



Article

Sensitive Voltammetric Detection of Chloroquine Drug by Applying a Boron-Doped Diamond Electrode

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Abstract: In this research, a boron-doped diamond (BDD) electrode has been explored to detect the chloroquine drug. The electrochemical performance of BDD electrode towards the irreversible anodic response of chloroquine was investigated by subjecting this electrode to the cathodic (-0.5 A cm^{-2} by 180 s, generating a predominantly hydrogen-terminated surface) and anodic ($+0.5 \text{ A cm}^{-2}$ by 30 s, oxygen-terminated surface) pretreatments. The cathodically pretreated BDD electrode ensured a better-defined anodic peak and higher current intensity. Thus, by applying the cathodically pretreated BDD electrode and square-wave voltammetry (SWV), the analytical curve was linear from 0.01 to 0.25 $\mu\text{mol L}^{-1}$ (correlation coefficient of 0.994), with sensitivity and limit of detection of 12.2 $\mu\text{A L } \mu\text{mol}^{-1}$ and 2.0 nmol L^{-1} , respectively. This nanomolar limit of detection is the lowest recorded so far with modified and unmodified electrodes.

Keywords: chloroquine; square-wave voltammetry; boron-doped diamond; electroanalysis; pharmaceuticals

1. Introduction

Chloroquine is a widely used antimalarial drug, and it is part of the World Health Organization (WHO) model list of essential medicines [1]. This drug belongs to the class of 4-aminoquinolines and acts as a weak base due to the presence of a base side chain in its molecular structure (Figure 1) [2]. This base character seems to be important to justify its biological activity, although the mechanism of action of this molecule has not yet been fully elucidated [2]. Chloroquine is commercialized as a phosphate salt and reaches the peak plasma concentration 4–12 h after an individual dose [3]. It is considered a safe drug, although the margin between the therapeutic and toxic dose is narrow, and the common side effects of this drug include nausea, vomiting, diarrhea, and abdominal discomfort [4]. Furthermore, the most worrying effect of chloroquine administration is the development of retinopathy, i.e., damage of retina with the consequent partial or complete loss of vision [5,6]. Nowadays, although the efficiency is not clear, the use of this drug in treating coronavirus 2019 (COVID-19) has been studied; however, self-medication is strongly not recommended [7,8]. In view of the relevance of chloroquine, high-performance analytical methods are required for its rigid control in pharmaceutical formulations.

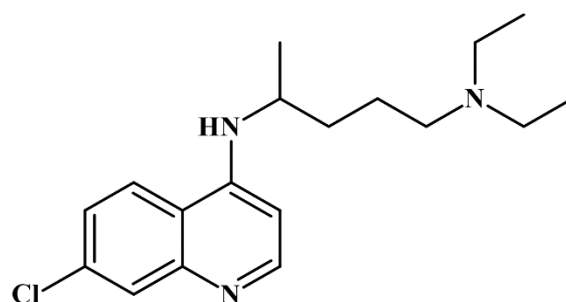


Figure 1. Molecular structure of chloroquine.

Among the analytical techniques, voltammetric analysis is one of the most powerful and versatile, which allows providing qualitative and quantitative information about a given analyte and can be used with different types of sensors. In this context, carbon electrodes are interesting alternatives for electroanalytical chemistry, such as graphite [9], carbon nanotubes [10], graphene [11], carbon black [12], nanodiamonds [13], and boron-doped diamond electrode [14]. Our research group has recently published some revisions about carbon conductive materials to highlight the importance of these materials [15–18]. They are highly conductive and inert, which can be modified or not [19,20] and can be applied for the determination of different compounds, including for environmental, medical, and food purposes.

An electrode material that has stood out significantly in recent years for applications in the field of electroanalysis is the boron-doped diamond electrode (BDD). The deposition of BDD films as a p-type semiconductor is routinely carried out by chemical vapour deposition methods from mixture gases (hydrocarbon (typically methane)/hydrogen) using different activation sources, for example hot filament, low-pressure direct current plasma, radio frequency plasma, or microwave discharge. A detailed description of the different growth methods of BDD films and their characterization can be found in specialized reviews [21–24]. This carbon material has a wide range of favourable characteristics, including excellent morphological and microstructural stability at high temperatures, weak adsorption of organic and inorganic compounds (reduced fouling), minimal and stable background current, fast charge-transfer kinetic, and wide potential window in aqueous and nonaqueous solvents [25–29]. Additionally, the anodic and cathodic electrochemical treatments of the BDD surface can generate functional groups that can increase the analytical signal and promote better electron transfer between the interface electrode/bulk solution [25]. As a result, BDD has become promising for the electrochemical detection of different analytes of interest, such as drugs [30,31] and environmental contaminants [14]. Lourencao et al. performed the individual and simultaneous voltammetric determination of paracetamol and caffeine by using a cathodically pretreated BDD electrode. The individual determination of these analytes was carried out by square-wave voltammetry (SWV) and the simultaneous determination by differential pulse voltammetry (DPV). This specific determination by voltammetric tools is critical because of the high oxidation potential of caffeine. However, the stable and low background current of BDD ensured the individual determination of caffeine in the linear range of $0.3 \mu\text{mol L}^{-1}$ to $91.0 \mu\text{mol L}^{-1}$, with a limit of detection (LOD) of $0.14 \mu\text{mol L}^{-1}$. Regarding the simultaneous determination, the following analytical parameters were achieved: linear ranges of $0.5 \mu\text{mol L}^{-1}$ to $83.0 \mu\text{mol L}^{-1}$ (both paracetamol and caffeine) and LODs of $0.49 \mu\text{mol L}^{-1}$ (paracetamol) and $0.035 \mu\text{mol L}^{-1}$ (caffeine). When applied to pharmaceutical samples, the DPV method based on the cathodically pretreated BDD provided quantitative data statistically equivalent to those from a high-performance liquid chromatography (HPLC) method. The use of BDD electrodes and pulsed voltammetric techniques is a highly successful combination, with outstanding analytical performance parameters for the detection of compounds of pharmaceutical [32,33], food [34], environmental [14,35], forensic [33,36] interest, etc. Additionally, its robustness as an electrode material was paramount in the establishment of high-frequency analytical methods based on the coupling of

flow injection analysis and amperometric detection (especially the multiple pulse amperometry for simultaneous determinations) [37–39]. The research group led by Richter and Munoz also proposed many innovative works using batch injection analysis [40–42].

Based on the above context, a new voltammetric procedure has been developed to detect the chloroquine drug using a BDD as the electrochemical sensor.

2. Materials and Methods

2.1. Reagents and Solutions

Analytical degree chemical reagents were employed in all the experimental stages, without any additional purification or treatment. Standard of chloroquine (purity > 99%) was purchased from Calendula Pharmacy (São Carlos-SP, Brazil). The electrochemical measurements were performed in aqueous media, and therefore, ultrapure water supplied by a Milli-Q system (Millipore, São Paulo, Brazil) (electrical resistivity > 18 Ω M cm) was used. A standard aqueous solution of 1.0×10^{-3} mol L⁻¹ chloroquine was daily prepared. Addition and recovery experiments were carried out by using manipulated drug obtained from a local drugstore. For this purpose, 1.0×10^{-3} mol L⁻¹ of this drug was prepared at 0.1 mol L⁻¹ Britton-Robinson buffer solution (pH 6.0).

2.2. Instrumentation

The voltammetric analysis (cyclic and square-wave voltammetries) were carried out using an Autolab PGSTAT-30 potentiostat/galvanostat (Metrohm Autolab, Utrecht, The Netherlands) controlled by the GPES 4.9 software. A three-electrode electrochemical cell with a volume of 20 mL was used. Thus, the electrodes system was composed of a BDD working electrode, a Pt wire counter electrode and an Ag/AgCl (3.0 mol L⁻¹ KCl) reference electrode. The BDD film was synthesized on p-silicon wafers and with a boron content of 8000 ppm in the Centre Suisse d'Electronique et de Microtechnique SA (CSEM), Neuchâtel, Switzerland, using the hot filament chemical vapour deposition technique, as described elsewhere [43–45]. To enable its use as a working electrode, the as-received BDD plate was fixed on conductive copper support using silver ink (Figure 2). The BDD electrode (0.32 cm² exposed area) was subjected to a previous electrochemical pretreatment. In this sense, the anodic and cathodic pretreatments were applied and compared. To achieve these pretreatments of the BDD surface, the current density of -0.5 A cm⁻² (cathodic) or $+0.5$ A cm⁻² (anodic) in a 0.50 mol L⁻¹ H₂SO₄ solution was applied by 180 s (cathodic) or 60 s (anodic), respectively. The morphological analysis of BDD was performed using a Supra 35-VP FEG-SEM equipment (Carl Zeiss, Oberkochen, Germany) with electron-beam energy of 20 keV.

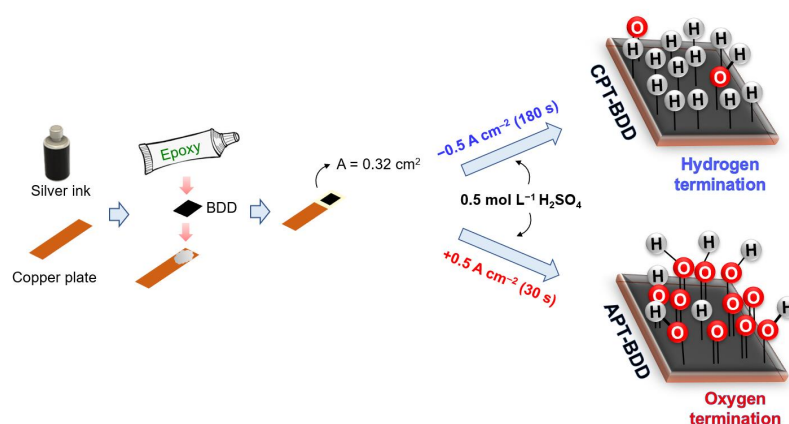


Figure 2. Scheme of boron-doped diamond (BDD) electrode assembly for electrochemical measurements and obtaining of cathodically pretreated (CPT-BDD) and anodically pretreated (APT-BDD) BDD electrodes.

3. Results and Discussion

3.1. Working Electrode Characterization

Morphology of BDD film growth on p-Si wafer with a B-doping level of 8000 ppm was evaluated by SEM. The SEM micrographs of the BDD surface are shown in Figure 3a,b. It was registered as a uniform morphology based on a regular pyramidal structure, following previous literature [46]. This regular pyramidal structure morphology refers to the preferential textured growth along the (111) planes direction [45,47,48]. Additionally, the electrochemical quality of the used BDD was investigated by exploring the response of an inorganic redox probe.

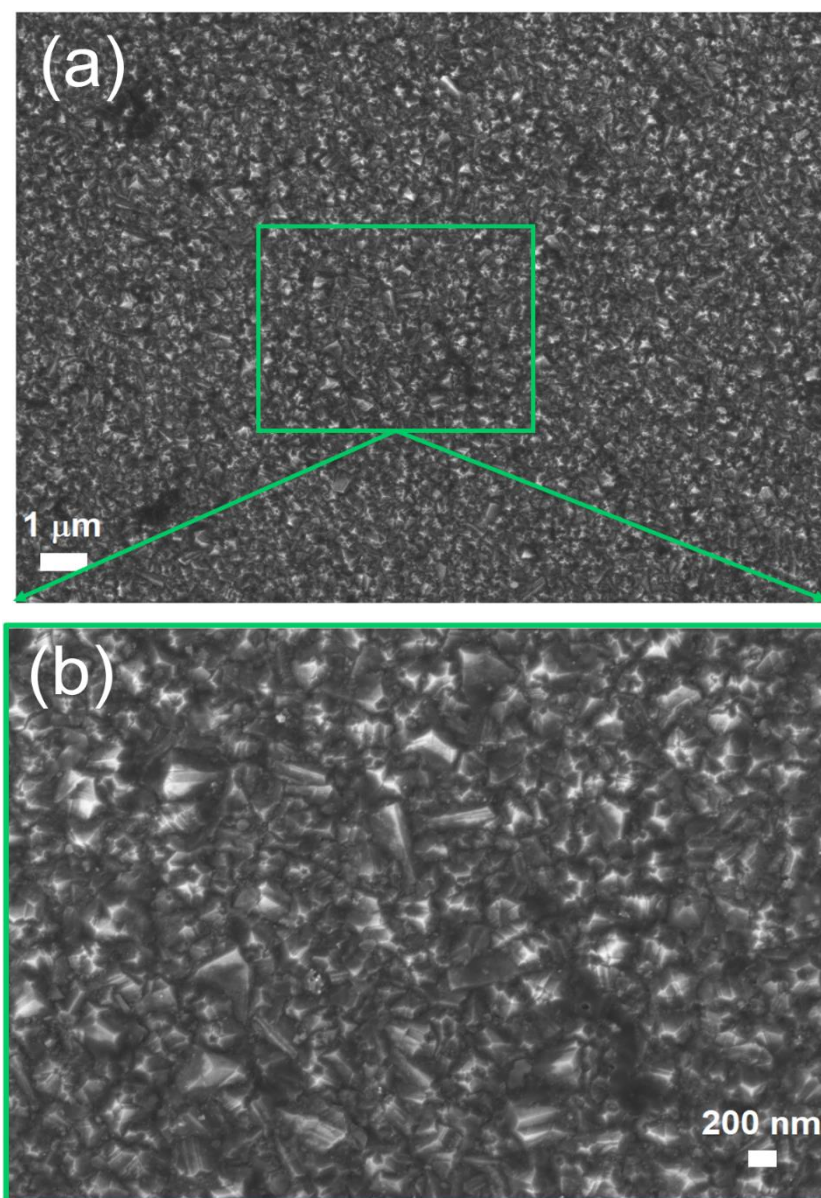


Figure 3. SEM micrograph recorded for BDD surface at different magnifications: (a) 17,000× and (b) 50,000×.

Figure 4a shows the cyclic voltammograms recorded for the cathodically pretreated BDD for a $1.0 \times 10^{-3} \text{ mol L}^{-1}$ $\text{K}_3\text{Fe}(\text{CN})_6$ solution. A well-defined pair of anodic and cathodic peaks was observed, related to the oxidation–reduction semi-reactions of $\text{Fe}^{3+/2+}$ redox pair. The peak-to-peak

potential separation (ΔE_p) was equal to 83 mV, which is a value close to the theoretical value of 59.2 mV, typical for a reversible redox process involving the transfer of one electron. Additionally, the ratio of anodic and cathodic peak currents (I_{pa}/I_{pc}), another parameter to evaluate the reversibility of redox processes, was calculated as being 1.04. Once again, the experimental I_{pa}/I_{pc} parameter was in concordance with its theoretical value of 1.0 reported to reversible redox reactions [49]. These data served to state the good electron transfer kinetic property of the BDD electrode. Moreover, the electroactive surface area of the employed electrode was predicted by cyclic voltammetry assays carried out in a wide scan rate range (10 to 500 mV s⁻¹) towards the same redox probe. The cyclic voltammograms collected in the mentioned scan rate range are shown in Figure 4b. From them, the plots of I_{pa} and I_{pc} versus square root of the scan rate ($v^{1/2}$) were constructed (Figure 4c). Comparing the slope of the linear I_{pa} vs. $v^{1/2}$ curve with the theoretical slope of the Randles–Sevcik equation for reversible and diffusion-controlled electroodic reactions, the electroactive surface area was calculated. Considering $n = 1$ (number of electrons), $D = 7.6 \times 10^{-6}$ cm² s⁻¹ (diffusion coefficient of Fe(CN)₆³⁻) and $C = 1.0 \times 10^{-6}$ mol cm⁻³ (concentration of Fe(CN)₆³⁻), the estimated electroactive area was 0.285 cm².

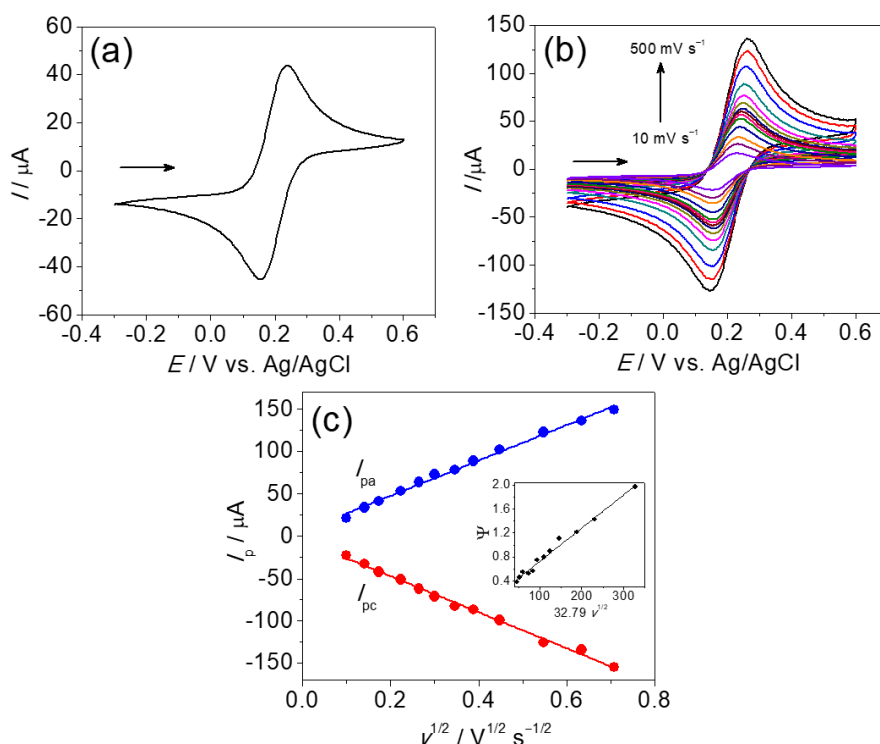


Figure 4. Cyclic voltammogram recorded at (a) 50 mV s⁻¹ and (b) different potential scan rates (10–500 mV s⁻¹) for 1.0×10^{-3} mol L⁻¹ K₃Fe(CN)₆ in 0.1 mol L⁻¹ KCl solution using the cathodically pretreated BDD electrode. (c) Plot of anodic (I_{pa}) and cathodic (I_{pc}) peak currents versus square root of scan rate ($v^{1/2}$). Inset: plot of Ψ vs. $32.79 v^{1/2}$.

Then, the heterogeneous electron transfer rate constant (k^0) was also predicted from the data of cyclic voltammetry at different scan rates. In this case, as the redox probe figures a reversible and diffusion-controlled electron transfer, the method reported by Nicholson [50,51] was applied. Nicholson's equation to calculate k^0 in this case is:

$$\Psi = k^0 [\pi D n v F / (RT)]^{-1/2} \quad (1)$$

where Ψ is a kinetic parameter, F is the Faraday's constant (96,485 C mol⁻¹), $R = 8.314$ J K⁻¹ mol⁻¹, $T = 298.15$ K, and the other terms have been previously defined. The kinetic parameter Ψ is dependent on the scan rate, and it was calculated from the ΔE_p values verified in the different scan rates using

the equations proposed by Lavagnini et al. [52]. Knowing the Ψ values, k^0 is directly determined as being the slope of a Ψ vs. $32.79 v^{1/2}$ plot (Inset of Figure 4c) [51]. The 32.79 factor is equivalent to the term $[\pi D n F / (RT)]^{-1/2}$ of Nicholson's equation (Equation (1)). Thus, the obtained k^0 constant was 0.0056 cm s^{-1} . This constant is comparable or better to the same parameter obtained for traditional carbon-based working electrodes, as glassy carbon [53] and carbon paste electrodes [54].

3.2. Chloroquine Electrochemistry

The electrochemical activity of chloroquine drug was explored by cyclic voltammetry. In this study, the influence of the predominant BDD surface termination on the analyte response, i.e., hydrogenated (for cathodic pretreatment) or oxygenated (for anodic pretreatment), was also accessed. Thus, the cyclic voltammograms collected towards a $1.5 \times 10^{-4} \text{ mol L}^{-1}$ chloroquine solution prepared in 0.1 mol L^{-1} phosphate buffer solution (pH = 7.0) as the supporting electrolyte using the CPT- and APT-BDD electrodes are available in Figure 5a. The potential scanning was started at +0.8 V and in the anodic direction. During the anodic scanning, in both cases, a well-discriminated peak was recorded. However, after the inversion of the potential scanning direction, no cathodic peaks to form a couple with the previous anodic peak were verified, indicating the property of chloroquine molecule to undergo an irreversible oxidation reaction. This irreversible oxidation reaction has been related to the aminoquinoline moiety [55], in a one-electron oxidation step, as outlined in Figure 5b. Comparatively, it is interesting to notice that the cathodically pretreated BDD provided a better chloroquine response. By using this pretreatment, the anodic peak of chloroquine was better defined and occurred at a less positive potential, which is a contributing factor in reducing possible interferences. This enhanced voltammetric response of chloroquine molecule on the cathodically pretreated BDD is in line with several other studies [14,32,56–60], which was selected for further studies.

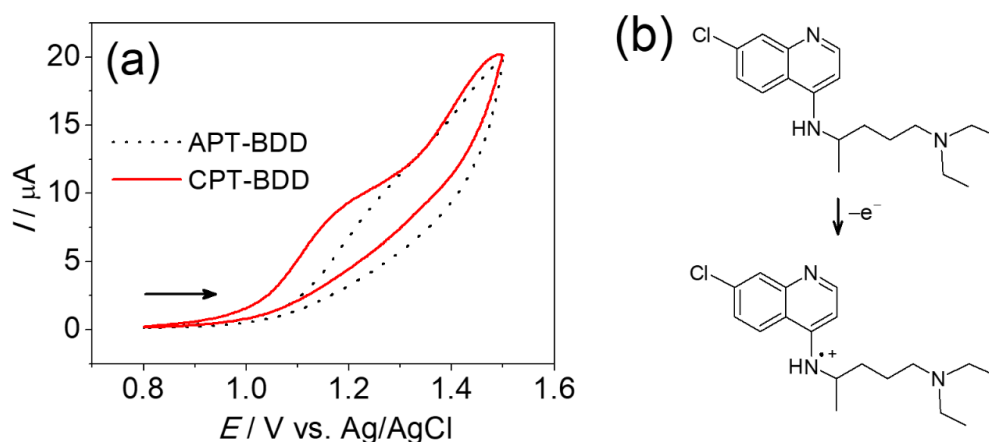


Figure 5. (a) Cyclic voltammograms ($v = 50 \text{ mV s}^{-1}$) obtained for $1.5 \times 10^{-4} \text{ mol L}^{-1}$ chloroquine solution in 0.1 mol L^{-1} phosphate buffer (pH = 7.0) electrolyte after different BDD pretreatments: cathodic (–) and anodic (—). (b) Possible electrooxidation mechanism of chloroquine.

3.3. Square-Wave Voltammetry (SWV) Detection of Chloroquine

The optimization for SWV detection of chloroquine was performed by using the parameters and conditions, as presented in Table 1. The selected conditions were 0.1 mol L^{-1} Britton–Robson buffer (pH = 6.0), frequency of 100 Hz, the amplitude of 50 mV, and potential increment of 5 mV. All these optimum values were recorded from obtaining a well-defined and high-intensity anodic peak as the analytical signal for chloroquine.

Table 1. Optimization of experimental conditions for chloroquine detection by square-wave voltammetry (SWV).

Parameter	Evaluated Conditions	Optimum Condition
Supporting electrolyte	0.1 mol L ⁻¹ H ₂ SO ₄ 0.1 mol L ⁻¹ Britton–Robson buffer 0.1 mol L ⁻¹ acetate buffer	0.1 mol L ⁻¹ Britton–Robson buffer
pH (Britton–Robson buffer)	2.0 to 8.0	6.0
SWV frequency (<i>f</i>)	10 to 150 Hz	100
SWV amplitude (<i>A</i>)	5 to 60 mV	50
SWV potential increment (ΔE)	1 to 10 mV	5

Under the optimum experimental conditions, the analytical curve for chloroquine was constructed. For this, different aliquots of a standard chloroquine solution were added to the electrochemical cell, homogenized by stirring, and the SWV reading was performed for the different obtained concentrations. By doing this, the BDD response was linear in the chloroquine concentration range of 0.01 to 0.25 $\mu\text{mol L}^{-1}$ (Figure 6), in accordance with the following linear regression equation: $I_p (\mu\text{A}) = 0.29 \mu\text{A} + 12.32 \mu\text{A L } \mu\text{mol}^{-1} C_{\text{Chloroquine}} (\mu\text{mol L}^{-1})$, with a correlation coefficient of 0.994 ($n = 3$). The LOD of 2.0 nmol L⁻¹ was estimated ($\text{LOD} = 3 \sigma / m$, i.e., three times the standard deviation of ten measurements of blank solution (σ) divided by the analytical sensitivity (m)) [61].

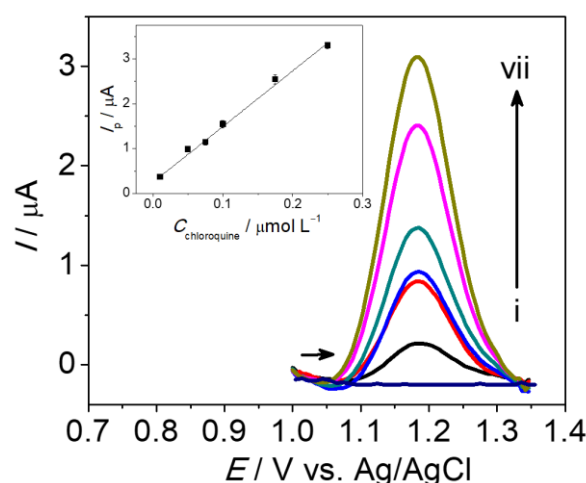


Figure 6. Square-wave voltammograms recorded using a cathodically pretreated BDD electrode for different concentrations of chloroquine in 0.1 mol L⁻¹ Britton–Robinson buffer solution (pH 6.0): (i) blank (purple); (ii) 0.01 (black); (iii) 0.05 (red); (iv) 0.075 (blue); (v) 0.10 (green); (vi) 0.20 (pink); and (vii) 0.25 (yellow) $\mu\text{mol L}^{-1}$. SWV parameters: $f = 100$ Hz, $A = 50$ mV, and $\Delta E = 5$ mV. Inset: Corresponding analytical curve (I_p vs. $C_{\text{Chloroquine}}$).

The repeatability study was performed for a 1.7×10^{-7} mol L⁻¹ chloroquine in 0.1 mol L⁻¹ Britton–Robinson buffer solution (pH 6.0) by using SWV in the selected experimental conditions. The value of 4.2% of relative standard deviation was obtained for $n = 3$. The addition and recovery experiments were also performed for a 1.0×10^{-3} mol L⁻¹ chloroquine (prepared by manipulated drug obtained from a local drugstore) in 0.1 mol L⁻¹ Britton–Robinson buffer solution (pH 6.0). After appropriate dilution, an average value of 102% was obtained for six determinations.

There are few studies in the literature reporting on the quantification of chloroquine by voltammetric analysis. Table 2 shows these works, providing a comparison of the main analytical parameters of the voltammetric procedures [55,62,63]. For the quantification of chloroquine using the rGO@WS₂-QDs nanomaterial, it was necessary to prepare reduced graphene oxide (rGO) by

Hummer's method [64] and tungsten disulphide quantum dots (WS₂-QDs) by hydrothermal method; both methods taking the time and employing toxic reagents. The electrode modified with biological material, double-stranded DNA (dsDNA) [62], required an accumulation potential of +0.35V for 60 s to perform the voltammetric measurements. In contrast, the proposed method using a BDD electrode, the voltammetric measurements were direct and without any preconcentration step. It is also important to note the LOD of the proposed voltammetric method was 10 times lower than the best LOD previously reported, allowing the detection of chloroquine at nanomolar levels. The analytical parameters obtained by applying the BDD electrode and the SWV technique indicate the great potential applicability of the method for the practical quantification of chloroquine. However, due to the current high demand for chloroquine, the Brazilian Health Regulatory Agency (ANVISA) decided to restrict its sale in pharmacies and ban export. Therefore, it was not possible to carry out studies on commercial pharmaceutical samples.

Table 2. Comparison of analytical parameters obtained with the BDD electrode and other modified electrodes from literature for the detection of chloroquine.

Electrode	Linear Range ($\mu\text{mol L}^{-1}$)	LOD ($\mu\text{mol L}^{-1}$)	Reference
rGO@WS ₂ -QDs/ GC	0.5–82.4	0.04	[28]
dsDNA/CP	0.1–10.0	0.03	[33]
CuNW/CP	0.13–13.3	0.02	[34]
BDD	0.01–0.25	0.002	This work

rGO@WS₂-QDs/GCE: reduced graphene oxide@tungsten disulfide quantum dots/glassy carbon electrode; dsDNA/CP: double-stranded DNA/carbon paste; CuNW/CP: copper nanowires/carbon paste electrode.

4. Conclusions

In this work, chloroquine electroactivity on a BDD electrode was investigated for the first time, whereas an irreversible oxidation reaction was diagnosed. Chloroquine is an antimalarial drug that has been presented an irreversible anodic behaviour by using the BDD electrode. The BDD electrode showed once again its efficiency as an electrode material for applications in the voltammetric determination of organic compounds. The influence of the chemical termination of the BDD surface (predominantly in hydrogen in the case of cathodic pretreatment and predominantly in oxygenated groups in the case of anodic pretreatment) was studied, with a more well-defined electrochemical response of chloroquine on the cathodically pretreated BDD. From this observation, the influence of several technical parameters on the anodic response of chloroquine was evaluated (a type of supporting electrolyte, pH, and SWV parameters), and the optimum experimental conditions recorded. Thus, from an analytical point-of-view, promising analytical parameters were registered, including a linear concentration range at submicromolar levels and an LOD at a nanomolar level (2.0 nmol L^{-1}). All these results indicate the capacity of the BDD electrode to quantify chloroquine sensitively, and future studies should be carried out to determine this active principle in real samples.

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