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Carbonised *Typha* tassel-modified enzymatic electrodes for ferrocene-mediated glucose biosensor and glucose/air biofuel cell applications

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ABSTRACT

This study demonstrates the application of carbonised *Typha* tassel (CTT) in ferrocene-mediated enzymatic glucose biosensing and enzymatic biofuel cell (EnBFC) applications. *Typha* tassel was carbonised under an inert atmosphere to obtain conductive CTT which was then mixed with an effective electron transfer mediator, ferrocene (Fc) obtaining a redox-active electrode material. The successful immobilisation of the glucose oxidase (GOx) enzyme was performed on a CTT-Fc modified screen-printed electrode followed by a chitosan protective coating. The resulting enzymatic electrode was electrochemically characterised as a glucose biosensor with a working range of 0–10 mM and LOD and LOQ values of 0.19 mM and 0.56 mM, respectively. The developed glucose biosensor also showed good reproducibility and reusability with RSD% values of 6.68 % and 8.75 %, respectively. Furthermore, a real sample demonstration was performed using commercial jam samples with good recovery values. Finally, an EnBFC demonstration was performed using the enzymatic biosensor as an anode and a non-enzymatic cathode prepared using platinum black on gas diffusion carbon electrodes reaching a maximum power density of 3.6 μW cm^{-2}. This study shows the promise of CTT as an alternative to conventional materials in enzymatic biosensor and bioelectronic applications as a suitable, cheap, and sustainable material.

1. Introduction

Biomass to bioelectronics can be defined as using biomass-derived carbonaceous materials (BCMs) in bioelectronic applications such as biosensors and fuel cells. Approximately 100 billion metric tons of biomass waste are generated globally causing environmental pollution [1]. Hence, transforming waste or unwanted biomass into carbon-based useful products can be very promising as an environmentally friendly approach. BCMs can be a cheap and sustainable alternative to conventional carbon materials, especially for single-use applications. Considering the notable effort to reach the sustainability goals we all desire for our planet, electrode material development using biomass waste can certainly contribute to this vision.

The most widely used materials in fabricating electrochemical devices are usually expensive and complicated to produce such as nanotubes, graphene, and their derivatives [2,3]. Although their performance is good, it can be costly for single-use applications such as glucose testing strips. Therefore, research has been moved towards exploring different carbonaceous materials that can be produced using sustainable and relatively cheaper methods [4–6]. BCMs have several more advantages as electrode materials such as structural stability, electrical conductivity, and high surface area, therefore, they have been utilised in many applications [7–9]. Moreover, the properties of the BCMs were reported to enhance the electrochemical activity hence becoming important in electrochemical applications such as batteries [10], supercapacitors [11], and electrochemical sensors [12].

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Several studies have demonstrated BCM as enzyme immobilisation support due to its highly porous structure and large surface area [13–15]. Recently, our group has been developing strategies to implement BCMs in bioelectronic applications. Initial studies showed that enzymatic glucose oxidation can be achieved using simple electrode configurations prepared with carbonised *Typha* tassel (CTT) [16]. More complex electrode architectures were then explored using CTT and carbonised pussy willow for a comprehensive understanding of the different enzyme immobilisation strategies on the performance of BCMs-based enzymatic electrodes [17]. These studies paved the way towards using BCMs as electrode materials for enzymatic biosensors and bioelectronics. Using *Typha*, a fast-growing and short-lived plant, could be useful for different applications that provide a cheap and efficient alternative as an electrode material.

Herein, we report the performance of a CTT-modified enzymatic electrode as a glucose biosensor and an anode for a glucose/air enzymatic biofuel cell (EnBFC). Glucose oxidase was immobilised on ferrocene-modified CTT screen-printed electrodes using a simple and highly effective strategy. Chitosan (Chit) was used as a protective layer on top of the enzymatic electrode because of its biocompatibility and suitability for enzyme immobilisation [18]. The fabricated enzymatic electrode was first used as a glucose biosensor and validated using cherry, strawberry, and peach jam samples. Finally, it was demonstrated as an EnBFC anode coupled with a gas diffusion electrode (GDE)-based platinum black (PtB) cathode. This study is the first comprehensive demonstration of the use of CTT in biosensing and biofuel cell applications. The highly stable biosensor performance using CTT shows great promise for different enzyme-based biosensors and opens opportunities for its use in other bioelectronic applications.

2. Experimental

2.1. Materials

All chemicals were obtained at analytical grade from Sigma-Aldrich or Merck and used as received.

2.2. Preparation of enzymatic electrodes

Electrochemical cleaning of screen-printed electrodes (SPEs, Methrohm Dropsens, Switzerland) was performed before any modification to remove the impurities on the electrode surface. The SPEs were stored at room temperature in a dry place when not in use. First, 0.1 M KCl containing 0.1 M phosphate buffer solution (PBS, pH 7.4) was dropped on the electrode and linear sweep voltammetry (LSV, Ivium Potentiostat/Galvanostat, Netherlands) was performed between 0 and −2 V (Ag/Ag^{+}) at 20 mV s^{-1} scan rate until stable voltammograms were obtained (Fig. 1(a)). The cleaned SPEs were washed with ultra-pure water (UPW, MP Minipure, 18.2 MΩ-cm) and dried at room temperature before use.

CTT was synthesised as previously reported [17,19]. Briefly, *Typha* tassel was placed in a tube furnace at a heating ramp of 10 °C min^{-1} until reaching 1000 °C for 1 h under nitrogen. After the carbonisation, CTT dispersions were prepared in dimethylformamide (DMF) containing different amounts of ferrocene (Fc) and 10 mg mL^{-1} CTT, followed by 3 h of sonication. The dispersions were stored in a sealed amber vial at room temperature when not in use and sonicated for 15 min before experiments. Enzyme immobilisation was performed as illustrated in Fig. 1(b). 6 μL of 10 mg mL^{-1} CTT containing Fc was drop-coated on SPEs (1 mg cm^{-2}) to obtain a redox-active carbonaceous layer on the

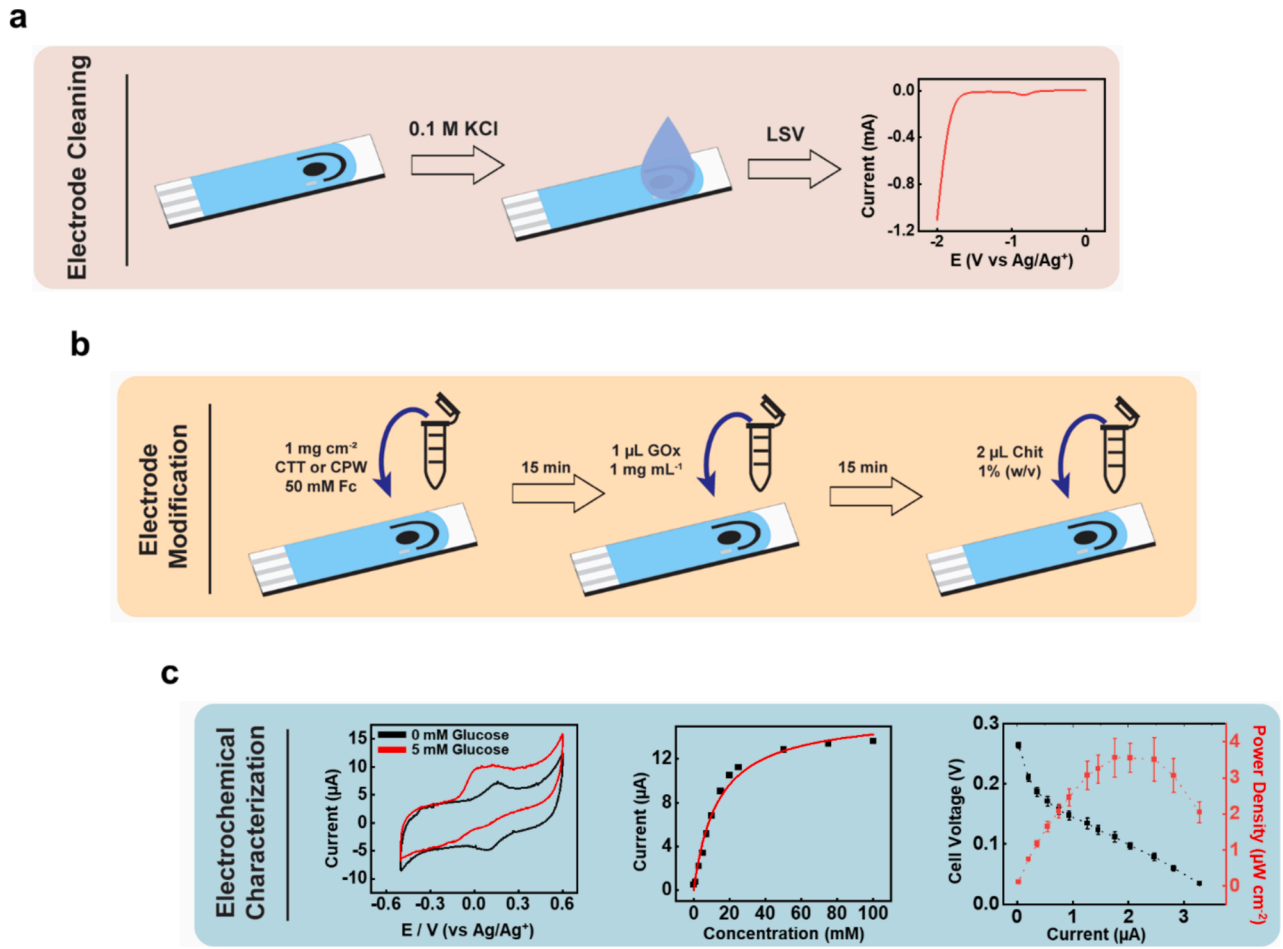

Fig. 1. Schematic representation of (a) electrode cleaning (b) electrode modification, and (c) electrochemical characterisation steps.

electrode and dried for 15 min. 1 μL of GOx enzyme was then drop-coated on the SPE/CTT-Fc and 2 μL of 1 % (w/v) Chit was added on top of the electrode as a protective layer to complete the enzyme immobilisation procedure (SPE/CTT-Fc/GOx/Chit). A control electrode was also prepared without CTT to demonstrate the effect of CTT on the performance of the enzymatic electrode (SPE/Fc/GOx/Chit). All prepared electrodes were kept at 4 °C for 24 h and soaked in 0.1 M PBS (pH 7.4) to stabilise the electrodes before use.

2.3. Electrochemical characterisation

SPEs were used in all electrochemical experiments with a working electrode surface area of 0.059 cm^2. The working and counter electrodes were carbon-based, and the reference electrode was silver (~74 mV vs SHE, data is obtained from the manufacturer). All electrochemical experiments were performed at 23 ± 2 °C using four independently prepared electrodes (N = 4) unless otherwise stated. The modified electrodes were electrochemically characterised using cyclic voltammetry (CV) and chronoamperometry (CA) (Fig. 1(c)).

The preparation steps of the enzymatic electrodes were performed using different enzyme concentrations, Fc concentrations, and applied potential for CA experiments. GOx concentrations of 1, 10, 20, and 40 mg mL^{-1}, Fc concentrations of 25, 50, and 75 mM, and applied potentials of 0.05, 0.1, and 0.3 V (vs Ag/Ag^+) were tested. The calibration curve was obtained using CA applied on the enzymatic electrodes prepared with the best-performing conditions. The limit of detection (LOD) was calculated as 3.3σ/Slope and the limit of quantification (LOQ) was calculated as 10σ/Slope as reported elsewhere [20].

2.4. Reproducibility, reusability, shelf-life, interference effects, and real sample analysis

Reproducibility, shelf-life, reusability, interference effects, and real sample tests were determined for biosensor validation (N = 4 samples). The reproducibility of the enzymatic electrodes was tested on 10 different electrodes prepared individually. The reusability of the electrodes was tested on the same electrode 25 times. The shelf-life of the electrodes was determined using individually prepared electrodes tested on different days for a month (tests performed on the 1st, 7th, 14th, and 30th days). The biosensor was washed with PBS and kept at + 4 °C until the next test. The effect of the interfering substances such as insulin (0.15 nM), uric acid (0.15 mM), and ascorbic acid (0.45 μM) and the mixture containing 5 mM glucose, insulin (0.15 nM), uric acid (0.15 mM), and ascorbic acid (0.45 mM) was determined by evaluating the change in the CA current values.

The real sample analysis was performed to demonstrate the potential of the prepared enzymatic electrodes using commercially available jams. For this purpose, cherry, strawberry, and peach jams were tested for their glucose content and they were prepared using a standard method according to the literature [17]. Briefly, 10 g of each jam sample was diluted with 100 mL of PBS (pH 7.4) and mixed using a magnetic stirrer. Then, diluted samples were centrifuged at 4000 rpm for 6 min and the supernatant was filtered using a 0.45 μm filter. The stock solution was kept in suitable glass vials and diluted as appropriate for real sample analysis.

2.5. EnBFC preparation and characterisation

The enzymatic electrodes using GOx were used as an anode for the EnBFC experiments. A non-enzymatic cathode was prepared using GDE (1 × 1 cm, Freudenberg, Weinheim, Germany) by drop coating and drying 15 μL of PtB dispersed in DMF at room temperature (GDE/PtB). The electrochemical characterisation of the cathode was tested in nitrogen and air-saturated solutions of 0.1 M PBS (pH 7.4) using LSV between 0.7–0.0 V (vs Ag/Ag^+) at 20 mV s^{-1}. The anode (SPE/CTT-Fc/GOx/Chit) and the cathode (GDE/PtB) were put together in a Perspex® cell with a solution volume of ca 2 mL. The polarisation experiments were performed using a resistor box (range: 1–10 MΩ) to apply external load and the output stabilised voltage was recorded with a multimeter. The current and the power of the EnBFC were calculated using Ohm's Law (V = I × R and P = I × V, respectively) (Fig. 1(c)). All EnBFC experiments were performed at 23 ± 2 °C using four independently prepared electrodes (N = 4 samples). All prepared electrodes were kept at 4 °C for 24 h and soaked in 0.1 M PBS (pH 7.4) before any tests to stabilise the electrodes.

3. Results and Discussion

3.1. Electrochemical characterisation

The enzymatic electrodes prepared using CTT-Fc can be a suitable substrate for enzyme immobilisation due to its polycrystalline carbonaceous structure supported by graphite-like interconnections. Such structural properties of CTT have been shown in the literature demonstrating the highly carbonised content confirming the successful carbonisation of the biomass [17]. GOx was successfully immobilised on the electrodes modified with CTT-Fc using a drop-casting technique owing to its highly carbonaceous structure, a layer of Chit was also applied as a protective layer to increase enzyme stability.

Glucose can be enzymatically oxidised using GOx and the produced electrons can be mediated to the electrode using Fc. Fig. 2(a) shows the possible electrochemical route for the enzymatic oxidation reaction using the SPE/CTT-Fc/GOx/Chit electrode. The successful electron transfer mechanism was confirmed using CV when 5 mM glucose was added to PBS as shown in Fig. 2(b). When no glucose is present in the solution two reversible peaks corresponding to the oxidation and reduction of Fc are observed with an I_{pa}/I_{pc} value of ca. 1.45 and a peak separation of ca. 60 mV. This suggests that a chemically quasi-reversible and electrochemically reversible reaction occurred at the electrode with the Fc mediator [21]. This quasi-reversible chemical reaction is observed due to a higher anodic peak current indicating that not all oxidised Fc can be reduced. This might be because of the mass transfer limitations inside the CTT-Fc composite. An increase in the oxidation peak and disappearance of the reduction peak was observed upon 5 mM glucose addition. This is the typical response of fast electron transfer kinetics due to the glucose-driven enzymatic reaction [22]. Therefore, successful co-immobilisation of Fc and GOx has been achieved using CTT and Chit as the conductive and protective layers, respectively.

Different enzyme loadings were tested using the SPE/CTT-Fc/GOx/Chit electrode. Fig. 2(c) shows the calibration curves obtained from the CA experiments conducted in 0.1 PBS (pH 7.4) while the current data was collected over 120 s for each glucose concentration. Although an increased sensitivity (from 0.499 μA mM^{-1} to 0.623 μA mM^{-1}) was observed with the increasing enzyme loading, the best-performing enzyme loading was chosen as 20 mg mL^{-1} because the sensitivity didn't increase significantly when twice the enzyme load was used (from 0.499 μA mM^{-1} to 0.609 μA mM^{-1}). Then, different Fc concentrations were tested using 25, 50, and 75 mM Fc-containing dispersions of CTT-Fc coated on SPEs when an enzyme concentration of 20 mg mL^{-1} was used. It was noted that while there is a slight increase in the current values between the 25 and 50 mM Fc concentrations in the performance of the enzymatic electrode, a remarkable difference was spotted when 75 mM Fc was used (Fig. 2(d)). Further experimental investigations regarding this behaviour later revealed that the electrochemical reversibility of the electrodes was significantly changed when 75 mM Fc was used (Fig. S1). Increasing peak separation with increasing concentrations of Fc suggests electrochemical irreversibility which might indicate sluggish electron transfer reaction for Fc due to the high barrier to electron transfer. The best electrochemical reversibility was seen for 25 mM Fc (ΔE_p = 0.1 V).

Finally, different applied potentials for the CA experiments were tested using 20 mg mL^{-1} GOx and 25 mM Fc concentrations. The tested

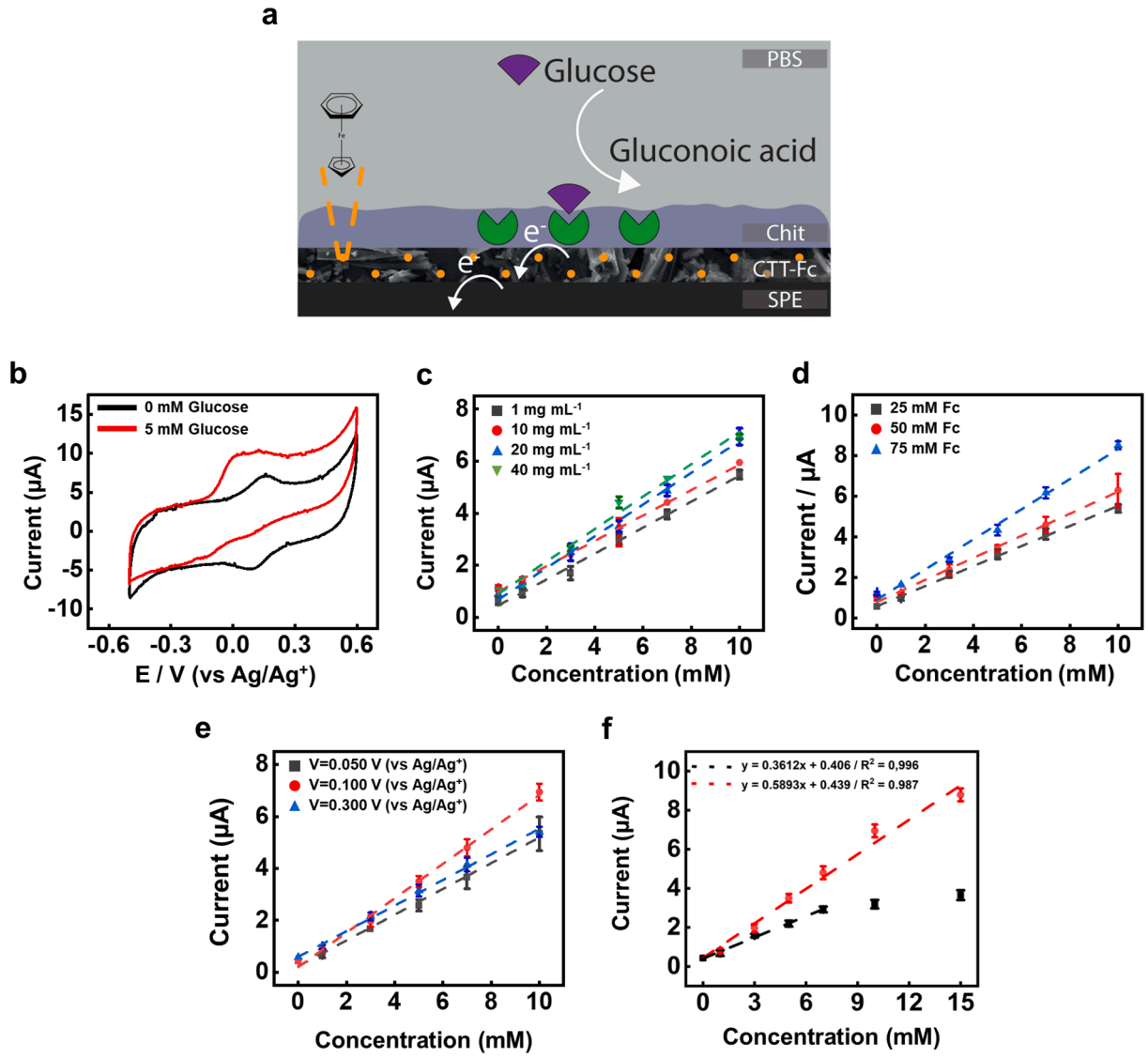

Fig. 2. (a) Schematic representation of the enzymatic glucose oxidation using SPE/CTT-Fc/GOx/Chit electrode (b) CVs (50 mV/s) showing the successful enzymatic glucose oxidation on SPE/CTT-Fc/GOx/Chit electrode (Fc: 50 mM) (c) the effect of enzyme loading (Fc: 50 mM) (d) the effect of Fc loading (e) the effect of applied voltage (Fc: 25 mM) (f) the effect of CTT using SPE/CTT-Fc/GOx/Chit (red) and SPE/Fc/GOx/Chit (black) electrodes (N = 4 samples, (Fc: 25 mM, applied voltage: 0.1 V vs Ag/Ag^+). All experiments were conducted in PBS (pH 7.4) containing different glucose concentrations. The experiments for choosing the best electrode modification conditions were performed as duplicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

values were determined based on the CV response as shown in Fig. 2**(b)**. The onset potential for the kinetic reaction set off ca. −0.15 V (vs Ag/Ag^+) and reaches near the maximum value ca. 0.03 V (vs Ag/Ag^+). Therefore, three different regions for the applied potential were used as 0.05, 0.1, and 0.3 V (vs Ag/Ag^+). Fig. 2(e) shows the calibration curves of the different applied voltages where 0.1 V (vs Ag/Ag^+) showed the best response. As a result, the final values of enzyme concentration, Fc concentration, and applied voltage were chosen as 20 mg mL^{-1}, 25 mM, and 0.1 V (vs Ag/Ag^+), respectively. A control experiment was also conducted using an electrode configuration without CTT modification where Fc was modified alone. Fig. 2(f) shows the calibration curves of the electrodes modified with and without CTT. The incorporation of CTT significantly increased the analytical performance of the enzymatic electrode with a linear response at a wider range of glucose (0–15 mM) than electrodes without CTT (0–7 mM). Furthermore, the sensitivity of the enzymatic electrode was increased by ca. 63 % demonstrating the superior performance of the CTT compared to the bare carbon electrode.

3.2. Electroanalytical performance of the enzymatic electrodes

The enzymatic electrodes were constructed using chosen conditions for glucose determination to demonstrate the potential of the CTT in biosensing applications. In enzymatic electroanalytical applications, the catalytic performance of the enzyme is important. Therefore, the electrochemical response of the enzymatic electrode was evaluated using different glucose concentrations. Fig. 3(a) shows the successful electrochemical oxidation of glucose. The Michaelis-Menten type of behaviour was obtained for the SPE/CTT-Fc/GOx/Chit electrode tested in PBS solution containing glucose up to 100 mM. The nonlinear regression analysis was used based on the Michaelis-Menten model to calculate the parameters of affinity (K_m) and maximum rate (V_{max}) as 13.46 ± 1.6 mM and 16.16 ± 0.64 µA, respectively.

The calibration curve is shown in Fig. 3(b) with a linear range between 0–10 mM glucose with a sensitivity value of 0.6797 µA mM^{-1}. The LOD and LOQ of the biosensor were calculated as 0.19 mM and 0.56 mM, respectively. The values of the analytical performance parameters

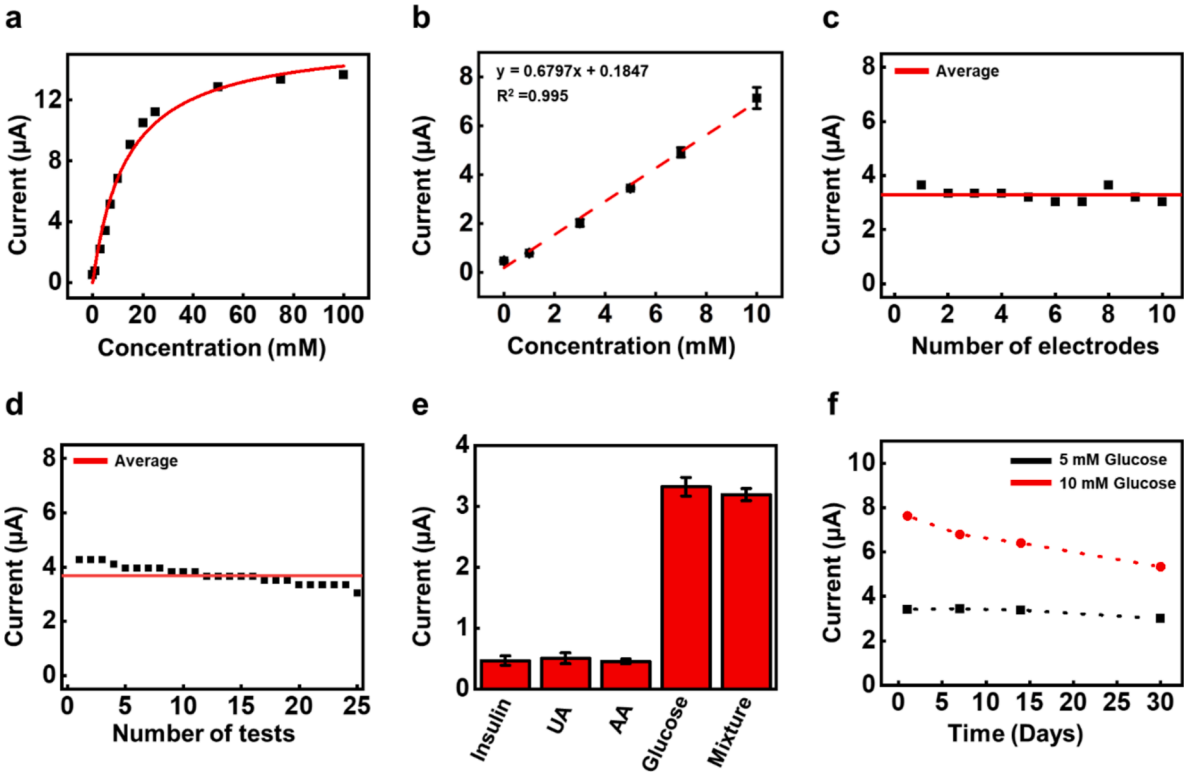

Fig. 3. (a) The current response of SPE/CTT-Fc/GOx/Chit electrode demonstrating Michaelis-Menten type kinetics (b) the calibration curve of the biosensor (c) reproducibility of the biosensor (d) reusability of the biosensor (e) the response of interfering substances compared to glucose response (f) the stability of the biosensor. All experiments were conducted in PBS (pH 7.4), and the calibration curve was obtained using 10 individual samples (N = 10).

were also summarised in Table 1. These analytical performance results indicated that the developed biosensor using CTT can be utilised in many applications such as glucose determination in food samples. However, other parameters are also important to assess the performance of the biosensor such as the reproducibility, reusability, shelf-life, response to possible interfering substances, and the performance in a real sample analysis.

The reproducibility of the biosensor was shown by testing 10 individual electrodes to detect 5 mM glucose in PBS (pH 7.4) (Fig. 3(c)). A good reproducibility was achieved with an RSD % of 6.68 % for the SPE/CTT-Fc/GOx/Chit electrode. Furthermore, the biosensor was tested 25 times for 5 mM glucose with an RSD % value of 8.75 % indicating that it can be reused multiple times (Fig. 3(d)). Some of the interfering substances that might be present in blood and food samples such as insulin (0.15 nM), uric acid (0.15 mM), and ascorbic acid (0.45 mM) were also tested to demonstrate the selectivity of enzymatic biosensors towards glucose. Fig. 3(e) shows the current responses of interfering substances separately and as a mixture with 5 mM glucose indicating good selectivity towards glucose. The shelf-life of the biosensor was also tested using 5 and 10 mM glucose detection tests over 30 days (Fig. 3(f)). The relative change % was calculated after 30 days as 12.33 % and 30.12 % for 5 and 10 mM glucose, respectively. It shows that under average glucose concentration of the detection range of the biosensor (5 mM), the current didn't significantly change, however, high glucose concentrations had a more adverse effect on the biosensor performance.

To demonstrate the use of the developed biosensor, a food sample analysis was preferred based on its simplicity compared to clinical samples. Different jam samples were obtained from a commercial company (KOSKA) in Turkey to test the glucose concentrations. Three different glucose additions were performed, and the glucose amount was calculated using the calibration curve. The recovery values with the diluted jam samples showed good recovery values for the biosensor (Table S1). Therefore, the developed biosensor could be used in glucose determination in jam sample analysis.

Table 1
Analytical performance parameters of the biosensor.

Analytical parameters	Values
Linearity range	0–10 mM
Regression equation, *i* (μA) and [Glucose] (mM)	$i = 0.6797[\text{Glucose}] + 0.1847$
Standard error of the slope, ±	0.0243
Standard error of the intercept, ±	0.1347
R^2	0.995
Sensitivity (μA mM^{-1})	0.6797
LOD, mM	0.19
LOQ, mM	0.56

3.3. EnBFC characterisation

Fuel cell tests were conducted using SPE/CTT-Fc/GOx/Chit and GDE/PtB as anode and cathode, respectively, to demonstrate the use of CTT in fuel cell applications. The schematic representation of the fuel cell set-up and the reactions involved is summarised in Fig. 4(a). The cathode material was chosen as PtB to maintain stable performance as Pt can be considered a reference material for the oxygen reduction reaction. Furthermore, GDEs were also used to maintain stable oxygen for the cathode reaction.

Fig. 4(b) shows the cathode response in nitrogen and air-saturated PBS (pH 7.4) to demonstrate the oxygen reduction performance. The catalytic cathode response was increased by ca. 5-fold when oxygen was present in the solution. The fuel cell polarisation curve can be seen in Fig. 4(c) along with the I-V curve. The results demonstrate the generic fuel cell polarisation behaviour with an open circuit potential (OCP) of 0.218 V and a maximum power density of 3.6 μW cm^{-2}. The decrease in the low and mid-current regions indicates that power is controlled by activation and ohmic losses. This could be due to the simple fuel cell

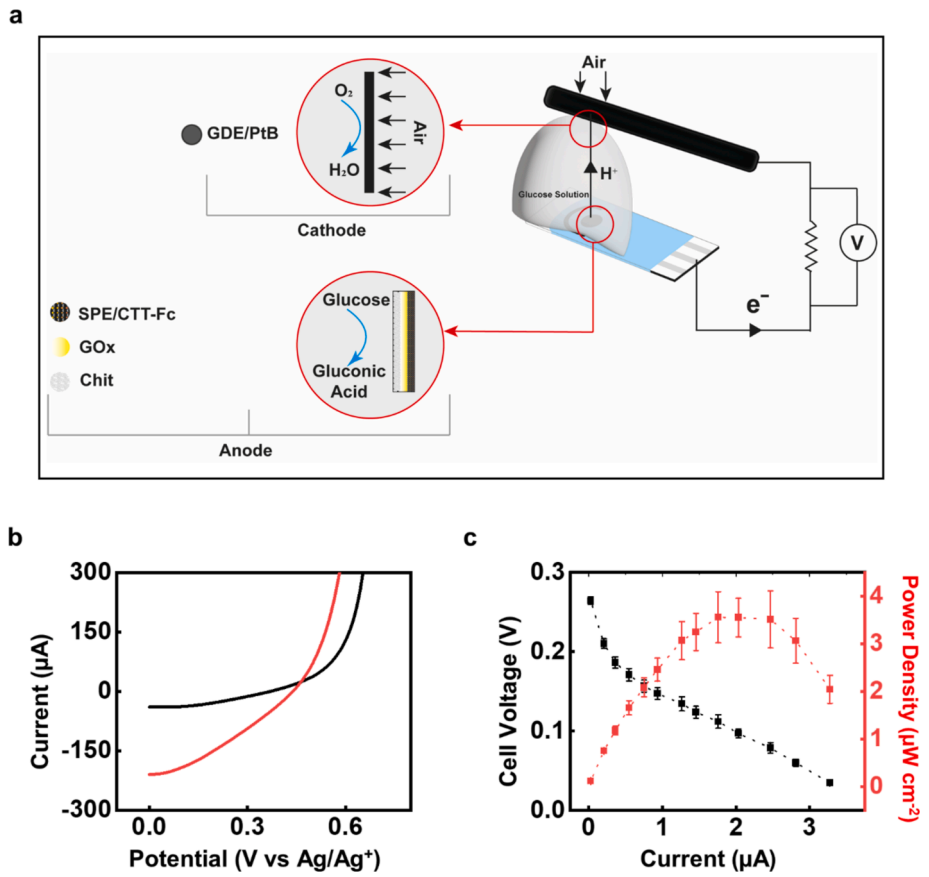

Fig. 4. (a) The schematic representation of the fuel cell set-up and the simplified reactions on electrodes (b) the LSV response of the GDE/PtB cathode in nitrogen and air-saturated PBS (pH 7.4) (c) EnBFC polarisation and I-V curves obtained in PBS 7.4 containing 5 mM glucose using SPE/CTT-Fc/GOx/Chit and GDE/PtB as anode and cathode, respectively.

design and the anode configuration as it wasn't optimised for EnBFC applications. This could be avoided by further optimising the anode configuration and the design of the fuel cell to reduce the internal resistance of the fuel cell. As a result, it was demonstrated that CTT-modified enzymatic electrodes can be used in glucose/air EnBFC applications.

Although BCMs are promising for enzymatic applications, their use in biosensors and bioelectronics is limited especially for glucose detection. One of the notable studies, GOx is immobilised on hierarchical nanoporous carbon using *Cinnamomum platyphyllum* leaves [23]. The hierarchical nanoporous nature of the carbonaceous provides direct electron transfer from the enzyme. The biosensors showed a LOD value of 0.19 mM with a linear response concentration up to 1 mM. Although promising results were obtained for direct electron transfer, the biosensor is limited to applications containing low amounts of glucose. In a recent study, dried kenaf stem was carbonised to construct a glucose biosensor with a linear range from 0.58 μM to 16 mM with a LOD of 0.19 μM [24]. The 3D porous structure of the electrode provided an effective loading of a large number of GOx molecules leading to enhanced performance. These studies demonstrate a slow but strong improvement in developing enzymatic biosensors and bioelectronics using BCMs.

4. Conclusion

In conclusion, this study successfully demonstrated the potential of CTT as a sustainable and effective electrode material for bioelectronic applications, specifically in enzymatic glucose biosensing and EnBFC anodes. The simple and efficient immobilisation strategy using Fc-modified CTT and glucose oxidase, with chitosan as a protective layer, resulted in highly stable and reproducible enzymatic electrodes. The enzymatic electrodes showed promising results in glucose detection, with excellent reproducibility, reusability, and resistance to interference. Furthermore, their application in glucose/air EnBFCs as anodes showcased the versatility of CTT-based materials in bioelectronic devices. Our work also has several limitations such as further investigation for clinical samples, more work on continuous sensor operation, and design optimisation to increase the power output of the fuel cell. Using CTT, derived from a fast-growing and abundant biomass source, highlights the potential for BCMs to replace conventional, expensive carbon materials in single or multi-use applications, paving the way for more sustainable and cost-effective bioelectronic technologies.

CRediT authorship contribution statement

Şevki Furkan Küçükayar: Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Şevval Kaya:** Writing – review & editing, Methodology, Investigation. **Veli Şimşek:** Supervision, Project administration, Investigation. **Mustafa Oguzhan Caglayan:** Supervision, Methodology, Conceptualization. **Zafer Üstündağ:** Writing – review & editing, Resources, Methodology, Conceptualization. **Samet Şahin:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Samet Sahin reports financial support was provided by Bilecik Seyh Edebali University. If there are other authors, they declare that they

have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Mustafa Oguzhan Caglayan, Zafer Üstündağ and Samet Şahin are co-founders of an R&D company, CSense Chemistry Inc., that develops technologies for screen-printed electrodes including biomass-derived inks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2024.112213.

Data availability

Data will be made available on request.

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