

**VOLTAMMETRIC BEHAVIOR OF 3,5-DINITROBENZOIC ACID IN SOLUTION
ON GCE AND ENCAPSULATED ON MULTIWALLED CARBON NANOTUBES
MODIFIED ELECTRODE.**

By

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ABSTRACT

The cyclic voltammetric behavior of 3,5-dinitrobenzoic acid (3,5-DNB) in 0.1 M PBS of pH 7 was examined at a glassy carbon electrode (GCE). 3,5-DNB was found to produce two irreversible reduction peaks corresponds to the reduction of each nitro group in the 3,5-DNB molecule. Our results contradict previous studies (P.Gopal et al. Journal of Molecular Liquids 178 (2013) 168-174) wherein the same peaks are assigned as, the first, to the reduction of the nitro group to hydroxylamine and the second, to the subsequent reduction to amine derivative.

Also we report that GCE modified with multiwalled carbon nanotubes (MWCNTs) can be derivatized with 3,5-DNB. The derivatization procedure involves simple immersion of the MWCNT-modified electrode in a solution containing 3,5-DNB.

SEM images reveals that the network of nanotubes form a homogeneous, twisted, densely packed, three-dimensional array that remains attached to the GCE surface. Both electrochemical and SEM measurements indicate that 3,5-DNB is encapsulated on the electrode, most probably by being trapped within the pockets of the mentioned three-dimensional array, without formation of covalent bonding.

Keywords:

MWCNT, 3,5-dinitrobenzoic acid, Modified electrode, Voltammetry, SEM.

1.- INTRODUCTION

The use of carbon nanotubes (CNTs) as working electrode has attracted the interest from the electrochemistry community in the last ten years. An analysis in the database of the Web of Science between 2004 and 2014 using as search criteria "Voltammetry & Carbon nanotubes" retrieved 6034 articles, increasing from 84 in 2004 to 1038 in 2014. This explosive increase in publications is due to the excellent characteristics of CNT to be used as working electrode in voltammetry. Their electronic characteristic and their specific properties such as surface-to-volume and porosity provide many interesting applications as electrode material. One of the most interesting applications is based in its feasibility to be modified according the requirement of the researcher.

Different authors have treated the modification of CNTs with nitro groups or nitrocompounds. Wang et al., [1] have efficiently introduced nitro groups on the surface of multiwalled carbon nanotubes (MWCNTs) by conventional nitration procedures. Compton et al. [2] reported the derivatization of MWCNTs by chemical reduction of 4-nitrobenzenediazonium tetrafluoroborate with hypophosphorous acid. The formed 4-nitrophenyl-MWCNTs (NB-MWCNTs) were abrasively immobilized onto the surface of a basal plane pyrolytic graphite (BPPG) electrode and characterized by cyclic voltammetry. Another study [3] showed the derivatization of graphite powder or MWCNTs by 4-nitrobenzylamine (4-NBA) simply by stirring the graphite powder or MWCNTs in a solution of acetonitrile containing 10 mM 4-NBA. The resulting 4-NBA-MWCNT powder was abrasively immobilized onto the surface of a clean BPPG electrode.

In a previous paper, Kubota et al., [4] revealed a modified electrode wherein 3,5-dinitrobenzoic acid (3,5-DNB) was covalently attached to a nanostructured platform based on MWCNTs and polyethylenimine (PEI). Then, a nitroso / hydroxylamine derivatives couple was electrogenerated *in-situ* by cycling the potential between 0.15 and -0.55 V vs SCE. This couple acts as a mediator for NADH electrooxidation. Furthermore, in other previous paper the voltammetric behavior of 3,5-DNB has been studied at a glassy carbon electrode (GCE) [5].

All the above procedures to modify MWCNT are based on a covalent attachment of the nitro compound with the MWCNTs. Recently [6,7] we developed a somewhat different method that does not involve covalent bond formation with the nanostructure but only adsorption on MWCNT causing encapsulation of the nitro compound in the MWCNT nanostructured network. The present work describes the voltammetric behavior of 3,5-DNB in the conventional way, i.e. the electroactive species 3,5-DNB is dissolved in a solution and reduced on a GCE. Also we developed a somewhat different strategy that involves encapsulation of the nitro compound in the MWCNT nanostructured network and then reduced in the adsorbed state.

2.- Experimental

2.1. Apparatus and Reagents

Electrochemical measurements were carried out in a conventional three-electrode cell. Voltammetric curves were recorded on a CHI 900 (CH Instruments Inc., USA) instrument. We used a GCE measuring 3 mm in diameter (Model CHI104, CH Instruments) as the working electrode. A platinum wire (BASiMW-1032) and an Ag/AgCl/NaCl electrode (0.3 M) (BASi MF-2052) were used as the auxiliary and reference electrodes, respectively. All potentials are referred to the Ag/AgCl reference electrode.

MWCNTs (1.5 μm in length and 10 nm in diameter) were obtained from Dropsens S.L., Spain.

All voltammetric experiments were performed after bubbling N_2 into the test cell for 10 min before each run. The temperature was held constant at 25 ± 0.1 $^\circ\text{C}$.

We used a 0.1 M phosphate buffer solution (PBS), pH 7, as the aqueous medium.

3,5- dinitrobenzoic acid (3,5-DNB) and 3-nitrobenzoic acid (3-NB) (Figure 1) were obtained from SIGMA ALDRICH.

All other reagents were of analytical grade.

Stock solutions of 3,5-DNB and 3-NB were prepared at a constant concentration of 2×10^{-2} M in ethanol.

2.2. Preparation of MWCNT Suspensions and Modified GCEs

Before each modification, the GCE was cleaned by polishing with 0.3 μm and 0.05 μm alumina and then was washed thoroughly with water. The MWCNTs were dispersed in 1,3-dioxolane by sonicating for 5 min. The sonication procedure was repeated three times. The MWCNTs were immobilised by casting 5 μL of the

MWCNT dispersion on the GCE. Then the modified electrodes were obtained by drying the dispersion at room temperature.

2.3.- Encapsulation and Voltammetry of nitro compounds

The voltammetric determination was carried out on GCE modified with MWCNTs (MWCNTGCE) and nitrocompound encapsulated. The encapsulation process involved the dipping of the MWCNTGCE in an ethanol solution containing 2 mM of nitrocompound. The optimum dipping time was 4 second, and no potential was applied during the dipping step. After the dipping, the nitrocompound was encapsulated on the modified electrode. The electrode was washed with milliQ water yielding the modified electrode 3,5-DNB-MWCNTGCE or 3-NB-CNTGCE. The modified electrode was immersed in an electrochemistry cell with aqueous medium (PBS), pH 7, and then potential was scanned between 1.0 V and – 1.2 V vs. Ag/AgCl to register the voltammetric curve.

2.4.- Scanning Electron Microscopy (SEM)

For SEM measurements, glassy carbon discs (TED Pella brand, INC (N 16524)) measuring 12.7 mm in diameter were used. The discs were previously polished with suspensions of alumina particles measuring 0.05 and 0.1 mm in diameter. The morphology and dispersion of the 3,5-DNB-MWCNTGCE was investigated by SEM using an Inspect Scanning Electron Microscope F-50 operated at 20kV.

3.- Results and Discussion

The cyclic voltammetric behaviour of 3,5-DNB in 0.1 M PBS of pH 7 was examined at a GCE, as is displayed in figure 2A. In the first cathodic scan, starting at 0.0 V, we can observe two irreversible reduction peaks, I and II, with peak potentials of $E_p^I = -0.68$ V and $E_p^{II} = -0.97$ V. The peaks I and II corresponds to the reduction of each nitro group in the 3,5-DNB molecule, according to the well-known general equation:



The difference in the peak potentials of both nitro groups is as follows. The reduction of the first nitro group is facilitated by the electron-acceptor effect of the second nitro group, however, the second nitro group reduction is hindered by the electron donating effect of the NHOH group produced in the reduction of the first nitro group.

In the reverse scan, oxidation of the hydroxylamine derivative to nitroso derivatives produce a wide anodic peak III with a potential peak of $E_p^{III} = 0.40$ V. Furthermore, in the second cathodic scan a peak IV corresponding to the reduction of nitroso to hydroxylamine derivative appear at a potential peak $E_p^{IV} = -0.30$ V. Consequently peaks III and IV form a quasi-reversible couple according to the well-known equation:



In order to confirm the above couple RNHOH / RNO we run a different cyclic voltammetric experiment wherein the anodic scan was shortened at 0.0 V avoiding the oxidation of the hydroxylamine derivative, peak III, and consequently the peak IV disappeared (see figure 2B).

Furthermore in order to compare the assignment of voltammetric peaks for 3,5-DNB we also explore the cyclic voltammetric behavior of 3-NB which have only the nitro substitution in 3-position. According to the voltammogram displayed in figure 3A we can observe one irreversible reduction peak, I, with peak potential of $E_p^I = -0.74$ V corresponding to the reduction of the nitro group in the 3-NB molecule. In the reverse scan, oxidation of the hydroxylamine derivative to nitroso derivative produce a wide anodic peak III with a potential peak of $E_p^{III} = 0.17$ V. In the second cathodic scan a peak IV corresponding to the reduction of nitroso to hydroxylamine derivative appear at a potential peak $E_p^{IV} = -0.24$ V. Also we run a different cyclic voltammetric experiment wherein the anodic scan was shortened at 0.0 V avoiding the oxidation of the hydroxylamine derivative, peak III, and consequently the peak IV disappeared (see figure 3B).

According to the above results, peaks I and II corresponds to the reduction of each nitro group in the 3,5-DNB molecule. The above interpretation is totally different to the previously proposed by Gopal et al., [5]. In fact, in such paper the authors explained that the first peak I refers to the reduction of nitro groups into corresponding hydroxylamine and the second peak II refers to reduction of hydroxylamine into amino groups. There are a lot of evidences that contradict the Gopal's proposal. The most direct evidence is that if the Gopal's proposal is correct the voltammograms corresponding to 3-NB would have two reduction irreversible peaks and not just one as we found in this study. Another weak point of the Gopal's interpretation is that it is very unlikely that both nitro groups are reduced at

the same peak potential. In fact, in the reduction of dinitrobenzene derivatives the real question arises as to which of the two nitro groups is reduced first [8,9].

Recently [6,7] we developed a somewhat different strategy to study the voltammetric behavior of aromatic nitro compound that involve encapsulation of the nitro compound in the MWCNT nanostructured network. In this case the electroactive compound is fixed in the electrode phase instead of being dissolved in the solution phase as is the case of traditional voltammetry.

In the case of the voltammetry with 3,5-DNB encapsulated in the modified electrode the situation is rather different as is displayed in the cyclic voltammograms of figure 4A. In the first cathodic scan, starting at - 0.23 V, two sharp peaks (peaks I, II) with peak potentials of $E_p^I = -0.54$ V and $E_p^{II} = -0.66$ V can be observed. Both peaks correspond to the reduction of each nitro group present in the compound. The nitro groups are reduced to the hydroxylamine derivatives, which are subsequently oxidized to nitroso derivatives in the reverse anodic scan, producing peaks III and IV at peak potentials of $E_p^{III} = -0.09$ and $E_p^{IV} = -0.02$ V, respectively. In the second cathodic scan, two peaks (III' and IV') corresponding to the reduction of nitroso to hydroxylamine derivatives appear at - 0.14 and -0.06 V, respectively. Furthermore, if the cathodic sweep is restricted until -0.57 V, so that only one group is reduced, the obtained cyclic voltammogram is shown in Figure 4B. In the first cathodic scan, starting at 0.00 V, one sharp peak (peak I) with peak potential of $E_p^I = -0.54$ V can be observed. The nitro group is reduced to the hydroxylamine derivative, which is subsequently oxidized to nitroso derivative in the reverse anodic scan, producing peak III with peak potential $E_p^{III} =$

0.00 V. In the second cathodic scan, one peak (IV) corresponding to the reduction of nitroso to hydroxylamine derivative appear at - 0.07 V.

In the second cathodic scan, the nitro reduction peaks disappear entirely, showing that the trapped nitro compounds were reduced in the first scan. In the subsequent cycles, only the redox couples of the nitroso / hydroxylamine derivatives is detected.

As observed (Fig. 4A), well-resolved peaks of 3,5-DNB are obtained when the compound is reduced entrapped on the MWCNTs compared with the situation when 3,5-DNB in solution is reduced in the bare GCE (Fig. 2A). However, the main difference is the extraordinary increase in the peak current for all the peaks (four times higher compared with the bare electrode). Consequently, the use of our new proposed electrode based on trapped 3,5-DNB in the MWCNT array allows a considerable increase in sensitivity and selectivity of the voltammetric response.

When the above cyclic voltammetric experiment (figure 4) was continued by several scans the nitroso / hydroxylamine couple was maintained but the persistence or stability of the couple was strongly dependent of the number of the nitro group reduced in the 3,5-DNB molecule. When two nitro groups were reduced in the first scan the stability of the nitroso / hydroxylamine couples was displayed in figure 5A. On the other hand when only one nitro group in the 3,5-DNB molecule was reduced in the first scan the nitroso / hydroxylamine couple follow the behaviour displayed in figure 5B. From these experiments is clear that the nitroso / hydroxylamine couple originated from the 3,5-DNB molecule wherein only one nitro

group was reduced results to be more persistent after several measurements. In Figure 6A we can observe the time evolution after successive scans of both, the anodic peak current due to the oxidation of the hydroxylamine group to nitroso and the cathodic peak current due to the reduction of the nitroso group to hydroxylamine, from the nitroso / hydroxylamine couple. In figure 6B we compare the analogue behaviour obtained for a compound as 3-NB which contains only one reduced nitro group. The persistence of the nitroso / hydroxylamine couple obtained from the successive scans for the case of 3-NB results to be very low when compared with the case of 3,5-DNB wherein only one nitro group was reduced. The above results appear to show that the existence of an unaltered nitro group in the molecule allows better anchorage of the molecule in the network of nanotubes making more persistent the existence of the nitroso / hydroxylamine couple trapped on the electrode.

Consequently according to our results the most stable and persistent nitroso / hydroxylamine derivatives couple was electrogenerated *in-situ* by cycling the potential between -0.20 and 0.20 V starting from 3,5-DNB with only one nitro group reduced in the first scan. We are currently working on using this couple as a redox mediator as proposed by Kubota et al.,[4] for his system.

The results presented here have a certain qualitative similarity to those previously described by Kubota et al.[4] but there are strong differences mainly in the preparation of the nanostructured platform and the intensity of the obtained peak currents. In the case of the Kubota's paper the procedure involved dropping an aliquot of 5 μ L of a dispersion of MWCNT with PEI and then dried under nitrogen

atmosphere. After that, the electrode was dipped in ethanol containing N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 3,5-DNB for 1.5 h. After this procedure the 3,5-DNB was covalent attached to nanostructured platform based on MWCNTs. On the other hand, our procedure involved dropping an aliquot of 5 μ L of a dispersion of 3 mg/mL of MWCNT with 1,3-dioxolane and then dried at room temperature. After that, the electrode was dipped in a solution containing 2mM 3,5-DNBA, in 100% ethanol for 4 sec. and then the electrode was retired of the solution containing the nitro compound, washed and immersed in a solution containing only PBS, pH 7, obtaining a voltammogram according to figure 4A. Consequently our described procedure is much easier, less time consuming and without addition of other components different than 3,5-DNB.

The morphology of the MWCNT modified electrode without and with 3,5-DNB encapsulated were studied by SEM, as displayed in Figure 7A,B respectively. SEM images reveals that the network of nanotubes form a homogeneous, twisted, densely packed, three-dimensional array that remains attached to the GCE surface. Also it is possible to observe that the encapsulation or immobilization of the 3,5-DNB does not change significantly the morphology of the nanostructure.

In contrast to the nanostructure previously developed by Kubota et al., [4] in our case we do not have a covalent bond between 3,5-DNB and the nanostructured platform. Probably this fact causes the extraordinary large peak current values obtained in our system when compared with those obtained in the prior art. It is a well-known fact [10] that the formation of covalent bonds in the wall of the CNT

change the configuration of carbon atoms from sp^2 to sp^3 . This change affects the conductivity properties of the CNTs due to loss of π electrons of the nanostructure. Consequently one of the main advantages of the encapsulation method is that covalent bonds do not occur which change the conductive properties of the nanotubes.

4.- Conclusions

As opposed to previous findings by Gopal et al. [5] we explained the irreversible peaks I and II in the reduction of 3,5-DNB on GCE as the reduction of each nitro group in the 3,5-DNB molecule. On the other hand the previous authors assumed that the first peak I refers to the reduction of nitro groups into corresponding hydroxylamine and the second peak II refers to reduction of hydroxylamine into amino groups. We have included strong evidences supporting our interpretation.

We revealed a new strategy to study the voltammetric behaviour of 3,5-DNB encapsulated on MWCNTs modified electrode, most probably by being trapped within the pockets of the MWCNTs three-dimensional array, without formation of covalent bonding. In this case 3,5-DNB is fixed in the electrode phase instead of being dissolved in the solution phase as is the case of traditional voltammetry. The use of this new strategy based on trapped 3,5-DNB in the MWCNT array allows a considerable increase in sensitivity and selectivity of the voltammetric response.

Furthermore, a R-NO / R-NHOH couple was electrogenerated *in-situ* from 3,5-DNB at the modified electrode by cycling the potential in the range between -0.2 and 0.2 V at pH 7, indicating that the redox couple is immobilized in the electrode network.

This redox couple could be used as a mediator in the electrocatalysis of NADH, as has been previously shown by Kubota's group [4]. With this purpose, work is currently being conducted in our laboratory. In contrast to the nanostructure previously developed by Kubota et al., [4] in our case we do not have a covalent bond between 3,5-DNB and the nanostructured platform.

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FIGURE CAPTIONS

Figure 1.- Molecular structure of 3,5-DNB and 3-NB.

Figure 2.- Cyclic voltammograms obtained from a 2 mM solution of 3,5-DNB in 0.1 M PBS, pH 7 on a GCE. 100 mV/s. (A) Potential sweep window 1.0 to -1.25 V; initial potential 0.0 V. 100 mV/s. (B) Two scans at potential sweep window 0.0 to -1.25 V; initial potential 0.0 V. 100 mV/s.

Figure 3.- Cyclic voltammograms obtained from a 2 mM solution of 3-NB in 0.1 M PBS, pH 7 on a GCE. 100 mV/s. (A) Potential sweep window 0.4 to -0.90 V; initial potential -0.2 V. 100 mV/s. (B) Two scans at potential sweep window -0.20 to -0.90 V; initial potential -0.20 V. 100 mV/s.

Figure 4.- Cyclic voltammograms of 3,5-DNB entrapped on a MWCNTGCE modified electrode in 0.1 M PBS, pH 7. 100 mV/s. (A) Potential sweep window 0.20 to -0.80 V; initial potential -0.23 V. 100 mV/s. (B) Potential sweep window 0.20 to -0.57 V; initial potential 0.00 V. 100 mV/s.

Figure 5.- Time evolution of successive cyclic voltammograms of the redox couple R-NO / R-NHOH obtained from 3,5-DNB encapsulated obtained after: (A) two nitro groups were reduced in the first cathodic scan, and (B) only one nitro group was reduced in the first cathodic scan.

Figure 6.- Time evolution of peak currents from the redox couple R-NO / R-NHOH obtained after successive CV at 100 mV/s from: (A) 3,5-DNB encapsulated and after reduction of only one nitro group in the 1st cathodic scan, (B) 3-NB encapsulated.

Figure 7.- SEM images with 50,000 x magnification of the nanostructured platform of MWCNTs (A) without 3,5-DNB encapsulated and (B) with 3,5-DNB encapsulated.

Figure 1

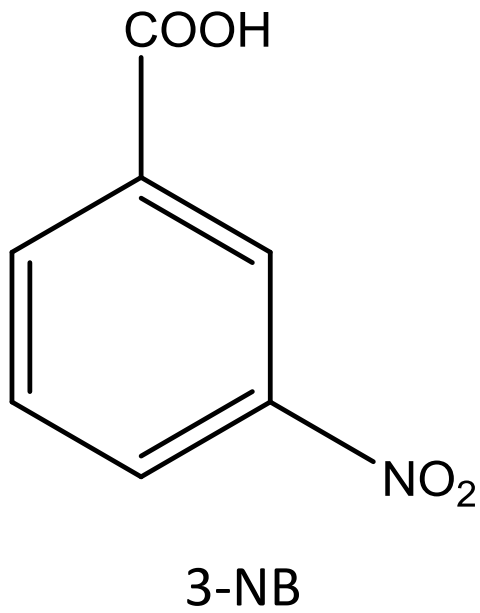
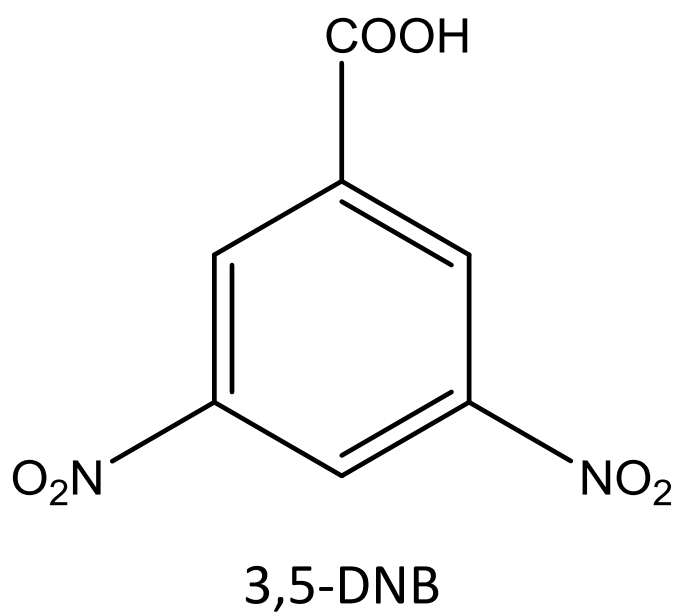


Figure 2

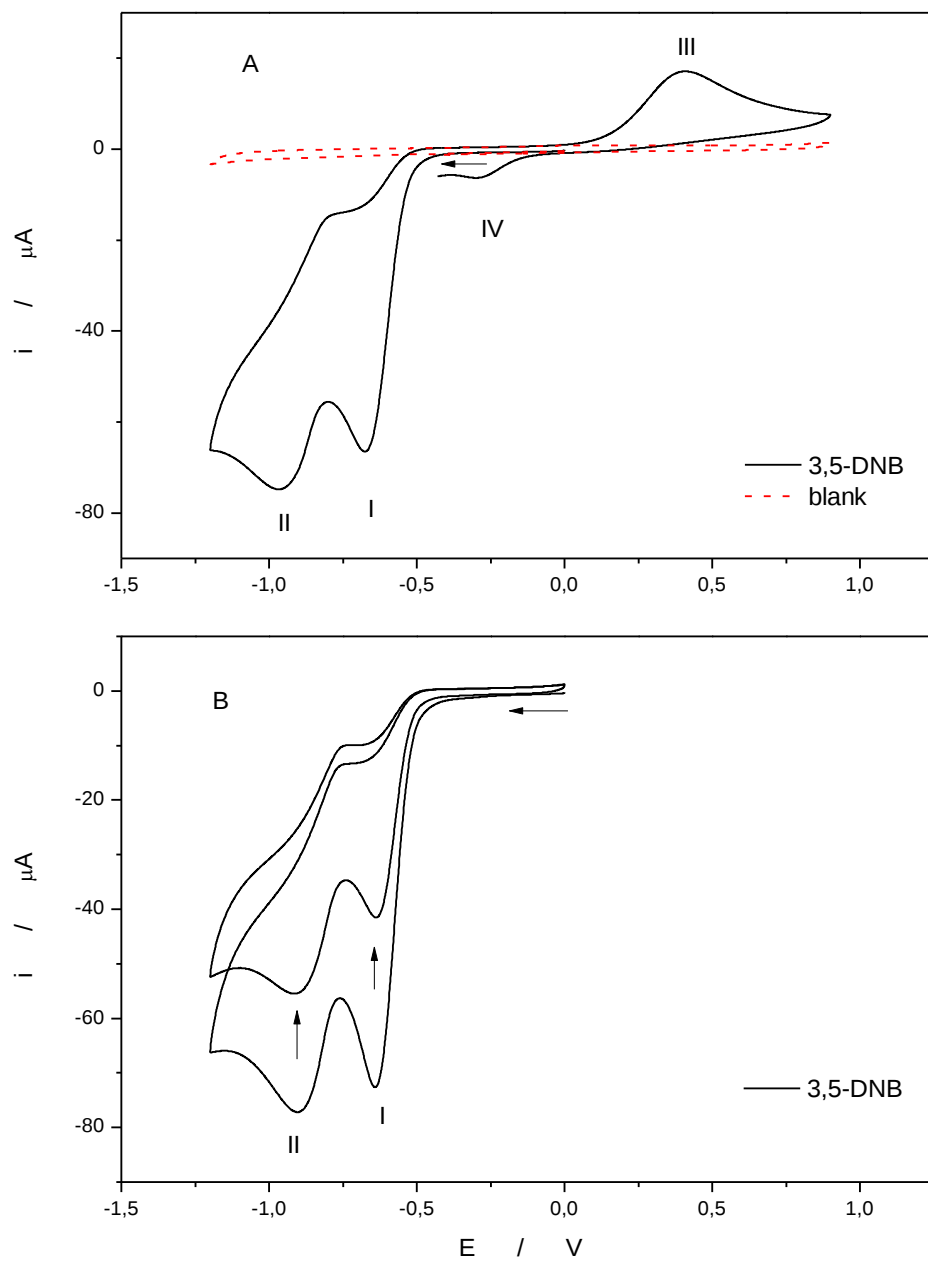


Figure 3.-

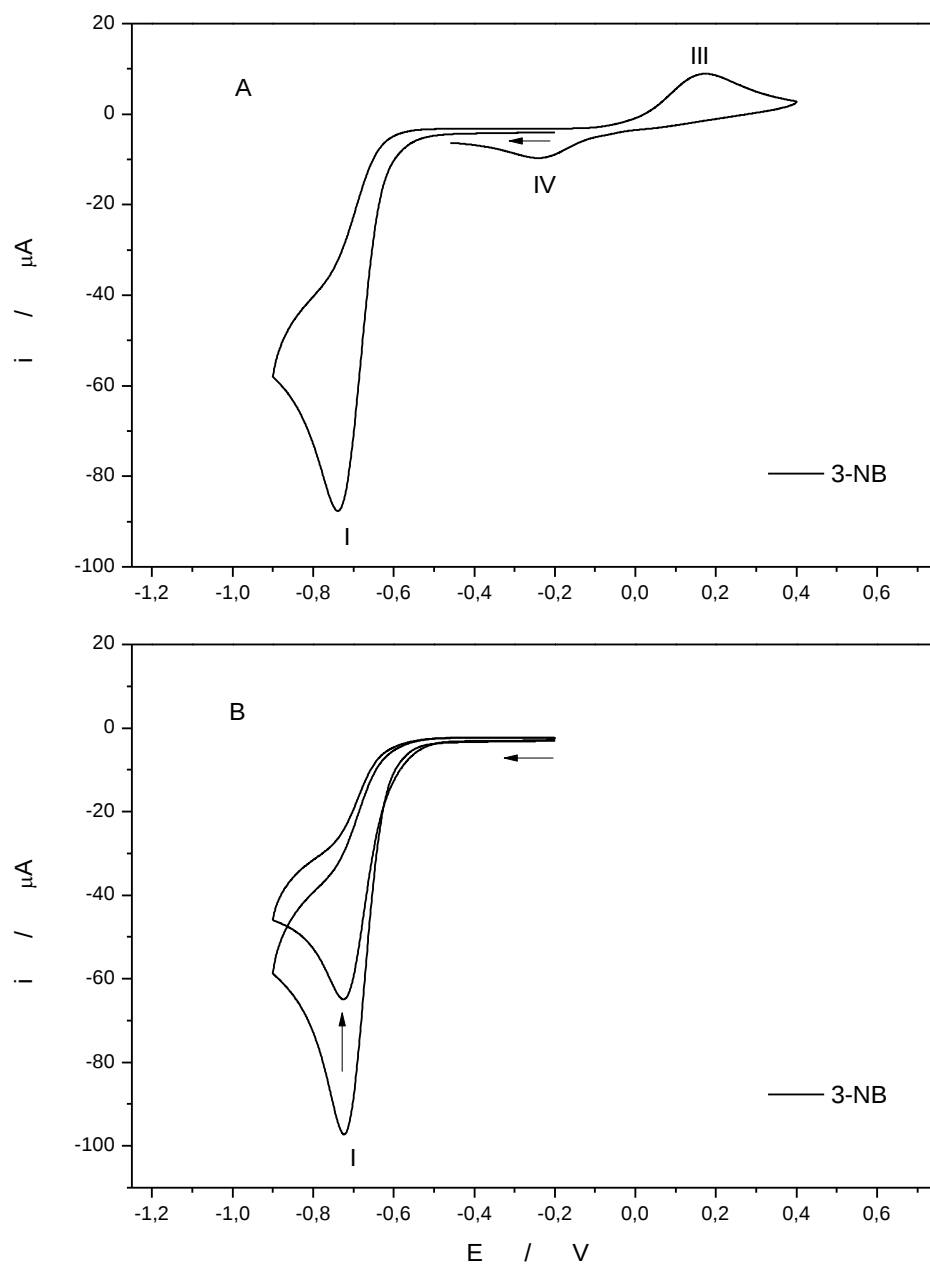


Figure 4.-

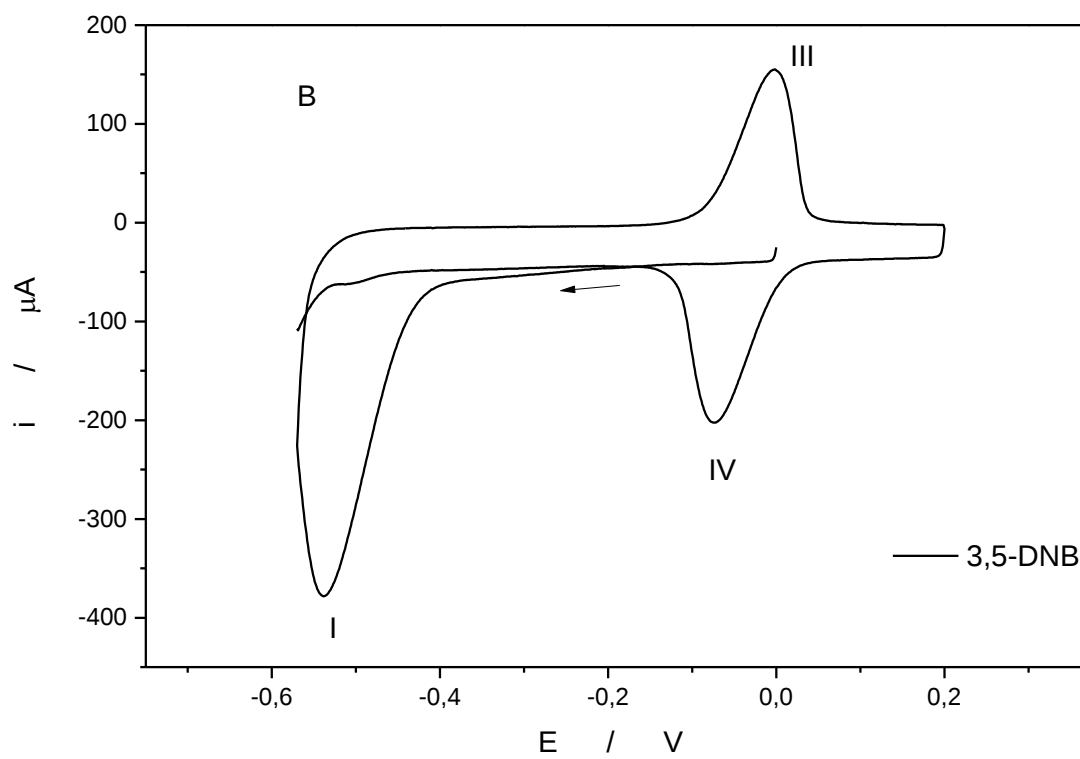
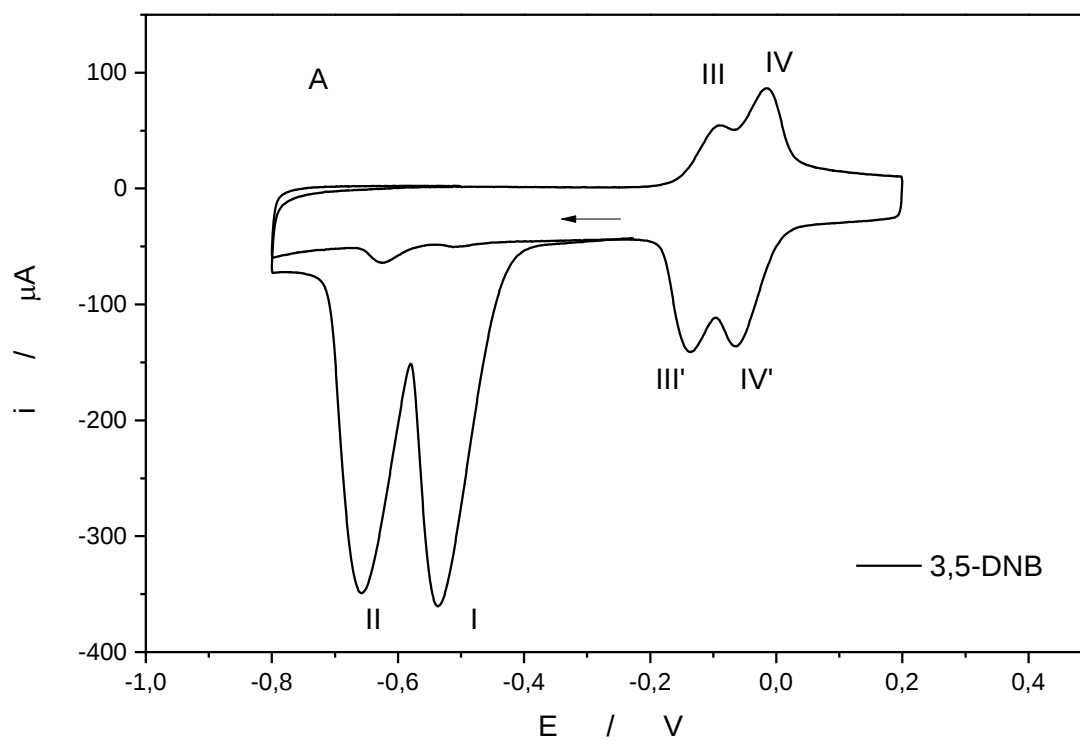


Figure 5.-

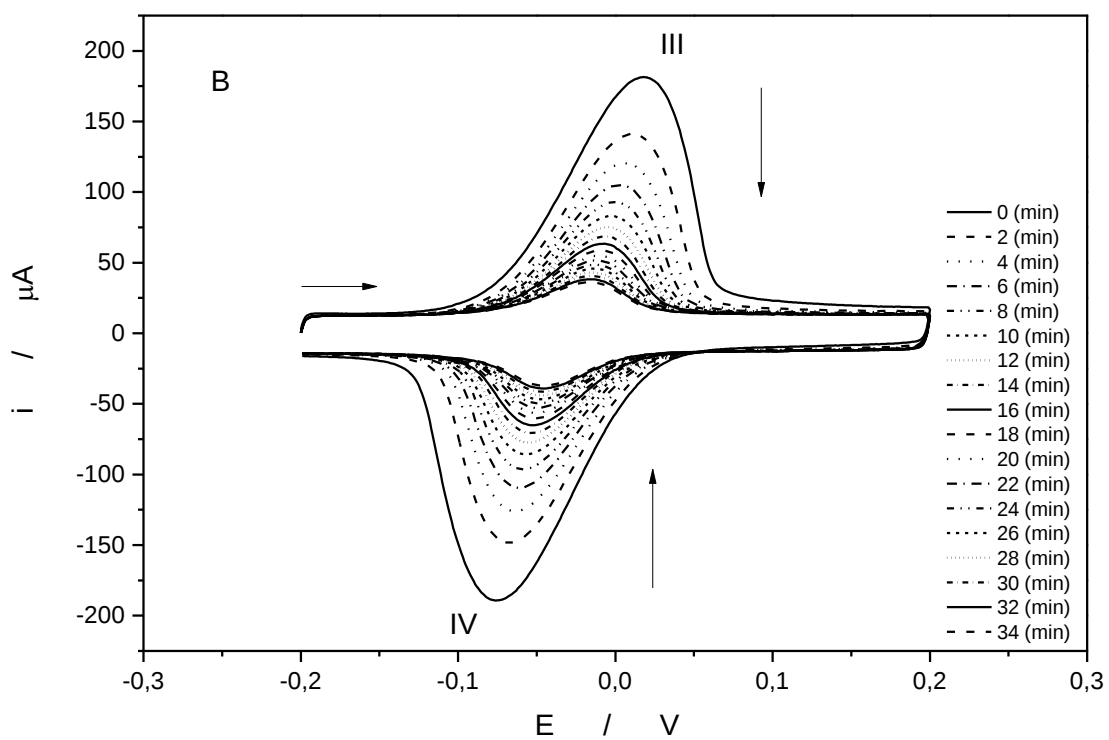
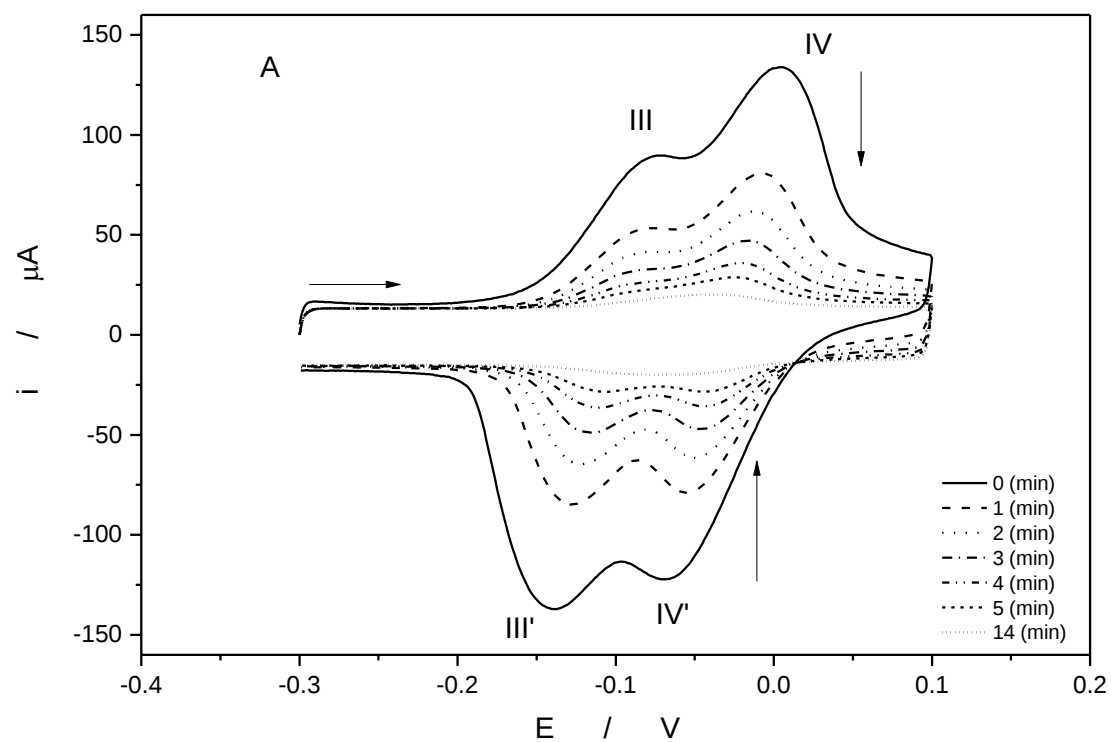


Figure 6.-

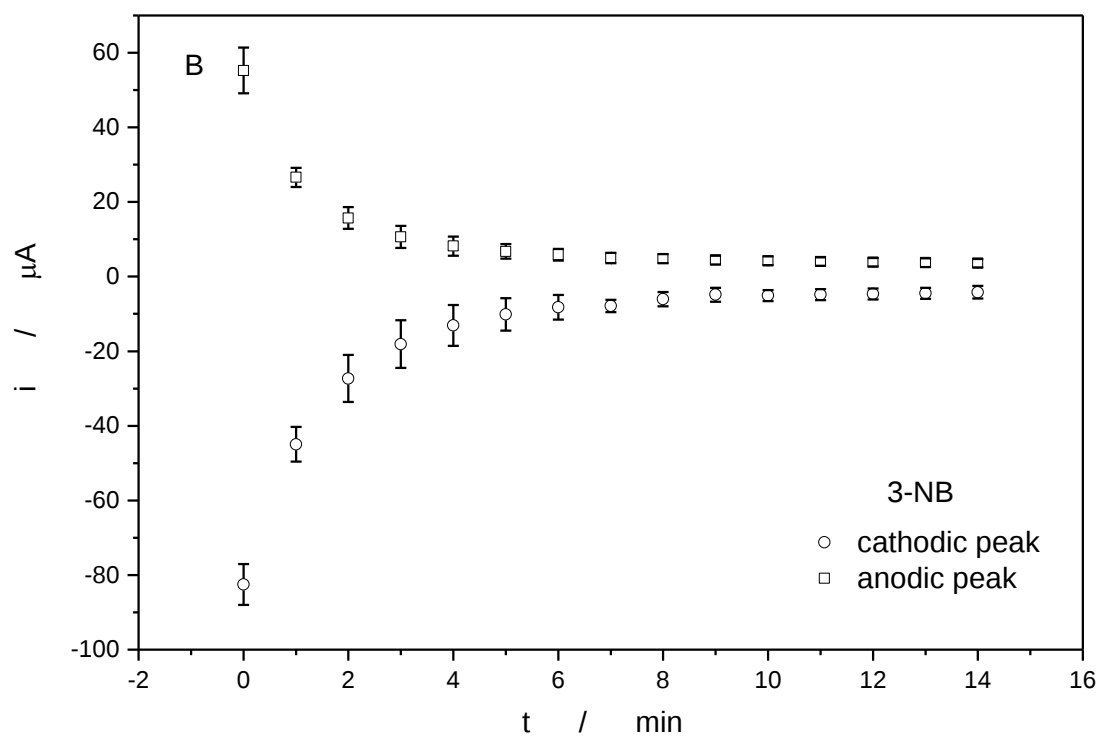
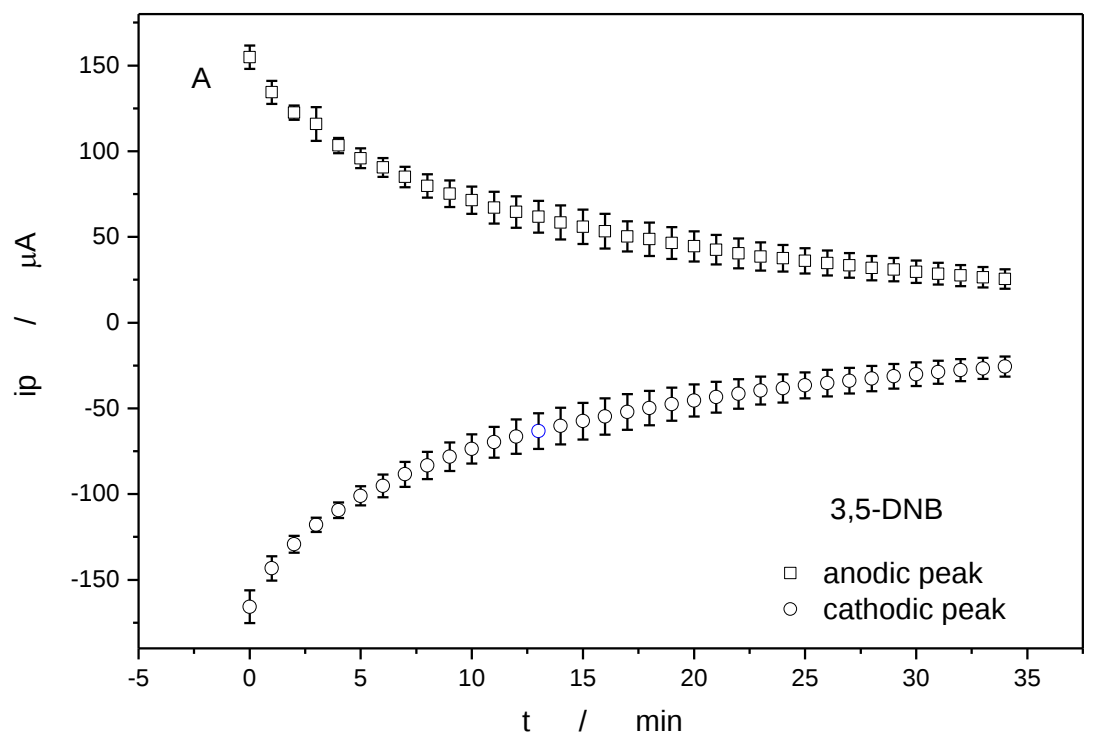


Figure 7.-

