GREEN SYNTHESIS OF GOLD NANOPARTICLES USING PEPPERMINT EXTRACT FOR POTENTIAL PLASMONIC PHOTOTHERMAL THERAPY

[1]Romana SMOLKOVÁ, [1]Ruslan MARIYCHUK, [1]Sergej IĽKOVIČ, [2]Liudmyla M. GRISHCHENKO, [2]Vladyslav V. LISNYAK

[1]University of Presov, Faculty of Humanities and Natural Sciences, Presov, Slovakia, EU,
romana.smolkova@unipo.sk

[2]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

https://doi.org/10.37904/nanocon.2023.4805

Abstract

In the search for a suitable and biocompatible material with high photothermal efficacy for hyperthermic application in nanomedicine, gold (Au) nanocolloid solutions with a surface plasmon resonance located in the near-infrared spectral region were prepared using a one-step environmentally friendly phytosynthetic process. The room-temperature process involves a liquid extract of *Mentha* × *piperita* leaves and Au(III) aqueous solutions, and no additional reagents or surfactants are required. The one-step green process yields Au nanocolloid solutions that are stable for weeks. UV-Vis spectroscopy measurements of the obtained Au nanocolloid solutions show that the position of the near-infrared surface plasmon resonance maximum can be controlled by the synthetic conditions in the range of 720-1100 nm. Transition electron microscopy shows that irregularly shaped Au nanoparticles (Au-NPs), in the form of triangles and hexagons, are phytosynthesized together with regular (spherical) Au-NPs in varying ratios. These ratios depend on the composition of the mixture used and the phytosynthesis conditions. When plasmonic Au nanocolloid solutions are exposed to laser light at wavelengths of 808, 850 and 980 nm, they produce a reliable and consistent photothermal response without any photoinduced decay. The optical properties and potential biocompatibility of the gold nanocolloids make them promising for hyperthermia or bioimaging.

Keywords: Peppermint, gold nanoparticles, green synthesis, surface plasmon resonance, photothermia

1. INTRODUCTION

Nanoparticles (NPs) have attracted considerable attention due to their enhanced optical, electrical, magnetic, and catalytic properties that exceed those of bulk materials. The widespread use of NPs in various fields underscores the importance of their characterization and regulation to prevent potential harm to human health and the environment. Currently, various NPs are widely used in electronics, photonics, catalysis, and medicine. The size, shape, structure, and composition, as well as the refractive index in the surrounding medium, are the keys to the unique optical properties of gold nanostructures [1]. Typically, Au-NPs have a surface plasmon resonance (SPR) that can be tuned by the size and shape of the Au-NPs. This property makes them valuable in biomedicine, particularly for plasmonic photothermal therapy (PTT) [2]. Currently, researchers are investigating the potential use of NPs with photothermal (PT) activity in the treatment of cancer and other diseases. Typically, tumors are more sensitive to heat than normal tissue due to the reduced blood supply. Targeted heating of a specific area can be achieved by accumulating PT agents within the tissue and exposing them to a laser beam. This PTT method prevents damage to the surrounding healthy tissue [3]. However, PT Au-NPs must absorb light within one of the two optical windows in the near-infrared range of 650-850 nm or 950-1350 nm in order for laser light to safely pass through the healthy tissue to reach the Au-NPs localized within tumor tissue. Spherical Au-NPs have a peak maximum in their surface plasmon resonance (SPR) at

about 530 nm, which is below the range of medical laser devices operating in the first optical window. However, when spherical Au-NPs are reshaped, the SPR peak position is significantly shifted to the near-infrared region [3,4]. Although there are several methods to prepare non-spherical gold nanoparticles, including nanorods, nanoshells, and nanostars, some of them require harmful chemicals or even hazardous solvents to reduce Au(III) and stabilize Au-NPs [4,5]. The preparation of biocompatible Au-NPs using plant extracts through green synthesis is a safer alternative to chemical processes.

Phytosynthesis of gold nanoparticles is an easy, fast, and widely accepted technique that avoids hazardous substances that may interfere with medical applications [6-8]. The addition of herbs such as peppermint, which have natural antioxidant, antimicrobial, fungicidal, and antiviral properties, can increase the efficiency of the resulting nanoparticle solutions. The composition of aqueous peppermint extract is very different from that of an essential oil and depends mainly on the polarity of the dissolved compounds. The essential oil of peppermint contains menthol, menthone, menthofuran, and 1,8-cineol as the main components, while the aqueous extract consists of a number of phenolic compounds, including luteolin-O-rutinoside, eriocitrin, rosmarinic acid, and jasmonic acid [9,10]. The phenols in the plant extract have high antioxidant activity and can reduce $HAuCl_4$, which is used as an Au(III) precursor. In addition, the phenolic compounds can stabilize the resulting Au-NPs without the need for additional chemical stabilizers [6,7].

Based on our previous research on the preparation of metal nanoparticles by phytosynthesis, herein we present the rapid and environmentally friendly (green) synthesis of PT Au-NPs using *Mentha* × *piperita* extract. We evaluated the shape and light-related properties of the prepared Au-NPs by means of transition electron microscopy (TEM) and UV-Vis spectroscopy. Moreover, we investigated the photothermal properties of the prepared Au-NPs for their potential use as PT agents.

2. MATERIALS AND METHODS

Peppermint plants (cv. *Piperita*) were obtained from the experimental field of the University of Presov in Slovakia and dried at room temperature. To prepare the extract, 10 g of dry leaves were ground and placed in a boiling flask. Then 100 ml of distilled water was added, and the mixture was boiled for 1 hour. After filtering, the remaining plant fragments were removed from the filtrate by centrifugation at 3,000 rpm for 30 min. The Au-NPs were synthesized by adding varying amounts of an aqueous solution containing 1 mM $HAuCl_4$ to the water-diluted plant extract to obtain final Au concentrations ranging from 0.375 to 0.625 mM. The final solutions contain 0.25 mg/mL of dry organic matter. The synthesis was performed at room temperature in the presence of air. Absorption spectra of the obtained Au nanocolloids were recorded in the range of 300-1100 nm using a Shimadzu UV 1800 spectrophotometer. The stability of Au nanocolloids up to three weeks was monitored by the UV-Vis measurements. A JEOL JEM-2100F TEM instrument was used to obtain TEM images. To observe the PT effect, Au nanocolloids (3 mL) in a PT quartz cuvette (10×10×40 mm) were exposed to lasers with λ_{ex} of 808 nm (0.5 W), 850 nm (1 W), and 980 nm (0.5 W) (provided by Laserland and Jolooyo, China), and the temperature changes were measured using an infrared thermometer (MLX90614, Melexis, Belgium) [10]. To follow the stability of the nanocolloid solutions, three heating-cooling cycles were applied to all samples, namely 10 min of heating and 40 min of cooling.

3. RESULTS AND DISCUSSION

The different peppermint extracts have been used to synthesize Au-NPs reported in the literature; however, the methods used to prepare the extracts do not significantly affect the composition of the aqueous extract. The major components of the extract identified by the previous studies are phenolic compounds, including luteolin-O-rutinoside, eriocitrin, rosmarinic acid, and other flavonoids, flavone, and flavanone glycosides. These components are responsible for its antioxidant activity and also act as reducing agents of $HAuCl_4$ during redox reactions [8-10].

The rapid production of Au-NPs was achieved using aqueous *Mentha* × *piperita* extract. The color of the solution changed from light yellow to purple-red, indicating the formation of stable Au-NPs. The reaction was completed in 60 min, yielding Au nanocolloids, and we followed their stability for three weeks using absorption spectroscopy without any noticeable changes in their spectra. The concentration of $HAuCl_4$ was adjusted between 0.375 and 0.625 mM. Lower concentrations of Au(III) produced only spherical Au-NPs, while higher concentrations of Au(III) stimulated the formation of large metal particles of less than 1 μm [8]. For the preparation of non-spherical Au-NPs colloids with their highest ratio relative to spherical Au-NPs, the optimal extract should be based on the dry matter content of 0.25 mg/mL.

The absorption spectra of the Au nanocolloids showed a distinct relatively narrow SPR peak at 530 nm. This feature was due to the formation of spherical Au-NPs in addition to pseudo-spherical Au-NPs. **Figure 1** (left panel) shows that a second broad SPR maximum (λ_{max}) related to non-spherical Au-NPs is registered in the range 724-972 nm. Increasing the concentration of Au(III) caused a bathochromic shift and broadening of the second SPR maxima. As expected, the absorption maxima of both SPR peaks increased with higher Au(III) concentrations. **Figure 1** (right panel) shows the comparison of the absorbance dependence on Au(III) concentration in the respective Au nanocolloids at the wavelength of the applied lasers.

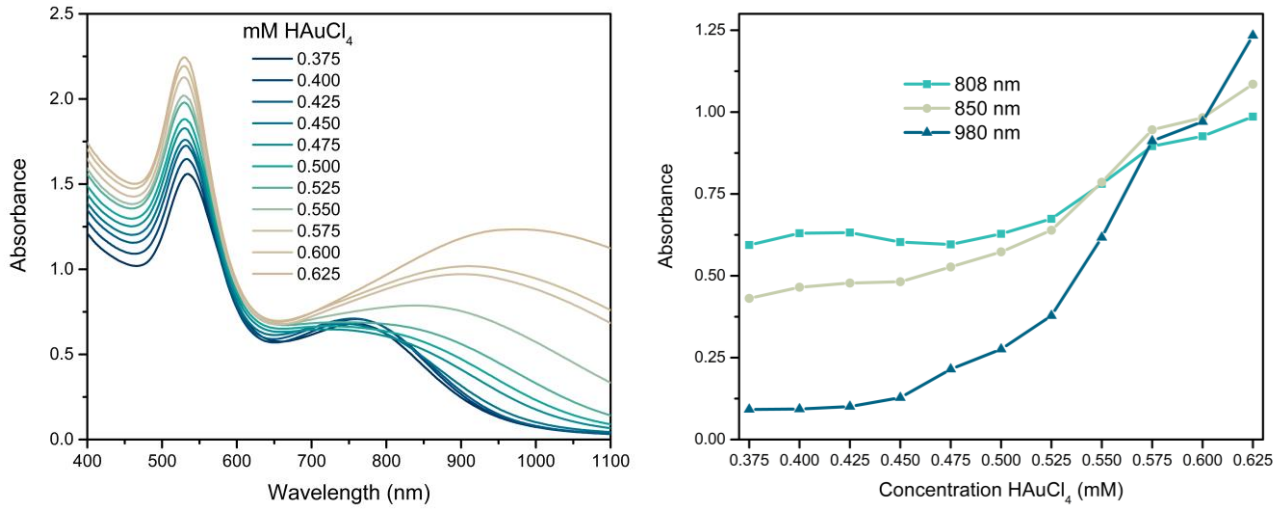

Figure 1 UV-Vis spectra of the prepared Au nanocolloids (left panel). The absorbance of the respective Au-NPs recorded at the wavelength of applied lasers (right panel)

The UV/Vis and NIR absorption spectra, daylight images, and TEM images of Au-NPs in water (**Figure 2**) confirmed that Au(III) can undergo phytochemical reduction to metallic Au(0) in the absence of an external reducing agent. The TEM images show the *in situ* formation of both visible-absorbing spherical Au-NPs and NIR-absorbing anisotropic Au-NPs within Au nanocolloids (**Figure 2**). Flat, thin nanotriangles and nanohexagons are visible along with a larger number of Au nanospheres and pseudospheres. The size of the Au spheres and pseudospheres is less than 20 nm, while the triangular and hexagonal Au prisms vary in size. The presence of different Au nanostructures is consistent with the two SPR maxima observed in the absorption spectra of Au nanocolloids. Presumably, phenolic compounds reduce Au(III) chloride to Au(I) chloride at room temperature, resulting in the formation of Au-NPs. Here, the possible transformation of Au nuclei into seed structures with stacking defects, which act as seeds and cause the growth of triangular prisms. The presence of surfactants and higher amounts of extract tends to direct the growth along the (111) plane, stimulating the formation of Au nanoprisms. In addition, the phytochemicals can bind with the growing Au-NPs, which restricts the growth along the high-energy crystal planes (200), (220), and (311), resulting in an important feature of the (111) plane growth as reported previously [7,12].

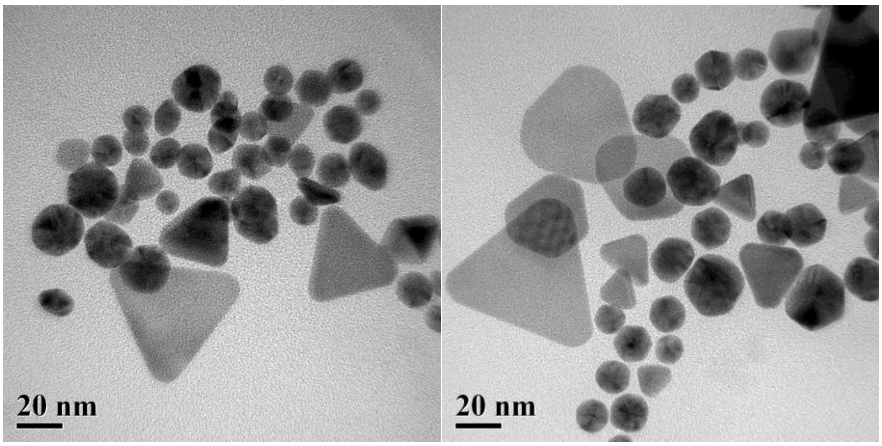

Figure 2 TEM images of the prepared Au-NPs

Since the Au nanocolloid solutions showed the second SPR maxima in either the first or second NIR optical window for potential PTT treatment, the respective nanocolloids could be tested by irradiation with lasers of different wavelengths within the above-mentioned bio-optical windows. For these experiments, we selected samples with the highest absorbance at the wavelength of the respective lasers (0.550-0.625 mM Au concentration). The samples showed a reliable and consistent photothermal response without any photoinduced decay (**Figure 3**).

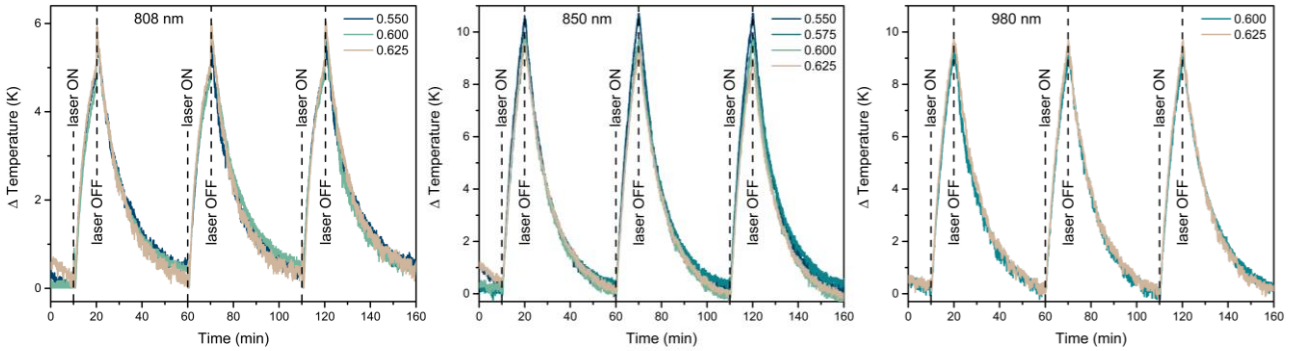

Figure 3 Temperature changes observed upon 808, 850, or 980 nm laser irradiation of the Au nanocolloids with different NIR SPR maxima

The laser-induced hyperthermic effect (ΔT) is summarized in **Table 1**. The highest ΔT was calculated for the Au nanocolloid with *c*(Au) = 0.550 mM, when irradiated with a 1 W laser of 850 nm. The synthesized Au nanocolloids show higher ΔT in comparison with previously reported spherical Au-NPs of comparable size [13].

Table 1 Absorption maxima (λ_{max}) and temperature changes (ΔT) recorded for selected Au nanocolloids.

Sample *c*(Au) mM	λ_{max} (nm)	ΔT (K) 808 nm	ΔT (K) 850 nm	ΔT (K) 980 nm
0.550	830	5.74 ± 0.12	10.68 ± 0.04	-
0.575	893	-	9.79 ± 0.06	-
0.600	905	5.84 ± 0.12	9.74 ± 0.08	9.28 ± 0.16
0.625	972	5.98 ± 0.05	9.34 ±0.24	9.74 ± 0.06

Near-infrared light within the 700-1500 nm spectral range penetrates tissue more efficiently than visible light due to the reduced absorption and scattering, although wavelengths above 950 nm are less efficient because

of the increased absorption by water and lipids [14,15]. Our Au-NPs exhibit higher hyperthermic effect within the more favorable first bio-optical window with the highest ΔT = 10.68 K upon irradiation with 850 nm laser.

4. CONCLUSION

We have prepared potentially biocompatible Au nanocolloid solutions using an environmentally friendly single-step phytosynthetic method. This was achieved by using an aqueous extract of *Mentha x piperita*, which can mediate the synthesis of colloid solutions of Au-NPs. The size, shape and distribution of gold nanoparticles can be tuned by the conditions of the synthesis, while simultaneously preserving significant control over their optical properties. These Au nanocolloid solutions remained stable at room temperature for several weeks. The resulting Au-NPs exhibit surface plasmon resonance within the near-infrared spectral range.

Furthermore, prepared Au nanocolloid solutions exhibited reliable and consistent photothermal response without photodegradation when exposed to lasers at 808 nm, 850 nm, and 980 nm. Upon irradiation with a 0.5 W 980 nm laser, the resulting temperature change (ΔT) is comparable to that generated by a 1.0 W 850 nm laser. Au nanocolloids represent a promising option for bioimaging or hyperthermic applications due to their advantageous optical properties and potential for biocompatibility.

ACKNOWLEDGEMENTS

This work was supported by the Scientific Grant Agency of the Ministry of Education, science, research and sport of the Slovak Republic and the Slovak Academy of Sciences, No. 1/0882/21.

REFERENCES

[1] SUNEEV, A.B.; VANISH, K.; JAVAD, K.; AMRINDER, P.S.; SURESH, K. Role of gold nanoparticles in advanced biomedical applications. *Nanoscale Advances*, 2020, vol. 2, pp. 3764-3787. Available from: https://doi.org/10.1039/D0NA00472C

[2] ABADEER, N.S.; MURPHY, C.J. Recent Progress in Cancer Thermal Therapy Using Gold Nanoparticles. *Journal of Physical Chemistry*, 2016, vol. 120, pp. 4691-4716. Available from: https://doi.org/10.1021/acs.jpcc.5b11232h

[3] HUANG, X.; JAIN, P. K.; EL-SAYED, I. H.; EL-SAYED, M. A. Plasmonic Photothermal Therapy (PPTT) Using Gold Nanoparticles. *Lasers in Medical Science*. 2007, vol. 23, pp. 217-228. Available from: https://doi.org/10.1007/s10103-007-0470-x

[4] VINES, J. B.; YOON, J.-H.; RYU, N.-E.; LIM, D.-J.; PARK, H. Gold Nanoparticles for Photothermal Cancer Therapy. *Frontiers in Chemistry*. 2019, vol. 7, pp.1-16. Available from: https://doi.org/10.3389/fchem.2019.00167

[5] RILEY, R. S.; DAY, E. S. Gold Nanoparticle-mediated Photothermal Therapy: Applications and Opportunities for Multimodal Cancer Treatment. *WIREs Nanomedicine and Nanobiotechnology*. 2017, vol. 9, pp.1-16. Available from: https://doi.org/10.1002/wnan.1449

[6] VALENCIA, G. A.; VERCIK, L. C. D. O.; GAVIAO FERREIRA, L.; LLANOS, J. H. R.; VERCIK, A. Synthesis and Characterisation of Gold Nanoparticles Using *Mentha Piperita* Leaf Extract: A Green, Non-Toxic and Rapid Method. *International Journal of Nano and Biomaterials*. 2014, vol. 5, pp. 181-192. Available from: https://doi.org/10.1504/ijnbm.2014.066905

[7] MARIYCHUK, R.; GRULOVA, D.; GRISHCHENKO, L.M.; LINNIK, R.P., LISNYAK, V.V. Green synthesis of nonspherical gold nanoparticles using *Solidago canadensis* L. extract. *Applied Nanoscience*. 2020, vol. 10, no. 12, pp. 4817-4826. Available from: https://doi.org/10.1007/s13204-020-01406-x

[8] MARIYCHUK, R.; SMOLKOVÁ, R.; BARTOŠOVÁ, V.; ELIAŠOVÁ, A.; GRISHCHENKO, L. M.; DIYUK, V. E.; LISNYAK, V. V. The Regularities of the *Mentha Piperita* L. Extract Mediated Synthesis of Gold Nanoparticles with a Response in the Infrared Range. *Applied Nanoscience*. 2021, vol. 12, pp. 1071-1083. Available from: https://doi.org/10.1007/s13204-021-01740-8

[9] KAPP, K., HAKALA, E., ORAV, A., POHJALA, L., VUORELA, P., PÜSSA, T., VUORELA, H., RAAL, A. Commercial Peppermint (*Mentha×piperita* L.) Teas: Antichlamydial Effect and Polyphenolic Composition. *Food Research International*. 2013, vol. 53, pp. 758-766. Available from: https://doi.org/10.1016/j.foodres.2013.02.015

[10] DORMAN, H. J. D., KOŞAR, M., KAHLOS, K., HOLM, Y., HILTUNEN, R. Antioxidant Properties and Composition of Aqueous Extracts from *Mentha* Species, Hybrids, Varieties, and Cultivars. *Journal of Agricultural and Food Chemistry*. 2003, vol. 51, pp. 4563-4569. Available from: https://doi.org/10.1021/jf034108k

[11] PAŚCIAK A., PILCH-WRÓBEL A., MARCINIAK Ł., SCHUCK P.J., BEDNARKIEWICZ A. Standardization of Methodology of Light-to-Heat Conversion Efficiency Determination for Colloidal Nanoheaters. *ACS Applied Materials and Interfaces*. 2021, vol. 13, pp. 44556 - 44567. Available from: https://doi.org/10.1021/acsami.1c12409

[12] MARIYCHUK, R., FEJER, J., PORUBSKA, J., GRISHCHENKO, L.M., LISNYAK, V.V. Green synthesis and characterization of gold triangular nanoprisms using extract of *Juniperus communis* L. *Applied Nanoscience*. 2020, vol. 10, no 8, pp. 2835-2841. Available from: https://doi.org/10.1007/s13204-019-00990-x

[13] MOUSTAOUI, H., SABER, J., DJEDDI, I., LIU, Q., DIALLO, A.T., SPADAVECCHIA J., DE LA CHAPELLE M.L., DJAKER, N. Shape and Size Effect on Photothermal Heat Elevation of Gold Nanoparticles: Absorption Coefficient Experimental Measurement of Spherical and Urchin-Shaped Gold Nanoparticles. *The Journal of Physical Chemistry C*. 2019, vol. 123, no. 28, pp. 17548-17554. Available from: https://doi.org/10.1021/acs.jpcc.9b03122

[14] SMITH, A.M., MANCINI, M.C., NIE, S. Bioimaging: Second window for in vivo imaging. *Nature Nanotechnology*. 2009, vol. 4, no. 11, pp. 710-711. Available from: https://doi.org/10.1038/nnano.2009.326

[15] SANOJ REJINOLD, N., CHOI, G., CHOY, J.-H. Recent trends in nano photo-chemotherapy approaches and future scopes. *Coordination Chemistry Reviews*. 2020, vol. 411, 213252, pp. 1-23. Available from: https://doi.org/10.1016/j.ccr.2020.213252