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Electrochemical characterization of bioactive hydroxyxanthones by cyclic voltammetry

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ABSTRACT

The present study reports the electrochemical behavior of several phenolic and catecholic-substituted 2,3-diarylxanthones on a glassy carbon electrode, challenged by cyclic voltammetry at different pH values (4.0, 7.4, and 11.0). Higher pH values required lower anodic and cathodic peak voltages. The oxidation of catecholic groups occurred at lower peak potentials in a reversible and pH dependent manner. Anodic peak potentials appeared at higher pH values and were attributed to the electrochemically irreversible oxidation of the phenolic groups. The number and position of hydroxyl substituents were the determinants for the electrochemical behavior and found to correlate with the scavenging activity for reactive oxygen (ROS) and nitrogen species (RNS). A xanthone with two catechol units presented the lowest anodic potential voltage ($E_{pa} = 0.15$ V) and proved to be the most effective ROS and RNS scavenger.

Xanthones are redox-active compounds with a tricyclic scaffold, identified in some higher plants, with most of the derivatives restricted to the *Guttiferae* and *Gentianaceae* families. Several derivatives can also be isolated from a variety of fungi and lichens.[1] These compounds are highly diverse in their chemical structure, including simple oxygenated and prenylated substituents, or appearing as more complex frameworks as xanthonolignoids or bis-xanthones.[1,2] Naturally occurring analogues exhibit several biological and pharmacological properties such as anti-allergic, anti-inflammatory, antimalarial, antimicrobial, and antitumour activities and also play an important role in the inhibition of several enzymes.[3]

Studies on the antioxidant activity of both natural and synthetic xanthones have provided interesting results, including: (i) scavenging activity against reactive oxygen species (ROS) and reactive nitrogen species (RNS), (ii) inhibition of pro-oxidant enzymes, and (iii) metal chelating capacity.[3–5] Structure/activity studies of phenolic compounds revealed that the number and position of hydroxyl substituents in their skeleton are features of great importance for a high antioxidant activity.[6]

In a previous work, concerning the putative scavenging effects of several hydroxylated xanthones against ROS and RNS, it was demonstrated that compounds with a catechol motif were the most effective scavengers, in some cases being more active than natural xanthones.[7,8] In other chemical assays, involving human blood LDL and human skin keratinocytes, we could also establish structure–activity relationships.[9] From these studies, it may be predicted that the investigation of the redox potential of hydroxylated xanthones can provide valuable insights into the pharmacological properties of these molecules.

Electrochemical methodologies have been successfully used to establish correlations between structure, oxidation potential, and biological activity of electroactive species. Their operating simplicity, higher sensitivity, short time of analysis, and lower reagent consuming as well as lower costs are some advantages presented by these techniques compared with chromatographic or spectroscopic methods.[10,11] Thus cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many relevant studies of redox processes, in organic and inorganic chemistry, to clarify the electrochemical behavior of complex chemical and biochemical systems, and to obtain information about all types of interfacial processes depending on electrochemical currents.[12] The aim of the present study is to study the electrochemical

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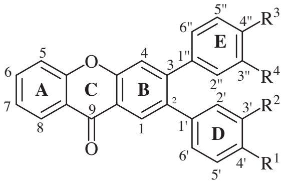


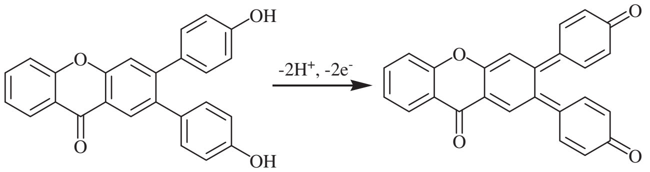
Compound	R^1	R^2	R^3	R^4	E_p (V) *versus* Ag/AgCl								
					pH 4.0			pH 7.4			pH 11.0		
					E_{p1a}	E_{p2a}	E_{pc}	E_{p1a}	E_{p2a}	E_{pc}	E_{p1a}	E_{p2a}	E_{pc}
XH1	H	H	H	H	-	-	-	-	-	-	-	-	-
XH2	OH	H	H	H	-	0.84	-	-	0.58	-	-	0.38	-
XH3	OH	OH	H	H	0.42	-	0.27	0.16	-	0.12	-0.04	-	-0.07
XH4	H	H	OH	H	-	0.87	-	-	0.64	-	-	0.40	-
XH5	OH	H	OH	H	-	0.84	-	-	0.56	-	-	0.37	-
XH6	OH	OH	OH	H	0.42	0.96	-0.18	0.16	0.69	-0.12	0.00	0.51	-0.08
XH7	H	H	OH	OH	0.41	-	0.34	0.18	-	0.14	-0.01	-	-0.05
XH8	OH	H	OH	OH	0.44	0.93	-0.07	0.18	0.65	-0.11	0.04	0.37	-0.03
XH9	OH	OH	OH	OH	0.48	-	0.19	0.15	0.37	-0.06	-0.08	0.12	-0.12

Scheme 1. Chemical structures and anodic and cathodic potentials of 2,3-diarylxanthones **XH1**–**XH9**.

behavior of a group of phenolic and catecholic-substituted 2,3-diarylxanthones[13] (Scheme 1), in aqueous solution, by cyclic voltammetry,[14] and compare the obtained results with their scavenging activities for ROS and RNS.

In order to simulate the physiological conditions, the cyclic voltammograms were performed at pH 7.4 (sodium phosphate). Assays were also performed under acidic and basic conditions, using acetate (pH 4.0) and carbonate–bicarbonate (pH 11.0) buffers, respectively. The study on the electrochemical behavior of xanthone **XH1** was limited by its solubility, which only permitted a final concentration of 0.05 mM, and no detectable peaks were found in the cyclic voltammograms, for all tested pH values (Scheme 1). This limitation did not allow to investigate the influence of the 2,3-diphenylxanthone motif in the electrochemical behavior of the studied xanthones **XH2**–**XH9**. Masek et al.[15] performed the cyclic voltammogram of the parent xanthone at 1 mM and detected three oxidation peaks. In that study, the cyclic voltammogram of the parent flavone at 1 mM showed two oxidation peaks with higher potential values. These results indicate that the xanthone oxidation occurs at lower oxidation potentials, compared to the flavone, an important feature to enhance the well-documented antioxidant ability of xanthone-type compounds.[4,5]

By increasing pH values, the anodic and cathodic peak voltages of hydroxylated xanthones **XH2**–**XH9** decrease, similarly to that reported by other authors for several phenolic compounds.[16–19] At pH 7.4, compounds **XH2** and **XH4** (final concentration of 0.05 mM) presented voltammograms with a single irreversible oxidation peak at 0.58 V and 0.64 V, respectively (Scheme 1). The cyclic voltammetric profile of these compounds is similar to that observed in *para*-substituted flavonoids. In fact, genistein, a 4′-hydroxyisoflavone, has a comparable oxidation peak (0.51 V) with that of **XH2** and apigenin, the corresponding 4′-hydroxyflavone, presented the same oxidation peak (0.64 V) of **XH4** at pH 7.4 versus Ag/AgCl.[20] Cyclic voltammograms of several hydroxybenzoic and hydroxycinnamic acids also presented single anodic peaks at higher potentials and the absence of a cathodic peak in the reverse scan.[21] Moreover, the rapid decrease of the oxidation peak after multiple cyclic scans, without polishing the electrode between the cycles, clearly indicates that **XH2** and **XH4** products adsorb on the surface, blocking the diffusion process and their oxidation in the working surface electrode. Interestingly, derivative **XH5** (two *para*-phenolic groups) undergoes only one irreversible oxidation peak (0.56 V), with a lower potential compared to analogues **XH2** and **XH4**, indicating that the two phenolic groups were oxidized at the same potential. We can suggest that compound **XH5** can be oxidized to a quinoide structure and, due to their instable nature, it decomposes in the voltammetric cell, making it difficult to observe the reduction peak (Scheme 2).



Scheme 2. Proposed oxidation of xanthone **XH5** in phosphate buffer pH 7.4.

Cyclic voltammograms of the other derivatives **XH3** and **XH6**–**XH9** showed reversible oxidation peaks from 0.15 to 0.18 V, corresponding to the catechol unit (3,4-dihydroxyphenyl substituent) oxidation (Fig. 1). This lower electrochemical anodic peak (generally lower than 0.50 V) has already been reported in the flavonoid family on a glassy carbon electrode at neutral pH versus Ag/AgCl.[10,21–23] The results suggest that the D-catechol unit of the studied xanthones is more susceptible to oxidation than the E-catechol moiety. Thus, compound **XH3** (D-catechol, E_{p1a} = 0.16 V) presented a lower oxidation peak than compound **XH7** (E-catechol, E_{p1a} = 0.18 V) and the same is observed for compounds **XH6** and **XH8** (E_{p1a} = 0.16 V and 0.18 V, respectively). Of note, compound **XH9** (two catechol groups) showed the lowest potential oxidation peak at 0.15 V (Scheme 1). Such low oxidation potential and their effectiveness to donate hydrogen atoms, make **XH9** a highly potent antioxidant with the most powerful reducing ability.[24] In this regard, it is shown that, among all the xanthones under study, **XH9** is the only derivative which can repair the α-tocopheroxyl radical thereby restoring α-tocopherol.[8] Overall, these results are consistent with our previous studies on the electrochemical behavior of flavones and 2-styrylchromones, indicating that a higher number of hydroxyl groups correspond to a lower anodic potential peak of the catechol unit.[25]