Synthesis of Ag/Au (core/shell) nanoparticles by laser ablation in liquid and study of their toxicity on blood human components

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Abstract: Q-switch Nd: YAG laser of wavelengths 532nm and 1,064nm with energy in the range 0.2 J to 1J and 1Hz repetition rate was employed to synthesis Ag/Au (core/shell) nanoparticles (NPs) using pulse laser ablation in water. In this synthesis, initially the silver nano-colloid prepared via ablation target, this ablation related to Au target at various energies to creat Ag/Au NPs. Surface Plasmon Resonance (SPR), surface morphology and average particle size identified employing: UV-visible spectrophotometer, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The absorbance spectra of Ag NPs and Ag/Au NPs showed sharp and single peaks around 400nm and 410nm, respectively. The average diameter achieved for Ag/Au NPs were as 30nm and 25nm corresponding to 532nm and 1064nm, respectively. The TEM images showed that Ag/Au NPs possess a spherical shape, while the samples average size were in range from 20 to 30nm. There was an obvious increase in size during the use of 532nm laser. As for the effects of toxicity, results on human blood components showed that these nanoparticles have no effect on RBCs, WBCs and HB Therefore; these particles considered promising in the biological and medical applications.

Keywords: Ag/Au nanoparticles; Core/shell; pulsed laser ablation; morphological; toxicity.

1. Introduction

NPs defined as small particles with (1-100) nm diameter; they show a distinctive characteristics comparing to bulk form properties of the same materials [1-3]. High reflective radiation, specific mechanical properties, high electrical and thermal conductivity are the features of bulk noble metals, such features generate due to their crystal structure as well as the presence of delocalized electron existence of the electron gas [4]. In addition, the bimetallic nanoparticles (CSNPs), which created from two different metallic elements acting as surface shell and inner core, appear as novel nanomaterial class [5]. In the case of NPs required considered toxic, this might result in troubles to the host organs and tissues. Also, the coating related to benign substance on the core’s top make NPs biocompatible and less toxic. Often, the shell layer is not just acting as nontoxic layer but also work on improving the base material properties [6]. To combine the two elements in single substance, particularly in NP, might have synergistic impact on the properties and thus providing access to novel materials with novel applications. For instance, using silver and gold nanoparticles might be an opening to a novel potential to couple anti-bacterial AgNPs with...
multiple biophysical molecules through covalent bonding related to the gold atoms [7]. AgNPs were of high importance due to their considerable role in living organisms, biological systems, as well as medicine in current medical industries. Ag and Ag NPs indicated to be very important to prevent any infections that may occur in open wounds and burns. AgNPs indicated to possess anti-platelet, anti-viral and anti-fungal activities, also they were significant as therapeutic agents and anti-microbials. Essentially, AgNPs developments as anti-bacterial agents is ongoing [8]. At the same time, AuNPs were considered as large surface area, nontoxic particles that might be altered with other molecules, also utilized in biomedical field. The importance of AuNPs in the field of biochemistry was because of the optical properties and compatibility. Generally, NPs specified as excellent therapeutic agents because of their simple transport in carrier loading drug and diseased cells [9].

Bottom-up and top-down are the two approaches used for synthesizing NPs. The engineering method is developing a bottom-up model with detailed description related to the technologic factors of energy system and the way it might develop in future. Typically, the energy demands were exogenously provided, while the models are analyzing the way that certain energy requirement must be achieved in cost optimal manner. The cost-effective method is building top-down models describing the economy, and showing the potential for substituting various production factors for the purpose of optimizing the social welfare, such models don’t involve a lot of technical aspects [10-11].

One of the fast, simple, and environmentally-friendly methods for synthesis nanomaterials is the pulsed laser ablation in liquid solutions (PLAL), in such method; a solid target immersed in liquid medium within reactor as well as being radiated via focused laser beams. In addition, the beam is reaching solid target’s surface, also vaporizes it and create ablation plume that contain particles, molecules, atoms, ions and clusters. Also, the intermediate reactive products in plume react with surrounding liquid molecules, creating nanostructures containing atoms of original in addition to the liquid target. On the other hand, the confinement created via liquid layer with high pressure and temperature resulting from focused pulse beam providing optimum condition for creating diastole phases, if possible the synthesis regarding various nanostructures [12-13]. Furthermore, PLAL used for preparing Au/Ag NPs and their composites. The final size related to core-shell nanoparticles along with their optical characteristics might be altered via either modifying the size of core particle or the shell’s thickness [14].

A study conducted by Dolgaev et.al. indicating the formations of Au and Ag NPs via pulsed laser ablation metal target in liquid environments (C2H4Cl2, C2H5OH, H2O) via utilizing Cu vapor laser (λ=510nm), such approach allows high rates of NPs formation [15]. In addition, a study conducted by Lu L et.al Synthesized Au-Ag core-shell NPs via chemical reaction in two-step process. Well defined and strong Ag absorption band recorded in the case when the content of Ag is exceeding 60 wt. %. Furthermore, it indicated that concentration regarding Cl(-) ions in Au precursor solution is of high importance in the bimetallic sol stability as well as the deposited Ag shell structure [16].

Zhi-Chuan Xu et.al attempted to synthesis Au/Ag a colloidal nano-alloy types through single metal targets’ laser ablation in water as well as re-irradiation regarding mixed colloidal suspensions [17]. In vitro antibacterial activities of the Au-NPs examined through a study conducted by Falah et.al [18] against Gram negative and positive. E. Coli and S. aureus and examined the toxicity of synthesized Au NPs on the blood cells of humans.

In the this study, Ag/Au core/shell NPs synthesized in water utilizing pulsed Q-switched Nd:YAG at 532 nm and 1064 nm wavelength where different laser energy employed to irradiate silver and gold metal. These synthesized core/shell NPs were encourage to be used in the biomedical applications.
2. Experimental details

Q-switch Nd:YAG laser, the Nd:YAG (neodymium doped yttrium aluminum garnet; Nd:Y3A1;O12) can be defined as one of the crystals utilized as lasing medium for the solid state laser. Nd:YAG laser provide pulses of 532nm and 1064nm (i.e. frequency doubled), with maximum power for each pulse reaching to 1000mJ, pulse width is equal to10ns, Transverse mode TEM00, maximum repetition rate is equal to 6Hz. The Nd:YAG laser beam has been focused to utilize a lens onto metallic target. The target fixed via a holder at the quartz container bottom, distance between the lens and sample is equal to 12cm, this was the system used for laser ablation. In addition, the vials and quartz beaker cleaned with the use of aqua regia (3 HCl:1 HNO3) as well as double distilled water, before the experiment. Furthermore, a manual x-y translator applied to position and translate the entire target holding set-up for avoiding a crater on target. The utilized Au/Ag targets have been in analytical grade with a 99.99% purity having thickness of approximately 1.5mm.

Samples of blood (2ml) collected into two tubes: the first tube was blood with Ag/Au NPs, while the second one was of whole blood. The samples incubated for 60mins at a temperature of 37Celsius. Hematological autoanalyzer (Orphee Mythic 22 Hematological Analyzer; Diamond Diagnostic; U.S.) utilized for determining various hematological parameters, for instance Hemoglobin (HB), White Blood Cells (WBCs) and Red Blood Cells (RBCs) [19-20].

3. Statistical Analysis

Comparison between groups of data of three replicates was made by unpaired t-test utilizing graphpad prism V-5.01. A $\rho$ value of <0.05 was considered as being significant [21].

4. Results and discussions

Figure (1) showed absorbance spectra of Ag NPs which are prepared at laser wavelengths of 532nm and 1064nm via PLAL with laser energy at 200, 400, 600, 800 and 1000mJ. The absorption peaks in the visible area were the characteristics of metals nanoparticles formation, whereas the confinement in nanoscale proved via blue shift in the SPR absorption peak relative to bulk. The SPR spectrum related to AgNPs solution, showing semi symmetric absorption band which is centered at 400nm, indicating that the NPs in growth solution were spherical. In addition, the absorption peak related to the samples synthesized at 1000mJ and was higher than that synthesized at 200mJ that was associated with higher NPs concentration. As for wavelength variation, there was an increase in absorbance a combined with a decrease in wavelength, i.e. an increase in laser.
Au/Ag NPs mixed in the same solution, also two Plasmon bands must be indicated, whereas for alloys, one Plasmon band anticipated. In Figure (2) absorbance spectra of Ag/Au NPs prepared by 532nm and 1064nm PLAL at different laser energy. There was a single Plasmon band; this indicates that the solution contains nanoparticles in the form alloys. Peaks position remaining practically constant at 410nm. An increase in absorbance can be observed in case of Ag/Au NPs compared to Ag pure NPs related to higher concentration of NPs. The existence of single surface Plasmon peak indicated that created NPs are fairly spherical; in cases of the ellipsoidal particles, the spectrum of the absorption might have 2 Plasmon peaks.

Surface imaging and elemental analyses of the samples carried out using FE-SEM. Ag/Au nanoparticles prepared at laser energy 1000mJ and laser wavelengths 532nm and 1064nm as can be observed in Figure (3). The Ag/Au core/shell nanoparticles prepared with 1000mJ and wavelength 532nm have spherical
shape and diameters approximately 30nm (Figure 3a). The Ag/Au core/shell NPs prepared with 1000mJ and wavelength 1064nm have spherical shape and diameters approximately 25nm (Figure 3b).

Fig. (3): SEM images of Ag/Au NPs prepared with (a) $\lambda_{\text{Laser}}$ 532nm (b) $\lambda_{\text{Laser}}$ 1064nm.

TEM utilized for deciphering the surface morphology as well as the particle size related to synthesize NPs, the TEM images indicating that bi-metallic NPs were core/shells structures, with Au as shells and Ag as core. There was a difficulty in distinguishing Ag and Au from TEM images as both posses’ identical lattice parameters. However, with the high electron density regarding Au atoms, Au shell was differentiated with Ag core. Figure (4) showed increasing magnifications of TEM image for Ag/Au NPs synthesized through pulsed laser ablation in DDW with laser energy at 1000mJ and laser wavelengths 532nm and 1064nm. Ag/Au NPs deposited on a silicon substrate

It can be seen that the nanoparticles were in a shell and core form; the subsequent particles were spherical with 30nm as average size. In the case when Au target ablated in Ag colloidal suspension, growth and formation of Ag/Au core-shell particles occurred. It must be indicated that these particles considered to be the proximity of geometry regarding the expansion of the plasma plume, became they were large particles in comparison to other particles. In addition, there was an increase in size which result in more ions of Au being attacked because of the increase of surface area.

5. Toxicity of Ag/Au nanoparticles

The aim of this experiment was to study analysis toxicity of Ag/Au NPs depending on changes induced in different dimensional hematological parameters like the RBCs, WBCs and HB count after treatment with Ag/Au NPs. The toxicity test of nanoparticles can help in selecting these particles as antibacterial or some other biological application.
5.1 Side effect of Ag/Au NPs in RBCs

Figure (5) exhibited a comparison between groups of treatment with Ag/Au NPs and control negative in RBCS. The results showed there is no side effect on RBCs count in treatment groups, no significant difference of treatment in comparison with control negative.

5.2 Side effect of Ag/Au NPs in WBCs

Figure (6) exhibited a comparison between groups of treatment with Ag/Au NPs and control negative in WBCS. The results showed there was no side effect on WBCs count in treatment groups, no significant difference of treatment in comparison with control negative.
Fig. (6): WBCs count in human blood after Ag/Au NPs treated with laser wavelengths (a) $\lambda_{\text{Laser}}$ 532nm and (b) $\lambda_{\text{Laser}}$ 1064nm.

5.3 Side effect of Ag/Au NPs HB concentration

Figure (7) exhibited a comparison between groups of treatment with Ag/Au NPs and control negative in HB. The results showed there is no side effect on HB count in treatment groups, no significant difference of treatment in comparison with control negative.

Fig. (7): HB count in human blood after Ag/Au NPs treated with laser wavelengths (a) $\lambda_{\text{Laser}}$ 532nm and (b) $\lambda_{\text{Laser}}$ 1064nm.

Recent researches showed that NPs adsorbed onto erythrocyte surface with no disturbance to RBC morphology or membrane Tathrocytes showing various approaches for protecting them from oxidative stress. This result indicated that Ag/Au NPs with these concentrations were biologically safe and might be utilized in medical treatment.

Conclusions

Based on the results from this study, a small-size Ag/Au nanoparticles via laser ablation in water was synthesized. In this work, different laser energy and wavelength used and tested. The surface Plasmon resonances obtained for the core-shell Ag/Au nanoparticles was 410 nm and for the silver nanoparticles was 400nm. Both SEM and TEM images indicate that the NPs have spherical shape and average diameter about 20 to 30nm. Furthermore, by testing the nanoparticles on human blood, there were no noticeable changes in the blood components, they were found to be non-toxic. There is a possibility of concluding that the core/shell Ag/Au NPs is one of the potential candidate materials for the utilization a biomedical industry and as a coating for surfaces of materials to protect them against microbial growth.
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