Journal of Electroanalytical Chemistry 772 (2016) 58–65

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Electrochemical evaluation of antioxidant capacity in pharmaceutical antioxidant excipient of drugs on guanine-based modified electrode

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 20 February 2016
Accepted 12 March 2016
Available online 15 March 2016

Keywords:
Hydroxyl radicals
Antioxidant capacity
Guanine biosensor
TiO_2 nanoparticles
Drug
Pharmaceutical antioxidant excipient

ABSTRACT

The aim of this study is to propose a new electrochemical method for the evaluation of the antioxidant capacity of the pharmaceutical antioxidant excipient in drugs. The evaluation was conducted by integrity testing of DNA bases, which were modified on an electrode. Guanine was selected as the electrochemical probe. Based on the changes of the oxidation current of guanine on a modified glassy carbon electrode (GCE), the protective capacity of the antioxidant for guanine was determined. TiO_2 nanoparticles (TiO_2NPs) adhering to maltiwalled carbon nanotubes (MWCNTs) were used for guanine oxidation via photo generating hydroxyl radicals. The MWCNTs were used, because of their unique ability to accelerate the electron transfer rate. Using a simple evaporation and drying process, the TiO_2NPs/MWCNTs, modified material was added to the GCE. In this report, sodium metabisulfite was selected as the object antioxidant for antioxidant capability determination. The detection results in real samples showed that the guanine/TiO_2NPs/MWCNTs/GCE biosensor is appropriate for evaluating the antioxidant capacity of antioxidants in drugs.

1. Introduction

The oxidation process is a major factor in drug deterioration. This results in the changes in the drug components, which are the main factors in maintaining the effectiveness of a drug. The oxidation of active ingredients in a pharmaceutical drug is caused primarily by atmospheric oxygen and a small amount of ozone, hydrogen peroxide, metal oxide and other agents. The hydroxyl radical (•OH), is one of the free radicals that possesses strong oxidation ability and is known to be influential in the pharmaceutical instability. Hydroxyl radicals can be neutralized by pharmaceutical antioxidant excipient. A very low concentration of antioxidant can significantly delay, inhibit or prevent the oxidation of the active ingredients in a pharmaceutical. Therefore, antioxidants are very important in maintaining the stability of the pharmaceutical active ingredients [1–3].

Various analytical methods have been used to determine the capacity of antioxidants. For example, high performance liquid chromatography (HPLC) employing salicylic acid as a scavenger has been employed in the Fenton process [4]. Capillary Zone Electrophoresis with Amperometric Detection (CZE-AD) has also been used [5]. Electronic Spin Resonance (ESR), employing phenyl-tert-butylnitrone (PBN) as a scavenger, has been used in the Fenton process [6]. Other •OH scavengers that have been employed include dimethyl sulfoxide (DMSO) [7], salicylate and 4-hydroxybenzoate [8]. Although these methods offer high accuracy, they have inherent disadvantages, such as complex operation and high cost. By contrast, electrochemical method offers high sensitivity, simplicity, speed, low cost, miniaturization and other advantages, making real-time, on-line analysis possible [9]. Since the discovery of the electrochemical activity of DNA on a mercury electrode by Palecek [10], a large number of experiments applicable to modern electrochemical methods for the direct determination of DNA have been suggested for interaction and hybridization studies.

There have been reports in literature of a series of electrochemical DNA biosensors that are based on the theory of free radical induced DNA damage, which have been used to evaluate the antioxidant capacity of food or biological samples. The double-stranded (ds) DNA [11–13] and purine bases [14–15] immobilized on an electrode were used as the oxidation markers. Quantitative detection of DNA is usually based on the electrochemical activity of guanine or adenine. When •OH attacks DNA, the primary site of the oxidative damage is guanine. Electrochemical methods have been used to study the mechanism of guanine oxidation on carbon based electrode [16]. Following the oxidation reaction of guanine, it is conversed into guanine groups. The intermediate product of the electrochemical oxidation process of guanine on carbon based surfaces is 8-oxoguanine (8-oxoG) [17] which is an irreversible $4e^-$ oxidation reaction. Finally, the specific product of the oxidative degradation of guanine is 8-hydroxy-guanine (8-OHdG). The addition of an antioxidant, will promote competition with DNA for •OH, so that the increased oxidation signal of DNA reflects the capacity of antioxidant. Considering the properties of conventional bare electrode surface, the direct oxidation of guanine is very difficult, because of the slow electron transfer kinetics and high oxidation potential [18–19]. One of the

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http://dx.doi.org/10.1016/j.jelechem.2016.03.008
1572-6657/

important tasks in electroanalytical science is the preparation of chemically modified electrodes. The main purpose for modifying the bare electrode is to improve the electron transfer rate between the electrically active material and the bare electrode. Carbon nanotubes (CNTs) are an ideal one-dimensional carbon based material, because they are highly conductive at room temperature. Ballistic transport is a special phenomenon of CNTs where the electrons move freely through the structural system without scattering [20]. Due to this unique property, CNTs are excellent electrode modifying materials and as such have attracted considerable attention [21–22].

The Fenton reaction ($Fe(II)/H_2O_2$) is the most commonly used reaction for generating •OH radicals. However, it is difficult to distinguish if the resulting electrochemical signal is due to •OH free radical scavenging or iron chelation, because many antioxidants can chelate iron ions. In addition, some antioxidants, such as ascorbic acid, can reduce Fe (III) to Fe(II) thus promoting the Fenton reaction. The Fenton reaction in antioxidants evaluation will cause these interferences, so a chemical environment that lacks metal ions is more suitable for •OH generation.

Recently, researchers have become interested in the application of TiO_2 photo-catalysis to induce free radicals [23–25]. Many workers have reported the use of TiO_2 to generate a variety of free radicals during photochemical reactions. The •OH radical is generally considered to be the main product of the photo-catalytic oxidation reaction. The mechanism of the TiO_2 photo-catalytic progress can be explained as follows. Initially, photo-catalytic generated holes are formed when TiO_2 particles are irradiated with UV light (Eq. (1)). Next, OH- or H_2O holes is oxidized at these holes to produce •OH radicals (Eq. (2), Eq. (3)), and •OH radicals have very strong destructive effect on organic species. Finally, oxygen effectively prevents the generation of holes and electrons (Eq. (4)), thus ending the chain reaction.

$$TiO_2 + h\nu \rightarrow TiO_2\ (e_{CB}^- + h_{VB}^+) \quad (1)$$

$$TiO_2(h_{VB}^+) + OH^- \rightarrow TiO_2 + {}^{\cdot}O{\cdot}H \quad (2)$$

$$TiO_2(h_{VB}^+) + H_2O \rightarrow TiO_2 + H^+ + {}^{\cdot}O{\cdot}H \quad (3)$$

$$TiO_2(e_{CB}^- -) + O_2 \rightarrow TiO_2 + O_2^{-\cdot} \quad (4)$$

The objective of this study was to investigate the antioxidant properties of pharmaceuticals. The process began with the establishment of a guanine electrochemical probe as the oxidation target. Second, the TiO_2 photo-catalytic system was used to generate •OH radicals. Third, the oxidation damage of guanine was accompanied by the generation of •OH. Finally, the performance of antioxidants in the drug was evaluated based on the variation of the guanine oxidation peak current. The capacity of antioxidants in real pharmaceutical samples was evaluated using the proposed guanine TiO_2NPs/MWCNTs/GCE biosensor (depicted in Scheme 1).

2. Experimental

2.1. Chemicals

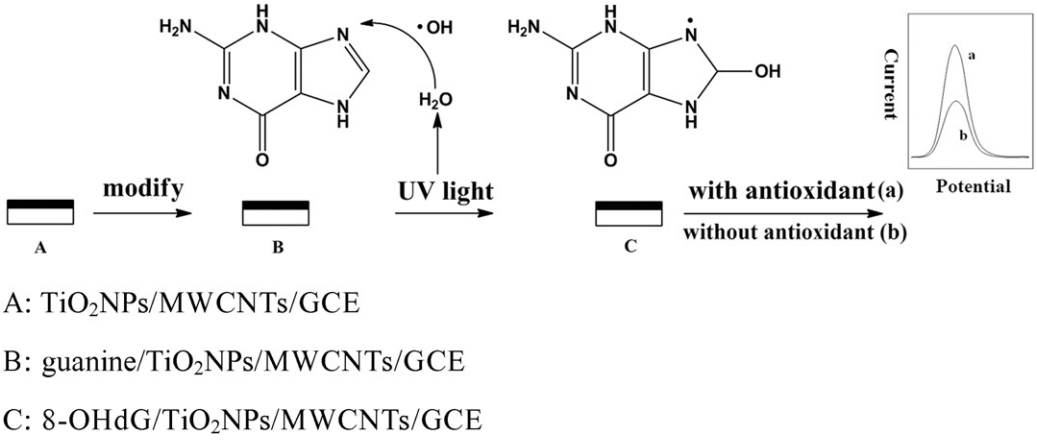
Guanine (G 6779, Bioultra), TiO_2 (10 nm, 99.5% trace metals basis) and sodium metabisulfite ($Na_2S_2O_5$, analytical standard) were purchased from Sigma. A concentrated guanine (1 g L^{-1}) solution was prepared by dissolving the solute in 1 mol L^{-1} NaOH. Guanine solutions of different concentrations were prepared by diluting the concentrated stock solution with PBS (0.1 mol L^{-1}) before use. A 0.5 g L^{-1} $Na_2S_2O_5$ standard solution was prepared daily and was immediately used after preparation. This solution was stored in a refrigerator and preserved to avoid light exposure. A 0.1 mol L^{-1} PBS (pH 7.4) solution was used as the electrolyte throughout the electrochemical experiments. Adrenaline Hydrochloride Injection was obtained from Sinopharm Group Co., Ltd. Sub boiling water (room temperature) was used in the experiments. The MWCNTs used in this investigation were obtained from Shenzhen Nanotech Co., Ltd. (Guangdong, China). Other chemicals used in the experiment were of analytical grade without any purification.

2.2. Apparatus

A CHI 660d electrochemical workstation (Shanghai Chenhua Instrument Corporation, China) was used for all the electrochemical measurements. A glassy carbon working electrode with effective area of 7.07 mm^2, a saturated calomel reference electrode and a counter electrode of platinum wire (length of 10 cm, diameter of 0.5 mm) completed the three-electrode system. The electrolytic cell was a 10 ml beaker. A Scanning Electron Microscopy (SEM) was used in this work; a su8010 SEM (HITACHI Corporation, Japan) operating at 10 kV was employed. An UV lamp (model of ZF7, Yuhua Instrument Corporation) was used for •OH generation. A pH-meter (pH S-3C, Leici Instrument Corporation, Shanghai) was used for determining the pH of the solutions.

2.3. Preparation of TiO_2NPs/MWCNTs

The modified materials, TiO_2NPs/MWCNTs, were prepared according to the literature [26]. This process entailed first, the MWCNTs suspension was obtained by dispersing 10 mg MWCNTs in distilled water (100 ml) and then sonicated for 10 min. Second, the MWCNTs suspension and TiO_2 powder with the mass ratio 1:20 were mixed and added to a 150 ml flask with sonication for 10 min. After the sonication, the



Scheme 1. The principle of guaninne/TiO_2NPs/MWCNTs sensor.