



Fabrication of paper-based analytical devices using a PLA 3D-printed stencil for electrochemical determination of chloroquine and escitalopram

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Abstract

In recent years, the use of prescribed and non-prescribed drugs has increased. Therefore, advances in new technologies and sensors for detecting molecules in natural environments are required. In this work, a 3D-printed polylactic acid stencil is used to fabricate paper-based analytical devices (ePADs). Herein, we report the use of carbon-based lab-manufactured conductive ink for the fabrication of sensors towards the detection of chloroquine and escitalopram. For each batch, eight ePADs were successfully fabricated. Firstly, the fabricated sensors were evaluated morphologically by scanning electron microscopy and electrochemically by cyclic voltammetry and electrochemical impedance spectroscopy experiments. The sensors displayed a well-defined voltammetric profile in the presence of the redox couple, when compared to a commercial carbon screen-printed electrode. Differential pulse voltammetry conducted the detection of chloroquine and escitalopram with detection limits of 4.0 and 0.5 $\mu\text{mol L}^{-1}$, respectively. The ePADs fabricated using the 3D stencil are here presented as alternatives for the fabrication of electrochemical analytical devices.

Keywords Electrochemical paper-based analytical devices (ePADs) · 3D-printing · Conductive ink · Chloroquine · Escitalopram

Introduction

Pharmaceuticals play a vital role in human day life, which may induce the consumption of prescription, and non-prescribed drugs have increased [1]. In addition, over the past two decades, the number of detected drugs and activated pharmaceutical ingredients in the natural environment has increased, especially in natural waters [2]. Quarantine and isolation can drastically affect our mental health, increasing adverse effects such as insomnia, anxiety, and depression [3], leading to the search for professional psychological treatments and anti-depressant pharmaceuticals. Escitalopram (EST) is a selective serotonin reuptake inhibitor (SSRI) [4, 5] commonly known as a prescription to the treatment of depression [6] and generalized anxiety

disorder. Another common pharmaceutical often searched and currently taken without medical prescription is chloroquine (CQ). This anti-malarial drug has been extensively used to treat malaria and eliminate prophylaxis [7]. Recently, the use of CQ has been reported as one of ten medications currently being evaluated as a potential treatment for COVID-19 [8]. However, until this point, there are no effective therapies for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Currently, the US Food and Drug Administration (USFDA) and the World Health Organization (WHO) do not have any supporting data on the effective role of CQ in this specific treatment [8]. High ingestion of pharmaceuticals leads to constant disposal of active pharmaceutical ingredients in aquatic environments through improper manufacturer disposal, hospital discharges, and domestic wastewater, which increases awareness of the unintended presence of these molecules in the aquatic environment (e.g., rivers, lakes, sediments, and biota) [9–11].

Electrochemical paper-based analytical devices (ePADs) have emerged as an opportunity to develop low-cost devices for monitoring possible water contaminants as CQ and EST.

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This technology has been applied in the field of electroanalysis due to suitable characteristics, such as manufacturing of different electrode arrangements, suitability for on-site (“in-loco”) monitoring, high sensitivity, and a wide range of applications [12–14]. Traditionally, an ePAD and a screen-printed electrode (SPE) consist of a set of three electrodes (working, reference, and counter) printed using a carbon ink or carbon paste [15]. The ink consists mainly of a conductive material, such as graphite or carbon black, complemented with a binder or plasticizer to input robustness to the printing technology [13]. Carbon-based inks can be fabricated either in a laboratory scale using graphite powder and common binders, such as nail polish [16] and glass varnish [17], or in an industrial scale.

Although there are different types of substrates (PET, polyester, PVC) that have been used for suitable screen-printing, ePADs have gained the attention of researchers for developing low-cost sensors [18, 19]. Paper is a common, cheap material, and the use of paper-based devices is in accordance with the directives of Industry 4.0 towards improvement of product quality [20]. Paper-based analytical devices offer a wide range of intrinsic characteristics, such as natural capillary driven fluidic flow, which excludes the need for pumps [14], low cost, and reduced consumption of reagents [21]. In addition, three-dimensional (3D) printing is an emerging technology that has been applied in the development and fabrication of various analytical chemistry applications [22].

In this context, the objective of this study was to evaluate the electrochemical paper-based electrodes fabricated using a PLA 3D-printed stencil for the electrochemical determination of the pharmaceuticals chloroquine and escitalopram.

Experimental procedures

Instrumental

Electrochemical experiments were recorded in a PGSTAT128N potentiostat with a FRA32M module (Metrohm) and data analysis was conducted using the Electrochemical Circle Fit at NOVA 2.1.4 software.

Morphologic characterization of the materials was acquired by scanning electron microscopy (SEM) using a JEOL MODELO JSM7500F equipped with a field emission gun (FEG) operated at 2 kV at the IQ-UNESP Araraquara, Brazil. The ePADs did not receive any coating for analysis.

3D stencil fabrication

The stencil’s design was conceptualized for printing 10 electrodes at each batch (Online Resource 1). The parts were designed using the open web service Tinkercad

(Autodesk, USA). The stencil is composed of two parts: the first is a plate (90 mm × 94 mm) printed in the format of two rows with five electrodes each. The 3-electrode structure (with working, reference, and auxiliary contacts) was modeled on the stencil. The second part of the stencil is a plate (90 mm × 94 mm) and was designed to support the paper for conductive ink adhesion deposition. The two parts of the stencil are fixed using conventional screws. The 3D stencil was printed by fused deposition modeling of a thermoplastic filament technique using a polylactic acid (PLA) filament (3D Fila, Brazil) with a diameter of 1.75 mm. PLA is a biodegradable polymer and has resistance to organic solvents, being a suitable material for applying lab-made conductive inks. Printing was performed using an ANET ET4-PRO printer (Anet Technology, China) and the following print parameters: print temperature: 200 °C; bed temperature: 65 °C; layer thickness: 0.1 mm. The slicing process for generating the G-CODE was performed using the open software Ultimaker Cura version 4.8.0 (Ultimaker B.V., Netherlands).

Paper-based electrode fabrication

The fabrication of the ePADs was conducted using a modified version of the method reported by Pradela-Filho et al. [1]. A carbon-based conductive ink was elaborated by hand mixing graphite powder (Sigma-Aldrich) and glass varnish (Acrilex®) as the binding agent, in the proportion of 20%. The mixture was homogenized with 1.0 mL of acetone to assure ink viscosity. A schematic representation of the fabrication of the electrodes is shown in Fig. 1.

The lab-made ink was spread on the PLA stencil with a brush and forced through the paper substrate (Online Resource 2). The printed electrodes were dried for 12 h at room temperature. The obtained electrodes were ~2.5 cm, with the working electrode (WE) giving a geometric radius of 1.5 mm. Approximately 120 mg of conductive ink was used for each fabrication batch, and loss of 20% of the electrodes was estimated.

Electrochemical experiments

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were conducted in a 0.2 mol L⁻¹ PBS (pH 7.4) solution containing 0.1 mol L⁻¹ of KCl and 5.0 mmol L⁻¹ of the redox couple Fe(CN)₆^{3-/4-}. CV data was recorded in the potential range of -0.5 to 1.0 V (scan rate of 50 mV s⁻¹) vs. Ag/AgCl. The EIS experiments were recorded in an open circuit potential (OCP) setup with 10 points per decade, frequency range of 10⁷ and 10⁻² Hz, and

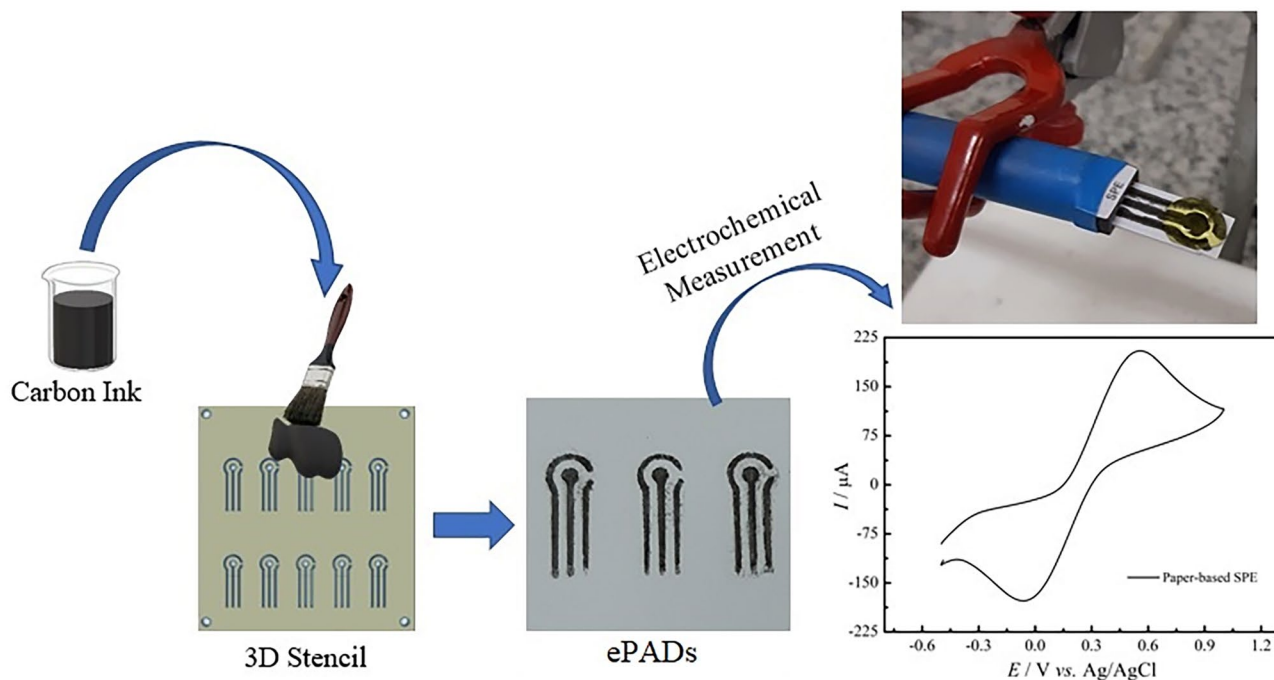


Fig. 1 Schematic representation of ePADs fabrication

amplitude of 10 mV. The Nyquist plots were fitted in Randles and Erschler electronic equivalent circuits [23].

Results and discussion

Morphology studies

Scanning electron microscopy (SEM) images of the fabricated ePADs are shown in Fig. S1. In Fig. S1A, it is possible to observe a few cellulose fibers mixed with graphite structures. In Fig. S1B, the graphite structures are randomly organized agglomerates with sizing ranging from 0.5 to 50 μm . This characteristic surface may be related to using a brush for the manual printing approach developed for this work.

Electrochemical characterization

Electrochemical characterization of the optimized ePADs (20%) was carried out by using CV and EIS experiments. In Fig. 2a, the ePADs (solid line) had a similar behavior compared to commercial carbon SPE (dashed line). The commercial electrode presented a peak separation (ΔE_p) of ~ 270 mV. Although the ePADs showed a higher peak separation for the redox processes, both had a similar outline. This behavior was also observed by a similar study developed by Pradela-Filho et al. [17]. In Fig. 2b, as anticipated, the paper-based electrode presented a higher resistance to charge transfer (R_{ct}) in the order of 3.5 k Ω in relation to the commercial one (500 Ω). For both experiments above, only the working electrode of the ePADs was used;

Fig. 2 Electrochemical characterization of the ePADs recorded in 0.2 mol L⁻¹ PBS having 0.1 mol L⁻¹ KCl and 5.0 mmol L⁻¹ [Fe(CN)₆]^{3-/4-}. **a** Cyclic voltammograms comparing commercial SPE (dashed line) and the ePADs (solid line). **b** Nyquist plots for commercial SPE (red triangle) and the ePADs (filled square)

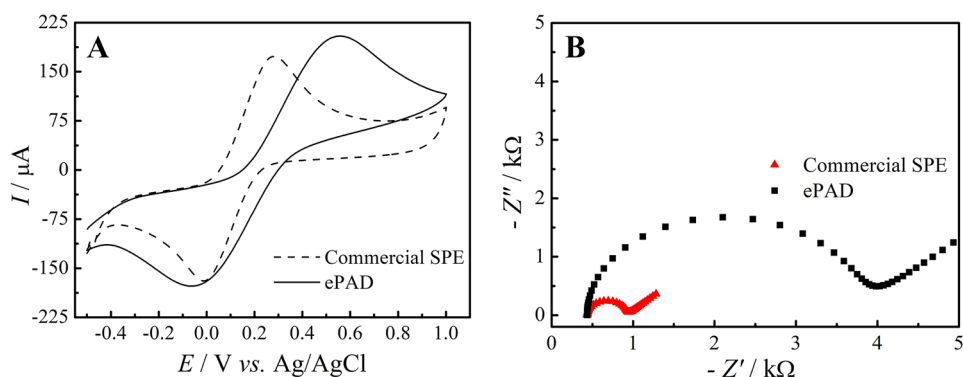
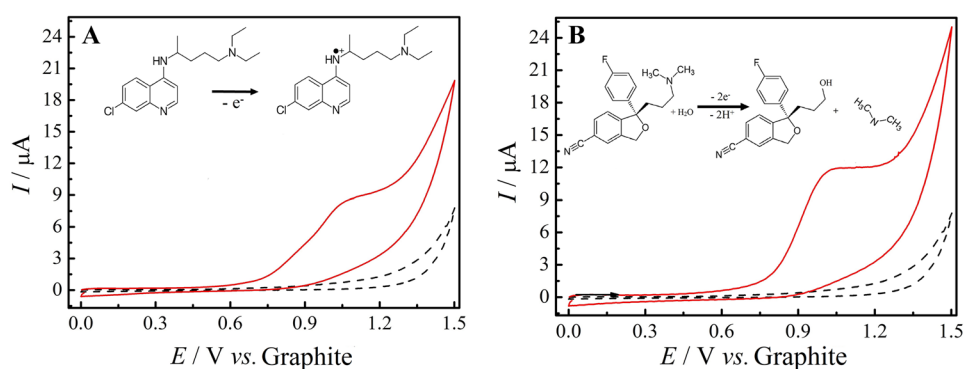


Fig. 3 Cyclic voltammograms recorded in 0.1 mol L⁻¹ PBS (pH 7.0) in the absence (dashed line), **a** in the presence of 200 μmol L⁻¹ of CQ, and **b** in the presence of 200 μmol L⁻¹ of EST



a platinum-plate was used as a counter electrode (CE) and Ag/AgCl as RE.

Electrochemical behavior of CQ and EST at ePADs

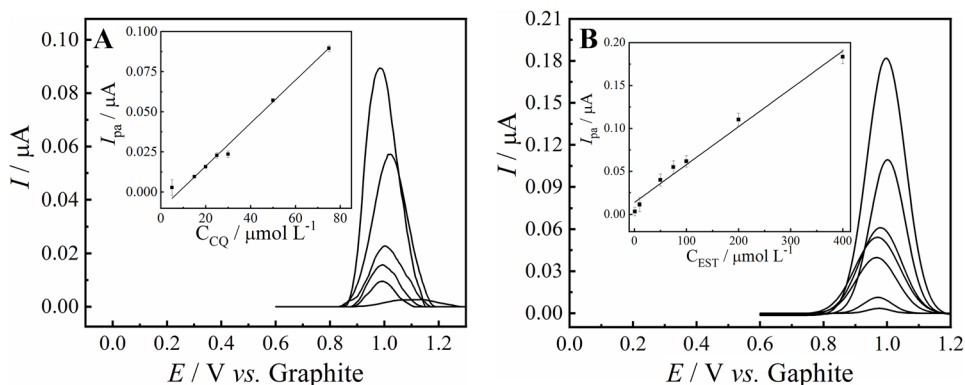
The electrochemical behavior of escitalopram and chloroquine on the ePADs was carried out in 0.2 mol L⁻¹ PBS pH 7.0, containing 200.0 μmol L⁻¹ of CQ or EST by cyclic voltammetry experiments (scan-rate = 50 mV s⁻¹). The results presented in Fig. 3a show that no electrochemical process was observed for the voltammetric response in the absence of CQ (dashed line). However, in the presence of CQ (solid line), an irreversible oxidation peak at $E_{pa} = +1.04$ V vs. graphite is observed. The irreversible oxidation process of chloroquine has been reported in the literature in a range of 0.8 to 1.2 vs. Ag/AgCl reference electrode. This anodic peak may be related to the irreversible oxidation of the N-heterocyclic nitrogen of the aminoquinoline portion and the nitrogen of the alkylaminoside group of the chloroquine molecule chain (inset Fig. 3a) [7, 24–26]. In Fig. 3b, escitalopram presented a similar irreversible oxidation peak at $E_{pa} = 1.02$ vs. graphite is observed. This anodic peak has been reported in the literature to be correlated to the transfer of two electrons in the terminal tertiary amine group. A single irreversible peak for escitalopram has been reported in previous

works towards the electrochemical detection of this anti-depressant drug [27–29] (inset Fig. 3b).

After electrochemical characterization by cyclic voltammetry, the ePADs were applied in the detection of CQ and EST using differential pulse voltammetry (DPV). These experiments were carried out in the potential range of 0.6 to 1.2 V with a scan rate of 10 mV s⁻¹ using freshly prepared solutions of the analytical standards of CQ and EST diluted in 0.2 mol L⁻¹ PBS (pH 7.0). In Fig. 4a, the electrochemical detection of CQ presented a linear response from 5.0 to 75.0 μmol L⁻¹ (calibration graph (I_{pa} (μA)) = $(-0.0215 \pm 0.0014) + (0.00268 \pm 0.00005)$ [CQ/μmol L⁻¹]; $N=7$; $r=0.995$), and in Fig. 4b, the detection of EST presented a linear response from 1.0 to 400.0 μmol L⁻¹ (calibration graph (I_{pa} (μA)) = $(0.014 \pm 0.004) + (0.00044 \pm 0.00002)$ [EST/μmol L⁻¹]; $N=7$; $r=0.98$) [30]. The obtained LOD of 4.0 μmol L⁻¹ for CQ and 0.5 μmol L⁻¹ for EST were calculated using a $3\sigma/\text{slope}$ ratio, where σ is the standard deviation of three consecutive voltammograms without the analyte (blank).

The proposed sensor presented similar limits for detection of those using graphite-based inks or using low-cost binders, such as nail polish [16] and glass varnish [17]. Hence, the use of other carbon materials in ink composition like multi-walled carbon nanotubes (MWCNT) [31], reduced graphene nanoribbons (rGNRs) [32], and commercial available carbon inks [33] may offer lower LODs and improved sensitivity for paper-based sensors.

Fig. 4 Baseline-corrected DPV voltammograms recorded with ePADs in 0.2 mol L⁻¹ PBS (pH 7.0) at different concentrations of **a** chloroquine ranging from 5.0 to 75.0 μmol L⁻¹ (inset: analytical curve for chloroquine) and **b** escitalopram ranging from 1.0 to 400.0 μmol L⁻¹ (inset: analytical curve for escitalopram). DPV conditions: step potential = 50 mV; modulation amplitude = 25 mV



Conclusion

This study showed that 3D-printed stencils are a promising alternative in the fabrication of electrochemical paper-based analytical devices. The composition of 80:20% of graphite/glass varnish based ink could produce up to eight electrodes ($N = 10$) for each batch of fabrication of the ePADs. The morphological characterization confirmed the adherence of the carbon ink onto the paper substrate with graphite agglomerates ranging from 0.5 to 50 μm . The freshly prepared ePADs showed well-defined voltammetric profiles in the presence of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple and sensitivity in determining pharmaceuticals. The novel approach of using a 3D stencil and a lab-made conductive ink allowed the confection of sensitive sensors with an acceptable reproducibility. The ePADs showed a linear response in small concentrations for chloroquine (5.0 to 75.0 $\mu\text{mol L}^{-1}$) and for escitalopram (1.0 to 400.0 $\mu\text{mol L}^{-1}$), respectively. The fabricated ePADs showed a suitable analytical performance comparable to those reported in the literature for the determination of different molecules, in that way advancing in the field of easy-to-fabricate devices with low-cost materials.

Supplementary information.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10008-021-05075-w>.

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Declarations

Competing interests The authors declare no competing interests.

References

- Lapostolle F, Vianu I, De SC et al (2021) COVID-19 epidemic: chloroquine, a French obsession? *La Press Médicale Open* 100007 <https://doi.org/10.1016/j.lpmope.2021.100007>
- Guo J, Liu S, Zhou L et al (2021) Prioritizing pharmaceuticals based on environmental risks in the aquatic environment in China. *J Environ Manage* 278:111479. <https://doi.org/10.1016/j.jenvman.2020.111479>
- Wang Y, Shi L, Que J et al (2021) The impact of quarantine on mental health status among general population in China during the COVID-19 pandemic *Mol Psychiatry* 1–10 <https://doi.org/10.1038/s41380-021-01019-y>
- Sánchez C, Bøgesø KP, Ebert B et al (2004) Escitalopram versus citalopram: the surprising role of the R-enantiomer. *Psychopharmacology* 174:163–176
- Wu YJ (2012) Heterocycles and medicine. A survey of the heterocyclic drugs approved by the U.S. FDA from 2000 to present. In: *Progress in heterocyclic chemistry*. Elsevier Ltd, pp 1–53
- Kasper S, Baldwin DS, Larsson Lönn S, Boulenger JP (2009) Superiority of escitalopram to paroxetine in the treatment of depression. *Eur Neuropsychopharmacol* 19:229–237
- Srivastava M, Tiwari P, Mall VK et al (2019) Voltammetric determination of the antimalarial drug chloroquine using a glassy carbon electrode modified with reduced graphene oxide on WS2 quantum dots. *Microchim Acta* 186:1–10. <https://doi.org/10.1007/s00604-019-3525-3>
- Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB (2020) Pharmacologic treatments for Coronavirus Disease 2019 (COVID-19): a review. *JAMA J Am Med Assoc* 323:1824–1836
- Ebele AJ, Abou-Elwafa Abdallah M, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam* 3:1–16
- Gogoi A, Mazumder P, Tyagi VK et al (2018) Occurrence and fate of emerging contaminants in water environment: a review. *Groundw Sustain Dev* 6:169–180. <https://doi.org/10.1016/J.GSD.2017.12.009>
- Yang Y, Ok YS, Kim KH et al (2017) Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review. *Sci Total Environ* 596–597:303–320. <https://doi.org/10.1016/J.SCITOTENV.2017.04.102>
- Couto RAS, Lima JLFC, Quinaz MB (2016) Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis. *Talanta* 146:801–814
- Squizzato AL, Almeida ES, Silva SG et al (2018) Screen-printed electrodes for quality control of liquid (Bio)fuels. *TrAC - Trends Anal Chem* 108:210–220
- Baharfar M, Rahbar M, Tajik M, Liu G (2020) Engineering strategies for enhancing the performance of electrochemical paper-based analytical devices. *Biosens Bioelectron* 167:112506. <https://doi.org/10.1016/J.BIOS.2020.112506>
- Mustafa F, Finny AS, Kirk KA, Andreescu S (2020) Printed paper-based (bio)sensors: design, fabrication and applications. *Compr Anal Chem* 89:63–89. <https://doi.org/10.1016/BS.COAC.2020.02.002>
- Pradela-Filho LA, Araújo DAG, Takeuchi RM, Santos AL (2017) Nail polish and carbon powder: an attractive mixture to prepare paper-based electrodes. *Electrochim Acta* 258:786–792. <https://doi.org/10.1016/j.electacta.2017.11.127>
- Pradela-Filho LA, Andreotti IAA, Carvalho JHS et al (2020) Glass varnish-based carbon conductive ink: a new way to produce disposable electrochemical sensors. *Sensors Actuators, B Chem* 305:127433. <https://doi.org/10.1016/j.snb.2019.127433>
- Batista Deroco P, de Giarola J, F, Wachholz Júnior D, et al (2020) Paper-based electrochemical sensing devices. *Compr Anal Chem* 89:91–137. <https://doi.org/10.1016/BS.COAC.2019.11.001>
- Arduini F, Moscone D (2020) Multifarious aspects of electrochemical paper-based (bio)sensors. *Compr Anal Chem* 89:139–161. <https://doi.org/10.1016/BS.COAC.2020.01.001>
- Arduini F, Micheli L, Scognamiglio V et al (2020) Sustainable materials for the design of forefront printed (bio)sensors applied in agrifood sector. *TrAC - Trends Anal. Chem.* 128:115909
- Scordo G, Moscone D, Palleschi G, Arduini F (2018) A reagent-free paper-based sensor embedded in a 3D printing device for cholinesterase activity measurement in serum. *Sensors Actuators B Chem* 258:1015–1021. <https://doi.org/10.1016/J.SNB.2017.11.134>
- Cardoso RM, Kalinke C, Rocha RG et al (2020) Additive-manufactured (3D-printed) electrochemical sensors: a critical review. *Anal Chim Acta* 1118:73–91. <https://doi.org/10.1016/J.ACA.2020.03.028>
- Wang J (2006) *Analytical electrochemistry*, 3rd edn. John Wiley & Sons Inc, Hoboken, NJ, USA

24. Lam K, Van Wyck SJ, Geiger WE (2017) One-electron oxidation of chloroquine, cymanquine, and related aminoquinolines in nonaqueous media. *J Electroanal Chem* 799:531–537. <https://doi.org/10.1016/J.JELECHEM.2017.06.041>
25. Radi A (2005) Accumulation and trace measurement of chloroquine drug at DNA-modified carbon paste electrode. *Talanta* 65:271–275. <https://doi.org/10.1016/J.TALANTA.2004.05.024>
26. Oliveira GG, Azzi DC, Silva TA et al (2020) Sensitive voltammetric detection of chloroquine drug by applying a boron-doped diamond electrode. *C* 6:75. <https://doi.org/10.3390/c6040075>
27. Gholivand MB, Akbari A (2016) A novel voltammetric sensor for citalopram based on multiwall carbon nanotube/(poly(p-aminobenzene sulfonic acid)/ β -cyclodextrin). *Mater Sci Eng C* 62:480–488. <https://doi.org/10.1016/j.msec.2016.01.066>
28. Baccarin M, Cervini P, Cavalheiro ETG (2018) Comparative performances of a bare graphite-polyurethane composite electrode unmodified and modified with graphene and carbon nanotubes in the electrochemical determination of escitalopram. *Talanta* 178:1024–1032. <https://doi.org/10.1016/j.talanta.2017.08.094>
29. Silva MKL, Leão AL, Sain M (2021) Cesarino I (2021) A functionalized renewable carbon-based surface for sensor development. *J Solid State Electrochem* 253(25):1093–1099. <https://doi.org/10.1007/S10008-020-04882-X>
30. Fitzer E, Kochling K-H, Boehm HP, Marsh H (1995) Recommended terminology for the description of carbon as a solid (IUPAC Recommendations 1995). *Pure Appl Chem* 67:473–506. <https://doi.org/10.1351/pac199567030473>
31. Araújo DAG, Camargo JR, Pradela-Filho LA et al (2020) A lab-made screen-printed electrode as a platform to study the effect of the size and functionalization of carbon nanotubes on the voltammetric determination of caffeic acid. *Microchem J* 158:105297. <https://doi.org/10.1016/J.MICROC.2020.105297>
32. Martins TS, Bott-Neto JL, Oliveira ON, Machado SAS (2021) Paper-based electrochemical sensors with reduced graphene nanoribbons for simultaneous detection of sulfamethoxazole and trimethoprim in water samples. *J Electroanal Chem* 882:114985. <https://doi.org/10.1016/J.JELECHEM.2021.114985>
33. Deroco PB, Fatibello-Filho O, Arduini F, Moscone D (2020) Electrochemical determination of capsaicin in pepper samples using sustainable paper-based screen-printed bulk modified with carbon black. *Electrochim Acta* 354:136628. <https://doi.org/10.1016/J.ELECTACTA.2020.136628>

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