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Research Article

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Electrochemical Characterization of Carbonized Typha Tassel Modified Screen-Printed Electrode and Its Enzymatic Glucose Oxidation Application

Baskı Devre Elektrotlara Modifiye Edilmiş Karbonize Hasır Otu Püskülünün Elektrokimyasal Karakterizasyonu ve Enzimatik Glikoz Oksidasyonu Uygulaması

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ABSTRACT

Converting biomass into cheaper but valuable products is very important for a more sustainable world. Especially with emerging technology, the use of hazardous materials in the synthesis of substances such as carbonaceous materials pose a threat to our environment. In this study, electrochemical performance of a carbonaceous material synthesized from typha tassel using a simple and cheap method without any hazardous substances was investigated. It was then used as an enzyme immobilization material for electrochemical glucose oxidation to demonstrate its potential application in bioelectronics. Physical and chemical characterization of raw typha tassel (RTT) and carbonized typha tassel (CTT) were performed using SEM and FTIR techniques. CTT, was then grounded into fine powder, dispersed in DMF and coated onto screen-printed electrodes (SPEs). CTT modified SPEs were electrochemically tested using cyclic voltammetry in 0.1 M phosphate buffer containing 1 mM ferrocene carboxylic acid as a redox mediator at pH 7.4 Finally, glucose oxidase enzyme was adsorbed on CTT modified SPEs to demonstrate its performance in electrochemical enzymatic glucose oxidation reactions. SPE/CTT/GOx system showed promising electrochemical activity and stability at physiological conditions as well as good activity with adsorbed enzyme. This study suggests that CTT is very promising for an easy, effective and cheap ‘biomass to bioelectronics’ construction material.

Key Words
Carbonaceous materials, bioelectronics, electrochemistry, typha.

ÖZ

Biyokütleyi daha ucuz ama değerli ürünlere dönüştürmek, daha sürdürülebilir bir dünya için çok önemlidir. Özellikle ilerleyen teknolojiyle, karbonlu malzemeler gibi ürünlerin sentezinde tehlikeli maddelerin kullanımı çevremizi tehdit etmektedir. Bu çalışmada, herhangi bir tehlikeli madde içermeyen basit ve ucuz bir yöntemle hasır otu püskülünden sentezlenen bir karbonize malzemenin elektrokimyasal performansı araştırılmıştır. Daha sonra biyoelektronik alanındaki potansiyel kullanımını göstermek için elektrokimyasal glikoz oksidasyonu için bir enzim immobilizasyon malzemesi olarak kullanılmıştır. Hasır otu püskülü (RTT) ve karbonize hasır otu püskülünün (CTT) fiziksel karakterizasyonu SEM ve FTIR teknikleri kullanılarak yapılmıştır. CTT, daha sonra ince bir toz halinde öğütülmüş, DMF içinde disperse edilmiş ve baskı-devre elektrotlar (SPE’ler) üzerine kaplanmıştır. CTT modifiye edilmiş SPE’ler, mediyatör olarak 1 mM ferosen karboksilik asit içeren 0.1 M fosfat tamponu (pH 7.4) ile elektrokimyasal olarak test edilmiştir. Son olarak, elektrokimyasal enzimatik glikoz oksidasyon reaksiyonu için performansını göstermek üzere, CTT modifiye SPE’ler üzerinde glikoz oksidaz (GOx) enzimi adsorbe edilmiştir. SPE/CTT/GOx sistemi, adsorbe enzimle iyi aktivite göstermenin yanı sıra fizyolojik şartlar altında iyi elektrokimyasal aktivite ve kararlılık göstermiştir. Bu çalışma, CTT’nin ‘biyokütleden biyoelektroniğe’ gidişte kolay, ucuz ve etkili bir yapı malzemesi olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler
Karbon malzemeler, biyoelektronik, elektrokimya, hasır otu.

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INTRODUCTION

Different carbonaceous materials (CMs) have been widely used in electrochemical studies because of their excellent electrical communication, electrocatalytic activity, high surface area and biocompatibility [1, 2]. Typical CMs used in electrochemical detection of analytes include carbon nanotubes, graphene and its derivates, graphitic carbon nitride and carbon dots [3]. Such materials need complex synthesis procedures involving hazardous materials which poses a threat to the environment. Although much effort has been shown to use green methods to produce these materials, the complexity of those systems is still a concern.

In recent years, carbonization of natural sources such as plants, fruits and nuts have been used as a cheaper source for a low-cost carbonaceous material [4-6]. Such materials can be used for different applications such as supercapacitor design, fluorescent materials for bioimaging and drug delivery, lithium ion batteries and electrochemical sensing [4, 5, 7, 8]. Among many different sensing methodologies, electrochemical techniques stand out because of their high sensitivity and selectivity as well as low cost device manufacturing possibilities [9]. However, electrode materials and their practicality play an important role in such techniques to differentiate desired signals for target analytes. As well as their structural properties, their physical properties such as size, surface area and usability for portable systems are also important.

There are many different electrode types used in electrochemistry such as glassy carbon, gold electrodes and screen-printed electrodes (SPEs) [10, 11]. Among all, SPEs have attracted many researchers due to their versatile design which makes them important for portable technologies [12]. Moreover, SPEs provides flexible modification platform for researchers to easily adapt these electrodes to their desired applications. Therefore, it is now one of the best candidates in the market for bioelectronic applications. Glucose Oxidase (GOx) is one of the most widely used enzyme for glucose biosensing due to its well-established structure and very good selectivity towards glucose [13-15]. It has been demonstrated in biofuel cells, biosensors, and even multi analyte sensing platforms indicating it has wide range of application areas [16-18]. Since it is very accessible in the market at reasonable cost, it can be considered as the model enzyme for glucose oxidation systems especially using electrochemical methods.

In electrochemical enzymatic systems, one of the most important issue is the transfer of the electrons from active center of the enzyme to the electrode. This is difficult because most enzymes such as GOx has their active center deep buried inside their protein shell [19, 20]. This makes the direct electron transfer more difficult for such enzymes. A number of mediators, on the other hand, poses a solution to this problem such as osmium, benzoquinone, poly-vinylferrocene, ferrocene (Fc) and its derivatives [21]. Since Fc is non-toxic to human body and its derivatives such as ferrocene carboxylic acid (FcCOOH) can be soluble in different solvents such as water, it is one of the most attractive mediator of all [22, 23].

In this study, the physical characterization and electrochemical performance of a carbonized typha tassel (CTT) has been investigated. SEM and FTIR techniques were used for physical characterization studies. CTT was grounded to fine powder, dispersed in DMF and coated onto screen-printed electrodes (SPEs). Cyclic voltammetry (CV) is then used for electrochemical characterization studies using a redox mediator FcCOOH at pH 7.4. Finally, glucose oxidase enzyme was adsorbed on CTT modified SPEs to demonstrate its performance for electrochemical enzymatic glucose oxidation reactions. This study is, to the best of author's knowledge, a first-time demonstration of the electrochemical characterization and glucose oxidation performance of CTT.

MATERIALS and METHODS

Materials

FcCOOH, dimethylformamide (DMF), GOx (from Aspergillus Niger lyophilised powder, ~200 U/mg) were purchased from Sigma-Aldrich (Dorset, UK) and used as received. Carbon SPEs, (model: DRP-C110 with working electrode diameter of 0.40 cm) were purchased from DropSens (Oviedo,Spain) and used without pretreatment. Heat gun (Model: Maestro JS-HG-12AII) was obtained from Tekzen (İstanbul,Turkey).

Preparation of CTT Mmodified SPE and Enzyme Immobilisation

CTT were prepared using a method published previously [24]. Briefly, 10 g of TT was carbonized in a tube furnace at 1000°C under flowing inert nitrogen at a heating ramp of 10°C/min for 1 h. After the carbonization procedure, SEM images were taken and FTIR analyses were performed for RTT and CTT. Planetary ball mill (Retsch, PM 100) at 500 rpm were then used to ground

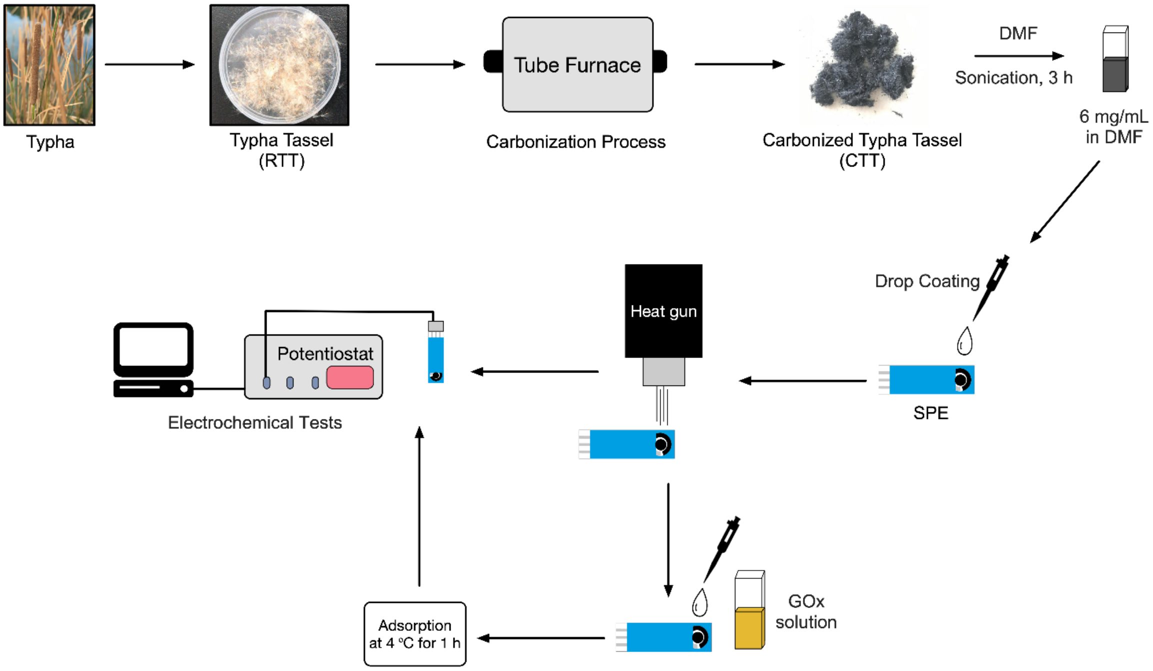

Figure 1. Schematic representation of the preparation of CTT modified SPE, enzyme immobilization and electrochemical tests.

CTT into fine powder. Finally, the resulting fine powder was dispersed in DMF to give 6 mg/mL CTT concentration and sonicated for 3 h in an ultrasonic bath. The obtained dispersion is kept at room temperature in a sealed vial and sonicated for at least 15 min before each use.

CTT modified SPEs were prepared drop-coating a small amount of 6 mg/mL dispersion onto working electrode surface and then applying a drying process using heat gun until the desired amount of material load is reached. Dried electrodes were then tested electrochemically in 150 µL of 0.1 M PBS solution containing 1 mM FcCOOH at pH 7.4.

Immobilization of GOx were performed by drop coating 10 µL of 10 mg/mL GOx solution in 0.1 M PBS at pH 7 on CTT modified SPE for 1 h at 4°C. Finally, the electrode is rinsed with ultra-pure water to remove weakly adsorbed enzymes and tested in 150 µL of 0.1 M PBS solution containing 1 mM FcCOOH at pH 7.4.

Electrochemical Characterisation

Electrochemical measurements were carried out using an Ivium potentiostat-galvanostat (Ivium Technologies B.V., Netherlands). CV measurements were carried out in 0.1 M PBS containing 1 mM FcCOOH at pH 7.4. Stock solution of glucose was allowed to mutarotate for minimum 24 h before use and were subsequently kept refrigerated at 4°C. CV experiments were performed at different scan rates from 200 mV/s to 5 mV/s. The schematic representation of the preparation of CTT modified SPE, enzyme immobilization and electrochemical tests are shown in Figure 1.

RESULTS and DISCUSSION

Physical and Electrochemical Characterizations of CTT Modified SPE

SEM and FTIR analyses were used for the physical characterization of the RTT and CTT samples to demonstrate their surface properties and molecular structure. Figure 2 shows the SEM images of TT before and after the carbonization procedure indicating the formation of carbonaceous fibers. It is important to note that, not only the fibers of TT gained carbonaceous structure, but also carbonaceous connections can be seen between fibers forming a polycrystalline-carbon-like structure. This interconnected carbonaceous fiber structure could be very important due to its high 2-D surface area and increased electrical conductivity for different applications such as bioelectronics.

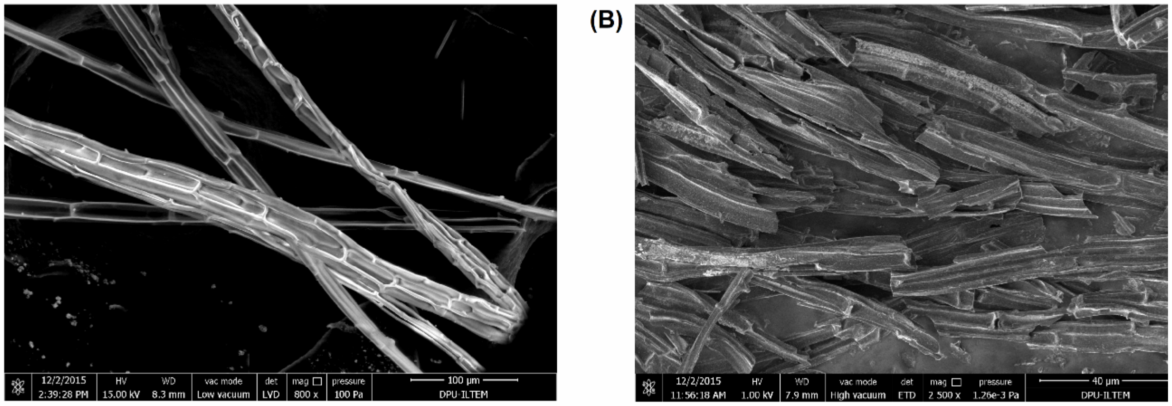

Figure 2. SEM images of (A) RTT and (B) CTT.

Figure 3 demonstrates the FTIR spectra of RTT and CTT samples. The peaks appeared at around 800, 1000, 1300, 2900 and 3390 cm^{-1} for RTT demonstrate characteristic behavior of cellulosic materials published in literature previously [25]. In detail, absorption band around 800 cm^{-1} refers to characteristic β-glycosidic linkage between glucose units. The band around 1000 cm^{-1} is a C-O stretch refers to secondary alcohols and ethers in cellulosic structure. The bands at 1300 and 2900 cm^{-1} indicates C-H bending and stretching in glucose structure. The adsorption band at 3390 cm^{-1} also represents the O-H stretching.

On the other hand, the FTIR spectra of CTT shows very similar characteristic peaks with carbonaceous materials such as graphene and graphite [26]. Moreover, the appearance of the peaks at around 650 and 1650 cm^{-1} indicates the formation of C=C formation which also supports the carbonaceous structure forming. There is also a peak shift regarding to hydroxyl groups between 3300-3700 cm^{-1} which is due to the removal of bound water removal post carbonization.

Electrochemical characterization of SPEs without any modification (bare electrode) and modified with different loadings of CTT was performed using CV in 0.1 M PBS containing 1 mM FcCOOH as redox probe at pH 7.4. Figure 4 (A) show that the anodic and cathodic peak currents were increased upon coating of CTT on SPEs. This indicates that the electrode surface gained more conductivity thus had enhanced electron transfer properties. Figure 4 (B) also shows the change in anodic peak currents relationship with CTT loading.

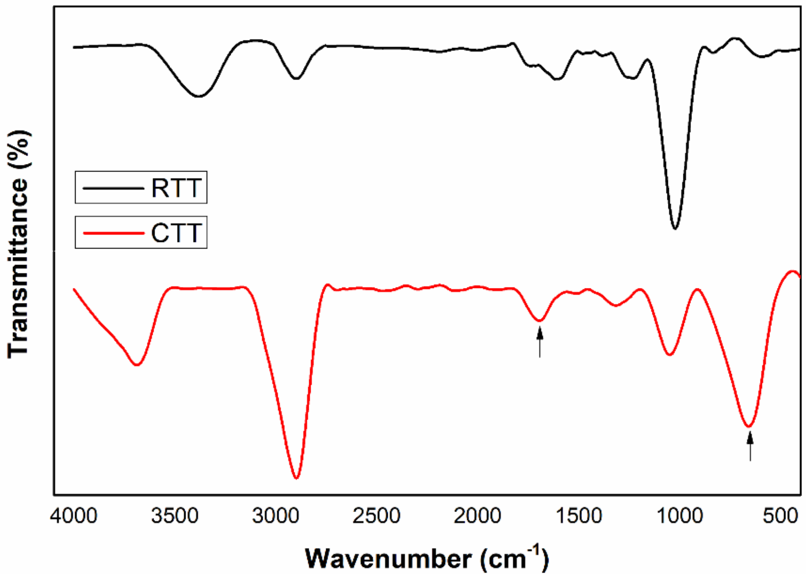

Figure 3. . FTIR spectra of RTT and CTT.

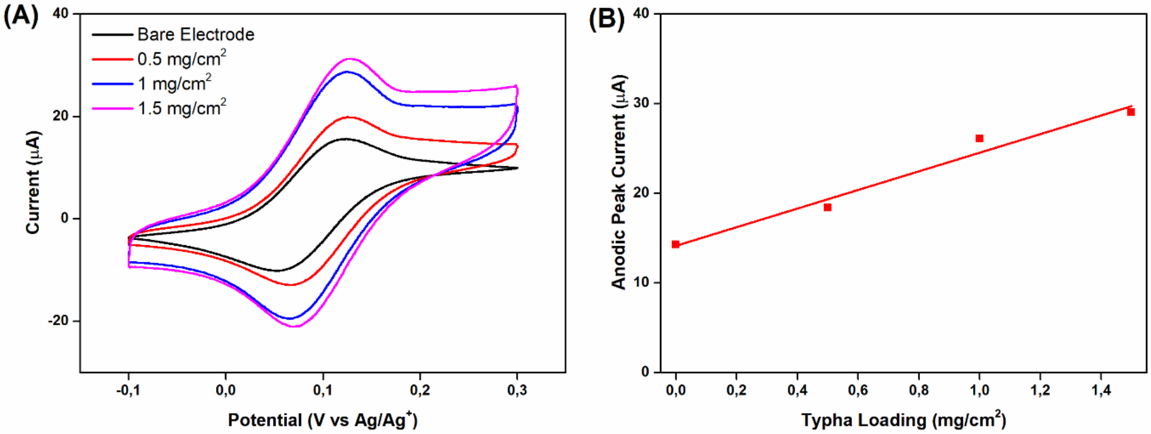

Figure 4. (A) CVs (50 mV/s) showing the effect of CTT loadings on SPE. (B) Anodic peak current values of CTT loadings obtained from (A). All experiments were conducted in 0.1 M PBS containing 1 mM FcCOOH at pH 7.4. Error bars are sample standard deviations and sample is defined as the number of electrodes tested. (n=3 samples).

The electrochemical current responses showed increasing values between 0.5 mg/cm^2 and 1.5 mg/cm^2 CTT loading. Less than 0.5 mg/cm^2 CTT loading were also applied but nonuniform films were obtained, on the other hand, higher CTT loading haven't been tested due to the oxidation and reduction peak currents haven't significantly increased more than previous CTT loading amount and the practical difficulties of the coating procedure. This is mainly because when excessive amounts of CTT were coated onto the confined electrode area, the material started to leach out resulting instable films due to weak interactions with electrode surface caused by excessive loading. Hence 1 mg/cm^2 was selected as optimum loading for further experiments. Error bars cannot be seen in Figure 4b as the values of standard deviation vary between 0.02-0.05 which are very small compared to anodic peak current values.

Figure 5 (A) shows the electrochemical performance of 1 mg/cm^2 coated CTT electrode using CV at various scan rates in 0.1 M PBS solution containing 1 mM FcCOOH at pH 7.4. Anodic and cathodic peaks have been obtained around 150 mV and 50 mV vs Ag/Ag+ respectively with an oxidation onset potential of around 25 mV, which is in parallel with previously reported redox potentials for ferrocene [24]. The fast, efficient and diffusion-controlled electron transfer processes have been confirmed with the linear relationship between peak current and square root scan as shown in Figure 5 (B). As FcCOOH is a diffusional mediator, this shows the successful and efficient electron transfer mechanism between CTT modified SPE and FcCOOH mediator has been successfully achieved [27]. Figure 5 (C) shows the stability of the CTT modified SPE after multiple CV scans have been applied. It is very usual to obtain electrocatalytic current drop after the first scan of electrochemical measurements with diffusional redox probes such as FcCOOH. This is because of the electrochemical stabilization of the redox active species between electrode surface and the electrolyte.

After the stabilization current drop between the 1st and 5th scan, the current response stabilized and even slightly increased up to 50th cycle. The electrocatalytic performance of the CTT modified electrode didn't show further current drop up to 100th cycle. However, the peak potentials shifted to more negative voltages about 30 mV between the 50th and 100th scans. This peak shift is not massive but could be due to some instabilities formed on the electrode-electrolyte interface after so many consecutive voltammograms applied. It also could be due to leaching of DMF into the electrolyte, which has lower pH value than 7.4, therefore could affect the potential where redox peaks appear.

Glucose Oxidation with CTT Modified SPE

CTT modified SPE shows very promising electrochemical activity at physiological conditions and its performance is very comparable with widely used nanomaterials such as nanotubes. Since it is significantly cheaper to produce CTT compared to its alternatives, it can be a very cheap option in bioelectronic applications such as biosensors. Incorporating biological species onto electrode materials is a very important aspect since signal differentiation is needed to detect the desired analyte. Therefore, not only highly conductive but also highly biocompatible materials such as CTT are great of interest in bioelectronics.

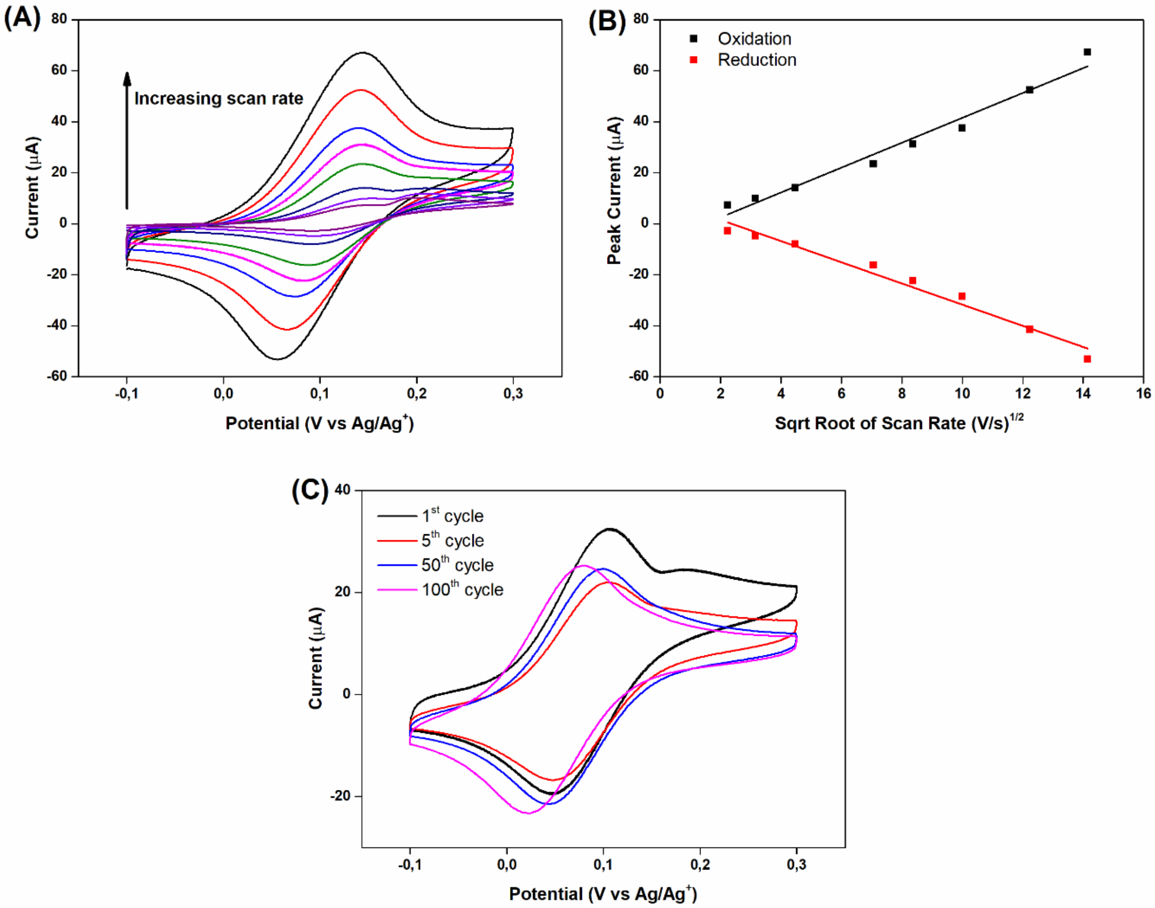

Figure 5. (A) CVs of CTT (material loading of 1 mg/cm^2) on SPE tested at various scan rates. (B) Anodic and cathodic peak current relationship with the scan rate derived from Figure 2 (A). (C) stability of CTT coated SPEs (cut-SWCNT loading: 1 mg/cm^2). CVs were conducted in 0.1 M PBS containing 1 mM FcCOOH at pH 7.4.

To demonstrate its potential in biosensing, a model electrode was prepared by adsorbing GOx enzyme onto TT modified SPE (annotated as SPE/CTT/GOx) and its glucose oxidation performance was tested. Figure 6 shows the performance of the prepared enzymatic electrode in the presence of 10 mM glucose. The TT modified SPE showed an increased oxidation current upon addition of 10 mM glucose at the same onset potential (around 25 mV vs Ag/Ag+) indicating;

I. Successful immobilization of the enzyme on CTT modified SPE,

II. The electrons produced from the enzymatic glucose oxidation reaction by GOx has been successfully transferred via FcCOOH mediator to the CTT modified SPE.

Consequently, SPE/CTT/GOx systems showed promising results for its potential in electrochemical glucose oxidation. This configuration could be used in many bioelectronic applications such as biosensors and biofuel cells. However, further optimizations on system parameters are needed.

CONCLUSION

This study is, to the best of author's knowledge, a first-time demonstration of the electrochemical characterization and glucose oxidation performance of CTT. It also shows that CTT is very promising for an easy, effective and cheap 'biomass to bioelectronics' construction material. Consequently, this study has the potential to pave the way for cheaper and effective glucose biosensing to replace more expensive state of the art.

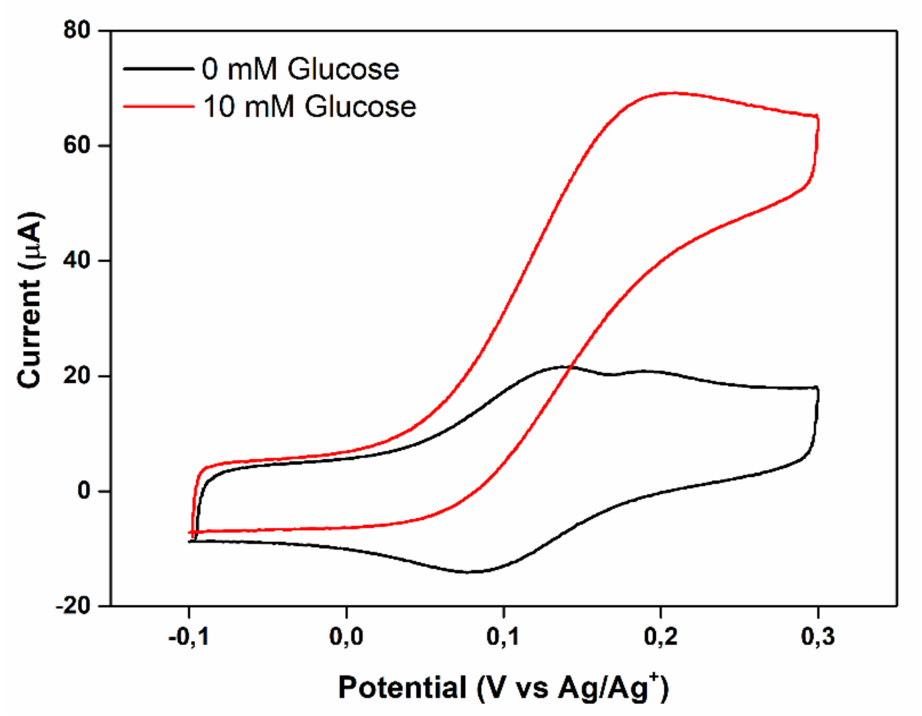

Figure 6. . CVs of SPE/CTT/GOx (material loading of 1 mg/cm^2) with 0 and 10 mM glucose. All experiments were conducted in 0.1 M PBS containing 1 mM FcCOOH at pH 7.4 A stock solution of glucose in 0.1 M PBS was used to test different concentrations.

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