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Cobalt phthalocyanine as a biomimetic catalyst in the amperometric quantification of dipyrone using FIA

Ana Carolina Boni[a], Ademar Wong[a], Rosa Amália Fireman Dutra[b], Maria Del Pilar Taboada Sotomayor[a,*]

[a] Department of Analytical Chemistry, Institute of Chemistry, State University of São Paulo (UNESP), 14801-970 Araraquara, SP, Brazil
[b] Laboratory of Diagnostic Research (LAPED), University of Pernambuco, 50100-130 Recife, PE, Brazil

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ABSTRACT

A biomimetic sensor for the determination of dipyrone was prepared by modifying carbon paste with cobalt phthalocyanine (CoPc), and used as an amperometric detector in a flow injection analysis (FIA) system. The results of cyclic voltammetry experiments suggested that CoPc behaved as a biomimetic catalyst in the electrocatalytic oxidation of dipyrone, which involved the transfer of one electron. The optimized FIA procedure employed a flow rate of 1.5 mL min^{-1}, a 75 μL sample loop, a 0.1 mol L^{-1} phosphate buffer carrier solution at pH 7.0 and amperometric detection at a potential of 0.3 V *vs.* Ag/AgCl. Under these conditions, the proposed method showed a linear response for dipyrone concentrations in the range 5.0×10^{-6}–6.3×10^{-3} mol L^{-1}. Selectivity and interference studies were carried out in order to validate the system for use with pharmaceutical and environmental samples. In addition to being environmentally friendly, the proposed method is a sensitive and selective analytical tool for the determination of dipyrone.

1. Introduction

Greater public awareness has resulted in heightened interest in the environmental impacts caused by the presence of industrial wastes and domestic sewage in rivers and other aquatic systems. Discharges of untreated industrial effluents contaminate natural waters, which can render them unsuitable for use, cause disease and even destroy entire ecosystems [1–3]. Pharmaceutical drugs excreted by humans and livestock can reach water bodies in discharges from sources including ineffective sewage treatment plants.

Many methods are available for the measurement of environmental pollutants. Most of these involve collection of the material to be analyzed and its retrieval to a laboratory in order to conduct the required analyses. Degradation of the sample can occur during transport, affecting the analytical results. It is therefore desirable to develop methodologies that enable measurements to be performed *in situ*, and that provide rapid and reliable real-time responses. Portable flow injection analysis (FIA) systems employing electrochemical sensors [4,5] can be developed for this purpose, and offer advantages including excellent sensitivity as well as speed and reproducibility.

Biomimetic sensors [6,7] are a class of electrochemical detectors that are prepared by the modification of electrodes [8–12] with metal complexes that have similar chemical structures as the active sites of oxide-reductase enzymes. Such sensors possess characteristics that make them more durable and stable than their analogues constructed using enzymes, including high stability over time as well as with drastic changes of pH and temperature (which could denature enzymes). They are cheap, simple to manufacture and may even be disposable (as in the case of screen-printed electrodes). Biomimetic sensors are more sensitive, compared to enzymatic biosensors, due to a smaller diffusion barrier and greater electron transfer between the biomimetic complex and the analyte [6,7]. This new and increasingly popular class of sensors has been used for the identification and quantification of various drugs [13–17], and has also been coupled to flow systems [18,19].

Among the oxide-reductase enzymes that have been frequently imitated are the P450, which have a common active site, protoporphyrin IX (or protohemin IX, an iron porphyrin) [20]. The reason to mimic the P450 enzymes, of which approximately 450 different types are currently known [21], is that these enzymes catalyze a wide range of chemical reactions in organisms, producing metabolites that are physiologically essential or beneficial to them. In addition, hydroxylation, oxidation and reduction reactions can degrade xenobiotics including drugs, pesticides and endocrine disruptors such as synthetic hormones and sunscreens [20,21].

* Corresponding author at: IQ/CAr – UNESP, Rua Prof. Francisco Degni S/N, Quitandinha, Araraquara 14800-900, Brazil. Tel.: +55 1633019620; fax: +55 1633019692.
E-mail address: mpilar@iq.unesp.br (M.D.P.T. Sotomayor).

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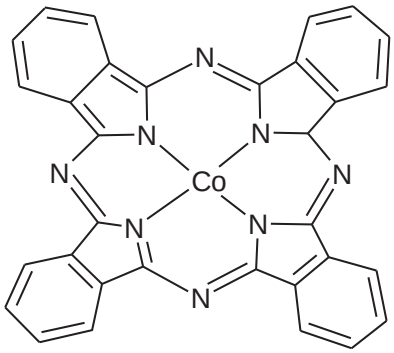

Fig. 1. Chemical structure of cobalt phthalocyanine.

Table 1
Types of electrodes modified with cobalt phthalocyanine that have been reported for the determination of relevant analytes.

Type of electrode	Analyte
Carbon paste	Hydrazine [24]
Carbon paste	Guanine [25]
Glassy carbon	Dopamine [26]
Screen-printed	Thiocholine [27]
Screen-printed	Citric acid [28]
Pyrolytic graphite	Glutathione [29]
Boron-doped diamond	Hydrogen peroxide [30]

Although all of the P450 enzymes contain a common active site, namely the iron porphyrin complex, the selectivity of each P450 is a consequence of the chemical environment surrounding the hemin group [20]. As a result, compounds derived from phthalocyanines and porphyrins of iron [13,18,19,22,23], or other metals such as manganese [14], nickel [15] and copper [17], have been successfully used in the construction of biomimetic chemical sensors for a variety of analytical purposes.

Sensors based on electrodes modified with cobalt phthalocyanine (Fig. 1) have also been reported in the literature for different analytes and applications, using electrode materials such as carbon paste [24,25] or boron-doped diamond [30] (Table 1). These devices have been shown to be chemically and mechanically stable, however not all of them either present electrocatalytic behavior [26] or give responses at very high potentials [25]. These characteristics are essential in biomimetic sensors in order to achieve a high degree of sensitivity and selectivity.

Dipyrone (Fig. 2) is a drug that is widely used, not only in Brazil but also in other countries including France, Germany, Hungary, Israel, Spain, Sweden and a number of developing nations. It is still one of the most popular and powerful analgesics [31]. However, the use of dipyrone was abolished more than thirty years ago in the United States of America, due to its controversial effect [32] on bone marrow function. Its usage has been associated with aplastic anemia and agranulocytosis [32–34]. Despite its possible toxicity, no maximum acceptable concentrations of the drug have been reported in the medical literature, while in Brazil no legal limits have been established for its concentration in aquatic environments. It is therefore evident that there continues to be a need for analytical methods able to quantify dipyrone in various different matrices.

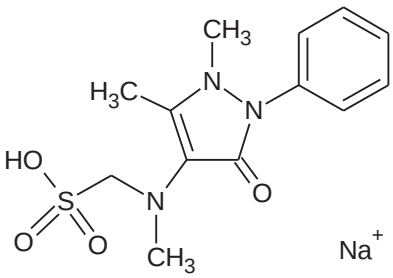

Fig. 2. Chemical structure of dipyrone.

This work describes the development of a sensor prepared with cobalt (II) phthalocyanine [CoPc], used as a P450 enzyme biomimetic catalyst in the sensitive and selective detections of dipyrone. The objective was to develop an analytical tool that was faster, cheaper and more reliable than existing techniques.

2. Experimental

2.1. Chemicals and solutions

All the chemicals used were analytical or HPLC grade. Cobalt (II) phthalocyanine [CoPc], dipyrone, mineral oil and graphite powder were purchased from Sigma–Aldrich. NaH_2PO_4 and NaOH were purchased from Synth-Brazil. TRIS [tris(hydroxymethyl)aminomethane], HEPES [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid] and PIPES [salt of sodium 1,4-piperazinediethanesulfonate] buffers were obtained from Merck. Methanol and phosphoric acid were purchased from Ecibra-Brazil. The dipyrone and buffer solutions were prepared with water purified using a Milli-Q (Direct-0.3) system, and pH was measured using a Thermo Scientific pH meter (Orion 3 Star Benchtop) fitted with a glass electrode.

2.2. Biomimetic sensor construction

The modified paste was prepared by homogenizing 12.5 mg of CoPc with 87.5 mg of graphite powder and 1.0 mL of 0.1 mol L^{-1} phosphate buffer solution (at pH 7.0). The material obtained was dried at room temperature, and then mixed with mineral oil to obtain a homogenous paste. The paste was placed into the cavity of a glass tube (4 mm internal diameter, 1 mm depth), and a platinum slide was inserted for electrical contact with the paste. The influence of the amount of CoPc employed in paste preparation on the response of the sensor was evaluated using three different pastes, prepared with weight percentages of CoPc varying from 7.5% to 20.0%.

2.3. Electrochemical measurements and flow manifold

The cyclic voltammetry experiments were conducted at room temperature in a conventional three-electrode cell, with the modified carbon paste electrode used as the working electrode. An Ag/AgCl(KCl_{sat}) electrode and a platinum wire were used as the reference and counter electrodes, respectively. The measurements were performed using a PalmSense potentiostat (Palm Instruments BV, The Netherlands), which was interfaced with a microcomputer running PSLite (v. 1.7.3) software (Palm Instruments BV) for control of potential and acquisition of data.

For coupling to a flow system, the biomimetic sensor was inserted into a flow-through wall-jet amperometric cell, and used as the working electrode (WE in Fig. 3). A homemade Ag/AgCl(KCl_{sat}) electrode was used as the reference, and a platinum wire was used as the auxiliary electrode. The electrodes were connected to the PalmSense potentiostat. The flow rate of the 0.1 mol L^{-1} phosphate buffer carrier solution was controlled using an Ismatec peristaltic pump. The standards and samples containing dipyrone were injected into the carrier using a sliding central bar sampling valve [35].

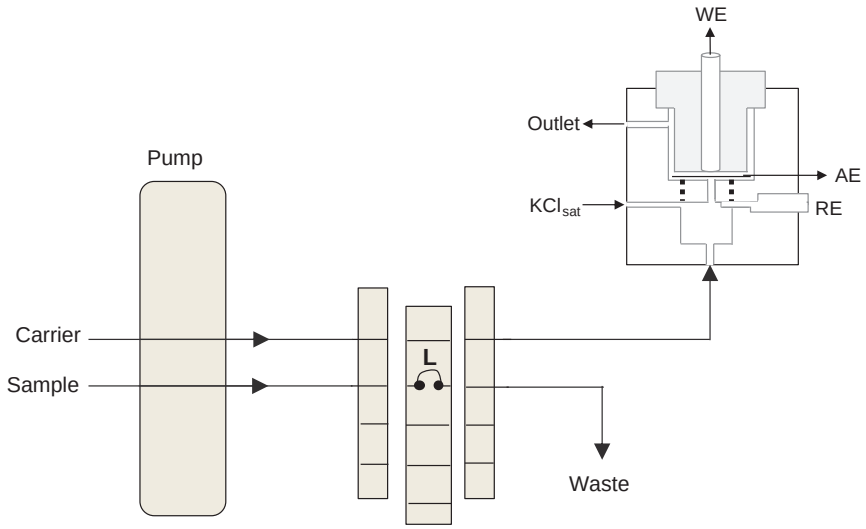

Fig. 3. Schematic diagram of the flow injection system with amperometric detection. L: sample loop; WE: working electrode (biomimetic sensor); AE: auxiliary electrode (platinum); RE: homemade reference electrode (Ag/AgCl(KCl$_{sat}$)).

2.4. HPLC analyses

Chromatographic analyses were performed using a Shimadzu Model 20A liquid chromatograph, coupled to an SPD-20A UV/Vis detector, a SIL-20A autosampler and a DGU-20A$_5$ degasser. The chromatography system was controlled by a microcomputer. A C8 column (250 mm × 4.6 mm, Shim-Pack CLC ODS) was positioned inside a Shimadzu CTO-10AS oven in order to maintain a constant temperature.

The procedure employed was in accordance with the reference method for the quantification of dipyrone [36]. The mobile phase was composed of a mixture of 280 mL of methanol and 720 mL of 0.1 mol L^{-1} NaH_2PO_4, at pH 7.0. The flow rate was 1.0 mL min^{-1} and the sample injection volume was 10 μL. The detection wavelength was 254 nm.

2.5. Application of the biomimetic sensor using the pharmaceutical formulations

Dipyrone was quantified in four commercial samples using the external standards procedure. Samples of two commercial solutions (1 and 2 in Table 5) were analyzed after dilution with deionized water, without any additional treatment. Two samples of tablets (3 and 4 in Table 5) were prepared for analysis by grinding and homogenizing in a mortar, after which a portion of the material was weighed out and dissolved in deionized water. The solution was filtered in order to remove any insoluble substances present in the tablets.

2.6. Application of the biomimetic sensor for analysis of aquatic samples

Five water samples from rivers in São Paulo State (Brazil) were enriched with dipyrone at two concentration levels (9.76×10^{-5} and 3.00×10^{-4} mol L^{-1}) and then analyzed using the proposed sensor in order to evaluate the influence of the matrix on recoveries. The enriched samples were also analyzed using the HPLC reference method [36], in order to determine the nominal concentrations of dipyrone in the samples. All of the river water samples were filtered prior to enrichment in order to remove any insoluble substances present.

3. Results and discussion

3.1. Electrochemical and biomimetic characteristics of the sensor

Cyclic voltammetry experiments (using a scan from 0 to 450 mV *vs.* Ag/AgCl, at a scan rate of 20 mV s^{-1}) were carried out in order to evaluate the effect of [CoPc] on the response of the sensor to dipyrone. The results (Fig. 4) showed that the peak corresponding to the oxidation of cobalt (II) in the complex disappeared in the presence of dipyrone, at a potential of 250 mV *vs.* Ag/AgCl (Fig. 4, curve a). At the same time, a high current intensity anodic peak was obtained, without the appearance of any cathodic peak, showing that dipyrone was irreversibly oxidized on the sensor surface at a potential of 340 mV *vs.* Ag/AgCl (Fig. 4, curve b). The response profile of the sensor was as expected for an enzymatic catalysis process in which an increase of current occurs in only one direction (either oxidation or reduction) [23].

Experiments were performed using different scan rates in order to determine whether the oxidation of dipyrone was due to an electrocatalytic process. The results obtained are shown in Figs. 5–7.

In cyclic voltammetry, it is known that when the reaction kinetics are controlled by diffusion of the species from the bulk solution to the electrode surface, the peak current is proportional to the square root of the scan rate, in agreement with the Randles–Sevcík equation [37,38]. Cyclic voltammograms were therefore obtained

Table 2
Parameters evaluated during the optimization of the biomimetic sensor for dipyrone determination using the FIA system.

Parameter	Value					
Amount of the complex in the paste (%, w/w)	7.5	12.5[a]	20.0	–	–	–
Buffer (electrolyte)	PHOSPHATE[a]	HEPES	PIPES	TRIS	–	–
Buffer pH	6.0	6.5	7.0[a]	7.5	8.0	8.5
Applied potential (mV *vs.* Ag/AgCl)	200	250	300[a]	350	400	–

[a] Optimized value.

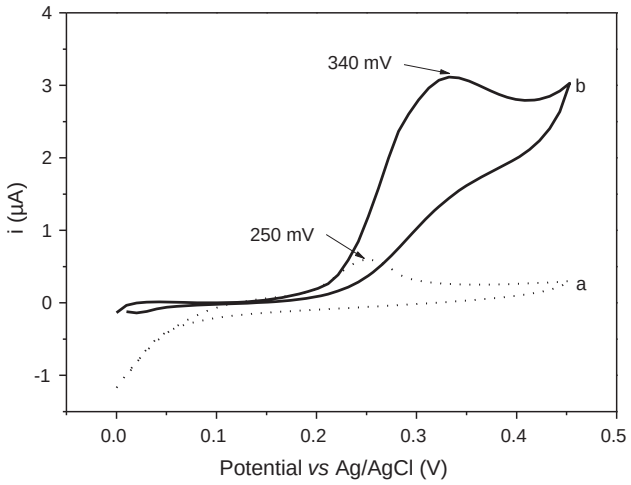

Fig. 4. Catalytic profile for dipyrone oxidation on the proposed sensor based on carbon paste modified with cobalt phthalocyanine: (A) 0.1 mol L^{-1} phosphate buffer solution (pH 7.0); (B) buffer solution containing 1.4×10^{-3} mol L^{-1} of dipyrone.

at different scan rates (Fig. 5) in order to investigate whether the dipyrone oxidation process was controlled by mass transport. The graph shown in Fig. 5 inset was plotted using these voltammograms. The straight line fit indicated that the process was controlled by diffusion.

The existence of an electrocatalytic process was confirmed by plotting the scan rate-normalized current ($i \cdot \nu^{-1/2}$) against the scan rate (ν). The form of the curve obtained (Fig. 6) indicated that the oxidation of dipyrone occurred by electrocatalysis, and involved an electrochemical process coupled with a chemical step [39]. In addition, a linear relationship was obtained between the anodic peak potential (E_p) and log ν (Fig. 7), indicative of a proportional electron transfer flow in the electrode.

Using the information obtained from the linear regression (shown in the inset of Fig. 5), together with the theoretic model described by Andrieux and Savèant (Eq. (1)) [40], it was possible to calculate a diffusion coefficient (D_0) of 5.9×10^{-7} cm^2 s^{-1}, corresponding to the average diffusion of dipyrone and its oxidation products during the catalytic process.

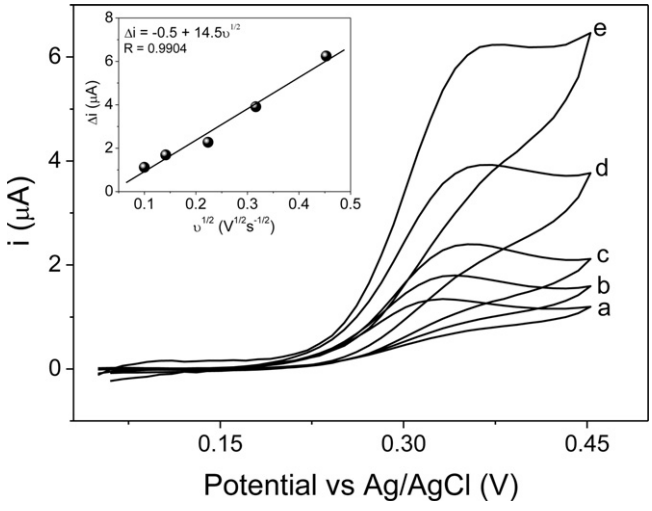

Fig. 5. Cyclic voltammograms recorded at different scan rates: (a) 0.010 V s^{-1}; (b) 0.020 V s^{-1}; (c) 0.050 V s^{-1}; (d) 0.100 V s^{-1} and (e) 0.200 V s^{-1}. The inset shows the linear profile of the variation of the current (Δi) as a function of the square root of the scan rate ($\upsilon^{1/2}$). Measurements were carried out in 0.1 mol L^{-1} phosphate buffer solution (at pH 7.0) containing 5.0×10^{-4} mol L^{-1} of dipyrone.

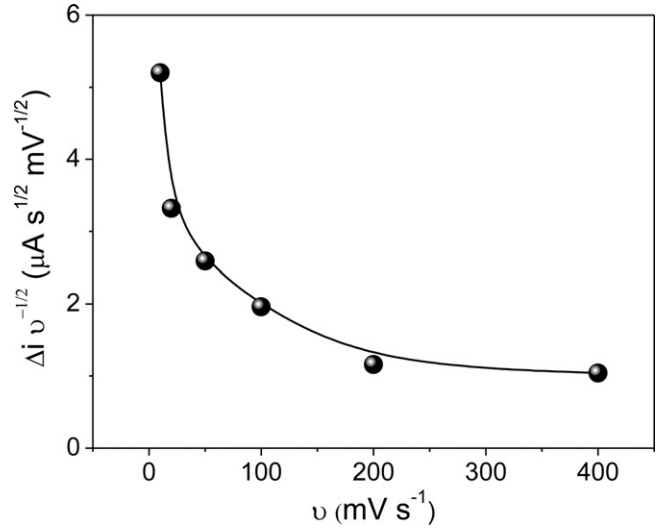

Fig. 6. A typical catalytic electrooxidation profile. A 5.0×10^{-4} mol L^{-1} solution of dipyrone was electrooxidized by the cobalt (II) complex immobilized on the carbon paste. Measurements were carried out in 0.1 mol L^{-1} phosphate buffer solution (at pH 7.0), used as the electrolyte.

$$i_p = 0.496FAC_S D_0^{1/2} \left(\frac{F}{RT}\right)^{1/2} \upsilon^{1/2} \quad (1)$$

In Eq. (1), C_S is the dipyrone concentration (5.0×10^{-7} mol cm^{-3}), D_0 is the diffusion coefficient of dipyrone, F is the Faraday constant, $R = 8.31447$ J mol^{-1} K^{-1}, $T = 298$ K and $A = 0.126$ cm^2. The dipyrone diffusion coefficient could therefore be obtained from the slope ($\times 10^{-6}$) of the line shown in Fig. 5 inset and the factor $0.496FAC_S D_0^{1/2} F^{1/2} (RT)^{-1/2}$.

Assuming that the electrocatalytic oxidation of dipyrone is an irreversible process, the Randles–Sevcík relationship (Eq. (2)) can be used to determine the number of electrons involved.

$$j_p = (2.99 \times 10^5) n[(1-\alpha)n_a]^{1/2} D_0^{1/2} C_S \upsilon^{1/2} \quad (2)$$

In Eq. (2), $j_p = i_p/A = 114.6$ μA cm^{-2} $V^{-1/2}$ $s^{1/2}$, n is the total number of electrons involved in the reaction, α is the coefficient of electron transfer, n_a represents the number of electrons involved in the determining step, D_0 (cm^2 s^{-1}) is the diffusion coefficient of the

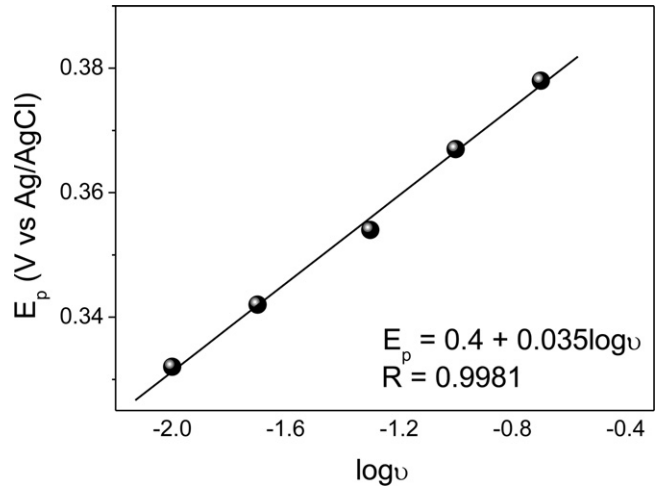

Fig. 7. Linear variation of the oxidation potential (E_p) *vs.* log υ. Scans were carried out in 0.1 mol L^{-1} phosphate buffer containing 5.0×10^{-4} mol L^{-1} of dipyrone.

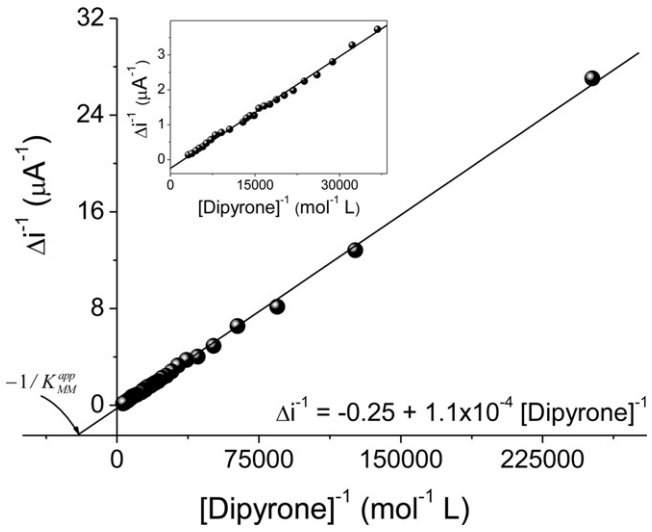

Fig. 8. Lineweaver–Burk plot for catalyzed dipyrone electrooxidation on the CoPc-based sensor. The first 25 points are shown in the figure inset. Measurements were carried out in 0.1 mol L^{-1} phosphate buffer solution (at pH 7.0), applying a potential of 340 mV *vs.* Ag/AgCl.

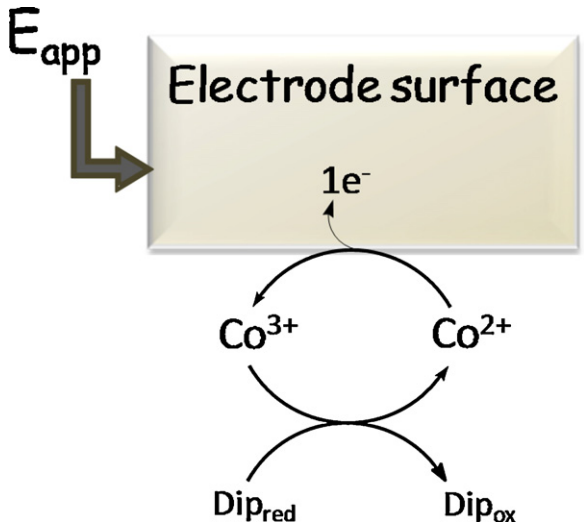

Fig. 9. Proposed mechanism for the sensor response, based on experimental evidence. Dip: dipyrone; red: reduced dipyrone; ox: oxidized dipyrone.

electroactive species, C_S (mol cm^{-3}) is the dipyrone concentration and υ is the scan rate.

In order to estimate the value of n, it is first necessary to calculate the value of $(1-\alpha)n_a$. In catalytic or irreversible systems, an approximate calculation is based on the linear relationship between the anodic peak potential (E_p) and log υ (Fig. 7). The relationship between the slope of Fig. 7 and the factor $(1-\alpha)n_a$ (Eq. (3)) gave a value of 0.83.

$$0.03536 = \frac{1.15RT}{[(1-\alpha)n_a]F} \quad (3)$$

After calculating the value of $(1-\alpha)n_a$, using Eq. (2) and the previously estimated dipyrone diffusion coefficient, it was possible to calculate the number of electrons involved in the catalytic electrooxidation of dipyrone on the surface of the CoPc-based sensor. The value obtained was 1.09, which was close to $1 \times e^-$.

In order to explore whether the behavior of the proposed sensor was similar to that of enzymatic biosensors, its response was monitored until the concentration reached the saturation region. The results obtained showed a hyperbolic profile, indicating that the cobalt compound in the carbon paste followed the Michaelis–Menten kinetic model. The Lineweaver–Burk graph (Fig. 8) enabled calculation of the apparent Michaelis–Menten constant (K_{MM}^{app}). A value of 4.4×10^{-4} mol L^{-1} indicated strong affinity between the cobalt complex and the dipyrone substrate. These results also strongly suggest that the detector possessed the characteristics of a biomimetic sensor.

Based on the findings described above, it was possible to propose a plausible mechanism for the sensor response (Fig. 9). In this mechanism, Co^{3+} is electrochemically generated on the electrode surface (electrochemical step), and is the species responsible for the oxidation of dipyrone (chemical step). After the reduction of the Co^{3+} ion, the current required for its re-oxidation will be proportional to the concentration of the analyte in the standards or samples.

3.2. Coupling of the biomimetic sensor to the FIA system

Following confirmation, using batch mode voltammetry, that the sensor possessed genuine biomimetic characteristics, it was coupled to a flow injection analysis system. Optimization of the system involved consideration of the following parameters: (i) amount of complex in the paste; (ii) applied amperometric potential; (iii) composition, concentration and pH of the carrier buffer solution (electrolyte). These parameters are summarized in Table 2, with the selected values indicated in bold type.

Table 3
Selectivity of the biomimetic sensor. The slopes of the analytical curves were used to calculate the response for each compound, relative to the response obtained for dipyrone.

Drug	Relative response (%)
Dipyrone	100.0
Captopril	14.5
4-AAP	12.5
Ascorbic acid	11.0
Paracetamol	3.2

Flow rate optimization (Fig. 10) employed a range of 0.7–2.1 mL min^{-1}. A flow rate of 1.5 mL min^{-1} was selected since this provided the best compromise between analysis frequency and sensitivity. The injection volume was investigated using loop volumes of 25, 50, 75 and 100 μL (Fig. 8). The signal increased with

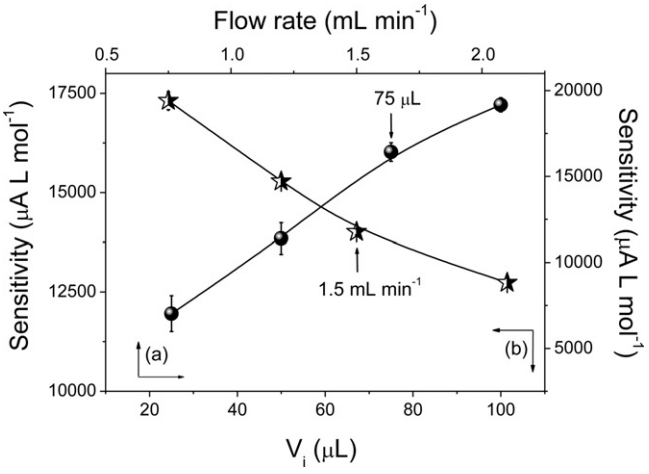

Fig. 10. Influence of the parameters in the proposed FIA system: (a) effect of injected sample volume (V_i) on the response to dipyrone, using a flow rate of 1.5 mL min^{-1}; (b) effect of flow rate on the response to dipyrone, using a V_i of 50 μL. The experiments were carried out with a carrier solution of 0.1 mol L^{-1} phosphate buffer at pH 7.0, and applying a potential of 300 mV *vs.* Ag/AgCl(KCl_{sat}). The error bars correspond to the standard deviation for three replicates.

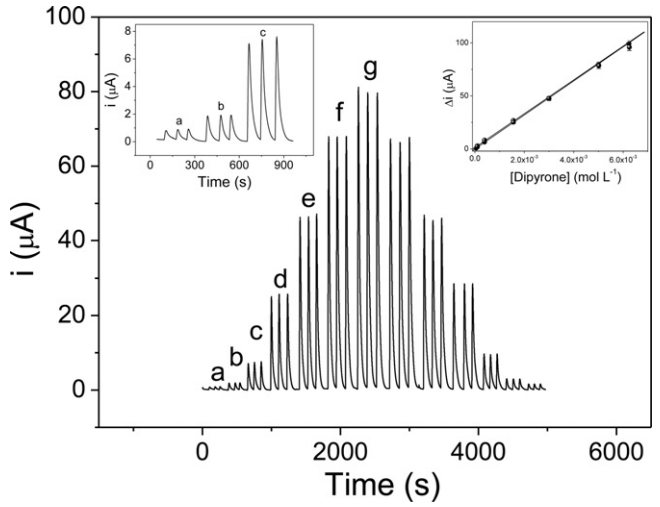

Fig. 11. FIA signals obtained for the biomimetic sensor under optimized flow conditions for different dipyrone concentrations. [Dipyrone]: $a = 5.0 \times 10^{-5}$ mol L^{-1}; $b = 9.5 \times 10^{-4}$ mol L^{-1}; $c = 3.9 \times 10^{-3}$ mol L^{-1}, $d = 1.5 \times 10^{-3}$ mol L^{-1}; $e = 3.0 \times 10^{-3}$ mol L^{-1}; $f = 5.0 \times 10^{-2}$ mol L^{-1}; $g = 6.25 \times 10^{-3}$ mol L^{-1}. Left inset: The three lower dipyrone concentrations. Right inset: Typical analytical curves for increasing and decreasing dipyrone concentrations.

increasing V_i, reaching a maximum at 100 μL. However, a volume of 75 μL was selected since this provided good sensitivity without unduly affecting the frequency of sampling.

3.3. Analytical characteristics of the FIA system

A calibration curve (Fig. 11) was generated after the FIA system parameters had been optimized. No memory effects were observed, and linear fits were obtained for both increasing and decreasing concentrations of dipyrone (Eqs. (4) and (5), respectively).

$$\Delta i/\mu A = -0.09(\pm 0.07) + 16\,062(\pm 647)[\text{Dipyrone}]/\text{mol L}^{-1} \quad (R = 0.9960,\ n = 7) \tag{4}$$

$$\Delta i/\mu A = 1.5(\pm 0.1) + 15\,876(\pm 407)[\text{Dipyrone}]/\text{mol L}^{-1} \quad (R = 0.9987,\ n = 7) \tag{5}$$

The limits of detection and quantification were 1.5×10^{-5} and 4.5×10^{-5} mol L^{-1}, respectively, calculated according to IUPAC recommendations [41] from the standard deviation of ten separate measurements of the blank.

The repeatability of the sensor was investigated using seven consecutive injections of 2.5×10^{-3} mol L^{-1} dipyrone solution, for which a relative standard deviation (RSD) of 0.5% was obtained. Under the conditions employed, the sensor could be operated at an analytical frequency of 36 injections per hour. The between-sensor reproducibility was 2.5%, calculated as the RSD of the analytical curves for six separate sensors. It should be pointed out that excellent reproducibility in the construction of the sensors was achieved due to the ease of preparation of the modified carbon paste.

3.4. Selectivity and interference studies

Good selectivity is one of the most desirable characteristics of analytical techniques. Hence, in addition to dipyrone, the response of the sensor was tested using sixteen other drugs: acetylsalicylic acid, 4-aminoantipyrine (4-AAP), amitriptyline, ascorbic acid, captopril, chloramphenicol, ciprofloxacin, clarithromycin, diclofenac, ketoprofen, naproxen, paracetamol, piroxicam, prednisone, ranitidine and tenoxicam. Table 3 lists those drugs to which the sensor showed a response. The response to 4-AAP was 12.5%, relative to the response to dipyrone, but this was expected because 4-aminoantipyrine is one of the active metabolites of dipyrone and has a similar chemical structure. Unlike other sensors based on carbon paste, the present sensor gave a small signal for ascorbic acid (11.0%). The results indicated that the proposed sensor was highly selective, as expected for a device designed to mimic an enzymatic biosensor.

Table 4
Relative responses (%) obtained in the FIA system interference study, using 5.0×10^{-4} mol L^{-1} of dipyrone and a 1:10 molar ratio of analyte/interferent.

Interferent	Relative response (%)
Caffeine	102
Cellulose	102
Lactose	100
Magnesium stearate	99
Stearic acid	102
Sodium bisulfite	103
Sodium phosphate	101

The interference study was performed considering the main application of the sensor to be in analyses of commercial pharmaceutical formulations. Table 4 shows the relative responses (%) obtained in the presence of each interfering substance (at an interferent/analyte molar ratio of 10:1), compared to the response for dipyrone alone. The results indicated that the sensor did not suffer from interferences due to the presence of these substances. Matrix interferences are therefore not expected to occur in analyses involving this class of substance.

3.5. Sensor application

The application of the proposed sensor was tested using two different types of sample. The first involved four commercial pharmaceutical formulations. The second employed water samples collected from five rivers in São Paulo State, which were enriched with dipyrone at two concentration levels in order to demonstrate the applicability of the proposed FIA system to environmental samples.

Table 5 shows the results obtained for the commercial formulations. The concentrations were determined using the FIA system, and then compared with the results obtained by the chromatographic reference method. There were no significant differences between the two techniques (at a confidence level of 95%).

Using the FIA technique, the average error for the river water analyses was 1.2%, relative to the HPLC reference method (Table 6). The results indicate that dipyrone was always present in these rivers, since the measured concentrations exceeded those expected from knowledge of the amounts of dipyrone added to the water samples.

Table 5
Determination of dipyrone in pharmaceutical formulations using the biomimetic sensor FIA technique and the chromatographic reference method.

Sample	Dipyrone amount			Error (%)
	Declared value	Reference method (HPLC)	Proposed method	
1	500[a]	438.0	435.0 ± 16.5[c]	0.7
2	50[a]	52.0	49.5 ± 0.7[c]	3.8
3	300[b]	296.4	297.0 ± 8.6[c]	0.3
4	500[b]	520.0	535.0 ± 5.6[c]	2.9

[a] mg L^{-1}.
[b] mg tablet^{-1}.
[c] Standard deviation for 3 replicates.

Table 6
Comparison between the values obtained for dipyrone in water from rivers, using the proposed FIA system and the reference method. The samples were enriched at two concentration levels (9.76×10^{-5} and 3.00×10^{-4} mol L^{-1}).

River	Measured concentration (mol L^{-1})		Error (%)
	HPLC method	Proposed FIA method[a]	
Tietê river at the *Usina da Barra* club	9.8×10^{-5}	$9.9 \times 10^{-5} \pm 2.6 \times 10^{-6}$	1.0
	3.4×10^{-4}	$3.4 \times 10^{-4} \pm 1.4 \times 10^{-5}$	0.0
Jacaré Guaçú	9.9×10^{-5}	$1.0 \times 10^{-4} \pm 4.2 \times 10^{-6}$	1.0
	3.4×10^{-4}	$3.2 \times 10^{-4} \pm 1.1 \times 10^{-5}$	5.9
Jaú	1.2×10^{-4}	$1.2 \times 10^{-4} \pm 3.6 \times 10^{-6}$	0.0
	3.0×10^{-4}	$3.0 \times 10^{-4} \pm 7.2 \times 10^{-6}$	0.0
Jacaré Pepira	1.0×10^{-4}	$9.9 \times 10^{-5} \pm 3.5 \times 10^{-6}$	1.0
	3.3×10^{-4}	$3.2 \times 10^{-4} \pm 1.2 \times 10^{-5}$	3.0
Tietê river by the bridge in *Barra Bonita* city	1.0×10^{-4}	$1.0 \times 10^{-4} \pm 3.5 \times 10^{-6}$	0.0
	3.6×10^{-4}	$3.6 \times 10^{-4} \pm 1.1 \times 10^{-5}$	0.0

[a] Standard deviation for 3 replicates.

4. Conclusions

This paper presents an alternative and environmentally friendly methodology for the determination of dipyrone, using a flow injection system with amperometric detection based on a biomimetic sensor modified with the CoPc complex. The new FIA system is fast, sensitive and inexpensive, and can be used for the analysis of dipyrone in media including pharmaceutical drug formulations and river water samples.

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References

[1] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81–90.
[2] M.E. Balmer, H.R. Buser, M.D. Muller, T. Poiger, Environ. Sci. Technol. 39 (2005) 953–962.
[3] M. Schlumpf, B. Cotton, M. Conscience, V. Haller, B. Steinmann, W. Lichtensteiger, Environ. Health Perspect. 109 (2001) 239–244.
[4] J. Ruzicka, E.H. Hansen, Flow Injection Analysis , John Wiley, New York, 1988.
[5] B.F. Reis, M.F. Giné, E.A.M. Kronka, Quim. Nova 12 (1989) 82–91.
[6] M.D.P.T. Sotomayor, L.T. Kubota, Quim. Nova 25 (2002) 123–128.
[7] M.D.P.T. Sotomayor, A.A. Tanaka, R.S. Freire, L.T. Kubota, C.A. Grimes, in: e.c. Dickey, M.V. Pishko (Eds.), Encyclopedia of Sensors, American Scientific Publishers, California, 2006, pp. 195–209.
[8] P.R. Moses, L. Wier, R.W. Murray, Anal. Chem. 47 (1975) 1882–1886.
[9] J.A. Cox, M.E. Tess, T.E. Cummings, Rev. Anal. Chem. 15 (1996) 173–223.
[10] M.A.T. Gilmartin, J.P. Hart, Analyst 120 (1995) 1029–1045.
[11] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold, Z. Yang, Electroanalysis 7 (1995) 5–22.
[12] J. Wang, Electroanalysis 3 (1991) 255–259.
[13] I.V. Batista, M.R.V. Lanza, I.L.T. Dias, S.M.C.N. Tanaka, A.A. Tanaka, M.D.P.T. Sotomayor, Analyst 133 (2008) 1692–1699.
[14] W.J.R. Santos, A.L. Sousa, F.S. Damos, S.M.C.N. Tanaka, L.T. Kubota, A.A. Tanaka, M.D.P.T. Sotomayor, J. Braz. Chem. Soc. 20 (2009) 1180–1187.
[15] L.F. Moreira, M.R.V. Lanza, A.A. Tanaka, M.D.P.T. Sotomayor, Analyst 134 (2009) 1453–1461.
[16] A.C. Boni, M.D.P.T. Sotomayor, M.R.V. Lanza, S.M.C.N. Tanaka, A.A. Tanaka, J. Braz. Chem. Soc. 21 (2010) 1377–1383.
[17] D.F. Gobatto, A. Wong, M.R.V. Lanza, M.D.P.T. Sotomayor, Open Chem. Biomed. Methods J. 3 (2010) 98–107.
[18] M.C.Q. Oliveira, M.R.V. Lanza, A.A. Tanaka, M.D.P.T. Sotomayor, Anal. Methods 2 (2010) 507–512.
[19] A. Wong, M.R.V. Lanza, M.D.P.T. Sotomayor, Comb. Chem. High Throughput Screen. 13 (2010) 666–674.
[20] M. Sono, M.P. Roach, E.D. Coulter, J.H. Dawson, Chem. Rev. 96 (1996) 2841–2888.
[21] A.S. Kalgutkar, R.S. Obach, T.S. Maurer, Curr. Drug Metab. 8 (2007) 407–447.
[22] F.S. Damos, M.D.P.T. Sotomayor, L.T. Kubota, S.M.C.N. Tanaka, A.A. Tanaka, Analyst 128 (2003) 255–259.
[23] A. Sigoli, M.D.P.T. Sotomayor, M.R.V. Lanza, A.A. Tanaka, L.T. Kubota, J. Braz. Chem. Soc 19 (2008) 734–743.
[24] C.D.C. Conceição, R.C. Faria, O. Fatibello-Filho, A.A. Tanaka, Anal. Lett. 41 (2008) 1010–1021.
[25] I. Balan, I.G. David, V. David, A.-I. Stoica, C. Mihailciuc, I. Stamatin, A.A. Ciucu, J. Electroanal. Chem. 654 (2011) 8–12.
[26] F.C. Moraes, M.F. Cabral, S.A.S. Machado, L.H. Mascaro, Electroanalysis 20 (2008) 851–857.
[27] E. Jubete, K. Zelechowsk, O.A. Loaiza, P.J. Lamas, E. Ochoteco, K.D. Farmer, K.P. Roberts, J.F. Biernat, Electrochim. Acta 56 (2011) 3988–3995.
[28] K.C. Honeychurch, L. Gilbert, J.P. Hart, Anal. Bioanal. Chem. 396 (2010) 3103–3111.
[29] N. Sehlotho, S. Griveau, T. Nyokong, F. Bedioui, Electroanalysis 19 (2007) 103–106.
[30] T. Kondo, A. Tamura, T. Kawai, J. Electrochem. Soc. 156 (2009) 145–150.
[31] F.A. Fehintola, Afr. J. Med. Sci. 34 (2005) 403–405.
[32] P. Danieli, M.B. Leal, Rev. Bras. Farm. 84 (2003) 17–20.
[33] I.M. Benseñor, Rev. Paul. Med. 119 (2001) 190–191.
[34] V.J. Dorr, J. Cook. South. Med. J. 89 (1996) 612–614.
[35] F.H. Bergamin, J.X. Medeiros, B.F. Reis, E.A.G. Zaggatto, Anal. Chim. Acta 101 (1978) 9–16.
[36] British Pharmacopoeia, Mental Health Act 1, 2007.
[37] J.E.B. Randles, Trans. Faraday Soc. 44 (1948) 327–338.
[38] A. Sevcík, Collect. Czech. Chem. Commun. 13 (1948) 349–377.
[39] A.J. Bard, L. Faulkner, Electrochemical Methods: Fundamentals and Applications , 2nd ed., Wiley, New York, 2001.
[40] C.P. Andrieux, J.M. Savéant, J. Electroanal. Chem. 93 (1978) 163–168.
[41] L.A. Currie, Anal. Chim. Acta 391 (1999) 105–126.