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Combination of electrochemical biosensor and textile threads: A microfluidic device for phenol determination in tap water

F.R. Caetano[a], E.A. Carneiro[a], D. Agustini[a], L.C.S. Figueiredo-Filho[b], C.E. Banks[c], M.F. Bergamini[a], L.H. Marcolino-Junior[a,*]

[a] Laboratório de Sensores Eletroquímicos (LabSensE), Departamento de Química, Universidade Federal do Paraná (UFPR), CEP 81.531-980 Curitiba, PR, Brazil
[b] Campus Paranavaí, Instituto Federal do Paraná, 87703-536 Paranavaí, PR, Brazil
[c] Faculty of Science and Engineering, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK

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ABSTRACT

Microfluidic devices constructed using low cost materials presents as alternative for conventional flow analysis systems because they provide advantages as low consumption of reagents and samples, high speed of analysis, possibility of portability and the easiness of construction and maintenance. Herein, is described for the first time the use of an electrochemical biosensor for phenol detection combined with a very simple and efficient microfluidic device based on commercial textile threads. Taking advantages of capillary phenomena and gravity forces, the solution transportation is promoted without any external forces or injection pump. Screen printed electrodes were modified with carbon nanotubes/gold nanoparticles followed by covalent binding of tyrosinase. After the biosensor electrochemical characterization by cyclic voltammetry technique, the optimization of relevant parameters such as pH, potential of detection and linear range for the biosensor performance was carried out; the system was evaluated for analytical phenol detection presenting limit of detection and limit of quantification 2.94 nmol L^{-1} and 8.92 nmol L^{-1} respectively. The proposed system was applied on phenol addition and recovery studies in drinking water, obtaining recoveries rates between 90% and 110%.

1. Introduction

The miniaturization of analytical systems began in the late 1970s, when Terry et al. (Reyes et al., 2002) presented a portable gas chromatograph, which was able to make small separations using thermal conductivity detection. From then on, microfluidic systems have experienced explosive growth since its development also they are known as micro total analysis system (μTAS). These devices have been fabricated using materials such as glass, polymers, hydrogels, paper and other materials (Burns et al., 1998; Neuži et al., 2012; Parolo and Merkoci, 2013).

In the last few years, cotton thread-based devices appear as a promising option to overcome some limitations imposed by paper-based microfluidic analytical devices (μPAD) such as low efficiency of sample delivery, low mechanical strength of wet paper, problems with low surface tension of the sample and the requirement of construction of hydrophobic barriers to delimit microchannels (Desmet et al., 2016; Santhiago and Kubota, 2013). Concerning properties for the use of cotton thread as low cost microfluidic analytical device, some advantages can be cited as liquid flow by capillary forces without external pumping, flexibility, high mechanical strength when wet, low cost, worldwide availability, disposability and not required construction of hydrophobic barriers to design microchannels (Mitchell et al., 2005; Nilghaz et al., 2015, 2013).

Most of the analytical work using cotton-thread devices consists in colorimetric detection which depends on ability of visual discrimination from analyst (Jia et al.; Nilghaz et al., 2015). The combination of cotton-threads and electrochemical detection is relatively new topic in electroanalytical procedures (Agustini et al., 2016). This first work related describes the construction of low cost microfluidic thread-based electroanalytical device (μ-TED) employing graphite electrodes which was used for simultaneous amperometric determination of acetaminophen and diclofenac and a second work describing the optimization of μ-TED devices (Agustini et al., 2017). Ochiai et al. (2017) described μ-TED using multiwalled carbon nanotubes (MWCNTs) modified screen printed electrodes (SPE) for electrochemical determination of estriol hormone using amperometry, obtaining a limit of detection of 5.3×10^{-7} mol L^{-1}.

The research in order to develop new biosensors for phenolic compounds determination has been increased in the last years, since

* Corresponding author.

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these compounds present potential hazard for aquatic life and human health (Mukherjee et al., 2013); usually appears in the environment via industrial wastes from a plenty of kinds of production such as plastics, dyes, drugs, resins, pesticides, and especially paper and cellulose (Villegas et al., 2016). Therefore, it is considered a priority pollutant by the American Environmental Protection Agency (EPA, 2016) which has established its limit concentration in drinking water as 1.0×10^{-8} mol L^{-1}.

In this present work, we report the first enzymatic biosensor employed as an electrochemical detector combined with μ-TED system. For this, Tyrosinase was covalently immobilized onto multiwalled carbon nanotubes and gold nanoparticles (GNPs/MWCNT) nanocomposite using a disposable carbon screen printed electrode (C-SPE) and the phenol detection was performed in tap water. The SPE provide disposable, planar, low-cost miniaturized size and incorporate the whole electrode system (working, reference and auxiliary) in a single device, which are suitable characteristics for the proposed microfluidic device (Mohamed, 2016). In electroanalysis, more specific for development of new biosensors architecture, gold nanoparticles have been highly employed due to some characteristics such as a good mechanical resistance and electrical properties, high surface area for enzyme immobilization and biocompatibility (Gevaerd et al., 2015; Lan et al., 2017; Vicentini et al., 2016; Vidotti et al., 2011). Tyrosinase (Tyr) is also known as polyphenol oxidase (PPO) or catechol oxidase (Shleev et al., 2005; Yaropolov et al., 1996), catalyzes the oxidation reactions, such as: the hydroxylation of monophenols to o-dihydroxy phenols, and subsequently the oxidation-dihydroxy phenols to o-quinones in the presence of molecular oxygen. The o-quinone can be easily detected by electrochemical techniques such as voltammetry or amperometry (Kochana et al., 2015; Tan et al., 2011).

2. Experimental section

2.1. Reagents

All the chemicals were analytical grade and used as received from Sigma–Aldrich. Multi-walled carbon nanotubes sample (MWCNTs) was purchase from Dropsens (purity of 95%, length 1.5 μm, diameter 10 nm). All solutions were prepared with deionized water (specific resistivity > 18 MΩ cm) obtained with a Millipore Direct-Q3 water purification system.

2.2. Synthesis of GNPs/MWCNT nanocomposite and SPE modification

The nanocomposite (GNPs-MWCNTs) used as platform for *tyrosinase* immobilization was synthesised following the procedure fully described in a previous work by Caetano et al. (2017). Briefly, the methodology consists in a biphasic system (water/toluene) where the reduction of gold precursor $H[AuCl_4]$ by $NaBH_4$ in presence of MWCNTs is performed. As a result, well dispersed gold nanoparticles (7 ± 4 nm) onto multiwalled carbon nanotubes were obtained.

Electrode printing process was carried out using the appropriate masks and a microdek 1670RS printer which the steps consisted of the deposition of graphite carbon inks (for working electrode and auxiliary electrode printing), Ag / AgCl (reference electrode) and dielectric ink (Gwent, Liverpool, UK) on a polyester substrate (Rana et al., 2017)

A 1.0 mg mL^{-1} suspension of GNPs-MWCNTs in isopropyl alcohol containing 0.05% (w/v) of Nafion® was subjected to ultrasonication for 20 min. The chemically modified electrode was prepared by drop casting using 3.0 μL of suspension onto the C-SPE (geometrical area = 0.07 mm^2) and let dry at room temperature for 1 h. Cyclic Voltammograms were recorded using a conventional reference electrode Ag/AgCl (KCl 3.0 mol L^{-1}).

2.3. Tyrosinase immobilization

Working electrode containing GNPs-MWCNTs was chemically modified with cystamine (CYS) and glutaraldehyde (GA) to link the Tyr covalently. Firstly, 20 μL of 50 mmol L^{-1} CYS solution was casted onto the electrode surface, and the solvent was evaporated at room temperature for 1 h, and then rinsed with 0.1 mol L^{-1} phosphate buffer solution (PB, pH 6.5). Subsequently, 20 μL of 2.5% (v/v) GA solution was casted onto surface of GNPs-MWCNTs modified electrode and the solvent was evaporated at room temperature for 1 h. The electrode was washed with 0.1 mol L^{-1} PB solution (pH 6.5). Finally, 20 μL of a solution containing 100 units of Tyr (5 KU mL^{-1} in PB solution 0.05 mol L^{-1}, pH 6.5) was casted onto the electrode surface and the solvent was evaporated at room temperature overnight. Biossensor (Tyr-GNPs-MWCNTs/SPE) was washed with with 0.1 mol L^{-1} PB solution (pH 6.5) and kept at −4 °C.

2.4. Construction of the thread-based electroanalytical device (μTED) and Electrochemical measurements

The material used as substrate was ABS (Acrylonitrile-butadiene-styrene) polymer using a GTMax3D printer - Graber i3 model (Americana – SP, Brazil). The proposed device (Fig. 1) was developed by printing a plastic support using a 3D printer (25 mm of wideness, 6.5 mm of thickness and length 90, 60 and 30 mm) were used as the substrate for assembly of the μTED. The scheme presented in Fig. 1 shows the device structure. The device construction steps consists in (i) placement of two pieces of double sided scotch tape near the inlet and outlet reservoirs; (ii) accommodation of Tyr-GNPs-MWCNTs/SPE on the double-sided tape next to the outlet reservoir; (iii) fixation of arrangement of microchannels which are formed hydrophilic threads (9 parallel threads without twisting) throughout the device, from the inlet reservoir to the outlet reservoir; (iv) placement of two pieces of double sided tape on the ends of the hydrophilic gauze. Detection zone was covered by pieces of cotton thread in order to maintain all electrodes immersed in solution during measurements.

2.5. Transmission electron microscopy, Scanning Electron Microscopy, X-ray diffraction and electrochemical analysis

The size and distribution of the GNPs were determined by means of transmission electron microscopy (TEM) using a JEOL JEM 1200 operated at 120 kV. The samples were prepared by dropping the nanohybrid suspension standard hole cupper grids covered by a thin parlodium film. Scanning electron microscopy (SEM) analysis were carried out in a TESCAN Vega 3 equipment, operated at 120 kV. X-ray diffraction measurements were carried out in a Shimadzu XRD–3A

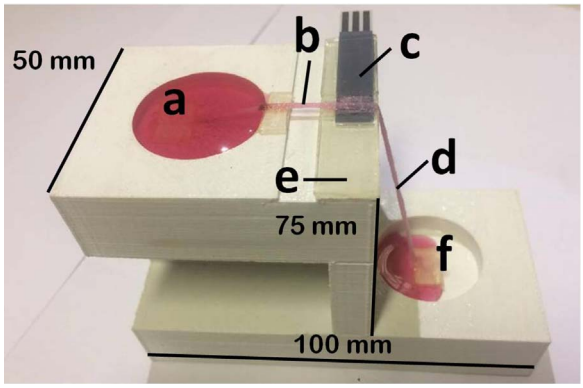

Fig. 1. Dimensions and constituents parts μ-TED a) Inlet reservoir b) injection zone c) SPE (Electrochemical detection zone) d) Hydrophilic textile thread e) Adhesive tape f) outlet reservoir. To demonstrate the solution flux on threads, a food coloring was employed.