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Carbon nanotube β-cyclodextrin modified electrode as enhanced sensing platform for the determination of fungicide pyrimethanil

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ABSTRACT

A sensitive electrochemical sensor was successfully developed based on a glassy carbon (GC) electrode modified by a combination of multi-walled carbon nanotubes (MWCNT) with β-cyclodextrin (β-CD) incorporated in a polyaniline film, and applied to detect and determine the fungicide pyrimethanil in pome fruit (apples). The β-CD/MWCNT modified GC electrode displayed a detection limit of 1.04 μM (0.21 mg/kg) which is below the maximum residue levels set for pyrimethanil in pome fruit and citrus fruit by EU regulations. The results indicate that the β-CD/MWCNT modified GC electrode exhibits efficient electrocatalytic oxidation of pyrimethanil with high reproducibility, repeatability and stability. Furthermore, the obtained results were in excellent agreement with those obtained using an established HPLC procedure.

1. Introduction

Plants make up the majority of the earth's living environment. Directly or indirectly, plants make up all the food on which humans and animals depend. The European and Mediterranean area is characterized by a great diversity of cultivated and wild species. Different species of cereals, fruit orchards and vineyards are grown over a large proportion of cultivated land in most European countries. The Mediterranean Diet, recently nominated as World's Intangible Cultural Heritage by UNESCO, emphasizes primarily plant-based foods such as fruits, vegetables, nuts, healthy fats, e.g. olive oil, and drinking red wine in moderation. Some of these food crops can be affected by a range of pests and diseases, causing significant losses to farmers and threatening food security. It is estimated that diseases, insects and weeds together interfere with the production of, or destroy, between 31 and 42% of all crops produced worldwide annually (Agrios, 2005). Over the last century, the control of plant diseases and other plant pests has depended increasingly on the extensive use of pesticides (Agrios, 2005). Some of these chemicals are applied to protect food crops from the pests at various stages of cultivation and during post-harvest storage. However, the risk of residues remaining on the food is of major concern in food safety issues. To regulate the safety of foods, government agencies have set tolerance levels or maximum residue limits (MRLs) for pesticide residues on food commodities (European Food Safety Authority [EFSA], 2015; Environmental Protection Agency [EPA], 2015).

Pyrimethanil, *N*–(4,6–dimethylpyrimidin–2–yl)aniline (Scheme 1), belongs to the anilino–pyrimidine class of fungicides and has been used worldwide for pre-harvest foliar application or for post-harvest commodity treatments (EFSA, 2011). This broad-spectrum fungicide is effective against diseases caused by *Botrytis*, *Monilinia*, *Venturia* and *Penicillium* spp. and other pathogens (Gullino, Leroux & Smith, 2000; Kanetis, Forster, & Adaskaveg, 2007; Sholberg, Bedford, & Stokes, 2005). The application rates of pyrimethanil during the growing season are in average 600 g ha^{-1} in apple orchards or 1 kg ha^{-1} in vineyards (EFSA, 2011). The metabolism of pyrimethanil in primary crops was investigated and it was shown that metabolites of pyrimethanil were present at levels significantly below those of the parent (EFSA, 2011). Consequently, the residue definition for foliar and post-harvest treatment in all crop groups was defined as pyrimethanil only, for both enforcement and risk assessment (EFSA, 2011). Currently,

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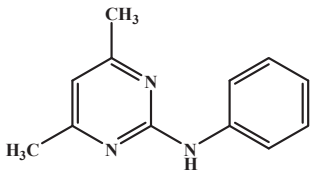

Scheme 1. Molecular structure of pyrimethanil (PYR).

pyrimethanil residues are most commonly monitored using gas chromatography or high performance liquid chromatography (HPLC) coupled with either selective detectors or mass spectrometry (Navickiene & Ribeiro, 2004; Gulaboski & Pereira, 2009; Vaquero-Fernández, Sanz-Asensio, Fernández-Zurbano, López-Alonso, & Martínez-Soria, 2013; Yáñez-Sedeño, Riu, Pingarrón, & Rius, 2010). However, these methods are in general time-consuming, labour-intensive and not environmentally friendly. Thus, it is very necessary to develop simple, rapid and low cost methods for the determination of pesticide residuals in fruits.

Electrochemistry provides powerful and versatile tools for food analysis owing to their simplicity, rapidity, affordability and miniaturization for on-site detection (Gulaboski et al., 2009). The use of electrochemical sensing approaches has been widely exploited as an inexpensive method to sensitively detect a variety of compounds, including pesticides. The physical and catalytic properties make carbon nanotubes (CNT) ideal for use as electrode materials in sensors. CNT-based sensors in general exhibit low limits of detection and fast response due to the signal enhancement provided by high surface area and rapid electrode kinetics (Vashist, Zheng, Al-Rubeaan, Luong, & Sheu, 2011; Yáñez-Sedeño et al., 2010). Composite materials formed with CNT have also been prepared with the aim of improving operational characteristics of the resulting sensors in terms of selectivity, stability or sensitivity. Nanocomposites of β-cyclodextrin (β-CD) and multiwalled CNT (MWCNT) deposited on a glassy carbon electrode (GCE) have recently been successfully used to study and quantify many organic molecules owing to the synergistic effect of both materials (Rahemi et al., 2012, 2013; Shen & Wang, 2009). The great significance of cyclodextrins lies in their ability to selectively form inclusion complexes (host–guest complexes) with many organic and biological molecules.

A number of reports have recently appeared highlighting the synergetic performance of PANI–CNT composites in certain applications including sensors (Gajendran & Saraswathi, 2008). The electrochemical properties of these PANI-CNT composites are greatly enhanced compared to the individual components (Gajendran and Saraswathi, 2008). Recently it has been shown that the sensitivity of PANI–CNT composite sensors can be improved by incorporation of CDs (Rahemi et al., 2012, 2013). The usefulness of CDs is related to their unique structures and to the fact that CDs retain their analyte-recognizing and entrapping properties under a relatively broad range of experimental conditions (Szente & Szemán, 2013). The interfacial architecture and electrochemical activity of PANI/CNT-modified electrodes after incorporating CDs and the resulting recognition effects were already reported (Rahemi et al., 2012, 2013; Shen & Wang, 2009).

The purpose of the present study is the establishment of an analytical methodology for the determination of pyrimethanil (PYR) residues in fruits using a polyaniline-β-CD/MWCNT-modified glassy carbon electrode. The chemical recognition of pyrimethanil by β-CD is combined with the added advantage of a faster electron transfer process due to the functionalised MWCNT, dispersed in the conducting PANI matrix. The new analytical methodology developed has been employed for the direct oxidative determination of pyrimethanil in apples by cyclic voltammetry.

2. Experimental

2.1. Reagents

Multi-walled carbon nanotubes (MWCNTs) were obtained from NanoLab (USA). Pyrimethanil, aniline and β-cyclodextrin (β-CD) were supplied by Sigma–Aldrich Química (Sintra, Portugal). All other chemicals and reagents (Sigma–Aldrich Química) employed were of analytical grade and were used as received without any further purification.

All solutions were prepared with deionised water (Milli-Q-50 18 MΩ cm). Buffer solutions employed for voltammetric determinations were 0.1 mol L^{-1} in the pH range 3–9.

HPLC–grade methanol was supplied by Carlo Erba. The solvents were filtered through a 0.45–μm filter before use.

2.2. Apparatus

Voltammetric experiments were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (Metrohm Autolab, Netherlands). All measurements were conducted in a one-compartment glass electrochemical cell equipped with a three-electrode system arrangement. The working electrode used was a bare or a modified glassy carbon electrode (GCE, d = 2 mm), the counter electrode was a platinum wire, with a saturated Ag/AgCl reference electrode completing the circuit. All measurements were carried out at room temperature.

The pH measurements were performed using a Crison pH-meter (Crison, Spain) equipped with a glass electrode.

HPLC analysis (Navickiene & Ribeiro, 2004) was performed using a Shimadzu LC-20AD Prominence Liquid Chromatograph (Shimadzu, Tokyo, Japan) with a diode array detector (SPD-M20A). Separation was performed on a prepacked Nucleosil 100-5 C18, analytical column (250 mm × 4.6 mm, 5 μm, Macherey–Nagel, Duren, Germany) and the mobile phase consisted of methanol–-water (70:30, v/v). It was delivered isocratically at 1 mL min^{-1} at room temperature.

The chromatographic data was processed using the software package LabSolutions (Shimadzu, Japan).

2.3. Preparation of MWCNT modified GCE

The preparation and characterization of the PANI-β-CD/fMWCNT modified GCE has been previously described (Rahemi et al., 2012). Briefly, two milligrams of (–COOH) functionalised MWCNT (fMWCNT) was dispersed by using ultrasonic agitation in 1 mL aqueous β-CD solution (2%) to give a 2 mg mL^{-1} black suspension. Before surface modification, the 2 mm bare GCE was carefully polished to a mirror finish with an aqueous slurry of alumina powder (BDH Chemicals, VWR, USA) on a microcloth pad and then ultrasonically cleaned in ultra-pure water and ethanol alternately to remove traces of alumina and possible contaminants. Subsequently, a solution of aniline (0.011 mol L^{-1}) was electropolymerized on the cleaned GCE, in a sulphuric acid aqueous solution (0.025 mol L^{-1}), sweeping the potential between −0.1 V and 1.0 V vs. Ag/AgCl at a scan rate of 50 mV s^{-1} for 50 cycles. After preparation of the polyaniline film on the GC electrode surface, an aliquot of 6 μL (2 mg mL^{-1}) of the MWCNT or fMWCNT dispersion was drop cast onto the GCE surface and dried in air at ambient temperature. Finally, the surface of the PANI-β-CD/MWCNT modified GCE was gently washed with water to remove the loosely attached β-CD/MWCNT.

The PANI-β-CD/fMWCNT film coated GC sensor was activated in acetate buffer solution (pH 4.0) by cyclic voltammetric sweeps between +0.5 and + 1.3 V vs Ag/AgCl until stable cyclic