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Utilization of polyvinyl amine hydrolysis product in enhancing the catalytic properties of Co_3O_4 nanowires: Towards potentiometric glucose bio-sensing application

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Abstract

In this study, we have used a concept of overnight hydrolysis of polyvinyl amine and its role towards the enhancement of catalytic properties of cobalt oxide (Co_3O_4) nanostructures. The use of the hydrolyzed products in the synthesis of controlled Co_3O_4 nanostructures was investigated by hydrothermal method. Both of hydrocarbon chain and amide groups were produced and exhibited strong impact on the morphology and catalytic properties of Co_3O_4. The hydrolyzed products supported the well-defined Co_3O_4 nanowires morphology. Additionally, the produced Co_3O_4 nanowires displayed high surface to volume ratio and they were loaded with high quantity of glucose oxidase, and consequently a sensitive potentiometric response towards glucose detection was observed. Importantly, the proposed biosensor was tested against several analytical parameters such as linear range, stability, reproducibility, repeatability, storage life time,

selectivity, and response time. Resulted attested that the fabricated Co_3O_4-based glucose biosensor exhibited a linear response over the concentration range of 0.0005 to 6 mM with a limit of detection(LOD) of 0.0001mM. The Nernstian slope of 42 mV/dec of fabricated glucose biosensor was estimated. The storage life was also studied for four weeks. Furthermore, the improved electrochemical sensing performance of Co_3O_4 nanowires was evidenced by electrochemical impedance spectroscopy measurements, which showed low charge transfer resistance of 2.2 x 10^3 Ohms. The produced glucose biosensor was successfully used to quantify the glucose concentration from real blood samples, thereby emphasizing its possible utilization as an alternative tool for monitoring of glucose levels. Thus, this new synthesis concept has a high potential for production of large scale well-defined morphology and improved catalytic properties of desired nanostructured materials to suit various electrochemical applications including batteries, supercapacitors, and water splitting.

Keywords: Hydrothermal method, Nucleation and Growth, Nanostructure, Oxidation

1. Introduction

Glucose is a fundamental need of living objects. The physiological glucose concentrations are in the range of 79.2-110 mg dl^{-1} at the fasting conditions in the human blood. The abnormal glucose concentration is an indicator of several metabolic problems and is considered as main causing agent for the different diseases including diabetes, obesity and cardiovascular. Therefore, the monitoring of glucose in human body is highly important [1] in order to prevent the life taking diseases. Several efforts have been put forwarded in this direction for the quantification of glucose with excellent sensitivity, efficiency, and swift response from the vitro and vivo conditions. The glucose biosensors have been developed and used for the detection of glucose including optical devices [2], colorimetric [3], fluorimetry[4], amperometry [5], and potentiometric methods [6] since several decades.

Among them, the electrochemical methods are versatile for the detection of glucose due to their numerous advantages such as high sensitivity, fast in response, stability, reproducibility and simplicity. Generally, glucose biosensors have been fabricated by using enzymatic approach [7, 8] since decades. The use of amperometry mode of electrochemical methods at high current bias produces the interference effect from easily oxidizing agents such as ascorbic acid and uric acid during the glucose sensing. Therefore, the selectivity and destruction of biological cells are the

major issues during amperometric methods. In contrast to amperometric glucose biosensors, the potentiometric measurement have high superiority because it does not use any current bias so the issue of selectivity and the destruction of biological samples is prevented during the analysis. Today, an advancement in the field of nanostructured materials made it possible to manipulate the materials close to the atomic level with outstanding physical and chemical properties. Therefore taking the advantage of high surface to volume ratio of nanostructured materials which can easily and densely host the glucose oxidase enzyme and generate a strong output potential when the glucose oxidation reaction could take. To add this, various precious metal and metal oxide nanostructures with extent sensing performance have be used for the development of glucose sensors [9-23]. Cobalt oxide (Co_3O_4)is one of the notable transition metal oxide which exhibits plenty of favorable offers for the development of biosensors such as good biocompatibility, and excellent stability, low cost and can be produced from earth abundant materials [24]. These potential properties of Co_3O_4 have been capitalized for the wide range of electrochemical applications such as electrocatalysis, supercapacitors, batteries and electrochemical sensing devices [25-30]. The point of care for using Co_3O_4 based material for the potentiometric glucose biosensor is that it should have high catalytic activity, surface area and can produce a high output potential signal, therefore these properties of Co_3O_4 should be improved before to use it for glucose bio-sensing applications. Keeping these challenges in mind, we have used a concept of hydrolyzing the polyvinyl amine as it could produce number of long chain of hydrocarbons, amide and hydroxide ions which further may play a vital role on the control of morphology and catalytic properties of Co_3O_4 nanostructures. The use of hydrolyzed products of polyamineand their contributing role on the well defined nanostructures ise not reported elsewhere.

In this study, we have used the reactive species produced from the overnight hydrolysis of polyvinyl amine for the growth of controlled Co_3O_4 nanostructures via hydrothermal method. These Co_3O_4 nanostructures were physically studied by SEM, EDS and XRD techniques. Then, the glucose oxidase was physically immobilized onto nanowires through electrostatic attraction for the development of potentiometric glucose biosensor. The proposed glucose biosensor has shown the linear response for the glucose concentrations from 0.0005 mM to 6 mM with a limit of detection of 0.0001mM. The fabricated glucose biosensor is stable, reproducible, selective and

sensitive, therefore it can be used as an alternative sensing device for the analysis of glucose form real samples.

2. Materials and Methods

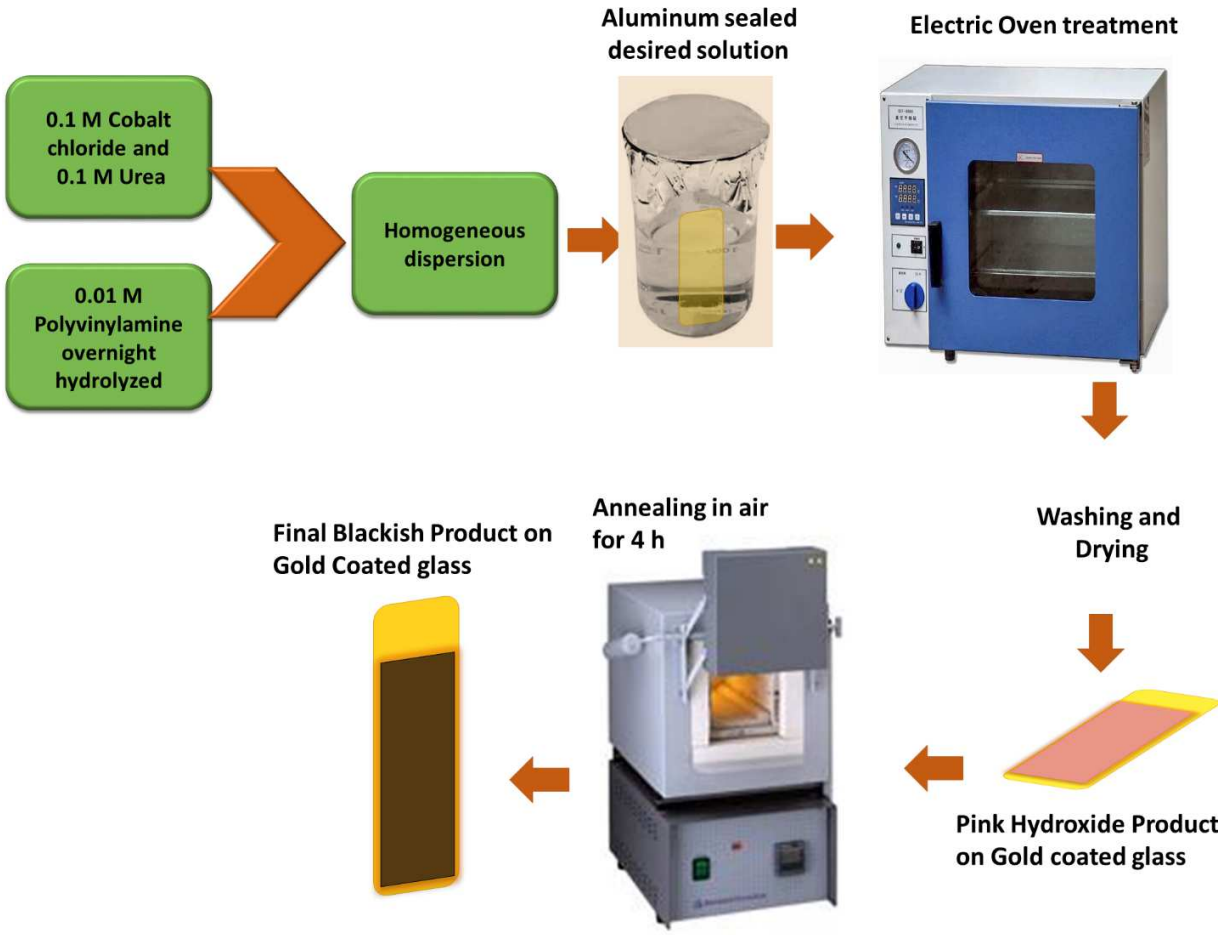
2.1. Materials

Cobalt chloride hexahydrate, urea, polyvinyl amine, glucose, uric acid, ascorbic acid, oxidase, 25% glutaraldehyde and saline phosphate buffer of pH 7.3. These chemicals were purchased from Sigma-Aldrich Karachi Pakistan. All the experiments were performed in the prepared phosphate buffer solution of pH 7.3 in deionized water.

2.2. Preparation of modified nanostructures of Co_3O_4 using polyvinyl amine via hydrothermal method

The nanostructures of Co_3O_4 were prepared by three steps methodology described as under: First, we have hydrolyzed the 0.01M polyvinyl amine in deionized water for overnight as its hydrolysis kinetics is slow. The aqueous solution could contain both the hydrocarbon chains, amide and hydroxide ions. The presence of hydrocarbon chain could accelerate the length of one dimensional Co_3O_4 nanostructures whereas the concentration of amide and hydroxide ions is enhancing the nucleation rate. The obtained nanostructures of Co_3O_4 have high surface area, excellent catalytic sites, thus produced an enhanced output potential during potentiometric measurements. The Co_3O_4 nanostructures were grown the gold coated glass substrate using hydrothermal method and the fabrication of gold coated glass electrode is described in our published work [31]. Secondly, the 0.1M concentrations of cobalt chloride hexahydrate and urea precursors were made in the hydrolyzed solution of polyvinyl amine. Then cleaned gold coated glass substrate was vertically hanged in the beaker. Then precursor's solution was sealed with aluminum sheet and hydrothermally treated at 95 ^{o}C for 5 hours in electric oven. Afterwards, the cobalt hydroxide nanostructures were washed several times with the deionized water and ethanol in order to wash out the unreacted amount of hydrocarbon and amide groups from the surface of materials. Then, the nanostructured fabricate substrate was dried in open air for overnight at room temperature. In the third step, we combusted the cobalt hydroxide nanostructures in the muffle furnace for 4 hours. Finally, we obtained a dark product of Co_3O_4 and it was used for the structural and functional characterization. The powder of nanostructured material was also obtained by these steps. The

pristine Co_3O_4 sample was prepared without the addition of hydrolyzed products of polyvinyl amine by the similar method. The synthesis process is further described by scheme 1.



Scheme 1: Illustrates the synthesis process for the production of Co_3O_4 nanowires in the presence of overnight hydrolyzed polyvinyl amine solution

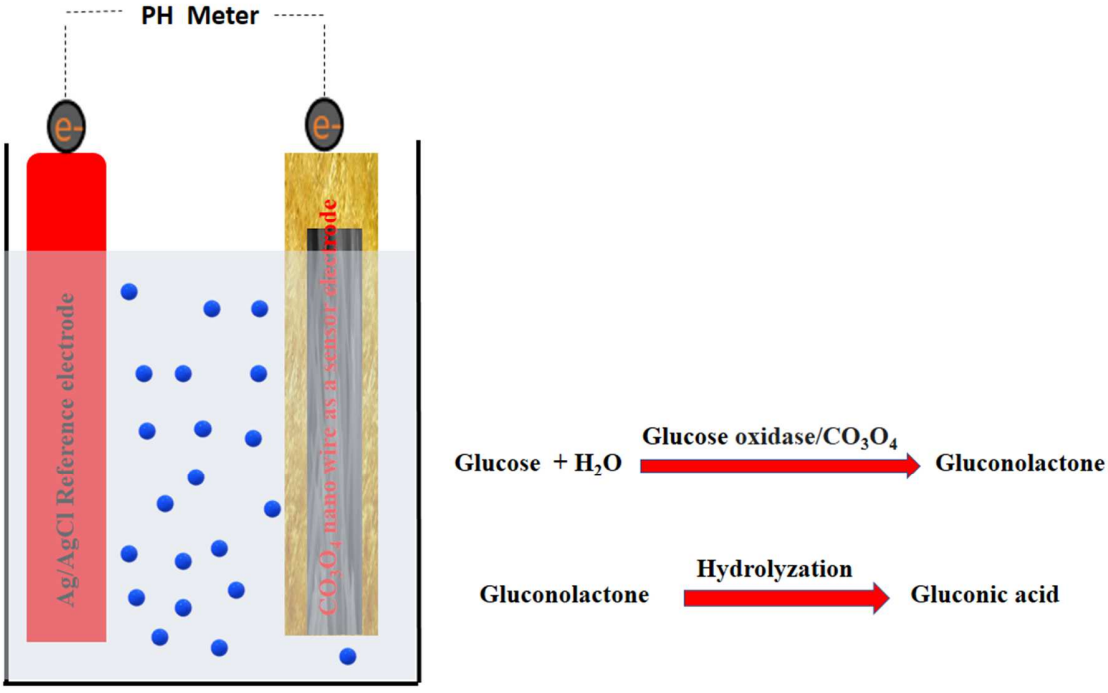
2.3. Characterization and bio-sensing application

The crystalline structure and morphology were studied by the powder X-ray diffraction and scanning electron microscopy techniques. For the structural studies, the Co_3O_4 material was scratched form the gold coated substrate. The composition of material was analyzed by the energy dispersive spectroscopy equipped with SEM. Scanning electron microscopy was carried out at 3kV with a ZEISS Gemini SEM 500 equipped with field emission gun. XRD was carried out under the

conditions using the source of CuKα radiation (λ = 1.54050 Å), current of 45 mA, and an accelerating voltage of 45 kV.

The fabrication of glucose biosensor based on glucose oxidase functionalized Co_3O_4 nanostructures was as followed:

Prior to the fabrication of glucose biosensor, we dissolved a 10 mg of glucose oxidase in 0.01M phosphate saline solution of pH 7.3 and 100 µL of 5% glutaraldehyde as a cross linker for the prevention of self-enzyme reaction. Then the nanostructures of Co_3O_4 were dispersed in the enzyme solution for 10 mints and dried for 30 min. Then, glucose oxidase was immobilized onto Co_3O_4 nanostructures and used as working electrode for the potentiometric determination of glucose in phosphate buffer solution. The potentiometric measurements were performed with two electrode cell set up. The reference electrode was silver-silver chloride (Ag/AgCl) filled with 3M KCl solution. A 0.1M stock solution of glucose was prepared in 0.01M phosphate buffer solution of pH 7.3. The fresh solutions of glucose were prepared in the phosphate buffer solution using dilution method and used for the functional characterization. All the potentiometric measurements were performed at room temperature. All the potentiometric measurements were performed with pH meter from METTLER TOLEDO and dynamic response and sequential experiment was done on the potentiotat supplied from Netherland. The charge transport during potentiometric response was evaluated in 0.5mM glucose solution prepared in phosphate buffer solution of pH 7.3 by electrochemical impedance spectroscopy (EIS) at sweeping frequency from 50 KHz to 5 KHz at 10 mV of sinusoidal potential and 0 open circuit potential. The practicality of fabricated glucose biosensor was evaluated via glucose determination in blood. Real samples of 5µL of human blood were collected from three subjects after breakfast and used without any treatment, but the dilution in 5 mL of phosphate buffer solution of pH 7.3 was made and stirred for 120 s. Then, each sample was analyzed by proposed glucose biosensor.



Scheme 2: Sensing mechanism of glucose ontto cobalt oxide nanowires coated with glucose oxidase

3. Results and discussion

3.1. Structure, and composition studies Co_3O_4 samples

The crystallographic analysis on the prepared Co_3O_4 samples was carried out by the powder XRD technique as shown in Figure 1. The pristine Co_3O_4 and polyvinyl amine assisted Co_3O_4 samples have shown well defined crystal geometries which are confirmed as cubic phase and well supported by the reference card no: 00-009-0418. The overnight hydrolysis of polyvinyl amine has shown a significant effect on the crystal arrays of Co_3O_4, therefore a strong potentiometric signal was shown by these nanostructures. Also, the polyvinyl amine assisted Co_3O_4 nanostructures have shown a strong compatible surface for the immobilization of glucose oxidase which further accelerated the glucose oxidation and an enhanced output potential was observed. The XRD study has revealed only pure cubic phase of Co_3O_4.

The morphology of pristine Co_3O_4 and polyvinyl amine assisted Co_3O_4 nanostructures were studied by SEM as enclosed in Figure 2. The pristine Co_3O_4 has possessed a randomly oriented morphology of platelet consisting a self-combination of nanoparticles as shown in Figure 2a-b. The length of platelets may be in the range of few microns and size of self-assembled Co_3O_4 could be less than 150 nm. However, the overnight hydrolysis of polyvinyl amine has changed the morphology of Co_3O_4 from platelets to nanowires with a proper orientation as shown in Figure 2c-d. The length of polyvinyl assisted Co_3O_4 could be 5-7 microns and the diameter of nanowires might be around 100 nm. Also, the hydrolyzed polyvinyl amine has shown Co_3O_4 nanostructures with high surface to volume ratio which is highly important for the loading of glucose oxidase enzyme and resulting an improved output potential signal during the potentiometric analysis. From SEM analysis, it is obvious that the polymer hydrolysis has played a very critical role in tuning the morphology of Co_3O_4 through the presence of large amount of hydrocarbon chains, and amide groups during the growth process. The growth kinetics of Co_3O_4 is well governed by the presence of these groups which strengthened the idea of controlling the morphology of metal oxides by using the overnight hydrolysis of polyvinyl amine. The chemical composition of both pristine and polyvinyl amine assisted Co_3O_4 samples was also investigated by EDS as shown in Figure 3a-b. Both materials were containing the Co and O as the main elements.

3.2.Potentiometric analysis of glucose using glucose oxidase immobilized polyvinyl amine assisted Co_3O_4 nanostructures

The glucose oxidase was immobilized on the polyvinyl amine assisted Co_3O_4 nanowires and they were tested as a working electrode for the potentiometric determination of glucose in 0.01M saline phosphate buffer solution. The working electrode was soaked in saline phosphate buffer solution of pH 7.3 for overnight in order to quantify the stable output potential signal. The glucose oxidase was also immobilized on the pristine Co_3O_4 for the comparative study for understanding the role of Co_3O_4 nanowires obtained from the overnight hydrolysis of polyvinyl amine. Figure 4a-b shows the output potential response of pristine and polyvinyl amine assisted Co_3O_4 and it can be seen that the well-defined morphology with high surface to volume ratio is very important for the development of potentiometric glucose biosensors. The performance of pristine Co_3O_4 sample is poor in terms of analytical parameters such as regression coefficient, linear range and limit of detection as shown in Figure 4a. However, the performance of polyvinyl amine assisted Co_3O_4 is highly improved in terms of analytical properties including, regression coefficient of 0.99, wide

linear range of 0.0005 mM to 6 mM, and low limit of detection of 0.0001mM. The improved performance of polyvinyl amine assisted Co_3O_4 could attributed to heavy loading of glucose oxidase, fast electron communication due to proper orientation of nanowires and also a highly biocompatible surface. Importantly, the Nernstian slope of 42 $mVdec^{-1}$ is also obtained.

The bio-sensing mechanism on the glucose oxidase immobilized Co_3O_4 nanowires is mainly controlled by the interaction of glucose oxidase with glucose substrate which was further strengthened by the nanowires of Co_3O_4 as a co-catalyst. The co-catalytic properties of Co_3O_4 have supported the main glucose oxidase reaction towards the glucose oxidation. The most important reactions involved during the potentiometric measurements are as described. The reaction of glucose on the immobilized glucose oxidase onto the surface of Co_3O_4 nanowires resulted into the formation of unstable glucono-lactone species, which further gives rise to gluconic acid. Then, gluconic acid interacts with water and produces the gluconate and hydronium ions in the electrochemical cell as shown under [32]:

$H_2O + O_2 + \beta$-D- glucose GOD $\rightarrow$ d-gluconolactone+H_2O_2 (1)

d-glucono lactone spontaneously $\rightarrow$ $gluconate^- + H^+$ (2)

It is the most probable the concentration of hydronium and gluconate ions moving on the surface of nanowires of Co_3O_4 which produces the output potential during the measurement. Various bio-sensing parameters were also studied on the glucose oxide immobilized Co_3O_4 nanowires such as repeatability, reproducibility, dynamic response time, selectivity, and effect of pH and temperature. The repeatability of glucose oxidase immobilized Co_3O_4 nanowires was investigated via the use of same electrode with span of 12 hour each time as shown in Figure 5. It is clear that the developed electrode has a high potential to maintain the linear range and limit of detection, thus it can be considered as an alternative analytical protocol for the determination of glucose. The reproducibility is another performance evaluation indicator for the bio-sensing device and for this purpose inter-electrode signal was noticed by using ten independent working electrodes fabricated at the same conditions as shown in Figure 6. The inter-electrode study was performed in 1 mM glucose solution. The potentiometric response of each electrode was found less than 3% of relative standard deviation which reveals an outstanding performance of Co_3O_4 nanowires during the potentiometric analysis of glucose. The storage life of the proposed device was studied for 4 weeks

and the performance of Co_3O_4 nanowires was found acceptable and significant as given in Table 1. The improved performance of biosensor is assigned to high isoelectric point of Co_3O_4 which strongly bound to the low isoelectric bio-membrane of glucose oxidase. The advantage of using potentiometric strategy is that it prevents the chances of interference form the easily oxidizing species like uric acid and ascorbic acid due to use of zero biasing current during the analysis. The potentiometric response of selectivity is shown in the Figure 7. It can be visualized that the biosensor signal is remained unaltered during the addition of common interfering agents like urea, uric acid, dopamine, cholesterol and ascorbic acid. The same concentration of glucose and interfering species was used during the selectivity study. This confirms that the biosensor is highly selective towards the quantification of glucose. The selectivity of glucose biosensor is only depending on two aspects such as enzyme-glucose reaction and selective signal to the glucose substrate. The enzyme based reactions are highly selective due to specific interaction with biomolecules and the glucose oxidase is known as the best example of enzyme only selective towards glucose. The potentiometric response is highly dependent on the pH of analyte solution and the most consideration during the development of biosensor is that it should be tested under the physiological pH values of human body, therefore we have studied the effect of pH. The pH study was carried out in 1mM glucose concentration. On other hand, the stability of Co_3O_4 should be verified as the metal oxides do not seem stable at certain pH values. The pH of analyte solution was adjusted using 0.1M HCl and NaOH solutions. The range of pH values between 3 and 13 was selected as shown in Figure 8a.

This study indicates that the Co_3O_4 nanowires has shown optimum output potential signal around pH 7 which confirms that material has high capability to work under physiological pH values. The signal is low at very high and a low pH values which reveals that Co_3O_4 material is unstable in highly alkaline and acidic pH conditions [33]. Also, the glucose oxidase enzyme deactivates at the low pH and high pH values, therefore the proposed configuration is most suitable for the quantification of glucose at normal pH values. Furthermore, the environmental conditions like temperature has also significant effect on the output potential signal. For this reason, we also studied the effect of temperature on the response of glucose biosensor as shown in Figure 8b. The activity of biosensor was enhanced with the increase in the temperature due to frequent collision of glucose molecules with immobilized glucose oxidase on the Co_3O_4 nanowires. The output

potential of biosensor was found higher at 40 ^{o}C and further increase in the temperature drastically lowered the performance of biosensor due to denaturation of enzyme as shown in Figure 8b. However, for the ease of measurement, we carried out all the measurements at room temperature. After the use of biosensor, it was stored at 4 ^{o}C in refrigerator in order to prevent the effect of environmental conditions. The dynamic response of time to glucose biosensor was measured in 1mM glucose solution as shown in Figure 9.

The biosensor exhibited response time less than 1 s and revealing high sensitivity towards the detection of glucose. Moreover, the multi-step potentiometric technique was used to record the calibration plot and it was found that with successive addition of glucose the output signal was stable and increasing rapidly as shown in Figure 10.

Figure 11 represents the typical Nyquist cruves estimated from EIS data for pure Co_3O_4 and polyvinyl assisted Co_3O_4 nanowires immobilized with glucose oxidase and without enzyme in phosphate buffer solution of pH 7.3. It is obvious from Figure 11 that charge transport is more favourable in the polyvinyl assisted Co_3O_4 nanowires compare to pure pure Co_3O_4 indicating the role of polyvinylamine in tuning the electrical communciation withing the material. However, after the immubilziation of gluxose oxidase, it has produced the barrier for the charge trnsfer due to the insulling proerpties of glutaradehyde and gluccosseoxidase itself [35, 36]. The measured charge transfer values for the pure Co_3O_4 and polyvinylamine assisted Co_3O_4 nanowires with and without glucose oxidase immoblization are enclosed in Table 2. It can be seen that polyvinylamine assisted nanowires with glucose oxidase immobilization has rvealed better charge transport properties compare to the pure Co_3O_4.

This study confirms that the proposed fabrication technology is accurate and has a high potential as an alternative tool to monitor the glucose levels from real samples. The analytical application of proposed glucose oxidase immobilized Co_3O_4 nanowires was also performed using standard addition method. This study is further illustrating the important aspect of proposed method about its practical suitability as shown in Table 3.

The comparative analysis of presented glucose biosensor was carried out in terms of different biosensor parameters such as linear range, sensitivity, response time, regression coefficient, limit

of detection as given in Table 4. The proposed configuration has better or comparable to some of the recently reported glucose biosensors. Particularly a fast response time, low limit of detection and excellent regression coefficient value of 0.99 are indicating the superior performance of proposed study.

4. Conclusions

In summary, we have studied the influence of overnight hydrolysis of polyvinyl amine on the morphology and catalytic performance of Co_3O_4 nanostructures. The material synthesis was carried out by the hydrothermal method. The Co_3O_4 nanomaterial exhibits nanowire like morphology with high surface to volume ratio, which enabled the high loading of glucose oxidase via electrostatic attraction. The potentiometric response of glucose oxidase immobilized biosensor was probed using 0.01M saline phosphate buffer solution of pH 7.3. The proposed glucose biosensor has demonstrated a wide linear range of 0.0005 to 6 mM, with limit of detection of 0.0001mM. Also, the repeatability, reproducibility, dynamic response time, multistep potentiometric response, effect of pH and temperature were investigated along with storage life time and selectivity. Significantly, the proposed configuration has a high potential to monitor the glucose in real samples with outstanding analytical accuracy. The developed methodology for the preparation of Co_3O_4 with tuned morphology and enhanced catalytic features could be used as alternative method for the fabrication of a wide range of nanostructured materials for various electrochemical-based applications such as batteries, supercapacitors, water splitting and other biomedical applications.

Acknowledgment

We extend our sincere appreciation to the Researchers Supporting Project (RSP-2022/79) at King Saud University, Riyadh, Saudi Arabia for partial funding of this work. We would like to thank the platform “Microscopies, Microprobes and Metallography (3M)” (Institut Jean Lamour, IJL, Nancy, France) for access to SEM facilities.

Conflict of interest statement

Authors declare there are not conflict of interest in this research work

Data Availability Statement

The datasets generated analysed during the current study are available from the corresponding author on reasonable request

Author contribution

Munirah D. Albaqami did electrochemical measuremnts
Asma Alothman, discussed the electrochemical results
Ayman Nafady, partially supervised and finace the work and edited the revised manuscript
Shymaa S. Medany, wrote the first draft of manuscript
Aqeel Ahmed Shah, did XRD measurement
Umair Aftab, did EIS measuremnt and anlyzed the results
Mazhar Hussain Ibupoto, synthesized materials
Arfana Begum Mallah, partially supervised work
Aneela Tahira, did XRD analysis
Matteo Tonezzer, edited the draft of manuscript
Brigitte Vigolo, did SEM measurement
Zafar Hussain Ibupoto, supervised the work and proofread manuscript

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Figure captions

Figure 1. Powder XRD of (a) pristine Co_3O_4, (b) polyvinyl amine assisted Co_3O_4 nanowires

Figure 2. Typical SEM images (a,b) pristine Co_3O_4 (b) polyvinyl assisted Co_3O_4 nanowires

Figure 3. EDS spectra (a) pristine Co_3O_4 (b) polyvinyl assisted Co_3O_4 nanowires

Figure 4. Calibration (a) pristine Co_3O_4 (b) polyvinyl assisted Co_3O_4 nanowires in saline phosphate buffer solution of pH 7.3

Figure 5. Repeatability calibration of polyvinyl assisted Co_3O_4 nanowires $_{in}$ saline phosphate buffer solution of pH 7.3

Figure 6. Reproducibility study of polyvinyl assisted $Co_3O_{4\ nanowires}$ in 1 mM glucose in saline phosphate buffer solution of pH 7.3

Figure 7. Interference study of polyvinyl assisted Co_3O_4 nanowires in saline phosphate buffer solution of pH 7.3 using different interfering agents like uric acid, dopamine, ascorbic acid, fructose, urea and glucose

Figure 8. (a) Effect of pH in 1.0 mM glucose, (b) Effect of temperature in 1.0 mM glucose prepared in saline phosphate buffer solution of pH 7.3

Figure 9. Dynamic response time of polyvinyl assisted Co_3O_4 nanowires in 1.0 mM glucose in saline phosphate buffer solution of pH 7.3

Figure 10. Sequential addition of glucose concentration from 0.005mM to 5 mM prepared in saline phosphate buffer solution of pH 7.3 and the successive potentiometric response of polyvinyl assisted Co_3O_4 nanowires

Figure 11: EIS analysis of pristine Co_3O_4 and polyvinyl assisted Co_3O_4 nanowires with and without immobilization of glucose oxidase in 0.5mM glucose prepared in phosphate buffer solution of pH 7.3.

Table 1: Life time study of glucose biosensor based on glucose oxidase immobilized polyvinyl assisted Co_3O_4 nanowires

No of weeks	Linear range (mM)	Slope ($mVdec^{-1}$)	Limit of detection (mM)
1	0.0005-6	42	0.0001
2	0.0006-6	44	0.0002
3	0.0003-6	43	0.0003
4	0.0005-6	44	0.0002

Table 2: The calculated charge transfer values of Co_3O_4 nanostructures with and without enzyme immobilization

Sample	**Charge transfer resistance (Rct)**
Pristine Co_3O_4	4.3 x 10^3 Ohms
Pristine + Glucose Enzyme	8.1 x 10^3 Ohms
Sample Co_3O_4	1.7 x 10^3 Ohms
Sample Co_3O_4 + Glucose Enzyme	2.2 x 10^3 Ohms

Table 3: Practicality of glucose oxidase immobilized polyvinyl assisted Co_3O_4 nanowires

Experiment no:	Proposed method	Commercial glucometer	% recovery of proposed method
1	5.2±0.02	5.15±0.01	100.97±0.05
2	5.1±0.04	5.08±0.001	100.39±0.06
3	5.3±0.03	5.23±0.01	101.33±0.04

Table 4: Comparative analysis obtained results of presented glucose biosensor with reported works

Materials	Linear range	Sensitivity	Regression coefficient	Response time	LOD	Reference
Nafion-GOx/GO/AZO/Ag	2 mM to 10 mM	15.44 mV/mM	0.997	26 s	1.89 mM	37
BSA-Nafion-GOx/ZnO NWs/Ag	0.5–1000 µM	35 mV/decade	-	1–4 s	-	38
GOx/ZnO NRs/Ag	1 µM to 10mM	2.51 mV/decade	0.980	-	-	39
AgNPs-GOx/Ag-ISE	0.1–3 mM	8.62 mV/decade	-	-	10 µM	40
MIP-based on MAA	0.02–7 mM	43.7 mV/mM	0.980	-	-	41

Co_3O_4/gold coated glass	0. 5 µM to 6 mM	42 mV/decade	0.99	1s	0.1µM	This work

Full names. GOx: glucose oxidase; GO: graphene oxide; AZO: aluminum-doped zinc oxide; ZnO: zinc oxide; NWs: nanowires; Ag: silver, NPs:nanoparticles, BSA: bovine serum albumin; MIP: molecularly imprinted polymer; MAA: methacrylic acid; NRs: nanorods.

Figure 1

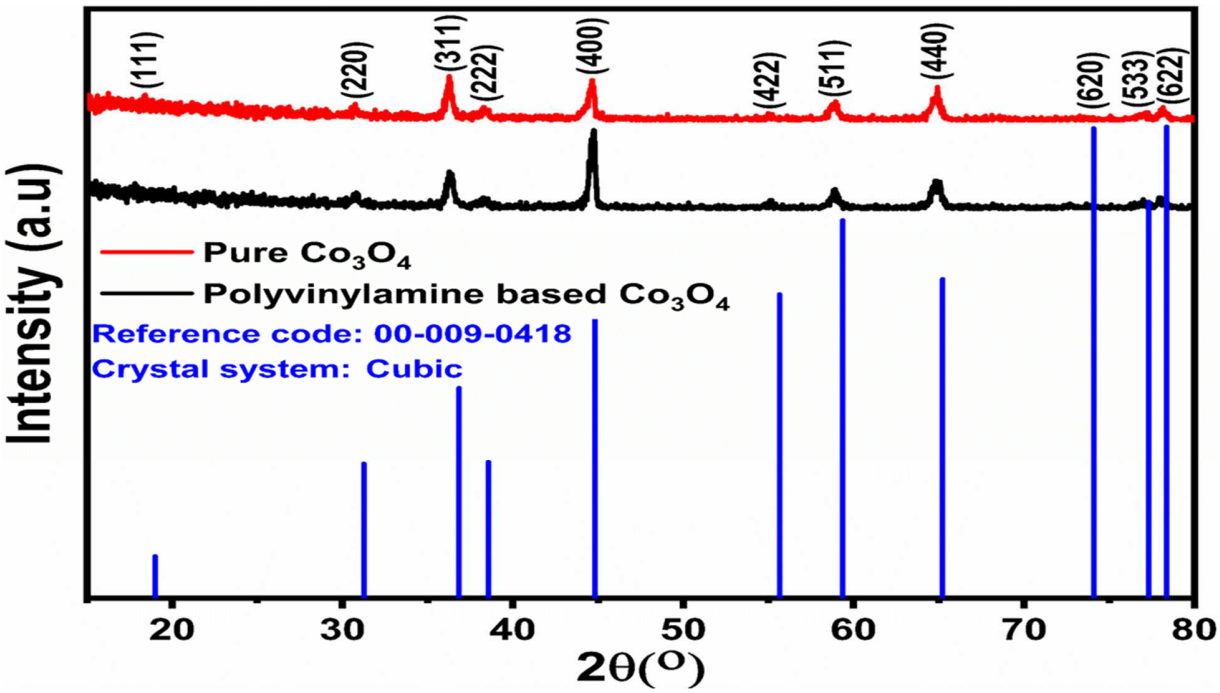


Figure 2

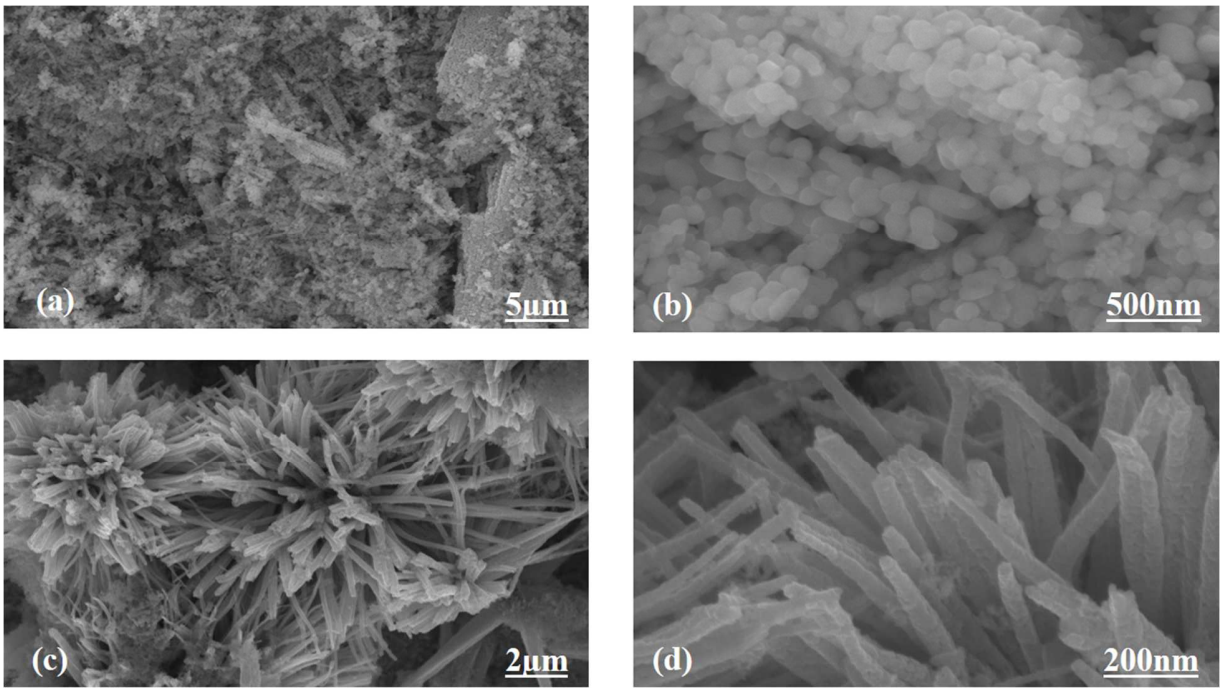


Figure 3

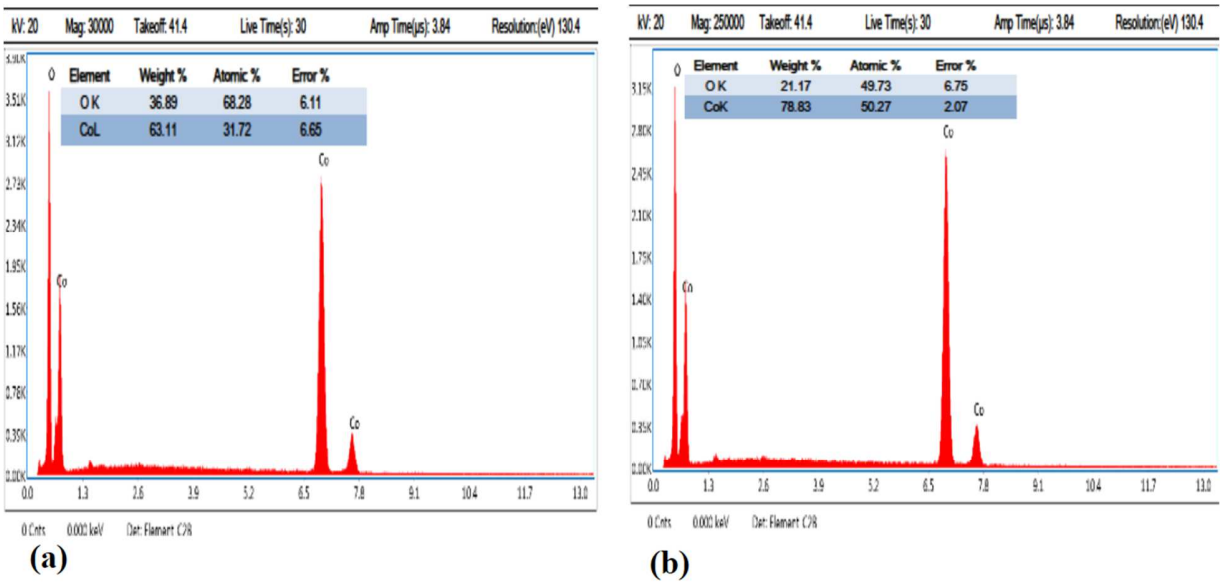


Figure 4

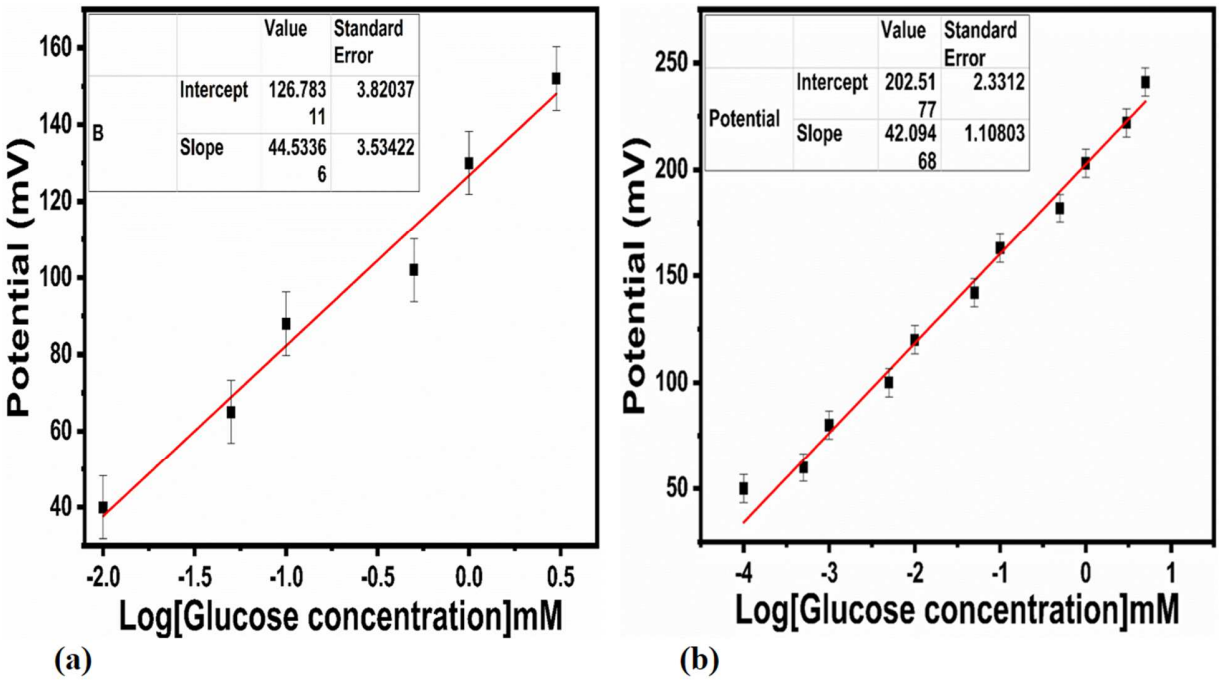
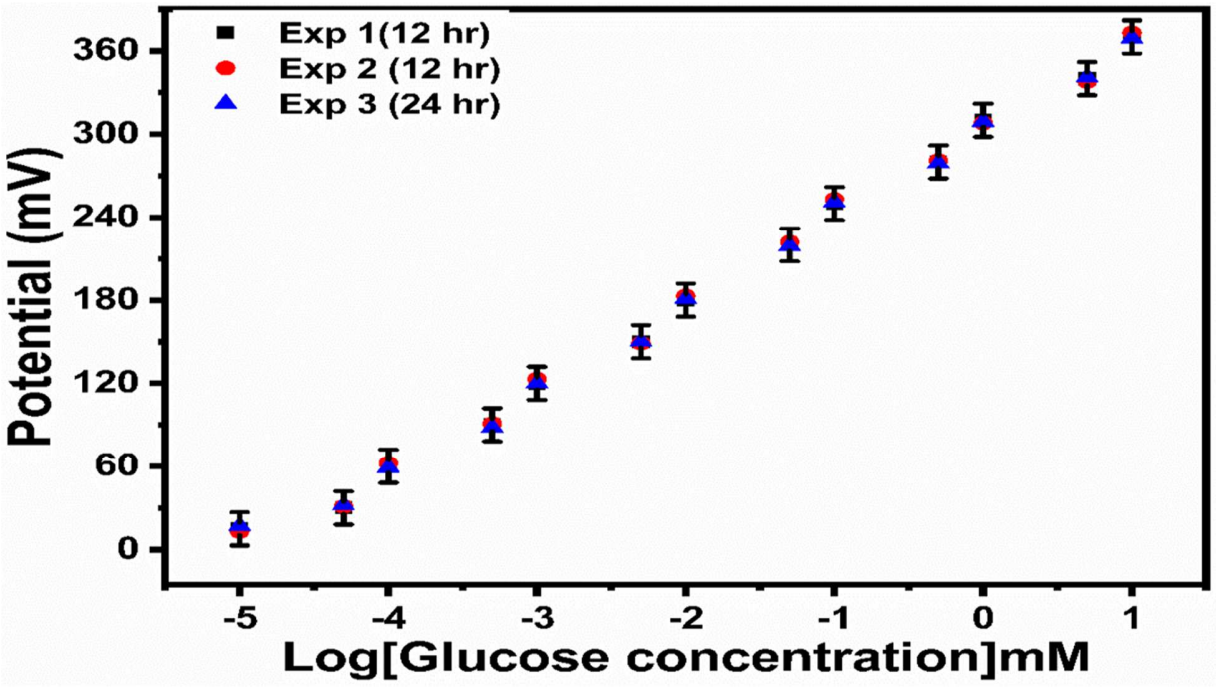


Figure 5



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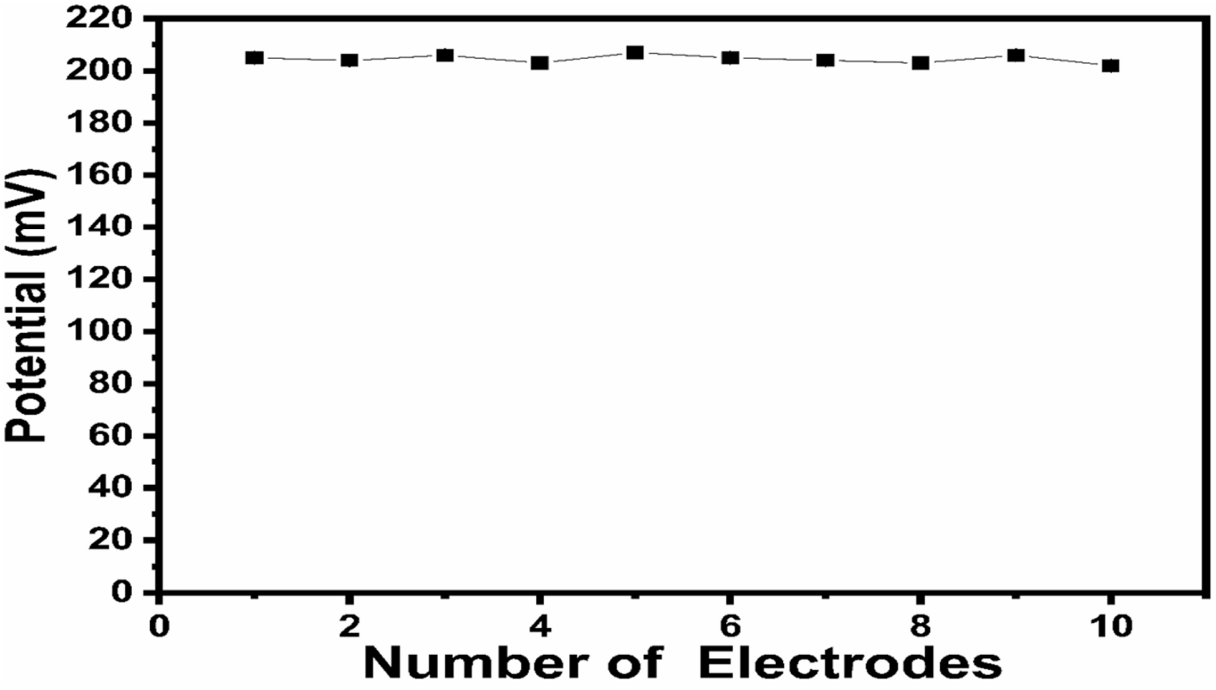

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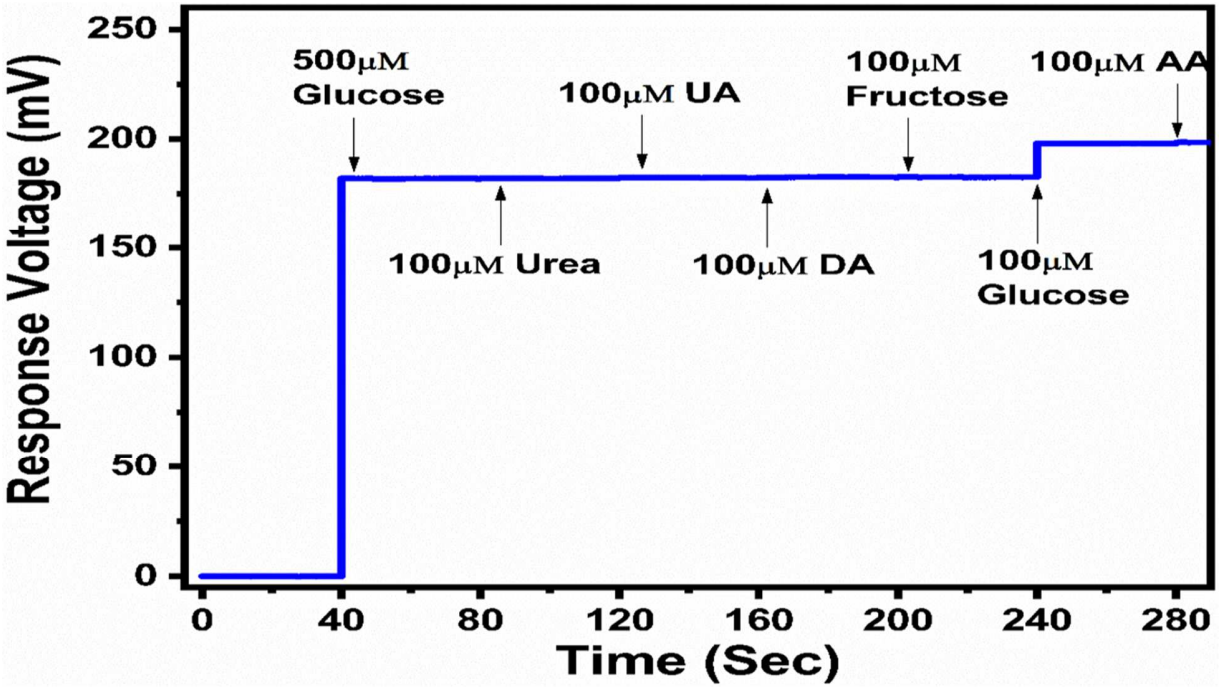

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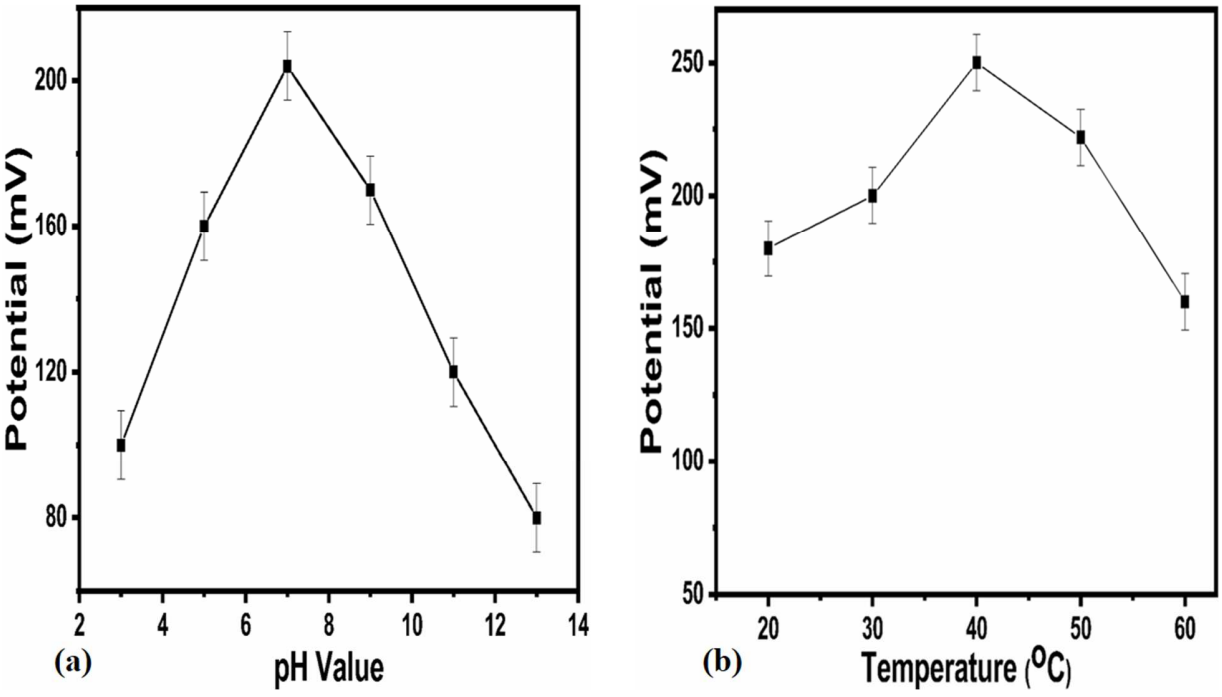

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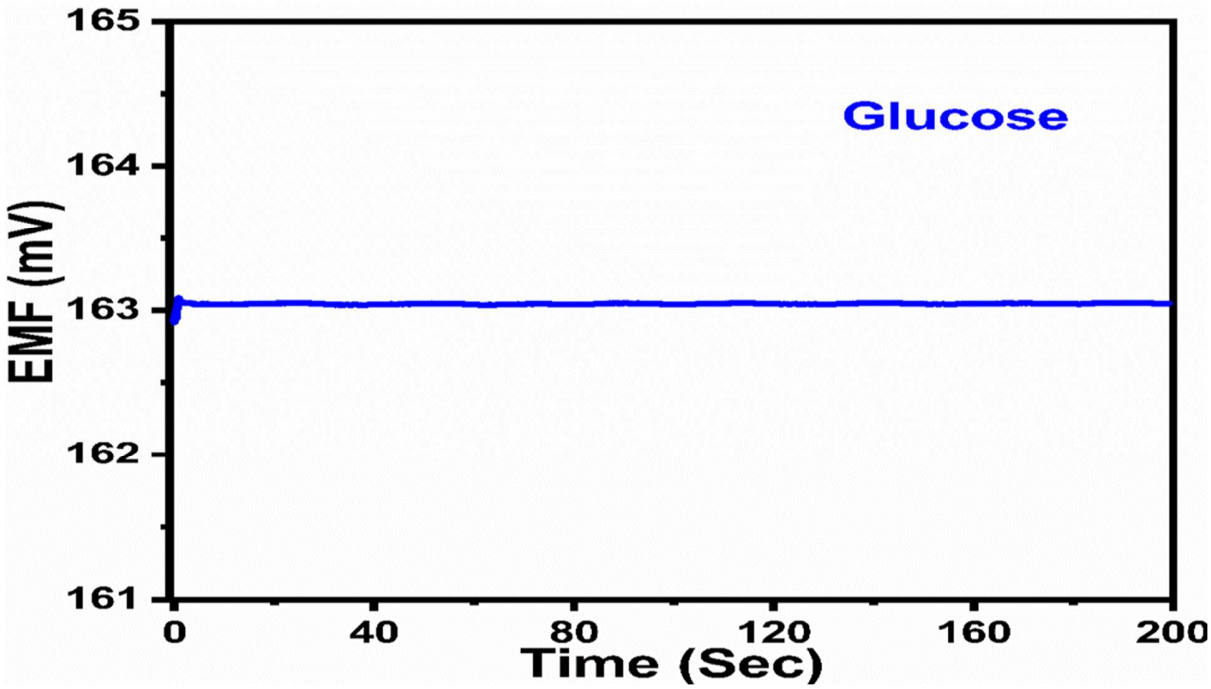

Glucose
EMF (mV)
165
164
163
162
161
0
40
80
120
160
200
Time (Sec)

Figure 10

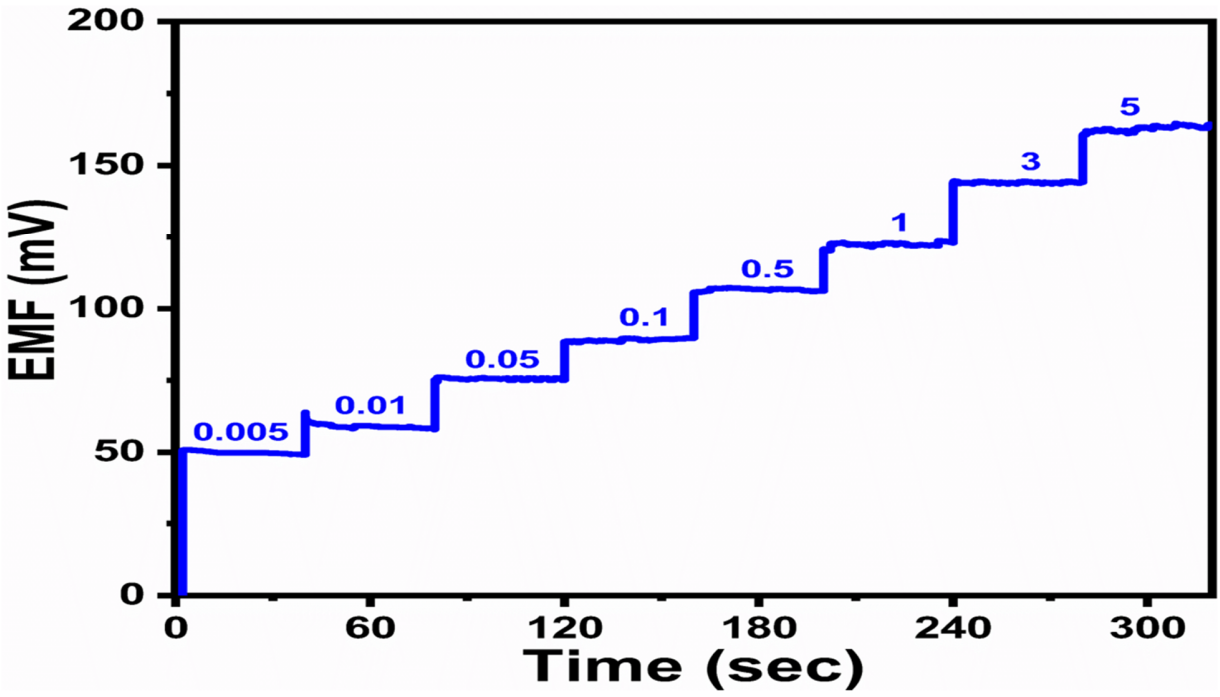
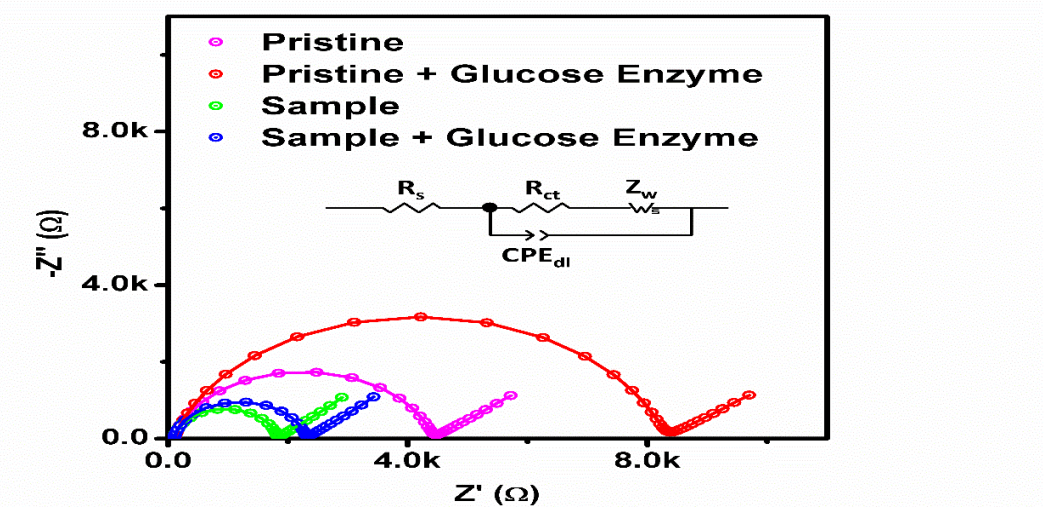

EMF (mV)
200
150
100
50
0
0.005
0.01
0.05
0.1
0.5
1
3
5
0
60
120
180
240
300
Time (sec)

Figure 11


Pristine
Pristine + Glucose Enzyme
Sample
Sample + Glucose Enzyme
R_s
R_{ct}
Z_w
CPE_{dl}
8.0k
4.0k
0.0
-Z'' (Ω)
0.0
4.0k
8.0k
Z' (Ω)