

Synthesis and Characterization of Fe_2O_3 Nanomaterials using HVPC Growth Technique for Glucose Sensing Application

Donna Marie B. De Mesa, Gil Nonato C. Santos, Reuben V. Quiroga

Abstract - This study investigated the capability of the grown Iron Oxide nanoparticles (NPs) for faster glucose detection. Horizontal Vapor Phase Crystal (HVPC) Growth Technique was utilized in the synthesis of Iron Oxide NPs from 99.99% purity Magnetite – Iron Oxide (Sigma- Aldrich) bulk material. Different parameters were varied such as the growth environment (without external magnetic field, with external magnetic field), size of the sealed tube (10cm, 12cm, 14cm), growth temperature (1000° C, 1100°C, 1200°C) and dwell time (8hr, 9 hr, 10 hr). Scanning electron Microscope (SEM) and Energy Dispersive X-ray (EDX) confirmed that the Iron Oxide nanowires and almost uniform size nanoparticles approximately 50nm in diameter were formed utilizing the optimum parameters such as 1000°C growth temperature, 8 hours dwell time, 12 cm size of sealed tube and the presence of external magnetic field in the growth environment. The application of the external magnetic field enhanced the Superparamagnetic (SPM) property of the grown Iron Oxide NPs above the Curie temperature. The net magnetic moment determined the direction of magnetic force of attraction present in the grown NPs. Particles to particles- chain assembly were observed in which some NPs were interconnected forming nanowires/ agglomerated nanowires in the vapor - solid phase nucleation. The magnetic field also lessened the effect of gravity which aligned the formation along the field. Glucose oxidase (GOx) and chitosan have been immobilized by physical adsorption onto electrode with Iron Oxide NPs. Amperometric – electrochemical circuit setup was used to determine the glucose sensing ability of the modified electrode with iron oxide NPs while varying some factors such as glucose concentration, applied DC potential and electrolyte solution. At $\alpha = 0.05$, since $F(27.52; 54.48) > FC(3.29; 4.49)$, two- way ANOVA reveals that there was a significant difference between the current responses while varying the electrolytes for different modified electrodes. Among the modified electrodes, G/FeO/CH/GOx and SS/FeO/GOx showed highest sensitivity and longest limit of detection correspondingly. The correlation coefficients (ave. $r = 0.74; 0.99$) indicates that there was a linear relationship between the response current versus varying concentrations and applied DC potential. Iron Oxide NPs integrated in modified electrode in an amperometric- electrochemical circuit also showed low detection limit (0.008mM), fast response time ($<5s$), usage repeatability (≈ 20 times) and longest detection limit range of 0.008mM to 32mM. Based on the results, modified electrode with Iron Oxide NPs showed high surface reaction and catalytic activity, large surface-to-volume ratio and strong adsorption ability that are beneficial in the immobilization of glucose oxidase. The Iron Oxide NPs' magnetic property was considered in the modification of the electrode for Amperometric- Electrochemical circuit for glucose sensing application since the magnetic field reinforces the attraction of the particles to the electrode's surface.

Keywords - Iron Oxide Nanomaterials, Horizontal Vapor Phase Crystal (HVPC), glucose sensing application, amperometric glucose sensing, biosensing applications

1 INTRODUCTION

Diabetes is one of the most alarming diseases worldwide. In the article entitled "Diabetes Warning" at the Inquirer.net (2008), Dr. Philip S. Chua mentioned that the victims of diabetes are expected to grow from 246 million to 380 million by year 2025 based from the report of the International Diabetes Institute in Australia. One way to prevent diabetes is through proper monitoring of the glucose concentration in the body. A person can still develop diabetes if the glucose levels in the bloodstream are not carefully regulated due to its building up in the blood instead of going to the cells (Mongillo, 2007). There are numerous ways used in monitoring glucose concentration. Some researchers today integrate nanotechnology in medical applications.

"There's a plenty of room at the bottom," a talk presented by Feynman not knowing that it did become the central point in the field of nanotechnology long before anything related to the word "nano" had emerged (Ashby *et*

al., 2009). Nanotechnology is the research and development of materials, devices, and systems that exhibit physical, chemical, and biological properties at dimensions ranges from 1 to 100 nanometers which are expected to bring lighter, stronger, smarter, cheaper, cleaner, and more durable products (Mongillo, 2007). Some of the major applications of the nanotechnology are energy storage, production, and conversion, agricultural productivity enhancement, water treatment and remediation, drug delivery systems and disease diagnosis and screening. Since then, many researches had materialized in the study of nanomaterials and one of these is the iron oxide nanomaterials that was utilized in disease diagnosis and screening.

Iron Oxide nanoparticles (NPs) have attracted extensive interest due to their magnetic properties and their potential applications in many fields. Even though cobalt and nickel are highly magnetic materials, they have limited applications due to their susceptibility to oxidation and toxicity. Some of the applications of Iron Oxide NPs include multi-tera bit storage device, catalysis, sensors, and a platform for high-sensitivity biomolecular magnetic resonance imaging (MRI) for medical diagnosis and therapeutics.

In the review "Biosensor fabrication based on metal oxide nanomaterials," magnetite (Fe_3O_4) iron oxides nanoparticles and thin films structures have potential

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applications in the field of biology, pharmacy, diagnostics, drug delivery, hyperthermia treatments, MRI contrast enhancement agents, purification of biomolecules, cell separation, biosensors and enzymatic assays (Umansakar *et al.* (Eds.), 2009). Cash and Clark (2010) indicated that the magnetic nanoparticles such as iron oxides have also been used independently (Kaushik *et al.*, 2008; Li *et al.*, 2009; Yang *et al.*, 2009) or in combination with other materials (Baby & Ramaprabhu, 2010; Luo *et al.*, 2010) for glucose sensing. The magnetic nature enables the formation of nanoparticle conductive wires on the electrode surface and simplifies the assembly of Glucose Oxidase (GOx) -labeled nanoparticles onto the electrode. Thus, magnetic nanoparticles are good in the fabrication of nanoparticle electrode assemblies since the magnetic field reinforces the attraction of the particles to the electrode's surface (Cash and Clark, 2010).

There are several techniques in synthesizing iron oxide nanoparticles such as co-precipitation (Mikhaylova *et al.*, 2004; Wu *et al.*, 2007; Vereda *et al.*, 2007; Kaushik *et al.*, 2008; Yang, L. *et al.*, 2009), simple annealing method (Kumar *et al.*, 2009), thermal decomposition, hydrothermal synthesis (Chen *et al.*, 2009; Wu *et al.*, 2010), microemulsion, forced hydrolysis (Zhang *et al.*, 2011), sonochemical synthesis, template assisted synthesis (Janardhanan *et al.*, 2007) and sonochemical synthetic route. In addition, these nanoparticles can also be prepared by the other methods such as electrochemical synthesis, laser pyrolysis techniques, microorganism or bacterial synthesis especially the Magnetotactic bacteria and iron reducing bacteria among others (MION).

In this study, Iron Oxide NPs were synthesized using the Horizontal Vapor Phase (HVPC) Growth technique varying some factors to determine the optimum growth parameter. The effects of the variation in the growth environment, size of the sealed tube, growth temperature and the dwell time on the surface topography and morphology and elemental composition were investigated using Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX). Iron oxide NPs grown at optimum parameters were embedded in the cathode- electrode connected to an Amperometric - Electrochemical circuit for glucose sensing application.

2 EXPERIMENTAL DETAILS

A. Synthesis of Iron Oxide Nanomaterials

1) *Preparation of Raw Materials:* The fused silica tube with dimensions 8.5 mm inner diameter, 11 mm outer diameter and 220 mm length was sealed at one end using a high temperature blowtorch (a mixture of LPG and oxygen). For all samples with substrate, a dent was formed approximately five centimeter from the one-end sealed tube. The sealed tube that served as the container of the material was cleansed using an ultrasonic cleanser for 30 minutes to remove the contaminants and was air-dried to remove the excess water inside the tube. Fifty grams of Magnetite - Iron

Oxide with purity rate 99.9% was weighed using the digital laboratory balance and was loaded into the dried tube. Then, the tube with material inside was connected to a Thermionic High Vacuum system to decrease its pressure to about 10-6 Torr. The tube was fully sealed at different points ending with varying sizes of the tube (10 cm, 12 cm, 14 cm). It was then detached from the vacuum system.

2) *Growth of Iron Oxide Nanomaterials:* Iron oxide nanomaterial was grown by thermal activation. The fully sealed fused silica tube with samples was inserted 5cm from one other end of the Thermolyne programmable furnace to achieve the temperature gradient necessary for the growth of the nanomaterial. The desired growth temperatures (1000°C, 1100°C, 1200°C), dwelling times (8 hr, 9hr, 10 hr) and ramp time (80 minutes) were set. A 40.8 mT Nakamura (B10 - 3200) external magnetic field was applied during the growing process.

B. Iron Oxide Nanomaterials Characterization

1) *Iron Oxide Nanomaterials Retrieval:* The fused silica tube was labeled into three zones namely: Zone 1 (Hottest Part), Zone 2 (Moderate), Zone 3 (Coldest Part). The tube was cracked under fumehood. The grown nanomaterial in the inner surface of the tube was characterized using the Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray.

2) *Investigation of Surface Morphology and Elemental Composition:* Iron oxide nanomaterials' structure and elemental composition were characterized using SEM and EDX respectively. The samples grown in the substrate and different parts of the tube were coated three times with gold using JEOL Gold Coater before it was placed inside the SEM.

C. Iron Oxide Nanomaterials for Glucose Sensing application

The Iron Oxide NPs were embedded on the electrode for the Amperometric - Electrochemical glucose sensing application. These NPs were grown in the stainless steel and graphite rod substrates utilizing the optimum parameters.

1) *Reagents, Materials and Equipments:* Reagents such as 99.99% Magnetite (518158), 10KU (55mg) Glucose Oxidase from *Aspergillus Niger* (G7141- 181300 units/g) and 75% Chitosan from shrimp shells (C3646) were purchased from Sigma Aldrich Company. Additional reagents like D-Glucose, Sodium Acetate, Acetic Acid, HCL, uric acid, ascorbic acid, and pure distilled water were obtained from DLSU - Chemistry and STRC - Solid State Laboratories. Different glasswares such as beaker, graduated cylinder, dropping pipette, watch glass and stirring rod were utilized in the preparation of different solutions needed. Equipments namely Fisher Scientific - Stirring hotplate, Sartorius Digital balance, Pasco - Explorer GLX with Voltage- Current Sensor, Temperature Sensor and pH sensor, PASCO Scientific SF-9500 Galvanometer, Lodestar DC Power Supply, Alligator

Clips were employed in the glucose sensing application of the grown iron oxide nanomaterials.

2) *Preparation of the different components needed in Amperometric - Electrochemical - Glucose Sensing:* About 820.34 mg Sodium Acetate ($\text{NaC}_2\text{H}_3\text{O}_2$) was added to 150 ml pure distilled water heated to 35°C . The pH was measured after letting the solution cool to room temperature. In order to obtain pH of 5.1, 1M HCL was carefully dropped in the solution. Additional distilled water was poured in the solution to complete the 200 mL solvent. Two (2) milligrams of Glucose oxidase was then dissolved in 1 ml of 50mM Sodium Acetate buffer solution with pH of 5.1. Other set-up solutions were used in liquefying chitosan and D-glucose ($\text{C}_6\text{H}_{12}\text{O}_6$). Ten (10) milligrams of chitosan was dissolved in 1 mL of 1M Acetic Acid (CH_3COOH). Varying amounts of D-glucose concentrations (0.008mM, 0.08mM, 0.8mM, 1mM, 2mM, 4mM, 8mM, 16mM, 32mM, and 64mM) were mixed with 100 ml pure distilled water as electrolytes for the electrochemical- amperometric glucose sensing application of the grown iron oxide NPs.

Instead of glass substrate, stainless steel and graphite rod with grown Iron Oxide NPs using the HVPC Growth Technique were utilized as cathode electrodes in the electrochemical circuit. Electrode surface with Iron Oxide NPs was modified by the application of the chitosan and glucose oxidase enzyme. Some electrodes were prepared with different layer coatings to determine the appropriate combination for further testing of the modified electrode. After the application of chitosan and glucose oxidase, modified electrodes were air dried for four hours and kept at cold temperature for one day before the testing process.

3) *Testing the Modified Electrodes Iron Oxide NPs for Amperometric - Electrochemical Glucose Sensing Application:* The schematic diagram of the circuit utilized in testing the different modified electrodes for Amperometric – Electrochemical Glucose sensing application was shown in Figure 1.

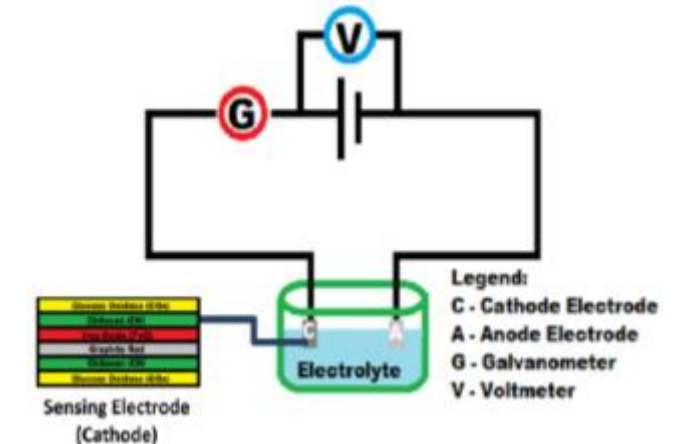


Fig. 1 Electrochemical-Amperometric Circuit

PASCO Scientific Galvanometer was connected in series in the circuit to measure the current while the PASCO –

Explorer GLX was connected in parallel with the circuit to measure the constant applied (bias) potential supplied by the Lodestar DC power supply. The cathode acts as the sensing (working) electrode with varying layer coatings of chitosan, glucose oxidase enzyme and the grown iron oxide NPs in the stainless steel/ graphite rod substrate. Different factors such as modified electrodes, glucose concentrations, applied DC potentials and electrolyte solutions were varied to determine the response current (μA) in the Amperometric- Electrochemical glucose sensing application of Iron Oxide NPs.

C. Statistical Tools

All data collected in the testing of the grown Iron Oxide NPs integrated in the cathode electrode of the electrochemical – amperometric circuit for glucose sensing application were subjected to some statistical tools such as correlation and two-way ANOVA. Correlation was utilized in order to determine the linear relationship between the response current as glucose concentration and applied DC potential were varied. Two-way Analysis of Variance (ANOVA) was used to analyze if there is a significant difference in the current reading when different modified electrodes were employed in several electrolyte solutions.

3 RESULTS AND DISCUSSION

A. Synthesis and Characterization of Iron Oxide Nanomaterials

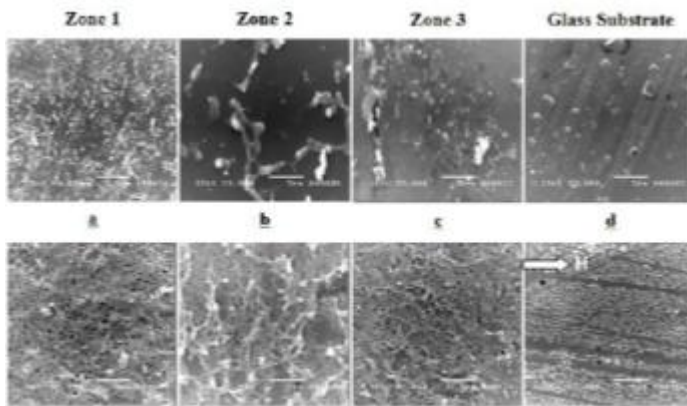


Fig. 2. Micrographs of nanostructures grown with different growth environments: (a-d) Without external magnetic field (e-h) With external magnetic field

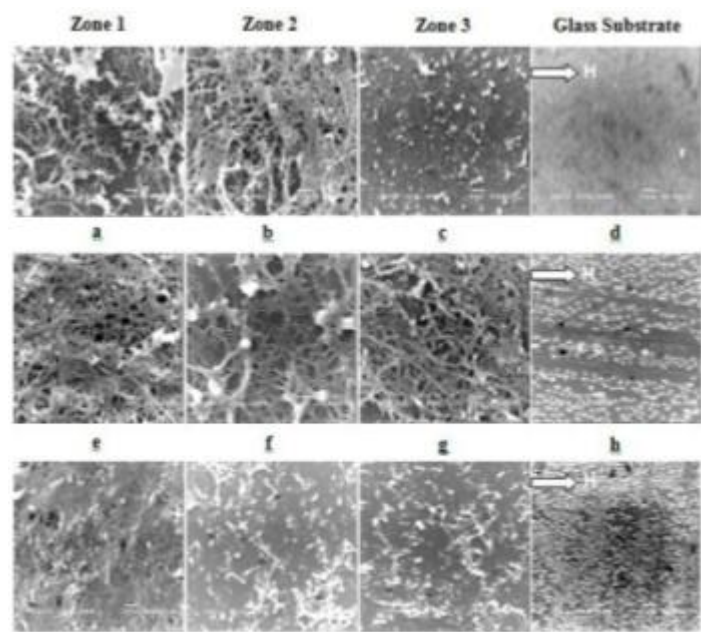


Fig. 3. Micrographs of nanostructures grown with different sizes of sealed tube: (a-d) 10 cm (e-h) 12 cm (i – l) 14 cm

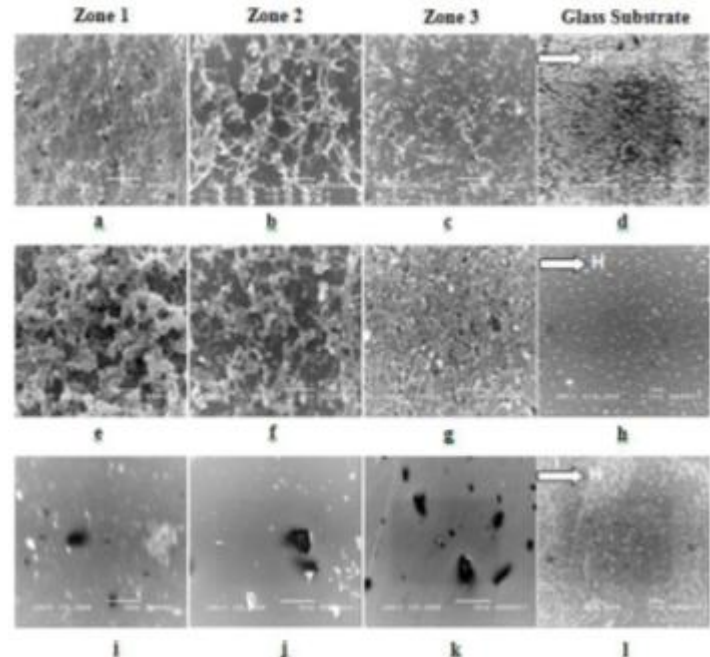


Fig. 4. Micrographs of nanostructures grown with different growth temperatures: (a-d) 1000°C (e-h) 1100°C (i – l) 1200°C

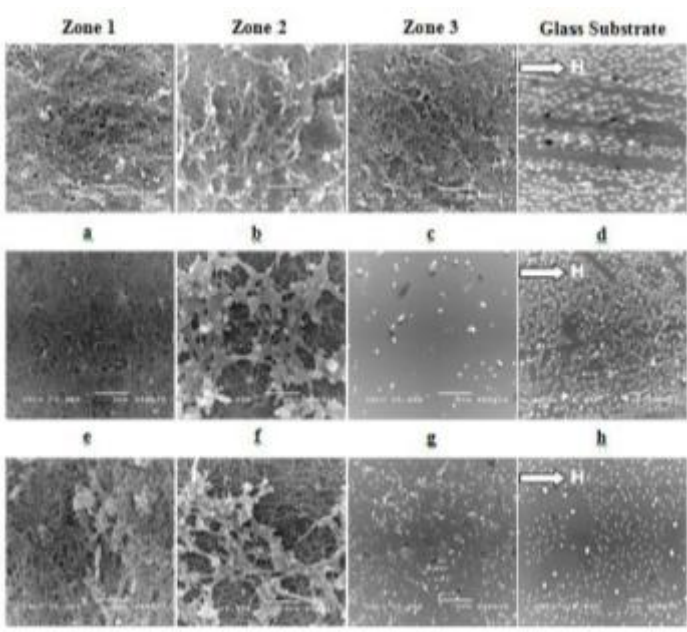


Fig. 5. Micrographs of nanostructures grown with different dwelling times: (a-d) 8 hours (e-h) 9 hours (i – l) 10 hours

Table I shows the summary of the grown Iron Oxide nanomaterials in the different zones of the fused silica tube and glass substrates. The addition of external magnetic field in the growth environment aligns the magnetic moment of Iron Oxide particles which determines the magnetic force on the particles which drive, transport and assemble particles into particle- chain along the magnetic field lines of force. The newly formed nanoparticles enhanced its internal magnetization which attracts other magnetic particles in the zone where these were deposited upon nucleation (Wu *et al.*, 2010).

Table I: Summary of the grown Iron Oxide in different zones after varying different parameters

Parameters	Zone 1	Zone 2	Zone 3	Glass Substrate
A. Varying the GROWTH ENVIRONMENT				
Without External Magnetic Field	Nanowires	microwires	microparticles	microparticles
With External Magnetic Field	Nanowires	nanowires	nanowires	nanoparticles
B. Varying the SIZE OF SEALED TUBE				
10 cm	Nanowires	nanowires	nanowires	nanoparticles
12 cm	Nanowires	nanowires	nanowires	nanoparticles
14 cm	Nanowires	nanowires	nanowires	nanoparticles
C. Varying the GROWTH TEMPERATURE				
1000 °C	Nanowires	nanowires	nanowires	nanoparticles
1100 °C	agglomerated nanowires	agglomerated nanowires	agglomerated nanowires	nanoparticles
1200 °C	-	-	-	nanoparticles
D. Varying the DWELL TIME				
8 hours	Nanowires	nanowires	nanowires	nanoparticles
9 hours	Nanowires	agglomerated nanowires	nanowires	nanoparticles
10 hours	agglomerated nanowires	agglomerated nanowires	nanowires	nanoparticles

A vapor – solid phase nucleation happens during the growth process. These grown NPs interconnected with each other which generated the nanowires/ agglomerated nanowires deposited in different zones of the fused silica tube. The application of an external magnetic field in the growth environment makes the material superparamagnetic

that improves its internal magnetization even when growth temperature is above the Curie temperature (Faraji, M. *et al.*, 2010). Magnetostatic interaction plays an important role in the growth mechanism of the magnetic nanomaterials since the presence of the external magnetic field was also used to lessen the effect of gravity which aligned the growth of the material in the direction of the field.

The optimum parameters in synthesizing Iron Oxide nanomaterials were able to form nanowires and almost uniform size nanoparticles with diameter approximately 50nm. These parameters were with external magnetic field growth environment, 12 cm sealed tube, 1000oC growth temperature and 8-hour dwell time. Thus, superparamagnetism occurs when there is a size reduction of the materials resulting in the single domain particles with diameter in the range of 3- 50 nm.

B. Elemental Composition of Iron Oxide Nanomaterials

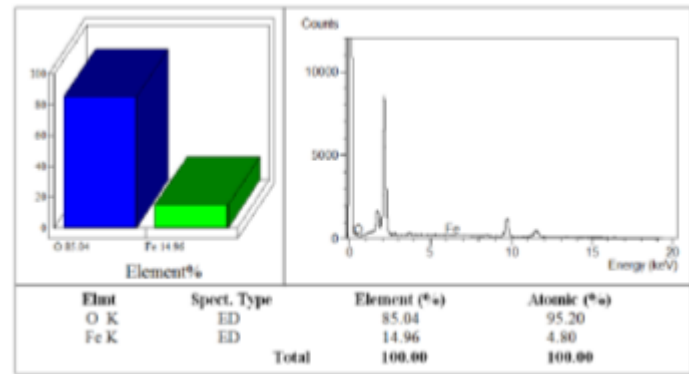


Figure 6. Iron Oxide nanowires grown at the fused silica tube (a) Elemental Composition graph (b) EDX Spectrum (c) Elemental/ Atomic Compositions

Fig. 6 shows composition of the oxygen and iron for the grown nanowires at the fused silica tube. More oxygen content was observed for nanowires than in the nanoparticles grown and deposited in the substrate.

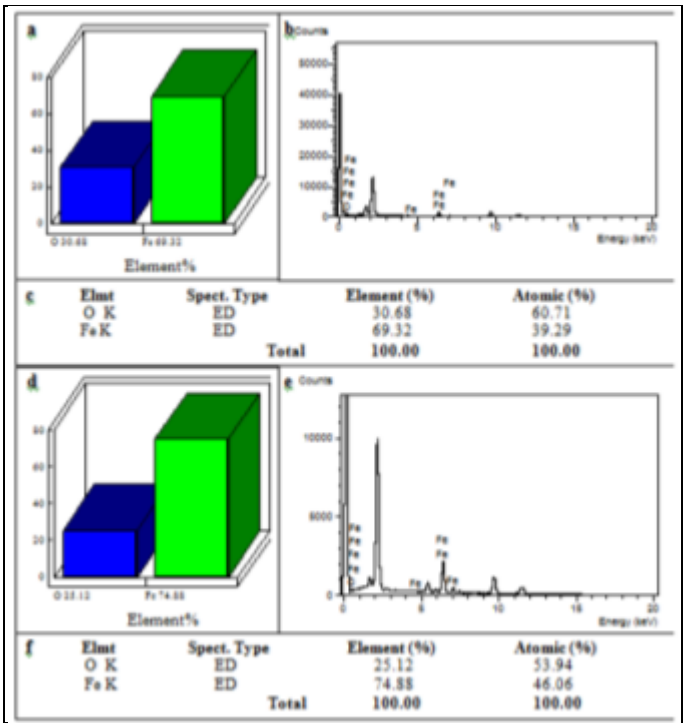


Figure 7. Iron Oxide NPs grown at the different parts of the stainless steel substrate (a, d) Elemental Composition graph (b, e) EDX Spectrum (c, f) Elemental/ Atomic

Different elemental and atomic compositions were observed for nanostructures grown. Compositions vary depending on the type of substrate iron oxide was deposited under external magnetic field. Magnetite – iron oxide NPs were found in the stainless steel (a) / graphite rod (d) substrates as shown in Fig. 7.

C. Grown Iron Oxide Nanoparticles (NPs) utilized in modified cathode electrode of an Amperometric – Electrochemical circuit for glucose sensing application

1) Evaluating the performance of the grown Iron Oxide NPs integrated in the cathode electrode: The Iron Oxide NPs were synthesized utilizing the optimum parameters such as with external magnetic field growth environment, 12 cm sealed tube, 1000°C growth temperature and 8-hour dwell time. These NPs with an average size of about 50 nm indicate its Super Paramagnetic property which promotes faster electron transfer and β – D glucose detection via glucose oxidase enzyme. The grown NPs were embedded in the cathode electrode which was connected to an amperometric – electrochemical circuit as shown in Fig. 8. The current readings were observed and recorded against varying different factors such as modified cathode electrodes, glucose concentrations, applied DC potential, and electrolyte solutions. Some factors that may interfere in the data gathering were held constant.

Table II. Current Reading for different Modified Cathode Electrodes

Modified Cathode Electrode	Current Reading (µA)		
	Glucose Solution (8mM)	Distilled Water	Difference
SS	0.00	0.00	0.00
SS/FeO	3.00	1.00	2.00
SS/CH	2.50	2.00	0.50
SS/GOx	4.00	2.00	2.00
SS/FeO/GOx	5.00	2.00	3.00
SS/FeO/CH	4.50	3.00	1.50
SS/FeO/GOx/CH	6.00	3.00	3.00
SS/FeO/CH/GOx	5.00	3.50	1.50
SS/FeO/CH/GOx/CH	5.00	3.00	2.00
G	1.00	0.50	0.50
G/FeO	1.50	1.00	0.50
G/FeO/GOx	3.00	1.50	1.50
G/FeO/GOx/CH	8.00	5.00	3.00
G/FeO/CH/GOx	9.00	6.00	3.00
G/FeO/CH	6.00	4.00	2.00
G/CH	3.50	2.00	1.50
G/GOx	3.50	2.00	1.50

Legend: SS - Stainless Steel; FeO - Iron Oxide NPs; GOx - Glucose Oxidase; CH - Chitosan; G - Graphite rod

Table II shows the current reading for each modified cathode electrode. The silver plate anode, 0.60 V applied DC potential, distilled water and 8mM glucose concentration were held constant. Current reading increases until reaching the maximum value then gradually decreases with time. This happens when the glucose concentration becomes less in the analyte (β -D Glucose) – enzyme complex, less electrons will be produced. Based from the gathered data, some modified electrodes with Iron Oxide NPs (e.g. SS/FeO/GOx/CH, G/FeO/CH/GOx) enhanced the enzyme’s ability of sensing its analyte (β -D Glucose) since it promotes faster electron transfer from analyte to electrode’s surface. Thus, glucose oxidase enzymes detects β -D Glucose in less than 5s response time with highest current reading of 9µA in G/FeO/CH/GOx electrode.

Table III. Current Reading after varying the glucose concentration

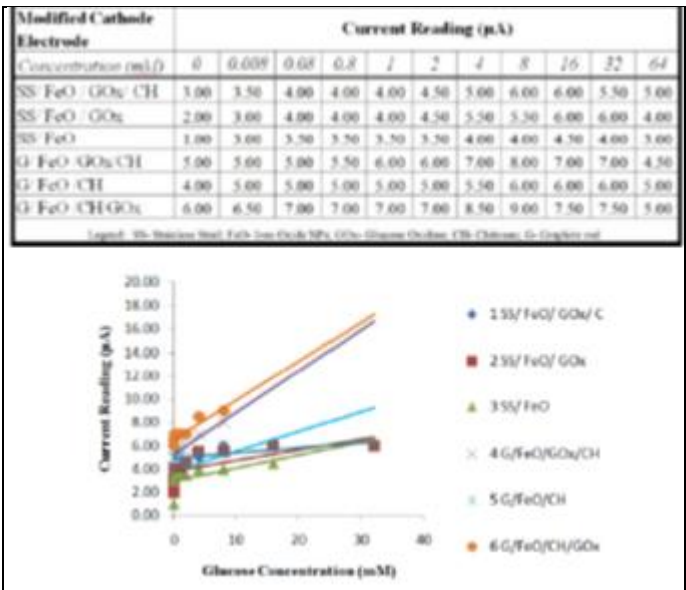


Fig. 8. Current Reading for different Glucose Concentrations within detection limits

Table III and Fig. 8 describe the effect of varying the concentration of glucose to the current reading. Only six best-modified cathode electrodes were used in this setup and some controls were 0.60 V DC applied potential and silver plate anode electrode. When the glucose concentration increases, the response current also increases until reaching the maximum limit that glucose can be detected by the modified electrode in an amperometric – electrochemical circuit.

Table IV and Fig. 9 show the direct relationship of current and applied DC potential. Some controls in this setup were six best-modified cathode electrodes, silver plate anode electrode, and 8mM glucose concentration. As potential increases, current also increases for every electrode. Maximum current reading recorded was 13.5 µA from G/FeO/CH/GOx electrode.

Table IV. Current Reading after varying the applied DC potential

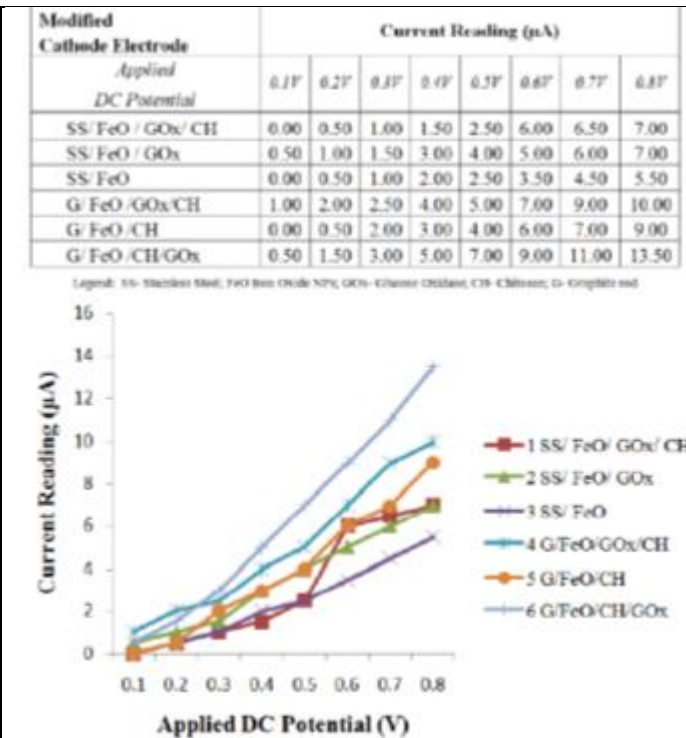


Fig. 9. Current Reading for different applied DC Potential

Table V. Current Reading for different electrolytes in each Modified Electrode

Modified Cathode Electrode	Current Reading (µA)			
	Electrolytes(8mM)	Distilled Water*	Glucose Oxidase	Uric Acid
SS/FeO/GOx/CH		3.00	6.00	4.00
SS/FeO/GOx		2.00	6.50	4.00
SS/FeO		1.00	3.00	4.00
G/FeO/GOx/CH		5.00	8.00	5.50
G/FeO/CH		4.00	6.00	5.00
G/FeO/CH/GOx		6.00	9.00	5.50

*except Pure Distilled Water

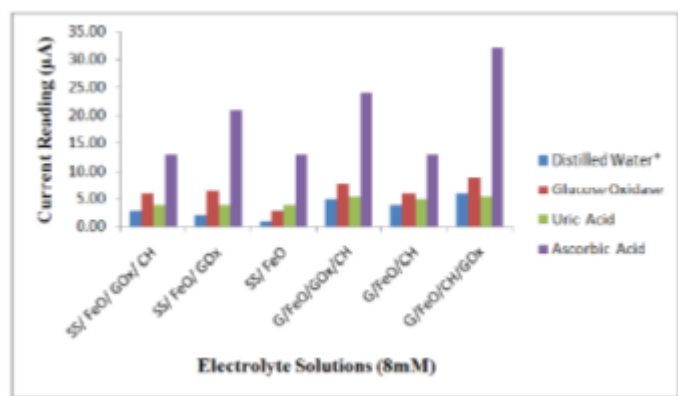


Figure 10. Current Reading for different Electrolyte Solutions

Table V and Fig. 10 illustrate the different current readings for several electrolytes for six best electrodes. This setup further verifies that the electrode with iron oxide NPs, glucose oxidase and chitosan specifically determines β -D glucose. Uric acid was undissolved in 100ml distilled water that produced the same current responses for each type of substrate (SS/ G). The difference between the current responses for ascorbic acid and glucose was obviously seen. Higher values for current were tabulated for ascorbic acid since it is an ionic solution compared to non-ionic glucose having the same concentration. Distilled water was used as reference value without any concentration for comparison.

2) *Comparison to Commercial Glucose Meter*: A commercial glucose meter was also evaluated to determine its performance in determining the concentration of glucose. Some of its characteristics were compared to the setup with Iron Oxide NPs integrated in the modified electrode of an amperometric - electro chemical circuit. The glucose meter includes a test strip that employs the enzyme glucose oxidase (A. Niger – 7%) that is specific for glucose measurement from 20 to 600 mg/dL (1.1 – 33.3 mM) in the blood samples. Other reagent compositions present in a test strip are electron shuttle (53.3%) and Non- Reactive agents (39.1%). The enzyme in the test strip reacts with glucose in blood and produces an electrical signal which measured by Glucose Meter and test results is displayed in 6 seconds (Apex Biotechnology Corp., 2010)

Table VI: Glucose Meter Reading and Amperometric - Electrochemical Current Reading

Glucose Concentration(mM)	0	0.008	0.08	0.8	1	2	4	8	16	32	64
Commercial Glucose Meter Reading											
Ave. Glucose Rdy. (mg/dL)			320*	20*		20	42*	114	149	340	600*
(mM)			17.60	1.10		1.10	2.30	6.30	8.20	18.70	33.00
Ave. Response Time (min)	4.00	6.00	10.00	8.50	7.50	4.50	4.00	10.5	5.00	7.00	6.50
Deviation(mM)			17.52	0.30		-0.9	-1.7	-1.7	-7.8	-13.3	-31
Iron Oxide NPs integrated in the modified electrode of an Amperometric - Electrochemical Circuit											
Current Rdy. (µA)**	3.00	3.50	4.00	4.00	4.00	4.50	5.00	6.00	6.00	5.50	4.50
Calibrated Glucose Rdy(mM)	0.00	0.008	0.08	0.8	1.00	2.00	4.00	8.00	16.0	32.0	64.0
Ave. Response Time (s)	3.00	2.00	2.00	2.00	3.00	3.00	3.00	3.00	4.00	4.00	4.00

Table VI shows the average concentration readings of the commercial glucose meter which were compared to the

calibrated glucose concentration of the modified electrode (SS/FeO/GOx/CH) of an Amperometric - Electrochemical Circuit. There are some deviations in the readings made by the commercial glucose meter compared to the actual concentration of the substance since it is intended to measure the amount of glucose within the blood sample. Some limitations of the commercial glucose meter are longer response time (ave. of 6.64 minutes), unable to detect glucose with low concentrations and one-time usage only. On the other hand, Iron Oxide NPs integrated in the modified electrode of an Amperometric- electrochemical circuit showed fast response time (<5s), low detection limit (0.008mM) and longer detection limit range (0.008mM to 32mM). Same modified electrodes with Iron Oxide NPs were utilized from the previous variations (glucose concentration, applied DC potential and electrolytes solution) which indicated the usage repeatability for about 20 times.

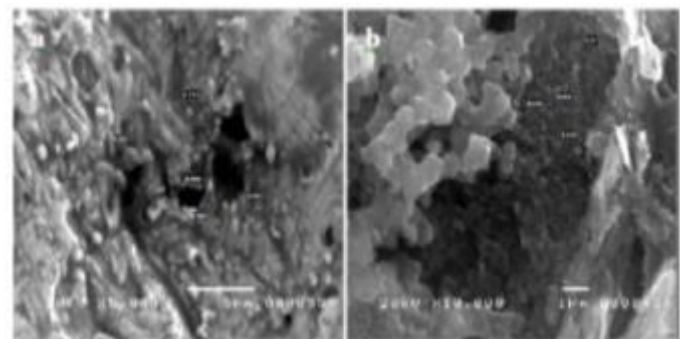


Figure 11. SEM Images of the Iron Oxide NPs integrated in the Cathode Electrode of an Amperometric - Electrochemical for Glucose Sensing Application: (a) Stainless substrate before the application of Glucose Oxidase and Chitosan (b) Graphite rod Substrate after the experimentation process with the application of Glucose Oxidase and Chitosan.

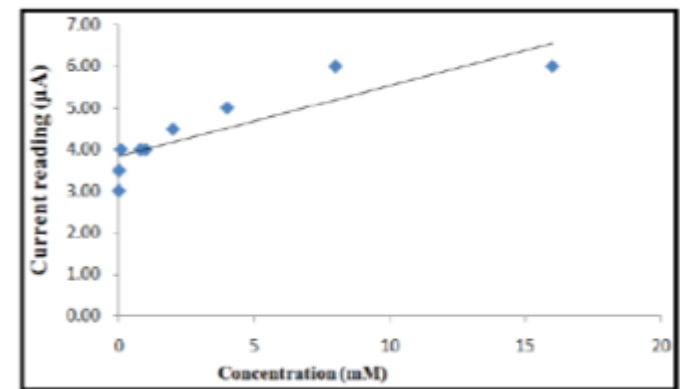


Figure 12. Concentration Calibration Curve of Iron Oxide NPs integrated in the Cathode Electrode of Amperometric - Electrochemical for Glucose Sensing

Figure 12 shows the calibration curve of the concentration – current reading of SS/FeO/GOx/CH modified electrode of an amperometric-electrochemical glucose sensing application of Iron Oxide NPs. As current increases, concentration also increases for Iron Oxide NPs integrated in the cathode electrode of the Amperometric – Electrochemical Circuit for Glucose Sensing.

The integration of the Iron Oxide NPs in the electrode for amperometric – electrochemical circuit in sensing glucose demonstrates high surface reaction and catalytic activity, large surface-to-volume ratio and strong adsorption ability that are beneficial in the immobilization of glucose oxidase (Kaushik *et al.*, 2008; Cash and Clark, 2010). Magnetic Iron Oxide NPs are good in the modification of electrode for Amperometric – Electrochemical circuit since the magnetic field reinforces the attraction of the particles to the electrode's surface (Li *et al.* 2009; Cash and Clark, 2010).

Superparamagnetic Iron Oxide NPs also acts as supramolecular assembly unit with advance functional properties that enhances the electrochemical sensing interface (Ansari *et al.*, n.d.). Iron Oxide NPs serve as nano-connectors to activate the redox- enzyme that speeds up the reaction which resulted to faster response time of detection. Higher sensitivity electronic signal transduction was generated since the modified electrode with Iron Oxide NPs has lower electroanalytical limit of detection.

3) *Statistical Analysis*: Two- way ANOVA were utilized in order to determine if there is a significant difference in the response current for variations made such as modifying the electrode and electrolyte solution. Correlation coefficients were computed to find out if there is a linear relationship between glucose concentration- current and applied DC potential - current.

Table VII: Two-way ANOVA for different modified electrodes variation

SUMMARY	Count	Sum	Average	Variance
SS	2	0.00	0.00	0.00
SS/ FeO	2	4.00	2.00	2.00
SS/ CH	2	4.50	2.25	0.12
SS/ GOx	2	6.00	3.00	2.00
SS/ FeO GOx	2	7.00	3.50	4.10
SS/ FeO CH	2	7.50	3.75	1.13
SS/ FeO GOx CH	2	9.00	4.50	4.50
SS/ FeO CH GOx	2	8.50	4.25	1.13
SS/ FeO CH GOx CH	2	8.00	4.00	7.00
G	2	1.50	0.75	0.13
G/ FeO	2	2.50	1.25	0.13
G/ FeO GOx	2	4.50	2.25	1.13
G/ FeO GOx CH	2	15.00	7.50	4.50
G/ FeO CH GOx	2	15.00	7.50	4.50
G/ FeO CH	2	10.00	5.00	2.00
G/ CH	2	5.50	2.75	1.13
G/ GOx	2	5.50	2.75	1.13
8mM Glucose Solution	17	79.50	4.67	5.49
Distilled Water	17	41.50	2.44	2.47

ANOVA	Source of Variation	SS	df	MS	F	P-value	F crit
	Modified Electrodes	129.06	16	7.50	16.53	0.000001	2.33
	Electrolytes	24.74	1	24.74	54.48	0.000002	4.49
	Error	7.26	16	0.45			
	Total	152.06	33				

Since $F(16.33) > F_c(2.33)$ at $\alpha = 0.05$, there is a significant difference in the response current for different modified electrodes. The addition of the magnetic Iron Oxide NPs in the electrode promotes faster electron transfer from the analyte to the electrode's surface. There is also a significant difference in the current readings for different electrolytes (distilled water, 8mM glucose) since the $F(54.48) > F_c(4.49)$ at $\alpha = 0.05$. Current reading in the distilled water is equivalent to zero concentration of glucose that will serve as reference values in this study.

To show the linear relationships between the current reading and the glucose concentration/ applied DC potential for different modified electrodes, correlation coefficients were obtained as can be seen in Tables VIII and IX.

Table VIII: Correlation Coefficients for current reading while varying glucose concentrations

Modified Cathode Electrode	low DL mM	high DL mM	r	r ²
SS/ FeO/ GOx/ CH	0.008	16.00	0.45	0.87
SS/ FeO/ GOx	0.008	32.00	0.25	0.70
SS/ FeO	0.008	16.00	0.07	0.64
G/ FeO/ GOx/ CH	0.800	8.00	0.13	0.59
G/ FeO/ CH	0.008	32.00	0.24	0.71
G/ FeO/ CH/ GOx	0.008	8.00	0.43	0.93
AVERAGE			0.26	0.74

r² - correlation coefficient within detection limit (DL) range
 r - correlation coefficient outside detection limit (DL) range

Table VIII shows the correlation coefficients for varying glucose concentration given the range from lowest to highest detection limits for each modified electrode. The average correlation coefficient ($0.74 \approx 1.0$) obtained for different electrodes suggests that there is a positive linear relationship between the glucose concentration and the response current within the detection limit range.

Table IX. Summary of Correlation Coefficients for current reading while varying DC Applied potential

Modified Cathode Electrodes	r
SS/ FeO/ GOx/ CH	0.96
SS/ FeO/ GOx	0.99
SS/ FeO	0.99
G/ FeO/ GOx/ CH	0.99
G/ FeO/ CH	0.99
G/ FeO/ CH/ GOx	1.00
AVERAGE	0.99

As can be seen in Table IX, the average correlation coefficient ($r = 0.99$), there is a positive linear relationship shown between the DC applied potential and the response current. Fig. 12 illustrates a straight line graph for different modified electrodes indicating that it follows an Ohmic relationship.

Table X. Two-way ANOVA for varying electrolytes solution for different modified electrodes

SUMMARY	Count	Sum	Average	Varianse
SS: FeO: GOx: CH	4	20.00	0.50	20.33
SS: FeO: GOx	4	33.50	8.38	74.23
SS: FeO	4	21.00	5.25	28.25
G:FeO:GOx:CH	4	42.50	10.63	81.23
G:FeO:CH	4	28.00	7.00	16.67
G:FeO:CH:GOx	4	92.50	23.13	160.73
Distilled Water	6	21.00	3.50	3.50
Glucose Oxidant	6	38.50	6.42	4.24
Uric Acid	6	28.00	4.67	0.57
Ascorbic Acid	6	116.00	19.33	61.07

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Modified Electrodes	170.93	5	34.19	2.91	0.05	2.90
Electrolytes	908.50	3	322.79	27.52	0.00	3.29
Error	175.05	15	11.73			
Total	1315.34	23				

Since the $F(2.91) > F_c(2.90)$ at $\alpha = 0.05$, there is a significant difference in the response current for six best modified electrodes. The variation in the electrolyte solutions also produced a significant difference in the values of current with $F(27.52) > F_c(3.29)$ at $\alpha = 0.05$. This verifies that each modified electrode specifically detects β -D glucose as can be seen in the different current responses for each electrolyte solution.

4 CONCLUSION

Iron Oxides NPs have been successfully synthesized using Horizontal Vapor Phase Crystal (HVPC) Growth Technique which was utilized in glucose sensing application. These NPs immobilized the glucose oxidase enzyme and improved the electron transfer between the glucose analyte to the modified electrode. Iron oxide NPs integrated in the modified electrode of an Amperometric – Electrochemical Circuit for sensing glucose showed low detection limit which indicates a large surface to volume ratio, fast response time which suggests that the catalytic property of the immobilized glucose enzyme was enhanced and usage repeatability as Chitosan prevents the aggregation of Iron Oxide NPs when being utilized in an amperometric electrochemical circuit. In

addition, high sensitivity was also observed within detection limit range of 0.008mM to 32mM.

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