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Development of a simple, rapid and validated square wave voltametric method for determination of promethazine in raw material and pharmaceutical formulation using DNA modified multiwall carbon nanotube paste electrode

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ABSTRACT

A simple voltammetric method based on a composite biosensor MWCN/SiO_2/Al_2O_3/Nb_2O_5/DNA (MWCN/SiAlNb/DNA) was developed and validated for the determination of promethazine (PHZ) in raw material and pharmaceutical formulation. The best results were obtained by using square wave voltammetry under measurements at 0.30 mol L^{-1} phosphate buffer pH 7.0 and DNA immobilization onto SiAlNb surface using 0.8 mg mL^{-1} concentration. Validation parameters such as: selectivity, linearity, precision, accuracy, and stability presented results within the acceptable range. The sensitivity of sensor was found to be 0.210 μA μmol^{-1} L. Therefore, the developed method was successfully applied for the PHZ determination in real samples of pharmaceutical formulations and can be used for routine quality control analysis of bulk pharmaceutical and pharmaceutical formulations containing PHZ.

1. Introduction

Promethazine (PHZ), which belongs to the phenothiazine group, is widely used as an antihistaminic for the symptomatic relief of hypersensitivity reactions and for enhancing the analgesic, anesthetic and sedative effect of other drugs [1]. However, PHZ can cause adverse effects in humans, such as endocrinal, cardiac and reproductive alterations [1]. PHZ, as other phenothiazines, easily undergoes oxidation in acidic medium in the presence of oxidizing agents. This reaction depends on acidity, concentration of phenothiazine derivatives, type of oxidant and its redox potential [2,3].

The official method presented in the United States Pharmacopoeia (USP) for PHZ consists of thin-layer chromatographic method with visualization in the ultraviolet region [4]. Various alternative methods have been reported for analysis of PHZ as titrimetry with different electrodes or in aqueous phase [5], spectrophotometry [6,7], spectrofluorimetry [8], high performance liquid chromatography [9–14], gas chromatography [15,16] and electrophoresis [17–19]. Electrochemical methods, which are simple, cost effective, and require relatively short analysis times, without the need for derivatizations or time-consuming extraction steps, have been also employed to determine PHZ, such as differential pulse voltammetry [20], differential pulse polarography [21], differential pulse stripping voltammetry [22] and voltammetry with flow injection analysis [23].

The electrochemical methods employ some electrodes to determine PHZ such as glassy carbon electrode [24,25], gold electrode [26] and electrodes modified with carbon nanotubes (CNs) [27], wax impregnated graphite electrode [28], highly boron doped diamond [29] and DNA modified carbon paste electrode [30] have already been used as working electrodes.

CNs possesses a special tube structure with unique properties such as good electrical conductivity, high chemical stability and extremely high mechanical strength [31,32]. In addition, the subtle electronic behavior of CNs reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials [33–35]. These properties make CNs as a suitable candidate for the modification of electrodes as DNA. The use of DNA as the recognition element of affinity biosensors has been an increasing interest in research [36–38]. DNA recognition layers can be employed for detecting analytes interacting with such layers. Small molecules as drugs can be detected via

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changes in their response (associated with their interaction with the recognition layer), through intrinsic changes in the DNA signal (due to such association) or via the displacement of certain markers [38,39].

Bearing in mind the aforementioned comments, this study shows the development of a biosensor for determining PHZ, which consisted of a composite based on the multiwall carbon nanotubes (MWCN) paste and an inorganic material based on silica – $SiO_2/Al_2O_3/Nb_2O_5$ (SiAlNb). The electrodes modified with inorganic materials practically form a new class of chemically modified electrodes. Moreover, the inorganic materials can be incorporated in the composites, generally with the aim of increasing the stability of the electroactive species immobilized. Several inorganic matrices have been used [40–42] as modifying agents conventional electrodes. These include silica gel obtained by sol–gel process, metal oxides and phosphates, γ-alumina, zeolites, and other types.

A variety of materials based on silica together the chemistry of their surface implies a great potential for the application of these species in electrochemistry. Some of the properties of this material such as adsorption capacity, acid–base chemical, thermal stability, can advantageously be exploited, for example, the accumulation of electroactive species prior to electrochemical detection [43,44]. Furthermore, the silica can be grafted with a variety of functional groups leading to a considerable enrichment of their surface properties. The high surface area synthetic silica, when combined with their surface chemistry, makes this material useful as supports for various catalysts.

Recently it was demonstrated the use of material SiAlNb for the preconcentration of Zn(II) and Cd(II) in solution [45,46]. The use of SiAlNb material becomes interesting, because it displays great potential to prepare chemically modified electrodes.

PHZ is usually administered in the form of injection liquid, oral solution, suppositories or coated tablets. Its determination in raw material and pharmaceutical formulations is extremely important [1,26]. Therefore the aim of this study was to develop and characterize a DNA modified multiwall carbon nanotubes paste electrode and to determine PHZ in raw material and coated tablets.

2. Experimental

2.1. Samples

Promethazine hydrochloride was obtained from Sigma Aldrich (St. Louis, MO, USA). The raw material pharmaceutical was obtained from Aventis-Pharma (São Paulo, SP, Brasil). Fenergan® (Sanofi Aventis) was obtained from a local market.

2.2. Solvents and reagents

Multiwall carbon nanotubes (MWCN) were acquired from CN Corporation Ltd. (Incheon, Korea) with diameter of 10–40 nm and length of 5–20 μm. Herring sperm deoxyribonucleic acid (DNA), piperazine-1,4-bis[2-ethanesulfonic acid] (PIPES), 4-[2-hydroxyethyl] piperazine-1-ethanesulfonic acid (HEPES), tris[hydroxymethyl]aminomethane (Trizma), sodium hydroxide, nitric acid, acetic acid and mineral oil were acquired from Sigma–Aldrich® (St. Louis, MO, USA). Sodium acetate and potassium chloride were obtained from Sial® (São Paulo, SP, Brazil). Dibasic sodium phosphate was acquired from Vetec® (Rio de Janeiro, RJ, Brazil). The material $SiO_2/Al_2O_3/Nb_2O_5$ (designated as SiAlNb), obtained by sol–gel process, was prepared according to previously described procedure [45,46].

All other chemicals were of analytical grade with the highest purity available. Water was distilled and purified using a Millipore Milli-Q Plus system (Bedford, MA, USA).

2.3. Instrumentation, electrochemical characterization and optimization of the biosensor

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 12, Eco Chemie, The Netherlands) interfaced with a personal computer for data acquisition and potential control. The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at $25 \pm 1\,^{\circ}C$. An Ag/AgCl/KCl ($3.0\,mol\,L^{-1}$) electrode, a platinum wire, and the MWCN–SiAlNb–DNA were used as the reference, auxiliary and working electrodes, respectively. The prepared electrodes were characterized by FT-IR model 8300 from Shimadzu (Tokyo, Japan). The KBr (1%) pellet technique was used to characterization of materials. The scanning electron micrograph images were taken using a JEOL model JSM 300 (Tokyo, Japan). The Al_2O_3 and Nb_2O_5 contents in the material were determined by using energy dispersive X-ray fluorescence analysis (EDFRX) on a model EDX 800 HS from Shimadzu (Tokyo, Japan). The analysis are specific surface area (S_{BET}) of the materials were performed on equipment Quantachrome Model Nova 1200e (Boynton Beach, FL, USA) and determined by the BET (Brunauer, Emmett and Teller) multipoint method by submitting the samples to previous activation at 250 °C in vacuum for 4 h. The measures of average pore size and average pore volume were obtained through the Barrett–Joyner–Halenda (BJH) method.

2.4. Preparation of the biosensor using $SiO_2/Al_2O3/Nb_2O_5$ (SiAlNb) and multi-wall carbon nanotubes

For the immobilization of the biological component (DNA) on the surface of the matrix sol–gel silica (SiAlNb), 320 mg of this material was added in 60 mL of a solution containing $0.8\,mg\,mL^{-1}$ of DNA prepared in $0.10\,mol\,L^{-1}$ phosphate buffer pH 7. This mixture was stirred for 15 min and allowed to stand by 12 h for adsorption of biological component in the matrix. The mixture was filtrated and then dried at room temperature. This mixture was called SiAlNb–DNA.

After the drying process, it was prepared a MWCN paste containing material SiAlNb–DNA prepared previously. This material (60 mg) consisted of a mixture 1:1 (w/w) between the SiAlNb–DNA and CNs in mineral oil. This final mixture (MWCN–SiAlNb–DNA) was introduced into the lower cavity of the glass tube which was employed as working electrode.

2.5. Preparation of reference solutions

Stock standard solutions of PHZ were prepared by dissolution of the drug in water to obtain a final concentration of $0.1\,mol\,L^{-1}$. This standard solution was diluted to give the following concentrations: 10, 20, 30, 40, 50, 60, 70, 90, 100, 110, 120, 130, 140 and 150 $\mu mol\,L^{-1}$ of active pharmaceutical ingredient. All of these solutions were stored at −20 °C in the absence of light. Working standard solutions were prepared daily by diluting the stock solutions to an appropriate concentration with $0.3\,mol\,L^{-1}$ phosphate buffer pH 7.0.

2.6. Preparation of sample solutions

2.6.1. Bulk pharmaceutical

PHZ bulk pharmaceutical was prepared by dissolution of 3.2 mg of drug in a 10 mL volumetric flask containing 10 mL of $0.3\,mol\,L^{-1}$ phosphate buffer pH 7.0. This solution was sonicated for 2 min, mixed on a vortex mixer for 2 min. After, it was used 500 or 600 μL of this solution and transferred into a 10 mL volumetric flask, diluted to volume with $0.3\,mol\,L^{-1}$ phosphate buffer pH 7.0