Full Paper

Determination of Piroxicam in Pharmaceutical Formulations Combining Amperometry and Multicommutation

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Abstract
This work describes the development of a multicommutated flow system with amperometric detection, applied in the determination of piroxicam in pharmaceutical formulations.
In order to prevent the electrode surface fouling caused by sample excipients, the tubular glassy carbon working electrode was modified with a Nafion film, which allowed to improve the repeatability of measurements, compared with the bare electrode. The developed unit was coupled to a multicommutated flow system, which was designed in a way to enable the analysis of different pharmaceutical formulations containing piroxicam and various hydrophilic or lipophilic excipients.
The automated flow system presented a linear range up to 5.00×10^{-4} mol L^{-1} and a detection limit of 1.0×10^{-5} mol L^{-1}. The system was applied in the analysis of several pharmaceutical formulations containing piroxicam and no statistically significant difference between the results obtained by the proposed and the reference methods was found, for a 95% confidence level. Repeatability in the analysis of samples (expressed in *RSD*) was lower than 5% ($n = 10$).

Keywords: Piroxicam, Amperometry, Modified tubular electrode, Nafion, Multicommutation

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1. Introduction

Usually, the quantification of a drug included in different pharmaceutical forms implies the use of distinct procedures, due to the variations in the composition and characteristics of the diverse forms. As a result, different sample processing leads to more complex and time-consuming methodologies, restraining process automation which is essential in routine analysis.

Flow analysis systems represent an advantageous alternative to the methodologies used in pharmaceutical analysis in terms of automation, reagent consumption, cost and simplicity of manifolds. Particularly, multicommutated flow systems [1] are characterized by the high versatility concerning the adaptation of the manifold configuration to any specific determination. The flow network formed by the commutation devices connected to each other in different configurations allows the flowing stream to be directed through distinct analytical pathways, according to the preset time-based computer routine.

Piroxicam is an antiinflammatory drug belonging to the oxicams group, and can be found in diverse pharmaceutical formulations. Its quantification in these samples has prompted the need for the development of analytical methodologies, namely potenciometric [2], spectrophotometric [3–7] and spectrofluorimetric [8–10].

The aim of this work was to develop a multicommutated flow system with amperometric detection, using a tubular glassy carbon working electrode modified with Nafion, for the determination of piroxicam in different pharmaceutical forms. The electrode modification with Nafion is intended to prevent the probable fouling of the electrode surface by the excipients, namely the surfactants and lipophilic macromolecules [11, 12], enabling the use of the same electrode for a larger period of time and reducing the frequency of cleaning steps.

2. Experimental

2.1. Reagents and Solutions

Reagents of p.a. quality or similar were used without having been subjected to any additional purification. In the preparation of solutions, water purified by the Millipore Milli Q system (conductivity < 0.1 μS cm^{-1}) was used.

As carrier solution in the flow system and, simultaneously, supporting electrolyte, a $C_6H_7O_7^-/C_6H_6O_7^{2-}$ buffer solution (pH 5.3), prepared by mixture of 10.0 mL of a 1.00 mol L^{-1} citric acid solution and 24.0 mL of a 1.00 mol L^{-1} NaOH solution, diluted to 100 mL, was used.

In the supporting electrolyte optimization studies, a $C_6H_7O_7^-/C_6H_6O_7^{2-}$ buffer solution, prepared from 2.00 mol L^{-1} citric acid and 2.00 mol L^{-1} NaOH solutions, a $H_2PO_4^-/HPO_4^{2-}$ buffer solution, prepared from 1.00 mol L^{-1} NaH_2PO_4 and 1.00 mol L^{-1} Na_2HPO_4 solutions, and a NH_4^+/NH_3 buffer solution, prepared from 2.00 mol L^{-1} NH_4Cl and 2.00 mol L^{-1} NH_3 solutions were used.

A 1.00×10^{-3} mol L^{-1} piroxicam stock solution was prepared by weighing and dissolution of piroxicam (Sigma) in dimethylsulfoxide (DMSO) (Fluka), and was kept protected from light. Standard solutions were prepared by diluting the piroxicam stock solution in DMSO.

For the working electrode surface coating, a 1% (v/v) Nafion solution in ethanol was used, being prepared from a 5% (m/v) Nafion perfluorinated ion-exchange resin solution (Aldrich).

Samples of pharmaceutical formulations containing piroxicam, commercially available in Portugal, were analyzed. For each sample, a solution with a piroxicam concentration of 2.00×10^{-4} mol L^{-1} was prepared, dissolving or diluting the sample in DMSO. Samples with lipophilic excipients were filtered before being inserted in the flow system.

2.2. Equipment

In the developed multicommutated flow system (Fig. 1) solutions and samples were aspirated by an automatic burette (Crison model Micro BU 2031) equipped with a 10 mL syringe (Hamilton 1010). To control the selection and direction of solutions and samples inside the manifold three 3-way solenoid valves (NResearch 161 T031) were used. A homemade power driver, based on an integrated ULN 2003 circuit, was used to operate solenoid valves [13]. Control of the analytical system was made through an interface card (Advantech PC-LABCard model PCL-711B) and a microcomputer. The software was developed in QuickBasic version 4.5 (Microsoft) and allowed to control the functioning of the burette and the solenoid valves. Connection between the components of the flow system was made with Teflon tubes (Omnifit), of 0.8 mm inner diameter. Amperometric measurements were carried out in an Autolab electrochemical system (Eco Chemie model PGSTAT 10) and data acquisition was accomplished through GPES software (version 4.6).

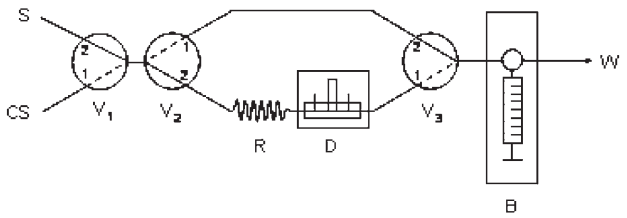

Fig. 1. Multicommutated flow system for piroxicam determination: V_1, V_2 and V_3, three-way solenoid valves; S: sample; CS: carrier solution ($C_6H_7O_7^-/C_6H_6O_7^{2-}$ buffer solution, pH 5.3); R: reactor; D: tubular amperometric detector; B: automatic burette equipped with a 10 mL syringe; W: waste.

To perform sample analysis by the reference method prescribed by the British Pharmacopoeia [14], a chromatograph (Merck model Hitachi LaChrom L-7100), with a column (Merck LiChrosorb RP-C18, 5 μm, 250 mm) and a UV-vis detector (Merck Hitachi L-7455) were used for the analysis of capsules, tablets and granules, and a UV-vis spectrophotometer (Perkin Elmer Instruments) was used for the analysis of ampoules.

2.3. Amperometric Detector with a Nafion Modified Tubular Electrode

Typically, in multicommutated flow systems, solutions are aspirated instead of being propelled, which simplifies the flow manifolds since, in this case, only one propulsion device is needed for the driving of all solutions. As a consequence, flow systems present an inner pressure lower than the atmospheric pressure, demanding that all manifold components, including the detector, are tightly fixed, in order to avoid air entrance. The construction of the amperometric detector was based in a tubular detector with modified electrodes recently described [15], which demonstrated to have the required robustness to be used in multicommutated flow systems in which solutions are aspirated.

For cleaning and modification (coating of the active surface) of the working electrode, this was withdrawn from the tubular detector and was firstly polished, using a cotton thread soaked in diamond spray (Ziesmer diamond spray 1 μm). Then, it was cleaned through ultra-sounds in ethanol, for 2 min, and finally washed with deionized water. The surface coating, by the droplet evaporation method, consisted in the deposition of 20 μL of a 1% (v/v) Nafion solution directly into the central orifice of the electrode. It was kept at room temperature (about 25 °C) until the complete evaporation of the solvent (about 30 min).

2.4. Automatic Procedure

The multicommutated flow system (Fig. 1) comprised three 3-way solenoid valves (V_1, V_2 and V_3). Valve V_1 was used to select the insertion of carrier solution (CS) or sample (S), while valves V_2 and V_3 were simultaneously operated in order to define two parallel analytical pathways, in which one included the reactor (R) and the tubular detector (D) and the other was intended to carry out the exchange of samples, avoiding their passage through the detector. By this means, the contact time between sample and working electrode was reduced and, thereby, the passivation of its surface was minimized. Moreover, it allowed a faster sample exchange, increasing the sampling rate. The automatic burette (B) with the syringe was placed at the end of the analytical manifold, after the detector, and was used to aspirate all solutions.

The analytical cycle was initiated with valves V_1 and V_3 in position 2 and valve V_2 in position 1, enabling the insertion of the sample into the manifold, being rejected to waste

without passing through the detector (20 s). Then, V_1 commuted to position 1 to permit the aspiration of carrier solution, cleaning the manifold in both analytical pathways (45 s). Subsequently, V_1 moved to position 2 and, with V_2 and V_3 in positions 2 and 1, respectively, the sample volume was aspirated (3.8 s). Finally, with V_1 and V_3 in position 1 and V_2 in position 2, the sample was directed to the detector (110 s).

3. Results and Discussion

3.1. Characterization and Behavior of the Nafion Modified Tubular Electrode

Since the purpose of the work was to develop an analytical system which allowed the analysis of different pharmaceutical forms containing piroxicam, with the minimum pretreatment, and considering the probable fouling of the working electrode surface owing to adsorption of excipients (namely surfactants and lipophilic macromolecules present in some formulations), the electrode surface was modified with a Nafion film. This polymer was used, not for its ion-exchange properties, but because it formed a semipermeable film at the electrode surface, constituting a barrier to the adsorption of excipients [11, 12].

The optimization of the parameters related to the modification procedure was performed with the aim of obtaining the maximum reproducibility of the measurements. All experiments were carried out with a 1.00×10^{-4} mol L^{-1} piroxicam solution, aspirating into the system a volume of 30 μL, which was directed towards the detector by the carrier solution, at a flow rate of 0.96 mL min^{-1}.

Considering, on one hand, the variability in the composition of the samples' matrices and, on the other, the relative difficulty to dissolve piroxicam, several solvents were tested and their ability to dissolve the samples was compared. It was shown that DMSO could dissolve easily piroxicam, even in high concentrations such as 10^{-3} mol L^{-1}, and also the different samples. Besides, it was miscible with aqueous solutions and presented a low toxicity [16], thus it was chosen for the following experiments.

The influence of the supporting electrolyte/carrier solution composition in the analytical signal intensity was also evaluated, being tested the buffer solutions of $C_6H_7O_7^-/C_6H_6O_7^{2-}$ in the pH range between 2.2 and 6.1, $H_2PO_4^-/HPO_4^{2-}$ (pH between 5.4 and 7.5) and NH_4^+/NH_3 (pH between 8.0 and 10.3). The results indicated that I_p increased with the increase of the pH, from 2.2 to 5.3, decreasing beyond that value. For that reason, the $C_6H_7O_7^-/C_6H_6O_7^{2-}$ buffer solution (pH 5.3) was chosen for the next experiments. As this buffer solution was prepared from citric acid and sodium hydroxide solutions, different concentrations of both solutions were tested (from 0.50 to 3.00 mol L^{-1}). It was shown that I_p increased with the increase of both concentrations up to 1.00 mol L^{-1}, being constant for higher values. Therefore, this value was selected for the following trials.

To evaluate the effect of the tubular electrode modification in the repeatability of the analytical signal intensity, consecutive measurements of two samples with a higher probability to passivate the electrode surface (a gel and a cream) were carried out, and the I_p values obtained for the bare electrode and the modified electrode with a 2% Nafion solution were compared (Fig. 2). The results allowed to conclude that the electrode surface modification with Nafion permitted to reduce its fouling, since the analytical signal intensity was more repeatable for the modified electrode, with *RSD* values 2 to 5-fold lower than those obtained for the bare electrode ($n = 12$).

The influence of the Nafion solution concentration (which affects the film thickness), used in the electrode coating, was then evaluated. Polymer solutions with concentrations of 0.5, 1.0, 2.0 and 3.0% (v/v) were prepared and a volume of 20 μL was deposited in the central orifice of the electrode. The obtained I_p value was less repeatable for the lower concentration, which originated a thinner film, and presented approximately the same repeatability for the other concentrations, being the 1% (v/v) solution used in the subsequent experiments.

To assess the reproducibility of the modification procedure, which was reflected in the reproducibility of I_p, four electrodes were modified in two different days and the I_p values obtained in both days were compared. It was shown that the modification procedure enabled to attain reproducible results since, for each modified electrode, the I_p values obtained with coatings performed in different days presented a *RSD* of about 4%.

The stability of the film formed at the electrode surface was evaluated by carrying out successive determinations of a 1.00×10^{-3} mol L^{-1} piroxicam solution. After 90 consecutive

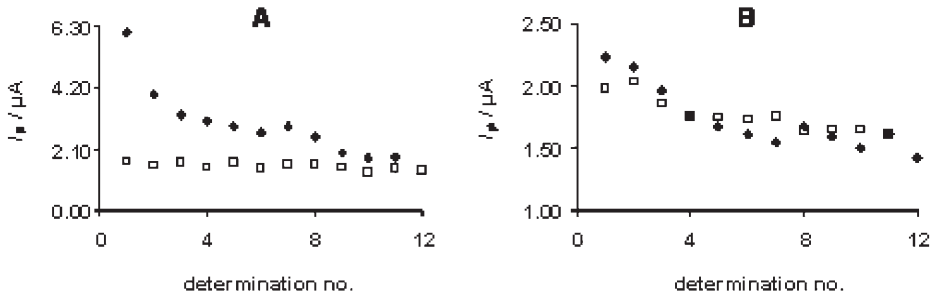

Fig. 2. Variation in I_p through successive determinations of piroxicam in pharmaceutical formulations (A: gel, B: cream), for the bare electrode (◆) and the Nafion modified electrode (□).

measurements, I_p values presented good repeatability, with *RSD* about 5%. These results demonstrated the high stability of the film, subjected to a repetitive contact with DMSO, in opposition to the swelling that occurs with the commercial Nafion membranes, after contact with this solvent, as previously referred [17]. This distinct behavior was, probably, due to the fact that, in this case, determinations were performed using a flow system which enabled the insertion of a reduced sample volume and the minimization of the contact time between the sample and the film, contrarily to what happened in the experiments with the Nafion membranes. Using the same modified electrode during several days, it was observed that I_p was approximately constant for five consecutive days, starting to decrease about 10 to 20% in the following days. Hence, the modified electrode was used throughout five successive days, after which it was withdrawn from the detector and submitted to a polishing to remove the film, followed by a new coating.

The optimization of the potential applied to the working electrode was carried out keeping the experimental conditions referred earlier, this is, aspirating 30 μL of a 1.00×10^{-4} mol L^{-1} piroxicam solution to the system, which was directed to the detector at a flow rate of 0.96 mL min^{-1}. Potential values between 0.6 and 1.0 V were tested and the results indicated an increase in I_p with the increase of the potential up to 0.9 V, remaining constant beyond this value, which was chosen for the next trials.

3.2. Optimization of the Multicommutated Flow System Parameters

Considering that DMSO is a non-aqueous solvent with a reduced electrical conductivity, and that the implementation of controlled-potential experiments requires a conducting solution containing ionic species to decrease the resistance of the solution, to eliminate electromigration effects and to maintain a constant ionic strength [18], the multicommutated flow system was optimized with the purpose to enhance the mixture between sample plug and carrier solution/supporting electrolyte, so that the sample could reach the detector fully homogenized with the supporting electrolyte.

The flow system parameters were optimized by using a factorial design at 2 levels, which enables to evaluate the effect of factors and their interactions, providing the necessary information with the minimal experimentation. Taking into consideration the characteristics of the flow system, four parameters were selected to optimize: the sample volume (V), the sample insertion mode (I), by binary sampling or by unique volume, the reactor length (R) between valve V_2 and the detector and the flow rate (F). A fractional factorial design 2^{4-1} (4 factors and 2 levels) was used to assess which factors most significantly influenced the mixture between sample and carrier solution.

Regarding the sample volume, the values of 30 and 150 μL were chosen for low (−) and high (+) levels, respectively. According to previous studies, volumes lower than 30 μL would significantly diminish I_p and originate less repeatable measurements, and volumes higher than 150 μL might cause noise in the baseline, since the homogenization of the sample plug with the supporting electrolyte would be more difficult to achieve. As for the sample insertion mode, the sample volume could be inserted by using a single plug (level −) or by binary sampling, dividing the volume in 3 smaller plugs, intercalated with carrier solution (level +), knowing that the insertion of the sample volume by binary sampling improves its mixture with the carrier solution. Concerning the reactor length, which was coiled to enhance the mixture, the values of 10 and 50 cm were selected for the (−) and (+) levels. In agreement with preliminary studies, the increase in the reactor length improved the mixture but, simultaneously, caused an unnecessary increase of the sample dilution and a decrease of the sampling rate. In relation to the flow rate, the values of 0.72 and 2.16 mL min^{-1} were chosen. Previous studies showed that lower flow rates enhanced the mixture but diminished the sampling rate.

Trials were performed in duplicate and in a random way to minimize the effect of uncontrollable factors. All experiments were carried out with a 1.00×10^{-4} mol L^{-1} piroxicam solution.

Analysis of the results (using the Yates algorithm) led to the conclusion that the four factors affected the analytical signal, and the sample volume (V) was the factor that most significantly influenced I_p. However, the obtained graphs indicated that the volume high level (150 μL) originated a noisier baseline, due to the incomplete homogenization of sample and supporting electrolyte. When V was at the low level, the baseline was stable. Therefore, the sample volume was fixed at the low level (30 μL) and a complete factorial design was performed to confirm the influence of the other factors. The factors' levels used were the same used for the fractional factorial design.

According to the obtained results, the factors that foremost influenced the analytical signal intensity were I and F. The graphs showed that the best mixture was obtained when R was at the high level, as expected. In view of these results, R was fixed at the high level (50 cm) and a supermodified simplex [19] was carried out to optimize the values of I and F. As the flow rates allowed by the automatic burette are discrete values, whenever the trial flow rate was not one of the allowed flow rates, it was used the nearest flow rate possible, never with a difference higher than 0.12 mL min^{-1} between them.

According to the results obtained with the supermodified simplex, the levels combination that enabled the best mixture between sample and carrier solution was $I = 1$ plug (sample inserted in unique volume) and $F = 0.48$ mL min^{-1}. The graphs showed that this flow rate value permitted to attain the most stable baseline.

Under the established conditions, the analytical cycle took about 3 min, which corresponded to a sampling rate of 20 determinations per hour.

With the flow system parameters optimized, piroxicam standard solutions with concentrations between 1.00×10^{-5}

mol L^{-1} and 4.00×10^{-3} mol L^{-1} were analyzed. A linear relationship was observed between piroxicam concentration and analytical signal intensity up to the concentration of 5.00×10^{-4} mol L^{-1}, occurring linearity loss beyond that concentration level. The detection limit, calculated experimentally, was 1.0×10^{-5} mol L^{-1}.

3.3. Interference Studies

Considering the application of the developed method in the analysis of diverse pharmaceutical formulations containing piroxicam, the effect of several excipients present in these samples (lactose, stearic acid, propyleneglycol, polysorbate 80, ethanol, glyceryl monostearate and triethanolamine) in the analytical signal was evaluated. Solutions containing piroxicam with a concentration of 1.00×10^{-4} mol L^{-1} and the excipient being studied in different concentrations were analyzed. The interfering concentration of each compound was considered as being that which caused a variation in I_p greater than or equal to $\pm 5\%$ in relation to the analytical signal obtained in its absence. According to the obtained results, it was possible to conclude that lactose didn't affect I_p when it was present in a concentration up to 100-fold higher than that of piroxicam. As for propyleneglycol, polysorbate 80 and ethanol, the maximum concentration without originating interference was 4% (v/v), for stearic acid was 2% (m/v), for glyceryl monostearate was 0.4% (v/v) and for triethanolamine was 0.005% (v/v), so it was not predictable that these excipients interfered in the determination of piroxicam, at the concentrations expected in the samples.

3.4. Sample Analysis

To evaluate the applicability of the developed method, several pharmaceutical formulations, existing in Portugal, were analyzed. Piroxicam was also determined by the reference methods , HPLC for capsules, tablets, dispersible tablets and granules and UV-vis spectrophotometry for ampoules. Table 1 shows the results obtained by the proposed and the reference methods.

The agreement between the results obtained by the proposed and the reference methods was evaluated through the Student *t*-test for paired samples, in which the calculated *t* value (-0.52) was lower than the critical *t* value (2.36, two tail), for a 95% confidence level ($n = 8$). The regression line between the results obtained by the developed and the reference methods presented a correlation coefficient (R) of 0.988, with $a = 0.41 \pm 2.84$ and $b = 0.97 \pm 0.15$, for a 95% confidence level.

For the suppositories, gels and cream, since there were no reference methods for these pharmaceutical forms, recovery studies were performed (Table 1). Satisfactory recovery percentages were obtained, which allowed to conclude that excipients didn't interfere in the determination.

Repeatability of the measurements carried out by the proposed method was estimated by carrying out 10 successive determinations of all samples, with a RSD lower than 5% being obtained. Figure 3 represents the graphs acquired in piroxicam determination in ampoules, suppositories, gel and cream, which demonstrate the high repeatability achieved.

Table 1. Results obtained in the determination of piroxicam in pharmaceutical formulations, by the proposed and the reference methods and in the recovery studies.

Sample	Nominal value (mg, mg/mL or mg/g)	Piroxicam found[a] (mg, mg/mL or mg/g)		Relative deviation (%)	Recovery studies		
		Proposed method	Reference method		Amount added (μg/mL)	Amount found (μg/mL)	Recovery (%)
Reumoxican capsules	20	19.8 ± 0.7	19.7 ± 0.5	$+0.5$	–	–	–
Flexar capsules	20	21.0 ± 0.5	20.3 ± 0.3	$+3.4$	–	–	–
Feldene dipersible tablets	20	19.0 ± 0.2	19.5 ± 0.1	-2.6	–	–	–
Reumoxican dispersible tablets	20	19.3 ± 0.8	19.7 ± 0.2	-2.0	–	–	–
Piroxicam Ratiopharm tables	20	20.0 ± 0.1	20.0 ± 0.1	0	–	–	–
Brexin granules	20	19.5 ± 0.5	20.0 ± 0.1	-2.5	–	–	–
Feldene ampoules	20	12.1 ± 0.3	11.7 ± 0.1	$+0.4$	–	–	–
Flexar ampoules	20	15.4 ± 0.2	15.9 ± 0.1	-3.1	–	–	–
Feldene suppositories	20	19.1 ± 0.4	–	–	16.6 33.1	16.8 33.4	1012 1009
Flexor gel	5	5.0 ± 0.1	–	–	16.6 33.1	15.8 34.0	95.2 1021
Feldene gel	5	4.7 ± 0.1	–	–	16.6 33.1	16.9 33.4	1018 1009
Reumoxican cream	10	9.7 ± 0.2	–	–	33.1 41.4	36.0 41.8	1088 1010

[a] Average ± standard deviation of 3 determinations.

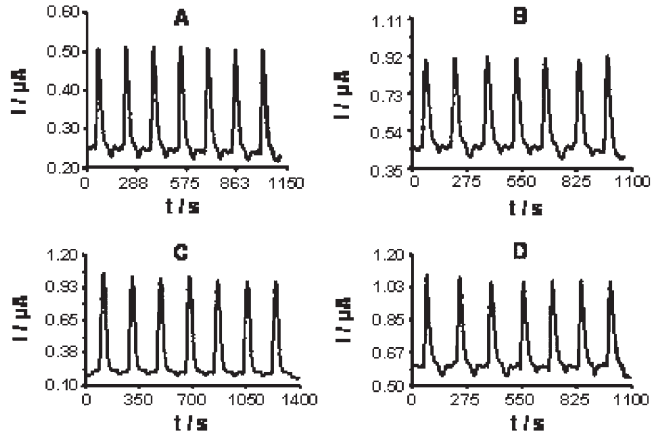

Fig. 3. Repetitive injections of pharmaceutical formulations (A: ampoule, B: suppository, C: gel, D: cream). Sample volume 30 µL, reactor length 50 cm, flow rate 0.48 mL min^{-1}, working electrode modified with 20 µL of a 1% Nafion solution.

4. Conclusions

The proposed automated system enables the determination of piroxicam in pharmaceutical formulations in a rapid, simple and inexpensive way, yielding similar results to those obtained with the reference methods. The capability of the proposed system to analyze samples with highly diverse matrices constitutes its major advantage when compared to the already existing methodologies for piroxicam quantification in pharmaceutical formulations, since those methods are, generally, only dedicated to the analysis of pharmaceutical solid forms.

On one hand, the relative difficulty to dissolve piroxicam in aqueous solutions and, on the other, the presence of lipophilic excipients in some samples, justified the choice of DMSO as the solvent of all samples. Its low electrical conductivity didn't impair its use in amperometric measurements because the flow system was optimized in order to enhance the mixture of the sample with the supporting electrolyte, allowing the sample to reach the detector completely homogenized with the supporting electrolyte.

The Nafion film deposited at the surface of the tubular glassy carbon electrode prevented the adsorption of sample excipients, conferring greater stability to the electrode and higher repeatability to measurements.

The use of the flow system allowed to perform measurements with a reduced sample volume, which was in contact with the detector for a very short period of time, contributing to the film stability by preventing the film swelling caused by DMSO. Therefore, a particular modified electrode could be used throughout 90 consecutive determinations without a significant change of the analytical signal intensity.

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6. References

[1] B. F. Reis, M. F. Giné, E. A. G. Zagatto, J. L. F. C. Lima, R. A. Lapa, *Anal. Chim. Acta* **1994**, *293*, 129.
[2] S. Khalil, N. Borham, M. A. El-Ries, *Anal. Chim. Acta* **2000**, *414*, 215.
[3] A. S. Amin, *J. Pharm. Biomed. Anal.* **2002**, *29*, 729.
[4] B. S. Nagaralli, J. Seetharamappa, M. B. Melwanki, *J. Pharm. Biomed. Anal.* **2002**, *29*, 859.
[5] M. I. Pascual-Reguera, M. J. Ayora-Cañada, M. S. C. Ruiz, *Eur. J. Pharm. Sci.* **2002**, *15*, 179.
[6] M. A. El-Ries, G. Mohamed, S. Khalil, M. El-Shall, *Chem. Pharm. Bull.* **2003**, *51*, 6.
[7] J. F. García-Reyes, E. J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz, *Talanta* **2006**, *68*, 1482.
[8] P. C. Damiani, M. Bearzotti, M. Cabezón, A. C. Olivieri, *J. Pharm. Biomed. Anal.* **1998**, *17*, 233.
[9] G. M. Escandar, A. J. Bystol, A. D. Campiglia, *Anal. Chim. Acta* **2002**, *466*, 275.
[10] S. M. Z. Al-Kindy, A. Al-Wishahi, F. E. O. Suliman, *Talanta* **2004**, *64*, 1343.
[11] P. Ugo, L. M. Moretto, F. Vezzà, *ChemPhysChem.* **2002**, *3*, 917.
[12] C. M. A. Brett, D. A. Fungaro, *J. Braz. Chem. Soc.* **2000**, *11*, 298.
[13] R. A. S. Lapa, J. L. F. C. Lima, B. F. Reis, J. L. M. Santos, E. A. G. Zagatto, *Anal. Chim. Acta* **1997**, *351*, 223.
[14] T*he British Pharmacopoeia 2005,* The Stationery Office under license from the Controller of Her Majesty's Stationery Office, Vol III, London *2731*, **2005**, 2823.
[15] M. L. S. Silva, M. B. Q. Garcia, J. L. F. C. Lima, E. Barrado, *Anal. Chim. Acta* **2006**, *573–574*, 383.
[16] R. Vignes, *American Chemical Society Annual Meeting 2000.*
[17] B. Chachulski, J. Gębicki, *Electroanalysis* **2006**, *18*, 433.
[18] J. Wang, *Analytical Electrochemistry*, 2nd ed., Wiley-VCH, New York **2000**, pp. 102–103.
[19] M. W. Routh, P. A. Swartz, M. B. Denton, *Anal. Chem.* **1977**, *49*, 1422.

