Electropolymerized Supramolecular Tetraruthenated Porphyrins Applied as a Voltammetric Sensor

Monize M. da Silva,* Gabriel H. Ribeiro,* Alzir A. Batista,† Anizio M. de Faria,* André L. Bogado* and Luis R. Dinelli*†,*

*Faculdade de Ciências Integrais do Pontal, Universidade Federal de Uberlândia, Rua Vinte, 1600, 38304-402 Ituiutaba-MG, Brazil
†Departamento de Química, Universidade Federal de São Carlos, Rodovia Washington Luís (SP 310), km 235, 13565-905 São Carlos-SP, Brazil

A porfirina 5,10,15,20-Tetra(4-piridil)manganês(III), [Mn-TPyP(H₂O)₂]PF₆, e a porfirina supramolecular eletropolimerizável (ESP), {Mn-TPyP(H₂O)₂[RuCl₃(dppb)]}₄PF₆ (dppb = 1,4-bis(difenilfosfina)butano), foram sintetizadas e caracterizadas. Um filme fino da PSE foi obtido na superfície do eletrodo de carbono vítreo utilizando o método de voltametria cíclica. Foi observado um aumento da corrente de pico com o aumento do número de ciclos voltamétricos, mostrando um comportamento típico de espécies sendo adsorvidas na superfície do eletrodo. O eletrodo modificado com a ESP foi utilizado para a quantificação de acetaminofeno por voltametria cíclica. O eletrodo modificado foi utilizado para a determinação de acetaminofeno em uma amostra comercial e os resultados foram satisfatórios, pois apresentaram concordância quando comparado pelo método de HPLC.

Keywords: tetraruthenated porphyrins, supramolecules, acetaminophen determination, voltammetric sensor

Introduction

The importance of porphyrins is not limited to their participation in the transport of oxygen to heme proteins and photosynthetic activities. In fact, they also have valuable contributions in various fields, such as liquid crystalline materials,1 due to their remarkable electro-optical properties, oxygen measurements in vivo,2,3 photodynamic therapy,4,5 malaria treatment,6 molecular wires,7,8 energy conversion,9,10 nonlinear optical,11 optical limiter,12 Langmuir and Langmuir-Blodgett (LB) films,13,14 as well as switching fluorescence.15 Porphyrins are also present in many applications of chemical analyses,16 such as electrochemical and optical sensors,17,18 modified electrodes,19,20 spectrophotometric reagents,21,22 stationary phase in the HPLC column,23,24 and as modifiers in open tubular electrochromatography.25 Metalloporphyrins are the most accepted modifiers of electrodes, since they are known as excellent and selective catalysts. The immobilization of metalloporphyrins through...
electrochemical deposition allow for film formation on small surface electrodes as well as on electrodes with complex geometry.

The use of modified electrodes has been considered the best strategy for the determination of several analytes, particularly active principles in pharmaceutical formulations. Accordingly, methods for acetaminophen determination in pharmaceutical preparations have been developed for many years due to the fact that an overdose of acetaminophen can cause fulminating hepatic necrosis and other toxic effects. Techniques typically employed for the determination of acetaminophen in clinical laboratories are titrimentry, chromatography, spectrophotometry and immunoassays. However, determination using electrochemical techniques has been demonstrated to be promising. One of the first studies reported the use of glassy carbon electrodes modified with 4-vinylpyridine. Shortly after, the electropolymerized porphyrin was already being used as the modified electrode for the determination of acetaminophen.

Porphyrins can also be modified by peripheral groups, which increase the size of the macromolecules. The 5,10,15,20-Tetra(4-pyridyl)-21H,23H-porphyrin (TPyP) has been used effectively in the formation of supramolecules due to their reaction with peripheral metal complexes. Such supramolecules have been used successfully as modifiers for preparation of electrochemical sensors.

The study of TPyP containing ruthenium peripheral complexes was boosted up by the discovery of exceptional electrocatalytic activity of 5,10,15,20-Tetra(piridyl) porphyrin cobalt (II) in the tetra electron reduction of O₂ to H₂O₂. This complex has four groups of pentamim ruthenium in peripheral positions, in the tetra electron reduction of O₂ to H₂O₂.

In a study conducted by our research group, we synthesized the tetraruthenated porphyrins {H₂TPyP[RuCl₃(dppb)]₄}₄ and {M-TPyP[RuCl₃(dppb)]₄}₄ where dppb = 1,4-bis(diphenylphosphino)butane and M = nickel or cobalt, from the reaction of 5,10,15,20-Tetra(4-pyridyl)-21H,23H-porphyrin with the mer-[RuCl₃(dppb)(H₂O)] complex in a ratio of 1:4. Additionally, the X-ray structure of tetraruthenated porphyrin [Ni-TPyP[RuCl₃(dppb)]₄] was reported. The great advantage of these series of complexes as modifiers was the possibility of film deposition through successive voltammetric cycling of different electrodes such as platinum, ITO and glassy carbon. The proposed mechanism for the formation of films, which necessarily depends on the peripheral group “RuCl₃(dppb)₄” involves the formation of binuclear mixed-valence complexes (Ru⁵/Ru⁴) with bridging chlorides between the molecules. The modified glassy carbon electrode with porphyrin (Co-TPyP[RuCl₃(dppb)]₄) was successfully used as an electrochemical sensor for the detection of many analytes such as catechol.

Understanding the mechanism of the film formation for these classes of molecules {M-TPyP[RuCl₃(dppb)]₄} (M = transition metal) allowed for the development of new classes of materials containing supramolecules. One great advantage in the use of electropolymerized electrode is the control of the number of layers deposited by controlling the number of voltammetric cycles. In towards of this view, the present work describes the synthesis of the new supramolecule {Mn-TPyP(H₂O)₃[RuCl₃(dppb)]₄}₄PF₆, which was immobilized on a glassy carbon electrode by electropolymerization and used as a sensor for acetaminophen.

**Materials and methods**

**Materials**

The chemicals employed were of reagent-grade quality (Aldrich or Fluka) and used as received. Reagent-grade solvents (Merck) were distilled prior to use. Doubly distilled deionized water was used for all aqueous solutions. Purified argon atmosphere was used in all procedures described herein for the removal of dissolved oxygen.

**Measurements**

UV-Vis spectra were recorded in CH₂Cl₂ on a Perkin Elmer (Lambda 25) spectrophotometer. Electron paramagnetic resonance (EPR) spectra were measured at -160 °C with a Varian E-109 Instrument operating at the X-band frequency, within a rectangular cavity (E-248) fitted with a temperature controller. Cyclic voltammetry was carried out at room temperature in freshly distilled dichloromethane containing 0.1 mol L⁻¹ Bu₄N⁺PF₆⁻ (TBAH) or 0.1 mol L⁻¹ sodium acetate (NaAc) solution, using a µautolab III potentiostat/galvanostat. The voltammetric measurements were performed in a cell with three electrodes, using a modified glass carbon or bare glass carbon (geometric diameter = 0.2 cm) as the working electrode (previously polished with alumina), a saturated Ag/AgCl as the reference electrode, a platinum electrode as the auxiliary electrode. The glass carbon and the platinum electrode were polished with alumina before use. Under these conditions, ferrocene is oxidized at 0.43 V (Fe⁺/Fe). Elemental analyses were performed at the Department of Chemistry of the Federal University of São Carlos, São Carlos (Brazil), using a FISIONS CHNS EA1108 microanalyzer. Conductimetry measurements were
were carried out with a CDM230 (Meter Lab). Solutions were prepared in CH₂Cl₂ or methanol at a concentration of 3.1 mol L⁻¹. Magnetic susceptibility measurements were used on a scale of Magnetic Susceptibility JM (Johnson Matthey). The value of magnetic susceptibility was found using the following equations: \( X_m = X_g \times MW \times \mu = K \times (X_m \times T)^{1/2} \); \( \mu = [n(n+2)]^{1/2} \times (X_m \times 10^3) \) (Xm: Molar susceptibility; Xg: susceptibility in grams (value obtained directly in the balance); MW: molecular weight; \( \mu \): magnetic moment; K: constant; 2.84; T: temperature in Kelvin and n: number of unpaired electrons).

Atomic-force microscopy (AFM) measurements were performed at the Department of Material Science of the Federal University of São Carlos, São Carlos (Brazil), using a Nanoscope V Veeco/Bräker with a scan assist. The chromatographic evaluations were performed using a Varian ProStar HPLC comprising a ProStar 210 binary pump, a ProStar 352 UV/Vis Detector (at 254 nm), and a Rheodyne model 7125 injection valve with a 5 µl loop. Experiments were carried out at 25 °C. Data were processed using Galaxie software for data acquisition. The mobile phases were prepared volumetrically from individually measured amounts of each solvent. All solvents were filtered and degassed before use. All measurements were performed in a reversed C₁₈ column phase (150x4.6 mm ID; particle size, 5 µm), with a mixture of methanol: H₂O (70:30, v/v) as the mobile phase for the detection of acetylphenone.

**Syntheses**

The mer-[RuCl₂(dppb)(H₂O)] [dppb = 1,4-bis(diphenylphosphino)butane] complexes were prepared according procedures reported in the literature.⁵⁶,⁵⁷ H₂TPyP was synthesized by a modification of a procedure described in the literature:⁴⁶ freshly distilled pyrrole (3.35 g, 0.05 mol) and 4-pyridinecarboxaldehyde (5.35 g, 0.05 mol) were added to 350 mL of refluxing reagent grade acetic acid. After refluxing for 2 h, the solution was cooled down to room temperature and filtered. The purple crystals were washed sequentially with methanol, hot water and dried in vacuum to remove the absorbed acid. The H₂TPyP was then purified in an alumina column using chloroform as a solvent and 5% methanol in chloroform as an eluent to yield 2.00 g (26%).

The [Mn-TPyP(H₂O)]PF₆ was synthesized by a modification of a procedure described in the literature:⁴⁶ 0.150 g (0.247 mmol) of 5,10,15,20-tetra(pyridyl)porphyrin was dissolved in 100 mL glacial acetic acid and had 0.149 g of manganese acetate (0.617 mmol) and then the same molar amount of KPF₆ (0.113 g), slowly added to it. The system was refluxed for 6 hours, which was followed by an UV/Vis spectroscopy. After that, the solvent was evaporated and the resulting product was dried under vacuum for 24 hours. To purify the porphyrin, the obtained solid was dissolved in distilled water at 62 °C, filtered off, and reprecipitated with a sodium acetate (2 mol L⁻¹) solution, washed with cold water and then dried under vacuum. Finally, the product was eluted through a chromatography column, using alumina as a stationary phase and the mixed solvent chloroform (95%)/methanol (5%) as eluent. Yield: 181 mg (85.5%); C₃wH₃₂F₆MnN₄O₂PF₇Cl₄ found (theoretical) / %: C 50.26 (50.66); H 3.69 (3.01); N 11.14 (11.53). UV/Vis (CH₃Cl) \( \lambda_{\text{max}}/\text{nm} \): 470 (\( \varepsilon = 8.93 \times 10^3 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)), Soret Band; 579 (\( \varepsilon = 9.19 \times 10^2 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)); 613 (\( \varepsilon = 7.04 \times 10^2 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)); IR (1% KBr solution) \( v_{\text{max}}/\text{cm}^{-1} \): 1610 (\( v_{\text{C}=\text{C}} \)); 1435 (\( v_{\text{C}=\text{C}} \)); 1011 (\( v_{\text{C}=\text{H}} \)); 843 (PF₆); 696 (\( v_{\text{C}=\text{H}} \)); 557 (\( v_{\text{C}=\text{H}} \)); 247 (\( v_{\text{Mn-N}} \)); cyclic voltammetry: redox pair Mn(II)/Mn(III), \( E_{1/2} = 107.5 \text{ mV} \), \( E_{\text{ap}} = 0.97 \) (ap = anodic peak; cp = cathodic peak).

The supramolecular tetraruthenated porphyrins \{Mn-TPyP(H₂O)₂[RuCl₃(dppb)]\}PF₆ was synthesized by a modification of a procedure described literature:⁴⁴ 15 mg (21.1 µmol) of \{Mn-TPyP(H₂O)₂\}PF₆ and 59 mg (91 µmol) of mer-[RuCl₃(dppb)H₂O] reacted in 10 mL of a mixture of chloroform (95%) and methanol (5%). The mixture was stirred for 4 h, then had its volume reduced under vacuum until approximately 2 mL and had diethyl ether added to it in order to result in a reddish-brown powder. The excess of mer-[RuCl₃(dppb)H₂O] was removed by dissolving the reaction product in CH₂Cl₂ followed by its filtration. The filtrate was reduced to 1 mL, and ether was added to achieve the desired compound. Yield: 58 mg (82%); C₁₅₂H₁₀₂₃F₁₂₂MnN₄O₆P₄Ru₄ found (theoretical) / %: C 54.17 (53.88); H 4.10 (4.16); N 3.36 (3.31); UV -Vis (CH₃Cl) \( \lambda_{\text{max}}/\text{nm} \): 522 (\( \varepsilon = 1.61 \times 10^4 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)); 575 (\( \varepsilon = 2.12 \times 10^3 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)); 615 (\( \varepsilon = 9.27 \times 10^2 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)). The band at 522 nm (\( \varepsilon = 1.98 \times 10^4 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1} \)) is characteristic of the peripheral complex: IR (1% KBr solution) \( v_{\text{max}}/\text{cm}^{-1} \): 1611 (\( v_{\text{C}=\text{O}} \)); 1433 (\( v_{\text{C}=\text{C}} \)); 697 (\( v_{\text{C}=\text{H}} \)); 514 (\( v_{\text{R}=\text{P}} \)); 340 (\( v_{\text{R}=\text{O}} \)); cyclic voltammetry: redox pair Ru(II)/Ru(III), \( E_{1/2} = 615.5 \text{ mV} \), \( E_{\text{ap}} = 1.01 \).

Electrode modified by electropolymerization of \{Mn-TPyP(H₂O)₂[RuCl₃(dppb)]\}PF₆

Electropolymerization of \{Mn-TPyP(H₂O)₂[RuCl₃(dppb)]\}PF₆ on the glassy carbon electrode surface was carried out in CH₂Cl₂ solutions containing 10⁻³ mol L⁻¹ of monomer and 0.1 mol L⁻¹ TBAH by cycling (100 mV s⁻¹) the GC working electrode potential repeatedly between
–0.4 V and +1.0 V (vs Ag/AgCl). Therefore, a film was obtained on the glassy carbon electrode surface after 4 voltammetric cycles. Finally, the modified electrode was washed with dichloromethane in order to remove the non-electropolymerized porphyrin on the electrode surface. This electrode is named ESPE (electropolymerized supramolecular porphyrin electrode).

Detection of acetaminophen

Acetaminophen was determined by cyclic voltammetry using the electropolymerized supramolecular porphyrin electrode (ESPE). Cyclic voltammograms were recorded in the range of 0.4 to 1.0 V at a scan rate of 100 mV s\(^{-1}\) in 0.1 mol L\(^{-1}\) acetate buffer. An analytic curve ranging from 0.05 to 1.0 mmol L\(^{-1}\) acetaminophen was prepared. Samples were analyzed by the standard addition method. Acetate buffer solution (pH 4.75) 0.1 mol L\(^{-1}\) was prepared from 0.1 mol L\(^{-1}\) of acetic acid (HAc) and 0.1 mol L\(^{-1}\) sodium acetate. The pH (2-8) of the solutions were adjusted to the required value by addition of aliquots of 1.0 mol L\(^{-1}\) HAc or 1.0 mol L\(^{-1}\) sodium hydroxide. Commercial acetaminophen (syrup, 100 mg mL\(^{-1}\) of acetaminophen) was obtained from a drugstore. The standard sample of acetaminophen was purchased from Aldrich. All electrochemical experiments were in triplicate.

Results and discussion

Characterization of manganese porphyrin – [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\)

Several studies in the literature show the syntheses and characterizations of porphyrins containing the manganese ion (III) as the central metal. Herein, only unpublished results about [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\) will be discussed.

The magnetic susceptibility measurements revealed a value of 1.08 × 10\(^{-5}\) (This value was obtained using the formula shown in the experimental section) for [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\), which is in agreement with the four unpaired electrons.

This measurement shows that the manganese ion (III) has a configuration of type \(t_{2g}^3e_g^1\), which is characteristic of the tetragonal geometry with a strong Jahn-Teller effect. The tetragonal geometry has been proposed because the elemental analysis suggests two additional water molecules in the experimental composition when compared to the theoretical formulation. The infrared spectrum of the complex showed bands related to the P–F bond at 844 cm\(^{-1}\), due to the PF\(_6\) stretching as the counter ion, which provides the ionic behavior of this specimen. Conductivity measurements (42.4 µS cm\(^{-1}\)) made in methanol confirmed that the ionic complex has a 1:1 ratio. Therefore, this result supports a tetragonal geometry for [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\).

Characterization of tetraruthenated porphyrin {Mn-TPyP(H\(_2\)O)\(_2\)[RuCl\(_3\)(dppb)]\(_4\)}PF\(_6\)

The UV/Vis spectroscopy data were useful for the characterization of porphyrins. The free base [TPyP] and the metalloporphyrin [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\) showed typical absorption spectra. The {Mn-TPyP(H\(_2\)O)\(_2\)[RuCl\(_3\)(dppb)]\(_4\)}PF\(_6\) complex showed a small difference in the electronic spectrum, when compared to the porphyrin [Mn-TPyP(H\(_2\)O)\(_2\)]PF\(_6\) spectrum, except for the band at 522 nm, that showed a small increase in the absorbance, which is due to the contribution of the “RuCl\(_3\)(dppb)(py)” moiety to the porphyrin complex. This implies that the peripheral complex does not interfere in the local symmetry (D\(_{4h}\)) of the porphyrin and, therefore, the electronic spectrum of the tetraruthenated porphyrin is the sum of the porphyrin with the ruthenium complex electronic spectrum. The structure of {MnTPyP(H\(_2\)O)\(_2\)[RuCl\(_3\)(dppb)]\(_4\)}PF\(_6\) is shown in Figure 1.

![Figure 1. Structure of {Mn-TPyP(H\(_2\)O)\(_2\)[RuCl\(_3\)(dppb)]\(_4\)}PF\(_6\) (P–P = dppb).](image-url)
the ruthenium (III) complexes have a configuration of type $t_{2g}^3$, $e_g^1$ and $t_{2g}^5$, respectively.

Modified electrode by electropolymerization of supramolecular tetraruthenated porphyrin.

The film was formed from repetitive voltammetric sweeps between the −0.4 V and +1.0 V range, with a scan rate of 0.1 V s$^{-1}$, using 1.0 × 10$^{-4}$ mol L$^{-1}$ {Mn-TPyP(H$_2$O)$_2$[RuCl$_3$(dppb)]$_4$}PF$_6$, which resulted in the behavior shown in Figure 2A. The peak current increased with the number of cycles, which shows a typical behavior of the species being adsorbed on the surface of the electrode.

The mechanism of the electropolymerization has already been reported in the literature and involves the reduction of “RuCl$_3$(dppb)” moiety (Ru$^{III}$→Ru$^{II}$) at 0 V, with the formation of a mixed binuclear valence complex (Figure 2B) (Ru$^{V}$/Ru$^{II}$) at $E_{1/2} = 0.55$ V.$^{44}$ The film thickness can be controlled by the number of voltammetric cycles and films formed with a very high number of cycles are very thick and have a passive electrode. Table 1 shows the behavior of the ESPE in the acetaminophen detection with different layers (between 1-7 voltammetric cycles). It is possible to observe the maximum $I_{ap}$ and minimum $E_{ap}$ when the modified electrode was obtained with four cycles. After that the voltammetric cycles does not improve those electrochemical parameters. For that reason, four voltammetric cycles were used to make the film on the voltammetric sensor to be used in the next measurements.

The film was also characterized by Atomic Force Microscopy (AFM) measurement (Figure 3) and its thickness can be estimated as thick as 4.5 nm, but unfortunately its thickness was not determined. However useful information may still be gleaned from it, especially the roughness of the film (1.16 nm in a 100 µm$^2$ area).

<table>
<thead>
<tr>
<th>Nº of voltammetric cycles</th>
<th>$I_{ap}$/µA</th>
<th>$E_{ap}$/mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.1</td>
<td>420</td>
</tr>
<tr>
<td>2</td>
<td>12.4</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>11.7</td>
<td>408</td>
</tr>
<tr>
<td>4</td>
<td>16.6</td>
<td>394</td>
</tr>
<tr>
<td>5</td>
<td>14.6</td>
<td>403</td>
</tr>
<tr>
<td>6</td>
<td>15.5</td>
<td>403</td>
</tr>
<tr>
<td>7</td>
<td>12.4</td>
<td>426</td>
</tr>
</tbody>
</table>

Figure 2. (A) Repetitive voltammograms (4 cycles) of {Mn-TPyP(H$_2$O)$_2$[RuCl$_3$(dppb)]$_4$}PF$_6$ and (B) electropolymerized supramolecular tetraruthenated porphyrins, 0.1 mmol L$^{-1}$ {Mn-TPyP[Ru(dppb)]$_4$(µCl$_3$)$_2$}$_2^{4n}$. $^{4n}$ TBAH, CH$_2$Cl$_2$, glassy carbon as working electrode, scan rate = 100 mV s$^{-1}$.

Figure 3. AFM image of a {Mn-TPyP[Ru(dppb)]$_4$(µCl$_3$)$_2$}$_2^{4n}$ film deposited on ITO electrode surface.

Electrochemical behavior of acetaminophen

Figure 4a shows the electrochemical behavior of acetaminophen using the modified and unmodified electrode. It was possible to observe in the modified electrode...
response a well defined oxidation peak of acetaminophen, with higher current and also a shift of the cathodic potential from 589 to 518 mV. Also the electrochemistry process seems to be more reversible ($\Delta E_p = 80$ mV) compared to the bare carbon glassy electrode (CG). As can be seen in Figure 4a the acetaminophen oxidation process associated is quasi reversible on the modified electrode, differently of the process showed by the bare glassy carbon electrode, showing that kinetically the oxidation processes on the electrode surfaces are different. Figure 4b shows the electrochemical behavior at ESPE before addition of acetaminophen in the electrolyte solution. It is possible to observe that the modified electrode does not show any electrochemical process in the electrolyte acetate buffer solution between $-0.4$ to $1.0$ V.

The variation of peak current with scan rate, from 20 to $500$ mV s$^{-1}$, was investigated using $7.05 \times 10^{-4}$ mol L$^{-1}$ acetaminophen in $0.1$ mol L$^{-1}$ acetate buffer solution pH 4.75 (Figure S1 in the Supplementary Information section). The results showed that anodic peak currents change linearly with the square root of the scan rate ($v^{1/2}$) for acetaminophen which indicates a diffusion-controlled process for electrooxidation of acetaminophen on the surface of the EPSE according to the following equation:$^{52}$

$$I_{ap} (\mu A) = 1.37258 + 2.7899v^{1/2} (mV s^{-1})^{1/2}, (R^2 = 0.999)$$

**pH dependence study**

The electrochemical behavior of acetaminophen was studied in sodium acetate as a function of pH as shown in Figure 5. It was observed (Figure 5A) that the oxidation potential ($E_{ap}$) of acetaminophen decreased as the pH increased. This behavior indicates that the acetaminophen is hydrolyzed in alkaline medium which brought more reducing compounds such as $p$-hydroxyaniline.$^{52}$ The dependence of the anodic peak potential ($E_{ap}$) with pH can be described by the following equation:

$$E_{ap} (mV) = -56.18 \text{ pH} + 883.55, (R = 0.999)$$

The slope of $-56.18$ mV pH$^{-1}$ was obtained in these experiments, which is very close to the theoretical Nernstian value of $-59$ mV for electrochemical processes involving the same number of protons and electrons. At pH higher than 9.0 the oxidation become kinetically less favorable. This may be explained by the partial formation of the phenoxide, which is negatively charged and for that reason is preferentially attracted to the positively polarized electrode surface.$^{28,53}$ Figure 5B shows the effect of pH on the peak current of acetaminophen, which the value of $I_{ap}$ increases with the increase pH, reaching a maximum at pH 4.75, and then decreases at alkaline pH. Therefore, pH 4.75 was selected for further studies.

**Figure 4.** (a) Cyclic voltammograms of 0.25 mmol L$^{-1}$ acetaminophen in 0.1 mol L$^{-1}$ acetate buffer solution (pH 4.75) at the ESPE (solid line); GC electrode (dot line). (b) Cyclic voltammogram at the ESPE in 0.1 mol L$^{-1}$ acetate buffer solution (pH 4.75).

**Figure 5.** (A) Effect of pH on the anodic peak potential (B) Effect of pH on the peak current of acetaminophen ($2.439 \times 10^4$ mol L$^{-1}$) using ESPE.
Determination of acetaminophen

Figure 6 shows cyclic voltammograms obtained from the increasing additions of acetaminophen in 0.1 mol L\(^{-1}\) acetate buffer solution (pH 4.75) at scan a rate of 0.10 V s\(^{-1}\) using the ESPE. A linear relationship was found between the anodic peak current and the acetaminophen concentration. Peak currents as a function of concentration in the range of 50 to 700 µmol L\(^{-1}\) is shown in inset of Figure 6 for which was obtained a regression equation of \(I_{ap} (\mu A) = 0.47 + 0.0416 C (\mu mol L^{-1}) (R^2 = 0.999)\). The estimated detection limit was 5.32 µmol L\(^{-1}\) (three times the blank standard deviation/slope). The relative standard deviation (RSDs) of 0.17% and 0.86 % for 10 measurements of 50 µmol L\(^{-1}\) and 700 µmol L\(^{-1}\) acetaminophen, respectively, suggested that the ESPE has a high level of reproducibility.

![Figure 6. Cyclic voltammograms of acetaminophen as a function of concentration in the range of 50-700 µmol L\(^{-1}\) in 0.1 mol L\(^{-1}\) acetate buffer solution (pH 4.75) at the ESPE. Inset: Anodic peak currents vs. acetaminophen concentration.](image)

The linear dynamic range (LDR), sensitivity, detection limit (LD) and relative standard deviation (RSD) of the proposed method were compared with other systems for determination of acetaminophen (Table 2). The results of ESPE show that the proposed method can be efficiently used for the identification and determination of acetaminophen by cyclic voltammetric. The RSD value for the present method suggests that the ESPE has a high level of reproducibility.

Application: acetaminophen detection in real samples

The modified electrode was used for the detection of acetaminophen in commercial drugs by cyclic voltammetry. A standard calibration curve was obtained with a commercial drug containing acetaminophen, and with the information contained on the bottle, a solution was prepared supposedly with 42.3 mmol L\(^{-1}\). From the regression equation, obtained by the standard addition method, the concentration of acetaminophen was found to be 40.45 ± 1.86 mmol L\(^{-1}\). The detection range obtained by \(t\)-test with a confidence interval of 95% varies from 35.83 to 45.08 mmol L\(^{-1}\). Therefore, the result found for the sample shows that the modified electrode is efficient for the quantitative determination of acetaminophen.

The voltammetric results obtained with the new sensor were compared with those from the HPLC method. Five different concentrations of standard acetaminophen solutions (132.3; 264.6; 396.9; 529.2 and 661.6 µmol L\(^{-1}\)) were analyzed by reversed-phase HPLC in order to plot the analytical curve, \(A_{acetaminophen peak (a.u.)} = 0.18 C_{acetaminophen (µmol L^{-1})} + 13.27, R^2 = 0.98\). The determination of acetaminophen in commercial drugs was performed by the injection, in triplicate, of an aqueous solution (1:100, v/v) previously prepared from commercial drug. The concentration of acetaminophen, determined by HPLC, was of (40.57 ± 1.31) mmol L\(^{-1}\). At a 95% confidence level, there was no significant difference in accuracy (evaluated by the Student \(t\)-value, confidence interval 37.32-43.82 mmol L\(^{-1}\)) and also no significant difference in precision (evaluated by

### Table 2. Comparison of voltammetry methods for determination of acetaminophen

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Electrode</th>
<th>LDR / (µmol L(^{-1}))</th>
<th>Sensitivity / (µA µmol(^{-1}) L)</th>
<th>LD / (µmol L(^{-1}))</th>
<th>RSD / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>GC/tetraruthenated porphyrin film</td>
<td>1-100</td>
<td>NR</td>
<td>0.11</td>
<td>NR</td>
</tr>
<tr>
<td>55</td>
<td>GC/Cu(II)-conducting polymer complex</td>
<td>20-5000</td>
<td>0.016</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>56</td>
<td>Nafion coated GC tubular</td>
<td>50-500</td>
<td>NR</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>36</td>
<td>Electropolymerized niquel porphyrin</td>
<td>5-200</td>
<td>NR</td>
<td>NR</td>
<td>2.0</td>
</tr>
<tr>
<td>28</td>
<td>Carbon film resistor electrode</td>
<td>0.8-500</td>
<td>0.024</td>
<td>0.4</td>
<td>3.1</td>
</tr>
<tr>
<td>57</td>
<td>Nanogold modified indium tin oxide (ITO) electrode</td>
<td>0.2-1500</td>
<td>0.01</td>
<td>0.18</td>
<td>2.4</td>
</tr>
<tr>
<td>58</td>
<td>PG/electropolymeterized-molecularly</td>
<td>5-500</td>
<td>NR</td>
<td>0.79</td>
<td>NR</td>
</tr>
<tr>
<td>This work</td>
<td></td>
<td>50-700</td>
<td>0.04</td>
<td>5.32</td>
<td>0.17</td>
</tr>
</tbody>
</table>

LDR: Linear dynamic range; LD: Detection limit; RSD: Relative standard deviation; NR: Not Reported.
the variance ratio F-value) between the cyclic voltammetry method and the HPLC method.

Conclusions

This work reports on the synthesis and characterization of a new supramolecule containing manganese (III) and peripheral ruthenium (III) complexes {Mn-TPyP(H2O)2[RuCl3(dppb)]}PF6. This polymetalated porphyrin was electropolymerized in a glassy carbon electrode by the cyclic voltammetry and subsequently used as a voltammetric sensor for detection and quantification of acetaminophen. The sensor presented high sensitivity and stability. The film was characterized by Atomic Force Microscopy, which revealed a thin film in the indium tin oxide surface (1.16 nm in 100 µm2 of area). When the modified electrode was used for the detection of acetaminophen in real samples, satisfactory results were obtained compared to other methods such as HPLC.

Supplementary Information

Supplementary information (Figure S1) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgements

The authors gratefully acknowledge the LERMAC (Laboratório de Energias Renováveis, Materiais e Catálise) and LCI (Laboratório de compostos Inorgânicos FACIP/UFU), the FAPEMIG, FINEP (ctinfra 03/2007) and Rede Mineira de Química for financial support and Professor Joesadaque José de Sene for English review.

References

49. Fagadar-Cosma, E.; Mirica, M. C.; Balci, I.; Bucovicean, C.; Cretu, C.; Armeanu, I.; Fagadar-Cosma, G.; *Molecules* 2009, **14**, 1370.

Submitted: June 7, 2013
Published online: September 11, 2013

FAPESP has sponsored the publication of this article.
Electropolymerized Supramolecular Tetraruthenated Porphyrins Applied as a Voltammetric Sensor

Monize M. da Silva, a Gabriel H. Ribeiro, a Alzir A. Batista, b Anizio M. de Faria, a André L. Bogado a and Luis R. Dinelli *a

a Faculdade de Ciências Integradas do Pontal, Universidade Federal de Uberlândia, Rua Vinte, 1600, 38304-402 Ituiutaba-MG, Brazil

b Departamento de Química, Universidade Federal de São Carlos, Rodovia Washington Luís (SP 310), km 235, 13565-905 São Carlos-SP, Brazil

Figure S1. CVs of 7.05 × 10⁻⁴ mol L⁻¹ acetaminophen in 0.1 mol L⁻¹ acetate buffer solution (pH 4.75) at the ESPE with different scan rates. The inset shows the relationship between anodic current and scan rate.