

EDN: NSEMSY

УДК 54.182; 546.55; 538.958

Analysis of the Effect of pH on the Stability and Optical Spectra of Au Nanoparticles Linked by Conductive BPE Molecule

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Received 10.08.2024, received in revised form 06.09.2024, accepted 06.10.2024

Abstract. The preparation of detergent-free gold NPs by the citrate method is shown. The procedure of functionalization of monomeric Au NPs with 1,2-bis(2-pyridyl)ethylene (termed BPE) molecule to obtain Au-BPE dimers is described. According to the results of TEM analysis, the shape of the obtained NPs is close to spherical with a diameter of 21.94 ± 0.28 nm. In dimers, the distance between NPs is controlled by the linker and is 0.75 ± 0.12 nm, which is in agreement with the dimensions of the BPE molecule. According to the results of Experimental absorption UV-Vis spectra, measured over time, in 0.15 M nitric acid solution, Au-BPE dimers are more stable than in an alkali solution of the same molarity. Long-term exposure leads to a decrease in the intensity of the LSP peak of the dimers.

Keywords: Au nanoparticles, citrate method, UV-Vis spectra, 1,2-bis(2-pyridyl)ethylene.

Citation: A.V. Lukyanenko, A.V. Tsarenko, A.S. Aleksandrovsky, A.S. Fedorov, A.Sh. Umarova, Analysis of the Effect of pH on the Stability and Optical Spectra of Au Nanoparticles Linked by Conductive BPE Molecule, J. Sib. Fed. Univ. Math. Phys., 2024, 17(6), 754–760. EDN: NSEMSY.



Introduction

Nowadays the development of nanotechnology has led to progress in many areas of human activity. Nanoparticles are the most widely used product of nanotechnologies because they have ultra-small sizes down to single nanometers with a variety of characteristics. Successes have been achieved in using gold nanoparticles (Au NPs) in various fields, including photonics, nanooptics,

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the creation of quantum devices, and high-performance sensors. Many chemical and biological sensing methods have been developed based on DNA or RNA aptamers [1, 2]. An aptamer is a series of artificial oligonucleotide chains with a predictable and tunable structure, allowing it to bind to a target with high affinity and specificity [3]. In nanoparticle-based tests, one method of detection is to detect changes in the decay or shift of the wavelength of the localized surface plasmon resonance (LSPR) λ_{max} . This allows chemical binding events on the surface of nanoparticles to be converted into optical signals that can be easily tracked [4].

The properties of NPs depend on their size, shape, and environment. The production of nanoobjects using a bottom-up approach and the improvement of traditional chemical synthesis methods remains in demand due to their efficiency, flexibility, scalability, and low cost. One-step citrate method allow for the production of spherical particles and have become widespread [5]. Chemical modification of the NP surface can expand their functionality. Linker molecules containing functional groups can bind to the surface of NPs and form well-defined and reproducible structures such as dimers, trimers, and higher-order aggregates. These forms have been shown to significantly enhance the intensity of surface-enhanced Raman scattering (SERS) signals due to the presence of "hot spots" — regions of high electromagnetic enhancement at the junctions between metal nanoparticles [6, 7]. The Wustholz and all. reported the creation of "hot spots" in the sub-nanometer proximity of dimers and trimers, achieving a significant enhancement of SERS [8].

Several studies have shown that gold dimer can serve as an excellent SERS substrate for ultrasensitive detection applications. In [9], the authors present an improved selective colorimetric sensor using the LSPR absorption interaction of AuNPs via 4-ATP functionalization. The authors present an improved selective nitrite sensor using the LSPR absorption interaction of Au NPs via 4-ATP functionalization. The use of dimeric Au NPs for SERS analysis of thrombin was demonstrated in [10], and the detection limit was only 1 fM. The use functionalized Au NPs has led to progress in ultrasensitive detection in comparison to assays using monomeric Au NPs.

Au NPs and structures based on them remain the most popular products of nanotechnology. The presence of a conductive material between two NPs leads to the appearance of a new mode, called charge-transfer plasmon (CTP) mode, with the charge periodically moving between pairs of NPs through a conductive bridge. Modified gold nanoparticles, in particular Au-BPE dimers, have become widely used for the development of highly sensitive analytical methods. From a practical perspective, it is important to study the stability of these structures at both low and high levels of pH.

1. Materials and methods

Chemicals. For the dimer manufacturing, the following chemicals were used as received without further purification: auric tetrachloric acid (99.9% HAuCl_4), sodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ 99.0%), and 1,2-bis(2-pyridyl)ethylene ($\text{C}_{12}\text{H}_{10}\text{N}_2$ 97.0%) were purchased from Sigma-Aldrich (<https://www.sigmaaldrich.com>). Silver nitrate (AgNO_3 99.9%), potassium hydroxide (KOH 85%) and nitric acid (HNO_3 high purity) was purchased from Dia-m (<https://www.dia-m.ru>). Distilled water (DI) was obtained locally by distillation.

Instrumentation. The shape and size of the resulting particles was characterized using transmission electron microscopy (TEM) in a Hitachi HT7700 microscope and by counting more than 140 particles in the ImageJ program. Optical UV-Vis absorption spectra of water solutions of Au NPs and Au-BPE dimers in a 2 mm thick quartz cuvette (from Hellma®Analytics) were recorded using a Shimadzu UV-3600 spectrometer with a spectral bandwidth of 1 nm.

2. Sample preparation

Preparation of detergent-free Au NPs. The particles were prepared using the common citrate method [5]. The following procedure was used to prepare Au NPs: 50 mL of a 1 mM aqueous HAuCl_4 solution were placed in a 100 mL glass vial. The vial with the solution was then placed on a heated magnetic stirrer and heated to active boiling. After that 3 mL of freshly prepared 38.8 mM sodium citrate solution were added. The solution immediately turned black and then gradually changed to red-wine. The scheme of the procedure is shown in Fig. 1a. The reaction was allowed to proceed at the same temperature for 20 min, and then the resulting colloid was cooled to room temperature ($T = 25^\circ \text{C}$). To ensure linkage to BPE molecules, the pH of the Au NPs solution was adjusted to 7. For this, the prepared Au NPs solution was centrifuged at 7000 RPM for 20 min three times and resuspended in DI H_2O (pH 7) to bring the volume to 50 mL.

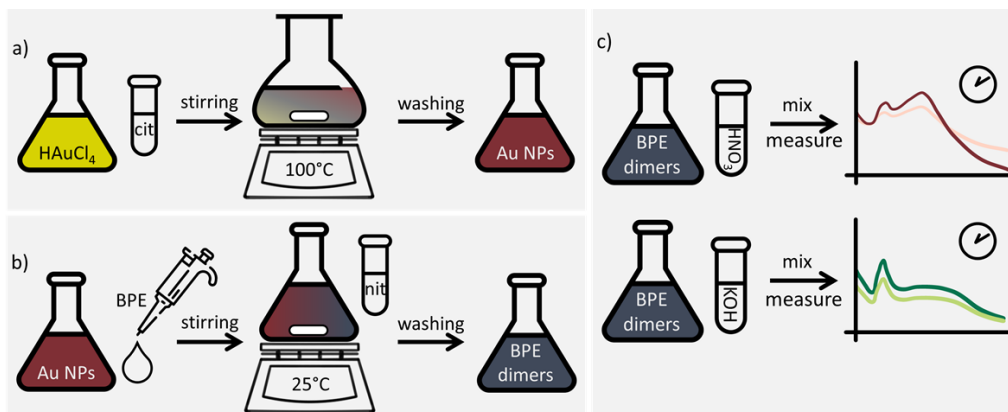


Fig. 1. (a) The scheme of the procedure for obtaining Au NPs and (b) the scheme of the functionalization procedure for obtaining Au-BPE dimers. (c) The scheme of the solution mixing and measurement)

Preparation of Au-BPE dimers. Au NPs dimers were prepared (shown in Fig 1b) according to the procedure described in [11]. To form Au-BPE dimers, $1.5 \mu\text{L}$ of a 0.05 mM BPE solution was added dropwise to 10 mL of freshly prepared Au NPs, under constant stirring. A sample of BPE crystals dissolved in water heated to 30°C was used to better dissolve the BPE. After some time, the NPs solution changed its color from red to dark purple, indicating the dimer formation. To interrupt the reaction, $0.85 \mu\text{L}$ of an aqueous solution of 0.4 mM silver nitrate was added to the mixture. Yellow illumination was used when preparing the silver nitrate solution since this substance is UV-light sensitive. The resulting mixtures were centrifuged for 20 minutes at 4000 RPM twice and resuspended in DI. The resulting dimer products were stored at 4°C for further characterization and use.

3. Results

Concentration calculation. It is assumed that all added HAuCl_4 is reduced to elemental gold and the entire amount of reagent is converted into spherical NPs, without taking into account losses during processing. According to the synthesis protocol, the total mass of gold in the form of nanoparticles in solution is $m(\text{Au}) = 9.85 \times 10^{-3} \text{ g}$. Based on the TEM study results, shown in Fig. 2a, NP diameter is $d = 21.94 \pm 0.28 \text{ nm}$, and based on that, can calculate NP mass $m(\text{NP}) =$

$(1.07 \pm 0.04) \times 10^{-16}$ g and then calculate concentration $N(\text{NPs}) = (18.5 \pm 07) \times 10^{12}$ nps/ml, which corresponds to a molarity of 3.08 nM. To form dimers, 45.2×10^{12} BPE linker molecules were added to the solution. Simple calculations show that there is 1 BPE molecule per pair of NPs, and after dimerization, about $N(\text{Au-BPE}) = 9.23 \times 10^{11}$ /ml dimers are obtained in water solution (Fig. 2b). The optical UV-Vis spectra of the absorption of monomer NPs and Au-BPE dimers was measured (Fig. 2c). Calculations based on experimental UV-Vis spectra, taking into account optical density (0.346), at maximum wavelength of $\lambda_{LSP} = 523$ nm give a lower concentration value of 1.88×10^{11} nps/ml, and accordingly, about 10 molecules per two NPs and 9.4×10^{10} /ml Au-BPE dimers, respectively.

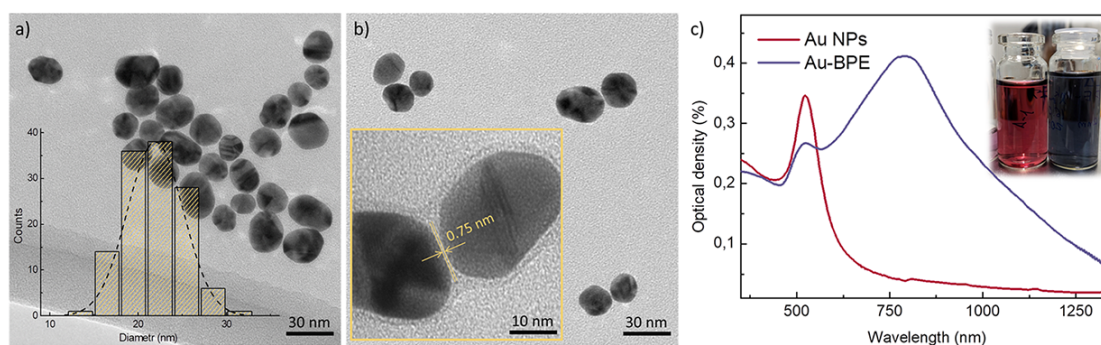


Fig. 2. (a) TEM image of Au NPs, the inset showing the histogram of the particle diameter distribution following the normal distribution (dashed line). (b) TEM image of Au-BPE dimers (the average distance between nanoparticles is noted). (c) Experimental absorption spectra of Au NP and Au-BPE dimers in water solution, a photo of the NPs and the BPE dimers is inset)

The discrepancy between the calculated and experimental values can be explained by the loss of non-stabilized (detergent-free) NPs and volume variations during dilution after centrifugation. During the formation of dimers, some of the linkers are in the interparticle space, and some molecules cover the surface of the NPs. The experimentally measured distance between connected spheres was 0.75 ± 0.12 nm, which is in agreement with the dimensions of the BPE molecule as shown in the inset in Fig. 2b.

The effect of pH. When using dimers for analysis or theranostic applications, it is practical to study the effects of acidic and alkaline environments on their stability. The procedure for mixing dimer solutions with acid (HNO_3) and alkali (KOH) is shown in Fig. 1c. A 0.15 M aqueous solution of nitric acid was added to some of the dimerized BPE (1:1 in volume fractions). After mixing the solutions, continuous recording of optical spectra was carried out for 50 minutes, at time intervals as shown in Fig. 3a. Another portion of BPE was mixed with a 0.15 M potassium hydroxide solution (also 1:1 in volume). Due to the rapid reaction, it was not possible to record the initial stages of the process and the spectra were measured after 5 and 10 minutes, respectively (Fig. 3b). The spectra were measured again after 20 hours, after the NPs had been washed from the solutions three times at 7000 RPM (20 minutes each). The NP sediment was collected and after dilution with water, the optical spectra were recorded again (Fig. 3c).

Initial absorption spectra of dimer solution contain the LSP resonance of individual nanoparticles of 22 nm diameter that is positioned at 540 nm, and CTP resonance due to electric conducting properties of BPE molecules, that maximizes at 810 nm. The amplitude of CTP resonance in the initial solution supersedes that of LSP resonance that is earlier reported in [11] as the formation of intense CTP resonance in contrast to weak CTP resonance reported in the

paper by Jiang.

Upon addition of nitric acid to the solution of dimers, amplitudes of both resonances experience slow but steady decrease (Fig. 3a). This means that Au-BPE dimers become unstable in the solution and their content decreases with the time passed after the addition of the acid. At the same time, we see that the optical density of the solution in the infrared, namely, at the wavelengths longer than 950 nm, experiences growth. This effect must be explained by partial destruction of BPE molecules followed by the formation of aggregates of closely spaced AuNPs with the interparticle distance smaller than that inside the dimers, i.e., smaller than 0.75 nm. Such aggregates may form continuous absorption spectrum in the infrared due to the interaction of LSP or CTP resonances of closely spaced AuNPs. Note that during the first hour of acid action the shape of both LSPR and CTPR remains the same in the vicinity of the peaks, and relation between LSP and CTP amplitudes under the action of nitric acid remains the same, that means that in the presence of acid the content of monomers do not increase with respect to content of dimers.

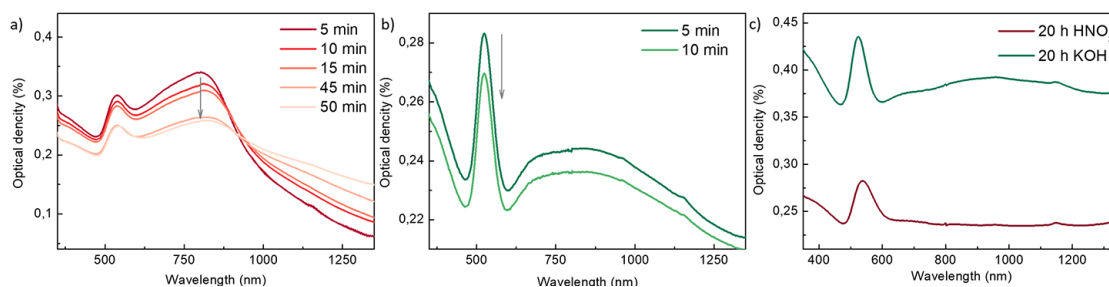


Fig. 3. Experimental absorption UV-Vis spectra (a) Au-BPE dimers with the adding of 0.15 M HNO₃, (b) Au-BPE dimers with the adding of 0.15 M KOH, (c) the spectra after exposing the solution for 20 hours

Upon addition of potassium hydroxide, the modification of the absorption spectrum of Au-BPE dimers solution proceeds, as we mentioned above, much faster at the initial minutes (Fig. 3b). The amplitudes of LSP and CTP resonances experience faster drop at the beginning of the process. The maximum of LSPR shifts to 524 nm while CTPR changes its shape and becomes more spectrally flat and broad. The amplitude of LSPR under the action of potassium hydroxide, in contrast to the action of nitric acid, becomes dominant over CTPR. Partially it could mean that destruction of dimers is accompanied by the formation of monomers that in the hydroxide ambience become stable and are not captured into aggregates.

After 20 hours of action, the situation with nitric acid and potassium hydroxide changes radically. The spectrum of dimers that undergone the action of hydroxide for 20 hours experiences minor changes with respect to those that undergone it for 10 min, except for further increase of the infrared wing. Oppositely, the action of nitric acid results in complete disappearance of CTP resonance and preserving LSP resonance, that becomes it almost identical to monomer solution, with the exception of flat continual aggregate background in the range from 600 to 1200 nm.

Conclusion

The Au NPs obtained by the citrate method have an estimated concentration of about $18.5 \pm 07 \times 10^{12}$ nps/ml and, according to TEM analysis, their shape is close to spherical with a diameter of 21.94 ± 0.28 nm. The properties of nanoparticles can be expanded by modifying

them with organic molecules. A procedure for functionalizing monomeric Au NPs with 1,2-bis(2-pyridyl)ethylene molecules to obtain Au-BPE dimers is described. Formation of complexes from nanoparticles is possible due to bonding with various organic molecules, leading to the formation of "hot spots". The enhancement factor for nanoparticles with hot spots does not correlate with aggregation state, but depends critically on the size of interparticle gap [8]. Therefore, the BPE linker presented in this work allows the formation of dimers with a short distance between nanoparticles. In the obtained Au-BPE dimers the distance between the NPs is 0.75 ± 0.12 nm, which agrees with the dimensions of the BPE molecule. The effect of the pH environment on the stability of Au-BPE dimers was studied. Aqueous solutions of nitric acid and potassium hydroxide were mixed with the dimers. Optical spectra were recorded for 50 minutes at different time intervals. According to the results of absorption UV-Vis spectra, measured over time, in 0.15 M nitric acid solution Au-BPE dimers are more stable than in the alkali solutions of the same concentration. Long-term treatment leads to a decrease in the intensity of the LSP peak of the dimers.

Study was supported by the Russian Science Foundation, Agreement no. 23-12-20007, and the Government of the Krasnoyarsk Territory and the Krasnoyarsk Territorial Foundation for Support of Scientific and R&D Activities, Agreement no. 256.

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Анализ влияния pH на стабильность и оптические спектры наночастиц Au, связанных проводящей молекулой ВРЕ

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Аннотация. Показано получение золотых наночастиц (Au НЧ) цитратным методом. Описана процедура функционализации мономерных Au НЧ молекулой 1,2-бис(2-пиридил)этилена (называемой ВРЕ) для получения димеров Au-ВРЕ. По результатам анализа ПЭМ форма полученных НЧ близка к сферической с диаметром $21,94 \pm 0,28$ нм. В Au-ВРЕ димерах расстояние между частицами контролируется линкером и составляет $0,75 \pm 0,12$ нм, что согласуется с размерами молекулы ВРЕ. По результатам экспериментальных UV-Vis спектров поглощения, измеренных от времени, в 0,15 М растворе азотной кислоты Au-ВРЕ димеры более стабильны, чем в щелочном растворе той же молярности. Длительное воздействие приводит к снижению интенсивности пика LSP димеров.

Ключевые слова: Au наночастицы, цитратный метод, UV-Vis спектры, 1,2-бис(2-пиридил)этилен.