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Development of a voltammetric sensor for diospyrin determination in nanomolar concentrations

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Abstract

A sensor based on glassy carbon (GC) electrode modified with cobalt tetrasulfonated phthalocyanine (CoTSPc) and a poly-L-lysine (PLL) film is proposed for diospyrin determination in nanomolar concentrations with differential pulse voltammetry (DPV) technique. The modified electrode showed excellent catalytic activity presenting much higher peak currents than those measured on a bare GC electrode. Linear response range, sensitivity and limit of detection (LOD) were of 1–120 nmol l^{-1}, 220.46 nA l $nmol^{-1}$ cm^{-2} and 0.3 nmol l^{-1}, respectively. The repeatability of the proposed sensor, evaluated in term of relative standard deviation (R.S.D.), was measured as 4.4% for 10 experiments in 50 μmol l^{-1} diospyrin samples. The developed sensor was applied for the determination of diospyrin in the crude extracts of the stem–bark of *Diospyros montana* Roxb. and the average recovery for these samples was 101.9 (±3.1)%.

Keywords: Diospyrin; Cobalt tetrasulfonated phthalocyanine; Poly-L-lysine

1. 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 by virtue of their capacity to undergo facile redox reactions [1].

Diospyrin [2,6′-bis(5-hydroxy-7-methyl-1,4-naphthoquinone)], a bis-hydroxynaphthoquinonoid plant product, was identified to be the tumour-inhibitory constituent of the stem–bark of *Diospyros montana* Roxb. [2], while its semi-synthetic derivatives were found to be more effective against Ehrlich Ascites Carcinoma (EAC), a murine tumour model. Some of these compounds possess significant antiparasitic and antimycobacterial activities [3–7]. Several derivatives of diospyrin were found to be cytotoxic against human cancer cell lines, inducing apoptotic cell death [8]. Furthermore, diospyrin was found to inhibit the topoisomerase I of the DNA from *Leishmania donovani* parasites in preference to the enzyme from a mammalian source [9]. Investigations were carried out on the capacity of diospyrin derivatives to antagonize the camptothecin-dependent topoisomerase I-mediated DNA cleavage, and to inhibit the kinase activity of the enzyme [10]. It was found that some of these compounds were able to stall the dynamic assembly of the spliceosome, indicating the possibility of using them to correct aberrant splicing in human genetic diseases.

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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 [11,12].

In the case of diospyrin and its analogues, the separation has been standardized by using a HPLC method with UV detection [13,14]. However, the chromatographic methods are expensive, deal with the difficulty of sample preparation and the necessity of molecules derivatization, which limit their utility. In contrast, electrochemical methods present the advantages of simplicity and low cost.

The use of bare electrodes for detection of organic compounds also 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 electronic transfer rate, also promoting an increase in the sensitivity of the system [15]. In this sense, a wide variety of compounds have been used as electron transfer mediators for electrooxidation or reduction of several target molecules [16–18].

Metallophthalocyanine complexes are a fascinating group of macrocyclic compounds due to their ability to exhibit excellent physico-chemical properties that are essential for various important applications, such as electrochemical sensors [19]. These complexes have acquired most interest due to their singular properties, including high thermal stability and catalytic efficiency for a greater number of molecules [20–23]. Based on these properties, this work reports the development of an efficient and stable sensor for diospyrin determination in nanomolar concentrations using a glassy carbon (GC) electrode modified with cobalt tetrasulfonated phthalocyanine (CoTSPc) and poly-L-lysine (PLL) film, where presumably a strong interaction is established by ion-pair formation between the amino group ($-NH_3^+$) of the PLL and the sulfonic group (SO_3^-) from the CoTSPc molecule.

2. Experimental

2.1. Chemical and solutions

All used chemicals were analytical grade. Diospyrin (Fig. 1) was isolated from the stem–bark of *D. montana* Roxb. collected from Bolangir district in Orissa, and purified, as described earlier [2]. Briefly, the plant material was extracted in a Soxhlet apparatus with petroleum ether (b.p. = 60–80 °C), followed by chloroform. The residue obtained by removing the solvent from the chloroform extract, under reduced pressure, was processed through repeated leaching with acetone and ethanol to yield a crude product which was crystallized from chloroform to yield pure diospyrin (m.p. 258–260 °C). It was then dissolved in dimethyl sulfoxide (DMSO) to prepare a solution of diospyrin (200 μg ml^{-1}). Cobalt(II) tetrasulfonated phthalocyanine, was prepared and purified according to the procedure of Weber and Busch [24]. Poly-L-lysine hydrobromide was purchased from Sigma, St. Louis, USA. Sodium acetate anhydrous (NaOAc) was purchased from Nuclear, São Paulo, Brazil. Acetic acid (HOAc) was purchased from Vetec, Rio de Janeiro, Brazil. Dimethyl sulfoxide was purchased from Synth, São Paulo, Brazil. The solutions 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 model 350.

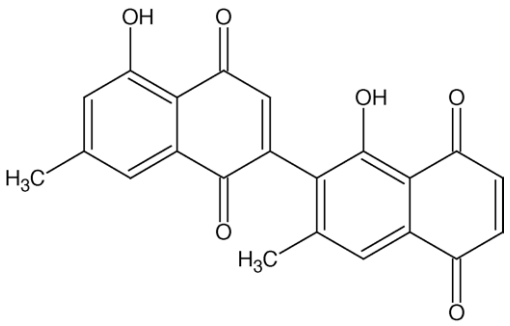

Fig. 1. Chemical structure of diospyrin.

2.2. Voltammetric measurements

The voltammetric measurements were obtained with a 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 acetate buffer solution + DMSO (1:1) with a Ag|AgCl|Cl$^-$ (saturated solution) electrode as reference, a Pt wire as auxiliary and the modified GC as working electrode were used for all measurements. Oxygen was removed by bubbling nitrogen through the solution.

2.3. Electrode preparation

The glassy carbon electrode, with geometrical area of 0.071 cm^2, was acquired from Metrohm-Switzerland and used for sensor construction. Prior to the modification, the electrode surface was treated according to the procedure described by Zhu and Nan-Qiang [25]. After cleaning the electrode, 10 μl of a solution, prepared by mixing CoTSPc and PLL solutions (1:1, v/v) with concentrations: 0.4, 0.6, 0.8, 1.0 or 1.5 mmol l^{-1} for CoTSPc and 0.25, 0.4, 0.5, 0.6 or 0.75 mmol l^{-1} for PLL, was put onto electrode surface and let to dry at 80 °C temperature for 8 min.

2.4. Sample preparation

For diospyrin quantification in crude plant chloroformic extract, 0.63 mg of each sample was weighed and diluted with 5 ml of acetate buffer + DMSO (1:1). An aliquot of 50 μl was added into the measurement cell. After this step, several additions of standard solution also prepared in acetate

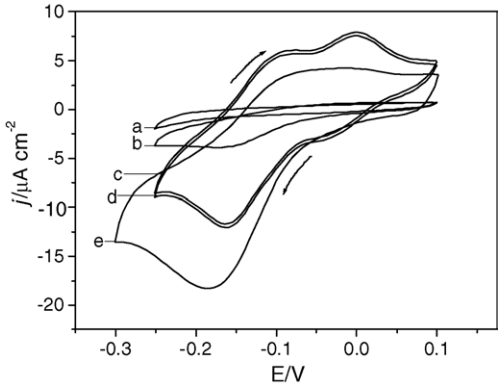

Fig. 2. Cyclic voltammograms performed in acetate buffer + DMSO (1:1) pH 5.4 for the GC bare electrode in absence (a) and presence of diospyrin (d), GC bare electrode + PLL, in the presence of diospyrin (c) and CoTSPc/PLL modified electrode in absence (b) and presence of diospyrin (e). Scan rate = 0.02 V s^{-1} and potential amplitude = 0.01 V.

buffer/DMSO (1:1), were performed and the current density values were interpolated in an analytical curve, previously obtained.

2.5. Analytical curve development

After optimising experimental parameters for the proposed sensor, the analytical curve was constructed by addition of aliquots of diospyrin (stock solution of 2×10^{-7} mol l^{-1}) into measurement cell containing acetate buffer + DMSO solution at pH 5.4. The technique employed was differential pulse voltammetry (DPV).

3. Results and discussion

3.1. Immobilization of CoTSPc on the electrode surface

CoTSPc was adsorbed on the electrode surface using PLL polymer. After this step, successive cyclic voltammograms were performed in a potential range between 0.100 and −0.300 V. The variation of the peak current was evaluated as the relative standard deviation (R.S.D.). After 20 cycles, a R.S.D. < 5% was observed, suggesting a good stability of the modified electrode. Based on these aspects, presumably the interaction between the polyelectrolyte and the four groups $-SO_3^-$ should be by ion pairing between the amino group ($-NH_3^+$) of the poly-L-lysine and the anionic moiety of the phthalocyanine.

3.2. Electrocatalytic reduction of diospyrin on the modified electrode

Fig. 2 shows the cyclic voltammograms recorded in acetate buffer + DMSO (1:1) pH 5.4 for the GC bare electrode (a) and the modified electrode with CoTSPc (b) in absence of diospyrin. For comparison, this figure also presents the behaviour of the modified electrode with PLL only (c), bare electrode (d) and modified electrode with CoTSPc (e), in presence of diospyrin. Fig. 2b shows a voltammetric wave at ∼−0.160 V, most probably associated to reduction of immobilized $[Co(II)TSPc]^{2-}$ into $[Co(I)TSPc]^{3-}$ species, since these reductions are known to occur at the central metal in Co(II) phthalocyanine complexes [26,27]. Fig. 2c and d shows the voltammetric behaviour of the diospyrin on modified electrode with only PLL and on the bare GC electrode, respectively. As can be seen, the voltammetric responses were similar and the modified electrode with PLL only does not promote a catalysed diospyrin reduction. In this range of potential the diospyrin presented two redox couple: one about −0.100 and −0.160 V and other at 0 and −0.05 V. These redox couples can be associated with the reduction and oxidation of one of the quinonoid groups present in diospiryn, leading to the corresponding hydroquinone. The former redox couple should be associated with the adsorbed diospyrin. On the other hand, the last redox couple can be attributed to the dissolved diospyrin. The complete investigation of the redox mechanism is out of the scope of the present paper and will be reported elsewhere. In Fig. 2e, a significant increase in the cathodic current peak (−0.160 V) was observed at the modified electrode with CoTSPc and PLL. The increase in this reduction potential caused the disappearance of the cathodic peak at −0.050 V and indicates that the film can suppress the adsorption of diospyrin. The increase in the peak current was about 60% of the peak current measured on the bare electrode (Fig. 2c) in presence of diospyrin. Therefore, the high activity of the modified GC electrode for the reduction of diospyrin in the mixed solution can be associated with the low charge transfer resistance of the CoTSPc/PLL film and presence of $[Co(II)TSPc]^{2-}$ as electroactive sites.

Table 1
Influence of the [CoTSPc] used in film preparation on the peak current density obtained with the sensor for 50 μmol l^{-1} diospyrin in acetate buffer + DMSO solution (pH 5.4) and 0.5 mmol l^{-1} PLL

[CoTSPc] (mmol l^{-1})	$-j$ (nA cm^{-2})
0.40	1742
0.60	1830
0.80	1124
1.00	899
1.50	729

Scan rate = 0.02 V s^{-1}; pulse amplitude = 0.09 V.

3.3. Influence of CoTSPc and PLL concentrations in the sensor response

The influence of the CoTSPc and PLL concentrations in the sensor response for diospyrin was investigated by modifying the electrode surface with films containing different compositions. Initially, the electrode surface was modified with films prepared from solutions containing different concentrations of CoTSPc solution (0.4, 0.6, 0,8, 1.0 and 1.5 mmol l^{-1}) and a fixed concentration of PLL at 0.5 mmol l^{-1} (Table 1).

Table 2
Influence of the PLL concentration used in film preparation on the peak current density obtained with the sensor for diospyrin in the same conditions as reported in Table 1

[PLL] (mmol l^{-1})	$-j$ (nA cm^{-2})
0.25	1200
0.40	1313
0.50	1832
0.60	1296
0.75	1245

[CoTSPc] = 0.6 mmol l^{-1}.

The results indicated that the best responses were obtained with 0.6 mmol l^{-1} CoTSPc solution and, therefore, this concentration was chosen for membrane preparations.

In the next step, with the CoTSPc concentration fixed at 0.6 mmol l^{-1}, the influence of the PLL solution concentration on the sensor (0.25, 0.4, 0.5, 0.6 and 0.75 mmol l^{-1}) was investigated. The results showed a better response of the sensor with a PLL concentration of 0.5 mmol l^{-1} (Table 2). Lower PLL concentrations produced smaller and unstable responses, which was attributed to CoTSPc leaching out the PLL film, once under such conditions, the amount of PLL may be insufficient for CoTSPc immobilization. In conclusion, glassy carbon surfaces modified from solutions prepared from a mixture of 0.6 mmol l^{-1} CoTSPc and 0.5 mmol l^{-1} PLL solutions allowed to obtain chemically modified electrodes with good sensitivity, repeatability and stability.

3.4. Influence of buffer concentration

The influence of different concentrations of acetate buffer was studied, using 50 μmol of diospyrin solution. The studied concentrations were 0.07, 0.10, 0.15 and 0.2 mol l^{-1}. Table 3 shows the results obtained from differential pulse voltammetry measurements. These results showed a better response of the sensor with a concentration of acetate buffer from 0.15 mol l^{-1}. In this sense, the concentration of 0.15 mol l^{-1} was chosen for further experiments.

3.5. Influence of the potential scan rate (ν) and potential amplitude (a)

The effect of the scan rate and potential amplitude on the differential pulse voltammetry response of the CoTSPc and PLL modified glassy carbon electrode, in acetate buffer + DMSO solution were verified.

Table 3
Influence of the acetate buffer concentration with DMSO on the peak current density obtained by DPV with the sensor for 50 μmol l^{-1} diospyrin

[Acetate] (mol l^{-1})	$-j$ (nA cm^{-2})
0.07	1830
0.10	2049
0.15	3410
0.20	2710

Scan rate = 0.02 V s^{-1}; [CoTSPc] = 0.6 mmol l^{-1}; [PLL] = 0.5 mmol l^{-1}.

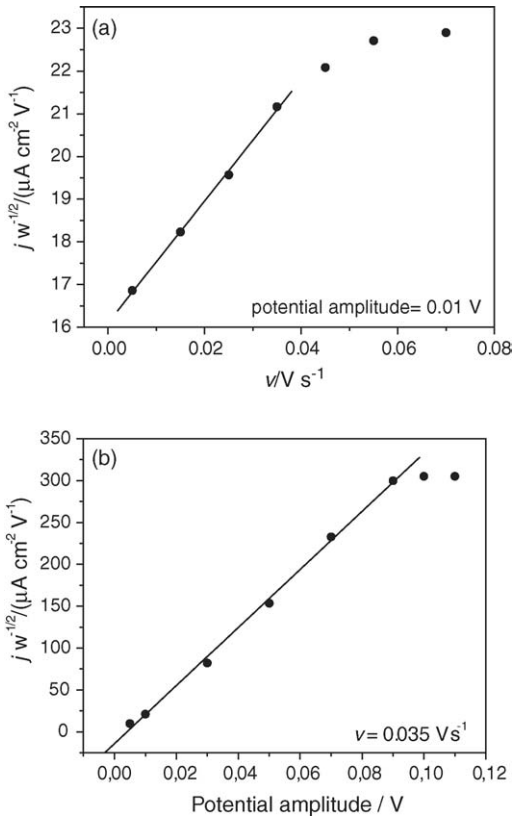

Fig. 3. (a) Plot of the potential scan rate (ν) vs. current, with fixed amplitude (0.01 V). (b) Plot of the potential amplitude vs. current, with a fixed scan rate (0.035 V s^{-1}). CoTSPc/PLL modified electrode, under optimized conditions.

The peak current/peak half width ratios ($j_p/W_{1/2}^{-1}$, μA cm^{-2} V^{-1}) values presented a linear increase with the scan rate variation from 0.005 to 0.035 V s^{-1} (Fig. 3a). On the other hand, when the scan rate is >0.035 V s^{-1}, the current peak value remained almost constant, accompanied by broadening and distortion of the peaks. As it sets the best voltammetric profile with higher sensitivity, the scan rate of 0.035 V s^{-1} was chosen and subsequently used throughout the present study. The current values of peak were also found to vary with pulse amplitude of 0.005–0.100 V (Fig. 3b) applied on DPV at a scan rate of 0.035 V s^{-1} for the modified electrode. The use of the pulse amplitude >0.090 V led to current peak values almost constant and to an increase in the capacitive current. In this sense, the best voltammetric sensitivity was obtained with 0.090 V and therefore, this value was chosen for further studies. As can be seen, the peak current increases rapidly by increasing the pulse amplitude and the step potential (scan rate) when small amplitudes and step potential are used, however the response soon level off when higher values of amplitude and step potential are used [28]. This behaviour has been verified by other researchers and has been attributed to exponential relation between the current density with the amplitude and the step potential [29,30].

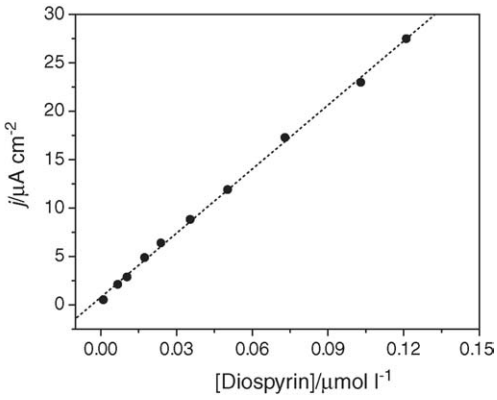

Fig. 4. Calibration plot obtained by DPV for diospyrin determination with CoTSPc/PLL modified electrode under optimized conditions at concentrations of: (1) 1, (2) 6.76, (3) 10.4, (4) 17.4, (5) 23.8, (6) 35.4, (7) 50.2, (8) 73.0, (9) 103.0 and (10) 120.0 nmol l^{-1}. Scan rate = 0.035 V s^{-1} and potential amplitude = 0.090 V.

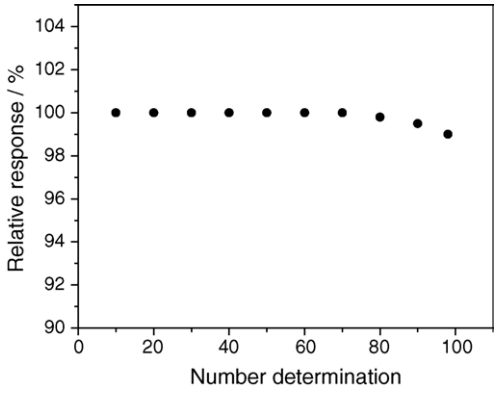

Fig. 5. Relative response (%) as a function of the number of determinations under optimized conditions.

4. Sensor characteristics

Under optimised conditions, in order to obtain an analytical curve for the developed sensor, differential pulse voltammograms for reduction of diospyrin were carried out at different concentrations in 0.15 mmol l^{-1} acetate buffer + DMSO solution at pH 5.4. The proposed sensor showed a good linear response range from 1 to 120 nmol l^{-1} (Fig. 4), which can be expressed according to the following equation:

$$j_p(\mu A\,cm^{-2}) = 0.8\,(\pm 0.2) + 220.5\,(\pm 2.8) \times [\text{diospyrin}]\,(\mu mol\,l^{-1}) \quad (1)$$

with a correlation coefficient of 0.999 (for $n = 10$) and sensitivity of 220.46 nA l $nmol^{-1}$ cm^{-2}. Such good sensitivity can be attributed to the efficiency of the electron transfer between the modified electrode and diospyrin due to the catalytic effect and low charge transfer resistance of the film. A limit of detection (LOD) of 0.3 nmol l^{-1} was determined using a 3σ/slope ratio and limit of quantification (LOQ) was 1.0 nmol l^{-1} using 10σ/slope, where σ is the standard deviation (S.D.) of the mean value for 10 voltammograms of the blank, determined according to the IUPAC recommendations [31].

The stability of the CoTSPc/PLL film modified electrode was checked by recording successive cyclic voltammograms. After 80 cycles, no change was observed in the voltammetric profiles of the modified electrode. Even in the presence of diospyrin, the modified electrode remained stable and the voltammograms reproducible after 80 successive cycles (Fig. 5). Furthermore, no significant change in the current response was observed in experiments performed with modified electrode stored in air for at least 1 month.

The modified electrode presented a good repeatability for diospyrin determinations. The relative standard deviation of the peak current for ten determinations in solutions containing 50 μmol l^{-1} diospyrin was 4.4%. These experiments indicate that the GC electrodes modified with CoTSPc/PLL films have good stability and repeatability, probably associated with the ability of the PLL to fix and protect the CoTSPc molecule on the electrode surface by strong electrostatic interactions.

4.1. Application to samples

This method was applied for diospyrin determination in two samples of the same crude chloroformic extract of *D. montana* Roxb. in triplicate (Table 4). The samples presented value of 20.2 (±3.0) ng g^{-1} and 36.3 (±1.9) ng g^{-1}, respectively. The concentration of diospyrin was determined using the standard addition method. The results suggest that the method is very effective for diospyrin determination in lower level of detection.

4.2. Recovery studies of diospyrin in the samples

For an additional check on the accuracy of the developed method and the matrix interferences, analytical recovery

Table 4
Addition and recovery of diospyrin in two crude extract samples ($n = 3$) obtained with the modified electrode

Samples	Diospyrin added (nmol l^{-1})	Diospyrin expected (nmol l^{-1})	Diospyrin found (nmol l^{-1})	Diospyrin found (ng g^{-1})	Recovery (%)
A	0	–	54 (±8)	(20.2 ± 3.0)	–
	100	154	160 (±14)		103.9 (±7.0)
B	0	–	97 (±5)	(36.3 ± 1.9)	–
	100	197	196 (±22)		99.5 (±8.1)

experiments were performed by adding known amounts of diospyrin in two samples of the crude chloroformic extract of *D. montana* Roxb. The percentage of the recovery values were calculated by comparing the concentration obtained from the samples with actual and added concentrations according to the equation:

$$\text{Recovery}\,(\%) = \frac{\text{concentration found}}{\text{concentration expected}} \times 100.$$

The recovery study for the samples is shown in Table 4. It can be clearly observed that the matrices did not influence in the sensor response.

5. Conclusion

This work demonstrated that glassy carbon electrode modified with CoTSPc/PLL film is a feasible alternative for analytical determination of diospyrin in a mixed aqueous + DMSO solution. The modified electrode exhibited high electrocatalytic activity when compared to the reduction on the bare electrode. Under optimized conditions differential pulse voltammetry measurements in acetate buffer + DMSO (pH 5.4) solutions yield limits of detection and of quantification for diospyrin of 0.3 and 1.0 nmol l^{-1}, respectively, and good sensitivity (220.46 nA l $nmol^{-1}$ cm^{-2}). The good sensitivity for diospyrin detection is similar to the data obtained previously from HPLC and RPLC analysis [13,32]. The modified electrode also showed good repeatability (S.D. = 4.4%) suggesting that the GC electrode modified with CoTSPc/PLL films is very promising for diospyrin determination in crude extract of *D. montana* Roxb., without the time-consuming step of preliminary separation [14].

It is possible to affirm that this method can be applied in the case of other biologically and commercially important quinones [14]. These studies are under investigations in our laboratories.

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