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Polarographic behaviour of meloxicam and its determination in tablet preparations and spiked plasma

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Abstract

Meloxicam is a new non-steroidal anti-inflammatory drug (NSAID), which has a higher activity cyclooxygenase-2 (COX-2) than against cyclooxygenase-1 (COX-1), with potentially high anti-inflammatory and analgesic action. The voltammetric behaviour of meloxicam was studied using direct current (DC), differential pulse polarography (DPP) and cyclic voltammetry (CV). The influence of several variables (including nature of the buffer, pH, concentration, modulation amplitude, scan rate, drop size, etc.) was examined in DPP method for meloxicam. The best DPP response was obtained in acetate buffer pH 4.88. The peak currents were measured with a static mercury drop electrode at -1.49 V versus Ag/AgCl. Calibration curve for meloxicam was linear at a concentration range from 0.38 to 15.0 μg ml^{-1}. The method was validated and applied to the determination of meloxicam in tablets, which were in two different dosage forms. A spectrophotometric method reported in the literature was utilized as a comparison method. There were no significant differences between the results obtained by two methods. DPP method is also available and applicable for the determination of mentioned substance in plasma. Mean recovery was $99.20 \pm 0.37\%$. It was concluded that the developed method was accurate, sensitive, precise, reproducible and useful for the quality control of meloxicam in pharmaceuticals and spiked plasma.

Keywords: Meloxicam; Polarographic behaviour; Differential pulse polarography; Tablet analysis; Plasma analysis

1. Introduction

Meloxicam (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazoly)-2*H*-1,2-benzo-thiazine-3-carboxamide-1,1dioxide) ($C_{14}H_{13}N_3O_4S_2$) (Fig. 1) is a new non-steroidal anti-inflammatory drugs (NSAIDs) of enolic acid class compounds [1,2]. Meloxicam is a novel NSAIDs with a favourable cyclooxygenase-2 (COX-2)–cyclooxygenase-1 (COX-1) selectivity has also been shown to have potent anti-inflammatory effects [3]. Meloxicam makes selective inhibition to COX-2 more than COX-1 [4].

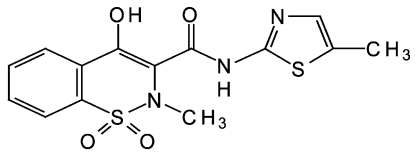

Fig. 1. Chemical structure of meloxicam.

Because of very low solubility of meloxicam in acidic medium, it may cause few local gastrointestinal adverse events [5].

In the literature, spectrophotometric [6–8], densitometric [7], high performance liquid chromatographic (HPLC) [8], flow injection [6,9] polarographic [10] and voltammetric [11,12] methods are reported for the analysis of meloxicam in pharmaceuticals. HPLC is the technique most commonly used for the determination of meloxicam in plasma [13].

The main purpose of this study is to certain the polarographic behaviour of meloxicam and to develop a simple, cheap and sensitive polarographic method for the determination of meloxicam in pharmaceuticals and also applying it to plasma. In the literature [10], the polarographic techniques were applied to the pharmaceutical preparations of meloxicam and the limit of detection (LOD) value has been found 3.51 μg ml^{-1}. In our study, developed method was more sensitive than this method (0.02 μg ml^{-1}). Therefore, our method can be applied to the plasma. There are no extraction and evaporation steps for the determination of meloxicam

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in either pharmaceuticals or plasma by the developed polarographic method.

2. Experimental

2.1. Apparatus

Electrochemical measurements were performed by a PAR 174A polarographic analyser. A PAR 303A was used in the static Hg drop electrode mode as a working electrode. A Ag/AgCl electrode was used as a reference electrode and auxiliary electrode was a Pt wire. Data were recorded on a Houston Omnigraphic 2000 X–Y recorder. Cyclic voltammetric measurements were carried out on a BAS CV 27 voltammograph. Hanging Hg drop electrode was used as a working electrode. All experiments were performed at 20 ± 0.1 °C.

Spectrophotometric measurements were carried out by using a Shimadzu 160-A double-beam UV–Vis spectrophotometer with 1 cm quartz cells at a scan rate 60 nm min^{-1} with a fixed slit width of 3 nm.

2.2. Materials and reagents

All experiments were performed with analytical-reagent grade chemicals and pure solvents. Meloxicam was donated from Nobel (Turkey) and used as received. Melox tablets (labelled to contain 7.5 and 15 mg meloxicam per tablet in two dosage forms) were obtained from local pharmacies.

2.2.1. Standard solutions

Stock standard solutions (250 μg ml^{-1}) of meloxicam were prepared in MeOH for pharmaceutical analysis and in MeCN for plasma determinations. These solutions were kept at $+4$ °C during 2 months and observed that meloxicam stock solutions were stable in this period. Standard solutions were prepared by appropriate dilutions of stock solution, daily.

Acetate buffer was prepared by mixing 49.2 ml glacial AcOH = CH_3COOH and completed to volume 1 l with distilled water. The pH of solution was adjusted with 0.1 F HCl or 0.1 F NaOH. Stock solutions of Fe(III)$Cl_3 \cdot 6H_2O$ were prepared 2×10^{-2} F in MeOH.

2.3. Procedure

2.3.1. For polarographic analysis

The supporting electrolyte solution (5 ml), containing pH 4.88 acetate buffer, was pipetted into polarographic cell. Dissolved oxygen was removed from the solution by a purified nitrogen gas stream through the cell for 8 min. Polarogram of this solution was recorded then standard solutions of meloxicam in MeOH were added. Nitrogen was passed through the solution for 30 s and directed above the solution during the scan. Polarograms of these solutions were recorded.

2.3.2. For spectrophotometric analysis (comparison method) [6]

Two millilitres of Fe(III)$Cl_3 \cdot 6H_2O$ solution was transferred into 10 ml volumetric flask. Different volume of standard solution of meloxicam in MeOH was added then completed to the volume with MeOH. The absorbance of meloxicam–Fe(III) complex was measured at 600 nm against 2×10^{-3} F Fe(III) solution in 1 h.

2.3.3. Analysis of tablets

Ten tablets were weighed from each dosage forms and powdered. Equivalent amount to one tablet was weighed and transferred to a 50 ml volumetric flask. Methanol = MeOH (30 ml) was added and the flask was sonicated for 15 min to effect complete dissolution and diluted to the mark with MeOH. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with MeOH. Then tablet sample solutions were analysed same as in Sections 2.3.1 and 2.3.2.

2.3.4. Analysis of spiked plasma

Drug free human blood was centrifuged (3500 rpm) in 30 min at room temperature and the plasma was separated. Different volumes of standard solution of meloxicam in MeCN (MeCN was used to precipitate the proteins) were added to the plasma. *After* centrifugation at 3500 rpm in 15 min and 100 μl of this supernatant was transferred into the polarographic cell containing 5 ml supporting electrolyte and analysed with differential pulse polarography (DPP) method.

3. Results and discussion

3.1. Polarographic behaviours

Direct current (DC), tast, pulse and DPP techniques were applied for assay of meloxicam. The DPP technique was most sensitive and its peak current was higher than the other techniques. According by DPP has been chosen.

The effect of pH was investigated in various acidic, basic and buffer solutions having a pH value range of 1–12. Acetate buffer at pH 4.88 was found to be the best supporting electrolyte. Meloxicam shows a distinct peak at -1.49 V versus Ag/AgCl in acetate buffer pH 4.88.

The studies showed that well-defined polarograms and relatively high peak currents were obtained when scan rate: 5 mV s^{-1}, pulse amplitude: 50 mV, drop time: 1 s and small drop size (0.010 cm^2) were used.

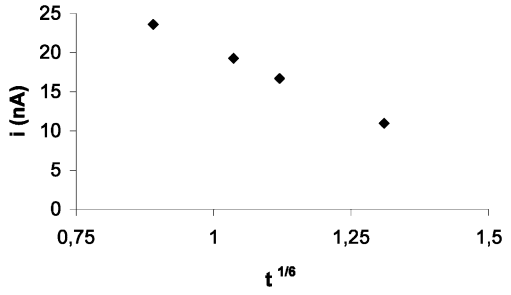

Fig. 2. Variation of peak current (i) with $t^{1/6}$ for 5.88 μg ml^{-1} meloxicam.

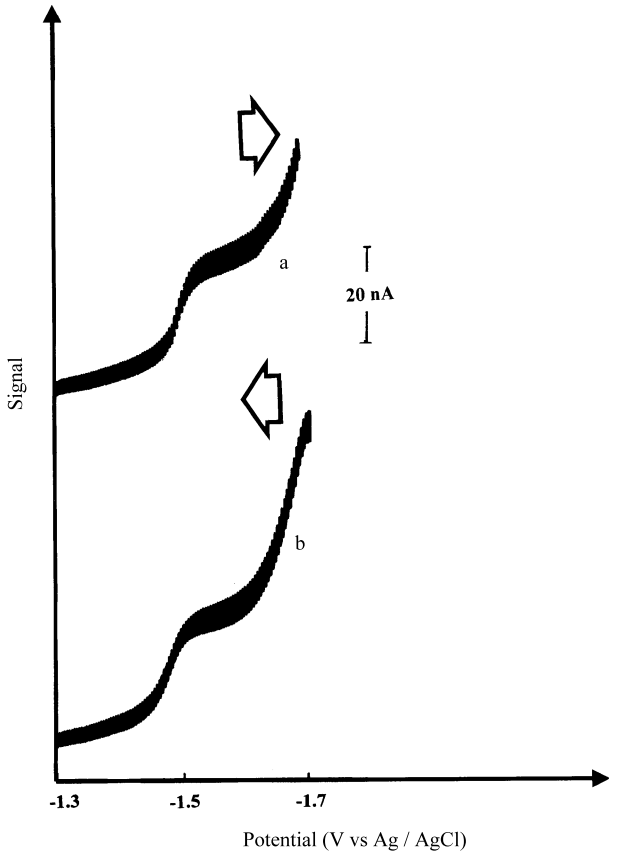

Fig. 3. DC polarogram of meloxicam (5.88 μg ml^{-1}): (a) anodic scanning; (b) cathodic scanning. (Supporting electrolyte = acetate buffer (pH 4.88), scan rate = 5 mV s^{-1}.)

The character of the limiting current was investigated by DC, DPP and CV techniques. In DC polarography, diffusion current decreases with increasing of $t^{1/6}$ (t is the drop time) [14]. A plot of limiting current versus $t^{1/6}$ gave a straight line and the equation of line was $y = -29.88x + 50.34$ ($r = 0.9984$) (Fig. 2).

The DPP studies at different temperature showed that limiting current increased with increasing temperature. Temperature coefficient of the meloxicam was found to be 0.72% deg^{-1} with standard deviation of 0.16. This value was lower than 2% and these results clearly indicated that the current was diffusion controlled [15].

The cyclic voltammograms were recorded 10 and 1000 mV s^{-1} scan rate (v). A plot of log i_p versus log v gave a straight line ($y = 0.73x - 1.36$) and correlation coefficient was 0.9993. Slope which was nearly 0.5 supported that the peak current was controlled by diffusion [16].

The reversibility test was performed by DC and CV. In DC, a plot of $\log[(i_d - i)/i]$ versus E gave a straight line ($y = 0.041x - 1.58$; $r = 0.9964$). The slope of this line was not similar to the theoretical Nernst voltage of 0.0295 V (25 °C), of 2.303 RT/nF for a reduction involving two electrons. Charge transfer coefficient, α (0.71) was found be < 1 (slope $= 0.059/\alpha n$) [17]. In DC polarograms the half-wave potentials of anodic and cathodic waves were compared. There was a 10 mV difference between the half-wave potentials. The ratio of anodic to cathodic current (I_a/I_c) was found 0.95 (Fig. 3). In DPP, the peak potentials shifted with drop time (0.5, 1, 2, 5 s). The shift value was very small as 0.03 V. In CV, no oxidation (anodic) peak was appeared on the reverse scan (Fig. 4). A plot of peak current versus square root of the scan rate did not give a straight line. The peak potentials shifted to negative values by increasing scan rate. All these DC and CV results showed that the electrode process was not reversible [16].

The reduction mechanism of meloxicam was investigated. The polarographic behaviours of piroxicam and tenoxicam have been explained [18,19]. DC polarograms of piroxicam and meloxicam solutions in 5.88 μg ml^{-1} were recorded. Half-wave potential $E_{1/2}$ and currents of the polarographic waves were found to be identical (Fig. 5). According to these observations the polarographic behaviour seemed to be similar [18].

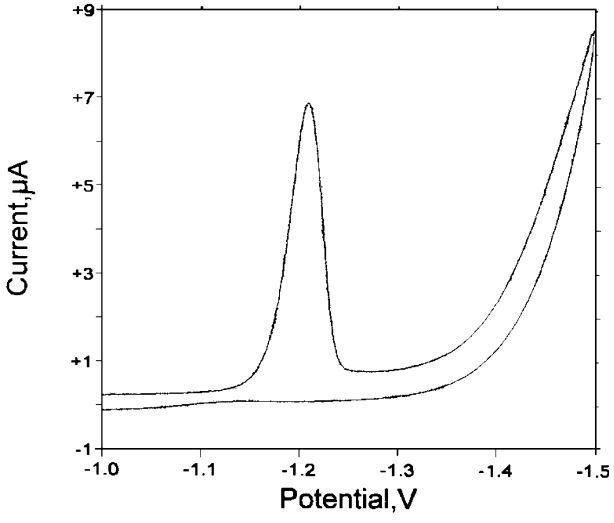

Fig. 4. Cyclic voltammograms (CV) of meloxicam (5.88 μg ml^{-1}). (Supporting electrolyte = acetate buffer (pH 4.88), scan rate = 800 mV s^{-1}.)

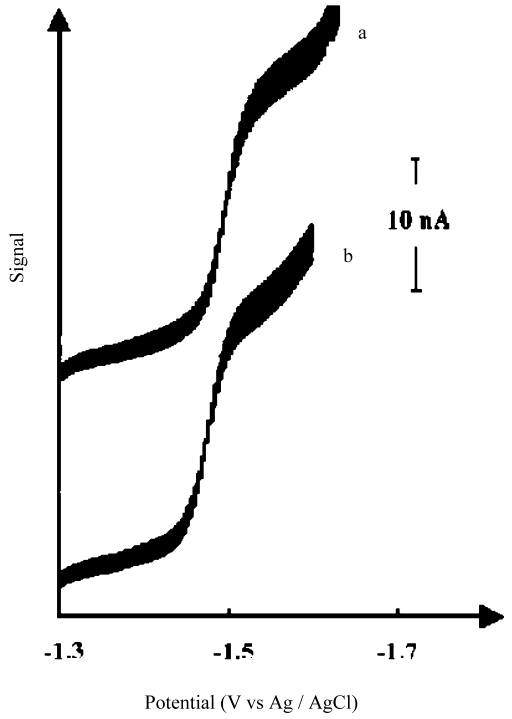

Fig. 5. DC polarograms of: (a) piroxicam (5.88 μg ml^{-1}); (b) meloxicam (5.88 μg ml^{-1}). (Supporting electrolyte = acetate buffer (pH 4.88).)

Meloxicam have a tiazole ring instead of a pyridine ring in piroxicam. The same situation has also validated for tenoxicam [19]. As a result, a two electron transfer corresponding to the reduction of the enol group in the cyclic benzene sulfonamide ring appeared at pH 4.88 was proposed as the mechanism of the electrode reaction.

3.2. Method validation [20]

3.2.1. Linearity

The effect of meloxicam concentration on the peak current were determined (Fig. 6). The calibration graphs of peak current versus meloxicam concentration were found to be linear over the range 0.38–15.0 μg ml^{-1}.

The regression equation was $y = (49.24 \pm 0.17)x + (48.12 \pm 0.79)$ ($n = 10$) were y is the peak current in nA and x is the concentration in μg ml^{-1} ($r = 0.9994$). The r value was found to be significant ($t_C = 1.03 < t_T = 2.13$, $P < 0.05$) and the intercept was not significantly different from zero (t_C: $0.0739 = 0.07$).

3.2.2. Sensitivity

The LOD for meloxicam was found as 0.02 μg ml^{-1}, at a signal to noise ratio of 3. The limit of quantification (LOQ) was 0.38 μg ml^{-1}, for meloxicam. The coefficient of variation was 2.35% for LOQ ($n = 6$).

3.2.3. Accuracy and precision

It was shown that the excipients such as sodium citrate dihydrate, lactose monohydrate, avicel, aerosile, magnesium stearate, PVP, which are commonly formulated in tablet dosage form, do not interfere with the proposed method. For this purpose, the determination of meloxicam in a synthetic tablet samples (the mixture of excipients and labelled amount of meloxicam) were prepared. The precision, accuracy and repeatability of DPP and UV spectrophotometric methods for meloxicam were determined in six replicate analysis on synthetic tablet samples and results were presented in Table 1. The percentage recoveries for meloxicam in tablets were $98.90 \pm 0.08\%$ for 7.5 mg and $98.88 \pm 0.27\%$ for 15 mg in DPP method.

The intraday and interday precision and accuracy were evaluated by analysing four different concentration of meloxicam solutions (Table 2).

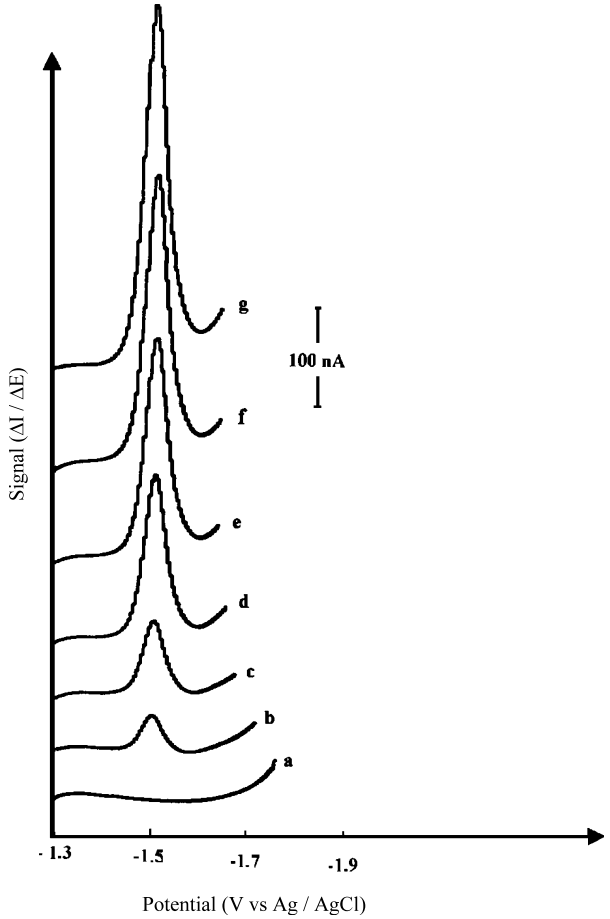

Fig. 6. The effect of concentration on the peak current of meloxicam by using DPP: (a) supporting electrolyte (acetate buffer pH 4.88) 5 ml; (b) 0.38 μg ml^{-1}; (c) 0.74 μg ml^{-1}; (d) 1.96 μg ml^{-1}; (e) 2.91 μg ml^{-1}; (f) 3.85 μg ml^{-1}; (g) 4.76 μg ml^{-1} of meloxicam. (Scan rate = 5 mV s^{-1}.)

Table 1
Studies of precision, accuracy and reproducibility for the analysis of meloxicam by the develop (DPP) and the comparison (UV) methods ($n = 7$)

	Nominal value	
	7.5 mg	15 mg
DPP		
$\bar{x}$	7.42 ± 0.01	14.83 ± 0.04
SD	0.02	0.11
RSD (%)	0.27	0.74
% Mean recovery	98.90 ± 0.08	98.88 ± 0.27
UV		
$\bar{x}$	7.52 ± 0.02	14.98 ± 0.02
SD	0.04	0.05
RSD (%)	0.53	0.33
% Mean recovery	100.32 ± 0.02	99.95 ± 0.13

$\bar{x}$, mean ± standard error; SD, standard deviation; RSD (%), percentage relative standard deviation.

3.2.4. Selectivity

In order to detect interactions of excipients in this method, the standard addition technique was applied to the same preparations which were analysed by calibration curve. The regression equation of standard addition curve was found to be $y = 49.37x + 235.02$ ($r = 0.9988$). y is peak current (nA), x is the concentration of meloxicam (μg ml^{-1}). There was no difference between the slopes of two methods with standard and standard addition. This data showed that there was no interaction of excipients in the analysis of meloxicam.

3.2.5. Analytical applications

Quantitative analysis of meloxicam in tablets at two different dosage forms (7.5 and 15 mg) were performed by using DPP. The results obtained for meloxicam were favourably compared with reference UV method [6]. The statistical comparison of two methods was done by Wilcoxon paired test $(P > 0.05 t_C > t_T) = (t_C > t_T,\ P > 0.05)$. The results showed that there was non-significant difference between developed (DPP) and comparison (UV) methods (Table 3).

Table 3
The results of DPP and UV analysis of tablets ($n = 7$)

Labelled amount	DPP	UV
7.5 mg Meloxicam		
$\bar{x}$	7.49 ± 0.04	7.54 ± 0.02
SD	0.12	0.05
RSD (%)	1.54	0.72
CI	7.39–7.59	7.49–7.59
	t_C: $6.5 > t_T$: 2 ($P > 0.05$)	
15 mg Meloxicam		
$\bar{x}$	15.16 ± 0.09	15.05 ± 0.01
SD	0.23	0.02
RSD (%)	1.52	0.16
CI	14.94–15.38	15.03–15.07
	t_C: $5.5 > t_T$: 2 ($P > 0.05$)	

$\bar{x}$, mean ± standard error; SD, standard deviation; RSD (%), percentage relative standard deviation; CI, confidence intervals ($\alpha = 0.05$); t_C, calculated t; t_T, tabulated t.

The DPP method could be successfully applied to the determination of the meloxicam in spiked plasma. Meloxicam standard solutions were spiked to the plasma in the final concentrations of 2, 8, and 12 μg ml^{-1}. This was verified using water instead of plasma. These samples were analysed to the procedure described previously. As seen in Fig. 7, meloxicam could be determined without interference. Mean recoveries of three meloxicam concentration in plasma were found to be 98.85 ± 0.95, 99.45 ± 1.58, 98.75 ± 1.95%, respectively ($n = 6$). We have seen that, the developed method (at the linear range 0.38–15.0 μg ml^{-1}) could be applied to the quantitation of meloxicam from plasma. After oral administration of a 7.5 and 15 mg dose of meloxicam to human subjects, the mean peak levels in plasma were 1.05 and 2.45 μg ml^{-1} (C_{max}), respectively; which were attained 5 h (t_{max}) after administration, and

Table 2
Intraday and interday precision and accuracy of meloxicam

Added concentration (μg ml^{-1})	Intraday [a]				Interday [b]			
	Measurement concentration (mean) (μg ml^{-1})	SD	RSD (%)	Bias (%)	Measurement concentration (mean) (μg ml^{-1})	SD	RSD (%)	Bias (%)
2.40	2.42	0.04	1.65	100.83	2.37	0.05	2.11	98.75
6.19	6.18	0.05	0.81	99.84	6.15	0.04	0.65	99.35
8.62	8.62	0.01	0.12	100.00	8.61	0.01	0.12	99.88
10.42	10.38	0.07	0.67	99.62	10.36	0.10	0.97	99.42

SD, standard deviation; RSD (%), percentage relative standard deviation; bias (%), (nominal concentration − observed concentration/nominal concentration) × 100.

[a] Mean values represent seven different meloxicam standard for each concentration.

[b] Interday was determined from seven different runs over a 4-week period. The concentration of each run was determined from a single calibration curve run on the 1st day of the study.

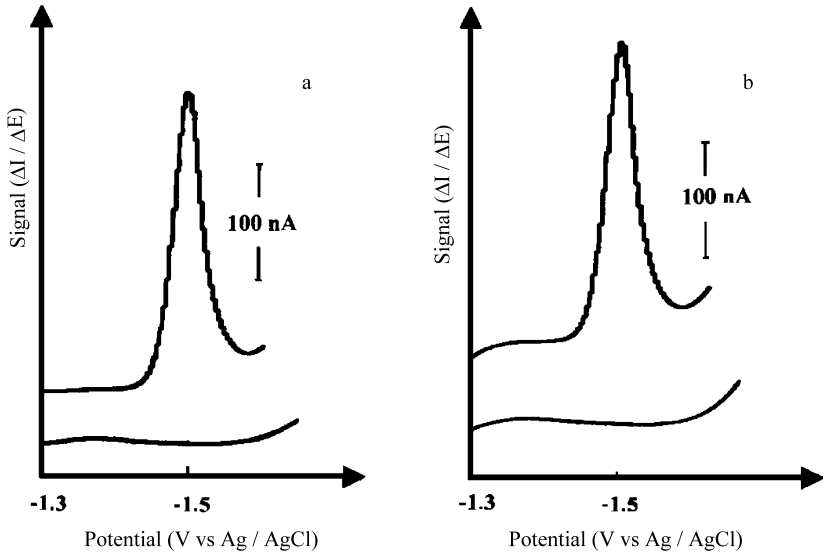

Fig. 7. Differential pulse polarograms of 3.38 μg ml^{-1} meloxicam in acetonitrile spiked to: (a) distile water; (b) human plasma. (Supporting electrolyte = acetate buffer (pH 4.88), scan rate = 5 mV s^{-1}.)

elimination half-life was about 20 h [4]. Thus, the proposed method proved to be satisfactory for the pharmacokinetic studies and routine estimation of meloxicam in human plasma.

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