Research Article

Ultrasensitive Detection of 2,4-Dinitrophenol Using Nanowire Biosensor

Yuri D. Ivanov,[1] **Kristina A. Malsagova** ⓘ**,**[1] **Tatyana O. Pleshakova** ⓘ**,**[1] **Rafael A. Galiullin,**[1] **Andrey F. Kozlov,**[1] **Ivan D. Shumov,**[1] **Irina A. Ivanova,**[1] **Alexander I. Archakov,**[1] **Vladimir P. Popov,**[2] **Alexander V. Latyshev,**[2] **Konstantin V. Rudenko,**[3] **and Alexander V. Glukhov**[4]

[1]*Institute of Biomedical Chemistry, Ul. Pogodinskaya 10, Build. 8, Moscow 119121, Russia*
[2]*Rzhanov Institute of Semiconductor Physics, Siberian Branch, Russian Academy of Sciences, Pr. Akad. Lavrent'eva 13, Novosibirsk 630090, Russia*
[3]*Physical Technological Institute, Russian Academy of Sciences, Nakhimovskii Pr. 36/1, Moscow 117218, Russia*
[4]*Novosibirsk Semiconductor Device Plant and Design Bureau, Ul. Dachnaya 60, Novosibirsk 630082, Russia*

Correspondence should be addressed to Kristina A. Malsagova; krisiki86@mail.ru

The method for the detection of 2,4-dinitrophenol (DNP) in solution is proposed. This method employs the sensors based on silicon nanowire field-effect transistors with protective layers of high-k dielectrics, whose surface is functionalized with an amino silane. Direct highly sensitive detection of DNP has been demonstrated, and the lowest detectable concentration of DNP was determined to be 10^{-14} M. Silicon-on-insulator nanowire (SOI-NW) sensors can well be employed for the rapid detection of a wide range of toxic and explosive compounds by selection of sensor surface modification techniques.

1. Introduction

2,4-Dinitrophenol (DNP) pertains to nitroaromatic compounds. It represents a crystalline solid, which is slightly soluble in water and readily soluble in boiling water and in organic solvents, such as ethanol and diethyl ether [1]. DNP is widely used in chemical industry [2] as a component of explosives and fungicides, as well as in textile industry as a dye for fabrics [3–6]. Regarding the physiological effect, DNP is reported to cause rapid loss of weight, but unfortunately is associated with an unacceptably high rate of significant adverse effects [7] due to its toxicity (lethal dose low 36 mg/kg (human) [8]). DNP poisoning can result in death in some cases [4, 7, 9, 10]. Clinical symptoms of DNP poisoning include anorexia, nausea, vomiting, headache, dizziness, feeling of suffocation, as well as general weakness, and weight loss [4, 11, 12]. In this respect, modern nanotechnology-based sensor platforms can well be of use in order to provide rapid monitoring of toxic organic compounds in human biomaterial. For instance, Maduraiveeran et al. recently developed a bimetallic nanoparticle-based amperometric sensor for the rapid detection of acetaminophen (a drug for the relief of fever and pain, which sometimes develops a fatal hepatoxicity and nephrotoxicity [13]) in human serum with subnanomolar concentration sensitivity [14].

Moreover, it is to be noted that DNP is highly explosive [15]. This is the reason why the development of highly sensitive, rapid, cheap, and easy-to-use detectors for the revelation of DNP in the environment is crucial. The optimal solution of this problem is the use of silicon nanowire (NW) field-effect transistor sensors. As the size of a nanowire is comparable with that of a biological macromolecule [16], most attention is paid to the use of silicon-on-insulator nanowire (SOI-NW) sensors for the detection of protein and nucleic acid disease markers [17–20]; the possibility of application of these sensors for cancer diagnosis in exhaled air was also shown [21]. With respect to biological

macromolecules, nanotechnology-based electrochemical methods allow one to attain very low detection limits [22], in contrast to small molecules and ions, when the detection limits are commonly in nanomolar to micromolar range [23].

Nevertheless, silicon NW-based devices are also successfully employed for the detection of small molecules and ions. In this regard, it is worth noting that SOI-NW sensor structures display pH sensitivity [24]. The silicon NW-based detection of mercury ions at 10^{-7} M concentration was demonstrated by Luo et al. [25]. The studies concerning NW-based gas-phase detection of small-molecule analytes, including inorganic gases and vapors, organic explosives, nerve agents, and volatile organic compounds, are reviewed in the paper by Cao et al. [26]. The liquid-phase detection of aromatic compounds was demonstrated by Talin et al. with examples of nitrobenzene and phenol in cyclohexane [27]; however, in this study, the analyte concentrations $<10^{-3}$ M were not tested. Engel et al. demonstrated the use of NW sensors for the detection of DNP, 2,4,6-trinitrotoluene and other nitroaromatic chemicals in solution with 5×10^{-13} M concentration sensitivity. These authors also showed that careful selection of surface chemistry of SOI-NW sensor can provide detection specificity [28]. For another nitroaromatic explosive, trinitrotoluene (TNT), even lower detection limit ($\sim 10^{-14}$ M) was attained by Lichtenstein et al. [29].

It is to be noted that the NW sensors used by Engel et al. [28] were fabricated by «bottom-up» method; with regard to mass production, this method is insufficiently feasible. In our present study, lower-submicron complementary metal-oxide-semiconductor (CMOS)-compatible technology employing subgate dielectrics with high dielectric constant (high-k) has been used for fabrication of SOI-NW sensors. This technology allows one to fabricate highly sensitive SOI-NW sensors. In this connection, it is to be noted that deposition of ultrathin dielectric layers onto NW sensor elements significantly improves their chemical stability (which directly influences the sensor performance). This was demonstrated in the study by Peled et al. with the example of deposition of 3 to 10 nm-thick Al_2O_3 layers onto silicon NW sensors [30].

Herein, we have demonstrated that, using such sensors, the lower limit of DNP detection in solution can be shifted down to 3×10^{-14} M; this value is an order of magnitude lower than that reported by Engel et al. [28], and comparable with that obtained for TNT by Lichtenstein et al. [29]. In this way, further development of SOI-NW-based sensing, including techniques of chemical modification of SOI-NW sensor surface, will provide selective detection of DNP and other toxic and explosive compounds with high concentration sensitivity.

2. Materials and Methods

2.1. Chemicals. 2,4-Dinitrophenol, ethanol (C_2H_5OH, 96%), and isopropanol (C_3H_7OH, 99.999%) were purchased from Reakhim (Russia). 3,3'-dithiobis (sulfosuccinimidyl propionate) (DTSSP cross-linker) was purchased from Pierce (USA). 3-aminopropyltriethoxysilane (APTES) was

purchased from Sigma-Aldrich (USA). Deionized water was obtained using the Milli-Q system (Millipore, USA).

2.2. Fabrication of SOI-NW Sensors. SOI-NW sensors with n-type conductivity were fabricated by optical lithography. The initial silicon layer thickness was 45 nm, and buried oxide (BOX) thickness was 200 nm. The SOI-NW sensor elements had the following dimensions: 250 nm width, 32 nm thickness, and 14 μm length; the number of SOI-NW sensors on the sensor chip crystal was 12 (Figure 1). The surface of SOI-NW sensor chip was entirely coated with a dielectric layer using the FlexAl reactor (Oxford Instruments, UK) by plasma-enhanced atomic layer deposition (PEALD). Prior to dielectric deposition, immediately before PEALD procedure, the surface of SOI-NW sensors was cleaned first in H_2O_2/NH_3 solution and then in NH_3 plasma at 500 W power and 50 mTorr working pressure for 2 min. Deposition of HfO_2 and Al_2O_3 was carried out by cycles in oxygen plasma. An 8 nm-thick HfO_2 layer was formed from tetrakis(ethylmethylamino) hafnium(IV) precursor at 270°C. A 4 nm-thick Al_2O_3 layer was formed from trimethylaluminum precursor at 300°C. After PEALD procedure, SOI-NW sensors were annealed in the forming gas ($N_2 : H_2 = 95 : 5$) at 425°C and 200 mTorr for 30 min. In the SOI-NW sensor system, a 500 μL measuring cell was employed, and the SOI-NW sensor chip served as its bottom. The diameter of the sensor area was ∼2 mm. The cell was equipped with a stirrer. The stirring rate was 3000 rpm.

2.3. Sensor Surface Modification. To remove organic contaminants, the surface of SOI-NW sensor chip was treated with isopropanol and then silanized in 3% APTES solution during 60 min at room temperature to provide amino functionality of the surface analogously to the technique described by Shehada et al. [31]. After silanization, the surface of SOI-NW sensor chip was treated with ethanol. The surface of control SOI-NW sensors was additionally modified with DTSSP cross-linker [20, 32]. Figure 2 displays the schematic representation of DNP detection with APTES-modified nanowires.

2.4. Preparation of Solutions. A series of test solutions of DNP with concentrations ranging from 10^{-12} M to 10^{-15} M were prepared from 0.05 M stock solution of DNP in ethanol by serial tenfold dilution. On each dilution stage, the solution was incubated in a shaker for 30 min at 10°C. Control experiments were carried out with solutions that did not contain DNP. All liquids used in the experiments were filtered using Vivaspin Turbo 15 centrifuge filters (3000 MWCO; Millipore, USA). The solutions were prepared directly before measurements.

2.5. Electrical Measurements. Electrical measurements were carried out using Keithley 6487 picoammeter (Keithley Instruments Inc., USA). During measurements, the support of SOI structures was used as a control electrode (transistor gate). The dependence of drain-source current on gate voltage $I_{ds}(V_g)$ for n-type SOI-NWs was obtained at
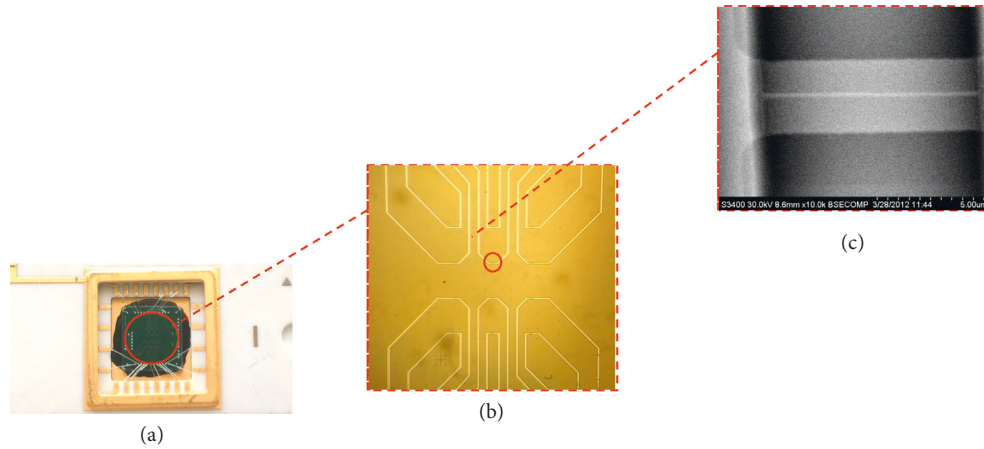
(c)

(a)

(b)

FIGURE 1: (a) Sensor chip with an array of SOI–NWs, (b) optical image of 12 SOI–NWs arranged in pairs on the chip surface, and (c) electron microscopy image of a single SOI-NW.
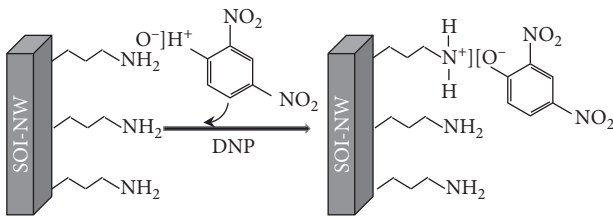

FIGURE 2: Scheme of DNP detection with APTES-modified nanowires.

$V_g = 0/70$ V and $V_{ds} = 0.2$ V. For the detection of DNP with the SOI-NW sensor, 150 μL of test solution of DNP was added into the measuring cell containing 300 μL of water. To account for the nonspecific signal, control experiments with use of DNP-free solutions were carried out in the same conditions. Time dependencies of normalized differential drain-source current signal $\Delta I_{ds}/I_{ds0}(t)$ were measured at $V_g = 20$ V and $V_{ds} = 0.2$ V. Normalized differential drain-source current was calculated as $\Delta I_{ds}/I_{ds0}$, where ΔI_{ds} is the difference between the signal obtained from working and control nanowires, and I_{ds0} is the value of the signal before DNP addition. To improve time stability of SOI-NW sensors' operation, an additional Pt electrode was immersed into the solution in the measuring cell analogously [33, 34]. Differential signal was obtained by subtracting the nonspecific signal from that obtained upon addition of test DNP solution.

3. Results and Discussion

Time dependencies of normalized differential drain-source current signal $\Delta I_{ds}/I_{ds0}(t)$ were obtained for DNP concentrations from 3×10^{-15} M to 3×10^{-13} M. Figure 3 displays typical time dependencies of the normalized differential signal from the SOI-NW sensor obtained upon DNP detection. Upon the addition of DNP into the measuring cell of the sensor system, an increase in the signal (due to the increase in nanowire conductivity caused by the influence of DNP) was observed (Figure 3). At that the signal

value decreases with the decrease of DNP concentration. The lowest detectable DNP concentration was 3×10^{-14} M.

Inset in Figure 3 displays typical $\Delta I_{ds}/I_{ds0}(t)$ curves obtained in control experiments, when either DNP-free ethanol solution or pure deionized water was added into the measuring cell instead of DNP solution. These curves clearly indicate that upon addition of DNP-free test solution, the response from the sensor was either indistinguishable or exceeded the initial signal level insignificantly by no more than 2% (what is comparable with the noise level, which made up ±5%). This fact indicates specificity of interaction between silane layer on the sensor surface and DNP molecules.

To demonstrate the reproducibility of DNP detection, experiments on repeated detection of DNP with recording the signal from one and the same SOI-NW sensor have been carried out. Figure 4 displays typical $\Delta I_{ds}/I_{ds0}(t)$ curves demonstrating the changes in the signal from one and the same SOI-NW upon repeated detection of DNP.

The curves displayed in Figure 4 indicate that upon repeated detection of DNP, the signal level changes by ~15–20% with sufficient reproducibility.

Thus, SOI-NW sensors fabricated using CMOS-compatible technology display the concentration limit of DNP detection at the level of 3×10^{-14} M. This value is an order of magnitude lower than that demonstrated by Engel et al. [28] and comparable with the detection limit attained for TNT by Lichtenstein et al. [29]. Engel et al. [28] employed sensors fabricated using «bottom-up» technology without any special protective coating. As is known, SOI-NW sensors devoid of protective oxide coatings are lacking stability, what was demonstrated in a number of studies (including the study by Peled et al. [30] and our previous paper [18]). In the present study, we have employed SOI-NW sensors with protective layers of high-k dielectrics. This has allowed us to obtain more stable and, at the same time, more sensitive structures [35]. The signal, observed upon the addition of DNP, is caused by the formation of complexes with a charge transfer between the DNP molecules and the amino groups of the silane layer; this leads to the change in surface potential of the silicon channel of the transistor [17].
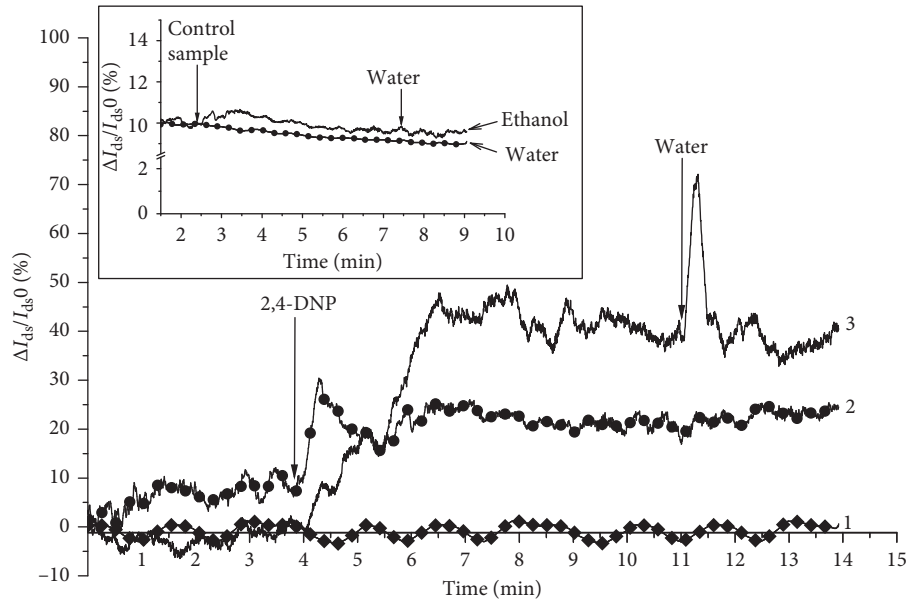
FIGURE 3: Time dependencies of the normalized differential signal from the SOI-NW sensor obtained upon DNP detection. DNP concentration 3×10^{-15} M (curve 1), 3×10^{-14} M (curve 2), and 3×10^{-13} M (curve 3) in deionized water. Inset displays examples of curves obtained in control experiments upon the addition of DNP-free solutions (ethanol or water) into the measuring cell. Experimental conditions: $V_g = 20$ V, $V_{ds} = 0.2$ V, and total solution volume in the cell 450 μL. Arrows indicate addition of DNP solution and washing with water.
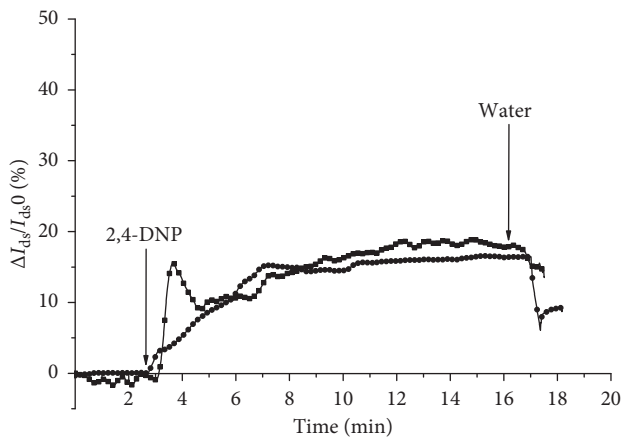

FIGURE 4: Time dependencies of the normalized differential signal from the SOI-NW sensor obtained upon repeated detection of DNP. Experimental conditions: DNP concentration 3.0×10^{-14} M in deionized water; $V_g = 20$ V, $V_{ds} = 0.2$ V total solution volume in the cell 450 μL. Arrows indicate addition of DNP solution and wash with water.

It is worth noting that high stability of SOI-NW sensors with a protective layer has allowed us to reveal the desired signal against the background of a nonspecific signal from the ethanol-containing solution, which increases the transistor current (Figure 3, curve 2).

As was demonstrated by Engel et al., careful selection of surface modification can lead to an increase in sensor specificity and in sensitivity against nitrophenol analyte to be detected [28]. Therefore, in the future, to provide the specificity and to increase the sensitivity of DNP detection, various modifications of SOI-NW sensor surface can also be used. Accordingly, due to low price and manufacturability,

the developed sensors based on lower-submicron CMOS-compatible technology can well find application in highly sensitive detection of poisons and explosives. Moreover, these sensors can be arranged in multichannel arrays, from which separate sensitive units for detection of different target compounds can be formed.

4. Conclusions

Our study has demonstrated that SOI-NW sensors, fabricated using CMOS-compatible technology, allow direct highly sensitive detection of DNP with 3×10^{-14} M detection limit. These sensors can well be used for rapid monitoring of toxic compounds. SOI-NW sensors are cheap, easy to use, and can be adapted for detection of a wide range of toxic and explosive compounds by selection of sensor surface modification techniques.

Data Availability

The data underlying the results of this research can be provided on request directly to the interested person.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.
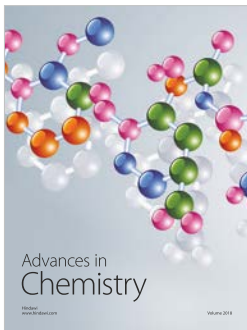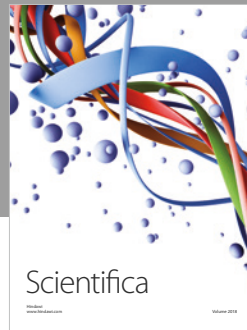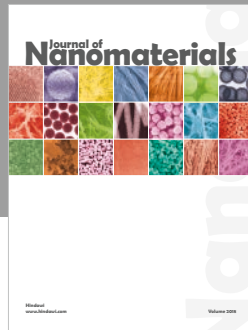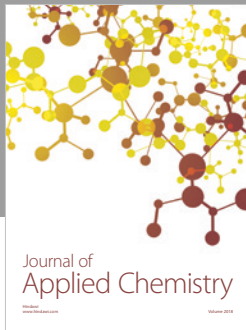
Acknowledgments

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