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Fabrication of an amperometric acetylcholine esterase-choline oxidase biosensor based on MWCNTs-Fe₃O₄NPs-CS nanocomposite for determination of acetylcholine

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Abstract

In this paper, a novel amperometric bienzymatic biosensor based on iron (II, III) oxide nanoparticles (Fe₃O₄NPs), multi-walled carbon nanotubes (MWCNTs) and chitosan (CS) modified glassy carbon electrode (GCE) was fabricated for the determination of acetylcholine (ACh). The CS was used to immobilize acetylcholine esterase (AChE) and choline oxidase (ChOx). The nanocomposites of Fe₃O₄NPs and MWCNTs were characterized by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The electrochemical measurements were based on the detection of enzymatically produced hydrogen peroxide (H₂O₂). The experimental parameters such as working potential, enzyme unit, pH and temperature were optimized. The linear ranges of the biosensor were 0.02 µmol L⁻¹ - 0.111 µmol L⁻¹ and 0.111 µmol L⁻¹ - 1.87 µmol L⁻¹. The detection limit was calculated as 0.61 nmol L⁻¹. In addition, the amperometric biosensor showed high sensitivity, good selectivity, repeatability, reproducibility and long-term stability. The fabricated biosensor was used to determine ACh levels in serum samples.

Keywords: Biosensor, Acetylcholine, MWCNTs, Fe₃O₄NPs

1. Introduction

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