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Electrochemical study of hydroxychloroquine and its determination in plaquenil by differential pulse voltammetry

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Abstract

Hydroxychloroquine (HCQ) is a halogenated aminoquinoline that presents wide biological activity, often being used as an antimalarial drug. The electrochemical reduction of HCQ was investigated by cyclic voltammetry and chronoamperometry using glassy carbon electrodes. By cyclic voltammetry, in acid medium, only the cathodic peak was observed. The electrochemical behavior of this peak is dependent on pH and the electrodic process occurs through an E_rC_i mechanism. The electron number (1e) consumed in the reduction of HCQ was obtained by chronoamperometry. A method for the electrochemical determination of HCQ in pharmaceutical tablets was developed using differential pulse voltammetry. The detection limit reached was 11.2 μg ml^{-1} of HCQ with a relative standard deviation of 0.46%. A spectrophotometric study of HCQ has been also carried out utilizing a band at 343 nm. The obtained detection limit and the relative standard deviation were 0.1 μg ml^{-1} and 0.36%, respectively. The electrochemical methods are sufficiently accurate and precise to be applied for HCQ determination, in laboratorial routine, which can be used to determine the drug at low level.

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Keywords: Hydroxychloroquine; Voltammetric behavior; Plaquenil; Electrochemical determination

1. Introduction

Hydroxychloroquine (HCQ) (**1**) is a potent antimalarial drug [1,2] also being used frequently as antirheumatic substance [3,4]. Determination of this compound includes extraction followed by nonaqueous titrimetric [5] or spectrophotometric methods based on reactions with iodide [6], quinones [7,8] and cobalt thiocyanate [9]. Because of the nonspecificity of most of these reactions, prior extraction of HCQ is commonly involved in the assay methods. Besides, a number of different HPLC methods have been proposed for the determination of HCQ in biological fluids using spectrophotometric [10] or fluorescence [11,12] detection. Current USP methods [13] utilizes a simple sample preparation analyzed using an isocratic HPLC system with UV detection.

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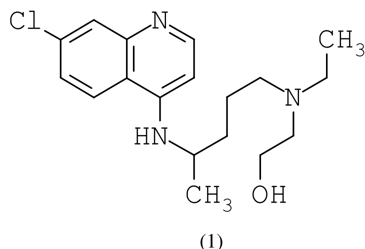


(1)

Few electrochemical methods has been used for determination of aminoquinolines. Among them can be mentioned the potentiometric determination of the chloroquine (CQ) in various pharmaceutical preparations using an ion selective membrane electrode [14]. However, no example of the electrochemical determination of HCQ was found in the literature.

The principal aim of present work was to develop an electrochemical method to detect HCQ in pharmaceutical formulations. A differential pulse voltammetry technique could be applicable to the analysis of these drugs containing HCQ.

For this purpose, the electrochemical behavior of HCQ was studied by cyclic voltammetry in similar conditions to that employed in the voltammetric determination.

2. Experimental

2.1. Reagents

Aqueous HCQ sulfate stock solutions were prepared by dissolving the pure HCQ sulfate (Sigma Chemical, USA). Buffered solutions were prepared by mixing a solution containing 0.1 mol l^{-1} orthophosphoric acid, 0.1 mol l^{-1} boric acid and 0.1 mol l^{-1} acetic acid with appropriate volume of 0.5 mol l^{-1} sodium hydroxide. Water was deionised using Milli-Q equipment.

2.2. Instrumentation

Electrochemical measurements were performed using a 273 EG&G PARC potentiostat equipped with a three-electrode system: a glassy carbon electrode as working electrode (0.5 cm^2), a platinum wire as an auxiliary electrode and a SCE as reference electrode. The pH was adjusted using a Micronal B372 pH meter. Absorbance measurements were made with a double beam HP 8452A diode array spectrophotometer. Quartz cells with 1.00 cm of path length were used for all measurements in the spectral range 200–500 nm, at a scanning rate of 200 nm min^{-1}, against suitable blank.

2.3. Voltammetric procedure

For voltammetric measurement, 5.0 ml of the electrolyte solution and the appropriate amounts of HCQ solution were added to the cell, and degassed with pure nitrogen (oxygen free) for 10 min. The cathodic potential sweep was carried under different operational parameters. Before each measurement, the glassy carbon electrode was polished with 0.05 μm alumina suspension. Residual polishing material was removed from the surface by sonication. All experiments were made at room temperature.

Optimum operational parameters for differential pulse voltammetry were obtained for HCQ determination (Table 1).

Calibration curves were obtained through sucessive additions of HCQ. Three measurements were carried out for each sample and the statistical treatment of the data was carried out according to Miller [15]. In this work, all weight-based expression of results says respect to the HCQ salt form.

2.4. Spectrophotometric procedure

HCQ was dissolved in 0.01 mol l^{-1} HCl, as recommended by British Pharmacopoeia [5], and

Table 1
Optimum operational parameters selected for the voltammetric determination of HCQ solutions

Parameters	Range	Pulse differential voltammetry
pH	2.0–12.0	4.0
Pulse time (ms)	5–100	5
Pulse width (mV)	5–100	50
Scan rate (mV s^{-1})	2–20	8

concentrations between 1×10^{-7} and 1×10^{-4} M were prepared. These solutions were directly added to the quartz cell to measure absorbance at 343 nm.

2.5. HCQ assay in formulations

Both voltammetric and spectrophotometric techniques were used to determine HCQ in pharmaceutical formulations. Twenty Plaquenil® tablets (Sanofi Winthrop, with declared amount of HCQ = 400 mg per tablet) were triturated in an agate mortar, pounded and 400 mg of the powder was finally dissolved in 250 ml of B-R buffer (pH 4.0) or 250 ml of 0.01 mol l^{-1} HCl with the aid of sonication (15 min). An aliquot of this solution was added to 5 ml B-R buffer (pH 4.0) and the mixture placed into the voltammetric cell. The voltammograms were recorded following the voltammetric procedure. The spectrophotometric determinations of HCQ were made by adding an aliquot of the HCQ per 0.01 mol l^{-1} HCl solution and completing the dilution to 5 ml with 0.01 mol l^{-1} HCl. For both methodologies, the standard addition method was used.

3. Results and discussion

3.1. Electrochemical behavior

HCQ is electrochemically reducible on glassy carbon electrode in B-R buffer under all pH range of 2–10 (Fig. 1). The effect of scan rate and pH were investigated in relation to the voltammetric behavior. An increase in pH causes a shift of 35 mV in the cathodic peak, in the pH range between 4 and 7 (Fig. 2a), indicating the influence of the protonation on the electrodic process. Probably, a predominance of the diprotonated form in acid medium and monoprotonated form in neutral medium should occur. In pH higher than 7.0, the neutral form is present and a diminution of solubility of the compound can be observed. Starting from the behavior of E_p vs. pH was possible to determine the pK_{a1} as being 3.4 for the quinoline ring nitrogen and pK_{a2} equals the 6.8 for the aliphatic amine.

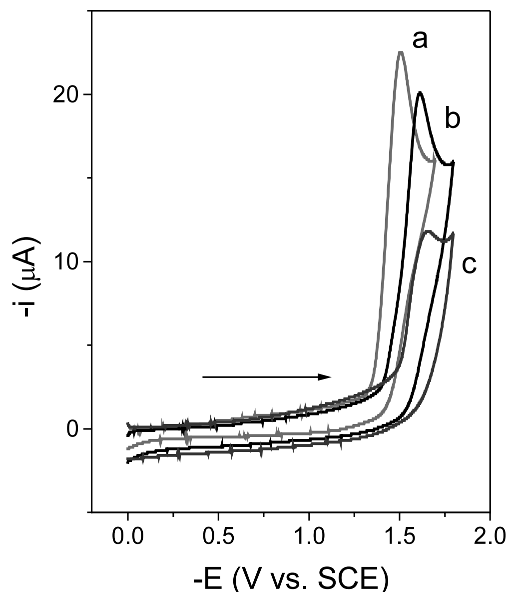


Fig. 1. Cyclic voltammograms of 1.0×10^{-3} mol l^{-1} of HCQ in B-R buffer pH: (a) 4.0; (b) 7.0; (c) 10.0 ($v = 0.1$ V s^{-1}).

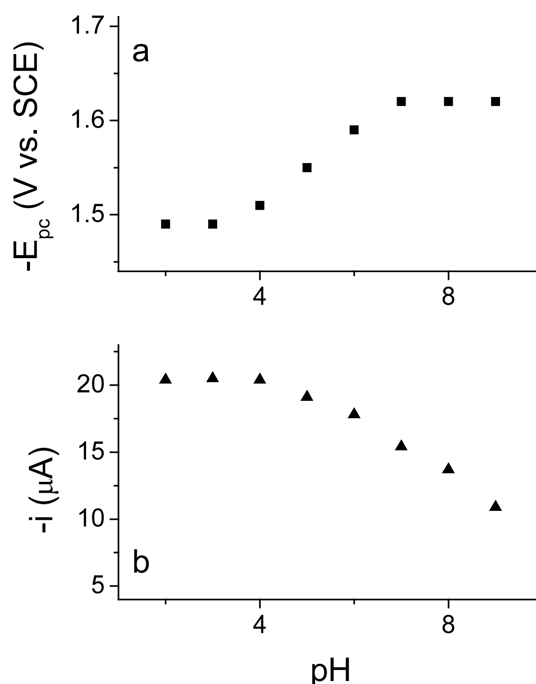


Fig. 2. Dependence of peak potential on pH to HCQ in B-R buffer, $v = 0.1$ V s^{-1}, in the potential intervals from 0 to −1.8 V vs. SCE. (a) Peak potential; and (b) peak current.

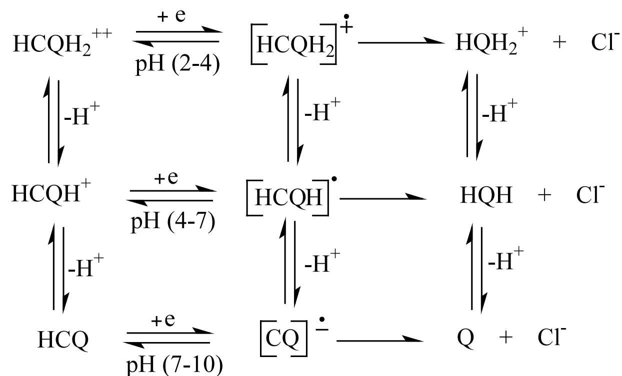
The cathodic current showed a gradual decrease with the increase of pH (Fig. 2b). This behavior is characteristic of electrodic processes influenced by protonic concentration of the solution indicating the presence of an acid–base equilibrium. According to literature [16], the rate of such deprotonation increases with increasing pH until a value sufficiently is attained when pratically no acid is produced and conjugate base can undergo reduction, usually at more negative potentials. However, as reduced species are often proton acceptors from vailable donors, the protonation occurs either before or/and after the eletronic transfer.

Cyclic voltammograms obtained in different scans rate were analyzed through the diagnostic approaches to electrochemical mechanism described by Nicholson and Shain [17,18]. In the interval of 0.01–1.0 V s^{-1}, the reduction of HCQ at pH 4.0, 7.0 and 9.0 was found to be an irreversible process, because no anodic current that can correspond to reoxidation is observed in the reverse scan.

Considering that so much the diprotonated species as the monoprotonated are electroactives, it was waited a linear plot of peak current as a function of $v^{1/2}$, indicating that the electrodic process is diffusion controlled in the range of pH investigated. The current function ($i_{pc}v^{-1/2}$) was independent of the scan rate and the peak potential shifts to more negative values with an increase in the scan rate. This behavior is indicative that the deprotonation reaction is fast enough to permit a diffusion control of the electrodic process. Nevertheless, the occurrence of an irreversible chemical reaction is also consuming the product electrogenerated (E_rC_i), which could be attributed to the subsequent cleavage of the C–Cl bond as observed in the reduction of other halogenated quinolines [19,20].

In all pHs studied chronoamperometric results acquired in the time range $0.5 < t < 5$ s for 1×10^{-3} mol l^{-1} compounds exhibit constant values of $it^{-1/2}/C$, indicating an electrodic process involving just one electron per molecule.

So, the voltammetric behavior of HCQ corresponds to an electrodic irreversible process, due the occurrence of a E_rC_i mechanism possibly related to the cleavage of C–Cl bond, subsequently to the monoelectronic reduction of the forms diprotonated and monoprotonated molecule. These informations about the electrodic behavior of the HCQ in acid medium can be visualized by the scheme:



3.2. Differential pulse voltammetry

Based on the voltammetric behavior of HCQ, a quantitative method was developed. In order to select the best electrochemical method, we compared the cathodic peak obtained by differential pulse, square wave and linear voltammetry and the best ratio of the peak to background currents was obtained with differential pulse voltammetry. In fact, the pulse techniques has been employed with success in the analytical determination of several chemotherapic compounds, due its better discrimination between background electrolyte current and faradaic current [21,22].

The best result of current was obtained in pH 4.0 similar to cyclic voltammetric results. At highest acid medium, this electrodic process is affected by hydrogen reduction.

The influence of scan rate in differential pulse voltammetry was investigated in the interval from 2 to 20 mV s^{-1}. Best results were obtained at scan rates between 2 and 8 mV s^{-1}. On the other hand, the increase of the pulse time promotes a reduction of the anodic current parallel to the decrease of the peak resolution, being the time of 5 ms being the most appropriate.

The pulse width was ranged in the interval of 5–100 mV showing a loss in the peak resolution in widths above 100 mV.

The technique of differential pulse voltammetry showed, under optimized conditions, a linear behavior of cathodic current peak in function of the HCQ concentration, in the interval from 2×10^{-5} to 5×10^{-4} mol l^{-1} (Table 2). The detection limit is given by $3S_{yx}/b$, the ratio of the standard deviation of y-residuals (S_{yx}) and the slope of the regression line (b). The detection limit obtained was 11.2 ± 2.6 μg ml^{-1} and standard deviation 1.1% ($n = 6$).

3.3. Spectrophotometry

Spectrophotometric measurements of HCQ in 0.01 mol l^{-1} HCl in the range 200–400 nm showed three maxima absorbance: 257, 329 and 343 nm (Fig. 3). The higher absorbance was obtained at 343 nm. According to literature, the absorption in this wavelength is attributed to the quinolinium group.

The absorbance measurements at 343 nm had a linear relationship with HCQ concentration between 4×10^{-6} and 2×10^{-5} mol l^{-1} (Table 2).

Table 2
Calibration lines for quantitative determination of HCQ by pulse differential voltammetry and UV spectrophotometry

Parameters	Pulse differential voltammetry	Spectrophotometry
Limit range (mol l^{-1})	2×10^{-5} to 5×10^{-4}	4×10^{-6} to 2×10^{-5}
Slope (b)	1.3×10^{-2}	2.4×10^{4}
Intercept (a)	2.9×10^{-7}	1.0×10^{-2}
Standard deviation of the slope (S_{yx})	2.6×10^{-5}	146.3
Standard deviation of the intercept (S_a)	6.5×10^{-9}	2.0×10^{-3}
Error standard deviation (S_b)	1.3×10^{-8}	2.0×10^{-3}
Limit detection (μg ml^{-1})	11.2 ± 2.6	0.01 ± 1.0
Correlation coefficient (r)	0.9999	0.9999
Data number (n)	6	5

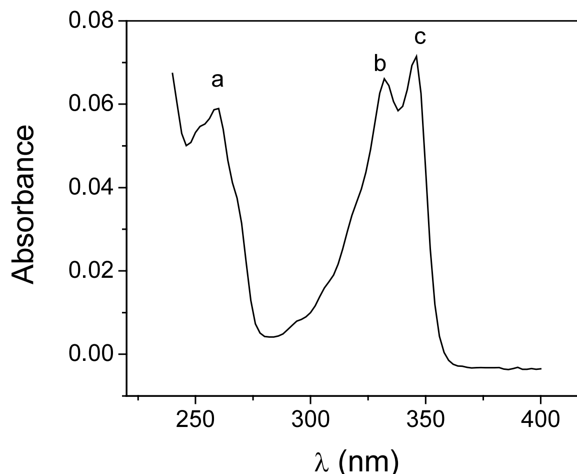


Fig. 3. Absorption spectrum of HCQ 4×10^{-5} mol l^{-1} in 0.01 mol l^{-1} HCl. (a) 257; (b) 329; and (c) 343 nm.

The relative standard deviation was 0.2% ($n = 6$) and the limit detection was 0.01 ± 1.0 μg ml^{-1}.

3.4. Pharmaceutical dosage form assays

Without any previous preparation, but just through adequate dilution of the analyte present in the solution of Plaquenil® tablets, pulse differential voltammetry and UV spectrophotometry can be used for the determination of HCQ. It is important to notice that in British Pharmacopoeia methods the alkaline extraction produces the neutral species that is not very soluble in a little aqueous, while in the acid extraction there is not change in the protonation of the present HCQ in the tablet.

Fig. 4 shows the voltammograms obtained for determination in tablets by means of the standard addition method. Six determinations were carried out at each concentration (Table 3), and results obtained gave a mean value by tablets of 386.7 ± 0.8 mg (spectrophotometry at 343 nm) and 384.5 ± 0.5 mg (pulse differential voltammetry) which is in accordance with the declared amount (400 mg).

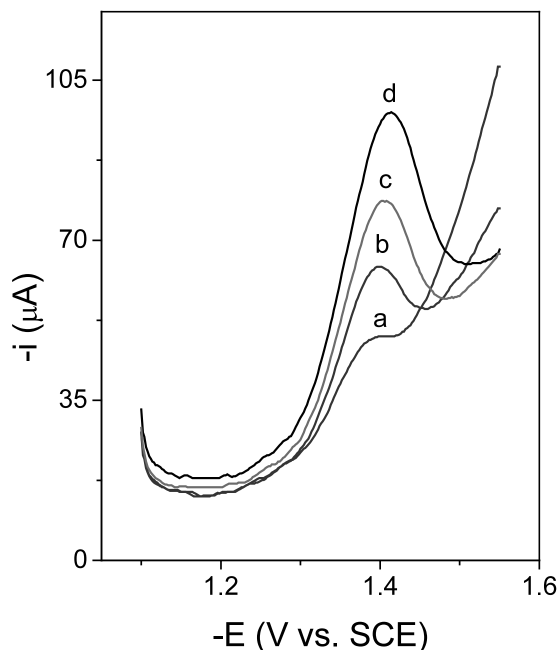


Fig. 4. Pulse differential voltammograms for HCQ in B-R buffer (pH 4.0), $v = 8$ mV s^{-1}, $\Delta E = 5$ mV and $t = 5$ ms. (a) Pure sample; (b) 3.5×10^{-5}; (c) 6.9×10^{-5}; and (d) 1×10^{-4} mol l^{-1}.

Table 3
Pulse differential voltammetry and UV spectrophotometry results on the assay of HCQ tablets

Modes	Pulse differential voltammetry	Spectrophotometry
Data number (n)	4	4
Limit range (mol l^{-1})	3.5×10^{-5} to 1.0×10^{-4}	4.0×10^{-6} to 1.5×10^{-5}
Slope (b)	4.4×10^{-1}	1.7×10^{4}
Intercept (a)	7.1×10^{-6}	5.5×10^{-1}
Standard deviation of the Slope (S_{yx})	2.0×10^{-3}	172.3
Standard deviation of the Intercept (S_a)	1.1×10^{-7}	1.6×10^{-3}
Error standard deviation (S_b)	1.3×10^{-7}	2.0×10^{-3}
Limit detection (μg ml^{-1})	8.9 ± 1.9	0.02 ± 0.05
Correlation coefficient (r)	0.9999	0.9999
No. of experiments	6	6
HCQ added (μg)	35.0	5.9
HCQ found (μg)	33.6	5.7

4. Conclusions

The pulse differential voltammetric results obtained in this work permit us to propose an effective method to determine HCQ in Plaquenil® tablets. This method is simple, precise and affordable; it also requires no complex pre-treatment of the active principle to be determined. The procedure and its application to pharmaceutical form of the HCQ represents a good alternative in the routine of the laboratorial analysis.

Acknowledgements

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