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Adsorptive stripping voltammetric determination of ambroxol

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An electrochemical procedure for the determination of ambroxol in mucolytics was described. The method was based on adsorptive accumulation of the species at the hanging mercury drop electrode (HMDE), followed by one of different modes of stripping sweep, viz. direct current tast (DCT), differential pulse (DP), square wave (SW) and first harmonic alternating current (AC1). The behaviour of adsorptive stripping response was studied under various experimental conditions, e.g. type of supporting electrolyte, pH, accumulation time, pulse amplitude, scan rate and mode of sweep. In Britton-Robinson buffer solution, an irreversible reduction process involving transfer of one electron and one proton was took place. The response was linear over the 0.2–6 μg/ml concentration range. Determination of the compound in oral dosage forms was achieved using the standard addition method. The average of determinations obtained by the square wave adsorptive voltammetric method with its relative standard deviation was 99.8 ± 2.40%.

1. Introduction

Methods for determining ambroxol, in biological fluids or in pharmaceuticals, include ion-selective potentiometric (Abdel-Ghani 2003), UV-spectrophotometric (Benli and Tancel 1998; Perez et al. 1996; Indrianto and Handajani 1993; Indrianto and Handajani 1994; Zafer et al. 2003), liquid (Brizzi and Pasetter 1990; Flores et al. 1989; Indrianto and Handajani 1993, 1994; Koundourellis 2000), gas chromatographic (Coloumbo et al. 1990; Indrianto and Handajani 1993; Schmid 1987) and capillary zone electrophoretic (Perez et al. 1997) techniques. No methods have so far been reported to the determination of ambroxol in tablets by adsorptive stripping voltammetry. Many organic compounds exhibit surface active properties that are manifested by their adsorption from solution onto the surface of a stationary phase. In the present work, the electrochemical behaviour of ambroxol on a mercury surface was investigated and the optimum conditions for the quantification analysis were studied.

2. Investigation, results and discussion

Ambroxol is adsorbed spontaneously on the surface of HMDE and reduced irreversibility by one electron with losing one proton in alkaline medium producing a single peak. The effect of the composition of the supporting electrolyte on the voltammetric measurement of ambroxol was examined by comparing the response in various electrolytes, such as phosphate, ammonia-ammonium chloride and modified Britton-Robinson buffer solutions. At pH 8–10, all the electrolytes gave apparently similar results. In all the forthcoming studies, the solution applied was contained 2 μg/ml ambroxol and modified Britton-Robinson buffer solution while the electrochemical parameters employed were programmed so that the time of accumulation t_a was 30 s at potential $E_a - 1000$ mV, the pulse amplitude ΔE was 30 mV and the scan rate α was 40 mV/s.

Increasing the pH values from 7 to 11 revealed parallel increasing in values of peak current and potential as shown in Fig. 1. Variation of differential pulse peak potential as a function of pH over the range 8–11 was given by the linear relation $E_p = 1.724 - 0.035$ pH with a correlation coefficient of $r = 0.984$. The peak height increased also with increasing pH values. It can therefore be concluded that the electrochemical reduction was facilitated on losing protons from the adsorbed molecules in the alkaline me-

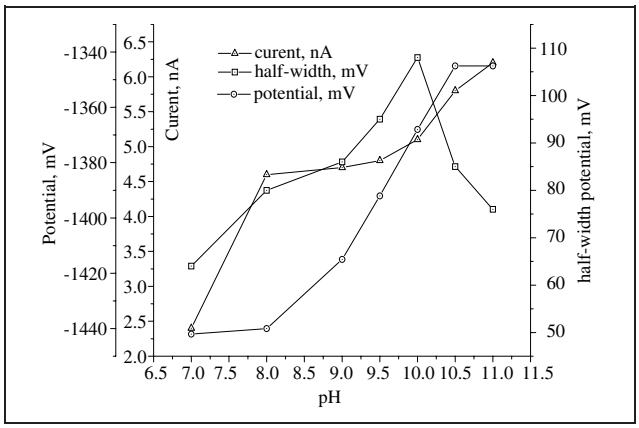

Fig. 1: Effect of Britton-Robinson buffer solutions pH 7–11 on adsorptive stripping voltammetric determination of 2 μg/ml ambroxol with $t_p = 30$ s at 130 mV, $\Delta E = 50$ mV, $\nu = 40$ mV/s

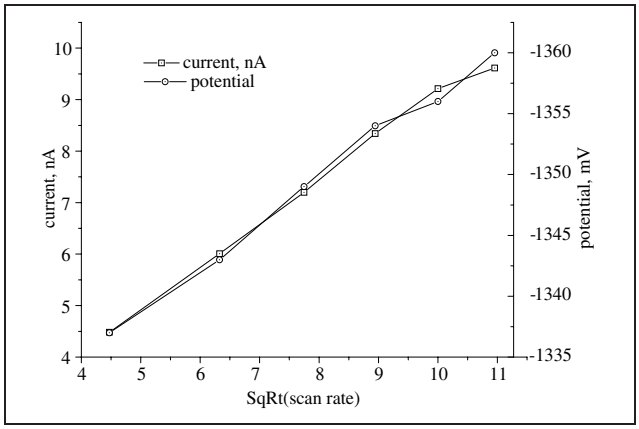

Fig. 2: Effect of square root of scan rate on 2 μg/ml ambroxol with tp = 30 s at 130 mV and pH 9

dium. The number of protons lost, *p*, was found approximately to be unity (p = 0.8) as concluded from electron transfer coefficient αn (0.76 ± 0.043) calculated at different pH values from 9 to 11 (Dick and Nadler 1983; San Martin et al. 1996; Zuman and Perrin 1969). The degree of irreversibility is increased by increasing the peak half-width potential with increasing the pH values from 7 to 10 (Fig. 1), but it re-decreased down to pH 11. The presence of a +I effect substituent in the ring, bromide in this case, caused the peak to be clearly defined over the pH range studied (Brand and Fleet 1968). Thus, the value pH 11 was selected for the rest of experiments giving up the best sensitivity.

The irreversibility of the reaction was also confirmed by variation of the potential with accumulation time, scan rate and pulse amplitude. The reduction peak was shifted to a more negative potential with increasing the amount of the adsorbed compound at the electrode surface or increasing the time of accumulation as shown in Fig. 2. This was an indication of irreversible character. The peak height also increased linearly with the accumulation time up to $t_a = 30$ s for all concentrations studied, 0.2–10 μg/ml. The deposition time longer than 30 s will lead to saturation of the mercury drop surface with the adsorbed species for a concentration of 10 μg/ml. Variation of the accumulation potential starting from −200 up to −1000 mV had a limited effect on the peak height which gave the same result if the pre-concentration step was carried out in an open circuit, taking into account that the total time of pre-concentration (accumulation before the scan, during the rest

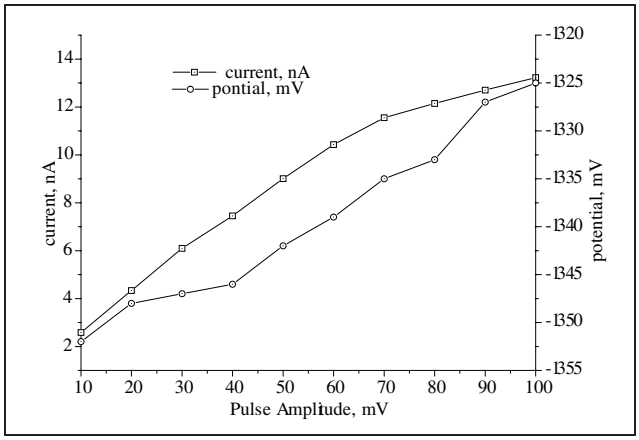

Fig. 3: Effect of pulse amplitude on 2 μg/ml ambroxol with $t_p = 30$ s at 130 mV and pH 9

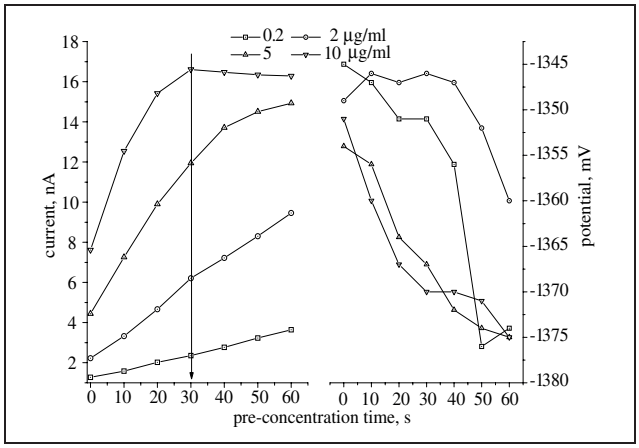

Fig. 4: Variation of current and potential with accumulation time at different concentration of ambroxol with $t_p = 30$ s at 130 mV, v = 120 mV/s, dE = 30 mV and pH 9

period and through scanning from the selected accumulation potential to −1000 mV) was fixed at 40 s.

The irreversibility was proved also by displacement of the reduction peak to a more negative potential with increasing the scan rate as shown in Fig. 3. The current was increased linearly with square root of scan rate with a correlation coefficient of r = 0.995.

The stripping current was increased linearly with increasing the pulse amplitude from 10 to 100 mV as shown in Fig. 4. The reduction peak was displaced at the same time linearly towards less negative potentials increasing the possibility of irreversibility relative to reversible electron reaction.

Modulating the direct current tast ramp with various wave forms minimized the large capacitive current component enabling the accurate recording of the smaller Faradaic current. This can be achieved by using superimposed differential pulse, square-wave or first harmonic a.c. modulation, with the result of a further increase in sensitivity over conventional DCT mode, as shown in Fig. 5. Based on these different cathodic stripping voltammetric behaviours of ambroxol, variations of current with the concentration were given as shown in Fig. 6 and a quantitative method has been developed. At pH 11 the calibration curves were described by the polynomial relationships as given in Table 1 over the concentration range 0.2–10 μg/ml, where i_p is the peak current for ambroxol in nA and c is the concentration in μg/ml. The relation can be described

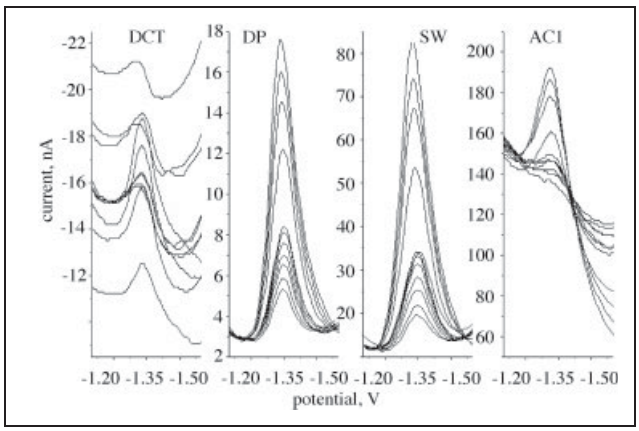

Fig. 5: Effect of different modes of scan over the concentration range of 2–10 μg/ml ambroxol with $t_p = 30$ s at −1000 mV and pH 9, scan rate = 40 mV/S and pulse amplitude of 30 mV

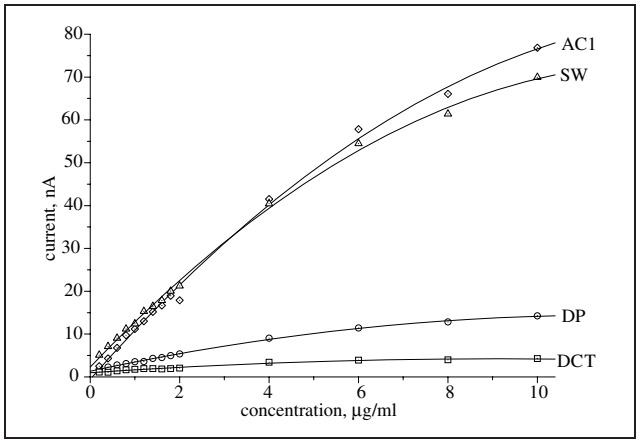

Fig. 6: Variation of current with concentration of 0.2–2 µg/ml ambroxol with $t_p = 30$ s at 130 mV, v = 120 mV/s, dE = 30 mV and pH 11

also as linear on the concentration range 0.2–6 µg/ml as given in Table 2. The method proposed here has yielded satisfactory results in the determination of ambroxol in the presence of filling materials in four different Egyptian pharmaceutical products: capsules of Ambroxol (Amoun) and Muco SR (STADA), tablets of Mucosolvan (Boehringer Ingelheim) and Ambroxol (Amoun). Results achieved with the standard addition technique are given in Table 3. The average of determinations obtained by the square wave adsorptive voltammetric method with its relative standard deviation was 99.8 ± 2.40% compared with that obtained by the reference method (Indrayanto and Handajani 1994) it was 100.64 ± 3.49%.

Table 1: Non-linear regression parameters obtained for calibration curves of ambroxol over the concentration range 0.2–10 µg/ml

Mode	R^2	Polynomial equation
DCT	0.98256	$I = 0.97971 + 0.70838\ C - 0.03869\ C^2$
DP	0.9984	$I = 1.34571 + 2.23529\ C - 0.09545\ C^2$
SW	0.99828	$I = 2.29385 + 10.97275\ C - 0.42425\ C^2$
AC1	0.9968	$I = -0.52775 + 11.81411\ C - 0.40999\ C^2$

Table 2: Linear regression parameters obtained for calibration curves of ambroxol over the concentration range 0.2–6 µg/ml

I	Intercept	Slope	R	LD
DCT	1.151	0.492 ± 0.031	0.981	1.048
DP	1.790	1.683 ± 0.052	0.995	0.514
SW	4.133	8.617 ± 0.153	0.998	0.295
AC1	1.157	9.604 ± 0.208	0.998	0.360

* LD = limit of detection calculated according to Miller and Miller (1988)

Table 3: Results obtained by standard addition method for the adsorptive stripping square wave voltammetric determination of ambroxol in different pharmaceutical products

Trade name	Claim (mg)	Proposed method* (mg)	Reference method[4] (mg)
Capsules			
Muco SR (SADA)	75	74.8 ± 1.51	76.07 ± 2.70
Ambroxol (Amoun)	75	74.6 ± 1.63	75.8 ± 1.61
Tablets			
Ambroxol (Amoun)	30	29.4 ± 0.74	28.22 ± 1.10
Mucosolvan (Boehringer)	30	30.6 ± 0.89	31.8 ± 1.39

* Average of 3 determinations

3. Experimental

3.1. Apparatus

All different modes of adsorptive stripping voltammograms (direct current tast DCT, differential pulse DP, square wave SW and first harmonic alternating current AC1) were recorded on a Metrohm VA 693 processor equipped with a Metrohm VA 694 stand involving three potentiometric electrodes. The working electrode was the hanging mercury dropping electrode (HMDE), while the auxiliary electrode was a platinum wire. The reference electrode was Ag/AgCl in 3 mol/L KCl. The instrumental settings applied to all modes were given in the following procedure.

3.2. Reagents

Unless otherwise stated, all reagents were of high analytical grade and doubly distilled water was always used. Buffer solutions were prepared by mixing 0.1 M of ammonia/ammonium chloride to give various pH values (7–10), mono and dihydrogen phosphate (pH 5–10) and modified Briton-Robinson buffer solutions (pH 3–11).

Capsules of Ambroxol (Amoun) and Muco SR (STADA), each containing 75 mg, tablets of Mucosolvan (Boehringer Ingelheim) and Ambroxol (Amoun) each containing 30 mg were used.

3.3. Procedure

An aliquot of ambroxol solution (2–100 µg/ml) was placed in a 10 ml flask containing 5 ml modified Britton-Robinson buffer pH 11. The flask was then completed to the mark, transferred to the electrode cell and deaerated by streaming pure nitrogen gas for 2 min. Adsorption was carried at −1000 mV for 30 s with continuous stirring at a speed of 2000 rpm. The stirrer was stopped and the solution was allowed to rest for 10 s, then the voltammogram was recorded using a Hanging Mercury Dropping Electrode (HMDE) with a drop size of ∼0.15 mm^2, a pulse amplitude "ΔE" of 30 mV (applied for all modes except DCT), frequency 25 Hz (for SW and AC1), a pulse duration of 20 ms (for DP), number of preparatory and measurement cycles was single (for SW and AC1), a potential scan rate "α" of 40 mV/s within a reduction potential range going from −1000 to −1600 mV. The triplicate measurements of content in the sample was averaged using standard addition method at room temperature (ca. 27 °C). To assay ambroxol in pharmaceutical products, a capsule or tablet was placed in a beaker containing distilled water which was then ultrasonated for 15 min. The beaker was transferred to a 100 ml measuring flask and completed to the mark with water. 0.1 ml of the solution was pipetted into a 10 ml-measuring flask and the procedure was completed as described previously. UV-spectrophotometry at 307 nm (Indrayanto and Handajani 1994) was performed as a reference method since the assay has not been described in any pharmacopeia yet.

This work (Habib IHI, Zayed SIM), has been presented in the IUPAC International Congress on Analytical Sciences, Waseda Univ., Tokyo, Japan, 6–10 Aug 2001.

References

Abdel-Ghani NT, Hussein SH (2003) Determination of ambroxol hydrochloride in pure solutions and some of its pharmaceutical preparations under FIA conditions. Farmaco 58: 581–590.

Benli P, Tuncel M (1998) Flow-injection analysis of ambroxol in pharmaceutical tablets. Pharmazie 53: 203–204.

Brand MJD, Fleet B (1968) Polarography in the study of classical organic functional group reactions: I. Polarographic behaviour of *cis*- and *trans*-cinnamic acid and substituted cinnamic acids. J Electroanal Chem 16: 341–350.

Brizzi V, Pasetti U (1990) High-performance liquid-chromatographic determination of ambroxol in pharmaceuticals. J Pharm Biomed Anal 8: 107–109.

Colombo L, Marcucci F, Marini MG, Pierfederici P, Mussini E (1990) Determination of ambroxol in biological material by gas chromatography with electron-capture detection. J Chromatogr B Biomed Appl 95: 141–147.

Dick JG, Nadler S (1983) Metrohm Monographs "Electrode Reaction Kinetics".

Flores Murrieta FJ, Hoyo Vadillo C, Hong E, Castaneda Hernandez G (1989) Assay of ambroxol in human plasma by high-performance liquid chromatography with amperometric detection. J Chromatogr Biomed Appl 82: 464–469.

Indrayanto G, Handajani R (1993) Quantitative determination of ambroxol hydrochloride in tablets. J Pharm Biomed Anal 11: 781–784.

Indrayanto G, Handajani R (1994) Quantitative determination of ambroxol hydrochloride in syrups by RP [reversed-phase]-HPLC and UV spectroscopy. Drug Dev Ind Pharm 20: 1639–1647.

Koundourellis JE, Malliou ET, Broussali TA (2000) High-performance liquid chromatographic determination of ambroxol in the presence of different preservatives in pharmaceutical formulations. J Pharm Biomed Analysis 23: 469–475.

Miller JC, Miller IN (1988) Statistics for Analytical Chemistry, Ellis Horwood Ltd., John Wiley & Sons NY, 115.

Perez-Ruiz T, Martinez-Lozano C, Sanz A, San-Miguel MT (1996) Automatic extraction-spectrophotometric method for the determination of ambroxol in pharmaceutical preparations. Talanta 43: 1029–1034.

Perez-Ruiz T, Martinez-Lozano C, Sanz A, Bravo E (1997) Determination of bromhexine and ambroxol in pharmaceutical dosage forms, urine and blood serum. J Chromatogr B Biomed Appl 692: 199–205.

San Martin Fernandez-Marcote M, Callejon Mochon M, Jimenez Sanchez JC, Guiraum Perez A (1996) Electrochemical reduction at a mercury electrode and differential-pulse-polarographic determination of dibucaine in pharmaceutical ointments. Analyst 121: 681–685.

Schmid, J (1987) Assay of ambroxol in biological fluids by capillary gas – liquid chromatography. J Chromatogr B Biomed Appl 58: 65–75

Zafer D, Hasan B, Nilgün GG (2003) Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC. J Pharm Biomed Anal 31: 867.

Zuman P, Perrin GL (1969) Organic Polarography. Wiley, NY, p. 95.