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Electrochemical Determination of Anaesthetic Drug Benoxinate Hydrochloride

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Abstract: The electrochemical oxidation of *Benoxinate* hydrochloride (Ben) has been studied in Britton-Robinson buffer at electrochemically pretreated glassy carbon electrode (EA-GCE). Ben exhibits a well-defined irreversible oxidation peak over the entire range of pH 2-11. Differential pulse voltammetry (DPV) was used to determine the compound of interest in pure form, when the peak current varied linearly over the range of $4.0 \times 10^{-7} - 3.2 \times 10^{-6}$ mol L^{-1}; the limits of detection and quantification being 8.3×10^{-8} mol L^{-1} and 2.8×10^{-7} mol L^{-1}, respectively. The recovery was found in the range of 99.3-100.7%, the relative standard deviation in the range of ±0.75-1.25%. The respective method proposed could be applied to determine Ben in pharmaceutical form.

Keywords: *Benoxinate* hydrochloride (Ben); Oxidation; Glassy carbon electrode; Differential pulse voltammetry; Determination; Pharmaceutical form.

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Introduction

The widespread adulteration of commercially available pharmaceutical preparations demand reliable method for drug quality control that are preferably selective, rapid and can be undertaken with simple equipment. Nevertheless, most of these methods involve several manipulation steps before the final result of the analysis, have poor selectivity or require expensive apparatus. Therefore the development of reliable low cost, simple, quick, and accurate methods for the active ingredient determination is welcomed.

Benoxinate hydrochloride (4-amino-3-butoxybenzoic acid 2-(diethylamino) ethyl ester, mono-hydrochloride; aka "*Oxybuprocaine* hydrochloride" – see Fig. 1) is one of the family of ester type local anaesthetics, being used in ophthalmology, otology, rhinology, and laryngology. Specifically, it is effectively applicable in short ophthalmologic procedures, such as minor eye surgery, tonometry, fitting of contact lenses, or local analgesia of the injured eye [1,2].

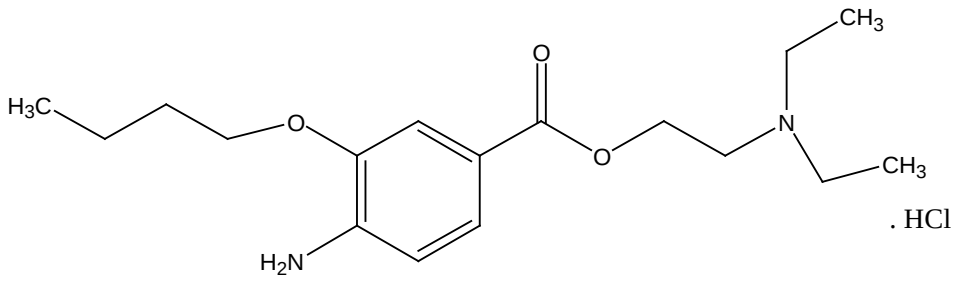

Figure 1. Chemical structure of *Benoxinate* hydrochloride (Ben)

For the determination (quantification) of *Benoxinate* hydrochloride / Ben, a number of various methods has been published and, among them, one can choose spectrophotometry [3,4], combination of gas chromatography with mass spectrometry [5-7], and high performance liquid chromatography [8-13]. Furthermore, some electroanalytical measurements are also feasible; for instance, non-aqueous titration [14] or the use of an ion-selective electrode [15].

In modern electroanalysis, carbon based electrodes are now in widespread use because of their broad potential window, low cost, rich surface chemistry, low background current, and chemical inertness. Glassy carbon electrode (GCE) is made of a class of non-graphitizing carbon that is widely used electrode material being also known as vitreous carbon. The GCE itself, usually in the disc configuration, is used very commonly because of its excellent mechanical and electrical properties, impermeability to gases, and extremely low porosity [16].

The anodization of the GCE at a high positive potential usually results in the stable peak currents and the whole process is an electrochemical activation of the carbon surface with the oxidized film containing various functional groups especially those of the carbon-oxygen type. Such functional groups increase the density of the active sites at the electrode surface and may significantly improve the electron transfer of the reaction. In addition, after

this treatment, the porous film modifies the electrode and its effective surface area is increased [17].

The literature survey performed exclusively for this study has revealed that there are no attempts made to investigate the electrode behavior of Ben via the oxidation of its electroactive sites. Therefore, the aim of this study — in a continuation to our previous work [18-21] — was to establish and optimize the experimental conditions for the determination of Ben in pure and pharmaceutical forms by using the appropriate voltammetric techniques.

Experimental

Chemicals and Reagents

Benoxinate hydrochloride and its commercially available pharmaceutical form, Benox Eye Drops (Batch No. 084842), were supplied from Egyptian International for Pharmaceutical Industries Company, 10th of Ramadan City, Egypt. Stock solution of Ben (1.0×10^{-3} mol L^{-1}) was prepared by dissolving an appropriate amount of Ben in bi-distilled water. The stock solution was stored in a refrigerator. Britton-Robinson (BR) buffer was prepared by mixing the acid mixture containing phosphoric acid (0.04 mol L^{-1}), acetic acid (0.04 mol L^{-1}), plus boric acid (0.04 mol L^{-1}). Buffer solutions were adjusted by adding the necessary amount of 2.0 mol L^{-1} NaOH in order to obtain the appropriate pH value. All chemicals were of analytical grade available and used without further purification.

Apparatus and Other Instrumentation

Either cyclic voltammetry (CV) or differential pulse voltammetry (DPV) was chosen to carry out the measurements using a computer-driven AEW2 analytical electrochemical workstation with ECProg3 electrochemistry software (Sycopel, England) in combination with a C-2 stand with a three-electrode configuration: a glassy carbon working electrode (model MF-2012, BAS), an Ag/AgCl/3M NaCl (model MF-2063, BAS) reference electrode, and a platinum-wire counter electrode (model MW-1032, BAS). Origin 7.0 software was used for the transformation of the initial signal. A cyberscan 500 (EUTECH Instruments, USA) digital pH-meter with a glass combination electrode served to carry out the pH measurements.

Pretreatment of the Glassy Carbon Electrode

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper. An electrochemical pretreatment of the glassy carbon electrode was performed by anodic oxidation at +1.70 V vs. ref. for 6 min in 0.04 mol L^{-1} BR buffer of pH 7.0. The electrode was then cycled between -0.80 and +1.10 V at a scan rate of 100 mV s^{-1} until a stable current-voltage profile was obtained.

Optimízation of Experimental and Instrumental Conditions for Determination of Ben

To obtain the optimum pH, an appropriate amount of Ben working solution (1.0 X 10^{-3} mol L^{-1}) was placed in the electrolytic cell containing 5 ml of BR buffer and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values and the optimum pH was obtained. In order to study the effect of scan rate (υ) on the peak current (Ip) of Ben, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Ben solution (0.001 mol L^{-1}) and the CV-scans were recorded at different scan rates over the range of 10-250 mV s^{-1}; resultant the "log I_P vs. log υ" plot could then be used to define the nature of the electrode reaction; i.e., whether there is a diffusion controlled transormation or an adsorption controlled process.

The optimum conditions of the DPV measuring mode for the determination of Ben were chosen based on studies of the peak current in dependence of the varied pulse amplitude, pulse width, and scan rate, when each parameter was changed while the others were kept constant. The respective variations were as follows: pulse amplitude: 25-100 mV, pulse width: 30-90 ms, and scan rate: 10-50 mV s^{-1}. All the measurements were carried out at the room temperature.

Procedures and Methods

General Procedure for the Determination of Ben in Pure Form. Three electrodes (see above) were immersed in 5 ml of BR buffer. Since dissolved oxygen had not interfered with the anodic voltammetry, no deaeration was needed.

Then, aliquots of the drug solution (1.0×10^{-3} mol L^{-1}) were introduced into the electrolytic cell and voltammetric analyses were performed and the corresponding voltammograms

recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram.

Determination of Benoxinate Hydrochloride in Benox Eye Drops. A portion of the solution needed to obtain 1.0 X 10^{-3} mol L^{-1} drug solution was transferred into a 100 ml volumetric flask which contains 70 ml of double distilled water. The content of the flask was sonicated for about 10 minutes and then made up to the volume with double distilled water. Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

Results and Discussion

Voltammetric Behaviour of *Benoxinate* hydrochloride a the Glassy Carbon Electrode

To elucidate the electrode reaction of Ben, the cyclic voltammograms at glassy carbon and electrochemically activated glassy carbon electrodes were recorded at different pH values and at different scan rates. As an example, Fig. 2 shows the cyclic voltammograms of 4.0 X 10^{-5} mol L^{-1} Ben solution in BR buffer of pH 7.0 in case of unmodified GCE and electrochemically activated glassy carbon electrode (EA-GCE) at a scan rate of 100 mV s^{-1}. Each voltammogram exhibits one well-defined anodic peak, with no peak on the reverse scan, suggesting the irreversible nature of the electrode reaction. It is obvious from the figure that EA-GCE is better than GCE for electrochemical determination of Ben.

Effect of pH

The influence of pH on Ben at EA-GCE is shown in Fig. 3, depicting the plot of peak current (Ip) vs. pH. From the graph drawn, it is obvious that the peak current reaches its maximum value at pH 7; i.e., neutral medium is the suitable medium for the determination of Ben by using DPV.

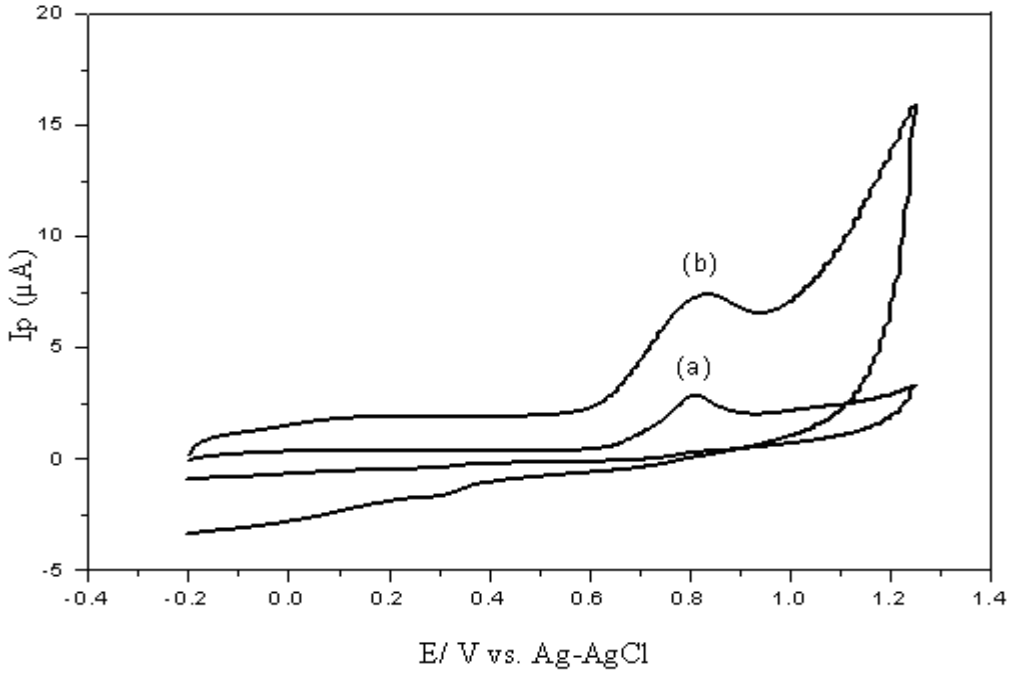

Figure 2. Cyclic voltammograms of 4.0×10^{-5} mol L^{-1} Ben solution in BR buffer (pH 7.0)
for GCE (a) and EA-GCE (b). Scan Rate 100 mV s^{-1}.

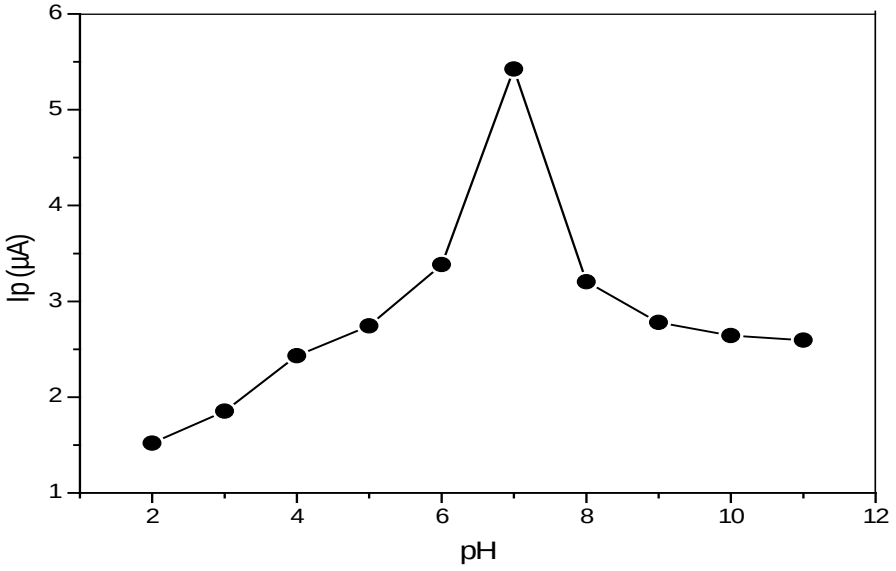

Figure 3. Effect of pH on peak current of 4.0 X 10^{-5} mol L^{-1} Ben solution in BR buffer at EA-GCE; can rate: 100 mV s^{-1}.

Effect of the Scan Rate

The effect of scan rate (υ) on the peak current (Ip) of Ben was shown in Fig. 4. Linear relationships were observed between log Ip and log υ over the scan range 10-250 mV s^{-1} and correspond to the following equation: log Ip = -0.36 + 0.41 log υ. The slope of 0.41 is close to the theoretically expected value of 0.50 for a diffusion controlled process [22].

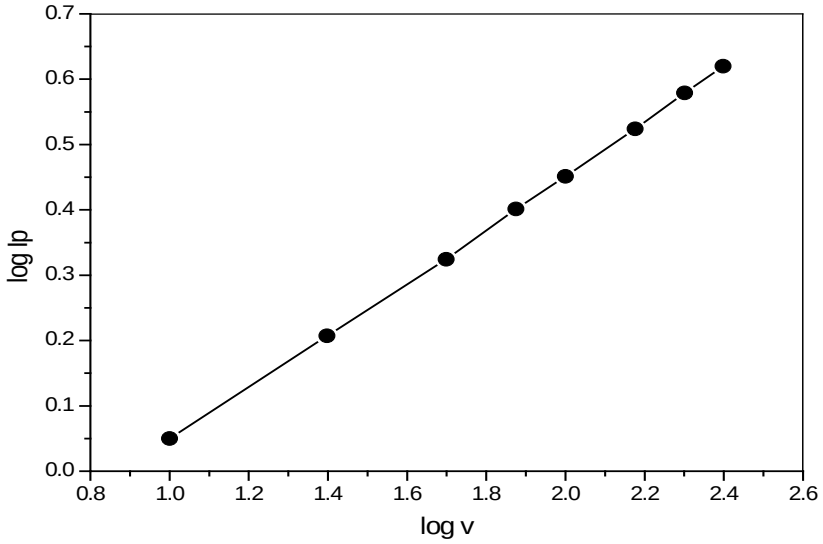

Figure 4. Anodic peak current response of 4.0 X 10^{-5} mol L^{-1} Ben solution as a function of scan rate (υ) in BR buffer of pH 7.0 at EA-GCE.

Effect of Instrumental Parameters

It was found that the peak current was increased with the increasing pulse amplitude and scan rate, while it decreased with the increasing pulse width. To obtain relatively high and narrow peaks the values of 50 mV, 30 ms, and 20 mV s^{-1} were finally chosen for pulse amplitude, pulse width and scan rate, respectively.

Determination of Benoxinate Hydrochloride in the Pure Form

Based on the electrochemical oxidation of Ben at EA-GCE, analytical method was developed involving DPV method to determine the drug under investigation. Linear relation between the peak current (Ip) and Ben concentration (C) was in the range of 4.0×10^{-7} -3.2×10^{-6} mol L^{-1}. The calibration plot illustrated in Fig. 5 can be described by the following equation and correlation coefficient:

Ip (μA) **= 0.219 C** (μM) **+ 0.184** (with $r^2 = 0.9998$)

Three replicates of the calibration curves were obtained over the concentration range of $4.0 \times 10^{-7} - 3.2 \times 10^{-6}$ mol L^{-1}. The limits of detection (LOD) and quantification (LOQ) estimated by means of the corresponding criteria ("S/N") were calculated with the aid of the following equations: **LOD = 3 SD/m** and **LOQ = 10 SD/m**, where "SD" is the standard deviation of the intercept of the calibration curve and "m" is the slope of the calibration curve [23]. Then, the respective LOD and LOQ were found to be 8.3×10^{-8} mol L^{-1} and 2.8 10^{-7} mol L^{-1}, respectively.

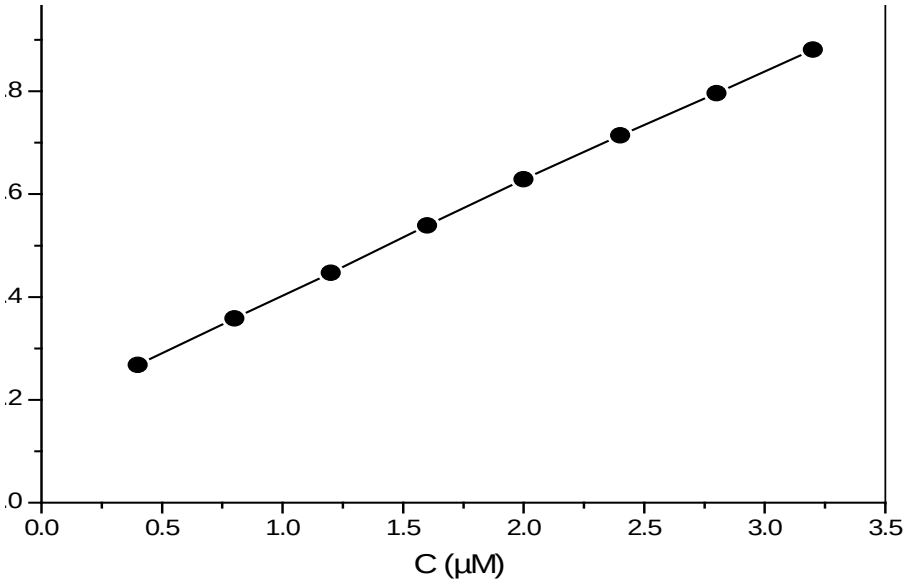

Figure 5. Calibration curve of Ben at EA-GCE by using DPV method, pulse amplitude of 50 mV and scan rate of 20 mV s^{-1}.

Accuracy and precision of the proposed method were determined by replicate analyses of five different concentrations of Ben and the results given are gathered in Table I. The recovery (R) were in the range of 99.3-100.7% and the relative standard deviation (RSD) oscillated in the range of ±0.75-1.25%.

The method proposed was found more sensitive than the spectrophotometric determinations when comparing the respective data: $2.90 \times 10^{-6} - 5.80 \times 10^{-5}$ mol L^{-1}[3] and $1.45 \times 10^{-6} - 4.35 \times 10^{-5}$ mol L^{-1} [4], ion-selective electrode method: $1.60 \times 10^{-5} - 0.13$ mol L^{-1} [15], and chromatographic method, having a higher LOD of 8.70×10^{-8} mol L^{-1} [13].

The results obtained by using the proposed method were compared with those of HPLC and the respective method [13]. The chromatographic analysis resulted in an average value of 100.03% with a relative standard deviation of 0.41%. These results show no significant difference between the performance of the two methods confronted with regard to the accuracy and precision, respectively. Moreover, the method developed seems to be simpler and less expensive, when offering sufficiently rapid procedure.

Table I: *Analytical parameters of the calibration plots for determination of Benoxinate Hydrochloride (Ben)*

Parameter	**EA-GCE**
Linearity range (mol L^{-1})	$4.0 \times 10^{-7} - 3.2 \times 10^{-6}$
Calibration curve equation	Ip (μA) = 0.219 C (μM) + 0.184
Correlation coefficient (r^2)	0.9998
LOD (mol L^{-1})	8.26×10^{-8}
LOQ (mol L^{-1})	2.75×10^{-7}
RSD* %	0.75-1.25
Recovery (%)	99.3-100.7

* Five different concentration of Ben.
* Number of replicates (n) = 5.

Determination of Benoxinate Hydrochloride in Benox Eye Drops

The proposed method was successfully applied to determine Ben in dosage form (Benox eye drops). The linearity range was 4.0×10^{-7}-3.2×10^{-6} mol L^{-1} with the average recovery of 100.2% and the average RSD of ±1.16%.

The results were compared with those obtained with the approved reference nonaqueous titration method [14]. The student t-test and variance ratio F-test excluded any significant differences between both methods with respect to accuracy and precision. The results are shown in Table II.

Table II: *Determination of Ben in Benox eye drops compared to the official method* [14].

Claimed mg/ml	**Official method** Recovery (%) ± SD[a]	**Method with EA-GCE** Recovery (%) ± SD[a]
4.0	99.65 ± 1.25	100.25 ± 1.14
	F-test [b] =	2.47
	t-test =	0.62

[a] Averaged from five determinations.

[b] Tabulated F and t values at 95% confidence level = 6.39 and 2.776, resp. (according to [23]).

Conclusions

Voltammetric determination of *Benoxinate* hydrochloride (Ben) has been studied at the electrochemically pretreated glassy carbon electrode, resulting in the development of the respective method for the determination of this pharmaceutical in selected samples. The main advantage of the method proposed is its simplicity and a rapid procedure compared to other similar methods. Furthermore, quite a low detection limit and a wide range of accessible concentrations are sufficient for routine analyses.

The new method with EA-GCE offers a good alternative to the existing procedures for the determination of *Benoxinate* hydrochloride because of its low cost, sensitivity, accuracy, and precision. Finally, the method proposed has shown clear advantages such as short period of real time for drug analysis and no time required for an extraction step prior to analysis.

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