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# Square-wave voltammetric determination of hydroxychloroquine in pharmaceutical and synthetic urine samples using a cathodically pretreated boron-doped diamond electrode

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ABSTRACT

A square-wave voltammetry method for the determination of hydroxychloroquine (HCQ) in pharmaceutical and synthetic urine samples with a cathodically pretreated (CPT) boron-doped diamond (BDD) electrode was successfully developed. Using cyclic voltammetry, the HCQ oxidation was shown to be a one-electron diffusion-controlled process. The obtained SWV analytical curve ($I_{pa}/\mu A = 0.033 + 1.24$ [HCQ/(μmol $L^{-1}$)]) for HCQ determination (using 0.1 mol $L^{-1}$ $H_2SO_4$ as supporting electrolyte) presented a linear response from 0.1 to 1.9 μmol $L^{-1}$, with a detection limit of 0.06 μmol $L^{-1}$, the best value attained yet with an unmodified electrode. The proposed method was successfully applied in the determination of HCQ in pharmaceutical (tablets) samples, with results similar at a 95% confidence level to those obtained using a reference spectrophotometric method. Additionally, adequate recovery results were obtained for the determination of HCQ in doped synthetic urine samples. The novel proposed method is inexpensive and fast, with no need of sample pretreatment.



## 1. Introduction

Hydroxychloroquine (HCQ), Fig. 1, is a drug derived from 4-aminoquinoline. HCQ is used as an antimalarial drug ever since World War II. It is also widely used in the treatment of lupus erythematosus, rheumatoid arthritis, and other inflammatory and skin diseases [1–5]. When ingested, HCQ is rapidly absorbed by the intestine, accumulating in organs such as the liver, spleen, lungs, and kidneys, being partially converted to active metabolites in the liver and excreted primarily via the kidney (15–25% of it unchanged) [6]. In the eyes, this drug is deposited in all tissues that contain melanin; this can cause changes in the iris, choroid, and especially in the retinal pigment epithelium, generating retinopathy [3–5]. The toxicity of HCQ is a cause of ophthalmic concern, because significant improvements in visual recovery are not observed even after the use of the drug is discontinued. According to studies reported in the literature [3,7–9], some risk factors increase the likelihood of retinopathy caused by HCQ; for example, the daily dosage (it should not exceed 6.5 mg $kg^{-1}$ body weight), the cumulative dose, and renal or liver disease, besides age and previous retinal diseases. Thus, pharmaceuticals containing HCQ must undergo strict quality control, which requires the development of simple, rapid, and accurate analytical procedures for the identification and quantification of this drug in both pharmaceutical and biological samples. For the quantification of HCQ in pharmaceutical tablets, the British Pharmacopoeia recommends the use of potentiometric titration in non-aqueous media [10]. Most of the described analytical procedures for the determination of HCQ in pharmaceutical formulations employ chromatographic techniques [11–15], which may have disadvantages such as the need of sample pretreatment, long analysis time, and large consumption of chemicals, thereby generating high amounts of waste.

Electroanalytical methods commonly have some advantages over chromatographic methods, such as lower consumption of chemicals, shorter analysis times, and lower cost of instrumentation. Nevertheless, so far only two such methods for the determination of HCQ have been reported in the literature, using glassy carbon (GC) electrodes, unmodified and modified [16,17]. Arguelho et al. [16] investigated the electrochemical reduction of HCQ at a GC electrode by cyclic voltammetry and chronoamperometry. Then, they developed an analytical method based on differential pulse voltammetry (DPV) and used it to assay HCQ in a pharmaceutical

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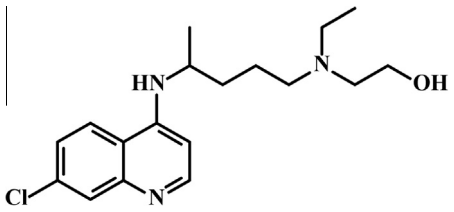


**Fig. 1.** Molecular structure of hydroxychloroquine ((RS)-2-[{4-[(7-chloroquinolin-4-yl)amino]pentyl}(ethyl)amino]ethanol).

formulation (tablets). Their obtained analytical curve was linear in the concentration range of $2 \times 10^{-5}$ to $5 \times 10^{-4}$ mol $L^{-1}$, with a detection limit of 25.8 μmol $L^{-1}$. Very recently, Ghoreishi et al. [17] reported on the use of a GC electrode modified with self-assembled monolayers (SAMs) of *N*,*N*′-bis[(*E*)-(1-pyridyl) methylidene]-1,3-propanediamine (PMPD) to determine HCQ also by DPV. They obtained an analytical curve with two linear concentration ranges, the first from 0.05 to 12.28 μmol $L^{-1}$ and the second from 12.28 to 111.11 μmol $L^{-1}$, with a detection limit of 4.51 nmol $L^{-1}$.

Boron-doped diamond (BDD) electrodes have distinct electrochemical properties from other electrodes commonly used for electroanalytical purposes, such as GC, pyrolytic graphite, and carbon fiber or paste [18–22]: a wide potential window, corrosion stability in highly aggressive media, low and stable background current, long-term stability, low adsorption of polar molecules, and low sensitivity to dissolved oxygen. However, for many analytes, the electrochemical behavior of BDD electrodes is extremely dependent on their surface termination (oxygen or hydrogen), which can be modified by appropriate electrochemical pretreatment (anodic or cathodic pretreatment) [23–28]. For instance, Suffredini et al. [24] evaluated the effect of electrochemical pretreatments on the electrochemical response of a BDD electrode for chlorophenols and reported a significant improvement of this response after the BDD electrode was cathodically pretreated. Due to the properties just described above, BDD electrodes have been increasingly used to develop electroanalytical procedures, especially for organic substances [22,29–37], because very frequently increased stability and accuracy are attained, along with low detection limits.

In this work, we report on the evaluation of the electrochemical behavior of HCQ on an electrochemically pretreated BDD electrode and on the development of a novel electroanalytical method for the determination of HCQ by square-wave voltammetry (SWV) in pharmaceutical and synthetic urine samples using a cathodically pretreated BDD electrode.

## 2. Experimental

### 2.1. Apparatus

All voltammetric measurements were carried out using an Autolab PGSTAT-12 (Ecochemie) potentiostat/galvanostat controlled by the GPES 4.9 software, coupled to a three-electrode electrochemical cell, using a BDD electrode (0.25 $cm^2$ exposed area) as working electrode, a Pt wire as counter electrode, and an Ag/AgCl (3.0 mol $L^{-1}$ KCl) reference electrode, to which all potentials are hereinafter referred to.

The BDD working electrode (a ca. 1 μm thick 8000 ppm BDD film on a silicon wafer, acquired from Adamant Technologies SA, Switzerland) was prepared as described elsewhere [38]. Prior to the electrochemical measurements, the BDD electrode was electrochemically pretreated in a 0.5 mol $L^{-1}$ $H_2SO_4$ solution: anodically by applying 1.0 A $cm^{-2}$ for 30 s and cathodically by applying −1.0 A $cm^{-2}$ for 120 s (this cathodic pretreatment was always preceded by an anodic one to guarantee a clean electrode surface).

For comparative purposes, HCQ determination was also carried out spectrophotometrically [14] using an UV–vis spectrophotometer (Shimadzu Model UV-2550) set at 342 nm with a quartz cuvette (optical path length of 10 mm) and a 0.01 mol $L^{-1}$ HCl solution as solvent.

### 2.2. Reagents and solutions

HCQ sulfate (purity ⩾ 98%) was purchased from Sigma–Aldrich and 95–97% pure $H_2SO_4$ from Merck. All other chemicals used were of analytical grade. All solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore®) (resistivity >18 MΩ cm). Unless otherwise noted, the supporting electrolyte solution in the electrochemical measurements was 0.1 mol $L^{-1}$ $H_2SO_4$, which was used to daily prepare a 10.0 mmol $L^{-1}$ HCQ standard solution (this was done to avoid any problems that could be caused by HCQ photodegradation, although an amber glass flask was used to keep the solution) [10].

### 2.3. Analytical procedure

The investigation of the electrochemical behavior of HCQ and several preliminary studies (such as the effect of electrochemical pretreatment, supporting electrolyte, and acid concentration) were carried out by cyclic voltammetry (CV). The use of SWV was evaluated for the determination of HCQ, when the values of the SWV parameters (frequency, *f*, amplitude, *a*, and scan increment, $\Delta E_s$) were explored and optimized to find the best conditions to develop an analytical method for the determination of this analyte in pharmaceutical and synthetic urine samples. The number of electrons (*z*) involved in the HCQ electrooxidation process was chronoamperometrically estimated by electrolyzing a stirred $2.0 \times 10^{-5}$ mol $L^{-1}$ HCQ in 0.1 mol $L^{-1}$ $H_2SO_4$ solution at an electrode potential of 1.56 V for 40 min and determining the final HCQ concentration by SWV (see below). For such, the following relationship (Faraday equation) was used [39]:

$$Q = z\ F\ n$$

where *Q* is the electric charge (C), *z* the number of electrons transferred, *n* the amount of HCQ (mol) consumed during the electrolysis, and *F* the Faraday constant (96,485 C $mol^{-1}$).

After optimization of the SWV parameters, SW voltammograms were obtained in the potential range of 1.3–1.6 V. For each HCQ concentration, all measurements were carried out in triplicate ($n = 3$). The analytical curve was constructed by addition of aliquots of the 10.0 mmol $L^{-1}$ HCQ standard solution to the electrochemical cell already containing 10.0 mL of the supporting electrolyte solution.

### 2.4. Preparation of the pharmaceutical and synthetic urine samples

One pharmaceutical formulation (tablets, acquired in the local market) with a HCQ sulfate label value of 400 mg was analyzed. For such, ten tablets were individually weighed and powdered, then their average mass (0.7236 g) was used for the following calculations. A mass of 0.3917 g was transferred to a 50.0 mL calibrated flask and the volume was completed with the supporting electrolyte solution. This solution was diluted to 10.0 mmol $L^{-1}$ and an aliquot of 500.0 μL was transferred directly to the electrochemical cell. The sample was analyzed using the standard addition method without any prior treatment or separation. Measurements were made in triplicate.