Short Communication

Voltammetric Oxidation of Ambroxol and Application to Its Determination in Pharmaceuticals and in Drug Dissolution Studies

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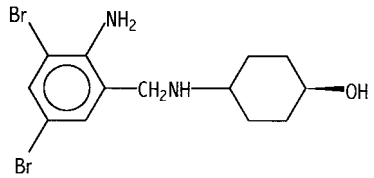
Abstract
A detailed study of the electrochemistry of ambroxol at a glassy carbon electrode was carried out in the pH range 1.8 – 11.0 in aqueous solution using cyclic and differential pulse voltammetry. The compound was oxidized irreversibly at high positive potentials resulting in the formation of a chemical product at less positive potentials, which was more readily oxidized than the parent compound. In addition, a differential pulse voltammetric method was proposed for the determination of the drug in different pharmaceutical formulations, and in drug dissolution studies.

Keywords: Ambroxol, Cyclic voltammetry, Differential pulse voltammetry, Glassy carbon electrode, Formulation analysis, Dissolution profile

Ambroxol, *trans*-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol, a pharmacologically active metabolite of bromhexine, is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics. It has been also reported to have antioxidative properties. Ambroxol is administered as a hydrochloride salt in daily doses of 30 – 120 mg using mostly oral formulations like tablets and syrups. Similar doses have been given by inhalation, injection or rectally [1]. Figure 1 shows the chemical structure of ambroxol with its pK_b values [2].

Several analytical methods for the determination of ambroxol are described, including high performance liquid chromatography [2 – 11], UV-visible spectrophotometry [10 – 12], flow injection analysis [13, 14], gas chromatography [15, 16] and capillary zone electrophoresis [17 – 19]. Some of the reported methods require time-consuming sample preparation or expensive instrumentation.

Reviewing the literature has given no information about the electrochemical redox properties of ambroxol. Consequently quantitative determination of this drug using electrochemical techniques is a non-explored matter up today except a HPLC method with amperometric detection [9].



$pK_{b1} = 1.39$

$pK_{b2} = 7.16$

Fig. 1. Chemical structure of ambroxol.

The purpose of this work is to investigate the electrochemical characterization of ambroxol on glassy carbon electrode and devise a suitable differential pulse voltammetric method for the analysis of ambroxol in pharmaceuticals and to study the dissolution profile of the drug in solid dosage forms.

Some typical linear sweep voltammograms of ambroxol in Britton-Robinson buffer at selected pH values are presented in Figure 2. The compound was oxidized in the strong acidic media yielding one main irreversible process. The presence of a secondary process was also observed at a more positive potential but became less distinct as the acidity was decreased and was not detected above pH 5. By the disappearing of this process, the main oxidation peak began to split into two overlapping peaks. As pH increased, the second peak was separated from the first peak, its shape becoming broader.

In the reverse scan, a reduction peak was observed; coupled with anodic peak on the second forward scan (Fig. 3a). The appearance of these closely spaced peaks located at considerably lower positive potentials than the parent compound supported that a chemical reaction subsequent to the electron step occurs, giving rise to species more readily oxidized than ambroxol. This was proven by successive scanning with a polished electrode to a potential lower than the oxidation potential of ambroxol, showing the absence of these couples. It is also seen that in Figure 3a, the peak currents of these couples increase slightly as the number of scans increase, while the peak at the more anodic potential decreases, which confirms that redox couples arise from the product of chemical follow-up reaction of am-

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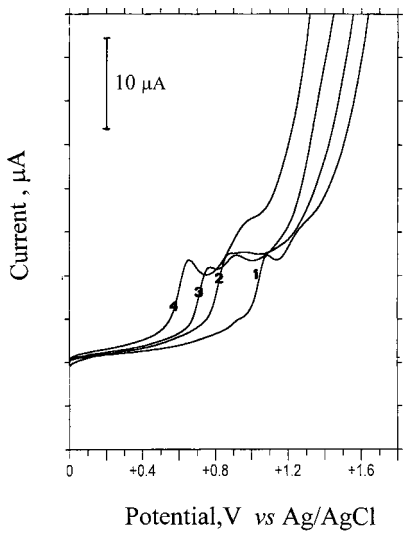

Fig. 2. Linear sweep voltammograms of 2×10^{-4} M ambroxol solutions in Britton-Robinson buffer at different pH values. Scan rate, 100 mV/s. 1) pH 2; 2) pH 6; 3) pH 8; 4) pH 11.

broxol oxidation [20]. These redox couples were observed in acidic media but their intensity decreased gradually by raising the pH and were not seen above pH 5 (Fig. 3b) at which the splitting of the primary (main) oxidation peak appeared. Such behavior was reported for the electrochemical oxidation of the amino group of clenbuterol molecule [21]. These couples, however, were not as pronounced as those observed for clenbuterol and may be due to the nature and position of the electrophilic substituents on the aromatic ring. From the cyclic voltammograms and bearing in mind the electrochemical oxidation of clenbuterol [21, 22], a mechanism can be proposed that the redox

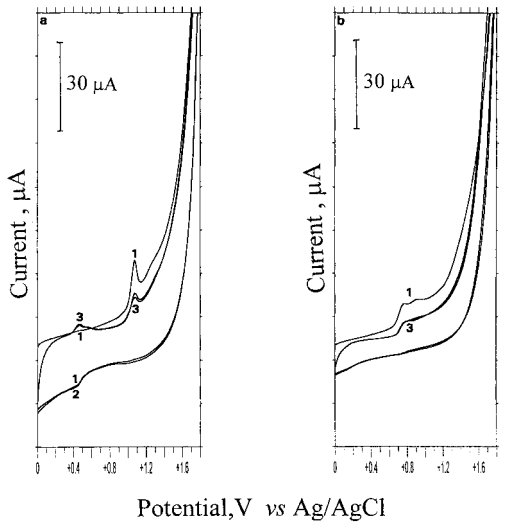

Fig. 3. Multisweep cyclic voltammograms of 2×10^{-4} M ambroxol solutions in Britton-Robinson buffer at different pH values. Scan rate, 100 mV/s. a) pH 3; b) pH 8. The numbers indicate the number of scans.

process of ambroxol occurs to yield dimer compounds, bonding the radical cations formed through the oxidation of the amine group.

The primary (main) anodic peak was easily measurable; hence all subsequent work was based on the measurement of the magnitude of this step.

The effect of potential scan rate between on the peak current and the peak potential of ambroxol was evaluated. As the scan rate was increased, the peak potential shifted to more positive values, which confirms the irreversibility of the process. The linear increase in the peak current with the square root of the scan rate, in the scan range 10 – 250 mVs^{-1} , with a slope of 0.37 (correlation coefficient 0.994), showed the diffusion control process. When the study of variation of logarithm of peak current versus logarithm of scan rate was made in all scan rate range, it was found that, the process had a diffusive component because the slope was 0.46 (correlation coefficient 0.989) close to the theoretical value of 0.5 for a diffusive process [23].

When the experiments carried out under the same conditions as the above study using different supporting electrolytes, it was observed that the shape and the number of the peaks in 0.2 M sulfuric acid and phosphate buffers at different pHs were similar to those obtained in Britton-Robinson buffers.

A linear response of peak potential against pH was observed in the pH range studied giving a negative slope of 52.8 mV per pH unit (correlation coefficient 0.993) (Fig. 4). The effect of the solution pH on the peak enhancement is also shown in Figure 4.

On the basis of the electrochemical investigation of ambroxol, an analytical method was suggested, and the necessary analytical parameters were established.

Quantitative measurements were made in 0.2 M H_2SO_4 so that the existence of a well-defined voltammetric peak made easy the quantitation and the peak currents were greater than those obtained in other supporting electrolytes.

When linear sweep and differential pulse voltammetry were used for determining the drug, the analytical signals provided showed that the oxidation peak is much more intense using the latter technique. For this reason, differential pulse mode (pulse amplitude, 50 mV; pulse width, 0.05 s; scan rate, 20 mVs^{-1}) was selected for ambroxol determination.

The intra-day reproducibility of peak potential and peak current was tested by repeating four runs on 4×10^{-5} M ambroxol; the relative standard deviations were calculated to be 0.19 and 1.29%, respectively. The inter-day reproducibility of peak potential and peak current was evaluated over 4 days by performing four measurements on each day. The relative standard deviations were found to be 0.28 and 1.75% for peak potential and peak current, respectively.

Using the optimum conditions described above, typical differential pulse voltammograms are obtained at various concentrations of ambroxol (Fig 5).

The use of ambroxol oxidation at +1.05 V as the analytical peak allowed quantification in the range of 6×10^{-6} -6×10^{-5} M. The slope, intercept and correlation

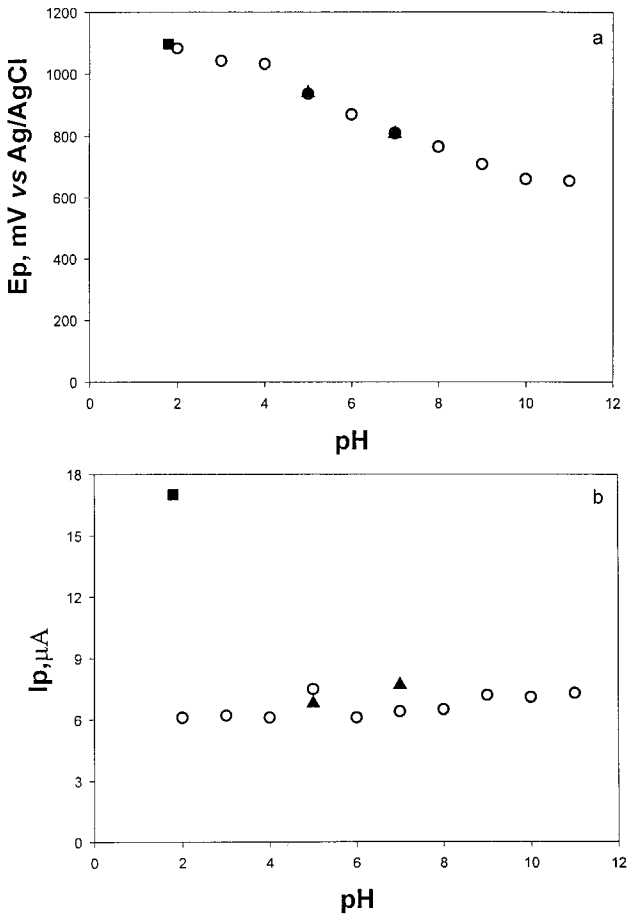

Fig. 4. Effects of pH on ambroxol peak potential a) and peak current b); ambroxol concentration, 2×10^{-4} M; scan rate, 100 mV/s. (■) 0.2 M H_2SO_4; (○) Britton-Robinson buffer; (▲) Phosphate buffer.

coefficient of the calibration plot were 6.7×10^4 μA/M (with a relative standard deviation of 1.08%), −0.16 μA (with a relative standard deviation of 1.24%) and 0.996, respectively.The detection limit and the limit of quantitation of the procedure were found to be 9.4×10^{-7} M and 3.2×10^{-6} M, which were calculated according to the 3 s/m and 10 s/m criterions, respectively, where s, is the standard deviation of

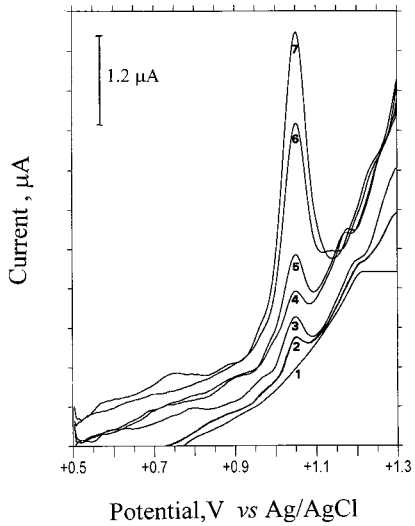

Fig. 5. Differential pulse voltammograms of different concentrations of ambroxol in 0.2 M H_2SO_4. Ambroxol concentration, 1) 0 M; 2) 6×10^{-6} M; 3) 8×10^{-6} M; 4) 1×10^{-5} M; 5) 2×10^{-5} M; 6) 4×10^{-5} M; 7) 6×10^{-5} M.

the peak currents (five runs) of the sample, m is the slope of the calibration plot.

On the basis of these results, the proposed differential pulse voltammetric method was applied to direct determination of ambroxol in different commercially available products, such as tablet, syrup and pediatric syrup. As far as we know, no official method is described in pharmacopeias related to the pharmaceutical dosage forms of ambroxol. For this reason, the UV-spectrophotometric method [10, 11] was choosen for comparison, since its simple and rapid analytical applicability. Using this technique, the calibration plot was prepared in concentration range 1×10^{-5}–1×10^{-4} M in 0.1 M HCl and the absorbances were determined at 306.6 nm. The results showed that the linearity range of calibration plot obtained by the proposed method could be expanded to about two times lower concentration than that obtained by UV-spectrophotometry.

Table 1 compares the results of the analysis of ambroxol between proposed and literature methods. Both methods involved no complex sample preparation but for the UV-

Table 1. Comparative and recovery studies for ambroxol pharmaceutical preparations using differential pulse voltammetry and UV-spectrophotometry.

Commercial products	*Differential pulse voltammetry*					*UV-spectrophotometry[10, 11]*				
	Mean[a]	*RSD (%)*	$t_{calculated}$	$F_{calculated}$	*Recovery[b] (%)*	*RSD (%)*	*Mean[a]*	*RSD (%)*	$t_{theoretical}$	$F_{theoretical}$
Tablet (30 mg per tablet)	30.12	0.53	2.08	0.06	99.4	0.32	29.96	0.18	2.31	2.60
Syrup (30 mg / 5 mL)	29.77	1.06	0.57	0.84	100.3	0.29	29.62	1.18	2.31	2.60
Pediatric syrup (15 mg / 5 mL)	14.82	0.45	1.06	0.22	100.1	0.14	14.75	0.89	2.31	2.60

[a] mean of five experiments, [b] mean of four experiments.

spectrophotometric studies in tablets, a separation by filtration was necessary.

For all the formulations examined the assay results were in good agreement with the declared content. The precision of compared spectrophotometric method, expressed as the relative standard deviation, is relatively more for tablets, whereas its precision is less for syrup formulations than the proposed voltammetric method, probably due to effect of excipients, stabilizers and flavoring substances in the matrix.

According to the Student's *t*- and variance ratio *F*-test, the calculated (experimental) *t* and *F* values were less than the tabulated (theoretical) values in either test at the 95% confidence level. This indicates that there is no significant difference between the performance of the literature and proposed method as regards to mean values and standard deviations (Table 1).

Moreover, in order to know whether the excipients show any interference with the analysis, the standard addition method was applied by adding increasing amounts of the pure drug to the earlier analyzed pharmaceutical formulations of ambroxol. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. As Table 1 shows, good results demonstrate the validity of the proposed method for the determination of ambroxol in commercial dosage forms.

Consequently, the above presented method is recommended for ambroxol analysis in pharmaceutical formulations.

It is essential to consider the in vitro dissolution tests as an important criteria for the quality of the pharmaceutical dosage formulation if it is obtained from various sources and can judge the suitability of this formulation to deliver the required active substance properly to the patient. Thus, the need of dissolution tests of drug formulations is indispensable to ensure good drug quality. The differential pulse voltammetric method was applied to the quantitation of ambroxol in dissolution rate samples from the tablets. Calibration plots were obtained at $+1.04$ V, and showed that the method is applicable over the range $4 \times 10^{-6} - 6 \times 10^{-5}$ M ($r = 0.999$) with a slope of 1.56×10^5 μA/M, intercept of -0.494 μA, relative standard deviation of slope 1.10%, and relative standard deviation of intercept 0.74%. For comparison spectrophotometric method at 306.6 nm, a linear relationship was also obtained in the range of $1 \times 10^{-5} - 1 \times 10^{-4}$ M. The results of the linear regression analysis are as follows: slope, 1.25×10^3 with a relative standard deviation of 1.32%, intercept, -0.0013 with a relative standard deviation of 0.76% and correlation coefficient, 0.998. The reproducibility of the methods were studied by analyzing a solution containing 2×10^{-5} M in three replicates. The relative standard deviations were calculated to be 0.55 and 1.01% for differential pulse voltammetric and for spectrophotometric method, respectively.

The release rate profiles were drawn as the percentage drug dissolved from the tablets versus time for both methods (Fig. 6).

Four different kinetics such as, zero order, first order, Hixson-Crowell, Weibull distribution (RRSBW) were applied to the results obtained from the dissolution studies and the results were evaluated kinetically [24, 25]. For releasing profile, best compliance according to the highest determination coefficient and lowest sum of weighed squared deviation values for ambroxol dosage form was found to be Weibull distribution [24] (Table 2). For this kinetic, $T_{63.2\%}$ results were obtained at 19.71 and 16.76 min for differential pulse voltammetric and spectrophotometric methods, respectively. The release of ambroxol from tablets was completed after 45 min in both methods. According to the RRSBW kinetic, low shape factor value ($\beta < 1$) corresponds with a steeper initial slope followed by a flattened tail in the final part.

The release percentages at all the time period are very similar in the two methods investigated, without any significative differences. On the other hand, the voltammetric measurements were more simple and rapid compared with the commonly used UV spectrophotometric

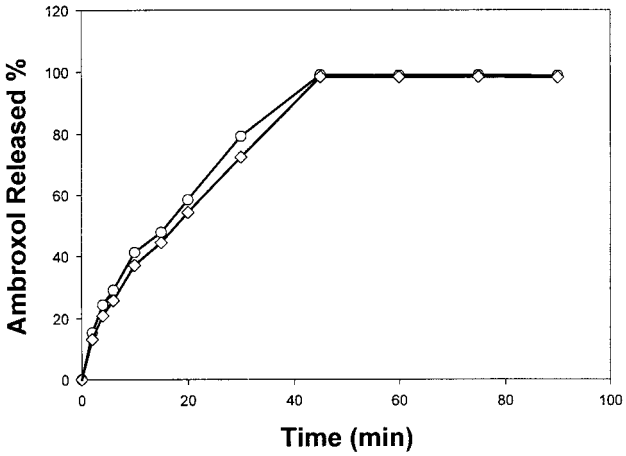

Fig. 6. In vitro dissolution profiles of ambroxol tablets. (□) differential pulse voltammetry, (○) UV-spectrophotometry.

Table 2. The kinetic assessment of release data of the paddle method from ambroxol tablet formulation (declared amount, 30 mg per tablet). k_r: Release rate constant of first order kinetic; k_r°: release rate constant of zero order kinetic; k: release rate constant of Hixson-Crowell kinetic; r^2: determination coefficient; SWSD: sum of weighed squared deviations; β: shape factor; $T_{63.2\%}$: value stands for the time for 63.2% release of the drug.

Method	*First order*			*Zero order*			*Hixson-Crowell*			*RRSBW*			
	k_r	r^2	*SWSD*	k_r°	r^2	*SWSD*	k	r^2	*SWSD*	$T_{63.2\%}$	β	r^2	*SWSD*
Differential pulse voltammetry	3.37	0.846	0.618	18.65	0.873	0.454	1.196	0.866	1.16	19.7	0.93	0.881	0.44
UV-spectrophotometry	3.65	0.839	0.51	17.55	0.865	0.63	1.26	0.865	18.73	16.76	0.91	0.897	0.43

method. No treatment of the sample such as filtration is required before the measurements.

Experimental

Electrochemical measurements were made with BAS 100W electrochemical analyzer (Bioanalytical System, USA). For voltammetric measurements a commercial glassy carbon working electrode (∅: 3 mm, BAS), a platinum wire auxiliary electrode and Ag/AgCl (NaCl 3M, BAS) reference electrode were utilized. Before each experiment, the glassy carbon electrode was polished manually with alumina (∅: 0.01 μm,) in the presence of double distilled water on a smooth polishing cloth.

The dissolution test was performed in a paddle dissolution apparatus recommended by the USP 24 [26].

Spectrophotometric measurements were carried out using a Shimadzu 1601 PC double beam UV-vis spectrophotometer.

Ambroxol HCl and its pharmaceutical preparations were kindly provided by Bilim Pharm.Ind. (Istanbul,Turkey). All the chemicals used were of reagent grade quality (Merck or Sigma) and they were employed without further purification.

Ambroxol stock solutions were prepared daily by direct dissolution in selected supporting electrolytes. Three different supporting electrolytes, namely sulfuric acid (0.2 M), phosphate buffer (0.2 M, pH 4.5, 6.5), Britton-Robinson buffer (0.04 M, pH 2 – 11) were used for the supporting electrolytes.

All solutions were protected from light and were prepared in doubly distilled water.

For dissolution studies, working solutions of 0.1 M hydrochloric acid, which is adequate to physiological conditions in gastric fluids, were used.

Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-3} M was weighed, transferred into a 100 mL calibrated flask and completed to the volume with 0.2 M H_2SO_4. The contents of the flask were sonicated for 15 min to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte.

Samples from syrup and pediatric syrup formulations, which contain paraben and benzoic acid as preservative, respectively, were also dissolved appropriately in 0.2 M H_2SO_4 and portions of the sample solutions were analyzed directly.

Voltammograms were recorded as in pure ambroxol. The content of ambroxol was calculated from the regression equation.

The dissolution methodology was carried out according to the USP dissolution procedures [26] for single-entity products with use of a USP paddle-stirrer type of apparatus in 900 mL of 0.1 M HCl, at a stirring rate of 75 rpm. The temperature of the cell was controlled at $37 \pm 0.5\,^{\circ}C$ by use of thermostatic bath. At each predetermined time interval, an exact volume of sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium. A correction factor was included in the calculations to account for the drug lost in the samples. Using the voltammetric technique, the current values were recorded and the amount of ambroxol released was determined from the related calibration plot. The dissolution test data were performed from the average of six parallel studies.

Furthermore, to obtain comparative results, an UV spectrophotometric method at 306.6 nm was also applied. This spectrophotometric method was very similar to those described in the USP for the single-entity products.

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