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Screen-printed enzymatic biosensor modified with carbon nanotube for the methimazole determination in pharmaceuticals formulations

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ABSTRACT

This paper describes the development of a screen-printed sensor, modified with carbon nanotubes for the rapid and sensitive quantification of methimazole (MT) in pharmaceuticals formulations. Tyrosinase [EC 1.14.18.1], immobilized on a rotating disk, catalyzed the oxidation of catechols to *o*-benzoquinone, whose back electrochemical reduction was detected on graphite screen-printed electrodes modified with carbon nanotubes at −150 mV. Thus, when MT was added to the solution, this thiol-containing compound participate in Michael type addition reactions with *o*-benzoquinone to form the corresponding thioquinone derivatives, decreasing the reduction current obtained proportionally to the increase of its concentration. This method could be used to determine MT concentration in the range of 0.074–63.5 μM ($r = 0.998$). The determination of MT concentration was possible with a detection limit of 0.056 μM in the processing of as many as 25 samples per hour. The biosensor has a reasonable reproducibility (R.S.D. < 3.50%) and a very stable amperometric response toward this compound (more than 1 month). The application of this analysis to different pharmaceutical samples containing MT supports the utility this biosensor.

1. Introduction

Methimazole (MT, 1-methyl-2-mercaptoimidazole, tapazole) is an orally active drugs used in the therapy of hyperthyroidism. MT is absorbed by the gastrointestinal tract and concentrates in the thyroid gland [1]. MT is widely used in medicine for treatment of hyperthyroidism and even as model substance for endocrine disruption in physiological and genomic studies. Its action is to slow iodide integration into tyrosine and thus inhibits the production of thyroid hormones.

Methimazole is used as a drug to manage hyperthyroidism associated with Grave's disease, but it has side effects as possible decrease of white blood cells in the blood [2]. MT has also been employed to promote growth in animals for human consumption.

In human body, methimazole is metabolized to *N*-methylimidazole and sulfite via sulfenic and sulfinic acid intermediates that are associated with the cytotoxic effects [3]. Substantial portion of orally taken drug is excreted with urine [4]. It has been reported that methimazole may also cause side effects, such as nephritis, liver cirrhosis, irritation of the skin, allergies and pharyngitis with fever [5].

Several analytical procedures have been described for the determination methimazole in different samples. Techniques used were gas chromatography–mass spectrometry (GC–MS) [6-8], high-performance liquid chromatography–mass spectrometry (HPLC–MS) [9,10], HPLC with ultraviolet detection [11,12], potentiometric [13], titrimetric [14] and flow-injection with ultraviolet detection [15].

Screen-printing technique seems to be one of the most promising approaches allowing simple, rapid and inexpensive biosensors production [16]. The biosensors based on screen-printed electrodes have been extensively used for detections of biomolecules, pesticides, antigens and anions [17]. Electrochemical biosensors based on screen-printed electrodes are in tune with the requirements of in situ screening devices, since all the equipment needed for the electrochemical analysis is portable. They have all the major performance characteristics of biosensors, among them the minimum sample preparation, the simplicity of the apparatus, the obtaining of fast results, moreover they are cost effective, small and becoming miniaturized with new technologies [18].

Carbon nanotubes (CNTs) are a novel type of carbon material and can be considered as the result of folding graphite layers into carbon cylinders. There are two groups of carbon nanotubes, multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes (SWCNT) [19]. The CNTs have generated great interest in future applications based on their field emission and electronic transport properties [20], their high mechanical strength and their chemical properties [21].

The research has been focused on their electrocatalytic behaviours toward the oxidation of biomolecules and their perfor-

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mance has been found to be much superior to those of other carbon electrodes in terms of reaction rate, reversibility and detection limit [22]. The uses of CNTs for preparation of biosensors based on CNT-modified screen-printed electrodes have been reported previously [23–27].

Tyrosinase a two copper-containing enzyme, catalyzes the *o*-hydroxylation of monophenols (monophenolase activity) and the oxidation of *o*-diphenols (Q) to *o*-quinones (P) (diphenolase activity) [28–30]. Over the past decades, several reports on the tyrosinase action mechanism have been published [31–34]. This enzyme has been used extensively in the development of biosensors for the detection of phenolic compounds [35–37]. To the best of our knowledge, no study involving an enzymatic biosensor behaviour for MT has been reported. Thus, in this paper, we present and discuss for the first time the electrochemical and enzymatic reaction for MT determination, resulting in a single, fast and inexpensive analytical method as well as very sensitive devise based on tyrosinase rotating biosensor systems.

In this paper, we performed a screen-printed enzymatic sensor modified with MWCNT for rapid and sensitive quantification of MT in pharmaceutical preparations. Tyrosinase immobilized on a rotating disk, catalyzed the oxidation of catechol (Q) to *o*-benzoquinone (P), whose back electrochemical reduction was detected on graphite screen-printed electrodes (GSPE) at −150 mV versus Ag/AgCl/NaCl 3 M. Thus, when MT was added to the solution, this thiol-containing compound participate in Michael type addition reactions with P to form the corresponding thioquinone derivatives, decreasing the reduction current obtained proportionally to the increase of its concentration. A large number of samples can be processed by means of the proposed method, which shows adequate sensitivity, low cost, versatility, simplicity and effectiveness. Our aim was to develop a new method able to analyze pharmaceuticals formulations, avoiding or minimizing the number of steps needed to assess the concentration of the MT.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical reagent grade. The enzyme tyrosinase (from mushroom, EC 1.14.18.1, 2000 U mg^{-1}) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The enzyme concentration was determined taking the value of M_r as 120,000. Glutaraldehyde (25% aqueous solution) was purchased from Merck, Darmstadt. 3-Aminopropyl-modified controlled-pore glass, 1400 Å mean pore diameter and 24 m^2 mg^{-1} surface area, was from Electro-Nucleonics (Fairfield, NJ) and contained 48.2 mol g^{-1} of amino groups. GSPE was purchased from Eco-BioServices&Researches S.r.l. (Fienze, Italy). Catechol and MT were purchased from Sigma Chemical Co., St. Louis, and all other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q-system.

2.2. Flow-through reactor/detector unit

The main body of the cell was made of Plexiglas. Fig. 1 illustrates the design of the flow-through chamber containing the rotating disk and the detector system. The GSPE is on the top of the rotating reactor. The rotating reactor is a disk of Plexiglas into which a miniature magnetic stirring bar has been embedded. Rotation of the lower reactor was effected with a laboratory magnetic stirrer with control of temperature (Metrohm AG, Herisau, Switzerland) and controlled with a variable transformer with an output between 0

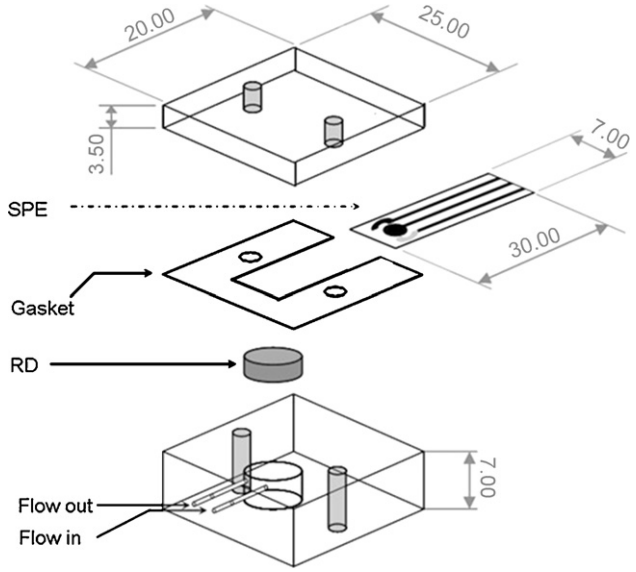

Fig. 1. Schematic representation of components in the bioreactor flow cell. SPE: Screen-printed electrode, RD: rotating disk. All measurements are given in millimetres. Gasket: Teflon, thickness: 0.3 mm.

and 250 V and maximum amperage of 7.5 A (Waritrans, Argentina). All solutions and reagents were conditioned to 37 °C before the experiment, using a laboratory water bath Vicking Mason Ii (Vicking SRL, Argentina).

Amperometric detection was performed using a BAS LC-4C potentiostat and BAS 100 B/W (electrochemical analyzer Bioanalytical System, West Lafayette, IN) was used to voltammetric determinations.

A pump (Wilson Minipuls 3 peristaltic pump, Gilson Electronics, Middleton, WI, USA) was used for pumping, introducing the sample, and stopping the flow. Fig. 2 illustrates schematically the components of the single-line continuous-flow setup. The pump tubing was Tygon (Fisher Accu Rated, 1.0 mm i.d., Fisher Scientific, Pittsburgh, PA, USA), and the remaining tubing used was Teflon (1.0 mm i.d. from Cole-Parmer, Chicago, IL, USA).

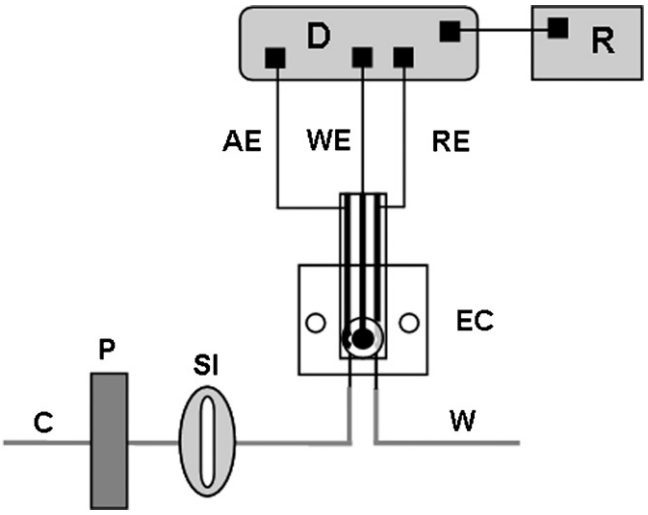

Fig. 2. Block diagram of the continuous-flow system and detection arrangement. P: Pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics, Inc. Middleton, WI); C: carrier buffer line; SI: sample injection; W: waste line; EC: cell containing the rotating disk and GSPE; WE: GSPE; RE: pseudo-reference electrode; AE: auxiliary electrode; D: BAS LC-4C potentiostat (Bioanalytical Systems, West Lafayette, IN, USA); R: recorder.