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Article

Assessment of Disulfide-linked Gold Nanoparticles as Colorimetric Sensor for Human Chorionic Gonadotropin Detection

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Abstract – **Gold nanoparticles (AuNPs) are a highly versatile compound to be used in sensing components due to their unique physicochemical and optical properties. Due to its flexibility for surface conjugation, glutathione disulfide-linked gold nanoparticle (AuNP-GSSG) was produced in this study to assess its sensing performance as a colorimetric sensor for human chorionic gonadotropin (HCG). Spectroscopic characterization of AuNP-GSSG confirmed the conjugation of GSSG onto the surface of AuNPs. AuNP-GSSG also showed high sensory performance with good linearity and low detection limit. However, due to the high percentage of interference, usage of AuNP-GSSG as an HCG sensor requires further pre-treatment step to reduce the interference and increase the sensor accuracy.**

Keywords – **gold nanoparticles, human chorionic gonadotropin, sensor**

I. Introduction

Nano metallic compounds often exhibit a particular set of unique properties that can be used for a diversity of applications. Featuring gold nanoparticles (AuNPs), researchers reported multitudes of advantageous properties such as dimension-related optoelectronic properties and the large surface-to-volume ratio [1], [2]. The toxicity of AuNPs was stated to be hugely dependent on the ligands attached while the central gold itself possessed no toxicity, thus showed high biocompatibility and low toxicity. Moreover, AuNPs also exhibit interesting optical properties; surface plasmon resonance (SPR) which occurred due to collective vibration of conduction electrons on the surface of the nanoparticle by the incident photons [2], [3]. SPR phenomenon can be observed through UV-vis spectroscopy, shown by surface plasmon band with peak maxima that can be found in 500 – 550 nm range.

AuNPs also have plenty of surface functionalization opportunities as surface stabilization provided binding sites for different types of ligands, each serving its purposes. Oftentimes, surface functionalization was used to develop a variety of chemical sensors for biochemical compound detections, namely DNA, tumor markers, toxins, and hormones [4]–[7]. The detection mechanisms often used to convey the presence of the analytes were SPR and fluorescence quenching properties of AuNPs as the optical responses generated were directly correlated by the number of analytes used during the study, thus making AuNP-based sensor an excellent detection compound.

This study focuses on the detection of human chorionic gonadotropin (HCG) that is generally used in pregnancy testing and oncology diagnostics. HCG is a hormone discharged by a female reproductive system in the beginning period of pregnancy as the zygote successfully implanted itself into the endometrium wall [8]–[10]. Therefore, HCG detected in a bodily sample, such as blood and urine can indicate the starting of the pregnancy [11]. However, abnormal HCG level in the body; including the confirmed presence of HCG in the male body is a discomforting indicator of a cancer diagnosis. Abnormal level of HCG in the female body along with other identified tumor markers in the female body can be a benchmark of gestational trophoblastic disease, a category of benign to malignant growth in the reproductive system [12]. On the other hand, the detected presence of HCG in the male body is a disquieting diagnosis of testicular cancer [13]. Detection of HCG in a medical field used sandwich enzyme-linked immunosorbent assay (ELISA) that comprises an anti-HCG antibody equipped with the capture and labeling enzyme [14]–[16]. Sandwich ELISA assay involves usage of capture and detecting antibody to specifically target and bind onto HCG, and marking agent to provide

electrochemical, electromagnetic radiation or fluorescence signals in response to the presence of HCG. Till recently, previous researches showed the development of sensors revolved around enzymatic reaction as the acting sensing mechanism [16]–[18]. Using antibody and enzymatic reaction, several AuNPs-based sensors developed as an immunoassay test unit and fluorometric markers with high sensitivity and specificity. Yet, usage of monoclonal antibodies, polymeric dendrimer, and peptide aptamer for sensing moiety incurred high procurement and production cost. Albeit the setbacks, disulfide-linked AuNPs can be utilized as a low-cost replacement for the sensing component. Disulfide bonds altered the aggregation state of AuNPs, in turn linearly gave out the corresponding colorimetric responses [19].

Therefore, this research went on another approach using produced AuNPs and introduced disulfide links to utilize the measurable changes in the aggregation state of AuNPs in presence of HCG. This research fully used the optical response of AuNPs with disulfide links upon the presence of HCG in solution. Furthermore, the performance aspect of AuNPs as HCG sensor was also assessed in an aspect of its linearity, sensitivity, and selectivity.

II. Experimental

A. Chemical and reagents

Chemicals used for the preparation and synthesis of AuNPs were hydrochloric acid (HCl), nitric acid (HNO_3), gold chloride ($AuCl_3$), and trisodium citrate ($Na_3C_6H_5O_7$), all obtained from Merck (Selangor, Malaysia). Meanwhile, chemicals used to coat the surface of AuNPs were 3,3'-dithiodipropionic acid (DTDPA), dimethyl sulfoxide, 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDC HCl), N-hydroxysuccinimide (NHS), and glutathione disulfide (GSSG), which were obtained from Aladdin Chemicals (Shanghai, China). Moreover, HCG standard and dithiothreitol (DTT) and sodium chloride (NaCl) for the sensory study were obtained from Merck (Selangor, Malaysia).

B. Synthesis of AuNPs and their sensory counterpart

AuNPs used in this research were synthesized using the altered Turkevich method, followed by adapting a reported procedure to functionalize the AuNPs for HCG sensing [7], [19], [20]. Every glassware used was cleaned using aqua regia (4:1 volumetric ratio of HCl: HNO_3) to remove any existing impurities on the surface of the glassware. The AuNPs were produced using a lower concentration of the reagents (1.0 mM $AuCl_3$, 1.0% $Na_3C_6H_5O_7$) and recommended reaction temperature (100°C). 20 ml of 1.0 mM $AuCl_3$ was constantly stirred and heated to 100°C. 2 ml of 1.0% $Na_3C_6H_5O_7$ was later added into the heated solution and continued stir. The reaction stopped when the color of the solution turned from pale yellow to dark red. Resulting AuNP was stored in 4°C storage before further uses.

Upon completing synthesizing AuNPs, the AuNPs were then treated with 1.0 ml of 3.0 mM DTDPA in DMSO and shaken for 1 hour in a 30°C water bath. The mixture was then mixed with 1.0 ml of 0.1 mM EDC HCl and 1.0 ml of 0.1 mM NHS, followed by similar shaking in a 30°C water bath for 1 hour. Consequently, the resulting solution was mixed with 1.0 ml of 1.0 mM GSSG and incubated at 30°C for another 1 hour. Final functionalized AuNP with GSSG (AuNP-GSSG) was used without any further purification.

C. Characterization and evaluation of AuNPs and their sensory counterpart

SPR absorption band of AuNP was observed using Varian Cary 50 UV-Vis spectrophotometer with the wavelength in the range of 300 – 800 nm with 1.0 nm resolution. Meanwhile, Varian Excalibur 3100 Fourier transform infrared (FTIR) spectrometer was used to observe the changes in the molecular structure of citrate ion.

The performance of AuNP-GSSG as an HCG sensor was verified through studying the linear range, sensitivity, and selectivity towards the presence of HCG using UV-vis spectroscopy. The linearity of the sensor was tested using a series of dilutions (0.1 – 0.5 ppm) and analyzed its absorbance value corresponding to the increasing concentrations of HCG. The testing was done using 2.5 ml of the sensor, added with 100 µl of each concentration of HCG. The interference effect from impurities was investigated by determining the changes that occurred upon adding the impurities. The impurities used in this research were 0.1 ppm solutions of DTT and NaCl. 50 µl of NaCl solution was added into the analysis solution (2.5 ml of AuNP-GSSG, 100 µl of 0.1 ppm HCG), followed by UV-vis spectroscopy analysis. Another 50 µl of NaCl solution was added into the same solution to reach the final volume of 100 µl, then analyzed again for its optical response. The procedure was repeated using DTT solution, instead of NaCl solution.

III. Results

A. Characterization of synthesized AuNPs and AuNP-GSSG

Synthesis of AuNPs *via* citrate reduction of $AuCl_3$ was proven successful as UV-vis spectroscopy revealed maximum absorbance (Abs_{max}) in the UV-vis spectrum (Figure 1) occurred at wavelength 518 nm. Abs_{max} achieved aligned with previous findings that showed SPR properties of AuNPs can be observed in the range of 500 – 600 nm [1], [3], [5], [21]–[23].

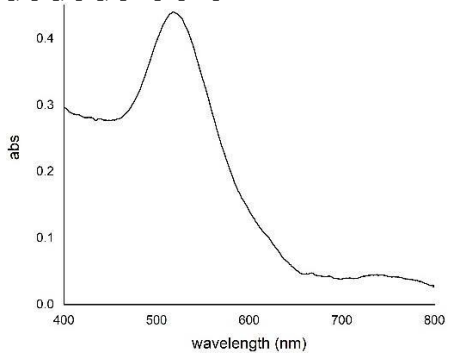

Figure 1. UV-vis spectrum of AuNP with Abs_{max} at 518 nm

Surface stabilization of AuNPs using citrate ion reduced the vibrational frequency of sodium citrate. Referring to *Figure 2*, the peaks at 2978, 1388, and 1157 cm^{-1} on the IR spectrum of sodium citrate diminished after the synthesis. The overall intensity of the IR spectrum of sodium citrate also dropped. Five assigned IR spectrum peaks of sodium citrate described the stretching C–H bonds (2978 cm^{-1}), O–H bonds (3363 cm^{-1}), C=O bonds (1635 cm^{-1}), bending C–H bonds (1388 cm^{-1}), and C-O bonds (1157 cm^{-1}). The peaks are shown in the IR spectrum of AuNPs only described the O–H bond and C=O bonds bonded onto the surface of AuNPs.

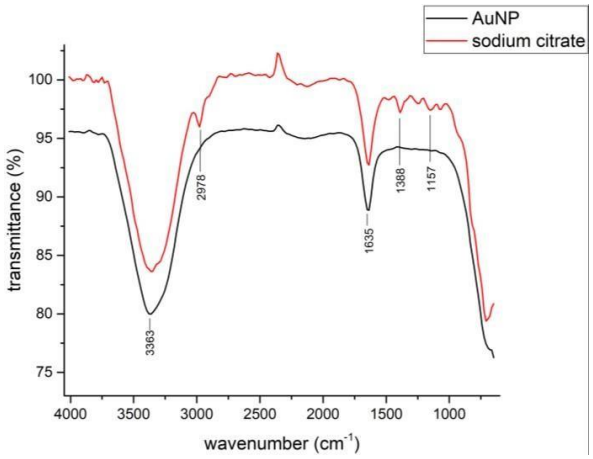

Figure 2. IR spectra of AuNPs and sodium citrate in distilled water

As the FTIR spectrometer incapable to perceive the changes in the molecular structure of the GSSG-conjugated AuNPs, the UV-vis spectroscopy was used to determine the presence of intended sensing moiety [19], [24]. The UV-vis spectra of the produced AuNPs and GSSG-conjugated AuNPs (Figure 3) shown red shifting from 518 nm to 715 nm due to successful conjugation of GSSG to the surface of AuNPs. It was caused by a significant change in the aggregation state of the AuNPs itself, resulting in shifting or diminishing of the SPR band [25].

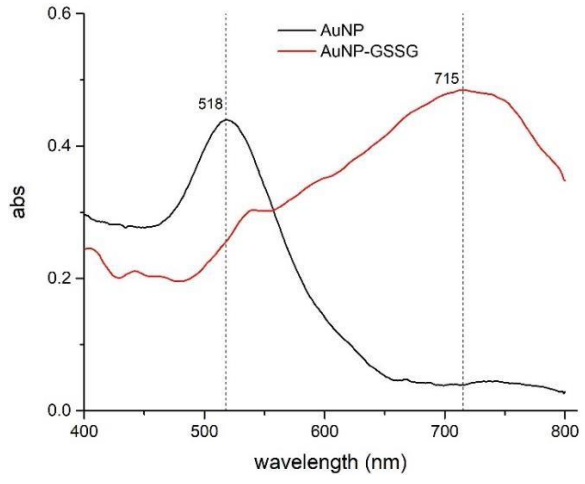

Figure 3. UV-vis spectra of produced AuNPs and GSSG-functionalized AuNPs

B. Performance assessment of AuNP-GSSG as HCG sensing material

From Figure 4, AuNP-GSSG showed a minimal response to the presence of HCG as the Abs_{max} of the SPR band blue-shifted from 715 nm to 701 nm. The minimal response was due to the high molecular weight of the HCG molecule that reacted to disulfide bonds at a lower rate, thus showed slight blue shifting [19], [25].

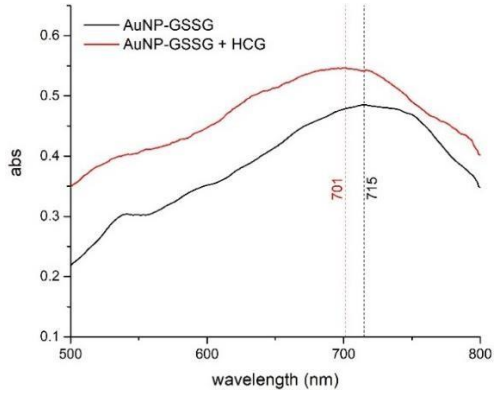

Figure 4. UV-vis spectra of the AuNP-GSSG and the response upon introduction of HCG into AuNP-GSSG

Linearity responses of AuNP-GSSG to the presence of HCG were observed at 701 nm as shown in Figure 5. The linear graph of the absorbance versus the concentration of HCG has been plotted along with its regression equation and its coefficient of determination (R^2). As shown, a good linear relationship can be observed between the absorbance and the HCG concentration using the regression equation of $y = 0.26505x + 0.28679$ with $R^2 = 0.9877$. The sensitivity of the sensor also calculated using the linear plot to find the limit of detection (LOD), which gave out LOD = 0.1577 ppm. The resulting LOD value shown that AuNP-GSSG can be purposed as a sensor to detect HCG in clinical samples without impurities as the lowest level HCG found in clinical samples was 0.2021 ppm [11]. Despite the excellent sensitivity, AuNP-GSSG was found to be less sensitive to HCG in lower concentrations as previous studies have shown even lower LOD values [18], [26].

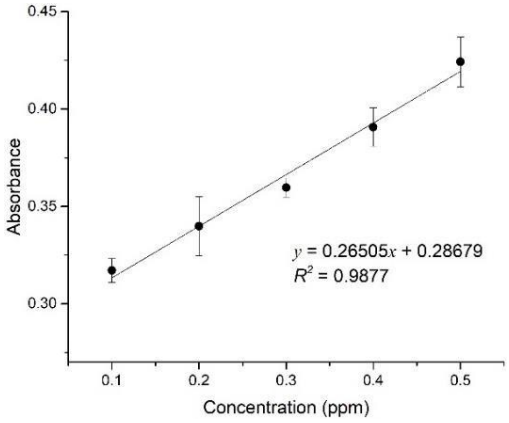

Figure 5. Linear plot of absorbance of AuNP-GSSG responses to HCG versus the concentration of HCG

The selectivity of AuNP-GSSG to HCG was studied via an interference study by adding impurities into the along with the calculation concentration of HCG and the percent of interference. It was observed that the percentages of interference exceeded the 5% threshold to declare the sensor as a good sensor [27]–[29]. The addition of NaCl and DTT interfered with the signal from the analyte solution [27]. The optical responses of AuNP- GSSG were tabulated in

10.08% to 37.67%, showing the impurities highly affected the detection of HCG. Thus, detection of HCG using AuNP-GSSG as the intended sensing component requires pretreatment of the sample beforehand to reduce the interference from the impurities.

TABLE I
PERCENTAGE OF INTERFERENCE FROM THE INTERFERENT TO ITS ADDED VOLUME INTO HCG SOLUTION (100 µL, 0.1 PPM)

Interferents Added	**The volume of Interferents Added (µl)**	**Absorbance**	**Calculated Concentration (ppm)**	**Percent of Interference (%)**
-		0.3171	0.1144	0.00
	50	0.2852	0.0062	10.08
	100	0.2462	0.1533	22.37
	50	0.2145	0.2727	32.35
	100	0.1977	0.3363	37.67

IV. CONCLUSION

As a conclusion to the study, AuNP produced and characterized using the analytical spectroscopic method was suitable for the development of the intended HCG sensor. Conjugation of AuNPs with GSSG for HCG sensing also successfully done like the red shifting of the SPR band confirmed the presence of GSSG-conjugated AuNPs. In terms of the sensing performance, AuNP-GSSG showed decent linearity ($R^2 = 0.9877$) with a low limit of detection (LOD = 0.1577 ppm). Albeit the spectacular detection capability, the selectivity of AuNP-GSSG was too diminutive from the interference study and requires further pre-treatment of the sample.

V. ACKNOWLEDGMENT

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