



Proceeding Paper Development of a Portable Electrochemical Platform with Chip-Integrated Gold Electrodes for Detection of Pharmaceutical Pollutants ⁺

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Abstract: Electrochemical portable sensing systems can offer viable support in the analysis of environmental contaminants due to the compactness of their electronic components and overall simplicity of their detection principles. In the present work, a new electrochemical portable platform (EPP) with miniaturized chip-integrated gold electrodes was developed and applied in the detection of the drug acetaminophen (APAP) as a model analyte. The produced miniaturized chip-integrated gold electrodes were first characterized via atomic force and scanning electron microscopy and integrated into the EPP, and subsequently the complete set-up was tested for electrochemical detection of APAP. The results showed adequate performance of the developed EPP when compared to a traditional electrochemical system under optimal conditions (pH 8, deposition potential 0.1 V, deposition time 240 s and scan rate of 50 mV.s⁻¹), with a sensitivity of 1.6 μ A.mM⁻¹ and limit of detection of 67 μ M. The EPP was validated in river and wastewater samples, achieving recoveries ranging from 93.0 to 96.6%.

Keywords: contaminants of emerging concern; pharmaceuticals; electrochemical portable platform; electroanalysis

1. Introduction

The development of small, versatile, and portable analytical systems is of utmost importance for the analytical and environmental sciences in order to perform simple, cheap on-site analyses. Electrochemical systems can offer a viable solution to this problem due to the compactness of the electronic components that compose the required equipment (potentiostat) and overall simplicity of detection principles [1,2]. Moreover, nanotechnology has been revolutionized the development of sensors by allowing for a wide diversification in the design of transducers as well as miniaturization and integration into small devices. Besides the improvement in versatility, a miniaturization approach also translates into lower environmental impact via reduction of the use of materials and reagents and decrease in generated waste; thus, sensors are becoming interesting alternatives to more traditional analytical techniques [1]. The main objective of the present work was the development and characterization of new miniaturized chip-integrated gold (ECCs) electrodes applied to a portable platform (EPP) for the detection of the drug acetaminophen (APAP). APAP was chosen as a model analyte for the validation since it is one of the most commonly prescribed drugs frequently detected in the environment [3,4]. As is known, pharmaceutical compounds are an important class of contaminants of emerging concern due to their



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). potential persistence and bioaccumulative and toxic nature, causing negative effects on ecosystems and consequently to human health [5]. Although several sensors have been designed for APAP, the development of gold-based transducers for this drug has been limited and usually consists of a bulkier independent three-electrode configuration system with a conventional electrochemical equipment [6–9].

2. Materials and Methods

2.1. Reagents and Solutions

Analytical grade chemicals were used without further purification. Britton-Robinson buffer (BRB, 0.1 M) was prepared using ortho-phosphoric acid (Sigma-Aldrich, Steinheim, Germany), boric acid, potassium chloride (VWR, Leuven, Belgium), sodium hydroxide (Labkem, Barcelona, Spain), and acetic acid glacial (Carlo Erba Reagents, Val-de-Reuil, France). A stock solution of 50 Mm acetaminophen (Sigma-Aldrich, Steinheim, Germany) was prepared in BRB. A 5 mM $[Fe(CN)6]^{3-/4-}$ ((Sigma-Aldrich, Steinheim, Germany) solution was prepared in 0.01 M phosphate buffer saline with a pH of 7.4 using disodium hydrogen phosphate and sodium dihydrogen phosphate (Sigma-Aldrich, Steinheim, Germany). Piranha solution was prepared with a 3:1 (v/v) proportion of sulfuric acid 95% and hydrogen peroxide 130 vol. (Sigma-Aldrich, Steinheim, Germany). All aqueous solutions and electrolytes were prepared with ultrapure water obtained from a Milliporewater purification system (18 M Ω , Milli-Q, Millipore, Molsheim, France).

2.2. Fabrication and Characterization of Chip-Integrated Gold Electrodes2.2.1. Design and Development of Electrochemical Cell-Chips

The ECCs consist of an array of four independent electrochemical cells (ECs), with each cell composed of a working electrode (WE) 1000 μ m in diameter, a pseudo-reference gold electrode (PRGE), and an auxiliary gold electrode, allowing for multiplex and independent detection with statistical significance (Figure 1).



Figure 1. Electrochemical cell chip design (**A**) and fabrication (**B**) using microfabrication techniques; custom-made electrochemical portable platform (**C**) and complete set-up (**D**).

The ECCs were designed using AUTOCAD 2020 and produced, using standard microfabrication technologies, on 200 mm diameter silicon wafers (crystalline silicon coated with 100 nm thermal oxide from Si-Mat, Kaufering, Germany). The devices were fabricated with a thin layer of gold (100 nm) deposited via physical vapor deposition with a multi-target confocal sputtering tool (Kenosistec, Binasco, Italy). The photolithography was performed via direct write laser lithography (Heidelberg DWL 2000, Heidelberg, Germany) followed by an ion milling etching process (Nordiko 7500, Hampshire, UK). An insulating layer of Al_2O_3 , 200 nm thick, was deposited via magnetron sputtering (Singulus Timaris FTM, Kahl, Germany) on top of the devices. A second step of photolithography and etching defined the active area and the contacts [10]. Finally, the silicon wafer was cut to obtain 21 mm by 24 mm silicon chips.

2.2.2. Characterization of the Surface Morphology

The surface morphology of the ECCs surface was analyzed with a commercial atomic force microscope (AFM) Dimension Icon, (Bruker, San Jose, CA, USA). The AFM measurements were performed in air using a standard tapping mode AFM probe (Nanosensors, spring constant of 42 N.m^{-1} , resonance of frequency of 240 kHz and nominal tip radius of 7 nm). The linear scanning rate was set as 0.7 Hz with scan resolution of 512 samples per line. The surface morphology of the electrode was also evaluated via scanning electron microscopy (SEM) using an FEI Quanta 650 FEG (FEI Europe B.V., Eindhoven, The Netherlands), operated under high vacuum. The electron acceleration voltage used was 10 kV, and a spot size of 5.0. Secondary electrons were detected via an Everhardt Thornley detector. The ECCs were characterized with cyclic voltammetry (CV) using both a commercial potentiostat (Metrohm Autolab PGSTAT302N, Metrohn, Herisau, Switzerland), and the developed EPP. Data acquisition and analysis were accomplished using NOVA software (version 2.0.2, Metrohm Autolab) and Electrolab software interface (developed by INL).

2.3. Electrochemical Characterization and Determination of Acetaminophen

The ECCs were first pre-treated both chemically and electrochemically before use to remove the photoresist film and to activate the electrodes. The photoresist layer was removed by washing ECCs sequentially with acetone and isopropanol (Sigma-Aldrich, Steinheim, Germany), followed by 60 min sonication in acetone. Next, the ECCs were rinsed with acetone, isopropanol, and finally with water and subsequently dried with a N₂ stream. Then, one drop of piranha solution was applied to each sensor of the ECCs and rinsed with water after 5 min. Electrochemical pre-treatment was then employed via application of CV in 0.1 M sulfuric acid (20 μ L covering the three electrodes) from -0.4 to 0.8 V at 100 mV.s⁻¹ for 15 scans. The ECCs were finally rinsed and N₂-dried and were then ready to be used. An initial characterization of the ECCs was first made in [Fe(CN)₆]^{3-/4-} solution using CV at 50 mV.s⁻¹. The calibration curve of APAP was obtained by CV at increasing concentrations of the analyte after a pre-concentration step performed at 0.1 V for 240 s.

The ECCs were validated in river- (collected from the Lis River, Portugal) and wastewater (collected from a wastewater treatment plant in Gaia City, North Portugal) samples. Each sample was diluted 1:1 (v/v) with 0.2 M BRB at pH 8, and 20 µL was dropped in one of the sensors comprising the ECC. Then, CV was applied under optimum conditions for APAP screening. In the absence of signal, the samples were spiked with known concentrations of APAP stock solution (6 mM for wastewater; 0.3 and 0.6 mM for river water).

3. Results and Discussion

3.1. Characterization of the the ECCs Surface by AFM, SEM, and CV

The morphology of the ECCs was characterized via AFM (Figure 2A) and SEM (Figure 2B). The gold electrode surface in Figure 2A showed a very regular and homogeneous surface with an average roughness of 0.92 ± 0.02 nm. The black spots in Figure 2B suggest the presence of photoresist residues from the microfabrication process.



Figure 2. AFM image (**A**) with the roughness Ra of 0.92 ± 0.02 for the bare electrode and corresponding SEM image (**B**) of the WE surface, scale bar 20 μ m. The roughness average (Ra, nm) is expressed as the average \pm standard deviation (n = 3).

Typical cyclic voltammograms of the ECCs obtained in a redox indicator with the commercial electrochemical platform and with the developed EPP are represented in Figure 3. The typical electrochemical behavior of an Au micro-fabricated electrode in $[Fe(CN)_6]^{3-/4-}$ can be seen in both Figure 3A,B, thus confirming that the fabrication process was successfully performed. The voltammogram curves also show the similar signals obtained by the two systems. A slight peak deformation can be seen in the reduction peak in Figure 3B, which may be due to solution decay. The results also confirm the adequate performance of the developed EPP, which was further used for the subsequent assays.



Figure 3. Cyclic voltammograms of the different sensors of an ECC acquired in AUTOLAB (**A**) and in the developed EPP (**B**) in 5 mM $[Fe(CN)_6]^{3-/4-}$ (PBS 10 mM, pH 7.4) at 50 mV.s⁻¹ using 5 mV voltage step.

3.2. Optimization of Acetaminophen Electroanalysis by the ECCs with the EPP

APAP voltametric behavior was first assessed at pH 7 based on the optimum pH identified in several works [11–13], where a well-resolved oxidation peak appeared at around +0.45 V (vs. PRGE). However, accordingly to the literature, the optimum pH can vary greatly from acidic [14] to basic pH [15]. Our own pH investigations (Figure 4) indicated a maximum oxidation peak at pH 8 (Figure 4B). Moreover, APAP's oxidation peak shifts towards less positive potentials, presenting a slope of -30 mV/pH (Figure 4C). An electrolyte at pH 8 was selected as the optimum and used in further studies.



Figure 4. Optimization of electrolyte (0.1 M BRB) pH for 1 mM APAP. (**A**) CV forward scan at 50 mV.s⁻¹. (**B**) Peak intensity as a function of electrolyte pH. (**C**) Peak potential as a function of electrolyte pH.

The deposition potential and time were also studied and optimized to increase the APAP signal. It is possible to observe in Figure 5A that peak intensity reaches maximum at an applied potential of 0.1 V (30 s) and was thus selected as the optimum value. The deposition time (Figure 5B) varied from 0 to 360 s and performed at 0.1 V, revealing a maximum peak at 240 s, which was then chosen as the optimum.



Figure 5. Optimization of APAP (1 mM in 0.1 M BRB at pH 8) deposition. (**A**) Peak intensity as a function of deposition potential (applied for 30 s). (**B**) Peak intensity as a function of deposition time for an applied deposition potential of 0.1 V.

Under the optimum conditions obtained above, the ECCs were then applied to detection of APAP at increasing concentrations from 0.1 to 2 mM (Figure 6). The linear range of this sensor varied from 0.1 to 1.5 mM, and the sensitivity retrieved from the calibration curve (Figure 6B) corresponds to 1.6 μ A.mM⁻¹ (203.8 μ A.mM⁻¹.cm⁻², based on the geometrical area of the working electrode of the sensor). The limit of detection (LOD) was calculated based on the standard deviation of the response and the slope of the calibration curve (LOD = $3.3\sigma_{residual-CC}/slope$) [16], which was 67 μ M. This value is higher compared with other literature works that employed miniaturized systems with three integrated electrodes for detection of APAP, such as those based on screen-printing [17–19] or paper-based technology [20,21]. Possible explanations for lower analytical performance of the EPP could be related to a very low geometrical area (0.0079 cm²) of the WE in the ECCs and the use of a less sensitive technique, as in the case of CV. Accordingly to the work of Fanjul-Bolado et al. [22], a comparison of electrochemical techniques used for APAP detection demonstrated a 5 times higher sensitivity for square wave voltammetry compared to CV. However, this technique was not available in the developed EPP.



Figure 6. Analysis of APAP with the EPP. (**A**) CV forward scan for various concentrations of APAP (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1, and 1.5 mM) recorded at 50 mV.s⁻¹ in 0.1 M BRB at pH 8, deposition time 240 s, deposition potential 0.1 V. (**B**) Respective calibration curve.

The EPP was finally applied to the analysis of APAP in river water and wastewater samples. Since no signal was obtained when the samples were directly analyzed, spiking was performed at two levels, namely, 0.3 and 0.6 mM. The obtained recoveries are acceptable, varying from 93.0% for river water to 96.6% for wastewater (Table 1).

Sample	Spiking Level (mM)	Recovery (%)	RSD (%)
Wastewater	0.6	96.6	4.1
River water	0.6 0.3	93.0 93.6	3.6 9.4

Table 1. Analysis of spiked water samples with acetaminophen (n = 3).

4. Conclusions

The developed EPP with chip-integrated gold electrodes demonstrated adequate performance to be applied in the future in the detection of pharmaceutical pollutants, although some improvements (e.g., type of electrochemical techniques available) could be made to enhance the sensitivity and operation of this new electrochemical platform. The development of portable and miniaturized electrochemical platforms may have a positive impact on the environmental field analysis not only due to their versatility but also their sustainable operation (e.g., reduced sample volume needed, in the vicinity of 20 μ L) when compared with traditional analytical techniques.

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