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Electrochemical behaviour of acemetacin*

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Introduction

Acemetacin or 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid carboxymethyl ester is an efficient anti-inflammatory compound, and a derivative of indomethacin. Synthesis [1, 2], metabolism and pharmacokinetics [3, 4] of acemetacin have been well established. In the field of quantitative analysis of acemetacin, thin layer chromatographic [5], liquid chromatographic [6], UV-spectroscopic [5], and fluorimetric [5] methods have been reported. No references on its electrochemical behaviour and related analytical methods have been found.

However the electroactivity of acemetacin can be predicted because one reducible C = O group is present in the molecule. The general model for the reduction of amides to α-hydroxyamines can be applied in this case. The aim of this work was to verify that possibility and to develop some polarographic quantitative procedures based on it. In the examination of the nature and features of the electrodic process tast polarography, linear sweep voltammetry and potential controlled coulometry were used; as a quantitative analytical technique, pulse polarography was also considered.

Experimental

A Tacussel PRG5 polarograph equipped with an EPL2B recorder was used in polarographic measurements. Coulometric and linear sweep voltammetric experiments were carried out with an Amel 551 potentiostat–amperostat and a Tacussel GSTP signal generator. In this case a Metrohm EA 290 hanging mercury drop electrode was used as the working electrode. Experimental curves were recorded on a Tektronix 5115 memory oscilloscope and a Philips PM 8133 X–Y recorder.

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Acemetacin (drug standard grade) and Oldan™ were kindly supplied by Europharma. All the other chemicals were analytical grade, and used without additional purification.

Potentials given in the text were measured against saturated calomel electrode (SCE). Current values reported were corrected for the residual current. All measurements were made at 25 ± 0.02°C, unless otherwise stated.

Because of the slight solubility of acemetacin in water, the study was carried out in water–acetonitrile solutions, the behaviour of the system being conditioned by the exact ratio of organic solvent in the medium. Reserve solutions were made in 100% acetonitrile. The stabilities of these solutions were verified over a period of some weeks.

Dissolved O_2 interferes strongly with the development of polarographic curves. It was removed from the solutions by bubbling N_2 (99.997%) for at least 15 min before each determination.

pH of the solution has proved to be the most important factor affecting the electrodic reaction. Britton–Robinson buffers were used as supporting electrolytes.

Results and Discussion

(a) *Polarographic behaviour*

Three different media were tested: 2% acetonitrile–water, 20% acetonitrile–water, and 100% acetonitrile. Tast polarographic curves obtained in 100% acetonitrile were ill-defined and will not be considered here. Table 1 summarises the results obtained in a range of pH from 2 to 12. In accordance with these results only one polarographic wave appeared when the pH was lower than 5.5, corresponding to the reduction of the protonated form of acemetacin. A second wave started to develop at more negative potentials and grew with the pH when this ranged from 5.5 to 8.5, while the first wave decreased. At pH = 8.5 only the second wave remained. In strongly basic media the products of hydrolysis are polarographically inactive. When the acetonitrile concentration of the medium was increased, the two waves appearing in the medium region of pH coalesced into one ill-defined wave.

Kinetic, diffuse or adsorptive characteristics have been established for each polarographic wave taking into account the evolution of limiting current with (a) concentration of acemetacin; (b) temperature; (c) height of mercury column; and (d) drop time. Both the wave appearing in acid media (pH < 5.5) and that appearing in basic media (pH > 8.5) showed limiting currents controlled by diffusion. In the medium range of pH the waves showed a more complicated behaviour caused by the protonation equilibrium of acid and basic forms of acemetacin and by the adsorption of electroactive species.

Controlled potential coulometric measurements allow us to assign two electrons (experimental mean value 2.19) for the overall electrodic reaction.

A logarithmic analysis of the polarographic curves revealed that only the reduction of acid form occurred in a reversible way. Transfer coefficients for electrochemically irreversible reduction of the basic form are given in Table 1.

With the aim of assuring the correct selection of suitable pH range for the analytical procedure, linear sweep voltammetry (LSV) has been used to confirm the diffuse or non-diffuse characteristics of the aforementioned reactions. The linear relationship between peak current and square root of scan rate confirmed the limiting current to be diffusion-controlled in the same conditions given in polarography.

Table 1
Main features of polarographic waves of acemetacin in acetonitrile–water media

	pH < 5.5	5.5 < pH < 8.5	pH > 8.5
(a) 2% Acetonitrile–water medium			
Number of waves	1	2	1
Half-wave potential	−1.20 V (pH = 3.50) −1.25 V (pH = 5.30)	1st wave: −1.26 V (pH = 5.56); −1.30 V (pH = 7.55) 2nd wave: −1.42 V (pH = 7.08); −1.45 V (pH = 8.44)	−1.46 V (pH = 8.74) −1.51 V (pH = 11.95)
Aspect	well-defined	slightly overlapped	well-defined
Current control	diffusive	1st wave: kinetic-adsorptive 2nd wave: diffusive-kinetic-adsorptive	diffusive
Transfer step	fast (revers.)	1st wave: fast (revers.) 2nd wave: slow (irrevers.) α = 0.5–0.6	slow (irrevers.) α = 0.5
(b) 20% Acetonitrile–water medium			
Number of waves	1	1	1
Half-wave potential	−1.18 V (pH = 2.36) −1.28 V (pH = 5.39)	−1.28 V (pH = 5.57) −1.45 V (pH = 8.50)	−1.45 V (pH = 8.88) −1.48 V (pH = 11.15)
Aspect	well-defined	ill-defined	well-defined
Current control	diffusive	diffusive	diffusive
Transfer step	fast (revers.)	slow (irrevers.) α = 0.3–0.4	slow (irrevers.) α = 0.5

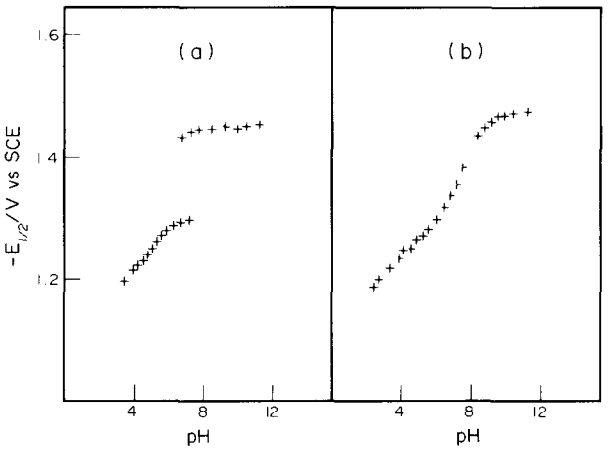

Figure 1
pH-Dependence of half-wave potential for the polarographic reduction of acemetacin in (a) 2% acetonitrile–water; and (b) 20% acetonitrile–water.

(b) *Scheme of the electrodic reaction*

Figure 1a shows the pH-dependence of the two reduction waves found in 2% acetonitrile medium. The half-wave potential of the first wave was shifted with increasing pH to more negative potentials. This was caused by the acid–base equilibrium preceding the electron transfer. From the slope of this plot the number of H^+ taking part in the reaction was calculated, proving to be equal to one. On the other hand, the half-wave potential of the wave appearing at more negative potentials was practically pH-independent. Very similar results were obtained in 20% acetonitrile medium (Fig. 1b), in spite of the ill-definition of the waves in the medium pH-range. In agreement with all the previously stated results, the following overall scheme can be proposed for the electrode reduction of acemetacin (Fig. 2):

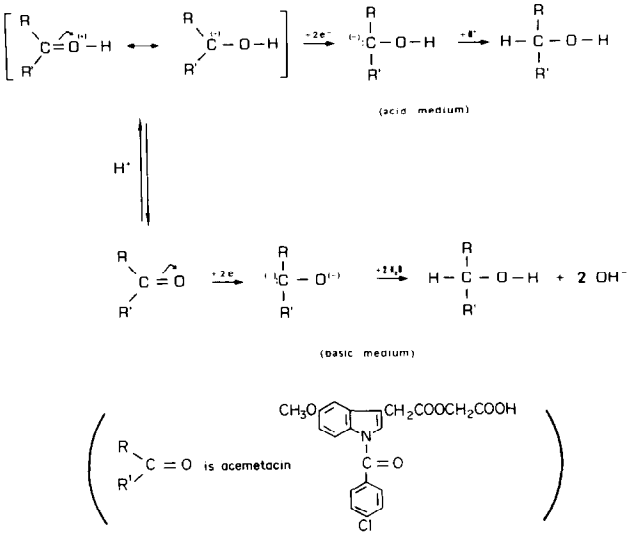

Figure 2
Reaction mechanism for the reduction of acemetacin in acid and basic media.

(c) *Analytical results*

Values of pH at which the process is diffusion-controlled were chosen, i.e. pH < 5.5 and pH > 8.5. As has been pointed out before, very strong basic media (pH > 12) must be excluded because products of hydrolysis of acemetacin are electrochemically inactive.

Three polarographic techniques were used: tast polarography (TP), differential pulse polarography (DPP) and normal pulse polarography with differential detection of current (NPP) also called pseudoderivative pulse polarography. The following experimental conditions for each technique were selected from systematic analysis of response as a function of several variables. Tast polarography: Potential range from −1.0 to −2.0 V; scan rate, 4 mV s^{-1}; drop time, 1 s.

Differential pulse polarography: Potential range, from −1.0 to −2.0 V; scan rate, 4 mV s^{-1}; drop time, 0.7 s; pulse amplitude, 20 mV, pulse length, 0.048 s.

Pseuderivative pulse polarography: potential range, from −1.0 to −2.0 V; scan rate, 4 mV s^{-1}; drop time, 0.7 s.

Regression analyses of calibration graphs are given in Table 2 for two different values of pH. In agreement with this TP is the most sensitive technique, with NPP giving the best linear response.

Table 2

Calibration results for the determination of acemetacin by tast polarography (TP), differential pulse polarography (DPP) and normal pulse polarography with differential detection of current (NPP). Medium, 2% acetonitrile–water; concentration of acemetacin, 0–10^{-3} mol dm^{-3}; temperature, 25°C. Results are given for two different pH

		TP	DPP	NPP
Slope/μA $mmol^{-1}$ dm^3	pH = 4.81	5.97	4.76	2.56
	pH = 9.39	5.22	2.11	1.01
Intercept/μA	pH = 4.81	0.004	0.029	−0.001
	pH = 9.39	0.015	−0.007	−0.016
Correlation coefficient	pH = 4.81	0.999	0.998	0.999
	pH = 9.39	0.998	0.998	0.997
Standard error of slope	pH = 4.81	0.089	0.112	0.043
	pH = 9.39	0.116	0.047	0.034
Detection limit/mmol dm^{-3}	pH = 4.81	0.0071	0.0112	0.0080
	pH = 9.39	0.0122	0.0121	0.0196

The experimental procedure was applied to acemetacin determination in a commercial product (OLDAN from Europharma) using NPP. Reproducibility was checked by a series of 10 determinations on identical samples. One capsule was dissolved in 25 ml of acetonitrile. 0.5 ml of this solution were taken in the electrolysis cell with 10 ml of buffer and 2 ml of acetonitrile. The variance of the results was 4.19×10^{-4} and its normality was verified by the d'Agostino test [7].

By the same procedure a series of 10 different capsules were checked. A mean value of 0.212 mmol l^{-1} was found for the acemetacin concentration with a variance of 3.53×10^{-4}, the results following a normal distribution. By applying the appropriate significance test no difference with the value given by the product maker was found, for a significance level $\alpha = 0.05$.

Conclusion

Polarography proves to be an easy and reliable method for the determination of acemetacin in capsules. Its main advantage lies in that no previous separation from other constituents is required. The proposed procedure is valid for quality control and could serve as a basis for a later procedure of acemetacin determination in biological fluids.

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