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# DETECTION OF GLUCOSE USING GOLD NANOPARTICLES PREPARED BY GREEN SYNTHESIS

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**Abstract:** Gold nanoparticles were synthesized using the cost-effective, eco-friendly green synthesis method. In the process, tetrachloroauric acid was used as a precursor and extract from flower leaves of the Hibiscus Tiliaceus plant was used as a reducing agent. Different concentrations of glucose were mixed with the synthesized gold nanoparticles and their effects were investigated. UV-Vis spectrometer was used to record the absorption spectra of the samples. Transmission electron microscopy (TEM) analysis was performed to determine the size and shape of the nanoparticles. The results showed that there is a linear relationship between the glucose concentration and the surface plasmon resonance absorption peak. This relationship occurred in the glucose concentration range of 1-15mM. The ability of this system to detect glucose in complex biological solutions was also investigated, and the results showed that it was still able to effectively detect glucose in the concentration range of 1-15mM.

**Key words:** gold nanoparticles, green synthesis, glucose.

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### 1. INTRODUCTION

It is well documented that there are approximately 422 million diabetics worldwide and the global prevalence of this chronic disease is in continual rise [1]. The treatment for diabetes includes following a

certain diet control agenda, exercising, and monitoring blood glucose levels [2]. The current daily standards for blood glucose monitoring involve glucose oxidation by the enzyme glucose oxidase and subsequent electrochemical

detection [3]. This quantitative method is considered accurate, but it relies on high-cost glucose strips in addition to battery-operated meters [4]. The enzymatic system is also sensitive to temperature and pH changes. Consequently, the glucose strips cannot be stored for long periods of time and undergo degradation upon prolonged exposure to humidity, air, and light [5-6]. Therefore, it is important to search for enzyme-free glucose detection methods.

In recent years, there has been a growing interest in research on nanoparticles due to their potential application in the fields of medicine and optoelectronic devices. These various applications include the use of nanoparticles in drug delivery, diagnosis, cancer treatment, and optical switching [7-11]. Furthermore, nanoparticles exhibit unique optical and photo-stability characteristics [12-13]. Such properties enable the use of these nanoparticles in the development of novel, enzyme-free glucose detection devices [14-18], which can be used for monitoring diabetics' blood glucose levels [19].

Nevertheless, the production of nanoparticles via the widely used chemical and physical methods is generally expensive and poses serious environmental threats [20-22]. The alternative green synthesis method has proven to be inexpensive, efficient, and environmentally safe [23-25]. Among the various routes used in the green synthesis method, the use of plants

is considered to be most cost-effective due to the plant's low cost of cultivation and maintenance [26-27]. *Hibiscus Tiliaceus* is an evergreen herbaceous tree, which contains large yellow flowers [28]. Extract from flower leaves of *Hibiscus Tiliaceus* is opted for the synthesis of gold nanoparticles due to the presence of phenolic hydroxyl groups [29], which act as natural reducing agents.

In this study, we report a novel fabrication of gold nanoparticles using  $\text{HAuCl}_4$  as a precursor and an extract from flower leaves of *Hibiscus Tiliaceus* tree as a reducing agent. The ability of the synthesized gold nanoparticles to detect glucose was also explored. The absorption spectra of the samples were recorded using a UV-Vis spectrometer, and transmission electron microscopy (TEM) was used to identify their morphology and size distribution of the nanoparticles.

## 2. METHODS

### 2.1- Synthesis of gold nanoparticles

The methodology for the preparation of gold nanoparticles using *Hibiscus* plant extract as a reducing agent has been adapted from previous research articles [30-31].

Briefly, leaves of flowers from the *Hibiscus Tiliaceus* were collected from the trees in the university campus. The leaves were washed with tap water and rinsed with distilled water and were left to dry in a 60°C oven for a duration of 24 hours.

Subsequently, the leaves were finely ground. Subsequently, 5.0 g of finely ground hibiscus flower leaves was dissolved in 200mL of double distilled water. Then, the solution was boiled for a duration of 5 minutes. After allowing it to cool down, the solution was filtered using Whatman's No. 1 filter paper.

In order to prepare 1 M of tetrachloroauric acid solution, 0.034 g of  $\text{HAuCl}_4$  was dissolved in 100mL of distilled water and stirred gently for 1-2 minutes. Thereafter, 10 mL of the prepared plant extract was mixed with 2 mL of the prepared tetrachloroauric acid solution. In addition, 10 mL of NaOH (1 M) was added into the plant extract and chloroauric acid mixture. Hence, the mixture was microwaved at 1,250 Watt for 20 seconds. The reduction took place as indicated by the purple colour change of the solution. The solution was found to be stable for a month with no colour changes and showed no precipitation.

### **2.2- Preparation of pure glucose solutions**

All glucose solutions were prepared by dissolving D-glucose in varying volumes of distilled water to obtain each respective glucose concentration (1, 2, 4, 7, 10, 12, 15 mM). Subsequently, 1.0 mL of the prepared gold nanoparticles were added to a series of 1.5 mL Eppendorf tubes. Then, 0.5 mL of each glucose concentration was added into a separate Eppendorf tube and all tubes were placed in a vortexer at speed 3 for a duration of 5 minutes.

### **2.3- Preparation of complex solutions**

According to a number of articles, the most notable reducing agents present in blood serum include ascorbic acid, fructose, galactose, lipoic acid, and glutathione[32]. A stock solution that contains twice the physiological concentrations of these agents was prepared. The next step involved the preparation of new glucose solutions that are twice the concentration of the original glucose solutions (i.e. 2, 4, 8, 14, 20, 24, 30 mM). Subsequently, 1.0 mL of the prepared gold nanoparticles were added to a series of 1.5 mL Eppendorf tubes. Then, 0.25 mL of the stock solution was combined with 0.25mL of each of the newly prepared glucose solutions and added into a separate Eppendorf tube. This produced a solution that contains the same glucose concentrations as the original glucose solutions as well as other serum reducing agents at physiological concentrations. All tubes were placed in a vortexer at speed 3 for a duration of 5 minutes.

### **2.4- Sample characterization**

The absorption spectrum of each sample was recorded using Shimadzu's UV-1800 UV-Vis spectrometer, and the Surface Plasmon Resonance (SPR) band of each sample was identified. The morphology and size distribution of gold nanoparticles (GNPs) were analyzed by transmission electron microscopy (TEM). TEM samples were prepared by depositing a small droplet of colloidal solution onto carbon support TEM grids. Grids

were dried in open air for a period of one hour and installed in the TEM for examination. TEM (FEI, Morgagni 268, Czech Republic) was operated at accelerating voltage of 80 kV and recorded several images for each specimen to perform statistical analysis. The particle size was measured using the Gatan digital software. The size histograms and the average size of the particles were also obtained.

### 3. RESULTS AND DISCUSSION

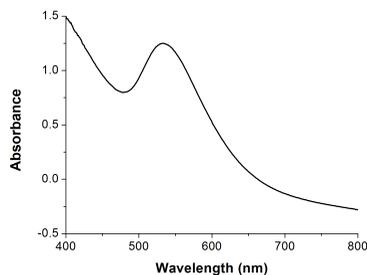
#### 3.1- Formation of gold nanoparticles

The hibiscus flower contains a number of bioactive agents, the most notable ones being the phenolic hydroxyl groups [28]. The dissociation of phenolic hydroxyl groups occurs in alkaline environments ( $pK_{a2} > 8$ ) [33-35]. Hence, with the addition of NaOH, the phenolic hydroxyl groups in the hibiscus extract reduced the oxidation state of  $HAuCl_4$  from a +3 to 0, resulting in the formation of colloidal gold nanoparticles [36].

#### 3.2-Confirming the identity of the synthesized nanoparticles

The identity of the gold nanoparticles was confirmed by recording the absorption spectra over a wavelength range of 400-800 nm. Due to the collective oscillation of free electron in the conduction band, gold nanoparticles demonstrate a characteristic peak surface plasmon resonance (SPR) band, which ranges from 515 to 570 nm [37]. Fig.1 shows UV-Vis

absorption spectra of the synthesized nanoparticles. The observed SPR peaks at a wavelength of 535 nm, which indicates the formation of gold nanoparticles.



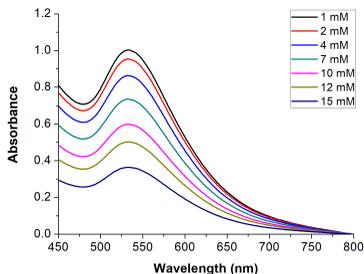
*Figure 1: UV-Vis absorption spectra of colloidal gold nanoparticles synthesized using Hibiscus plant extract.*

Repeated measurements of the absorption spectra were taken up to duration of 1 month. In all these measurements, the characteristic surface plasmon resonance band remained present, which indicates that the synthesized nanoparticles are stable.

#### 3.3-Detection of glucose in pure glucose solutions

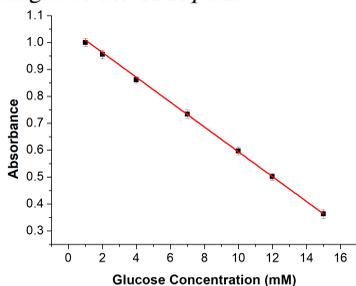
The absorption spectra of gold nanoparticles mixed with different glucose concentrations were recorded over a wavelength range of 450-800nm and are shown in Fig.2. As the amount of glucose increased, the surface plasmon peak at 535nm decreased. The stability of the SPR peak position indicates that the Au nanoparticles do not aggregate possibly due to the interactions of Au with glucose over the range of

concentrations used for this experiment. If the particles aggregate, the SPR band position will shift to a longer wavelength (red shift).



*Figure 2: UV-Vis absorption spectra of gold nanoparticles synthesized using Hibiscus plant extract mixed with different glucose concentrations. The arrow indicates the reference point for the determination of the height of the absorbance.*

Fig.3 shows a negative linear relationship between the glucose concentration and the SPR absorbance peak. The absorbance point at 800nm in Fig.2 was used as a reference for determining the height of the SPR peak.



*Figure 3: Variations in the SPR peak absorbance of gold*

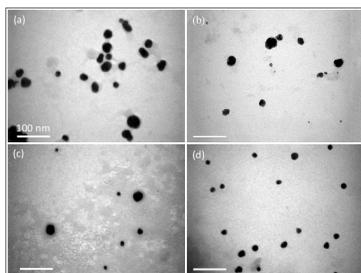
*nanoparticles mixed with different glucose concentrations.*

The negative linear relationship between the glucose concentration and absorbance peak can be attributed to the nature of the interaction between the glucose molecules and nanoparticles. Upon the addition of glucose, the glucose molecules bind to the surface of the gold nanoparticles, acting as what is known as a capping agent [38]. Capping agents influence the size of the nanoparticle by restricting its growth [39]. Hence, as the concentration of the capping agent (i.e. glucose) increases, the size of the nanoparticles will decrease. The TEM images and analysis further confirm these results.

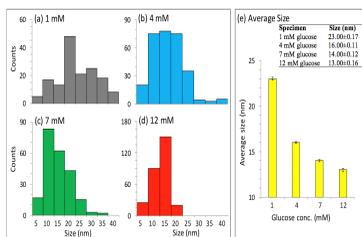
Fig.4 shows TEM images of the Au particles mixed with different glucose concentrations (1, 4, 7, 12 mM). It can be seen from the figure that Au nanoparticles are spherical in shape and that the size of the prepared particles is reduced by increasing the concentrations of glucose. It is worth mentioning that the TEM images were taken three weeks after synthesis and no aggregation was observed.

The size distribution of colloidal gold nanoparticles mixed with different glucose concentrations (1, 4, 7 and 12 mM) is shown in Fig.5.a-b. Fig.5.e shows the average size of colloidal gold nanoparticles mixed with different glucose concentrations. It can be seen from the figure that the average size of gold nanoparticles reduced from 23.00 nm to 13.00 nm as the glucose

concentration increased from 1 to 12mM.



*Figure 4: Morphology and size examination of colloidal gold nanoparticle (GNPs) by transmission electron microscopy. The GNPs were mixed with different concentrations of glucose; (a) 1 mM glucose, (b) 4 mM glucose, (c) 7 mM glucose and (d) 12 mM glucose. Notably: the size of the prepared particles is reduced with increasing the concentrations of glucose. All scale bars are 100 nm.*



*Figure 5: (a-d) Size histograms and (e) average size of colloidal GNPs mixed with different Glucose concentrations (1, 4, 7 and 12 mM), measured from transmission electron microscopy images shown in Figure 4. A reduction in average size was noted with increasing concentration of glucose.*

The linear relationship between the glucose concentration and the absorbance peak holds true in the glucose concentration range of 1-15mM. Table 2 presents the NICE target pre-prandial (before meals) and post-prandial (at least 90 minutes after a meal) blood glucose level ranges for non-diabetics, persons with type 1 diabetes, and persons with type 2 diabetes.

*Table 1: The NICE recommended target blood glucose level ranges [40]*

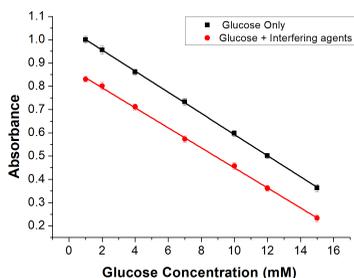
	Blood glucose target levels	
	Pre-prandial (mmol/L)	Post-Prandial (mmol/L)
Non-diabetic	4.0-5.9	<7.8
Diabetic (type 1)	4.0-7.0	<8.5
Diabetic (type 2)	4.0-7.0	5.0-9.0

By examining the values in table 1, the glucose detection range of this nanoparticle system is useful not only for monitoring diabetics' (type 1 & 2) blood glucose levels, but can also be used for the diagnosis of diabetes. Additionally, by being able to detect glucose levels as low as 1 mM, this assay can be used to test for hypoglycemia. Attempts have been made to detect glucose concentrations lower than 1 mM. Yet, the surface plasmon resonance absorbance peak varied significantly in these trials, indicating that this system's limit of detection is close to 1 mM. Glucose concentrations higher than 15 mM have been tested out, but they did not result in any significant changes in the absorbance of the SPR peak. The reason for this may be due to the finite surface area of the nanoparticle[41]. Excess

glucose molecules will not be able to directly bind to the surface of the nanoparticle at glucose concentrations higher than 15mM. Therefore, no further changes in the absorbance peak will be observed.

### 3.4 - Detection of glucose in complex solutions:

Our nanoparticle system has proven to be effective in detecting glucose in pure glucose solutions, with a glucose sensitivity range of 1-15 mM. In human blood serum, however, there are a number of reducing agents present, which might alter the size and morphology of the nanoparticles and hence influence their absorption spectra[42]. Most notably, these reducing agents are fructose, ascorbic acid, galactose, glutathione, lipoid acid, and uric acid[32]. A comparison between gold nanoparticles mixed with glucose and gold nanoparticles mixed with glucose in addition to these reducing agents has been carried out in order to ascertain the effect of such reducing agents. It is worth noting that only 5 out of the 6 reducing agents listed have been included in the comparative analysis. Uric acid has been purposely exempted since it leads to sampling precipitation problems[43]. Fig. 6 compares the variations in the peak absorbance of gold nanoparticles mixed with glucose only to gold nanoparticles mixed with glucose and the prominent reducing agents present in the blood serum.



*Figure 6: Variations in the SPR peak absorbance of gold nanoparticles mixed with different glucose concentrations vs. variations in the SPR peak absorbance of gold nanoparticles mixed with different glucose concentrations and other interfering agents*

As it is evident from the graph, our gold nanoparticle system is still able to detect glucose concentrations in the range of 1-15mM even with the presence of interfering agents. However, the samples containing glucose and other interfering agents showed a larger decrease in the peak absorbance compared to samples containing glucose only. Such decrement can be attributed to the ratio of reducing agents in samples containing glucose only to the samples with other interfering agents. The samples containing other interfering agents have a higher concentration of reducing agents compared to samples containing pure glucose solutions. Higher concentration of reducing agents results in the decreased size of the gold nanoparticle due to their

absorption and stabilizing effect upon being oxidized[44].

#### 4. CONCLUSIONS

We developed an eco-friendly, enzyme-free, and an inexpensive method for the detection of glucose. This method is based on the greensynthesis of gold nanoparticles (using flower leaves of Hibiscus Tiliaceustree extract as a reducing agent). The nature of the interaction between the synthesized gold nanoparticles and glucose molecules were described. The results have shown that the glucose detection range of this method (1-15 mM) is

useful for monitoring diabetics' blood glucose levels, diagnosing diabetes, as well as testing for hypoglycemia. The simplicity of this method opens the doors for the development of new, enzyme-free devices for the detection of glucose.

#### 5. ACKNOWLEDGEMENT

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