MULTIWALLED CARBON NANOTUBES MODIFIED ELECTRODES WITH ENCAPSULATED 1,4-DIHYDROPYRIDINE-4-NITROBENZENE SUBSTITUTED COMPOUNDS.

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ABSTRACT

We report a voltammetric study of several nitroaromatic compounds such as the drugs: Nifedipine, Nitrendipine, Furnidipine and Nisoldipine in multiwalled carbon nanotubes (MWCNT) modified electrodes.

All the compounds are strongly encapsulated in the three dimensional structure of the MWCNT and then reduced to the corresponding hydroxylamine derivatives. In the case of the modified electrode with MWCNT the answer in current are remarkably increased (approximately 40 times). This current multiplier effect is due to the nitro compounds are not only superficially adsorbed but this really encapsulated in the nanotube network covering the GCE.

The nitro reduction peak was linearly dependent with the concentration of the nitroaromatic compound with detection limit (LOD) of $1.2 \cdot 10^{-8}$ mol·L^{-1} and a quantification limits (LOQ) of $3.9 \cdot 10^{-8}$ mol·L^{-1} for the case of Nitrendipine. All the studied nitrocompounds followed equivalents behaviours. Obviously from this result it is possible to postulate this technology as a very useful tool to develop analytical methods to determine these nitroaromatic drugs in real samples such as biological fluids.

From the obtained results it is possible to conclude that for the studied 1,4-dihydropyridine-4-nitrobenzene substituted compounds a greater difficulty of reduction implies a greater ability to encapsulation.

INTRODUCTION

The remarkable electronic and structural characteristics [1-7] of carbon nanotubes (CNTs) have attracted the attention of many electrochemical researchers making them a material of choice as electrode phase. In fact many research efforts have been focused into elucidate its properties and to develop applications based on them.

One of the most interesting characteristics of the CNT is its feasibility to be modified and consequently the possibility to design electrodes according to the needs of the researcher. In general the strategies for modifying CNTs [7] can be resumed into three main areas: a) Chemisorption, with the covalent bonding of the modifier to the CNTs either through chemical or electrochemical activation; b) Physisorption, with the physical adsorption of the modifier to the CNT; and c) Miscellaneous methods. For applications with electrocatalytic purposes modifications are useful for anchoring active objects and/or creating catalytic centres [8, 9]. For bioelectrochemical applications the modification of CNT imply functionalize them in order to interact immobilizing bio-guests [10,11]. The most common organic functionalities introduced on CNTs are carboxyl (–COOH) [12,3], carbonyl (-CO) [3], fluorine (–F) [13], amino groups (–NH_2) [14–16], hydroxyl (–OH) [12] and nitro (–NO_2) [17], among others.

Modification of electrodes with nitro groups has been described on conventional electrodes, including Au and glassy carbon electrodes (GCEs)[18–20]. Ortiz et al. [18] described the electrochemistry of 4- nitrophenyl modified GCEs in aqueous media using aromatic diazonium salts; in addition, Gui et al. [19] recently published a comparative study of the electrochemical reduction of 4-nitrophenyl covalently grafted on gold and GCE surfaces by electrochemical reductive adsorption of the corresponding diazonium salt. Mano et al. [20] used nitrofluorenone derivatives to modify the surface of GCEs. Different authors also have treated the modification of CNTs with nitro groups or nitrocompounds. Wang et al., [17] have efficiently introduced nitro groups on the surface of multiwalled carbon nanotubes (MWCNTs) by conventional nitration procedures, and they can be subsequently reduced readily to amino groups. Compton et al. [21] 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 [22] 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 mmol·L^{-1} 4-NBA. The resulting 4-NBA-MWCNT powder was abrasively immobilized onto the surface of a clean BPPG electrode. The results of the cyclic voltammetric characterization for both 4-NBA-MWCNT- and NB-MWCNT-modified electrodes were qualitatively identical, producing a quasi-reversible couple after the first sweep, attributed to the two-electron, two-proton oxidation/reduction of the aryl- hydroxylamine/aryl-nitroso moieties.

Furthermore, we report [23] that GCE modified with MWCNTs can be derivatized by the nitroaromatic drug Nitrendipine (NTD) ((RS)-ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4- dihydropyridine-3,5-dicarboxylate) simply by dipping the electrode in a solution of 0.1 mol·L^{-1} Britton– Robinson buffer containing 0.1 mmol·L^{-1} NTD (pH 2) for 4min at open circuit. The derivatized electrode is thus reduced, producing the corresponding hydroxylamine derivative-modified electrode, which can be further oxidized to the nitroso derivative. A stable nitroso/hydroxylamine derivative couple appears if the modified electrode is conveniently cycled. With proper selection of an electrode potential, the derivatized electrode can be modified as nitroso or hydroxylamine derivatives.

In this paper we include the extension to other compounds in order to study the effects of some structural changes in the encapsulation of these nitroaromatic compounds. Specifically we include some compounds of the same family that follows the general structure described in Figure 1. We have selected compounds such as Nifedipine, Nisoldipine, and Furnidipine in order to compare the results obtained with Nitrendipine. The selected compounds combine changes in the position of the nitro group (*orto* or *meta*) and different substituents. In fact the comparison would reveal the importance of the structure and their incidence in the encapsulation step.

EXPERIMENTAL

Apparatus and reagents

Multiwalled carbon nanotubes (MWCNTs) were obtained from DropSens S.L. Spain.

We used a 0.1 mol·L^{-1} Britton–Robinson buffer, pH 2 (6.74 mL boric acid, 6.183 g phosphoric acid and 5.72 mL of 0.1 mol·L^{-1} acetic acid and filled to 1 L with H_2O), as the aqueous medium. All aqueous solutions were prepared with deionized water purified with a Milli-Q Ultrapure water system.

The 1,4-dihidropyridine-4-nitrobenzene substituted nitrocompounds (Fig. 1): Nitrendipine (NTDP), Nifedipine (NFDP) and Nisoldipine (NSDP) were obtained from Sigma-Aldrich® and Furnidipine (FNDP) was obtained from Laboratorio Chile S.A. All of the other reagents were of analytical grade.

Stock solutions of nitrocompounds were prepared at a constant concentration of $1 \cdot 10^{-3}$ mol·L^{-1} in ethanol. The working solutions were buffer Britton–Robinson buffer, pH 2 without nitrocompound.

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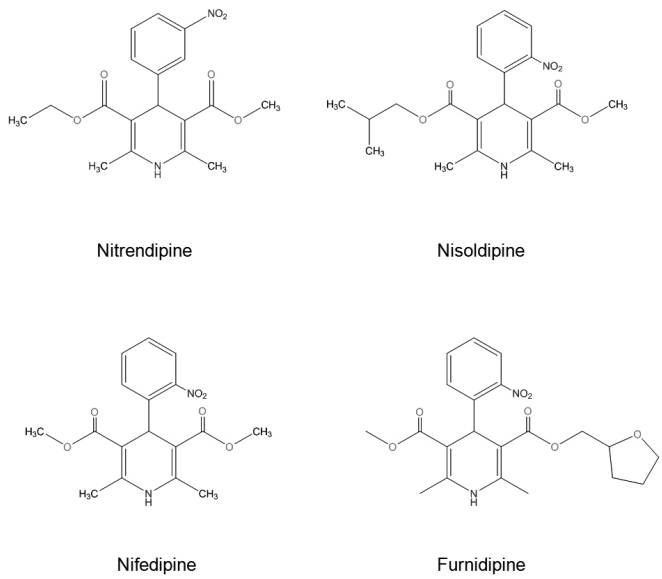

Figure 1.- Molecular structures of some 1,4-dihydropyridine-4-nitrobenzene substituted compounds.

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 glassy carbon electrode (GCE) with a 3-mm diameter (Model CHI104, CH Instruments) as the working electrode. A platinum wire (BASiMW-1032) and an Ag/AgCl/NaCl electrode (0.3 mol·L^{-1}) (BASi MF-2052) were used as the auxiliary and reference electrodes. All potentials are referred to relative to the Ag/AgCl reference electrode.

All voltammetric experiments were obtained after bubbling with N_2 for 10 min in the cell before each run. The temperature was held constant at 25±0.1 °C.

Procedure to modify GCE with MWCNT.

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 at 3 mg/mL with water by sonication for 5 min. The sonication procedure was repeated three times. Casting the GCE with 5 μL of the MWCNTs dispersion performed immobilization of MWCNTs. The optimum conditions were obtained by drying the dispersion dropped onto the GCE in an oven.

The general procedure to modified the GCE is showed in the scheme of Figure 2.

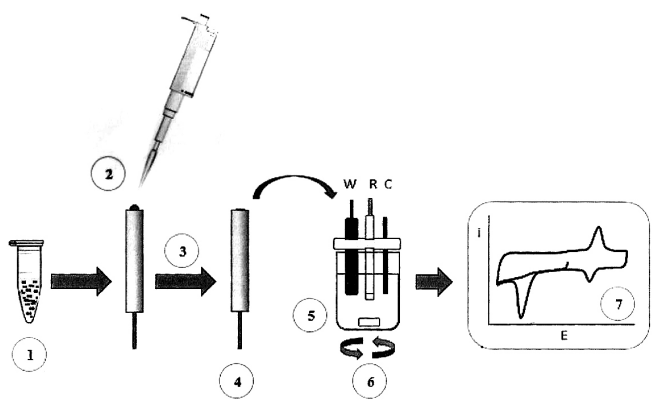

Figure 2.- General procedure for the preparation of modified electrodes. 1. Dispersion of MWCNT. 2. Casting of 5 μL MWCNT dispersion. 3. Drying in oven. 4. Modified electrode. 5. Cell with nitrocompound. 6. Stir. 7. Cyclic voltammetry.

Nitrocompund encapsulation: The modified MWCNTGCE was dipping in a solution of 0.1 mmol·L^{-1} of nitrocompound in ethanol. No potential was applied during this step, yielding the modified electrode Nitrocompound-MWCNTGCE.

Voltammetric transduction: After the dipping process, the nitrocompound-MWCNTGCE was submitted to cyclic voltammetry (CV). The modified electrode was introduced in a cell containing only a buffer solution; next, the CV experiment was run using the following potential parameters: Ei=0 V; E_L=−0.7 V and E_H=+0.6 V and starting with a negative sweep potential.

RESULT AND DISCUSSION

We prepared a modified MWCNTGC electrode by casting a dispersion of MWCNTs on a GCE according to the above-described procedure. Next, the MWCNTGCE was dipped in a voltammetric cell containing a solution of 0.1 mol·L^{-1} Britton–Robinson buffer, 0.1 mmol·L^{-1} nitrocompound, at pH 2 for 4 min at open circuit. Next, we carried out a cyclic voltammetric experiment according to the above-described procedure. Figure 3 shows cyclic voltammograms for NTDP in a naked GCE and Figure 4 shows cyclic voltammograms after dipping the MWCNTGCE electrode in the NTDP solution. Consequently the voltammogram after the dipping corresponds to the encapsulated NTDP on the MWCNTGCE. In the first scan (solid line), a large sharp peak (Ep = -0.48 V), which is due to the irreversible 4-electron 4-proton reduction (Eq. (1)) of the nitro group of the NTDP molecule to the corresponding hydroxylamine derivative, was observed. When the scan direction was reversed, we observed only one oxidation peak at 0.32 V, with its corresponding reduction peak at 0.2 V (dotted line). This redox couple is due to the quasi-reversible 2-electron, 2-proton oxidation/reduction couple (Eq. (2)) of the hydroxylamine/nitroso derivatives. In the second scan, the peak due to the reduction of the nitro group to the hydroxylamine derivative completely disappeared in spite of the homogeneous medium contained the nitro compound. On the other hand the quasi-reversible was maintained in successive scans.

$$R\text{-}NO_2 + 4e^- + 4H^+ \rightarrow R\text{-}NHOH \quad (1)$$

$$R\text{-}NHOH \leftrightarrow R\text{-}NO + 2e^- + 2H^+ \quad (2)$$

All the studied nitrocompounds follow a qualitatively similar behaviour according to the above equations (1) and (2). From the comparison of the voltammograms carried out on GCE and MWCNTGCE it is clear that in the case of the modified electrode with the MWCNT the answer in current (peak current, ip) are remarkably increased (approximately 40 times). This current multiplier effect is due to the nitro compounds are not only superficially adsorbed but this really encapsulated in the nanotube network covering the GCE.

Due to the increased answer in peak current for the nitro compounds is obvious to think this procedure as an analytical tool in order to quantitatively determine these types of compounds. In this scope we found a linear relation between the peak current due to the reduction of the nitro group with the concentration of the nitrocompound.

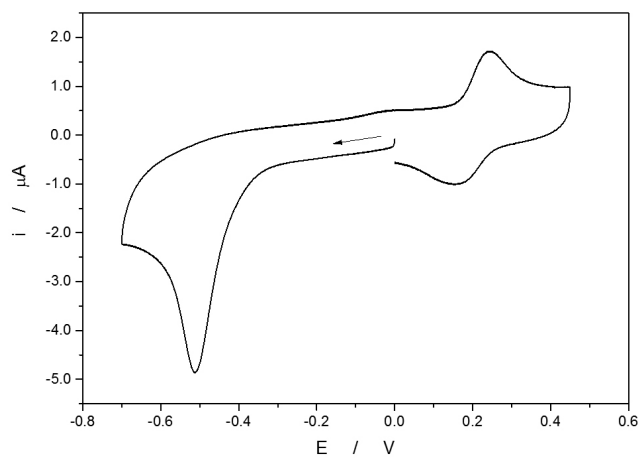

Figure 3.- Cyclic voltammogram of 0.1 mmol·L^{-1} Nitrendipine in buffer Britton-Robinson, pH 2 on GCE. E_i: 0 (V), E_L: -0.7 (V), E_H: 0.6 (V). v = 100 mV/s.

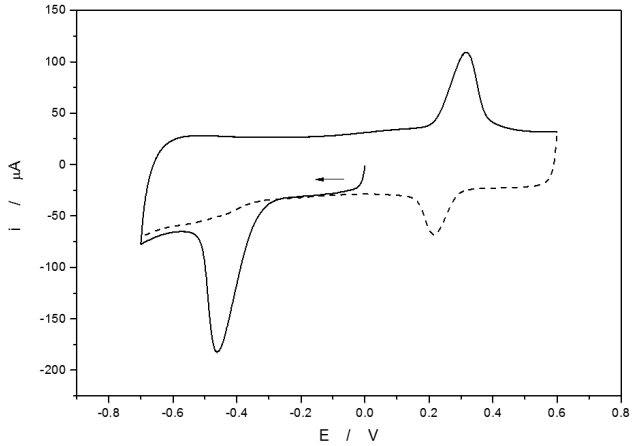

Figure 4.- Cyclic voltammograms of 0.1 mmol·L^{-1} Nitrendipine in buffer Britton-Robinson, pH 2 on GCE modified with MWCNT. E_i: 0 (V), E_L: -0.7 (V), E_H: 0.6 (V). v = 100 mV/s.

In figure 5 we show the calibration curve (Ip = 26.7 C + 9.2·10^{-7}; R = 0.9993) obtained for the case of Nitrendipine. From this curve we obtained a variation coefficient of 3.81 %, a detection limit (LOD) of 1.2 ·10^{-8} mol·L^{-1} and a quantification limits (LOQ) of 3.9·10^{-8} mol·L^{-1}. All the studied nitrocompounds followed equivalents behaviours. Obviously from this result it is possible to postulate this technology as a very useful tool to develop analytical methods to determine these nitrocompounds drugs in real samples such as biological fluids.

As the main difference between the reduction of these nitrocompounds in GCE or in modified GCE with carbon nanotubes was the current response, we hypothesize that the key was in the active sites of both electrodes. Being the electrode covered with carbon nanotubes possessor of more active sites for the reduction of these nitrocompounds. With the aim of verify this hypothesis we determined the active surface of both electrodes for these nitrocompounds. The active surface of the electrodes was determined by voltammetry. The area of the reduction signal of the nitro group corresponding to Nitrendipine (NTDP), Nisoldipine (NSDP), Furnidipine (FNDP) and Nifedipine (NFDP) was measured. All measurements were done in triplicate and to ensure good saturation of the electrode the accumulation time was 4 minutes. The concentration of the nitrocompounds in the work cell was 0.1 mmol·L^{-1}, pH = 2, Britton buffer - Robinson 100%.

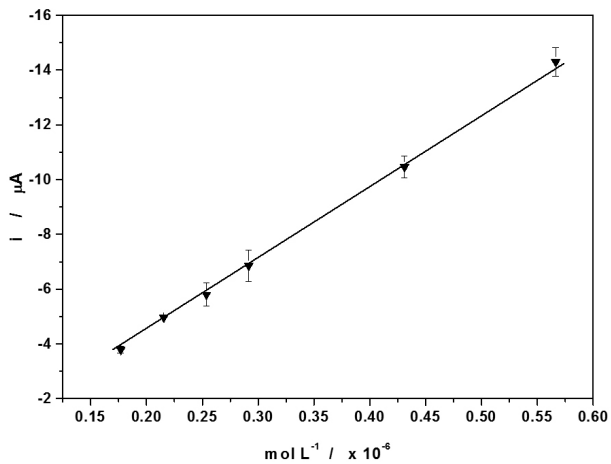

Figure 5.- Calibration curve of NTD in buffer Britton-Robinson, pH 2. RSD = 3.81 %; LOD = 1.2 ·10^{-8} mol·L^{-1}. LOQ = 3.9·10^{-8} mol·L^{-1}.

In Figure 6 we show the voltammograms corresponding to the reduction of the nitro group to the four studied nitrocompounds on the MWCNTGC electrode. From this curves we obtain the following potential peak values (Ep) : -570 mV, -540 mV, -510 mV and -500 mV for FNDP, NSDP, NFDP and NTDP, respectively. Consequently the most easily reducible compound was NTDP which is the only one of the nitrocompound having nitro substituent in the *meta* position to the DHP ring. In the case of the *orto* substituted the reduction of the nitro group is hindered by steric effect due to the DHP ring in *orto* position.

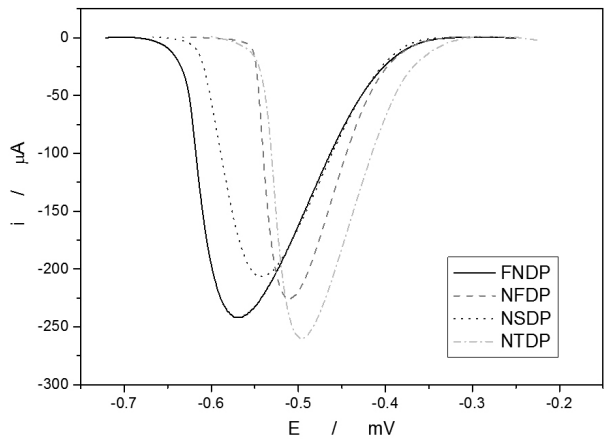

Figure 6.- Linear voltammograms showing the peaks due to nitro reduction for different 1,4-dihydropyridine-4-nitrobenzene substituted compounds.

The surface covering on an electrode (Γ) is the amount of compound (in mol) per unit area of the electrode (cm^2) area. The covering of an electroactive species (Γ) can be calculated from the area of the electrochemical signal from the integration of the charge Q according to the following equation:

$$\Gamma = \frac{Q}{nFA} \qquad (3)$$

where:

Q = charge corresponding to the integration of the area under the reduction signal; n = number of electrons exchanged in the reduction reaction; F = Faraday constant; A = Geometric area of the GCE and MWCNTGCE (0.07 cm^2).

The "Microcal Origin" software was used to plot the baseline, eliminating the capacitive current and integrating the area under the reduction signal. From equation 3, the surface covering of the nitrocompounds on the glassy carbon electrode was obtained. As the covering per unit area is equal to the electrode modified with carbon nanotubes, it can use the same expression to calculate the active area on the modified electrodes.

$$A = \frac{Q}{n\,F\,\Gamma} \qquad (4)$$

The results obtained when calculating the active area of the electrode and encapsulated compounds per unit area (mol/cm^2) are showed in Table 1. From the density of encapsulated compound (mol/cm^2) and the geometric area of the GCE we obtained the moles of encapsulated compound. Also the results obtained using electrodes with and without carbon nanotubes are compared. The order of encapsulation for the different nitrocompounds was FNDP > NSDP > NFDP > NTDP.

Table 1. Encapsulated compounds per unit area (mol/cm^2) in both GCE and MWCNTGCE and active area of the MWCNTGCE.

	FNDP (mol/cm^2)	**NSDP (mol/cm^2)**	**NFDP (mol/cm^2)**	**NTDP (mol/cm^2)**
GCE	1.20·10^{-11} ± 4.78·10^{-12}	6.57·10^{-12} ± 1.97·10^{-12}	4.87·10^{-12} ± 3.17·10^{-13}	4.80·10^{-12} ± 1.74·10^{-13}
MWCNT-GCE	1.37·10^{-9} ± 1.19·10^{-10}	1.06·10^{-9} ± 1.02·10^{-10}	7.86·10-10 ± 7.83·10-11	5.92·10^{-10} ± 4.25·10^{-11}
	FNDP (cm^2)	**NSDP (cm^2)**	**NFDP (cm^2)**	**NTDP (cm^2)**
Active area MWCNT-GCE	10.8 ± 1.13·10^{-5}	11.3 ± 4.82·10^{-6}	11.3 ± 1.16·10^{-5}	8.50 ± 3.12·10^{-5}

The data in the Table 1 show the amount of the compound that contains

each electrode in terms of area. Electrodes modified with MWNTC can encapsulate more moles of compound per cm^2, compared with the non-modified electrodes due to that modified electrodes considered a three dimensional structure and the calculations are considered to be the geometric area of the electrode, which in both cases is the same.

According to the data of Table 1 it can be concluded that the modified electrodes may contain up to two orders of magnitude more moles of nitro compound than the unmodified electrodes. Due to the fact that the structures of the dihidropyridine (DHP) moiety are similar is expected a similar packaging for all the electrodes, i.e. that have a similar active area. The data of the table shows that the 2-nitrophenyl DHP has an active area close to 11 cm^2, only NTDP presents a lower area, this being a 3-nitrophenyl-DHP.

The above results show that the structure of the studied different nitrocompounds affects both encapsulation and reduction process. Consequently, we have found a surprisingly linear relationship between the peak potential (Ep) for the reduction of the nitro group and the encapsulation of the nitroaromatic compound expressed as the quantity of encapsulated moles (Figure 7). From this figure it is possible to conclude that a greater difficulty of reduction implies a greater ability to encapsulation.

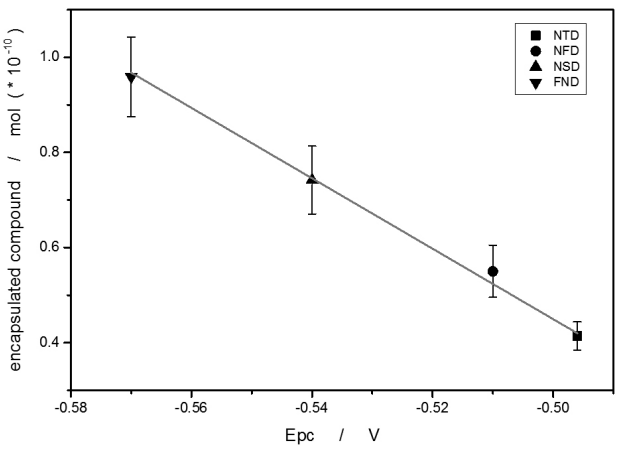

Figure 7.- Linear behaviour between peak potential (Ep) for the reduction of the nitro group and moles of the encapsulated 1,4-dihydropyridine-4-nitrobenzene substituted compounds.

ACKNOWLEDGEMENTS

This work was supported by Grant Nº 1130160 from FONDECYT.

REFERENCES

1.- S.Iijima and S. Ichihashi. *Nature* 363, **1993**, 603.
2.- R.H. Baughman, A.A. Zakhidov and W.A. de Heer. *Science* 297, **2002**, 787.
3.- D. Tasis, N. Tagnatarchis, A. Bianco and M. Prato. Chemical Reviews 106, **2006,** 1105.
4.- J.M. Nugent, K.S.V. Santhanam, A.Rubio and P.M. Ajayan. *Nano.Lett*.**,** 1, **2001,** 87.
5.- J. Wang, M. Musameh and Y. Lin. *J. Am. Chem. Soc.* 125, **2003,** 2408.
6.- H. Luo, Z. Shi, N. Li, Z. Gu and Q. Zhuang. *Anal. Chem.* 73, **2001**, 915.
7.- G. Wildgoose, C. Banks, H. Leventis, R. Compton. *Microchim. Acta* 152, **2006,** 187.
8.- J.L. Figueiredo, M.F. Pereira. *Catal.Today* 150, **2010**, 2.
9.- G. Wildgoose, C. Banks, R. Compton. *Small* 2, **2006**, 182.
10.- W.Huang, S.Taylor, K.Fu, Y.Lin, D.Zhang, T.W.Hanks, A.M.Rao, Y.P.Sun. *Nano Lett.* 2, **2002**, 311.
11.- W.Yang, P.Thordarson, J.Gooding, S.Ringer, F.Braet. *Nanotechnology* 18, **2007**, 412.
12.- S. Banerjee, T. Hemraj-Benny, S.S. Wong. *Adv. Mater*. 17, **2005**, 17.
13.- E. Mickelson, C. Huffman, A. Rinzler, R. Smalley, R. Hauge, J. Margrave. *Chem.Phys. Lett.* 296, **1998**, 188.
14.- A. Gromov, S. Dittmer, J. Svensson, O.A. Nerushev, S.A. Perez-García, L. Licea-Jiménez, R. Rychwalski, E.E.B. Campbell. *J. Mater. Chem.* 15, **2005**, 3334.
15.- L. Shao, Y. Bai, X. Huang, Z. Gao, L. Meng, Y. Huang. *J. Ma. Mater. Chem. Phys*.116, **2009**, 323.
16.- C. Chen, B. Liang, D. Lu, A. Ogino, X. Wang, M. Nagatsu. *Carbon* 48, **2010**, 939.
17.- L. Wang, S. Feng, J. Zhao, J. Zheng, Z. Wang, L. Li, Z. Zhu. *App. Surf. Sci.* 256, **2010**, 6060.
18.- B. Ortiz, C. Saby, G. Champagne, D. Bélanger, *J. Electroanal. Chem.* 455, **1998**, 75.
19.- A. Gui, G. Liu, M. Chockalingam, G. Le Saux, E. Luais, J. Harper, J. Gooding, *Electroanalysis* 22, **2010**, 1824.
20.- A. Mano, A. Kuhn, *J. Electroanal. Chem.* 477, **1999**, 79.
21.- C. Heald, G. Wildgoose, L. Jiang, T. Jones, R.G. Compton, *Chemphyschem* 5, **2004**, 1794.
22.- G. Wildgoose, S. Wilkins, G. Williams, R. France, D. Carnahan, L. Jiang, T. Jones, R.G. Compton, *Chemphyschem* 6, **2005**, 352.
23.- R. Moscoso, J. Carbajo, M. López, L.J. Núñez-Vergara, J.A. Squella, *Electrochemistry Communications* 13, **2011**, 217.