Screen printed electrodes based on metalloporphyrins and SWCNT for neurotransmitters detection: Raman spectroscopy and cyclic voltammetry investigation

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In this article, we explore the Raman characteristics and the electrochemical response of SWCNT-metalloporphyrins biohybrids. The immobilization of metalloporphyrins on the SWCNT surface has proven to result in novel electron donor–acceptor biohybrid structure with a good selectivity for neurotransmitters. SWCNT and porphyrin functionalization of gold-based screen-printed electrodes allows tailoring of the original sensing characteristics by decreasing the oxidation potential and lowering concentration limits, properties that can further be employed in the diagnosis of neurotransmitter-associated diseases. It is shown that only a specific Zn-porphyrin-SWCNT couple is appropriate for an electrochemically sensitive response when they are immobilized on gold screen-printed electrodes (Au-SPE).

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1. Introduction

Neurotransmitters (such as dopamine, serotonin, epinephrine, nor-adrenaline, etc.) are responsible for the transmission of signals from nerves to muscles, organs, and glands. Dopamine is responsible for the reward-motivated behavior of the brain [1]; epinephrine controls pulmonary and cardiac functions [2]; serotonin is a neuromodulator that controls the peripheral nervous system, smooth muscles, and platelets [3], [4]; and nor-epinephrine acts as the "stress hormone" and is responsible for nervousness, memory and the "flight" reaction of the "fight-or-flight" response to acute stress [5]. Different neurological disorders are correlated with abnormal levels of neurotransmitters. Thus, variation in the concentration of dopamine (normal level between 10^{-7} – 10^{-3} M) [1], [6], epinephrine (normal level 0 – 140 pg/mL) and nor-epinephrine (normal level 70 – 1700 pg/mL) can be an indicator for Parkinson's disease, Alzheimer's disease, multiple sclerosis or depression [7], [3].

The conventional protocols for analyzing neurotransmitters require special equipment, are time-consuming and high-cost. Moreover, they can only be assessed in hospitals or professional laboratories. An alternative to these methods are electrochemical methods, which rely on modified carbon paste electrodes. They are intensively explored for the development of disposable sensors, which are easy-to-use, less time consuming and allow real-time analysis of biological samples (blood, saliva) [8]. Thus, electrochemical detection methods address a key issue in healthcare, which is real-time monitoring of biologically-active chemical compounds [9]. Disposable sensor devices that can assess the level of neurotransmitters several times a day can be successfully commercialized to both health specialists (doctors, hospitals, clinics) and to demand-based customers (market – e.g.: pharmacies).

Other applications of electrochemical detection methods are the determination of uric and ascorbic acid [10], DNA base [11], heavy metals [12], essential amino acids [13] and even viruses such as Covid-19 [14].

Many naturally occurring compounds are used in research for green synthesis [15], [16], [17]. Among the materials used as sensitive materials, porphyrins are recognized as molecular building blocks due to their presence in all biological systems [18]. Doping single wall carbon nanotubes (SWCNT) [19], with different porphyrins modulates the charge transfer between the two components [20] and can lower overpotentials, improving the corresponding sensitivity. This behavior could expand the limits of detection of the biological analytes [21]. The unique optical and electrical properties of SWCNTs, such as high photoconductivity and intense phonon scattering, lead to extremely high electron mobility and provide a strong inelastic light scattering, which can enhance the Raman signal [22]. In addition, advances in electrode miniaturization allow for designing various configurations of working electrodes [23].

The present work aims to investigate the interaction between SWCNTs and metalloporphyrins via Raman

spectroscopy and to electrochemically characterize via cyclic voltammetry the metalloporphyrin-modified screen-printed electrodes (SPE) for the detection of several neurotransmitters (dopamine, serotonin and epinephrine) in phosphate buffer solution (PBS, pH=7.4).

2. Experimental

2.1. Materials

The employed three metalloporphyrin powders [a – (5,10,15,20-Tetraphenyl-21H,23H-porphine manganese(III) chloride) (abridged as Mn-porphyrin,) b – Zinc 5,10,15,20-tetra(4-pyridyl)-21H,23H-porphine (abridged as Zn-porphyrin) and c – 5,10,15,20-Tetrakis(4-methoxyphenyl)-21H,23H-porphine cobalt(II) (abridged as Co-porphyrin)] were procured from commercial vendors (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Subsequently, these metalloporphyrins were dispersed in N,N-dimethylformamide (99%, Merck) at a concentration of 1%.

SWCNTs were purchased from Shenzhen Nanotech Port Co. Ltd., Shenzhen, China. The PBS buffer solution was prepared daily from 20.2 g monosodium phosphate ($NaH_2PO_4 \cdot 2H_2O$) and 3.4 g disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$), pH=7.4. The employed neurotransmitters were: dopamine (98.5% puriss, Merck), serotonin (98% purris, Fluka), and epinephrine (1 mg/1 ml aqueous solution, provided by Terapia Bucharest). For each type of neurotransmitter we prepared solutions of 10^{-7} mol/l in PBS and stored them in the refrigerator at 4°C.

The SWCNTs and metalloporphyrins were deposited onto screen-printed electrodes (SPE-AT-220 from Metrohm DropSens), which are composed of three planar electrodes on a ceramic substrate. The working (WE) and auxiliary (AUX) electrodes are made of Au, while the pseudoreference electrode is made of silver. WE is a thin disc of gold with area 0.125 cm^2. The AUX and the reference electrodes are screen printed in a circle surrounding the WE at a distance of 1 mm.

Other solvents used in the preparation of the sensitive materials were H_2SO_4 (98%, Redox), HNO_3 (60%, Redox), ethanol (99%, Redox), distilled water.

2.2. Purification of SWCNTs

SWCNTs were cut and chemically modified by carboxylation using the following adapted recipe [24], [25]: 12 ml of 3:1(V/V) H_2SO_4 (98%) and concentrated HNO_3 (60%) were mixed with 24 mg of SWCNTs. The mixture was ultrasonicated for 2 h at 20°C by ice cooling, then diluted with 1000 mL of distilled water. The solution was repeatedly filtered (40 μm PTFE filter) and washed until pH=5. Finally SWNTs were washed in ethanol and afterwards dried in vacuum.

2.3. Fabrication of metalloporhyrin/SWCNT biohybrids

Carboxylated SWCNTs were functionalized with porphyrins as follows: 5 mg of SWCNT and 5 mg of metalloporphyrins (Co-, Mn, or Zn-porphyrins) were dispersed into 4 mL N,N-dimethylformamide (DMF) for 20 minutes in the ultrasound bath.

2.4. Functionalization of the screen printed electrode

The functionalization of the SPEs was performed by dropcasting 5 μl of SWCNT/metalloporphyrinin DMF dispersion on the gold working electrode surface, in separate batches of 1 μl. The solution was then left to dry at 50°C under IR light.

2.5. Characterization methods

SWCNT – porphyrin -DMF dispersion was characterized by Raman spectrometry. The Raman spectra were recorded by Jasco NRS 3100 with dual laser beams, 532 nm and 785 nm. Cyclic voltammetry (CV) was performed on a Voltalab 40 system (Radiometer Analytical) adapted for SPE. The oxidation potential was recorded within the range -0.8 V to +0.8 V) . For each experiment 100 μl of solution was dispersed on the modified SPE to cover in a thin film the WE, AUX and reference electrodes.

3. Results

3.1. Raman spectroscopy characterization

The Raman spectra for the three biohybrid SWCNT/metalloporphyrins systems are presented in Fig. 1. The spectra are similar because the metalloporphyrins have a similar structure. The fine, individual traits of the metalloporphyrins (Fig. 2, Fig. 3 and Fig. 4) are noticed in the Raman spectra: (a) the Mn-porphyrin has four phenyl functional groups which restrict the vibration and reduce the intensity of the spectrum; (b) the Co-porphyrin has four methoxyphenyl functional groups, which are imprinting in the spectrum as well defined peaks at ≈1500 cm^{-1} and ≈1600 cm^{-1}; (c) the Zn-porphyrin has four pyridyl functional groups, which show up in the 1200 cm^{-1} region [26]; (d) in all spectra, the SWCNT is present below 500 cm^{-1} (this represents the Radial Breathing Mode and is specific to the SWCNT) [27];

In Fig. 2, the peak at 409 cm^{-1} represents the symmetric vibration of the Mn-N bond [28]. The phenyl functional groups are observed in the spectrum of Mn-porphyrin at 1500 cm^{-1} and 1575 cm^{-1}. The methoxyphenyl groups in the spectrum of Co-porphyrin are shown at similar wavenumbers, 1500 cm^{-1} and 1565 cm^{-1} (Fig. 3). What is interesting is that the Raman spectra of the biohybrids are significantly modified after the functionalization process.

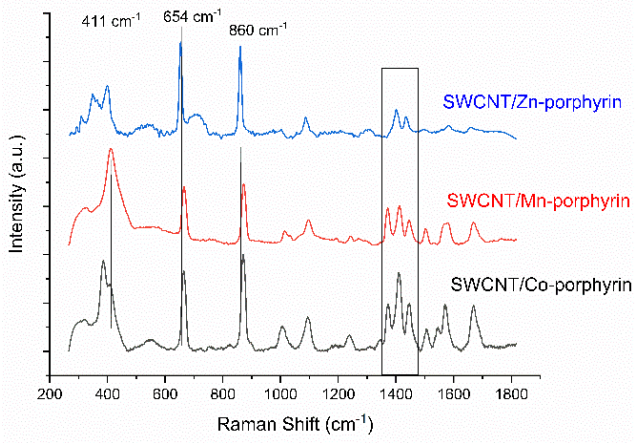

Fig. 1. Raman spectra of the SWCNT/metallo-porphyrins system (color online)

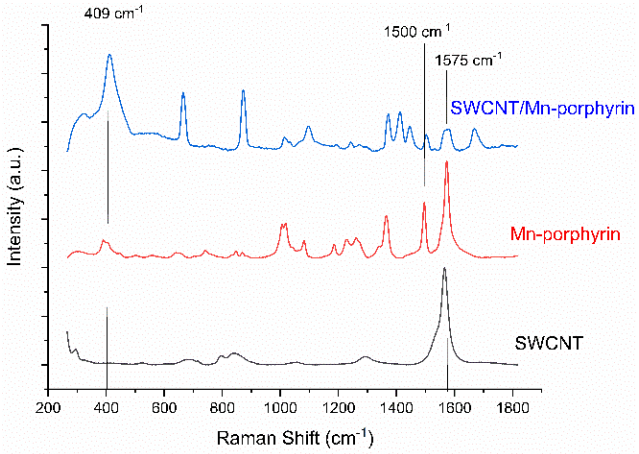

Fig. 2. The Raman spectra of the SWCNT, Mn-porphyrin and the SWCNT/Mn-porphyrin (color online)

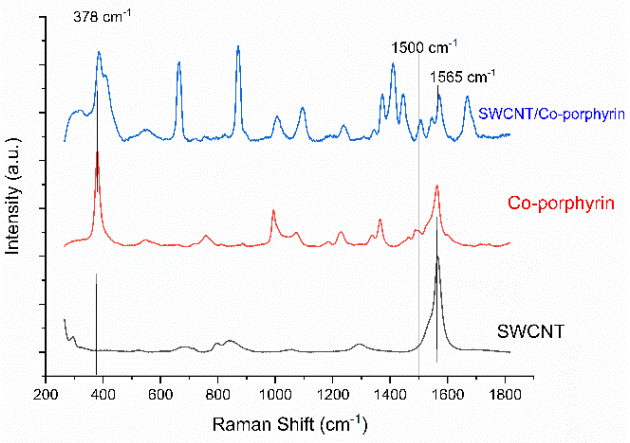

Fig. 3. The Raman spectra of the SWCNT, Co-porphyrin and the SWCNT/Co-porphyrin (color online)

When the bands of the SWCNT/Co-porphyrin and SWCNT/Mn-porphyrin biohybrids are compared with the pristine corresponding metallo-porphyrins and SWCNT, we notice that the bands are enhanced, but identical or close to the bands observed for the pure product, which confirm that the spectra of functionalized SWNTs/metallo-porphyrins are mainly a combination of the spectra of SWNTs and metallo-porphyrin molecules.

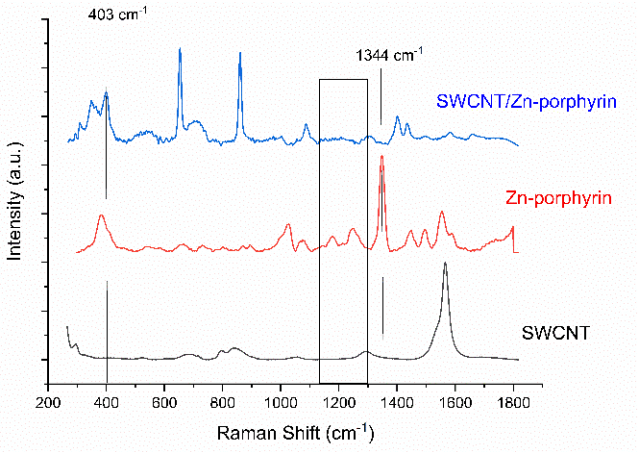

Fig. 4. The Raman spectra of the SWCNT, Zn-porphyrin and the SWCNT/Zn-porphyrin (color online)

In Fig. 4 we present the Raman spectra of the Zn-porphyrin and the SWCNT/Zn-porphyrin in order to assess the effectiveness of functionalization. We can see that the spectrum for the system shows the peaks for the porphyrin in an attenuated manner (1344 cm^{-1}) due to the physical interaction with the SWCNTs.

3.2. Cyclic voltammetry studies

The obtained results for the electrochemical measurements are presented below.

The bare Au electrode presents a low electrochemical signal for the investigated neurotransmitters. Dopamine and epinephrine showed similar curves, with a low intensity reduction peak at ≈ 370 mV. For serotonin, the shape of the CV was similar to the other two neurotransmitters, but it exhibited also a low intensity oxidation peak at ≈ 290 mV (Fig. 5).

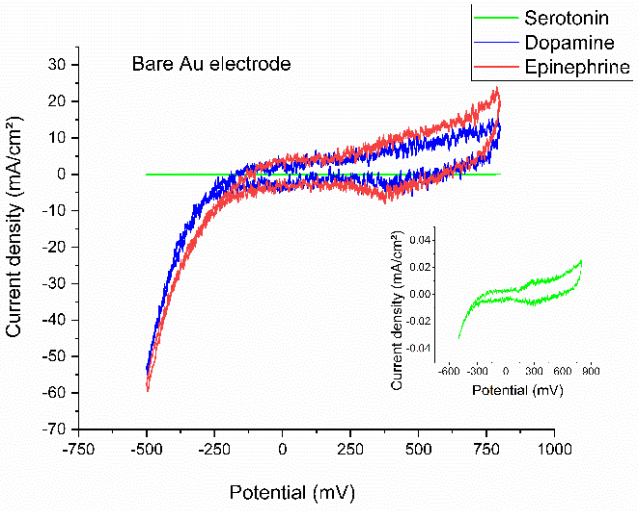

Fig. 5. The CVs for the bare Au electrode in neurotransmitters solution (10^{-7}M) (color online)

When the functionalized SWCNT-metalloporphyrins sensors were tested, the variation of electrochemical signals was obvious. SWCNT/Co-porphyrin, serotonin and epinephrine show similar voltammograms, with a low reduction peak at -470 mV and a low oxidation plateau at 290 mV and 405 mV. However, the results obtained for dopamine are different: for dopamine, the voltammogram exhibits a quasi-reversible redox reaction, with a reduction potential at 350 mV and a reduction potential at -97 mV. The intensity of the redox peaks is still low, proving the slow kinetics of the reaction (Fig. 6).

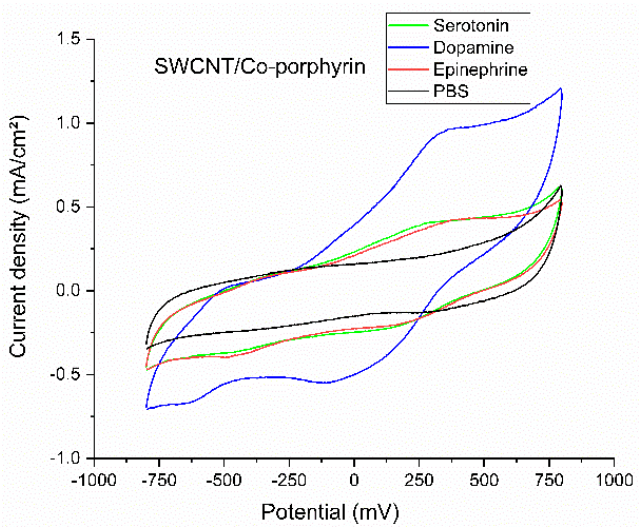

Fig. 6. SWCNT/Co-porphyrin CVs for the three neurotransmitters at 10^{-7}M (color online)

The reversibility of the reaction is improved for the SWCNT/Mn-porphyrin and SWCNT/Zn-porphyrin. In the CV for the SWCNT/Mn-porphyrin system dopamine is still the major contributor, followed by epinephrine and serotonin (Fig. 7). If dopamine (which is positively charged) is easily electrochemically oxidized, serotonin has a reactive oxidation product that develops an insulating layer on the electrode surface, therefore decreasing sensitivity over time. Epinephrine is still more difficult to be identified by CV due to its inappropriate specific molecular recognition elements [5]. This can be observed in the cyclic voltammetry diagram (Fig. 6) by following the oxidation direction of epinephrine: when the potential increases and oxidation begins, this forward scan covers with the maximum of oxidation for serotonin. When the oxidation stops and the potential begins to drop, this decreasing scan overlays the decreasing oxidation curve of dopamine. Even if the kinetics of the reaction are improved, the low dissociation of the oxidation peaks (between serotonin, dopamine and epinephrine) represents a major drawback for its use as an electrochemical sensor.

The reduction curves show two similar peaks for the serotonin and epinephrine and a single broad reduction peak for dopamine.

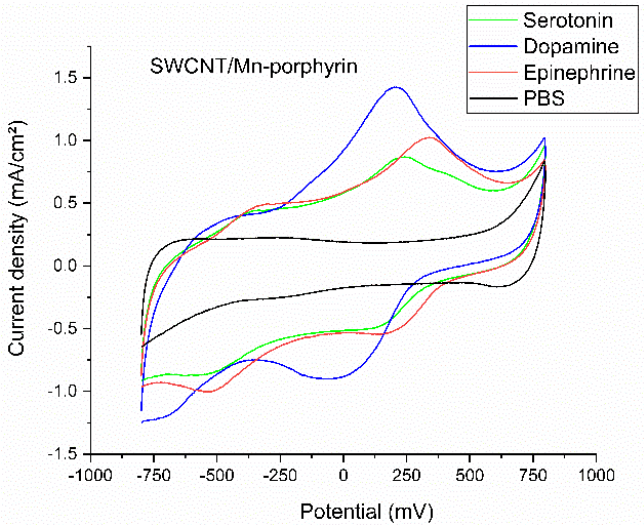

Fig. 7. SWCNT/Mn-porphyrin CVs for the three neurotransmitters at 10^{-7}M (color online)

The SWCNT/Zn-porphyrin shows the most accurate identification of neurotransmitters (Fig. 8). The oxidation potential for the three neurotransmitters can be easily determined (275 mV for dopamine, 326 mV for serotonin and 225 mV for epinephrine). Although the reduction peaks for serotonin and epinephrine have a low intensity and indicate a quasi-reversible to irreversible reaction, the reduction peak corresponding to dopamine is similar to the corresponding oxidation peak.

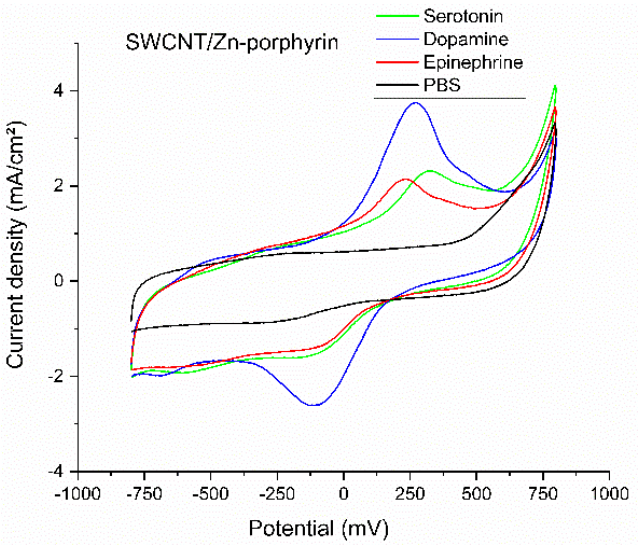

Fig. 8. SWCNT/Zn-porphyrin CVs for the three neurotransmitters at 10^{-7}M (color online)

4. Conclusions

Herein we explore the electrochemical response of SWCNT-metalloporphyrins biohybrids. The immobilization of metalloporphyrins on the SWCNT surface has proven to result in novel electron donor–acceptor biohybrid structure with a good selectivity for

neurotransmitters. SWCNT and porphyrin functionalization of gold-based SPE allows tailoring of the original sensing characteristics by decreasing the oxidation potential and lowering concentration limits, properties that can further be employed in the diagnosis of neurotransmitter-associated diseases. We demonstrate that only a specific Zn-porphyrin-SWCNT couple is appropriate for an electrochemically sensitive response when they are immobilized on gold screen printed electrode (Au-SPE).

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