ABSTRACT

Diabetes mellitus is a chronic disease requiring accurate and continuous monitoring of blood glucose levels. This monitoring is commonly performed using conventional glucometers based on glucose oxidase (GOx); however, the accumulation of hydrogen peroxide (H_2O_2) as a byproduct of the enzymatic reaction can gradually inactivate GOx, thereby degrading biosensor performance. This study investigates the effect of GOx and catalase layer sequencing in a layer-by-layer system based on carbon nanotubes (CNT)/polyethylenimine (PEI) on electron transfer efficiency, catalytic activity, and glucose biosensor stability, as well as determining the optimal configuration. Four electrode configurations (CNT/PEI/GOx, CNT/PEI/GOx–Cat, CNT/PEI/GOx/PEI/Cat, and CNT/PEI/Cat/PEI/GOx) were fabricated on a glassy carbon electrode (GCE) and characterized using SEM-EDX, FTIR, cyclic voltammetry (CV), and chronoamperometry (CA). Biosensor performance was evaluated through Michaelis-Menten kinetics, selectivity testing, reusability, and storage stability.

The results demonstrate that enzyme layer sequencing significantly affects biosensor performance. The CNT/PEI/GOx/PEI/Cat configuration was optimal for operational performance, exhibiting the highest electron transfer rate constant (ks) of 1.07 s^{-1}, a Michaelis-Menten constant (Km) of 6.55 mM, maximum reaction velocity (Vmax) of 22.06 $\mu A \cdot cm^{-2}$, sensitivity of 3.34 $\mu A \cdot cm^{-2} \cdot mM^{-1}$, limit of detection (LOD) of 44.84 µM, and 100% sensitivity retention after 4 cycles. Positioning GOx closer to the electrode shortens the electron transfer distance, while catalase in the outer layer decomposes H_2O_2 and regenerates O_2. For storage stability, the CNT/PEI/Cat/PEI/GOx configuration performed best, retaining 80% sensitivity after 8 weeks. All configurations demonstrated good selectivity against NaCl, uric acid, and ascorbic acid. This study confirms that structured layer sequencing significantly influences the performance of CNT/PEI-based glucose biosensors.

Keywords: *glucose biosensor, layer-by-layer, glucose oxidase, catalase, carbon nanotube*