Isatin halogen-derivatives redox behaviour

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A B S T R A C T
Isatin halogen-derivatives like other isatin derivatives have several pharmacoterapeutic applications, such as antibacterial, antitubercular, and anticancer activities. The electrochemical behaviour, at a glassy carbon electrode, of some mono- and di-fluoro, chloro, bromo and iodo isatin derivatives, by cyclic, square wave and differential pulse voltammetry, over a wide pH range, was investigated, and compared with isatin electrochemical behaviour. The presence of one or two halogens in the benzene ring affected the oxidation processes. The oxidation mechanism of isatin monohalogen-derivatives, with only one halogen at the position C5 or C7, was an irreversible, pH-dependent, adsorption-controlled process, and occurred in three consecutive charge transfer reactions, first on the benzene ring with the production of one hydroxyl group attached to the ring, and the electroactive oxidation product formed was oxidized to para- and/or ortho-quinone derivatives and polymeric products. The isatin dihalogen-derivatives oxidation was also irreversible, in two consecutive charge transfer reactions, with the formation of polymeric products, and occurred at more positive potentials. The reduction mechanism of isatin halogen-derivatives was a pH-dependent two consecutive charge transfer reactions. The first process was the reversible reduction of the carbon-halogen bond and the second the irreversible cleavage of the carbonyl group at the position C3 in the heterocyclic ring. The halogens substituents in the isatin benzene ring gave rise to different redox processes, depending on the number and halogen position.

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1. Introduction

Isatin (1H-indole-2,3-dione) (ISA), one of the most important derivatives of indole, is an endogenous compound identified in many organisms, present in mammalian tissues and body fluids, and also as a natural product of plants, for example genus Isatis, in Culinthe discoulour, Lindl, and in Couroupita guianensis, Aubl [1–3]. Isatin is a very important molecule due to its broad range of biological and pharmacological properties, and also because it is a synthetically versatile substrate. Isatin and its derivatives are extensively used as important raw materials for designing potential bioactive agents [3–5]. Recently the study of isatin derivatives have been shown to demonstrate antiparameal, antibacterial, antifungal, antiviral, anti-HIV, anti-convulsant, antitumoral, anti-inflammatory, antihelmintic activities, to influence neurodegenerative diseases, and to participate in metabolism [3,6–8].

Isatin halogen-derivatives have also been reported to exhibit several pharmacoterapeutic activities, such as antibacterial, antituberculor, anticancer and antineoplastic activities [9–20].

Isatin fluoro-derivatives are used in the synthesis of new compounds that may belong to a class of chemotherapeutic agents for the treatment of various bacterial infections, act by inhibiting DNA gyrase, the principal target in gram-negative bacteria, and also the topoisomerase IV, the principal target in gram-positive bacteria [13]. The isatin 5-fluoroderivatives were also synthesized and the antitubercular activity was evaluated and some of the compounds presented complete inhibition against the Mycobacterium tuberculosis H37Rv strain [14]. In 2006, an isatin 5-fluoro-derivative (sunitinib) was approved by FDA for the treatment of gastrointestinal stromal tumours and advanced renal cell carcinoma [15–16].

Isatin bromo-derivatives have been shown to exhibit anticancer activity [17–19]. The in vitro cytotoxic activities of isatin bromo-derivatives were determined against the human monocyte-like, histiocytic lymphoma cell line (U937), showing that the introduction of electron-withdrawing groups at positions C5, C6, and C7 significantly increased the anticancer activity when compared with isatin, the substitution at the 5-position being the most favourable [18]. The C5 substitution in the isatin ring has been associated with increased biological activity for a range of indole-based compounds [18]. Both chloro and bromo substituted isatin derivatives presented antifungal and antibacterial activity but the isatin 5-chloro-derivatives, when compared with the
isatin 5-bromo-derivatives presented a better antibacterial activity [21]. The 5-chloro-isatin ketals, such as the dioxolane ketal of 5-chloro-isatin, have significant anticonvulsant and anxiolytic activities [22].

Electrochemical techniques have been widely used to study the structure, reactivity and mechanism of action of pharmaceutical and biological compounds. Also, other parameters can be evaluated using electrochemical data, such as stereoechemistry, diffusion, solubility and metabolism. It should be considered that many physiological processes are depending on redox reactions, and it is easy to find complementary electrochemical and biological reactions, providing useful information on the mechanism of the compounds in living systems. These reactions are most often studied with electrochemical techniques, cyclic, square wave and differential pulse voltammetry, since they have high sensitivity and selectivity [23–27].

There are some studies concerning the redox behaviour of isatin and of its derivatives in aqueous and non-aqueous media, using voltammetry at glassy carbon or mercury electrodes [28–33]. Since isatin halogen-derivatives, in general, are having increasing applications in the synthesis of new molecules with pharmaceutical interest, the investigation of their in vitro redox behaviour is very important in order to predict the in vivo redox reactions.

The aim of the present study is focused on the redox behaviour of a series of eleven isatin mono- and dihalogen-derivatives, Scheme 1. The influence of the number and halogen atoms (F, Cl, Br and I), substituents in the isatin benzene ring, in the redox properties, for a wide range of solution conditions, using cyclic, square wave and differential pulse voltammetry at a glassy carbon electrode, was investigated and a redox mechanism proposed.

2. Experimental

2.1. Materials and reagents

The isatin and all isatin halogen-derivatives were synthesized according to methods described in the literature [5,34–37], Scheme 1. Stock solutions of isatin and all isatin halogen-derivatives, with a concentration of 1 mM, in ethanol, were prepared and stored at 4 °C.

Supporting electrolyte solutions, with ionic strength $I = 0.1$ M, of different pH composition: pH 2.0 (HCl + KCl), pH 3.3 (HOAc + NaOAc), pH 4.5 (HOAc + NaOAc), pH 5.2 (HOAc + NaOAc), pH 5.9 (NaH2PO4 + Na2HPO4), pH 7.2 (NaH2PO4 + Na2HPO4), pH 8.0 (NaH2PO4 + Na2HPO4), pH 9.2 (NaOH + Na2B4O7), pH 11.2 (NaOH + Na2HPO4), using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity < 0.1 μS cm$^{-1}$) according to the literature, were prepared [38].

Nitrogen saturated solutions were obtained by bubbling high purity N2 for a minimum of 10 min in the solution, and continuing with a N2 flow over the solution during the voltammetric experiments.

Microvolumes were measured using electronic pipettes (EP), EP-10 μl and EP-100 μl Plus Motorized (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison microphp 2001 pH-meter with an Ingold combined glass electrode.

All experiments were done at room temperature, $T = 298$ K (25 °C).

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a μAutolab running with GEPES 4.9 software, Metrohm/Autolab, Utrecht, The Netherlands. Measurements were carried out using a glassy carbon working electrode (GCE) (d = 1.5 mm), a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) as reference electrode, in a 1 mL one-compartment electrochemical cell (eDAQ Europe). The experimental conditions for cyclic voltammetry (CV) were scan rate 100 mV s$^{-1}$, and 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 50 mV s$^{-1}$.

The GCE was polished using diamond spray (particle size 1 μm, Kement, Kent, UK) 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.

2.3. Acquisition and presentation of voltammetric data

All DP Voltammograms presented were baseline-corrected using the moving average application with a step window of 2 mV included in the GEPES version 4.9 software. This mathematical treatment improved the visualization and identification of peaks over the baseline without introducing any artefact, although the peak current is in some cases reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original DP voltammograms was used in the presentation of all experimental DP voltammograms for a better and clearer identification of the peaks.

3. Results and discussion

Initial studies concerning the voltammetric behaviour of eleven isatin halogen-derivatives, Scheme 1, with substituents at C4, C5, C6 or C7 positions, were carried out in 0.1 M phosphate buffer pH = 7.0, N2 saturated solutions, in 200 μM isatin halogen-derivatives, by CV, scan rate 100 mV s$^{-1}$, at a GCE. During the voltammetric measurement

<table>
<thead>
<tr>
<th>Compound</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISA</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>5-F-ISA</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>5-Cl-ISA</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>5-Br-ISA</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>5-I-ISA</td>
<td>H</td>
<td>I</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>7-Cl-ISA</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
</tr>
<tr>
<td>7-I-ISA</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>I</td>
</tr>
<tr>
<td>4,6-Bi-ISA</td>
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<td>H</td>
<td>Br</td>
<td>H</td>
</tr>
<tr>
<td>5,7-diCl-ISA</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>Cl</td>
</tr>
<tr>
<td>5,7-diBr-ISA</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>Br</td>
</tr>
<tr>
<td>5,7-F-Cl-ISA</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>Cl</td>
</tr>
<tr>
<td>5,7-Br-Cl-ISA</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>Cl</td>
</tr>
</tbody>
</table>

Scheme 1. Chemical structures of isatin and isatin halogen-derivatives.
a constant flux of N₂ was kept over the solution surface in order to avoid the diffusion of atmospheric oxygen into the solutions.

All isatin halogen-derivatives were electroactive and some oxidation and reduction peaks occurred, Figs. 1 and 2, Tables 1 and 2. Nevertheless, the reduction and the oxidation mechanisms for all eleven isatin halogen-derivatives were independent of each other, so they were investigated separately.

3.1. Oxidation

The oxidation of isatin and eleven isatin halogen-derivatives was investigated by CV, and the oxidation of 5-F-ISA and 7-I-ISA, by DP and SW voltammetry, was also investigated.

3.1.1. Cyclic voltammetry

CV experiments, at GCE, in the potential range 0.0 V till +1.3 V, in physiological 0.1 M phosphate buffer pH = 7.0, containing 500 μM of isatin and 200 μM isatin halogen-derivatives, Scheme 1, Figs. 1 and 2, and Table 1, were carried out to study the influence of the halogen substituent(s) on the electrochemical oxidation of isatin.

In general, the CVs obtained in 200 μM solutions of all isatin halogen-derivatives showed the occurrence of two consecutive irreversible oxidation peaks 1a and 2a, Figs. 1 and 2, and Table 1. However, the presence of one or two halogens in the benzene ring considerably affected the oxidation process.

The isatin monohalogen-derivatives, 5-F-ISA, 5-Cl-ISA, 7-Cl-ISA and 7-I-ISA, Scheme 1, anodic peaks 1a and 2a occurred at less positive potentials and, when compared with the 5-Br-ISA, 5-I-ISA and isatin dihalogen-derivatives oxidation behaviour, a new oxidation process, peak 3a, was detected, Figs. 1 and 2, and Table 1.

3.1.1.1. Isatin monohalogen-derivatives. CVs obtained in the supporting electrolyte 0.1 M phosphate buffer pH = 7.0, and in solutions of 200 μM of (5,7)-(Cl, Br, I)-isatins, are presented in Fig. 1 and Table 1. CVs in 200 μM 5-fluoro-isatin (5-F-ISA), in 0.1 M phosphate buffer pH = 7.0, Fig. 3, presented three irreversible oxidation peaks, peak 1a, at \( E_{p1a} = +0.62 \text{ V} \), peak 2a, at \( E_{p2a} = +1.01 \text{ V} \), and peak 3a, at \( E_{p3a} = +1.20 \text{ V} \). Successive scans recorded in the same solution, without cleaning the electrode surface, showed a decrease of the oxidation peak current of the three anodic peaks 1a, 2a and 3a, due to the adsorption of 5-F-ISA oxidation products on the GCE surface.

CVs were also obtained for different scan rates. Increasing the scan rate, the peak 1a current also increased. However, it was not a diffusion-controlled oxidation process because there was not a linear relationship between \( I_{pa} \) of peak 1a and the square root of the scan rate [23]. This is explained taking into consideration the strong adsorption of 5-F-ISA molecules on the hydrophobic GCE surface, in agreement with the highly hydrophobic character of isatin [33].

The oxidation of isatin monohalogen-derivatives led, in general, to the appearance of three anodic oxidation peaks and a decrease in the peak currents, when successive scans were recorded, showing the same behaviour when compared with the 5-F-ISA, and the isatin monohalogen-derivatives, at ortho- or para-halogen substituent positions, have the same oxidation mechanism. However, in the electrooxidation of 5-Br-ISA and 5-I-ISA, only two anodic peaks were detected in agreement with ISA oxidation [31].

3.1.1.2. Isatin dihalogen-derivatives. CVs obtained in the supporting electrolyte, and in solutions of 200 μM of isatin dihalogen-derivatives, 4,6-diBr-ISA, 5,7-diCl-ISA, 5,7-diBr-ISA, 5,7-F-Cl-ISA, 5,7-Br-Cl-ISA, in 0.1 M phosphate buffer pH = 7.0, are presented in Fig. 2 and Table 1.

The isatin dihalogen substitution caused the appearance of only two anodic oxidation peaks and a decrease in the peak currents when successive scans were recorded, showing the same behaviour when compared with non-substituted ISA, Fig. 2A.

The isatin dihalogen-derivatives oxidation potentials depended on the nature and position of the halogen atoms in the ring: 5,7-diCl-ISA > 5,7-F-Cl-ISA > 4,6-diBr-ISA > 5,7-Br-Cl-ISA > 5,7-dibr-ISA.

as the presence of the second halogen atom in the benzene ring hampered, as expected, the isatin dihalogen-derivatives oxidation processes, when compared to the isatin monohalogen-derivatives, Figs. 1 and 2 and Tables 1 and 2.
3.1.2. Differential pulse voltammetry

Successive DP voltammograms were recorded in a solution of 20 μM of 5-F-ISA, in 0.1 M phosphate buffer pH = 7.0, Fig. 4A and Table 3. In the first DP voltammogram oxidation peak 1a, at $E_{p1a} = +0.62$ V, peak 2a, at $E_{p2a} = +1.04$ V, and peak 3a, at $E_{p3a} = +1.16$ V, occurred. In the second DP voltammogram, two new peaks were detected, peak 4a, at $E_{p4a} = +0.18$ V, and peak 5a, at $E_{p5a} = +0.39$ V, and both peak currents increased with the number of scans.

Table 1

<table>
<thead>
<tr>
<th>Isatin halogen-derivatives oxidation</th>
<th>Potential/V (vs. Ag/AgCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scan 1</td>
</tr>
<tr>
<td>ISA</td>
<td>$E_{p1a} = +1.10$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.24$ V</td>
</tr>
<tr>
<td>5-F-ISA</td>
<td>$E_{p1a} = +0.64$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.03$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p3a} = +1.17$ V</td>
</tr>
<tr>
<td>5-Cl-ISA</td>
<td>$E_{p1a} = +0.63$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.00$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p3a} = +1.16$ V</td>
</tr>
<tr>
<td>5-Br-ISA</td>
<td>$E_{p1a} = +1.02$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.18$ V</td>
</tr>
<tr>
<td>5-I-ISA</td>
<td>$E_{p1a} = +1.03$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.18$ V</td>
</tr>
<tr>
<td>7-Cl-ISA</td>
<td>$E_{p1a} = +0.62$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.02$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p3a} = +1.23$ V</td>
</tr>
<tr>
<td>7-I-ISA</td>
<td>$E_{p1a} = +0.62$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.01$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p3a} = +1.20$ V</td>
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<td>$E_{p2a} = +1.21$ V</td>
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<td>$E_{p2a} = +1.20$ V</td>
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<td>$E_{p1a} = +1.03$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p2a} = +1.22$ V</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Isatin halogen-derivatives reduction</th>
<th>Potential/V (vs. Ag/AgCl)</th>
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<tbody>
<tr>
<td></td>
<td>Scan 1</td>
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<tr>
<td>ISA</td>
<td>$E_{p6c} = −0.58$ V</td>
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<tr>
<td></td>
<td>$E_{p7c} = −0.78$ V</td>
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<tr>
<td>5-F-ISA</td>
<td>$E_{p6c} = −0.50$ V</td>
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<tr>
<td></td>
<td>$E_{p7c} = −0.78$ V</td>
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<tr>
<td>5-Cl-ISA</td>
<td>$E_{p6c} = −0.51$ V</td>
</tr>
<tr>
<td></td>
<td>$E_{p7c} = −0.80$ V</td>
</tr>
<tr>
<td>5-Br-ISA</td>
<td>$E_{p6c} = −0.51$ V</td>
</tr>
<tr>
<td>5-I-ISA</td>
<td>$E_{p6c} = −0.49$ V</td>
</tr>
<tr>
<td>7-Cl-ISA</td>
<td>$E_{p6c} = −0.49$ V</td>
</tr>
<tr>
<td>7-I-ISA</td>
<td>$E_{p6c} = −0.49$ V</td>
</tr>
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<td>4,6-diBr-ISA</td>
<td>$E_{p6c} = −0.47$ V</td>
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<tr>
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<td>$E_{p7c} = −0.74$ V</td>
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</tr>
<tr>
<td></td>
<td>$E_{p7c} = −0.67$ V</td>
</tr>
<tr>
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<td>$E_{p6c} = −0.40$ V</td>
</tr>
<tr>
<td>5,7-F-Cl-ISA</td>
<td>$E_{p6c} = −0.48$ V</td>
</tr>
<tr>
<td>5,7-Br-Cl-ISA</td>
<td>$E_{p6c} = −0.42$ V</td>
</tr>
</tbody>
</table>
In a new experiment, with a clean GCE, in 50 μM 5-F-ISA, in 0.1 M phosphate buffer pH = 7.0, two successive DP voltammograms, were performed. The first DP voltammogram was scanned just until before peak 2a, and in the second DP voltammogram the peaks 4a and 5a were not detected, Fig. 4B.

This experiment was repeated again, with a clean GCE, and again two successive DP voltammograms were performed. The first DP voltammogram was scanned just until before peak 3a, and in the second DP voltammogram the peaks 4a and 5a appeared, Fig. 4C. Consequently, the peaks 4a and 5a were undoubtedly assigned to the 5-F-ISA oxidation products formed in the 5-F-ISA peak 2a oxidation process.

The effect of pH on the electrochemical oxidation of lower concentrations 20 μM 5-F-ISA, using DP voltammetry, at a GCE, in different electrolytes 0.1 M ionic strength, over a wide range 2.0 < pH < 11, was investigated.

For 2.0 < pH < 9.0 the potential of all three anodic peaks 1a, 2a and 3a were shifted to more negative values with increasing pH, Fig. 5A and B. In the $E_{pa}$ vs. pH plot, Fig. 5B, the slope of the dotted line, $-59$ mV per pH unit, showed that the mechanism of all oxidation processes involved the same number of electrons and protons. The number of electrons transferred, $n$, was determined by the peak width at half height, $W_{1/2} \approx 70$ mV for peak 1a, $W_{1/2} \approx 93$ mV for peak 2a, and $W_{1/2} \approx 65$ mV for peak 3a, each corresponding to the electrochemical oxidation reaction involving the transfer of one electron and one proton. Therefore, 5-F-ISA was oxidized in three successive steps and with one-electron and one-proton transfer in each step. The plot of peaks 1a, 2a and 3a current vs. pH, Fig. 5A, showed that 5-F-ISA peak currents were much higher for $3.0 \leq pH < 7.0$. So, it is demonstrated that the 5-F-ISA oxidation is significantly affected by the pH. Consequently, it is important to consider the 5-F-ISA and the 5-F-ISA oxidation products predominant form at each pH which means structural changes, for example, in the phenolic groups, Scheme 2, due to its acid-base equilibrium and variations on the hydrophilic character, strongly influencing, as expected, the 5-F-ISA electrooxidation process (peak potentials and currents) on the GCE surface.

For pH > 9.0, the oxidation peak 1a was pH-independent corresponding to an electrochemical reaction involving one electron transfer. The investigation in different pH environments allowed the determination for 5-F-ISA of $pK_a$ ~ 9 from the data in Fig. 5B.

The strong adsorption of 5-F-ISA and its oxidation products at the GCE surface was investigated for different pHs 3.5, 6.9 and 9.0. The electrode was immersed for 10 min in a 20 μM 5-F-ISA solution, in each pH, and after the electrode was washed with a jet of deionized water and transferred to the buffer electrolyte solution. The strong adsorption on GCE in acid and neutral media was confirmed because peaks 1a, 3a and 4a, appeared in acid electrolyte solution, in neutral electrolyte solution only peak 1a occurred, and for pH 9.0 no peaks were found.

The DP voltammograms recorded in a solution of 20 μM 7-I-ISA also showed an oxidation process with three irreversible pH-dependent oxidation steps, peak 1a, at $E_{p1a} = +0.80$ V, peak 2a, at $E_{p2a} = +0.88$ V, and peak 3a, at $E_{p3a} = +1.17$ V.

![Fig. 3. CVs in N₂ saturated supporting electrolyte (•••) 0.1 M phosphate buffer pH = 7.0, of 200 μM 5-F-ISA, (●) first and (▬) second scans, $ν = 100$ mV s⁻¹.](image1)

![Fig. 4. Successive DP voltammograms, in three different potential windows, in 0.1 M phosphate buffer pH = 7.0 of 5-F-ISA: (A) 20 μM and (B-C) 50 μM, (●) first, (▬) second and (•••) third scan, $ν = 5$ mV s⁻¹.](image2)
For $3.0 < \text{pH} < 9.0$ the potential of both peaks 1a and 2a were shifted to more negative values with increasing pH. In the $E_{pa}$ vs. pH plot the slope of the dotted line, $-59$ mV per pH unit, showed that the mechanism of both oxidation processes involved the same number of electrons and protons. The number of electrons transferred $n$, was determined by the peak width at half height, $W_{1/2} \approx 70$ mV for peak 1a, and $W_{1/2} \approx 87$ mV for peak 2a, close to the theoretical value of $90$ mV, both peaks oxidation reaction involving the transfer of one electron and one proton. For $\text{pH} > 8$, the oxidation peaks 1a and 2a were pH-independent, corresponding to an electrochemical reaction involving the transfer of one electron. The value of $pK_a - 8$ for 7-I-ISA was determined.

Successive DP voltammograms recorded in 20 μM 7-I-ISA showed a similar behaviour to 5-F-ISA. However, in the second DP voltammogram the peaks current increased with the number of scans, and only one new peak 4a, at $E_{pa} = +0.23$ V, corresponding to the oxidation of 7-I-ISA oxidation products, formed at the GCE surface after the first positive-going cycle, occurred Table 3.

### 3.1.3. Square wave voltammetry

One of the most important advantages of SW voltammetry is the possibility to verify the reversibility of the electron transfer reaction during only one scan. Since the current is sampled in both positive and negative-going pulses, peaks corresponding to the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same scan. Other advantages are greater speed of analysis, lower consumption of the electroactive species in relation with DP voltammetry and reduced problems with the adsorption on the GCE surface [23]. The SW voltammetry experiments enabled to confirm the oxidation mechanisms of 5-F-ISA and 5-I-ISA.

The first SW voltammogram in 20 μM 5-F-ISA, in 0.1 M phosphate buffer pH = 7.0, showed two oxidation steps, peak 1a, at $E_{pa} = +0.64$ V, and peak 2a, at $E_{pa} = +1.03$ V, Fig. 6A. The irreversibility of 5-F-ISA peaks 1a and 2a was confirmed by plotting the forward and backward components of the total current, Fig. 6A. The anodic peak 3a occurred at very high positive potential, near the potential corresponding to oxygen evolution, and consequently was not detected by SW voltammetry. The second SW voltammogram showed the occurrence of reversible peak 4a, at $E_{pa} = +0.19$ V, and reversible peak 5a, at $E_{pa} = +0.34$ V, corresponding to the 5-F-ISA oxidation products reversible oxidation, Fig. 6B.

The first SW voltammogram, in 20 μM 5-I-ISA, in 0.1 M phosphate buffer pH = 7.0, showed two oxidation steps, peak 1a, at $E_{pa} = +0.64$ V, and peak 2a, at $E_{pa} = +1.03$ V. In the first SW voltammogram the irreversibility of 7-I-ISA peaks 1a and 2a was also confirmed, and the third SW voltammogram showed the occurrence of the reversible peak 4a, at $E_{pa} = +0.27$ V, corresponding to the 7-I-ISA oxidation product reversible oxidation, Fig. 6C and D.

### 3.1.4. Oxidation mechanism of isatin halogen-derivatives

The oxidation of isatin halogen-derivatives with different halogen substituents, such as fluorine, chlorine, bromine and iodine, attached to the isatin ring structure, Scheme 1, followed in general a similar oxidation processes. The oxidation mechanism, in physiological pH = 7.0, based on CV, DP and SW voltammetry, is proposed in Scheme 2.

In order to propose the oxidation mechanism of the isatin halogen-derivatives, the oxidation mechanism of isatin [31,33] and phenolic compounds [33,39–45], was revisited.

The oxidation of all isatin halogen-derivatives proceeded in successive steps on the benzene ring, and was influenced by the halogen number and position in the isatin molecule, and some isatin halogen-derivatives oxidation products were electroactive. To simplify the analysis of the oxidation mechanisms, the isatin halogen-derivatives according to the halogen number and position in the molecule, isatin 5 (or 7)-monohalogen-derivatives and isatin dihalogen-derivatives, were considered separately.

### 3.1.4.1. Isatin 5 (or 7)-monohalogen-derivatives

The oxidation reactions in isatin 5 (or 7)-monohalogen-derivatives proceeded in three successive steps on the benzene ring. In the first step, peak 1a, one electron was removed from the benzene ring, following deprotonation and direct nucleophilic attack by water with the production of the 7-hydroxy-5-monohalogen-isatin or 4-hydroxy-7-monohalogen-isatin. The second step, peak 2a, corresponded to the oxidation of the 7 (or 4)-OH group, produced in the first oxidation step, with the production of the peak 4a, para-quinone derivative, and peak 5a, ortho-quinone
derivative. The peak 3a, due to the oxidation of an oxidation product formed in the second peak 2a, was confirmed by DP voltammetry, Fig. 4A and B.

The phenol oxidation involved the formation of a phenoxy radical that was oxidized in two pathways. In one pathway phenol was oxidized to a para and/or ortho-quinone derivative that was reversibly reduced. In another pathway phenol initiated polymerization, leading to strongly adsorbed products on the electrode surface [31,33,39,41–45]. It was found that the relative reaction rates of these two pathways depended on the halogen position on the ISA molecule. An oxidation mechanism for isatin 5 (or 7)-monohalogen-derivatives was proposed, Scheme 2.

However, the electroxidized 5-Br-ISA and 5-I-ISA on GCE surface followed the same pathway of ISA, showing only two anodic peaks at higher potentials, and they were more stable compared with the other mono-halogen-ISA derivatives, 5-F-ISA, 5-Cl-ISA, 7-Cl-ISA and 7-I-ISA. So, the oxidation mechanism of 5-Br-ISA and 5-I-ISA is in agreement with the oxidation mechanism of ISA and with the isatin dihalogen-derivatives described in the section below.

3.1.4.2. Isatin dihalogen-derivatives. The presence of the second halogen atom in the isatin benzene ring hampered the oxidation processes, peaks 1a and 2a, when compared to the isatin monohalogen-derivatives oxidation. The main isatin dihalogen-derivatives oxidation reaction, due to competitive electronic and steric effects, corresponded to the formation of the phenoxy radical polymeric products, but by CV, DP and SW voltammetry, no quinone derivative peaks were detected. The withdrawal of the negative charge from the oxygen atom of the phenoxy anion by two halogen atoms make the isatin dihalogen-derivatives stronger acids compared to isatins monohalogen-derivatives. The effect of the electron withdrawal is stronger for the isatin dihalogen-derivatives isomers with halogen atoms next to the OH group. The shift in the isatin dihalogen-derivatives oxidation peaks 1a and 2a relative to the isatin monohalogen-derivatives was due to the stabilizing effects caused by the second halogen attached on the benzene ring, making it more difficult to remove electrons from the isatin dihalogen-derivatives [45,46].

In all compounds studied the phenoxy radical initiated polymerization which resulted in several oxidation products that were adsorbed on the electrode surface or were oxidized to para and/or ortho-quinone derivatives, which were reversibly reduced [39]. The relative reaction rates of this two phenoxy radical pathways depended on the number and halogen position on the ISA molecule.

3.2. Reduction

The reduction of ISA and eleven isatin halogen-derivatives was investigated by CV, Figs. 1 and 2, and the reduction of 5-F-ISA was also investigated by DP and SW voltammetry, Figs. 7 and 8.

3.2.1. Cyclic voltammetry

CVs were obtained in the potential range 0.0 V till −1.2 V, in N2 saturated solutions, in 200 μM isatin halogen-derivatives, in physiological 0.1 M phosphate buffer pH = 7.0, Figs. 1 and 2, and Table 2.

The reduction of all halogen-derivatives showed one reversible reduction peak 6c. In the second scan peak 6c current decreased due to strong adsorption of the isatin halogen-derivatives reduction products on the GCE surface. The reduction peak 6c is due to the electron transfer to the quinoid system following the electron transfer to the C-X group with subsequent cleavage of the carbon-halogen bond at positions C5, C7 or C6 [45,47].

CVs obtained in solutions of 200 μM of 5-Cl-ISA, 5-F-ISA, 7-I-ISA and 4,6-diBr-ISA also showed a new irreversible cathodic peak 7c, Figs. 1 and 2 and Table 2, due to the reduction of the carbonyl group at the position C3 in the heterocyclic ring [31,33]. In the isatin halogen-derivatives: 5-Br-ISA, 5-I-ISA, 5,7-diCl-ISA, 5,7-diBr-ISA, 5,7-F-Cl-ISA and 5,7-Br-Cl-
ISA the cathodic peak 7c was not detected, showing that the presence of the halogen atom in the benzene ring hampered the reduction of the carbonyl in the isatin halogen-derivatives when compared to ISA.

The reduction of $200 \mu M$ 5-F-ISA, Fig. 3, showed two successive cathodic peaks, reversible peak 6c, at $E_{p6c} = -0.50$ V, and an irreversible peak 7c, at $E_{p7c} = -0.78$ V. The second CV showed a decrease of peaks 6c and 7c current, due to the 5-F-ISA reduction products adsorption on the GCE surface, Fig. 3.

The isatin halogen-derivatives reduction CVs were compared with the ISA reduction CV [31], where two reduction peaks were detected, at $E_{p6c} = -0.48$ and $E_{p7c} = -0.60$ V, and with the 500 $\mu$M ISA reduction CV, Figs. 1A and 2A. However, in the 500 $\mu$M ISA CVs, Figs. 1A and 2A, the peak at $-0.48$ was shifted towards a more cathodic potential at $-0.58$ V and, as expected, the small peak at $-0.60$ V was not detected. This is explained because the 500 $\mu$M ISA concentration used was much higher than the 300 $\mu$M ISA concentration in [31], and the reduction products associated with the first reduction process will be adsorbed on the GCE hindering the electroreduction of the second process. The ISA electroreduction was also investigated using SW voltammetry for a lower ISA concentration and the results, in Section 3.2.3, are in agreement with [31].

3.2.2. Differential pulse voltammetry

The electrochemical reduction of 5-F-ISA was studied using DP voltammetry in different 0.1 M ionic strength electrolytes over a range between 2.0 < pH < 10.

The DP voltammograms of 20 $\mu$M 5-F-ISA showed two pH-dependent reduction peaks, peak 6c and 7c, and their potential shifted to more negative values with increasing pH. Peak 7c only occurred for electrolytes with pH > 8.0, Figs. 7A and B.

For 2.0 < pH < 8.0 the 5-F-ISA pH-dependence was linear for both peaks 6c and 7c, Fig. 7A. In the $E_{pc}$ vs. pH plot, Fig. 7B, the slope of the dotted line was $-59$ mV per pH unit, and the width at half-height of peak 6c was $W_{1/2} \approx 74$ mV, and of peak 7c was $W_{1/2} \approx 92$ mV, so, both reduction processes occurred with the transfer of one electron and one proton. For pH > 8.0, the one electron transfer reduction peak 6c was pH-independent.

3.2.3. Square wave voltammetry

The 5-F-ISA and ISA electrochemical reduction was studied using SW voltammetry, Fig. 8A and B.

The first SW voltammogram recorded in a solution of 20 $\mu$M 5-F-ISA, in 0.1 M phosphate buffer pH = 7.0, showed two reduction steps, peak 6c, at $E_{psc} = -0.41$ V, and peak 7c, at $E_{p7c} = -0.70$ V, Fig. 8A, in agreement with the CV and DP voltammetry results. Plotting the forward and
The electrochemical behaviour of a group of eleven isatin halogen-derivatives, with a large spectrum in clinical applications, by CV, DP and SW voltammetry, over a wide pH range using a GCE, was investigated. The results showed that the halogen groups attached to the isatin benzene ring strongly influenced the oxidation and reduction mechanisms, and new redox processes, when compared with isatin, occurred. The electrochemical results demonstrated that the monohalogen substitutions, attached to the isatin benzene ring at C5 or C7 position, decreased the electron-donor character, while the dihalogen substitutions, attached to the isatin benzene ring at C5, C7 or C4, C6, decreased the electron-donor character, when compared with isatin. The halogens attached on the benzene ring in the isatin structure gave rise to different redox mechanisms depending on the nature, the number, the halogen-derivatives when compared to ISA.

4. Conclusions

The electrochemical behaviour of a group of eleven isatin halogen-derivatives, with a large spectrum in clinical applications, by CV, DP and SW voltammetry, over a wide pH range using a GCE, was investigated. The results showed that the halogen groups attached to the isatin benzene ring strongly influenced the oxidation and reduction mechanisms, and new redox processes, when compared with isatin, occurred. The electrochemical results demonstrated that the monohalogen substitutions, attached to the isatin benzene ring at C5 or C7 position, increased the electron-donor character, while the dihalogen substitutions, attached to the isatin benzene ring at C5, C7 or C4, C6, decreased the electron-donor character, when compared with isatin. The halogens attached on the benzene ring in the isatin structure gave rise to different redox mechanisms depending on the nature, the number, and the position of the halogen substituents on the isatin molecule.

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