A COMPARATIVE STUDY OF GOLD NANOPARTICLES SYNTHESIZED WITH A 'GREEN' APPROACH

SILVESTRI Daniele[1], CHŁĄD Zuzanna[1], WACŁAWEK Stanisław[1*], ADACH Kinga[1], FIJAŁKOWSKI Mateusz[1], PADIL Vellora Thekkae Vinod[1], ČERNÍK Miroslav[1*]

[1]*Centre for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Czech Republic, EU, phone +420 485 353 006*

* stanislaw.waclawek@tul.cz, miroslav.cernik@tul.cz

Abstract

Nowadays 'green' synthesis of gold nanoparticles (Au NPs), utilizing organic compounds as a reducing and stabilizing agents (e.g. plant extracts, biodegradable polymers), is gaining considerable attention. This approach of nanoparticle synthesis gives the opportunity to avoid often expensive and harmful substances for the environment. In this paper, Au NPs were synthesized, for the first time, by two natural materials - tarragon plant extract and PHB-chitosan polymer conjugate. The analysis of gold nanoparticles was provided by Vis spectroscopy and scanning electron microscopy (SEM). Moreover, the stability of Au NPs was determined by zeta potential analysis whereas their size by differential centrifugal sedimentation (DCS) technique. It was found that the mean size of Au NPs was 7 and 30 nm for the particles reduced with PHB-chitosan polymer conjugate and tarragon plant extract, respectively. Furthermore, the catalytic properties of thus synthesized nanoparticles were compared toward 4-nitrophenol reduction. It was determined that at the optimal conditions the pseudo first-order kinetic rate constant was 0.74 and 0.29 min^{-1} for the processes involving Au NPs synthesized by PHB-chitosan polymer conjugate and tarragon plant extract, respectively.

Keywords: Gold nanoparticles, green synthesis, polymers, plant extract, catalysis

1. INTRODUCTION

The methods for the preparation of gold nanoparticles (nAu) are generally based on the reduction of Au(III) using toxic or dangerous reducing agents such as sodium borohydride, hydrazine hydrate or hydrogen [1]. Therefore these considerations resulted in numerous publications suggesting new synthesis routes of nAu in which green reduction and protection agents were used [2]. Most of these reducing and stabilizing agents were obtained from plants [2], algae [3], bacteria [4] and fungi [5].

The green synthesis of metal nanoparticles (mainly nAu) employing plant extracts are cost-efficient, and consequently can be applied as an alternative for the large-scale production of metal nanoparticles. Plant extracts can act as both reducing and capping agents [6]. Those agents mostly are vitamins, amino acids, phenolic and flavonoids compounds, enzymes, polysaccharides and organic acids such as citrates [7,8].

Other eco-friendly reducing and stabilizing agent is chitosan (chit), with excellent biodegradable and biocompatible characteristics, moreover it is a naturally occurring polysaccharide. Using chitosan for the reduction and stabilization of gold nanoparticles has been already performed [9]. The other biopolymer - polyhydroxybutyrate (PHB) is a polymer produced as a carbon reserve in certain bacteria [10]. PHB was already used in synthesis and other chemical processes e.g. for stabilization of nanoscale zero-valent iron [11]. To improve the reduction of gold precursor and stability of nAu (moreover to enhance solubility properties of PHB in water), two polymers (polyhydroxybutyrate and chitosan) have been conjugated. Furthermore, particles of nAu have been found to be an excellent catalysts. They are often employed as a catalyst for 4-nitrophenol (4-NP) reduction (to 4-aminophenol (4-AP)) by sodium borohydride ($NaBH_4$). This model reduction

is often used to study the catalytic efficiency of metal nanoparticles. The mechanism of the reduction is explained that nAu are able to catalyze the reaction by facilitating electron transfer from BH_4^- to 4-NP [12,13].

In this paper, two different 'green' approaches for the synthesis and stabilization of nAu by polyhydroxybutyrate-chitosan (PHB-chit) conjugate and by tarragon pant extract (PE) were shown. In order to highlight how different ways of synthesis and stabilization can affect nanoparticle properties and their catalytic activity several characterization methods were used (visible spectrophotometry, differential centrifugal sedimentation (DCS), scanning electron microscope (SEM)). The stability of nAu was estimated by zeta potential analysis and the reduction of 4-nitrophenol by sodium borohydride was used as a model system to evaluate the catalytic activity of nAu synthesized by PHB-chit and PE.

2. MATERIALS AND METHODS

2.1. Chemicals

Chitosan (Physical form 75-85% deacetylated, low molecular weight 50,000-190,000 Da), tetrachloroauric(III) acid (99.999%) were purchased from Sigma-Aldrich. Polyhydroxybutyrate (PHB, Biomer® P209) was purchased from Biomer (Krailing, Germany). For pH adjustment NaOH 0.1 M (LACH-NER, s.r.o., Czech Republic) was applied. During the extraction of tarragon, C_2H_5OH (VWR BDH Prolabo Chemicals, Belgium) was used. The tarragon (*Artemisia dracunculus*) extract was prepared from dried herbs (Kawon, Poland). To perform catalyses sodium hydroxide (LACH-NER, s.r.o., Czech Republic), sodium borohydride (99%, Sigma-Aldrich, Czech Republic) and 4-nitrophenol (99%, Sigma-Aldrich, Czech Republic) was used.

2.2. Synthesis of spherical gold nanoparticles

In order to obtain homogeneous solution of spherical gold nanoparticles (without morphological dysfunctions) it was necessary to create appropriate synthesis conditions i.e. time of the reaction, dose of the gold precursor as well as the reducing agent. After this preliminary studies the synthesis conditions were selected as follows.

2.2.1. Synthesis of spherical gold nanoparticles with PE

The synthesis of nAu with PE was carried out in 5 mL of 1 mM $HAuCl_4$ aqueous solution. The solution was hearted to 80 °C and then 120 µL of 0.1 M NaOH aqueous solution was added in order to obtain pH of 5. The reduction of gold particles was obtained by slowly pipetting 5 mL of 3% tarragon plant extract (20 g/L in 30% of ethanol solution). The reaction was stopped after 15 minutes and the final concentration of gold was 0.5 mM.

2.2.2. Synthesis of spherical gold nanoparticles with PHB-chit

To synthesize gold nanoparticles 0.5 mM of $HAuCl_4$ and 1g/L of PHB-chit was used. The solution was put in a closed glass tube and placed in the microwave (Royal-Lux MW 750) for 30 second at 800 W power, thereafter the reaction was stopped placing the glass tube in ice.

2.3. Analytical

Synthesized nAu were taken from the reaction mixture and subsequent analytical procedures were undertaken. Vis spectra's were measured within the wavelength range of 400-900 nm by an ultraviolet-visible LAMBDA™ 35 UV-Vis spectrophotometer (PerkinElmer, USA) with matched 1 cm quartz cells (cuvettes).

The composition and morphology of the synthesized nAu were studied using a scanning electron microscope (SEM) with a beam current between 12 nA and 40 nA and acceleration voltage of 10 kV and with a complete detection system comprising In-lens energy and angle selective backscatter detector (EsB); 4-quadrant solid-state backscattered detector (AsB) and conventional secondary electron detector (Everhardt-Thornley, ZEISS, Ultra/Plus, Germany).

The zeta potential values of nAu were determined in freshly prepared suspension by Zetasizer ZS (Malvern Instruments Ltd, UK). Measurements were performed (ten runs each) with autocorrelation functions of 10 seconds. Each result was the average of triplicate measurements.

The differential centrifugal sedimentation technique (CPS Disc Centrifuge, DC24000UHR, CPS Instruments Inc., USA) was used for particle size distribution analysis. Measurements were made at a disc rotation speed of 24000 RPM. The particle sedimentation was carried out in an 8−24% (w/w) sucrose density gradient. Prior to each sample measurement, the instrument was calibrated using a PVC nanosphere standard (238 nm).

3. RESULTS AND DISCUSSION

3.1. Vis spectrophotometric analysis

In both cases the yellow color of gold precursor ($HAuCl_4$) and reducing agent mixture solution has changed to red wine color [14] (indicating the formation of nAu (**Figure 1**).

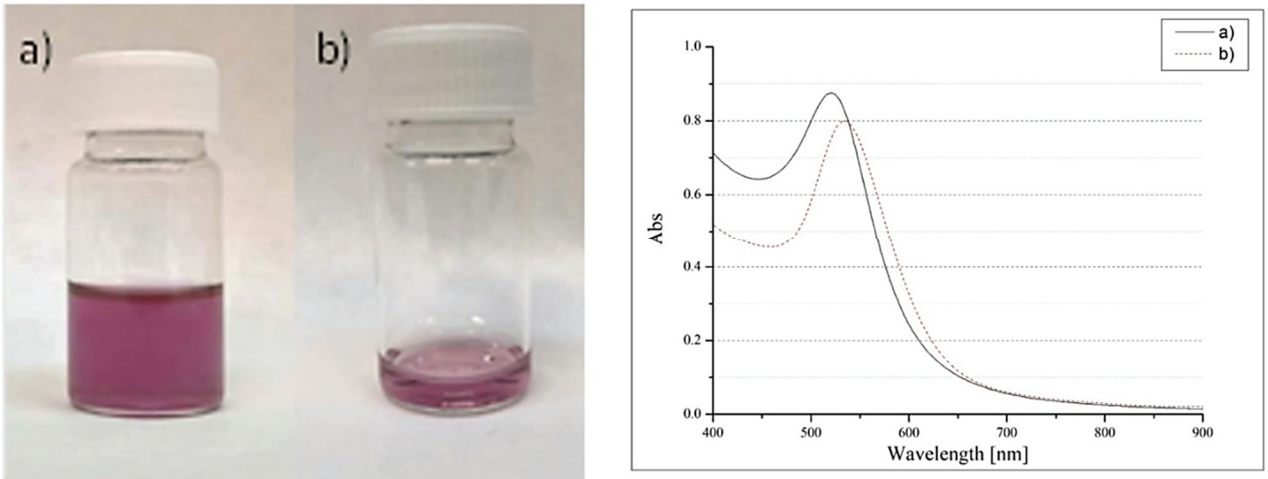

Figure 1 On the left side: Digital images of nAu synthesized with: a) PHB-chit and b) PE. On the right side: Vis spectrum of nAu synthesized with: a) PHB-chit and b) PE

The nAu (nanoparticles of gold) synthesized by PHB-chitosan conjugate (PHB-chit) and tarragon plant extract (PE) were characterized by visible spectroscopy (from 400 nm to 900 nm; **Figure 1**). The surface plasmon (SRP) resonance of nAu synthetized by a polymer conjugate is located at 520 nm and synthetized by plant extract at 530 nm. From the SRP situated in this area of visible spectrum, one can conclude that the gold particle size varies between 4 to 40 nm which was confirmed by further analysis. The peak of nAu synthesized with PE is located at longer wavelength, indicating in average larger particle size [2]. The analysis shows that nAu synthetized by PE has lager particle size in comparison to the particles synthetized by PHB-chit. Future analysis such as scanning electron microscope (SEM) and differential centrifugal sedimentation (DCS) confirmed this hypothesis.

3.2. DCS (differential centrifugal sedimentation) analyses

The differential centrifugal sedimentation technique (DCS) was used for an accurate determination of the particle size distribution of nAu synthesized by PHB-chit and PE (**Figure. 2**). The median size distribution of nAu synthesized by PHB-chit was found to be ~7 nm. As for the nAu synthesized by tarragon plant extract the median particle size distribution was determined to be approximately 30 nm. The **Figure 2** shows a large difference between the size distributions of nAu synthesized with PHB-chit conjugate and the nanoparticles

synthesized with PE, the visible difference is not only in the median size distribution of these particles but also in the narrowness of their distribution.

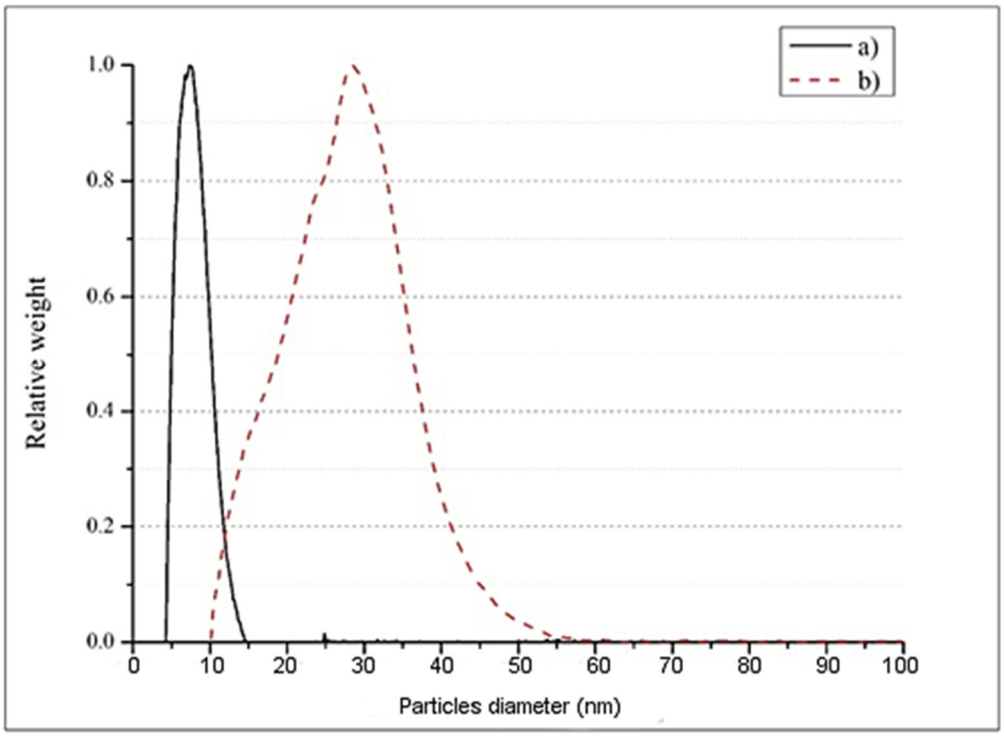

Figure 2 The particle size distributions of nAu synthesized by: a) PHB-chit and b) PE

3.3. SEM analysis

In **Figure 3** the SEM pictures of gold nanoparticles synthesized with PHB-chit conjugate a) and nAu synthesized with PE b) are being shown.

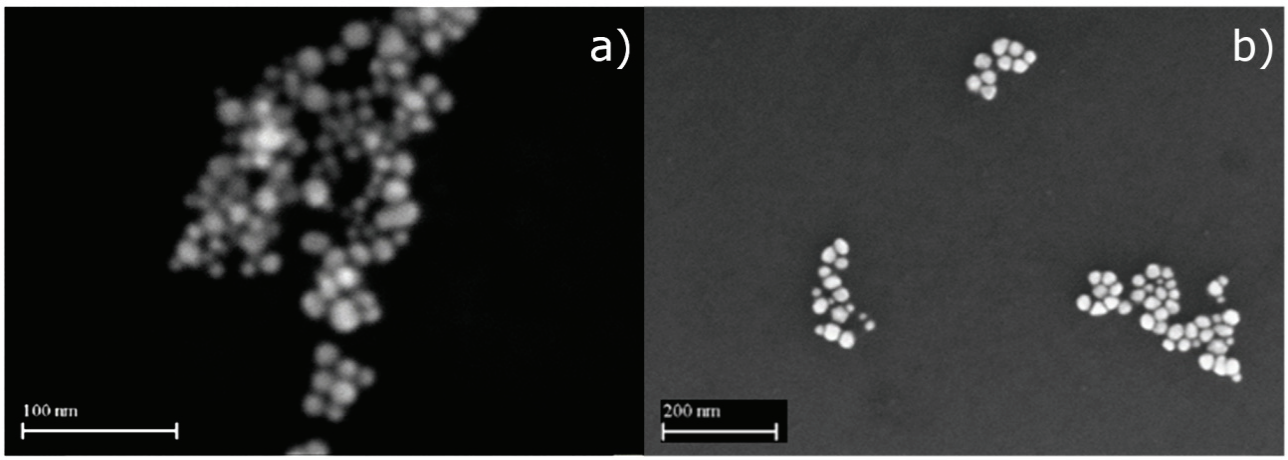

Figure 3 Scanning electron microscope morphological analysis of synthesized nAu by: a) PHB-chit and b) PE

The observable particle size and morphology were in agreement with the analyses obtained by the DCS and Vis spectrophotometry. As it can be seen, the spherical nAu synthesized with the PHB-chit, are considerably smaller in comparison to the nAu synthesized with PE. Some of the particles synthesized by the PHB-chit

conjugate seem to have significantly smaller diameter than 10 nm (**Figure 3a**) in contrary to the larger particles synthesized by a plant extract (**Figure 3b**).

3.4. Zeta potential analysis and catalytic reduction of 4-NP

The zeta potential value for nAu synthesized by PHB-chit was about -27 ± 0.1 mV and by PE was around -37 ± 1 mV (**Table 1**). The zeta potential value of a colloidal solution can be an indicator of its stability. The greater the value of zeta potential the higher is the repulsion between the nanoparticles [15]. This means that nAu synthesized by PE can be considered as more stable in comparison to the nAu synthesized by PHB-chit.

Furthermore, the reduction of 4-nitrophenol (4-NP) by sodium borohydride ($NaBH_4$) to 4-aminophenol (4-AP) was used as a model system in order to evaluate the catalytic activity of spherical nAu synthesized by two different agents: PHB-chit and PE (**Table 1**).

Table 1 The zeta potential values and pseudo first-order kinetic rate constant of 4-NP reduction by nAu synthesized by PHB-chit and PE

nAu type	Size of the particles (nm)	Zeta potential (mV)	k_{app} (min^{-1})
PHB-chit	7	-27.1 ± 0.1	0.74
PE	30	-37 ± 1	0.29

The reduction of 4-NP to 4-AP could be assumed as a pseudo first-order owing the presence of $NaBH_4$ in excess. It has been found that the pseudo first-order kinetic rate constant was 0.74 and 0.29 min^{-1} for the processes involving au NPs synthesized by PHB-chitosan polymer conjugate and tarragon plant extract, respectively. It can be assumed that greater catalytic performance of nAu synthesized by PHB-chitosan can be due to their smaller size (and therefore larger specific surface area) as well as narrower size distribution.

4. CONCLUSIONS

Particles of nAu were successfully synthetized by two different eco-friendly reducing and stabilizing agents such as PHB-chit and PE. The size of nAu synthesized by PHB-chit and PE was measured by centrifugal particle sedimentation technique and was found to be 7 nm and approximately 30 nm, respectively. This observation was further confirmed by UV-Vis spectrophotometry and scanning electron microscope (SEM) analyses. Zeta potential analysis revealed that nAu synthesized by PE could be more stable in comparison to the nAu synthesized by PHB-chit (their zeta potential value was found to be -37 mV and -27 mV, respectively). It was further determined that the pseudo first-order kinetic rate constant of 4-nitrophenol reduction was 0.74 and 0.29 min^{-1} involving Au NPs synthesized by PHB-chitosan polymer conjugate and tarragon plant extract, respectively.

ACKNOWLEDGEMENTS

The research presented in this article was supported by the Ministry of Education, Youth and Sports in the framework of the projects the National Programme for Sustainability I (LO1201), Research Infrastructure NanoEnviCz (LM2015073) and the OPR&DI project "Institute for Nanomaterials, Advanced Technologies and Innovation", CZ.1.05/2.1.00/01.0005.

REFERENCES

[1] WU X., LU C., ZHOU Z., YUAN G., XIONG R., ZHANG X. Green synthesis and formation mechanism of cellulose nanocrystal-supported gold nanoparticles with enhanced catalytic performance. Environmental Science Nano, 2014, vol.1, pp.71-79.

[2] ELIA P., ZACH R., HAZAN S., KOLUSHEVA S., PORAT Z., ZEIRI Y. Green synthesis of gold nanoparticles using plant extracts as reducing agents. International Journal of Nanomedicine, 2014, vol. 9, no.1, pp.4007-4021.

[3] RAMAKRISHNA M., RAJESH BABU D., GENGAN R.M., CHANDRA S., NAGESWARA RAO G. Green synthesis of gold nanoparticles using marine algae and evaluation of their catalytic activity. Journal of Nanostructure in Chemistry, 2016, vol. 6, no.1, pp. 1-13.

[4] HE S., GUO Z., ZHANG Y., ZHANG S., WANG J., GU N. Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Materials Letters, 2007, vol. 61, no.18, pp.3984-3987.

[5] KITCHING M., RAMANI M., MARSILI E. Fungal biosynthesis of gold nanoparticles: mechanism and scale up. Microbial Biotechnol. 2015, vol.8, no.6 pp.904-917.

[6] PADIL V.V.T., WACŁAWEK S., ČERNÍK M. Green Synthesis: Nanoparticles and Nanofibres Based on Tree Gums for Environmental Applications. Ecological Chemistry and Engineering S. 2016, vol.23, no.4, pp.533-557.

[7] SAHU N., SONI D., CHANDRASHEKHAR B., SATPUTE D.B., SARAVANADEVI S., SARANGI B.K., PANDEY R.A. Synthesis of silver nanoparticles using flavonoids: hesperidin, naringin and diosmin, and their antibacterial effects and cytotoxicity. International Nano Letters. 2016, vol. 6, no.3, pp.173-181.

[8] SHANKAR S.S., AHMAD A., PASRICHA R., SASTRY M., EL-SAYED M.A., ZHENG J.G., SASTRY M., AJAYAKUMAR P. V., ALAM M., KUMAR R., SASTRY M., KUMAR R. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. Journal of Materials Chemistry. 2003, vol.13, no.7 pp.1822-1826.

[9] HUANG H., YANG X. Synthesis of Chitosan-Stabilized Gold Nanoparticles in the Absence/Presence of Tripolyphosphate. Biomacromolecules. 2004, vol. 5, no.6, pp.2340-2346.

[10] HUANG R., REUSCH R.N. Poly(3-hydroxybutyrate) is associated with specific proteins in the cytoplasm and membranes of Escherichia coli. Journal of Biological Chemistry. 1996, vol.271, no.36, pp.22196-202.

[11] WACŁAWEK S., CHRONOPOULOU L., PETRANGELI PAPINI M., PADIL V.V.T., PALOCCI C., KUPČÍK J., ČERNÍK M. Enhancement of stability and reactivity of nanosized zero-valent iron with polyhydroxybutyrate. Desalination and Water Treatment. 2017, vol.69, pp.302-307

[12] MA T., YANG W., LIU S., ZHANG H., LIANG F. A Comparison Reduction of 4-Nitrophenol by Gold Nanospheres and Gold Nanostars. Catalysts. 2017, vol.7, no.2, pp.1-10.

[13] ZHAO P., FENG X., HUANG D., YANG G., ASTRUC D. Basic concepts and recent advances in nitrophenol reduction by gold- and other transition metal nanoparticles. Coordination Chemistry Reviews. 2015, vol.287 pp.114-136.

[14] MERZA K.S., AL-ATTABI H.D., ABBAS Z.M., YUSR H.A. Comparative Study on Methods for Preparation of Gold Nanoparticles. Green and Sustainable Chemistry. 2012, vol.2, no.1, pp.26-28.

[15] VIJAYAKUMAR S. In vitro stability studies on gold nanoparticles with different stabilizing agents. International Journal of Current Scientific Research. 2014, vol.11, pp.84-93.