SYNTHESIS OF IRREGULARLY SHAPED GOLD NANOPARTICLES USING NATURAL POLYPHENOLS

[1]Ruslan MARIYCHUK, [1]Romana SMOLKOVA, [2]Lyudmyla GRISCHENKO, [2]Vladyslav LISNYAK

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

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

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Abstract

The extensive studies in nanoscience in the last decades are dedicated to the development of new protocols for the preparation of nanoparticles (NPs) with controlled size and shape. The dimensions and steric parameters influence the reactivity, catalytic, electrical, and optical properties and define the application of NPs in various fields: catalysis, sensing, medicine, electronics, etc. The combination of the green synthesis approach in the preparation of noble metal NPs, which defines their biocompatibility, environmental, cost, and time efficiency in synthesis, together with the control of optical properties such as near-infrared (NIR) response through the regulation and control of both size and shape, opens new possibilities in nanomedicine to develop new effective drug delivery systems, to improve the selectivity and efficiency of optical photothermal therapy, etc. Protocols for the synthesis of gold NPs using phytochemical plant extracts are one of the most promising ways in the design of biocompatible gold NPs with different shapes.

In this study, we review the recent achievements in the synthesis of gold nanoparticles with different morphologies such as nanotriangles, nanohexagons, nanourchins, and nanoshells using natural polyphenols. We consider previously isolated compounds, such as gallic acid, and rosmarinic acid, as well as whole plant extracts of peppermint, goldenrod, juniper, lemon balm, etc. The results of NIR spectroscopy and transmission electron microscopy studies are discussed. We show the possibility of synthesizing gold NPs with a size below 60 nm with a response in the NIR region by influencing the shape of gold NPs.

Keywords: Plasmonic nanoparticles, green synthesis, phytosynthesis, biocompatible, near infrared range

1. INTRODUCTION

Nanoparticles (NPs) are of significant interest as they serve as crucial building blocks for nanomaterials. Their distinct properties in optics, chemistry, magnetism, and catalysis greatly differ from those of bulk materials, making them well-suited for novel applications in electronics, technology, agriculture, food production, catalysis, pharmacy, and medicine. Extensive research has led to the development of various techniques for synthesizing NPs, categorized as "top-down" and "bottom-up" methods [1]. The former entails breaking down bulk materials into smaller fragments, with drawbacks being inadequate control over size and shape morphology, and dependence on expensive, complicated equipment. Therefore, bottom-up methods that assemble the smallest parts, atoms, molecules, and ions into NPs have garnered the most attention in recent years owing to their time and cost efficiency, non-toxic nature, and ability to offer complete control over the physical and chemical properties of NPs. These properties are contingent on the size, shape, composition, and crystallinity of NPs. The tendency for nanoparticles to aggregate and agglomerate in a solution affects the synthesis protocols and the selection of stabilizing agents. Recent research has focused on eco-friendly synthesis methods for nanoparticles, which utilize non-toxic reducing and stabilizing agents [2, 3]. These methods have been extensively studied and are proving to be promising alternatives. The special group of sustainable techniques encompasses biological methods utilizing whole organisms, such as plants, mushrooms, seaweeds, and microbial cells, as well as chemicals derived from biological sources, including cell-free extracts from plants, microorganisms, macrofungi, and macroalgae [4,5].

Phytochemicals, such as polysaccharides, membrane proteins, peptides, polyols, polyphenols, and carotenoids extracted from various plant sources such as vegetables, fruits, seeds, grains, or leaves, are commonly studied for their roles as reducing and capping agents in the synthesis of nanoparticles [6-8]. The group of polyphenols is particularly recognized and has demonstrated potential beneficial effects on multiple diseases. Natural polyphenols have reducing properties, making them ideal for the synthesis of nanoparticles, especially for therapeutic purposes. Several studies have investigated the green synthesis of nanoparticles utilizing polyphenols [9]. The various structures of polyphenols, such as flavonoids, phenolic acids, stilbenes, or lignans, allow for the manipulation of optical properties by adjusting their shape. The size of spherical AuNPs affects the presence of surface plasmon resonance (SPR) absorbance maximum, which occurs in the visible range between 530–560 nm. Non-spherical nanoparticle shapes, such as nanotriangles, nanohexagons, nanourchins, and nanoshells, exhibit a response in the near-infrared (NIR) range [10,11]. This opens up new opportunities for biomedicine, as the NIR range encompasses two therapeutic windows [12].

In this study, we examine the potential of natural polyphenolic compounds to be used to produce near-infrared responsive, photo-stable Au NPs with irregular shapes for future applications.

2. EXPERIMENTAL

Materials. The reagents used in this study were purchased and used as is, and solutions were prepared using double distilled water. Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) was obtained from Thermo scientific (USA), gallic acid from Merk (Germany), and rosmarinic acid from Sigma-Aldrich (Germany). Prior to each experiment, the glassware and magnetic bars were cleaned using a freshly prepared aqua regia solution (a mixture of HCl and HNO_3 in a 1:3 ratio). Peppermint leaf and elderberry fruit extracts were prepared using previously published procedures [13] and [14], respectively.

Synthesis of the gold nanoparticles. Polyphenols were used as mediators to synthesize AuNPs by combining either gallic or rosmarinic acid with $HAuCl_4$ in an aqueous solution. The final concentration of the gallic or rosmarinic acid and the estimated Au concentrations were 0.5 mg/mL and 0.125 to 0.5 mM, respectively. Each solution was stirred at 400 rpm using a magnetic bar and was maintained at room temperature (23 °C). Plant extract-mediated syntheses were conducted according to previously published protocols utilizing peppermint [13] and elderberry [14] fruit extracts.

AuNPs characterization. The characterization of AuNPs was carried out through UV-Vis spectroscopy of Au nanocolloid solutions using a Shimadzu UV-1800 spectrophotometer (Japan) in a quartz cuvette with a 1 cm optical path in the range of 300−1000 nm. The morphology analysis of AuNPs was conducted via transmission electron microscopy (TEM) using a JEOL JEM-2100F instrument.

3. RUSULTS ANS DISSCUSSION

One of the most important benefits of green synthesis approaches for AuNPs in addition to low price and fast reaction time is biocompatibility, which comes from the suggestion that if no toxic compounds were used as reactants, then no toxic compounds will be present in the resulting nanocolloid solution. Another benefit of phytosynthesis, synthesis using plant extracts, consists of the wide availability of reactants. Therefore, it is no surprise that many researchers focused their research on the search for efficient plant extracts or their isolated components for reproducible protocols of the synthesis of stable and biocompatible AuNPs. Extracts of many different plants were examined. However, the utilization of plant extracts meets several problems. First, the plant extract, in general, is a complex composition of hundreds, if not thousands, of organic compounds with different chemical and physical properties with different reducing and capping properties. Another problem is a standardization of the synthesis protocols due to the difference in the composition of plant extracts depending on the year locality of the harvesting, ending with weather conditions in certain seasons.

The diverse arrangement of polar and nonpolar groups in natural polyphenol molecules make them excellent candidates for synthesis experiments aimed at controlling size and shape. The incorporation of specific molecules plays an integral role in the generation of non-spherical AuNPs with responsiveness in the NIR range. To ensure reliable synthesis of Au nanorods, cetyltrimethylammonium bromide (CTAB) is utilized [15]. To explore less toxic alternatives, experiments were conducted using different polyphenols. For example, gold nanoparticles with flat triangular and hexagonal shapes were formed through the use of salicylic acid [16]. Similar outcomes were observed with the use of juniper *Juniperus communis* L. [17] or *Solidago canadensis* [18]. Despite the response in the NIR range, the obtained colloidal solutions of Au nanoparticles were an inhomogeneous mixture of both spherical and non-spherical particles. The use of catechol as a reducing and capping agent produced the same outcome as previously observed [19]. Contrastly, the use of ascorbic acid led to enhanced response of AuNPs in NIR range [20]. Despite successes in green synthesis, the creation of nanorods depends upon the usage of a toxic stabilizing agent such as CTAB. Stable AuNPs were achieved through the use of natural eugenol, which was obtained from the essential oil *Syzygium aromaticum* L. Nonetheless, while the structure appeared promising, only spherical AuNPs were produced [21]. In this study, AuNPs samples were synthesized using a green approach, which involved the use of phytochemicals obtained from plants such as peppermint leaves extract, elderberry fruits extract, and isolated gallic and rosmarinic acid that were discovered in plant extracts. The synthesis with plant extracts followed previously established protocols for peppermint (*Mentha piperita* L.) [13] and elderberry (*Sambucus nigra* L.) [14].

For the synthesis with isolated polyphenolic acids, the concentration 0.5 mg/mL was used as a typical concentration of organic matter in the successful synthesis using plant extracts.

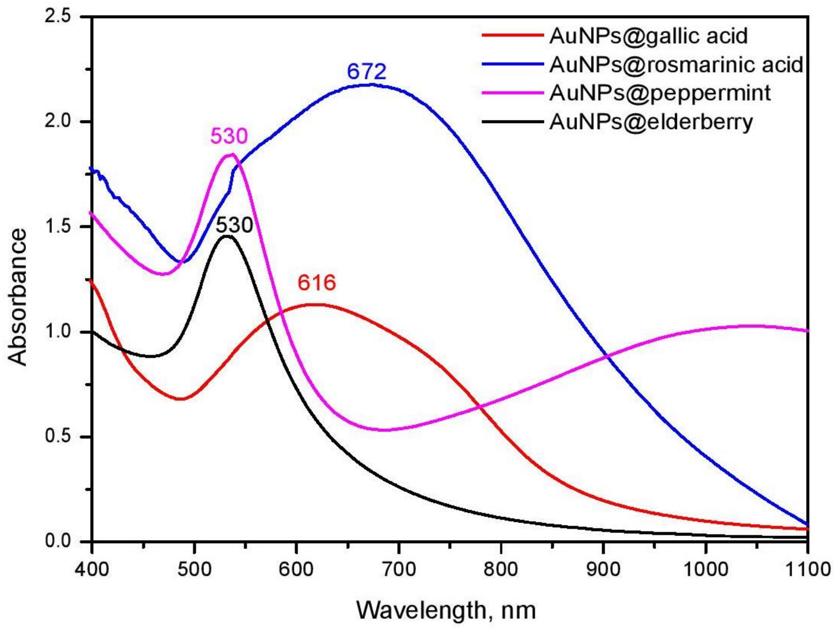

Figure 1 UV-Vis spectra of AuNPs aqueous nanocolloid solutions prepared using gallic acid, rosmarinic acid, an extract of peppermint's leaves and a polyphenolic fraction of elderberry fruits

Depending on the organic material used, AuNPs with different optical properties were obtained (**Figure 1**). For example, the use of a polyphenolic fraction (mainly anthocyanins) isolated from fruits led to the formation of nearly spherical AuNPs with maximum absorption at the SPR peak at 530 nm [13]. The anthocyanins presented in different extracts contain both polar and non-polar groups and show a temperature-dependent instability. Therefore, the formation of irregularly shaped AuNPs was expected. The formation of nearly spherical AuNPs can be explained by the effect of elevated temperature decomposing anthocyanins. Previous research has indicated the impact of reaction temperature on the shape of AuNPs [18].

The use of peppermint extract allows the preparation of a complex mixture. This is indicated by the presence of two SPR absorption maxima at 530 nm generated by spherical AuNPs and at 1038 nm generated by non-spherical (triangular and hexagonal) AuNPs. It is important to note that the position of the SPR can be tuned by the ratio of the reactants in the range from 530 to deep NIR (1300 nm).

Since the plant extracts are a complex mixture of phytochemicals, the next part of the study was focused on the synthesis experiments with using the main components of peppermint extract: rosmarinic and gallic acids.

Rosmarinic acid is one of the major polyphenols found in the extracts of plants of the *Lamiaceae* family, such as peppermint or lemon balm (*Melissa officinalis*). The UV-Vis spectra of AuNPs showed a distinct maximum at 672 nm. The formation of non-spherical AuNPs was confirmed by the TEM method (**Figure 2, a**). The obtained AuNPs have the shape of nanourchins and this deviation from the spherical shape produces the NIR absorbance. Similar AuNPs have been obtained using ethylenediaminetetraacetic acid [22].

Gallic acid was used in an attempt to substitute another triol, pyrogallol, which showed the possibility of preparing AuNPs with SPR maximum up to 880 nm, but without the involvement of a toxic stabilizing agent (CTAB) [23]. UV-Vis spectra of AuNPs showed a clear SPR peak maximum at 616 nm with strong absorption at 700 nm (**Figure 1**).

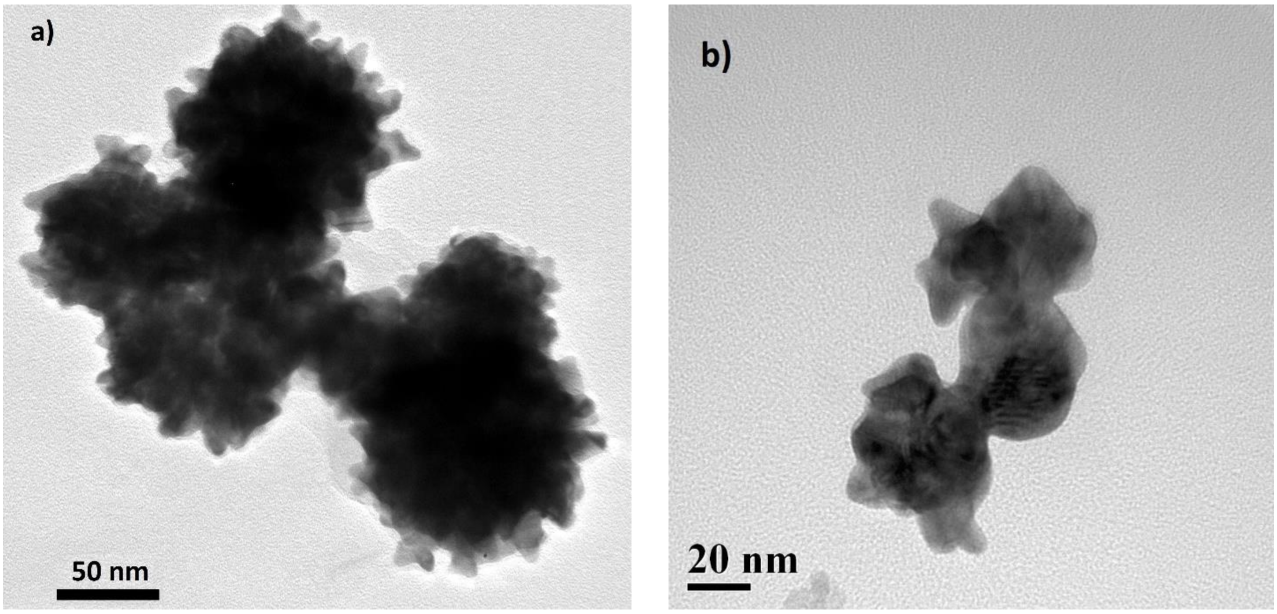

Figure 2 TEM images of AuNPs prepared using rosmarinic (a) and gallic (b) acids with initial concentrations of polyphenolic acids of 0.5 mg/mL and concentration of Au^{3+} of 0.5 mM Au^{3+}

Figures 2a and **2b** show nano-urchins prepared with rosmarinic acid and gallic acid. **Figure 2b** shows a TEM micrograph of individual nano-urchins at an early stage of formation. It is observed that the nano-urchins have a tweened structure and a small diameter, estimated to be ~20 nm based on several similar micrographs. Presumably, the interior of the nano-urchins consists of numerous pseudospheres, most of which are fused together. **Figure 2a** shows Au giant aglomerate nanostructures consisting of many self-organizing nano-urchins based on pseudospheres from which nanocones nucleate and grow. Nanocones emanate from the edges of the pseudospheres and grow radially outward. They are slightly curved and become progressively thinner as they grow. The most typical size of the aglomerate varies in the range of 150-250 nm. **Figure 2a** shows nanoagglomerates characterized by radial growth of nanocones in all directions. It has also been observed that aglomerated nanostructures may have a hollow center. In fact, imaging of several individual nano-urchins indicates that each could be fused nanocrystals.

The structure of nano-urchins or nano-flowers [24] looks promising when adjustment synthesis conditions would allow increasing the deviation of the shape from spherical. However, the natures of the Au NPs, which are inter-grown twins, suggest certain instability of such structure [25]. This was confirmed by laser heating studies: the raising temperature decreased after each heating-cooling cycle [24].

4. CONCLUSION

In this study, we tried to develop new green synthesis protocols for the preparation of AuNPs with controlled size and shape using phytochemical plant extracts as one of the most promising ways for the design of biocompatible AuNPs with different shapes. We reviewed the recent advances in the field achieved by using natural polyphenols and considered previously isolated compounds such as gallic acid and rosmarinic acid and used them in "green" synthesis, as well as an extract of peppermint leaves and a polyphenolic fraction of elderberry fruits. The results of NIR spectroscopy and transmission electron microscopy showed that we synthesized AuNPs with a size below 60 nm with a response in the NIR region by influencing the shape of Au pseudospheres. The combination of the approach in the preparation of AuNPs with the presumed biocompatibility makes the synthesis of Au nano-urchins prepared using rosmarinic and gallic acids, with low cost and high time efficiency in synthesis, very promising and opens up new possibilities in nanomedicine. The control of optical properties such as NIR response through the regulation and control of both size and shape make nano-urchins prepared with rosmarinic and gallic acids quite prominent agents for possible photothermal therapy applications.

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REFERENCES

[1] KHAN I., SAEED K., KHAN I. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*. 2019, vol. 12, pp. 908-931. Available from: https://doi.org/10.1016/j.arabjc.2017.05.011

[2] SINGH P., KIM Y.-J., ZHANG D., YANG D.-C. Biological Synthesis of Nanoparticles from Plants and Microorganisms. *Trends in Biotechnology*. 2016, vol. 34, pp. 588-599. Available from: https://doi.org/10.1016/j.tibtech.2016.02.006

[3] PEROTTI G.F., PEREIRA DA COSTA, L. Chapter 12: Biological Materials. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 316-332. Available from: https://doi.org/10.1039/9781839163623-00316

[4] YONEZAWA T., ZHU S., NGUYEN M.T. Chapter 15: Miscellaneous Reductants. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 393-459. Available from: https://doi.org/10.1039/9781839163623-00393

[5] AHMED S., AHMAD M., SWAMI B.L., IKRAM S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*. 2016, vol. 7, pp. 17-28. Available from: https://doi.org/10.1016/j.jare.2015.02.007

[6] OGUNDARE S.A., VAN ZYL W.E. Chapter 10: Polysaccharides. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 249-275. Available from: https://doi.org/10.1039/9781839163623-00249

[7] HALDAR D. Chapter 8: Amino Acids and Peptides in Colloidal Nanoparticle Synthesis. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 184-218. Available from: https://doi.org/10.1039/9781839163623-00184

[8] ZHENG G., LIANG E., WANG S. Chapter 13: Proteins Engineer the Size and Morphology of Noble Metal Nanoparticles. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 333-354. Available from: https://doi.org/10.1039/9781839163623-00333

[9] KINAYYIGIT S. Chapter 4: Role of Phenols and Phenol Derivatives in the Synthesis of Nanoparticles. *RSC Nanoscience and Nanotechnology*. 2021, vol. 50, pp. 73-96. Available from: https://doi.org/10.1039/9781839163623-00073

[10] MITCHELL M.J., BILLINGSLEY M.M., HALEY R.M., WECHSLER M.E., PEPPAS N.A., LANGER R. Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery*. 2021, vol. 20, pp. 101-124. Available from: https://doi.org/10.1038/s41573-020-0090-8

[11] LOPATYNSKYI A.M., MALYMON Y.O., LYTVYN V.K., MOGYLNYI I.V., RACHKOV A.E., SOLDATKIN A.P., CHEGEL V.I. Solid and Hollow Gold Nanostructures for Nanomedicine: Comparison of Photothermal Properties. *Plasmonics*. 2018, vol. 13, pp. 1659–1669. Available from: https://doi.org/10.1007/s11468-017-0675-1

[12] SMITH, A.M., MANCINI, M.C., NIE, S. Bioimaging: Second window for in vivo imaging. *Nature Nanotechnology*. 2009, vol. 4, pp. 710-711. Available from: https://doi.org/10.1038/nnano.2009.326

[13] 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*. 2022, vol. 12, pp. 1071-1083. Available from: https://doi.org/10.1007/s13204-021-01740-8

[14] MARIYCHUK, R., PORUBSKÁ, J., OSTAFIN, M., ČAPLOVIČOVÁ, M., ELIAŠOVÁ, A. Green synthesis of stable nanocolloids of monodisperse silver and gold nanoparticles using natural polyphenols from fruits of Sambucus nigra L. *Applied Nanoscience*. 2020, vol. 10, pp. 4545-4558. Available from: https://doi.org/10.1007/s13204-020-01324-y

[15] 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

[16] MALIKOVA N., PASTORIZA-SANTOS I., SCHIERHORN M., KOTOV N. A., LIZ-MARZAN L. M. Layer-by-Layer Assembled Mixed Spherical and Planar Gold Nanoparticles: Control of Interparticle Interactions. *Langmuir*. 2002, vol. 18, pp. 3694-3697. Available from: https://doi.org/10.1021/la025563y

[17] 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, pp. 2835-2841. Available from: https://doi.org/10.1007/s13204-019-00990-x

[18] MARIYCHUK, R., GRULOVA, D., GRISHCHENKO, L.M., LINNIK, R.P., LISNYAK, V.V. Green synthesis of non-spherical gold nanoparticles using *Solidago canadensis* L. extract. *Applied Nanoscience*. 2020, vol. 10, pp. 4817-4826. Available from: https://doi.org/10.1007/s13204-020-01406-x

[19] LUO X., XIE X., MENG Y., SUN T., DING J., ZHOU W. Ligands dissociation induced gold nanoparticles aggregation for colorimetric Al^{3+} detection. *Analytica Chimica Acta*. 2019, vol. 1087, pp. 76-85. Available from: https://doi.org/10.1016/j.aca.2019.08.045

[20] VIGDERMAN L., ZUBAREV E. R. High-Yield Synthesis of Gold Nanorods with Longitudinal SPR Peak Greater than 1200 nm Using Hydroquinone as a Reducing Agent. *Chemistry of Materials*. 2013, vol. 25, pp. 1450-1457. Available from: https://doi.org/10.1021/cm303661d

[21] FIZER, M.M., MARIYCHUK, R.T., FIZER, O.I. Gold nanoparticles green synthesis with clove oil: spectroscopic and theoretical study. *Applied Nanoscience*, 2022, vol. 12, pp. 611-620. Available from: https://doi.org/10.1007/s13204-021-01726-6

[22] NHAT HANG N. T., PHUONG PHONG N. T. Facile synthesis of urchin-like gold nanoparticles using binary reducing agent composed of EDTA and hydroquinone. *Vietnam Journal of Chemistry*. 2018, vol. 56, pp. 667-671. Available from: https://doi.org/10.1002/vjch.201800067

[23] HUANG Y., XIA K., HE N., LU Z., ZHANG L., DENG Y., NIE L. Size-tunable synthesis of gold nanorods using pyrogallol as a reducing agent. *Science China Chemistry*. 2015, vol. 58, pp. 1759-1765. Available from: https://doi.org/10.1007/s11426-015-5437-3

[24] SANTOS O, CANCINO-BERNARDI J, PINCELA LINS PM, SAMPAIO D, PAVAN T, ZUCOLOTTO V. Near-Infrared Photoactive Theragnostic Gold Nanoflowers for Photoacoustic Imaging and Hyperthermia. *ACS Applied Bio Materials*. 2021, vol. 4, pp. 6780-6790. Available from: https://doi.org/10.1021/acsabm.1c00519

[25] EL KORAYCHY E.Y., RONCAGLIA C., NELLI D., CERBELAUD M., FERRANDO R. Growth mechanisms from tetrahedral seeds to multiply twinned Au nanoparticles revealed by atomistic simulations. *Nanoscale Horizons*. 2022, vol. 7, pp. 883-889. Available from: https://doi.org/10.1039/d1nh00599e