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Monitoring of intermediates of clioquinol electro-oxidation by thin-layer spectral and electrophoretic electrochemistry

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ABSTRACT

Monitoring of unstable intermediates is very important for clarifying complex electrochemical reaction pathways. A combination of thin-layer spectral and electrophoretic electrochemistry, both based on fast thin-layer electrolysis, has been developed to monitor the intermediates of electro-oxidation of clioquinol in acidic, physiological and alkaline buffer media. Two thin-layer electrochemical cells were fabricated and coupled with a UV-vis spectrometer and an electrophoresis apparatus, respectively. Double-potential thin-layer UV–vis spectroelectrochemistry, double-wavelength thin-layer cyclic voltabsorptometry and thin-layer electrophoretic electrochemistry provided mutually-supporting information on the redox pathway of clioquinol. Two intermediates, a disproportionatable semiquinone anion radical and a labile quinone derivative, were detected. The chemical activity of the semiquinone anion radical is highly pH-dependent. The final soluble products of clioquinol are pH-dependent but irrelevant to whether the oxidation reaction is electrochemical or chemical in nature. A complex reaction pathway is proposed for the oxidation of clioquinol, which may help to better understand the metabolism mechanism and chemical toxicity of this anti-neurodegeneration drug.

1. Introduction

Reactive intermediates are generally produced during a complex electrochemical process. The experimental observation of these highly reactive species is very important for elucidating the reaction pathways. Electrolysis in a common-volume electrolytic cell is usually time-consuming, leading to the disappearance of the intermediates that are not stable enough, and probably to the deactivation of working electrode due to the accumulated adsorption/deposition of the products onto the electrode surface. Thin-layer electrochemical cells, usually with a volume in a microliter scale, allow almost 100% conversion of electroactive species in several seconds to minutes. Based on thin-layer electrolysis, various spectroscopic methods such as UV-vis [1], ATR-FTIRS [2], SERS [3] and ESR [4] have been used for *in situ* monitoring of the intermediates and products. A new application of thin layer cell has been recently developed in our group by coupling it with high-performance capillary electrophoresis (CE) and UV–vis detection [5]. This technique allows rapid exhaustive electrolysis, direct sampling and online electrophoretic separation of the electrolysis products. It can be further used to separate and detect some reactive intermediates, as demonstrated in the present work for the investigation of the electrochemical oxidation of clioquinol (CQ).

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, Scheme 1) is a halogenated derivative of 8-hydroxyquinoline that acts as bioactive metal chelator. It was initially used as an antibiotic for the treatment of diarrhea and skin infection. Recently, CQ has received new attention due to its ability to dissolve copper from amyloid-β peptide (Aβ) aggregates associated with Alzheimer's disease (AD) [6,7]. It is effective in preventing the accumulation of insoluble Aβ in the brain by hindering the interaction of Aβ with metal ions [8]. By reducing Cu and Zn ions, CQ also acts as an antioxidant [9]. Similarly, natural antioxidants curcumin and ginkgo extract have modest but positive effects in slowing AD development. Therefore, drugs that target the oxidative pathways in AD could have genuine therapeutic efficacy [10].

On the other hand, CQ has been withdrawn from the market as an oral agent in the early 1970s because of its association with neurotoxicity, a syndrome called subacute myelo-optic neuropathy [9,11,12]. The toxicity mechanism is not fully understood. Exposure of murine cortical cultures to 1–3 μM CQ for 24 h resulted in the formation of malondialdehyde and death of approximately 40% of neurons [13]. One report provided evidence that chronic treatment with CQ may alter the tissue homeostasis of vitamin B_{12} in the brain

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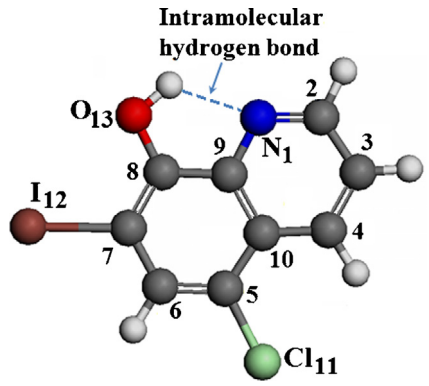

Scheme 1. Molecular structure of CQ with atomic numbering.

[14], while another report suggested that therapeutically relevant concentrations of CQ are toxic to cultured neurons by an oxidative mechanism that is unrelated to vitamin B_{12} deficiency [13]. The latter study suggests that CQ at low micromolar concentrations is an oxidative neurotoxin.

It therefore seems that oxidation of CQ may be related to its actions whether as a therapeutic agent for AD or as a neurotoxin. The oxidation pathway of CQ is currently unclear and deserves further study for better understanding its conversion and role in the involved aerobic metabolism processes. Because of the apparent parallelism between chemical and electrochemical oxidation [15], it is likely that the study of electro-oxidation of phenol derivatives could help, at least in part, in understanding their complex oxidation mechanisms. Oliveira-Brett et al. recently studied the electro-oxidation mechanism of CQ using cyclic, differential pulse and square-wave voltammetry over a wide pH range [16]. The obtained results revealed that the anodic oxidation of CQ is pH-dependent and involves one electron and one proton transfer to produce a radical, which after a chemical reaction produces a quinone-like structure. Another electrochemical report on CQ is about its determination in plasma and tissues of hamsters by HPLC and electrochemical detection [17].

In the present work, the electro-oxidation of CQ was studied in acidic, physiological and alkaline pH media by thin-layer spectral and electrophoretic electrochemistry, based on two self-made thin-layer electrochemical cells. The results provided *in situ* and online information not only on the products but also on the intermediates formed during the oxidation of CQ. A total of four oxidation products were separated by CE, including two unstable intermediates. The potential-independent disproportionation of a free radical intermediate was followed by thin-layer cyclic voltabsorptometry (CVA) [18,19].

2. Experimental

2.1. Chemicals and solutions

Clioquinol (98% pure) was purchased from Afla Aesar China (Tianjin) and was used as received. Spectrograde graphite powder (320 mesh) and spectrograde paraffin wax (solidification point 62–65 °C) from Shanghai Chemical Works were used for preparing the graphite paste electrode. Pyrolytic graphite sheet was from Aidahengsheng (Tianjin, China). Other chemicals were of analytical grade. All solutions were prepared using doubly-distilled water from an all-glass distillatory apparatus.

A stock solution of 1.0 mM CQ for voltammetric and spectroelectrochemical measurements was prepared in ethanol and stored at 4 °C in a refrigerator. The supporting electrolytes were 0.2 M Britton-Robinson (BR) buffered solutions with various pH values (plus 0.2 or 0.5 M KCl) in ethanol and water solvents (v/v 1:4). The stock solution was diluted to various concentrations by mixing with the supporting electrolyte solutions. High pure N_2 was used to deoxygenate the electrolytes.

The CQ solutions for the electrophoretic electrochemistry were prepared in ethanol and BR buffers (v/v 3:2, containing 0.1 M KCl). The high ethanolic content is necessary for preparing higher concentrations of CQ for effective photometric detection at the end of the capillary. The background electrolyte (BGE) for CE was a solution of 20 mM $Na_2B_4O_7$ with a pH value of 10.5. All samples and solutions were filtered with 0.45 μm nylon syringe filter (Shanghai Xingya purification materials Co., China) before injection.

2.2. Apparatus, cells and electrodes

Cyclic voltammetry and spectroelectrochemistry were carried out on a CHI660 C electrochemical workstation (Chenhua, Shanghai, China). UV-vis spectroscopic and photometric measurements were carried out on an UV-2500 spectrophotometer (Shimadzu, Japan) to monitor the soluble reactants and products under potentiostatic and potentiodynamic conditions.

A conventional single-compartment cell was used for voltammetric measurements. A thin-layer spectroelectrochemical cell was self-made, using a standard quartz photometric cell with 10 mm optical path length as the cell body. A quadrate graphite electrode with a PVC substrate was prepared as the working electrode (working area 0.80 cm^2). The incident light beam parallels to the working electrode and goes through the thin-layer electrolyte solution (10 mm long, 0.2 mm thick) on the electrode surface. The schematic view of the thin-layer spectral cell can be found in the literature [19].

A thin-layer cell for electrophoretic electrochemistry was self-made on a single plastic chip, just 2.5 cm by 3.5 cm. The schematic view of the chip cell can be found in the literature [5]. It has a typical double layer structure consisting of a 1.5 mm-thick plastic cover slip on top of a 4 mm-thick plastic substrate. The centerpiece of the electrolysis part of the cell is a quadrate graphite electrode (working area 0.80 cm^2), which is held just above the plastic substrate by 0.2 mm-thick spacers, creating a thin-layer chamber under the electrode. The capillary then runs directly below this chamber, with a small hole in the bottom of the chamber providing a way for any liquid injected into the chamber to pass into the capillary. After loading the sample, the open end of capillary is pushed to the inlet of a BGE reservoir for CE.

The three-electrode system was composed of a graphite working electrode, a Ag/AgCl/KCl reference electrode and a platinum wire counter electrode. The graphite working electrode used in conventional single-compartment cell was a disk solid graphite paste electrode with a smaller geometrical area of 0.062 cm^2, whereas those used in both the thin-layer cells were quadrate graphite sheet electrodes with a larger area of 0.80 cm^2. The preparation of the graphite paste electrode was described previously [15,20]. The Ag/AgCl electrode contained a KCl saturated aqueous solution, except in the chip thin-layer cell, where the Ag/AgCl wire was directly inserted to the electrolyte solution containing 0.1 M KCl.

2.3. Procedures

Clioquinol solutions were deoxygenized with high pure N_2 for about 15 min before experiment. The working electrode was polished carefully with 800 and 2000 grit emery papers, respectively. Before each run, the working electrode was cleaned and activated by repetitive cyclic scans between–0.4 and 1.6 V in 0.1 M KOH solution, until only the background current remained. Considering the adsorption of CQ on graphite electrode, a pre-accumulation step