SHAPE VARIABILITY OF PHYTOSYNTHESIZED GOLD NANOPARTICLES

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Abstract

Biomedical applications of nanomaterials depend on the size and morphology of nanoparticles and their biocompatibility. However, the armory of trusted protocols usually includes reducing agents, including sodium borohydride, methoxy polyethylene glycol, potassium tartrate, etc., and capping agents, such as sodium dodecyl benzyl sulfate, polyvinylpyrrolidone, etc., which are usually toxic. The unavoidable presence of toxic compounds in nanocolloid systems limits the use of nanoparticles for biological systems. This study aims to synthesize non-spherical gold nanoparticles using plant extracts and characterize their properties for biomedical applications. Gold nanoparticles were obtained through the direct interaction of Au^{3+} cations with aqueous extracts of selected plants (elderberry and peppermint) under different synthetic conditions. Ultraviolet and visible spectroscopic measurements confirmed the response of the obtained Au nanocolloid solutions to near-infrared radiation. This response opens the prospect of using the obtained nanoparticles in nanomedicine for antitumor therapy, targeted drug transport, diagnostics, or drug delivery systems. The position of the maximum near-infrared radiation can be adjusted by the synthetic conditions (extract composition, concentration, reagent ratio, pH, and temperature). Transmission electron microscopy and atomic force microscopy showed the formation of a mixture of circum-spherical Au nanoparticles and non-spherical Au nanoparticles: nanotriangles with sizes from 20 nm and nanohexagons with sizes > 100 nm.

Keywords: Plasmonic nanoparticles, phytosynthesis, non-spherical nanoparticles, biocompatible nanoparticles

1. INTRODUCTION

Nanomaterials have become an important branch of modern science due to their unique chemical and physical properties. Due to their unusual optical, electric, magnetic, and catalytic properties, nanomaterials were recognized as new and efficient components that can be used for the creation of novel advanced materials.

Biocompatibility and optical properties (surface plasmon resonance, SPR) of noble metal nanoparticles (NPs) open the possibility of their applications in modern nanomedicine, which is currently being actively developed. Typically, the NPs of noble metals, especially gold (Au NPs), are employed to deliver therapeutics and mediate heat and light to specific types of tissues [1]. The structure, composition, size, and shape of metal NPs define the optical and electronic properties which can be adjusted for utilization for advanced photothermal therapy [2] and controlled drug release systems [3].

Au NPs with their excellent chemical stability and biocompatibility are suitable for biomedical applications. However, Au NPs with reasonably small size (< 60 nm) exhibit their surface plasmon resonance in the range of 510–560 nm, far beyond the biological transparency window of 650–1350 nm. This region is divided into

two optical near-infrared ranges, at λ = 650–850 nm and 950–1350 nm, respectively. The effective utilization of Au NPs for photothermal therapy due to the deeper penetration of long-wave radiation requires NPs with a response in the second near-infrared range [4]. This is the reason, why the significant interest of scientists is focused on the synthesis of Au NPs with different shapes – spheres [5,6], nanorods [7], nanoshells [8], nanostars [9], nanoprisms [10], nanotriangles [11], and nanohexagons [12,13].

Various methods for synthesizing metal NPs were developed in recent decades: hydrothermal synthesis, co-precipitation, microemulsion, inert gas condensation, ion sputtering scattering, microwave, pulse laser ablation, sol-gel, spark discharge, sonochemical, and biological synthesis. Physical methods usually require the utilization of highly expensive equipment and do not provide the control over parameters of final nanoparticles (size and shape). From this point of view, traditional wet chemistry methods are preferable. However, the most efficient protocols involve the use of toxic reducing and capping agents which remain in the resulting nanocolloid solutions. As result, such as Au NPs show high cytotoxicity towards living organisms and are unsuitable for application in biomedicine. Biological synthetic methods, including the application of biological materials, e.g., plants, fungi, and bacteria, can be considered to be a prospective alternative. Such methods are also economical and time-effective and environmentally friendly [14,15]. One of the routes for obtaining biocompatible non-spherical Au NPs in the greenest manner is phytosynthesis - the use of plant extracts and natural surfactants [16]. Despite the extensive studies, the wide application of photosynthetic protocols is limited because the plant extracts are complex systems and the knowledge of the formation mechanism of non-spherical Au NPs is limited [17].

This study aims to consider the current state in the development of phytosynthesis protocols for the preparation of Au NPs with optical activity in the near-infrared region.

2. EXPERIMENTAL

2.1. Preparation of nanoparticles

Au NPs were synthesized using aqueous extracts of elderberry (*Sambucus nigra*) and peppermint (*Mentha piperita*) according to protocols [6] and [18], respectively. Syntheses were performed at an ambient laboratory temperature (20–25 °C) by the direct interaction of the plant extracts with 1 mM of $HAuCl_4$ aqueous solution under continuous stirring.

2.2. Characterization of nanoparticles

The obtained nanocolloid solutions were characterized by UV-Vis spectroscopy using Shimadzu UV-1800 spectrophotometer with matched 1-cm quartz cells. Transmission electron microscopy (TEM) imaging was conducted with a JEOL JEM-2100F microscope. TEM specimens were prepared by dropping a sonicated aqueous suspension of Au nanoparticles on a carbon-coated copper grid and followed by drying it under the infrared lamp. TEM images of different magnifications were captured at a maximum acceleration voltage of 200 kV.

3. RESULTS AND DISCUSSION

UV–Vis spectroscopy was applied to follow the formation of the Au NPs through the observation of the SPR peak position and shape (**Figure 1**). The collected UV–Vis spectra of Au NPs prepared using different extracts are different in principle. To the best of our knowledge, the presence of the SPR peak maximum can be seen in the spectral range from 530 to 570 nm. This SPR peak is true evidence of the formation of spherical Au NPs, as confirmed by the literature [5, 6]. Besides, we should notice that in rare cases, the second maximum can be seen in the near-infrared range of the recorded spectra.

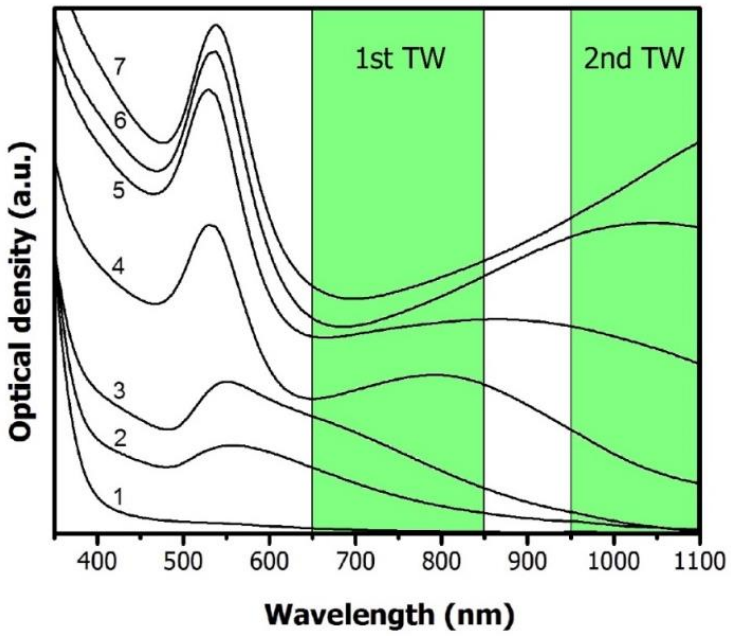

Figure 1 UV-Vis spectra of *Mentha piperita* mediated Au NPs prepared with different concentrations of Au^{3+}: 1 – 0.0250; 2 – 0.1250; 3 – 0.3125; 4 – 0.3750; 5 – 0.5000; 6 – 0.5625 and 7 – 0.7500 mM compared with the first and second therapeutic windows (TW). Spectra in scale but shifted along *y*-axis for better view

The size and shape of Au NPs have a real influence on the position of SPR absorption maximum. In many cases, the growth of the spherical Au NPs leads to a small shift of absorption maximum in the infrared range, however, this dependence for non-spherical Au NPs is very different [19].

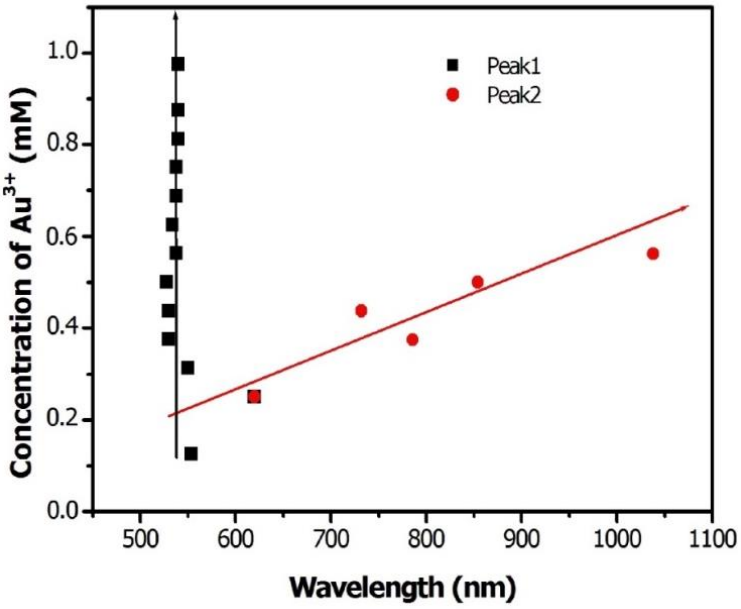

Figure 2 Dependencies of the positions of first and second absorption maxima for Au nanocolloids prepared with different initial concentrations of Au^{3+}

Here slight changes in the size or in the aspect ratio, which is a geometrical presentation of shape deviation from an ideal sphere, results in a considerable shift of the SPR absorption maximum in the near-infrared

spectral range [20]. On the basis of UV-Vis spectra, show that, depending on the composition, almost perfect spherical Au NPs (*Sambucus nigra* extract) [6] and non-spherical Au NPs (*Mentha piperita*) can be obtained from the extracts. And although in the second case, a mixture is formed, it is possible to adjust the adsorption and position to a maximum in the NIR.

Figure 2 shows that two surface plasmon resonance (SPR) peaks form in all samples. The first SPR peak, at 528 nm, corresponds to the contribution of the spherical Au NPs, definitely, its position is unchanged. The second SPR peak is formed because of the presence of non-spherical Au NPs in nanocolloid solutions. When increasing the concentration of Au^{3+} in the reaction mixture, Au NPs can increase in size, which is manifested a rapid shift of the maximum in the IR range, and the concentration of larger newly formed Au NPs increases.

Figure 3a shows the synthesized spherical Au NPs with an average diameter of 6.6 ± 1.2 nm. A TEM image shows a solitary spherical Au nanoparticle with size of 7.7 nm (**Figure 3b**), and one can see here the morphology of the seed, with an interplanar spacing characteristic of the {111} plane in face-center cubic Au structure. Based on TEM images, one can suggest that the composition of extracts impacts the morphology of the resulting Au NPs.

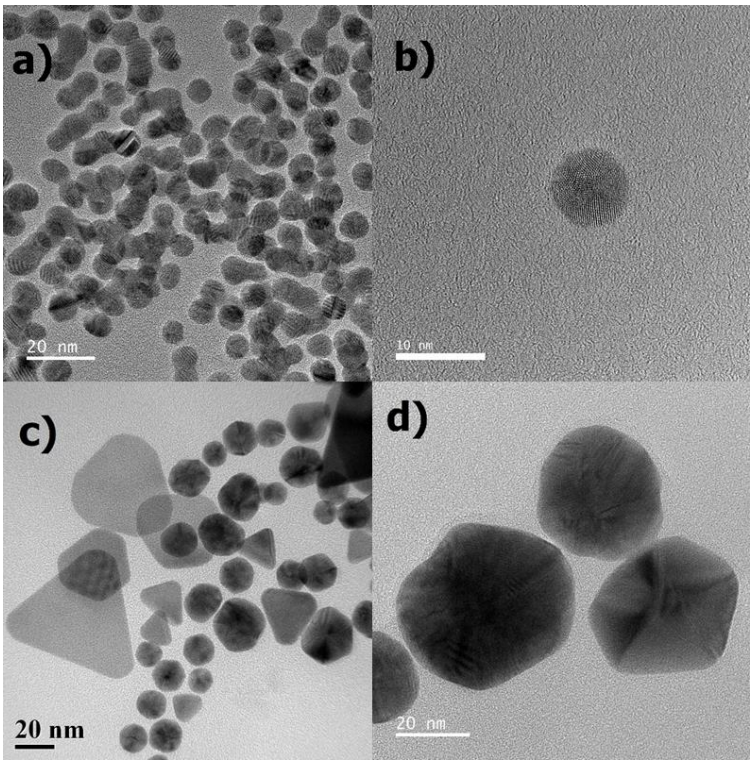

Figure 3 TEM images of Au NPs prepared using polyphenolic fraction of *Sambucus nigra* extract (a, b) and aqueous extract of *Mentha piperita* (c, d)

Whereas in some cases almost perfect Au nanospheres are produced (extracts of *Sambucus nigra*), in other cases, a mixture of spherical and non-spherical Au NPs (extracts of *Mentha piperita*) is formed. Moreover, depending on the amount of Au^{3+} ions in the reaction mixture, the formation of thin Au nanotriangles and also thin Au nanohexagons are registered. The contribution of the latter shifts the absorption maximum of SPR further into the near infrared spectral range. Besides, it should be noticed that the spherical Au NPs are not quite spherical but with ribs, which indicates a presence of a special organic substance, which probably, under optimal conditions, could increase the yield of non-spherical Au NPs. Separation by centrifugation or electrophoresis could be principal methods for the preparation of mono-disperse irregular shape Au NPs from the multicomponent colloidal solutions of gold. Spherical Au NPs are among the well-studied nanoobjects

considering their synthesis and characterization; their surface usually is modified extensively with ligands. The affinities of extract components to the surface of spherical Au NPs cause the translation of the affinity into the final morphology of particles when different phyto-organics were used. Typically, the extract components with low binding affinity can support stabilization of large spherical Au NPs in colloidal solutions. In such conditions, the polyphenolic fraction of *Sambucus nigra* extract enables just a growth of gold seeds to bigger spherical Au NPs, without influencing their shape. We should point out that when using the extract of *Mentha piperita*, a mixture of crystallographic habits is usually obtained, including single-crystalline, mono-twinned, and pentatwinned populations of pseudospherical Au NPs. In fact, seed-mediated growth of non-spherical Au NPs is strongly dependent on the crystal structure of the seeds, which are mono-twinned or multiplies twinned. The pseudo-spherical single-crystalline seeds may produce triangles or hexagons (**Figure 3c**), in good yields. Increasing the negative charge on the Au seeds at the presence of excess amount of reducing agent can be a reason for the migration of Au atoms to low-energy facets, for example, to {111}. Conversely, at low concentrations of reducing agents, a slower process of Au^{3+} reduction takes place, resulting in penta-twinned gold intermediates which are appropriate for the growth of the six-branched Au NPs. These results strongly suggest that while crystalline defects may be necessary for such branching anisotropic growth, such twinning can be both induced and specifically tuned by dosing one of reactants. So, it is not surprising, that we can also capture the Au NPs with truncated tetrahedral morphology, as well as the Au NPs decahedral with additional tetrahedral growth and with icosahedral morphologies (**Figure 3d**).

4. CONCLUSION

Based on the results of our studies, we have considered the optical characteristics in the region of SPR and the morphological characteristics of Au NPs, both spherical and specific irregularly shaped Au NPs, prepared by using green protocols. It was found that the use of extracts of some plants (*Sambucus nigra*) leads to the formation of almost perfectly spherical Au NPs. The details of the types of defects observed by the TEM method are presented. According to the obtained data, we do not rule out the possibility of obtaining the required non-spherical Au NPs with a response in the near-infrared range using extracts of selected plants, for example, *Mentha Piperita*. The synthesis of Au NPs makes it possible to obtain biocompatible metal NPs for biomedical purposes; however, there are still quite complex issues related to the mechanisms of growth of non-spherical particles. Herein, we have proposed a view of the growth of such particles, considering the formation of twinning and multi-twinning planes of Au seeds.

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