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Descreve-se, no presente trabalho, o desenvolvimento de um sensor voltamétrico altamente sensível para a oncocalixona A, utilizando-se eletrodo de carbono vítreo modificado com uma bicamada de ftalocianina tetrasulfonada de ferro(II) (FeTSPc) e tetra-(N-metil-4-piridil)-porfirina de ferro(III) (FeT\textsubscript{4}MPyP). O eletrodo modificado apresentou alta atividade catalítica e estabilidade em relação à redução da oncocalixona, proporcionando deslocamento anódico de ca. 30 mV e amplificação da corrente de pico, em relação a iguais parâmetros obtidos em eletrodo de carbono vítreo não modificado. Um ampla faixa linear de resposta entre 0.005-1.2 µmol L\textsuperscript{-1}, com sensibilidade de 8.11 µA L µmol\textsuperscript{-1} e limites de detecção (LOD) e quantificação (LOQ) de 1.5 e 5 nmol L\textsuperscript{-1} foram obtidos com o uso desse sensor.

The development of a highly sensitive voltammetric sensor for oncocalyxone A using a glassy carbon electrode modified with a bilayer iron(II) tetrasulfonated phthalocyanine (FeTSPc) and iron(III) tetra-(N-methyl-4-pyridyl)-porphyrin (FeT\textsubscript{4}MPyP) is described. The modified electrode showed high catalytic activity and stability for the oncocalyxone A reduction, provoking the anodic shift of the reduction peak potentials of ca. 30 mV and presenting much higher peak currents than those obtained on the bare GC electrode. A wide linear response range between 0.005-1.2 µmol L\textsuperscript{-1}, with a sensitivity of 8.11 µA L µmol\textsuperscript{-1} and limits of detection (LOD) and quantification (LOQ) of 1.5 and 5 nmol L\textsuperscript{-1} were obtained with this sensor.

Keywords: voltammetric sensor, oncocalyxone, quinone, chemically modified electrodes, catalysis

Introduction

Plants are repositories for bioactive organic molecules. Several classes of natural compounds are pharmacologically active ingredients and serve as drugs and/or leads for drug developments. Among them, quinones, ubiquitous secondary metabolites, play essential roles, mainly in the biochemistry of energy production, serving as vital links in the electron transport.\textsuperscript{1} They form an important class of toxic metabolites and paradoxically, can be either, potentially carcinogenic or effective anticancer agents. The cytotoxic activity of quinones can often be correlated to their chemical behavior. The striking feature of quinone chemistry is the ease reduction and therefore their ability to act as oxidizing or dehydrogenating agents, the driving force being the formation of a fully aromatic system.\textsuperscript{2-5}

\textit{Auxemna oncocalyx} Taub. is a native Brazilian tree, characteristic of the xerophytic vegetation present in the northeastern region. It is commonly called “pau branco.” The chemical investigation of \textit{A. oncocalyx} heartwood extracts resulted in the isolation and characterization of several unusual terpenoid quinones and hydroquinones of C\textsubscript{16} framework.\textsuperscript{6-9} These secondary metabolites seem to be typical of the genus and, interestingly, were isolated in a homolog series differing in their degree of oxidation, oxygenation (ether or alcohol), rearrangement and intramolecular cyclization of the terpenoid side chain. As a general pattern, all compounds possess a methoxyl group in the C-2 position and, several of them, an angular methyl group β-oriented attached to C-8a, besides a \textit{cis} configuration for the B/C rings. Oncocalyxone A (1)
(Figure 1), \( \text{rel-8α-Hydroxy-5-hydroxymethyl-2-methoxy-8aβ-methyl-7,8,8a,9-tetrahydro-1,4-anthracenedione} \), a deep red powder, was the first compound isolated from the EtOH extract of \( A. \) oncocalyx. This secondary metabolite, which was obtained in significant amounts (0.13\% crude extract), is responsible for the dark color of the heartwood of this plant.

\[
\text{H}_2\text{CO} \quad \text{O} \quad \text{CH}_3 \quad \text{OH} \\
\text{O} \quad \text{OH}
\]

**Figure 1.** Chemical structure of oncocalyxone A (I).

Oncocalyxone A (I) exhibited a series of pharmacological properties, such as cytotoxic, analgesic, anti-inflammatory, antioxidant and causative of inhibition of platelet activation.

The potential pharmacological activity presented by oncocalyxone A (I) and the unusual and varied structural character of the other compounds isolated from \( A. \) oncocalyx provide important models for the synthesis of new potential biological active compounds.

Due to the importance of phytotherapy, a major impetus was generated towards improving the techniques of separation and analysis of plants of commercial importance for their standardization in terms of these recognized molecular entities.

Electrochemical methods have been widely used for quantification of quinones in vegetal extracts and present the advantages of simplicity and low cost.

The use of bare electrodes for detection of organic compounds presents a number of limitations, such as low sensitivity and reproducibility due to the slow electron transfer reaction between the electrode surface and the analyte, and lower stability. As an alternative, the use of chemically modified electrodes has been proposed, which improve the electron transfer rate, also promoting an increase in the sensitivity of the system. In this sense, a wide variety of compounds have been used as electron transfer mediators for electrooxidation or reduction of several target molecules.

The modification of electrodes with alternating deposition of functional compounds has been attracting much interest because of its potential application, and it is a very simple way to experimentally produce complex layered structures with precise control of layer composition and thickness. Additionally, porphyrins and metalloporphyrins have demonstrated good features as analytical sensors because they can provoke electrocatalysis, increasing the sensitivity and selectivity of the electrode.

In this context, the present work reports the development of an efficient and stable sensor for oncocalyxone A (I) determination, based on a glassy carbon electrode modification with a bilayer produced by drop-casting of iron(II) tetrasulfonated phthalocyanine (negatively charged) and iron(III) tetra-(N-methyl-4-pyridyl)-porphyrin (positively charged), where presumably a strong interaction is established by ion-pair formation between the ammonium group of the porphyrin and the sulfonic (–SO\(_3\)-) group of the phthalocyanine.

**Experimental**

**Chemical and solutions**

All used chemicals were analytical grade. Iron(II) tetrasulfonated phthalocyanine (FeTSPC) was synthesized and purified according to the procedure of Weber and Busch. Iron(III) tetra-(N-methyl-4-pyridyl)-porphyrin (FeT\(_4\)MPyP) was purchased from Porphyrin Products Inc. (UT, USA). Oncocalyxone A (I) (Figure 1), which data is presented, was isolated from wood parts of \( Auxemma \) oncocalyx collected in June 2005, at Pentecoste County, State of Ceará, Brazil. The plant was identified by Professor Edson P. Nunes, and a voucher specimen (No. 18459) is deposited at the Herbarium Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil. The extract preparation, methods of isolation and chemical characterization were previously described by Pessoa et al.

\[ \text{rel-8α-Hydroxy-5-hydroxymethyl-2-methoxy-8aβ-methyl-7,8,8a,9-tetrahydro-1,4-anthracenedione (I)} \]
132.7 (C9a), 111.5 (C10), 135.1 (C10a), 61.3 (C11), 20.9 (C12), 56.4 (OMe).

Monobasic sodium phosphate (NaH$_2$PO$_4$), dibasic sodium phosphate (Na$_2$HPO$_4$), citric acid (C$_6$H$_8$O$_7$), acetonitrile (CH$_3$CN), sodium hydroxide (NaOH), hydrochloric acid (HCl) were acquired from Merck, Rio de Janeiro, Brazil, PIPES [piperazine-$N,N'$-bis(2-ethanesulfonic acid)] and HEPES [N-(2-hydroxyethyl)piperazine-$N$-(2-ethanesulfonic acid)] were acquired from Sigma, St. Louis, USA. Phosphate, McIlvaine, HEPES and PIPES buffers and solutions of FeT$_2$MPyP and FeTSPc were prepared with water purified in a Milli-Q Millipore system and the actual pH values of the buffer solutions were determined with a Corning pH/Ion Analyzer 350 model.

**Voltammetric measurements**

The voltammetric measurements were carried out in an Autolab PGSTAT-30 potentiostat from Echo Chemie (Utrecht, The Netherlands) coupled to a PC microcomputer with GPES 4.9 software. An electrochemical cell containing 5.0 mL of phosphate buffer solution with a saturated calomel electrode (SCE) as reference, a Pt wire as auxiliary and a modified GC as working electrode were used for all measurements. Oxygen was removed by bubbling nitrogen through the solution, during 5 to 10 min. The optimized parameters in Square Wave Voltammetry (SWV) were set up using a step potential ($\Delta E$) 0.002 V, pulse amplitude ($\Delta E_{sw}$) 0.05 V and frequency (f) 100 Hz.

**Construction of the sensor**

The glassy carbon electrode, with geometrical area of 0.071 cm$^2$ was acquired from BAS-USA, and used for all measurements. Prior to the modification, the electrode surface was treated according to the procedure described by Zhu and Nan-Qiang. After cleaning the electrode, an electrostatically formed layer was prepared on the electrode surface by drop-casting, transferring 15 µL of a FeTSPc solution, in the concentrations 0.01, 0.05, 0.1, 0.15 and 0.19 mmol L$^{-1}$ and let to dry at 80 °C during 10 min. After 10 min, 15 µL of the FeT$_2$MPyP solution, in the concentrations 0.01, 0.05, 0.1, 0.15 and 0.19 mmol L$^{-1}$, respectively, were added to the electrode surface and also let to dry at 80 °C during 10 min. The bilayers were prepared by repeating the procedure described above. Presumably the interaction between FeTSPc and FeT$_2$MPyP complexes occurs by ion-pairing between the amino group (–C$_4$H$_3$N$^+$) of the iron porphyrin and the anionic –SO$_3$ of the tetrasulfonated phthalocyanine. For the sensor optimization, a bilayer of FeTSPc + FeT$_2$MPyP complexes was prepared.

**Analytical curve**

After optimizing experimental parameters for the proposed sensor, the analytical curve was built by addition of aliquots of I (stock solution of 1.0 µmol L$^{-1}$ in acetonitrile/water, 1:1 v/v, kept under nitrogen and dark) into measurement cell containing phosphate buffer solution (0.05 mol L$^{-1}$) at pH 7.0. The technique employed was square wave voltammetry (SWV), and the SWV characteristic parameters were: step potential ($\Delta E$) 0.002 V, frequency (f) 100 Hz and pulse amplitude ($\Delta E_{sw}$) 0.050 V.

**Results and Discussion**

**Electrocatalytic reduction of I on the modified electrode**

Figure 2 shows the cyclic voltammograms for the unmodified GC electrode in absence (a) and presence (b) of I ($5 \times 10^{-6}$ mol L$^{-1}$) and the voltammograms for the modified GC electrode with a layer of FeTSPc alone in presence of $5 \times 10^{-6}$ mol L$^{-1}$ I (c) and a layer of FeT$_2$MPyP (e) in presence of I ($5 \times 10^{-6}$ mol L$^{-1}$). For comparison, this figure also presents the cyclic voltammograms for the modified GC electrode with a bilayer of FeTSPc/FeT$_2$MPyP in the absence (d) and presence (f) of I ($5 \times 10^{-6}$ mol L$^{-1}$) in 0.1 mol L$^{-1}$ phosphate buffer solution (pH 7.0). In presence of I (Figure 2b), a typical quinone reduction behavior is
shown, represented by a reversible pair of waves, with uptake of 2 e− and 2 H+, generating the hydroquinone.231-32

As can be seen, the best response for I was obtained with the FeTSPc/FeT_MPyP modified GC electrode (Figure 2f). On this modified electrode, an anodic shift of the reduction peak potential of ca. 30 mV and a much higher peak current for I than those obtained on the bare GC electrode were observed (Figure 2b compared to 2f). Additionally, on this electrode, the peak current for the reduction of I was also higher than that ones showed on the electrode modified with only FeT_MPyP or FeTSPc (Figures 2c or 2e, respectively, compared to 2f).

The reduction of I on FeT_MPyP modified electrode (Figure 2e) occurred next to the reduction current of the FeTSPc/FeT_MPyP modified GC electrode (Figure 2d). When only FeT_MPyP was used, a poor response was observed (Figure 2e). This suggests that the FeT_MPyP complex effectively increases the reduction rate of I, while the FeTSPc layer must be improving the assembly of the active sites of FeT_MPyP resulting in a better catalyst for I.

The interaction of the FeTSPc/FeT_MPyP complexes and the increase in the peak current of I (Figure 2f), when compared to the bare electrode (Figure 2b), can be associated to the redox reactions of the immobilized [Fe(III)T_MPyP]+ and [Fe(II)TSPc]+ giving [Fe(II)T_MPyP]+ and [Fe(III)TSPc]+ species. [Fe(II)T_MPyP]+ is oxidized back, reducing the quinone (I), originating the catalytic cycle (equations 1 and 2). [Fe(III)TSPc]+ species is electrochemically reduced to [Fe(II)TSPc]1+ leading to a cycle. Therefore, the high activity of the modified GC electrode for the reduction of I in aqueous solutions can be associated with the low charge transfer resistance of the FeTSPc/FeT_MPyP bilayer as well the presence of FeT_MPyP as an electrocatalyst. The combination of these points proportionates a powerful catalytic property for the reduction of I. This effect results from the changes in the chemical environment around the active sites, induced by the contrasting molecular packing interactions with FeT_MPyP. The catalytic properties and the use of phthalocyanines and porphyrin bilayers have been described33 and in the present case can be written as follows:

\[
[Fe(III)T_MPyP]^+ + [Fe(II)TSPc]^+ \rightarrow [Fe(II)T_MPyP]^+ + [Fe(III)TSPc]^+ \quad (1)
\]

\[
2[Fe(II)T_MPyP]^+ + \text{oxygen}_{\text{air}} \rightarrow 2[Fe(III)T_MPyP]^+ + \text{oxygen}_{\text{red}} \quad (2)
\]

Influence of the concentration of FeTSPc and FeT_MPyP in the sensor response

The concentrations of FeTSPc and FeT_MPyP are a control factor and influence the electrochemical behavior in larger extent. Thus, the dependence of the complexes concentration on the electrode response for I was investigated by preparing a bilayer with different concentrations of FeTSPc as well as for FeT_MPyP: 0.01, 0.05, 0.1, 0.15 and 0.19 mmol L−1. The results showed in Table 1 indicate that the best response was obtained using 0.1 mmol L−1 of both complexes.

<table>
<thead>
<tr>
<th>[FeTSPc]/[FeT_MPyP] (× 10−4 mol L−1)</th>
<th>−I/µA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1:1.9</td>
<td>1.40</td>
</tr>
<tr>
<td>0.5:1.5</td>
<td>1.43</td>
</tr>
<tr>
<td>1.0:1.0</td>
<td>2.29</td>
</tr>
<tr>
<td>1.5:0.5</td>
<td>1.23</td>
</tr>
<tr>
<td>1.9:0.1</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Lower current values were obtained when the amount of FeTSPc was higher than FeT_MPyP, and vice-versa: when the amount of FeT_MPyP increases, a similar trend was observed. This trend suggests that the better ratio for FeTSPc/FeT_MPyP assembly is 1, ideal for the kinetic of electron transfer. In this condition, high current values were obtained. The concentration of 0.1 mmol L−1 for each constituent of the complex FeTSPc/FeT_MPyP was chosen for further experiments.

Influence of pH, buffer solution and buffer concentration

The influence of the solution pH in the electrochemical reduction response of I using 0.1 mol L−1 phosphate buffer at pH 6.0, 6.4, 7.0, 7.4 and 8.0 was studied (Figure 3). The peak current increased with pH in the range from 6.0 up to 7.0. The highest peak current was obtained in pH 7.0. A decrease in the current is observed when the solution pH is higher than 7.0. Thus, the optimum pH for further studies was set in 7.0. In addition, this study showed that the oncocalyxone reduction peak potential undergoes, as expected, a slight cathodic displacement when the pH solution increases. The influence of the buffer solution on the sensor response was tested for four different buffer solutions (HEPES, McIlvaine, PIPES and phosphate) with concentrations of 0.1 mol L−1. The best responses were obtained in phosphate buffer (Figure 4) that was chosen as the buffer solution.

Figure 5 shows the influence of different concentrations of phosphate buffer (0.025, 0.050, 0.10, 0.15 and 0.20 mol L−1) for the measurement of I. The best response was obtained for the concentrations of 0.05 and 0.1 mol L−1;
outside this range, a current decrease has been observed. Based on this, the concentration of 0.05 mol L$^{-1}$ was chosen for further experiments.

**Influence of the frequency (f) and potential amplitude (a)**

The effects of the frequency and potential amplitude on the square wave voltammetry (SWV) response of the FeTSPc/FeT$_4$MPyP modified GC electrode, in phosphate buffer solution were verified. The peak current values presented a linear increase with the frequency variation from 20 to 140 Hz with $\Delta E_s = 0.002$ V. However, when the frequency is $>100$ Hz, the current peak value remained almost constant, accompanied by distortion of the peaks. As it sets the best voltammetric profile with higher sensitivity, the frequency of 100 Hz was chosen and subsequently used throughout the present study. The current values of peak were also found to vary with pulse amplitude of 0.010–0.080 V applied on SWV at a frequency of 100 Hz for the modified electrode and $\Delta E_s = 0.002$ V. The use of the pulse amplitude $>0.050$ V led to the peak current values almost constant and an increase in the background current. In this sense, the best sensitivity was obtained with 0.050 V and therefore, this value was chosen for further studies.

**Analytical characterization**

In order to obtain an analytical curve for 1, with the developed sensor, under optimized conditions (phosphate buffer, 0.05 mol L$^{-1}$, pH 7.0), experiments were carried out adding aliquots of 1 from a stock solution (Figure 6), kept in dark and protected from oxygen. Under optimized conditions the proposed sensor showed a linear response ranging from 5.0 nmol L$^{-1}$ up to 1.2 $\mu$mol L$^{-1}$, which can be expressed according to equation 3:

$$I_p (\mu A) = 0.23 (\pm 0.90) + 8.11 (\pm 0.17) [1] (\mu mol L^{-1})$$

with a correlation coefficient of 0.996 (for $n = 8$) and with good sensitivity and limit of detection which can be attributed to the efficiency of the electron transfer.
between the FeTSPc/FeT₄MPyP film and 1. Additionally, the proposed sensor presented a relatively wide linear response range, which can be related with the high affinity between the catalyst and the analyte. The limit of detection of 1.5 nmol L⁻¹ was determined using a 3σ/slope ratio and limit of quantification was 5.0 nmol L⁻¹ using 10σ/slope, where σ is the standard deviation of the mean value for 10 voltammograms of the blank, determined according to IUPAC recommendations.³⁴

Conclusions

This work demonstrated that glassy carbon electrode modified by alternating FeTSPc and FeT₄MPyP, layer by layer, is a feasible alternative for the analytical determination of 1. Optimization of the experimental conditions for square wave voltammetry furnished a low limit of detection and high sensitivity for oncocalyxone. In this sense, this work demonstrated that the glassy carbon electrode modified with FeTSPc/FeT₄MPyP is a sensitive, robust and stable sensor showing great potential for oncocalyxone determination and additionally to be employed as an electrochemical detector in HPLC.

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