2013

In our days it is a growing interest in the development of nanotechnology, the science which deals with the production, characterization and manipulation of materials at the nanoscale. The noble metal nanoparticles (Au, Ag, or Pt) play an important role in scientific research due to their applications in medicine, biology, optoelectronics and material science. From the noble metals, silver is one of the most studied, due to its unique properties and use, especially in the bio-medical field.

This study aims to demonstrate that rosemary (*Rosmarinus officinalis*) extract, commonly known as an ornamental or medicinal plant, can be also used to phytosynthesize silver nanoparticles.

The potential ability of this plant for the bioreduction of Ag^+ to Ag^0 was investigated by spectral methods (UV-VIS, FTIR, XRF, DLS). The stability of the phytosynthesized silver nanoparticles was checked by zeta (ξ)-potential measurements. DLS analysis and zeta potential were analyzed on the ultrasounds treated samples (30 min).

Addition of rosemary extract to $AgNO_3$ aqueous solution led to the appearance of green dark color after 24 hour, which indicates the formation of colloidal silver nanoparticles. These colors arise due to excitation of surface plasmon vibrations of metal nanoparticles.



INTRODUCTION

Over the past centuries, the application of natural bioactive compounds is growing due to their benefits in the herbal pharmacy or food industries. The natural components that can be extracted from plants can find applications in the biomedical area, due to their different functional activities (antioxidant or antimicrobial activity, anti-hypertensive, anti-cancer or neurodegenerative diseases prevention).[1]

The consumption of foodstuffs with a high amount of antioxidant components has an important positive impact on human health, is capable to reduce the risk of heart diseases and some neurological diseases, and to prevent cancer and other inflammatory diseases due to their good sources of antioxidants. [1,2]

New advances in the nanotechnology domain and the emerging needs of the biomedical area led to development of new nanoparticle-based materials;[3,4] the nanoparticles are used as drug carriers, as their features (easy formulation using organic/inorganic materials, easy modification of the targeted molecules, drugs or other molecules on them, effective delivery to target places,

* Corresponding author: raluca_bunghez@yahoo.com

controlled drug release trough external/internal stimuli) can offer great development opportunities.[5]

From the last decades, silver nanoparticles have been also used for wound dressings, textiles materials and disinfectants, due to their antioxidant and antimicrobial properties.[6,7]

Rosmarinus officinalis is a small perennial evergreen shrub belonging to the Lamiaceae family; its name derives from the Latin for "dew" (ros) and "sea" (marinus), or "dew of the sea".[8] The leaves are used to flavor various foods, or for medical or dermal purposes.

Silver nanoparticles (AgNPs) have become one of the important topics due to their antimicrobial applications, discovered for over 2000 years, in many areas. In the last decades, many advances have been made in drug delivery system. On this line, fruits and plants play an important role in this domain. Silver nanoparticles have new or improved properties depending on their size, morphology, and distribution. Significant reports have been published dealing with the synthesis of metal nanoparticles using plant or fruit extracts.[9,10,11]

The first goal of our work was to extract rosemary components. The second goal of this work was to synthesise AgNP using rosemary leaf extracts.

The synthesis of rosemary extract-silver nanoparticles was confirmed by UV-VIS, Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, Dynamic Light Scattering (DLS) and X-Ray Fluorescence *(*XRF) analysis.

The antioxidant properties of these nanomaterials were evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

MATERIAL AND METHODS

1. Materials

Silver nitrate ($AgNO_3$) was purchased from Merck (Germany); DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical) was supplied by Sigma-Aldrich and methanol by Scharlau. *Rosmarinus officinalis* was purchased from Roumanian local source.

2. Methods

2.1. Synthesis of rosemary-silver nanoparticles

Rosemary (*Rosmarinus officinalis*) leaves were washed in distilled water and dried at 50°C. So, 10 g of this plant was weighed and transferred into a 100 mL Erlenmeyer flask containing 125 mL of distilled water and boiled for 5 minutes in order to release the intracellular material into solution. The rosemary aqueous extract obtained, was then cooled and filtered through a filter paper to obtain a clear green extract.

The rosemary-silver nanoparticles were prepared by mixing 5 mL of rosemary leaf aqueous extract with 5 mL of aqueous solution of 10^{-3}M $AgNO_3$ and kept overnight at room temperature. The colloidal silver nanoparticles were separated from the biomass using centrifugation (10 min, 5000 rpm, SIGMA 2-16 K centrifuge).

The rosemary silver nanoparticles were subjected to 30 min ultrasonic irradiation on an ultrasound bath. After ultrasound treatment, the samples were centrifuged (10 min, 7000 rpm) and the supernatants were kept and used in experiments.

2.2. Characterization methods

For the UV-VIS determinations, a M42 Carl Zeiss Jena UV spectrophotometer from 200 to 600 nm, with a 1 nm slit width and 1 nm step size, 0.3 nm/s average scan rate, deuterium lamp, double beam, microprocessor and quartz cell was used to measure the aqueous solution absorbance and the molar absorption spectra for each sample at 22°C.

Fourier transformed IR spectroscopy (FT-IR) standard spectra were collected using a Perkin Elmer Spectrum GX spectrometer with Attenuated Total Reflectance (ATR) diamond crystal. Scans in the range of 400–4000 cm^{-1} were collected for each spectrum at a spectral resolution of 4 cm^{-1}.

The obtained data were processed using specific data analysis software (Origin Pro 8.0).

The physical stability of samples was evaluated using the processes zeta potential (ξ-potential) and DLS – Dynamic Light Scattering. The ξ-potential and DLS determination of the samples was performed using a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) by measuring the electrophoretic mobility of the samples in an electric field.

The X-ray fluorescence technique is of special interest for the analysis of silver nanoparticles because the technique is not only fast, sensitive and capable of simultaneous multi-element analysis, but also ensures that the sample can be quantitatively analyzed without damage. The equipment used is a PW4025 – MiniPal – PANalytical energy dispersive XRF Spectrometer with a rhodium anode. The XRF determinations have been carried out in Helium atmosphere, for a period of 300 seconds, without any filter, at proper voltage and current intensity.

2.3. Antioxidant activity

The antioxidant activity of the extracts was evaluated spectrophotometrically using the DPPH method described by literature[12,13] with minor changes. Each plant extract was evaluated at 100 mg/L concentration, by mixing 0.5 mL of them with 1 mL of DPPH solution (2 mg/100 mL). Each sample was mixed 30 minutes and then kept in the dark for 30 minutes, at room temperature. After that, each mixture sample was tested for the DPPH radical-scavenging activity by measuring the absorbance at 517 nm on a UV-VIS spectrophotometer (Specord M 42).

As blank was used a solution prepared by mixing 0.5 mL of bidistilated water with 1 mL of the DPPH solution (2 mg/100 mL). The antioxidant activity (AA%) was calculated using the formula:

$$AA\% = [(A_{Control} - A_{Extract}) / A_{Control}] \times 100 \quad (1)$$

where: $A_{Control}$ is the absorbance of a DPPH solution without extract, $A_{Extract}$ is the absorbance of the plant extract with DPPH (2 mg/100 mL).

As positive control it was used hydroquinone at 100 mg/L.

RESULTS AND DISCUSSION

The synthesis of silver nanoparticles was confirmed by UV-VIS absorption and ATR-FTIR spectroscopy. The aqueous rosemary extract was used as a reducing agent for Ag^+ as well as a capping agent for silver nanoparticles.

The bioreduction of the Ag^+ ions was detected by recording the UV-VIS spectra of the samples. UV–VIS spectrum of the rosemary-AgNPs presented an absorption band at 440 nm (Fig. 1).

Fourier transform infrared spectroscopy (FTIR) was used to study the obtaining of AgNPs using rosemary leaves and to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping the bioreduced silver nanoparticles synthesized by the plant extract. The ATR-FTIR spectrum (Fig. 2) absorption bands, indicates the presence of active functional groups in the synthesized silver nanoparticles. In order to obtain good signal/noise ratio, the FTIR transmission spectra of aqueous rosemary extract before and after bioreduction of Ag^+ ions were recorded in the region 400-4000 cm^{-1}.

In rosemary aqueous extract, peaks from 1632, 1384 and 1115 cm^{-1} have been attributed to amides, proteins and enzymes, which seems to be responsible for the reduction of metal ions when it is using vegetable materials for the synthesis of metal nanoparticles.[6]

The structure of sample does not suffer many changes, which demonstrated the fact that metal nanoparticles does not intervene in modifying the structure of rosemary plant.[7,9,14,15]

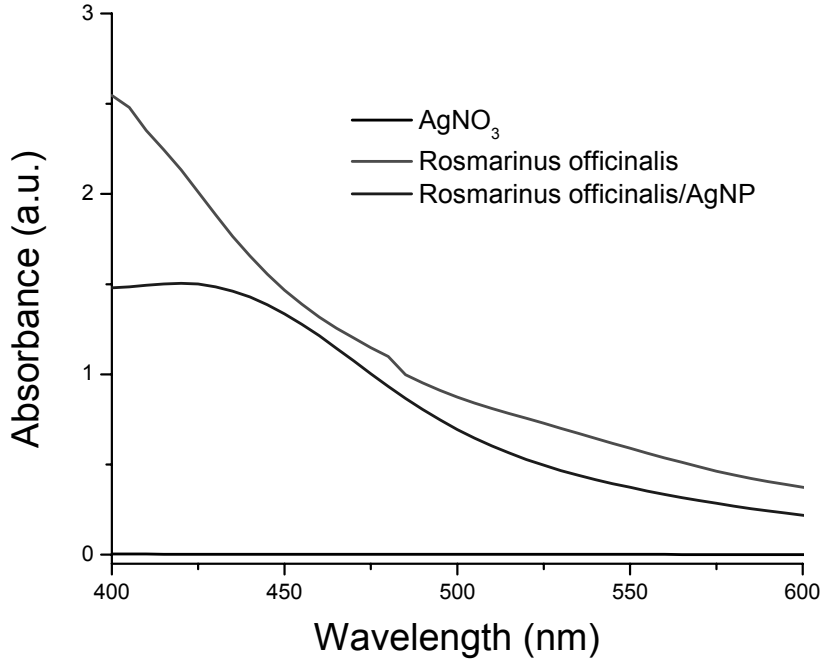


Fig. 1 – The UV–VIS spectra of $AgNO_3$, rosemary extract and AgNPs in rosemary extract; only the region of interest (400-600 nm) is presented.

Some IR bands common to rosemary water extract appeared in AgNPs, but transmittance level of the plant extract bands was weakened after interaction with silver nanoparticles and shifted to 3429 cm^{-1} (O–H stretching), 2324 cm^{-1} (alkyls C–H stretching), 1632 cm^{-1} (assigned to amide I, arising due to carbonyl stretch in proteins) and 1115 cm^{-1} (corresponding to C-O, C-N stretching vibrations of the aliphatic amines or alcohols/phenols, representing the presence of polyphenols in the rosemary extract).[16,17]

The silver ions will be surrounded by various phytochemical constituents present in *Rosmarinus officinalis*, creating a coating on silver ions which receive electrons from these phytocompounds that acted as electron donors resulting in bioreduction of silver ions.

ATR-FTIR results demonstrated that bioactive compounds responsible for silver bioreduction could be proteins and polyphenols (of rosemary *aqueous extract*) presumed to act as reducing and capping agents for the silver nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.

The antioxidant properties of samples: *Rosmarinus officinalis* leaves aquoeus extract and AgNP -rosemary, were determined by DPPH method. The herbal silver nanoparticles exhibited high values of antioxidant activity (AA = 99.6 %) than rosemary aqueous extract (AA%=93.5%).

The DLS results indicated that all the particles are nano-sized with average diameters of 76.7 nm and the zeta potential values (-34.2 mV) of rosemary silver nanoparticles revealed a good stability of the samples.

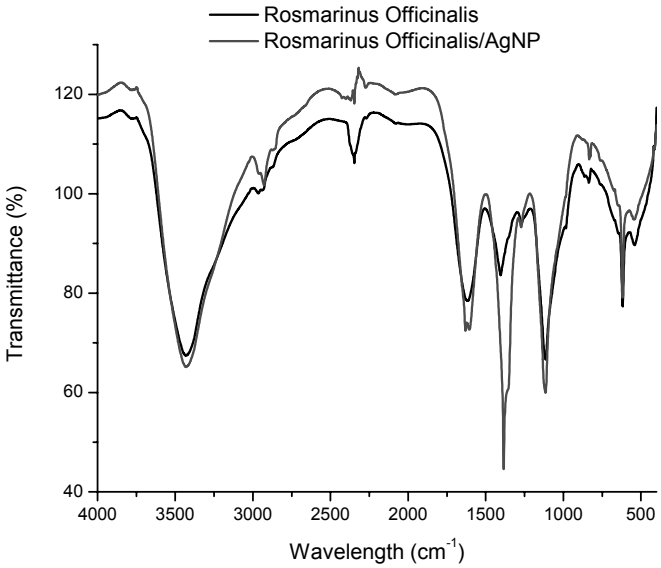


Fig. 2 – ATR-FTIR spectra of rosemary aqueous extract and rosemary-AgNP samples.

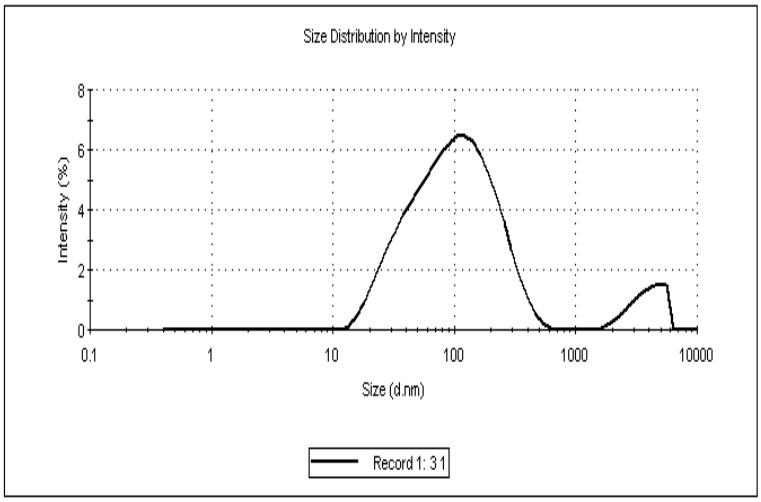


Fig. 3 – DLS analysis of AgNP rosemary suspension.

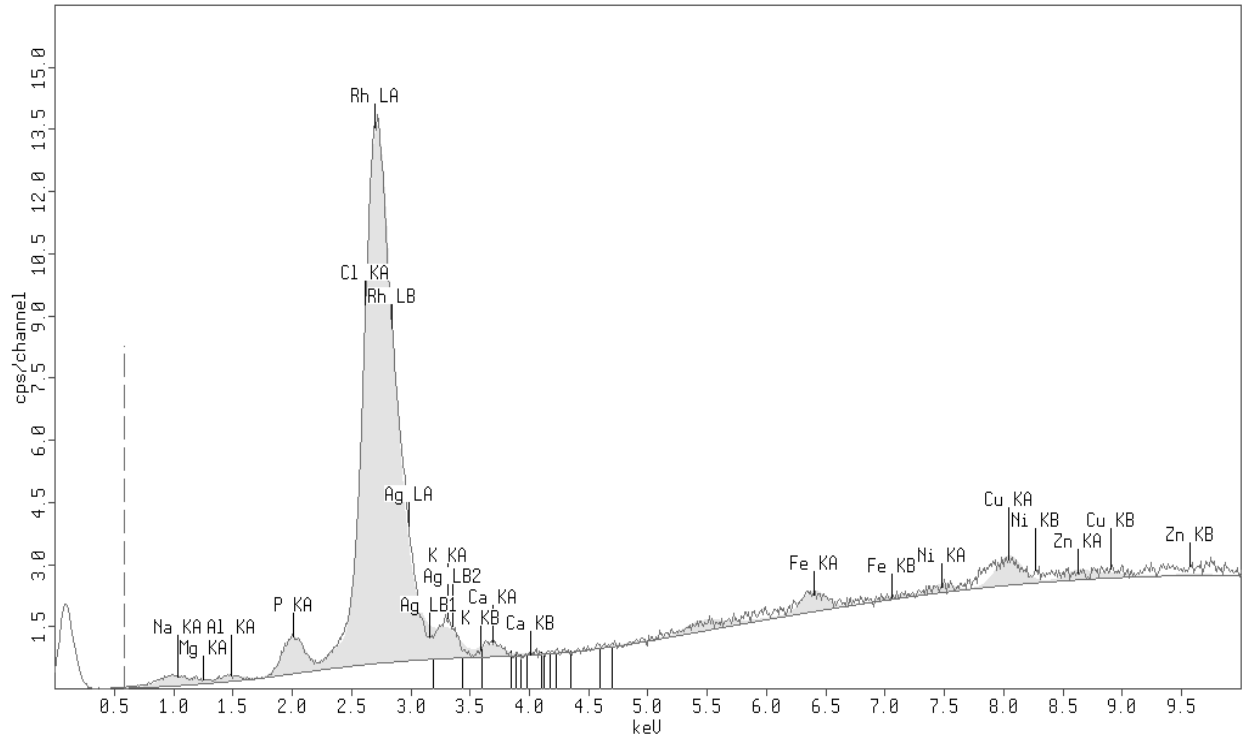


Fig. 4 – XRF investigation of silver presence in rosemary-AgNP.

X-ray fluorescence analysis was used to identify the silver existence in the rosemary-AgNP nanomaterials.

Using this analysis, it was observed (Fig. 4) the presence of silver in the rosemary-AgNP sample. As expected, the blank, rosemary aqueous extract used as a reference, doesn't contain silver.

CONCLUSIONS

The bio-reduction of aqueous Ag^+ ions by the *Rosmarinus officinalis* leaf extract has been demonstrated. This green ecofriendly synthesis, used to obtain silver nanoparticles, has many advantages as an easy way to obtain nanoparticles and to save material resources.

We reported an aqueous rosemary plant extract which was used to phytosynthesize silver nanoparticles. *Rosmarinus officinalis* plant extract proved to have strong reducing properties for "green synthesis" of silver nanoparticles.

The phytosynthesis of rosemary-AgNPs was observed by the appearance of a green dark color and confirmed by spectral analyses (UV-VIS and ATR-FTIR spectroscopy) that revealed the presence of the polyphenols and proteins in the plant extract, bioactive compounds responsible for bioreduction of silver ions and for stabilization of AgNPs.

UV-VIS analysis confirmed the formation of silver nanoparticles (AgNP), by the presence of specific absorption peak at 440 nm. XRF analysis confirmed the existence of silver in the samples.

The antioxidant properties were determined by DPPH method, the herbal silver nanoparticles exhibiting high values of *antioxidant activity* (AA = 99.6%) higher than rosemary aqueous extract (AA%=93.5%).

It was demonstrated the fact that the *Rosmarinus officinalis* extract could be utilized as a good natural source of antioxidants and a possible food supplement or as an antioxidant agent in pharmaceutical industry.

ATR-FTIR results demonstrated that bioactive compounds responsible for silver bioreduction could be proteins and polyphenols from rosemary extract presumed to act as reducing and capping agents for the silver nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.

These eco-friendly silver nanoparticles can be applied in different medical operations, such as treatment of wound healing or to discover new studies in biology, medicine or material science.

REFERENCES

1. S. Moreno, T. Scheyer, C.S. Romano and A.A. Vojnov, *Free Radic Res.*, **2006**, *40*, 223-231.
2. S. Tavassoli and Z. Emam Djomeh, *Global Veterinaria*, **2011**, *7*, 337-341.

3. M. Jahanshahi and Z.Babaei, *Afr. J. Biotechnol.*, **2008**, *7*, 4926-4934.
4. G. Bao, S. Mitragotri and S. Tong, *Annu. Rev. Biomed. Eng.*, **2013**, *15*, 253-282.
5. E.K. Lim, E. Jang, K. Lee, S. Haam and Y.M. Huh, *Pharmaceutics*, **2013**, *5*, 294-317.
6. R. Podila, R. Chen, P.C. Ke, J.M. Brown and A.M. Rao, *Appl. Phys. Lett*., **2012**, *101*, 263701 - 263701-4.
7. C. Malarkodi, S. Rajeshkumar, M. Vanaja, K. Paulkumar, G. Gnanajobitha and G. Annadurai, *J. Nanostr. Chem.*, **2013**, *3*, 30-36.
8. A. Room, "A Dictionary of True Etymologies", Routledge, USA, 1980, 150
9. A.M. Awwad, N.M. Salem and A.O. Abdeen, *Int. J. Ind. Chem.*, **2013**, *4*, 1-6.
10. S.Prabhu and E. K.Poulose, *Int.Nano Lett.*, **2012**, *2*, 1-10.
11. V.K. Sharma, R.A. Yngard and Y. Linet, *Adv. Colloid. Interfac*., **2009**, *145*, 83-96.
12. S.P. Wong, L.P. Leong and J.H.W. Koh, *Food Chem*., **2006**, *99*, 775-783.
13. O.M. Mosquera., Y.M. Correra and J. Nino, *Braz. J. Pharmacogn.*, **2009**, *19*, 382-387.
14. R. Sathyavathi, M. Balamurali Krishna, S. Venugopal Rao, R. Saritha and N. Rao, *Adv. Sci. Lett.*, **2010**, *3*, 1-6.
15. S.C.G. Kiruba Daniel., R. Kumar, V. Sathish, M. Sivakumar, S. Sunitha and T.A. Sironmani, *Int. J. NanoSci. Nanotechn.*, **2011**, *2*, 103-117.
16. Y. He, Z. Du, H. Lv, Q. Jia, Z. Tang, X. Zheng, K. Zhang and F. Zhao, *Int. J. Nanomed.*, **2013**, *8*, 1809-1815.
17. A. Mubayi, S. Chatterji, P.M. Rai and G. Watal, *Adv. Mat. Lett*., **2012**, *3*, 519-525.