

INFLUENCE OF DIFFERENT PLANTS EXTRACTS ON SILVER NANOPARTICLES GREEN SYNTHESIS

Three plants extracts were used for biosynthesis of Ag nanoparticles (AgNPs). AgNPs nucleation process requires effective reduction agents which secure Ag^+ to Ag^0 reduction and also stabilizing/capping agents. The UV-vis and TEM observation revealed that the best results were obtained by *R. officinalis* leaf extract. The strong SPR band peak appeared at the wavelength 418 nm. Synthesized AgNPs were globular, fine (~20 nm), uniform and stable throughout the experiment. A rapid rate of AgNPs synthesis was also significant and economically advantageous factor. Fine (10-20 nm) and globular nanoparticles were synthesized also by *U. dioica* leaf extract, but the stability of nanoparticles was not permanent. Despite *V. vitis-idaea* fruit extract contains a lot of reducing agents, UV-vis did not confirm the presence of AgNPs in solution. Synthesized Ag particles were very unstable, Ag particles agglomerated very fast and clearly indicated sediment was formed.

Keywords: nanoparticles, silver, biosynthesis, reduction agents, TEM

1. Introduction

Metal nanoparticles, due to their specific magnetic, electrical, chemical, optical and mechanical properties are currently used in many fields, such as medical sector for drug delivery and diagnosis [1], in the chemical sector for catalysis [2], for environmental protection and energy conversion [3-4]. There is lot of conventional methods of nanoparticles preparation: physical (vapour deposition, molecular beam epitaxy, arc-discharge, etc.), chemical (chemical reduction, electrochemical techniques, irradiation-assisted chemical methods, pyrolysis, etc.) and biological methods. Physical methods are efficient but they require expensive and complicated technologies. Chemical methods are more affordable but they are not environment friendly. Biological or green synthesis is considered as a perspective, clean and economical method [5]. Moreover, this method has advantage over conventional in easy technology and not being toxic therefore, this method can be also considering as environmental friendly. Green synthesis allows to use a lot of plants extracts, algae, fungi and others biological substances which are suitable for synthesis of the nanoparticles. The biological substances present in plants extracts act as reducing, stabilising and capping agents and lead to the formation of nanoparticles with variety of shapes [6]. Among various inorganic NPs silver nanoparticles are very interesting and special, and lot of scientific groups are currently engaged in AgNPs research [7-14]. Silver is a nontoxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing by microorganisms

[15-18]. Therefore, silver is a main research subject in toxic and antimicrobial nanotechnology field.

In this paper, we present a green synthesis of silver nanoparticles using *Rosmarinus officinalis* (*R. officinalis*), *Urtica dioica* (*U. dioica*) leaf extracts and *Vaccinium vitis-idaea* (*V. vitis-idaea*) fruit extract. Plants contain a number of phytochemicals and other beneficial substances, which are important for nanoparticles synthesis [19,20]. The substances presented in plants differ in type, chemical bonds and amount. Therefore, they affect the synthesis of nanoparticles in various ways.

The purpose of this study was to prepare AgNPs by easy, cheap and eco-friendly technique using aqueous extracts of three different plants and observe the influence of plant extracts dissimilarity on AgNPs synthesis rate, shape, size and stability. As there are only a few works that describe the connection between plant composition and nanoparticle formation, we have tried to shed light on this issue.

2. Materials and methods

The fresh leaves of *R. officinalis*, *U. dioica* and fresh fruits of *V. vitis-idaea* were used for the extracts preparation. To remove impurities, the leaves and the fruits were washed several times with distilled water. Cleaned leaves and fruit were grinded separately in a grinding mortar and then added to 125 ml of distilled water. Mixtures were heated for 5 min at 70°C, then shaken for 45 min at 45°C. Thereafter, filtered and centrifuged at

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6000 rpm for 20 min. Prepared supernatants were used for AgNPs synthesis. The leaves and fruits were harvested in autumn. The presence and the amount of compounds found in extracts can vary considerably, even in the same plant or fruit from season to season and batch to batch. Therefore, to ensure repeatability of the experiment all experiments (plants extracts from autumn harvest and AgNPs synthesis) were performed in triplicate.

AgNPs were synthesized using three different plants extracts (*R. officinalis*, *U. dioica* and *V. vitis-idaea*). For each experiment, 10 ml of extract were added dropwise to 100 ml of heated (70°C) silver nitrate (AgNO_3) water solution (concentration of Ag 50 mg/l). The mixtures were then stored in daylight at room temperature. It is generally known that silver nitrate is the least expensive salt of silver, it offers several other advantages as it is non-hygroscopic, relatively stable to light, easy dissolves in water. Therefore, we used AgNO_3 as precursor for Ag^+ .

The AgNPs stability was monitored by measuring the UV-vis spectra of the solutions in 10-mm optical-path-length quartz semi-micro cuvettes (UNICAM UV-vis Spectrometer UV4). The size and morphology of the nanoparticles were studied by means of a Transmission Electron Microscope (JEOL model JEM-2000FX microscope operated at an accelerating voltage of 200 kV).

3. Results and discussion

3.1. Green synthesis of AgNPs by *R. officinalis* leaf extract

Adding of *R. officinalis* extract to AgNO_3 solution led to immediate change of solution colour to brown (as a result of excitation of Surface Plasmon Resonance). The pH of the solution after mixing was 7.81 and did not change significantly after AgNPs formation. Immediate formation of AgNPs was

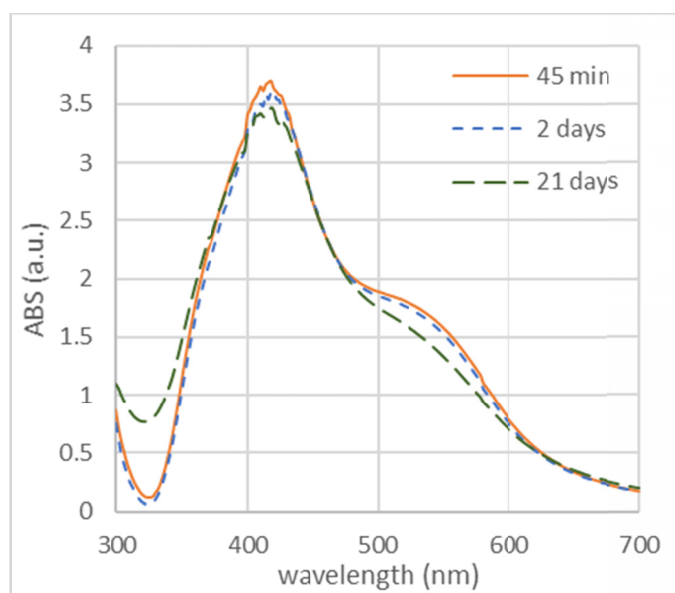


Fig. 1. The UV-vis absorption spectra measured for the AgNPs solution prepared by *R. officinalis* extract

confirmed also by UV-vis spectroscopy, typical strong peak at wavelength 418 nm was observed (Fig. 1).

It is evident, that AgNPs formed very fast and retain the same quality throughout the experiment (Fig. 1). The SPR band is toll and slim, the ABS value is high with negligible changes (Fig. 2), no peak shifting to high/low value of wavelength (Fig. 3) indicates creation of small, uniform nanoparticles without changes over time. Day light exposing (for 21 days) did not cause the degradation of AgNPs. It can be assumed that *R. officinalis* contain not only very efficient reducing but also very effective stabilizing agents.

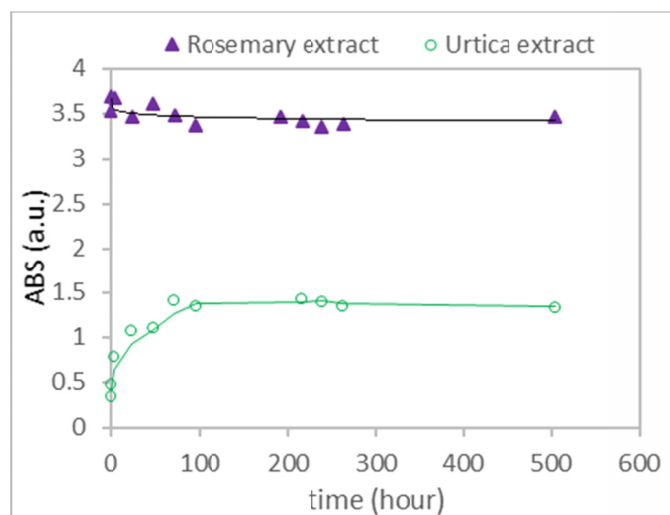


Fig. 2. Maximum value of absorbance variations as a function of time during NPs production

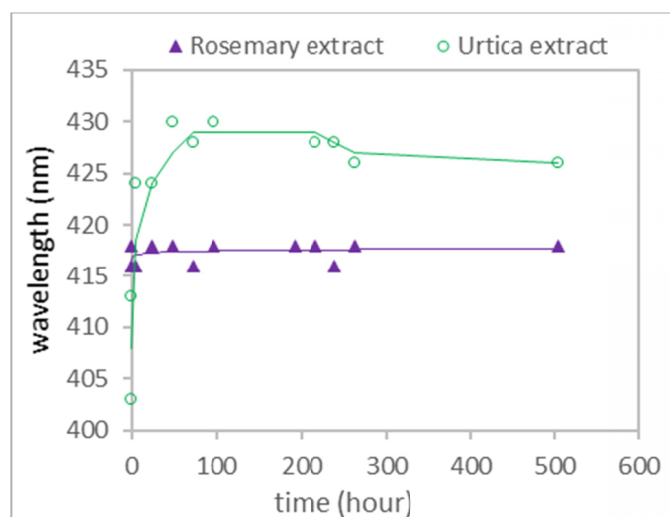
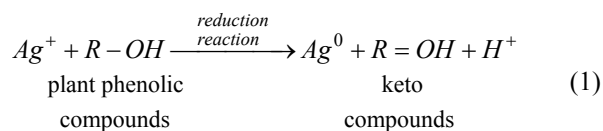


Fig. 3. Wavelength variations at maximum value of absorbance as a function of time during NPs production

It is well known that *R. officinalis* is one of the major sources of natural antioxidants. Chemical structures of the main compounds present in rosemary leaves can be subdivided into two categories: antioxidants and essential oils [21]. Many authors [22-24] have reported various bioactive molecules in *R. officinalis* plant extract, which are responsible for a synthesis of

silver nanoparticles. Ghaedi et al. and Dzimitrowicz et al. [22,23] confirmed, that this extract contains high amount of the hydroxyl groups of phenolic compounds (rosmarinic acid, carnosic acid, carnosol and rosmanol), which are the main reducing agents (Fig. 4). The reduction reaction mechanism of AgNPs synthesis can be written as follows:



According to [21] the most important antioxidants presented in *R. officinalis* extract are phenolic diterpene carnosic acid and the phenolic rosmarinic acid. Carnosol and rosmanol are formed by oxidative degradation of carnosic acid therefore are not contained initially in the leaves. Thus, these compounds are artefacts of the extraction process [21].

Additional impact on AgNPs formation have essential oils presented in *R. officinalis* leaves. Many authors screened the constituents of the essential oils [25-27]. More than 25 compo-

nents were identified, some of them perform the role of reducing agent, some role of stabilizing agent but some may also act as both stabilizers and reductants. Several authors [25-28] agreed on four main constituents of essential oils: 1,8-cineol (40.0%), camphor (17.9%), α -pinene (10.3%), and camphene (6.3%) (Fig. 4).

Analysis of Dzimitrowicz et al. [23] showed that the essential oils of *R. officinalis* are rich in monoterpenoids, ketones and alcohols. Above mentioned compounds of essential oils contain lower amount of reducing agents than antioxidants, but essential oils also contribute to AgNPs synthesis [23]. A comparison of the effect of antioxidants and essential oils is presented in [23]. Smaller NPs (~8.7 nm) were synthesized using antioxidants, but their fast coalescence was observed. Essential oils caused formation of bigger (~60 nm), well-dispersed and stabile NPs. It is likely that also in our case mainly essential oils have contributed to the formation of uniform and very stable nanoparticles of Ag.

In this study, formation of fine and stabile nanoparticles was confirmed also by the TEM observation. TEM micrograph of AgNPs synthesized by *R. officinalis* extract immediately after extract adding (Fig. 5a), after 11 days of exposition (Fig. 5b) and

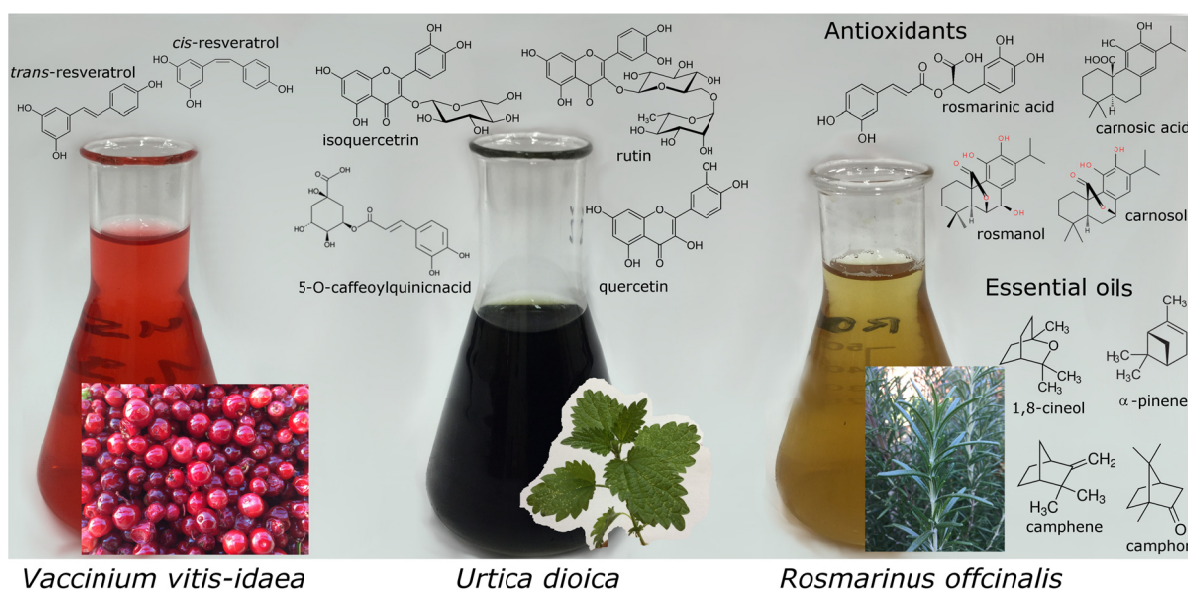


Fig. 4. Plants extracts and the chemical structures of main of *R. officinalis* antioxidants and essential oils, *U. dioica* bioactive phenolic compounds and *V. vitis-idaea* antioxidants

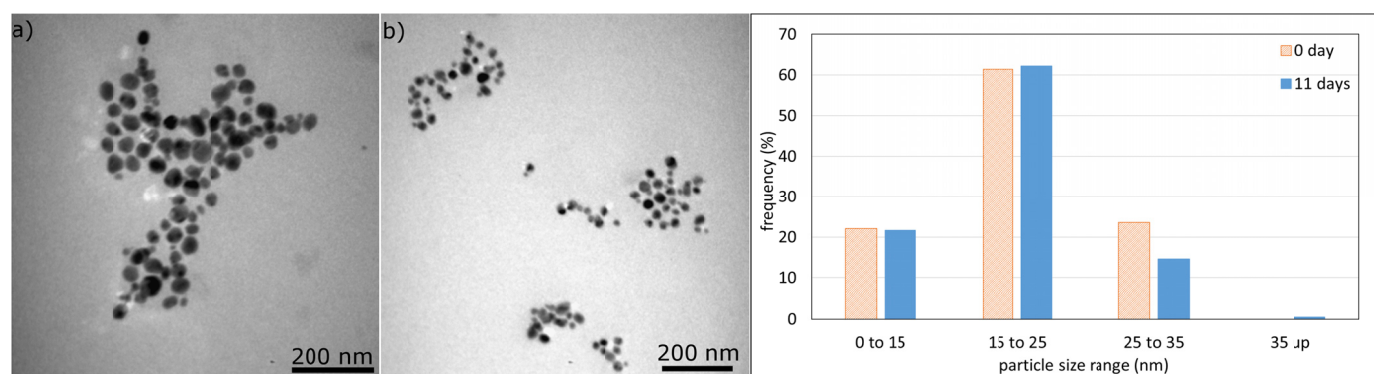


Fig. 5. TEM micrograph of AgNPs synthesized by *R. officinalis* extract immediately after extract adding a); after 11 days b); and size distribution histograms of AgNPs

relevant size distribution histograms of AgNPs (Fig. 5c) show that the size of nanoparticles did not change at all. The AgNPs are spherical, with diameter in the size range of 15-20 nm.

3.2. Green synthesis of AgNPs by *U. dioica* leaf extract

U. dioica has good antioxidant activity because it is a good source of polyphenols and phytochemicals. Leaves extract contain alcohol, triterpenoids, alkaloids, polyphenols and tannins [29, 30], therefore this plant was chosen as a one of the reducing agent for the synthesis of Ag nanoparticles.

Corresponding to literature mainly –OH groups of phenolic compounds in *U. dioica* leaves extract are responsible for the reduction of Ag^+ into Ag^0 . The rutin, quercetin, 5-O-caffeoylquinic acid and isoquercitrin (Fig. 4) were identified as a most abundant bioactive phenolic compounds exhibiting antioxidant activity [31].

Similar findings were reported by K. Jyoti et al. [29], which also indicated the presence of C-N and C-C groups in proteins

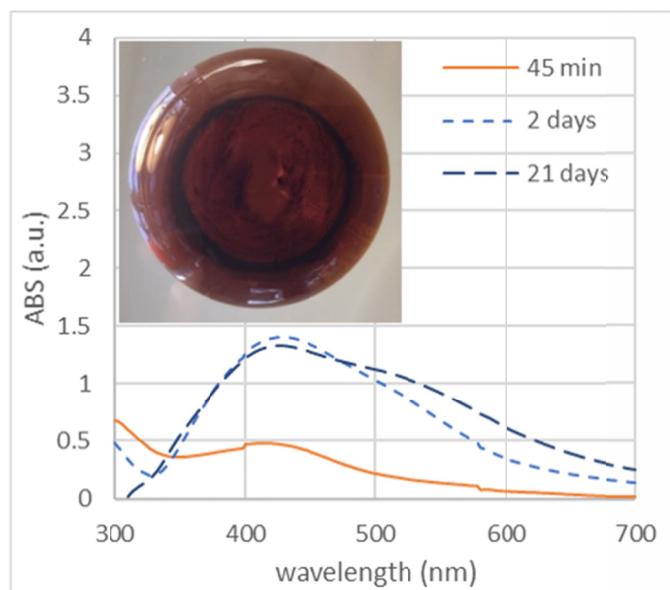


Fig. 6. The UV-vis absorption spectra measured for the AgNPs solution prepared by *U. dioica* extract; the inset shows sediment of AgNPs

and N-H groups present in amide linkages of the proteins. These functional groups fulfil the role as stabilizer of AgNPs and/or capping agent.

Using *U. dioica* extract did not cause such rapid AgNPs formation (the light brown coloration appeared after ~5 hours, pH 7.44) and the peak (408 nm) was not so noticeable (Fig. 6). Gradual synthesis of nanoparticles is evident also on Fig. 2, maximum of ABS was reached on the third day. At the same time the broadening of SPR band started, what indicates beginning of NPs agglomeration. Presence of slight sediment on the bottom of Erlenmeyer flask was well visible on the 5th day of the experiment (Fig. 6). Red-shift of wavelength values (Fig. 3) in combination with SPR band's broadening and loss of symmetry can indicate: increasing of particle size, loss of uniform nanoparticles, loss of narrow particles size distribution or combination of these changes.

The AgNPs synthesized by *U. dioica* leaves extract are globular and surrounded by an organic tin layer (Fig. 7a, b). The comparison of the AgNPs TEM images and size distribution histograms (Fig. 7c) shows change in particles size in dependence on time. Nanoparticles formed during the first day of the experiment show wide interval of size (5-50 nm) but AgNPs exposed to daylight for 11 days show decrease of particles size interval and increase of NPs percentage in diameter at range of 10-15 nm.

It can be assumed that growth and agglomeration of nanoparticles started around the third day (Fig. 3) and continued throughout the experiment, which was also confirmed by the formed sediment (Fig. 6). Therefore, at the end of experiment only small nanoparticles (~15 nm) remained in solution. It is possible to say that the red-shift of wavelength values confirmed the loss of narrow particles size distribution at the same time with particles agglomeration.

3.3. Green synthesis of AgNPs by *V. vitis-idaea* fruit extract

Various functional groups assigned to the free –OH in the molecule and –OH group forming hydrogen bonds, carbonyl

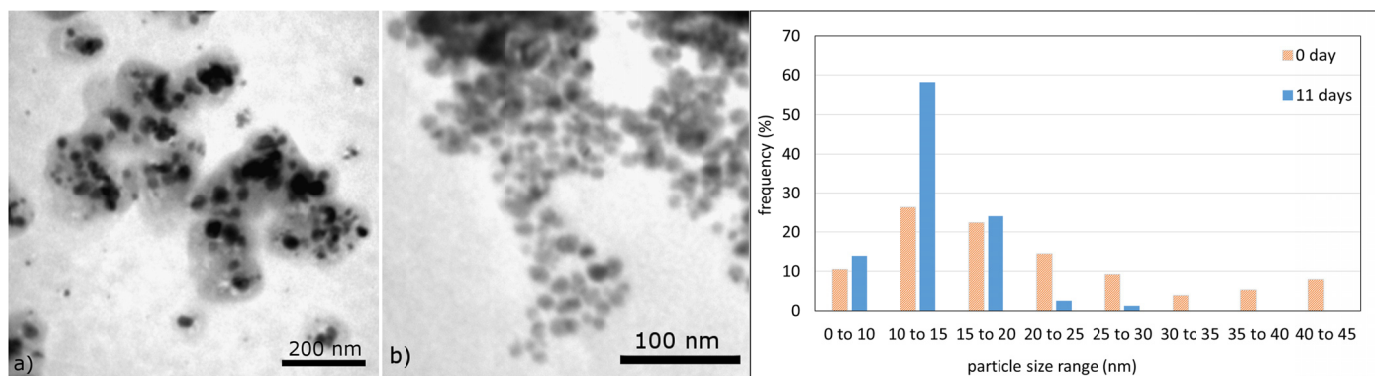


Fig. 7. TEM micrograph of AgNPs synthesized by *U. dioica* extract after extract adding a); after 11 days b); and size distribution histograms of AgNPs

group (C=O), groups C=C aromatic ring and C-OH groups were identified in aqueous fruit extract of *V. macrocarpon* [32]. The presence of significant constituents as an antioxidant flavonoid and phenolic acids was confirmed [33,34]. This organic compounds acts as reducing and capping agents. Mainly, presence of antioxidants such as polyphenols (resveratrol *trans*- and *cis*-form) [35,36] are associated with formation of Ag⁰ from Ag⁺ (Fig. 4).

Khodadadi et al., [32] has shown that using of *V. macrocarpon* fruit extract is suitable and effective method of AgNPs synthesis. Our results are not in agreement with results obtained by Khodadadi et al. The UV-vis of solution with *V. vitis-idaea* fruit extract did not show the absorption peak at all (Fig. 8). In our case, unstable AgNPs were formed, particles agglomerated very fast. The sediment was observed already two hours after the start of the experiment (Fig. 8).

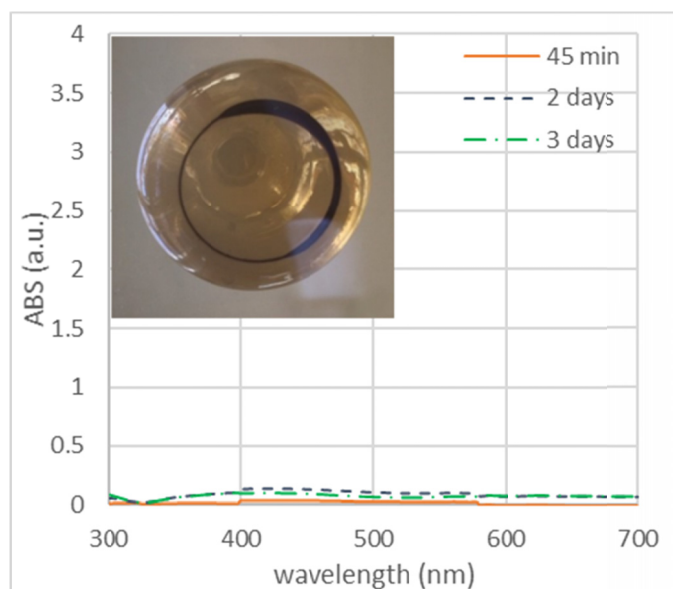


Fig. 8. The UV-vis absorption spectra measured for the AgNPs solution prepared by *V. vitis-idaea* extracts; the inset shows sediment of AgNPs (*V. vitis-idaea* extracts)

Composition of different *Vaccinium* species or geographic location may not be so significant factors to affect the synthesis of AgNPs so strongly, but it is obvious that both the extract preparation process (in our experiment the same method was used for leaves and fruit extract preparation) and the fruit condition (collection time, freshness of fruit) can influence the extract composition. In our case, freshly harvested cranberries (*V. vitis-idaea*) were used, Khodadadi et al. used dried *V. macrocarpon*. Also the low pH of the solution (pH 3.52 after adding extract to AgNO₃ solution) could have a significant effect and contribute to fast agglomeration of Ag. It is obvious that there is a need for at least extract pH treatment to prevent AgNPs agglomeration.

4. Conclusions

The main aim of our work was to prepare AgNPs by plants extracts and to observe the influence of extracts composition on AgNPs synthesis, for that reason three different plants have been chosen (*R. officinalis*, *U. dioica* and *V. vitis-idaea*). The leaves of *R. officinalis* and *U. dioica* were chosen because of their different composition. *R. officinalis* is one of the major resources for natural antioxidants and essential oils. *U. dioica* has good antioxidant activity but there are no essential oils. *V. vitis-idaea* fruit is well known for their strong antioxidant effect but at the same time *V. vitis-idaea* belong to naturally sour fruit therefore, also extract has low pH. In order to compare and analyse the results of experiments, extracts were prepared in the same way (without pH adjustment), the same concentration of silver nitrate solution were used and conditions (temperature, time, light conditions) of AgNPs synthesis were the same.

Uniform, spherical, with diameter in range 15-20 nm and stable Ag nanoparticles were prepared by *R. officinalis* leaves extract. *U. dioica* leaves extract supported synthesis of AgNPs but nanoparticles were not so uniform (particle diameter changed during experiment) and stable as in case of *R. officinalis* leaves extract. Adding of *V. vitis-idaea* fruit extract to silver nitrate water solution supported formation of Ag nanoparticles, but they were not stable, rapid coalescence of Ag particles occurred and sediment of agglomerated particles was evident on bottom of Erlenmeyer flask.

Obtained results also shown that *R. officinalis* leaves contain very effective reducing and stabilizing/capping agents. Probably combination of antioxidants and essential oils presented in leaves guarantee formation of fine and stable nanoparticles. Other benefit of this extract was very rapid, nearly immediate synthesis of AgNPs. Leaves of *U. dioica* also contain reducing agents and they can be used for AgNPs synthesis but leaves substances are not so effective for stabilizing of created nanoparticles. *V. vitis-idaea* fruit extract contain a lot of antioxidant flavonoid and phenolic acids which are responsible for Ag⁺ to Ag⁰ reduction, but it is obvious that the method of extract preparation has to be adapted to particular species of plant. Also the low pH is one of the reasons why particles agglomerated so fast.

The results showed that for complete understanding of plants extracts impact on AgNPs formation there is needed further study of extract composition and reaction which take place in process of synthesis. These preliminary results also showed that the use of plants extracts is a perspective method of nanoparticles production.

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REFERENCES

- [1] S. Parveen, R. Misra, S.K. Sahoo, *Nanomed-Nanotechnol.* **8** (2), 147-166 (2012).
- [2] V.V. Gulians, N.R. Shiju, *Appl. Catal. A Gen.* **356**, 1-17 (2009).
- [3] J.R. Lead, Y. Ju-Nam, *Sci. Total Environ* **400**, 396-414 (2008).
- [4] W. Kim, K. Kim, B. Jung, J. Kim, *Sol. Energy Mater. Sol. Cells* **94**, 1835-1839 (2010).
- [5] A.K. Mittal, Y. Chisti, U.C. Banerjee, *Biotechnol. Adv.* **31**, 346-356 (2013).
- [6] M. Noruzi, D. Zare, K. Khoshnevisan, D. Davoodi, *Spectrochim. Acta* **79**, 1461-1465 (2011).
- [7] S. Schultz, D.R. Smith, J.J. Mock, D.A. Schultz, *Proc. Natl. Acad. Sci. Unit. States Am.* **97**, 996-1001 (2000).
- [8] S. Eckhardt, P.S. Brunetto, J. Gagnon, M. Pribe, B. Giese, K.M. Fromm, *Chem. Rev.* **113**, 4708-4754 (2013).
- [9] B. Kumar, K. Smita, L. Cumbal, A. Debut, R.N. Pathak, *Bioin. Chem. and Appl.* 784268 (2014).
- [10] D.I. Gittins, D. Bethell, R.J. Nichols, D.J. Schiffrin, *J. Mater. Chem.* **10**, 79-83 (2000).
- [11] B. Bhattarai, Y. Zaker, T.P. Bigioni, *Current Opinion in Green and Sustainable Chemistry*, (2018), doi: 10.1016/j.cogsc.2018.06.007.
- [12] T. Souza, L.R.R. Souza, L.P. Franchi, *Ecotoxicology and Environ. Safety* **171**, (2019) 691-700.
- [13] N. Roy, A. Gaur, A. Jain, S. Bhattacharya, V. Rani, *Environmental Toxicology and Pharmacology* **36** (10), 807-812 (2013).
- [14] L. Wei, J. Lu, H. Xu, A. Patel, Z.S. Chen, G. Chen, *Drug Discovery Today* **20** (5), 595-601 (2015).
- [15] S.H. Jeong, S.Y. Yeo, S.C. Yi, *J. Mat. Sci.* **40**, 5407-5411 (2005).
- [16] O. Choi, K.K. Deng, N.J. Kim, L. Ross Jr., R.Y. Surampalli, Z. Hu, *Water research* **42**, 3066-3074 (2008).
- [17] M. Akter, Md.T. Sikder, Md.M. Rahman, et al., *Journal of Advanced Research* **9**, 1-16 (2018).
- [18] S.P. Deshmukh, S.M. Patil, S.B. Mullani, S.D. Delekar, *Materials Science & Engineering C* **97**, 954-965 (2019).
- [19] A. Vallverdú-Queralt, J. Regueiro, M. Martínez-Huélamo, J.F. Rinaldi Alvarenga, *Food Chemistry* **154**, 299-307 (2014).
- [20] <https://www.drugs.com/npp/rosemary.html>, accessed: 6.11.2018.
- [21] A. Wollinger, E. Perrin, J. Chahboun, V. Jeannot, D. Touraud, W. Kunz, *Comptes Rendus Chimie* **19** (6), 754-765 (2016).
- [22] M. Ghaedi, M. Yousefinejad, M. Safarpour, H. Zare Khafri, M.K. Purkait, *J. Industrial and Engineering Chemistry* **31**, 167-172 (2015).
- [23] A. Dzimitrowicz, S. Berent, A. Motyka, P. Jamroz, K. Kurbach, W. Sledz, P. Pohl, *Arabian Journal of Chemistry* (2016), in press.
- [24] Romo Vaquero M, Yáñez-Gascón M-J, García Villalba R, Larrosa M, Fromentin E. et al., *PLoS ONE* **7** (6), e39773 (2012).
- [25] Jafar Akbari, Majid Saeedi, Davood Farzin, Katayoun Morteza-Semnani, Zahra Esmaili, *Pharmaceutical Biology* **53** (10), 1442-1447 (2015).
- [26] Y. Zaouali, T. Bouzaine, M. Boussaid, *Food Chem. Toxicol.* **48**, 3144-3152 (2010).
- [27] W. Wang, N. Wu, Y.G. Zu, Y.J. Fu, *Food Chem.* **108**, 1019-1022 (2008).
- [28] I.H. Hameed, I.A. Ibraheam, H.J. Kadhim, *J. Pharmacogn. Phytother.* **7**, 90-106 (2015).
- [29] K. Jyoti, M. Baunthiyal, A. Singh, *J. Radiation Res. and Appl. Sci.* **9** (3), 217-227 (2016).
- [30] H.J. Al-Tememe, *J. Pharmacognosy and Phytotherapy* **7** (10), 238-252 (2015).
- [31] Khan Nadiya Jan, Khan Zarafshan, Sukhcham Singh, *Food Measure* **11**, 423-433 (2017).
- [32] B. Khodadi, M. Bordbar, A. Yeganeh-Faal, M. Nasrollahzadeh, *J. Alloys and Compounds* **719**, 82-88 (2017).
- [33] M. Atarod, M. Nasrollahzadeh, S.M. Sajadi, *J. Colloid Interf. Sci.* **462**, 272-279 (2016).
- [34] M. Sajjadi, M. Nasrollahzadeh, S.M. Sajadi, *J. Colloid Interf. Sci.* **497**, 1-13 (2017).
- [35] Laima Česonienė Remigijus Daubaras: *Nutritional Composition of Fruit Cultivars, Chapter 8 – Phytochemical Composition of the Large Cranberry (Vaccinium macrocarpon) and the Small Cranberry (Vaccinium oxycoccos)*, Elsevier Inc. ISBN 978-0-12-408117-8, (2016).
- [36] E. Pappas, K.M. Schaich, *Crit. Rev. Food Sci. Nutr.* **49**, 741 (2009).