Investigation of electrolysis-related modification of graphene films in biosensors

I A Eliseyev¹, A S Usikov²³, S P Lebedev¹, A D Roenkov³, M V Puzyk¹, Yu M Makarov², E V Gushchina¹, G A Oganesyan¹, A A Voronina², E I Shabunina¹, N M Shmidt¹

¹Ioffe Institute, 26 Politekhnicheskaya, 194021 St. Petersburg, Russia
²Nitride Crystals Inc., 9702 Gayton Road, Suite 320, Richmond, VA 23238, USA
³Nitride Crystals Group, 27 Engelsa pr., 194156 St. Petersburg, Russia
⁴Herzen University, 48 Moika emb., 191186 St. Petersburg, Russia
⁵St. Petersburg Electrotechnical University "LETI", 5 Professora Popova, 197376 St. Petersburg, Russia

E-mail: zoid95@yandex.ru

Abstract. In this work, the modification of the surface parameters of graphene chips after electrolysis treatment in a NaClO₄ aqueous solution has been studied. Two electrolysis modes have been analysed. In the first one, a negative potential (-0.2 V) is applied to the graphene chips, while in the second one the potential is positive (0.8 V). Investigation using a number of techniques including atomic force microscopy, Kelvin probe force microscopy, Raman spectroscopy, measurements of current-voltage characteristics and low-frequency noise has shown that the electrolysis mode with application of a positive potential on graphene chips decreases the 1/f noise and allows one to obtain a uniform surface potential distribution while leaving the graphene structure undamaged. The results of this study help to understand the efficiency and reproducibility of the procedure for electrolysis treatment of graphene chips.

1. Introduction

Graphene is a promising material for creating biosensors capable of diagnosing extremely low concentrations of biomolecules associated with various socially significant diseases at their early stages, including HIV, hepatitis, cancer, influenza, and COVID-19. Graphene films are known to be able to adsorb various molecules and biological agents on the surface [1]. Experiments recently carried out at the Smorodintsev Institute of Influenza have shown that the biosensors based on graphene films grown at the Ioffe Institute allow the detection of orders of magnitude lower concentrations of influenza viruses in comparison with traditional methods of enzyme immunosorbent assay [2].

Selective sensitivity of graphene films is achieved by special processing creating additional covalent bonds that provide chemical reactions with detected biomolecules. The fabrication of graphene-based biosensors usually includes a number of preparatory stages: surface potential modification, functionalization (attachment of the phenylamine group), and immobilization (attachment of antibodies related to antigens).

Each stage has an influence on the sensitivity of biosensors. At the first stage, it is necessary to ensure a uniform distribution of the surface potential across the sample, which enables one to achieve
complete functionalization of the entire graphene surface and consequently helps to enhance the sensitivity of biosensors and improves reproducibility of the results of the biomolecule detection. At the same time, it is crucial that the modification procedure would not damage the structure of graphene. The aim of this study is to investigate the effect of the stage of surface potential modification by different electrolysis modes on the distribution of the surface potential of graphene, as well as on its crystalline quality.

2. Experimental
In this work, we studied the modification of the surface parameters of graphene chips after electrolysis in 0.1 M (0.1 mol/l) NaClO₄ aqueous solution. Two electrolysis regimes were under investigation. In Mode I, a negative potential (-0.2 V) is applied on graphene chips, which is known to cause removal of the oxygen groups from the graphite surface [3]. In Mode II, a positive potential (0.8 V) is applied on graphene chips during electrolysis. According to the literature data, such treatment results in electrochemical oxidation and formation of oxygen groups on the surface of unoxidized graphite [4].

The films of graphene under study were grown by thermal decomposition of 4H-SiC substrates [5]. The investigated chips were obtained from four plates and planted on holders with metallic contacts. The sensor area of the graphene surface was 1 x 1.5 mm². Details of sensor fabrication and their appearance can be found in our previous work [6]. To monitor reproducibility of the biosensor’s parameters after electrolysis treatment we used a number of techniques that included atomic force microscopy (AFM), Kelvin probe force microscopy (KPFM), Raman spectroscopy, measurements of current-voltage (I-V) characteristics and low-frequency noise.

AFM and KPFM measurements were carried out on Ntegra AURA setup (NT-MDT, Russia). Raman spectra were measured at room temperature in the backscattering geometry using a T64000 spectrometer (Horiba Jobin-Yvon, France) equipped with a confocal microscope, which allowed obtaining information from the region of a graphene film 1 µm in diameter. Along with local measurements, the measurements of sample areas of 7 × 7 µm² with a step of 0.5 µm with subsequent plotting of Raman maps of spectral lines parameters were performed. A YAG: Nd solid-state laser with a wavelength of 532 nm was used as an excitation source. The laser power on the sample was limited to 4.0 mW to prevent damaging and modification of graphene films.

The I-V measurements were measured using the KEITHLEY 6487 power source. The power spectral density of voltage fluctuations was measured in the frequency range of 1 Hz - 50 kHz. For noise measurements, the studied samples were connected in series with a low-noise load resistor \(R_L\) whose resistance varied from 100 Ohm to 13 kOhm depending on the current through the chip. The voltage fluctuations \(S_V\) at the resistors \(R_L\) were amplified by a low-noise preamplifier SR560 (Stanford Research Systems, USA) and subsequently measured by SR 770 FET NETWORK Analyzer (Stanford Research Systems, USA). The background noise of the preamplifier does not exceed 4 nV/√Hz at 1 kHz, which is approximately equivalent to Johnson–Nyquist noise of a 1000 Ohm resistance.

3. Results and discussion
The resistance measurements were carried out on all chips obtained from graphene/SiC plates. The resistance spread for the chips processed from the first plate (Plate 19) was 4% with an average value of 8.3 kOhm. The chips of the second plate (Plate 35) demonstrated lower average resistance values ~ 4.9 kOhm and their spread was near 2%. The I-V characteristics of all the chips were linear.

Before and after electrolysis treatment, the frequency dependences of the spectral density of voltage \(S_V\) and current \(S_I\) fluctuations had a \(1/f\) character typical for graphene (Figure 1). However, the \(S_V\) values at 1 Hz obtained before and after electrolysis were significantly different for both modes. It can be seen that electrolysis treatment in mode I leads to an increase in \(S_V\) relative to the initial values by 5 times. On the contrary, use of Mode II led to a striking decrease in \(S_V\) by more than an order of magnitude.
Figure 1. Dependence of the spectral density of voltage fluctuations ($S_V$) on frequency for the graphene chips from plate 19 (curves 1 and 2) and plate 35 (curves 3 and 4) after the electrolysis at Mode I (curves 2 and 4) and at Mode II (curves 1 and 3).

Table 1. Estimated parameters of the samples under study.

| Chip name | $S_V$, V$^2$/Hz (LF-noise at 1.22 Hz) |
|-----------|-------------------------------------|
|           | Before electrolysis | After Mode I (-0.2 V) | After Mode II (0.8 V) |
| 35-61     | 5.0*10^{-11}       | 1.26*10^{-11}          |                        |
| 35-21     | 4.4*10^{-11}       | 5.2*10^{-10}           |                        |
| 19-21     | 7.8*10^{-12}       | 2.5*10^{-11}           |                        |
| 19-31     | 9.0*10^{-12}       | 1.60*10^{-12}          |                        |

Figure 1 and Table 1 point out the changes in the degree of defectiveness of the graphene surface after treatment since the $S_V$ values integrally characterize the defectiveness of the material. As it was mentioned above, graphene electrolysis treatment with negative potential applied on it may lead to an increase of oxygen group concentration on its surface. In this regard, a decrease in the graphene defectiveness degree after treatment in Mode II is in good agreement with the well-known mechanism of reducing the density of surface states at the Si-SiO$_2$ surface, when oxygen groups are introduced during its formation.

The KPFM measurements provided an assessment of the homogeneity of the surface potential before and after electrolysis. Variation of the surface potential on the initial samples was 25-50 mV (Figure 2, curve 2). After treatment of the surface of these samples in an aqueous electrolyte in mode II, we observed a significant decrease in the surface potential variation down to 5 mV (Figure 2, curve 3). However, treatment of the samples in mode I was accompanied by an increase in the variation of the surface potential values up to 150 mV (Figure 2, curve 3). In the context of the fact of an increase in low-frequency noise such an increase in the surface potential variation can be interpreted as an increase in the degree of defectiveness of the graphene film.
Figure 2. Typical surface potential profiles of the graphene chips before treatment in the electrolyte (black curve); after treatment in mode II (red curve); after treatment in mode I (blue curve). The surface potential distribution profiles were extracted from the surface potential distribution maps obtained from $10 \times 10 \mu m^2$ areas.

Figure 3 shows typical Raman spectra of two samples subjected to different electrolysis treatment procedures. The spectrum of the sample 1 after treatment in Mode II is characteristic of high-quality graphene grown on 4H-SiC and is composed of sharp $G$ ($\sim 1590$ cm$^{-1}$) and 2$D$ ($\sim 2700$ cm$^{-1}$) lines and a contour in the range of 1200-1650 cm$^{-1}$ related to the buffer layer [7,8]. In case of the sample after treatment in Mode I, we see an intensive defect-related $D$-line in its spectrum. Analysis of Raman spectroscopy data can not only give us information on the presence of defects, but also provides information on their nature. Particularly, one can distinguish between point (0D) and line (1D) defects since appearance of the latter leads to stronger phonon localization and hence faster increase of the $G$-line FWHM with increase in defect concentration. For this, the well-known method of analyzing the ratio of the $D$- and $G$-line intensities ($I(D)/I(G)$) and the $G$-line FWHM can be used [9]. In case of sample after Mode I, the $G$-line FWHM is 21 cm$^{-1}$, and the $I(D)/I(G)$ ratio is 0.3, which corresponds to the domination of the line defects and the average crystallite size of 64 nm [9,10]. Thus, the results of Raman spectroscopy measurements confirm the appearance of defects after treatment in Mode I, which was assumed based on the analysis of the low-frequency noise measurements data.

Figure 3. Raman spectra of the samples after treatment in modes I and II. The spectra are presented upon subtraction of the second-order 4H-SiC spectrum contribution.
Properties of graphene surface and its interaction with adsorbates are extremely dependent on the number of graphene layers [11]. Thus, we analyzed the shape of the 2D line in the Raman spectra of the samples under study to figure out the share of bilayer graphene inclusions on the surface of each chip and the distribution of bilayer domains. The 2D line has a symmetric Lorentzian shape in case of monolayer graphene, and an asymmetric shape described by four Lorentzians in case of bilayer, whose 2D line FWHM is twice that in the case of a monolayer [12]. Thus, the FWHM distribution map of the 2D line was analyzed to determine the fraction of bilayer graphene for every sample. According to this analysis, the samples can be divided into two groups: with larger (~30 %) and smaller (~5 %) share of bilayer graphene. The maps for two characteristic samples showing the distribution of bilayer inclusions in the areas of 7 × 7 µm² are shown in Figure 4.

Figure 4. Raman maps of the 2D line FWHM distribution for two chips under study: (a) for the chip with ~30 % of bilayer inclusions, (b) for the chip with ~5 % of bilayer inclusions.

After treatment of the chips in mode II, the variation of the surface potential was approximately 10 mV (Figure 5, curve 2). With a larger amount of bilayer graphene (up to 30%), the variation of the surface potential is higher - up to 20-40 mV (Figure 4, curve 2). This can be connected with smaller chemical reactivity of bilayer graphene in comparison to monolayer [13].

Figure 5. Typical surface potential profiles of the graphene chips with different share of bilayer graphene inclusions: 30% (black curve) and less than 5% (red curve) after treatment at mode II. The surface potential distribution profiles were extracted from the surface potential distribution maps obtained from 10 × 10 µm² area.
4. Conclusion
Influence of two modes of electrochemical treatment of the surface of graphene in a 0.1 M NaClO₄ electrolyte was investigated by a number of techniques. It was shown that the mode of electrolysis with a negative potential applied on graphene results in formation of defects and increased inhomogeneity of the surface potential of graphene. On the contrary, treatment of the graphene surface with positive potential applied to it increases the uniformity of the surface potential distribution without causing significant disturbances in the crystal structure of graphene. Such an increase in surface potential uniformity may be connected with an increase of the concentration of oxygen on the surface of graphene. However, for graphene chips containing a considerable amount of bilayer graphene inclusions, the efficiency of this mode is significantly lower due to lower chemical reactivity of bilayer graphene. Thus, it is revealed that use of the electrolysis mode with a positive potential applied on graphene for the modification of the surface potential of monolayer graphene chips contributes to the successful subsequent steps required to obtain biosensors with reproducible parameters and more uniform attachment of antibodies and viruses over the chip area.

Acknowledgments
This research was supported within the State Assignments from the Ministry of Science and Higher Education of the Russian Federation to the Ioffe Institute (0040-2019-0006)

References
[1] Li X, Tao L, Chen Z, Fang H, Li X, Wang X, Xu J-B and Zhu H 2017 Graphene and related two-dimensional materials: Structure-property relationships for electronics and optoelectronics Appl. Phys. Rev. 4 021306
[2] Lebedev A A, Davydov S Y, Eliseyev I A, Roenkov A D, Avdeev O, Lebedev S P, Makarov Y, Puzyk M, Klotchenko S and Usikov A S 2021 Graphene on SiC Substrate as Biosensor: Theoretical Background, Preparation, and Characterization Materials (Basel). 14 590
[3] Zhou M, Wang Y, Zhai Y, Zhai J, Ren W, Wang F and Dong S 2009 Controlled synthesis of large-area and patterned electrochemically reduced graphene oxide films Chem. - A Eur. J. 15 6116–20
[4] Yakovlev A V., Yakovleva E V., Tseluikin V N, Krasnov V V., Mostovoy A S, Rakhmetulina L A and Frolov I N 2019 Electrochemical Synthesis of Multilayer Graphene Oxide by Anodic Oxidation of Disperse Graphite Russ. J. Electrochem. 55 1196–202
[5] Lebedev A A, Davydov V Y, Usachov D Y, Lebedev S P, Smirnov A N, Levitskii V S, Eliseyev I A, Alekseev P A, Dunaevskiy M S, Rybkin A G, Novikov S N and Makarov Y N 2018 Study of properties and development of sensors based on graphene films grown on SiC (0001) by thermal destruction method Journal of Physics: Conference Series vol 951
[6] Eliseyev I A, Usikov A S, Lebedev S P, Roenkov A D, Puzyk M V., Zubov A V., Makarov Y N, Lebedev A A, Shabanina E I, Dementev P A, Smirnov A N and Shmidt N M 2020 Raman scattering and low-frequency noise in epitaxial graphene chips J. Phys. Conf. Ser. 1697
[7] Ferrari A C and Basko D M 2013 Raman spectroscopy as a versatile tool for studying the properties of graphene. Nat. Nanotechnol. 8 335–46
[8] Wang T, Huntzinger J R, Bayle M, Robin C, Decams J M, Zahab A A, Contreras S, Paillet M and Landois P 2020 Buffer layers inhomogeneity and coupling with epitaxial graphene unravelled by Raman scattering and graphene peeling Carbon N. Y. 163 224–33
[9] Gustavo Cançado L, Gomes da Silva M, Martins Ferreira E H, Hof F, Kampioti K, Huang K, Pénicaud A, Alberto Achete C, Capaz R B and Jorio A 2017 Disentangling contributions of point and line defects in the Raman spectra of graphene-related materials 2D Mater. 4 025039
[10] Cançado L G, Takai K, Enoki T, Endo M, Kim Y A, Mizusaki H, Jorio A, Coelho L N, Magalhães-Paniago R and Pimenta M A 2006 General equation for the determination of the
crystallite size $L_a$ of nanographite by Raman spectroscopy. \textit{Appl. Phys. Lett.} \textbf{88} 163106

[11] Seekaew Y, Phokharatkul D, Wisitsoraat A and Wongchoosuk C 2017 Highly sensitive and selective room-temperature NO$_2$ gas sensor based on bilayer transferred chemical vapor deposited graphene. \textit{Appl. Surf. Sci.} \textbf{404} 357–63

[12] Ferrari A C, Meyer J C, Scardaci V, Casiraghi C, Lazzeri M, Mauri F, Piscanec S, Jiang D, Novoselov K S, Roth S and Geim A K 2006 Raman spectrum of graphene and graphene layers. \textit{Phys. Rev. Lett.} \textbf{97} 187401

[13] Yamamoto M, Einstein T L, Fuhrer M S and Cullen W G 2012 Charge inhomogeneity determines oxidative reactivity of graphene on substrates. \textit{ACS Nano} \textbf{6} 8335–41