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Article

Piroxicam voltammetric determination by ultra low cost pencil graphite electrode

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Piroxicam (PRX) was determined in pharmaceutical capsules with differential pulse voltammetry (DPV) in a three electrode system consisting of a pencil graphite electrode (PGE) as working electrode, a Pt wire and a reference electrode of Ag/AgCl/KCl 3 M. An irreversible oxidation peak was observed in E_{pa} *c.a.* 0.6 V, which correlates to the oxidation of PRX. The coefficient of linear correlation obtained was 0.9946, with limit of detection of 2.1 µM and limit of quantification of 4.7 µM. PGE assays showed good analytical performance compared to high performance liquid chromatography and spectrophotometry, showing the potential to be further developed and employed in quick and simple analyses.

Keywords: Electroanalysis. Pencil graphite electrode. Piroxicam. Voltammetry.

INTRODUCTION

Piroxicam (PRX) is an oxicam non-steroidal anti-inflammatory drug (NSAID) widely used as anti-inflammatory, analgesic and antipyretic agent. It exerts biological effect through dual cyclooxygenase (COX) inhibition, *i.e.,* inhibition of both COX-1 and COX-2 isoforms (Sangha, Yao, Wolfe, 2005; Rai, Sarkar, Raha, 2005).

Given the high prevalence of inflammatory illnesses, NSAIDs therapy is widespread in medicine (Roddy, Choi, 2014; Perkins *et al.,* 2015). These drugs have been stated to also exhibit chemo suppressive and chemo protective properties (Sporn, Suh, 2000), which supported their inclusion in the treatment of some cancer types such as colorectal cancer (Schror, 2011). Hence NSAIDS relevance in therapeutics, the assessment of drugs such as PRX in pharmaceutical forms is a mandatory step for quality control analysis.

As described in official compendia, methods such as gas, liquid or high performance liquid chromatography (HPLC), as well as spectrophotometric methods are the main indicated approaches to assess NSAIDs in pharmaceutical forms (Anvisa, 2010; USP, 2016). Despite their notoriety in pharmaceutical analyses, these methods are prone to drawbacks such as high equipment cost, strenuous sample pre-preparation, time consuming analysis and high solvent use. Therefore, the development of innovative analytical methods capable of mitigating the drawbacks of standard assessment tools is a thriving field in chemistry.

Amongst the available approaches used to assess pharmaceutical drug products are electrochemical methods such as voltammetry. These techniques offer the possibility of a low cost drug determination without compromising analytical performance (Gupta *et al*., 2011). Electroanalytical determination of oxicam NSAIDs has been successfully described in literature, leading to limits of quantification (LOQ) around mM (10^{-6} mol.L^{-1}) (Mohamed, 2016). In literature, the determination of PRX with the use of many modified electrodes is reported. Some of these modified electrodes show high sensibility with LOD and LOQ values of nM scale (10^{-9} mol.L^{-1}), however, the preparation of these electrodes require specific materials, expensive equipment, qualified instrumentalists and are often strenuous (Shahrokhian, Jokar, Ghalkhani, 2010; Macêdo, 2018; Gholivand, Malekzadeh, Derakhshan, 2014; Shaikh *et al*., 2017).

Although cheap and requiring minute solvent volumes, voltammetry can be prone to low reproducibility hence electrode fouling. Therefore, disposable electrodes composed of matrixes susceptible to easy surface renewal

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are highly regarded for quantification assays. Henceforth, pencil graphite electrodes (PGE) stand as an excellent tool in the quality control of pharmaceuticals (David, Popa, Buleandra, 2017). These electrodes can easily be polished in sandpaper, what allows quick electrode preparation between analyses. Moreover, these devices have demonstrated LOD values akin to those observed in conventional electrodes (Dilgin, Karakaya, 2016; Saglam *et al*., 2016).

To the best of our knowledge, the majority of studies of graphite electrode in pharmaceutical forms determination use ultrapure graphite (99% graphite). Therefore, the aim of this work was to employ nude pencil graphite electrode in the voltammetric assessment of PRX capsules and to compare obtained results with analytical methods from official compendia.

MATERIAL AND METHODS

MATERIAL AND REAGENTS

4B 2.5 mm diameter nude graphite pencils were purchased from Koh-I-Hardmuth (Czech Republic). All other chemicals and solvents were of reagent grade and were used without further purification. Electrolyte solutions were prepared by using high analytical grade salts, which were diluted in double distilled Milli-Q water (conductivity ≤0.1 μScm−1) (Millipore S. A., Molsheim, France). PRX of analytical grade (98% purity) was purchased from Sigma (Saint Louis, USA) and the correspondent stock solutions were prepared immediately prior to the experiments.

PGE preparation

PGE were prepared simply by fitting the nude graphite rod into a plastic tube ca. 2.5 mm, to serve as a casing, in such way, that the pencil was firmly placed and only 2.5 mm diameter circular area was in contact with the analytical solution. The pencil was polished in a Jet401 Norton 47F 1200 sandpaper after each individual assay.

Electroanalytical assays

Voltammetric experiments were carried out with a potentiostat/galvanostat μAutolab III® integrated to the GPES 4.9® software, Eco-Chemie, Utrecht, The Netherlands. The measurements were performed in a 5.0 mL one-compartment electrochemical cell, with a three-electrode system consisting of a PGE, a Pt wire and the Ag/AgCl/KCl 3 $mol.L^{-1}$ (both purchased from Lab solutions, São Paulo, Brazil), representing the working, counter and reference electrode, respectively. The experimental conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 $mV\ s^{-1}$. The experimental conditions for square wave voltammetry (SWV) were: pulse amplitude 50 mV, were frequency 50 Hz and a potential increment of 2 mV, corresponding to a scan rate of 100 $mV\ s^{-1}$. The experimental conditions for cyclic voltammetry (CV) were: scan rate of 100 $mV\ s^{-1}$ and scan range from 0 to 1 V. All experiments were done at room temperature (21 ± 1 ºC) in triplicate (n = 3). The electrolytes used were 0.1 $mol.L^{-1}$ acetate (ABS) (pH 3.0, pH 4.0 and pH 5.0) and phosphate buffer solutions (PBS) (pH 6.0, pH 7.0, pH 8.0 and pH 9.0), the pH values were corrected with 1 mM HCl and NaOH solutions. The DP voltammograms were background-subtracted and baseline-corrected, and then all data were analyzed and treated with the software Origin 8®.

Determination of piroxicam in capsules

In order to quantify PRX content in capsule pharmaceutical form, three approaches were taken for comparative reasons: spectrophotometry, HPLC and the proposed PGE electroanalytical voltammetric assay. In the electroanalysis, stock solutions for the PRX capsules were prepared by weighting 20 capsules, and then transferring powder equivalently to produce a 1 $mmol.L^{-1}$ solution in a 200 mL volumetric flask. The flask was then completed with 1 $mmol.L^{-1}$ aqueous NaOH solution. The resulting solution was sonicated in room temperature (22 ± 1 °C) for 20 minutes and then filtered in filter paper. Stock solution was prepared shortly prior to analysis.

The spectrophotometric measurements were carried out by using a UV-vis spectrophotometer (Quimis, model Q-798U2VS, Brazil). All samples were analyzed in a 1 cm glassy cell length at room temperature. 20 capsules were weighted, their contents were removed and then, weighted again. After homogenization, an amount equivalent to 25 mg of PRX was transferred to a 250 mL volumetric flask and the volume was completed with 0.1 $mol.L^{-1}$ aqueous sodium hydroxide solution. Dilution was performed in order to yield a final concentration of 10 μg/mL. The standard solution was prepared in the same manner. Absorbances were measured in 354 nm, using 0.1 $mol.L^{-1}$ aqueous sodium hydroxide solution as a blank.

HPLC coupled to ultraviolet detector was performed at 248 nm with a columm (300 mm of length and 3.9 mm of diameter) packed with C_{18} (10 μm) kept at room temperature. The mobile phase (flow rate was 2 mL/minute) was a mixture (6:4) of methanol and dibasic sodium phosphate

buffer. 20 capsules were weighted, their contents were removed and, then, weighted again. After homogenization, quantity equivalent to 10 mg of piroxicam was transferred to a 200 mL volumetric flask, then added 150 mL of 0.01 mol.L^{-1} methanolic cloridric acid. The resulting solution was stirred and left in ultrasound, at room temperature, for 30 minutes. The volume was completed with 0.01 mol.L^{-1} methanolic cloridric acid solution. The standard solution was prepared in the same manner, in 0.01 mol.L^{-1} methanolic cloridric acid.

RESULTS AND DISCUSSIONS

Electrochemical performance of PGE

In order to evaluate and ascertain optimal analytical parameters, the DPV assays were performed for PRX in different acetate and phosphate buffer solutions pHs. Acidic pH values showed higher anodic peaks, as PRX pKa stands between 3 and 4, nevertheless, the best signal was observed in pH 3 (Figure 1), and therefore this pH buffer solution was employed in all further assays.

Cyclic voltammetries with PGE and glassy carbon electrode (GCE) showed a single anodic peak, as shown in Figure 2A. Moreover, SWV did not show cathodic peaks with significant difference from the blank assays (Figure 2B). PRX seems to have an irreversible electro-oxidation reaction in acidic pHs ($I_{pa}/I_{pc} > 1.0$), as presented in previous studies and in the SWV herein conducted (Shaikh et al., 2017; Torriero *et al*., 2006).

As presented in Figure 2, an anodic peak was observed in E_{pa} *c.a*. 0.6 V which correlates to the oxidation of PRX over PGE surface. Due to the potential associated to the faradaic peak herein reported and the particularities

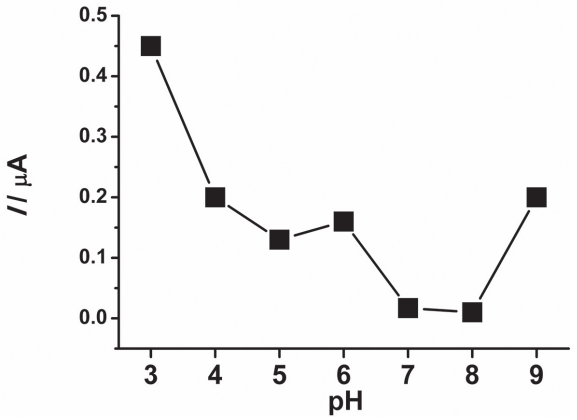

FIGURE 1 - Plot of DP voltammetry peak current values of PRX in different pH acetate/phosphate buffer solutions.

of PRX chemical structure, it can be implied that the oxidative process occurs in the phenolic moiety of PRX. Nonetheless, literature reports that a Nernstian single proton/electron oxidative process is associated to PRX under voltammetries taken at anodic scans, which further corroborates to the results herein discussed (Gholivand, Malekzadeh, Derakhshan, 2014; Gholivand, Karamian, 2011; Macêdo, 2018).

Concerning PRX electrochemical dynamics, the observed redox process seems to be controlled by diffusional effects, hence the linearity observed in the I_{pa} (A) *vs.* $v^{1/2}$ (mV.s^{-1})$^{1/2}$ plot, where I_{pa} stands for the anodic peak current and $v^{1/2}$ stands for the scan rate of each CV performed (Figure 3).

Furthermore, sequential analyses performed without PGE polishment showed decrease in analytical signal (Figure 4), as expected, due to the adsorptive effect of organic molecules on the electrode surface.

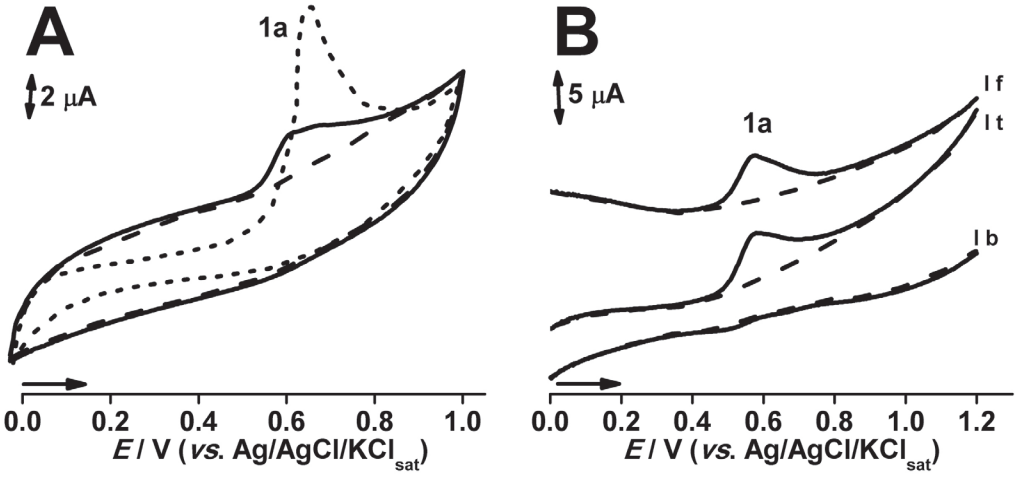

FIGURE 2 - 0.1 mmol.L^{-1} PRX cyclic voltammograms with PGE (——) and glassy carbon electrode (• • •) (A) and square wave voltammetry (B), likewise, depicting an anodic peak (I_t as total current, I_f as forward current and I_b as backward current) (blank assays are depicted in – – –).

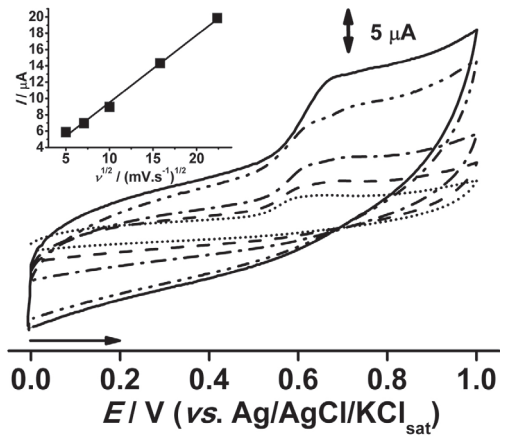

FIGURE 3 - 0.1 mmol.L^{-1} PRX cyclic voltammograms performed at different scan rates (25 mV.s^{-1} • • •; 50 mV.s^{-1} - - -; 100 mV.s^{-1} – • –; 250 mV.s^{-1} – • •; 500 mV.s^{-1} ——) (*Inset*: Linear plot of peak current *vs*. ν $^{1/2}$).

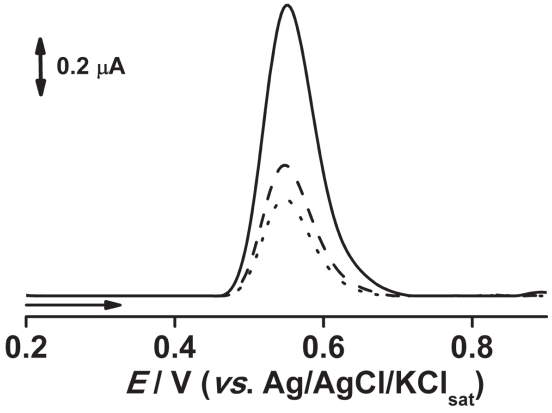

FIGURE 4 - Graph displaying three sequential 0.1 mmol.L^{-1} PRX DP voltammograms, displaying the electrode area adsorption effect on response signal (Scan 1 ——; Scan 2 - - -; Scan 3 • • •).

PRX capsules determination and comparison between approaches

The electroanalytical, spectrophotometric and chromatographic methods herein performed were validated according to RDC n° 166/2017 (Ministério da Saúde, 2017) and ICH guidelines (2005) for validation of analytical procedures.

Several statistical and analytical parameters, such as linear correlation coefficient (r), LOQ, LOD and concentration range were calculated. Moreover, recovery assays were performed with HPLC, spectrophotometry and voltammetry.

Intra-day and Inter-day assays were performed with excipient (placebo) added to the analytical solution, in proportion of 1:1000 (drug: excipient).

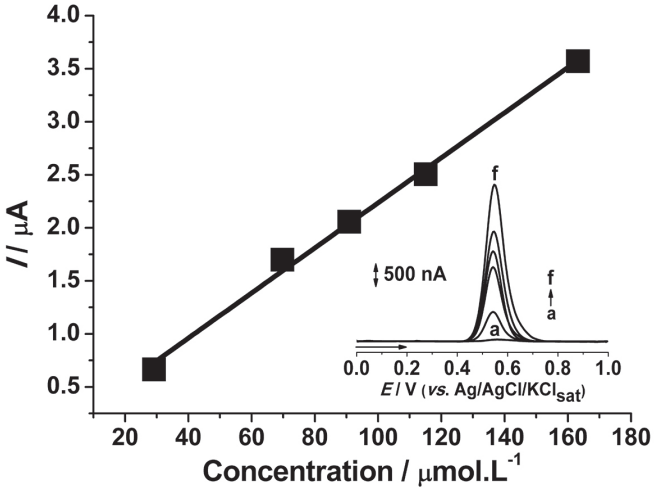

FIGURE 5 - Calibration curve and linear regression calculated by DPV assays for PRX in the following concentrations (a- blank assay): b- 29.13, c- 69.77, d- 90.91, e- 115.04 and f- 163.18 μmol.L^{-1}.

The proposed determination method showed accuracy, robustness and precision within required parameters of official compendia (below 5 % of variation in recovered value and RDS). These parameters with the voltammetric approach were quite similar to the values found for the chromatographic method and showed variations smaller than those found in the spectrophotometry.

TABLE I - Analytical parameters calculated in the voltammetric, spectrophotometric and chromatographic assays for PRX capsules.

Avaliated Parameter	Voltammetry	Chromatography	Spectrophotometry
Precision Repeatability* (RSD)(%)	1.5	0.9	2.1
Intermediary Precision** (RSD)(%)	1.8	1.1	3.4
Limit of Detection (LOD) (μmol.L^{-1})	2.10	0.18	19.83
Limit of Quatification (LOQ) (μmol.L^{-1})	4.70	0.59	65.44
Linearity (r)	0.9946	0.9978	0.9912
Range (μmol.L^{-1})	29.13 – 163.18	10.14 – 50.78	20.12 – 105.24

* Intra-day assays are shown in RSD(%), n = 3. **Inter-day are shown in RSD(%), n = 6.

Chromatography showed a slightly better performance than the voltammetric method in the intra and inter-day assays, probably due to the intrinsic variations in the PGE surface area after each polishment procedure.

The voltammetric assays performed for the piroxicam capsules determination showed recovery of 98.9 ± 0.04 % (mean ± SD) of the labeled value.

PRX capsules determination reported in studies with electrochemically modified electrodes (EQMs) showed LOD and LOQ with values lower than 10 nmol.L^{-1} (Gholivand, Malekzadeh, Derakhshan, 2014; Gholivand, Karamian, 2011; Norouzi, Ghaheri, 2011). However, given the simpler handling and preparation of PGE, the LOD value of 2.1 µmol.L^{-1} and LOQ value of 4.7 µmol.L^{-1} (Table I) seem adequate when the assay does not demand analytical scales lower than µmol.L^{-1}, which is the of determination in capsules.

CONCLUSION

PGE voltammetric assessment of PRX showed analytical performance similar to the chromatography. Chromatographic determination still demonstrated superiority in aspects such as accuracy, selectivity and precision. Also, voltammetric determination of PRX capsules with EQMs have higher sensitivity than with PGE.

Therefore, the overall performance of the proposed method is outstanding, and given the cost/benefit associated to voltammetrical assessment, it is reasonable to say that PGE is an excellent alternative assess PRX in pharmaceutical formulae.

The proposed PGE was cheap, easy to use and simple to clean, as well as providing results that are equivalent to official methods, showing a high potential to be applied in simple and fast assays.

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