Bioelectrochemistry 80 (2011) 175–181

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Anodic behavior of clioquinol at a glassy carbon electrode

Masoumeh Ghalkhani [a,b], Isabel P.G. Fernandes [a], Severino Carlos B. Oliveira [a], Saeed Shahrokhian [b], Ana Maria Oliveira–Brett [a,*]

[a] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal
[b] Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form 8 October 2010
Accepted 10 October 2010
Available online 16 October 2010

Keywords:
Clioquinol
Oxidation mechanism
Adsorption process
Phenoxy radical
Voltammetry
Glassy carbon

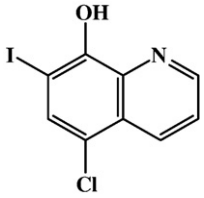
ABSTRACT

Clioquinol is an antifungal, antiprotozoal and an Alzheimer's disease drug with cytotoxic activity toward human cancer cells. The electrochemical behavior of clioquinol and its oxidation product was studied using cyclic, differential pulse and square-wave voltammetry over a wide pH range on a glassy carbon electrode. The results revealed that the oxidation of clioquinol is an irreversible pH-dependent process that proceeds with the transfer of one electron and one proton in an adsorption-controlled mechanism and results in the formation of a main oxidation product, which adsorbs very strongly on the glassy carbon surface. The charge transfer coefficient was calculated as 0.64. The adsorbed oxidation product presented reversible redox behavior, with two electron and two proton transfer. The electrochemical oxidation of clioquinol as a phenolic compound involves the formation of a phenoxy radical which reacts in at least two ways: in one pathway the radical initiates polymerization, the products remaining at the electrode surface, and in the other the radical is oxidized to a quinone-like structure. A mechanism for the oxidation of clioquinol is proposed.

1. Introduction

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ), Scheme 1, is a halogenated derivative of 8-hydroxyquinoline, belonging to the family of drugs called anti-infectives. CQ is an antibacterial and antifungal medication widely used as a topical cream for the treatment of skin infections such as eczema, athlete's foot, and other fungal infections [1].

In the period between 1950 and 1970, CQ was used orally as an antiparasitic agent for the treatment of amoebiasis. However, its oral consumption was banned in 1970 after it was thought in Japan in the 1960s to cause neurotoxicity, a syndrome called subacute myelo-optico-neuropathy (SMON) [1–3]. Recent epidemiologic analysis did not support the relation between SMON and CQ, and instead decreased levels of vitamin B12 could play a role in this syndrome [4].



Scheme 1. Chemical structure of clioquinol (CQ).

The formation of extracellular senile plaques composed of amyloid β-peptide (Aβ) may be one of the causes of Alzheimer's disease. Recent clinical studies demonstrated Alzheimer's disease improvement by reducing or removing Aβ, a metalloprotein, from the brain [5]. The solubilization and clearance of Aβ by using metal protein compounds which chelate with copper and zinc ions, present promising results for the treatment of Alzheimer's disease [6]. The metal-binding properties of CQ have led to a renewed interest in its use for treatment. CQ has been successfully used, in combination with vitamin B12, for the treatment of Alzheimer's disease [7–9].

CQ has a relatively low affinity but high selectivity for Zn^{2+} and Cu^{2+}, and its hydrophobicity may allow penetration through the blood–brain barrier and concentration in the Aβ mass. CQ's metallobiology properties can affect the metal–Aβ equilibria, promoting the dissolution of Aβ aggregates and inhibiting their neurotoxic effects [10]. Furthermore, by reducing copper and zinc ions, CQ also can act as an antioxidant.

CQ has been used to ameliorate the neurotoxicity of 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) in an animal model of Parkinson's disease [11] and also inhibits aggregation of mutant huntingtin protein and mitigated disease pathology in a murine model of Huntington's disease [12].

The cytotoxicity and genotoxicity of CQ have been described. CQ is a phenolic compound and free phenoxy radical intermediates [13–15], which are important in the toxicity caused by phenolic compounds,

* Corresponding author. Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal. Tel./fax: +351 239 835295.
E-mail address: brett@ci.uc.pt (A.M. Oliveira–Brett).

1567-5394/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2010.10.001

can be formed. The enzymes expressed in the skin, such as cyclooxygenase (COX-2), prostaglandin synthase, lipoxygenase, and tyrosinase [16–18], may provide a favorable redox environment for the *in-vivo* oxidation of the phenolic compounds.

Despite the success in the treatment of Alzheimer, Parkinson and Huntington diseases, the mechanisms involved in the neuroprotective effects of CQ *in vivo* remain largely unknown. Consequently, a knowledge of the redox properties of CQ and of its derivatives is very important for a better understanding of their biological activity and toxicity.

Research on the redox behavior of pharmaceutical compounds using electrochemical techniques has the potential for providing valuable insights into the redox reactions of these molecules. Due to their high sensitivity, voltammetric methods were used to study the redox behavior of various pharmaceutical compounds [19–21]. The electrochemical reduction behavior of CQ has been studied by polarography at the hanging mercury drop electrode [22], but its electrochemical oxidation behavior has never been investigated.

The present paper describes the electrochemical oxidation mechanisms of CQ and its oxidation product, for a wide range of solution conditions, using cyclic, differential pulse and square wave voltammetry, at a glassy carbon electrode. In order to propose the CQ oxidation mechanism a group of six phenol substituted derivatives was also investigated. The information on the electrochemical behavior of CQ obtained from the results at different pHs may play a crucial role in understanding its properties as well as its metabolism in biological systems.

2. Experimental

2.1. Materials and reagents

CQ was obtained from Sigma and used without further purification. A stock solution of 1.00 mM CQ was prepared in ethanol and was stored at 4 °C. All supporting electrolyte solutions, Table 1, were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1}). Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison microph 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C).

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Metrohm/Autolab, Utrecht, The Netherlands. Measurements were carried out using a glassy carbon electrode (GCE) (d = 1.5 mm) working electrode, a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) as reference, in a 1 mL one-compartment electrochemical cell. The experimental conditions for differential pulse (DP) voltammetry were pulse amplitude 50 mV, pulse width 70 ms, and scan rate 5 mV s^{-1}. For square wave (SW) voltammetry, the experimental conditions were frequency 25 Hz and potential increment 2 mV, corresponding to an effective scan rate of 100 mV s^{-1}. The GCE was polished using diamond spray (particle size 1 μm) before every electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

Table 1
Supporting electrolytes, 0.1 M ionic strength.

pH	Composition
2.0	HCl + KCl
3.3	HAcO + NaAcO
4.4	HAcO + NaAcO
5.2	HAcO + NaAcO
5.9	NaH_2PO_4 + Na_2HPO_4
6.9	NaH_2PO_4 + Na_2HPO_4
8.0	NaH_2PO_4 + Na_2HPO_4
9.2	NaOH + $Na_2B_2O_7$
11.6	NaOH + KCl

2.3. Acquisition and presentation of voltammetric data

All DP voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 2 mV included in the GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak current is in some cases reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all graphs were determined from the original untreated voltammograms after subtraction of the base line.

3. Results and discussion

3.1. Cyclic voltammetry

Cyclic voltammetry was used to investigate the anodic behavior of CQ using a glassy carbon electrode, Fig. 1. Consecutive cyclic voltammograms of 50 μM CQ in pH 2.0 0.1 M HCl/KCl at scan rate of 100 mVs^{-1} show only one oxidation reaction and the formation of an electroactive product.

The first scan presents an anodic peak 1_a, at $E^1_{pa} = +0.82$ V, and on the reverse scan, a cathodic peak 2_c, at $E^2_{pc} = +0.60$ V. This peak 2_c corresponds to the reduction of the CQ oxidation product formed after CQ oxidation in the first cycle at the GCE surface. In the successive scans a new anodic peak 2_a, at $E^2_{pa} = +0.63$ V, appeared showing the reversibility of peak 2_c.

The peak 2c and 2a currents are nearly equal and the peaks separation is about 30 mV. Considering the equation $E_{pa} - E_{pc} = 57 / n$ mV, two electrons are involved in this reversible reaction.

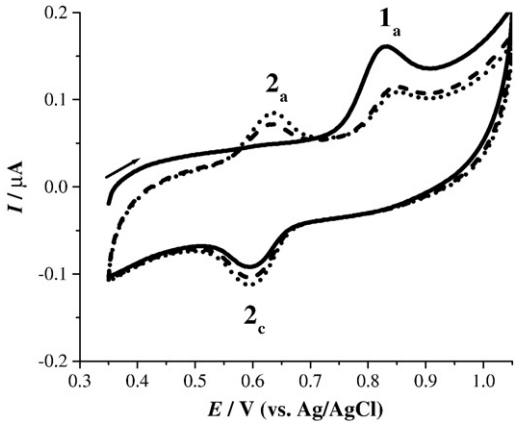


Fig. 1. Cyclic voltammograms of 50 μM CQ in pH 2.0 0.1 M HCl + KCl: (—) first, (---) second and (•••) third scan, $\nu = 100$ mV s^{-1}.