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An extensive study of electrochemical behavior of brimonidine and its determination at glassy carbon electrode

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ABSTRACT

The electrochemical behavior of brimonidine (BRIM), an antiglaucoma agent applied in therapy for lowering high intraocular pressure, was investigated by cyclic voltammetry, differential pulse voltammetry and square wave voltammetry using a glassy carbon electrode (GCE).

The reduction of BRIM occurs as one-step quasi-reversible reaction in acid and neutral medium, reaching the full reversibility in alkaline solutions. Reduction process involves the transfer of two electrons and two protons at the pyrazine ring of quinoxaline moiety, forming a dihydro-derivative. In acid and neutral solutions, brimonidine reduction product is partly oxidized to its hydroxy-derivative. BRIM is also oxidized irreversibly with the transfer of one electron and one proton at secondary amine moiety. The effects of pH of the electrolyte solution, scan rate and BRIM concentration were monitored. The nature of the electrode process was found to be controlled by the adsorption at pH > 6 and the total surface concentration of brimonidine adsorbed onto the GCE surface at pH 7, $\Gamma_{BRIM} = 1.35 \times 10^{-10}$ mol cm^{-2} was obtained. Based on this study, differential pulse voltammetric method was developed, validated and suggested for rapid electroanalytical determination of the low concentration of brimonidine. The linearity was achieved within the concentration range from 5×10^{-7} to 5×10^{-6} M with LOD = 1.6×10^{-7} M and LOQ = 5.3×10^{-7} M. The method was applied for brimonidine determination in pharmaceutical dosage form, eye drops.

1. Introduction

Glaucoma is a group of eye diseases characterized with high intraocular pressure (IOP). If not treated, the pressure within the eye may cause permanent vision loss. An antiglaucoma agent brimonidine (BRIM, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)quinoxaline-6-amine) (Scheme 1), is effective α_2-agonist, with very high potency in lowering IOP and thus preventing optic nerve damage. BRIM is applied as the eye drops, therefore it may act directly to the point of disorder. Therapeutic acting of BRIM consists of suppressing the rate of aqueous humor flow and enhancing uveoscleral outflow, and thus lowering the eye pressure [1,2].

Although topically applied, ophthalmic medications may achieve sufficient serum levels through absorption into conjuctival and nasal mucosas to obtain systemic effects potentially interacting with the other drugs [3]. This correlates with low concentration levels in biological fluids, demanding the use of very sensitive methods. Therefore, a couple of the hyphenated techniques like gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) were reported [4,5], aiming to BRIM detection and determination in human plasma, blood serum and ocular fluids. Tzovolou et al. proposed capillary electrophoretic (CE) analysis of BRIM in the aqueous humor of the eye and blood serum [6].

On the other hand, the high performance thin layer chromatographic (HPTLC) method was reported in the literature for brimonidine tartrate estimation as a bulk drug and in ophthalmic solution [7]. This approach is of a great interest from the view point of the drug quality control. Stability indicating the assay method using hydrophilic interaction liquid chromatography (HILIC) has also been developed and validated for the quantitative determination of brimonidine tartrate formulated as an ophthalmic solution [8]. Recently, we have reported the electroanalytical method at boron doped diamond electrode (BDDE) for BRIM determination in pharmaceutical dosage form, eye drops [9]. Based on the electrochemical study of BRIM at BDDE, it was shown that the reduction occurred in one-step quasi-reversible mechanism, involving the transfer of two electrons and two protons. Accordingly, it was concluded that the reduction happened at the quinoxaline ring confirming the same mechanism as for other quinoxaline

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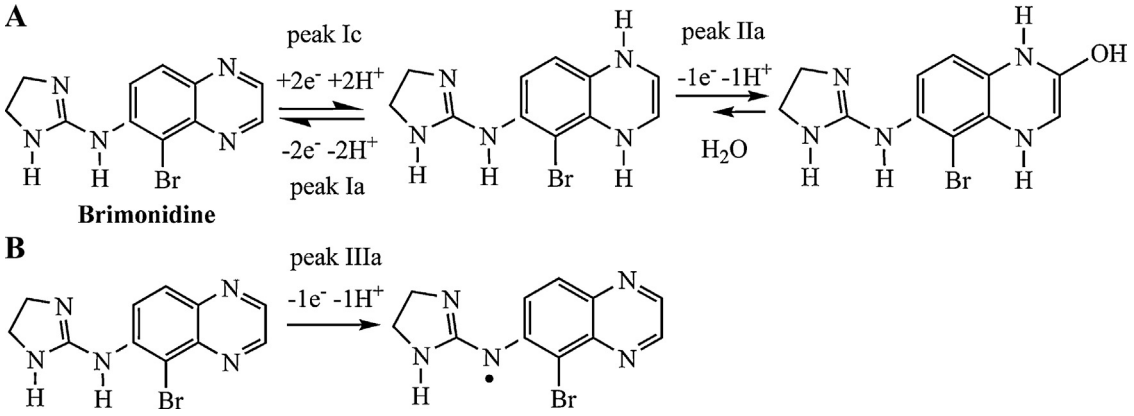

Scheme 1. The proposed electrochemical mechanism of BRIM at a glassy carbon electrode. (A) Reduction; (B) oxidation.

derivatives [10,11]. We have also reported electrochemical study and the method for determination of varenicline, the quinoxaline derivative, in tablets and in spiked plasma, at BDDE, glassy carbon (GCE) and hanging mercury electrode [10].

No literature data were found on the voltammetric study of BRIM at GC electrode up to now.

In this work an extensive study of voltammetric behavior of BRIM at GCE is presented. Also, the adsorption phenomenon of BRIM was confirmed at GCE surface, as well as it was already established at the mercury surface. The redox mechanism of BRIM at GC electrode was proposed, and the new voltammetric method for BRIM determination in pharmaceutical dosage form of eye drops was established.

2. Experimental

2.1. Chemicals

Brimonidine tartrate was kindly donated by the Agency of Drugs and Medical Devices, Belgrade, Serbia. Alphagan eye drops were produced by Allergan Pharmaceuticals Ireland. A stock solution of 6×10^{-4} M BRIM was prepared in bi-distilled water. The solutions of different concentrations were obtained by diluting the stock solution with different supporting electrolytes [12], and prepared from the chemicals of analytical grade quality. The following supporting electrolytes were used: HCl + KCl for pH 2.2; acetate buffer for pH 3.6, 4.6 and 5.4; phosphate buffer for pH 6.2, 7.0 and 8.0, and ammonia buffer for pH 8.5, 9.2 and 10.2. Ionic strength of all solutions was adjusted to 0.1 M. All the experiments were done at room temperature ($25 \pm 1\,^{\circ}C$).

2.2. Apparatus

The voltammetric measurements were performed with a μAUTOLAB analyzer (EcoChemie, Utrecht, The Netherlands) running with the GPES 4.9 software.

In all the experiments the three electrode system was used: a glassy carbon electrode (GCE, produced by CH Instruments, Inc., USA, $d = 3$ mm) as working electrode, a Ag/AgCl as reference electrode (3 M KCl), and a Pt wire as auxiliary one.

A SCALTEC SBC 31 balance, Ultrasonic bath "Iskra" UZ 4R and Radiometer pH meter, PHM 220, with combined pH electrode Radiometer GK2401B and appropriate standard buffer solutions were used.

The GCE was manually polished using the aqueous slurry of Al_2O_3 powder (particle size 0.05 μm) on a smooth polishing pad before each experiment. The electrode was rinsed with bidistilled water and then sonicated in absolute ethanol for 2 min.

2.3. Procedures

An appropriate volume of BRIM stock solution was diluted with an adequate buffer solution in the cell yielding the final concentration of 1×10^{-4} M for the cyclic voltammetry (CV), 2×10^{-5} M for the differential pulse voltammetry (DPV), and 5×10^{-5} M for the square wave voltammetry (SWV) study, in a total volume of 15.00 mL. The determination in dosage form of Alphagan eye drops was performed by standard addition method. First, the DP voltammogram of Alphagan solution corresponding to the concentration level of 1×10^{-6} M BRIM was recorded. Then, the appropriate volume of standard solution corresponding to concentration 1×10^{-6} M of BRIM was added into solution and voltammogram was recorded. No preparation of dosage form prior to the determination was needed in spite of its complex composition.

The test solution was deoxygenated by bubbling with high purity nitrogen for a minimum of 10 min to remove any oxygen interferences, when experiments were performed in cathodic region. The cyclic voltammograms were recorded between −1.2 V and +1.6 V, at scan rate ranged from 10 to 100 mV s^{-1}.

The experimental parameters for differential pulse voltammetry (DPV) were: pulse width 50 ms, scan rate 5 mV s^{-1} and pulse amplitude 50 mV.

For square wave voltammetry (SWV) the following conditions were selected: frequency 25 Hz and potential increment 2 mV, corresponding to an effective scan rate of 50 mV s^{-1}, and the pulse amplitude of 100 mV.

3. Results and discussion

3.1. Cyclic and differential pulse voltammetry

The redox behavior of BRIM was initially studied by CV in the solution of 1×10^{-4} M in 0.1 M acetate buffer pH 3.6 bubbled with nitrogen. The cyclic voltammograms were recorded in three successive scans starting from 0.0 V, towards −1.2 V and reversing to the positive potential limit of +1.6 V, at a scan rate $\nu = 100$ mV s^{-1}. On the first negative-going scan, one cathodic peak (peak Ic) was obtained at $E_{p,Ic} = -0.454$ V. Changing the scan direction, one main anodic peak (Ia) appeared at $E_{p,Ia} = -0.356$ V and two additional anodic peaks (IIa and IIIa) at potentials of $E_{p,IIa} = +0.356$ V and $E_{p,IIIa} = +1.250$ V (Fig. 1A) were noticed.

Recording the cyclic voltammograms in the opposite direction starting from 0.0 V, going to +1.6 V and reversing to −1.2 V under the same conditions, the second anodic peak (IIa) was not present in the first scan, but it appeared in the second and third scan (Fig. 1B). The third anodic peak (IIIa) is permanently present in all the scans.