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Derivatization of SWCNTs with Cobalt Phthalocyanine Residues and Applications in Screen Printed Electrodes for Electrochemical Detection of Thiocholine

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Abstract

Single-walled carbon nanotubes (SWCNTs) derivatized with cobalt phthalocyanine (CoPh) were applied onto screen-printed graphite electrodes (SPEs) to be used for the low-potential electrochemical oxidation of thiocholine (TCh). Covalent attachment of CoPh to SWCNTs *via* stable sulfonamide bonds was confirmed by Raman/FT-IR spectroscopy and thermogravimetric analysis (TGA) coupled with FT-IR detection. The resulting modified SPE surfaces (CoPh-SWCNT-SPEs) were characterized by cyclic voltammetry and Electrochemical Impedance Spectroscopy (EIS) with the redox probe $[F_3(CN)_6]^{3-/4-}$. Detection of TCh was accomplished using cyclic voltammetry and amperometry; a lower overpotential (100 mV vs. Ag/AgCl pseudoreference electrode) was obtained using CoPh-SWCNT-SPEs as compared to unmodified SPEs and SPEs modified with non-functionalized SWCNTs (SWCNT-SPEs). The linear range for TCh detection was 0.077 - 0.45 mM, with a sensitivity of 5.11 10^{-1} µA mM^{-1} and a limit of detection of 0.038 mM according to the 3s/m definition.

Keywords: SWCNT, cobalt phthalocyanine, thiocholine, SPE

1. Introduction

Thiocholine (TCh) is an electroactive product obtained from thiocholine ester hydrolysis catalyzed by acetylcholinesterase (AChE). TCh oxidation is widely used to evaluate the activity of AChE and to develop biosensors for organophosphate and carbamate pesticide detection based on AChE inhibition [1]. When pesticides are present in solution, the enzyme activity is inhibited, resulting in a decrease in TCh production. From this, the electrochemical detection of TCh can be correlated to pesticide concentration. Significant improvements in TCh detection have been achieved through the use of electrode mediators to enhance electron transfer and minimize the overpotential required for TCh oxidation, ca. +700 mV vs. Ag/AgCl. For example, electrodes with mediators such as tetracyanoquinodimethane (TCNQ) [2], Prussian Blue [3], cobalt phthalocyanine (CoPh) [4], cobalt hexacyanoferrate[5] and poly(3,4-ethylenedioxythiophene)[6] have been already reported.

In 2004, it was shown that multi-walled carbon nanotubes (MWCNTs) can also be applied to electrodes to decrease the overpotentials involved in the enzymatic detection of pesticides and nerve agents [7]. The reported bi-enzymatic system detected the presence of H_2O_2 using cholinesterases and choline oxidase, at 500 mV. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was used to bridge the CNT carboxyl groups to enzyme amine residues *via* amide linkage. Similarly, the anodic detection of enzymatically - generated *p*-nitrophenol was shown to be enhanced by using electrodes modified with multiwalled carbon nanotubes (MWCNTs) [8].

The electrocatalytic activity of MWCNTs has also been demonstrated in mediator-less TCh detection but in most cases, MWCNT were used to modify glassy carbon electrodes (GCEs) or carbon paste electrodes (CPE); Very recently Rotariu et al. [9] combined MWCNT and ionic liquids at CPEs. Previously, Liu et al. used MWCNT dispersions in DMF to facilitate the casting of GCEs [10, 11]. Du et al. [12] and Kandimalla et al. [13] prepared mixtures of chitosan and glutaraldehyde to disperse and cast MWCNTs onto GCEs; the latter approach served as a network for subsequent immobilization of cholinesterase. Zhang et al. [14] deposited the first layer of MWCNTs by electrodeposition over GCEs followed by alternating layers of PDDA [poly(diallyldimethylammonium chloride)] and MWCNTs dispersed in borate buffer.

Single walled carbon nanotubes (SWCNTs) have been recently proved to be advantageous *versus* MWCNTs in several sensing applications [15, 16]. However, in contrast with the multiple examples found for TCh and pesticide detection involving MWCNTs, very few pieces of work have been found where SWCNTs have been employed in that field [17, 18].

Screen printed technology is a rapid and cost effective way to fabricate robust solid electrodes. It offers several advantages, such as versatility of the design, reproducibility in the sensor preparation and low cost production [19, 20], which permits that the sensors can be disposed after a single use. Screen printing electrodes (SPEs) are frequently used in TCh based sensors; in most of the cases the working electrode was made with an ink/paste containing high mediator loadings (around 5% in commercial pastes). Very few examples have been found where nanotubes (in all cases MWCNTs) have been used instead of these mediating pastes to lower the overpotencial of TCh oxidation [21, 22].

The use of metallophthalocyanines in the fabrication of electrochemical sensors evolved from their unique physicochemical, electronic and electrocatalytical properties. Combination of these two remarkable π -electron species, nanotubes and transition metal phtalocyanines, may open new possibilities for their application as electrocatalyst/mediators suitable for electrochemical sensors. There are some pieces of work where CoPh has been applied to modify SWCNT by electropolymerization [23] or electrosorption [24], and used in sensors aimed to detection of nitrite [25] and nerve agents [26]. However, chemical modification of the nanotubes by covalent binding with phthalocyanines is not very frequent [27] and to the best of the authors knowledge, it has never been used to decrease the overpotential of thiocholine detection.

The present work describes a simple way of binding functionalized cobalt phthalocyanine tetrasulfonyl residues to SWCNTs *via* stable sulfonamide linkages, and the application of these CoPh-SWCNTs into SPEs for TCh detection. Enhancement of the enzymatic TCh oxidation was reached by using CoPh-SWCNTs as a reaction mediator while the amount of modified nanotubes was minimal comparing to the amount of traditional mediators present in commercial pastes for SPEs.

2. Experimental

2.1 Materials and reagents

Pristine SWCNTs, with an external diameter between 1-2 nm and length between 5 and 30 μm, were purchased from CheapTubes (Brattleboro, USA). All reagents were purchased from Sigma-Aldrich. Thionyl chloride, chlorosulfonic acid, ethylenediamine and aniline were distilled before use. Pyridine was dried over potassium hydroxide pellets and distilled. Nafion® 117 was employed, which consists of a 5% solution in a mixture of lower aliphatic alcohols and water. CoPh, hydrochloric acid, methanol, sulfuric and nitric acid were used as purchased. Acetyl cholinesterase (AChE, 317 U/mg, from Electric Eel) and acetylthiocholine chloride (ATCh) were used as received. TCh standard solutions were prepared by the enzymatic reaction of AChE and acetylthiocholine chloride solutions for 3 hours at room temperature. The final TCh concentration was determined spectrophotometrically following the Ellman´s method [28], after reaction with dithio-bis-(nitrobenzoic acid).

2.2 Apparatus and testing conditions.

Characterization of the pristine and derivatized nanotubes was performed by spectroscopic, gravimetric and electrochemical techniques.

Raman spectra were collected using a Witec confocal Raman microscope system (Ulm, Germany) equipped with a fiber-coupled Melles Griot (Carlsbad, CA) argon ion laser operating at 514.5 nm focused though a 60x objective. The collected light was dispersed through a triple monochromator (600 g/mm, 500 nm blaze) and detected with a thermoelectrically cooled (-60°C) charged coupled device. Sample preparation consisted of placing a small amount of carbon nanotubes in powder form between a microscope slide and a coverslip.

FT-IR analysis was accomplished using a Genesis II FT-IR, MATTSON. 3 mg of pristine nanotubes were ground with 100 mg of dry potassium bromide powder in agate mortar and the mixture was pressed to form pellets. The same procedure was also performed with the CoPh-modified nanotubes (CoPh-SWCNTs).

TGA measurements were performed using NETZSCH TG 209 instrument coupled with BRUKER FT-IR IFS 66 spectrometer, to identify and quantify bonded phthalocyanine residues on SWCNTs. TGA measurements for CoPh-SWCNTs **3** (Figure1) were done in an argon atmosphere at a heating rate of 15°/min in a temperature range up to 600°C.

Electrochemical studies were conducted using an ECO Chemie Autolab PSTAT 128N potentiostat-galvanostat (KM Utrecht, The Netherlands), equipped with a FRA module and connected to a personal computer. All electrochemical experiments were performed at room temperature (21±1°C) and potentials are always referenced to an Ag/AgCl pseudoreference electrode.

The electrochemical characterization of the modified electrodes containing unmodified SWCNT or CoPh-SWCNTs, was performed using the redox pair $Fe(CN)_6^{-3}$ /$Fe(CN)_6^{-4}$, by Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). Both CV and EIS measurements were carried out after deposition of 80 µl of a $K_3Fe(CN)_6$/$K_4Fe(CN)_6$, 2.5 mM solution in 0.1 M KCl. Voltammograms were recorded between -0.3 and +0.7 V with a scan rate of 0.050V/s. Impedance was measured at the equilibrium potential of the $Fe(CN)_6^{4-}$/$Fe(CN)_6^{3-}$ couple, with a 0.01 V (rms) sinusoidal excitation waveform. Measurements were made at 40 steps per decade in the appropriate frequency range, five times at each frequency and averaged during each run.

Voltammetric characteristics of TCh were investigated for the screen-printed electrodes (CoPh-SWCNT-SPE, SWCNT-SPE, and bare SPE) in the potential range from -300 to +700 mV, with a scan rate of 50 mV/s. In all cases, voltammograms were recorded in phosphate buffer (pH 7.0) using five different TCh standard solutions (0.077, 0.143, 0.250, 0.330, 0.40 and 0.45 mM). Amperometric responses were recorded by successive additions of TCh in phosphate buffer pH 7.0.

2.3 Derivatization of SWCNTs

The preparation of amine-modified SWCNTs (compound **1)** is illustrated in Figure 1(A): First, pristine SWCNTs were oxidized by means of a mixture of sulfuric and nitric acid (3/1, v/v) to produce carboxylic groups at the ends/defect sites of the nanotubes. The mixture was sonicated for 4 h and then stirred overnight at room temperature. The carboxylic groups were converted into chlorides by refluxing for 5 hours with thionyl chloride. Thionyl chloride was then removed under reduced pressure and the nanotubes product was treated with excess of ethylenediamine solution in methanol, sonicated and maintained for 8 hours at 40°C. The mixture was diluted with methanol and centrifuged at 13 000 rpms for 10 minutes. The sediment was exhaustively washed with methanol and vacuum dried [15, 16, 29-31].

The preparation of cobalt phthalocyanine tetrasulfonyl chloride starting material (compound **2;** a mixture of isomers) was then performed as illustrated in Figure 1(B): A mixture of cobalt phthalocyanine (1 g) and an excess of chlorosulfonic acid (7 ml) was stirred for 5 hours at 140°C. The mixture was cooled and thionyl chloride (3 ml) was added prior to refluxing for 3 hours. The mixture was poured onto ice to form a precipitate that was washed with cold water and then air dried [32]. The yield of compound **2** was 1.7 g.

Amine-modified SWCNTs were reacted with phthalocyanine tetrasulfonyl chloride to yield tetrasulfonylphthalocyanine anilide-SWCNTs (compound **3**, mixture of isomers**)** as shown in Figure 1 (C). In short, cobalt phthalocyanine tetrasulfonyl chloride (100 mg) was added to a sonicated suspension of SWCNTs-amine **1** (20 mg) in 2 ml of dry pyridine. The mixture was maintained at 50°C and sonication was continued for 2 days. Aniline (1 ml) was then added with further sonication at 50°C for another day. The product mixture was diluted with methanol, centrifuged, and the sediment was exhaustively washed with methanol and then dried.

Preferred position for Figure 1

2.4 Dispersion of SWCNTs and CoPh-SWCNTs

Dispersions of both pristine (SWCNTs) and cobalt phthalocyanine derivatized SWCNTs (CoPh-SWCNTs) were prepared in a similar manner; 0.5 mg of nanotubes were suspended in 1 ml of mixture containing 490 µl of ethanol, 490 µl of water and 20 µl of Nafion® 117 solution, and the resulting dispersion was sonicated in an ultrasonic bath during an hour. In both cases, 3 µl of suspension were required for applying onto the screen printing working electrode.

2.5 Preparation and modification of screen-printed electrodes

Screen-printed graphite electrodes (SPEs) were prepared at CIDETEC using a Thieme 110E screen-printing machine, and a three electrode configuration with a Ag/AgCl pseudoreference electrode, a graphite counter electrode and a graphite working electrode (diameter = 3.6 mm). Before any modification the SPEs were pretreated by application of a constant potential of 1.20V in H_2SO_4 0.1M, during 120 s. Subsequently, the SPEs were casted with the dispersions of SWCNTs (SWCNT-SPE) or CoPh-SWCNTs (CoPh-SWNCNT-SPE). A drop (3 µl) was used to cast every working electrode. Solvent was evaporated under ambient conditions. Pretreated SPEs were used as control experiments (and will be denoted as bare SPEs).

3. Results and discussion

3.1 Characterization of pristine and derivatized SWCNTs

3.1.1 Determination of cobalt phthalocyanine residues bonded to SWCNTs by spectrophotometry

A sample of modified carbon nanotubes was suspended in an excess of diluted 1:1 hydrochloric acid, sonicated and heated at 90°C for 24 h. The mixture was centrifuged; the clear solution was collected and combined with aqueous washings of the sediment. The solution was evaporated to dryness and dissolved in a known volume of water. The concentration of cobalt phthalocyanine tetrasulfonic acid was determined by comparing its absorbance at ~660 nm with the absorbance of a standard solution. The amount of liberated cobalt phthalocyanine from 1.0 mg of modified SWCNTs was roughly 10^{-8} mol.

3.1.2 Raman spectroscopy

Raman spectra for both SWCNTs and CoPh-SWCNTs are shown in Figure 2. The radial breathing modes (RBM), disorder D mode, tangential/graphite mode (G-band), and the overtone G' mode were all monitored as indicators of covalent derivatization with CoPh. The spectra were normalized to the tangential G band at ~1580 cm^{-1}. In the Raman spectrum of the derivatized SWCNTs, the radial breathing modes have disappeared when compared to the spectrum of pristine SWCNTs. The decay of these modes is consistent with the disruption of the oscillator strength that gives rise to these modes. Similar results were reported by Fantini et al. [33], where spectral shifts, broadening, and reduction in RBM intensity were attributed to displacement of the Fermi level due to the added functional group on the CNT side-wall. As well, the D/G peak intensity ratio increases from 0.06 for pristine SWCNTs to 0.51 for CoPh-derivitized SWCNTs which indicates the formation of covalent bonds at the surface of the carbon nanotube through conversion of sp^2-hybridized carbon atoms to sp^3-hybridized carbons on the nanotube surface. The relative decrease in the tangential mode (G-band) is consistent with the loss of electronic resonance as a result of the covalent attachment of the substituent.

The G' mode at ~2680 cm^{-1} was also consistent with reported values for pristine SWCNTs [34]. Although the two-phonon G' band has been shown to be relatively insensitive to functionalization [35], a comparison of pristine to derivatized SWCNTs can yield valuable information about the type of nanotube starting material and reaction products. The double-resonance G' mode originates as an overtone of the disorder-induced D band in graphite. In SWCNTs, the G' band can either be a single Lorentzian peak or as a bimodal peak with contributions from semiconducting and metallic SWCNTs. In semiconducting SWCNTs, the splitting of the G' band is attributed to phonon dispersion in opposite directions to the anisotropic phonon dispersion found in metallic nanotubes. As shown in Figure 2, the G' band is bimodal in the case of pristine SWCNTs, suggesting a mixture of several types of SWCNTs in the starting material. However, after reaction with CoPh the resultant G' mode Raman band was mainly single-mode peaks with a slight blue-shift (~ 5 cm^{-1}) for CoPh-SWCNTs. This indicates preferential binding of CoPh substituents to a specific type of nanotubes.

Preferred position for Figure 2

3.1.3 FT-IR studies

In the spectrum of CoPh-SWCNT a strong, sharp peak at 1676 cm^{-1} is observed, referring to carbonyl group stretching vibrations of amides. The presence of amide groups confirms chemical functionalization. Additionally, new peaks are observed in the IR spectrum of CoPh-SWCNTs (lower spectrum) as compared to pristine SWCNTs (upper spectrum) and were attributed to different types of C-N bonding (see Figure 3). A sharp peak at 1719 cm^{-1} is in the region of double C=N bonds (stretching) and a broad peak at around 1140 cm^{-1} which corresponds to additional C-N bonding. Lastly, CoPh-SWCNTs showed stronger, more pronounced C-H stretching at 2970-2858 cm^{-1} as compared to pristine nanotubes.

Preferred position for Figure 3

3.1.4 Determination of cobalt phthalocyanine residues bonded to SWCNTs by TGA-FT-IR

The amount of bonded phthalocyanine residues was calculated according to previously reported methods [36]. The initial mass loss of ~5% below 200ºC [derivative thermogravimetry (DTG) minimum at ~86°C] corresponds to removal of residual water and methanol used for final washing. In the range of 200 - 400ºC (8.3% mass loss) (DTG minimum at 425°C) the volatile decomposition product shows IR peaks at around 2300 cm^{-1} and 1370 cm^{-1}. At 400 - 600°C (16% mass loss) (DTG minimum at 425°C) strong peaks at 2300 and 600 cm^{-1} are present in the IR spectrum, probably corresponding to –CNS residues.

Preferred position for Table 1

After the removal of adsorbed solvent (below 200°C), the mass of derivatized CoPh-SWCNTs reduced from 100 mg to 95 mg. In the range of 200–600°C the mass loss corresponding to detached moieties was ~24 mg leaving ~71 mg carbon. The molecular weight of the attached cobalt phthalocyanine anilide moiety including ethylenediamine residue (cobalt phthalocyanine residue bonded to SWCNTs *via* aminoethylamide group; $C_{53}H_{36}N_{13}O_8S_4Co$) is 1169 g/mol. The amount of detached phthalocyanine derivative is 24/1169, i.e. 0.02 mmol per 100 mg of untreated sample of SWCNTs **3**.

71 mg of SWCNTs that remain after heating the sample to 600°C corresponds to 5.9 mmol of elemental carbon. The amount of CoPh moieties detached from 12 mg (1 mmol) of SWCNTs carbon equals 0.02/5.9 = 0.0035 mol moiety/mol carbon.

The amount of cobalt phthalocyanine tetrasulfonic acid liberated from 1.0 mg of derivatized SWCNTs and determined by UV-Vis spectroscopy was roughly 10^{-8} mol. It is 10^{-3} mmol per 100 mg of the sample. This value divided by 5.9 mmol of SWCNTs carbon gave 0.0017 mol CoPh moiety per mol carbon. The order of magnitude of both determinations is roughly the same.

3.1.5 Electrochemical characterization

Bare SPEs, CoPh-SWCNT-SPEs and SWCNT-SPEs were characterized by cyclic voltammetry and electrochemical impedance spectroscopy (EIS), with the redox couple $[Fe(CN)_6]^{4-/3}$, Figure 4 a) displays the cyclic voltammograms obtained with a 2.5 mM $[Fe(CN)_6]^{4-/3-}$, prepared in 0.1M KCl. The bare SPE presents a quasireversible voltammogram with a midpoint potential of 0.118 V and a peak-to-peak potential separation of 236 mV.

The modification of SPE with SWCNTs and with CoPh-SWCNTs induced a decrease in the cathodic to anodic peak potential separation (ΔEp), indicating an easier electronic transfer originated by the nanotubes. In the case of SWCNT-SPE, the ΔEp was reduced to 64 mV and in the case of CoPh-SWCNT-SPE to 70 mV. The modifications of the surface clearly cause an increase in anodic and cathodic currents.

Cyclic voltammetry results were corroborated by EIS studies. As shown in Figure 4b), SWCNT-SPE exhibits fast electron transfer as evidenced by a minimal semicircular portion at the Nyquist diagram. In contrast to that, bare SPE shows a much slower electron transfer and the impedance spectra follows the theoretical shape, including a significant semicircular portion, observed at higher frequencies, which corresponds to the electron transfer limited process, followed by a linear part, characteristic of the lower frequencies and that could be attributable to a diffusion limited electron transfer This fact proves that the nanotubes enhance the electron transfer. CoPh-SWCNT-SPE present a slight increase in the electron transfer resistance in comparison with SWCNT-SPE, which is reflected in the larger semicircular part of the spectrum. This increase could be attributable to an additional barrier for the redox probe access to the CoPh-SWCNT modified electrode.

Preferred position for Figure 4

3.2 Electrochemical behavior of TCh oxidation on different modified SPE surfaces

3.2.1 Cyclic voltammetric characteristics of TCh

Cyclic voltammetry was used to determine the best anodic oxidation potential for TCh detection. Figures 5a), 5b) and 5c) show the voltammograms obtained for increasing TCh concentrations in 0.1 M phosphate buffer pH 7.0 at bare-SPE, SWCNT-SPE and CoPh-SWCNT-SPE respectively, with the parameters defined in Section 2.2. As it can be seen in all the cases, no oxidation peak was recorded when no TCh was present Growing concentration of TCh led to an increase in the electrochemical response in all cases, but with significant differences; the maximum oxidation potentials appeared at different positions depending on the electrode studied. For bare SPE this maximum was over +700 mV (Figure 5a)), while SWCNT-SPE resulted in a decrease of this maximum to +550 mV. These results prove the slight catalytical effect of the unmodified SWCNT for TCh detection. After modification with CoPh moieties, the catalytic effect of the resulting nanotubes (CoPh-SWCNTs) is much higher (Figure 5c)) and the potential of maximum oxidation is now drastically diminished to +100 mV (vs Ag/AgCl pseudoreference electrode). This proves the successful mediation reached by the newly modified nanotubes.

Thus, it can be concluded that CoPh-SWCNT-SPE systems can achieve a remarkable decrease in the necessary overpotential for effective oxidation of TCh, without the need of large concentration of mediator. This was attributed to an enhancement of electron transfer provided by synergistic effect between SWCNTs and the phthalocyanine, in agreement to what it was reported for other analytical applications[24] where, in that case, the attachment SWCNT and phthalocyanine was performed by electrosorption instead of covalent binding. It is worth remarking that in spite of the small amonts of CoPh bound in our case to the SWCNTs, the behaviour *versus* unmodified SWCNTs towards the catalytic oxidation of thiocholine is greatly improved.

Preferred position for Figure 5

3.2.2 Amperometric detection of TCh

As stated above, for SPE systems containing non-modified SWCNTs the optimum potential for the oxidation of TCh was +550 mV *vs*. Ag/AgCl pseudoreference electrode.

This is a relatively high potential for real samples, where the oxidation of interfering species would contribute to the electrochemical response. On the other hand, we found that with CoPh-modified SWCNTs, +100 mV would provide suitable oxidation of TCh. Thus, this potential was used for comparing the sensitivity of the amperometric detection of TCh over different modified SPEs.

Calibration plots were constructed for bare SPE, SWCNT-SPE and CoPh-SWCNT-SPE, for TCh concentrations in the 0.077 mM to 0.45 mM range (Figure 6), with the plateu values obtained after every addition. In the SPEs modified with nanotubes, fast responses were observed after few seconds of addition, while total stabilization of the signal took about two to three minutes. The response was much slower in bare SPEs. The obtained slope and intercept values are shown in table 2. As it can be seen SPE systems with CoPh-SWCNTs provided the greatest sensitivity to changes in TCh concentrations. Although the response with unmodified SWCNT-SPEs was still noticeable, these electrodes were less sensitive as reflected in the lower slope value. Bare SPEs did have a slight response to TCh but such response was minimal when compared with the modified electrodes; one order of magnitude lower than CoPh-SWCNT-SPEs.

The reproducibility in the fabrication of ten different CoPh-SWCNT-SPE sensors constructed in the same manner, for 0.25 mM TCh (measured amperometrically at +100 mV vs Ag/AgCl pseudoreference electrode) resulted in reproducible signals with a relative standard deviation (RSD) value of 6.5%. This result demonstrated the reliability of the sensor construction procedure, which is of great importance when working with single use SPEs, as is the case of acetyl cholinesterase (AChE) inhibition biosensors aimed to pesticide detection. In that case every point in the pesticide calibration plot, or in the samples to be measured, is recorded from a different SPE. The reason for this is that regeneration of the enzyme AChE trapped in the biosensor, although possible in case of organophosphate pesticides, is time consuming and requires the use of oximes or other compounds to break the bound pesticide/enzyme, so in most of the cases the use of one electrode for every pesticide measurement is preferred.

The repeatability of the measurements was assessed by taking the signal at +100 mV of 10 successive amperometric signals recorded at the same concentration previously mentioned, with a CoPh-SWCNT-SPE (same electrode for the ten measurements). The RSD obtained was 3.2% for CoPh-SWCNT-SPE, which indicated the good adhesion of the CoPh-SWCNT into the SPE surface. Repeatability is also very important at inhibition sensors, since the value of the current intensity generated by TCh oxidation needs to be recorded before and after exposure to the pesticide. Therefore, it is important to guarantee that the decrease in the response in those biosensors is effectively due to the pesticide exposure and not to changes in the working electrode surface (lost of nanotubes, enzyme, etc).

The limits of detection and determination for the CoPh-SWCNT-SPE were calculated according to the 3 s/m and 10 s/m respectively, where m is the slope of the calibration plot and s was estimated as the standard deviation (n=10) of the signal at a 0.077 mM concentration of TCh. These values were 0.038 and 0.127 mM respectively, similar to those obtained recently for carbon paste electrodes (not SPEs) [9], although in our case the reproducibility in the electrode fabrication is considerably improved (6.5% vs 15%). Although there may be other systems that could report lower LOD for TCh detection, this lower values are not a requirement for pesticide quantification, since in AChE inhibiton biosensors the concentration of acetyl thiocholine chloride employed (from which the TCh will be generated) is normally in the range from 1 to 10 mM [5, 6, 21]; concentrations in that range guarantee reaching the saturation of enzyme, as required if the inhibition of the catalytical action of this biocomponent needs to be accounted.

Preferred position for Table 2

Preferred position for Figure 6

4. Conclusions

We have illustrated that SWCNTs modified by covalent attachment of residues of cobalt phthalocyanine tetrasulfonic acid derivative (CoPh-SWCNTs) can be successfully prepared and effectively used on SPEs for the electrochemical detection of TCh. Raman spectroscopy, TGA, chemical hydrolysis and FT-IR were used to confirm the binding of the CoPh to the nanotube surface. Electrochemical impedance spectroscopy and cyclic voltammetry demonstrated the differences in electron transfer originated at the electrode surface as a result of the different nanostructuration of the working electrode.

This work also proved that the binding of CoPh moieties to the SWCNT improves the electrocatalytic effect towards TCh oxidation and permits the decrease in the oxidation overpotential of TCh from over +700 V for bare SPEs or +550 mV with pristine SWCNT-SPEs to +100 mV in the new CoPh-SWCNT-SPEs. Such electrodes exhibited good analytical performance for the detection of TCh, in terms of repeatability, reproducibility and detection limit and therefore hold great promise for pesticide detection.

The derivatization of these types of nanotubes with CoPh and their easy but durable deposition on screen-printed devices open new possibilities for the construction of disposable biosensors which work at low potentials, without the need of high mediator loadings, and with high sensitivity towards specific pesticides. In this direction, further efforts will be dedicated to effective entrapment of enzyme (AChE) on the surface of the newly prepared CoPh-SWCNT-SPEs to build AChE based biosensors for environmental analysis of organophosphate pesticides.

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Tables

Table 1. TGA analysis of anilide of SWCNT-cobalt phthalocyaninesulfonic acid (compound **3** in Figure 1).

Sample	Mass loss below 200°C	Mass loss 200 – 400 °C	Mass loss 400-600°C	Estimated % of SWCNT	Mol of moiety/ mol of carbon	Assumed M.W. of detached moiety
SWCNT-cobalt phthalocyanine anilide	5%	8%	16%	~70%	$3.5\cdot10^{-3}$	1169

Table 2: Analytical parameters for the amperometric response (100 mV vs Ag/AgCl) of the differently modified SPEs

Type of SPE ***Variable***	**Co-SWCNT-SPE**	**SWCNT-SPE**	**Bare-SPE**
Slope (μA mM^{-1})	$5.11\ 10^{-1}$	$2.33\ 10^{-1}$	$8.76\ 10^{-2}$
y-intercept (μA)	$7.40\ 10^{-2}$	$1.23\ 10^{-2}$	$5.79\ 10^{-3}$
R^2	0.991	0.986	0.992

Figures

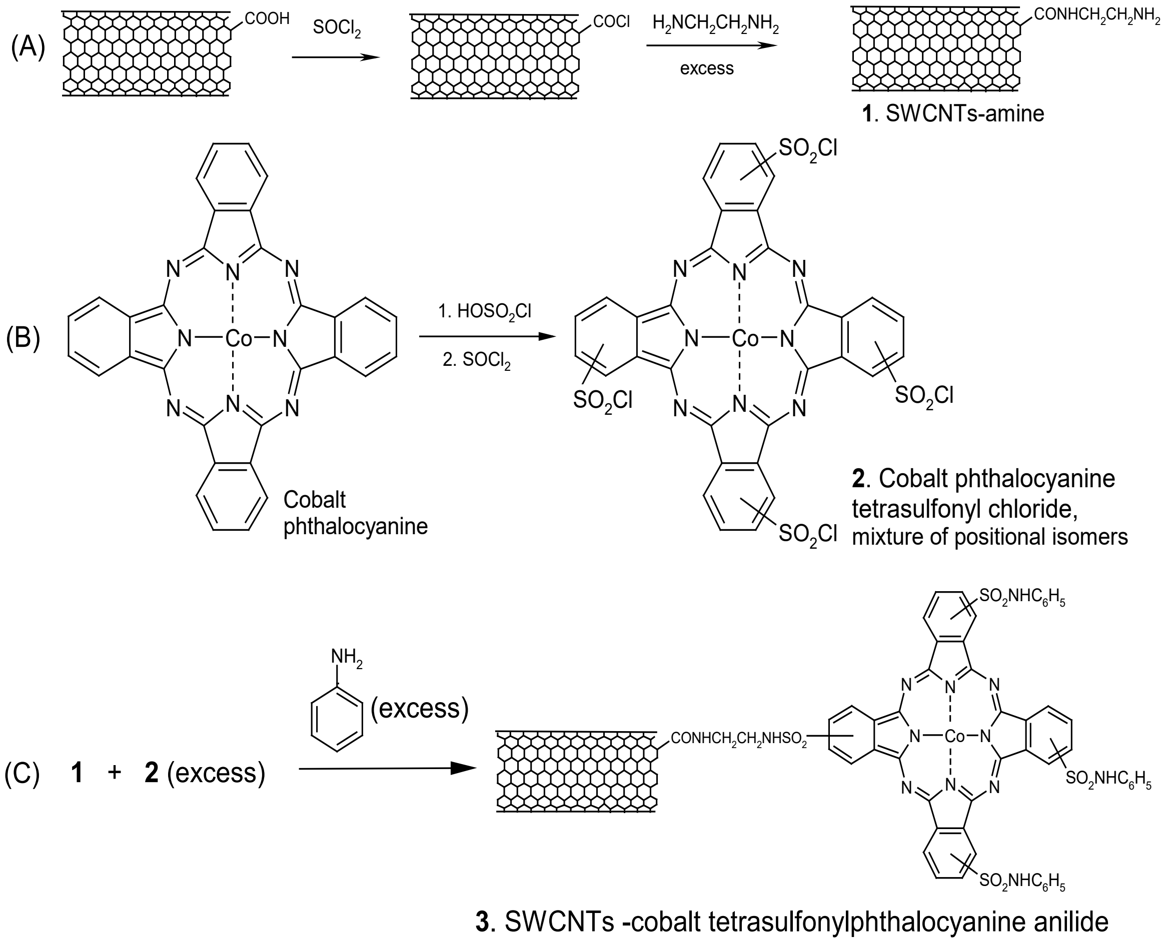

Figure 1. Scheme of the three-stage derivatization of SWCNT with CoPh moieties: (A) Synthesis of SWCNTs-amine **1,** (B) Synthesis of chlorosulfonated cobalt phthalocyanine **2**. (C) Modification of SWCNTs with cobalt phthalocyanine residues.

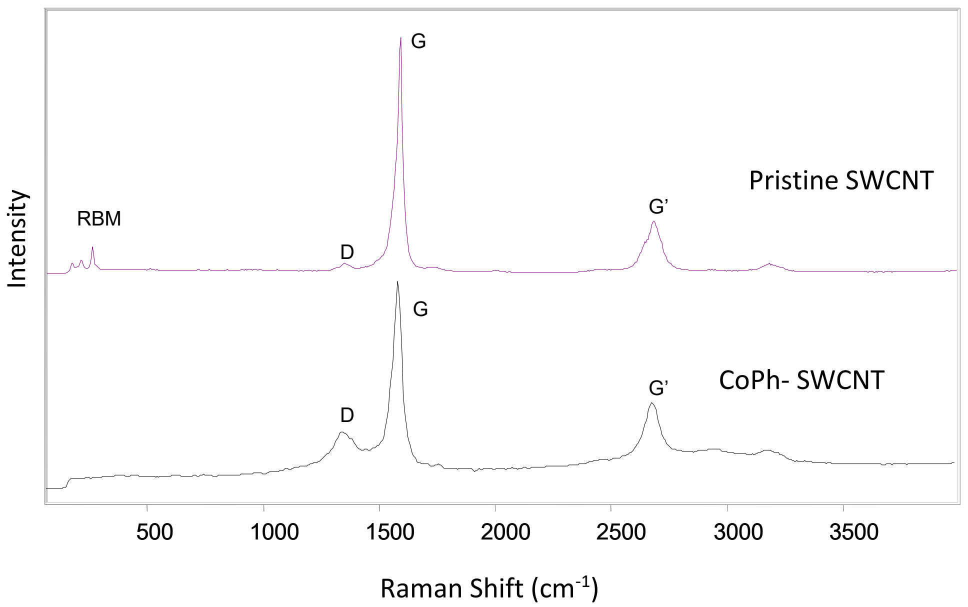

Figure 2. Raman spectra for Pristine SWCNT and derivatized SWCNTs (CoPh-SWCNT).

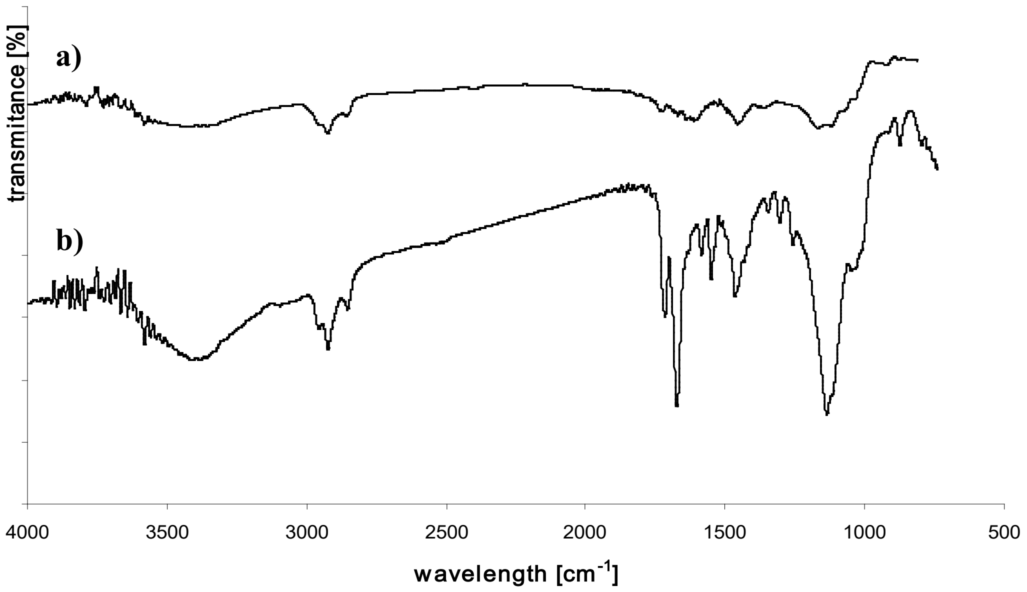

Figure 3. FT-IR spectra of a) pristine SWCNT and b) CoPh-SWCNT.

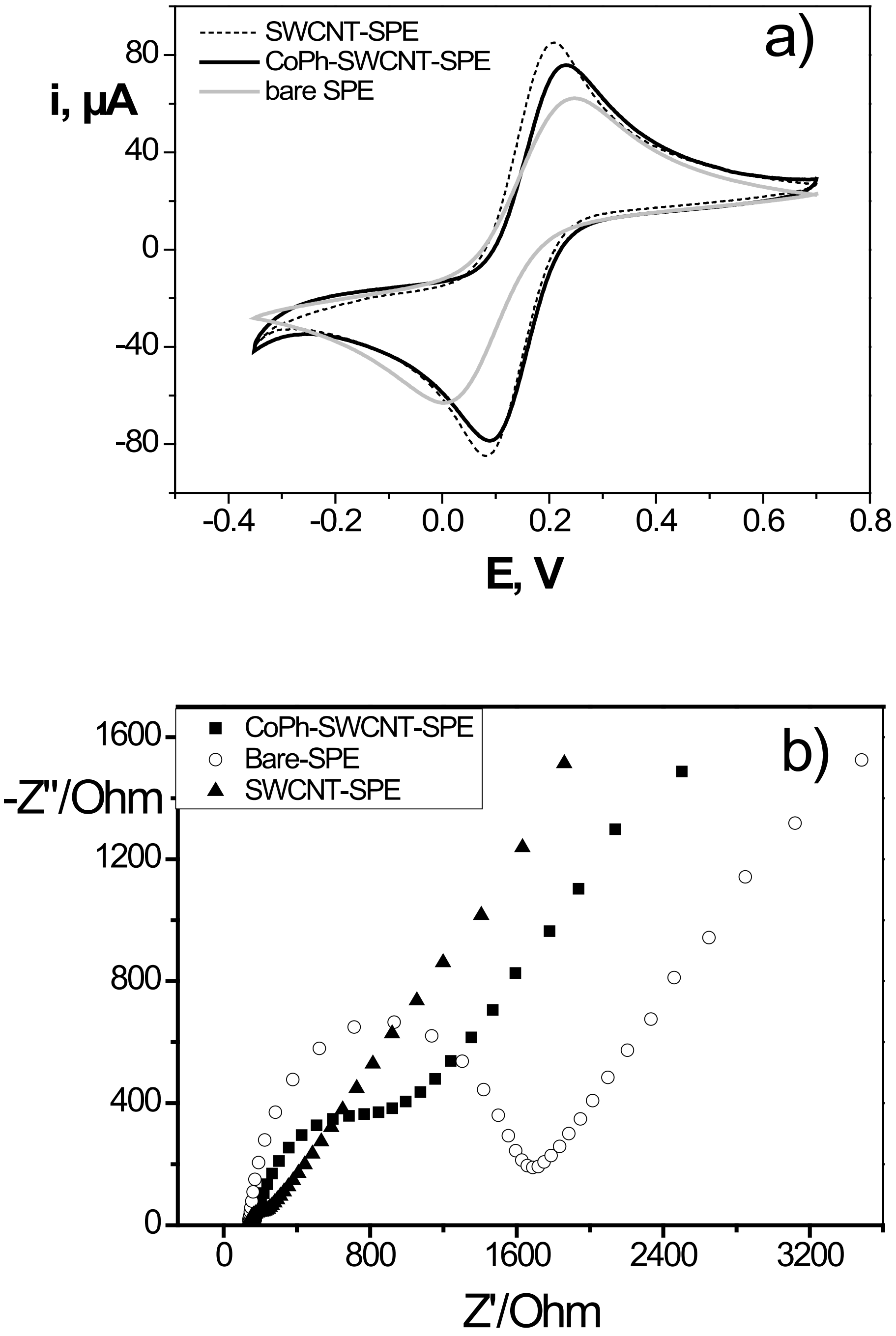

Figure 4. Characterization of the surface of the differently modified working electrodes a) by CV, and b) by EIS, with $[Fe(CN)_6]^{4-/3-}$.

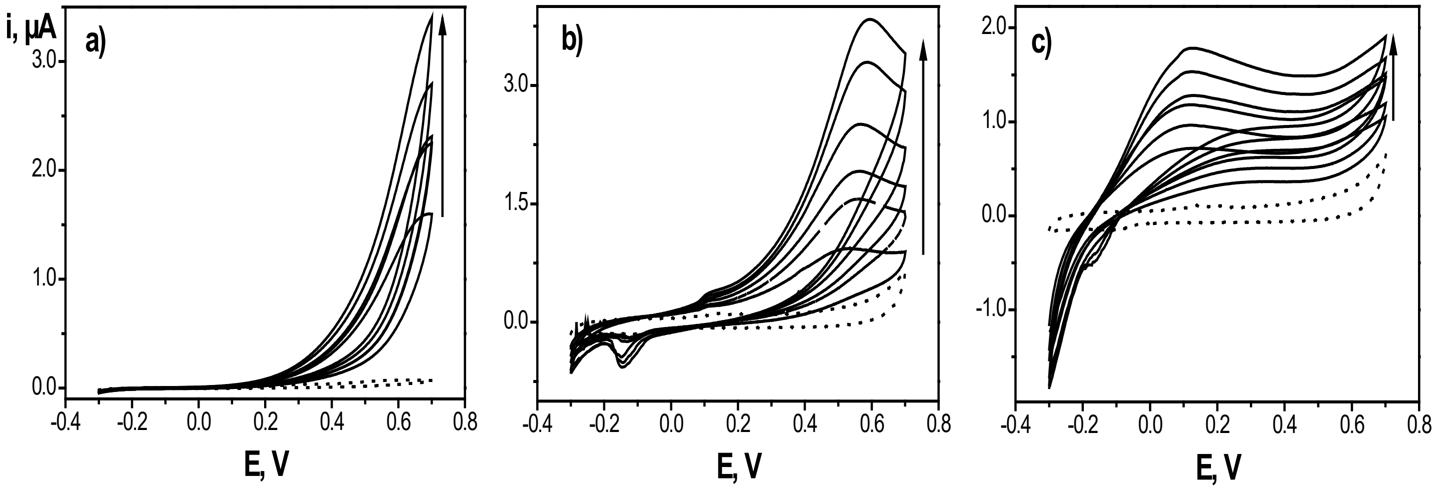

Figure 5. Cyclic voltammograms of: a) bare SPE, b)SWCNT-SPEs, c) CoPh-SWCNT-SPE . [TCh]; 0.07-0.45 mM. Supporting electrolyte: phosphate buffer 0.1 M, pH 7.0. Dotted lines represent the response for the 0.1M PBS buffer ph 7.0. Scan rate 50 mV/s.

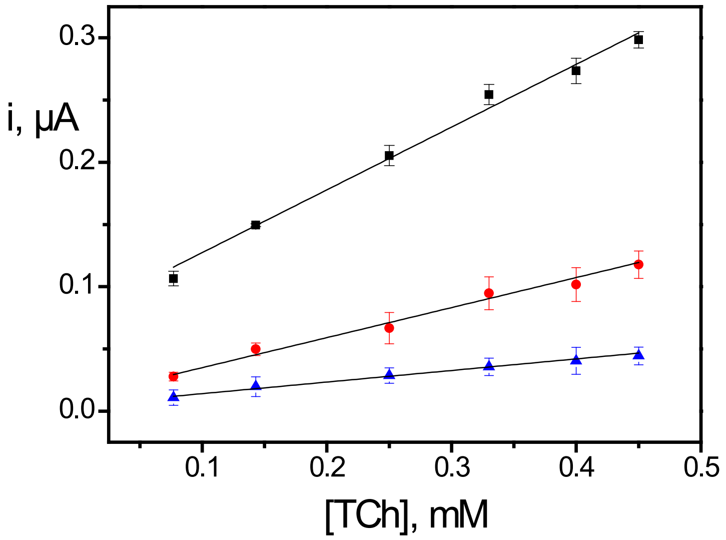

Figure 6. Amperometric response of bare-SPE (blue triangle), Co-SWCNT-SPE (black square), and SWCNT-SPE (red circle) at +100 mV vs Ag/AgCl. [TCh]: 0.077-0.45 mM. Supporting electrolyte: phosphate buffer 0.1 M, pH 7.0.