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Short communication

Mechanistic study of colchicine's reduction behavior

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ABSTRACT

Electrochemistry/mass spectrometry (EC/MS) using two different types of electrolytic cells was employed for the systematic mechanistic study of colchicine's reduction, both in aqueous and non-aqueous media. In aqueous media, at around −1 V vs. Ag/AgCl, colchicine suffers a single-electron reduction to a transient anion radical, which after a follow-up protonation leads to a neutral free radical (E_rC_i mechanism). Depending on the experimental conditions, the latter undergoes some dimerization. At more negative potentials (−1.4 V vs. Ag/AgCl) and pH < 7, the free radical is undergoing another single-electron reduction and a subsequent protonation. In the absence of protons (aprotic media), the one-electron reduction gives the anion radical. This process becomes fully reversible at high scan rates (≥10 V/s).

1. Introduction

Colchicine (Col) is a specific anti-inflammatory agent used for centuries in the treatment of acute gouty arthritis. Being inducted into therapy before the development of modern pharmacological studies, colchicine's multimodal mechanism of action is still in the focal point of the very recent biomedical and toxicological research [1–6]. Although Col is not used clinically to treat cancer due to its toxicity, however it represents a model molecule for the development of a series of novel anticancer drugs with improved toxicological profiles [7–11]. Nevertheless, the central administration of Col causes oxidative stress (with significant glutathione depletion) in animals leading to cognitive impairment [12], and its therapeutic use has been linked to sporadic Alzheimer's disease in humans [13].

The redox mechanism of Col has never been specifically targeted or experimentally confirmed, since the first assumptions made in the early '70s [14,15]. Nevertheless, its elucidation could play a crucial role in the confirmation and prediction of some of Col's relevant pharmacological and toxicological properties and ultimately could contribute in deciphering the beneficial and harmful implications of this ancient medication in the human body.

Therefore, the aim of the study was to undertake a systematic mechanistic study of Col's redox behavior, to clarify the existing inconsistencies and correlate the findings with its reported biotransformation and toxicokinetics.

2. Material and methods

Col, perchloric acid (70%, w/w), phosphoric acid (98%, w/w), and dimethylformamide (DMF) were purchased from Merck, Germany. Formic acid (96%, w/w) and tetrabutylammonium hexafluorophosphate (TBAHFP) were obtained from Fluka, Switzerland, whereas acetonitrile (ACN) was purchased from Promochem (Germany).

All EC assays were performed with a PGSTAT30 potentiostat (Metrohm Autolab) monitored by GPES 4.9 software.

Col solutions were infused (15–33.33 μL/min) by a syringe pump (SP2, Antec) through the electrochemical cell and into the ESI-MS system (Agilent 1100 series LC/MSD Ion Trap SL). The ionization (+ESI and −ESI; 4500 V, 300 °C, nitrogen as drying gas at 5 L/min) and mass spectrometric detection parameters were kept constant (full scan mode, m/z = 100–900). Two types of electrochemical cells were tested: a two-compartment cell made in-house as reported by Arakawa [16] and a one-compartment cell (ReactorCell, Antec), using a diamond based working electrode. Chronoamperometric experiments (Quadstat, eDAQ) were performed, first in an open circuit mode, followed by a stepwise polarization of the working electrode for 3.5 min at each potential, while recording the mass spectra (average of 2 min). Non-volatile buffer systems used in conventional voltammetrometric experiments were replaced by MS compatible electrolytes.

3. Results and discussion

A chemically irreversible, single wave reduction of Col was reported both in aprotic and protic media (up to 4 V s^{-1}) [14,17–20], assuming the formation of an intermediate anion radical [21]. Contradictory

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data is reported related to the total number of electrons involved in the reduction process of Col, where coulometric analysis performed in pure ACN suggested the transfer of one [14], while in dry DMF of two electrons [21]. The reversibility of the electron transfer was only reported in aprotic media using a high frequency triangular wave voltammetry, whereas in aqueous media (Aq) a follow-up chemical reaction (protonation) is suggested [14].

Earlier reports assumed in Aq the conversion of Col's anion radical to colchiceine or a pinacolic dimer [14,17]. Later on, the attempts at identifying the reduction products of Col by controlled bulk electrolysis in Aq (pH = 1–7) were hindered by the formation of an intractable red gummy material. Upon the reduction of Col in dry DMF only a small amount of 10-methylaminocolchiceine (15%) and unreacted colchicine (9%) has been isolated from the electrolysis mixture, ruling out the possibility of dimeric cathodic product formation [21].

3.1. EC analysis

The shape of Col's irreversible cathodic wave is strongly dependent on the used electrode material or the presence of an organic solvent [20,22]. The reduction process is independent of the pH at values above 9, however, the recorded peak potentials manifests a strong anodic shift (64.2 mV pH^{-1}) with the consecutive decrease of current intensity in more acidic solutions (Fig. 1A), indicating the participation of one proton for each transferred electron.

In acidic media (pH = 2.5), a linear variation of log *Ip* versus log *v* ($R^2 = 0.999$), with a slope of 0.46 is obtained. Furthermore, a linear peak current function $Ip/v^{1/2}$ with increasing scan rate (0.01–0.2 Vs^{-1}) and a linear dependence of peak current on Col's concentration are all pointing toward a purely diffusion controlled reduction process ($D = 8 \cdot 10^{-6}\ cm^2\ s^{-1}$). Upon increasing scan rate, the reduction peak potential (*Ep*) experiences a linear negative shift ($Ep = f(\log(v))$, slope 33.5 mV/decade, $R^2 = 0.967$) (Fig. 1B).

Digital simulations (GPES 4.9, Metrohm-Autolab) of the electrochemical process and double-potential step chronoamperometric measurements on boron-doped diamond electrode (Fig. 1C) have all pointed towards a reversible heterogeneous electron transfer (E_r), followed by an irreversible, first order chemical reaction (C_i) in protic media. The subunitary current ratio ($-ir/if = 0.462$) of the chronoamperometric plot recorded in Aq indicated a quick and irreversible protonation of the generated anion radical. However, in DMF, its protonation does not occur and the current ratio is close to unity ($-ir/if = 0.958$).

Therefore, the E_rC_i model for Aq media seems to be in agreement with the slope of the Ep vs. log *v* plot (33.5 mV/decade vs. the predicted value of 29.6 mV/decade at 25 °C [23]) and the recorded chronoamperometric data.

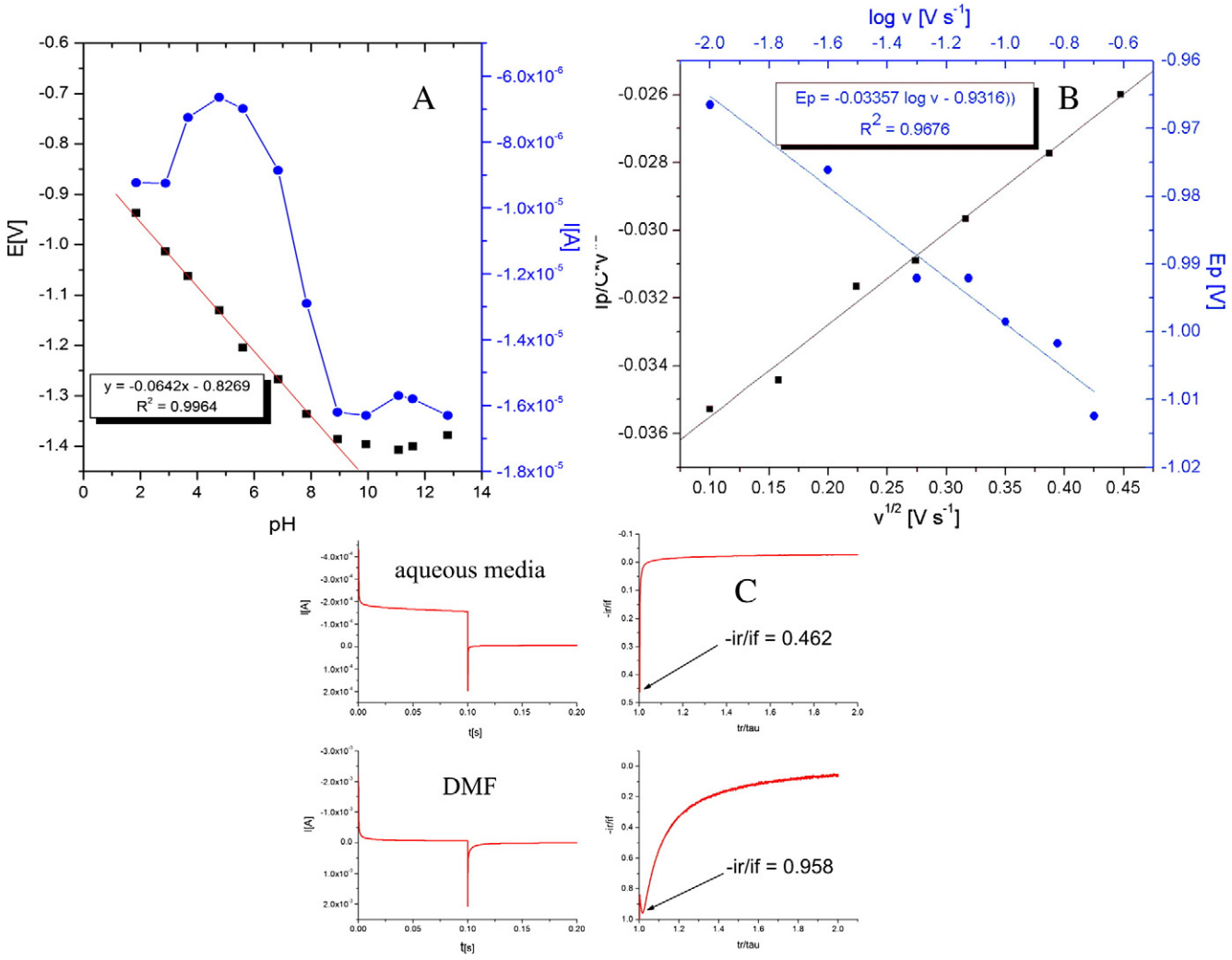

Fig. 1. pH (A) and scan rate (B) dependence of peak potential and cathodic current in aqueous media; glassy carbon working electrode, 100 mV s^{-1}. Double-potential step chronoamperometry (C) Aq, 0.1 M H_3PO_4/$HClO_4$, $E_i = -0.6$ V, $E_1 = -1.3$ V, $E_2 = -0.6$ V vs. Ag/AgCl (3 M KCl), 0.1 s polarization, 0.0002 s sampling rate; DMF, 0.1 M TBAHFP, $E_i = 0$ V, $E_1 = -1.5$ V, $E_2 = 0$ V vs. Ag/Ag^+, 0.1 s polarization, 0.00005 s sampling rate.