Development of amperometric glucose biosensor based on glucose oxidase co-immobilized with multi-walled carbon nanotubes at low potential

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ABSTRACT

The electrodes modified with multi-walled carbon nanotube (MWCNT) film containing adsorbed glucose oxidase (GOx) with respect to highly sensitive glucose detection are developed and demonstrated by electrochemical studies at low potential. Glucose-sensing properties were studied using cyclic voltammetric and chronamperometric techniques. A linear calibration plot was obtained in the concentration range between 1.0 and 500.0 μM, and the detection limit was determined to be 1.3 ± 0.1 μM. Interferences from other biological compounds were studied. The long-term stability, reproducibility, and sensitivity of the GOx biosensor were measured. The proposed glucose biosensor was successfully applied to human serum sample.

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1. Introduction

Carbon nanotubes (CNTs) have been extensively used in electrochemical and biosensing studies [1–4]. For example, CNTs have been used for electrocatalysis [5–8], immobilization of proteins [7–10], and promotion of direct electrochemistry of redox proteins [11,12], and so on. However, most CNT synthesis methods inevitably introduce some impurities. Treated purification is necessary, and metal catalyst residues still exist in CNTs even after purification. The impurities resulted in conflicting reports [13–15]. MWCNTs are cost-effectively produced by laser ablation of pure graphite with unparalleled purity without using any metal catalyst [16]. It is essentially metal-free and can be used directly for electrochemical and biosensing study. MWCNTs unique properties rapidly promote various applications [17,18]. In this study, the biosensing application of MWCNTs was exploited for glucose biosensor as a model, which based on the single-walled carbon nanotubes (SWCNTs) biosensing properties at low potential [19–22]. The biosensor was constructed by immobilizing GOx in Nafion–MWCNT film. Glucose investigation is very important in a number of ways, like food industry for quality control purposes, in fermentation, and as a clinical indicator for diabetes [23,24]. Self-monitoring of blood glucose is an important part of diabetes care [25,26] using various portable, economic, and sensitive glucose sensors. Under electrochemical investigation, an electron transfer in biological systems is one of the prominent areas in biochemical and biophysical sciences [27] and in recent years there has been substantial attention in the direct electron transfer between redox proteins and electroactive biomolecules with electrode surfaces [28–30]. However in the absence of mediating small molecules, the observation of well defined electrochemical behavior of immobilized flavoprotein oxidase systems such as GOx is rendered extremely difficult, because the flavin adenine dinucleotide (FAD) group is embedded deep within the protein structure thereby making the transmission coefficient for direct electron transfer between the second and a support electrode very small [31,32]. Various immobilization strategies [33,34] have been adopted to fabricate enzyme electrodes for biosensor applications. These strategies have exhibited variable degrees of success and many cases electron transfer mediators have been used to facilitate electronic communication between the active site of the protein and the underlying electrode. However, the potential at which an amperometric enzyme biosensor is operated depends on the redox potential of the mediator used rather than that exhibited by the active site of the redox enzyme.

Usually the difference in magnitude between the concluding potentials is significant and is a factor which acts against successful biosensor operation, since the more positive the operating potential, the greater the tendency is for the sensor to respond to