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Integrated p-NOI Structures on Nanoporous Material Designed for Biodetection

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Abstract

Pesticides are utilized to protect the crops, destroying or controlling any pest. Unfortunately, pesticides pollute the entire environment: plants, organisms, soil, and water. This chapter describes a paraoxon pesticide biosensor that includes nanostructures and porous materials integrated on silicon (Si), as convergent objectives of the green microelectronics strategy. The transducer element is in an interdigitated capacitive electrode that recently highlighted a special nanostructure—the planar nothing on insulator (p-NOI)—included in the capacitive detection system. The biodetection is based on the hydrolysis of the acetylcholinesterase (AChE) enzyme as biosensor receptor. So, the final application is an enzymatic biosensor that utilizes the nanoporous Si layer for the enzyme adsorption, with p-NOI capacitive transducer, for the environmental monitoring.

Keywords: biomaterials, electronics, nanotechnologies, biosensor tool, green environment

1. Introduction

Green industries imply few research directions: (i) an industry based on recycling the unwanted resources that take care from the beginning of the company construction to conceive a sustainable fabrication process flow; (ii) a traditional industry that becomes sensitive to environment protection, minimizing the waste and pollution; or (iii) a top industry that provides useful tools for the environmental conservation and monitoring.

The nano-biotechnology-based industry which falls into the last category can produce pesticide biosensors, wastewater detectors [1], micro-nano-filters for air-water-soil cleaning [2], and pathogen biosensors using nanomaterials such as metal nanoparticles, quantum

dots, magnetic nanoparticles, carbon nanotubes, and graphene due to their surface properties, excellent electron transfer, and a large ratio of surface area to volume, making them particularly attractive for use in labels or transducing platforms for optical or electrochemical sensors and biosensors.

The examples of such biosensors are the organophosphorus pesticides using liposome-based nano-biosensors [3]. Gold nanoparticles for pesticide detection using cyclic voltammetry [4], organophosphorous pesticide (OP) biosensor based on quenching of the fluorescence from CdTe QDs [5]. Acetylcholinesterase action is monitored using a localized surface plasmon resonance (LSPR) fiber optic biosensor [6]. AuNP-AChE conjugates for paraoxon electrochemical biosensor [7]. AuNP-AChE onto chemically reduced graphene nanosheets (cr-Gs) [8], graphene oxide/Nafion (RGON) nanohybrids electrochemical biosensor platform to detect organophosphorus hydrolase as an enzyme for the hydrolysis of Ops [9], pathogen detection in soils using nanobiosensors [10, 11].

Unfortunately, the pesticides used in this field not only spoil the soil but also infest the entire food chain. Less toxic new generations of pesticides may reduce the risks transmitted to people and environment, especially by water contamination. The pesticides reduce the nitrogen fixation in plants, consequently decrease the biodiversity, destroy habitats, and threaten jeopardized species [12]. Integrated biosensors usually contain onto the same chip of the semiconductor solid-state support, the transducer as an electronic device, and the biological detector as an enzyme [13] or an antibody [14].

This chapter describes a pesticide biosensors fabricated using nanoporous Si materials to entrap the receptor element, along with the transducer element consisting of an interdigitated capacitive electrodes to detect pesticides, like paraoxon. The novel detection scheme is using interdigitated capacitive electrodes which highlighted a special nanostructure called as the planar nothing on insulator (p-NOI) [15, 16]. The biodetection is based on the hydrolysis under the acetylcholinesterase (AChE) enzyme action, as biosensor-specific receptor [17]. The final product is an integrated biosensor that is constructed by microtechnological processes aided by biotechnological enzyme processing steps, having a nanoporous Si layer coupled to a p-NOI capacitive transducer, which is sensitive to the pesticide concentration.

The p-NOI structure that is integrated inside the biosensor transducer has another facet in this work: the first p-NOI structure still exists between top metal on insulator placed on silicon, and the second p-NOI structure is present between two adjacent lateral metal fingers. The first one must accomplish an isolation through the bottom nanoporous material. The second one has the distance between fingers high enough versus a nanometric p-NOI that allows a tunnel current flow [15]. Hence, the tunneling conduction is missing in this case. But the liquid droplet that connects two adjacent fingers by an ionic conductor offers a novel conduction route.

2. The work principle and simulation results for a p-NOI structure

Recently, the nothing on insulator (NOI) device, as the succession n-Si/Vacuum nanocavity/n-Si (nVn) on insulator, was proposed [18] and timely updated [19]. The horizontal variant implementation for the NOI transistor is unknown at the actual technology level, etching a

straight cavity in Si from 10 to 20 nm depth of only 2 to 3 nm width, without pipes which seem to be impossible [20].

If the nVn succession is used as the device body, we speak about a NOI (nothing on insulator) transistor [20]. If oxide (O) is used instead of the vacuum (V) cavity and, additionally, a metal is used instead of one semiconductor zone, we speak about a mOn succession as metal/oxide/n-Si. This structure still conserves the NOI work principle [21]. Also, if oxide is replaced by any insulator (I) placed between two metals placed on a Si wafer surface, we speak about MIM structure [22]. The mOn and MIM structures use the same thin insulator tunneling principle but benefit on materials placed on the front plan of the Si wafer. Both of them are associated with the planar variant of a NOI device, simply noted by p-NOI device [15].

Therefore, a vertical implementation of the p-NOI variant is more suitable for the integration of the biosensor transducer. The insulator can be oxide or sandwich of insulators of 10 nm up to 50 nm thickness to prevent the substrate tunneling [23]. The oxide is grown by the Si planar technology. Therefore, the presented p-NOI structure is a vertical simplified NOI variant, with the advantage to be inherent integrated on the Si wafer during the biosensor metallic electrode configuration on insulator. On the other hand, the Fowler-Nordheim tunneling through the bottom insulator is poor. Hence, more than 50 nm oxide thickness ensures an excellent dielectric insulation that is suitable for the biosensor transducer purposes. The explanation comes from two Fowler-Nordheim tunneling ways in this transducer: (i) the useful one that acts the p-NOI device at the surface of the device and (ii) the parasitic tunneling toward substrate that must be avoided. The transducer successfully interacts with bio-liquid on the top of the wafer, generating the capacitance variation, while efficiently prevents the leakage current toward substrate, for thick-enough oxide layer. However, a principle that must be checked is to simulate an exponential I-V dependence for a vertical p-NOI, to put in agreement the Fowler-Nordheim tunneling principle with the p-NOI conduction mechanisms [24].

Figure 1 presents the proposed vertical p-NOI structure with substrate as back-gate. The usual anodes play the gate role, and the cathodes are the source or drain. Therefore, the notations are kept as in a transistor configuration. Essentially, in **Figure 1** there are three vertical

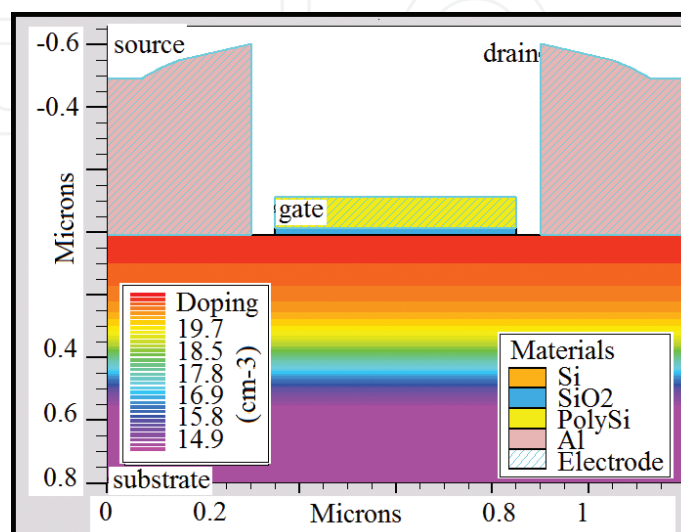


Figure 1. The basic p-NOI device in the planar configuration.

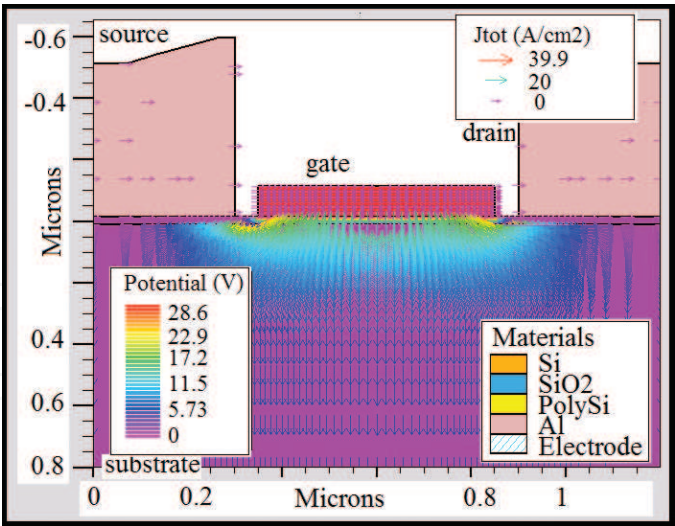


Figure 2. The p-NOI device biased at +29 V.

metal-oxide-semiconductor-metal structures as simultaneous three vertical p-NOI structures, similar to the adjacent fingers included in the next studied biosensor.

In **Figure 1** the p-NOI structure size and doping concentration are presented. On the polysilicon terminal, a voltage of 29 V is applied, and the other metallic contacts are grounded. **Figure 2** shows the potential distribution in the central p-NOI device and the current vectors through the structure, after ATLAS Running. The maximum current density is 39.9A/cm². In **Figure 3a** and **3b**, the current-voltage characteristics, I_G - V_G -type, are shown through the p-NOI structure when the gate voltage has increased from 0 to 29 V. It is demonstrated in this case that a tunnel current arises, after the exponential shape of the curves at linear and logarithmic scale, being a good start-up result.

For a p-NOI structure with adjacent metals, the applied voltage on the polysilicon electrode can be more favorable when the semiconductor is superficially doped with 10^{20} cm^{-3} .

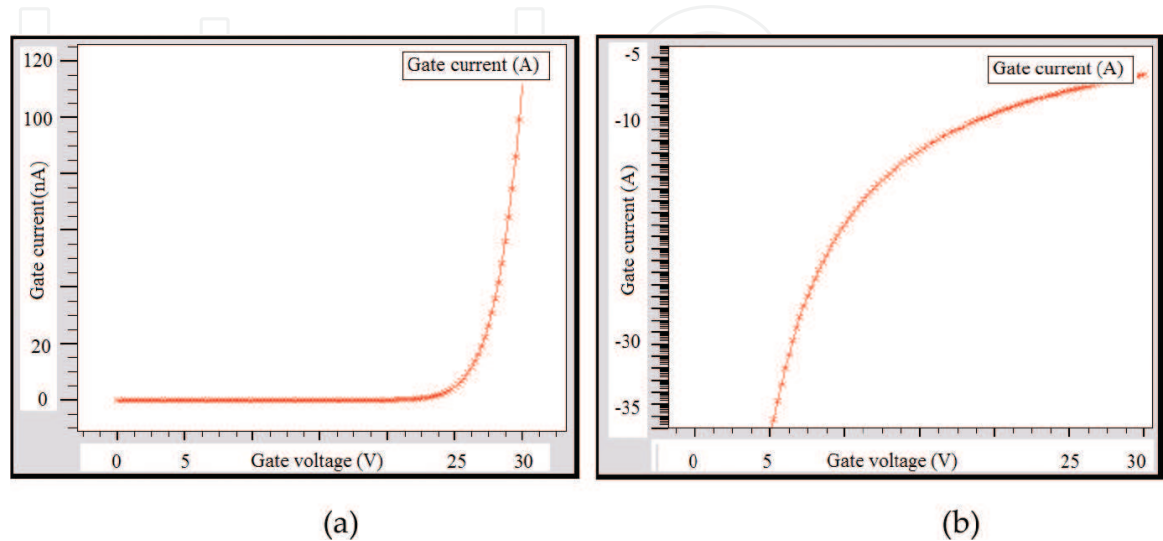


Figure 3. The simulated characteristics of the vertical p-NOI at scale: (a) linear and (b) logarithmic.

Figure 4 shows the distribution of the electron concentration in the p-NOI structure biased at 29 V. There does not seem to be significant carrier depletion.

It can be seen in **Figure 5** that the electric field inside the ultrathin oxide has an increased value up to approximative 8 V/cm, in agreement to the Si-SiO₂ boundary conditions with $\epsilon_{\text{Si}}/\epsilon_{\text{oxide}} \sim 2.7$.

The explanation is still associated to a strong tunneling for the main electrode and weak tunneling for the adjacent electrode, in agreement with the Fowler-Nordheim tunneling theory applied for the NOI device [24]. However, the higher distances between two adjacent metallic fingers inside the next pesticide biosensors foster rather the capacitive effect of p-NOI than the conductive effect. Therefore a capacitive analysis is performed for the extreme case of an ultrathin oxide thickness of 5 nm, when a AC signal is applied to the p-NOI structure (**Figure 6**).

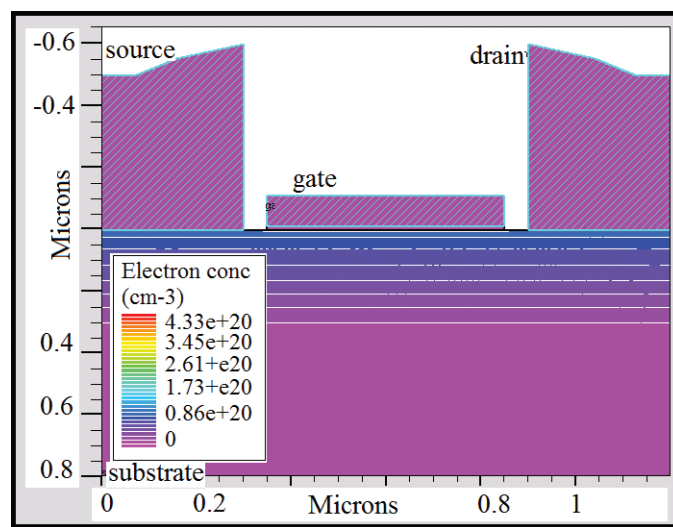


Figure 4. Distribution of the electron concentration in the p-NOI structure at 29 V.

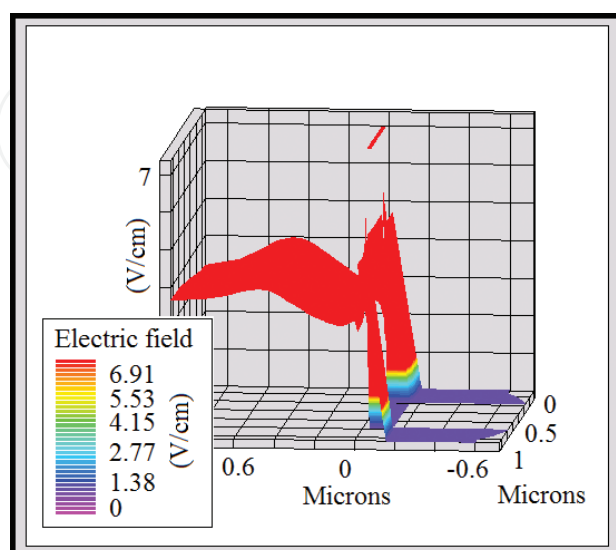


Figure 5. The electric field distribution in the p-NOI structure at 29 V.

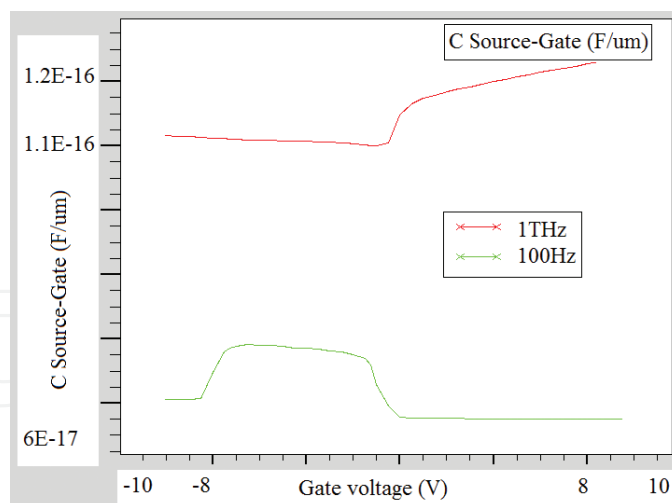


Figure 6. The CV analysis for a AC sweep for V_G between -10 V and $+10$ V for two adjacent metal fingers like source and gate of a p-NOI structure with 5 nm film thickness and similar size as in **Figure 1**, for low frequency (100 Hz) and high frequency (1 THz).

Obviously, the capacitive range is in agreement with the sizes of the p-NOI structure form (**Figure 1**) and varies between 6×10^{-17} and 1.2×10^{-16} F/ μ m (**Figure 6**).

3. The work principle of the pesticide biosensor with nanoporous Si layer

3.1. Work principle

The proposed pesticide biosensor works with acetylcholinesterase noted by AChE, with the code—EC 3.1.1.7. This receptor element is used to degrade agonists of the acetylcholine (ACh) neurohormone. In the living matter, ACh is present at neuromuscular junction and in the cholinergic nervous system, modulating the electrical pulse transmission at synaptic spaces, as other neurotransmitters [25]. The AChE has a very high catalytic affinity for acetylcholine and for its agonists as parasympathomimetic pesticides. This property opens the door of pesticide-selective detection by AChE-based enzyme biosensors [26].

The pesticides are intensively used in agriculture usually as organochlorines, carbamates, and organophosphate. Paraoxon belongs to the organophosphate class, being an oxon and the active metabolite of the parathion pesticide. Their working principle on the pests is based on the inhibition of AChE, allowing acetylcholine to transfer nerve impulses indefinitely and causing paralysis. Paraoxon is a novel generation of pesticide, which reacts as an inhibitor of AChE. Pesticides from this group act directly by stimulating the nicotinic receptors or indirectly by the inhibition of cholinesterase, as an acetylcholinesterase inhibitor, abbreviated as AChEI. Paraoxon is one of the most potent acetylcholinesterase inhibitor available in insecticide [27]. In water solvent, it stands for a high risk

of poisoning for humans or animals, due to its simply absorption through teguments in contact with the contaminated water from environment. As pesticide, the parathion is dissolved in water and usually is applied by treatment. It is frequently sprayed to rice and fruits. The usual concentrations are 0.05 and 0.1% [28]. After the rain, the pesticide is accumulated in water and soil. This parathion degradation in time produces multiple water-soluble products.

3.2. Nanoporous materials for enzyme entrapping

This section depicts the paraoxon biosensor starting from a Si wafer technology. Some intermediate nanoporous materials are used in the biosensor construction, for the enzyme entrapping. Among these materials, TiO_2 [29], Al_2O_3 [13], or porous Si still exists [30]. The porous material integration on a silicon wafer is starting by the first metal deposition, followed by subsequent processing steps, in order to convert them into compounds and finally into a porous matrix. The main steps of porous Si layer formation are:

- The Si start wafer is n-type, <100> orientation, and 2–9 Ωcm resistivity.
- The first process is a thermal oxidation that allows the pattern configuration.
- The next process is the boron ion implantation through the patterned mask in a high dose on the front wafer to convert the upper Si layer into p-type.
- Then, the Si p-type layer is converted in porous Si by anodization in the electrolyte $\text{HF}:\text{CH}_3\text{COOH}:\text{H}_2\text{O}$ with 180:60:60 ratios, at current density more than 2 mA/cm^2 .
- The last process consists in annealing at 550°C in H_2 and 850°C in N_2 to increase the film stability.

This porous Si technology provides usual porous Si layers with a porosity of 56, suitable for the enzyme entrapping process [30].

These intermediate porous materials augment the capillary, allowing the biomaterials entrapping in a liquid phase, during the pre-deposition technological stage. At the same time, the porous layer must be grown onto the Si wafer in order to be strongly anchored to substrate and in order to avoid accidental detachments. Nanoporous Si can be easily converted from a Si thin upper layer. Having a closer lattice constant with Si, the porous Si stands for an efficient intermediate material for the next technological steps. The nanoporous Si material preparation by anodization is a perfect compatible method with the microelectronics technology. The pore sizes can be simply adapted in respect with the anodization reaction parameters, changing the electrolyte composition. Due to an increased area, offered by the nanoporous Si material against the monocrystalline Si, an enhanced miniaturization with capacitive electrodes can be performed. Therefore, the porous Si was selected as intermediate layer for the AChE enzyme entrapping. This solution is also in agreement with the nowadays tendency.

4. Results of some key technological steps

4.1. Nanoporous Si characterization

The morphology of the synthesized porous Si film is characterized by SEM microscopy (**Figure 7**). When the anodization process is tested for 60 mA/cm², some multiple pores are crowded inside a single larger pore rather with a crater shape. When the anodization process is tested for 300 mA/cm², some huge pores reached 7 μm size. However, the current density of 50 mA/cm² offered the optimum porosity for the enzyme entrapping [22].

Figure 8 presents the designed mask for the nanoporous Si region configuration.

Then, **Figure 9** presents the next mask used for the metal deposition on nanoporous Si material.

4.2. Electrode design and processing

The biosensor transducer is represented by an electrical capacitance. From the design stage, the capacitor has a constant armature surface and a fix distance between electrodes, so that any variation in capacitance reflects the electrical permittivity change of the material. This permittivity variation is proportional with the quantity of ions accumulated after the enzymatic-assisted reaction of the pesticide hydrolysis. Therefore, in order to increase the sensor sensitivity, as high as possible, the active area is demanded high. In this sense, the electrodes are designed with an interdigitated geometry [31].

Now, **Figure 10** shows multiple metallic traces for a global view of the biosensor transducer.

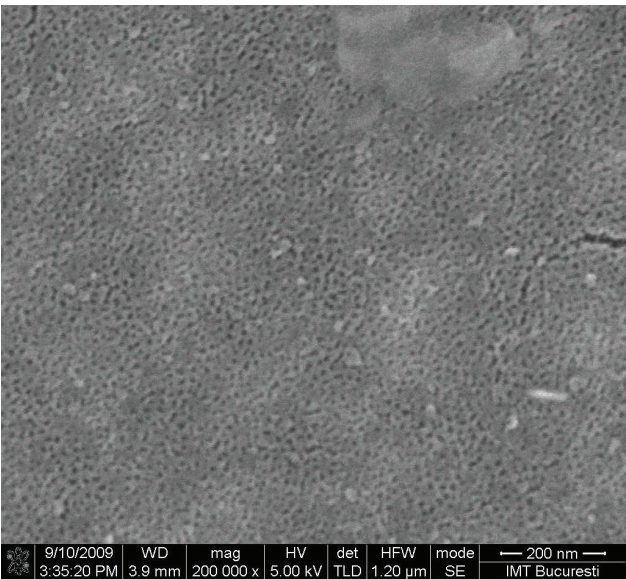


Figure 7. The SEM image reveals the nanoporous material.

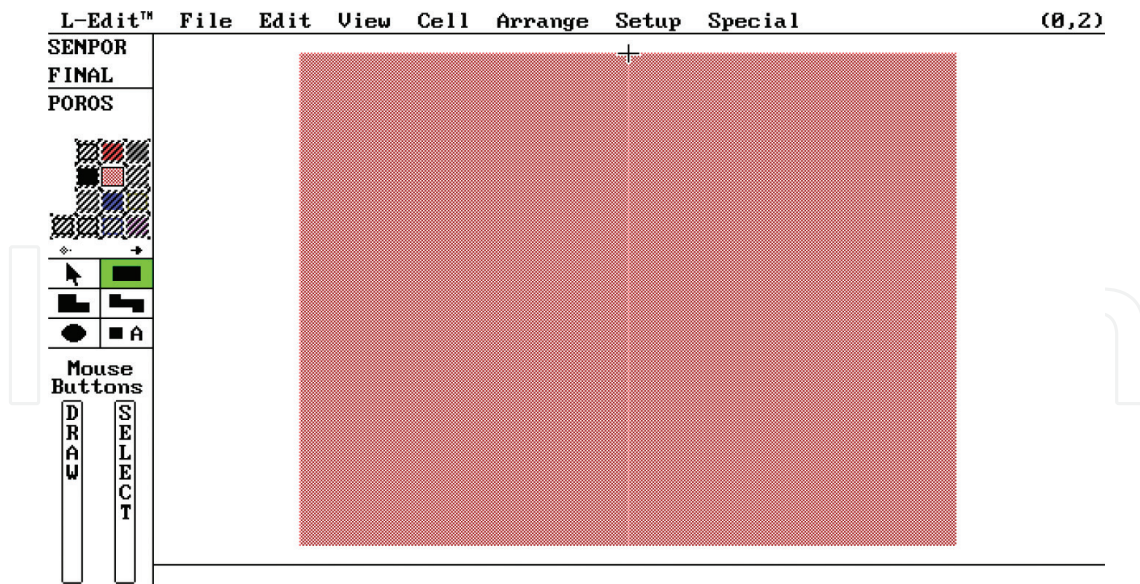


Figure 8. The porous Si mask.

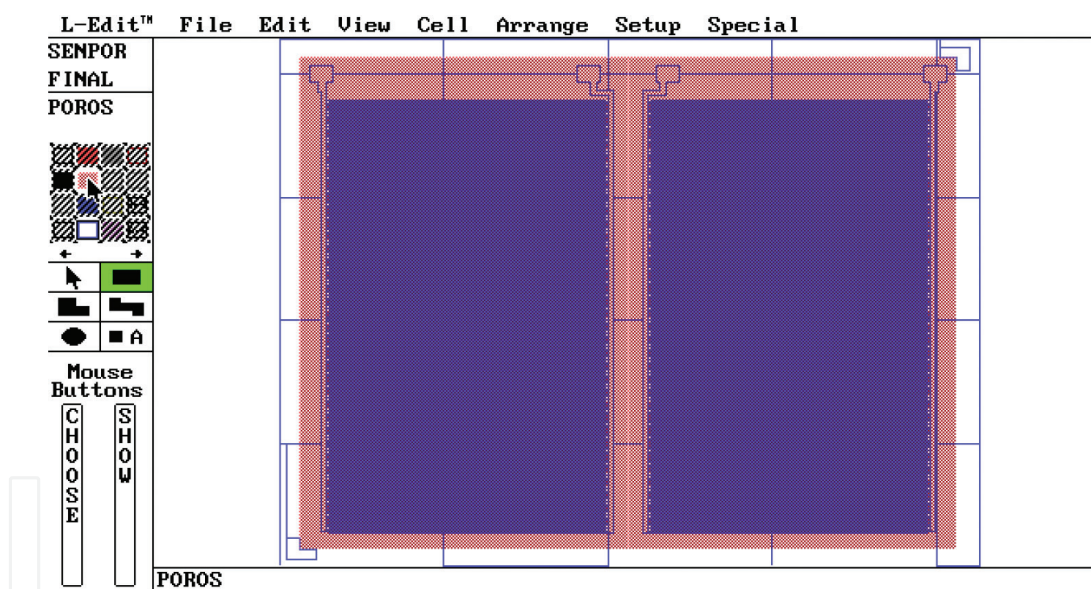


Figure 9. Mask of the metal overlap onto nanoporous Si material.

4.3. The enzyme processing step

In the first step, the powder of the acetylcholinesterase enzyme is blended with serum albumin, all being of Sigma provenience. A phosphate buffer solution keeps pH = 7.1 as constant pH. The mixture has helped to be entrapped on the Si wafer surface by the adsorption method. In this scope, the glutaraldehyde, in solution of 2.5% concentration, is added as cross-link agent.

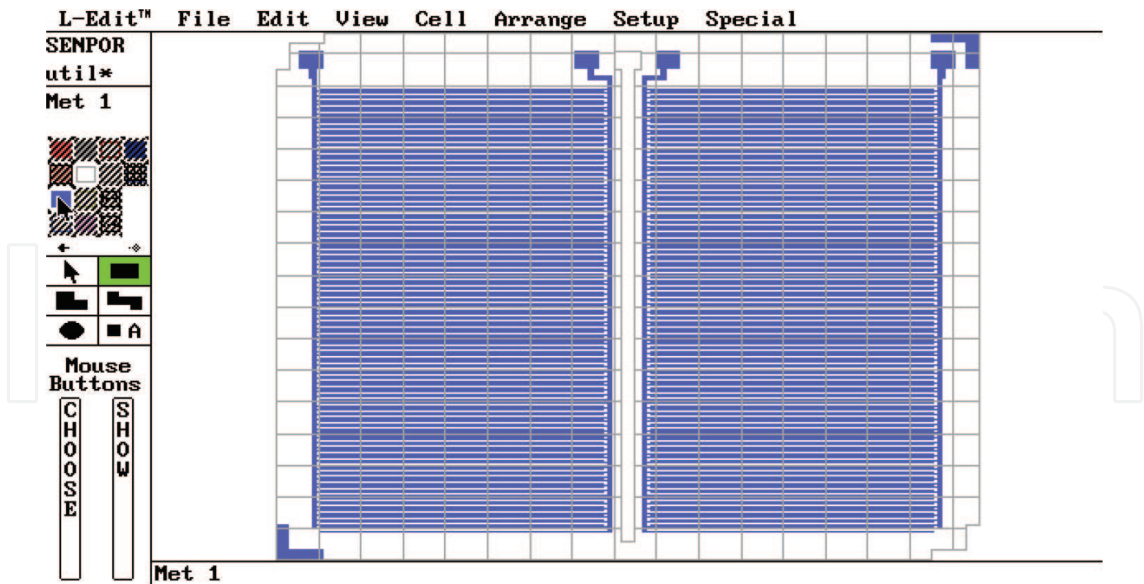


Figure 10. Final overlap of the metallic electrodes.



Figure 11. The top view of a piece of the enzymatic membrane.

The enzymatic area overlaps on the nanoporous Si area that facilitates the immobilization. The structure is introduced in drying stove for 24 hours. A top view of a piece of the processed AChE membrane is presented in **Figure 11**.

5. The final product: a tool for the environment monitoring

The final interdigitated structure comprises 98 horizontal metallic traces, which are starting from the central pillar for each electrode, detail in **Figure 12**.

After the enzyme entrapping, the integrated biosensor was tested by capacitance-voltage (C-V) analysis. The capacitance-voltage experimental curve is measured for the final product, after the enzyme entrapping, but in the absence of pesticide in measured solution.

The capacitance ranges from 45 nF up to a minimum of 7 nF, in agreement with p-NOI electrode structures with 8 μ m width and 10 μ m gap space. The shape of the C-V curve

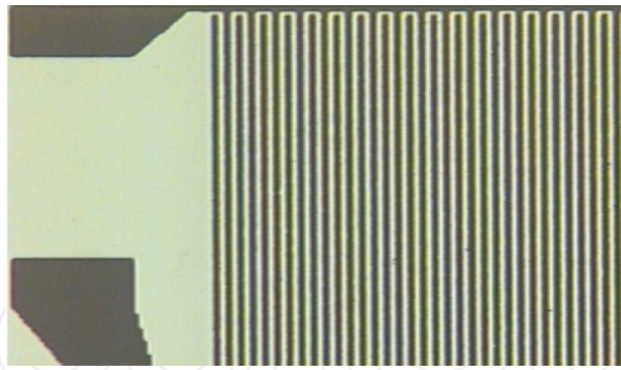


Figure 12. Detail of electrodes on the final product.

proves that our capacitive biosensor works as a p-NOI capacitor, with enzyme on nanoporous Si material on Si substrate. The voltage ranging from negative values toward positive values brings the capacitor from the accumulation regime through the depletion regime—the middle decreasing part of the curves—toward the inversion regime. The C-V curve is almost reproducible that indicates an adequate enzyme entrapping onto the silicon surface occurred.

6. Conclusions

The chapter presented a biosensor generated by micro-nanotechnology and biotechnology to serve as a monitoring tool to ensure a green environment condition. The fabrication steps of an integrated pesticide biosensor with AChE enzyme on nanoporous Si structure were presented. The structure of interdigitated electrodes was investigated as physical phenomena simulations inside the novel proposed planar nothing on insulator (p-NOI) structure. The contributions and purpose of this work were:

- Fabrication of nanoporous Si layer onto the Si surface and characterization by CV
- The enzyme membrane immobilization technique by adsorption onto the porous Si layer

The growing technological process of the nanoporous Si layer onto the Si surface was processed by the conversion of the n-type wafer into a p-type at the surface, followed by anodization. The enzyme membrane immobilization technique was by adsorption onto the porous Si layer and fixed with glutaraldehyde by the cross-link method. Finally, the preparation of the capacitive electrodes as an interdigitated structure comprised 98 horizontal metallic p-NOI traces. The primary C-V curves checked the sensor functionality.

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Conflict of interest

There are no conflicts of interest known.

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