

Development of Polymer-based Chemical Sensor to Study the Impact of Polymer Concentration and Freeze-Thaw Cycle on the Detection of Gallic Acid

Sundus Khan and Joo Kheng Goh*

School of Science, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia.

*E-mail: goh.joo.kheng@monash.edu

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A simple, inexpensive and efficiently sensitive sensor for voltammetric detection of gallic acid (GA) has been fabricated with 0.1% and 1.0% w/v of polyvinyl alcohol (PVA). Electrode responses were evaluated for the oxidation of 10 to 50 μM GA at bare glassy carbon electrode, a GCE modified electrochemically with PVA and PVA/MWCNT/GCE, PVA/ZnO/GCE, PVA/MWCNT/ZnO/GCE composites by solvent casting method. The PVA/GCE at 0.1% (w/v) has proven to be the best electrochemical sensor among the four electrochemical modified electrode as it has increased oxidative current to 26 folds as compared to a bare GCE. PVA/GCE 0.1% (w/v) has detected GA in the lowest concentration range of 50 μM with a high sensitivity of $11.03 \pm 1.61 \mu\text{A}$ as compared to bare GCE with 0.43 μA . The PVA/GCE 0.1% (w/v) modified chemical sensor has shown more pronounced current sensitivity of $21.85 \pm 1.14 \mu\text{A}$ after 5 freeze-thaw cycles, an effective casting method in this study. Freeze-thaw cycle method has strengthened PVA hydrogels enhancing the current sensitivity for the detection of GA to 51 folds as compared to bare GCE and 1.98 folds as compared to PVA/GCE (without freeze-thaw cycle). Satisfactory precision was obtained for PVA/GCE 0.1% (w/v) (without freeze thaw cycle) with %RSD of 15.58 whereas, %RSD for PVA/GCE 0.1% (w/v) (with 5 freeze-thaw cycle) was 5.24. Under optimal conditions, the polymer based electrochemical sensor PVA/GCE displayed a linear range to GA in the concentration of 1.6×10^{-6} to $4.9 \times 10^{-5} \text{ mol L}^{-1}$ with a detection limit of $4.95 \times 10^{-7} \text{ mol L}^{-1}$.

Keywords: Electrochemical modified electrode, freeze-thaw cycle, gallic acid, PVA hydrogel, square wave voltammetry

1. INTRODUCTION

Sensors have become popular on commercial level as well as wide range of applications of potentiometric, amperometric and conductometric electrochemical sensors have found in clinical,

environmental, industrial and agricultural fields [1]. The chemical sensor becomes extensively active and effective way to detect biomedical compounds in modern sensor technology. The electrochemical sensor depends on the electrochemical effect established between the fabricated electrodes and analyte [2]. Electrocatalysis is a type of application of modified electrodes importantly achieving great demand from the researchers where surface of electrode is modified to kinetically accelerate the reactions on the modified electrode which is not favorable on unmodified one. Preparation of polymer modified electrodes can be done by electrochemical methods, the parameters for instance, monomer concentration and applied anodic potential can be altered to direct functionalities and polymer surface thickness. Outstanding sensors are extremely selective, efficient sensitivity, fewer interferences, low detection limit, extensive shelf-life, inexpensive and have simple renewable surface [3].

Electrochemical methods are the alternate, efficient, simple, portable, low-cost and sensitive methods for the evaluation of antioxidant capacity. Electrochemical techniques are applicable in fundamental studies of oxidation and reduction processes to understand reaction mechanisms. The importance of this technique is that the electrochemical oxidation of antioxidants, for instance, polyphenols is based on the bond breaking of O-H bond which is the similar phenomenon of the antioxidants against free radicals [4].

Voltammetric technique, one of the electrochemical methods is useful in retrieving analytical, kinetic and thermodynamic information about the redox properties of analyte where target analyte is determined due to the electrochemical changes identified by working or indicator electrode however, the reference electrode have constant potential which is independent of properties of solution [5]. Pulse techniques such as square-wave voltammetry (SWV), and differential pulse voltammetry (DPV) are successful electroanalytical technique for chemically modified electrodes because they respond less to the matrix interferences and efficient in the reduction of background current. SWV works on the principle of differential multi pulse techniques giving enhanced current signal (ΔI) confirming highly resolved current peak. There are enormous benefits of chemically modified electrode (CMEs), such as, they are fabricated without difficulty having minimum background current and comprehensive potential range with speedy renewability [6, 7]. Oxidation potential is the measurable parameter to monitor the antioxidant capacity; low (less positive) oxidation potential in samples refers to high antioxidant capacity. Voltammetric methods are applicable for studying the design of new and important antioxidants in addition to explore the mechanism of action for characterization of natural and synthetic antioxidants. Voltammogram is like a mark of the redox mechanism as the voltammogram is expressed by waves and peaks showing the redox processes for potential window chosen for study [8, 9].

Polyphenols are the significant class of naturally occurring antioxidants which has numerous benefits in reducing the oxidative stress because of free radicals. The strongest triphenolic antioxidant among phenols is gallic acid (GA) and it has low molecular weight. GA (3, 4, 5-trihydroxybenzoic acid) is plant phenol usually found in the kingdom of plants and mainly occur in free state or in the form of derivative in various foods such as grapes, tea, nuts, and sumac (*Rhus coriaria* L.). GA comprises of an aromatic ring and planar geometry. Three hydroxyl groups bonded to the aromatic ring has been taken by ortho position [10]. GA is a metabolite of propyl gallate possessing powerful antioxidant, anticancer and anti-inflammatory activity to enhance biochemical pathways [11].

In our research, modification of bare GCE has been done with polyvinyl alcohol (PVA) and nanocomposites, multi-walled carbon nanotubes (MWCNTs) and zinc oxide (ZnO). PVA is a semi crystalline water-soluble, synthetic polymer comprising charge storage capacity, dielectric strength along with excellent properties being nontoxic and biodegradable [12]. PVA has remarkable water solubility and ease of fabrication making the composite processing feasible with water hence PVA has been approved as a suitable medium for CNT composites [13]. MWCNTs and PVA has been used in the electrospinning process to improve electron interactions for better electrical conductivity performances. PVA is popular and advantageous in wide applications due to their outstanding physical properties, chemical resistance, and biodegradability. Exceptional adhesion to porous and water-absorbent surfaces are served due to the binding characteristics of PVA [14]. ZnO nano-composites are versatile engineered nanomaterials as they are inexpensive, good optical, electrical, semiconductors, stable in hydrogen plasma environment, easily adaptable, antimicrobial and non-toxic. It has numerous functions in nanoelectronics and biosensors field [15].

In the past studies, there is a literature gap for an efficient polymer based electrochemical sensor which can optimize the sensitivity of polyphenols to its maximum. Furthermore, studies for the impact of significant parameters i.e., effect of polymer concentration and number of freeze thaw cycles on the polymer sensor for the oxidation of GA are also not seen. Our research will be highly beneficial to fill the knowledge gap and understand the significance of polymers in the sensor technology for the detection of GA.

For this purpose, the core area of this work is to develop a smart, sensitive and selective polymer based electrochemical sensor for GA determination based on chemical modification of electrode with polymer matrix (PVA) and nanocomposites, MWCNTs and ZnO as GA cannot be detected easily by bare GCE. Among all the four sensors fabricated with PVA 0.1% (w/v) (PVA/GCE, PVA/MWCNT/GCE, PVA/ZnO/GCE, PVA/MWCNT/ZnO/GCE), PVA/GCE (0.1% w/v) has shown the best and optimum current sensitivity $11.03 \pm 1.61 \mu\text{A}$ and lowest oxidation potential +205 mV for the oxidation of GA (50 μM). Satisfactory precision and accuracy was obtained with % relative standard deviation (RSD) of 15.58.

It is also important to highlight that this fabrication of four kinds of sensors was done for two concentrations of PVA 0.1% (w/v) and 1% (w/v). The study of variation of polymer concentration was carried out to demonstrate the effect of enhanced PVA concentration on the sensitivity of oxidative current of GA. Our findings have established the eminent features of this research affirming that PVA/GCE (0.1% w/v) is the best electrochemical sensor among all the experimented sensors in our study as well as the other sensors developed in the past research. PVA/GCE (0.1% w/v) has exposed the most promising and enhanced current sensitivity with low oxidation potential opening the new door for the efficient detection of GA as compared to PVA/GCE (1% w/v).

In our research, polymer based electrochemical sensor has also been analyzed after five freezing and thawing cycles to monitor efficiency of the polymer sensor to increase oxidative peak current of GA. An increase in freezing and thawing cycles serves to add stability to existing crystals [16]. This study has also established about additional worthy of attention aspect of PVA hydrogels in detection of GA. They are stabilized with improved mechanical strength after five freeze-thaw cycles for PVA/GCE

0.1% (w/v) by enhanced oxidative peak current whereas with slightly higher oxidation potential as compared to PVA/GCE 0.1% (w/v) (without freeze thaw cycle).

2. EXPERIMENTAL

2.1 Materials and standards

All chemicals were of analytical reagent grade and used without further purification. Supporting electrolytes 0.1 M [KH_2PO_4 , PBS, PBS/ KH_2PO_4 (1:1)] solutions were prepared. Fresh stock standard solution of GA (1.0 mM) was prepared each time. All solutions were made with deionized water and deaerated with oxygen-free nitrogen gas for 10 minutes before recording each measurement. hydrochloric acid solution HCl (2.0 M) (molar mass 36.46 g/mol) 37% Friendemann Schmidt Chemical and sodium hydroxide solution NaOH (1.0 M) (molar mass 40 g/mol) R&M Chemicals. Polyvinyl alcohol (PVA) (M_w Approx. 30000) Merck, Zinc Oxide ZnO (molar mass 81.37 g/mol) Merck. Black powder of MWCNTs (purity>95%, diameter~20-40 nm, length~5-15 μm) were purchased commercially from Shenzhen Nanotech and used without further purification.

2.2 Apparatus and electrodes

Cyclic and square wave voltammetric measurements were carried out by BAS (Bioanalytical systems, West Lafayette, Indiana, USA): CV-50W electrochemical workstation connected to an external computer was used. A conventional three-electrode electrolytic cell was employed. A bare GCE, a GCE modified with PVA, PVA/MWCNT, PVA/ZnO and PVA/MWCNT/ZnO composite as working electrodes. Silver-Silver chloride, Ag/AgCl in (3.0 M NaCl) as reference electrode; 1 mm diameter platinum wire as counter electrode. The morphology of PVA/MWCNT/ZnO composite was characterized on the surface of 5 mm diameter basal plane pyrolytic graphite electrode (BPPGE) before and after electrolysis by Field Emission Scanning Electron Microscopy (FE-SEM) (Hitachi SU8010). Polymer solutions were prepared on hot plate 78HW-1. The pH Meter F-71 (Horiba Scientific) was used for pH measurements. FTIR spectrometric readings were recorded using PerkinElmer Spectrum Two. All experiments were carried out at room temperature 25 °C.

2.3 Procedures

A bare working electrode (3 mm diameter) was polished with alumina slurry, ultrasonic grinded for one minute. After that it was rinsed with deionized water prior to use.

Stock solution of GA was prepared and dissolved with deionized water. Stock solution of the antioxidant GA spiked into 10.0 mL of supporting electrolyte (0.1 M) KH_2PO_4 /PBS (1:1) to make final concentrations (10, 20, 30, 40, 50 μM) and placed into an electrochemical cell followed by degassing with pure nitrogen for 10 minutes to deaerate the dissolved oxygen prior to the voltammetric measurements. Thus, the three electrodes were dipped in the solution. Unless otherwise mentioned, the

temperature will be (25 ± 2) °C. Thus, potential scan was done and the voltammogram was recorded by SWV.

Background data was kept and subtracted from the data which minimizes side effects such as double layer charging current. All potentials recorded in relevance to Ag/AgCl reference electrode.

2.4 Preparation of PVA solution

PVA 0.1% (w/v) solution was prepared with constant mechanical stirring by magnetic stirrer refluxing at 80.0 °C on hotplate for an hour. Afterwards solution was left to cool down at room temperature.

2.4.1 Fourier transform infrared spectroscopy (FTIR)

Development of the polymer modified electrode with PVA for determination of GA is confirmed by FTIR. Three solutions were prepared. Aqueous PVA 0.1 % (w/v), GA aqueous solution (1.0 mM) and GA-PVA 0.1% (w/v) solutions were made and FTIR was recorded to observe GA interaction with PVA. Same procedure was done with PVA 1% (w/v).

2.5 Preparation of the chemically modified polymer electrode by solvent casting method

2.5.1 PVA 0.1% (w/v) ($M_w=30000$ g/mol)

2.5.1.1 Preparation of PVA/ GCE electrode

A bare GCE polished consecutively with alumina slurry. It was then rinsed with deionized water followed by sonication in ethanol and deionized water for 5 min, separately. Once the electrode was clean, it was pre-treated in PBS/ KH_2PO_4 (1:1) (pH 7.0) by electrochemical scanning repetitively with the electrode potential between -0.2 and +2.0 V versus Ag/AgCl reference electrode at a rate of 20 mV s^{-1} for 15 min by Cyclic Voltammetry. With the completion of pre-treatment of electrode, it was scanned in PBS/ KH_2PO_4 (1:1) (pH 7.0) containing 0.1 % PVA between 0.0 and 1.0 V at a rate of 20 mV s^{-1} for four cycles. The electrode was then rinsed with acetone and distilled water followed by sonication for 5 minutes in water to eliminate any physically adsorbed traces. The PVA modified GCE was kept at 4.0 °C in 0.1 M PBS/ KH_2PO_4 (1:1) (pH 7.0) after it was used [17].

2.5.1.2 Preparation of PVA/MWCNT/GCE electrode

A GCE was polished to shiny surface and finished with alumina slurry. It was rinsed with deionized water repeatedly. Drying of the electrode was carried at room temperature. PVA 0.1% (w/v) solution was prepared by constant mechanical stirring of PVA by magnetic stirrer refluxing at 80.0 °C on hotplate for an hour. MWCNTs (10.0 mg) was dissolved with 5.0 ml of the PVA by ultra-sonication

for 3 h [18]. Uniform mixing of CNTs either single walled or multi-walled nanotubes in any solvent which has low viscosity as compared to high viscosity polymer solutions is facilitated by ultrasonication. To develop PVA-MWCNT composite film on the glassy carbon electrode, 4.0 μL of PVA-MWCNT composite uniform mixture was casted on the GCE and it was dried at room temperature for 1 h. A thin film was formed on GCE.

2.5.1.3 Preparation of PVA/ZnO/GCE electrode

The PVA 0.1% (w/v) was prepared by weight percentage of metal oxide nanoparticles by a simple and low-cost solution casting techniques. After the preparation of 0.1% (w/v) PVA solution in deionized water at 80.0 $^{\circ}\text{C}$ followed by mechanical stirring till a homogeneous solution was obtained, ZnO by percent weight (5.0 wt%) was added to the PVA solution (100.0 mL) followed by continuous stirring for one hour. The solution was sonicated for half an hour afterwards. The 4.0 μL aliquot of PVA/ZnO was casted on GCE and it was dried at room temperature for one day. Thin film of PVA/ZnO composite on GCE was obtained after evaporation of water and kept in a vacuum desiccator until used [12].

2.5.1.4 Preparation of PVA/MWCNT/ZnO/GCE electrode

PVA/ZnO (5.0 wt%) solution (0.2 mL) and (10.0 mg/5.0 mL of MWCNT/PVA [18]) was mixed with continuous stirring for 1 hour followed by sonication of the solution for 30 minutes. The 4.0 μL aliquot of PVA/MWCNT/ZnO was casted on GCE and dried at room temperature for one day. Thin films of PVA/MWCNT/ZnO composite on GCE was obtained after evaporation of water and kept in a vacuum desiccator until used.

2.5.1.5 Stability test for PVA/GCE modified electrode

Stability test was conducted on PVA/GCE polymer modified electrode 0.1% (w/v) 25 days old for GA in 0.1 M PBS/ KH_2PO_4 .

2.5.1.6 Preparation of PVA/GCE electrode with freeze thaw cycle

The PVA solution 0.1% (w/v) freezing was done at -20°C for 24 h followed by thawing at 23°C for 24 h to obtain the denser gel structures after five freezing-thawing cycles [19]. PVA/GCE electrode was then fabricated with the PVA solution after five freezing-thawing cycles in the same way as described in the above method in section 2.5.1.1.

2.5.1.7 Reproducibility test for PVA/GCE modified electrode

For accuracy and precision of the results, all the readings were recorded in triplicates for duplicate sets. Set 1 represents PVA/GCE electrode fabricated from freshly prepared PVA solution 0.1% (w/v) while set 2 represents PVA/GCE electrode fabricated from PVA solution 0.1% (w/v) (after two weeks).

2.5.2 PVA 1.0 % (w/v) ($M_w=30000$ g/mol)

All the electrodes were prepared in the same manner for PVA 1.0 % (w/v) ($M_w=30000$ g/mol).

2.6 Procedures for data collections for optimization of experimental parameters in detection of GA

2.6.1 Study of supporting electrolyte

The supporting electrolyte solutions KH_2PO_4 , PBS, $\text{PBS}/\text{KH}_2\text{PO}_4$ (1:1) were prepared at concentration of 0.1 M using deionized water. Voltammetric measurements of 1.0 mM GA were then conducted using each supporting electrolyte at bare GCE under neutral conditions to determine the best response. The supporting electrolyte that presented the best result was selected for this study.

2.6.2 Study of pH

The pH study was conducted at PVA/GCE polymer modified electrode. The pH of 10.0 mL 0.1 M $\text{PBS}/\text{KH}_2\text{PO}_4$ (1:1) with 1.0 mM GA analyte added was adjusted by 2.0 M HCl or 1.0 M NaOH before the voltammetric measurement. The pH of the solution was determined via pH meter. The pH was adjusted to be varied from pH 2 to pH 11.

2.6.3 Study of scan rate

Effect of scan rate on the square wave voltammogram was assessed for PVA/GCE polymer modified electrode in supporting electrolyte 10.0 mL 0.1 M $\text{PBS}/\text{KH}_2\text{PO}_4$ (1:1) with 1.0 mM GA analyte added. The effect of varying scan rate was studied at low 0.06 V s^{-1} and high scan rates 0.5 V s^{-1} .

3. RESULTS AND DISCUSSION

3.1 Optimized electrochemical parameters for GA

Relating to the results of supporting electrolyte study, three types of 0.1 M aqueous supporting electrolytes under neutral conditions were studied. In the presence of PBS, current sensitivity was low

for the oxidation of GA. On the other hand, the current sensitivity was enhanced in the presence of KH_2PO_4 but with more positive oxidation potential while PBS/ KH_2PO_4 (1:1) was suitable supporting electrolyte for oxidation of GA based on the highest oxidative current peak with less positive (low) oxidation potential as compared to KH_2PO_4 at bare GCE.

The study of pH was carried out to determine the behavior of the electrochemical sensor PVA/GCE polymer modified electrode for GA in the presence of PBS/ KH_2PO_4 (1:1). At low pH, when the pH~2 electrochemical sensor PVA/GCE polymer modified electrode was more sensitive in the determination of pro-oxidant behavior of GA rather than antioxidant behaviour and this pattern remained the same to pH~5. On the other side, when pH=6, PVA/GCE was only sensitive in the determination of antioxidant behavior of GA giving the optimum current sensitivity.

At high pH, of 10.5, PVA/GCE had given low current sensitivity for the oxidation of GA, whereas at pH7.4, PVA/GCE had more current sensitivity for the oxidation of GA. At basic pH (above 8.0), electrochemical sensor had shown no sensitivity for the oxidation of prooxidant behaviour of GA. From the above findings, PVA/GCE polymer modified electrode is the best electrochemical sensor under neutral conditions around pH6.4 for the determination of GA.

The effect of scan rate on anodic peak current of 10-50 μM GA in 0.1 M PBS/ KH_2PO_4 was studied at scan rates i.e. 0.06 V s^{-1} and 0.5 V s^{-1} at PVA/GCE polymer modified electrode. It was observed that oxidative peak current was enhanced significantly with the increase of scan rate, with a shift of oxidative peak towards less positive potential from +288 mV to +205 mV.

3.2 FTIR analysis

FTIR spectrums confirmed that there was no interaction between PVA and GA. Therefore it can be used as a binder for the fabrication of chemically modified polymer electrode.

3.3 Study of oxidation of gallic acid at bare GCE

Oxidative peak current was obtained for the oxidation of GA concentration at 50 μM in 0.1 M PBS/ KH_2PO_4 aqueous solution over the potential range of 0 to +750 mV versus Ag/AgCl (in 3M NaCl) at bare GCE by SWV. The oxidation process of GA is irreversible. The oxidation peak was recorded at more positive potential +320 mV and oxidative peak current was obtained at 0.43 μA for 50 μM GA.

3.4 Study of oxidation of gallic acid at polymer modified electrodes with polymer concentration 0.1% and 1% (w/v)

3.4.1 Comparative study of all polymer modified electrodes with PVA concentration at 0.1% (w/v)

Voltammograms were successfully recorded on the surface of unmodified and modified electrodes for 0.1% (w/v) PVA by SWV. The oxidative peak current was highly enhanced with pronounced anodic peak at PVA/GCE modified electrode as well as, the oxidation peak was efficiently

shifted from more positive potential +320 mV to less positive potential +205 mV at PVA/GCE electrode as low oxidation potential refers to high antioxidant capacity.

PVA/MWCNT/GCE has shown low oxidative peak current because MWCNT has been blocked in the layers of polymer hydrogel. This is the reason why MWCNT has decreased the sensitivity of the electrode rather enhancing the electrocatalytic activity with the combination of PVA as compared to PVA/GCE modified electrode. PVA/MWCNT/GCE modified electrode showed moderate sensitivity in the detection of GA based on the current values shown in Fig.1(a). In the same figure below, PVA/ZnO/GCE and PVA/MWCNT/ZnO/GCE showed weaker current of GA.

PVA/ZnO/GCE has displayed lowest current sensitivity with 0.1% (w/v) PVA compared to all the other sensors except that of the bare GCE because ZnO has obstructed the conductivity of PVA for the oxidation of GA thereby poor oxidative current has been obtained for GA (Fig. 1a).

PVA/MWCNT/ZnO/GCE has shown better results as compared to PVA/ZnO/GCE and bare GCE as MWCNT has shown electrocatalytic activity in enhancing the oxidative peak current and shifting the potential to less positive side for GA however, this electrode has not displayed improvement as compared to PVA/MWCNT/GCE and PVA/GCE as ZnO has reduced the electrocatalytic efficiency of PVA/MWCNT/ZnO/GCE modified electrode.

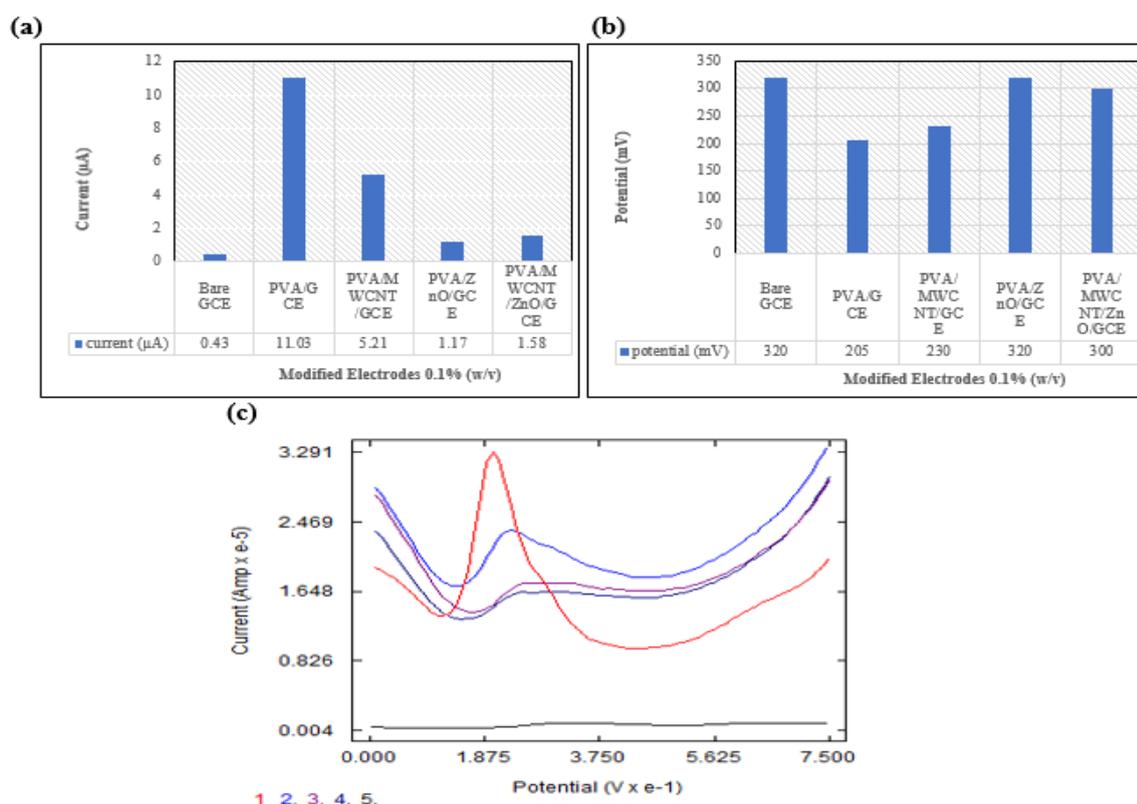


Figure 1. (a) Current profile of polymer modified electrodes for PVA 0.1% (w/v) at 50 µM of GA in PBS/KH₂PO₄ (0.1 M) by SWV, (b) Potential profile and (c) Square wave voltammogram obtained at the (1) PVA/GCE (without freeze thaw cycle); (2) PVA/MWCNT/GCE; (3) PVA/MWCNT/ZnO/GCE; (4) PVA/ZnO/GCE; (5) Bare GCE.

PVA/GCE polymer modified electrode has been established to be the best electrochemical sensor and has optimum current sensitivity to $11.03 \pm 1.61 \mu\text{A}$ for the oxidation of GA with low oxidation

potential which are the ideal parameters for the detection of antioxidant by increasing the current to 26 folds as compared to bare GCE as well as all other modified electrodes in our research (Fig. 1).

Fig. 1c depicts the summary of voltammograms obtained at polymer modified electrodes and bare GCE with 0.1% (w/v) PVA confirming that PVA/GCE polymer modified electrode has highest sensitivity with good detection limit.

3.4.2 Comparative study of all polymer modified electrodes with PVA concentration at 1% (w/v)

Voltammograms were successfully recorded on the surface of bare GCE and modified electrodes for 1% (w/v) PVA by SWV. In this case, the oxidative peak current was highly enhanced on PVA/ZnO/GCE and PVA/MWCNT/ZnO/GCE polymer modified electrodes whereas, the oxidation peak was efficiently shifted from more positive potential +320 mV (bare GCE) to less positive potential +240 mV and +250 mV at PVA/ZnO/GCE and PVA/MWCNT/ZnO/GCE electrodes respectively confirming that these two electrodes were highly sensitive electrodes for the oxidation of GA with 1% (w/v) PVA compared to all other modified and unmodified electrodes as shown in Fig. 2b.

PVA/MWCNT/GCE showed low oxidative peak current because MWCNT blocked in polymer hydrogel hence MWCNT played significant role in enhancing the electrocatalytic activity with the combination of PVA (Fig. 2a).

PVA/ZnO/GCE and PVA/MWCNT/ZnO/GCE modified electrodes with 1% (w/v) PVA displayed high sensitivity for the detection of GA as compared to other electrodes (Fig. 2). Hydrogels provide free spaces in their cross-linked network contributing to the development of nano particles which behaves as nanoreactors during swelling stage of polymer [21]. It was clearly observed that ZnO nanoparticles showed growth in the polymeric network of high concentration of PVA hydrogel as it has offered more free spaces within their polymeric network as compared to PVA/ZnO/GCE modified electrode with 0.1% w/v PVA.

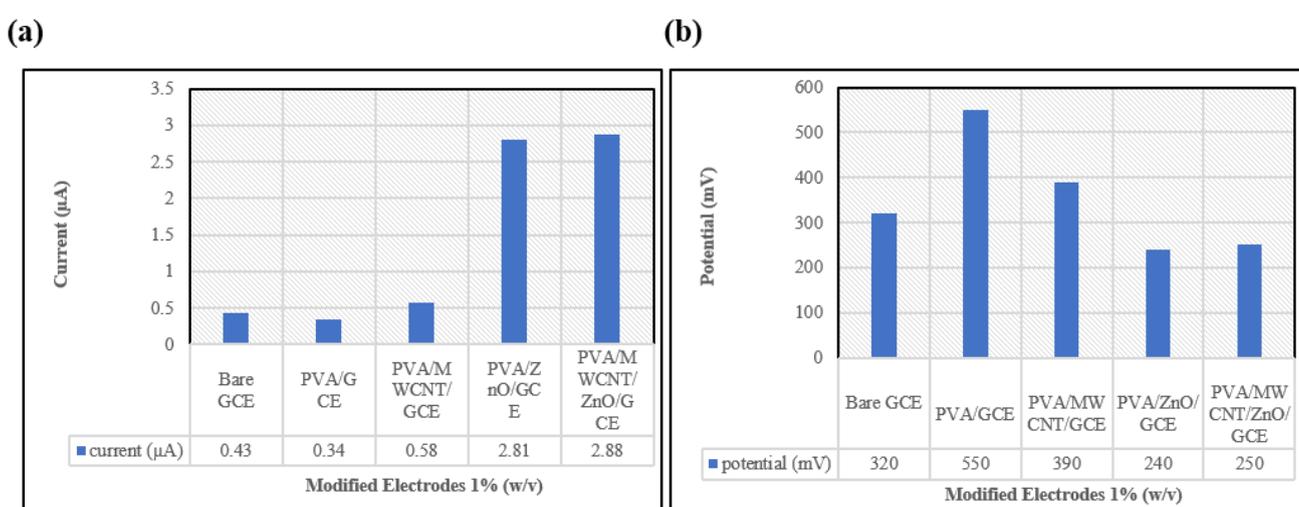


Figure 2. (a) Current profile of polymer modified electrodes for PVA 1% (w/v) at 50 µM of GA in PBS/KH₂PO₄ (0.1 M) by SWV, (b) Potential profile.

Whereas PVA/GCE modified electrode for 1% (w/v) exhibited the least sensitivity for the oxidation of GA as compared to other electrodes. Fig. 2b displayed that PVA/GCE polymer modified electrode has oxidation peak at more positive potential +550 mV i.e., not suitable for detection of GA.

3.4.3 Comparison of the effect of varying polymer concentration on the modification of electrodes; 0.1% (w/v) PVA/GCE modified electrode and 1% (w/v) PVA for oxidation of GA

Fig. 3 provides the comparison summary of the effect of varying polymer concentration on the modified electrodes; that are PVA/GCE modified electrode with 0.1% (w/v) and 1% (w/v) of PVA for oxidation of GA in 0.1 M PBS/ KH_2PO_4 by SWV. It is evident from the comparison that PVA/GCE modified electrode with 0.1% (w/v) PVA displayed the highest and distinct sensitivity for the oxidation of GA with lowest oxidation potential and good detection limit.

In this study, PVA modified electrode exhibited phenomenal approach towards sensing of GA. On the other hand, the efficiency of polymer based electrochemical sensor has also been assessed while increasing PVA concentration by 10% (w/v) of the original concentration of 0.1% (w/v) and studying its effect on the oxidation of GA. The effect of concentration has direct effect on fiber structure of polymer as the fiber diameter and inter-fiber spacing increases and flat fibers are obtained from high concentrated solution [22]. On that account, lower concentration of PVA tends to produce smaller diameter nanofibers which enhanced the electro-catalytic oxidation of GA at PVA/GCE 0.1% (w/v) as compared to 1% (w/v) owing large fiber diameter which decreased the electro-catalytic activity of GA.

The increase in concentration of PVA results in the increase of viscosity and density [23]. Consequently, electro-catalytic oxidation of GA decreased significantly at 1% (w/v) PVA/GCE modified electrode) as the concentrated PVA solution did not flow well due to hindrance in mobility and conduction of electrons. - 0.1% (w/v) PVA/GCE has less viscosity and density facilitating the mobility and conduction of electrons for the oxidation of GA.

Increase in PVA concentration impacts positively in the increase of degree of crystallinity [20]. The overlapping and advancement of PVA chain folding enhanced with the increase of concentration facilitating crystalline formation more effectively [16]. Therefore, polymers with high crystallinity have hardness, tensile strength, and are more resistance to solvents. Crystal formation is favored by robust intermolecular forces and a firm chain backbone because the molecules tend to arrange themselves in an orderly manner with high density to maximize the number of secondary bonds. Hence a crystalline structure is developed by the collaborative organization of molecules [24]. Due to above mentioned reasons, 1% (w/v) PVA/GCE with higher concentration and crystallinity as well as maximum density hindered its mobility therefore electro catalytic oxidation of GA was not enhanced. However, 0.1% (w/v) PVA/GCE improved the oxidation current of GA dramatically owing to low concentration and low crystallinity favoring the electro catalytic activity. Additionally, 0.1 % (w/v) PVA/GCE modified electrode produced more significant results as compared to 1 % (w/v) PVA/GCE modified electrode for the oxidation of GA as the associations could be overcome simply during flow as they were weaker in dilute polymer solutions as well as (ion-solvent) interactions between salt and aqueous polyvinyl alcohol were observed at lower concentration of polyvinyl alcohol whereas in concentrated polymer solutions these associations (polymer association to water) were tough and affected slightly during flow

process because the negative values of A-coefficient (ion-ion interaction) at higher concentration of polyvinyl alcohol established that the polymer association to water was highly strong [23].

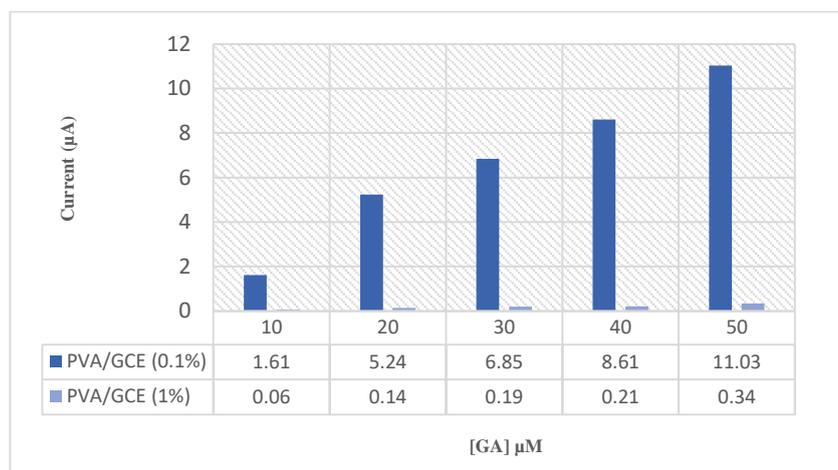


Figure 3. Comparison of current profile of PVA/GCE (0.1%) and PVA/GCE (1%) for the oxidation of GA

3.5 Significant tests of 0.1% (w/v) PVA/GCE modified electrode

3.5.1 Reproducibility test of PVA/GCE modified electrode (without freeze thaw cycle)

Reproducibility test for 0.1% (w/v) PVA/GCE polymer modified electrode conducted for GA in 0.1 M PBS/KH₂PO₄. This test was done to ascertain the reproducibility and reliability of the polymer based electrochemical sensor PVA/GCE.

Table 1. Reproducibility test of PVA/GCE modified electrode 0.1% (w/v)

Conc (µM)	Set A				Set B			Average	
	Current (µA)		Mean±SD (n=3)	Current (µA)		Mean±SD (n=3)	Mean±SD (Set A & B)		
	1	2		1	2		3		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	1.83	2.45	1.17	1.82±0.64	1.39	1.18	1.60	1.39±0.21	1.61±0.43
20	6.66	5.42	6.25	6.11±0.63	3.04	5.60	4.44	4.36±1.28	5.24±0.96
30	7.80	7.92	7.28	7.67±0.34	4.27	7.31	6.49	6.02±1.57	6.85±0.96
40	10.57	9.84	7.61	9.34±1.54	5.37	8.71	9.55	7.88±2.21	8.61±1.88
50	11.39	13.40	13.54	12.78±1.20	6.96	10.65	10.22	9.28±2.02	11.03±1.61

Set A: PVA/GCE electrode fabricated from freshly prepared PVA solution 0.1% (w/v); Set B: PVA/GCE electrode fabricated from PVA solution 0.1% (w/v) (after two weeks). For each set: Concentration study of GA was done in triplicates.

Readings were recorded in two sets for statistical analysis, set A was PVA/GCE electrode fabricated from freshly prepared PVA solution of 0.1% (w/v) and set B was PVA/GCE electrode fabricated from PVA solution of 0.1% (w/v) (after two weeks). For each set, concentration study of GA was done in triplicates to obtain standardized results.

Set A showed mean oxidative current for 50 μM of GA $12.78 \pm 1.20 \mu\text{A}$ whereas set B displayed mean oxidative current $9.28 \pm 2.02 \mu\text{A}$. It is obvious from the results that set A (PVA/GCE electrode with freshly prepared PVA) revealed more enhanced and reliable results with low %RSD of 9.39 as compared to set B except for 10 μM as shown in Table 1.

Definitively, excellent reproducibility and reliable results were obtained at PVA/GCE modified electrode (0.1%) with mean oxidative current $11.03 \pm 1.61 \mu\text{A}$ and %RSD 15.58 (Set A & B). Coefficient of determination, R^2 for average set (Set A & B) was 0.9871 explained adequate variability of the electrochemical data around its mean.

3.5.2 Stability test of PVA/GCE modified electrode

The stability of 0.1% (w/v) PVA/GCE polymer modified electrode was assessed. As shown in Table 2, it is interesting to note that oxidative current for the oxidation of 50 μM GA at freshly prepared PVA/GCE polymer modified electrode was 11.03 μA which was decreased to 7.51 μA with %RSD 9.29 at old PVA/GCE polymer modified electrode. This electrode has the stability for 25 days because its current sensitivity was decreased over the period of 25 days. Coefficient of determination, R^2 for average set (Set A & B) was 0.9920.

Table 2. Stability test of PVA/GCE modified electrode 0.1% (w/v)

Conc (μM)	Set A			Set B			Average		
	Current (μA)			Current (μA)			Mean \pm SD (n=3)		
	1	2	3	1	2	3	Mean \pm SD (Set A & B)		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
10	1.32	0.93	1.04	1.09 ± 0.16	1.69	1.72	1.31	1.57 ± 0.23	1.33 ± 0.20
20	3.81	3.88	3.92	3.87 ± 0.06	2.19	2.27	2.24	2.23 ± 0.04	3.05 ± 0.05
30	6.56	5.28	5.21	5.68 ± 0.76	4.41	4.64	4.74	4.59 ± 0.17	5.13 ± 0.47
40	6.92	7.13	7.27	7.11 ± 0.18	5.23	5.27	5.36	5.29 ± 0.07	6.20 ± 0.13
50	7.84	7.93	8.53	8.10 ± 0.38	6.16	6.58	7.99	6.91 ± 0.96	7.51 ± 0.67

Set A: PVA/GCE electrode fabricated from freshly prepared PVA solution 0.1% (w/v); Set B: PVA/GCE electrode fabricated from PVA solution 0.1% (w/v) (after two weeks) For each set: Concentration study of GA was done in triplicates

Readings were recorded in two sets for statistical analysis, set A was PVA/GCE electrode fabricated from freshly prepared PVA solution 0.1% (w/v) and set B was PVA/GCE electrode fabricated from PVA solution 0.1% (w/v) (after two weeks). For each set, concentration study of GA was done in triplicates to obtain standardized results.

Set A exhibited mean oxidative current for 50 μM of GA $8.10 \pm 0.38 \mu\text{A}$ with %RSD 4.69 whereas set B displayed mean oxidative current $6.91 \pm 0.96 \mu\text{A}$ with %RSD 13.89. It can be concluded that set A (PVA/GCE electrode with freshly prepared PVA) showed more reliable results with low %RSD as compared to set B.

3.5.3 Potential cycling test PVA/GCE modified electrode

The potential cycling for the oxidation of GA in 0.1 M PBS/ KH_2PO_4 was performed at PVA/GCE polymer modified electrode for 0.1% (w/v) PVA by SWV. It has revealed that GA was successfully oxidized but it was not reduced confirming that this process was ideally irreversible process of GA. From the first cycle of the voltammogram, the peak current for GA was reduced although the oxidation peak remained established even after 10th potential cycle.

3.5.4 Calibration curve for limit of detection

Table 3. Overview of sensors for GA detection.

Sensors	Oxidation Potential Volts (V)	Limit of detection (LOD) (mol. L ⁻¹)	Linear range (mol. L ⁻¹)	Supporting Electrolyte	Electrochemical Technique
CS-fFe ₂ O ₃ -ERGO/GCE	0.55 V	1.5×10^{-7}	1.0×10^{-6} – 5.0×10^{-5}	HAc–NaAc buffer (pH 2.0)	DPV [26]
Gel-RGONS/GCE	0.60 V	4.7×10^{-7}	1.0×10^{-6} – 1.1×10^{-4}	PBS solution (pH 2.0)	CV [27]
MWCNT/CPE	0.525 V	2.7×10^{-7}	2.5×10^{-3} – 7.5×10^{-3}	B–R buffer solution 0.2 M (pH 2.0)	DPV [28]
SPE-Au AuNPslinker/fullerenol-TvL	-0.1 V	6.0×10^{-6}	3.0×10^{-5} – 3.0×10^{-4}	Acetate buffer (pH 4.0)	Amperometry [29]
SiO ₂ nanoparticle-modified CPE	0.478 V	2.50×10^{-7}	8.0×10^{-7} – 1.0×10^{-4}	Phosphate buffer (pH 1.7)	DPV [30]
This work PVA/GCE	0.182 V	4.95×10^{-7}	1.6×10^{-6} – 4.9×10^{-5}	PBS/ KH_2PO_4 (pH 6.4)	SWV

Abbreviations: CS=chitosan, fFe₂O₃= fishbone-shaped Fe₂O₃, ERGO= electrochemically reduced graphene oxide, GCE=glassy carbon electrode, RGONS=reduced graphene oxide nanosheet, MWCNT=multiwalled carbon nanotube, CPE=carbon paste electrode, Britton–Robinson (B–R) buffer solution, SPE=screen-printed electrode, AuNPs= gold nanoparticles, TvL= *Trametes versicolor* Laccase, DPV-differential pulse voltammetry, CV-cyclic voltammetry, SWV-square wave voltammetry.

The limit of detection (LOD) for GA at 0.1% w/v PVA/GCE modified electrode () with a coefficient of determination R^2 (0.9176) has been obtained. The ks/m relation was used to calculate LOD [25], where $k = 3$ for LOD. High sensitivity and good detection limit of 4.95×10^{-7} mol. L⁻¹ was obtained. The PVA/GCE modified electrode revealed good linear range from 1.6×10^{-6} to 4.9×10^{-5} mol. L⁻¹ for detection of GA by SWV. Conclusively, outstanding analytical achievement displayed by our polymer sensor in oxidation potential and sensitivity compared to the reported sensors developed in the past [26-30] as shown in Table 3.

3.5.5 Study of the effect of freeze thaw cycle on PVA/GCE modified electrode 0.1% (w/v)

Oxidative current was obtained for the oxidation of GA concentration ranged from 10 μ M - 50 μ M in 0.1 M PBS/KH₂PO₄ aqueous solution over the potential range of 0 to +750 mV versus Ag/AgCl (in 3 M NaCl) at 0.1% (w/v) PVA/GCE modified electrode (with 5 freeze thaw cycles) by SWV.

The sensitivity of GA at 0.1% (w/v) PVA/GCE modified electrode (with 5 freeze thaw cycles) was found to be significantly maximum among all the modified electrodes in our research as the oxidation peak potential was observed at +225mV with highest oxidative peak current 21.85 ± 1.14 μ A for GA after freeze thaw cycles. Strong mechanical strength has been displayed by PVA gels prepared by freezing and thawing methods over PVA gels prepared by chemical cross-linking. PVA hydrogels prepared by freezing and thawing methods have potential applications in medicine and pharmacy [20].

In this research, preparation of excellent biodegradable, biocompatible, non-toxic and mechanically strong hydrogels is achieved by introducing a cryogenic method of solidification of PVA called freeze thaw method. Freeze thaw method is safe to control chemical cross linking as hydrogels that are prepared by redox, thermal or induction of radiation polymerization with inclusion of cross linking agent can give rise to the problems for example, loss in biocompatibility, toxicity due to cross linking agents; these issues confront in the biomedical applications of hydrogels. Pendant hydroxyl groups of PVA accelerate the formation of crystals by tough inter-chain hydrogen bonding that facilitates physical cross-linking by successive freezing thawing cycles [31]. Novel applications of cryogels made from natural and synthetic polymers have remarkable uses in the biomedicine and biotechnology fields owing to its great elasticity, possible bioactivity, non-toxicity, biocompatibility, non-carcinogenic behaviour, strong mechanics and porous nature of the polymer. Hydrogels are becoming popular in research because of its biocompatibility as they have hydrophilic polymeric linkage swallowing adequate amount of water while itself remain insoluble under normal physiological parameters like pH, ionic concentration and temperature [15]. Various bonding such as covalent, hydrogen bonds, electrostatic or dipole–dipole interactions are found in cross linking of polymeric chains [32]. Because of these outstanding interactions, hydrogels can be effectively valuable for developing biosensors, bioreactors, bio separators, tissue engineering, and drug delivery [33].

The physically crosslinked hydrogels display greater stability and firmness after freeze thaw cycles as degree of crystallinity and mechanical strength of hydrogel increases closely together due to increase number of hydrogen bonded PVA crystallites with their significant distribution of mechanical

load in polymeric network. PVA hydrogels prepared using freeze/thaw cycles display a great firmness, non-toxicity, efficient acceptability in the body and elastic nature [34].

Research findings as summarized in Table 4 focused on the fact of stabilization of PVA hydrogel owing to improve electrocatalytic oxidation of GA at PVA/GCE modified electrode (with 5 freeze thaw cycles). Hence, the polymer based electrochemical sensor after freeze thaw cycle was found to be a great potential sensor for the detection of GA in the electrochemical research. Average set of freeze thaw test for GA exhibited mean oxidative current of 21.85 ± 1.14 with %RSD 5.24. Coefficient of determination, R^2 for average set (Set A & B) was 0.9984.

Table 4. Freeze-thaw test PVA/GCE modified electrode 0.1% (w/v)

Conc (μM)	Set A			Mean \pm SD (n=3)	Set B			Mean \pm SD (n=3)	Average
	Current (μA)				Current (μA)				Mean \pm SD
	1	2	3		1	2	3		(Set A &B)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	3.09	4.39	4.27	3.92 ± 0.72	5.24	4.90	4.52	4.89 ± 0.36	4.41 ± 0.54
20	8.52	8.33	9.63	8.83 ± 0.70	8.02	7.86	8.53	8.14 ± 0.35	8.49 ± 0.53
30	13.16	13.36	13.71	13.41 ± 0.28	13.41	13.11	16.18	14.23 ± 1.69	13.82 ± 0.99
40	18.10	16.41	16.25	16.92 ± 1.03	19.08	19.43	18.04	18.85 ± 0.72	17.89 ± 0.88
50	19.86	22.38	21.77	21.34 ± 1.31	23.00	21.23	22.81	22.35 ± 0.97	21.85 ± 1.14

Set A: PVA/GCE electrode fabricated from freshly prepared PVA solution 0.1% (w/v); Set B: PVA/GCE electrode fabricated from PVA solution 0.1% (w/v) (after two weeks). Both set A and B electrodes were fabricated from PVA solution undergone to five freeze-thaw cycles. For each set: Concentration study of GA was done in triplicates.

The values of the peak current increased significantly on polymer modified electrode with 0.1% (w/v) PVA after freeze thaw cycles indicating a strong influence of this method in stabilizing and strengthening PVA hydrogel. It is also evident from the results that 0.1% (w/v) PVA/GCE modified electrode (with 5 freeze thaw cycles) improved the current sensitivity for 50 μM of GA at low oxidation potential to 51 folds efficiently as compared to bare GCE. However, PVA/GCE modified electrode 0.1% (w/v) (with 5 freeze thaw cycles) greatly enhanced the current sensitivity for 50 μM of GA to 1.98 folds as compared to the PVA/GCE (without freeze thaw cycle).

The reason of enhanced peak current at PVA/GCE modified electrode after freeze thaw cycle was that the aqueous PVA solution have the peculiar characteristics of crystallite formation upon repetitive freeze thaw cycles. The number and the stability of the crystallites were increased with the increased number of freeze thaw cycles. Physically crosslinked gels have strong mechanical strength, high degree of swelling in water, and elastic nature.. It was shown that the samples with maximum number of freeze thaw cycles produced the strong gel structure after five cycles [20]. Samples with

greater number of freeze thaw cycles express more stability as rearrangement within the structure is held to a minimum [16].

3.5.6 FESEM analysis

Fig. 4 shows FESEM image of 0.1% w/v PVA modified electrode before (a) and after (b) electrolysis in the presence of GA at 5 mm diameter BPPGE. The morphology of the PVA/GCE was obtained via solvent casting method by electrochemical scanning [17]. The images showed that PVA formed a homogenous film coating of polymer on the BPPGE surface (Fig. 4a). The electrolysis of GA can be seen in Fig. 4b by GA deposition on the surface which has confirmed the dissociation of GA with PVA on the electrode.

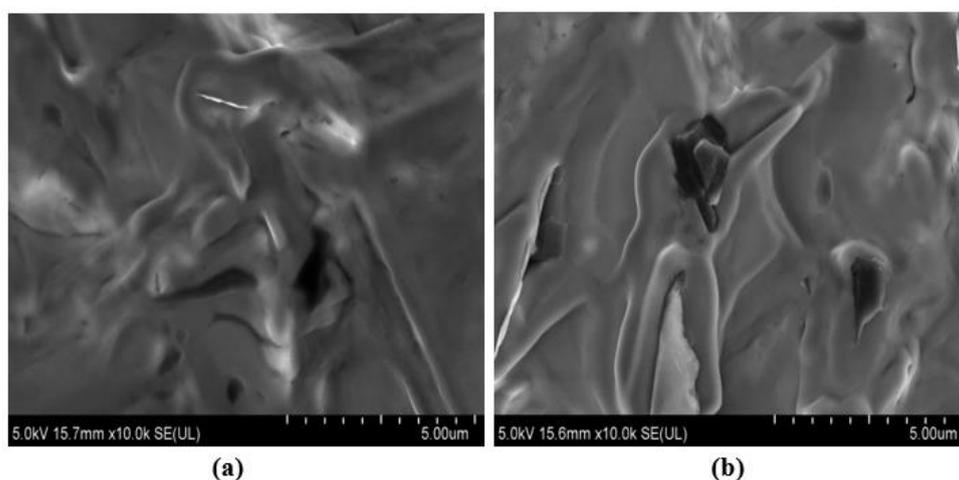


Figure 4. FESEM image of PVA solution on BPPGE surface before electrolysis (a) and after electrolysis (b) at magnification of 10,000 times.

4. CONCLUSION

We have demonstrated that 0.1% (w/v) PVA/GCE modified electrode (with 5 freeze thaw cycles) fabricated by solvent casting method via electrochemical treatment has shown the most promising results as appropriate and suitable electrochemical sensor for oxidation of GA with maximum enhancement of peak current and less positive potential. It shows high sensitivity with good detection limit and wide linear range compared to all other modified electrodes (0.1% and 1% w/v) and bare GCE in this study.

Electrode modification with increased concentration of PVA has not produced profound current enhancement for oxidation of GA due to hindrance in mobility and conduction of electrons within polymer network at high polymer concentration 1% (w/v).

Effect of freeze thaw cycle on PVA/GCE modified electrode 0.1% (w/v) reveals that PVA hydrogel remarkably stabilized to enhance the electrocatalytic oxidation of GA after 5 freeze thaw cycles

except slightly higher oxidation potential as compared to the PVA/GCE (without freeze thaw cycles). Consequently, repetitive freeze thaw cycles reinforced the already existing crystals in polymer network and strengthening them with enhancement of anodic peak current for GA.

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