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Short communication

Accumulation and trace measurement of chloroquine drug at DNA-modified carbon paste electrode

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Abstract

The voltammetric behaviour of chloroquine was investigated at carbon paste and dsDNA-modified carbon paste electrodes in different buffer systems over a wide pH range using cyclic and differential pulse voltammetry. Chloroquine was oxidized in the pH range 2.0–11.0 yielding one irreversible main oxidation peak. A second peak was also observed only in the pH range 5.0–7.0. The modification of the carbon paste surface with dsDNA allowed a preconcentration process to take place for chloroquine such that higher sensitivity was achieved as compared with the bare surface. The response was characterized with respect to solution pH, ionic strength, accumulation time and potential, chloroquine concentration, and other variables. Stripping voltammetric response showed a linear calibration curve in the range 1.0×10^{-7} to 1.0×10^{-5} mol l^{-1} with a detection limit of 3.0×10^{-8} mol l^{-1} at the dsDNA-modified electrode. Application of the modified electrode to serum, without sample pretreatment, resulted in good recovery higher than 95% and the higher standard deviation was 3.0%.

Keywords: Voltammetry; Chloroquine; DNA–drug interaction; DNA-modified electrode; Serum analysis

1. Introduction

Chloroquine, 4-(7-chloro-4-quinolylamino) pentyldiethylamine (**I**) has been used extensively for several decades as a suppressant in the prophylaxis and treatment of clinical attacks of malaria, which is probably the most widespread disease to afflict mankind. It is also used in the treatment of rheumatoid arthritis and similar collagen diseases, and for amoebic hepatitis [1].

Several studies suggested that interactions of chloroquine with DNA might underlie the antimalarial activity of this drug. This idea was derived from the demonstrated interaction of the 4-aminoquinolines, especially chloroquine, with DNA [2–4] and the ability of a number of quinolines to inhibit DNA replication and RNA synthesis.

Determination of drug concentrations in different body fluids is important for prophylaxis and treatment of malaria cases, investigation of pharmacokinetics as well as help to decide true resistance of malaria parasites to different antimalarials. The drug has been determined in biological fluids with methods such as colorimetric tests [5,6] spectrophotometry [7], spectrofluorometry [8,9], high-performance liquid chromatography with fluorescence detection [10–13], thin-layer chromatography [14,15], gas chromatographic methods [16,17], and capillary zone electrophoresis [18].

Electrochemical methods for the analysis of drug–DNA binding were demonstrated [19–26]. So far, few electrochemical techniques have been attempted for the purpose of the study of DNA interaction with chloroquine [26,27]. In this paper, a sensitive transfer stripping voltammetric procedure was developed for the determination of the chloroquine drug with carbon paste electrode (CPE) modified with dsDNA, with the view of the association of chloroquine with dsDNA immobilized at electrode surface.

2. Experimental

2.1. Apparatus

All voltammograms were obtained with a computer-driven AEW2 Analytical Electrochemical Workstation with ECprog3 Electrochemistry software (Sycopel, England) in

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combination with C-2 stand with a three-electrode configuration: a carbon paste electrode (BAS Model MF-2010, 3 mm diameter) or DNA-modified carbon paste working electrode, an Ag/AgCl/3 M KCl (BAS Model MF-2063) reference electrode, and a platinum wire (BAS Model MW-1032) counter electrode. Microcal Origin (v.6.1) software was used for the transformation of the initial signal. A CG 808 (Schott Geräte, Germany) digital pH meter with glass combination electrode served to carry out the pH measurements.

2.2. Reagents

Double-stranded calf thymus dsDNA was purchased from Sigma and used as received. Chloroquine as diphosphate was supplied by Egypt Cid Company (purity > 95.0%). The stock solution of chloroquine (1×10^{-3} mol l^{-1}) was prepared in bidistilled water. The stock solution of DNA (1000 μg ml^{-1}) was prepared in phosphate buffer (0.02 mol l^{-1}, pH 8.0). Dilution was done just prior to use. All solutions were prepared from analytical-grade chemicals and sterilized Milli-Q deionized water.

2.3. Procedure

The carbon paste was prepared in the usual way by hand-mixing of graphite powder (Aldrich, Milwaukee, WI; ø = 1–2 μm) and 1.8 ml of Nujol (Sigma; $d = 0.84$ g ml^{-1}). The ratio of graphite powder to mineral oil was 70:30. The CPE surface was pre-treated by applying +1.70 V for 1 min in phosphate buffer solution (0.05 M, pH 7.0) without stirring, creating a surface on which dsDNA adsorbed through electrostatic interaction with carboxyl moieties [28]. The dsDNA was adsorbed on the surface of the pre-treated CPE by applying a potential of +0.50 V for 5 min in 10 ppm DNA solution with stirring. The electrode was then rinsed with deionized water. Chloroquine was accumulated at the dsDNA-CPE by immersion in a buffer containing chloroquine for selected times with stirring at 1200 rpm. After the accumulation, the electrode was then removed from the solution and washed with distilled water for 15 s (to eliminate non-specifically adsorbed substances) and with the background electrolyte for 15 s (to keep the electrolyte concentration in the voltammetric cell constant). The electrode was finally placed in the voltammetric cell with a background electrolyte (not containing any dissolved drug). Cyclic voltammogram or differential pulse voltammogram was recorded. The dsDNA-modified electrode was regenerated by removing the accumulated drug, by scanning it in solution of high-ionic strength under stirring, and checked by recording a blank voltammogram.

2.3.1. Recovery studies in human serum

Serum samples, obtained from healthy samples were stored frozen until assay. Known amounts of chloroquine and 0.1 ml perchloric acid (0.1 mol l^{-1}) were added to the drug-free human serum (1.0 ml). The mixture was centrifuged for 10 min at 5000 rpm. Then, the protein precipitate was discarded and an aliquot (0.5 ml) of the clear supernatant was completed to 5.0 ml of acetate buffer (pH 4.0, 0.02 mol l^{-1}) and transferred to an accumulation cell and the voltammetric procedure was continued as for pure drug.

3. Results and discussion

3.1. Voltammetric behaviour at carbon paste electrode

Preliminary cyclic voltammetry experiments for 5.0×10^{-5} mol l^{-1} chloroquine in Britton–Robinson buffer background solutions over the pH range 2.0–11.0 were carried out by using bare-carbon paste electrode. Fig. 1 shows some representative cyclic voltammograms obtained at carbon paste electrode. Chloroquine is oxidized in the pH range 2.0–11.0, yielding one main oxidation peak. A second peak was also observed at less positive potential only in the pH range 5.0–7.0. Both oxidation processes involved are irreversible, as no cathodic peak was found at scan rates between 10 and 500 mV s^{-1}. The anodic peaks may be attributed to the irreversible oxidation of the *N*-heterocyclic nitrogen of the aminoquinoline moiety [29] and the nitrogen of alkylamino side chain grouping of chloroquine molecule.

The potentials of chloroquine oxidation peaks shifted toward less positive values by increasing the pH. When potentials of chloroquine oxidation peaks are plotted as a function of pH, it follows that, between pH 5.0 and 7.0, the slope dE_p/dpH for the less positive peak of chloroquine is about −0.073 V/pH, whereas that for the more positive peak about −0.099 V/pH until pH 9.0, then remains practically pH independent. The intersection point observed at pH 9.0 can be attributed to the pK_a value of 8.4 reported in the literature for the *N*-heterocyclic nitrogen of the aminoquinoline moiety of chloroquine molecule [30].

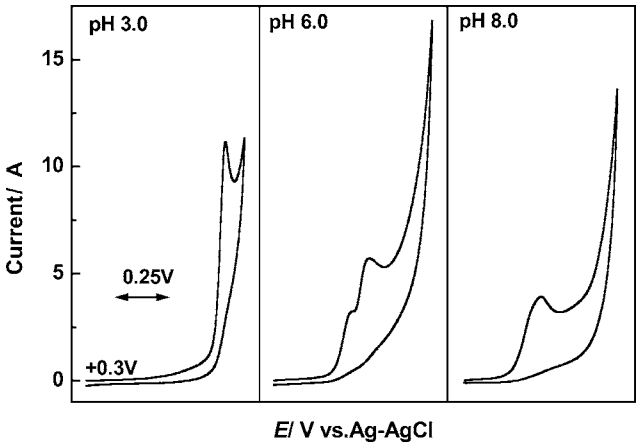

Fig. 1. Cyclic voltammograms (CVs) for 5.0×10^{-5} mol l^{-1} chloroquine on carbon paste electrode in Britton–Robinson buffer at different pH values; scan rate, 100 mV s^{-1}.

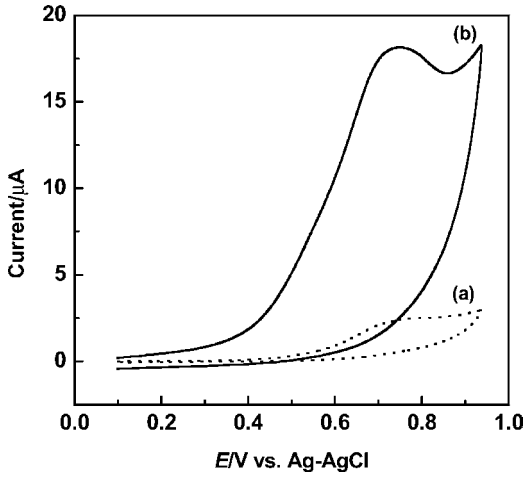

Fig. 2. Cyclic voltammograms (CVs) obtained after accumulation of chloroquine at pre-anodized bare CPE (a) and dsDNA-CPE (b) from a stirred 5.0×10^{-5} mol l^{-1} chloroquine in acetate buffer (0.02 M, pH 4.0) solution for 60 s at +0.35 V, followed by washing the electrode, and transfer into a voltammetric cell containing phosphate buffer blank solution (0.02 M, pH 8.0); scan rate, 100 mV s^{-1}.

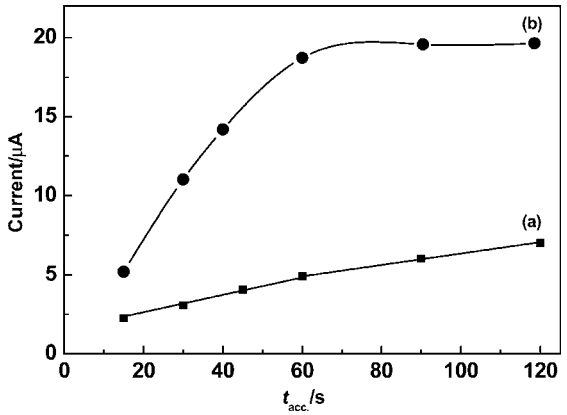

Fig. 3. Accumulation curves of (a) 1.0×10^{-5} mol l^{-1} and (b) 5.0×10^{-5} mol l^{-1} chloroquine obtained with a dsDNA-CPE using the same experimental conditions as in Fig. 2.

Cyclic voltammograms for 5.0×10^{-5} mol l^{-1} chloroquine in B–R buffers at pH 3.0, 6.0, and 8.0 at different potential scan rates were then recorded. Plots of peak current versus the square root of scan rate for the two peaks are linear in the range 20–250 mV s^{-1}, which provide evidence that these oxidative processes involve diffusion of the electroactive species [31]. The peak potential shifted towards more positive values as the scan rate was increased, a further evidence for irreversible processes.

3.2. Voltammetric behaviour at dsDNA-modified carbon paste electrode

Fig. 2 displays cyclic voltammograms recorded in blank phosphate buffer (0.02 M, pH 8.0) solution after dipping the pre-anodized bare-carbon paste (a) and dsDNA-modified carbon paste (b) electrodes in a stirred 5.0×10^{-5} mol l^{-1} chloroquine in acetate buffer (0.02 mol l^{-1}, pH 4.0) at an accumulation potential of +0.35 V for 60 s. The bare-carbon paste shows a single anodic peak, associated with the oxidation of the adsorbed drug. In contrast, the dsDNA-modified electrode exhibits a substantially larger (about 10-fold) signal, reflecting its stronger affinity to chloroquine. Such higher affinity might be attributed to the intercalative and electrostatic binding of the drug with the surface-confined dsDNA layer [2–4]. As a result, trace level of chloroquine can be quantified by means of stripping analysis.

The pH plays an important role on the preconcentration step. The stripping peak current recorded in the blank phosphate buffer (0.02 mol l^{-1}, pH 8.0) solution after 60 s accumulation from buffer solutions (phosphate, acetate, borax–HCl, and Britton–Robinson buffer) in the pH range 2.0–11.0 showed the maximum in acetate buffer solution (0.02 mol l^{-1}, pH 4.0), which was selected for accumulation step. Chloroquine is positively charged at low pH values [32], allowing interaction with the negatively charged phosphate backbone DNA. The pH of background electrolyte in measurement cell has also a significant effect. A phosphate buffer (0.02 mol l^{-1}, pH 8.0) was found to be optimum.

The response of the stripping peak at various accumulation potentials was also carried out in the range −0.2 to +0.4 V or at open circuit potential following 60 s accumulation from 5.0×10^{-5} M chloroquine solution at pH 4.0. No real difference in stripping peak current was recorded in the range −0.2 to +0.1 V, but from +0.2 V onwards a significant increase in the response was observed. Such behaviour could be attributed to the partial desorption of the adsorbed dsDNA which may occur at less positive accumulation potentials. An accumulation potential of +0.35 V was chosen as the optimum accumulation potential for further study.

Fig. 3 shows the accumulation curves of the chloroquine at two concentration: 1.0×10^{-5} and 5.0×10^{-5} mol l^{-1} on dsDNA-modified electrodes. This figure demonstrates that at low concentration of chloroquine (1.0×10^{-5} mol l^{-1}) there is a linear response up to 60 s after which the slope changes. For higher concentration of chloroquine, i.e. 5.0×10^{-5} mol l^{-1}, the current increased rapidly within 60 s and levelled off at longer accumulation times, suggesting a fast rate of interaction of chloroquine with the modified electrode. The influence of ionic strength on the efficiency of the accumulation of 5.0×10^{-5} mol l^{-1} chloroquine solution was studied at dsDNA-CPE using linear sweep voltammetry. The ionic strength was varied by changing the NaCl from 0.10 to 0.50 mol l^{-1} in acetate buffer of pH 4.0. The results suggest that the reaction process is highly dependent on salt concentration. There was an apparent decrease in current response as the salt concentration increased, varying from approximately 13 μA at 0.10 mol l^{-1} to 2 μA at 0.50 mol l^{-1} NaCl. At low-ionic strength conditions, it might be assumed that the reaction mechanism includes the electrostatic attraction of the negative phosphate groups on the exterior of the DNA double helix with chloroquine cations. Thus, a high

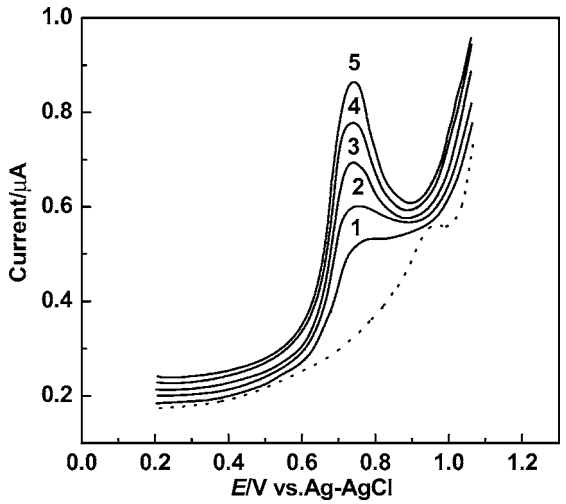

Fig. 4. Differential pulse voltammograms (DPVs) obtained after medium exchange for increasing concentration of chloroquine; (1) 2.0×10^{-7} mol l^{-1}, (2) 4.0×10^{-7} mol l^{-1}, (3) 6.0×10^{-7} mol l^{-1}, (4) 8.0×10^{-7} mol l^{-1}, and (5) 1.0×10^{-6} mol l^{-1}. Dotted lines (. . .) represent the blank. Pulse amplitude, 50 mV; pulse width, 30 ms; scan rate, 10 mV s^{-1}.

signal of chloroquine was found at low-ionic strength. In a high-ionic strength solution, the ionic shielding of the negative charges on the DNA was achieved and thus chloroquine may no longer interact with DNA electrostatically, and the electrochemical signal might only be attributed to chloroquine cations that intercalated into the DNA double helix between stacked base pairs of DNA. This is in agreement with the experimental data obtained for the affinity of DNA for chloroquine by equilibrium dialysis using tritiated chloroquine [4]. Advantageously, the chloroquine signal decreased with an increase of ionic strength, which can be utilized for a removal of the reversibly bound chloroquine.

Fig. 4 illustrates differential pulse voltammograms of five different concentrations of chloroquine at DNA-modified carbon paste electrode following 60 s stirring at +0.35 V in acetate buffer (0.02 mol l^{-1}, pH 4.0) and after medium exchange for phosphate buffer blank solution (0.02 mol l^{-1}, pH 8.0). The response showed calibration curve covering two orders of magnitude, 1.0×10^{-7} to 1.0×10^{-5} mol l^{-1}. The following calibration equation was obtained: $i(\mu A) = 0.0825 + 0.4361C(\mu mol\,l^{-1})$ ($n = 10$; $r = 0.9990$). A limit of detection of chloroquine (calculated using a signal-to-noise ratio of three) was 3.0×10^{-8} mol l^{-1}. The repeatability of the DNA-modified electrode was evaluated at concentration level of chloroquine 5.0×10^{-7} M resulting in acceptable relative standard deviations, for 10 consecutive runs, of ±2.2%. The DNA-modified electrode was renewed prior to each assay.

In order to check the applicability of the method to biological materials, the recovery studies were performed in human serum. Serum samples were fortified with chloroquine to achieve final concentrations of 2.0×10^{-6}, 6.0×10^{-6}, and 1.0×10^{-5} mol l^{-1}, respectively. The amount of chloroquine was determined in triplicate in serum extract using standard addition method. There are no extraneous peaks in voltammograms obtained from serum samples. The average recovery was higher than 95% and the higher standard deviation was 3.0%, which indicated good recovery from serum samples

Following administration, chloroquine is rapidly dealkylated into pharmacologically active desethyl-chloroquine, bis-desethylchloroquine, 7-chloro-4-aminoquinoline, and 7-chloro-4-hydroxyquinoline. Other metabolites include chloroquine side chain *N*-oxide, chloroquine di *N*-oxide and carboxylic acid metabolite [33]. The metabolites could not be differentiated from the intact compound using voltammetric method since metabolism of the chloroquine does not modify the oxidation potential of the electroactive aminoquinoline moiety. For application of the proposed to the analysis of chloroquine in serum, a separation step may be needed to avoid interference of the drug metabolites. Further work is necessary to determine the relative affinity of various metabolites to DNA.

4. Conclusion

Application of the dsDNA-CPE allows the preconcentration of chloroquine and increases the sensitivity as against the bare-carbon paste electrode. Convenient quantitation at the micromolar levels is feasible using short accumulation periods. At an effective dosage of 250 mg per day, steady-state plasma concentrations are in the range, varying between 0.8 and 2.0×10^{-6} mol l^{-1} [34]. The voltammetric measurement of dsDNA-CPE for serum samples after medium exchange is, thus, sufficiently sensitive to determine the levels expected in serum after therapeutic doses. The proposed voltammetric technique has the advantages of being, simpler, faster and less expensive than HPLC methods described in literature. This work also offers further information regarding the chloroquine–DNA interaction.

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