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The characterization of the immobilized active substrates on screen-printed electrodes designed for response to the biochemical agents

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SWCNT functionalized with Mn-Porphyrins were deposited by dropcast respective by MAPLE on gold substrate of the screen printed electrodes (SPE). The structure of the immobilized active substrate was investigated by Raman and UV-VIS spectroscopy. The degree of immobilization is correlated with electrochemical response to few chemical/biochemical agents. The study shows a correlation between repeated washes and cleanings and the efficency of the active substrate.

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1. Introduction

The porphyrin is the most important ligand in the biochemistry of inorganic compounds. The porphyrin is a macrocyclic tetrapyrrolic system , with π conjugated double liaisons and different subtitutes connected to the periphery. These substitutes influence the delocated molecular orbitals of the complex and, by doing so, they modify the properties of the porphyrin. The complex can accept or yield two hydrogen ions, the last possibility leading to porphyrin systems with metalic ions, called metalloporphyrins. The stability of the metalloporphyrins decreases from Ni^{2+}>Cu^{2+}>Co^{2+}>Fe^{2+}>Zn^{2+} (Irving-Williams series) [1]. The importance of these compounds in biological systems is influenced by two diferent points of view:

a. The metalloporphyrins can develop diferent functions by modifying the metalic ion, the subtitutes to the porphyrinic cycle or the oxidation state of the metalic ion.

b. The evolution of the biological systems modifies the structure and the function of the molecules already present in the organical structure, rather then producing new systems.

Based on the first point of view, the latest research has focused on the porphyrin properties of chemiluminescence. In a toxic medium, the porphyrins change their color, due to an alteration in the electronic state as a result of the interaction between the porphyrin and a certain molecule in the medium [2]. This property has led to a developement in sensory industry, but it is not the only remarkable capacity of the porphyrins. Recent progress in malaria infections treatment has shown that organometallic complexes and metalloporphyrins have *in vitro* activity against *Plasmodium falciparum* [3].

During the last few years, the porphyrins and their derivated complexes have been studied by diferent methods [10] and the measurements indicate from optical properties, observed by absorbtion spectra, to fluorescence transitions [4], heat of interactions with diferent neutral molecules, thermodynamic stability and physicochemical properties by calorimetric titration method [5]. The spectrophotometrical data show that derivative systems from porphyrin, like 5,10,15,20-tetrakis(4-carboxylphenyl)porphyrin, can be used as sensors in the determination of Zn in natural waters and nutritional supplements [6]. Also, the use of light and Zn-protoporphyrins inhibits respiration and phosphorylation in mitochondria, characteristic used today as a new widely practice in dermatology [7-9]. Manganese pophyrins, deposited onto chemically modified electrodes, epoxidates the styrene with molecular oxygen [11] and MnTPP (manganese tetraphenylporphyrin) with tetracyanoethylene anion presents a magnetic moment [12].

In accord with their multi-properties, the metalloporphyrins are used in lays, layered semiconductors, nanotubes, and mesoporous materials [13] and in the developement of new types of soluble and insoluble oxidation polymer catalysts [14].

Less studied are porphyrins used as substrate or mediator in biosensors and their interaction with different analytes. In addition the electron transfer from porphyrins to detection system is not well-understood due to less information related to the interface properties electrode-porphyrins.

The present work shows an immobilization of the active substrate based on Mn-porphyrins on screen-printed electrodes by two different deposition methods, dropcast and matrix assisted pulsed laser evaporation (MAPLE). The degree of immobilization is investigated by electrochemical response via cyclic volammetry.

2. Experimental

2.1 Materials

Porphyrin: *(5,10,15,20-tetraphenyl)porphinato manganese (III) chloride* (MnTPPCl), chemical synthesized according to previous well-established protocols [15-20] (Fig. 1). The (MnTPPCl) is soluble and stable in acetonitrile, *N*,*N*-dimethylformamide, dimethylsulfoxide, dichloromethane, dichloroethane, THF, chloroform, under the form of intense green solutions.

SWCNTs: purchased from NTP, China.

Buffer solution: Monosodium Phosphate (NaH_2PO_4*$2H_2O$) and Disodium Phosphate (Na_2HPO_4*$12H_2O$) for chemical analysis. Dopamine (98.5% puriss) has been supplied by Fluka. Serotonin (98% puriss) and N,N-dimethylformamide (99%) have been bought from Merck.

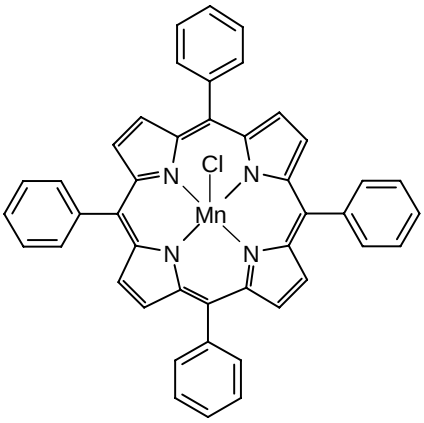

Fig. 1. Structure of (5,10,15,20-tetraphenyl)porphinato manganese (III) chloride (MnTPPCl);

Planar screen-printed electrodes: SPE-AT-220 (DropSens), consisted of: (i) a working electrode (WE) gold thin layer with 0.125 cm^2 area, screen printed in the central part on the ceramic substrate, (ii) counter electrode (gold, auxiliary electrode) and (iii) a silver pseudo-reference electrode (Ag), (Fig. 2).

Fig. 2. Dropsens SPE-Au 220AT

2.2 Experimental details

SWCNTs have been cutted by oxidation in 3:1(V/V) H_2SO_4 and concentrated HNO_3 [21]. SWCNT functionalization with porphyrin was performed following these steps: 5 mg of SWCNT and 5 mg of metalloporphyrin (Mn-porphyrins) were dispersed into 4 mL N,N-dimethylformamide (DMF) by 20 minutes ultrasonic agitation [22]. Modified SPE was performed by depositing 10 µl of SWCNT – porphyrins – DMF-resin dispersion on the gold working electrode surface. The dispersion was left to dry at 50°C.

2.3 Maple experimental conditions

A copper target holder was filled with ~5 ml SWCNT-porphyrin-DMF dispersion, pipetted into the target holder and frozen by immersing in liquid nitrogen (LN). The frozen target was mounted on a refrigerated assembly which can be cooled down to 173 K. MAPLE depositions of Mn(III)-metalloporphyrin were performed using a pulsed excimer KrF* laser (λ = 248 nm, t_{FWHM} = 25 ns, pulse repetition rate = 10 Hz, laser fluence = 300 mJ/cm^2). The fluence threshold was about 300 mJ/cm^2 for Mn(III)-metalloporphyrin. The incident angle of the laser beam was 45º. The target–substrate distance was set at 4 cm. The spot size was within the range 8 mm^2 and the beam was scanned over the entire surface of the 3 cm diameter target rotating at 4.9 Hz. The number of subsequent laser pulses applied for the deposition of one structure was 20,000. The background pressure in the chamber during deposition was ~ 4.86-6.76 10^{-2} torr. Evaporated materials were collected onto gold screen-printed electrodes (Au-SPE) and kept at room temperature for post-deposition analyses.

2.4 Characterization methods

SWCNT – porphyrins -DMF dispersion was characterized by Raman and UV-VIS spectroscopy. Raman spectra were recorded by Jasco NRS 3100 with dual laser beams, 532 nm and 785 nm, respectively. UV-

VIS spectra were recorded by Jasco V-570 spectrophotometer. Cyclic voltammetry were performed with a Voltalab 40 system (Radiometer Analytical) adapted for Screen printed electrodes (SPE). The oxidation potential was recorded within the range (−0.8 - 0.8) V.

3. Results and discussions

3.1 Raman

Typical Raman spectra of pristine SWCNT and purified and cutted SWCNT are given in Fig.3. The diameter, d (nm), of cutted and purified SWCNTs in Fig. 3 was estimated from a widely used relationship, $d=248/\omega$, where ω (cm^{-1}) is the RBM Raman shift [23,24], and the value equals 0.975 nm. The D-band (1298 cm^{-1}) is observed and is ascribed to raman mode of the amorphous carbon and/or to the disordered grapheme sheet. The high intensity of the G-band (1581 cm^{-1}) compared to the D-band indicates a high purity of the specimen.

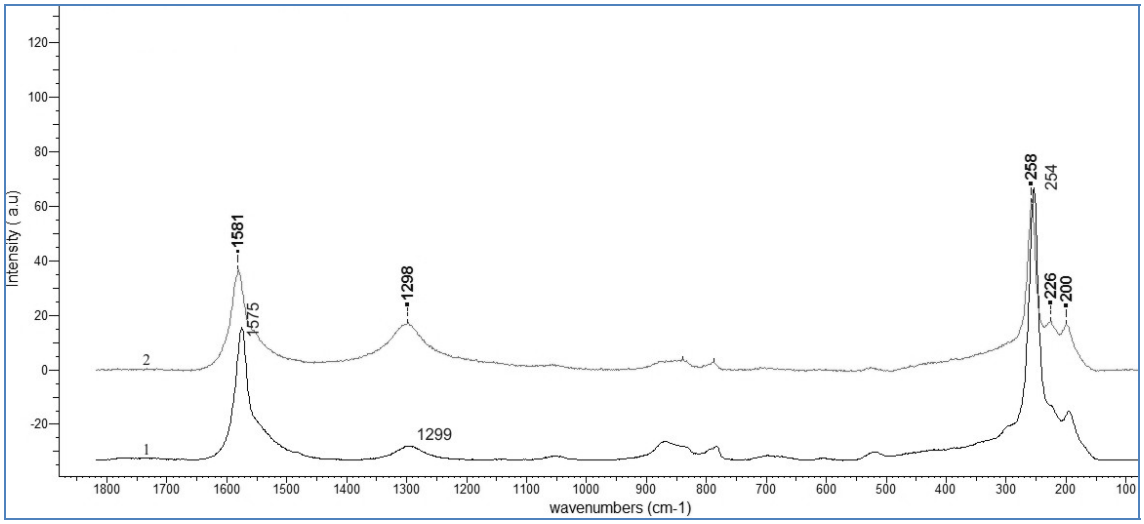

Fig. 3. Raman spectra of (1)pristine SWCNT and (2) purified and cutted SWCNT.

Raman spectra for DMF (1), (MnTPP)Cl (2) and SWCNT- (MnTPP)Cl-DMF dispersion (3) are shown in Fig. 4. Raman spectra for porphyrins and metalloporphyrins are complicated because of their complex structures. The band of 1,015 cm^{-1} belonging to porphyrin ligands is ascribed to both vibration of pyrrole breathing and phenyl stretching that do not shift in Mn complexes. The bands group 1,272 and 1,236 cm^{-1} is assigned to C_m-Phenyl. The porphyrin ligands are assigned to both 1,371 cm^{-1} and 1,343cm^{-1} and are usually associated with symmetric stretch pyrrole half-ring vibrations (C-N). The band centered at 416 is associated to Mn-N vibrations.

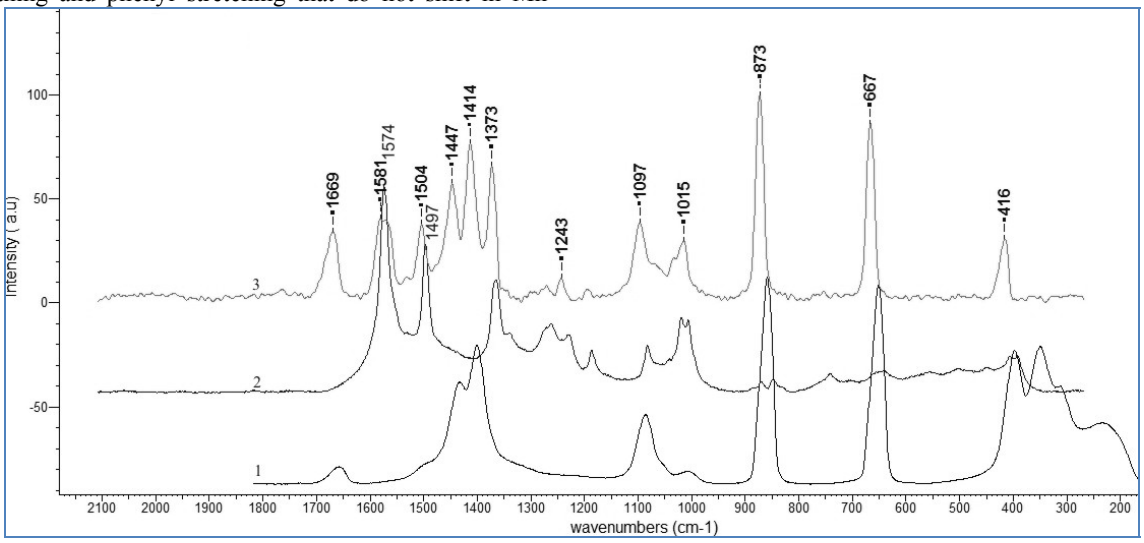

Fig. 4. Raman spectra of (1)DMF, (2) (MnTPPCl) and (3) SWCNT-(MnTPPCl)-DMF dispersion.

3.2 UV-VIS

The absorption spectra of Mn-metalloporphyrins are complex *d-hyper* type, because of the existence of unoccupied orbitals in metals with symmetries *eg*(*d*(p))–*dxz* and *dyz*. The intense band in the range 440–480 nm in the spectra of the above complexes is assigned to the charge transfer from the $a1u(\pi)$ and $a2u(\pi)$ orbitals of porphyrin to the *eg*(*d*(π)) orbitals of the metal. The respective band in the spectrum is called the charge transfer band (Fig. 5. figure in foreground, band V)[25].

The stability of the manganese porphyrin was investigated by varying pH in the range 1.5-8.0 as displyed in Fig. 6., the position of the bands III, IV and V remain the same, but the intensity of absorption is decreased in acid media and even stronger decreasing is noticed in basic environment. The absorption spectra of SWCNT-(MnTPP)Cl-DMF dispersion is shifted to left compared to the absorption spectra of Mn-metalloporphyrins.

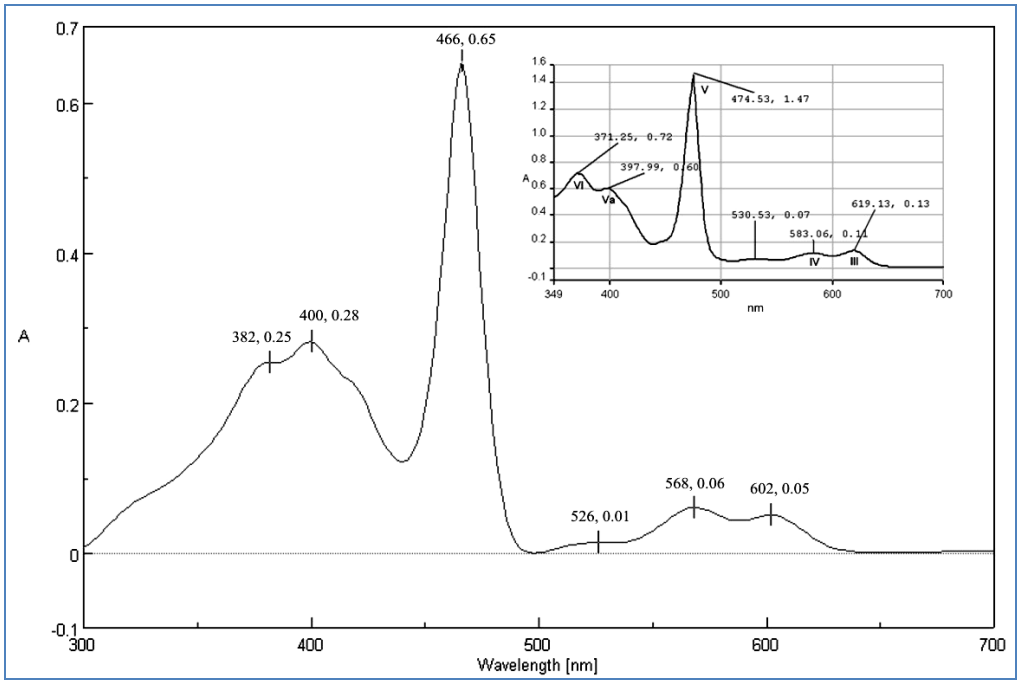

Fig. 5. UV-VIS spectra of SWCNT- (MnTPPCl) – DMF suspension and (MnTPPCl)-DMF in background.

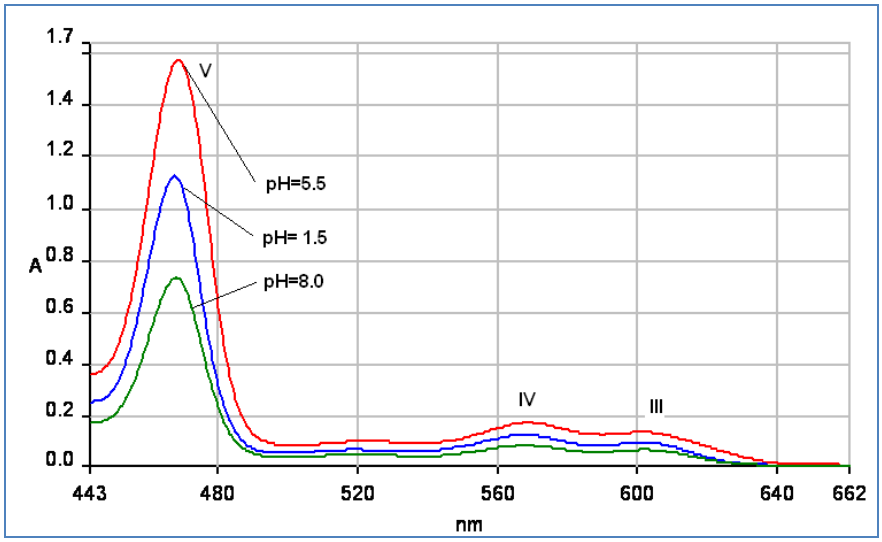

Fig. 6. The influence of pH upon the shape of UV-Vis spectra of (5,10,15,20-tetraphenyl)porphinato manganese (III) chloride in acetone-water system

3.3 Cyclic voltammetry

The voltammetric behavior of dopamine and serotonin, two major neurotransmitters, was compared for SPE-Au with *SWCNT-(MnTPPCl)* deposited on working electrode by dropcast and MAPLE. Fig. 7.and fig 8. exhibits the characteristics of these two cases. The response of dopamine (10^{-7}M) at SPE-Au with dropcast *SWCNT-(MnTPPCl)* electrode displayed a well-defined oxidation peak at 325.5 mV and a reduction peak centered around of -250 mV (Fig. 7, curve 1). The response of serotonine (10^{-7}M) at SPE-Au with dropcast *SWCNT-(MnTPPCl)* electrode displayed an oxidation peak at 268.4 mV and a reduction peak centered around of 156 mV (Fig. 7, curve 2). The response of dopamine (10^{-3}M) at SPE-Au covered with MAPLE-deposited *SWCNT-(MnTPPCl)* has one oxidation peak centered at 244 mV (Fig. 8, curve 1). The response of serotonine (10^{-3}M) at SPE-Au covered with MAPLE-deposited *SWCNT-(MnTPPCl)* has one oxidation peak centered at 70 mV (Fig. 8, curve 2).

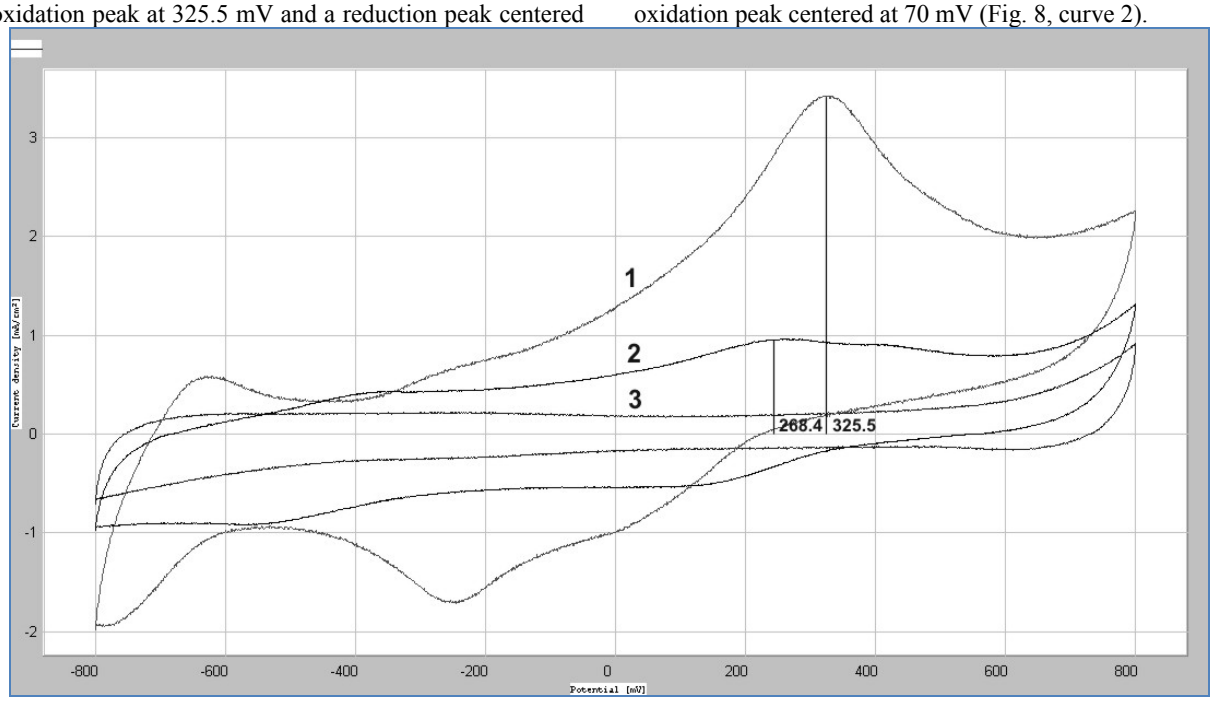

Fig. 7. Cyclic voltammograms of SPE-Au modified electrode with SWCNT-(MnTPPCl)-DMF by dropcast:(1)Dopamine, (2) Serotonin and (3)Buffer solution pH 7;

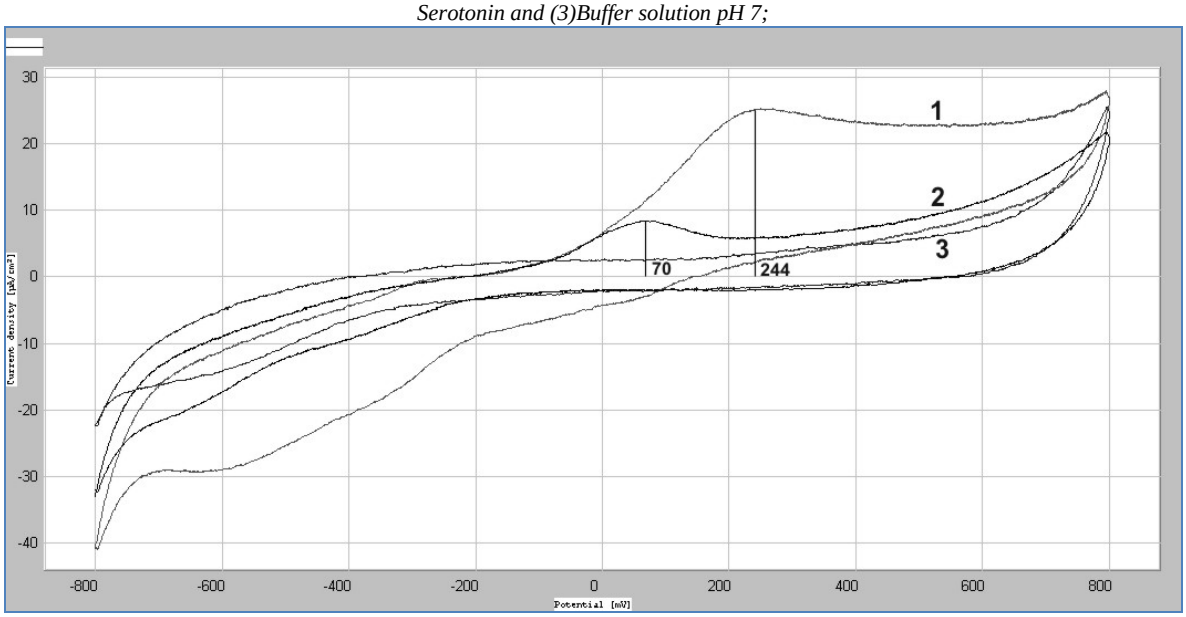

Fig. 8. Cyclic voltammograms of SPE-Au modified electrode with SWCNT-(MnTPPCl)-DMF by maple:(1)Dopamine, (2) Serotonin and (3)Buffer solution pH 7;

4. Conclusions

Raman and UV-Vis spectroscopy shows the functionalization of the SWCNT with (MnTPPCl). Voltammetric measurements indicated that SWCNT-(MnTPPCl) deposited on SPE-Au by MAPLE are not adequate for neurotransmitters detection, while SWCNT-(MnTPPCl)- deposited by dropcast on SPE-Au have a good response for both neurotransmitters, dopamine and serotonin.

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