CHAPTER 5

CHARACTERIZATION OF DISULFIDE-LINKED GOLD NANOPARTICLES AS GOLD NANOPARTICLE-BASED SENSOR

5.1 Experimental

5.1.1 Materials

The performance evaluation study was carried out using HCG standard, while sodium chloride (NaCl) and dithiothreitol (DTT) were used for selectivity evaluation. All chemical used was obtained from Merck (Selangor, Malaysia).

5.1.2 Linearity

Linearity study of the sensor used dilutions of HCG standard, ranging from 0.1 to 0.5 ppm of HCG. The analysis was done using 100 µl of every concentration of HCG standard with fixed 2.5 ml of AuNP-based sensor and analysed using UV-Vis spectroscopy. Optical responses of the sensor to the amount of HCG in solution were plotted in a linear plot of absorbance versus concentration of HCG.

5.1.3 Sensitivity

Sensitivity of sensor were studied by determining its limit of detection (LOD) and limit of quantification (LOQ), which calculated using the equations below in Microsoft Excel (Microsoft, USA) software.

$$LOD = 3.3 \times \frac{\sigma_{linear\ plot}}{m_{linear\ plot}} \quad \text{Eq. 8}$$

$$LOQ = 10 \times \frac{\sigma_{linear\ plot}}{m_{linear\ plot}} \quad \text{Eq. 9}$$

where, $\sigma_{linear\ plot}$ is the standard deviation of the linear plot, and $m_{linear\ plot}$ is the gradient of the linear plot.

5.1.4 Selectivity

Determination of the selectivity of the sensor was done using interference study adapted from Muhammed Maasus (2019). The interference study was carried out by analysing the optical response from 2.5 ml of sensor mixed with 100 µl of 0.1 ppm HCG without interferent added. Then, 50 µl of 0.1 ppm NaCl was introduced into the sensor-HCG mix to obtain its optical response. Upon completion, another 50 µl of 0.1 ppm NaCl was added to achieve the 100 µl of NaCl in the analyte solution and its optical responses were recorded. The procedure was then repeated with 0.1 ppm DTT solution replacing NaCl solution. The interference percentage was calculated using the equation below

$$\%\ \text{interference} = \left| \frac{Abs_{standard} - Abs_{test}}{Abs_{standard}} \right| \times 100\% \quad \text{Eq. 10}$$

5.2 Results and discussions

5.2.1 Linearity and sensitivity

The response of AuNP-GSSG to HCG as AuNPs-based sensor were measured via UV-Vis spectroscopy as the colour change upon adding HCG solution into AuNPs-based sensor was not significantly visible through naked eyes. From the UV-Vis spectra

obtained as shown in Figure 5.1, the peak shifting can be observed as changes upon introducing HCG into the sensor.

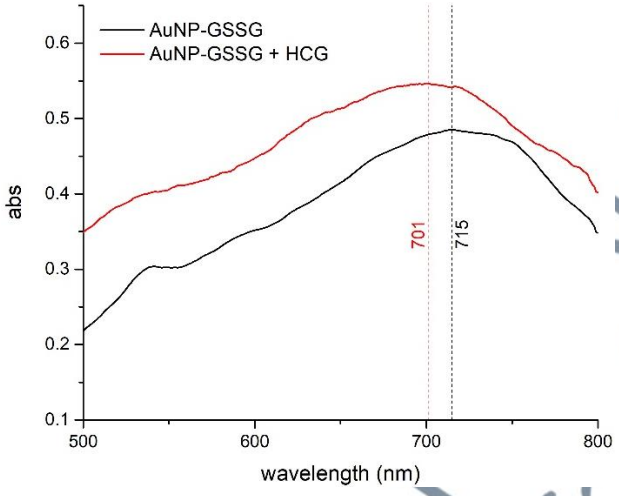

Figure 5.1: UV-Vis spectra of AuNP-GSSG as AuNPs-based sensor along with the optical response upon introducing HCG into sensor solution

Figure 5.1 shows the UV-Vis spectra of AuNP-GSSG functioning as AuNPs-based sensor with its optical response upon addition of HCG. It was observed that the peak maxima had blue shifted by 14 nm in response to addition of HCG. Minimal blue shifting effect was also observed by Durocher et al. (2009) as the peak shifting was affected by the size or molecular mass of the targeted molecule. GSSG-functionalized AuNPs was found to react at significantly lower rate and showing minimal peak shifting when subjected to higher-weighted thiol compounds. Usage of HCG with far more higher molecular weight than the samples used by Durocher et al. (2009) in this study explained the minimal to no visible colour change.

Following the UV-Vis spectra in Figure 5.1, the linear correlation plot was constructed using the absorbance of AuNP-GSSG in presence of HCG at the selected wavelength of 701 nm with varied concentrations. Figure 5.2 shows the linear plot constructed from the UV-Vis spectroscopy data obtained.

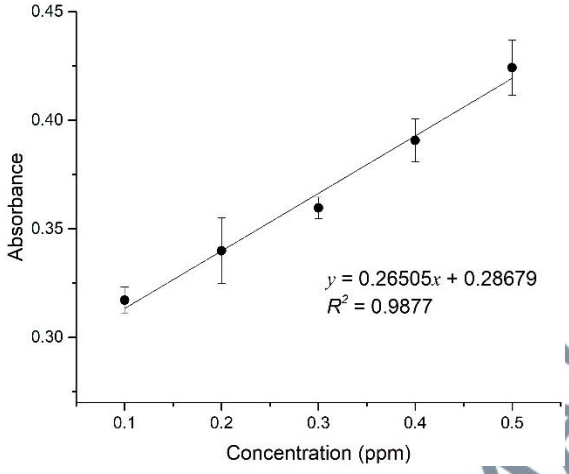

Figure 5.2: Linear plot of absorbance of AuNP-GSSG response to HCG versus the concentration of HCG

Figure 5.2 shows the linear plot of AuNP-GSSG response to HCG in form of absorbance versus the concentration of HCG used. From the spectrometric data collected from the UV-Vis spectroscopy analysis, a linear function of $y = 0.26505x + 0.28679$ with decent linearity ($R^2 = 0.9877$) was constructed to depict the linearity of the response of AuNP-GSSG to HCG. In the sensitivity aspect, both LOD and LOQ calculations were derived from the same spectrometric data obtained as the aforementioned linearity analysis. LOD and LOQ calculations yielded the value of 0.1577 ppm (3.9008 mIU/ml) and 0.4778 ppm (11.8186 mIU/ml). Cole (2015) stated HCG can be detected as low as 5 mIU/ml (0.2021 ppm) in human body, making the sensor capable to detect the lowest limit of HCG from bodily samples. Several previous studies had proven to surpass both the linearity and sensitivity of the functionalized AuNPs as shown in Table 5.1.

Table 5.1: List of recently developed AuNPs-based HCG sensor along with its R^2 and LOD value

Author (Year)	Detection method	Sensing moiety	R^2	LOD (ppm)
Zhang et al. (2019)	Spectrophotometry	Monoclonal antibody	0.9872[a] 0.9831[b]	0.3638
Wang et al. (2018)	Spectrophotometry	Polyamidoamine dendrimer	0.996	0.0012
Cao et al. (2017)	Amperometry	Monoclonal antibody	0.991	0.0146
Xia et al. (2017)	Colorimetry	Peptide aptamer	0.98	0.6064
Chang et al. (2014)	Colorimetry	Peptide	0.98	1.0107

[a]Low concentration (0.3638 – 11.6432 ppm); [b]High concentration (11.6432 – 93.1454 ppm)

Table 5.1 shows the performance of AuNPs-based sensor for HCG detection in terms of its linearity and sensitivity, along with its detection method. Recent studies shown every developed sensor resulted in decently high linearity ($R^2 > 0.9$) regardless of its detection method or sensing moiety. However, high sensitivity of sensing performance were shown by usage of polymeric dendrimer and monoclonal antibody by Cao et al. (2017) and Wang et al. (2018). Both sensing components also shown higher sensitivity to HCG compared to previous studies and this research.

5.2.2 Selectivity

Selectivity of the sensor was evaluated from the interference study by calculating its percentage of interference of each interferent using Equation 10. The calculated percentage of interference is tabulated in Table 5.2.

Table 5.2: Percentage of interference from the introduced interferent with respect to its added volume into 100 µl of 0.1 ppm HCG solution.

Interferent	Volume added (µl)	Absorbance test	Measured concentration (ppm)	Percentage of interference (%)
-	-	0.3171*	0.1144	0.00
NaCl	50	0.2852	0.0062	10.08
	100	0.2462	0.1533	22.37
DTT	50	0.2145	0.2727	32.35
	100	0.1977	0.3363	37.67

*Absorbance standard

Table 5.2 shows the percentage of interference from the interferent along with its added volume into 100 µl of 0.1 ppm HCG solution. The result showed high interference from NaCl and DTT. High percentage interference exceeding 5%, as agreed by Harmita (2004), Muhammed Maasus (2019) and Wulandari et al. (2013), did not qualify the sensor as a good chemical sensor. High percentage interference also indicated the sensor would not give out good detection results from real clinical samples, which may contain one of the interferents if not both. Putnam (1971) proved salt content reached 14157 ppm in typical human urine, especially NaCl at 8001 ppm. Higher NaCl content in urine can heavily interfere with detecting of HCG in urine as clinical sample. Meanwhile, DTT have been known to assist on myeloma treatment by denaturing antigens that delay the timely drug delivery *via* blood transfusion. Barrientos-Soto et al. (2017) mentioned DTT was able to denature the Kell antigen and other antigens that can cause haemolytic transfusion reaction. The target CD38 antigen also highly susceptible to denaturing by DTT without any implications onto other important clinical antigens. DTT also shown to cleave the disulfide bonds with higher rate than proteins as discussed by Durocher et al. (2009). From the aforementioned findings by Barrientos-Soto et al. (2017) and Durocher et al. (2009), it can be concluded

that as low as 10 μg of DTT in 50 μl solution, DTT were able to interfere with the detection of HCG using disulfide linked functioned sensor. Aside from high interference resulted from NaCl and DTT, the epitope of HCG did not respond readily to disulfide linkage as disulfide linkage was not as selective as antibody-antigen reaction. Birken et al. (1988) and Cole (1997) agreed that subunits of HCG responded specifically to antibodies, namely monoclonal anti-HCG antibody. Minimal blue shifting of the UV-Vis spectra was caused by thiol groups in the HCG structure cleaved the disulfide linkages of GSSG, however findings of Durocher et al. (2009) explained that heavier thiol compounds such as HCG cleaved disulfide link with lower rate compared to disulfide linkage cleaving by DTT that has lower molecular mass than HCG. Therefore, presence of DTT can interfere severely with detection of HCG using AuNP-GSSG as sensor.

5.3 Conclusion

Minimal blue shifting from 715 nm to 701 nm showed AuNP-GSSG response to presence of HCG in solution. The responses of AuNP-GSSG to presence of HCG was plotted with decent linearity (R^2 = 0.9877) and sensitivity (LOD = 0.1577 ppm; LOQ = 0.4778 ppm). However, high percent of interference ($\%_{interference}$ > 5%) indicated AuNP-GSSG was unable to be used for real clinical samples that may contain the interferents in higher concentrations.