Materials Science and Engineering C 59 (2016) 136–144

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Enhanced amperometric detection of metronidazole in drug formulations and urine samples based on chitosan protected tetrasulfonated copper phthalocyanine thin-film modified glassy carbon electrode

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ARTICLE INFO

Article history:
Received 16 May 2015
Received in revised form 25 July 2015
Accepted 27 August 2015
Available online 3 September 2015

Keywords:
Differential pulse voltammetry
Metronidazole
Tetrasulfonated copper phthalocyanine
Chitosan
Urine samples

ABSTRACT

An enhanced electrocatalytic reduction of metronidazole antibiotic drug molecule using chitosan protected tetrasulfonated copper phthalocyanine (Chit/CuTsPc) thin-film modified glassy carbon electrode (GCE) has been developed. An irreversible reduction occurs at −0.47 V (vs. Ag/AgCl) using Chit/CuTsPc modified GCE. A maximum peak current value is obtained at pH 1 and the electrochemical reduction reaction is a diffusion controlled one. The detection limit is found to be 0.41 nM from differential pulse voltammetry (DPV) method. This present investigation method is adopted for electrochemical detection of metronidazole in drug formulation and urine samples by using DPV method.

1. Introduction

Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (MTZ), is one of the nitroimidazole derivatives well-known for its antimicrobial properties [1,2]. It is effective against *Trichomonas* [3], Vincent's organisms [4], and anaerobic bacteria [5,6]. Veterinarians also use MTZ to treat bacterial infections specifically giardia in dogs and cats [7]. The chemical structure of the drug is shown in Fig. 1. Though MTZ is typically well tolerated at <2 g daily dosage and beyond, a level of toxicity has been reported [8]. Longer-term use for severe infection is often limited by the development of peripheral neuropathies [9], as well as optic neuropathy [10], neutropenia and central nervous toxicities including cerebellar ataxia, encephalopathy and seizures. Therefore, it is necessary to monitor MTZ concentration in patients under high antibiotic therapy.

The toxicity of MTZ drug is dose-dependent on biological fluids like serum, urine and saliva. It is well absorbed in serum as well as tissue concentrations usually at high levels [11]. Several analytical methods have been reported for determination of MTZ, which include spectrophotometry [12], polarography [13], liquid chromatography [14], gas chromatography [15], high performance liquid chromatography [16] and gas chromatography–mass spectrometry (GC–MS) [17]. However, the above reported methods could not mention the importance of selectivity of MTZ determination. Hence, it is primary importance to develop an alternative method for MTZ determination with a high degree of selectivity and sensitivity.

Chemically modified electrodes (CMEs) have continued to be a major concern during the past decade. CME has been widely considered for selective and sensitive electrochemical determination such as environmentally [18–20] and biologically important compounds [21–23]. CMEs can catalyze the electrode reaction by significantly decreasing the required over-potential, pH independent, fast reacting and stable in redox behavior [24,25]. Modification of CME with suitable materials to facilitate electrochemical reactions of redox compounds to proceed without any hindrance [26,27]. Notably, certain transition metal complexes of porphyrins and metal phthalocyanines are used to catalyze electrooxidation or electroreduction of chemical and biological compounds. Mostly, metal phthalocyanine modified electrode is very attractive because of less soluble compounds and its simple modifications of molecular structure [28,29]. It can be used extensively in catalysts, semiconductors and anticancer medicine.

Chitosan is one of the naturally available biopolymers and it is widely used in various applications, due to its poor solubility and high

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http://dx.doi.org/10.1016/j.msec.2015.08.063

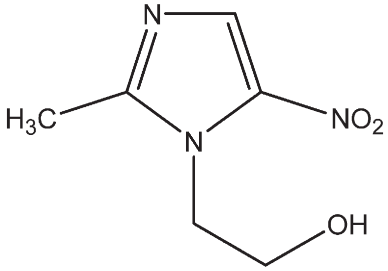

Fig. 1. Structure of MTZ.

thermal stability [30,31]. Nowadays, chitosan is considered as one of the most important polymer to stabilize modified electrode substrates. Chemically modified chitosan is an efficient sorbent material for heavy metals, carbon electrode coated with carbon nanotube/chitosan composites for detection of polyphenols [32], paracetamol and uric acid [33]. Furthermore, the electrochemical reduction of MTZ at deoxyribonucleic acid (DNA) modified GCE [34], metalloporphyrin modified carbon paste electrode [35], activated GCE [36], nanomaterial thin film coated GCE [37], carbon fiber microdisk electrode [38], Ni/Fe-LDH modified GCE [39], MIP/MWCNT/GCE [40] and Cu-Poly(Cys) [41] has been reported.

With this above background, in the present study we report a novel voltammetric sensor based Chit/CuTsPc/GCE which has been developed for selective determination of MTZ. The prepared MTZ sensor was characterized in terms of selectivity, sensitivity and reproducibility. A relatively low applied potential and improved selectivity for MTZ detection has been realized. In addition, the designed sensor was successfully applied for determination of MTZ in commercially available tablets and urine samples.

2. Experimental

2.1. Chemicals

All reagents and chemicals used were of analytical grade and without further purification. Metronidazole was obtained from Vanavile Chemicals, Pvt. Ltd., Cuddalore, Tamil Nadu, India. Flagyl tablets at 200 mg and 400 mg were purchased from local medical stores. Copper phthalocyanine tetrasulfonic acid tetrasodium salt (CuTsPc) and chitosan were purchased from Sigma Aldrich, USA. Sodium hydroxide was purchased from Fisher Scientific, India. All other chemicals used in this study were purchased from SRL Chemicals, Pvt. Ltd., India.

2.2. Instrumental methods

UV–Visible spectral studies were observed by using a Shimadzu UV–Visible Spectrophotometer, Japan (Model UV-1800). Morphological and structural investigations were carried out with field emission microscopy (JEOL-JSM-6360 Instrument, USA) and atomic force microscopy images were obtained using AFM Park Systems, XE-70, South Korea. The cyclic voltammetric experiment was carried out using a CHI 660A electrochemical instrument, Gamry model 330, USA. A conventional three electrode system comprising of a glassy carbon electrode (GCE) 3 mm in diameter was purchased from BAS. Pvt. Ltd., USA. Ag/AgCl and platinum wire were used as a reference electrode and counter electrode, respectively. A Bioanalytical System (BAS, USA) polishing kit was used to polish the GCE surface.

Electrochemical impedance spectroscopy (EIS) measurements were performed with the use of the CHI-660A electrochemical instrument. Electrolyte was prepared by using 0.1 M KCl containing a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe. All solutions were purged with high purity nitrogen gas for about 10 min before performing all electrochemical experiments. All experiments were carried out at room temperature.

2.3. Preparation of the Chit/CuTsPc thin-film

The Chit/CuTsPc composite was prepared by two different methods viz. infiltration and direct mixing method. The 1% (*w*/*v*) chitosan solution was poured onto a glass plate and left to dry through evaporation for 24 h and then heated at 60 °C for 6 h. Later, the thin film was neutralized with 1 M NaOH, washed thoroughly and dried. The dry film was immersed in 0.01 M CuTsPc solution for 24 h followed by washing with deionized (DI) water and stored in deionized water prior to different measurements. Alternatively, CuTsPc solution (2 mg mL^{-1}) was prepared by dissolving 10 mg CuTsPc in 10 mL water. Chitosan (1% *w*/*v*) was prepared by dissolving 1 g of chitosan in 100 mL of DI water and then 1 mL of glacial acetic acid was added. The mixture was allowed to stir for few minutes until the complete dissolution of chitosan. The whole reaction mixture was concentrated by using a rota-evaporator to reach a final volume of 10 mL. The viscous matrix was drop-casted over GCE and used for the electrochemical studies.

2.4. Preparation of the Chit/CuTsPc modified GCE

The GCE surface was mechanically cleaned by polishing with 500 μm alumina powder, washed with double distilled (DD) water and then sonicated for 5 min in ethanol, followed by DD water. GCE substrate was modified with Chit/CuTsPc solution (5 μL) by drop-casting method using a micro-syringe and dried under room temperature before use.

3. Results and discussion

Recently, polymer stabilized metal phthalocyanine was demonstrated for construction of many types of chemically modified electrodes which are widely used in various applications. For example, chitosan [42], poly-L-lysine [43], polyallylamine [44] and natural gum [45] are used to stabilize metal phthalocyanine and these hybrid nanocomposite modified electrodes can be used for electrochemical determination of biologically important molecules. In the present study, chitosan is chosen as a dispersing agent to stabilize the CuTsPc film because of the strong electrostatic interaction between chitosan with CuTsPc [46]. Stability and film forming performance of CuTsPc dispersed chitosan were investigated through UV–Visible spectral studies. CuTsPc peak appeared at 614 nm and 695 nm corresponding to dimeric and monomeric species due to the π–π* transition on macrocycle [47]. In Fig. S1, the short band position is slightly red shift when compared to the CuTsPc solution because of molecular aggregation. The molecules were uniformly distributed and formed CuTsPc nanocrystals within the chitosan polymer matrix. So the drop-casting method [48] is highly preferred for loading copper phthalocyanine type of electron transfer mediators within the

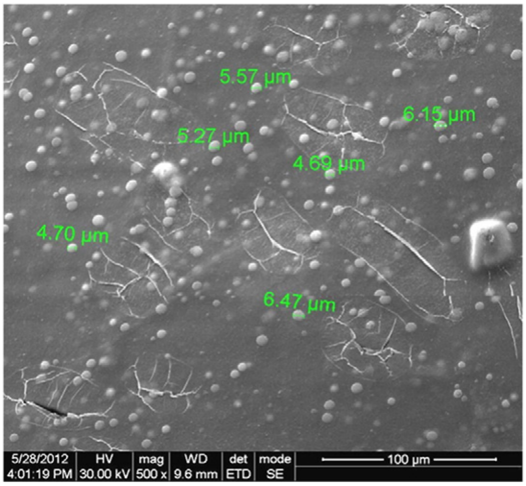

Fig. 2. SEM image of Chit/CuTsPc.