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Glassy carbon electrode modified with a nanocomposite of multi-walled carbon nanotube decorated with Ag nanoparticles for electrochemical investigation of Isoxsuprine

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ABSTRACT

A highly sensitive voltammetric sensor is prepared using multi-walled carbon nanotube decorated with silver nanoparticles (AgNPs-MWCNTs) and is applied for the determination of Isoxsuprine (ISOX). The characterization of the prepared nanocomposite is performed using energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), atomic force microscopy (AFM), transmission electron microscope (TEM), scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry techniques. The electrochemical behavior of ISOX is studied on the surface of the modified electrode by linear sweep and cyclic voltammetry methods. Under the optimized conditions such as pH of the supporting electrolyte, accumulation time and amount of the casted modifier, a significant electrochemical improvement is observed toward the electro-oxidation of ISOX on the surface of AgNPs-MWCNTs glassy carbon electrode (AgNPs-MWCNTs/GCE), in comparison to MWCNTs/GCE and bare GCE. At the surface of the modified electrode two oxidation peaks are observed for ISOX at potentials around 0.63 V (peak I) and 0.82 V (peak II) in BR buffer solution of pH 7.0. By using two anodic peaks of ISOX, two linear dynamic ranges of 0.04–5.0 μmol L^{-1} and 0.2–5.0 μmol L^{-1} with detection limits of 12 nmol L^{-1} and 60 nmol $L^{-1}$are obtained for peaks I and II, respectively. The modified electrode showed a simple fabrication procedure, high sensitivity, stability and good reproducibility in response to ISOX. The AgNPs-MWCNTs/GCE is successfully applied for the accurate determination of trace amounts of ISOX in pharmaceutical and clinical preparations.

1. Introduction

Isoxsuprine hydrochloride (ISOX), under the trade name Duvadilan is a benzyl alcohol derivative (Scheme 1). It is a beta-adrenergic agonist that causes direct relaxation of uterine and vascular smooth muscle, and may also produce positive inotropic and chronotropic effects on the myocardium. This drug is used along with other treatment for certain blood vessel diseases (e.g., arteriosclerosis obliterans, Raynaud's disease, Buerger's disease, cerebrovascular insufficiency) [1,2]. It works by widening blood vessels to help increase blood flow (improve circulation) to certain parts of the body (e.g., hands/feet, brain). This effect may help decrease symptoms such as cold hands and feet, numbness, tingling, and decreased memory or judgment [3]. Also it is used in the treatment of peripheral vascular disease and in premature labor [4]. The mechanism of action of ISOX is controversial, because ISOX has beta-adrenergic activities that could not be offset by beta-adrenergic blockers [5]. Although stimulation of the beta-adrenergic receptor increases blood flow to produce vasodilatation, this agent may also have direct effects on the contractility of smooth muscle. Also Isoxsuprine is most commonly used to treat hoof-related problems in the horse, most commonly for laminitis and navicular disease, as its effects as a vasodilator are thought to increase circulation within the hoof to help counteract the problems associated with these conditions [6]. Except under special circumstances, this medication (ISOX) should not be used immediately postpartum or when the following medical problems exist; For use in management of premature labor only: Cardiac disorders, especially those associated with arrhythmias, or maternal hyperthyroidism (Isoxsuprine may precipitate arrhythmias or heart failure; occult cardiac disease may be unmasked) or chorioamnionitis (intrauterine infection) or hemorrhage or intrauterine fetal death or known abnormality (immediate delivery required) or eclampsia (toxemia) and severe pre-eclampsia or pulmonary hypertension [7]. The drug has an

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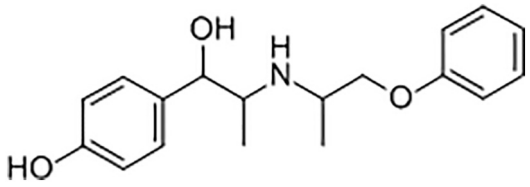

Scheme 1. Chemical structure of ISOX.

elimination half-life under 3.0 h and its adverse effects are including trembling, nervousness, weakness, dizziness, flushing, transient palpitation, tachycardia, chest pain, hypotension, abdominal distress, nausea, vomiting, intestinal distention, and severe rash. Therefore, the exact determination of this drug is of special importance [8].

Several analytical methods including spectrophotometry [9–11], chromatography [12,13] and fluorimetry [14,15] have been employed for the determination of ISOX in pharmaceutical and clinical preparations. Although ISOX seems to be an electroactive compound, which can electrochemically get oxidized, only one work has found in the literature describing the electrochemical analysis of ISOX based on our knowledge [16].

Nowadays, with the pharmaceutical development, such as formulation and stability studies, quality control, toxicology and pharmacological testing in animals and human, the development of simple, sensitive, rapid and reliable analytical methods for the determination of drug species becomes very important [17]. Techniques based on classic chromatography have some drawbacks, such as expensive instruments and maintenance, intensive solvent usage, time-consuming sample pretreatment and optimization of chromatogram conditions. In comparison to chromatography, the electroanalytical techniques by using the chemically modified electrode (CME) have preferable advantages like low cost, simplicity procedure, high sensitivity, rapid response, simple fabrication, portable and low detection limit. Therefore, CMEs have been extensively studied and widely used for the determination of many electroactive components in pharmaceutical analysis [18–20].

Among recent years, carbon nanostructures and metal nanoparticles are in the spotlight of various research fields especially in electroanalytical investigations due to special properties [21,22]. Multi-walled carbon nanotubes (MWCNTs) are one of the most commonly used carbon nanostructures that have received a remarkable attention in a wide range of applications, especially in electrochemical sensors. The broad application area for MWCNTs is due to their fabulous physical and chemical properties, including an extremely large surface area to volume ratio, appropriate conductivity, high porosity and loading, nontoxicity, hollow structure, small diameter, special optical and electrical properties and exceptionally high tensile strength [23–25]. Therefore, these unique properties of MWCNTs allow using as a template for preparing metal nanoparticle-MWCNTs nanohybrids [26]. Metal nanoparticles with respect to some significant advantages such as facile synthesis, easy surface functionalization, efficient catalytic role in electrochemical reactions and the enhancement of electron transfer processes have key role in the design and construction of the electrochemical sensor and biosensor platforms [27–29]. Over last two decades decoration of CNTs with noble metal nanoparticles such as Au, Pd, Pt and Ag has received a considerable attention toward the design of novel analytical procedures especially in construction of electrochemical-based sensor [30–34]. Ag nanoparticles due to high conductivity, effective catalytic behavior, amplified electrochemical signals and excellent biocompatibility is considered as a very good candidate for the decoration on CNTs [35,36]. Therefore, combination of AgNPs and MWCNTs characteristics causes producing novel hybrid nanostructures, which exhibit the properties of the two nano-sized composites as well as reducing the overvoltage and improving the kinetics of the electrode processes. Moreover, formation of a nanocomposite from these nanostructures has a progressive effect on the mass transfer through the modifier film and enhances the sensitivity and selectivity of modified electrode response [37,38]. Recently AgNPs-MWCNTs composite has been used for the determination of nitrite [38], clonazepam [39], nonenzymatic hydrogen peroxide [40] and also for the sensitive detection of superoxide anion released from living cells [41].

In the present work, MWCNTs decorated with silver nanoparticles (AgNPs) by a simple chemical plating method were applied for electrochemical investigation and determination of ISOX. Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to characterize the electrode surface characteristics. The prepared modified electrode was successfully applied for voltammetric determination of ISOX in pharmaceutical and clinical samples. A good linear relationship was obtained between the anodic peaks currents and ISOX concentrations in two linear dynamic ranges of 0.04–5.0 μmol L^{-1} and 0.2–5 μmol L^{-1} with detection limit values of 12 nmol L^{-1} and 60 nmol L^{-1}, respectively with good repeatability and reproducibility.

2. Experimental

2.1. Chemicals and reagents

ISOX (> 99.0% purity) kindly was prepared from Mehr Darou pharmaceutical company (Tehran, Iran). MWCNT, synthesized by catalytic chemical vapor deposition (CVD, purity N 95%) with outer diameter (o.d.) of 10–20 nm, inner diameter (i.d.) of 5–10 nm and tube length of 0.5–200 nm, was purchased from Nanostructured & Amorphous Materials (Houston, TX, USA). All other chemicals were of analytical reagent grade and obtained from Merck. In all electrochemical experiments, the pH of all solutions was fixed by using Britton–Robinson (BR) buffer solutions (containing 0.04 M of acetic acid, orthophosphoric acid and boric acid) by the addition of 0.20 M sodium hydroxide under a pH-meter. Doubly distilled deionized water was used for all aqueous solution preparations (Zolalan Co, Iran). Stock solutions of ISOX were prepared daily in ethyl alcohol (EtOH) solution. The working solutions were prepared by diluting the stock solution of ISOX with Britton–Robinson buffer solutions. Tablets of ISOX (10 mg per tablet) were purchased from local pharmacies. Fresh frozen human blood serum was obtained from the Iranian Blood Transfusion Organization. Two percent (v/v) of pure methanol was added to the serum sample. After vortexing each sample for 2 min, the precipitated proteins were separated by centrifugation for 10 min at 10,000 rpm. Then, the sample was diluted, spiked with different amounts of standard ISOX without extraction or further treatment and applied for the recovery tests in the voltammetric determination.

2.2. Apparatus

Voltammetric experiments were performed with a Metrohm Computrace Voltammetric Analyzer model 757 VA. A conventional three-electrode system was used with a glassy carbon working disk electrode (2.0 mm in diameter, purchased from Azar electrode Co., Urmia, Iran), an Ag/AgCl (saturated KCl) reference electrode, and a Pt wire as the counter electrode. Ultrasonic KODO model JAC 1002 was used for cleaning the surface of electrodes and suspension agitation in modifier preparations. A digital pH/mV/Ion meter (Metrohm, pH Lab 827) was applied for preparing the buffer solutions, which was used as the supporting electrolyte in the voltammetric experiments. Field-emission scanning electron microscope (FE-SEM MIRA 2 TESCAN, 15 kV, Republic Czech) equipped with an energy-dispersive X-ray spectroscopy (EDS) detector was used for studying the structure and surface morphology of the modified electrodes. The transmission electron microscope (TEM) images were obtained using a PHILIPS CM 200 instrument. Electrochemical impedance spectroscopy (EIS) measurements were performed with a Potentiostat/Galvanostat/Frequency

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