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Label-free disposable immunosensor for detection of atrazine

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ABSTRACT

This work reports the construction of a fast, disposable, and label-free immunosensor for the determination of atrazine. The immunosensor is based on a field effect transistor (FET) where a network of single-walled carbon nanotubes (SWCNTs) acts as the conductor channel, constituting carbon nanotubes field effect transistors (CNTFETs). Anti-atrazine antibodies were adsorbed onto the SWCNTs and subsequently the SWCNTs were protected with Tween 20 to prevent the non-specific binding of bacteria or proteins. The principle of the immunoreaction consists in the direct adsorption of atrazine specific antibodies (anti-atrazine) to SWCNTs networks. After exposed to increasing concentrations of atrazine, the CNTFETs could be used as useful label-free platforms to detect atrazine. Under the optimal conditions, a limit of detection as low as 0.001 ng mL^{-1} was obtained, which is lower than that of other methods for the atrazine detection, and in a working range between 0.001 and 10 ng mL^{-1}. The average recoveries obtained for real water samples spiked with atrazine varied from 87.3% to 108.0%. The results show that the constructed sensors display a high sensitivity and could be useful tools for detecting pesticides like atrazine at low concentrations. They could be also applied to the determination of atrazine in environmental aqueous samples, such as seawater and riverine water.

1. Introduction

Due to the increasing worldwide use of pesticides, there is a perceived threat of environmental damage as well as health issues. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, ATZ) is one of the most used pesticides in agriculture, in order to optimize yields of crops such as corn, sugar cane, and sorghum [1]. It is considered a persistent environmental contaminant, occurring at trace levels in groundwater, and it has been recognized as mutagenic and teratogenic besides having effects on the reproductive, endocrine, central nervous, and immune systems [1]. According to Lasserre et al. [1], ATZ has effects on the expression of several proteins located in different cellular compartments (nucleus, cytosol, membrane) and it is involved in various processes such as oxidative stress, DNA damages, shape of the cell, gene expression regulation or spermatogenesis. In 2004, ATZ was banned in the European Union because of its persistent groundwater contamination [2,3].

Many analytical methods have been developed for the determination of ATZ based on high performance liquid chromatography (HPLC) coupled to diode array detection (HPLC-DAD), HPLC coupled to tandem mass spectrometry, LC coupled to mass spectrometry (LC–MS), and gas chromatography coupled also with mass spectrometry (GC–MS) [4–9]. Techniques, such as GC–MS requires extraction using organic solvents and expensive equipment. Moreover, these methods cannot be used for continuous and on-site analysis [5]. Recently, the enzyme-linked immunoassays, such as ELISA and other immunosensors to ATZ have also been reported [10–12]. The binding properties of an antibody to an antigen have been used for the development of a broad variety of analytical techniques and sensors for clinical and environmental monitoring [13–15]. Besides, other immunosensors namely electrochemical immunosensors [16–19], surface plasmon resonance (SPR) biosensors [20,21], and optical immunosensors [22] have been also constructed for ATZ detection.

The incorporation of nanomaterials in sensors for the development of a wide variety of nano-electronic systems with

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applications on environmental, food and clinical analysis has been reported, since such nanostructures display particular electrical, chemical and transport properties [23]. For example, SWCNTs are one-dimensional nanostructures which show high electron transfer reactions due to the presence of all carbon atoms in their surface [24]. Thus, their large surface area together with the possibility of functionalization with different molecules allowed turning them into ideal sensors for the specific detection of various analytes and even viruses [24,25]. CNTFETs have become a suitable system for immunosensor applications because they combine the principles of molecular recognition through the recognition layer with the transduction capabilities of the carbon nanotubes and they have been used to detect various compounds such as proteins [26–28]. In this work, a simple disposable immunosensor based on CNTFETs principle was constructed for the label-free detection of ATZ with potential application in seawater and riverine water analysis.

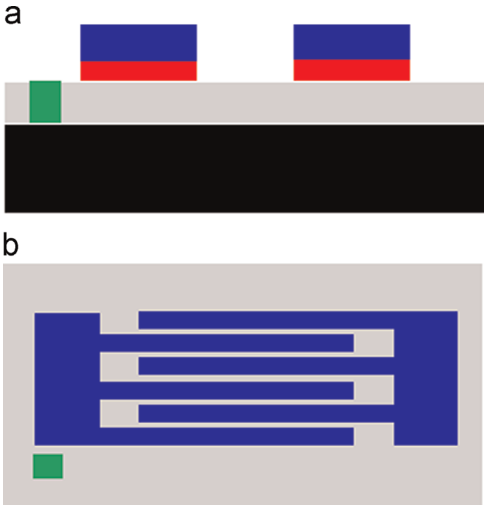

Fig. 1. Schematic (a) cross view and (b) top view with final architecture of fabricated FET (at blue: Au layer; at red: Ti layer; green: Cr/Au layer-gate electrode; at gray: SiO_2 layer; at black: Si wafer). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Reagents

SWCNTs synthesized through cobalt-molybdenum catalysis (CoMoCAT, Southwest Nanotechnologies), sodium cholate (SC), Dulbecco's saline phosphate buffer (PBS, pH 7.4), Tween 20, acetone, 1-propanol, desethylatrazine, hydroxyatrazine, and ATZ (purity: 99.1%) were purchased from Sigma-Aldrich. Anti-atrazine (Anti-ATZ) was purchased from Antibodies-online (1.17 mg mL^{-1}). All chemicals and solvents were of commercially available analytical reagent grade. Anti-ATZ was dissolved with 0.01 M phosphate buffer solution (PBS, pH 7.4) and stored at 4 °C. ATZ was dissolved with PBS containing 25 μl of Tween 20 (PBST).

2.2. Preparation and characterization of SWCNTs dispersion

SWCNTs (approximately 4 mg) were dispersed in 14 mL of aqueous solution of SC (0.2% *w*/*v*). Based on the work of Justino et al. [29], where the experimental conditions for the dispersion of SWCNTs were optimized through the application of an experimental design, the SWCNT dispersion in this work was obtained after 60 minutes of sonication (Branson 2510) and then 7 min of centrifugation (Benchtop centrifuges K2015) at a relative centrifugal force of 2000 × *g* at 20 °C. UV-visible spectra (Shimadzu UV-2101PC) were obtained were obtained between 400 and 800 nm.

2.3. FET fabrication

The final FET architecture is shown in Fig. 1.

The FET were fabricated in three inch silicon wafer (in black, Fig. 1a) and in the final configuration, they were based on ten interdigitated electrodes of 1.5 μm of width and 1000 μm of length (in blue, Fig. 1b). For that, various steps were followed: (a) passivation of wafer with SiO_2 (400 nm-thick layer, in gray, Fig. 1a) by plasma enhanced chemical vapor deposition (Electrotech Delta CVD System); (b) deposition of Ti (10 nm-thick, in red, Fig. 1a) and Au (100 nm-thick, in blue, Fig. 1a) on Si/SiO_2 substrate through physical vapour deposition by sputtering (Alcatel Magnetron Sputtering System); (c) definition of source and drain metal electrodes through optical lithography (Direct Write Laser 2.0, DWL, Heidelberg Instrument), using a 1.5 μm-thick photoresist layer (PFR 7790 G-27 cP, JSR Corporation) coated in a standard spin coating system; (d) upon development, photoresist layer defines the mask for the ion beam etching (Nordiko 3000 Series) of the Au film; (e) removal of the photoresist film through immersion of the wafer in a stripper solution (Microstrip® 2001, Fujifilm) at 65 °C; (f) coating of a photoresist film (1.5 μm) on the wafer and definition of a second mask (non-inverted) by optical lithography (DWL system) in order to define the back gate electrode (at green, Fig. 1b), with definition of an opening in the resist (as shown in Fig. 1a); (g) removal of 500 nm of SiO_2 by reactive ion etching (LAM Rainbow 440 System); (h) deposition of Cr/Au (50 nm-thick/100 nm-thick) films by ion beam deposition (Nordiko 3000 Series) immediately after ion beam etching (without vacuum break)-the photoresist and the Cr/Au films were removed by liftoff, immersing the wafers in a microstrip solution (Microstrip® 2001, Fujifilm) at 65 °C. After such processing steps, the wafers were cleaned (isopropanol and distilled water), dried with N_2, and the silicon wafer was diced (DISCO DAD 321) in order to promote individualized FET (~3 × 2 mm^2). Each FET was then mounted into a printed circuit board, fixed and wirebonded with Al wires (25 μm Ø), which were protected with a silicone gel (Elastosil E41, Wacker) also to produce an open chamber (~1 mm Ø) for further sensing experiments. Electrical measurements were made using a 4155 C Agilent semiconductor parameter analyzer, which was linked to a closed test fixture (Agilent 16442 A) where the devices were positioned; in the test fixture, the drain, gate, and source of each FET were connected to respective terminals to provide electrical circuit for sensing measurements.

2.4. Immunosensor surface functionalization

Immunosensor surface functionalization was performed in two steps. Firstly, the FET surface was washed with acetone, 1-propanol, distilled water and dried under N_2 and then, 2 μl of SWCNTs dispersion was deposited on the clean FET surface for 15 min at room temperature, in order to obtain the CNTFETs. Anti-ATZ, which is the recognition molecule, was immobilized over the surface of the CNTFETs by dropping 2 μL of an anti-ATZ solution in PBS (10 mg L^{-1}) onto the surface of CNTFETs. After adsorption of the anti-ATZ, the CNTFETs were dried with N_2 and electrically characterized the I_D*vs.* V_D, between 0 and +2 V.

2.5. Detection of atrazine

In order to detect the atrazine, 2 μl of ATZ solution at different concentrations from 0.001 to 10 ng mL^{-1} (5 decimal concentrations) were dropped into five individual CNTFETs per