Voltammetric Reduction of a 4-Nitroimidazole Derivative on a Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrode

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Abstract

We report the electrochemical behavior of a 4-nitroimidazole derivative, 1-methyl-4-nitro-2-hydroxymethylimidazole (4-NImMeOH), on glassy carbon electrode (GCE) modified with multiwalled carbon nanotubes (MWCNT). As dispersing agents, dimethylformamide (DMF) and water were used. The electrochemical response of the resulting electrodes was evaluated using linear sweep, cyclic and square-wave voltammetry (LSV, CV and SWV). Several parameters such as medium pH, nature and concentration of the CNTs dispersion and accumulation time were tested. The optimal conditions determined for obtain better response were: pH 2, dispersion concentration = 4 mg/mL of CNT in water, accumulation time = 7 min. The MWCNT-modified GCE exhibited attractive electrochemical properties producing enhanced currents with a significant reduction in the overpotential and good signal-to-noise characteristics, in comparison with the bare GCE. The modified electrode is highly repeatable for consecutive measurements, reaching a variation coefficient of 2.9% for ten consecutive runs.

Keywords: Carbon nanotubes, Nitro compound, 4-Nitroimidazole, Cyclic voltammetry, Square-wave voltammetry

1. Introduction

Nitroimidazole derivatives are heterocyclic drugs widely used as antibacterial, antiprotozoal and anticancer agents [1] but, it is also well established that these molecules present several adverse effects [2].

Most, if not all, of the biological characteristics of nitroimidazole depend on metabolic reactions that lead to the reduction of the nitro group and the formation of a highly reactive intermediate, the nitro radical anion species, which is responsible not only for the antimicrobial and radio-sensitizing properties of these compounds, but also for their cytotoxic and mutagenic properties. However, both the cell toxicity of the metabolites of the various nitroimidazoles and the possible therapeutic importance of the different substituents in positions 1 and 2 of the imidazole ring require further investigation.

The reported studies about the electrochemistry of nitroimidazoles are mainly focused on the analytical determination of some pharmacologically important 5-nitroimidazoles, such as metronidazole, ornidazole, secnidazole, tinidazole and megazol [3 – 8]. However, electrochemical studies of 4-nitroimidazole derivatives are scarce and restricted to a polarographic study of several 1,2-dialkyl-4-nitroimidazoles [9] and some electrochemical studies about the cyclic voltammetric behavior of 4-nitroimidazole in aprotic [10] and protic media [11]. Furthermore, in a previous paper [12], we studied the nitro radical anion formation from a new 4-nitroimidazole derivative and more recently the micellar effects on the reduction of the same 4-nitroimidazole derivative was also evaluated [13].

The use of carbon nanotubes (CNT) as electrode modifiers have attracted much attention during the last time due to the unique behavior of CNT, including their remarkable electrical, chemical, mechanical and structural properties [14]. Furthermore, recent studies have shown that carbon nanotubes (CNTs) modified electrodes exhibit attractive electrochemical properties producing electrocatalytic effects [15]. There are only few papers devoted to applications of carbon nanotubes on nitro compounds. Simple and sensitive electrochemical methods for the determination of metronidazole and chloramphenicol at a CNT coated glassy carbon electrode were published [16, 17]. More recently the electrochemical reduction of nitrobenzene at a pyrolytic graphite electrode modified with CNTs was studied in order to construct a two-compartment flow reactor packed with CNTs modified electrodes for removal nitrobenzene from wastewater [18]. On the other hand, nitro compounds as (2,5-dimethoxy-4-[nitrophenyl)azo] benzenediazonium chloride [19] and 4-nitrobenzylamine [15] have been used to derivatize multiwalled CNTs electrodes.

In the present study we have centered our attention on the reduction of a recently described 4-nitroimidazole derivative in order to study its behavior on a multiwalled CNTs-film coated glassy carbon electrode.

Experimental

2.1. Reagents and Solutions

1-Methyl-4-nitro-2-hydroxymethylimidazole (4-NIm-MeOH) (Fig. 1) was synthesized and characterized in our laboratory according to the procedure previously described [12]. Multiwalled carbon nanotubes (MWCNT) $1-5$ μm long and (30 ± 15) nm diameter were obtained from Nano-Lab (USA). CNTs were oxidized by chemical treatment with a mixture $3:1$ of 3 M $H_2SO_4 : 3$ M HNO_3 by refluxing for 3 hours. After that, the suspension was filtered and washed with water until neutral pH. All the employed reagents were of analytical grade and were used without prior purification. Nitrogen gas was obtained from AGA Chile S. A. with maximum impurities of $H_2O < 3$ ppm; $O_2 < 2$ ppm; $C_nH_m < 0.5$ ppm.

All the voltammetric experiments were obtained after bubbling with N_2 for 10 min. in the cell before each run. Temperature was kept constant at $25 \pm 0.1\,°C$ in all experiments.

0.1 M Britton–Robinson buffer was prepared dissolving 6.18 g of boric acid, 5.7 mL of glacial acetic acid, 6.7 mL of phosphoric acid and completing up to 1000 mL with water, and the desired pH was adjusted with concentrate solutions of NaOH. The used water was purified with Milli-Q Ultra-Pure Water System.

4-NImMeOH stock drug solution was prepared dissolving 7.85 mg and diluted up to 5 mL with 0.1 M Britton-Robinson buffer, to obtain a final concentration of 0.01 M. Work solution was prepared taken an aliquot of the stock solution and diluted to 10 mL with 0.1 M Britton–Robinson buffer.

2.2. Apparatus

Voltammetric curves were recorded on a CHI 900 (CH Instruments Inc., USA) attached to a PC computer with appropriate software, for total control of the experiments and data acquisition and treatment. Glassy carbon electrode (GCE) of 3 mm diameter (Model CHI104, CH Instruments) were used as working electrode, a platinum wire (BASi MW-1032) as the counter electrode and an Ag|AgCl|NaCl
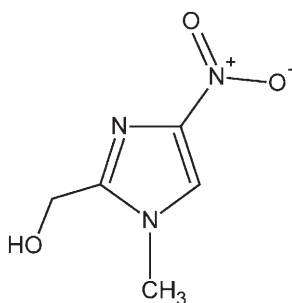

Fig. 1. Molecular structure of 1-methyl-4-nitro-2-hydroxymethyl-imidazole (4-NImMeOH).

(3 M) (BASi MF-2052) as the reference electrode, was used. A magnetic stirrer was utilized for accumulation of 4-NImMeOH on electrode surface.

2.3. Preparation of the Working Electrode

Before each modification, the GCE was cleaned by polishing with 0.3 μm and 0.05 μm alumina, and then was washed with abundant water. The oxidized CNTs were dispersed at different concentrations (mg/mL) with water or DMF by sonication for 5 min. The sonication procedure was repeated three times. The immobilization of CNTs was performed by casting the GCE with 5 μL of the CNTs dispersion (DMF, H_2O). The optimum conditions were obtained drying the dispersion dropped onto the GCE for 15 min at $50\,°C$ in a stove. The resulting modified electrodes were called GCE/CNT_{DMF} and GCE/CNT_{H2O} if the dispersion was carried out on DMF or water, respectively.

2.4. Procedure

4-NImMeOH adsorption: The modified GCE electrode was immersed in a stirred supporting buffer solution containing 4-NImMeOH waved 250 rpm; the accumulation time was variable and no potential was applied during this step.

Voltammetric transduction: was performed by LSV or SWV. The cathodic current at around -0.3 V, corresponding to the nitro reduction, was used as analytical signal.

3. Results and Discussion

According to previous studies 4-NImMeOH was easily reducible in Hg electrodes in aqueous, mixed and non-aqueous media [12] but we are not aware of any publications describing the use of carbon electrodes for the study of this 4-nitroimidazole derivative. The reduction of 4-NImMeOH on a GCE occurs in a similar way to that reported on Hg, i.e., one irreversible peak or wave in aqueous media is observed (Fig. 2). This reduction process is attributed to the four-electron reduction of nitro group to the corresponding hydroxylamine derivative according to the well known accepted overall mechanism (Eq. 1) for the electroreduction of aromatic and heterocyclic nitro compounds:

$$RNO_2 + 4e^- + 4H^+ \rightarrow RNHOH + H_2O \tag{1}$$

Our current interest is to study the behavior of this nitro compound on a GCE modified with CNTs. Figure 2 shows the comparison of voltammograms of 4-NImMeOH obtained at two different electrodes, i.e. bare GCE and MWCNT/GCE electrodes prepared using two different dispersions. Using a 5.21×10^{-6} M solution, in 0.1 M Britton–Robinson buffer at pH 2 it was found that no redox signals were observed when bare GCE was used, but very good signals, approximately 7 and 13 μA were obtained

using MWCNT/GCE dispersed in DMF and water, respectively. Only using more concentrated solutions, approximately 1×10^{-4} M of 4-NImMeOH, was possible to obtain a voltammetric signal on bare GCE. In all cases only one reduction peak was observed, indicating that below these experimental conditions the redox mechanism remains unaltered.

As indicated from Figure 2, the MWCNT-modified electrodes exhibit not even an increased in current response if not also a substantial positive shift of the cathodic peak potential. The decrease in overpotential seen at the MWCNT-modified electrode can be attributed to a more facile electron transfer occurring on the nanotubes in comparison to the GCE.

Furthermore, the electrochemical behavior of 4-NImMeOH at different scan rates was investigated by using CV (Fig. 3). Only one reduction peak appears at all sweep rates studies and no corresponding oxidation peaks are observed on the reverse scan suggesting that 4-NImMeOH is irreversibly reduced on both bare and MWCNT modified electrodes. Also, the cathodic peak shift with increasing scan rate, observed in Figure 3, is indicative of the irreversibility of the process.

A slope of 0.58 was revealed for the linear relation between the log of peak current and the log of sweep rate (Inset of Fig. 3) indicating a mixed adsorption-diffusion controlled process.

In order to study the optimal response for the reduction peak of 4-NImMeOH we have studied and optimized some parameters such as: pH of the solution, nature of the dispersing agent, concentration of CNT in the suspension and accumulation time.

The preparation of the modified electrode involves the dispersion of MWCNT to form a film on the GCE.
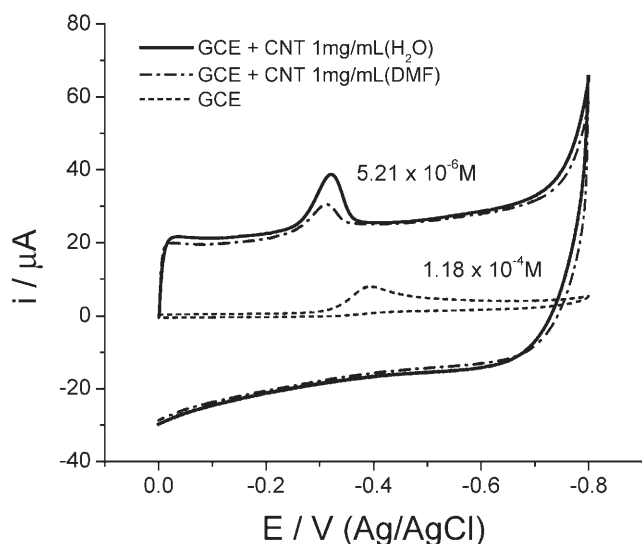

Fig. 3. Cyclic voltammograms of 4-NImMeOH at GCE/CNT-(H$_2$O) electrode at different sweep rates (20–250 V s^{-1}). Inset: logarithmic dependence of the peak current from the sweep rate of the experiment. CNT concentration: 4 mg/mL; 0.1 M Britton–Robinson buffer, pH 2.

Obviously the nature and the thickness of the film will depend on the solvent used to prepare the dispersion. Thus, we have tested water and DMF as dispersing agents obtaining a slightly higher peak current when water was used, as is observed in Figure 2. A homogeneous covering of GCE surface is obtained when CNT are dispersed using water. For further studies we have selected water to prepare the CNT suspension. On the other hand the film homogeneity and thickness can be regulated with the concentration of the dispersion. In this way, the voltammograms showing the effect of using two different concentrations of CNT is displayed in Figure 4. From this figure, it is clear that at low CNT concentrations, the GCE is not entirely covered being possible to observe two electrochemical signals due to the reduction of 4-NImMeOH on MWCNT-GCE and GCE, respectively. In Figure 5 the CNT concentration effect of the MWCNT dispersion on the reduction peak current of 4-NImMeOH is showed. The results suggested that the film formation is a progressive process that reaches a saturation point at about 4.0 mg/mL. On the other hand, more concentrated CNT dispersions produce unstable films which increase RSD of the measurement. Consequently a recommended concentration for the dispersion is 4.0 mg/mL of MWCNT in water for obtaining a better current signal.

The considerable enhancement in the reduction peak current is in part attributed to the strong adsorptive ability of the MWCNT film on GCE. This fact can be corroborated studying the dependence of peak current with the accumulation time of the 4-NImMeOH nitro derivative. Figure 6

Fig. 2. Cyclic voltammograms of 4-NImMeOH at GCE (dashed line), GCE/CNT$_{H2O}$ (solid line), and GCE/CNT$_{DMF}$ (dashed-dotted line). CNT concentration: 1 mg/mL. 0.1 M Britton Robinson buffer, pH 2; scan rate: 0.100 V s^{-1}; accumulation time: 120 s.
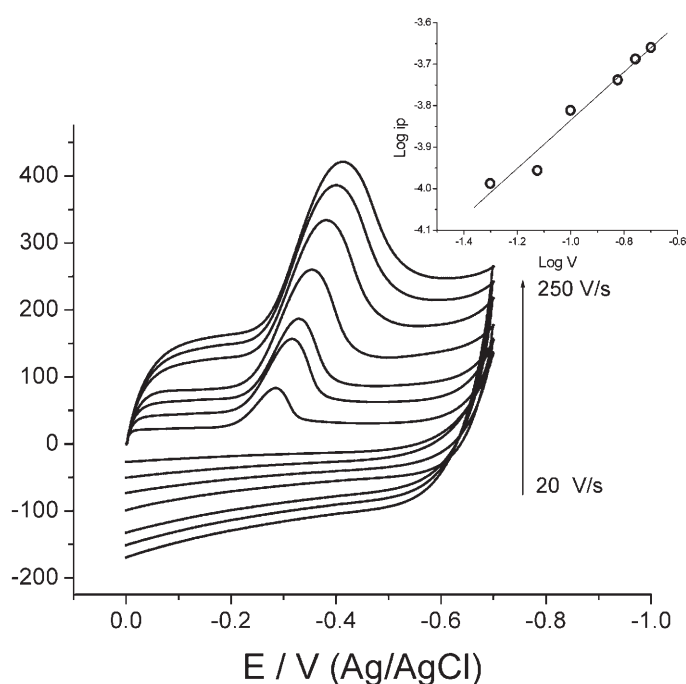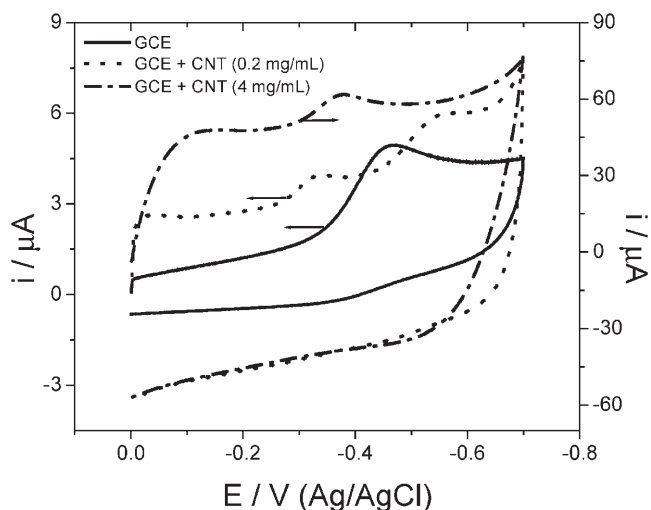
Fig. 4. Cyclic voltammograms of 4-NImMeOH at GCE (solid line) and GCE/CNT$_{H2O}$ using two CNT concentrations: 0.2 mg/mL (dashed line) and 4 mg/mL (dashed-dotted line) 0.1 M Britton–Robinson buffer, pH 2 . Scan rate: 0.100 V s^{-1}.
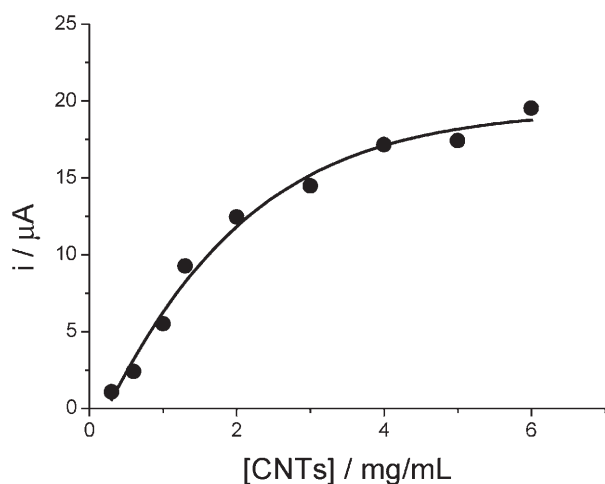

Fig. 5. Peak current dependence from the CNT concentration. 4-NImMeOH concentration: 3×10^{-6} M. CNT dispersed in water. Other experimental conditions are as in Figure 2.

shows the effect of accumulation time at open-circuit on the reduction peak current of 4-NImMeOH (5×10^{-4} M) using a CNT concentration of 4 mg/mL. The reduction peak current increases greatly within the first 300 s and then reaches a plateau indicating saturation at the MWCNT electrode. On the other hand the potential peak value did not change at different accumulation times. On the other hand, according to the reduction mechanism of 4-NImMeOH (Eq. 1) the peak potential of 4-NImMeOH would be strongly pH-dependent. In order to corroborate that dependence we have studied the behavior of the peak potential at different pHs between 2–12. In Figure 7, the E_p vs. pH plot shows that the peak potential is pH-dependent shifting to more negative potentials when pH increased. The slope of the acidic part of the E_p vs. pH line is 65 mV/pH. At pH above
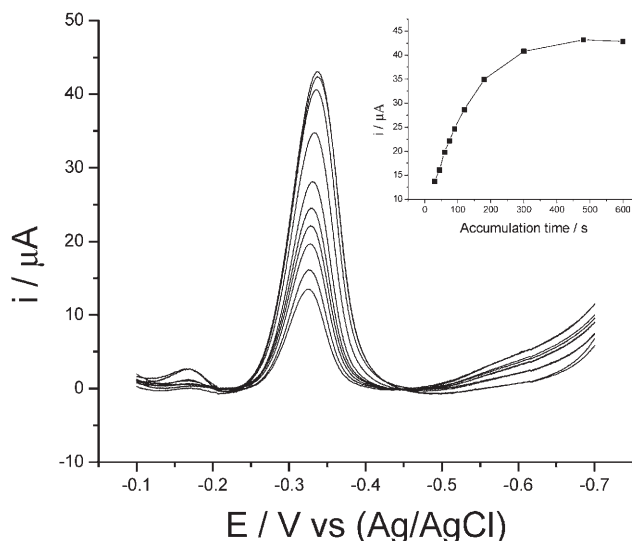

Fig. 6. Linear sweep voltammograms of 4-NImMeOH at GCE/CNT(H$_2$O) electrode at different accumulation times. Inset: Dependence of the peak current from the accumulation time. [4-NImMeOH]: 5×10^{-4} M. CNT$_{H2O}$: 4 mg/mL. 0.1 M Britton–Robinson buffer, pH 2 .

pH 7 the slope changes because the mechanism changes to a different situation wherein there are no protons involved before the rate determining step. A similar pH-dependence was obtained from a different electrode as Hg [13]. From this similarity is clear that the voltammetric behavior of 4-NImMeOH follows the same overall mechanism on MWCNT modified GCE than mercury.

The reduction peak current of 4-NImMeOH resulted to vary linearly with the 4-NImMeOH concentration when submitted to a LSV experiment. The peak current versus concentration curve in pH 2 Britton–Robinson buffer at two different accumulation times is displayed in Figure 8. As can be seen from the figure a linear behavior is obtained for both accumulation times. Furthermore, if the slope of the lines is used as a sensitivity criterion we can appreciate that the increase of the accumulation time from 2 to 7 minutes produce a change in the sensitivity from 3.80 to 6.42 μA/μM.

The repeatability of one MWCNT-film coated GCE was evaluated by measuring the current responses over ten consecutive runs. The result was 20.12 ± 0.59 μA with a variation coefficient of 2.9%, for a fixed 4-NImMeOH concentration of 3.6×10^{-6} M, showing a very good repeatability and short-term stability. When the repeatability was checked from ten independently prepared electrodes the variation coefficient was 7.0%.

On the other hand if the experiment was carried out using square-wave voltammetry with the following optimal parameters: accumulation time = 7 minutes, concentration of the MWCNT dispersion = 4 mg/mL, pH 2.0, square-wave amplitude = 25 mV and 75 Hz of frequency, the sensitivity was increased up to 23.5 μA/μM. Detection and quantification limits of 1.14×10^{-7} and 3.8×10^{-7}, respectively, were obtained.
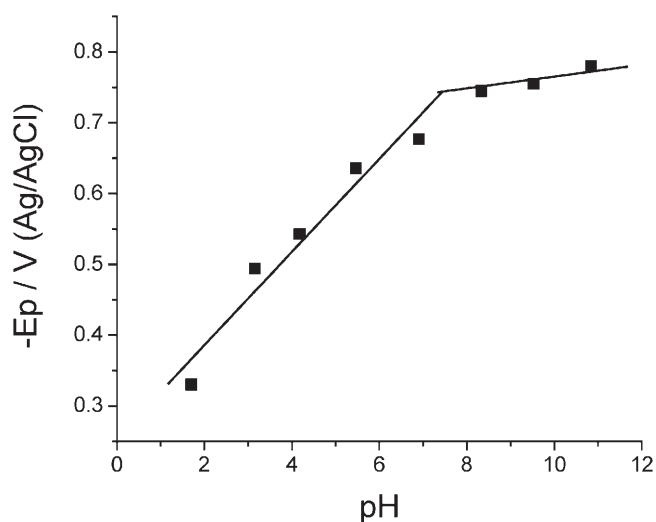
Fig. 7. Peak potential dependence with pH for the electro-reduction of 4-NImMeOH at GCE/CNT(H₂O). [4-NImMeOH]: 5×10^{-4} M. CNT_{H2O}: 4 mg/mL. 0.1 M Britton–Robinson buffer.

Our results reveal that the MWCNT-modified GCE exhibited attractive electrochemical properties for the reduction of 4-NImMeOH producing enhanced currents with a significant reduction in the overpotential and good signal-to-noise characteristics, in comparison with the bare GCE.

In spite of we have demonstrated that it is possible to apply a MWCNT-modified GCE for detection of a specific 4-nitroimidazole such as 4-NImMeOH, however, the same procedure could be extended to other 4-nitroimidazole derivatives.

5. Acknowledgements

6. References

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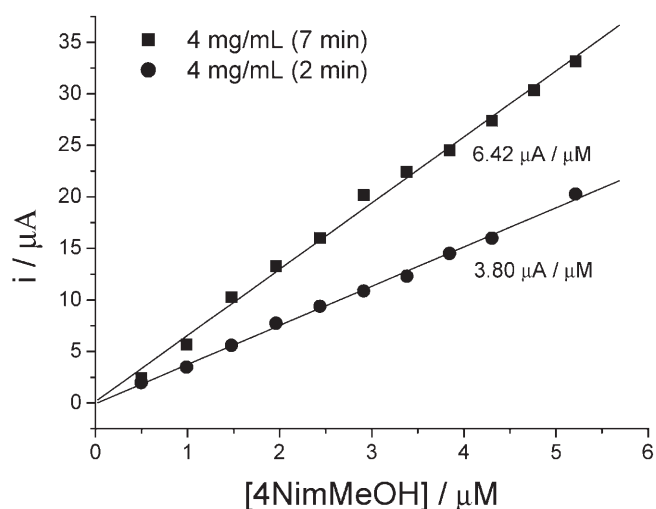

Fig. 8. Calibration curves of 4-NImMeOH obtained at GCE/ CNT_{H2O} at two accumulation times (2 and 7 min). CNT_{H2O}: 4 mg/mL. 0.1 M Britton–Robinson buffer.

4. Conclusions

We have studied the 4-NImMeOH compound as a prototype of 4-nitroimidazole derivatives. The reduction of 4-NImMeOH in aqueous medium on carbon electrodes (bare GCE or modified GCE) occurs via one irreversible 4-electron 4-proton wave in a similar way that on mercury electrodes.