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ORIGINAL PAPER

Electrochemical study of some non-steroidal anti-inflammatory drugs: solvent effect and antioxidant activity

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Abstract The electrochemical behavior of 12 non-steroidal anti-inflammatory drugs (NSAIDs) was studied by means of cyclic voltammetry at a glassy carbon electrode. The underlying solvent had a considerable effect to the oxidation potentials of the investigated NSAIDs due to the alteration of their polar intermediates' solvation. Oxicams were more capable of electrochemical oxidation, and the influence of both specific and non-specific solute–solvent interactions in their reactivity was confirmed by means of Kamlet–Taft's analysis. Oxicams were further studied by chronoamperometry at the potentials of 300, 500, and 800 mV. The results obtained by the employed electroanalytical techniques were compared with the reactivity of oxicams towards 1,1- diphenyl-2-dipicrylhydrazyl (DPPH). The study showed a correlation of oxicams' amperometric signal at 800 mV with their absolute reaction rate, z with DPPH.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are of great interest in medicine as they are widely used for mild to moderate pain relief as well as in the treatment of osteoarthritis and rheumatoid arthritis. Their action is attributed to the inhibition of the cyclooxygenase enzyme, which in turn prevents the biosynthesis of certain prostaglandines [1]. Their anti-inflammatory action can be associated with their antioxidant properties, in particular with their interaction with reactive oxygen species since an uncontrolled release of these potentially damaging intermediates during inflammation has been documented [2]. Such interactions can be studied by means of in vitro cell free experimental protocols, among others the reducing ability against the stable 1,1-diphenyl-2-dipicrylhydrazyl (DPPH) free radical, which is considered as a rapid and general measure of free radical scavenging ability [3, 4]. This popular test has been extensively used to assess the antioxidant capacity of phenols, aromatic amines, coumarin-like compounds, and indole derivatives [5–7], and the obtained results can be related to the inhibition of lipid peroxidation [8]. It should be mentioned that the solvent used may affect the underlying oxidation mechanism in the case of ionizable compounds like phenols, promoting radical reaction or electron transfer, while in polar organic solvents, even ionization may be possible [9, 10].

The antioxidant activity of a compound is also related to electrochemical parameters, especially its oxidation potential,

which provides an estimate of the energy required to donate an electron. Indeed, the lower the oxidization potential, the more easily will the compound donate an electron and the higher will its expected antioxidant activity be [11]. The determination of the oxidation potential and, generally, the investigation of the electrochemical behavior of a compound can easily be performed by applying cyclic voltammetry. The latter has been considered in recent years as a conventional methodology for evaluating the antioxidant capacity of human and horse plasma, animal tissues, edible plants, wines, and different types of tea and coffees [12–14]. Cyclic voltammetric results can be correlated with those coming from antioxidant protocols [14] because redox behavior of an antioxidant at an electrode is related to its behavior in chemical redox reaction with a radical [13]. Nevertheless, oxidation potentials and, hence, oxidation convenience in an electrode is affected in most cases by the solvent used due to its interactions with reactants or their intermediates [15].

Electrochemical techniques have been applied to a limited number of NSAIDs (especially piroxicam and meloxicam) for their quantification mainly in pharmaceutical formulations. However, voltammetric studies concerning other well-known NSAIDs, such as mefenamic, tolfenamic, and niflumic acid, are still very limited, or even absent in the case of lornoxicam. Furthermore, comprehensive comparative studies of the electrochemical behavior of NSAIDs, effect of solvent polarity, and comparison of electrochemical data with antioxidant protocols' results are still missing.

In light of the above considerations, we found it interesting to carry out a comparative study of the electrochemical behavior of several structurally diverse NSAIDs. The investigated NSAIDs involved four oxicams (piroxicam, meloxicam, tenoxicam, and lornoxicam), three fenamic acids (mefenamic, niflumic, and tolfenamic acid), two arylpropionic acids (ibuprofen and ketoprofen), a sulfoanilide (nimesulide), an indolacetic acid derivative (indomethacin), and acetylsalicylic acid. As solvents, methanol, ethanol, acetonitrile, and mixtures of the latter with water were used in order to unravel the solvent effect to their electrochemical reactivity. Chronoamperometry was also employed in the investigated solvents at three different potentials (300, 500, and 800 mV), for further study. The obtained electrochemical results were further compared with the reactivity of the NSAIDs towards DPPH, measured under different solvent conditions, and an attempt was made for their correlation.

Experimental

Instrumentation

The electrochemical investigation of the non-steroidal anti-inflammatory drugs was performed using the polarograph 747 VA Stand (Metrohm) connected with the 746 VA Trace Analyzer (Metrohm) microprocessor. The working electrode was a glassy carbon electrode, the reference a Ag/AgCl one, filled with 3 M KCl (≥99.5% p.a., Merck) in high-purity water (HPW) supplied by an EASYpure II (Model D 7381, Barnsted International) water purification system, and the auxiliary, a Pt electrode. The glassy carbon working electrode was polished at the beginning of each measurement with alumina powder (0.3 μm) for 3 min, using a polishing cloth, and it was rinsed with deionized water. Furthermore, in the end of each working day, it was sonicated for 5 min firstly in distilled water and, secondly, in acetone. The state of the electrode was checked using a 0.1 M $Fe(CN)_6^{4-}$ solution. Spectrophotometric measurements were carried out with a Cary 1 E UV–Vis spectrophotometer (Varian), using 1-cm quartz cuvettes.

Reagents

Ibuprofen, ketoprofen, acetylsalicylic acid, nimesulide, mefenamic acid, tolfenamic acid, niflumic acid, piroxicam, tenoxicam, meloxicam, lornoxicam, and indomethacin were of pharmaceutical grade and were provided by local pharmaceutical companies. Their structures can be found in various handbooks. For reasons of comparison, the structure of the four oxicams, investigated and discussed in greater extent, are presented in Fig. 1. L(+) ascorbic acid (≥99.7%, Carlo-Erba) and mannitol (≥98%, Sigma-Aldrich) were used as reference compounds. As electrolyte for the investigation of the electrochemical behavior of the non-steroidal anti-inflammatory drugs, $LiClO_4$ (≥99.0%, p.a., Fluka Biochemica) was used in a concentration of 0.1 M in methanol (≥99.9%, HPLC grade, Sigma-Aldrich), absolute ethanol (≥99.8%, p.a., Riedel-de Haen), acetonitrile (≥99.8%, HPLC grade, Merck), and its mixtures with HPW. DPPH (≥95%, Fluka Biochemica) was used for the evaluation of antioxidant properties of NSAIDs.

Electrochemical measurements

All studies were carried out in room temperature (22± 1°C). The electrochemical investigation, involving cyclic voltammetry and chronoamperometry, was performed by spiking micro-amounts of NSAIDs, corresponding to a final concentration of 0.5 mM, in a 10-mL Metrohm polarographic cell, containing as blank 0.1 M $LiClO_4$ in MeOH, EtOH, CH_3CN, or its mixtures with HPW. Cyclic voltammograms were recorded between −100 and 1,000 mV with a scanning rate of 20 mVs^{-1}, while for chronoamperometric measurements, the potentials of 300, 500, and 800 mV were selected. All measurements were carried out at least in triplicate, and the mean value was considered.

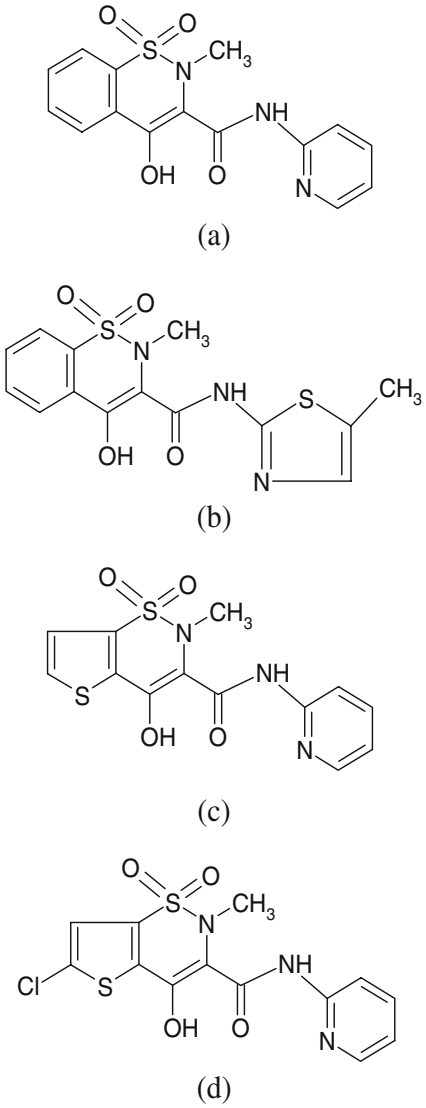


Fig. 1 Structures of **a** piroxicam, **b** meloxicam, **c** tenoxicam, and **d** lornoxicam

Study of the interaction of NSAIDs with DPPH

The reactivity of NSAIDs towards DPPH was studied using the method described in details elsewhere [6]. The percent reducing ability against DPPH was measured spectrophotometically at 517 nm at different time points from the fifth to 60 min, at time intervals of 5–10 min at room temperature under different solvent conditions, methanol, ethanol, and acetonitrile. The concentration of DPPH was 0.1 mM. IC_{50} values, corresponding to the drug concentration producing a 50% decrease in DPPH at steady state, were determined by non-linear fitting of Eq. 1.

$$\frac{1-A}{A_0} = \frac{[C]}{[C] + \mathrm{IC}_{50}} \tag{1}$$

where C is the drug concentration, A is the absorbance of DPPH in the presence of drug, and A_0 is the absorbance of DPPH at time zero.

The kinetic parameter, z, was derived by linear regression of individual rate constants k, determined at different drug/DPPH ratios during the first minutes of reaction time, against the [Drug]/[DPPH] ratio. Details are described in Ref. [8].

Statistical analysis

The statistical treatment of experimental data was carried out using the Statistica-Axa 7.0 software package (StatSoft, Tulsa, OK, USA).

Results and discussion

Cyclic voltammetric study of NSAIDs in different solvents

The electrochemical behavior of the investigated NSAIDs was studied using cyclic voltammetry at a glassy carbon electrode, which has been the working electrode of choice in such studies [11, 16]. As this electrochemical investigation was performed in respect to NSAIDs antioxidant activity, voltage scanning was not recorded in potentials over than 1,000 mV. For reasons of comparison, two known antioxidants, namely ascorbic acid and mannitol, were used. Initially, the study was performed in the aqueous phase with the addition of acetonitrile in a fraction of 10% in order to increase NSAIDs' solubility. As electrolyte, 0.1 M $LiClO_4$ in the investigated media was selected because not only had it been used for the voltammetric determination of NSAIDs [17] but it had also been successfully applied in our laboratory for the quantification of various organometallic compounds in organic phase [18–21], showing good compatibility with a wide range of organic solvents.

In the medium of water/acetonitrile, 9:1, acetylsalicylic acid, ibuprofen, ketoprofen, nimesulide, and mannitol gave no voltammetric response, which means that their electrochemical oxidation requires a potential over 1,000 mV vs. Ag/AgCl electrode. This difficulty in oxidation of those NSAIDs has also been described by other authors, using either a glassy carbon electrode or its modifications [22, 23]. On the other hand, piroxicam, tenoxicam, meloxicam, lornoxicam, mefenamic acid, tolfenamic acid, niflumic acid, indomethacin, and ascorbic acid gave one well-defined anodic peak with no associated cathodic peak in the reverse scan, indicating that their oxidation at the glassy carbon electrode is an irreversible process. This observation is in agreement with other relevant studies concerning some of the currently investigated drugs [17]. This behavior can

be representatively shown in the cyclic voltammogram of lornoxicam (Fig. 2a). The red line shows the corresponding voltammogram in the absence of lornoxicam.

By the first cycle curves of cyclic voltammograms, anode peak voltage (E_{ap}) values were determined in the case of electroactive compounds, while their half-peak voltage ($E_{1/2}$) was calculated as the voltage at which the current equaled half of the maximum anode peak current. In Table 1, the peak potentials (E_{ap}), half-wave potentials ($E_{1/2}$), and their difference, $\Delta E=E_{ap}-E_{1/2}$, are presented. As it is shown, oxicams were found to have the lowest oxidation potentials from all investigated NSAIDs, and, therefore, they can donate electrons faster. However, their antioxidant activity can be characterized as intermediate to weak and, of course, much lower than those of the strong antioxidant ascorbic acid. The latter, as it was expected, has very low $E_{1/2}$, about 240 mV, close to the values reported in other media [24]. Weak antioxidant properties can also be attributed to mefenamic and tolfenamic acid. For the electroactive compounds in the water/acetonitrile medium, ΔE values are around 70 mV, which corresponds to the involvement of one electron in the first step of their oxidation reaction [14]. A linear dependence of the peak current intensity upon the square root of the scan rate was found, demonstrating a diffusional character of the electrochemical process [25].

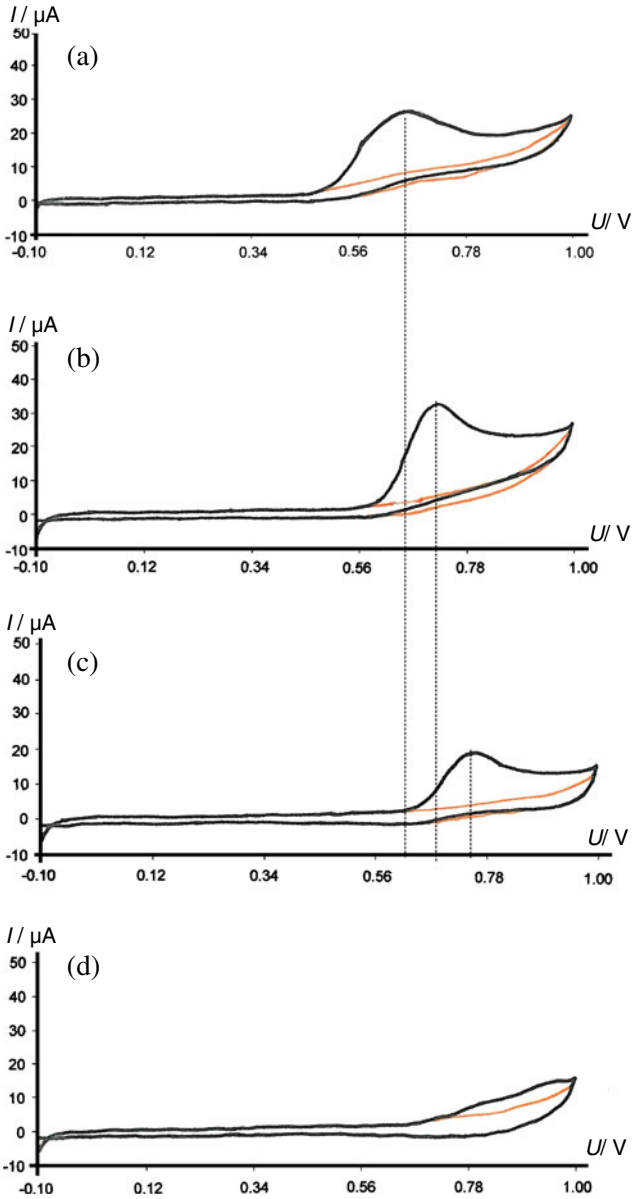


Fig. 2 Cyclic voltammograms of lornoxicam obtained in **a** 10% CH_3CN/90% H_2O, **b** MeOH, **c** EtOH, and **d** CH_3CN. *Red lines* show the corresponding (blank) voltammogram in the absence of lornoxicam. Conditions: lornoxicam concentration, 0.5 mM; electrolyte, 0.1 M $LiClO_4$; sweep rate, 20 mVs^{-1}

Solvent effect to electrochemical oxidation of NSAIDs

In order to study the solvent effect to the oxidation reactivity of the investigated compounds, methanol, ethanol, and acetonitrile were also tested, keeping all other parameters (electrolyte kind and concentration, solute concentration, and voltage sweep rate) constant. The peak potentials (E_{ap}), half-wave potentials ($E_{1/2}$), and their difference, ΔE, of all studied species in each solvent are included in Table 1. As it is shown, by decreasing the solvent polarity, the oxidation potential increases, and, therefore, the oxidation becomes more difficult. In the case of NSAIDs presenting weak antioxidant properties, such as tolfenamic, niflumic, and mefenamic acid as well as indomethacin, no anodic peaks were observed at a potential up to 1,000 mV. This can be explained on the basis that the oxidation mechanisms involve the formation of intermediates, more polar than the reactants. Hence, a decrease in polarity of the medium provokes the instability of such intermediates, and, consequently, the oxidation requires higher potential [15]. This influence is stronger in the case of ascorbic acid, whose E_{ap} values in the organic media reach those corresponding to oxicams and its anodic peak becomes substantially broad, implying complex electrochemical behavior [14].

Within oxicams, the sensitivity towards the solvent replacement is not similar. Indeed, as indicated by E_{ap} differences, piroxicam's oxidation is considerably affected by replacing the aqueous medium with methanol, while for the three other oxicams, the voltage change becomes most significant only when ethanol is replaced by the less polar acetonitrile. In all cases, no cathodic peaks were observed in the reversed scan even at higher scan rates investigated up to 200 mVs^{-1}, showing that the oxidation of oxicams is an irreversible process. This behavior is representatively depicted for lornoxicam in Fig. 2a–d. The red lines represent the corresponding voltammograms in the absence of lornoxicam. Especially, in the presence of acetonitrile, in

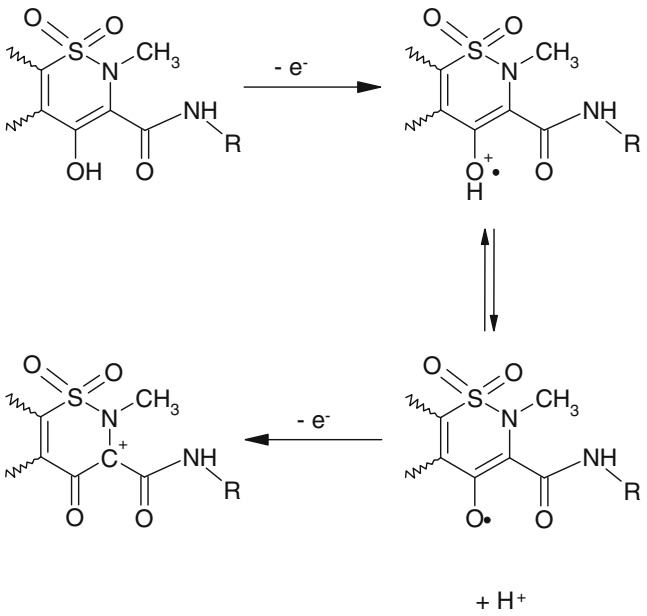
Table 1 Anodic peak voltages (E_{ap}), half-wave potentials ($E_{1/2}$), and their difference ΔE (E_{ap}–$E_{1/2}$) of non-steroidal anti-inflammatory drugs and antioxidative agents in different media

Compound	Media, 0.1 M $LiClO_4$ in											
	90% H_2O+10% CH_3CN			MeOH			EtOH			CH_3CN		
	E_{ap}	$E_{1/2}$	ΔE	E_{ap}	$E_{1/2}$	ΔE	E_{ap}	$E_{1/2}$	ΔE	E_{ap}	$E_{1/2}$	ΔE
Piroxicam	0.68±0.02	0.63±0.02	50±5	0.83±0.02	0.71±0.02	115±10	0.86±0.02	0.78±0.02	83±5	0.94±0.02	0.85±0.02	90±5
Tenoxicam	0.71±0.02	0.65±0.02	65±5	0.75±0.02	0.64±0.02	110±10	0.77±0.02	0.69±0.02	84±5	0.92±0.02	0.84±0.02	80±5
Meloxicam	0.70±0.02	0.62±0.02	80±5	0.77±0.02	0.71±0.02	55±5	0.80±0.02	0.72±0.02	80±5	0.78–0.98		
Lornoxicam	0.68±0.02	0.60±0.02	83±5	0.72±0.02	0.66±0.02	53±5	0.74±0.02	0.67±0.02	65±5	0.79–0.94		
Mefenamic acid	0.77±0.02	0.72±0.02	50±5	>1.00	0.95±0.02	–	>1.00	>1.00	–	>1.00	>1.00	–
Tolfenamic acid	0.78±0.02	0.72±0.02	60±5	>1.00	1.00±0.02	–	>1.00	>1.00	–	>1.00	>1.00	–
Niflumic acid	0.85±0.02	0.78±0.02	75±5	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–
Indomethacin	0.87±0.02	0.80±0.02	70±5	0.89±0.02	0.82±0.02	70±5	>1.00	>1.00	–	>1.00	>1.00	–
Acetylsalicylic acid	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–
Nimesulide	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–
Ibuprofen	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–
Ketoprofen	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–
Ascorbic acid	0.33±0.02	0.24±0.02	90±5	0.78±0.03	0.61±0.02	170±10	0.81±0.02	0.66±0.02	150±10	0.93±0.03	0.82±0.02	110±10
Mannitol	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–	>1.00	>1.00	–

the cases of lornoxicam and meloxicam, the peak is broadened and its position becomes disputable (Fig. 2d for lornoxicam), eventually due to the presence of a second peak. In this case rather, an E_{ap} range can be characterized. By contrast, tenoxicam and piroxicam give a defined single peak. The presence of two peaks may be justified on the basis of the oxidation mechanism. In general, oxicams' oxidation can be performed in two steps, according to the mechanism illustrated in Scheme 1.

The first step refers to the oxidation of the enolic –OH to form the corresponding free radical, which can be deprotonated (chemical step). The second step is the further oxidation of the deprotonated derivative, and the formation of a carbonyl and a positive charge localized on a carbon atom, which is stabilized by the lone pair of the neighboring nitrogen. This cation is of course susceptible of nucleophilic attack by the solvent (H_2O, MeOH, EtOH, and CH_3CN). When acetonitrile is used as solvent, a final acetamide derivative can be obtained through further reaction with acetonitrile and of trace amounts of water (Ritter reaction) [26]. In this aspect, the peak range of meloxicam and lornoxicam observed in the presence of acetonitrile indicates that the two oxidation steps become distinct, since the first oxidation step requires relatively lower energy starting at lower potential, while the second oxidation, demanding higher energy, takes place at higher oxidation potential. On the contrary, the single peak of piroxicam and tenoxicam at higher potential implies that both oxidation steps require higher energy, taking place simultaneously at higher potentials.

In order to better explore the effect of solvent polarity to the electrochemical reactivity of oxicams, different mixtures of acetonitrile with water were tested. The differences in voltammetric peak shape of oxicams were confirmed especially in higher acetonitrile fractions. In all cases, the irreversible character of the oxidation mechanism of oxicams was also tested and confirmed by the absence of any peak even by increasing the scan rate at the reversed scan up to 200 mV s^{-1}. Cyclic voltammograms of piroxicam obtained in acetonitrile fractions (φ) in water of 0.1, 0.5, 0.85, and 1 are depicted in Fig. 3. As it is shown, by increasing acetonitrile fraction, the anode peak of piroxicam is shifted towards more positive potentials, while a peak broadening containing eventually two non-distinct peaks is observed for $\varphi>0.65$. When $\varphi\rightarrow 1$, a rapid increase in current intensity produces again one distinct peak at higher potential as



Scheme 1 Electrochemical oxidation mechanism of oxicams

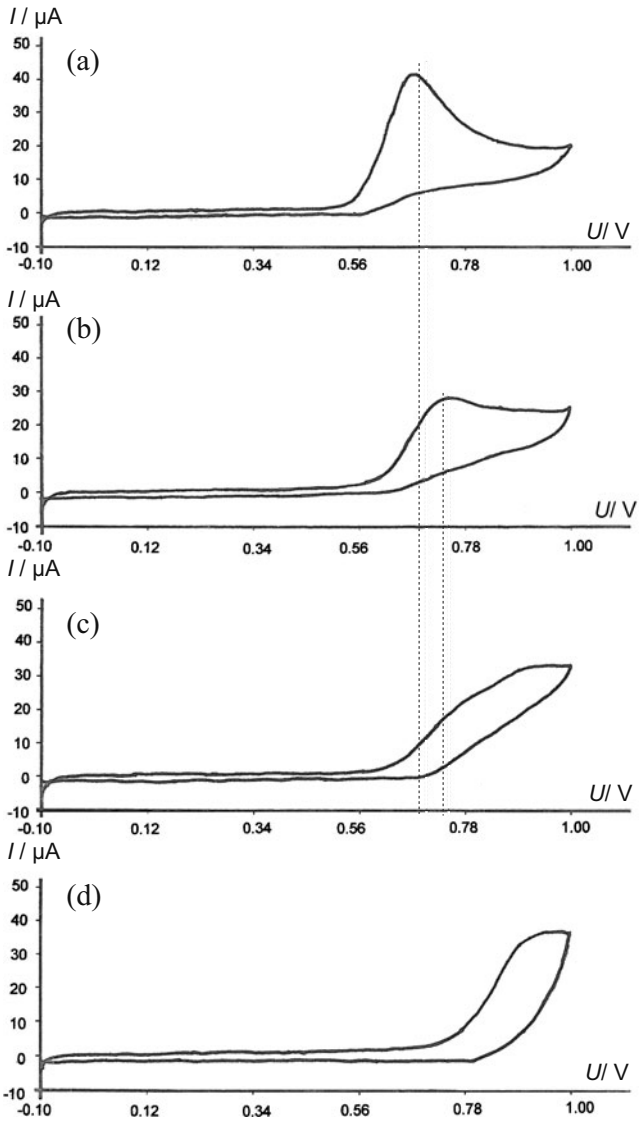


Fig. 3 Cyclic voltammograms of piroxicam obtained in **a** 10% CH_3CN/90% H_2O, **b** 50% CH_3CN/50% H_2O, **c** 85% CH_3CN/15% H_2O, and **d** 100% CH_3CN. Conditions: piroxicam concentration, 0.5 mM; electrolyte, 0.1 M $LiClO_4$; sweep rate, 20 mV s^{-1}

already discussed. Analogous behavior was observed for tenoxicam. However, in the case of meloxicam and lornoxicam, only a E_{ap} range can be determined for φ over 0.80 and 0.90, respectively, and no distinct peak at φ=1 is observed. Thus, it is clear that the shifting of oxicams' oxidation potentials at higher voltages by decreasing solvent polarity is governed by the increase of the E_{ap} of the second oxidation step (due to the higher energy required) leading to the formation of a broadened peak range. Further, decreasing the solvent polarity shifts E_{ap} of the first oxidation step at more positive potentials, leading to one defined peak again. Piroxicam and tenoxicam are stronger affected by solvent replacement, while the corresponding phenomenon is observed for meloxicam and lornoxicam at less polar solvents. The effect of acetonitrile fraction to the anode peak voltage of oxicams is depicted in Fig. 4, where the data correspond only to well-defined single peaks. Dashed lines correspond to the region of not well-defined peaks. It is interesting that in the case of piroxicam, a nearly linear $E_{ap}=f(\varphi)$ relationship was observed.

Study of solute–solvent interactions in the reactivity of oxicams using Kamlet–Taft's analysis

The observed variances in electrochemical behavior of oxicams indicate that a single macroscopic solvent parameter, like polarity, may not account for the multitude of solute–solvent interactions on the molecular microscopic level. Therefore, it may fail to describe the microenvironment around the reacting species, which governs the stability of the reactive intermediate. Selective solvation is more likely to occur, including non-specific solute–solvent association and specific solute–solvent association, such as hydrogen bonding and electron pair donor or acceptor interactions. The influence of specific and non-specific solute–solvent–solvent interaction to the reactivity of oxicams has been studied by employing the so-called Kamlet–Taft solvatochromic method [27],

$$\log k = A_0 + s \cdot \pi^* + a \cdot \alpha + b \cdot \beta \quad (2)$$

where π^* is an index of solvent dipolarity/polarizability accounting the ability of the solvent to stabilize a charge or a dipole by virtue of its dielectric effect (non-specific interaction), α is the solvent hydrogen bond donor (HBD) acidity, β is the solvent hydrogen bond acceptor basicity (specific interactions), and A_0 is the regression value of the solute property in the reference solvent cyclohexane. The regression coefficients s, a, and b reflect the relative susceptibilities of the solvent-dependent solute property $\log k$ (E_{ap} in the present case) to the indicated solvent parameter. For the Kamlet–Taft's analysis of oxidation potentials of oxicams, the corresponding E_{ap} values obtained with methanol, ethanol, and mixtures of acetonitrile with water were used. It should be mentioned that E_{ap} values from not well-defined peaks were not taken into account because such cases imply more complex electrochemical phenomenon. The statistical results as well as the weighed percentage contributions of Kamlet–Taft's solvatochromic parameters π^*, α, and β to the E_{ap} values of oxicams are presented in Table 2. As shown, some coefficients of the terms π^*, α, and β are not statistically significant. Therefore, regression analysis was repeated, taking into account only significant values. In the case of tenoxicam and lornoxicam, two combinations of the significant coefficient a were obtained with either s or b significant values. As it is shown, oxidation potential data correlate very well with the solvatochromic parameters, implying that both specific and non-specific solute–solvent interactions govern reactivity. A common property of all investigated oxicams is the HBD coefficient a with statistically significant negative values in all cases, suggesting better solvation of the reactant through hydrogen bond donor interactions as compared with the transition state. This observation further confirms the essential role of enolic OH in the first step in the oxidation mechanism of oxicams (Scheme 1).

Besides the α parameter, the investigated oxicams exhibit quite different electrochemical behavior as presented in Table 2. Indeed, piroxicam shows an electrochemical reactivity mainly governed by non-specific interactions. In the case of meloxicam, the positive sign of b implies that hydrogen bond acceptor interactions of the reactant intermediate are predominant. Tenoxicam and lornoxicam revealed a similar behavior (both differ by a chlorine substitution at the thiophene ring), expressed by the prevalence of solvent hydrogen bond donor interactions in analogous proportion either with non-specific interactions (negative sign of s) or

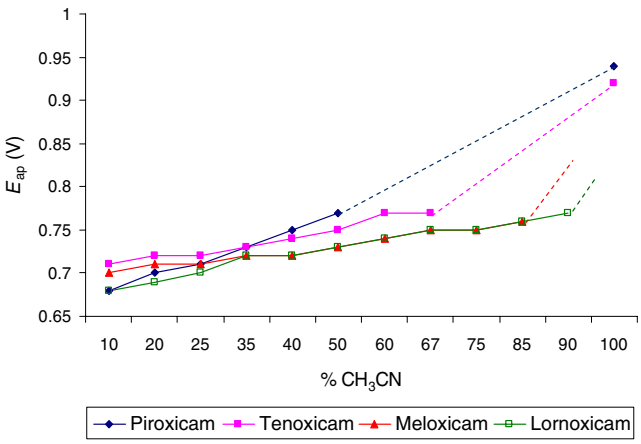


Fig. 4 Effect of the acetonitrile fraction in its mixture with water to the E_{ap} values of oxicams. Conditions: oxicams concentration, 0.5 mM; electrolyte, 0.1 M $LiClO_4$; sweep rate, 20 $mV\,s^{-1}$

Table 2 Statistical results and weighted percentage contributions for the correlation of E_{ap} for the electrochemical oxidation of oxicams with Kamlet–Taft's solvatochromic parameters α, β, and π^*

Compound	$100\times R^2$	SD	F	A_0	s	a	b	P_{π^*}	P_α	P_β
Piroxicam	100	0.004	353.6	0.16±0.06	−0.22±0.06	−0.09±0.02	−0.05±0.05	61	26	13
	100	0.004	547.9	0.11±0.01	−0.16±0.01	−0.10±0.01	–	62	38	–
Tenoxicam	88	0.013	15.4	0.02±0.20	−0.07±0.20	−0.10±0.05	−0.02±0.17	37	53	10
	88	0.013	25.9	−0.05±0.01	–	−0.11±0.02	0.04±0.02	–	73	27
	88	0.013	26.3	0.004±0.02	−0.05±0.02	−0.10±0.02	–	33	67	–
Meloxicam	100	0.001	485.6	−0.14±0.02	0.004±0.022	−0.04±0.01	0.09±0.02	3	30	67
	100	0.001	816.2	−0.13±0.002	–	−0.04±0.002	0.09±0.002	–	31	69
Lornoxicam	98	0.003	122.7	−0.12±0.04	0.01±0.04	−0.06±0.01	0.04±0.04	11	54	35
	98	0.003	196.2	−0.11±0.003	–	−0.06±0.003	0.03±0.01	–	67	33
	97	0.003	178.5	−0.07±0.01	−0.04±0.01	−0.05±0.003	–	40	60	–

with solvent hydrogen bond acceptor interactions with the reactant intermediate (positive sign of b).

Effect of excipients to cyclic voltammetric analysis

An investigation was carried out in order to study possible interferences in the cyclic voltammograms of the non-steroidal anti-inflammatory drugs being caused by excipients. For this purpose, voltammetric responses of the investigated NSAIDs were measured in the presence of the most common excipients, such as powdered cellulose, corn starch, sodium bicarbonate, sodium carbonate, lactose monohydrate, magnesium stearate, talc, and mannitol. It was found that all these excipients had no interference effect to the cyclic voltammograms of all investigated drugs in all solvents. Indeed, both $E_{1/2}$, E_{ap}, and peak current remained constant, while no additional peak was observed. This is in agreement with results from previous studies reporting the development of voltammetric techniques for the quantification of individual non-steroidal anti-inflammatory drugs in pharmaceutical formulations [17, 25, 28, 29]. Hence, the investigation of antioxidant activity of drugs using cyclic voltammetry can be performed directly in commercial solid pharmaceutical formulations, after their appropriate dissolution.

Chronoamperometric study of NSAIDs

Chronoamperometry was employed for further electrochemical study of NSAIDs. For this purpose, chronoamperometic measurements in methanol, ethanol, acetonitrile, and in 90:10 water/acetonitrile mixture in the presence of 0.1 M $LiClO_4$ as electrolyte were carried out at the potentials of 0.3, 0.5, and 0.8 V. These potentials can be used to characterize compounds according to high, intermediate, and weak antioxidant activity, respectively [16]. In all cases, the plot of I vs. $t^{-1/2}$ was linear, in agreement with Cottrell equation, implying a diffusion-controlled electrode procedure [30, 31]. Amperometric signal (I_a) of each investigated compound was read at t=120 s (a sufficient time period for obtaining a steady state and linearity), and the results were corrected by the corresponding current intensity of the blank. In the aqueous (90:10 water/acetonitrile) medium, oxicams gave measurable signal at 500 and 800 mV, while all other NSAIDs were detectable at 800 mV. This observation further confirms the classification of NSAIDs derived by cyclic voltammetry, according to which oxicams are moderate to weak antioxidants, while the remaining NSAIDs exhibit only very weak antioxidant properties. In methanol, oxicams, mefenamic, and tolfenamic acid were detectable at 800 mV, while in ethanol, only oxicams and mefenamic acid gave detectable signal. The decrease in amperometric signal by lowering the solvent polarity is obvious due to the increased difficulty in oxidation, as it has already been discussed. Generally, the plot of anodic currents vs. drugs' concentration showed good correlation in the investigated conditions. Especially, the linearity of the amperometric signal of oxicams at 800 mV was confirmed in a concentration range of 0.05 to 1 mM in methanol, ethanol, and acetonitrile.

Comparison of electrochemical data with the reactivity of NSAIDs towards DPPH

The electrochemical data described above were compared with the reactivity of NSAIDs towards the stable 1,1-diphenyl-2-dipicrylhydrazyl free radical, DPPH. In agreement with the electrochemical results, oxicams showed a moderate interaction with DPPH, reaching steady state after 60 min. All other investigated NSAIDs did not show any reactivity towards DPPH. In this view, electrochemical techniques can be considered as more sensitive to weak antioxidants since mefenamic and tolfenamic acid gave electrochemical signals despite their inactivity towards DPPH.

Table 3 Comparison of amperometric signal (I_a) of oxicams measured using chronoamperometry at 800 mV with their IC_{50}, z, and log$z$ values in methanol, ethanol, and acetonitrile

NSAID/ solvent	Methanol				Ethanol				Acetonitrile			
	I_a (μA)	IC_{50} (μM)	z ($M^{-1}s^{-1}$)	log$z$	I_a (μA)	IC_{50} (μM)	z ($M^{-1}s^{-1}$)	log$z$	I_a (μA)	IC_{50} (μM)	z ($M^{-1}s^{-1}$)	log$z$
Piroxicam	6.1±0.7	74±9	3.3±0.4	0.52	3.7±0.3	109±10	2.6±0.3	0.41	2.8±0.3	169±18	2.1±0.3	0.32
Tenoxicam	11.3±1.2	73±17	8.1±0.9	0.91	5.0±0.4	63±19	7.3±0.8	0.86	4.8±0.4	63±10	7.7±0.2	0.89
Meloxicam	1.3±0.4	–[a]	0.2±0.01	−0.70	2.9±0.3	–[a]	0.3±0.1	−0.52	2.2±0.3	382±42	1.5±0.2	0.18
Lornoxicam	2.0±0.5	–[a]	0.1±0.03	−1.00	3.1±0.3	–[a]	0.2±0.05	−0.69	2.4±0.4	577±113	0.8±0.1	−0.10
Mefenamic acid	1.2±0.3	–	–	–	0.6±0.2	–	–	–	–	–	–	–
Tolfenamic acid	0.8±0.2	–	–	–	–	–	–	–	–	–	–	–

All electrochemical measurements were performed at a concentration of 0.5 mM of each compound

[a] At steady state, 50% inhibition of DPPH was not reached

IC_{50} values of oxicams along with their amperometric signal at 800 mV, both referring at a concentration of 0.5 mM of each compound, are presented in Table 3. As expected, no correlation between I_a and IC_{50} can be derived because IC_{50} values refer to a fixed end-point of a reaction, without taking into account the reaction rate. Therefore, the kinetic approach of the reaction DPPH/oxicams was considered as a more representative measure of the antioxidant activity. Rate constants, k, of the reaction of oxicams with DPPH were calculated using second order kinetics for different concentration ratios $\frac{[\text{Oxicam}]}{[\text{DPPH}]}$ [8]. The very good linearity between the reciprocal of DPPH concentration, (C^{-1}), and time (t) verified the reaction order. As rate constants depend on the DPPH concentration, they also cannot be directly compared with electrochemical data. For this reason, absolute rate constants, z, were derived by linear regression of k against the concentration ratio $\frac{[\text{Oxicam}]}{[\text{DPPH}]}$ (correlation coefficients $r>$ 0.99), according to formula 3.

$$k = z \cdot \frac{[\text{Oxicam}]}{[\text{DPPH}]} + \text{intercept} \quad (3)$$

Absolute rate constants, z, and their logarithms, log$z$, of piroxicam, tenoxicam, meloxicam, and lornoxicam, are included in Table 3. Taking into account that strong antioxidants like trolox and ascorbic acid possess log$z$ values close to 3 log units [32], the results of Table 3 further confirm the moderate to weak antioxidant capacity of oxicams. The kinetic parameter z was found to correlate positively with the amperometric signal, I_a, of oxicams under all solvent conditions, with correlation coefficients r equal to 0.995, 0.995, and 0.986 in methanol, ethanol, and acetonitrile, respectively. A representative correlation plot is shown in Fig. 5. These findings support the relation between the oxidation reaction rate towards DPPH and the corresponding oxidation rate on the glassy carbon electrode; thus, the amperometric signal of NSAIDs can be considered as a suitable and friendly measure for the evaluation of their antioxidant activity.

Conclusions

The underlying solvent has a considerable influence to the oxidation potentials of the investigated NSAIDs and, hence, to their antioxidant activity, mainly by affecting the solvation of their more polar intermediates. The peak broadening of oxicams, observed for a certain fraction of acetonitrile in water, imply complex electrochemical behavior and the presence of an oxidation mechanism with two successive oxidation steps. Solvent effect is stronger in the case of piroxicam and tenoxicam compared with meloxicam and lornoxicam, while Kamlet–Taft's analysis confirms the contribution of both specific and non-specific solute–solvent interactions in reactivity. No interferences are caused by common excipients, allowing direct electro-

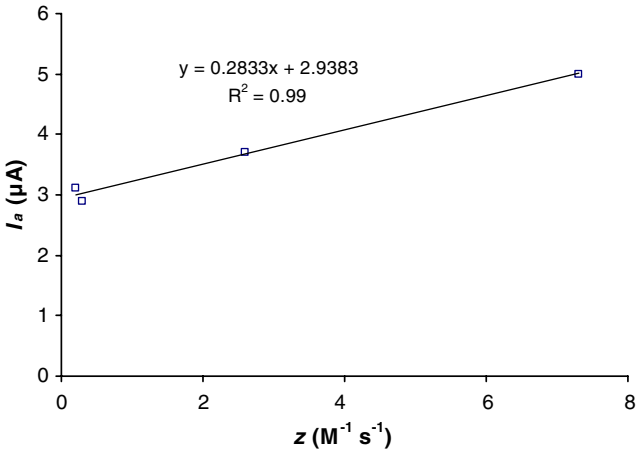


Fig. 5 Correlation $I_a=f(z)$ of piroxicam, tenoxicam, meloxicam, and lornoxicam in ethanol

chemical investigation of antioxidant activity of NSAIDs in commercial pharmaceutical formulations. Electrochemical data of NSAIDs are found to be consistent with their interaction with DPPH, and a positive correlation of oxicams' amperometric signal at 800 mV with their absolute rate constants towards DPPH, *z*, was achieved. In conclusion, the amperometric signal can be considered as a suitable and friendly alternative to evaluate the antioxidant activity with application to both pure substances and to pharmaceutical formulations.

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