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# Determination of 6-thioguanine based on localized surface plasmon resonance of gold nanoparticle

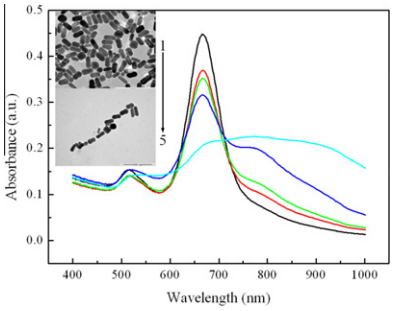
Ning Bi [a,*], Meihua Hu [b], Hongyu Zhu [a], Haibo Qi [c], Yuan Tian [c], Hanqi Zhang [c]

[a] School of Physical and Chemical, Henan Polytechnic University, Jiaozuo 454000, PR China
[b] School of Materials Science and Engineering, Henan Polytechnic University, Jiaozuo 454000, PR China
[c] College of Chemistry, Jilin University, Changchun 130012, PR China

## HIGHLIGHTS

- 6-TG was determined based on localized surface plasmon resonance (LSPR) using gold nanoparticles as the probe.
- The proposed method is simple and sensitive.
- The sensitivity of gold nanorods was higher than gold nanospheres.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Gold nanoparticles exhibit the optical properties of localized surface plamon resonance (LSPR) and are widely applied to the biosensors. The application of gold nanoparticles to the determination of anticancer drug 6-thioguanine (6-TG) was discussed. The binding of 6-TG molecule to the surface of gold nanoparticles alters the local refractive index in the vicinity of the nanoparticles and results in a shift of the LSPR spectrum. The experimental conditions were examined and optimized. Under the optimal conditions, the ratios of absorbances at two wavelengths are directly proportional to the concentrations of 6-TG. The developed method is simple, rapid, and sensitive. In addition, this method is particularly attractive because organic cosolvents, light-sensitive dyes, and sophisticated instruments are not required. This method was successfully applied to the determination of 6-TG in real samples and the results were satisfactory.



## Introduction

Noble metal nanoparticles exhibit striking optical properties due to the phenomenon of localized surface plasmon resonance (LSPR) [1,2]. LSPR arises from the resonant oscillation of conduction electrons on the surface of metal nanoparticles. At certain wavelengths of incident light, conduction electrons are set into resonant oscillation. The energy associated with this resonance is related to the composition, size, shape, and the surrounding dielectric environment of the nanoparticle [3–7]. The location and intensity of LSPR peak are sensitive to the local refractive index (RI) surrounding the nanoparticles. Analyte molecules binding to nanoparticles can alter the local RI in the vicinity of the nanoparticles and result in a shift of the LSPR spectrum. This shift can be described by measuring the change either in the peak intensity or in the peak location [8].

Among various nanoparticles, gold and silver nanoparticles have been extensively used for their remarkably optical properties which can be easily tuned by tailoring the size and shape of the nanoparticles. 10–100 nm gold nanoparticles exhibit a plasmon resonance at optical frequencies. Consequently, such nanoparticles exhibit a characteristic LSPR spectrum. The gold nanoparticles show one or more absorption peaks in the visible or near-infrared

* Corresponding author. Tel./fax: +86 391 3987811.
*E-mail address:* bining@hpu.edu.cn (N. Bi).

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(NIR) regions. For the spherical gold nanoparticles dispersed in water, the scattering and absorption spectra show sharp and narrow peaks at around 520 nm. For gold nanoshells, nanocages, and nanorods the spectra show the strong absorption properties in the NIR [9–12]. Gold nanorods show two plasmon resonance bands, the transverse plasmon band located at about 520 nm and the longitudinal plasmon band located at the NIR. The former is located in the visible region of the absorption spectrum, and the latter is related to the aspect ratio and changes from the visible to the NIR region of the absorption spectrum. The longitudinal absorption band is very sensitive to the aspect ratio. The longitudinal absorption band shifts to longer wavelength and the absorption intensity increases with the increase of the aspect ratio.

It is well known that purine compounds, such as adenine and guanine, are metabolites of nucleic acids and very important in the synthesis of DNA and RNA. The medicines containing purine compounds have been used to cure cancer. The anticancer drug 6-thioguanine (6-TG) is one kind of purine compound and used to influence immune response. Some methods have been reported for the determination of 6-TG, including voltammetry [13], fluorimetry [14], luminescence analysis [15], HPLC [16–20], and electrochemical method [21]. However, there are still some limits in these methods. One important limit of HPLC and spectrometric techniques is that 6-TG lacks sufficient UV absorption and thus to select a suitable mobile phase and a suitable reactant is required, which obviously increased the costs and analytical complexity. In addition, in the fluorimetry many substances interfere with the determination. 6-TG can be bound to the surface of gold nanoparticles strongly by Au–S. Therefore, to develop a powerful tool for the recognition and determination of 6-TG, the investigation of the interaction between 6-TG and gold nanoparticles is important.

In this work, the interaction between gold nanoparticles and 6-TG was investigated. Based on the LSPR of gold nanoparticles, the 6-TG was determined by absorption spectrometry [22,23]. The application of the proposed method in real samples was investigated, and the experimental results demonstrated the ability of gold nanoparticles as a new probe for determination of 6-TG in real samples. The experimental results also indicated that the proposed method has some advantages in sensitivity, simplicity, rapidity and stability.

## Experimental section

### *Materials*

Tetrachloroauric acid ($HAuCl_4{\cdot}4H_2O$, 99.99%), trisodium citrate dihydrate, sodium borohydride ($NaBH_4$, 99%), silver nitrate ($AgNO_3$, 99%), ascorbic acid (AA, 99.7%), cetyltrimethylammonium bromide (CTAB, 99%), goat serum, cysteine, glutathione, dithiothreitol and 6-TG were purchased from Beijing Ding Guo Biotech. Co. Ltd., China. BR buffer solution was used to control the acidity of the interaction system. Aqua regia solution was used to clean the glassware. Other chemicals used here are of analytical reagent grade and all the solutions used in this study were prepared with ultrapure water.

### *Equipments*

Absorption spectra were recorded on an Australian GBC Cintra 10e UV–vis–NIR spectrometer within the wavelength range from 400 to 1000 nm. The TEM image was obtained with a Hitachi H 800 transmission electron microscope operated at an accelerating voltage of 200 kV. The sample was prepared by dropping the gold nanoparticle solution on the carbon-coated copper grid and dried at room temperature.

### *Preparation of gold nanoparticles*

All glassware used for preparation of gold nanoparticles was thoroughly cleaned in aqua regia for 24 h, rinsed with ultrapure water and then dried in the oven prior to use.

### *Preparation of gold nanospheres*

Gold nanospheres were synthesized based on the reduction of $HAuCl_4$ with sodium citrate following the procedure described by Yu and coworkers with slight modification [24]. In a 250 mL round-bottom flask equipped with a condenser, 100 mL of 0.01% $HAuCl_4$ was heated to the boil with vigorous stirring. 3 mL of 1% sodium citrate was rapidly added into the boiling solution and the color of the solution changed from pale yellow to red–violet. The solution was kept in boiling for 10 min. Then the heating stopped, and the stirring continued for an additional 15 min. The solution was cooled down to room temperature, and filtered through a 0.22 μm filter membrane and stored at 4 °C in dark bottles. The resulting solution of gold nanospheres had an absorption maximum at 520 nm. The transmission electron microscope (TEM) image showed that the size of the gold nanospheres had a narrow distribution and the mean diameter of the gold nanospheres was about 18 nm.

### *Preparation of gold nanorods*

CTAB-stabilized gold nanorods were synthesized by the seed-mediated growth method improved by El-Sayed and coworkers [25,26]. The seed solution was prepared by mixing 5 mL of 0.2 mol $L^{-1}$ CTAB, 5 mL of 0.5 mmol $L^{-1}$ $HAuCl_4$ and 0.6 mL of freshly prepared ice-cold 10 mmol $L^{-1}$ $NaBH_4$. The solution was vigorously stirred continued for 2 min. The color of the solution changed from dark yellow to brownish yellow immediately after adding $NaBH_4$, which indicated the formation of the gold seeds. The resulting solution stood for 2 h at 25 °C and was used for the synthesis of gold nanorods.

In a flask, 50.0 mL of 0.2 mol $L^{-1}$ CTAB was gently mixed with 1.0 or 2.0 mL of 4 mmol $L^{-1}$ silver nitrate aqueous solution, and the resulting solution was referred as to the growth solution. Then 50.0 mL of 1 mmol $L^{-1}$ $HAuCl_4$ and 0.7 mL of 0.1 mol $L^{-1}$ AA were added into the growth solution with gently mixed. Finally 120 μL of the seed solution was added into the growth solution. The color of the solution gradually changed within 10–20 min. The solution was kept at 27–30 °C for 24 h. The solution was then centrifuged at 14,000 rpm for 20 min twice to remove the excess CTAB and the deposition containing the rods was redispersed in ultrapure water via the ultrasound to obtained gold nanorod solution. When 1.0 and 2.0 mL of silver nitrate solution were used, the obtained aspect ratios of the gold nanorods are 2.2 and 3.0, respectively.

### *Determination of 6-TG*

The stock solution of 10 mmol $L^{-1}$ 6-TG was prepared by dissolving 6-TG in ultrapure water. The working solution of 6-TG was prepared by diluting the stock solution with ultrapure water. First, gold nanoparticle solution was mixed with BR buffer solution. Then, the working solution of 6-TG was added into the mixture solution. The resulting solution was allowed to stand for appropriate time at room temperature and the absorption spectra of the solution were recorded with 1 cm path-length cell.

### *Preparation of sample solutions*

A kind of commercial tablet containing 6-TG was accurately weighed, ground and transferred into a 100 mL flask. 10 mL of 0.01 mol $L^{-1}$ NaOH was added into the flask and the flask was ultrasonically shaken for about 15 min. The solution was diluted