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Flow injection amperometric determination of dipyrone in pharmaceutical formulations using a carbon paste electrode

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Abstract

The behavior of a carbon paste electrode was investigated as an amperometric detector for the determination of dipyrone by flow injection analysis (FIA). The electrode presented low cost and easy construction by simple mixing of graphite powder and mineral oil. Initially, an electrochemical study of the dipyrone oxidation at a carbon paste electrode has been developed before its use in the FIA system. The oxidation currents monitored at +0.35 V versus Ag/AgCl, were proportional to the dipyrone concentrations. Experimental parameters, such as nature of supporting electrolyte, pH of the carrier solution, flow rate, sample volume injection and probable interferents were investigated. Under the best experimental conditions selected, the calibration curve for dipyrone was linear in the concentration range from 4.91×10^{-6} to 2.50×10^{-4} mol l^{-1} ($I_{anodic}/\mu A$) = 0.056 + 81.06 [dipyrone]) with a detection limit of 2.07×10^{-6} mol l^{-1}. Recoveries ranged from 93.8 to 100.8% and an analytical frequency of 130 h^{-1} was achieved. The proposed flow procedure has been satisfactorily applied to the determination of dipyrone in several pharmaceutical formulations.

Keywords: Carbon paste electrode; Dipyrone; Amperometric detection; Flow injection analysis

1. Introduction

The dipyrone (sodium salt of the 1-phenyl-2,3-dimethyl-4-methyl aminomethane sulfonate-5-pyrazolone), is a soluble white crystalline powder that presents analgesic and antipyretic activity [1]. Dipyrone acts at central and outlying level simultaneously, presenting fast, uniform and almost complete absorption. About 58% of the dose links to the plasma proteins, and the effect of the drug occurs approximately fifteen minutes after its administration. The biotransformation of the drug takes place at hepatic level, and the duration of its effect is approximately from 4 to 6 hours, and its elimination takes place via renal system. The drug can cause occasional or rare reactions, such as: transitory renal disturbances and inflammation of the renal tissue, mainly in patients with renal disease history or in the cases of overdose [2].

The methods commonly used for dipyrone determination in several pharmaceutical formulations are based on its reaction with iodide [3,4]. Spectral methods such as ultraviolet-visible absorption [5], fluorescence [6], and chemiluminescence [7,8] are also reported as alternatives for dipyrone determination.

Alternative automatic procedures based on flow injection technique with amperometric and voltammetric detectors have been widely suggested in pharmaceutical, food, forensic and clinical sciences [9,10]. The main factors that it contributes are low consumption of reagents and samples, better repeatability, high sample throughput, easier medium exchange after analyte accumulation, reduction of the risk of contamination during the analysis step, combined with good precision and high sensitivity, good selectivity, as well as relative low cost of the instrumentation. However, as far as it is

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known, the determinations of dipyrone using electroanalytical methods have been very few investigated. In a previous work, Perez-Ruiz et al. [11] employed a glassy carbon as working electrode as a detector in an amperometric flow cell. This flow injection procedure presented a rectilinear response in the dipyrone concentration range from 3.0×10^{-6} to 3.0×10^{-5} mol l^{-1} in an ammonium buffer solution at a potential of 0.4 V versus Ag/AgCl.

The flow injection amperometric simultaneous determination of dipyrone, ascorbic acid, dopamine and, epinephrine using modified microelectrodes, together with multivariate calibration analysis was proposed by Matos et al. [12]. The determination is based on a multichannel detection system, coupled in a flow cell containing an array of modified microelectrodes. Recently, the same authors developed a flow cell containing gold electrodes from recordable compact discs for the determination of dipyrone in pharmaceutical formulations [13].

In this paper, a FIA procedure with amperometric detection for the determination of dipyrone in pharmaceutical formulations is proposed, using a carbon paste electrode. The influence of several parameters (potential, pH and interference) besides the parameters of the flow system was studied.

2. Experimental

2.1. Apparatus

Cyclic voltammetric and amperometric measurements were carried out with an AUTOLAB PGSTAT-30 (Ecochemie) controlled by a personal computer using the GPES 4.8 software. The measurements were performed in a three-electrode flow cell configuration (Fig. 1) using a carbon paste electrode (CPE) as working, an Ag/AgCl as reference and a platinum auxiliary electrodes ($\phi = 3$ mm disk).

For cyclic voltammetry the potential was ranged from 0.0 to +1.3 V versus Ag/AgCl at a scan rate of 10 mV s^{-1}, stationary solutions were used in such case. The current measurements were performed using the GPES software (Ecochemie) by chronoamperometry (constant potential).

The body of the electrochemical flow cell (Fig. 1A) was fabricated with polyurethane resin from vegetable oil [14]. The effective volume of the flow cell was of 77 μl.

2.2. Reagents and solutions

All solutions were prepared using water from a Millipore Milli-Q system. All chemicals were of analytical reagent grade and were used without further purification. The supporting electrolyte used for all experiments was a 0.1 mol l^{-1} sodium acetate solution (pH 7.3). A 0.01 mol l^{-1} dipyrone stock solution was prepared daily by dissolving $C_{13}H_{16}N_3NaO_4S.H_2O$ (Merck) in 100 ml of the same sodium acetate solution and used to prepare dipyrone reference solutions in 0.10 mol l^{-1} sodium acetate solution (pH 7.3).

Pharmaceutical formulations containing dipyrone, such as Novalgina® (Hoeschst Marion Roussel), Anador® (Boehringer Ingelheim) and Magnopyrol® (Farmasa L.A.F.) were used in order to evaluate the FIA procedure performance in dipyrone determination.

2.3. Preparation and analysis of the pharmaceutical samples

For the analysis of tablet formulations, an accurate amount (0.2077 g) was transferred to a 50.0 ml volumetric flask and then the volume was completed with sodium acetate solution (pH 7.3). The liquid formulations (176 μl) were diluted with 0.10 mol l^{-1} sodium

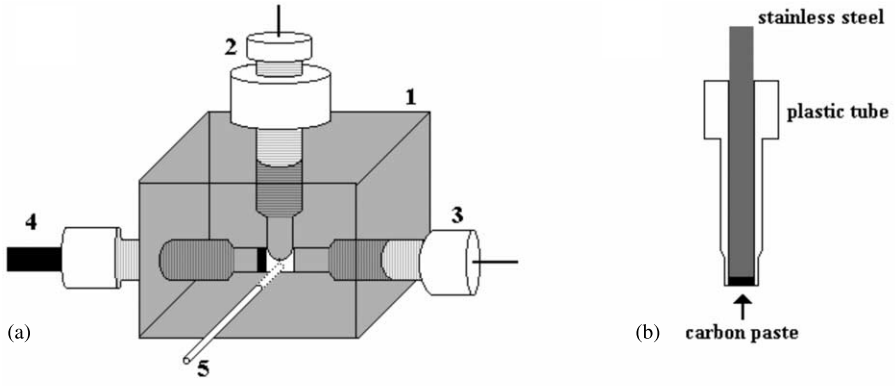

Fig. 1. Schematic diagram of the electrochemical flow cell used in the amperometric measurements in flow injection system. (A) 1. Polyurethane resin block; 2. reference electrode (Ag/AgCl); 3. platinum electrode; 4. carbon paste electrode; 5. polyethylene tubing (flow). (B) Carbon paste packed into an electrode body.

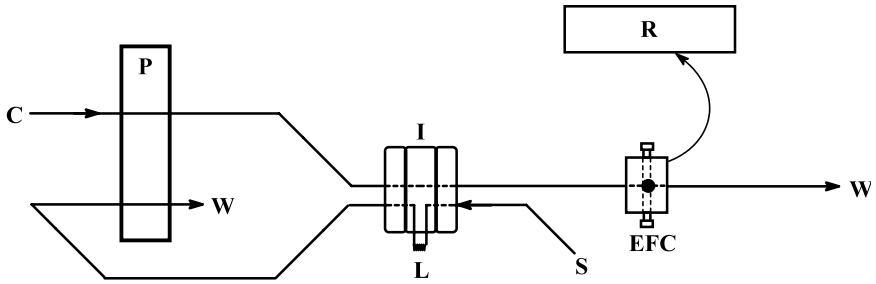

Fig. 2. Schematic diagram of the flow system used for evaluation of the carbon paste electrode for dipyrone determination. P, peristaltic pump; I, manual injector; S, sample or reference solutions; L, sample volume; C, carrier solution; EFC, electrochemical flow cell; R, amperometer (recorder); W, waste.

acetate solution. No additional treatment of the sample was required.

The content of dipyrone in these samples was determined by the standard addition method and compared with the iodimetric standard procedure [4].

2.4. Preparation of the carbon paste electrode

Carbon paste electrode was prepared by carefully mixing with 75% (m/m) of graphite powder (1–2 μm particle size, Aldrich) and 25% (m/m) of mineral oil (Aldrich). This resulting mixture was submitted to magnetic stirring in a beaker (50 ml) containing 20 ml of hexane. The final paste was obtained by the solvent evaporation. The carbon paste was packed into an electrode body (see Fig. 1B), consisting of a polyethylene cylindrical tube (o.d. 7 mm, i.d. 4 mm) equipped with a stainless steel rod serving as an external electric contact. Appropriate packing was achieved by pressing the electrode surface (surface area of 0.126 cm^2) against a filter paper.

2.5. Flow injection analysis system for dipyrone determination

The electrochemical cell was inserted in a one-channel flow injection system schematically represented in Fig. 2. The system was assembled with a peristaltic pump (Ismatec, model 7618-40, Switzerland) and a manual injector made of Perspex® with two fixed sidebars and a sliding central bar [15]. The manifold connections were made with polyethylene tubing (0.76 mm i.d.).

The 0.10 mol l^{-1} sodium acetate solution was used as the carrier solution (C) at a flow rate of 5.0 ml min^{-1}. The dipyrone reference in 0.10 mol l^{-1} sodium acetate solution contained in the sample volume loop (l, 408.6 μl) was injected and transported by the carrier stream after the baseline had reached a steady-state value. The analytical path was 30 cm and the entire flow injection system was kept at room temperature.

3. Results and discussion

3.1. The electrochemical oxidation reaction of the dipyrone at carbon paste electrode

The carbon paste was chosen as working electrode is in view of the fact that this electrode does not require electrochemical pretreatment as the glassy carbon electrode do [16,17]. Other advantages of the carbon paste electrode are renewability, simplicity, quick preparation and low cost.

The oxidation of dipyrone was studied by cyclic voltammetry in order to elucidate its electrochemical behavior. The electrochemical oxidation of dipyrone is represented by irreversible peaks at +0.33 V (peak 1), +0.57 V (peak 2) and +1.0 V (peak 3) versus Ag/AgCl/3

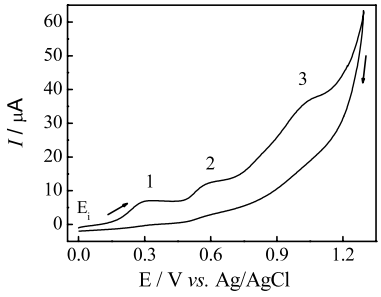

Fig. 3. Cyclic voltammogram for 1.0×10^{-3} mol l^{-1} dipyrone in sodium acetate solution (pH 7.3) at the carbon paste electrode. Scan rate 10 mV s^{-1}; Scan potential 0.0–1.3 V. Peak 1 = +0.33 V, peak 2 = +0.57 V and peak 3 = +1.0 V.

mol l^{-1} KCl in 0.10 mol l^{-1} sodium acetate solution (pH 7.3) as shown in Fig. 3. The magnitude of peak currents decreased with increasing of the number of cycles. The effect of square root of potential scan rate ($mV^{1/2}$ $s^{-1/2}$) on the peak current was linear in the potential scan rate from 1 to 100 mV s^{-1}, revealing a diffusion controlled rate reaction.

The effect of the pH on the oxidation of dipyrone (1.0×10^{-4} mol l^{-1}) was investigated over a pH range between 3.0 and 8.0 The results are presented in Fig. 4 which presents the cyclic voltammograms of dipyrone in three differents pHs at the CPE. Increasing the pH from

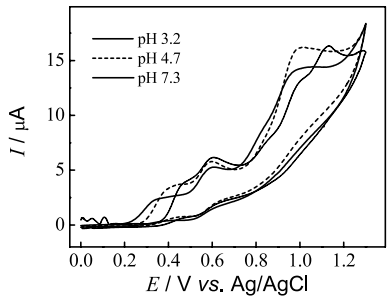

Fig. 4. Voltammetric behavior of 1.0×10^{-4} mol l^{-1} dipyrone in sodium acetate solution on a carbon paste electrode in different pHs. Scan rate 10 mV s^{-1}. PH 3.2 (dot line); pH 4.7 (dash line) and pH 7.3 (solid line).

3.2 to 7.3 it was observed a negative shift in the peak potential, becoming practically constant above pH 7.3. Although in more acid conditions higher oxidative currents have been observed, the pH 7.3 was chosen for further studies since a lower peak potential (ca. 200 mV) is observed which is favorable for the amperometric detection required in flow procedures, minimizing interferences. Perez-Ruiz et al. [11] also investigated the pH effect in dipyrone oxidation, but using a glassy carbon electrode. They also concluded that the alkaline pH was more suitable for the determination of the analite.

3.2. Flow injection analyis (FIA)

Fig. 5 presents the hydrodynamic voltammograms for 1.0×10^{-3} mol l^{-1} dipyrone in 0.10 mol l^{-1} sodium acetate solution (pH 7.3) under several potentials (+0.10 to +0.90 V vs. Ag/AgCl) and the effect of the sample volume from 81.7 to 612.8 μl at a fixed potential of +0.35 V.

The peak currents increased with increasing working potential from +0.20 V and above (Fig. 5A). The working potential of +0.35 V versus Ag/AgCl was chosen for FIA measurements. It is important to point out that the optimized working potential of +0.35 V matches exactly with the first oxidation peak (see Fig. 3).

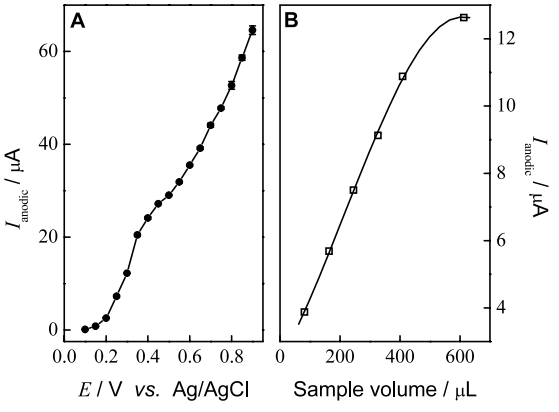

Fig. 5. Hydrodynamic responses for FIA do dipyrone on the CPE with to (A) applied working potential and (B) sample volume.

The effect of the sample volume from 81.7 to 612.8 μl, on the analytical signal was also investigated by changing the length of the sample loop (10–75 cm, 0.76 mm i.d.) for a 1.0×10^{-4} mol l^{-1} dipyrone in 0.10 mol l^{-1} sodium acetate solution at pH 7.3. As can be observed in Fig. 5B, the amperometric response increased with the increase of sample volume from 81.7 to 408.6 μl and became almost constant for sample volumes higher than 408.6 μl. Therefore, a sample volume of 408.6 μl was selected for showing better engagement between sensitivity and analytical frequency.

The effect of the flow rate on the magnitude of amperometric response was investigated by applying the working potential of +0.35 V and sample volume of 600 μl. The results showed that the flow injection current response is dependent of the flow rate. The current increases with flow rate reaching a maximum value at 5.0 ml min^{-1}. Increasing in flow rates causes a change in the diffusion profile at the electrode surface and consequently an increase of the diffusion current or a more efficient mass transport [18]. The limited electrode response at flow rates higher than 5.0 ml min^{-1} is caused by the dispersion of the sample and residence time in the detector. A flow rate of 5.0 ml min^{-1} was used in the further experiments, which maintained a good sensitivity and stability of amperometric response. The analytical path (manual injector to electrochemical flow cell) was studied in the range of 20–60 cm under the same experimental conditions as selected before. The results obtained showed that the amperometric response of the CPE was practically constant in that interval. Therefore, an analytical path of 30 cm was selected.

3.3. Analytical curve and reproducibility

After optimizing the best operating conditions for the FIA procedure, amperometric measurements were carried out in sodium acetate solution (pH 7.3) containing different dipyrone concentrations in order to obtain the analytical curve. Fig. 6 illustrates the FI current–time response for different dipyrone concentrations. The current values (at +0.35 V) obtained gave a linear relationship with the dipyrone concentrations from 4.91×10^{-6} to 2.50×10^{-4} mol l^{-1} (see inside Fig. 6). This plot could be represented by the equation (I_{anodic}/μA) = 0.056 + 81.06 [dipyrone]) with a correlation coefficient of 0.9997 ($n = 6$). To higher concentrations (> 2.5×10^{-3} mol l^{-1}) occurs the deviation of linearity. The detection limit was 2.07×10^{-6} mol l^{-1} (three times the standard deviation of the intercept/slope) and quantification limit of 6.91×10^{-6} mol l^{-1} of dipyrone (ten times the standard deviation of the intercept/slope). The relative standard deviation (RSD) for ten replicates

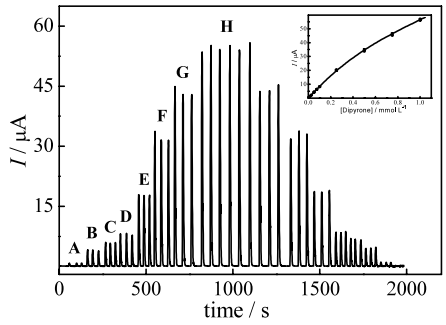

Fig. 6. Transient current signals obtained in triplicate for dipyrone solutions: (A) 0.025; (B) 0.050; (C) 0.075; (D) 0.10; (E) 0.25; (F) 0.50; and (G) 0.75 and (H) 1.0 mmol l^{-1} dipyrone. The inset shows the analytical curve for dipyrone. Applied working potential = 0.35 V vs. Ag/AgCl; sample volume = 408.6 µl of dipyrone; flow rate = 5.0 ml min^{-1}.

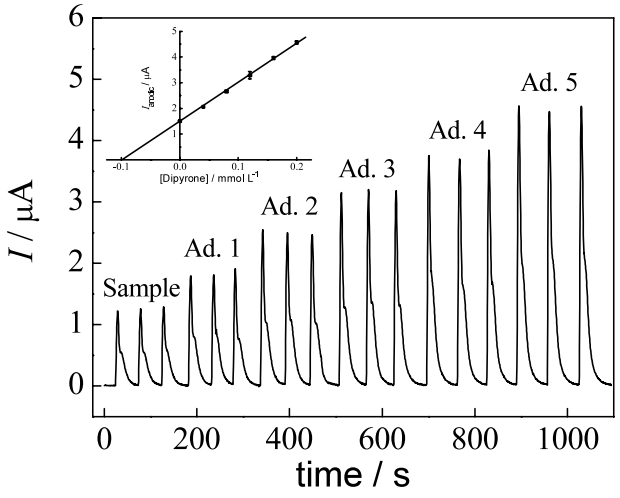

Fig. 7. Transient current signals obtained in the determination of dipyrone in Novalgin® using multiple standard addition procedure. The inset shows the standard addition curve. Applied working potential = 0.35 V vs. Ag/AgCl; sample volume = 408.6 µl of dipyrone; flow rate = 5.0 ml min^{-1}.

of 5.0×10^{-5} mol l^{-1} dipyrone was 3.7% and the analytical frequency was 130 determinations per hour.

3.4. Effect of concomitant substances, recovery and analysis of dipyrone in pharmaceutical samples

In order to investigate the analytical application of this method, the effect of the other components of the pharmaceutical formulations was investigated by carrying out the determination of 1.0×10^{-4} mol l^{-1} dipyrone in the presence of each excipient (potassium metabisulfite, saccharine, EDTA, caffeine, ascorbic acid and propylene glycol) at concentrations that can be found in those formulations. No significant interference in the flow injection procedure was observed up to 10fold excess for those excipients. The Table 1 shows the results of addition-recovery of the dipyrone in pharmaceutical formulations containing 0.1 mmol l^{-1} dipyrone. Recoveries ranging from 93.8 to 100.8% of dipyrone were obtained using FIA amperometric procedure. This is a good evidence of the accuracy of the proposed method.

Table 1
Results of addition-recovery experiments using FI amperometric procedure for three different standard concentrations of dipyrone in pharmaceutical formulations containing 0.1 mmol l^{-1} dipyrone

Sample	Dipyrone/mmol l^{-1}		Recovery (%)
	Added	Found	
Novalgina®	0.040	0.039	97.5
	0.080	0.078	97.5
	0.120	0.118	98.3
Anador®	0.040	0.039	97.5
	0.080	0.078	97.5
	0.120	0.121	100.8
Magnopyrol®	0.040	0.039	97.5
	0.080	0.075	93.8
	0.120	0.116	96.7

The proposed FIA amperometric procedure was applied for the determination of dipyrone in pharmaceutical formulations (see Table 2). The dipyrone content was determined by the standard addition method (see in Fig. 7 Novalgin® analysis) and compared with that obtained by official method (iodimetric titration) [4]. The iodimetric method consists in the dipyrone titration in hydrochloric acid medium with a standardized solution of iodine using starch as indicator. The statistical calculations for the assay results showed good precision of the FIA amperometric method. The results obtained were also compared by applying the F-test and t-test at 95% confidence level. In either case the calculated F or t values not exceed the theoretical values ($F_{3,3} = 9.28$, $t_6 = 2.45$), confirming that there are no significant differences between the results obtained by both procedures confirming the importance of the proposed flow injection presented in this paper.

Table 2
Mean results obtained for the determination of dipyrone in pharmaceutical formulations by FI amperometric procedure in comparison with the official method [4]

Samples	Label value	Iodimetric [a]	FIA method [a]	E_r
Novalgina® [b]	500	505±2	509±2	+0.8
Anador® [b]	500	486±3	503±2	+3.5
Magnopyrol® [c]	500	520±2	527±2	+1.3

E_r, relative error = FIA method vs. iodimetric method.
[a] Average of five determinations ± SD
[b] Milligrams of dipyrone per milliliter.
[c] Milligrams of dipyrone per tablet.

4. Conclusions

This work demonstrates the use of a CPE as an amperometric detector in FIA for dipyrone determination in pharmaceutical formulations. The method showed to be fast, simple and precise among other interesting features of the method proposed which make it applicable to dipyrone analysis and quality control of pharmaceutical samples. However, as far as it is known, there are few papers reporting the oxidation mechanism of the dipyrone [11]. In our laboratories the oxidation products of dipyrone is being studied by nuclear magnetic resonance after exhaustive electrolysis experiments. Different from the techniques mentioned in the introduction, the addition of an oxidant to the reaction system is unnecessary in the proposed method. Sample pre-treatment is also not needed. In comparison to the other electrodes the carbon paste showed to be very stable in usage times of several hours without requiring chemical or mechanical surface renovation. Cyclic voltammetric studies show the superiority of carbon paste electrode over glassy carbon electrode [11] in terms of reproducibility, sensitivity and low background current (capacitive current).

Acknowledgements

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