Facile Electrostatic Immobilization of Glucose Oxidase onto Citrate Capped Gold Nanoparticles for Surface-enhanced (Bio)-Catalysis

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Abstract

Diabetes mellitus is a serious life-time health issue which has been increasing among the greater population, approximately 285 million people carrying this disease worldwide. In this study, we have functionalised the gold nanoparticles (AuNPs) with biocatalytic enabled optical property, it is the subject of the study for detecting glucose towards the development of photometric nano-transducer. The citrate capped AuNPs were used to warrant the electrostatic self-assembly of glucose oxidase (GOx) in the colloidal state. Glucose biocatalysis was studied through the nano-optical function of glucose on the surface of AuNPs. Using surface plasmonic resonance as analytical technique, we have determined the molecular binding interaction between glucose molecule and AuNPs surface. Based on the visible spectrum, successful immobilization of GOx onto AuNPs was demonstrated. The GOx functionalized AuNP exhibits catalytic activities for the oxidation of glucose and resulting change in the absorption peak of colloidal bio-assembly. It was observed that the absorbance at 520 nm was proportional to the concentration of glucose in the test samples. The Lambert-Beer law expresses the linear relationship between the absorbance and glucose concentration at a fixed wavelength, i.e., λmax at 520 nm. The precise detection of glucose is essential to monitor the biological level of glucose in the body. It can be concluded that the nano-(bio) gold surface exhibits a rapid photometric response with changes of glucose concentration in the test samples.

Keywords: Gold nanoparticle; glucose oxidase; self-assembly; photometric analysis, biocatalysis.

Introduction

Diabetes mellitus is commonly referred to as diabetes, it is caused by blood glucose being excluded by pancreas because pancreatic hormone insulin is not efficiently produced or not used properly by the body. Insulin is an essential hormone which allows the body to regulate amount of glucose in the blood and convert it into energy needed for the body.

There are two forms of diabetes mellitus, which are Type 1 and Type 2. Diabetes is characterized as a chronic metabolic disorder with hyperglycemia (high blood sugar) and abnormal energy metabolism. It has number of complications and can occasionally be life threatening [**1**]. Type 1 diabetes generally affect 5-10% of all people due to destruction of insulin producing beta-cells by immune system in the pancreas which prevent efficient production of insulin [**2**]. The most common cause of type 1 is genetics and environmental factors. Type 2 diabetes makes up about 90-95% people affected, and it is predominant compare to type 1 [**3**]. It is caused by body being resistance to insulin and unable to respond to its action. It is associated with a person's lifestyle and diet since commonly obesity results the rise of this type diabetes. In present diagnosis, health professionals have reported greater number of children being diagnosed with diabetes.

This has concerned researchers and has led them to overcome the issue by early diagnosis of diabetes using the self-monitoring glucose meters for monitoring glucose concentration in the blood. In general, it is an electrochemical device configurated on the digital platform. The strip has enzyme electrode containing glucose oxidase. The enzyme is reoxidized with an excess of a mediator reagent, by the reaction of reoxidization on the electrode which generates an electric current. Thereby, the total charge passing through the electrode is proportional to the amount of glucose in the blood that has reacted with the enzyme [**4**].

However, precise detection of glucose is essential to monitor the biological level of glucose in the body. Therefore, nanotechnology has essential application in medicine and considers development for advanced treatments and diagnosis of diabetes. Nanotechnology is a field of encompassing nano-sized structures or geometry, materials and particles [**5**]. Nanoscales are

used to increase the capability of observing changes and properties of materials and analyse in depth involving atoms and molecules, describe their surface area, how they are bonded, geometry of the material etc. It is an interdisciplinary field and includes areas of biology, chemistry, physics, and engineering but since our consideration is chemistry, therefore, we choose to explore the field of nano-enzymatic chemistry [**6**]. The essential application of above-mentioned field is one of the most researched areas known as nano-(bio) technology, which is a combination of nanotechnology and biotechnology [**6,7**].

Researchers are paying much attention on developing effective and accurate diagnostic devices based on nano-(bio) technology and its application [**6,8**]. Several attempts have been made in the nanomedicine using their application to develop advanced techniques that can be applied to detect or control the disease such as fabrication of glucose biosensor by covalent attachment of glucose oxidase (GOx) to a gold nanoparticle monolayer modified Au electrode [**6,9**]. Gold nanoparticles (AuNPs) are widely used in the area of nanotechnology based on their wide range surface functionality and bioconjuagtes coupled with outstanding physical properties [**10**]. The spherical AuNPs has the optical and geometrical properties, size and shape-related exhibit a range of colours in aqueous solution as the core size increases from 1 to 100 nm and generally show a size relative absorption peak from 500 – 550 nm [**11**]. This absorption band arises from collective oscillation of the conduction electrons due to resonant excitation by the incident photons which is called a surface plasmon band. Functionalized AuNP provides a versatile platform for nanobiological assemblies, the binding event between the analytes and the AuNPs can alter the physicochemical properties of AuNPs such as Plasmon resonance [**11,12**]. It is influenced not only by size but also by shape, solvent, surface ligand, core charge and temperature. There are wide varieties of applications of gold nanoparticles such as lateral flow, optical sensing, light scattering applications, drug delivery and cancer therapy. These application of AuNPs are essential for the nanotechnology to develop advanced techniques such as sensors, probes, diagnostics, catalysis etc. [**13**].

In this investigation, citrate capped AuNPs was used to warrant the electrostatic self-assembly of the enzyme glucose oxidase (GOx) in the colloidal state. This minimizes the risk of destabilizing during the binding process that is aggregation – forms a cluster of particles, which causes plasmon shift and colour change. There are several other factors which could prevent aggregation such as store particles at the recommended temperature and maintain pH of the colloidal solution [**14**].

Aspergillus Niger secreted glucose oxidase (GOx) was taken in this study. It catalyses the oxidation of Beta D-glucose present in the plasma to D-glucono-1,5-lactone with the formation of hydrogen peroxide; the lactone is then slowly hydrolysed to D-gluconic acid. GOx produces gluconic acid [**15**]. Due to production of gluconic acid, absorption level is changed. Therefore, GOx is used as an analytical reagent. The enzymatic assay is specific for glucose allowing more accurate determination. The enzyme is to create the desired functionalities for specific applications of the AuNPs.

The enzyme-catalyzed reaction for the determination of glucose shown in **Fig. 1**.

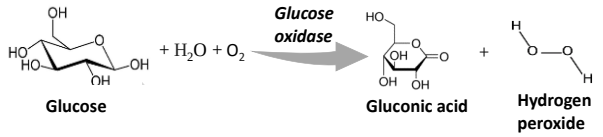

Fig. 1. Enzyme-catalyzed reaction of glucose into gluconic acid and H_2O_2.

The subject of this study was to functionalize and modulate AuNPs with an enzyme, i.e., GOx for detection of glucose concentration towards the development of photometric nano-transducer. Using surface plasmonic resonance as detection technique, we observed the molecular binding interaction between analyte molecule and AuNPs surface. Glucose biocatalysis was studied through the nano-optical function of glucose on the surface of AuNPs. On the basis of visible spectrum, a successful immobilization of GOx on AuNPs and biocatalysis of glucose were demonstrated. Thus, the resulting change in the absorption peak exhibited the response of oxidation of different concentrations of glucose on the surface of GOx/AuNPs. Thus, this study concerns investigation on spectrophotometric detection of glucose concentration, using ionically immobilized GOx onto AuNPs measured as absorption peak at 520 nm.

Experimental

Reagents

A 25 ml of citrate-stabilized AuNPs with diameter of 5 nm, OD 1 nm and absorption at 510 – 525 nm was used in this study. The AuNPs colloidal solution was stored at 2 - 8°C. Glucose oxidase (GOx) was obtained from fungi, *Aspergillus Niger*, 100,000 - 250,000 units/g in solid form, stored at -20°C and D-glucose in solid form, all the products were purchased from Sigma-Aldrich.

Preparation of the solutions

Seven volumetric flask of citrate buffer with concentration of 0.1 $moldm^{-3}$ were prepared at pH 5.4 as stock. To prepare the solution 0.1 M of citric acid with the volume of 16 ml and 0.1 M sodium citrate of 34 ml was needed [**16**]. This equation gave the mass of citric acid powder:

(Citric acid) mass (g) = Molar mass (g/mol) × Concentration (M) × Volume (l), i.e., 210.14 g/mol × 0.1 M × 0.016 l = 0.336224 g

So, 0.3362 g (±0.0001, 0.03%) was added to a 25 ml of graduated cylinder, distilled water was poured till the mark of 16 ml (±0.4 ml, 2.5%) was reached.

In similar way mass of sodium citrate powder was calculated:
(sodium citrate) mass(g) = Molar mass (g/mol) × Concentration (M) × Volume (l), i.e., 294.10 g/mol × 0.1 M × 0.034 l = 0.99994 g

A 0.9994 g (±0.0001, 0.01%) was added in a 100 ml of graduated cylinder, distilled water was poured till the mark of 34 ml (±1, 2.94%), was reached.

Thereafter, a stir bar was placed in the beaker with the solution of 16 ml of citric acid. The beaker was put on the magnetic stirrer and it was turned onto start stirring the solution. Added approximately 34 ml of sodium citrate to make sure the pH meter displayed the value 5.4 (±0.1, 1.85%) pH electrode was placed in the solution and waited 20 to 30 seconds. Switched off the magnetic stirrer, took off the pH electrode and poured the solution into a volumetric flask. Added distilled water into the solution and made final volume of 100 ml (±0.1, 0.1%). Repeated the procedure seven times to make 700 ml of citric buffer. In seven volumetric flasks 100ml of citric buffer was poured.

The other solution, glucose oxidase was prepared by pouring 0.012 g (±0.001, 0.83%) of GOx in 10 ml (±0.2, 2%) of citrate buffer solution, pH 5.4. Due to its highly inflammable nature, gloves and safely glasses were worn throughout the whole procedure.

Stock solution of glucose (100 mg/dl, 0.00106) was prepared in citrate buffer pH 5.4. It was prepared by adding 0.1044 g (±0.0001, 0.96%) of glucose in a volumetric flask, then citrate buffer was poured till the 100 ml (±0.1, 0.1%) mark had reached. Using the glucose stock and addition of citrate buffer, six different concentrations of glucose solution was prepared shown in **Table 1**. All the apparatus and equipment were thoroughly cleaned to reduce random errors.

Table 1. Preparation of six different concentrations of glucose solution, converted from mg/100 cm^3 to mol/ dm^3, their absolute uncertainties are also stated.

Volume of glucose (±0.1cm^3)	Volume of citrate buffer (±0.1cm^3)	Glucose, (mg/ 100 cm^3)	Glucose, (mol/ dm^3)	Absolute uncertainty (%)
25.0	75.0	25.0	1.39×10^{-5}	1.59
20.0	80.0	20.0	1.11×10^{-5}	1.69
15.0	85.0	15.0	8.33×10^{-6}	1.85
10.0	90.0	10.0	5.55×10^{-6}	2.17
5.0	95.0	5.0	2.77×10^{-6}	3.17
2.5	97.5	2.5	1.39×10^{-6}	5.16

Uncertainty calculations

- All the percentage uncertainties are calculated for all the values using this formula.

$$\frac{actual\ uncertainty}{measurement} \times 100$$

- Absolute uncertainty of stock solution of glucose is calculated by adding percentage uncertainty of the added solute and added volume of citrate buffer and then multiplied by concentration it has reached.
(0.96% + 0.1%) × 0.001 g/ml = 0.0106)
- Absolute uncertainty of different concentrations of glucose is calculated in this certain way:

% Uncertainty of mass of glucose + % Uncertainty of volume of stock of glucose + % Uncertainty of glucose volume added + % Uncertainty of citric buffer added.

- 0.96% + 0.1% + 0.4% + 0.13% = 1.59%
- 0.96% + 0.1% +0.5% + 0.13% = 1.69%
- 0.96% + 0.1% + 0.67% + 0.12% = 1.85%
- 0.96% + 0.1% + 1% + 0.11% = 2.17%
- 0.96% + 0.1% + 2% + 0.11% = 3.17%
- 0.96% + 0.1%+4% + 0.10% = 5.16%

Preparation of glucose oxidase functionalized AuNPs

In seven eppendorfs, using a micropipette with volume of 100 µl, 100 µl (±0.8 µl, 0.8%) of AuNPs was added with 200 µl (±0.8 µl, 0.4%) of GOx. Both reagents reacted for 2 hours at approximately 37°C (±0.5, 1.35%) in the incubator. The reaction was stopped with addition of 25 µl (±0.0175, 0.07%) of glucose in 6 eppendorfs with varied concentration of glucose, the solutions stayed in the incubator for approx. 20 mins at 37 °C. AuNPs and GOx had a volumetric ratio of 1:2 v/v which is an ideal ratio for glucose catalysis [**17**]. This procedure was repeated three times to obtain average absorption value.

Spectrophotometric detection of glucose

UV-vis absorption spectroscopy was done with a Genesis 10 UV-vis spectrophotometer. Fingerprints from cuvettes were removed with tissue to avoid false reading. Spectrophotometer was used to determine the absorbance of the enzyme glucose oxidase (GOx) [**18**]. Thereby, spectrum of AuNPs was carried out to make comparison in the absorbance. The absorbance of the fabricated bioconjugate complex was monitored in the absence of glucose and presence of various concentrations of glucose.

Results and discussion

Fabrication process of the biocongugate complex

Fig. 2 shows the stepwise electrostatic self-assembly of GOx onto AuNPs which was followed by incubation of citrate stabilized gold nanoparticles (100 µl) with glucose oxidase (200 µl, pH 5.4) at 37 °C for 2 hours. The incubation caused absorption of the enzyme glucose oxidase onto the gold nanoparticles due to GOx have opposite charges with respect to surface charge of AuNPs. Thus, electrostatic attractive force between the GOx molecules and the AuNPs surface caused strong interaction of the molecules. GOx has been exploited as a stabilizing agent at pH 5.4 which minimized the risk of aggregation.

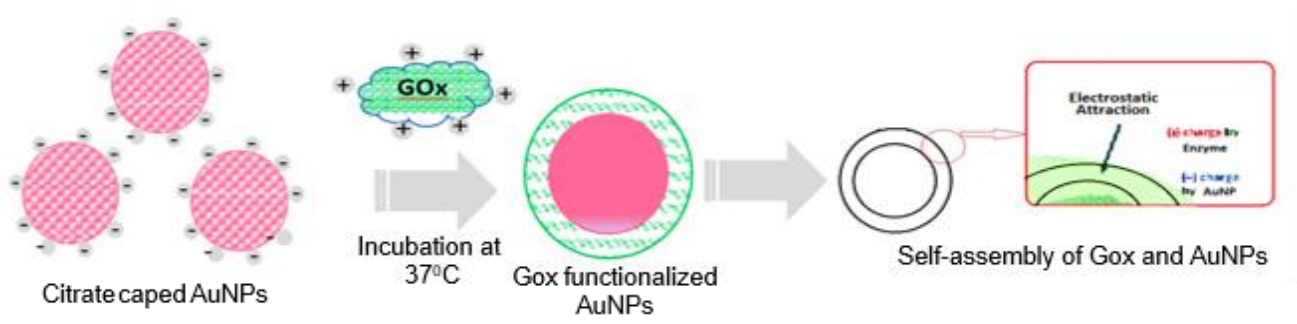

Fig. 2. Facile electrostatic self-assembly of GOx onto AuNPs.

Due to Plasmon resonance, binding event between the analyte and the GOx/AuNPs can alter the physicochemical properties of gold nano-surface [**11**]. **Fig. 3** shows the absorption band exhibited by bare AuNPs (diameter of 5.0 nm) at 520 nm is the characteristic surface Plasmon resonance band and demonstrates the visible spectrum of successful immobilization of glucose oxidase onto gold nano-particles' surface. The attachment of the enzyme glucose oxidase was monitored by the spectrophotometer, it showed the significant difference in the absorption peak at 520 nm. The drastic change in absorption band was caused by attachment of inactive GOx on the surface of the particles which slows down the light intensity and less light penetrated through glucose oxidase. Low light permeability on AuNPs and less light being absorbed compared to bare AuNPs therefore there is a decrease in plasmonic band after the immobilization of GOx onto the AuNPs. Additionally, the graph explained the assembly of gold nanoparticles and GOx were consistent and GOx molecules are firmly attached on the surface of the gold nanoparticles via electrostatic/ionic interactions. The surface of gold nanoparticles was fully or incompletely covered by GOx function which regulates the plasmonic peaks during UV-visible measurements in presence/absence of glucose. It was observed that during the experiment when eppendorfs of one of the trial were taken out after two hours' incubation, the solution still had some stances of yellow colour which indicates there were still some glucose oxidase left in the solution and all were not completely immobilized onto AuNPs.

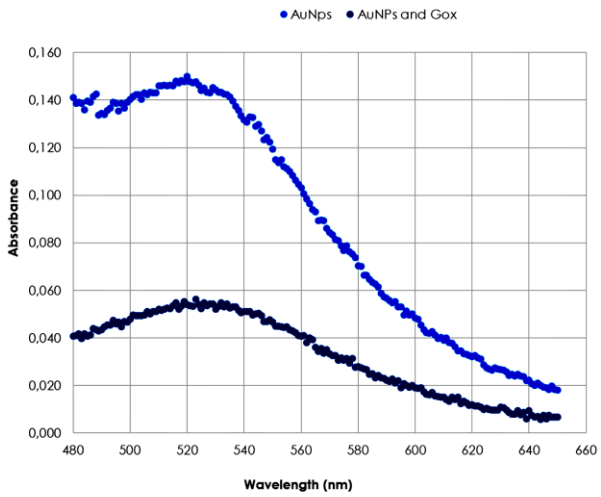

Fig. 3. Spectrophotometry of fabricated bioconjugate complex in the absence of glucose.

Performance of the nano-photometric transducer

The coupled nanoparticle system could be used in a potential nano-photometric transducer application. Spectrophotometry detection of the immobilized enzyme was investigated to monitor nano-photometric transducer's performance. Therefore, various concentration of substrate (glucose) were added to check the oxidization. The surface of glucose oxidase onto gold nanoparticles reacted with glucose and exhibited the catalytic activities for the oxidation of glucose. **(Fig. 4)** represents the enzyme catalyzes the oxidation of

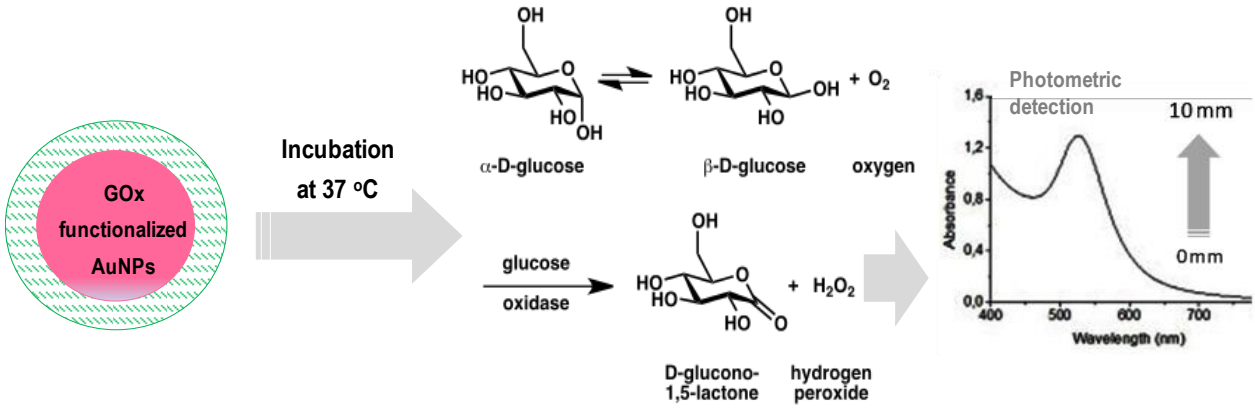

Fig. 4. Self-assembly of GOx and AuNPs, and enzymatic reaction of glucose followed by photometric detection of glucose.

D-glucose to D-glucono-lactone and it is oxidized by molecular oxygen to produce hydrogen peroxide. D-gluconolactone is then slowly hydrolyses spontaneously to produce gluconic acid. Production of gluconic acid in the medium may provide the possible reattachment of GOx present in the reacting solution on the surface of AuNPs. In the enzyme-catalysed reaction there is increase in substrate concentration (glucose) and the amount of GOx or covered area of GOx onto AuNPs decreases so AuNP's pority increases. Coverage area on AuNPs decreases and light penetration increases. On the addition of glucose concentration, the size of the particle has increased. Increase in the lambda max which indicates the formation of a thicker monolayer around the nanoparticles, resulting change in the absorption peak of nano-assembly. (**Table 2**) Absorption spectrum of the nano-photometric detection of different glucose concentrations in numerical form.

Table 2. Averaged absorption of AuNPs, GOx/AuNPs with and without varying glucose concentrations ranging from 2.5 to 25 mg/100dl at wavelength, 480 - 514 nm.

λmax, nm	AuNPs	AuNPs and GOx	Glucose, 2.5 mg/100dl	Glucose, 5.0 mg/100dl	Glucose, 10.0 mg/100dl	Glucose, 15.0 mg/100dl	Glucose, 20 .0 mg/100dl	Glucose, 25.0 mg/100dl
480	0.141	0.041	0.041	0.052	0.076	0.036	0.033	0.039
481	0.139	0.041	0.042	0.056	0.081	0.037	0.033	0.041
482	0.139	0.042	0.044	0.056	0.082	0.037	0.033	0.043
483	0.139	0.040	0.041	0.053	0.077	0.036	0.033	0.040
484	0.136	0.042	0.040	0.050	0.070	0.036	0.033	0.040
485	0.140	0.041	0.038	0.047	0.067	0.037	0.032	0.037
486	0.139	0.041	0.039	0.046	0.066	0.038	0.032	0.037
487	0.142	0.044	0.041	0.049	0.069	0.040	0.035	0.039
488	0.143	0.043	0.040	0.049	0.070	0.039	0.034	0.039
489	0.134	0.043	0.041	0.049	0.069	0.040	0.034	0.039
490	0.134	0.043	0.040	0.048	0.067	0.038	0.034	0.038
491	0.134	0.045	0.041	0.051	0.069	0.040	0.036	0.040
492	0.136	0.045	0.043	0.050	0.068	0.041	0.037	0.040
493	0.137	0.045	0.042	0.050	0.068	0.042	0.037	0.039
494	0.139	0.047	0.043	0.051	0.069	0.044	0.038	0.042
495	0.139	0.046	0.043	0.052	0.070	0.043	0.037	0.040
496	0.135	0.047	0.045	0.055	0.074	0.042	0.038	0.042
497	0.139	0.045	0.043	0.054	0.074	0.041	0.037	0.042
498	0.137	0.047	0.046	0.055	0.076	0.043	0.040	0.044
499	0.139	0.047	0.045	0.054	0.075	0.043	0.038	0.044
500	0.140	0.048	0.046	0.052	0.074	0.044	0.039	0.043
501	0.142	0.050	0.046	0.055	0.076	0.045	0.041	0.045
502	0.142	0.049	0.046	0.054	0.076	0.045	0.040	0.045
503	0.142	0.049	0.048	0.056	0.076	0.045	0.041	0.045
504	0.140	0.049	0.047	0.056	0.077	0.045	0.040	0.045
505	0.143	0.050	0.049	0.058	0.079	0.047	0.042	0.047
506	0.142	0.051	0.049	0.060	0.080	0.046	0.043	0.048
507	0.143	0.050	0.048	0.059	0.079	0.045	0.041	0.047
508	0.143	0.051	0.049	0.059	0.078	0.047	0.042	0.047
509	0.143	0.051	0.051	0.060	0.081	0.046	0.042	0.049
510	0.146	0.052	0.051	0.060	0.080	0.047	0.042	0.048
511	0.146	0.052	0.049	0.059	0.079	0.046	0.043	0.048
512	0.146	0.052	0.049	0.059	0.079	0.046	0.043	0.047
513	0.146	0.053	0.049	0.059	0.080	0.048	0.043	0.048
514	0.146	0.052	0.050	0.060	0.079	0.047	0.043	0.047

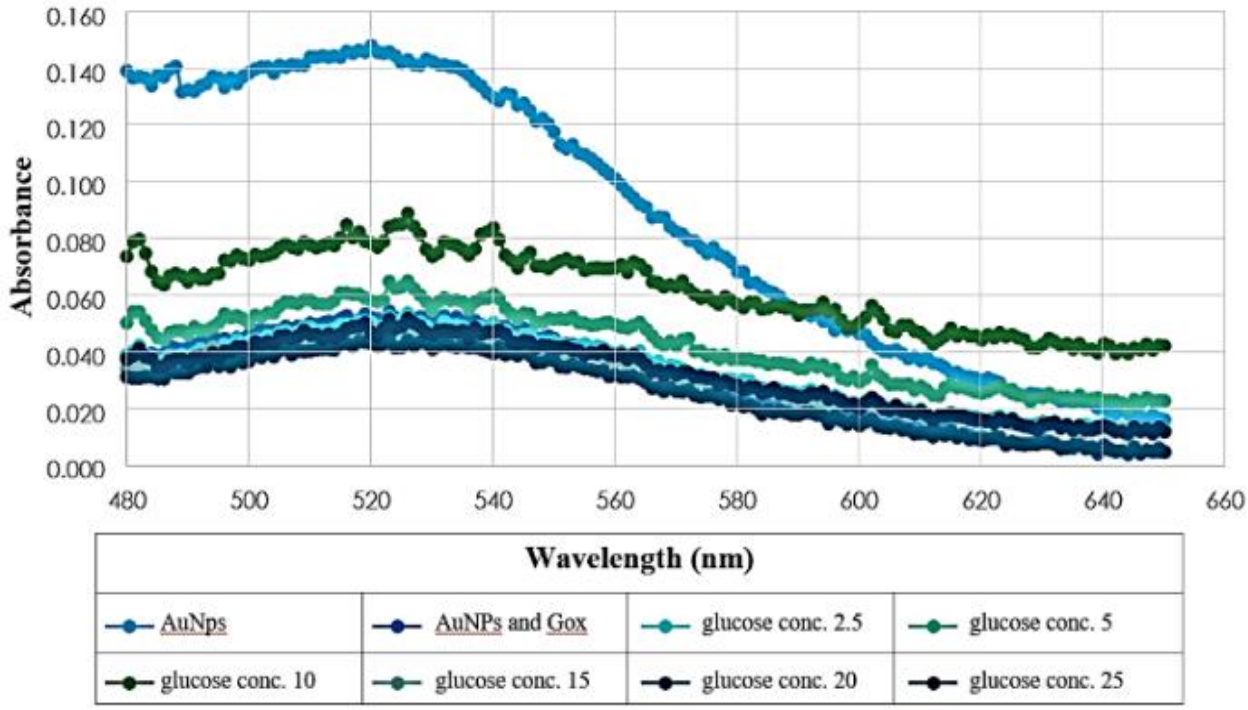

Fig. 5. UV-visible absorption spectra changes of bioconjugate complex after addition of various concentrations of glucose.

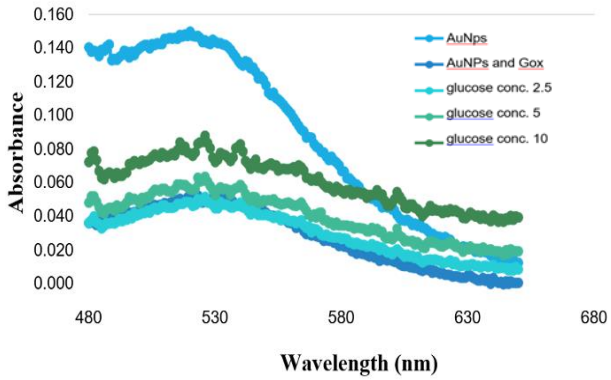

Fig. 6. UV-visible absorption spectra addition of glucose concentration in the range of 2.5- 10 mg/ 100dl.

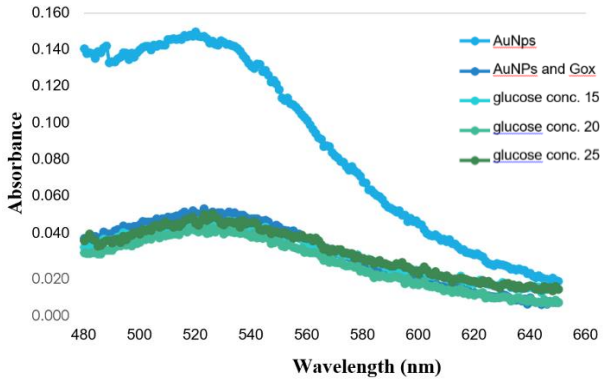

Fig. 7. UV-visible absorption spectra addition of glucose concentration in the range of 15- 25 mg/ 100dl.

Whereas, **(Fig. 5)** represents the UV-visible absorption spectra changes of bioconjugate complex after addition of various concentrations of glucose. It demonstrates as there is increases in the concentration of glucose there is increase in the plasmonic surface. However, the **(Fig. 6)** shows the pattern is followed only between the range of 2.5 - 10mg/ 100dl. Glucose concentration in the range between 15 - 25 mg/ 100dl does not show any changes in the lambda max even though there is increase in the glucose concentration in the solution, this is presented by the **(Fig. 7)** the oddity is due to reactivity of GOx because glucose seems to be saturated, this means that all glucose particles fully covered the surface of AuNPs therefore, low light permeability on AuNPs and less light being absorbed which could reflect in the plasmonic peaks (i.e., there is no increase in the λmax) of GOx functionalized AuNPs. The graphs also represent that there is no shifting in the absorption peaks which means aggregation has not formed during the whole procedure.

Standard calibration curve

Using UV-vis absorption spectra quantitative measurements have been obtained. Six dilutions of a standard of known concentrations are prepared. we have converted the unit of the glucose concentration from mg/ 100 cm^3 to mol/ dm^3 since blood sugar level is measured in mg/dl but in chemistry more commonly measurement of concentration is mol/ dm^3. The absorbance of glucose concentration is generated by reacting on immobilized GOx onto AuNPs. Absorbance of various concentrations

of glucose at Lambda max was plotted on a graph to obtain calibration curve (**Table 3**). **(Fig. 8)** represents calibration plot of the absorbance vs glucose concentration, the intensity absorption bands at 520nm gradually increases which indicates increase in glucose concentration leading to higher rate of reaction and more gluconic acid produced and causing increase in the absorption peak. The Beer-Lambert graph shows recordings of the nano- photometric transducer on successive changes of glucose concentration. The nano-photometric transducer exhibited a rapid response to changes of glucose concentration in the range between $1.39 \times 10^{-5} – 8.33 \times 10^{-6}$ mol/ dm^3. Since Beer-Lambert law expresses the linear relationship between the absorbance and concentration of a compound at a fixed wavelength. However, Lambda max of GOx/AuNPs vs. concentration of glucose ranging $5.55 \times 10^{-6} – 1.39 \times 10^{-6}$ mol/dm^3 does not follow the trend and there is a decrease in absorption which is said to be because of saturation.

Table 3. Lambda max of GOx/AuNPs vs. concentration of glucose ranging $1.39 \times 10^{-5} – 1.39 \times 10^{-6}$ mol/dm^3.

S. No.	Glucose, (mol/dm^3)	λmax, GOx/AuNPs and with Glucose	Δλmax
1	1.39×10^{-5}	0.054 - 0.052	0.0017
2	1.11×10^{-5}	0.054 - 0.060	0.0060
3	8.33×10^{-6}	0.054 - 0.080	0.0263
4	5.55×10^{-6}	0.054 - 0.050	0.0037
5	2.77×10^{-6}	0.054 - 0.045	0.0090
6	1.39×10^{-6}	0.054 - 0.049	0.0053

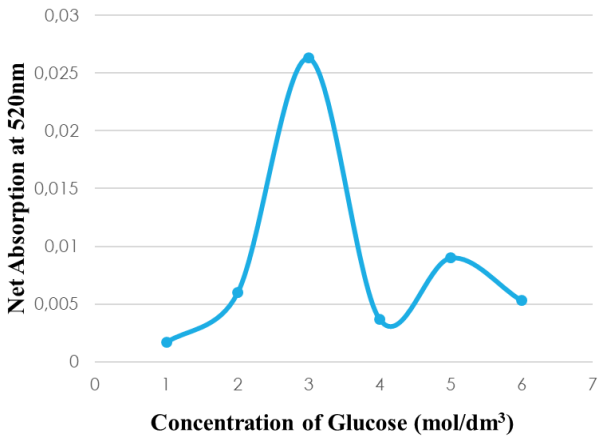

Fig. 8. Calibration curve of the absorbance of GOx/AuNPs vs glucose concentration.

We have investigated and discussed that nanoparticle-based technologies have played important roles in providing opportunities for the development of a new generation of sensing tools [**17**]. Because of their unique optical, chemical, electrical, and catalytic properties. The use of surface functionalized AuNPs in this investigation is a small step towards fabricating smart sensors that can detect glucose. Since, Gold nanoparticles (AuNPs) have been extensively studied for biological and chemical detections as well as analytical applications. AuNP-based sensors are expected to change the foundations of sensing and detecting biomolecules and metal ions [**19**]. Although, fabrication of glucose biosensor through various methodologies have diversified implications for quality and quantity mentioned in **Table 4**. However, the ease of surface functionalization of AuNPs allows chemists to create the desired functionalities for specific applications. The reusability of this method could attribute to good biocompatibility and highly efficient GOx immobilization and retention. The recovery of the nano- transduces could potentially measure for the repetitive use this as a reagent.

Table 4. Fabrication of glucose biosensor using various methodologies.

SN	Fabrication of glucose biosensor	Reference
1	Using graphene-polyethyleneimine-gold nanoparticles hybrid	**[20]**
2	Based on inserted barrel plating gold electrodes	**[21]**
3	Using Additive Processes through polyimide substrates	**[22]**
4	Using an electrochemically reduced graphene oxide–glucose oxidase bio composite	**[23]**
5	By Piezoelectric inkjet printing	**[24]**
6	Using Enzymes Immobilized in Exfoliated Graphite Nanoplatelets Nafion Membrane	**[25]**
7	Based on a Glassy Carbon Electrode Modified with Polythionine and Multiwalled Carbon Nanotubes	**[26]**
8	Based on PANI-GRA Nanocomposite Film Decorated with Pt Nanoparticles	**[27]**
9	Based on a novel PtPd bimetallic nanoparticle decorated multi-walled carbon nanotube catalyst	**[28]**

Conclusion

In this study, we have functionalized the gold nanoparticles (AuNPs) with an enzyme, i.e. glucose oxidase for detection of glucose towards the development of photometric nano-transducer. The photometric nano-transducer was studied through the nano-optical function of glucose on the surface of AuNPs. Using surface plasmonic resonance as detection technique, we have determined the molecular binding interaction between analyte molecule and gold nanoparticle surface. Since binding event between the analyte and the AuNPs has altered its Plasmon resonance. The incubation of (100 µl) with glucose oxidase (200 µl, pH 5.4) at 37 °C for 2 hours had caused absorption of the enzyme glucose oxidase onto the gold nanoparticles due to GOx carrying opposite charges with respect to surface charge of AuNPs. Thus, electrostatic attractive force between the GOx molecules and the AuNPs surface caused strong interaction of the molecules. Based on the visible spectrum successful

immobilization of glucose oxidase onto gold nanoparticle was demonstrated. The attachment of the inactive enzyme glucose oxidase had caused drastic change in absorption band, it showed the significant difference in the absorption peak at 520 nm.

To monitor nano-photometric transducer's performance, different concentrations of glucose was incubated in the solution of coated AuNPs with GOx. Since the GOx functionalized aAuNP exhibits catalytic activities for the oxidation of glucose and resulting change in the absorption peak of nano-assembly. It was observed that the absorbance at 520 nm is proportional to the concentration of glucose in the test samples. The Lambert-Beer law expresses the linear relationship between the net absorbance and glucose concentration at a fixed wavelength, i.e., λmax at 520 nm. The precise detection of glucose is essential to monitor the biological level of glucose in the body. It can be concluded that the nano-photometric transducer exhibits a rapid photometric response with changes of glucose concentration in the test samples. Finally, feasible and cost-effective point of care (POC) applications, for any glucose biosensor is suitable. The proposed novel glucose biosensor possesses advantages due to fast response measurements with a high degree of reliability for future clinical applications.

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References

1. Hu, F.B.; *Diabetes Care;* **2011**, *34*, 1249.
2. American Diabetes Association; Diagnosis and Classification of Diabetes Mellitus; *Diabetes Care;* **2009**, *32*, S62.
3. Zhang S.; Wang N.; Yu H.; Niu Y.; Sun C.; *Bioelectrochemistry*, **2005**, *67*, 15.
4. Norman, J.; Symptoms, Diagnosis and Treatments of Type 1 Diabetes; Type 1 Diabetes **2017** April.
5. Jensen, S.; Ask an engineer; How to glucometer work; MIT School of Engineering. Available from https://engineering.mit.edu/engage/ask-an-engineer/how-do-glucometers-work/ (Accessed 18th October **2017**)
6. DiSanto, R.M.; Subramanian, V.; Gu, Z.; *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **2015**, *7(4)*, 548.
7. Nagamune, T.; *Nano Convergence*, **2017**, *4*, 9.
8. Taguchi, M.; Ptitsyn, A.; McLamore, E.S.; Claussen, J.C.; *J. Diabetes Sci. Technol.*, **2014**, *8*, 403.
9. Putzbach, W.; Ronkainen, N.J.; *Sensors*, **2013**, *13*, 4811.
10. Yeh, Y.C.; Creran, B.; Rotello, V.M.; *Nanoscale*, **2012**, *4*, 1871.
11. Jain, P.K.; Lee, K.S.; El-Sayed, I.H.; El-Sayed, M.A.; *J. Phys. Chem. B* **2006**, *110*, 7238-7248.
12. Jain, P.K.; Huang, X.; El-Sayed, I.H.; El-Sayed, M.A.; *Acc. Chem. Res.*, **2008**, *41*, 1578.
13. Rudoi V. M.; Dement'eva, O. V.; *Inorg. Mater.*, **2018**, *9*, 134.
14. "Why do gold nanoparticles aggregate? How do I prevent them from aggregating? What do I do if I see aggregation?" Available from: https://nanohybrids.net/pages/understanding-gold-nanoparticle-aggregation (Accessed 18th October **2017**)
15. McDowall, J.; glucose Oxidase and Biosensors; Protien of the Month; May **2006**.
16. Ruzin; 1999; Plant Microtechnique and Microscopy; Buffers. Available from: http://microscopy.berkeley.edu/Resources/instruction/buffers.html (Accessed 18th October **2017**).
17. Tiwari, A.; Turner, A.P.F.; Biosensors Nanotechnology, ISBN: 978-1-118- 77351-2, July **2014**. pp.552.
18. Noorbehesht, N.; Seifkordi, A.A.; Otady M.; Amiri, R.; *Int. J. Adv. Biotechnol. Res.*, **2016**, *7*, 1226.
19. Zeng, S.; Yong, K.-T.; Roy, I.; Dinh, X.-Q.; Yu, X.; Luan, F.; *Plasmonics;* **2011**, *6*, 491.
20. Parvin R.; Mojtaba T.; Behzad H.; *Sens. Actuators B*, **2016**, *232*, 454.
21. Hsu, C.-T.; Chung, H.-H.; Tsai, D.-M.; Fang, M.-Y.; Hsiao, H.-C.; Zen, J.-M.; *Anal. Chem.*, **2009**, *81*, 515.
22. Du, C.; Durgan, C.J.; Matthews, D.J.; Motley, J.R.; Tan, X.; Pholsena, K.; Arnadottir,´ L.; Castle, J.R.; Jacobs, P.G.; Cargill, R.S.; Kenneth Ward, W.; Conley Jr., C.F.;. Herman, G.S.; *ECS J. Solid State Sci. Technol.*, **2015**, *4*, P3069-P3074.
23. Vijayaraj, K.; Hong, S.W.; Jin, S.-H.; Chang, S.-C.; Park, D.-S.; Anal. Methods., **2016**, *8*, 6974-6981.
24. Wang, T.; Cook, C.C.; Serbanb, S.; Alia, T.; Dragob, G.; Derby, B.; Fabrication of glucose biosensors by inkjet printing. arXiv:1207.1190 [cond-mat.mtrl-sci] https://arxiv.org/abs/1207.1190
25. Lu, J.; Drzal, L.T.; Worden, R.M.; Lee, I.; *Chem. Mater.*, **2007**, *19*, 25, 6240-6246.
26. Tang, W.; Li, L.; Wu, L.; Gong, J.; Zeng, X.; *PLoS ONE*, **2014**, *9*, e95030.
27. Zheng, H.; Wang, M.; Chen, J.; Liu, M.; Ye, Y.; Yan, Z.; *Int. J. Electrochem. Sci.*, **2018,** *13*, 6272-6285.
28. Chen, K.-J.; Lee, C.-F.; Rick, J.; Wang, S.-H.; Liu, C.-C. Hwang, B.J.; *Biosens. Bioelectron.*, **2012**, *33*, 75-81.

