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Electrochemical sensor based on rhodium nanoparticles
stabilized in zwitterionic surfactant for *p*-coumaric acid analysis

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Abstract

A new sensor was developed for the analysis of phenolic compounds by coating the surface of a glassy carbon electrode (GCE) with rhodium nanoparticles stabilized in 3-(1-tetradecyl-3-imidazolium) propanesulfonate (ImS3-14), a zwitterionic surfactant. The modifier was found to lower the electron transfer resistance by enhancing the electrical conductivity on the electrode surface. Some phenolic compounds were tested to determine the increase in the electroanalytical response when employing the proposed sensor in comparison to the bare GCE. *p*-Coumaric acid showed the greatest peak enlargement and, thus, it was selected as the target analyte. Chronocoulometry studies indicated that the increase in the peak is due to the adsorption of the *p*-coumaric acid on the sensor surface. The optimized analysis conditions were obtained in acetate buffer pH 4.0 employing differential pulse voltammetry, which provided a limit of detection of 472 nmol L⁻¹. The recoveries ranged from 98.3 to 104.4%, indicating satisfactory accuracy for the proposed method. The sensor exhibited good repeatability and reproducibility (both intraday and interday) and was applied successfully in the determination of *p*-coumaric acid in a cellulosic matrix.

Keywords: electrochemical sensor, *p*-coumaric acid, rhodium nanoparticles, voltammetry

Introduction

Phenolic compounds have attracted interest from the scientific community due to their properties and applications. Phenolic acids constitute a class of phenolic compounds with carboxylic groups as substituents^{1,2}, an example being *p*-coumaric acid (Fig. 1A), a hydroxycinnamic acid present in fruits, vegetables and beverages such as wine³. The properties of this acid include antioxidant,^{4,5} antimicrobial,⁶ anticancer⁷ and anti-inflammatory activity.⁸ Research has also led to its use as a platelet inhibitor,⁹ antidiabetic¹⁰ and lipid oxidation inhibitor.¹¹ Therefore, *p*-coumaric acid presents great potential for use in pharmaceutical formulations.

Fig. 1 Chemical structure of *p*-coumaric acid (A) and ImS4-14 (B)

Several methods for the analysis of *p*-coumaric acid have been reported, including chromatographic and electrophoretic methods coupled with mass spectrometry¹² and photodiode array detectors^{13,14} as well as spectrophotometric methods.¹⁵ In addition, electrochemical methods have been used for the determination and quantification¹⁶ of this acid along with antioxidant assays.⁵

In order to improve the analytical parameters in electrochemical methods, electrode modification has been intensely researched. A wide variety of materials have been used as modifiers, including metallic nanoparticles such as Pd, Ag and Au,¹⁷⁻¹⁹ resulting in lower limits of detection (LOD) and quantification (LOQ), besides providing an enhancement in the analytical signals.

Metallic nanoparticles need stabilizers to prevent agglomeration, which turns them into bulk metal. Stabilizers also have other functions, for instance, contributing to the nanoparticle size during synthesis and modifying the physicochemical properties of the solution (e.g., the solubility). In this regard, polymers, ionic liquids and quaternary ammonium salts, among other substances, have been used.²⁰ Additionally, surfactants have been reported to be efficient stabilizers for metallic nanoparticles which are organized in normal or reverse micelles. Reverse micelles work as nanoreactors in the synthesis of nanoparticles in organic solvents and are much smaller than the micelles formed in aqueous medium. Approximately 10 surfactant molecules may form a reverse micelle, whereas a minimum of 30–50 surfactant molecules are required for a normal micelle.²¹ In addition, the use of organic solvents in the synthesis process ensures rapid evaporation when applying the resulting solutions in the construction of electrochemical sensors, which makes the process much faster.

ImS3-14, 3-(1-tetradecyl-3-imidazolium) propanesulfonate (Fig. 1B), a zwitterionic surfactant, has been employed in the synthesis of metallic nanoparticles such as Pd and Au, which have also been successfully used as modifiers in sensors for the electrochemical analysis of hydroquinone and catechol.^{19, 22} The aim of this study was to investigate the application of reverse micelles formed by rhodium nanoparticles stabilized in surfactant (Rh-(ImS3-14)) for the construction of a new electrochemical sensor for the determination of phenolic compounds.

Experimental

Reagents and Solutions

All reagents, that is, *p*-coumaric acid, paracetamol, vanillic acid, rosmarinic acid, ferulic acid, caffeic acid, hydroquinone and potassium ferrocyanide, were analytical grade, (Sigma-Aldrich) and used without further purification. All solutions were prepared in ultrapure water produced by a Milli-Q System (Millipore, USA) with a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$. The *p*-coumaric acid capsules were purchased in a local compounding pharmacy in the concentration of 5 mg per capsule in a cellulose matrix. The Rh-(ImS3-14) solution was synthesized according to a procedure previously described in the literature.²³

Instrumentation

The voltammetric experiments were carried out using an Autolab PGSTAT204 potentiostat (Eco Chemie, The Netherlands). The measurements were performed in an electrochemical cell containing 10 mL of supporting electrolyte using a three-electrode system: platinum wire as the auxiliary electrode, Ag/AgCl (3.0 mol L^{-1}) as the reference electrode, and the proposed sensor as the working electrode (Rh-(ImS3-14)/GCE). All measurements were carried out at room temperature (25°C) and no deoxygenation procedures were taken.

Electrochemical impedance spectrometry (EIS) experiments were performed using an Autolab PGSTAT128N potentiostat (Eco Chemie, The Netherlands). The software Nova 1.10 was used to collect and evaluate the electrochemical data acquired with the potentiostats. Chronocoulometry experiments were carried out in acetate buffer (0.1 mol L^{-1} , pH 4.0) at 0.8 V using an Autolab PGSTAT128N potentiostat (Eco Chemie, The Netherlands) and the software

General Purpose Electrochemical System (GPES) 4.9 was used to collect the chronocoulometric data. Spectrophotometric measurements were performed with a Cary 60 UV-Vis spectrometer (Agilent Technologies, USA).

TEM analysis was performed using a JEOL JEM-1011 transmission electron microscope operating at 100 kV at LCME/UFSC, Florianópolis, Brazil. The average particle size was determined using ImageJ software.

Preparation and characterization of the rhodium nanoparticles in zwitterionic surfactant ImS3-14

The Rh-(ImS3-14) nanoparticles were obtained through the reduction of rhodium ions entrapped in reverse micelles. The nanoparticles were prepared by rapidly mixing equal volumes of solution A (containing 0.05 mol L⁻¹ ImS3-14, 0.20 mol L⁻¹ water and 7.2 x 10⁻⁴ mol L⁻¹ RhCl₃) with solution B (containing 0.05 mol L⁻¹ ImS3-14, 0.20 mol L⁻¹ water and 7.2 x 10⁻³ mol L⁻¹ NaBH₄). The color change of the resulting solution indicated the formation of metallic nanoparticles. The surfactant-stabilized Rh NPs were characterized by Transmission Electronic Microscopy (TEM). The sample for TEM analysis was prepared by deposition of the chloroform

Fig 2. TEM image (A) and size distribution (B) of rhodium nanoparticles formed in reverse micelles.

Scheme 1. Representation of the proposed sensor and electron transfer reaction for *p*-coumaric acid at the CGE surface covered with Rh-(ImS3-14).

125 Rh-(ImS3-14) dispersion on a carbon-coated copper grid.

126 Rh-(ImS3-14) characterization was performed by TEM. Figure 2A shows the TEM image
 127 of the Rh-(ImS3-14) nanoparticles and it can be observed that the use of ImS3-14 reverse
 128 micelles allowed the formation of nanoparticles, which are well dispersed in the grid and show no
 129 signs of aggregation. The TEM image shown in Fig. 2A indicates that the nanoparticles are
 130 polydispersed in terms of size and roughly spherical in shape, with the diameters of the Rh
 131 nanoparticles mostly ranging from 13 to 18 nm, based on the measurement of around 300
 132 particles (Fig. 2B).

133

134 *Rh-(ImS3-14) Sensor Construction*

135 A glassy carbon electrode (GCE) was polished using an aqueous alumina (0.05 μm)
 136 suspension for 2 min. It was immersed in deionized water and sonicated for 5 min. The electrode
 137 was then dried and 2 μL of the Rh-(ImS3-14) solution were deposited on the clean surface
 138 employing the drop coating method. The solvent, i.e., chloroform (CHCl_3), was left to evaporate
 139 at room temperature and the modified GCE was used as the working electrode. In the operation

of the resulting sensor (Scheme 1), a potential is applied to the electrode to oxidize the analyte (*p*-coumaric acid) on the electrode surface, which is covered with Rh nanoparticles stabilized in the surfactant (ImS3-14).

Sample preparation for p-coumaric acid determination

The content of three capsules of *p*-coumaric acid was dissolved in 50 mL of ultrapure water, under agitation. In order to determine the optimum extraction time, aliquots (10 μL) of the solution were analyzed by spectrophotometry in 3 mL of acetate buffer (0.1 mol L^{-1} , pH 4.0) at 300 nm. The measurements were taken at time intervals of 5 min and the procedure was carried out for a total of 20 min. The maximum absorbance occurred within 10 min and no further changes were observed. Therefore, the agitation time selected was 10 min.

The application of ultrasound was found to be ineffective and thus it was not used as part of the extraction procedure. The aqueous phase, without centrifugation, was used for the *p*-coumaric acid determination applying the method of standard addition.

Results and Discussion

Analyte studies

An initial comparative study was carried out in order to identify which phenolic compounds the proposed sensor can detect and which yields the greatest response. The electrode response in terms of peak current was evaluated for *p*-coumaric acid, hydroquinone, paracetamol and vanillic acid in the same concentration (4.98 $\mu\text{mol L}^{-1}$) and under the same analysis conditions (0.1 mol L^{-1} acetate buffer pH 5.0) using differential pulse voltammetry (DPV) - increment of 7 mV, pulse amplitude of 100 mV and time of 0.5 s.

The results show (Fig. 3A) that the peaks corresponding to hydroquinone and vanillic acid are broader. More defined peaks can be observed for paracetamol and *p*-coumaric acid. It is also clear that, despite a small difference, the greatest absolute response is observed for *p*-coumaric acid, which showed the highest peak current at the most positive potential (0.69 V).

On comparing the bare GCE and the Rh-(ImS3-14)/GCE (Fig. 3 B), it can be noted that for all of the compounds tested the proposed sensor provided a higher electrochemical response. Nevertheless, it can be observed that *p*-coumaric acid shows the greatest improvement in the response when the proposed sensor is employed, since its peak more than doubles under these conditions. Therefore, *p*-coumaric acid was chosen as the analyte for subsequent studies.

Electrochemical studies

The influence of pH on the oxidation peak currents for *p*-coumaric acid was investigated (Fig. 4A). The pH was varied from 2.0 to 8.0 using acidified acetate buffer (pH 2.0- 3.0), acetate buffer (pH 4.0-5.0) and phosphate buffer (pH 6.0-8.0) in a concentration of 0.1 mol L⁻¹ employing DPV (increment of 3 mV, pulse amplitude of 120 mV and time of 0.6 s). The optimum response was observed with

Fig. 3. (A) DPV voltammograms for different phenolic compounds: hydroquinone (a), paracetamol (b), vanillic acid (c) and *p*-coumaric acid (d) and (B) electroanalytical response for the same phenolic compounds as (A) obtained with the bare GCE and the proposed sensor.

Fig. 4 Peak currents obtained for 9.9 μmol of *p*-coumaric acid in 0.1 mol L⁻¹ acetate buffer pH 4.0 using DPV (A) and potential shift in relation to pH (B).

acetate buffer pH 4.0, at 0.76 V and, thus, this was chosen as the supporting electrolyte for the following essays.

The dependence of the oxidation peak potential on the solution pH was investigated (Fig. 4B) and a linear relation was observed ($r = 0.993$), with a slope of -50.9 mV/pH, which is close to the theoretical value of 59 mV/n and indicates an equal number of protons and electrons exchanged during the electron transfer reaction.

The number of electrons transferred in irreversible electrochemical reactions can be determined by the peak width at half height and was found to be 101 mV for the investigated system, a number close to the theoretical value of 90 mV for electron transfer reactions involving one electron¹⁶. Based on the results, it can be concluded that the oxidation of *p*-coumaric using the proposed sensor occurs with the transfer of one proton and one electron, according to the overall mechanism proposed in Fig. 5. The oxidation peak can be attributed to the oxidation of the hydroxyl group attached to the aromatic ring of molecule.²⁴ and under optimum conditions, the electron transfer reaction takes place in an acidic medium below the pK_a of *p*-coumaric acid (i.e., 4.7),²⁵ which means that the molecule is protonated during the process.

Fig. 5 Representation of the mechanism for the oxidation of *p*-coumaric acid.

195 Cyclic voltammetry (CV) was performed to investigate whether the irreversible behavior
 196 described in the literature also occurs when using the proposed sensor (Fig. 6).^{5,17,24,26} It can be
 197 observed that there is no change in the reversibility of *p*-coumaric acid when using the proposed
 198 sensor, despite the peak enhancement.

199 The effect of each component present in the Rh-(ImS3-14) solution was investigated with respect to
 200 the analytical response to *p*-coumaric acid (Fig. 7). The experiments were conducted in acetate buffer (0.1
 201 mol L⁻¹, pH 4.0) containing 59 μmol L⁻¹ of *p*-coumaric acid. According to the results, a small signal
 202 enhancement is observed in the presence of the surfactant (ImS3-14) despite the potential shift towards
 203 more positive values, an effect also observed in Fig. 6. The presence of the Rh nanoparticles, however,
 204 greatly enhances the peak current for the oxidation of *p*-coumaric acid.

Fig. 6. Comparison of peak currents obtained in the cyclic voltammetry at 25 mV s⁻¹ for 59 μmol L⁻¹ of *p*-coumaric acid using the bare GCE and Rh-(ImS3-14) - the proposed sensor.

Fig. 7. Differential pulse voltammograms for $9.9 \mu\text{mol L}^{-1}$ of *p*-coumaric acid in acetate buffer (0.1 mol L^{-1} ; pH 4.0) for different sensors.

CV was also performed with multiple cycles in order to examine the variation in the oxidation peak after several measurements (Fig. 8). A series of 10 consecutive experiments was carried out in acetate buffer (0.1 mol L^{-1} , pH 4.0) containing $49 \mu\text{mol L}^{-1}$ of *p*-coumaric acid using the proposed sensor as the working electrode. It can be seen that there is a decrease in the analytical signal after each measurement, probably due to the fouling of the film, since the surfactant (ImS3-14) is only slightly soluble in water but is soluble in electrolytic solutions.²⁷ The

Fig. 8 Consecutive cyclic voltammograms (A) and the peak currents (B) for $49 \mu\text{mol L}^{-1}$ *p*-coumaric acid in acetate buffer (pH 4.0) at 25 mV s^{-1} .

peak current drops after each measurement until the fifth measurement, after which the current stabilizes, an effect that can be explained by the adsorption of the analyte on the surface of the electrode.

An investigation was performed in order to determine the causes of the increase in the current response for *p*-coumaric acid when employing the proposed sensor. An electrochemical impedance spectroscopy assay was performed in order to compare the resistance of the surface of the Rh-(ImS3-14)/GCE to that of the bare GCE in the presence of $\text{Fe}(\text{CN})_6^{3-/4-}$ (Fig. 9). It can be observed that the resistance decreases when using the proposed sensor, which facilitates the electron transfer process. The values for the electron transfer resistance (R_{ct}) are 2.55 k Ω and 1.38 k Ω for the bare GCE and the Rh-(ImS3-14)/GCE, respectively. This effect can be attributed to the presence of the metallic nanoparticles, which enhances the electrode surface conductivity.

Another possible explanation for the increase in the response is a catalytic effect of the metallic nanoparticles, based on several reports in literature.^{18,19,28,29} However, as mentioned above, the oxidation potential for *p*-coumaric shifts towards more positive values when using the

Fig. 9. Electrochemical impedance spectra for bare GCE and Rh-(ImS3-14)/GCE in a solution containing 10 mmol L⁻¹ of probe $\text{Fe}(\text{CN})_6^{3-/4-}$ in KCL 0.1 mol L⁻¹, open circuit mode, 10 mV amplitude and frequency range of 0.1-100000 Hz.

proposed sensor in comparison to the bare GCE (Fig. 7), which shows that the surfactant presents a thermodynamic barrier to the oxidation of *p*-coumaric acid. In the presence of the metallic nanoparticles the oxidation potential remains the same as that obtained in the presence of the surfactant. It can thus be concluded that the surfactant is the component causing the potential shift and the addition of the metallic nanoparticles does not reverse this effect. Therefore, the main cause of the peak enhancement is unlikely to be a catalytic effect.

Electrode modifiers frequently cause an enlargement of the electrode surface area, which leads to an increase in the electrochemical response.^{30,31} The electrode area for the proposed sensor was determined by cyclic voltammetry at scan rates varying from 10 to 200 mV s⁻¹ using Fe(CN)₆^{3-/4-} in 0.1 M KCl as the probe and applying the Randles-Sevcik formula:

$$I_p = 2.69 \cdot 10^5 A D^{1/2} n^{3/2} C v^{1/2}$$

where I_p refers to the peak current, A is the surface area of the electrode, D is the diffusion coefficient ($D = 6.70 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$),³² C is the concentration (5 mmol L⁻¹) and n is the number of electrons ($n=1$). From the slope of the plot I_p vs $v^{1/2}$ (Fig. S1), the electroactive area was calculated for the proposed sensor ($A=0.0273 \text{ cm}^2$) and for the bare GCE ($A=0.0271 \text{ cm}^2$). Based on the results, it can be concluded that the proposed sensor does not significantly enhance the electrode area and, therefore, it is not the main factor causing the peak enhancement.

A further explanation for the increased response when using a modified electrode is the adsorption of the analyte on the electrode surface, which can be evaluated by Chronocoulometry.³³ The integrated Cottrell equation describes the relationship between Q and $t^{1/2}$ as follows:

$$Q = 2nFACD^{1/2}t^{1/2}\pi^{-1/2} + Q_{dl} + Q_{ads}$$

where A is the area of the electrode, C is the concentration of *p*-coumaric acid, Q_{dl} is the double layer charge, Q_{ads} is the adsorption charge and the other symbols have their usual meanings. Q_{dl}

can be determined by performing the experiment using the supporting electrolyte alone. Both Q_{dl} and Q_{ads} are time-independent variables which can be calculated from the intercept of the Anson plot.³⁴ Thus, chronocoulometric experiments were performed and the corresponding Anson plots were constructed (Fig. S2). The experiments were carried out in acetate buffer (0.1 mol L⁻¹, pH 4.0) using the bare GCE ($Q = 2.56 \times 10^{-6} + 6.77 \times 10^{-6} t^{1/2}$) and the proposed sensor ($Q = 1.83 \times 10^{-6} + 0.84 \times 10^{-6} t^{1/2}$) and also with the same supporting electrolyte containing 1.0×10^{-5} mol L⁻¹ of *p*-coumaric acid using the bare GCE ($Q = 3.33 \times 10^{-6} + 9.13 \times 10^{-6} t^{1/2}$) and the proposed sensor ($Q = 8.55 \times 10^{-6} + 1.99 \times 10^{-6} t^{1/2}$). On subtracting Q_{dl} , the Q_{ads} value for *p*-coumaric acid was calculated as 0.77 μ C for the bare GCE and 6.72 μ C for the Rh-(ImS3-14)/GCE. The surface coverage was then determined from the Faraday Law:

$$Q_{ads} = nFA\Gamma$$

where $n = 1$, A = electrode area, $F = 96\,485$ C mol⁻¹ and Γ is the surface coverage. From the calculations, it was determined that the values for the surface coverage were 2.94×10^{-10} and 2.55×10^{-9} mol cm⁻² for the bare GCE and the proposed sensor, respectively. Therefore, the amount of analyte absorbed is almost 9 times higher for the proposed sensor in comparison to the bare GCE. Based on these results, it is clear that the peak enhancement observed for the oxidation peak of *p*-coumaric acid is due to adsorption on the electrode surface caused by a pre-concentration of the analyte on the Rh-(ImS3-14) nanoparticles.

Optimization of sensor construction and experimental parameters

The number of layers of the Rh-(ImS3-14) solution deposited on the electrode surface in relation to the electrochemical response to *p*-coumaric acid was evaluated. It was observed that with an increase in the number of layers the analytical response to *p*-coumaric acid decreased, which can be attributed to a blocking effect caused by the surfactant. Therefore, one layer of

Table 1. Figures of merit for different electroanalytical techniques using the proposed sensor

Technique	Analytical Equation	R ²	LOD (mol L ⁻¹)	LOQ (mol L ⁻¹)
LSV	$i = 9.73 \times 10^6 [p\text{-coumaric acid}] - 15.9 \pm 1.97$	0.988	0.60 $\mu\text{mol L}^{-1}$	2.02 $\mu\text{mol L}^{-1}$
SWV	$\Delta i = 1.17 \times 10^7 [p\text{-coumaric acid}] - 23.18 \pm 2.39$	0.992	0.60 $\mu\text{mol L}^{-1}$	2.05 $\mu\text{mol L}^{-1}$
DPV	$i = 1.63 \times 10^7 [p\text{-coumaric acid}] - 3.27 \pm 2.60$	0.997	0.47 $\mu\text{mol L}^{-1}$	1.57 $\mu\text{mol L}^{-1}$

surfactant, i.e., one aliquot (2 μL) of the Rh-(ImS3-14), was considered as the optimum amount to be applied on the GCE surface.

Three electroanalytical techniques were evaluated in order to select that which would provide the best analytical parameters: Linear Sweep Voltammetry (LSV), Square-Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV), all optimized under the same conditions (9.9 $\mu\text{mol L}^{-1}$ of *p*-coumaric acid, in acetate buffer pH 4.0, in triplicate).

For the LSV evaluation, the parameter investigated was the scan rate (10, 25, 50 and 75 mV s^{-1}). The scan rate of 25 mV s^{-1} was selected since this provided the greatest response and the best peak profile. For the SWV optimization, three parameters were studied: scan increment (1-7 mV), pulse amplitude (10-90 mV) and frequency (10-40 Hz). Noise was observed at higher values, thus limiting the study range. A scan increment of 5 mV, pulse amplitude of 60 mV and frequency of 10 Hz were selected as the optimized parameters, since these values provided the best results in terms of peak current and voltammetric profile. Lastly, the DPV technique and its parameters were assessed: scan increment (1-5 mV), pulse amplitude (10-170 mV) and time (0.1-0.7 s). The best results were obtained at a scan increment of 3 mV, pulse amplitude of 120 mV and time of 0.6 s.

Calibration curves were constructed for all three techniques using the optimized parameters and the figures of merit are shown in Table 1. The limits of detection and quantification were calculated according to the following equations³⁵:

$$\text{LOD} = 3s / m \quad \text{LOQ} = 10s / m$$

where s is the standard deviation of the y -intercept and m is the slope of the calibration curve. In comparison to LSV, SWV presents higher sensitivity, but the differences between the LOD and LOQ values for the two techniques were small.

These results were expected since a fast sweeping technique such as SWV offers minimal benefits when applied to a system with slow charge transfer kinetics.³⁶ Moreover, SWV does not provide significant enhancement in the case of irreversible systems, such as the oxidation of the *p*-coumaric acid, since sweeping in the direction of reduction is ineffective. In summary, DPV provides the greatest sensitivity and the lowest LOD and LOQ values, and thus it was selected as the electroanalytical technique.

Precision studies

Repeatability was evaluated by constructing sensors using the same GCE on an interday/intraday basis in acetate buffer (0.1 mol L⁻¹, pH 4.0) containing 9.9 μmol L⁻¹ of *p*-coumaric acid using DPV (increment 3 mV, pulse amplitude 120 mV and time 0.6 s). Interday repeatability was assessed by evaluating the response of 8 different sensors in assays carried out on the same day. For each measurement a new sensor was constructed and the RSD was 6.2%. Intraday studies were carried out under the same conditions as the interday studies, performing 3 experiments on 3 different days, making a total of 9 measurements. The RSD was 4.4%.

Reproducibility was assessed by constructing sensors using three different GCEs, also on an interday/intraday basis, under the same conditions as those described above. Interday reproducibility was evaluated considering the responses of 6 different sensors in assays carried out on the same day and the RSD was 6.5%. Intraday studies were carried out under the same conditions as the interday studies, performing 6 experiments on 3 different days, giving a total of

18 measurements and the RSD was 9.5%. Therefore, the results demonstrate that the proposed sensor has good repeatability and reproducibility.

Interference studies

A study was carried out to investigate the interference of other compounds in the analysis of *p*-coumaric acid using the proposed sensor, by comparing the response to *p*-coumaric acid when adding other substances in fixed amounts. Caffeic acid, rosmarinic acid and ferulic acid were tested in proportions of 1:1, 1:5 and 1:10. These substances were selected as possible interferents given their conjoint presence in plants such as oregano (*Origanum vulgare* L.).³⁷ Caffeic acid and ferulic acid are of special interest in evaluating possible interferences given their structural similarity to *p*-coumaric acid.

The results show that the variations in the *p*-coumaric acid peaks in the presence of caffeic acid were below 10%. Rosmarinic acid interfered significantly in the proportion of 1:10 (with an increase of up to 112%), but this was not observed for lower proportions. However, ferulic acid interfered significantly (>40%) at all proportions tested, reaching 86.7% in the case of a proportion of 1:10, which can be attributed to its structural similarity to *p*-coumaric acid. Based on these results, the proposed method is not likely to offer selectivity when applied to complex samples, such as vegetable extracts, unless separation techniques are employed.

Cellulose, a known non-interferent in the determination of phenolic compounds, was then tested and the results did not indicate significant interference in the *p*-coumaric acid peaks.

Analytical application of the proposed sensor

The proposed sensor was applied in an analysis of *p*-coumaric acid in a cellulose matrix prepared by a local compounding pharmacy and, in order to verify the accuracy of the proposed

method, spectrophotometric analysis was also carried out. The results for the content of *p*-coumaric acid were 5.49 ± 0.03 and 5.14 ± 0.01 for the proposed sensor and for the comparative method (spectrophotometry), respectively. Hence, there is a good correlation between the values obtained using the proposed sensor and the comparative method (<10% difference).

Sample recovery studies were performed by adding standard solutions of *p*-coumaric acid in three different concentrations. The percentage recovery ranged from 98.3 to 104.4%, which shows satisfactory accuracy for the proposed method.

Conclusions

In this study, a new sensor was constructed by applying Rh-(ImS3-14) to the surface of a GCE as a modifier. This approach decreased the electron transfer resistance and enhanced the analytical signals for different phenolic compounds, but mainly for *p*-coumaric acid, which was chosen as the target analyte. The voltammetric peak enlargement is due to the adsorption of *p*-coumaric acid on the sensor surface.

In comparison with other electrochemical sensors used for the analytical determination of *p*-coumaric acid, the sensor presented satisfactory results. A modified GCE with multi-walled carbon nanotubes (MWCN/GCE) gave an LOD of 630 nM³⁸ while the value obtained in this study was 472 nM. Nevertheless, determinations using the bare GCE¹⁶ and a bare gold electrode³⁹ yielded LOD values of 83 and 2 nM, respectively. It should be noted that for the sensor proposed herein and in the study performed with a MWCN/GCE the LODs were calculated by statistical methods while the LODs in other studies performed with a bare GCE and gold electrodes were determined considering the signal-to-noise ratio. The available literature regarding the development of electrochemical sensors for *p*-coumaric acid determination is still

limited and further studies on this subject would certainly be useful in order to establish a more precise parallel. Apart from the study performed with a gold electrode, in which amperometry was employed, all other authors used voltammetry as the electroanalytical technique to perform the analytical determination of *p*-coumaric acid. In all cases, the voltammetric technique employed was DPV, which is more suitable for irreversible systems, as mentioned above.³⁶

In conclusion, this study describes a fast and suitable method for *p*-coumaric acid analysis, showing satisfactory analytical performance. The Rh-(ImS3-14) sensor showed good precision and accuracy and its successful application represents a relevant contribution to the use of this class of versatile materials in electroanalytical chemistry.

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