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# Electrochemical Hybrid Bio-sensors for Neurotransmitters Analysis

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***Abstract*** **-** Biosensors are devices that detect and transmit information regarding a physiological change or the presence of various chemical or biological materials in a given environment. A new concept of electrochemical biosensors, directly related to the biological redox processes at the cellular and subcellular level, is proposed for testing microfluidic probes, in order to investigate and treat neurological disorders. The micro-electrode features at nanoscale are based on incorporating via electropolymerization of oxidized conductive conjugated polymers at the surface of carbon structures with predefined architectures at nano-scale. The electrochemical characterization of novel micro-electrodes was performed by using reference redox molecules. The final sensorial features regarded the concept sensitivity and reproducibility at different concentrations of dopamine, epinephrine and norepinephrine in acid medium (0.1 M HCl). In order to tailor the hybrid bio-sensors for extended medical applications, potential interferences within biological samples of ascorbic acid, uric acid etc. were also taken into account. Finally, a functional model was developed, which is now under testing for medical sector exploitation.

## I. Introduction

The development of biosensor-based medical devices for diagnostic and therapeutic applications in healthcare may be crucially enabled by the present parallel development of new technologies: nano- and micro-scale structures, biocompatible materials etc. The medical market is interested in promoting new concepts of nano-bio-sensors specialised mainly in the detection of biologically important neurotransmitters/markers. The neurotransmitters are involved in the functioning of renal, cardiovascular, hormonal and nervous systems, but were also related to neurological diseases such as Parkinson's, Alzheimer's and schizophrenia, were found relevant in neurodegeneration, neuroblastomas and adrenal gland cancer, and are supposed to play a role in drug addiction and some manifestations of HIV. The microelectrodes to be developed should allow subsecond measurements during neurotransmission with minimal tissue damage and must be related to the biological redox processes at the cellular and subcellular level - intended to be put in evidence. Therefore, the R&D efforts have been orientated towards finding a sensitive, selective and reproducible method for the quantification mainly of dopamine derivates. Several analytical methods for biological applications are now in use to determine dopamine. These techniques usually work by simultaneously detecting dopamine and a wide range of other compounds and some of them are used in conjunction with each other and with electrochemical techniques. Examples of such techniques include flow injection analysis, microchip capillary electrophoresis, high performance liquid chromatography, fluorescence spectroscopy. Since the normal level of dopamine in blood or in urine is 0.01-1 μML-1 and 65-400 mcg/24 hours respectively, determination of this molecule is quite difficult in practice, and timely dependant. Dopamine is present not only at low concentrations in the human body, but also together with other electroactive compounds e.g. such as ascorbic acid, which may interfere with the dopamine signal due to their often higher concentrations and lower oxidation potentials. Electrochemical detection of dopamine should be preferred, because it offers advantages such as simplicity, speed, and sensitivity. The technique must be also quick, inexpensive to run, and have a high degree of selectivity under different bio-chemical media. Electrochemical sensors are usually seen as inexpensive to manufacture, highly sensitive and easy to employ. The actual dopamine biosensor concept is 98% based on metallic electrodes, i.e. Au and Pt in different configurations, [1]. In the last 5 years, new attempts occurred in order to increase the performance of such biosensors, i.e. mainly by modifying the electrode surface to increase the sensitivity for low/very low dopamine contents around 1 nM. Different approaches have been tested, such as use of protective films (Nafion), overoxidizing of polypyrrole, of poly(1,2-phenylenediamine), of poly(3-methylthiopene), or of polythiophene, and eventually use of self-assembled monolayers of poly(2-picolinic acid) etc. Unfortunately, such solutions are not entirely reliable and reproducible when associated to metallic electrodes. The drawbacks of actual attempts are in summary: the complicate concept of electrode, hard to be made technologically reproducible; large variation of electrode sensitivity and precision within uncertainly defined media; very

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expensive, but impossible of being reused by cleaning (i.e. surfaces are very sensitive and can be detrimentally affected during manipulation); hard to be submitted to Lab-on-chip concept; practically impossible of performing clear endurance tests, including mechanical, fatigue and adhesion – essential for market implementation. When dopamine is oxidized on metallic electrodes, the oxidation products irreversibly bound to the electrodes surface during the analysis. As a result, the regeneration of electrode surface becomes a crucial problem. A concept of cost-effective disposable electrodes may overcome this drawback. On the other hand, the carbon fibers have been shown to be compatible with physiological tissues and not toxic to cells. The use of carbon fibers for dopamine detection was tried by some research groups in the last 3-4 years, but the large scale solutions mainly resumed to simple carbon fibers, that provided not enough sensitive and selective, in spite of their clear advantages comparing to metal or metal/hybrid similar electrodes. If made technologically available, hybrid carbon/polymer micro-sensors may have clear advantages as regards measurements sensitivity and reliability, comparing to conventional metallic or carbon electrodes, and may represent a consistent step forward. It was shown that carbon nano-structures can be formed with unique architecture and tailored dimensions, which together with their specific chemical, electronic and mechanical properties make them interesting for development of new micro-electrodes. Typical dimensions of such carbon fibers range from 7-20 µm in diameter and 400-800 µm in length, but by controlled chemical vapor deposition techniques, nano-meter size fibers can be also obtained. On the other hand, conductive polymers provided already a high electrocatalytic capacity, by reducing the necessary potential for oxidation or reduction of numerous molecules. Accordingly, the incorporation via electropolymerization of such polymers - as charge-balancing (e.g. pyrrole derivatives, poly (3,4-ethylenedioxythiophene), poly(styrenesulfonate) and their copolymers), at surface of carbon nano-structures represents an important solution on the way of developing new sensing concepts for neurotransmitters.

## II. Experimental results and Discussions

The main method of synthesis was the tailored electropolymerization at carbon nano-structure level. An oxidation current was applied, resulting in free-radical cations that are coupled together and give rise to polymer repetition, further being chemically bonded to the carbon electrode surface. The electropolymerization is a reproducible process, with high control on the architecture and thickness of the polymer film, [2].

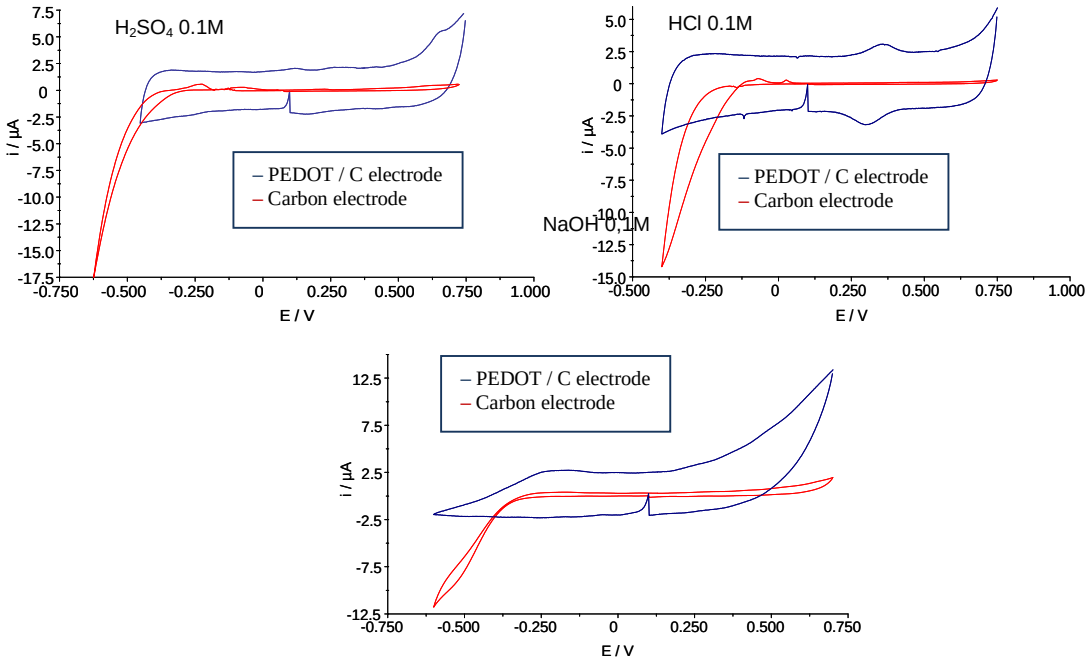


Figure 1. Electrochemical behavior of PEDOT/carbon electrode (blue line) and carbon electrode (red line) in different experimental electrolytes.

In Figure 1, the electrochemical activity of hybrid PEDOT - poly (3,4-ethylenedioxythiophene)/carbon electrode vs. carbon electrode are comparatively presented within different chemical media, emphasising the superior

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sensitivity of hybrid electrodes. The mechanical, fatigue and adhesion properties of the experimental coatings were assessed by using nanoindentation, high frequency impact and scratch specific tests. The images of microelectrodes structures for different conductive polymers and different electrocoating conditions are presented in Figure 2, emphasising the variety and stability of architectures at nano-scale. The electropolymerization is obviously a simple and effective process (may be done even at room temperature) and reproducible ar regards micro-electrode architecture, offering a very high control on the layout and thickness of the polymer film (by controlling the application time of the current or potential during electrocoating process).

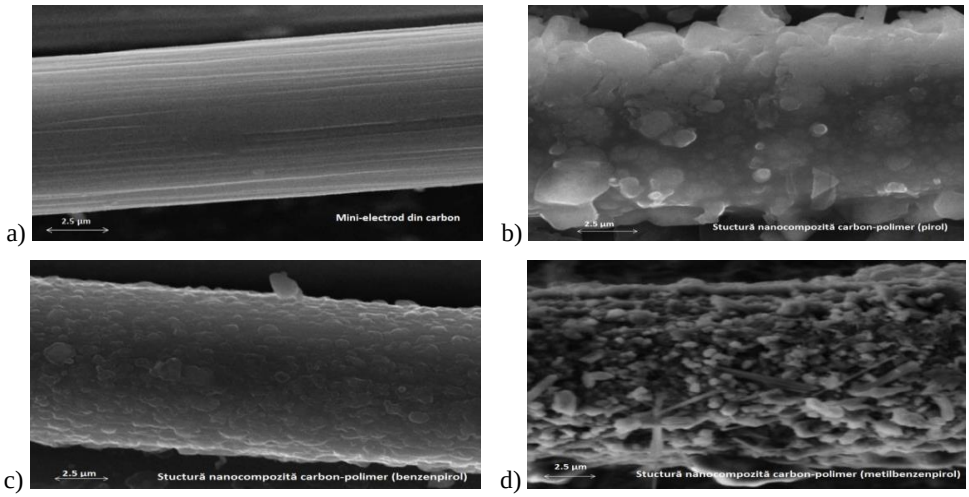


Figure 2. SEM images of hybrid micro-electrodes with different conductive polymer and electrocoating conditions (a. – carbon fiber; b. – d. different pyrrole derivatives)

The electrochemical characterization of working microelectrodes (via Potentiostat/Galvanostat cyclic voltammetry method) was performed by using reference redox molecules, i.e. the reference electrode was externally calibrated using 5mM ferrocene electrolyte solution. The electrochemical oxidation of dopamine on hybrid microelectrode was recorded in 0.1M phosphate buffer solution, pH = 7, using cyclic voltammetry by 2 scans. In Figure 3, the electrochemical responses of normal and hybrid polymer / carbon microelectrodes in buffer, and respectively buffer with dopamine solution, related to the potential range between -1V and 1V, are comparatively analysed. The preliminary results were very spectacular, i.e. the hybrid electrode is sensitive at dopamine, unlike original normal carbon microelectrode which is completely insensitive.

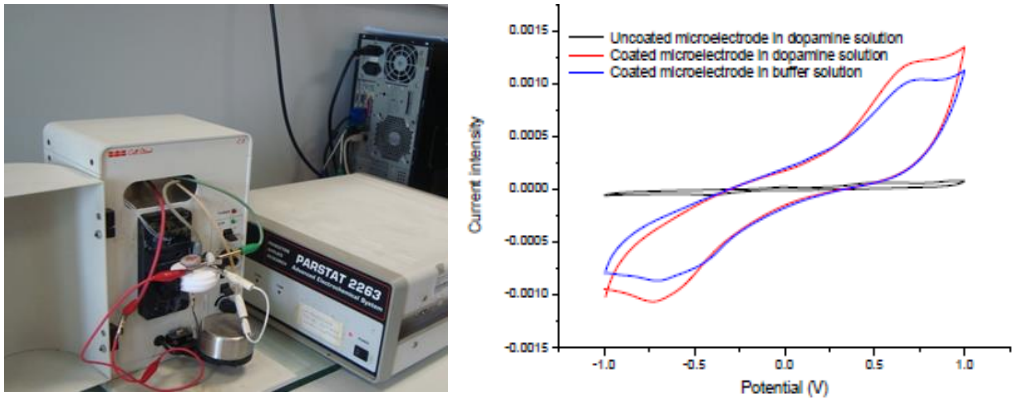


Figure 3. Cyclic voltammograms for: uncoated carbon microelectrode in dopamine solution (black line); hybrid microelectrode in dopamine solution (red line) and hybrid microelectrode in buffer solution (blue line).

Extended tests on different types of hybrid electrodes emphasized a high sensitivity and reproducibility at different concentrations of dopamine, epinephrine and norepinephrine in acid medium (0.1 M HCl), but provided also selectivity to each studied molecule. The adsorption is sensitive to a variety of kinetic responses, which cause different preconcentrations of analyte on the electrode surface, prior to oxidation. Therefore, by use of

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adequate conductive polymer coating and chemical conditions of testing, such electrodes can be used in the detection of other neurotransmitters too, e.g. catecholamine. Similar examples are provided in [3-5].

## III. Sensors manufacturing

In order to finally tailor the hybrid bio-sensors for medical applications, the potential interferences within biological samples of ascorbic acid, uric acid etc. were also taken into account, and the results emphasized once again the superiority of hybrid electrodes comparing to metallic or conventional carbon ones. An example of experimental sensor realised as a micro-array of 140 hybrid microelectrodes of an average of 150 µm length and 7µm thick, with about 10 µm between two neighbor electrodes, was tested via cyclic voltametry for dopamine concentration between 0.02 mM and 20 mM. The results are presented in Figure 4. The obtained results for all laboratory experiments have finally shown a high electrocatalytic capacity, higher signal intensities and improved analytical electron transfer and versatility of the concept.

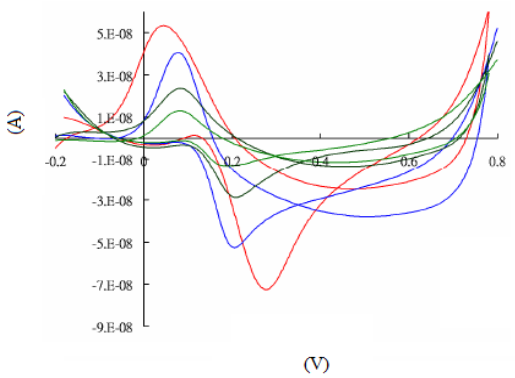


Figure 4. Example of cyclic voltamogrames of hybrid sensor for dopamine concentration of: 0.02 mM (green line), 0.2 mM (black line), 2 mM (blue line) and 20 mM (red line)

The scaling up of the technology for sensors microproduction was an important step, presuming the support material (may be thermoplastic or ceramic) generation with embedded electrodes, carbon array functionalization with conductive polymers [6], final product finishing and testing. An example of manufacturing chain is presented in Figure 5, and tested in partnership with an important biosensor company. The sensors, realized under 'disposable' concept allow the direct access to the three electrodes (reference, counter and respectively working hybrid electrode), the electrochemical detection test circuit being briefly presented in Figure 6.

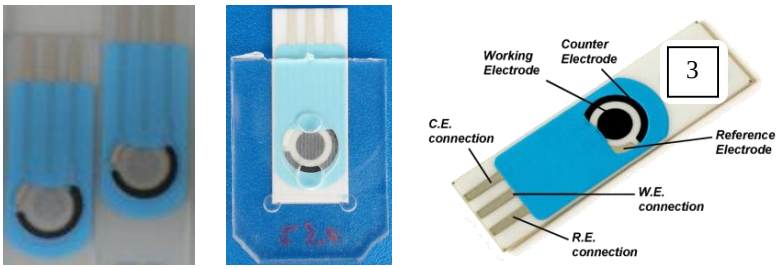


Figure 5. Example of Disposable hybrid microelectrodes manufacturing

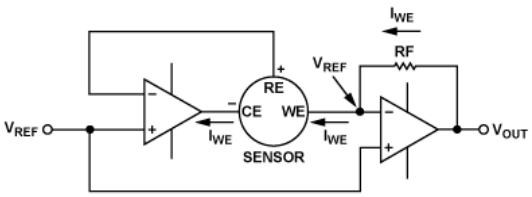


Figure 6. Example of a Simplified electrochemical detection circuit

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The final step in developing the biomedical application was the integration of hybrid sensor with a simplified electrochemical sensing circuitry, to achieve the portability and a reasonable cost, comparing to the actual laboratory variants of potentiostats. At present, a very good result was achieved by use of LMP91000 - programmable Analog Front End Potentiostat, Figure 7. The functional model is now under testing for medical sector exploitation, but the same concept can open also new perspectives for forensic medicine, or security / environment applications. The future research interests are oriented on superior functionalisation of carbon structures al nano-scale, by use of carbon nano-tubes or graphene instead of microfibers, as in [7].

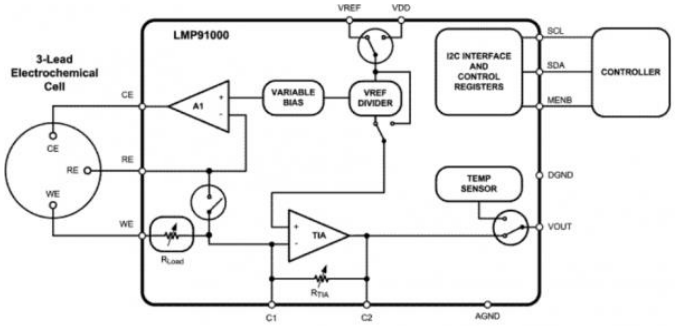


Figure 7. The description of LMP91000 - programmable Potentiostat

## IV. Conclusions

The study has shown that the incorporation via electropolymerization of conductive polymers - as charge-balancing (e.g. pyrrole derivatives, poly (3,4-ethylenedioxythiophene), poly(styrenesulfonate) and their copolymers), at surface of tailored carbon nano-structures represents an important solution on the way of developing new electrochemical microelectrodes concepts. The tests have shown that the obtained hybrid morphology exhibits smooth curved surfaces, and the architecture and electrochemical features are clearly dependent on experimental parameters of electrocoating, offering an important breakthrough to diverse bio-medical applications. Electrochemical characterization of selected neurotransmitters oxidation on hybrid microelectrodes can be successfully optimized using fast scan cyclic voltammetry. The hybrid disposable bio-sensors are suitable for integration with simplified electrochemical circuitry, e.g. programmable potentiostats, to achieve the portability and a reasonable cost for detecting neurotransmitters under different chemical and clinical conditions - comparing to the actual laboratory variants of potentiostats.

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