

Influence of Multiple Cleaning on the Detection Capabilities of ISFET Structures

Kinga Kondracka, Piotr Firek, Marta Grodzik, Maciej Szmidt, Ewa Sawosz-Chwalibóg, and Jan Szmidt

Abstract—The method of cleaning the ISFET structures after application of a biological substance was developed. There are few references in the literature to cleaning methods of this type of structure for biological applications, but they are relatively complex and difficult to automate. We decided to use resources commonly available in technological laboratories and methods that could be relatively easily automated, which would enable the full potential of ISFET transistors to be used. During the experiments, both acetone and deionized water were tested. The cleaning method was modified and it was checked whether it is possible to use such a method on one transistor more than once and how it affects the transistor's detection capabilities. We managed to obtain an effective method of cleaning ISFETs from biological substances. This method does not allow for obtaining exactly the same state as the original state of the transistor, but it ensures its correct operation and determining the influence of the tested biological substance on the transistor based on the results.

Keywords—ISFET, sensor, I-V characteristics, cells, cleaning

I. INTRODUCTION

DUE to the continuous and intensive development of biotechnology, medicine and pharmacology, it is also necessary to develop methods of examining the state of cells, which are commonly used in research in these fields. These methods face a number of requirements, such as: long-term high stability and the ability to detect even with very small amounts of the detected agent (the so-called low detection limit) [1] – less than 200 μl [2]. Due to the need to preserve the original condition of some samples, speed up the measurement and convenience, it is advantageous to avoid complex procedures in sample preparation. Another obvious criterion is a quick response - maximum analysis time of several minutes, as well as automatic execution of routine procedures [2]. The most popular currently used methods, such as staining cells with trypan blue [3], flow cytometry [4], or the impedance method [5] have properties that make it impossible to measure several samples at once, or significantly affect them or even irreversibly modify.

Biosensors are of great importance not only in the field of medical diagnostics, but also for national security, the forensic industry and environmental monitoring. The literature indicates the possibility of using ISFET (Ion Sensitive Field Effect Transistor) type transistors for this purpose [6-8]. These are ion-sensitive field effect transistors, the operation of which is

determined, among others, by the properties of the substance applied to their gate area [9]. Due to its high sensing potential, this transistor has been modified with highly specialized applications: FGFET (Floating Gate FET used for DNA detection, it has two gates, with one gate serving as the detection gate and the other as the control gate, electrically both gates are analogous to each other and are capacitively connected to a common floating gate. The basic principle of its operation is based on the assumption that charged DNA molecules induce a threshold voltage change on a floating gate without the presence of a reference electrode), EGFET (Extended-gate FET) ensuring higher stability due to the separation of the dry and wet environment, DGFET (Dual Gate FET), which allows you to eliminate the problem of time shift and hysteresis, ENFET (Enzyme FET which follows a common principle of detection: enzyme molecules that are immobilized on the surface of a semiconductor structure transform the corresponding substrate to obtain a charged product. This product is detected by the ion-sensitive surface layer of the sensor, and the resulting surface charge modulates the charge area at the insulator-semiconductor interface) and many more [10][11]. Interest in field-effect transistor (FET) biosensors is fueled by their highly desirable features, such as fast electrical detection without the need to label biomolecules, low energy consumption, portability, inexpensive mass production, and the ability to integrate sensors into circuits and entire measurement systems [12]. Due to the fact that for economic reasons it would be most advantageous to use such transistors many times, it turned out to be necessary to develop a method of cleaning ISFET transistors from biological substances applied to their active area. There are only a few references to transistor cleaning for this type of application in the literature. For example, cleaning of sensors prior to cell culture can be done by rubbing the surface with swabs to remove cell debris from the previous cell culture, sonication in detergent, boiling cell culture containers in 20% H_2SO_4 at 80°C or using mild argon plasma, and finally sterilizing the chips by heating at 150°C or soaking in 70% ethanol for 30 minutes [13]. However, the method is relatively complex given the possibility of massive simultaneous use of ISFET transistors and does not take into account all the possibilities of functionalizing the surface of the transistor, which may not be suitable for exposure to high temperatures or swabbing.

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The experiment was aimed at checking the effectiveness of cleaning ISFET transistors from biological substances in order to reuse them, which, however, will be more simplified and easier to automate. In addition, it was decided to use agents commonly available in laboratories where microelectronics is manufactured and used, which would enable the widespread use of the proposed method.

II. MATERIALS AND METHODS

The fabrication of ISFET (Fig. 1) is similar to the typical semiconductor field effect transistor (MOSFET) manufacturing process (Fig. 2-4). In the case of ISFET, the gate electrode is separated from the canal by an ion-sensitive barrier and a gap that allows the test substance to contact a sensitive barrier (Fig. 5) [14]. They are devices used as electrochemical sensors, the operation of which is based on the principle of the field effect, similar to the MOSFET transistor.

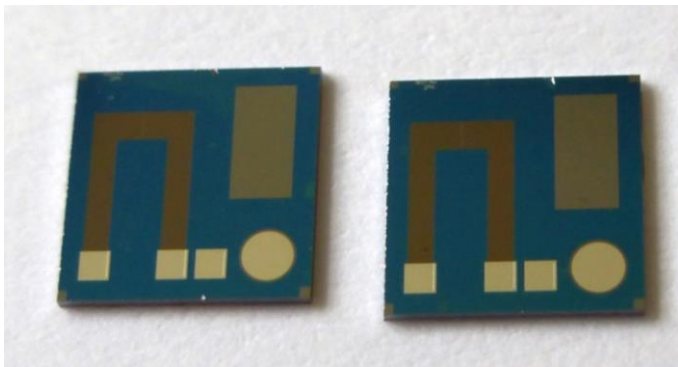


Fig 1. ISFET transistor

The realization of the early ISFET structures involved post-processing of MOS transistors and this was done by removing the gate metallization and depositing an ion-sensitive material on top of the gate dielectric [15].

As previously mentioned, ISFET transistors are manufactured in standard MOS technology. At the beginning, all impurities and natural oxide, formed on the surface of the single crystal silicon in the presence of oxygen, are removed. Then silicon oxide is realized in the atmosphere of water vapor in thermal oxidation process. This layer in subsequent stages will serve as a mask for the diffusion process into the areas of sources and drains. The first photolithography allows to obtain shapes of sources and drains and then this shapes are exposed due to the etching process in hydrogen fluoride solution. In the doping process, phosphorus is diffused to produce drains and sources and the next step is excess phosphorus enamel removal.

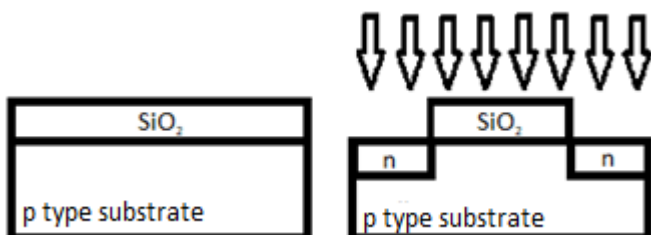


Fig 2. Substrate after oxide deposition (left) and phosphorus diffusion (right)

The second photolithography reveals channel areas, then the wafer is cleaned and a gate dielectric was produced, in this case SiO_2 . The next stage is third photolithography, which prepares the existing structures for the formation of contacts and in the next step windows in oxide are etched.

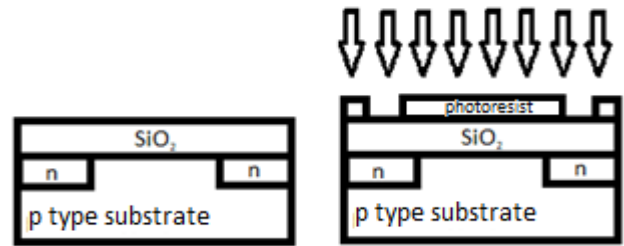


Fig 3. Substrate after deposition of gate oxide (left) and after photolithography enabling the formation of contacts (right)

Then the aluminum is evaporated to create contacts, the shape of which is given by subsequent photolithography.

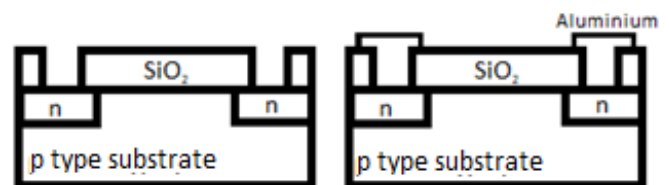


Fig 4. Transistor before making contacts (left) and finished ISFET (right)

Among the various potentiometric techniques, ISFET-based sensors have attracted considerable attention due to their potential for miniaturization, parallel measurements, fast response times, seamless integration into electronic manufacturing processes such as CMOS [10] [15] and the possibility of building more complex measurement systems from them [16]. Due to the widespread use in electronics and technological processes, it was decided to test different variants of cleaning transistors in acetone and deionized water.

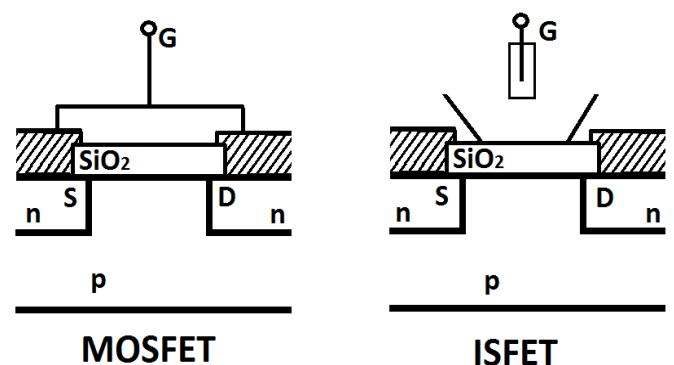


Fig 5. Comparison of the structure of MOSFET and ISFET transistors.

In order to enable the application of small amounts of biological substances and multiple quick measurements of the results, the transistors were soldered to the PCB plate (Fig. 6) with the control system, which was connected to the Source Measure Unit, which was the voltage source and the current meter.

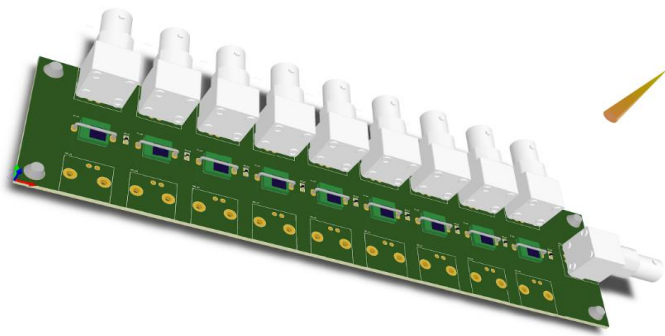


Fig 6. PCB board for ISFET transistors assembly for measurements with biological substances

The experiment was carried out in two stages; the first step was to develop a cleaning method, the second to check its effectiveness in multiple applications on the same transistor. In the first stage, two ISFET transistors were used, each of them was first rinsed in deionized water, then in acetone, dried with warm air and allowed to cool for 10-15 minutes. Then, the biological substance was applied alternately (applications were marked with letters A and consecutive ordinal numbers) and the described cleaning procedure was applied (numbers 1-4 refer to successive iterations of structure cleaning). In the second stage, 12 transistors previously used for experiments with biological substances and 6 new transistors were used. First, I-V characteristics were determined and on this basis they were divided into 6 groups of 3 transistors with the most similar current-voltage characteristics.

For each of the transistors 3 μl (Fig. 7) of the solution (biological substance with a lower concentration of products resulting from cell decay - hereinafter referred to as Y and biological substance with a higher concentration of products resulting from cell decay - hereinafter referred to as X) was applied. The measurement time was 4 minutes (5 measurements were made - immediately after applying the substance and after 1, 2, 3, 4 minutes). After the first test series, it turned out that the time (to such an extent) did not affect the measurement results.



Fig 7. ISFET transistors with applied biological substance

The cleaning process was as follows: after applying the cells, the transistors were rinsed in deionized water (by immersion), then in acetone (also by immersion), and then the transistors were allowed to dry for 10 minutes. Hot air drying is eliminated to facilitate and improve the process.

The results were compared on two levels: comparison of the same structures for different substances and second compare the same structures for the same substance. The first series was the reference for the evaluation of subsequent series.

III. RESULTS

The results of the first experiment (Fig. 8, 9) showed the correct operation of the cleaning procedure used, the current values after cleaning returned to their original values, even if they did not match, it was sufficient to clearly state that the transistor returned to its original state and it was clear distinguish when there is a test sample on the transistor and when not, in one of this two cases the reaction of the transistor to the test sample almost remained at the same current level. Subsequent applications of the biological substance indicated that this procedure does not damage the transistor and does not affect its reaction with other substances. Curves marked with numbers with the letter "A" represent the current for the transistor with the applied biological substance, curves with only the number represent the current for the transistor after the cleaning procedure.

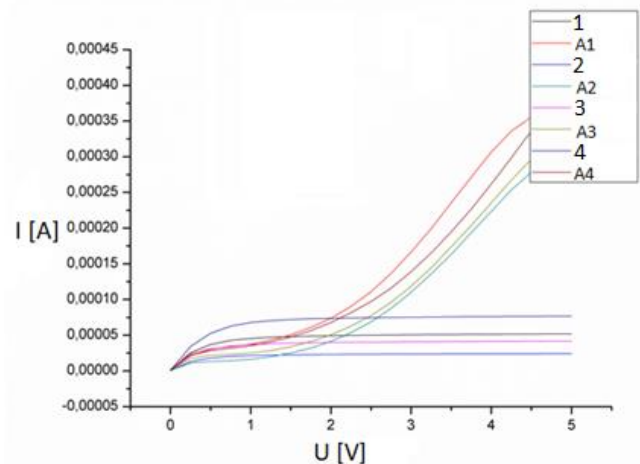


Fig.8. Results of the first stage of the experiment for first transistor

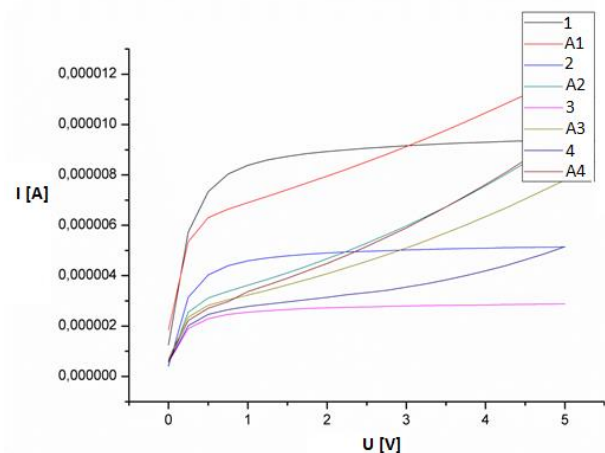
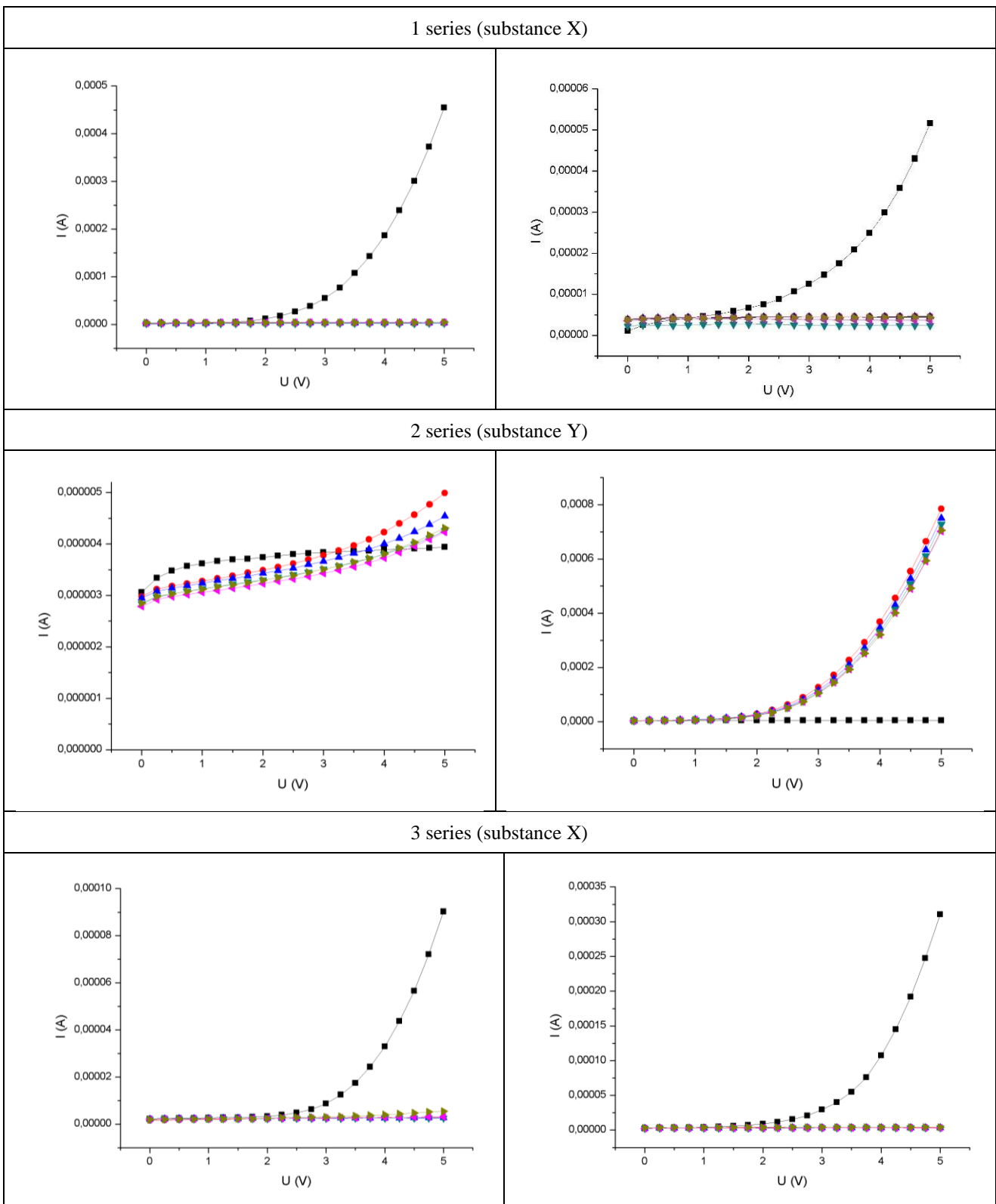
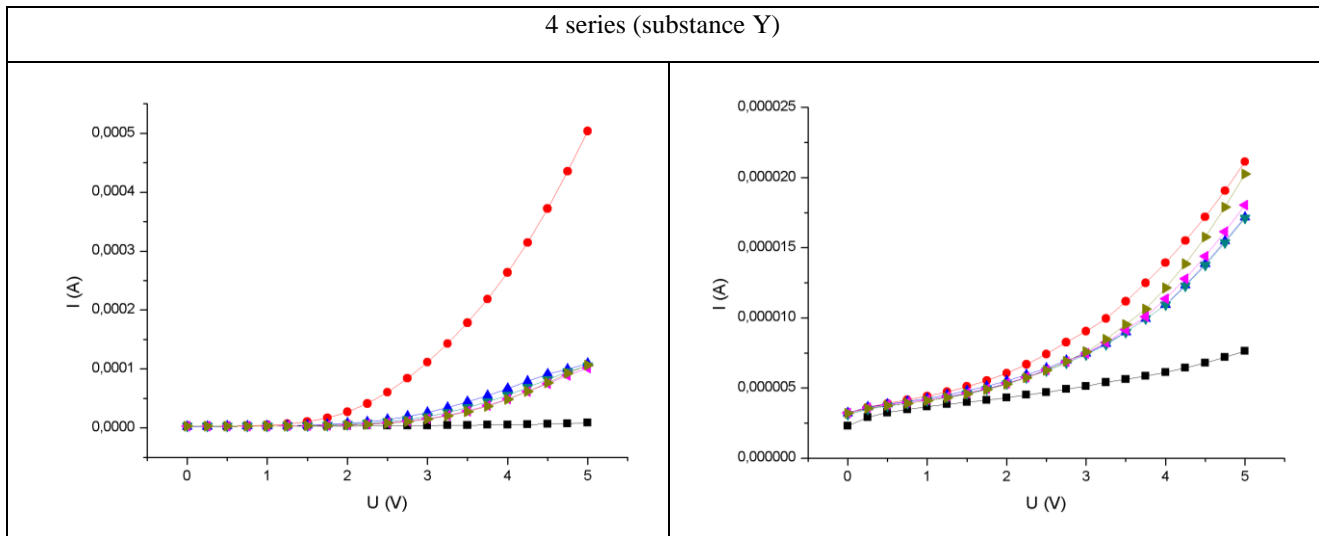


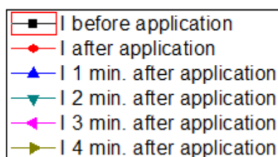
Fig.9. Results of the first stage of the experiment for second transistor

TABLE I
RESULTS FOR MULTIPLE CLEANING OF ISFET TRANSISTORS





Legend:



Due to the large number of results obtained during the second stage of the experiment, only a representative group is presented (Table 1). In each subsequent series, a pair of exactly the same transistors is presented, subjected to successive iterations of cleaning and applying biological substances to them.

In the case of every second series, the effect of the same substance on the transistor can be observed, and it can be concluded that the cleaning was effective. The entire second series, despite the correctness of the result, differs from the rest with the level of interference, this is an issue that will be subject to separate work and research in the future, but the very fact that the current level increased after the application of substance X, indicates the correct operation of the transistors in this series. Warm air drying of the transistors after cleaning with acetone and deionized water, and prior to application of the biological substance, is allow a return to measurement-specific current levels prior to any cleaning. It should also be remembered that due to the specificity of measurements that are made with the use of ISFET transistors, it is very advisable, but it is not completely necessary for reliable research. The return of the current level to the same values after the next cleanings of the transistors still needs to be refined, but the reaction to the given biological substances remains correct.

CONCLUSION

The proposed cleaning method allows the ISFET transistor to be reused to test biological substances. It is likely that the elimination of the additional warm air drying step caused, in the case of some transistors, a decrease in the currents for subsequent biological substance applications. This effect is not observed in the first stage of the experiment. Taking this into

account and standardizing the procedure, it is likely to obtain a much better repeatability. The introduction of a mechanical cleaning element is also considered, but this may make the automation somewhat difficult and limit the applicability of the method, because the ISFET transistor can be used in a variant with additional membranes on its surface, and in this case mechanical cleaning may cause a number of complications. Both the substances and methods used can be easily automated, which allow to clean many transistors at once and reuse them. Currently, research is also conducted on the use of other substances [17] or possible mechanical methods in the process of cleaning transistors.

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