Understanding the role of glucose oxidase on carbon felt as electrodes in biocapacitor studies

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Abstract. In this work, we have reported glucose oxidase incorporated carbon felt bioelectrodes (GOx/CFE) as biocapacitors for energy storage. Glucose oxidase (GOx) was incorporated into a carbon felt electrode (CFE) and the electrode was characterized using X-ray diffraction, scanning electron microscope and Fourier transform infrared spectroscopy. As a result, it was found that GOx was successfully incorporated into a bare CFE and enhances the specific capacitance of the electrode and it was stable up to 500 charge–discharge cycles. Consequently, it was observed that GOx/CFE exhibits enhanced energy storage capacitance compared to that of pristine carbon felt. The capacitance of GOx/CFE is found to be 4.21 mF cm^{-2} (23 F g^{-1}) while the bare CFE shows 3.68 mF cm^{-2} in a phosphate buffer solution ($\text{pH} = 7.0$). Albeit the capacitance values are small compared to conventional supercapacitors, the utility of these biocapacitors is expected to have a significant impact on glucose monitoring. Columbic efficiency obtained with the GOx/CFE matrix is 89%, and the electrode is stable up to 225 cycles with 100% retention of capacitance. After 225 cycles, the electrode loses the capacitance up to 12% retaining the capacitance of 88% up to 500 cycles. Cyclic voltammetric studies revealed that GOx/CFE is capable of energy storage with a $200 \mu\text{A}$ higher capacitive loop than the bare CFE at a scan rate of 10 mV s^{-1} . Electrochemical impedance analysis measurements also confirmed that GOx/CFE possess minimum resistivity. Moreover, it is very eco-friendly due to which unwanted pollution can be avoided. From the proposed matrix, it is believed that a green, eco-friendly, clean, renewable material for energy storage could be realized.

Keywords. Glucose oxidase (GOx); carbon felt; specific capacitance; biocapacitor; cycle-life.

1. Introduction

Supercapacitors are known for their high power and energy density which make them an essential component in electronic devices and vehicles. They play a very important role in mobile technologies [1]. The use of electrical double layer supercapacitors (EDLSCs) is more pronounced due to their enhanced performance in energy storage in the dielectric medium. They can store energy at the electrode/electrolyte interface by irreversible adsorption of ions at the electrode. Therefore, focus is on the improvement of the activity of supercapacitors by using materials like carbon [2–4], metal oxides and hydroxides [1,5,6] and carbon materials along with conducting polymers [7]. They were tested in different electrolytes such as aqueous [5] and non-aqueous [3,8], redox-active [4] and neutral electrolytes [6].

A biocapacitor has been identified as synonymous with health care applications. Unlike conventional energy storage devices such as batteries, capacitors and supercapacitors, biocapacitors will play a vital role in every individual's health.

Because of their biocompatibility, they can be implanted into a human system for the improvement in the functioning of organs in several unwanted situations like surgery. Even though the presently available energy storage devices offer more power density than biocapacitors, due to their lack of biocompatibility it is not possible to use them in healthcare applications. On the other hand, an energy storage device in the presence of biomolecules is an attractive one which has gained attention from several fields. Particularly in the sector of healthcare, those devices are expected to play a critical role in increasing the average lifespan of human beings. Therefore, in spite of the availability of high energy storage supercapacitors, these biomolecule incorporated devices are very much essential in health care applications such as biosensors and bioelectronics [9–11]. Because of the implantation of those devices into biosources, it is possible to harvest energy from them [12–16]. As a consequence, integration of biomolecules into the electrode matrix is becoming attractive. Therefore, research studies have tried to incorporate biomacromolecules such as human serum albumin [17], safranin O

[18], phenothiazine and flavin adenine dinucleotide [19], enzymes [20], proteins [21] etc., into the electrode surface for different applications. However, there is an immediate necessity to develop biocapacitors with biocompatibility, ecofriendly nature and long cycling ability. In this regard, the development of biosupercapacitors has been attempted using biorecognition elements such as enzymes, fibroins and peptides [22–28].

In this way, glucose oxidase (GOx) has received much attention due to its broad applications in the field of chemical, food, pharmaceutical, biotechnology and textile industries [29]. So, it is aimed to develop a biocapacitor by using of an electrode having the enzyme, GOx. Therefore, the present work focuses on the development of an innovative, ecofriendly bioelectrode in which GOx is incorporated. The GOx incorporated carbon felt as a 3D networked (bio)electrode for biocapacitors is shown to unveil the fabrication process (scheme). The electrode is found to be more stable and capable of storing energy up to 4.21 mF cm^{-2} .

2. Experimental

2.1 Materials required

All the chemicals used in this work were of analytical grade and used without any further purification. Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck. GOx from *Aspergillus niger* was purchased from Sisco Research Laboratories. All the aqueous solutions were prepared with the help of Millipore water with a resistivity of $18.2 \text{ M}\Omega$. All the electrochemical measurements were carried out at room temperature ($25 \pm 1^\circ\text{C}$).

2.2 Physico-chemical characterization techniques

X-ray diffraction (XRD) analysis was performed with an X-ray diffractometer procured from Bruker, Netherlands. The surface morphologies of the bare carbon felt electrode (CFE) and GOx/CFE were examined with the help of a scanning electron microscope (SEM) using a model from TESCAN. The Fourier transform infrared (FTIR) spectrum was recorded from a spectrometer obtained from Bruker Optik GmbH, TENSOR 27, Germany.

2.3 Electrochemical characterization and preparation of GOx/CFE

All the electrochemical measurements such as cyclic voltammetry, electrochemical impedance analysis (EIS) and charge–discharge measurements were carried out in PARSTAT MC from Princeton Applied Research. EIS was carried out in the frequency range of 0.1 Hz to 100 kHz with an amplitude of 10 mV . A three electrode system consisting of GOx/CFE, Ag/AgCl electrode and platinum spiral wire were used as working, reference and counter electrodes, respectively, in the

Figure 1. XRD pattern of the (a) bare CFE and (b) GOx/CFE.

electrolyte containing 0.1 M phosphate buffer solution (PBS) of $\text{pH} = 7$.

GOx/CFE was prepared according to the procedure [30]. In brief, a piece of carbon felt with the dimensions of $0.5 \times 1 \times 0.3 \text{ cm}$ (3D network) was cleaned in a hot H_2O_2 (10% , 90°C) solution for 3 h . It was further thoroughly rinsed with deionized water and dried at 60°C . A brass rod having a diameter of 1 mm was inserted into the CF to allow the external electrical circuit connection with CF. The prepared CF was immersed in 1 ml PBS containing 1 mg GOx for 24 h in a dry and cold environment ($< 10^\circ\text{C}$) due to which successful incorporation of GOx into the system was achieved. The cold environment preserves GOx from denaturing.

3. Results and discussion

3.1 XRD analysis

XRD measurement was performed in order to investigate the crystal structure of the bare CFE and GOx/CFE. Accordingly, figure 1a shows the peaks around 25° and 45° which were found to be common for CF [31] and they are corresponding to the different planes of (002) and (101) CF respectively. In the case of GOx/CFE, new waves appear around 32° and 35° (figure 1b) in addition to the existing peaks that are found to be the characteristic peaks of GOx [32]. From this analysis,

Figure 2. SEM images of the (a) bare CFE and (b) GOx/CFE.

it can be concluded that GOx was successfully incorporated into carbon felt and remains on the surface of carbon felt.

3.2 SEM analysis

The surface characterization of the CFE and GOx/CFEs was examined by SEM at 3000 $\times$ and shown in figure 2. The images explain the surface morphologies of bare carbon felt and GOx/CFE. Figure 2a illustrates the morphological features of the bare CFE where a smooth surface is observed whereas, in the case of carbon felt which is soaked in GOx, a non-uniform surface is observed as shown in figure 2b. This is due to the loaded GOx in the CFE. Because of the incorporation of GOx, bare carbon felt loses its smooth surface. The coagulated surface of CF confirms the presence of GOx in the system. From the images it can also be confirmed that GOx was successfully incorporated into the bare CFE.

Figure 3. FT-IR spectra of the (a) bare CFE, (b) GOx/CFE and (c) GOx.

3.3 FT-IR studies

FT-IR has further been utilized in order to confirm the presence of GOx in the modified electrode. Figure 3 compares the transmittance spectrum of the bare CFE (a) and GOx/CFE (b) with GOx (c). From figure, one can clearly observe that GOx and GOx/CFE produce a shoulder peak at a wave number of $\sim 2900\text{cm}^{-1}$ which is identified as the NH bending vibration present in the amine group of GOx. In the case of GOx/CFE, a minor shift in the peak is observed which may be due to the intermolecular interaction between carbon felt and GOx. The unmodified bare CFE fails to obtain the signal around the region which confirms the absence of GOx in the system. As a result of spectroscopic analysis, it was confirmed that GOx is successfully incorporated into the system.

3.4 Cyclic voltammetric studies

With an aim to understand the capacitive behaviour of the CFE and GOx/CFE, cyclic voltammograms were recorded in an electrolyte of PBS (pH = 7.0) which are shown in figure 4. Figure 4a compares the cyclic voltammograms of the bare CFE and GOx/CFE in an electrolyte of 0.1 M PBS (pH = 7.0). As can be seen in figure, cyclic voltammograms of both the electrodes are rectangular in shape without any significant redox peaks indicating the ideal capacitive

Figure 4. (a) Cyclic voltammetric responses of the (i) bare CFE and (ii) GOx/CFE at a scan rate of 10 mV s^{-1} in 0.1 M PBS . (b) Comparison of the cyclic voltammetric response of GOx/CFE at various scan rates ranging from 10 to 100 mV s^{-1} (i to v) in an electrolyte of 0.1 M PBS . (c) Cyclic voltammetric responses of the CFE loaded with different amounts of GOx: (i) 1 mg , (ii) 0.5 mg , (iii) 2 mg and (iv) 4 mg at a scan rate of 10 mV s^{-1} in an electrolyte containing 0.1 M PBS .

behaviour of the CFE and GOx/CFE. In the presence of GOx, a broader capacitive behaviour is observed with the current value of $230 \mu\text{A}$ which is $45 \mu\text{A}$ higher than that of the bare CFE. The enhanced current value is due to the incorporation of GOx into the CFE. The improved capacitance behaviour of GOx/CFE is mainly attributed to the active site of GOx (i.e., FADH_2 to FAD). It is also noted that there is a significant impact on specific capacitance with the modified electrode.

Figure 4b depicts the cyclic voltammograms recorded at various scan rates between 10 and 100 mV s^{-1} . Very interestingly, there is no change in the shape of the voltammograms even at higher scan rates indicating the rate capability of the modified electrode. The electrode produces linear variation in the current responses which are measured at an overpotential of 0.5 V . The capacitance of the CFE loaded with the different amounts of GOx was measured by using of cyclic voltammetry which is shown in figure 4c. It can be observed that the current response of the CFE loaded with 1 mg GOx is higher than that of 0.5 mg GOx. In the case of carbon felt loaded with

a higher amount (i.e. $>1 \text{ mg}$) of GOx a decrease in current is observed. This behaviour can be reasoned as follows. On comparing CFE containing 0.5 mg of GOx with the electrode of 1 mg of the biomolecule, the modified electrode carrying 1 mg of GOx carries the molecule throughout the lattice of the carbon felt. But the electrode modified with 0.5 mg of GOx is unable to spread over throughout the lattice of carbon felt. Based on this, it is believed that a significant amount of GOx is present and the same is exposed in the electrolyte. In the case of the CFE loaded with the higher amount of GOx, the active sites of the enzyme are deeply buried. Hence, the electronic conductivity between the redox active centre of GOx and the electrode is prevented by the thick insulating layer called glycoprotein [33,34]. The amount of GOx exposed towards the electrolyte is very low due to which there is a decrease in the current response at a higher amount of GOx in the system. From this experiment, it can be justified that an optimum level of GOx was incorporated into the carbon felt to achieve the maximum current and the system can behave as a bioelectrode.

Figure 5. (a) Galvanostatic charge–discharge curves of the (i) bare CFE and (ii) GOx/CFE at 1 A g⁻¹ of first 5 cycles. (b) Specific capacitance of GOx/CFE with various amounts of GOx load. (c) Specific capacitance of GOx/CFE at different current densities.

3.5 Charge–discharge measurement

To evaluate the specific capacitance and rate capability of the CFE and GOx/CFE, galvanostatic charge–discharge measurements were carried out in the solution, 0.1 M PBS (pH = 7.0) at a current density of 1 mA g⁻¹ in the potential range of 0 to 1 V. The experiment was carried out for 500 cycles in order to identify the stability of the electrodes. Figure 5a compares the first five cycles of the typical triangular charge–discharge profile of the CFE and GOx/CFE. From figure, one can observe that the discharge time for GOx/CFE is comparatively higher than that for the bare CFE which resembles the cyclic voltammetric profile of the electrode. Using the measurement, electrochemical stability and specific capacitance (C_{sp}) of GOx/CFE were determined. C_{sp} was obtained from the following equation

$$\text{Specific capacitance} = It/m\Delta V \quad (1)$$

where I is the discharge current in ampere, t is the discharge time in seconds, m is the amount of GOx loaded in CF in grams and ΔV is the difference in potential applied in volts.

From the calculation, it can be agreed that GOx/CFE unveils an excellent gravimetric capacitance of 23 F g⁻¹ (4.21 mF cm⁻²) at an operation rate of 1 mA g⁻¹ and is quite larger than the CFE. This value is comparable with the previous reports 4.68 [27] and 2.9 mF cm⁻² [35]. The increased capacitance of 530 μF is mainly attributed to the incorporation of GOx in the CFE.

An increase in GOx loading in CF leads to a decrease in capacitance drastically. This phenomenon is proved once again by charge–discharge measurements in addition to the cyclic voltammetric measurements. GOx has the active centre of the flavin adenine dinucleotide (FAD) group. The depth of the active centre in GOx is almost 8.7 Å which is relatively larger than that of other enzymes like laccase (4.3 Å), flavocytochrome b2 (4.2 Å) and peroxidase

Figure 6. (a) Coulombic efficiency of the (i) bare CFE and (ii) GOx/CFE. (b) Retention of capacitance of the GOx incorporated CFE up to 500 cycles.

(4.1 Å) [36]. Therefore, the rate of electron transfer between the electrode and electrolyte becomes slow. As a result, the capacitance value decreases as shown in figure 5b.

Furthermore, gravimetric capacitance was measured with different current densities and the comparison graph is shown in figure 5c. At low current densities (0.5 and 1 mA g⁻¹), the observed specific capacitance decay is 5.9%. At high current densities, there is a sudden decrease in capacitance with a specific capacitance decay of 47%. From figure, it was discovered that at low current densities, high capacitance values are obtained. In the case of higher current densities, it drastically decreases due to speedy charge–discharge characteristics. From the charge–discharge measurements, the amount of charge stored was found to be 8 mC from equation (2)

$$Q = C_{sp} \times \Delta E \times m \quad (2)$$

where Q is the charge stored by the bioelectrode, C_{sp} the specific capacitance, ΔE the potential window and m the mass of the electrode. Coulombic efficiency (CE) can also be calculated from charge–discharge measurements by using equation (3)

$$CE = \frac{t_d}{t_c} \times 100 \quad (3)$$

where t_d and t_c are the discharging and charging times, respectively. From the calculation, it was observed that 89% CE was achieved for GOx/CFE which is higher than that of the bare CFE, where it was found to be 75% only. Figure 6a relates CE of the CFE and GOx/CFE with the cycle number. The CE becomes constant after the second and fourth cycles in the CFE and GOx/CFE, respectively.

The loss in electrostability (LES) was also measured from the difference in voltammetric charge between the first and last cycle (equation 4).

$$LES = \frac{\Delta Q}{Q} \times 100 \quad (4)$$

where ΔQ is the difference between charge of the first and last cycle and Q is the charge of the first cycle stored in the electrode. From the calculated value, it is found that there is no LES for 500 cycles. However, in order to evaluate the capability of the electrode for practical applications, the electrochemical performance of the GOx/CFE is further measured under multiple cycles. Figure 6b shows the cycle life performance of the CFE and GOx/CFE indicating 88% of capacitance retention after 500 cycles. This capacity retention is mainly attributed to the active sites of GOx which is incorporated in CF through a 3D network structure of the GOx decorated CFE.

3.6 Impedance measurements

The impedance measurement was carried out to investigate the resistivity of the bare CFE and GOx/CFE in the electrolyte of PBS (pH = 7.0) which is shown in figure 7. As shown in the Nyquist plots of figure, the incorporation of GOx decreases the solution resistance from 25 to 10 Ω. This reduction in the internal resistance of the electrolyte results in improving the transportation and collection of electrons within the electrode. Also, charge transfer resistance between the electrode and the electrolyte is reduced. In a low-frequency region, the fall of the nearly vertical straight line of GOx/CFE indicates capacitance applications. The slope of 45° is called Warburg impedance and is the result of frequency dependence of diffusion in the electrode structure. Nyquist plots were analysed by fitting the equivalent circuit model.

Figure 7. EIS response of the (i) bare CFE and (ii) GOx/CFE and their corresponding equivalent circuit fit.

Figure 8. (a) XRD pattern and (b) SEM morphology of GOx/CFE after 500 charge–discharge cycles.

3.7 Post-mortem analysis

The GOx/CFE was characterized after 500 charge–discharge cycles using XRD and SEM in order to know the stability of GOx in the matrix and are shown in figure 8. From figure 8a, XRD measurement shows the distinctive peaks of GOx around 32 and 35°. It is observed that the matrix is able to retain GOx even after 500 charge–discharge cycles. SEM images of GOx incorporated carbon felt also show the presence of GOx after 500 charge–discharge cycles. From figure 8b, one can clearly observe the presence of GOx in the system. As a result, it can clearly be understood that the electrode is more stable in terms of retaining GOx on its surface.

4. Conclusion

The present work demonstrated the fabrication of a biocapacitor with the incorporation of GOx into CF through a 3D network structure as a green, eco-friendly energy storage system. The bioelectrode was characterized with several characterization techniques and electroanalytical tools. From them, it is observed that the electrode is incorporated with GOx and possesses higher capacitance values compared to the pristine CFE. The present fabrication method may open a new avenue for manufacturing a simple green electronic energy storage device with high performance. This proposed surface treatment makes a dramatic improvement in capacitor applications. Furthermore, in addition to the improved electrochemical performance, this method brings additional advantage of green energy storage.

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