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Functionalizing Gold Nanostars with Ninhydrin as Vehicle Molecule for Biomedical Applications

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Abstract - In recent years, there has been an explosion in Gold NanoParticle (GNP) research, with a rapid increase in publications in diverse fields, including imaging, bioengineering, and molecular biology. GNPs exhibit unique physicochemical properties, including surface plasmon resonance (SPR) and bind amine and thiol groups, allowing surface modification and use in biomedical applications. Nanoparticle functionalization is the subject of intense research, with rapid progress being made towards developing biocompatible, multi-functional particles. In the present study, the photochemical method has been done to functionalize various-shaped GNPs like nanostars by the molecules like ninhydrin. Ninhydrin is bactericidal, virucidal, fungicidal, antigen-antibody reactive, and used in fingerprint technology in forensics. The GNPs functionalized with ninhydrin efficiently will bind to the amino acids on the target protein, which is of eminent importance during the pandemic, especially where long-term treatments of COVID- 19 bring many side effects of the drugs. The photochemical method is adopted as it provides low thermal load, selective reactivity, selective activation, and controlled radiation in time, space, and energy.

Keywords: Gold NanoParticle (GNP); Surface modification; Nanoparticle functionalization; Photochemical method; Nanostars, Ninhydrin; Amino Acids

1. Introduction

To be precise, a nanometer is an extremely small-one billionth of a meter. That is 0.000000001m, 80,000 times smaller than the width of a human hair. Properties of materials can be very different at this level; for instance, they can be more chemically reactive and conduct electricity more effectively. They have potential like faster, lighter, can get into small spaces, cheaper, more energy-efficient, and have desirable properties.

Common oxidation states of gold include +1 (Au[I] or aurous compounds) and +3 (Au[III] or auric compounds). GNPs, however, exist in a non-oxidized state (Au[0]). GNPs are not new; in the 19th century, Michael Faraday published the first scientific paper on GNP synthesis, describing the production of colloidal gold by reducing aurochloric acid with phosphorous. Common methods of GNP production include citrate reduction of Au[III] derivatives such as aurochloric acid ($HAuCl_4$) in water to Au[0] [1].

In the present study, photochemical methods have been done to functionalize various shaped gold nanoparticles like rod, star and sphere by the molecules like ninhydrin. Ninhydrin is bactericidal, virucidal, fungicidal, antigen-antibody reactive, and used in many biomedical applications. The photochemical methods provide less instability due to heat, selectivity, and specificity, and the radiation can be modulated in terms of time, space, and energy.

2. Materials & Methods

0.1 M $HAuCl_4.3H_2O$, 0.01 M $AgNO_3$, 0.1 M CTAB (cetyltrimethylammonium bromide), 0.1 M Ascorbic Acid, 0.01 M ice-cold $NaBH_4$, Ninhydrin, Ethanol, Millipore Water

2.1 Preparation of Gold NanoStars (GNS)

1. 15 ml of centrifuge tube was taken and cleansed with soap water followed by tap water and Millipore water and rinsed several times.
2. After they were dried, 1.750 ml of water + 3.0 ml of CTAB + 200 µl of hydrated gold salt + 20 µl of freshly prepared ascorbic acid was added into the centrifuge tube.

3. Finally, 20 µl of silver nitrate solution was added dropwise, keeping the tube slanted. The reaction solution was mixed by gentle inversion 5 to 6 times.
4. When $AgNO_3$ (silver nitrate) was added, the colorless reaction solution slowly developed a faint purple color, changing to bluish purple. It indicated the formation of multi-spiked gold nanoparticles. Finally, the solution turned into light brown color.
5. UV visible spectra were taken by taking the CTAB as a reference.
6. The sample was centrifuged at 75 000 rpm for 15 minutes. The supernatant was discarded, and the pellet was diluted by 1 ml Millipore water and divided into three parts: samples 1, 2 and 3

2.2 Preparation of ninhydrin solution

Solution 1 is 0.0175 grams of ninhydrin in 5 ml of Millipore water, and Solution 2 is 0.0087 grams of ninhydrin in 5 ml of Millipore water

2.3 Preparation of reagents

The solutions were made to infer whether the change is particularly because of ninhydrin and no other substance. Table 1 represents the solutions made using Ninhydrin solution 1 and ethanol (EtOH). Table 2 represents the solutions made using Ninhydrin solution 2 and Millipore water.

Table 1: Reagents prepared using Ninhydrin solution 1 and ethanol (EtOH)

No.	Solution	Reagents
1	A	Only Ninhydrin solution 1
2	B	30 µl of $HAuCl_4.3H_2O$ in 3 ml of ninhydrin solution 1
3	C	1 µl of GNS in 3 ml of ninhydrin solution 1
4	D	6 µl of CTAB in 3 ml of ninhydrin solution1
5	E	1 µl of GNS in 3 ml of EtOH
6	F	30 µl of $HAuCl_4.3H_2O$ in 3 ml of EtOH

Table 2: Reagents prepared using Ninhydrin solution 2 and Millipore water

No.	Solution	Reagents
1	G	30 µl of $HAuCl_4.3H_2O$ in 3 ml of Ninhydrin solution 2
2	H	Only Ninhydrin solution 2
3	I	30 µl of $HAuCl_4.3H_2O$ in 3 ml of water

2.4 Photoirradiation

All the samples were prepared and kept at room temperature for two hours, followed by UV Visible spectroscopy. The reagents were irradiated at 254 nm for 15 minutes, followed by UV Visible spectroscopy. The wavelength was selected based on the reported wavelength for excitation of a similar molecule, Phthalimide. The observations for their color change and spectrum were noted.

3. Results & Conclusions

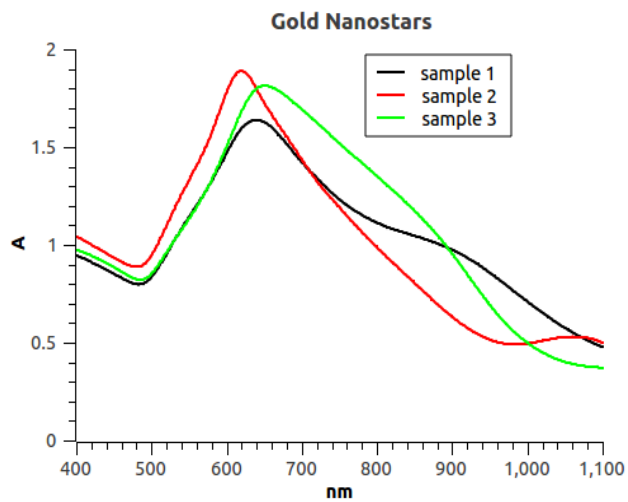
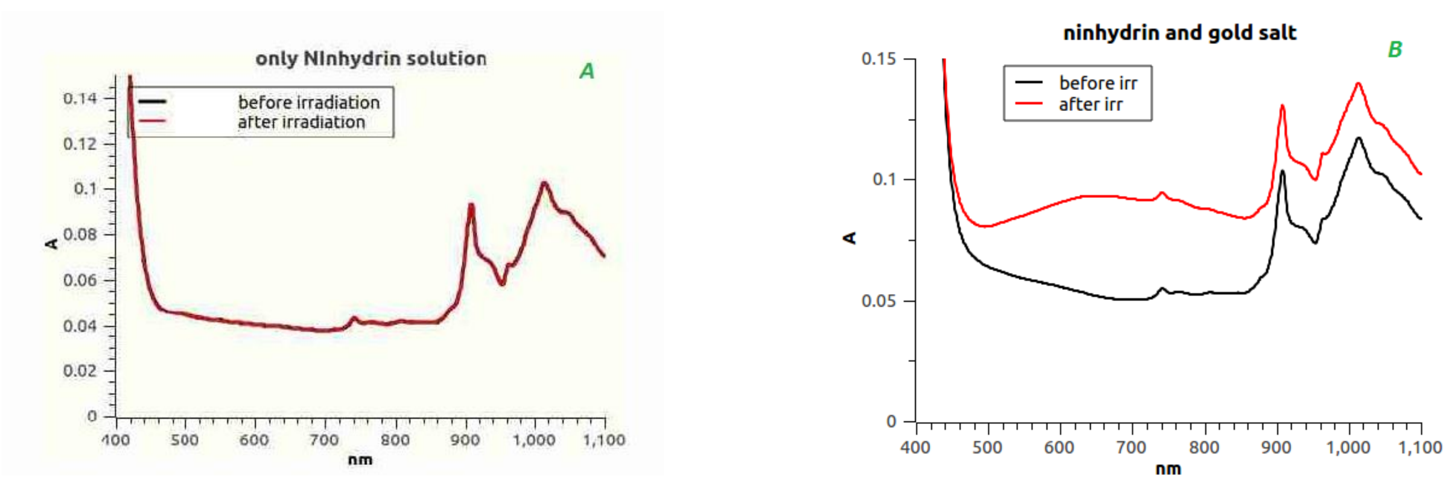
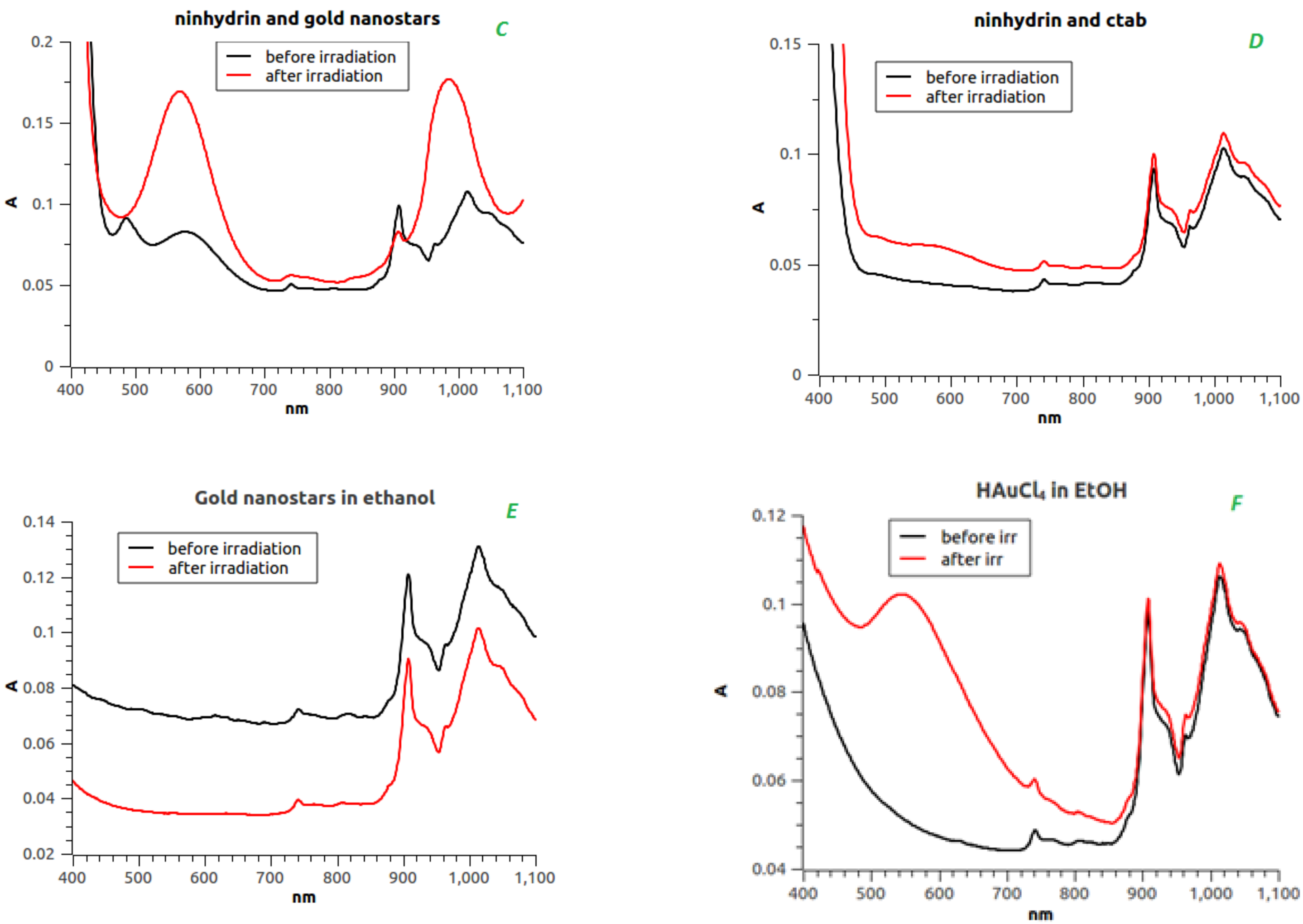


Fig. 1: Absorption spectra of Gold Nanostars (GNS) in samples 1, 2, and 3




ninhydrin and gold nanostars
C
before irradiation
after irradiation
A
nm
ninhydrin and ctab
D
before irradiation
after irradiation
A
nm
Gold nanostars in ethanol
E
before irradiation
after irradiation
A
nm
HAuCl4 in EtOH
F
before irr
after irr
A
nm

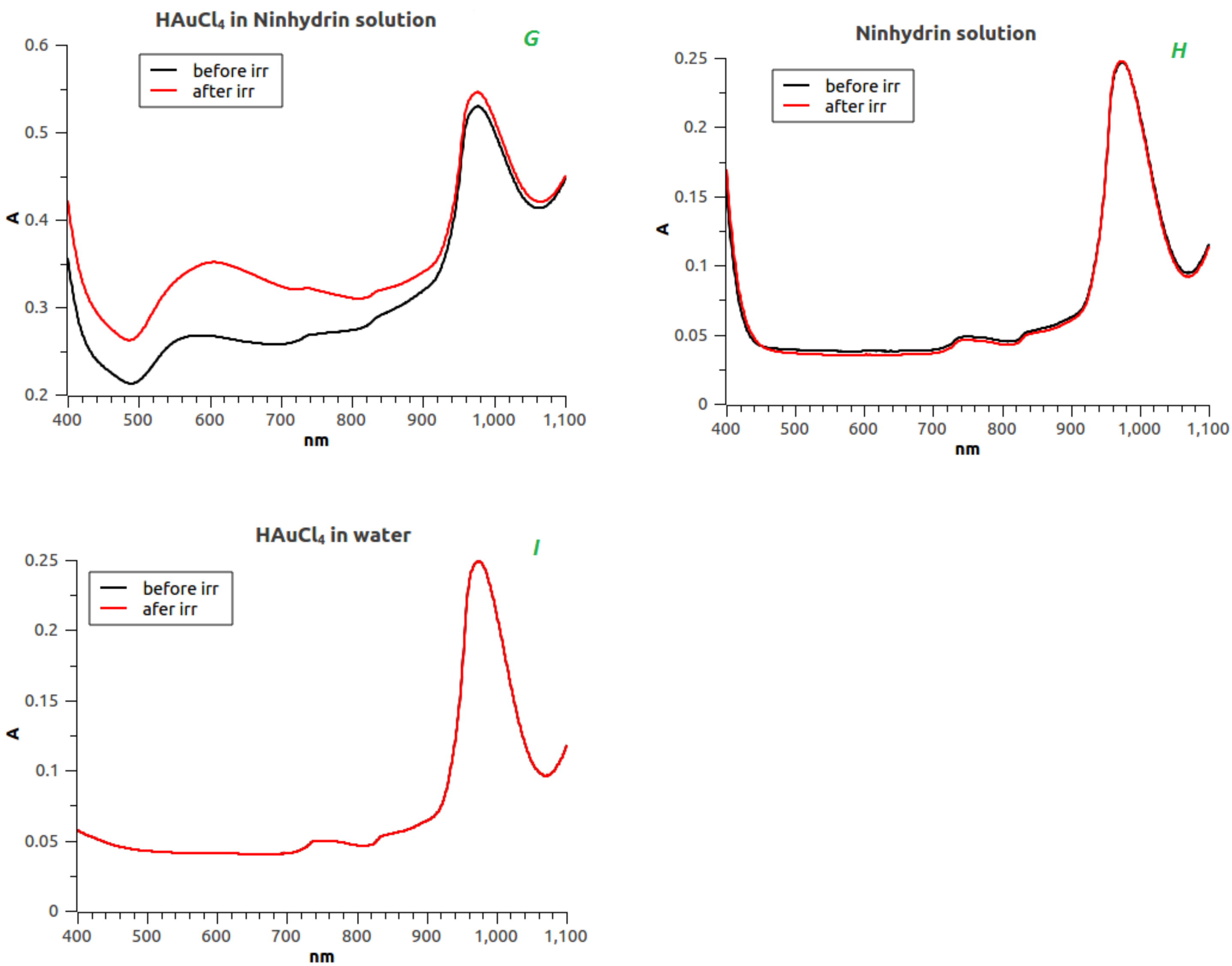


Fig. 2: Absorption Spectrum of Reagents A,B,C,D,E,F,G,H, and I before and after Photoirradiation

It was observed from Fig. 2 that the reagents B and G deviate around 600 nm, while C peaks distinctively at 567.25 nm and 983.9 nm. Though it is tough to say about the chemical reaction happening, ATR-FTIR of reagents will ensure that ninhydrin does not form Rhumann purple in the absence of amino acids. Therefore, through these experiments, we achieved the functionalization of gold nanostars with ninhydrin corroborated by the deviation in the spectrum obtained in a mixture of GNPs and ninhydrin irradiated with UV light. It prepares them as carriers to take up amino acids for targeted delivery or germicidal action.

References

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