

Cellulose Nanofiber Platform for Electrochemical Sensor Device: Impedance Measurement Characterization and Its Application for Ethanol Gas Sensor

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ABSTRACT

Bioplastics such as cellulose nanofibers (CNF) are widely used in daily life as an alternative to plastics. CNF is also considered a biodegradable, readily disposable, and low-cost material, making it suitable as a sensor platform. The mechanically treated CNF has both hydrophobic and hydrophilic properties where the hydrophobic sides bond strongly with MWCNTs to provide conductivity on the CNF sheet, while the hydrophilic sides allow the target component to be adsorbed on its laminated structure and react with the electrode surface. From an electrochemical measurement of ferricyanide solution indicated that the MWCNTs modified CNF could act well as electrode. Furthermore, the thin-sheet electrode successfully detected ethanol based on a direct electron transfer (DET) between PQQ-ADH and the MWCNTs on the electrode surface. Aside from its lightweight properties, the developed CNF electrode possesses excellent stretchability, flexibility, and adjustability to human skin, making it ideal for a future wearable skin gas sensor.

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1. INTRODUCTION

Polymeric matrices and plastics in general have been widely used as the electrode material for sensor devices. Plastic materials are mainly used for their easy manufacturing process and resistance to corrosion making them can be used for long time. However, excessive employment of plastic creates new problem of microplastic waste pollution which is a global problem and dangerous if not handled properly [1]. Not only bad for environment, but health impacts resulting from the bioaccumulation and biomagnification of microplastics inside the human body can lead to serious problems such as skin irritation, respiratory problems, digestive problems, reproductive problems, and even cancer [2]. Bioplastics such as cellulose nanofibers (CNF) are environmentally friendly plastics derived from plants that do not pollute the environment as an alternative to replace plastics [3]. CNF has been widely used in a variety of applications to support life, including as a plastic substitute for food packaging [4], pharmaceutical and biomedical applications [5], as well as a promising material for electrode platform [6].

CNF as electrode framework is important as they have good thermal and chemical stability [7, 8]. CNF is also biodegradable and readily disposable, making the electrode low cost and suitable for mass production [9]. Since CNF is a material without conductivity, the sheet must be modified with other materials that have high conductivity such as multi-walled carbon nanotubes (MWCNTs), to be able to be used as an electrode for electrochemical sensor. Moreover, MWCNTs are considered as promising conductive material, with extremely high electrical conductivity of $8 \times 10^8 \text{ S cm}^{-1}$, and easily to be mass produced through several manufacturing methods [10]. CNTs have a well-defined 1-D physical structure that contributes to their high flexibility and their ability to create long, continuous electronic conductive paths [11]. Additionally, CNF can bind strongly with carbons that are highly conductive including carbon nanotubes or graphene [12]. This makes it an ideal candidate for flexible electrodes.

The increase in wearable, handheld and portable consumer electronics has attracted researchers to the field of flexible sensor devices. Among various applications, flexible devices are becoming an emerging area of critical importance for the development of wearable gas sensors since human body emits various biological gasses through skin gas and exhaled gas that can be used for the early detection of diseases [13-15]. Non-invasive health sensors using skin gas are preferred since the patient will not feel uncomfortable with how the samples are taken from the body [14, 16, 17]. However, since the gas excreted from the skin is a mixed gas of various kinds and the concentration is extremely low at the ppm (parts per million) to ppb (parts per billion) level [18, 19], it is extremely difficult to detect a single gas with high sensitivity. Hence, enzyme electrodes are preferable due to the low concentration of the emitted skin gas [13, 14]. Skin gas production also strongly depends on the individual making it difficult to monitor the disease without continuous and constant measurement. For such analysis and health monitoring, it is necessary to develop skin gas detection device having the best balance between cost and detection sensitivity. Detection of alcohol vapor is one of the most desired daily necessities for human. The range of 74–112 ppb min⁻¹ are designated for individual with drinking problem and range as low as 4–80 ppb min⁻¹ for healthy individual [13, 14, 20].

The present technology used for skin gas measurements are still considered not portable, requires high temperature operation, and has low detection characteristics. Other tools available with better sensitivity are very inefficient in terms of size and cost. Electrochemical method is preferable for gas detection among others since they offer great sensitivity and low power consumption [21]. The previously developed CNF sheet for enzymatic fuel cell [22] was able to retain the target substrate enabling long-term power generation. Furthermore, specific enzyme reacted with the target substrate was adsorbed into the electrode. In this study, a wearable electrochemical enzymatic sensing device is developed utilizing CNF thin sheet modified with MWCNTs utilizing the reaction of dehydrogenase enzyme to target ethanol.

2. MATERIAL AND METHODS

2.1 Materials

Nanoforest-S BB made from bamboo using the aqueous counter collision (ACC) method was used as the cellulose nanofiber (CNF) and was kindly supplied by Chuetsu Pulp and Paper Co., Ltd. (Tokyo, Japan). The ACC-treated 2.5 wt% CNF has both surface properties of hydrophobic and hydrophilic [23, 24]. Pyrroquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) was kindly gifted by Prof. Osamu Shirai of Kyoto University (Kyoto, Japan). MWCNTs (purity: ≥98%, length: 3–6 μm, outer diameter: 10 nm ± 1 nm, inner diameter: 4.5 nm ± 0.5 nm) were purchased from Sigma Aldrich (St. Louis, MO, USA). Potassium hexacyanoferrate (III) (K₃[Fe(CN)₆]) was obtained from Fujifilm Wako Pure Chemical Industries (Osaka, Japan). Dimethylacetamide (DMAc), sodium acetate (CH₃COONa), acetic acid (CH₃COOH) and calcium chloride (CaCl₂) were purchased from Fujifilm Wako Pure Chemical. The pH of 0.1 M acetate buffer solution as supporting electrolyte was adjusted to 5.5 and supplemented with 2 mM CaCl₂ as it has been widely used for molecular biology applications. All solutions were prepared with deionized water (resistivity >18.2 MΩ cm) obtained using a Milli-Q water purification system (Millipore, Billerica, MA).

2.2 Fabrication of MWCNTs modified CNF sheet electrode

The CNF sheet was prepared by dispersing 1 mg of the 2.35 wt% CNF with 20 mL of Milli-Q water using an ultrasonic homogenizer for 20 min. Next, 10 mL of this CNF dispersion was spread on a teflon plate (4 cm × 10 cm) and heated at 140 °C on a hotplate until dry, as shown by figure 1. An electrical conductivity for the CNF sheet was given by casting a dispersed MWCNTs solution on its surface. The working electrode was then modified by drop-coating the PQQ-ADH enzyme on the electrode surface. For ethanol detection, 100 μL of 0.1 M ethanol was evaporated. All electrochemical measurements were done in atmospheric condition.

2.3 Instrumentations

Dispersed solutions were homogenized using ultrasonication probe-type BRANSON 5520 (Kanagawa, Japan). All potentials are reported with respect to Ag|AgCl|saturated KCl at 25 °C (+199 mV vs. the normal hydrogen electrode). Electrochemical measurements were conducted using an electrochemical analyser (model 660A, ALS Co., Ltd, Tokyo, Japan). pH was recorded using a pH meter (AUT-501, DKK-TOA Corp., Tokyo, Japan). Cyclic voltammetry (CV) measurements were performed using a three-electrode system included on the CNF electrode. All electrochemical measurements were done in atmospheric condition.

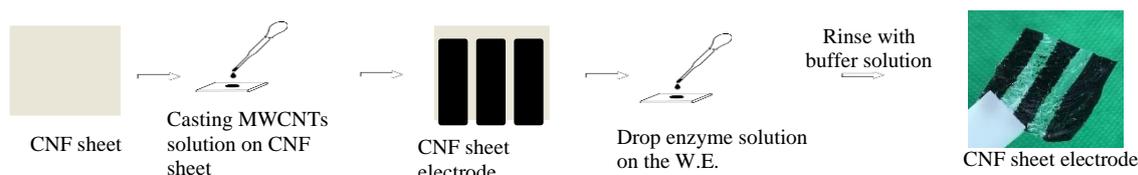


Figure 1 : Scheme of CNF electrode preparation.

3. RESULTS AND DISCUSSION

3.1 Electrochemical characteristic of MWCNTs modified CNF electrode

Figure 2 shows the SEM images of the surface and a cross section of the CNF sheet prior to modification with MWCNTs. The morphology of the surface area was relatively flat making it suitable for electrode modification. The morphology of cross-section area (Figure 2b) shows layered-like structure with nanometer-scale thickness. This layered structure is believed to have high conductivity in the horizontal dimension when the CNF sheet further modified with MWCNTs [22].

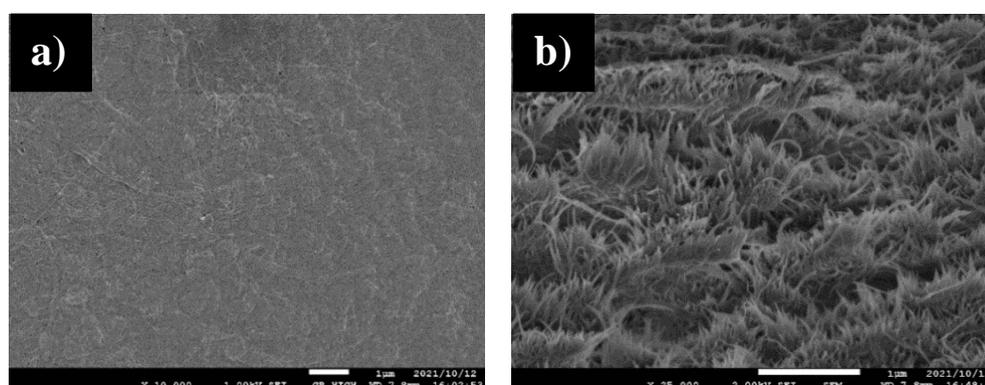


Figure 2 : SEM images of the surface (a) and cross section (b) of the CNF sheet.

To evaluate the electrochemical performance of modified CNF electrode, CV measurements were conducted by using $1 \text{ mmol dm}^{-3} \text{ Fe(CN)}_6^{3-}$ in $1 \text{ mol dm}^{-3} \text{ KCl}$ solution pH 7 (Figure 3). The oxidation and reduction peak potential of ferrocyanide was observed around $+0.46 \text{ V}$ and $+0.08 \text{ V}$ at potential sweep rate of 100 mVs^{-1} , respectively. The separation between the anodic and cathodic peak potentials was approximately 380 mV , resulting in a slow redox reaction. The separation of two peak potentials is considered as fast electron-transfer kinetics if it's equal to $\sim 60 \text{ mV}$ per electron [25]. Despite the slow reaction, the CNF conductive sheet is still able to transfer electrons and act as an electrode sufficiently. The redox reaction observed was diffusion-controlled process as plots of cathodic peak current (i_{pc}) versus the square root of the potential sweep rate is linear (Figure 4). The Randles-Sevcik equation (Eq. 1) was used to analyze the electrochemically active surface area (ECSA), where i_p is the peak current (A), v is scan rate (V s^{-1}), n is the number of electrons transferred in the redox event, D_0 ($\text{cm}^2 \text{ s}^{-1}$) is diffusion coefficient and C_0 (mol cm^{-3}) is the bulk concentration of the analyte. The value of D_0 used in this calculation was $1.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, referring to the previous work [22]. Meanwhile, the heterogeneous electron transfer rate (k°) was calculated by Eq. 2, where ψ is dimensionless rate parameter in CV, F is faraday constant $96,485 \text{ C mol}^{-1}$, R is gas constant $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$, and T is absolute temperature (K).

$$i_p = (2.69 \times 10^5) n^{3/2} D_0^{1/2} v^{1/2} C_0 A \quad (1)$$

$$\psi = k^\circ [\pi D n v F / (RT)]^{-1/2} \quad (2)$$

The modified CNF electrode had a smaller electrochemically active surface area (ECSA) of 0.03 cm^2 compared to the apparent electrode surface area of 0.28 cm^2 . On the other hand, the rate constant for surface redox-electrode reaction (k°) was estimated to be $4.98 \times 10^4 \text{ cm s}^{-1}$.

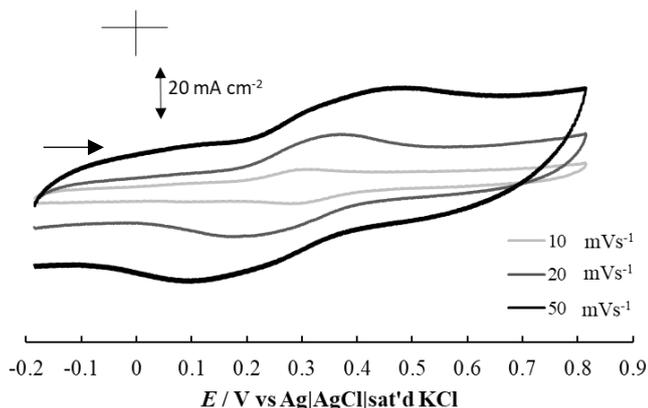


Figure 3: CV curves of $1 \text{ mmol dm}^{-3} \text{ Fe(CN)}_6^{3-}$ in $1 \text{ mol dm}^{-3} \text{ KCl}$ solution pH 7 recorded at modified CNF sheet electrode with various potential sweep rates.

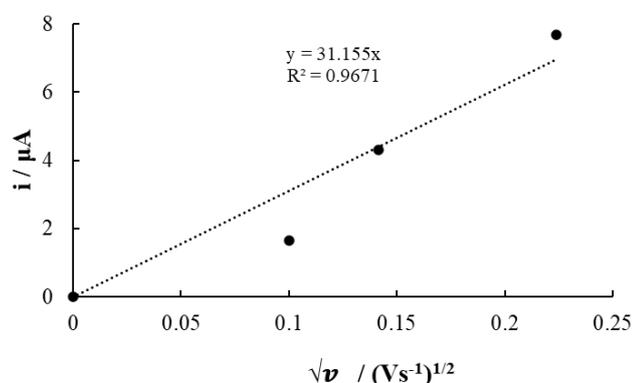


Figure 4: Plots of oxidation peak current versus the square root of potential sweep rate.

3.2 Ethanol detection

To detect the ethanol, a modified CNF sheet electrode was used where the working electrode had been modified by the PQQ-ADH enzyme. The target gas was generated by evaporating an ethanol solution. Figure 5 shows the CV results of ethanol detection at the PQQ-ADH enzyme/MWCNTs modified CNF electrode in 0.1 M acetate buffer pH 5.5 with 2 mM CaCl_2 at atmospheric condition. In the absence of alcohol, there was no catalytic oxidation current detected at the modified electrode. The oxidation current was then detected starting from -0.1 V (vs $\text{Ag}|\text{AgCl}|\text{saturated KCl}$) in the presence of ethanol. This result was in good agreement with previous works reported for the ethanol detection [26]. This proves that the physically adsorbed enzyme on the working electrode was active and able to transfer electrons as the ethanol was oxidized.

The reverse side of the CNF sheet directly faced the target component, allowing the target component to be adsorbed and react with the electrode surface due to the direct interaction between the hydrophilic component of the CNF and the ethanol. The laminated morphology of CNF sheet as shown on Figure 2 b will also increase the amount of target component adsorbed on its surface. The ethanol was then oxidized by the enzyme via direct electron transfer between the enzyme and the MWCNTs electrode surface.

Overall, the modified CNF electrode is capable to detect ethanol. The CNF is suitable for a future wearable sensor, as the CNF have bio-degradable, flexible, eco-friendly, disposable, and small size characteristics. However, the skin gas excreted from human body is a mixed gas of various kinds and in the extremely low concentration (ppb level), hence the detection range and limit detection for ethanol need to be established. It is expected that the modified CNF electrode can satisfy the required range of $75\text{--}112 \text{ ppb}$ for an individual with drinking problem. In addition, the real time of ethanol skin gas measurement is needed in order to confirm the sensor performance for daily life. The sensor is expected to allow remote healthcare monitoring in real time, assess individual health conditions, and obtain feedback from distant facilities.

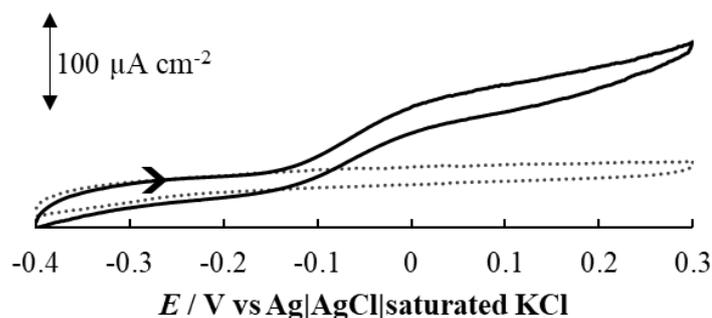


Figure 5: CV results at modified CNF sheet electrode in 0.1 M acetate buffer (pH 5.5) with 2 mM CaCl_2 in the presence (solid line) and absence (dashed line) of 0.1 M ethanol. Potential scanning rate: 10 mV s^{-1} .

The estimated ethanol gas concentration produced from 0.1 M ethanol solution was 344 ppm.

4. CONCLUSION

The fabricated CNF sheet possessed very thin and lightweight property with average thickness of ca. 25 μm , making the CNF sheet as promising framework for electrochemical sensor as the CNF can easily be adjustably attached human skin. The observation of redox reaction at MWCNTs modified CNF electrode using ferrocyanide showed that the modified CNF sheet proved to have enough electrochemical performance as an electrode. In addition, the PQQ-ADH modified CNF electrode was able to detect the ethanol evaporated from 0.1 M ethanol solution under atmospheric condition. Oxidation catalytic current was detected starting from -0.1 V vs Ag|AgCl|saturated KCl) which was based on the direct electron transfer between the enzyme and the electrode surface. Therefore, CNF sheet electrodes can be considered as a promising candidate for a future wearable sensor.

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