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Amperometric choline biosensor based on multiwalled carbon nanotubes/zirconium oxide nanoparticles electrodeposited on glassy carbon electrode

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ABSTRACT

A bienzymatic choline biosensor was constructed by coimmobilizing acetylcholinesterase (AChE) and choline oxidase (ChO) onto nanocomposite of carboxylated multiwalled carbon nanotubes (c-MWCNTs) and zirconium oxide nanoparticles (ZrO_2NPs) electrodeposited on the surface of a glassy carbon electrode (GCE) and using it (AChE–ChO/c-MWCNT/ZrO_2NPs/GCE) as working electrode, Ag/AgCl as reference electrode, and Pt wire as auxiliary electrode connected through a potentiostat. The enzyme electrode was characterized by scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and cyclic voltammetry (CV) studies, optimized, and evaluated. The biosensor exhibited optimum response within 4 s at +0.2 V, pH 7.4, and 25 °C. The detection limit and working range of the biosensor were 0.01 μM and 0.05 to 200 μM, respectively. The half-life of the enzyme electrode was 60 days at 4 °C. The serum choline level, as measured by the biosensor, was 9.0 to 12.8 μmol/L (with a mean of 10.81 μmol/L) in apparently healthy persons and 5.0 to 8.4 μmol/L (with a mean of 6.53 μmol/L) in persons suffering from Alzheimer's disease. The enzyme electrode was unaffected by a number of serum substances.

Choline is a precursor of acetylcholine, a neurotransmitter that is involved in the signal transmission among nerves, muscles, and organs. Monitoring the levels of acetylcholine and choline in serum is very important to detect neurodegenerative diseases such as Alzheimer's and neuromuscular diseases, myasthenia gravis, and impaired cholinergic neurotransmission [1,2]. Among the various methods available for measurement of choline, biosensing methods are comparatively simpler and more sensitive, rapid, and specific. These acetylcholine/choline biosensors employed acetylcholinesterase (AChE)[1] and choline oxidase (ChO), which catalyzed the following electrochemical reactions [3–5]:

(1) Hydrolysis of acetylcholine:

$$\text{Acetylcholine} + H_2O \xrightleftharpoons{\text{Acetylcholinesterase}} \text{Acetate} + \text{Choline} + H^+$$

(2) Oxidation of choline:

$$\text{Choline} + 2O_2 + H_2O \xrightleftharpoons{\text{cholineoxidase}} \text{Betaine} + 2H_2O_2$$

(3) Electrolysis of H_2O_2:

$$2H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

The flow of electrons (i.e., current) is directly proportional to the acetylcholine/choline concentration. A number of amperometric choline biosensors have been reported based on immobilization of AChE/ChO onto photopolymerized polyvinyl alcohol (PVA)–SbQ [6], methacrylate–vinylene carbonate copolymer [7], polyvinylferrocenium [8], poly-5,2′:5′,2″-terthiophene-3-carboxylic acid (poly-TTCA) [9], poly(2-hydroxyethyl methacrylate) films [10], PVA–SbQ and covered with Nafion (perfluorosulfonated membrane) [11], PVA cryogel membrane [12], poly(pyrrole)/poly(2-naphthol) bilayer membrane [13], and Prussian blue on the surface of a Pt electrode by crosslinking with bovine serum albumin and glutaraldehyde [14]. All of these biosensors had low storage stability due to leakage of enzymes and sensitivity.

Nanomaterials have attracted much attention for designing novel biosensing systems to improve their bioanalytical performance. Multiwalled carbon nanotubes (MWCNTs) are one promising

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[1] *Abbreviations used:* AChE, acetylcholinesterase; ChO, choline oxidase; PVA, polyvinyl alcohol; MWCNT, multiwalled carbon nanotube; ZrO_2NP, zirconium oxide nanoparticle; c-MWCNT, carboxylated MWCNT; GCE, glassy carbon electrode; DW, double distilled water; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; TEM, transmission electron microscopy; FTIR, Fourier transform infrared; SEM, scanning electron microscopy; ChCl, choline chloride; HPLC, high-performance liquid chromatography.

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nanomaterial that has been explored for chemical and biological sensing applications. These nanotubes have been employed in biosensors as effective catalyst supports due to their large surface area, unique structural and electromechanical properties, good biocompatibility, ease of preparation, and surface renewability [15–17]. Recently, nanocomposites of conducting polymers [18] and nanoparticles [19,20] have attracted potential interest for such purposes. Zirconium oxide nanoparticles (ZrO_2NPs) are nontoxic due to their excellent chemical inertness and biocompatibility and, thus, are an ideal support for immobilization of biomolecules. We describe here the construction and application of a bienzymatic choline sensor by covalently immobilizing AChE and ChO onto nanocomposite of carboxylated MWCNTs (c-MWCNTs) and ZrO_2NPs electrodeposited on a glassy carbon electrode (GCE).

Materials and methods

Chemicals and reagents

AChE (EC 3.1.1.7, type VI-S, from electric eel, activity 200–600 U/mg solid), ChO (EC 1.1.3.17, from *Alcaligenes* species, activity 10 U/mg solid), acetylcholine chloride, and choline chloride were obtained from Sigma Chemical Co, St. Louis (USA). c-MWCNTs (functionalized MWCNTs, 12 walls, length 15–30 mm, purity 90%, no metal content) obtained from Intelligent Materials (Panchkula (Haryana), India), ZrO_2 nanopowder obtained from Sisco Research Laboratory (Mumbai, India), and GCE (disk diameter 3 mm) obtained from Metrohm–India (Delhi, India) were used. All other chemicals were of analytical reagent grade. Double distilled water (DW) was used throughout the experiments.

Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements in a potentiostat/galvanostat (Autolab, model AUT83785, Eco Chemie, The Netherlands) with a three-electrode system consisting of enzyme electrode (AChE–ChO/c-MWCNT/ZrO_2NPs/GCE) as working electrode, Ag/AgCl as reference electrode, and Pt wire as auxiliary electrode, ultrasonication in Misonix Ultrasonic Liquid Processors (model XL-2000 series), transmission electron microscopy (TEM) images of ZrO_2NPs (1 mg/ml) in a transmission electron microscope at Punjab University (Chandigarh, India), Fourier transform infrared (FTIR) spectra in an FTIR spectrometer (model iS10, Thermo Electron, USA), and scanning electron microscopy (SEM) images of modified electrode in a scanning electron microscope (model Joel JSM-6510, Japan) were recorded.

Construction of c-MWCNT/ZrO_2NPs modified GCE

Prior to the electrodeposition, the bare GCE was polished with alumina slurry (diameter 0.05 μm) and then cleaned ultrasonically in ethanol and water, followed by thorough rinsing with DW. The powder of c-MWCNTs was then sonicated in 5.0 mM ZrO_2 solution containing 100 mM KCl (0.5 mg/ml) for approximately 15 min to form uniform c-MWCNT black-colored solution. The cleaned GCE was dipped into this ZrO_2 NPs/c-MWCNT suspension. A nanocomposite film of c-MWCNT/ZrO_2NPs was electrochemically deposited on the surface of polished GCE at a constant potential of −1.1 V for 5 min. The prepared electrode was rinsed gently with DW and dried in air [21].

Preparation of enzyme electrode

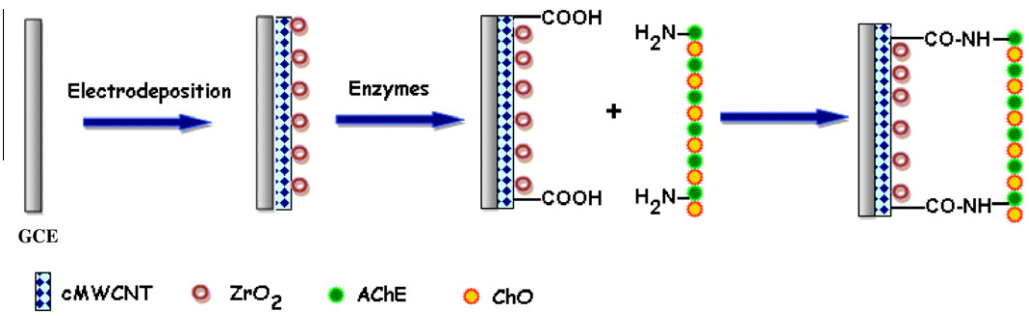
To prepare the enzyme electrode (AChE–ChO/c-MWCNT/ZrO_2NPs/GCE), a mixture of 10 μl of AChE solution (2 mg/ml) and 10 μl of ChO (10 mg/ml) was mounted on the surface of c-MWCNT/ZrO_2NPs modified GCE and kept at 4 °C for 24 h. The prepared enzyme electrode was rinsed with DW clearly, dried, and stored at 4 °C until use (Scheme 1).

Characterization of enzyme electrode

The enzyme electrode was characterized by SEM, FTIR, and EIS at different stages of its construction. EIS studies were carried out in a potentiostat/galvanostat in the frequency range of 0.01 Hz to 10 kHz with amplitude +0.2 V.

CV study, response measurements, and optimization of enzyme electrode

A cyclic voltammogram of AChE–ChO/c-MWCNT/ZrO_2NPs/GCE was recorded in the potential range of +0.0 to +0.6 V at a scan rate of 50 mV s^{-1} versus Ag/AgCl as reference electrode and Pt as auxiliary electrode in 15 ml of 0.1 M phosphate buffer (pH 7.0) containing 1 ml of choline chloride (ChCl). The maximum response was observed at +0.2 V; hence, subsequent studies were carried out at this voltage. To test the functioning of the biosensor, the three-electrode system was immersed into 15 ml of 0.1 M phosphate buffer (pH 7.0) containing ChCl (1 ml of 0.5 mM solution) in a 50-ml beaker, and the current (mA) generated at +0.2 V was recorded. The effect of pH of the buffer was studied over the pH range 5.0 to 9.0 at an interval of pH 0.2 using 0.1 M sodium succinate buffer for pHs 5.0 to 5.6, sodium phosphate for pHs 5.8 to 8.0, and borate buffer for pHs 8.2 to 9.0. The effect of incubation temperature on AChE–ChO/c-MWCNT/ZrO_2NPs modified GCE was studied by incubating the reaction mixture at different tempera-



Scheme 1. Scheme for preparation of AChE–ChO/c-MWCNT/ZrO_2NPs/GCE.