Pulse perturbation technique for determination of piroxicam in pharmaceuticals using an oscillatory reaction system

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Abstract: A simple and reliable novel kinetic method for the determination of piroxicam (PX) was proposed and validated. For quantitative determination of PX, the Bray−Liebhafsky (BL) oscillatory reaction was used in a stable non-equilibrium stationary state close to the bifurcation point. Under the optimized reaction conditions (T = 55.0°C, [H₂SO₄]₀ = 7.60×10⁻² mol L⁻¹, [KIO₃]₀ = 5.90×10⁻² mol L⁻¹, [H₂O₂]₀ = 1.50×10⁻¹ mol L⁻¹ and j₀ = 2.95×10⁻² min⁻¹), the linear relationship between maximal potential shift ΔE, and PX concentration was obtained in the concentration range 11.2−480.5 µg mL⁻¹ with a detection limit of 9.9 µg mL⁻¹. The method had a rather good sample throughput of 25 samples h⁻¹ with a precision RSD = 4.7% as well as recoveries RCV ≤ 104.4%. Applicability of the proposed method to the direct determination of piroxicam in different pharmaceutical formulations (tablets, ampoules and gel) was demonstrated.

Keywords: Piroxicam • Perturbation technique • Bray-Liebhafsky oscillatory reaction • Pharmaceutical preparations

1. Introduction

Piroxicam (hereinafter referred to as PX), 4-hydroxy-2-methyl-N-(pyridine-2-yl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a non-steroidal anti-inflammatory drug from the oxicam family with analgesic and anti-pyretic activities. This effective analgesic and anti-inflammatory agent is used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute pain in musculoskeletal disorder and acute gout [1].

Different analytical methods for determination of PX in various real samples from 1990 to 2008 were summarized by Starek and Krzek [2]. Apart from potentiometric, voltammetric and fluorimetric techniques, chromatographic and spectrophotometric techniques have been used most extensively. Some of those methods are cumbersome, expensive or time-consuming. Therefore, some new methods based on relatively simple and inexpensive equipment were desirable. In this regard, a kinetic method based on employment of the analyte pulse perturbation technique [3] (APP) was considered. The Bray-Liebhafsky (BL) oscillatory reaction [4,5] as a very nonlinear system, with potentiometric monitoring of analyte perturbation, showed promise as an alternative to some instrumental methods, due to its low cost instrumentation and relatively rapid detection procedure.

During the last two decades the development of analytical methods based on the use of oscillatory
reactions as matrices, realized in the continuously stirred tank reactor [6] (CSTR), has made steady progress. The application of oscillating chemical reactions to analytical determination has been reviewed in three papers [7-9]. Interest in the development of these methods has derived from the evident advantages of the experimental technique that has combined the CSTR, the APP technique, and potentiometric detection. In this way, various organic compounds, particularly biologically and pharmaceutically important ones [7-13], some ions [7-9,14-16], and gases [9] ions [17] may be determined at micromolar level in a simple, rapid manner. Moreover, compared to instrumental analysis the application of oscillatory chemical reaction as a simple analytical tool offers advantages such as wide linear concentration range (ca. 10$^{-7}$–10$^{-3}$ mol L$^{-1}$) and high sensitivity, i.e., low detection limit (ca. 10$^{-9}$–10$^{-6}$ mol L$^{-1}$; occasionally down to 10$^{-12}$ mol L$^{-1}$).

The kinetic methods applied in these analyses are based on the relationship between the response of a regular oscillating system (such as its oscillatory amplitude, oscillatory period, etc.) to the perturbation and analyte concentration [3,7,9,12,13,16]. On the other hand, when the methods are applied with the matrix in a stable stationary state, the analytical signal has the maximal potential shift ($\Delta E_m$) [8,10,11,14,22]. This paper reports the determination of piroxicam (PX) by its perturbation of the BL matrix [4,5], with the aid of the CSTR [18-21]. Thus, injecting various amounts of PX into the BL matrix in a stable steady state near a bifurcation point causes substantial changes in the potential of the matrix dynamic state ($\Delta E_m$) that is proportional to the PX concentration. In addition, this kinetic method was applied for PX determination in pharmaceutical formulations, and gave the satisfying results.

2. Experimental procedure

2.1. Reagents

Only analytically graded reagents without further purification were used for preparing the solutions. Potassium iodate, sulfuric acid, hydrogen peroxide and methanol were obtained from Merck and picroxam from Sigma. For preparing the solutions of KIO$_3$, H$_2$SO$_4$, and H$_2$O$_2$, deionized water ($\rho = 18$ MΩ cm, Milli-Q, Millipore, Bedford) was used.

A 4.8×10$^{-3}$ mol L$^{-1}$ PX standard stock solution was prepared by dissolving 0.0400 g of PX in 25 mL of methanol (Merck). This solution was stored in a refrigerator in the dark. Working standard solutions for calibration were obtained by convenient dilutions of this stock standard solution with methanol in order to obtain concentrations in the range 5.6–1590.5 µg mL$^{-1}$.

Several pharmaceutical formulations (tablets, injections and gel) containing PX (Pfizer) and other excipients (lactose anhydrous, cellulose microcrystalline, hypromellose, sodium stearyl fumarate in the case of tablets, benzyl alcohol, ethanol, hydrochloric acid, nicotinamide, propylene glycol, monobasic sodium phosphate, sodium hydroxide and water for injection in the case of injection, as well as carbopol 980, propylene glycol, ethanol, benzyl alcohol, diisopropanolamine, hydroxyethylcellulose and purified water, in the case of gel), were bought at Greek pharmacies.

2.2. Pharmaceutical preparations

Samples of pharmaceutical formulations (tablets, injections and gel) containing PX were analyzed by the procedure proposed.

In the case of tablets (one tablet contains 20 mg of PX, according to the manufacturer), the contents of 10 finely ground tablets were weighed and the average mass of one sample was evaluated. A sample solution was prepared by dissolving the amount of powder equivalent to 20 mg of the PX in a 50-mL volumetric flask made up to volume with methanol, and mixture was filtered through Whatman No. 1. filter paper. For injections (1 mL of injection contains 20 mg of PX; according to manufacturer) the contents of five ampoules were mixed. Accurately measured portions of the injection solution equivalent to 20 mg of the PX were transferred to a 50-mL volumetric flask and made up to volume with methanol. In the case of piroxicam gel (1 g of gel contains 5 mg of PX, according to manufacturer), 4 g of gel was dissolved in a 50 mL volumetric flask, made up to volume with methanol, and the mixture was filtered through Whatman No. 1 filter paper.

2.3. Apparatus and equipment

The instrumental set-up used to perturb the matrix was shown schematically in [22]. The various components included the following: 50-mL glass CSTR vessel (Metrohm model 876–20), thermostat ((Julabo, series ED, Germany), a magnetic stirrer ((M. Zipperer GmbH, Cat_ECM5, Denmark), a potential measuring system and peristaltic pumps ((ISMATEC, REGLO Analog, Model MS-4/06, Glattbrugg, Switzerland), A PC-multilab EH4 16-bit analog-to-digital converter (EH4, Serbia) electrochemistry analyzer was directly connected to the reactor through two electrodes, a platinum working electrode (Metrohm model 6.0301.100, Herisau, Switzerland) and a double junction Ag/AgCl electrode (Metrohm model 6.0726.100), and used to record the potential changes. It is known [23] that the platinum
electrode may be used for determination of I\(^-\) in low acidic iodide solutions, which is supported and confirmed in our research [14].

The CSTR and three tanks containing reagent aqueous solutions (H\(_2\)O\(_2\), KIO\(_3\) and H\(_2\)SO\(_4\)) were fitted with a water recirculation jacket connected to a thermostat. Peristaltic pumps controlled the flows (inflow and outflow) of reactants. Tygon tubing (Tyg R3603 ID 1.75 mm, Ismatec, Glattbrugg, Switzerland) was used for transporting the solutions of KIO\(_3\), H\(_2\)SO\(_4\) and H\(_2\)O\(_2\). These tubes were connected to Teflon tubes (Varian, Darmstadt, Germany), and the reagents were introduced to the reaction vessel through them. The volume of the reaction mixture was kept constant at 22.2±0.2 mL by removing the surplus volume of the reaction mixture.

The analyte was introduced using micropipettes (Brand, Wertheim, Germany). A 50-µL shot is estimated to last about 0.5 s. The intensity of the perturbation corresponds to the injected amount (in µg mL\(^{-1}\)) of PX standard samples.

### 2.4. Procedures for determination of PX

For quantitative determination of PX, matrix reaction systems with BL oscillatory reaction realized at T = 55.0°C (start-up procedure A), and at T = 58.0°C (start-up procedure B) was used.

#### 2.4.1. Procedure A

Thermostated at 55.0±0.1°C and protected from light, the reaction vessel was filled up with the three separate inflows of the reactant solutions, 5.90×10\(^{-2}\) mol L\(^{-1}\) KIO\(_3\), 6.47×10\(^{-2}\) mol L\(^{-1}\) H\(_2\)SO\(_4\), and 1.50×10\(^{-1}\) mol L\(^{-1}\) H\(_2\)O\(_2\), at a maximum flow rate of 5 mL min\(^{-1}\). Under these conditions, within 3.5 min, about twice the required volume of the reaction mixture entered the reaction vessel. Then the inflows were stopped, the stirrer was turned on (900 rpm), and the excess of the reaction mixture was sucked out through a U-shaped glass tube, in order to achieve an actual reaction mixture volume, 22.2±0.2 mL. Hence, the reaction commenced under the batch conditions. After two batch oscillations (after about 20 min) the inflows were turned on at the required specific flow rate, 2.95×10\(^{-2}\) min\(^{-1}\), and the inflow concentration of sulfuric acid was varied at intervals in the range, 5.38×10\(^{-2}\) mol L\(^{-1}\) ≤ [H\(_2\)SO\(_4\)] \(\leq\) 8.06×10\(^{-2}\) mol L\(^{-1}\). After two batch oscillations obtained under these conditions, in the subsequent routine analysis, the inflow concentration of sulfuric acid was immediately adjusted to the selected operation value (7.08×10\(^{-2}\) mol L\(^{-1}\), 7.20×10\(^{-2}\) mol L\(^{-1}\) and 7.60×10\(^{-2}\) mol L\(^{-1}\)). The preparatory procedure took about 50 min. We used similar conditions of the BL reaction for the development of the procedure for the determination of dynamic states of the BL reaction [21].

#### 2.4.2. Procedure B

This procedure was similar to that described for A, except for higher temperature, T = 58.0°C and different inflow concentrations of sulfuric acid as operational values (7.50×10\(^{-2}\) mol L\(^{-1}\), 7.69×10\(^{-2}\) mol L\(^{-1}\) and 8.06×10\(^{-2}\) mol L\(^{-1}\)). As we have already mentioned, we used similar conditions of the BL reaction for the development of the procedure for the determination of dynamic states of the BL reaction [21].

### 3. Results and discussion

Generally, all processes in this application of the BL oscillatory reaction involve bifurcation analysis, i.e., determination of a suitable dynamic state of the matrix that is the stable steady state near bifurcation point that will be perturbed, perturbation analysis, determination of the analyte under consideration and the application to real samples. A simple schematic presentation of these processes in an applied method for quantitative determination of analyte is presented in Fig. 1.

In this work, as the matrix reaction system suitable for the determination of PX, the Bray–Liebhafsky (BL) oscillatory reaction [4,5] (the reaction where hydrogen peroxide decomposes into water and oxygen in the presence of both iodate and hydrogen ions) under CSTR conditions was chosen. By variation of the sulfuric acid concentration in inflow as the control parameters different dynamic states are obtained: from stable non-equilibrium stationary states to the simple single-peak periodic and chaotic oscillations. In the vicinity of the transitions between these dynamic states known as the bifurcation points, the matrix system is extremely sensitive to perturbation. For analytical purposes, the investigation of dynamic characteristics of the matrix as a function of the control parameter (bifurcation analysis) must be performed, but only once.

#### 3.1. Bifurcation analysis (dynamic of the matrix reaction system)

With the aim of locating different stable non-equilibrium stationary states of the BL matrix in the vicinity of the bifurcation point, the dynamic states necessary for analytical purposes, we examined the dynamics of the BL matrix as a function of the control parameter (inflow concentration of sulfuric acid). The parameter may vary slightly for different setups in different laboratories. As
we have already described in detail [8,19-21], location of a bifurcation point between sustained oscillations and a stable non-equilibrium stationary state, requires some of the control parameters (temperature, specific flow rate, inflow concentration of KIO₃ or H₂O₂, etc.) to be varied while other parameters remained unchanged. In this work, the inflow concentration of sulfuric acid, was gradually increased from 5.38×10⁻² mol L⁻¹ to 8.55×10⁻² mol L⁻¹, while other parameters temperature, specific flow rate and inflow concentration of KIO₃ and H₂O₂ remained unchanged. From the dynamic structures observed under these conditions, and temperatures T = 55.0°C (procedure A) or T = 58.0°C (procedure B), we constructed the bifurcation diagrams presented in Figs. 2a and 2b. Although chaotic states are found between dynamic states with sustained oscillations and stable steady states, by linear extrapolation of a plot of the square of the amplitude of the limit cycle oscillations versus inflow concentration of sulfuric acid (Figs. 3a and 3b), a kind of Andronov-Hopf bifurcation point was found. In particular [H₂SO₄]₀ at a bifurcation point at T = 55.0°C and BPₐ = 7.20×10⁻² mol L⁻¹ (procedure A) and [H₂SO₄]₀ at a bifurcation point at T = 58.0°C and BPₐ = 7.57×10⁻² mol L⁻¹ (procedure B).

3.2. Perturbation analysis (selection of the dynamic state suitable for analytical purposes)

For developing the analytical method for PX determination, we need to select the non-equilibrium stable stationary states of the matrix system that will be used for perturbation with PX. Thus, we tested several dynamic states (indicated by arrows in Figs. 2a and 2b in order to find the maximum response to the analyte, i.e., the optimal injection point. In particular, at T = 55.0°C (procedure A) in the vicinity of the bifurcation point observed at BPₐ = 7.20×10⁻² mol L⁻¹ we perturbed the non-equilibrium stationary states that are realized for [H₂SO₄]₀ = 7.08×10⁻² mol L⁻¹, 7.25×10⁻² mol L⁻¹ and 7.60×10⁻² mol L⁻¹, while at T = 58.0°C (procedure B) in the vicinity of the bifurcation point observed at BPₐ = 7.57×10⁻² mol L⁻¹, values used for [H₂SO₄]₀ were 7.50×10⁻² mol L⁻¹, 7.69×10⁻² mol L⁻¹ and 8.06×10⁻² mol L⁻¹.

Typical traces of the BL matrix in the absence, and in the presence of different amounts of perturbing PX realized in the selected non-equilibrium stable stationary states (procedure B) are presented in Fig. 4. Thus, before perturbation, the system was in a stable...
stationary state, while its corresponding potential was denoted as $E_s$ (Figs. 4b and 4c). When a trace amount of PX was injected into the BL matrix, both an initial abrupt change in potential and overshoot-decay response was observed. The difference, $\Delta E_m = E_p - E_s$ where $E_p$ is the minimal value of the potential after the applied perturbation (Figs. 4b and 4c), is used for analytical purposes as representative potential values. The difference between maximum and minimum values of a potential in one oscillation after perturbation could be also considered for analytical purposes, but nothing new would be obtained.

It should be emphasized that we have chosen to work as close as possible to the bifurcation points we found, but sufficiently far from them, so that small spontaneous perturbations will not induce transition to the oscillatory states. This latter behaviour was observed when we investigated the sensitivity of the BL matrix to the perturbations with PX for infow concentration $[H_2SO_4]_0 = 7.50 \times 10^{-2}$ mol L$^{-1}$, that is in vicinity of BP of (Fig. 4a). In this case (Fig. 4a), the responses of the matrix system were significantly different from the cases shown in the Figs. 4b and 4c. Moreover, an injection of PX ($c_{inj} \geq 9.70 \times 10^{-5}$ mol L$^{-1}$) leads to the excitation of aperiodic oscillations followed by a different relaxation route. Perturbations performed under these conditions, were not appropriate for quantitative determination of PX, when $\Delta E_m$ was used as analytical signal. On the other hand, the number of oscillations in that case (Fig. 4a) is also sensitive to PX, but that is a different type of response to the one analyzed at the other operation points. In this connection, some of the other characteristic dynamic properties of the matrix are likely to be correlated with concentration of PX, and this will be the target of future investigation.

However, as can be seen from Fig. 5, maximum responses of the matrix system to the selected concentrations of PX (255.1 µg mL$^{-1}$, 397.6 µg mL$^{-1}$ and 563.3 µg mL$^{-1}$) at temperatures 55.0°C and 58.0°C were obtained for $[H_2SO_4]_0 = 7.60 \times 10^{-2}$ mol L$^{-1}$ at $T = 55.0°C$, and for $[H_2SO_4]_0 = 8.06 \times 10^{-2}$ mol L$^{-1}$ at $T = 58.0°C$. Consequently, the dynamic states at those acidities were chosen as optimal injection points for PX determination.

### 3.3. Approach to the determination of PX

Under the optimal experimental conditions described above, the BL matrix was perturbed with various concentrations of PX. Typical response curves, obtained after perturbations of the chosen stable non-equilibrium stationary state at $T = 58.0°C$ and $[H_2SO_4]_0 = 8.06 \times 10^{-2}$ mol L$^{-1}$, are presented in Fig. 4c. Similar responses were obtained when the non-equilibrium stationary state of BL matrix at $T = 55.0°C$ and $[H_2SO_4]_0 = 7.60 \times 10^{-2}$ mol L$^{-1}$ was perturbed with PX. In both cases, it was found that the maximal potential displacement, $\Delta E_m = E_p - E_s$, was proportional to the intensity of the perturbation. A good linear relationship between the $\Delta E_m$, and the PX concentration was found.

In accordance with ICH guidelines [24] linearity, limit of detection (LOD), limit of quantification, precision and accuracy as well as sample throughput were analyzed and summarized in Table 1. Under the optimized conditions, PX standard solutions with concentration between 5.6–1590.5 µg mL$^{-1}$ were analyzed. For both procedures, an excellent linear relationship was observed between PX concentration and $\Delta E_m$ from 11.1 µg mL$^{-1}$ to 480.5 µg mL$^{-1}$, with linearity loss occurring at higher concentration levels. The limit
of detection (LOD) and the limit of quantification (LOQ) [25] were experimentally verified according to the ICH guidelines [24]. The obtained LOD and LOQ are, respectively, 9.9 μg mL\(^{-1}\) and 30.2 μg mL\(^{-1}\) (procedure A), as well as 9.6 μg mL\(^{-1}\) and 28.8 μg mL\(^{-1}\) (procedure B).

In order to determine the precision and accuracy of the proposed methods, solutions containing three different concentrations of PX (23.2 μg mL\(^{-1}\), 99.4 μg mL\(^{-1}\) and 298.2 μg mL\(^{-1}\)) were prepared and analyzed in triplicate. Precision was expressed by the relative standard deviation (RSD\(^*\) = \(\frac{\text{RSD}}{\sqrt{n}}\) with a theoretical t-value at 95% confidence limit for two degrees of freedom). Average relative standard deviations are 3.9% (procedure A) and 4.2% (procedure B) (Table 1). The percent recoveries obtained as 100.4% (procedure A) and 99.6% (procedure B) indicate good accuracy of the method.

Sample throughput defined as the number of sample measurements per hour, was 25 samples h\(^{-1}\) for both procedures.

It is clear that both experimental procedures perform well in determination of PX, but for procedure A, the method provides both higher precision and greater instrumental sensitivity (the slope obtained from regression curve) than method B although it has almost equal analytical sensitivity (assessed as LOD). Also, experimental procedure A consumes less sulfuric acid than procedure B, since the non-equilibrium stationary states are realized for lower acidities. Hence, procedure A is used for analysis of real samples.

In comparison to the analytical performances of some previously reported methods (Table 2), the proposed method for the determination of PX shows a wider range than some of the methods [29,31,32], but higher detection limit than those reported in [26-28,30-33]. Nevertheless, our method involves neither sophisticated instruments, nor any time-
The processes required to establish an application of the BL oscillatory reaction to an analytical method are shown as A → B → C → D→ E in Fig. 1. But once a bifurcation point has been determined (step B, Fig. 1), in the subsequent routine analysis it is sufficient to reduce the inflow concentration of sulfuric acid to the working value just immediately after achieving the sustained oscillations at given experimental conditions. In other words, the bifurcation analysis is a step that can be overlapped in a particular simple scheme (Fig. 1). Moreover, when B, C and D, are already known, the procedure for analytical determination of a particular analyte requires only steps A and E. This greatly simplifies and shortens the estimated time for a full analysis of a sample.

In the case of perturbation of BL matrix with PX, it seems likely that there is interaction between PX and HIO based on the following considerations. In preliminary potentiometric examinations, we tested whether PX reacted with the individual reactants of the BL matrix or with their binary mixtures. We found that the injection of PX into the CSTR containing potassium iodate in sulfuric acid medium provided a response similar to that obtained upon its incorporation in the BL matrix. This suggests that PX oxidation by interaction with hypoiodous acid may be the first step after perturbation. Subsequently, the ratio between iodine intermediates ([HOI]ss and [I−]ss), established in the stationary state before perturbation is altered, resulting in a sudden increase in iodide concentration determined by the reaction (similarly to the PX reaction with HOBr):

$$PX + HIO \rightarrow PXOH^+ + I^-$$

The potential generation mechanism of the BL matrix interaction with PX, can be built on the known BL model, consisting of eight reactions (Table 3) [35], with the addition of Reaction P.

### 3.4. Application to real samples

To evaluate the applicability of the developed method, several commercial pharmaceutical preparations were analyzed under the optimized conditions by procedure A. Analyzed pharmaceutical dosage forms containing PX either in tablets, ampoules or gel, were prepared as given in section 2.2. The response curves obtained by perturbation of the BL matrix with sample solutions (tablets, injection and gel) are shown in Figs. 6a–6c. The average concentrations were calculated from five replicate measurements of two

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### Table 1. Features of the calibration plots and analytical figures of merit for the determination of PX at different temperatures and acidities.

| T (°C) | Linear range (µg mL⁻¹) | Regression equation | LODb (µg mL⁻¹) | LOQc (µg mL⁻¹) | RSDd (%) | STe |
|--------|------------------------|---------------------|----------------|----------------|----------|-----|
| 55.0   | 7.60×10⁻² 11.2−480.5   | log(Em) = 4.4−0.09 9 9.90 | 30.2           | 2.9            | 25       |
| 58.0   | 8.06×10⁻² 11.2−480.5   | log(Em) = 7.1−0.08 9.89 | 28.8           | 2.4            | 25       |

*Correlation coefficient; *Limit of detection established at a signal−to−noise ratio of 3; *Limit of quantification established at a signal−to−noise ratio of 10; *Average relative standard deviations obtained from three determinations of 23.2 µg mL⁻¹, 99.4 µg mL⁻¹ and 298.2 µg mL⁻¹; *Sample throughput is the number of sample measurements per hour.

### Table 2. Comparison of analytical performances of the previously reported method with the proposed method.

| Method                   | Linear range | Detection limit | Reference |
|--------------------------|--------------|-----------------|-----------|
| Amperometry              | 10 – 500 µmol L⁻¹ | 10 µmol L⁻¹     | [26]      |
| Potentiometry            | 52 – 10000 µmol L⁻¹ | 2.4 µmol L⁻¹    | [27]      |
| Voltammetry              | 0.2 – 25 µmol L⁻¹ | 0.65 µmol L⁻¹   | [28]      |
| Spectrophotometry        | 0.05 – 2.40 µg mL⁻¹ | /               | [29]      |
| Spectrofluorimetry       | 0.33 – 4.0 µg mL⁻¹ | 0.22 µg mL⁻¹    | [30]      |
| Capillary zone electrophoresis | 40 – 500 µmol L⁻¹ | 10 µmol L⁻¹    | [32]      |
| Liquid chromatography    | 0.1 – 6.0 µg mol⁻¹ | 0.02 µg mL⁻¹   | [33]      |
| Pulse perturbation technique | 11.2 – 480 µg mL⁻¹ | 9.9 µg mL⁻¹ | [34]      |

(P)
independent solutions of the same pharmaceuticals. In addition, recovery tests were done for the evaluation of the proposed methods. In all instances, the standard addition method is performed by accurate addition of 165.7 µg mL\(^{-1}\) of PX in the dilute sample. The average recovery values (RCV), 104.2% (injection), 100.4% (gel) and 95.1% (tablets), indicate that the method developed is free from any interference, and provides accurate results. This method can be considered to be applicable to real analysis of contents PX in ampoules, gel and tablets. Piroxicam in the same pharmaceutical samples was also determined by a spectrophotometric method [29]. As listed in Table 4, the results obtained correspond to those obtained using the reference method.

### 4. Conclusion

The kinetic analytical method with simple potentiometric detection has been established to determine piroxicam. The proposed method is based on perturbing the Bray–Liebhafsky matrix in a stable non-equilibrium

| Reaction | Kinetic model |
|----------|---------------|
| I | \(\text{IO}_3^- + \text{I}^- + 2\text{H}^+ \rightleftharpoons \text{HIO} + \text{HIO}_2\) |
| II | \(\text{HIO}_2 + \text{I}^- + \text{H}^+ \rightarrow \text{I}_2\text{O} + \text{H}_2\text{O}\) |
| III | \(\text{I}_2\text{O} + \text{H}_2\text{O} \rightleftharpoons 2\text{HIO}\) |
| IV | \(\text{HIO} + \text{I}^- + \text{H}^+ \rightarrow \text{I}_2\text{O} + \text{H}_2\text{O}\) |
| V | \(\text{HIO} + \text{H}_2\text{O}_2 \rightarrow \text{I}_2\text{O} + \text{H}_2\text{O}\) |
| VI | \(\text{I}_2\text{O} + \text{H}_2\text{O} \rightarrow \text{HIO} + \text{HO}_3\) |
| VII | \(\text{HO}_3\text{O} + \text{H}_2\text{O}_2 \rightarrow \text{HO}_3\text{O} + \text{H}_2\text{O}\) |
| VIII | \(\text{IO}_3^- + \text{H}^+ + \text{H}_2\text{O}_2 \rightleftharpoons \text{HIO}_2 + \text{O}_2 + \text{H}_2\text{O}\) |

**Table 3.** Mechanism of the model of the BL reaction.

**Table 4.** Determination of PX in pharmaceutical preparations.

| Pharmaceutical formulation (stated amount of PX) | Content | Proposed method | Reference method [28] |
|-----------------------------------------------|---------|----------------|-----------------------|
| | | PX found\(^a\) (g) | RCV\(^\pm\)RSD\(^\pm\) (%) | PX found (mol L\(^{-1}\)) | RCV\(^\pm\)RSD (%) |
| PX-injection\(^d\) (20 mg mL\(^{-1}\)) | 1 | 20.80 | 104.0±4.6 | 19.93 | 99.7±1.7 |
| | 2 | 20.88 | 104.4±2.8 | 19.72 | 98.6±1.3 |
| PX-gel\(^f\) (0.5%) | 1 | 20.26 | 101.3±2.5 | 20.40 | 102.0±1.9 |
| | 2 | 19.89 | 99.4±2.6 | 20.61 | 103.1±1.5 |
| PX-tablets\(^g\) (20 mg per one tablet) | 1 | 18.94 | 95.0±4.7 | 20.14 | 100.7±1.8 |
| | 2 | 19.05 | 95.2±3.9 | 20.09 | 100.5±1.7 |

\(^a\)Mean value of five determinations; \(^b\)Performed as accurate addition of 5.0×10\(^{-4}\) mol L\(^{-1}\) PX to the real samples; \(^c\)Relative standard deviation of recovery; \(^d\)Also contains (from Pfizer): benzyl alcohol, ethanol, hydrochloric acid, nicotinamide, propylene glycol, monobasic sodium phosphate, sodium hydroxide and water for injection; \(^e\)carbopol 980, propylene glycol, ethanol, benzyl alcohol, disopropylamine, hydroxyethylcellulose and purified water; \(^f\)lactose anhydrous, cellulose microcrystalline, hypromellose, sodium stearyl fumarate.

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Figure 6. Typical response curves obtained after perturbing the stationary state in the BL reaction under optimized experimental conditions by addition of 50 µL of PX solution in (a) tablets; (b) injection and (c) gel.
stationary state in the vicinity of a bifurcation point. It has rather good analytical attributes (detection limit and precision are 9.9 µg mL⁻¹ and 3.9%, respectively) and an excellent sample throughput (25 samples h⁻¹). Method applicability to real samples was demonstrated by analyzing piroxicam in pharmaceuticals (injection, gel and tablets). In addition, it involves neither expensive equipment nor any time-consuming extraction procedures.

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