Cathodic Adsorptive Stripping Square-Wave Voltammetry of the Anti-inflammatory Drug Meloxicam

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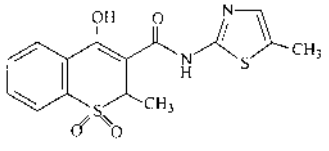
The adsorptive behavior of the anti-inflammatory drug meloxicam was studied by cyclic, differentia-pulse and square-wave voltammetry on a hanging mercury drop electrode (HMDE). The drug was accumulated at HMDE and a well-defined stripping peak current was obtained at −1.42 V *vs.* Ag/AgCl (saturated KCl) electrode in acetate buffer solution (pH 5.0). A voltammetric procedure was developed for the determination of meloxicam using square-wave cathodic adsorptive stripping voltammetry (SW-CASV). The optimum working conditions for the determination of the drug were established. The analysis of meloxicam in human plasma was carried out satisfactorily.

Key words meloxicam; square-wave; cathodic adsorptive stripping voltammetry

Meloxicam (I), 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a highly potent non-steroidal anti-inflammatory drug of enolic acid class of oxicam derivatives. It is indicated for the treatment of rheumatoid arthritis, osteoarthritis, and other joint diseases. Its therapeutic benefits combined with a good gastrointestinal tolerability are well documented.[1—4] Studies of the therapeutic and toxic effects of this anti-inflammatory drug require a sensitive method for its determination at a trace level. Many assay systems involving high-performance liquid chromatographic methods have been developed to determine meloxicam levels in biological samples.[5—8]

Adsorptive stripping voltammetry has been demonstrated as a sensitive analytical method for a wide range of pharmaceutical compounds adsorbing on the electrode surface.[9,10] A survey of the literature reveals that no attempt has been made to study the polarographic behavior of meloxicam. However, the polarographic and adsorptive behavior at the hanging mercury drop electrode (HMDE) of two oxicams, piroxicam and tenoxicam have been reported.[11—15] Differential-pulse polarography has been used for the piroxicam determination in drug formulation and in urine.[12] Square wave and square-wave adsorptive stripping voltammetric determination at the HMDE has also been employed for the determination of piroxicam and tenoxicam in urine.[13] The detection limits were 0.7 nM piroxicam and 0.1 nM tenoxicam. Differential-pulse polarographic determination of tenoxicam in pharmaceutical and blood has recently been developed.[15]

Presented here is a stripping procedure for trace measurement of meloxicam based on its controlled adsorptive accumulation at the HMDE followed by square-wave measurement of the surface species in order to improve the sensitivity and rapidity of the measurements.



(I)

Experimental

Chemicals A stock solution of 1.0×10^{-3} M meloxicam (Boehringer Ingeheim, International, GmbH Ingeheim am Rhein, Germany) was prepared in 0.05 M sodium hydroxide solution and stored in the dark at 4 °C. More dilute solutions were prepared daily with deionized water just before use. Supporting electrolyte solutions were prepared from double-distilled water using analytical grade reagents.

Apparatus Cyclic voltammograms were obtained with a PAR 273A analyzer and differential-pulse and square-wave voltammograms with an 394 electrochemical trace analyzer, coupled with a PAR 303A static mercury drop electrode (surface area = 0.026 cm^2). The polarographic cell bottom (PAR Model K0060) was fitted with an Ag–AgCl saturated KCl reference electrode and a platinum wire as a counter electrode. A magnetic stirrer (PAR 305) and stirring bar provided the convective transport during pre-concentration. The peak heights are automatically measured using the "tangent fit" capability of the instrument.

Procedure A 10 ml volume of the supporting electrolyte solution (usually 0.1 M acetic acid–sodium acetate buffer, pH 5.0) was added to the cell and de-aerated with nitrogen for 10 min (and for 30 s before each adsorptive cycle). The pre-concentration potential was then applied to a new drop for a selected time, while the solution was stirred at 400 rpm. The stirring was stopped, and after 15 s the voltammogram was recorded by applying a negative-going square-wave scan. After background voltammograms had been recorded, aliquots of the drug standard were introduced and the adsorptive stripping cycle was repeated using a new mercury drop. All data were obtained at ambient temperature.

Procedure for Plasma Plasma sample (0.5 ml) acidified with 1 ml of 2 M H_2SO_4, was transferred into a 5-ml glass tube. After addition of 3 ml of chloroform, the tube was vortexed for 10 min at 3500 rpm. The aqueous layer and the protein precipitate were discarded and the organic layer was transferred into another tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 200 μl of 0.05 M sodium hydroxide solution, completed to 10 ml with acetate, buffered and transferred to the voltammetric cell. For calibration graph, plasma samples, 0.5 ml each, were spiked with varying amounts of meloxicam to obtain final concentrations ranging from 50 to 500 ng ml^{-1}. The voltammetric procedure was continued as described above. A calibration graph was constructed by plotting the stripping peak current against the corresponding concentration of the drug. To carry out recovery experiments a known amount of meloxicam was added to plasma sample prior to its preparation, then the sample was pretreated. With an aliquot of the pretreated sample the voltammetric procedure was continued as described before. The concentrations of meloxicam in plasma were derived from the calibration graph.

Results and Discussion

The interfacial accumulation of the drug is indicated from repetitive cyclic voltammograms for 1×10^{-6} M of meloxicam in 0.1 M acetate buffer recorded following stirring for 45 s at −0.6 V (Fig. 1a). The short pre-concentration time results in

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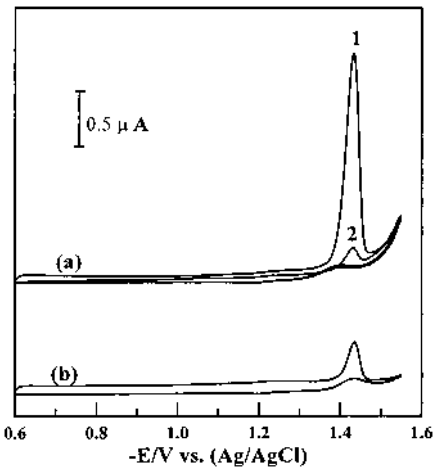

Fig. 1. a) Repetitive Cyclic Voltammograms for Meloxicam in 0.1 M HOAc–NaOAc Buffer at pH 5.0 and Scan Rate 100 mV s^{-1} after Pre-concentration for 45 s at −0.6 V Pre-concentration Potential in Solution Stirred at 400 rpm and b) an Analogous Voltammogram without the Accumulation

a large cathodic peak at −1.42 V (scan 1). This peak is attributed to the reduction of the double bond of the enol function.[11] A substantial decrease of the cathodic peak is observed in subsequent scans. Such behavior indicates rapid desorption of meloxicam from the electrode surface. The fact that no peaks were observed in the anodic branch scans suggests that the process is an irreversible one. The voltammogram in Fig. 1b represents the analogous response without accumulation; the voltammetric peak is substantially smaller than those obtained following accumulation. Adsorption-stripping cycles carried out for increasing values of the scan rate (v) under the above conditions gave rise to a reduction peak with intensities that showed linear increase with the scan rate between 0.025 and 0.5 V s^{-1}, according to the relationship: $i_{pc}=1.088\ (v/\mathrm{V\,s^{-1}})+0.156$; $r=0.996$; and $n=7$. This relation was as expected for an adsorption-controlled process.[16] Moreover, the peak potential shifted linearly to a more negative potential when the scan was increased.

Using a 1×10^{-6} M meloxicam solution, surface saturation was observed following stirring for 60 s. The response for surface-adsorbed meloxicam at saturation was used to determine the surface coverage. The surface coverage can be measured from the amount of charge consumed by the surface process as calculated by the integration of the area under the peak, corrected for residual current.[17] Division of the number of coulombs transferred, 1.824 μC, by the conversion factor (nFA) yielded coverage of 3.64×10^{-10} mol cm^{-2}. Each adsorbed meloxicam molecule therefore occupies an area of 0.46 nm^2. This area is close to that of tenoxicam and higher than that of piroxicam.[13] It is therefore likely that the thiazole substituent on the amide group is responsible for the different orientation of the adsorbed meloxicam molecules at the electrode surface.

The spontaneous adsorption of meloxicam at the HMDE can be used as an effective pre-concentration step, prior to the voltammetric measurement. Figure 2A shows square wave voltammograms at a hanging mercury electrode that has been immersed in a 1×10^{-8} M meloxicam solution for a) 0, b) 30, c) 60 and d) 90 s. The longer the pre-concentration time, the more meloxicam is adsorbed on the surface and the

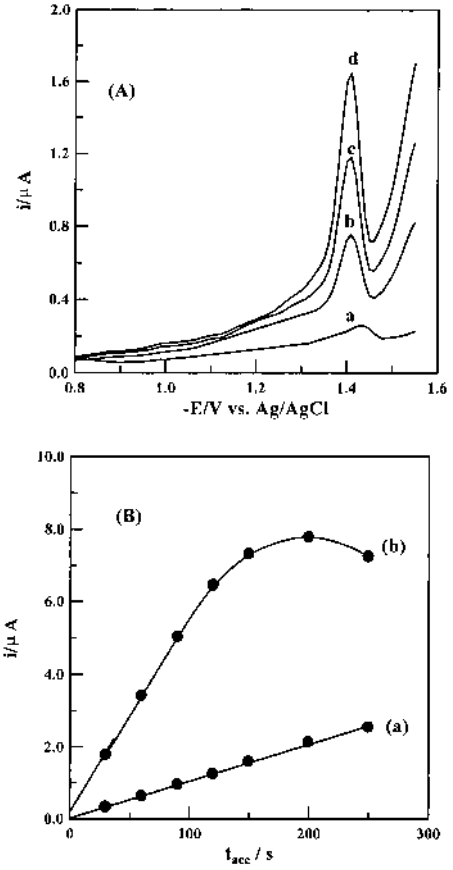

Fig. 2. A) Voltammograms for 1×10^{-8} M Meloxicam Following Pre-concentration Periods

a) 0, b) 30, c) 60 and d) 90 s

(B) Dependence of Peak Current on Pre-concentration Time at Meloxicam Concentrations

a) 1×10^{-8} M and b) 5×10^{-8} M; square waveform with frequency $f=100$ Hz, scan increment $\Delta s=4$ mV and pulse amplitude $E_{sw}=25$ mV. Other conditions as in Fig. 1.

larger is the peak current. For a 60 s pre-concentration, a 9-fold enhancement of the peak current is observed over that attained without pre-concentration. As a result, quantification of meloxicam is feasible down to the nanomolar concentration level. Figure 2B shows the dependence of the peak current on the pre-concentration time at two concentration levels. At 1×10^{-8} M meloxicam, the peak current increases linearly with increasing pre-concentration time; in contrast and as expected for a process limited by adsorption, the 5×10^{-8} M meloxicam shows a curvature for a period longer than 120 s, indicating that full surface coverage is approached. Obviously, the choice of pre-concentration time requires compromise between sensitivity and speed.

The nature, pH and concentration of the supporting electrolyte all influence the voltammetric response. Various supporting electrolytes such as Britton–Robinson, acetate and phosphate buffers were tested. It was found that acetic acid–sodium acetate buffer at pH 5.0 resulted in the highest signal. The stripping peak current for 1×10^{-8} M of meloxicam was measured in acetate buffer at pH 5.0 with ionic strengths in a range from 0.05 to, 0.25 M. The enhancement of peak current is decreased with increasing ionic strength. A well defined and sharp adsorptive reduction peak with small background current was observed in 0.1 M acetate buffer (pH 5.0). Thus,

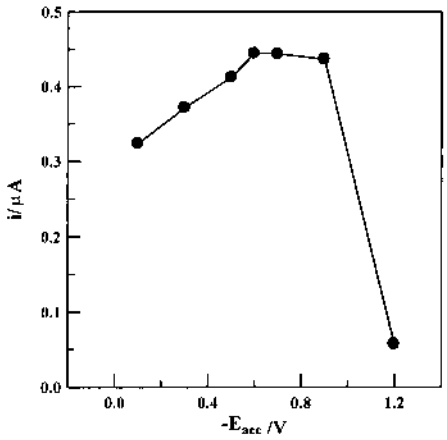

Fig. 3. Effect of Pre-concentration Potential, on the Square-Wave Stripping Signal for 1×10^{-8} M Meloxicam after Pre-concentration of 30 s, f= 120 Hz, Δs=4 mV, E_{sw}=25 mV

this medium was selected for analytical purposes.

The effect of accumulation potential was also evaluated over the range −0.1 to −1.2 V. As shown in Fig. 3, the stripping peak was low at −0.1V, then increased rapidly to maximum value between −0.5 to −0.7 V and finally decreased. A pre-concentration potential of −0.6 V was used in all subsequent works.

Figure 4 compares the differential-pulse and square-wave stripping voltammetry for 5×10^{-8} M meloxicam solution (60 s pre-concentration time at −0.6 V in each case). Although both stripping modes yield significant peak current enhancement compared to the response without pre-concentration, the square-wave stripping offers about 50-fold enhancement of the peak height over that attained by differential-pulse stripping mode. Square-wave mode offers improved performance in terms of sensitivity and speed and so this mode was used throughout.

The effects that frequency f, pulse amplitude E_{sw} and scan increment Δs had over the adsorptive signal have been studied. Frequency was varied from 20 to 120 Hz using a scan increment of 4 mV, pulse amplitude of 25 mV and 30 s pre-concentration time in 1×10^{-8} M meloxicam solution. A linear relationship was obtained between the peak current and the frequency of the signal up to 120 Hz, which is the frequency chosen to improve the sensitivity without any distortion of the peak or the baseline. At this frequency the pulse amplitude was varied between 25 and 100 mV. Although the current increases linearly with the amplitude, peak distortion in this signal was observed, resulting in a poorer resolution. Pulse amplitude of a value of 25 mV was applied as it was better for analytical purposes. When the scan increment (Δs) was tested, the increase of this parameter produced an increase in the peak intensity. A value of 8 mV was the most appropriate. A frequency of 120 Hz, of 25 mV pulse amplitude and a scan rate of 960 mV s^{-1} (Δs frequency) were used throughout the study.

Analytical Application Figure 5 illustrates the adsorptive stripping response to successive standard additions of meloxicam, each addition effecting a 2×10^{-8} M concentration; a 150 s pre-concentration time was employed. Even at these low concentrations the peaks are well defined. Also summarized in Table 1 are the characteristics of the calibration plots established with different pre-concentration times. The limit of linearity extends up to 1×10^{-7} M. Detection limit (corresponding to a signal-to-noise ratio of 3) near 2×10^{-11} M can be estimated, following 150 s pre-concentra-

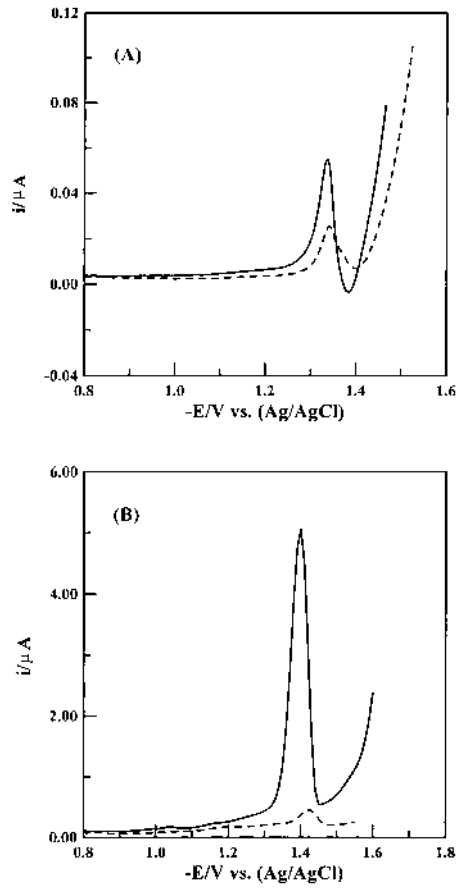

Fig. 4. Differential-Pulse and Square-Wave Adsorptive Stripping Voltam-Mograms for 5×10^{-8} M Meloxicam in 0.1 M HOAc–NaOAc Buffer at pH 5.0 after Pre-concentration for 60 s at −0.6 V Pre-concentration Potential

A) Differential pulse waveform with scan rate=10 mV s^{-1} and pulse amplitude= 25 mV and B) square waveform with f=120 Hz, Δs=8 mV, E_{sw}=25 mV. Broken lines represent the response without accumulation.

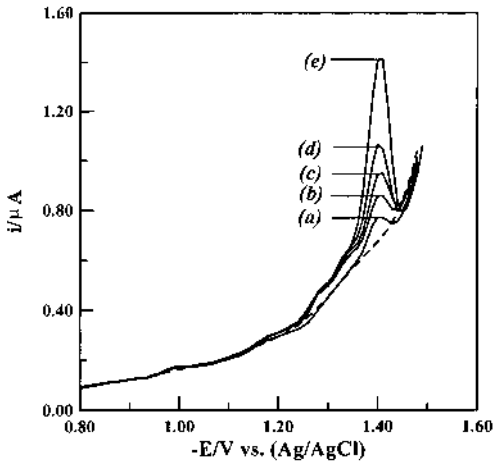

Fig. 5. Square-Wave Adsorptive Stripping Voltammograms Obtained after Increasing the Meloxicam Concentration

a) 2×10^{-10} M, b) 4×10^{-10} M, c) 6×10^{-10} M, d) 8×10^{-10} M, e) 1×10^{-9} M. Broken line represents supporting electrolyte without meloxicam. Square waveform as in Fig. 4.

Table 1. Characteristics of the Calibration Plots of Meloxicam

Pre-concentration time (s)	Linearity range (M)	Equation (slope in nA/nM)	Correlation coefficient	Detection limit (M)
0	5×10^{-8} —1×10^{-7}	$y=4.903x+0.696$	0.998	1.0×10^{-8}
30	1×10^{-9} —5×10^{-8}	$y=37.603x-8.419$	0.999	1.4×10^{-10}
60	1×10^{-9} —5×10^{-8}	$y=77.247x-40.305$	0.997	1.1×10^{-10}
150	2×10^{-10}—5×10^{-8}	$y=188.357x+4.784$	0.996	2.0×10^{-11}

tion time. Lower detection limits are expected following longer pre-concentration times.

The reproducibility was calculated from ten successive measurements on a stirred 1×10^{-8} M meloxicam after 45 s pre-concentration. The mean peak current was 269 nA with a range of 261—275 nA and relative standard deviation of 1.9%. Such precision indicates a reproducible adsorption process.

Meloxicam Assay in Plasma When adsorptive stripping voltammetry was performed directly in plasma without the pretreatment step, a significant depression of the stripping peak was observed owing to high protein content in human plasma. The protein may compete with meloxicam for the adsorption sites on the electrode and thus hamper the pre-concentration of meloxicam at the electrode surface. Thus, an extraction procedure as described above was proposed, and square-wave adsorptive stripping voltammograms for extracted meloxicam from plasma spiked at a concentration ranged from 50 to 500 ng meloxicam per 1 ml of plasma were recorded. Using the optimized conditions, the stripping peak current depends linearly on the meloxicam concentration between 50 and 250 ng ml^{-1} with a typical linear regression equation: i_p (nA)$=1.412\,C$ (ng ml^{-1})$+0.696$ ($r=0.998$). Plasma samples with concentration higher than 250 ng ml^{-1} were diluted prior to analysis. The detection limit (estimated as the concentration corresponding to a signal-to-noise ratio of 3) was 0.14 ng ml^{-1} of plasma, which is much lower than that achieved by the HPLC–UV technique (29 ng ml^{-1}).[6] The recovery of meloxicam from human plasma was determined in triplicate at concentrations within the range of the calibration graph (50, 100, 150 ng ml^{-1}). The mean recovery at these concentration levels was 99.4 ± 0.3%.

Meloxicam is extensively metabolized to four pharmacologically inactive metabolites through oxidation of the 5-methyl group of the thiazolyl rings to form hydroxy and carboxylic acid metabolites or oxidative cleavage of the benzothiazine ring to yield 2 oxoacetic acid metabolites, which are excreted in urine and faeces. Negligible amounts of meloxicam are eliminated unchanged in urine and faeces.[7] The electroreduction methods are not able to differentiate the main metabolites (also electroactive at the same potential) from the parent compound. If the determination in urine is intended the total amount is thus determined. But for plasma samples, over 90% of the plasma represented parent meloxicam so the interferences of metabolites could be ignored.

Conclusion

Application of the adsorptive striping square-wave voltammetry method to determine meloxicam in plasma samples shows clear advantages such as short period of real time analysis and low detection limit. This is evidently attributable to the relatively short accumulation times and the rapidity of the measurements deriving from the use of square-wave form. Because of its inherent sensitivity and accuracy, the adsorptive voltammetric method may be an effective alternative to the HPLC procedure.[5—8]

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