Voltammetric Study of Nimesulide and Its Differential Pulse Polarographic Determination in Pharmaceuticals

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Abstract

Nimesulide, *N*-(4-nitro-2-phenoxyphenyl)methanesulfonamide is an antiinflamatory analgesic agent that is both reducible at the mercury electrode and oxidizable at the glassy carbon electrode. Nimesulide in hydroalcoholic solution, presents cathodic response in a wide range of pH (2–12), both, by differential pulse and tast polarography techniques.The obtained results show only one main well-defined peak or wave in all the pH range studied. This peak (or wave) corresponds to the nitro group reduction in position 4. The voltammetric oxidation shows one well-resolved signal in all the pH range studied. This anodic signal could be attributed to the methylsulfonamide group oxidation. For analytical purposes, a very well resolved diffusion controlled differential pulse polarographic peak obtained at pH 7 was selected. This peak was used to develop a new method for the determination of nimesulide in pharmaceutical dosage forms. The recovery study (104.8% with a RSD of 1.3%) shows that the method is sufficiently accurate and precise to be applied in the individual tablet assay of commercial samples.

Keywords: Nimesulide, Voltammetry, Drug analysis, Differential pulse polarography

1. Introduction

Nimesulide, *N*-(4-nitro-2-phenoxiphenyl)methanesulfonamide (Fig. 1), is a drug that belongs to the non steroidal analgesic antiinflamatory group, and like others compounds of its therapeutic family is a weak acid of pka 6.5, but from the chemical point of view presents a novel structure, belonging to the methylsulfoanilides group [1].

Nimesulide exerts its actions by means of a series of mechanisms, including the selective inhibition of the prostaglandine synthesis, superoxide anion generation reduction from polimorfonuclears leukocyties, plattelet agregant factor synthesis inhibition and hipoclorous acid trapping [1, 2].

Nimesulide is administered both oral or rectally for a series of painful and inflammatory states. In the main of the short term studies, it is effective reducing the pain associated with osteoartritis, cancer, tromboflebitis, oral surgery and dismenorrea in adult, reducing the pain associated with general surgery in adult and children, and pain, fever and inflammation traumatic in adult and children [1].

Nimesulide is widely metabolized (1 to 3% of the dose is excreted inalterated in the urine), to several metabolites, which are excreted mainly in the urine (70%) and in the dregs (20%). The drug is almost thoroughly biotransformed to 4-hydroxynimesulide in the conjugated and free forms and this metabolite seems to contribute to the antiinflamatory activity of the parent compound. The maximum plasmatic concentrations of 4-hydroxynimesulide vary from 0.84 to 3.03 mg/L and are obtained within 2.61 to 5.33 hours after the oral administration of 50 to 200 mg nimesulide, in adults. The mean life elimination of 4-hydroxynimesulide varies from 2.89 to 4.78 hours and it is similar or slightly superior to the nimesulide (1.56 to 4.95 hours) [1, 3].

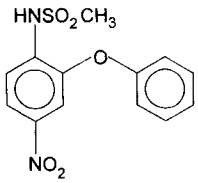

Fig. 1. Chemical structure of nimesulide.

The inaltered drug in plasmatic samples [4], as well as the simultaneous determination with its main metabolite have been quantified by HPLC with UV detection [5]. Also a HPLC method for the separation and quantification of nimesulide and its potential impurities in raw material have been described [6].

Nimesulide is not described in any Pharmacopeia and there are no offcial methods for the determination of purity and pharmaceutical forms assay of this drug. Furthermore, up to date no eletrochemical study has been devoted to nimesulide.

In the scope of our investigations, aimed at finding new electrochemical determinations of drugs [7-9], we have studied the electrochemical redox behaviour of nimesulide tending to develop a methodology that permits the nimesulide determination both as raw material and in pharmaceutical forms.

2. Experimental

2.1. Reagents and Solutions

Nimesulide (100% chromatographically pure, Pharma Investi Laboratories, Santiago, Chile). All reagents employed were of analytical grade.

The solutions under study were buffered using 0.04 M Britton–Robinson buffer, adjusting the ionic strenght at 0.1 M with KCl.

Stock solutions of nimesulide were prepared at a constant concentration of 1×10^{-3} M in ethanol.

An aliquot of stock solution was taken and diluted with ethanol/0.04 M Britton–Robinson buffer mixture (30/70) to obtain a final concentration of 1×10^{-4} M.

A series of 10 solution were prepared containing nimesulide concentration ranging between 5×10^{-6} and 5×10^{-5} M in ethanol / 0.04 M Britton–Robinson buffer mixture (30/70) at pH 7.0.

Excipients (corn starch, magnesium stearate, lactose and talc) were added to the drug for recovery studies, according to manufacturer's batch formulas for 100 mg nimesulide per tablet.

2.2. Equipment

An Unicam UV3 spectrophotometer with 1 cm quartz cell was used. A 25 mL thermostate Metrohm measuring cell, with dropping

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mercury electrode as a working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE) were employed. The operating conditions were: sensitivity 5–10 μA; drop time 1 s; potential range from 0 to −1700 mV; $\Delta Ep = -5$ mV; pulse retard 40 ms; pulse height −50 mV.

Differential pulse voltammetric experiments were carried out in an Inelecsa Model PDC 1212 assembly coupled for control acquisition and treatment of data with an ACER 500 PC. A CPRA Tacussel polarographic cell with a glassy carbon electrode (EDI type Tacussel) was used as working electrode. After each recording the working electrode was cleaned with a chromic-sulfuric acid mixture to heavily oxidize the surface and then thoroughly rinsed with water according to Adam's recommendations [10].

Cyclic voltammetry was carried out on totally automated Inelecsa assembly, similar to one previously described [11].

2.3. Procedures

2.3.1. Polarography

Ten series of one tablet of Aldoron (Pharma Investi Laboratory, amount declared of nimesulide per tablet, 100 mg) were suspend in ethanol, sonicated and diluted to 50 mL. A 2.5 mL aliquot of each solution was taken and diluted to 50 mL with ethanol/0.04 M Britton–Robinson buffer mixture (30/70), pH 7.0. Each sample solution was transferred to a polarographic cell, degased with nitrogen for 5 min and recorded at least twice from −300 mV to −600 mV, and the mg nimesulide in the sample solution were calculated from prepared standard calibration plot.

2.3.2. Spectrophotometry

The same general procedure described above was employed to obtain the 50 mL ethanol solution. Then, this solution was centrifuged for 10 min at 4000 rpm. A 0.3 mL aliquot of the supernatant was taken and diluted to 50 mL with ethanol/0.04 M Britton–Robinson buffer mixture (30/70), pH 7.0. The obtained solutions were measured at 402 nm, and mg nimesulide in the sample solution were calculated from the prepared standard calibration plot.

2.3.3. Apparent pK_a determination (pK_a')

For this purpose, both, the 302 and 402 nm UV bands were used. The pH solution was changed each 0.5 units and near the pK_a zone it was varied each 0.25 units. The temperature was kept constant at 25 °C and the used nimesulide concentrations was 5.5×10^{-5} M for all the pH range.

3. Results and Discussion

3.1. Cathodic Behavior

Nimesulide in hydroalcoholic solution (ethanol/0.04 M Britton–Robinson buffer 30/70, 0.1 M KCl), presents cathodic response in a wide range of pH (2–12), both by differential pulse and tast polarography techniques.

The obtained results show only one well defined peak or wave in all the pH range studied (Fig. 2). This peak (or wave) corresponds to the reduction of the nitro group in position 4, which is the only possible electroreducible group in the molecule.

The peak potential (E_p) shows a strong pH dependence behaviour between pHs 2 and 10 (Fig. 3), shifting cathodically when the pH increased, indicating that this compound would be more easily

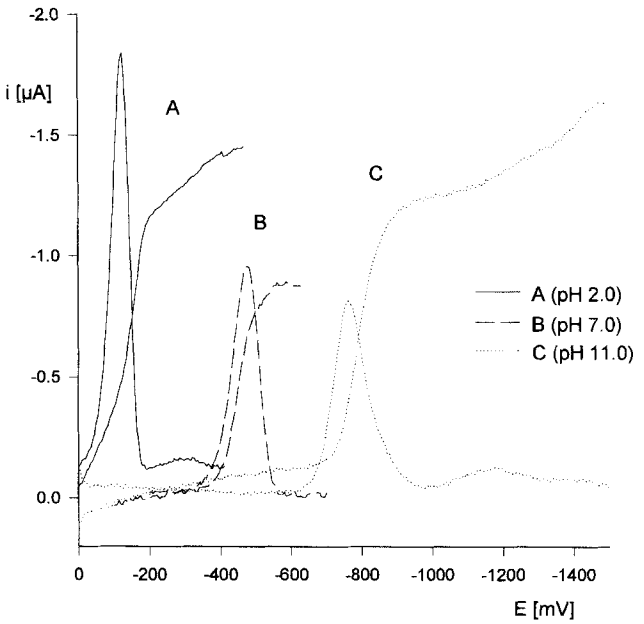


Fig. 2. Differential pulse and tast polarograms of a 1×10^{-4} M nimesulide solution at different pH (ethanol/0.04 M Britton–Robinson buffer 30/70).

reducible in acidic media. In the strong alkaline range (pH 10–12), the reduction of nimesulide presents a pH-independent behavior, that would indicate that in such zone the transfer of the first electron would be the slow step in the reduction process, without protonations steps before this electron transfer. From Figure 3 the presence of 3 breaks can be observed at approximately pH 4, 8.5 and 11, corresponding to changes in the mechanism, probably due to a) changes in the kind of electroactive specie(s), caused by protonation-deprotonation equilibria in the molecule, or b) changes in the relative rates of the different proton or electron transfer steps.

From tast polarograhic experiments we have obtained a half-wave potential ($E_{1/2}$) pH dependence totally analogous with those obtained by differential pulse polarography, confirming the validity of the results.

Furthermore, from the plot of the limiting current vs. pH (inset

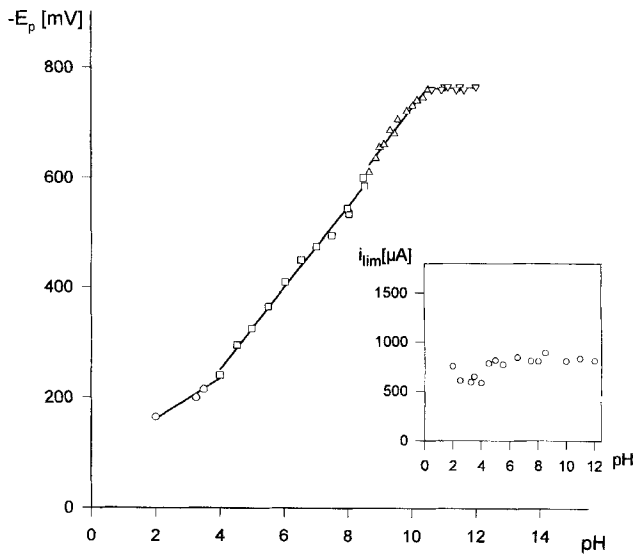


Fig. 3. Peak potential (DPP) and limiting current (tast) dependence of a 1×10^{-4} M nimesulide solution with pH (ethanol/0.04 M Britton–Robinson buffer 30/70).

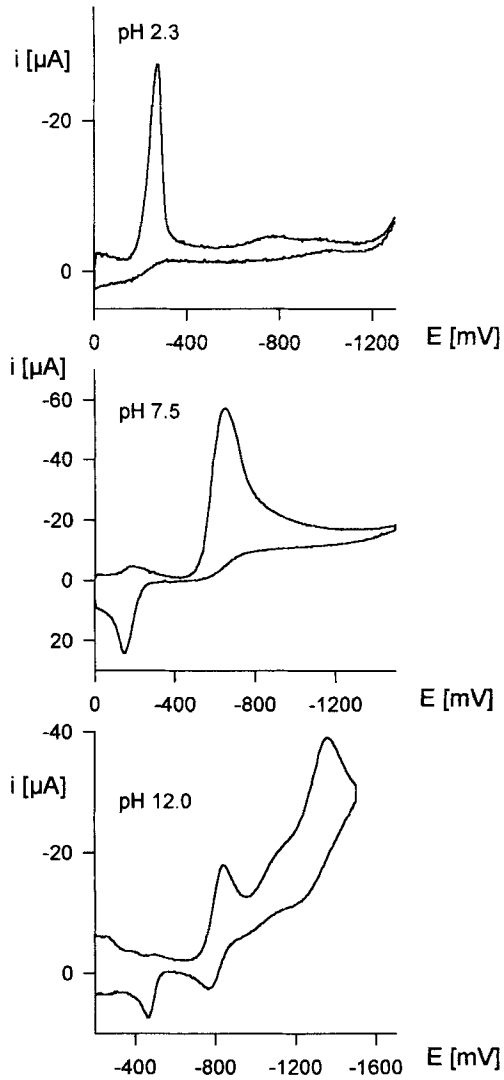


Fig. 4. Cyclic voltammograms of a 1×10^{-3} M nimesulide solution at different pH (ethanol/0.04 M Britton–Robinson buffer 30/70).

Fig. 3), two pH-independent zones between pH 2–4 and 5–10 were observed, showing a characteristic behavior for diffusion controlled limiting currents.

With the aim of learning more about the cathodic behavior of nimesulide, a cyclic voltammetric study was carried out. This study was accomplished in a wide range of pH (2–12), and the cyclic voltammograms found are summarized in Figure 4. In general, the obtained behavior follows the same general pattern of the nitroaromatic compounds [12]. The cyclic voltammetric response can be explained with reference to Figure 4.

At acidic pH (pH 2.3 in Fig. 4) a quite acute and irreversible peak that indicates the adsorptive character of the response was observed. This peak is due to the 4-electron reduction of the nitro group. Also one more cathodic wave is observed, due to the protonated hydroxylamine derivative reduction.

At pH 7.5 only one irreversible peak is observed, due to the 4-electron nitro group reduction, but in the return sweep (at approximately −300 mV) an anodic peak appears, corresponding to the oxidation of the hydroxylamine derivative formed in the forward sweep, according to

$$\mathrm{ARNHOH} \rightarrow \mathrm{ARNO} + 2\mathrm{H}^+ + 2\mathrm{e}^-$$

At strong alkaline pH (12.0) a reversible couple ($Ep_c = -843$ mV and $Ep_a = -769$ mV) was observed, that corresponds to the nitro radical anion formation, according to

$$\mathrm{ARNO_2} + \mathrm{e}^- \rightleftharpoons \mathrm{ARNO_2^{\cdot-}}$$

Furthermore, at more cathodic potential ($Ep_c = -1400$ mV) a second peak appears due to the 3-electron radical anion reduction

$$\mathrm{ARNO_2^{\cdot-}} + 3\mathrm{e}^- + 4\mathrm{H}^+ \rightleftharpoons \mathrm{ARNHOH}$$

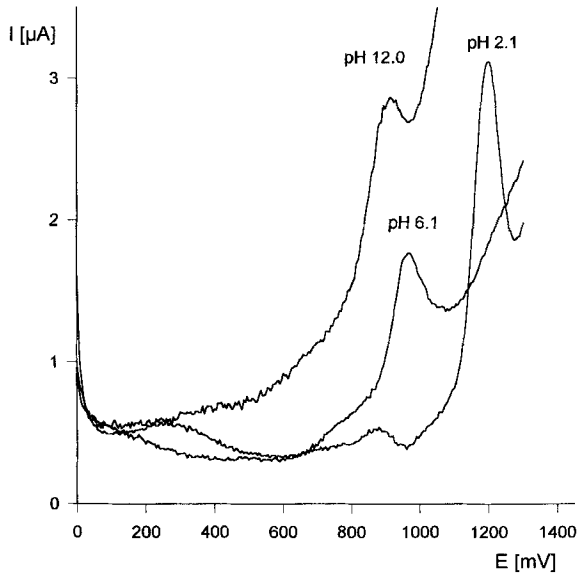


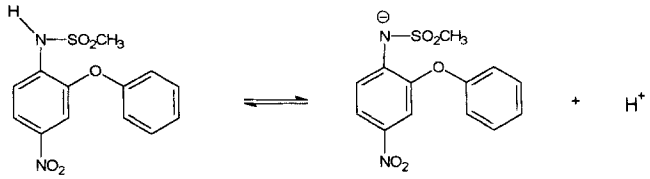
Fig. 5. Differential pulse voltammograms of a 1×10^{-4} M nimesulide solution at different pH (ethanol/0.04 M Britton–Robinson buffer 30/70).

3.2. Anodic Behavior

On the other hand, it was feasible for nimesulide to be electrochemically oxidized. Consequently, a study by differential pulse voltammetry in the same hydroalcoholic media, employing glassy carbon as working electrode was accomplished. It was found that nimesulide, in such experimental conditions, shows one well-resolved anodic signal in all the pH range studied (Fig. 5).

In Figure 6 the anodic peak potential with pH dependence is observed. It shows a pH-dependent behavior between pH 2 and 8, with a slope of −56.17 mV/pH ($r = 0.994$). However, at pH higher than 8.0 the peak potential shows a pH-independent behavior, indicating a notoriously change in the mechanism. Thus, in acid media the oxidation of nimesulide follows a proton-dependent mechanism while in alkaline media protons are not involved in the rate determining step or before. Considering that the described pK_a for nimesulide is 6.5, probably the change in the mechanism may be related to the pK_a of the molecule.

From the break of E_p vs. pH curve, we can define a voltammetric pK_a of approximately 7.2, which is related to the following balance, indicating that the oxidation is dramatically affected by this equilibria (Scheme 1).



Scheme 1.

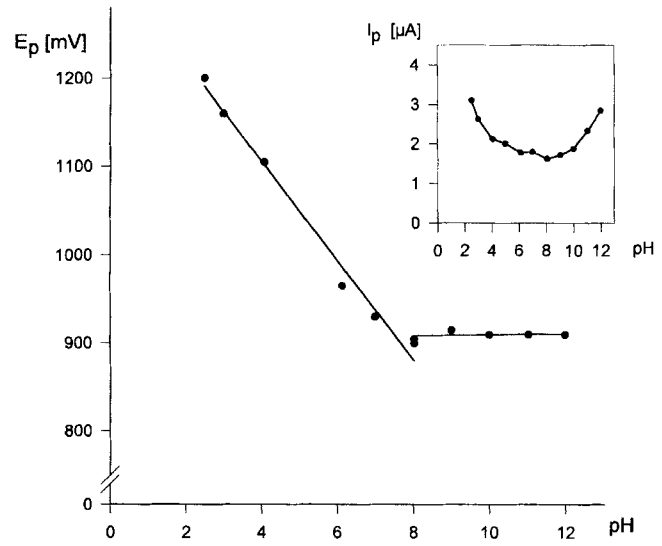


Fig. 6. Peak potential and peak current dependence of a 1×10^{-4} M nimesulide solution with pH (ethanol/0.04 M Britton–Robinson buffer 30/70).

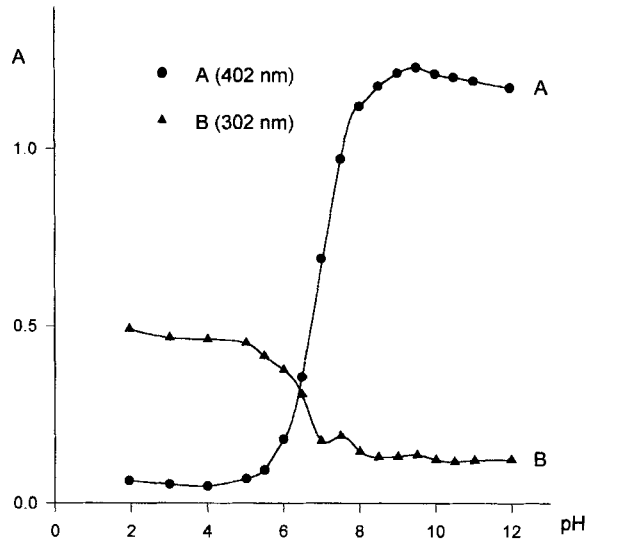


Fig. 8. Absorbance dependence with pH of a 5.5×10^{-5} M nimesulide solution at different wavelengths (302 and 402 nm).

Furthermore, the peak current vs. pH behavior has a close relationship to what was observed in the E_p vs. pH plot, where in the acid range a decrease of the peak current with the increase of pH was observed. On the other hand, in the basic range an increase in the peak current with the increase of pH was observed (Fig. 6, inset). This anodic signal could be attributed probably to the methylsulfonamide group oxidation contained in the structure of the nimesulide [13]. However, in acidic media the predominant specie is I, and in basic media the main specie is II, generating different oxidation mechanisms.

3.3. UV Spectroscopy

With the aim of both, learning more of the chemistry in solution of this drug and developing a comparative spectrophotometric method, an UV-vis spectrophotometric study was accomplished.

As can be seen in Figure 7, the drug presents at acid pHs a maximum wave length at 302 nm. In turn to neutral and basic pHs, a bathochromic shifting is produced, with a maximum wave length at 402 nm. From the absorbance–pH dependence of both wavelength at 302 and 402 nm, respectively (Fig. 8), it was found that the ranges between pH 2–5 and pH 8–10 show a pH-independent behavior (not ionized and ionized species, respectively). In turn, there was a pH-dependent zone between pH 5–8, that indicates the presence of a spectrophotommetric apparent pK_a. The data obtained in this study were useful to realize the apparent pK_a calculation of nimesulide. The value of pK_a' for nimesulide was calculated by the linear regression method, where the pH was considered the dependent variable and the $\log[(A_{max} - A)/(A - A_{min})]$, as the independent variable [14, 15]. The pK_a' obtained was 6.5 (using both wave lengths) (Fig. 9), similar to the value described in the literature [1] and relatively consistent with that given previously by polarography.

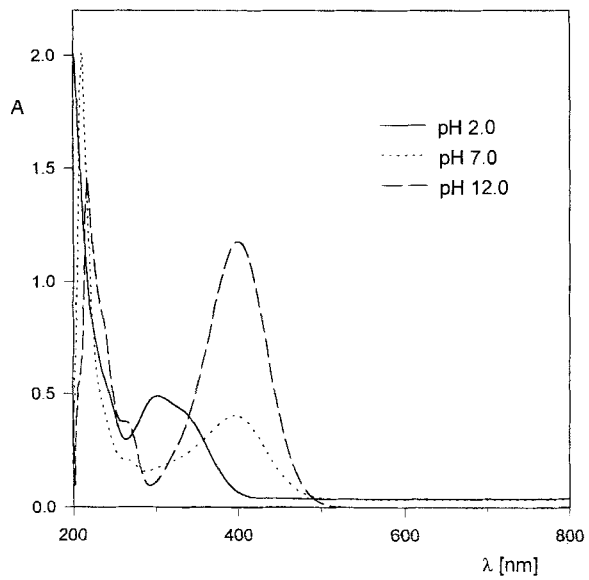


Fig. 7. UV spectra of a 1×10^{-4} M nimesulide solution at different pH.

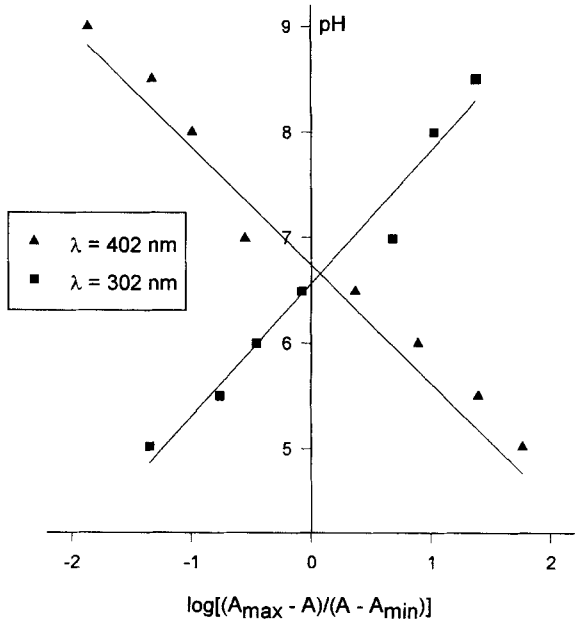


Fig. 9. pK_a' calculation plot at two different wavelengths (302 and 402 nm). ■) $pH = 6.56 + 1.256 \log[(A_{max} - A)/(A - A_{min})]$, $r = 0.995$; ▲) $pH = 6.45 - 0.92 \log[(A_{max} - A)/(A - A_{min})]$, $r = 0.994$.

3.4. Quantitative Application

Considering the above described basic knowledge of the electrochemistry and the spectrophotometric behavior of nimesulide, we tried quantitative applications.

We selected DPP as the electrochemical technique to develop a method to quantize nimesulide. For quantitation the calibration plot method, with concentration ranging between 5×10^{-5}–5×10^{-6} M of nimesulide solutions in ethanol/Britton–Robinson buffer (pH 7) was used. The calibration plot is described by the following regression curve:

$$i_p[\mu A] = 11588.49 \times C\,[M] - 7.3\times10^{-4}$$

(correlation coefficient = 0.999, n = 10, pH 7, t = 25 °C), where i_p is the peak current and C is the nimesulide concentration. The repeatability of the measurement was calculated from ten independent runs obtaining a RSD of 0.36%. The detection limit was 2.5×20^{-6} M and was calculated as the blank response plus three times the blank standard deviation divided by the slope of the calibration plot. In order to check the accuracy and precision of the developed method, we carried out a recovery study, obtaining an average recovery of 104.8% with a RSD of 1.3%, concluding that the proposed method is sufficiently accurate and precise in order to be applied to pharmaceutical forms. The results for the individual tablet assay for commercial tablets containing 100 mg nimesulide show a mean of 96.9% with a RSD of 4.1% for ten individual tablets.

Furthermore, to obtain comparative results an UV spectrophotometric method was also developed. The maximum at 402 nm was selected for quantitation. For analytical determination the calibration plot method, with concentrations ranging between 5×10^{-5} and 5×10^{-6} M, was used. This plot is described by the following regression line:

$$A = 8331.4\times C\,[M] - 3.6\times10^{-2};$$

(correlation coefficient = 0.9999, n = 10, t = 25 °C), where A is the absorbance of the pH 7 solution of nimesulide at 402 nm. The results of the recovery study (99.8, 1.5% RSD) and the individual tablet assay (102.8 mg, 1.8% RSD) are in accordance with the polarographic results. The quantitation of the tablets (uniformity content test) permits one to evaluate the homogeneity and distribution of the active principle. If we refered to the general limits for antiinflamatories described in Pharmacopoeia [16,17] (specifically nimesulide is not described in Pharmacopoeia), the analized formulation was found to b within the theoretical Pharmacopeia limits of a good manufacture.

Although both, polarographic and UV spectrophotometric methods, showed similar accuracy and precision, the principal advantage of the proposed polarographic method over the spectrophotometric one is that the excipients do not interfere and the centrifugation procedure is not necessary.

4. Acknowledgements

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