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ELECTRICAL CHARACTERIZATION OF THE EPINEPHRINE SOLUTION ON NATURAL DIAMOND WITH POSSIBLE BIOMEDICAL APPLICATIONS

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The natural diamond, frequently used in the jewelry industry, presents a large color spectrum, starting with light transparent blue and finishing with black diamond. In fact, the color is straight related to the boron content, starting from 10^{15} cm^{-3} up to 8×10^{21} cm^{-3}. Additionally, a special electrical conductivity was demonstrated for the diamond surface. This was the main reason to investigate in this paper, the current over-voltage characteristics in DC regime, using a test device with epinephrine or a buffer NaCl aq. solution, placed on a light transparent blue diamond. This natural Boron Doped Diamond (BDD) produced a sensitive and quite reproducible I – V curves. The stability was superior to a metallic electrode device, but still weak, very probable due to the poor conditions of the biodevice construction. However, from the stabile regions, some parameters of the epinephrine / Diamond biodevice were extracted. Finally, some applications of the diamond microelectrodes in biomedical field are discussed.

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1. Introduction

The approaching between electrochemistry and electronic devices provides molecular devices with atypical electrical characteristics. In a previous work, a two terminal biodevice using the electrochemical chain Sn/Epinephrine aq. solution/ Sn, was studied [1]. Interesting characteristics with negative differential resistance were achieved. Unfortunately a high non-reproducibility and instability were remarked. The chemical inertness of the diamond and gold electrodes suggest us to study the DC regime behavior of a biodevice based on the following electrochemical chain: Au/Biosolution/Diamond /Au. The molecular part of the biodevice consists in epinephrine or aqueous salt solutions with the same concentrations for comparison.

On the other hand, the natural diamond presents insulator behavior for Boron concentration under $N_B=5\times10^{14}$ cm^{-3}, semiconductor p-type properties for $N_B=10^{15}\div10^{19}$ cm^{-3}, semi-metallic behavior for $N_B=8\times10^{19}\div8\times10^{21}cm^{-3}$, [2]. Heavily B-doped diamond synthesized by HPHT technological method reaches $N_B= 10^{21}\div8\times10^{21}$ cm^{-3} Boron concentration and exhibits a type II superconductor activity at T=2.3K (zero resistance), [3]. Additionally, Boron Doped Diamond (BDD) crystals indeed show a dependence of the surface electrochemical activity on the crystallographic planes [2]. For instance, <111> crystals presents a better conductivity than <100> type, due to a higher carriers density. The diamond used in this work was micromachined in the jewelry industry, having a multifaceted surface.

Carbon – the main living being element – makes from Diamond an excellent biocompatible material. It was demonstrated that a Hydrogen terminated diamond surface is suitable for the covalent protein (amine) binding, while an Oxygen terminated diamond surface is excellent to immobilize some enzyme (catalase) without any damage in the enzymatic activity [4].

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Epinephrine is a neurohormone secreted by the adrenal medulla, which belong to the catecholamine class. Chemically, adrenaline (the Latin name of this neurotransmitter) comprises an aromatic cycle – "catechol" – to which is attached a nitrogen containing group – "amine" the condensed formula being: $C_9H_{13}NO_3$. This is an additional reason to believe that this kind of catecholamine is covalently binding to the natural diamond surface, [4].

2. Diamond microelectrodes for biomedical applications

Diamond microelectrodes were successfully used in the epinephrine concentration monitoring from the blood plasma. They can be directly implanted in small vessel, making possible the contact with blood components for in vivo measurements, [5]. Due to its semiconductor property, the diamond electrodes can produce an electrical innervations at neuro-muscular junction in order to stimulate the epinephrine secretion. In this case, the BDD microelectrodes work as electrochemical biosensors and must fulfill specific conditions: sensitive, reproducible and stable responses. Diamond satisfied these requirements for the oxidative detection of norepinephrine (another neurohormone, precursor in the epinephrine metabolism), with no evidence of response deactivation by the biomolecule adsorption.

In this paragraph, another type of application will be discussed for the diamond electrodes: as probes for some biosignals registering. In medical applications, the electrodes must fulfill some conditions for good working.

2.1 Reduced noise.

Obviously, the electrodes that comprise semiconductors instead metals will benefit from lower noise. The thermal noise (Nyquist) can be reduced using lower sizes for semiconductors, and higher doping concentration in order to minimize the series resistance series, R_S: $I^2_{Nyquist} \sim 1/R_S$.

All others noises (shot noise, generation-recombination noise, flicker noise) are depending on the carriers concentration. Therefore an increasing doping can favorite other type of noise then thermal. A trade-off is required. Among semiconductors diamond has the minimum amount of intrinsic carriers, hence most protected at noise; unfortunately it is the most expensive.

2.2 As small as possible. The bioliquid/electrode impedance, Z.

The identical problem in the electronic devices area is manufacturing of the ohm contacts. For in vivo biosignals recording, the impedance has a real part - a resistance that models the Electrode/Tissue current flow, R_{ET} and an imaginary part - a capacitive reactance that models the Double-Layer formed at the electrode / bioliquid junction, C_{DL}. For the skin biosignal registering, like EEG, EKG, a chemical mediator is used to minimize the resistance R_{ET}. By skin washing with alcohol and NaCl aq. solution (0.9%) used as mediator and Ag as electrodes, R_{ET} decreases from 800kΩ in wet skin case to 500 Ω, [6].

A microelectrode Ag/Silicon/Bioliquid/Ag type develops a native SiO_2 layer at Si-surface, passivating the Si surface and increasing the impedance. Diamond has the advantage to be a semiconductor, but it is chemical inert, in opposite with silicon. BDD presents an enhanced conduction at surface, consequently with a lower resistance R_{ET}.

Because the biosignals recording needs low-frequencies of operation (f<1kHz) (e.g. for EEG), the capacitive reactance can be minimized just increasing the capacitance, C_{DL}. Traditionally, there was an unique method: to increase the metallic electrodes area. Concerning the microelectrodes for biomedical applications must be taking into account semiconductors materials. The main competitor is silicon, [8]. A higher dielectric constant of diamond versus silicon is one of the advantages. The classical metallic electrodes can present higher values for C_{DL} (e.g.10÷100μF/cm^2) using trench etching in metal in order to increase the electrode area. Diamond technology lends oneself to multifaceted surface. Even our biodevice with jewelry diamond has multifaceted shape. These mean an increased area compared to a plate silicon surface.

3.Chemical inertness

Diamond represents a very stable electrode without corrosion or other secondary reactions. During electro-measurements, (e.g. brain surgery that uses special spherical Ag electrodes since to provide the cortex biopotentials), a lot of secretions are released at tissue/electrode interface, like glucose or urea or neurotransmitters, producing electrode corrosion. In order to avoid this drawback for measurements in DC regime, some non-biasing electrodes from glass are used, [7]. Unfortunately, the electrode impedance is depending on the material. At low frequencies, specific for living beings, were demonstrated the impedances values: Z = 80 kΩ for Ag, Z = 300 kΩ for Cu, Z = 800 kΩ for stainless steel, or Z = 100 MΩ for glass, great disadvantage for glass. The industrial solutions was microelectrodes from non-reactive metals like Pt, Ag, Au, steel, covered with thin layers of insulators like glass, which give rise Z = 100 kΩ.
BDD is an ideal candidate for chemical inertness and low impedance material for biomedical electrodes.

4. Optical properties

One of the main disadvantages of the conventional electrodes is that metals (Pt, Au, Ag) or Si are opaque for X-rays and didn't allow a good visibility during some procedures, like cardiac catheterizing The good transparency of diamond recovers this deficiency.

5. The miniaturization possibilities

Besides to silicon micro-machined electrodes, diamond is suitable for the Integrated Technologies, making possible the manufacturing of some MEMS or NEMS complex systems with eventual integrated devices in the diamond film.

6. As linear as possible. The transducer function current-concentration

Our previous experiments with Ag, Sn and stainless steel electrodes in contact with NaCl and epinephrine aqueous solutions have demonstrated a high non-linearity, especially in the zones with very different, but stables slopes in the diamond/Au case. Hence, this 6-th target was proved in diamond favor with this paper.

7. A price as low as possible

A biodevice based on a diamond electrode has a price proportional with the diamond quantity. Taking into account the general tendency toward nano films, can be expected that in a few years a nano-electrode with diamond becomes comparable as price with its classical relatives due to its superiors properties. The diamond biodevice cost will decrease from nanometer quantities of diamond, up to a few monocrystal atomic layers.

8. The biodevice construction

The biodevice comprises a jewelry diamond fixed on a gold ring, fig.1. In order to avoid contacts liquids/Au socle, a layer of insulator has covered the gold edges. A drop of hot and liquid paraffin was let to drip on entire surface with diamond and gold socle. After 2 minutes the paraffin is frozen and completely adheres to all the surface non-regularities. With a needle is scratched the insulator just over the diamond surface. Then with a piece of cotton imbued with metilic alcohol and a thin pin, the diamond surface is cleaned. The cavity on diamond has a width equal with 2 mm since to sustain in good condition a drop of bioliquid, Fig.2. Above this structure a gold electrode

was placed at different distances d=3 or 4 or 5 mm. This entire structure was electrically insulated being fixed in a plastic material, fig.1.

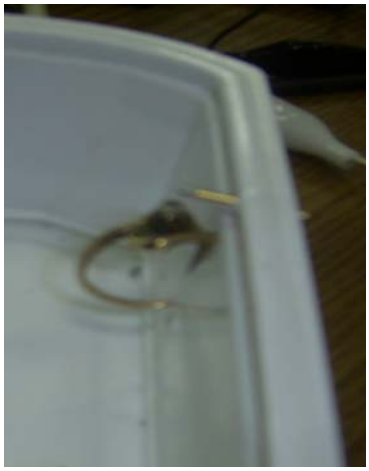


Fig.1. The biodevice ensemble picture.

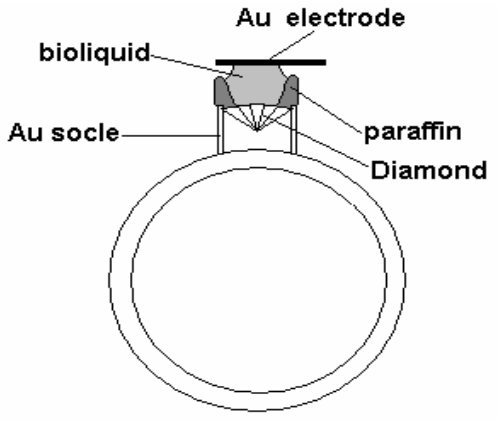


Fig.2. Details of the main components of the test biodevice.

The ring was grounded while the upper Au electrode, in series with a high value resistance was connected to the high potential borne of a DC power supply.
An epinephrine ampoule from pharmaceutical usage was broken in the moment of experiment. Besides to a NaCl aqueous solution, they were uploaded in two syringes, while the air was completely evacuated. The prospectus of a SICOMED S.A ampoule indicates a concentration of 1 mg epinephrine hcl/ml aqueous solution. The NaCl aqueous solution was prepared solving 1 mg of salt in 1ml distilled water.

9. The biodevice stimulation with DC signal

In the moment of experiment a small quantity of epinephrine/salt solution let drop from syringe in the cavity, so that the liquid quantity ensures contacts both with diamond and upper Au electrode. Immediately the external DC power supply was raised from 0V up to 1V with 0.1V/5 sec and from 1 V up to 20 V with 1V/10 s.

A HAMEG HM 8001-2 multi-meter measured the potential drop over the resistance R = 110 kΩ (Fig.3). Knowing the DC power supply voltage, V_{DC}, the potential over biodevice, ε_b, results, fig.3. The current that pass the biodevice is computed from the Ohm's law:

$$I_b = \frac{V_{DC} - \varepsilon_b}{R} \tag{1}$$

The dependence of the biodevice bias versus the independent parameter is represented in fig.4.a. A very poor difference occurs when V_{DC} was scanned from 0 V to 20 V for epinephrine and NaCl aq. solutions. Also no hysteresis occurred. This feature is an additional proof of very stable behavior of the biodevice in the case of diamond / epinephrine electrode in respect with the case of Sn/epinephrine electrode, fig 4b.

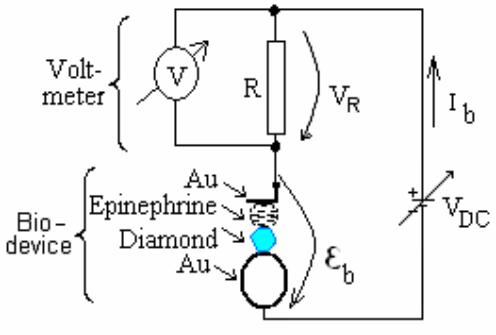
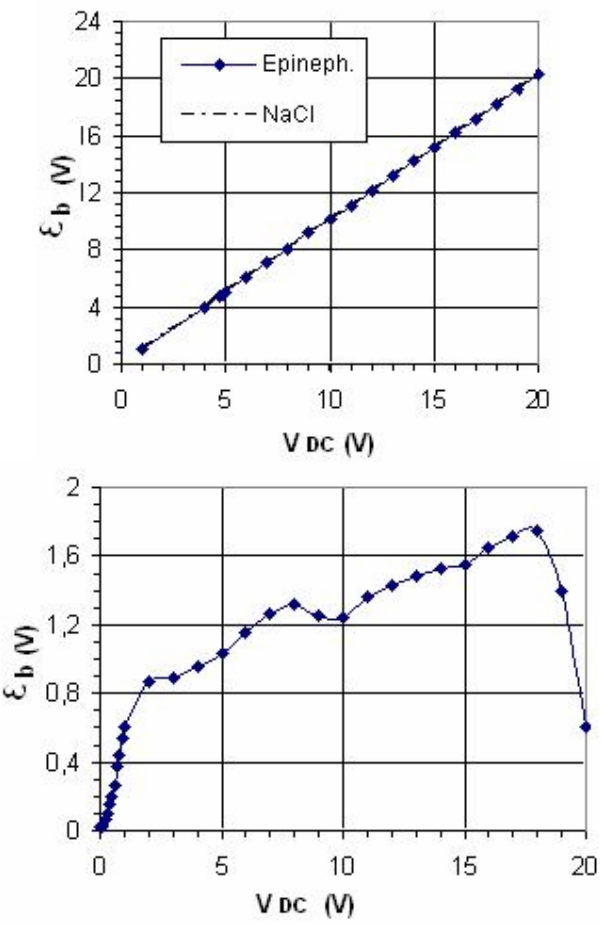


Fig.3. The measuring circuit.



(a) (b)

Fig. 4. The dependence of the electrode over-voltage (the electrochemical term) or biodevice bias (the microelectronics term) versus the DC power supply voltage: a) for Au/diamond/epinephrine/Au chain, b) for Sn/epinephrine/Sn chain.

The distance of the upper electrode was fixed firstly at d = 3 mm, so that the minimum quantity of liquid are uploaded in this case. Then the distance was raised at 4 and 5 mm, increasing the liquid volume in biodevice. Fortunately, this distance variation roughly didn't affect the I_b current during the V_{DC} scanning, fig.5.

Then the distance d was fixed at d = 3 mm and the biodevice bias monitories by turns for epinephrine and salt solutions, scanning V_{DC} between 0 V÷20 V. Figure 6 comparatively presents the DC characteristics measured for the biodevice with the Au/diamond/solutions/Au chain, where solutions were previously mentioned.

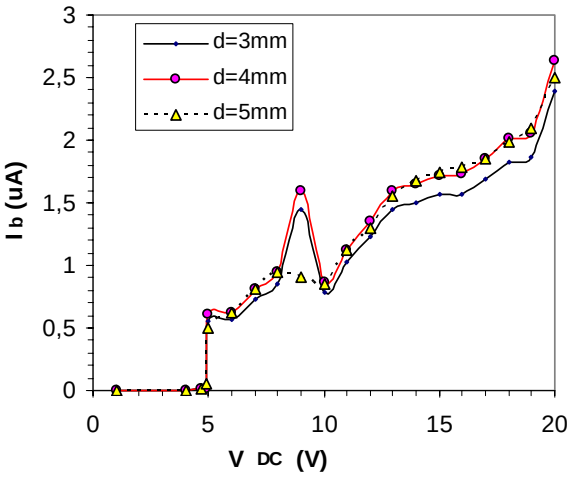


Fig.5. a) The I_b- V_{DC} variation for d= 3, 4, 5mm.

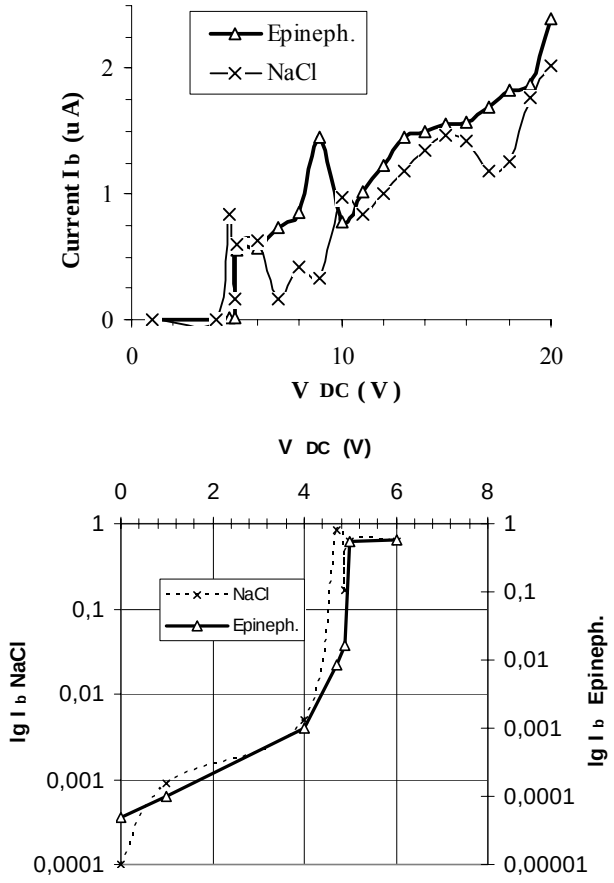


Fig.6. The dependence I_b- V_{DC}: (a) at linear scale for whole range; (b) detail at logarithmic scale, low voltage.

As was expected, the I_b – V_{DC} curves presents high non-linearity. Some peaks are simultaneously registered in fig.6.a for epinephrine and salt solution, in the V_{DC}=9V and 20V vicinity, very probably due to the biodevice construction (multifaceted diamond surface or others particularities). Avoiding these peaks the I_b – V_{DC} curves were very stable, having a constant slope for $V_{DC} \in (10,19)$V. Interesting is the characteristic zone under V_{DC}=5.2V, where I_b seems to be zero. In fact it decreases with high orders of magnitude as in the pn junction case, fig.6.b. This similar

behavior is due to the double layer forming at the diamond/bioliquids interface that acts as two opposite electrical charge sheets, as in the case of the space charge region arisen in a pn semiconductor junction vicinity of a pn semiconductor structure.

10. Extraction of some parameters

From the experimental data provided in fig. 6.a. can be traced the logarithmic dependence ln I_b- V_{DC} in fig.7. Selecting a straight line among these points by the regression method, it can be computed some parameters.

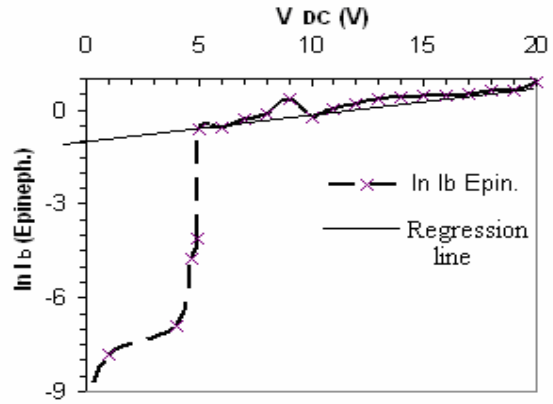


Fig.7. The experimental points ln I_b- V_{DC} and the regression line among these points.

An analytical model of the lnI_b- V_{DC} for a redox reaction is given by the Tafel's relation, [5]:

$$\ln I_b = \ln I_0 + \frac{\alpha z q}{KT} \cdot V_{DC} \tag{2}$$

where I_0 is the saturation current, ε is the overvoltage, z = 2 represents the electron number exchanged by adrenaline during the oxidation process, RT/F=kT/q=0.025 V at usual temperature T=300K, α is the transfer coefficient. From the intercept of the regression line with the vertical axis, the saturation current yields: I_0=0,36μA. From the slope of the same line, which is represented in fig.7, the transfer coefficient yields: α=0,17. From the electrochemistry theory, a model for the saturation current is [5]:

$$I_0 \cong F \cdot z \cdot k_{ox} \cdot [c_{ox}] \tag{3}$$

Knowing the Faraday's constant $F = q \cdot N_A = 9.6368 \cdot 10^4 C \cdot molec / mol$, z=2, [$c_{ox}$]=1mg/ml – the analyte concentration, from the relation (3), the oxidation rate for adrenaline can be computed: k_{ox}=0.286 nl/s that is inferior in respect with the Sn/Epinephrine cell, where k_{ox} was 0,465nl/sec, [1]. This proves a better inertness of this electrode. The epinephrine concentration was expressed in molarity as [c_{ox}]=5.46 × 10^{-3}M.

From electrical point of view, a relevant parameter is the differential resistance of the biodevice, defined as:

$$R_{diff} = \frac{dV_{DC}}{dI_b} \tag{4}$$

The first order derivative represents the slope of the measured curve I_b – V_{DC}. From the experimental values from Fig.6 ⇒ R_{diff} ≈ 7MΩ for V_{DC}>5V and R_{diff} ≈ 2GΩ for V_{DC}<5V. This makes from the proposed biodevice a commanded resistance in voltage.

11. Conclusions

In this paper a molecular device using epinephrine aq. solution 1mg/ml or physiological serum from pharmaceutical usage, placed on Diamond from the jewelry industry, was experimentally investigated. A non-linear response is expected due to the double layers developed at Diamond-Bioliquid and Au-Bioliquid interfaces. The experimental static characteristics available in Fig. 6 proved that till 5V the current is essentially zero, then a non-monotonically increasing was observed. In the case of Sn electrodes and Epinephrine placed on Plexiglas, a huge hysteresis occurred. A poor hysteresis occurred in the case of Biosolution of epinephrine on Diamond if the overvoltage was scanned up and down.

On the other hand, for in vivo medical applications the diamond electrodes with some bioliquids presented a better linearity then simple metals. This target is desired if some weak biosignals usually affected by noise, must be recorded without distortions. In this way, the concentration variation is correctly translated in non-distorted current variations in order to be transferred toward an input amplifier stage. The diamond is the most biocompatible material with the living matter and avoids a lot of drawbacks of the classical microelectrodes: the impedance can be modulated by boron doping concentration, the electrode surface can be increased by multifaceted planes, the optical transparency is high enough and the costs tend to decreases in the following years due to ultra-low quantities of diamond that have to be used in nanoelectrodes, nanodevices or NEMS.

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