



Development of bimetal-grown multi-scale carbon micro-nanofibers as an immobilizing matrix for enzymes in biosensor applications



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ABSTRACT

This study describes the development of a novel bimetal (Fe and Cu)-grown hierarchical web of carbon micro-nanofiber-based electrode for biosensor applications, in particular to detect glucose in liquids. Carbon nanofibers (CNFs) are grown on activated carbon microfibers (ACFs) by chemical vapor deposition (CVD) using Cu and Fe as the metal catalysts. The transition metal-fiber composite is used as the working electrode of a biosensor applied to detect glucose in liquids. In such a bi-nanometal-grown multi-scale web of ACF/CNF, Cu nanoparticles adhere to the ACF-surface, whereas Fe nanoparticles used to catalyze the growth of nanofibers attach to the CNF tips. By ultrasonication, Fe nanoparticles are dislodged from the tips of the CNFs. Glucose oxidase (GOx) is subsequently immobilized on the tips by adsorption. The dispersion of Cu nanoparticles at the substrate surface results in increased conductivity, facilitating electron transfer from the glucose solution to the ACF surface during the enzymatic reaction with glucose. The prepared Cu-ACF/CNF/GOx electrode is characterized for various surface and physicochemical properties by different analytical techniques, including scanning electron microscopy (SEM), electron dispersive X-ray analysis (EDX), Fourier-transform infrared spectroscopy (FTIR), BET surface area analysis, and transmission electron microscopy (TEM). The electrochemical tests show that the prepared electrode has fast response current, electrochemical stability, and high electron transfer rate, corroborated by CV and calibration curves. The prepared transition metal-based carbon electrode in this study is cost-effective, simple to develop, and has a stable immobilization matrix for enzymes.

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1. Introduction

For more than two decades, amperometric biosensors using enzymes are an active area of research for the detection of glucose in human serum [1,2]. In such biosensors, the enzyme is directly immobilized onto the electrode. As a result, the amperometric output signal is amplified because of direct electron transfer between the redox center of the enzyme and the electrode. In this context, physically or chemically modified carbon nanotube (CNT)- and carbon nanofiber (CNF)-based electrodes have shown substantial improvements in their amperometric performance. A review of such CNT- and CNF-based biosensors can be found in the literature [3,4]. Some examples are the Pt nanoparticle-supported CNTs [5,6], CNTs dispersed in glassy carbon [7], and Pt nanoparticle-graphene nanocomposite [8]. However, these electrodes are complicated to fabricate and are

relatively expensive because of the noble metals, including gold and platinum, used in the fabrication. Herein, it may be mentioned that there are electrodes based on non-carbon materials also, including polymers and silica, prepared for glucose biosensors [9–11]. However, such electrodes are coated with expensive gold or platinum noble metals as well.

Activated carbon microfibers (ACFs) have been used as adsorbents and support for metal catalyst nanoparticles in several environmental remediation applications [12–16]. Recently, ACF has been used as a substrate to grow carbon nanofibers (CNFs) by catalytic chemical vapor deposition (CCVD) using a suitable transition metal (Ni/Fe/Cu). The multi-scale web of carbon micro and nanofibers (ACF/CNF) has been found to be a more efficient adsorbent than ACFs alone [17–21]. The ACF/CNF composite is also a good thermal and electrical conductor. Therefore, this material, which is the focus of the present study, can potentially be used as the working electrode of glucose biosensors.

In this study, the ACF/CNF composite is prepared using a bimetal of Cu and Fe instead of either metal alone. Fe nanoparticles catalyze the growth of CNFs by the tip-growth mechanism, whereas Cu nanoparticles remain dispersed in the ACF substrate. The hierarchical

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web of ACF/CNF is ultrasonicated to open the CNF tips by dislodging the Fe nanoparticles. Glucose oxidase (GOx) is immobilized on the tips by adsorption. With Cu nanoparticles dispersed in the substrate, the prepared composite material is shown to exhibit significantly high electron transfer rate during the enzymatic reaction with glucose in liquid and thus is a potential candidate for the working electrode of glucose biosensors. Thus, the bimetal has different roles in the detection of glucose. Fe-nanoparticles act as the CVD catalyst for growing CNFs. The grown nanofibers are used to immobilize GOx by adsorption. The CNFs also act as the electrode to directly transfer electrons during the enzymatic reaction with glucose. Cu-nanoparticles dispersed on the substrate ACF enhance the conductivity of the substrate, facilitating electron transfer from the glucose solution to the ACF surface.

2. Materials and method

The phenolic resin precursor based micron size-ACF was procured from Nippon Kynol Inc., Japan. The chemicals used for the impregnation of ACFs, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (purity >99%), $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (purity >99.9%), $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and the surfactant reagent, sodium dodecyl sulfate (SDS) (Purity >99%) were purchased from Merck, Germany. Different gases, hydrogen (purity >99.999%), nitrogen (purity >99.999%), and acetylene (C_2H_2) (purity >99.999%) were purchased from BOC Gases, India. GOx derived from *Aspergillus niger* (activity >300 U/mg) were purchased from Calbiochem, Germany. The high purity grades Bradford reagent and β -D-Glucose (>80%) were purchased from Sigma-Aldrich, Germany and TCI, Tokyo, respectively. O-dianisidine (>99%) and peroxidase (>250 U/mg) were purchased from Alfa Aesar, UK. The remaining reagents required for preparing buffer solutions, KH_2PO_4 , K_2HPO_4 , NaCl crystals, glacial acetic acid and sodium acetate, were purchased from Merck, India. All chemicals were prepared in de-ionized (DI) water.

2.1. Synthesis of bimetal ACF/CNF

Fig. 1 describes the steps involved in the preparation of the ACF/CNF-based working electrode for glucose biosensors. Fe-metal nanoparticles (shown in red) that are used to catalyze the CNF growth on the ACF substrate are on the CNF tips, whereas Cu-metal

nanoparticles (shown in black) are dispersed in the ACFs (Fig. 1a). The hierarchical web of ACF/CNF is ultrasonicated to open the CNF tips by dislodging the Fe nanoparticles (Fig. 1b). Next, glucose oxidase (GOx) (shown in yellow) is immobilized on the tips (Fig. 1c). The Cu nanoparticles, which remain adhered to the ACF-substrate, render the composite material more electrically conductive. As a result, the electron transfer rate of the composite material used as the counter electrode of the biosensor is higher during the enzymatic reaction with glucose in solution (Fig. 1d), as shown later in this manuscript.

2.1.1. Pretreatment of ACFs

The as-received samples of ACF were leached for 2 h in 0.03 M nitric acid at 80 °C to remove any undesirable ions, such as chloride, nitrate, and phosphate ions. After leaching, the ACFs were washed several times with DI water until the surface of the ACFs became neutral (pH ~7). Next, the ACFs were dried for 6 h in static air at room temperature (30 ± 5 °C), then for 12 h in an oven at 120 °C and for another 12 h in vacuum at 200 °C to remove any entrapped gases from the pores of ACFs.

2.1.2. Impregnation of ACF with metal nitrates

The impregnation of ACFs was conducted by the wet incipient method. The salts of Fe and Cu nitrates, used as the precursors of Fe and Cu metals in ACF, respectively, were dispersed in DI water. Based on previous results [20], the concentration of metallic salts in the solution was adjusted to 0.4 M for impregnating ACFs. At concentrations in excess of 0.4 M, the pores of ACFs were found to be plugged with the salts. SDS (0.3% w/w) was added to the impregnating solution to produce mono-dispersed nanoparticles of the metallic salts and to increase the transfer of the salts without agglomeration to the ACF surface.

The set-up for impregnation consisted of a glass tube (I.D. = 20 mm, L = 90 mm) mounted inside a cylindrical glass shell (I.D. = 35 mm, L = 13 cm). The inner tube was perforated. ACF was wrapped on it. One end of the tube was sealed. The impregnating salt solution was continuously delivered into the tube via a peristaltic pump (speed = 110 rpm). The solution flowed into the tube and then radially outward into the shell through the wrapped ACF. The impregnation was conducted for 6 h. Some samples of ACFs impregnated with Fe and Ni or Ni and Cu, were also prepared for comparison purposes. As shown

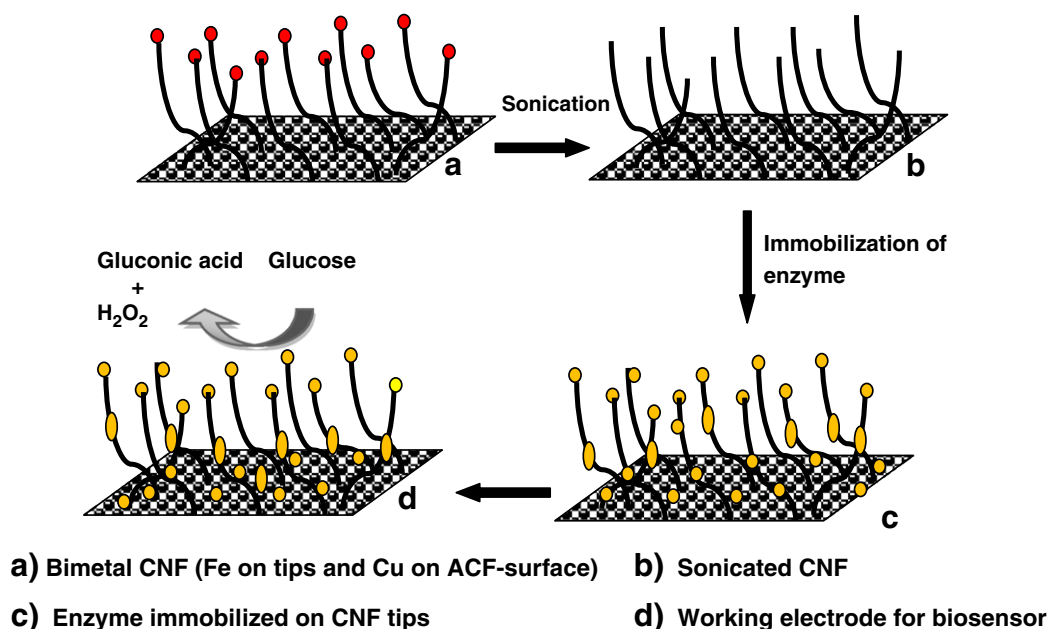


Fig. 1. Schematic of the proposed bimetal CNF-based electrode for glucose biosensor.

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