Ex-situ Generation of the Gold Nanowire Networks Bovine Serum Albumin Bio-Conjugated System Using Pulsed Laser Ablation in a Harsh Environment

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Abstract. The experimental conditions were investigated to fabricate gold nanowire networks (AuNNWs) by pulsed laser ablation in saline solutions. The study revealed that the production of alternative nanoparticles (or aggregates) or nanowire networks at the fundamental wavelength of a Nd:YAG laser (1064 nm) depend on the medium media surrounding Au nanoparticles during the process of synthesis. The aggregates and precipitates of the gold nanoparticles (AuNPs) in saline solutions were caused by the harsh environment of the anions with Na⁺ cations in the solution. The ex-situ was used for the preparation of bovine serum albumin (BSA) bio-conjugates. Enhanced colloidal stability was dramatically achieved when BSA was adsorbed onto Au nanowire networks. The optical extinction spectra of the colloids in the UV/vis region were obtained to evaluate the structure of the dispersed Au phase. Transmission electron microscopy (TEM) was applied to visualize the size and morphology of the colloidal particles. Au nanowire networks compatibility can be prepared and applied in real environments.

1. Introduction

Noble metal nanocrystals have attracted considerable attention from a fundamental point of view and for technological applications mainly due to the position of their surface plasmon resonance lying in the visible spectral range. Moreover, the strong dependence of the electronic, optical, catalytic, and magnetic properties of the nanoparticles on their shape [1] and size [2] provides opportunities of creating nanostructures for specific applications by way of controlling the preparation conditions to produce nanoparticles having the desired shape and size.

The profile of the optical transmission spectrum of the corresponding colloid obtained in the UV/Vis range allows their indirect assessment. For example, spherical nanoparticles (up to a certain size) are characterized by a single surface plasmon resonance (SPR) peak in their extinction spectrum. The appearance of two peaks arising from the transverse and longitudinal SPRs is observed in extended shapes, e.g., spheroids or nanorods [3]. The positions of these peaks in the extinction spectrum depend on the aspect ratio of the nanostructure considered [4]. The existence of aggregates or nanowires in the colloid influences the corresponding extinction spectrum in a specific way. One-dimensional nanostructures excite substantial interest regarding the fundamental properties of surface plasmon
polaritons (SPPs) propagation in the nanoscale structured matter [5]. The gold nanowires exhibit excellent electric-conductivity, chemical and thermal inertness, and biocompatibility. Their properties depend on substances absorbed on the surface. Pulse Laser ablation (PLA) technique has attracted increasing interest and is extensively employed to synthesize new novel materials [6]. It has inherent advantages compared to the different chemical methods used, especially when biological applications are considered. Bovine serum albumin (BSA) often serves as a model protein for nano-particle–protein studies, as it shares 98% of its amino acid sequence with its human variant [7] Although this protein is negatively charged at physiological pH, it also contains 11 hydrophobic binding domains, 66 positively charged lysines, and an unpaired sulfhydryl group (cysteine) [8].

When a saline solution has a NaCl concentration less than that of blood plasma or bodily fluids, AuNP cations form a complex with Cl- anions, resulting in aggregation and precipitation of the AuNPs. Hence, it is difficult to maintain a stable dispersion of colloidal AuNPs within any saline solution for a relatively long time. This is because particle agglomeration and sedimentation may affect the biodistribution of the material, which may lead to harmful side effects [9].

A large variety of chemical methods for fabricating gold nanowires have been reported in the literature. A general disadvantage of the suspensions produced by these methods is the presence of residual ions as byproducts and their potential toxicity which precludes the biological applications [10-12]. In this study, we used the method of nanosecond laser ablation of an Au disc immersed in a harsh environment to produce contamination-free nanowire networks.

2. Materials and Methods

AuNNWs were prepared via the laser ablation technique in two different solutions. The first was a solution of deionized distilled water (DDW) and highly concentrated NaCl (150 mM), while the second contained similar components, but after PLA process directly the addition of BSA (375 µM). Both solutions were prepared at room temperature.

To synthesize AuNNWs, a gold target was placed at the bottom of a 10-mL quartz vessel filled with 3 mL of both solutions independently. The target was irradiated with a Q-switched Nd: YAG laser operated with a peak emission wavelength of 1064 nm, a repetition frequency of 5 Hz, and a pulse duration of 6 ns. A schematic diagram of the pulse laser ablation in liquid (PLAL) system used in this experiment is illustrated in Figure 1.

The focusing lens with a focal length of 100 mm was used to focus the laser beam on the gold target. The energy per pulse 650 mJ was used to ablate the Au target for 4 min in both solutions.

The optical properties of the solution of synthesized colloidal AuNPs were characterized using an ultraviolet/visible (UV-vis) spectrophotometer (Ocean Optics; SD1024DW, USA). Transmission electron microscopy (TEM, FEI CM 12, USA) operated at 120 kV were employed was to study the structural properties of the samples.
3. Results and discussion
To ensure sample reliability, tests were performed to characterize the samples. After the laser ablation of the Au target in the DDW with NaCl, and ex-situ BSA-conjugated AuNNWs in saline solutions. Some changes in the coloration of the solutions were observed; the solution changed from colorless to transparent red. The change in color could be an indication of the production of colloidal AuNPs. Notably, after the ablation with laser fluences to obtain AuNPs in the 150 mM NaCl solutions, the color of the solution changed to different hues of color [13].

3.1 Absorption spectra
UV-vis absorption spectroscopy is the most commonly used technique to study the optical properties of AuNNWs, determine the relationship between the laser parameters and productivity, and evaluate the AuNNP-corona interaction. Furthermore, absorption spectroscopy is faster than other standard optical technologies. In colloidal GNNWs, the SPR band and its intensity depending on the size, shape, and distribution of the AuNNWs. It has been noticed after performing the ablation process to get Au NPs in 150 mol of NaCl solution, that decrease the values of the intensity; the solution color changed into transparent very light purple. Then, after an hour AuNPs started to aggregate and precipitate at the bottom of the sample container. Colloidal AuNNWs precipitate in NaCl solution at a physiological pH. The SPR absorbance is at a maximum at a maximum wavelength $\lambda_{\text{max}} = 530$ nm and decreases with NaCl concentration. Colloidal AuNPs were aggregated and precipitated to the bottom of the sample container at room temperature. Figure 2 (solid line) shows the SPR of colloidal AuNNWs.

The ex-situ AuNP–BSA system, the absorption spectrum was acquired after laser ablation and the addition of BSA at the physiological concentration (375 µM) after AuNPs adsorb BSA.
The solution color was transparent very light blue and insignificant color changes even after long period of time. Therefore, we believe that AuNNWs in this state was stable. The SPR absorption spectrum decreases, broadening the plasmon band and a red-shift of the peak occurs as a result of plasmon coupling with AuNP aggregates or bigger size. See figure 2 (dash line).

![Figure 2: UV-vis spectra of a colloidal dispersion containing particles synthesized by means of laser ablation of an Au target in DDW + NaCl solution (solid line) and DDW+BSA+NaCl solution (dash line).](image)

3.2. TEM observations

TEM was employed to measure the sizes, size distributions, and shapes of the AuNNWs. The TEM image shows an increment of aggregation and agglomeration along with larger particles. This is indicated by the larger particles, agglomeration, and aggregation in the NaCl physiology concentration, which was confirmed by the TEM image shown in figure 3 (a).

When the ex-situ BSA conjugated with the AuNNWs, it control and stop aggregation. BSA can stable the sample which can be attributed to the insignificant aggregation and intensive stability and build chains of AuNNWs shows figure 3 (b).
4. Conclusion
The study emphasized the important role of the self-absorption in creating AuNNWs by PLA in a Harsh Environment. Prepared AuNNWs by PLA technique in a harsh environment was investigated. The experimental results revealed that it is difficult to maintain colloidal AuNNWs for a long time in a solution containing only NaCl. After PLA process directly addition BSA to the NaCl solution was found to be an effective approach to producing AuNNWs within a short period in such harsh environments. Ex-situ technique in producing AuNNWs conjugated with BSA was successfully achieved by pulsed Nd:YAG laser ablation of the gold target in saline solution. The TEM image showed the development of investigating BSA effects on size, morphology, growth, and composition. The importance of the method of pulsed laser ablation for producing contamination-free AuNNWs suitable for medical and research aims was thus confirmed.

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