

PREPARATION OF GRAPHENE OXIDE COMPOSITES
CONTAINING NANOMETALS AND OXIDES FROM GRAPHITE
FOIL WASTES AND STUDY OF THEIR BIOCIDAL ACTIVITY

T. Dundua

Georgian Technical University
Faculty of Agricultural Sciences and Biosystems Engineering
Tbilisi, Georgia
dondua.tamar@gtu.ge

Accepted 2021 December 24

Abstract

Graphene oxide (GO) is obtained using improved Hummers method by oxidation of powdered graphite foil wastes (pGFW) at low (~ 0 °C) and relatively high (~ 50 °C) temperature. GO–Me(NO₃)_x complexes are synthesized by the interaction of GO and metal nitrates; further heat treatment in vacuum (exfoliation) leads to the production of composites containing reduced graphene oxide (rGO), nano-sized metals (Cu and Ag), and oxides (Fe₂O₃). The rGO–TiO₂ (nano) composite was obtained by hydrolysis of titanium alkoxide and subsequent heat treatment in the presence of GO. Composites have been shown to have high antibacterial activity both against gram-positive and negative bacteria.

1. Introduction

Since the discovery of graphene at the beginning of the 21st century, special attention has been paid to compounds with carbon 2D and 3D structures for their great importance and perspective [1]. Now they are widely used in many fields of science and technology, what is reflected in the scientific and patent literature. There are many organic–organic and organic–inorganic derivatives containing GO and rGO structures and they have a wide area of applications [2 – 6]. Graphite oxide was first prepared in the 19th century. In all methods of its synthesizing strong acids and oxidants are used [7 – 9].

Graphite and graphene oxides (GO) and reduced graphene oxide (rGO) are graphite single layer (graphene) oxidation products. Discussion about their structure in scientific literature continues. Below reviews are summarized by the data of various authors on the GO structure. It has been established that graphite oxidation first produces graphite oxide and then after sonication GO is obtained. rGO is a reduced product of GO. Different types of organic and inorganic compounds are used as reductants [10, 11]. Accordingly, GO and rGO physical-mechanical properties or chemical composition changes within a wide range. GO and rGO have organic functional groups (carboxylic, carbonyl, hydroxyl, epoxide). Their future functionalization or joining (immobilization) in organic or inorganic precursors may be performed. Below we discuss some methods of obtaining GO and rGO:

1. Hummers method: KMnO_4 ($\sim 0^\circ\text{C}$) is added to the mixture of graphite– NaNO_3 – H_2SO_4 by portions [9];
2. The improved Hummers method: KMnO_4 is added to the mixture of graphite– H_2SO_4 – H_3PO_4 ($\text{H}_2\text{SO}_4\text{:H}_3\text{PO}_4 = 9\text{:}1$) at 50°C [12];
3. Optimized improved Hummers method: KMnO_4 is added to graphite– H_2SO_4 mixture at $30 - 50^\circ\text{C}$ [13];
4. Mixture of expanded graphite ($< 30\ \mu\text{m}$)– KMnO_4 at $\sim 0^\circ\text{C}$ is added with H_2SO_4 [14, 15];
5. Pretreatment of graphite powder or its intercalation by H_2SO_4 – P_2O_5 – $\text{K}_2\text{S}_2\text{O}_8$ system and further oxidation to the GO [16];
6. NaNO_3 -free methods, e.g. adding with K_2FeO_4 – H_2SO_4 [17].

Currently, flake graphite is used for the obtaining GO and rGO by their reduction (in process of chemical or thermal treatment). Graphene is obtained with some structural defects [18, 19]. Obtaining methods and areas of application of GO, rGO, and graphene are well described in the scientific literature [20]. GO and rGO were obtained from commercial expanded graphite powders (with grain size $\text{D}_{50} \sim 15$ and $1 - 30\ \mu\text{m}$). Unlike other methods, the authors have proposed a new method of preparation of the reaction mixture that have also used ultrasonic cleaner [21, 22]. It was found that graphite foil and waste powders can be successfully used as precursors (pGFW) [23, 24]. Flexible graphite foils in various thickness and width are used in modern technologies. The remaining graphite foil pieces (wastes) are expanded graphites and their chemical oxidation to GO or graphene can be conducted using known methods with some adjustments [18]. Information on the properties and the fields of the application of the products and graphite foil manufacturer companies are available on websites [25]. What is graphite foil? It is obtained from graphite intercalated compounds (GIC). The GIC are obtained by intercalation of graphite with various compounds (gaseous compounds, HNO_3 , H_2SO_4 , CrO_3 , KMnO_4 , H_2O_2 , FeCl_3 , AlCl_3 , etc.). In the result of intercalation, expandable graphite is obtained. By thermal shock treatment of GIC high pressure is created between the graphite layers and the crystalline structure of graphite collapses in layers ($1 - 20$ layers). By its further treatment with pressure, graphite foil products and materials are received, which are used in various fields of technology [25].

Thus, natural and synthetic graphites, graphite intercalated with different compounds, expanded graphite, and graphite foil are used to obtain GO, rGO, and graphene. It is established that the reduction of the GO obtained from the samples of the different types of graphite are formed graphenes, in which the number of layers are very different ($1 - 10$ layers or more) [19, 26 – 30]. Graphene oxides and materials obtained from them have several unique properties. Currently, much attention is paid to the creation of new types of biologically active composites and their use both for solving environmental problems and for the production of medicines.

The purpose of our work is to use graphite foil wastes to obtain GO and rGO and composites containing nano metals and oxides from them and study their biocidal activity.

2. Materials and methods

Graphite flake, natural, 325 mesh – 99.98 % (metals basis), was purchased from Alfa Aesar, KMnO_4 , NaNO_3 , H_2SO_4 (98 %), HCl (37 %), HI (57 %) and ascorbic acid were purchased from Sigma Aldrich. By grinding graphite foil or its wastes, a powder particle size of $140\ \mu\text{m}$

was obtained. It was used to obtain graphene oxides. The morphology of the samples was studied by optical and scanning electron microscopes (Nikon ECLIPSE LV 150, LEITZ WETZLAR, and JEOL JSM-6510 LV-SEM). XRD patterns were obtained with DRON-3M (Cu $K\alpha$, Ni filter, 2 °/min) and XZG-4 (Cu $K\alpha$, $\lambda = 1.5418 \text{ \AA}$) diffractometers. FTIR spectra were recorded on Agilent Cary 630 spectrometer (350 – 5000 cm^{-1}).

Thermal treatment of GO and rGO (< 1500 °C) was implemented in high temperature vacuum furnace (Kejia furnace). For the ultrasound treatment and homogenization of suspensions was used Ultrasonic cleaner (45 KHz) and JY92-IIDN Touch Screen Ultrasonic Homogenizer (20 – 25 kHz, 900 W). GO and rGO particle sizes were determined by Winner802 DLS Photon correlation nano particle size analyzer.

2.1. Graphene oxide synthesis

1 g of NaNO_3 and 40 ml of 98 % sulfuric acid were added to 1 g of pGFW. The reaction mixture was cooled up to 0 – 1 °C and stirred for 30 min. Over the next 50 min, 4 g of KMnO_4 was added. The temperature of the reaction mixture is raised to 35 – 40 °C under stirring for 3 h. The reaction mixture was diluted with 100 ml of cold water keeping the temperature below 90°C, and then stirred at this temperature for 30 min. The mixture was diluted to 500 and 2 ml of 30 % of H_2O_2 solution was added. A yellowish suspension of graphite oxide was obtained. During the washing of the sediment its color gradually changed to dark brown. 20 min later solution was removed by decantation. This operation was repeated twice. For the rapid precipitation of graphite oxide from the suspension, a 5 % solution of hydrochloric acid (500 ml) was added. Decantation was repeated 3 times in 10 min intervals. An aqueous gel-like mass was obtained. Centrifugation and washing of this precipitate continued until the pH is 5 – 6. The sediment was washed with acetone 3 times and is dried in vacuum at 70 °C for 4 h.

GO and rGO synthesis was carried out by the third and fourth methods as described above. The synthesis procedure is given in our earlier papers with slight modifications [24, 31].

2.2. Synthesis of rGO–Ag composite by thermal exfoliation method

20 ml of an aqueous solution, containing 80 mg of silver nitrate was added to 60 ml graphene oxide suspension (4.2 mg GO/ml) under stirring. The mixture was stirred at room temperature for 30 min and the reaction mixture was left for 24 h. The GO– AgNO_3 complex was then separated from the reaction medium by centrifugation, further washed with water until the Ag^+ was removed and dried in a vacuum at 40 °C. The resulting black mass was placed in a glass reactor (L = 30 and D = 4 cm) and heated in a vacuum to 120 – 125 °C. At this temperature there is an instantaneous increase in the volume of the powder. The reactor is vacuumed for 10 min and cooled in argon. Black rGO–Ag composite puff powder is obtained. rGO–Cu– Cu_2O and rGO– Fe_2O_3 composites were obtained similarly.

2.3. Synthesis of rGO– TiO_2 composite by thermal exfoliation method

To a 60 ml of graphene oxide suspension (4.2 mg GO/ml) was added 2 drops of hydrochloric acid and 0.3 ml of titanium diisopropoxide bis (acetylacetonate) (70 % solution in isopropanol) dissolved in 10 ml of absolute ethyl alcohol (30 min). The reaction mixture was

stirred at room temperature for 4 h and left for 24 h. By centrifugation the GO-Ti(OH)_x was removed from the mixture, which was washed 3 times with ethyl alcohol and dried in vacuum at 70 °C. Thermal exfoliation of dry exfoliation of dry composite is carried out at 135 – 140 °C as described in case of rGO-Ag composite. Black rGO-TiO₂ composite powder was obtained.

2.4. Assessment of antibacterial activity: Microbial cultures

The inhibition effect was measured using the viable cell count method, in which the nanoparticles formed prior were incubated with the test bacteria via a suitable technique and the bacterial growth of *S. aureus* and *E. coli* was measured. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 isolates, obtained from the collection of the George Eliava Institute of Bacteriophage, Microbiology, and Virology, were used in this study.

For the experiments, a single colony was inoculated into 5 ml trypticase soy broth (TSB; oxide) and incubated at 37 °C overnight in aerobic condition. Cultures were refreshed for 2 h at 37 °C in an orbital shaker in aerobic condition and standardized at colony-forming units (CFU) of 5·10⁶ CFU/ml in TSB diluted 1:5 in phosphate-buffered saline (PBS) [32].

2.5. Antimicrobial activity

The standardized broth cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 isolates, prepared as indicated above, were incubated with different concentrations of GO nanocomposites (20 and 40 µg/ml) in saline at 37 °C for 24 h to evaluate the antimicrobial effect. As controls, bacteria were incubated with fresh diluted TSB (1:10 in PBS). The CFU were counted from each plate and the antibacterial activity was expressed as a function of cell viability loss. The loss of cell viability was determined using the number of colonies found in the experimental mixture incubated with GO nanocomposites and the number of colonies found in the sample, incubated without nanocomposites. The experiments were done in triplicate and the average values were reported.

2.6. Bacterial cell culture and disc diffusion assay

Bacterial cell cultures were inoculated in a fresh nutrient agar medium overnight in an incubator. Then 1 ml of these cultures of bacterial strains was transferred to solidified nutrient agar. Once the plates were ready, various concentrations (50, 100, and 150 µl) of nanoparticles loaded discs were placed over the nutrient agar plates. All the plates were left to diffuse the sample and kept in an incubator at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured [33, 34].

3. Results and discussions

3.1. Synthesis and characterization of graphene oxide from pGFW

Graphite foil wastes from various technological processes are contaminated with various types of organic and inorganic compounds. We have developed methods for the removal of waste impurities based on graphite production and purification processes, also well known

methods used to produce active carbon. The objects of study were the wastes that were under long- or short-term thermal treatment during the ceramic powders sintering process (1400 – 2100 °C). Waste powders with particle size < 50 and 50 – 140 µm were obtained after grinding the wastes. Earlier by XRD analysis we confirmed that in the samples we used it appears that the diffraction maximum of graphite and graphite foil match each other. The XRD pattern of the natural flake graphite exhibited peaks located at 26.44, 44.30, and 55.11 ° corresponding to planes (002), (101), and (004), respectively. In case of graphite foil, peak (101) did not appear at all and a peak of increased intensity (004, 25200) appeared. The same peak intensity reaches 11100 in case of graphite [24, 31]. At the same time, the graphite foil and its wastes are easily grinded. For GO synthesis powders with particle size < 50 and 50 – 140 µm were used. Below are pictures of industrial graphite foil, its wastes and grinded wastes (**Figure 1**).

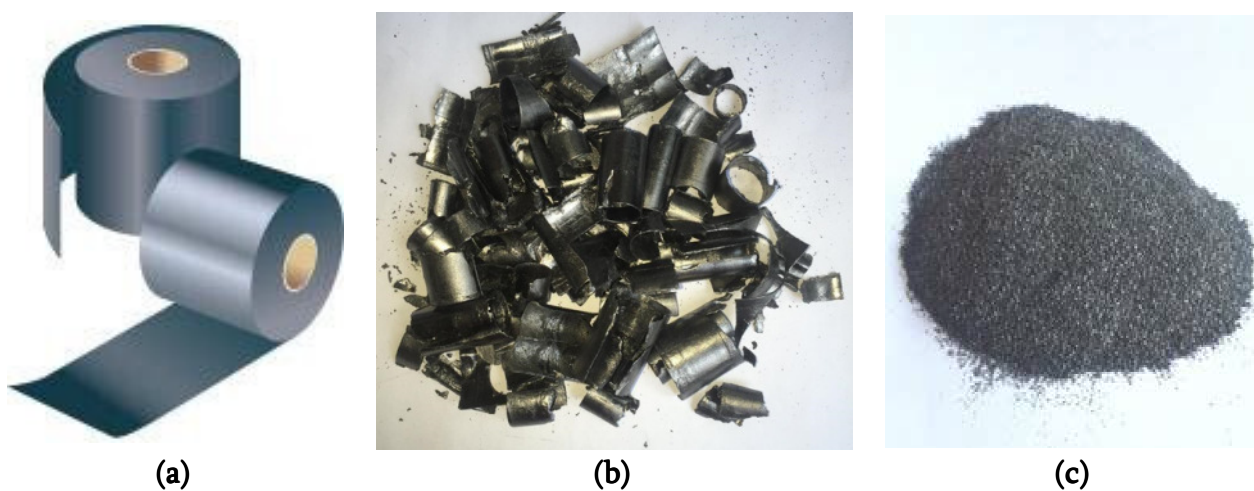


Figure 1. (a) Industrial graphite foil (<http://www.toyotanso.com/Products/index.html>), (b) Graphite foil wastes obtained during the high-temperature sintering of ceramics, and (c) pGFW obtained as a result of their grinding.

By EDX analysis it is established, that pGFW samples contain large amounts of inorganic impurities, which are removed by traditional methods of graphite purification. The basic processes for obtaining GO, rGO, and graphene from graphite foil and its wastes powders are given in **Figure 2**.

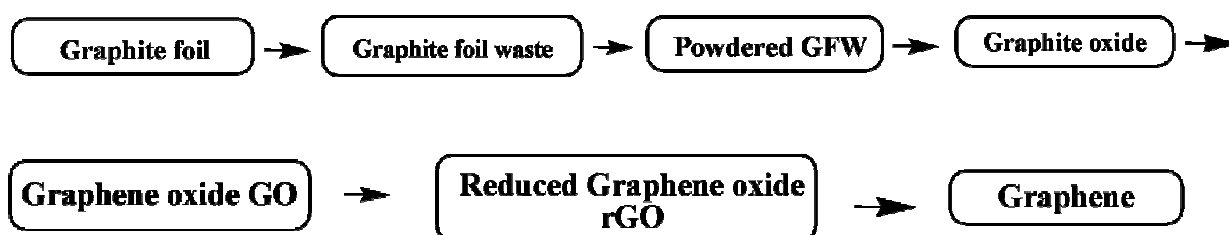


Figure 2. Simplified scheme for obtaining GO, rGO, and graphene from pGFW.

Following methods have been used for graphite foil and its wastes powder oxidation: Hummers method; optimized improved Hummers method and adding of H₂SO₄ to the mixture pGFW–KMnO₄. Experimental part provides detailed methods for pGFW oxidation (Methods 1, 3, and 4). Oxidation of pGFW results in the formation of yellow graphite oxide. The completion of the reaction and the production of the target product were checked by physical-chemical research methods. pGFW oxidation to GO using KMnO₄–NaNO₃–H₂SO₄ and KMnO₄–H₂SO₄ systems is easily fixed by XRD analysis. In particular, the characteristic XRD peak of pGFW at

$2\theta = 26.46^\circ$ completely disappears during the oxidation process due to complete oxidation of pGFW into graphite oxide. XRD analysis also confirmed that characterizing of GO and rGO phase diffraction peaks depends on the method of pGFW oxidation and sample thermal treatment processes. Characterizing of GO peaks are registered within $2\theta = 10.07 - 12.30^\circ$ interval. Typical diffractograms of GO, rGO and graphene obtained by thermal treatment at 1000°C are shown in **Figure 3**. The obtained results correspond to the available data of processes used flake graphite or expanded graphite as a precursor [19, 21, 27].

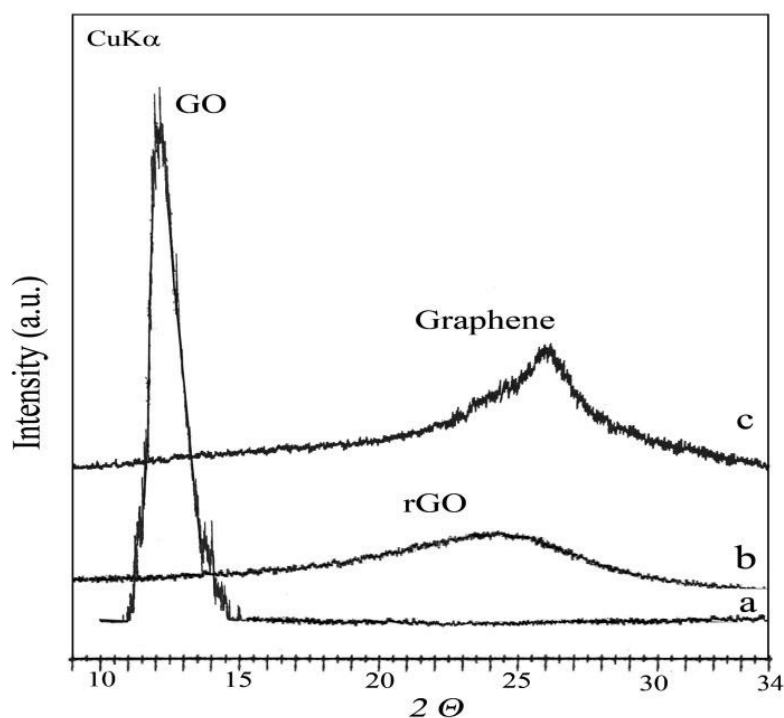


Figure 3. Typical XRD patterns of GO (a), rGO (b), and graphene obtained by thermal exfoliation GO at 1000°C (c).

Our earlier studies have shown that in case of low-temperature mode oxidation ($\sim 0^\circ\text{C}$, $\text{KMnO}_4\text{--NaNO}_3\text{--H}_2\text{SO}_4$) of pGFW, the C:O ratio (at.%) was found to be 1.61. During its reduction with ascorbic acid, the C:O ratio was changed to 4.26. The peak of GO at completely disappeared during the reduction process and appeared a broad diffraction maximum for rGO at $2\theta = 23.80^\circ$ [23].

According to the literature data, during the reduction of GO to rGO by the different methods the diffraction peak maximums appears at different values of 2θ between 20.0 and 26.64° [28, 35, 36]. By the reduction of GO with various reagents, obtained from the pGFW, diffraction peak maximums appeared: hydrazine – 26.13° , the hydroiodic acid (57 %) solution – 23.72° , Alder extract – 25.03° , and thermal treatment (250°C) – 23.58° . According to the reduction methods, peak intensity varies from 1292 to 9790. GO obtained from pGFW by optimized improved Hummers method ($\sim 50^\circ\text{C}$, $\text{KMnO}_4\text{--H}_2\text{SO}_4$) C:O (at.%) ratio was found to be 1.83, which increases by its reduction with ascorbic acid (C:O = 4) because oxygen-containing functional groups are partially removed from graphene oxide.

FTIR method has confirmed that obtained GO contains OH groups ($3200 - 3700\text{ cm}^{-1}$), aromatic ring (1635 cm^{-1} , C=C stretching), carbonyl group (C=O, 1734 cm^{-1}) and C–O groups (1231 cm^{-1}).

As mentioned in the experimental part, a large amount of acidic solutions are obtained during the separation of GO from reaction solution and its purification processes: solutions

contained ~ 20 wt.% of GO. Separation of GO from such solutions is a time-consuming process and it is preferable to reduce it to rGO with different reductants. Ascorbic acid, zinc powder, hydroiodic acid, hydrazine and plant extracts were used as reductants. Metal (Al, Zn, Mg, etc.) powders and organic compounds were particularly effective. These are capable of reducing both graphite and graphene oxides to rGO under low pH. The resulting rGO quickly precipitates, filtered and impurities easily separated from rGO during the washing process. It should be noted that separation of GO from the reaction mixture by industrial method and removal of impurities are considered to be difficult processes requiring further optimization [20].

We partially have corrected the method of synthesis of GO and its separation from the reaction mixture so that the experiments ended only after 10 h. Sulfuric acid and ions (K^+ , Na^+ , and Mn^{+2}) were removed with decanting (2 times with H_2O , 2 times with 5 % HCl solution, and 3 times with H_2O). 5 % solution of HCl precipitates GO-flakes in 10 min. Thus, the process of removing the main impurities could be accelerated [23]. Although in the process of accelerated decantation small particles of GO pass into the decanted liquid, it is very easy to separate them in the form of rGO. Using photon correlation particle sizer analyzer it is confirmed that in the decanted solutions their sizes varies in wide range with average diameter of 547nm. (Figure 4).

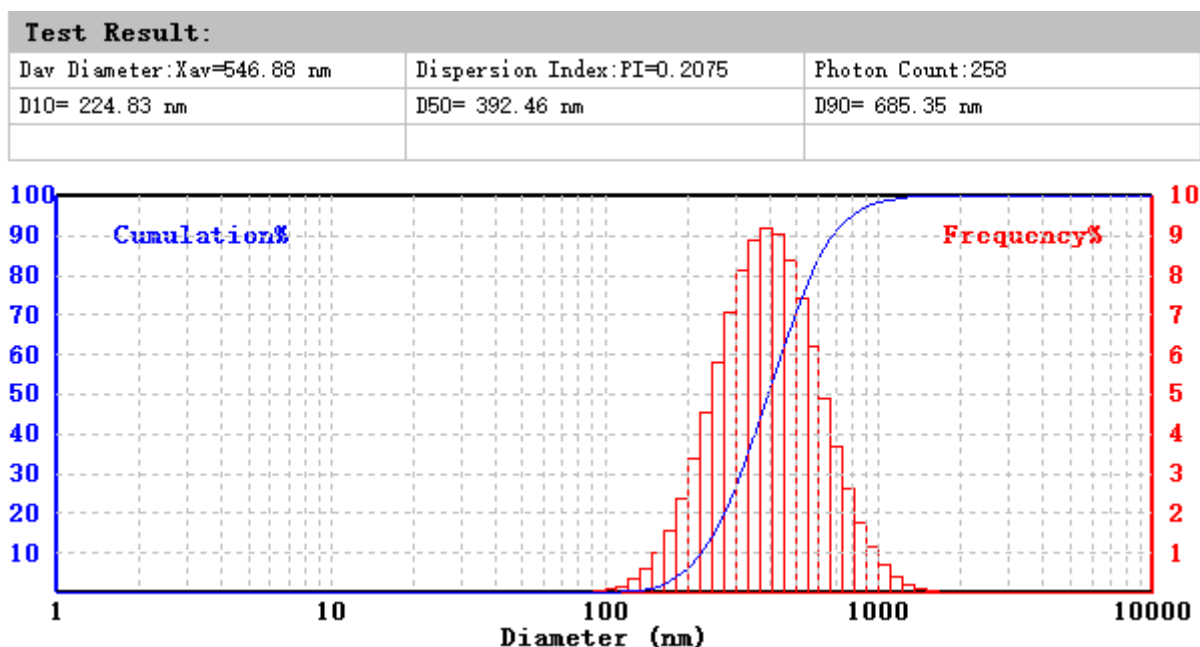


Figure 4. Particle size distribution in GO obtained from decanted solution.

By the reduction of GO with chemical compounds, rGO powders are obtained which are often used for production of organic and inorganic composites. rGO films have also great potential for use. The easiest way to get it is thermal treatment of GO films on air or in different gas flows. From rGO films are made filtration membranes, mechanical seals or protective layers. rGO film is good electric and heat conductor, therefore is widely used in electronics and optoelectronic devices. The process of heat treatment of GO films and the quick method of production the rGO films under controlled conditions is described in papers [35, 36].

It can be concluded that there is no difference between the oxidation processes of graphite and pGFW powders. In both cases, graphite oxide is obtained by further ultrasonication and reducing GO and rGO are obtained accordingly. The values of the diffraction maximum of GO and rGO depend on both the oxidizing systems, the nature of the reductant, and the heat treatment conditions.

3.2. Synthesis and study of rGO–metal and rGO–metal oxide composites

We have previously studied thermal treatment of GO powders and GO films obtained from pGFW were implemented at 20 – 1000 °C under argon flow and in vacuum, and at 20 – 300 °C in air. XRD analysis revealed that the GO diffraction peak disappeared at $2\theta = 12.22^\circ$ and two peaks of low intensity of rGO appeared at $2\theta = 20.3$ and 23.78° . By the thermal treatment of the resulted powder at 1000 °C yields graphene with defective structure having low intensity peak at $2\theta = 26.16^\circ$ [24, 31]. According to EDX spectrum the graphene obtained under these conditions does not contain any metal impurities and consists only carbon and oxygen in the ratio of 1:20, while in the initial rGO the same ratio was 1:4.7. This result is fully in line with the literature [37] showing that the C:O ratio varies as 1:8 – 1:246 depending on thermal treatment modes. The interesting fact is that during the vacuum thermal exfoliation of GO at 250 °C rGO is obtained with the same morphology as has the expanded graphite, which is obtained by the thermal shock treatment of graphite intercalated compounds (GIC). The difference is in mechanisms: when rGO is formed, chemical decomposition of GO occurs, as a result of which gases and water vapors spontaneously release, which leads to the collapse of its crystallites. In the case of GIC, during shock heat treatment, the intercalant creates a high pressure between the layers of graphite, damages its structure and leads to the obtaining of expanded graphite.

The rGO–Ag composite is obtained by two methods: reduction of the GO–AgNO₃ suspension with ascorbic acid and thermal treatment of the GO–AgNO₃ complex in a vacuum at 110 – 120 °C. As the XRD patterns show, the characteristic peak of GO at $2\theta = 10.27^\circ$ (upper) disappears and the amorphous complex GO–AgNO₃ (middle) by its thermal exfoliation forms the obtained (Figure 5a) rGO–Ag composite (lower). As can be seen from the SEM image, the amorphous complex of GO–AgNO₃ film is disintegrated as a result of thermal exfoliation in multiple layers, on which 5 – 12 nm silver particles are deposited (Figure 5b).

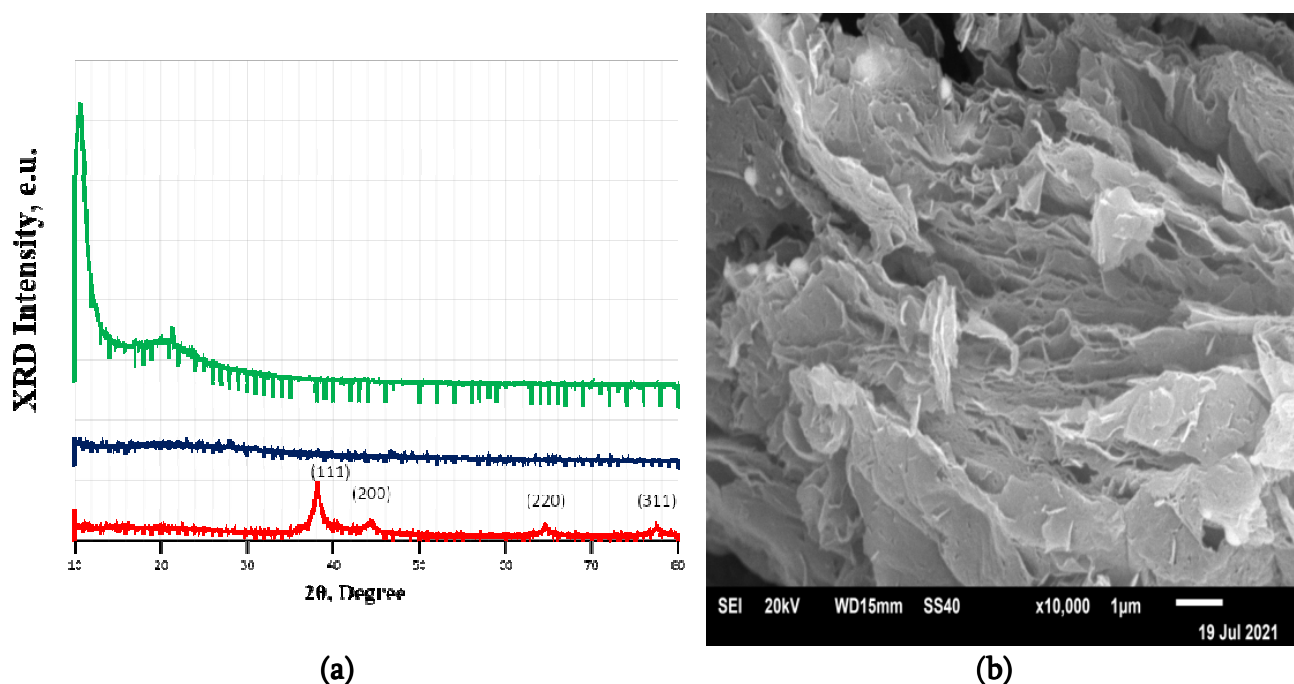


Figure 5. (a) XRD patterns of GO (upper), GO–AgNO₃ (middle) and rGO–Ag (lower), and (b) SEM image of rGO–Ag composite obtained by thermal exfoliation of GO–AgNO₃.

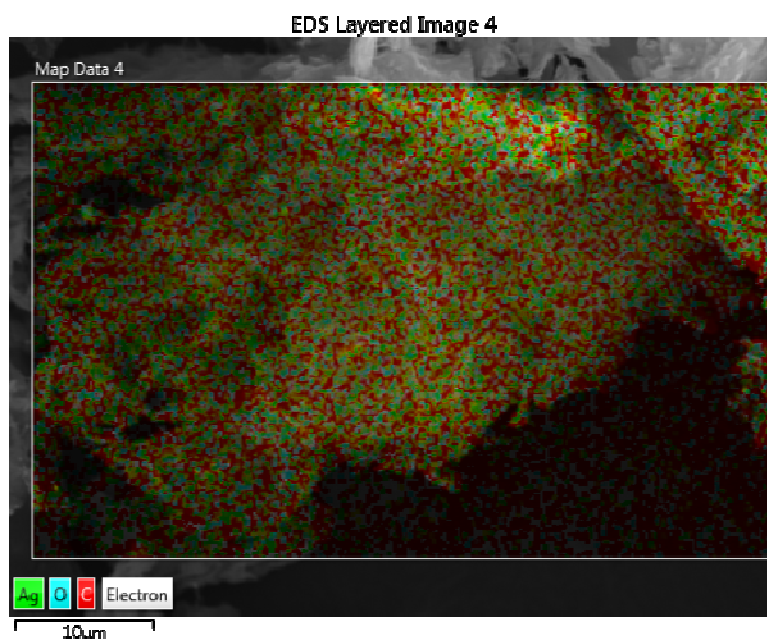


Figure 6. EDX mapping analysis of composites rGO–Ag.

EDX mapping analysis of rGO–Ag composites shows that the silver particles are evenly distributed over the entire surface of the sample (**Figure 6**). The silver content in the rGO–Ag composite depends on the GO:AgNO₃ ratio. We prepared composites that contain 1 – 16 wt.% of silver. The rGO–Cu–Cu₂O composite was obtained similarly. Their XRD patterns and SEM images are given in **Figure 7**. Comparing the SEM images of rGO–Ag and rGO–Cu–Cu₂O composites, it is clear that the same processes of thermal exfoliation of nitrate complexes take place and therefore the morphology of the layered composites is similar.

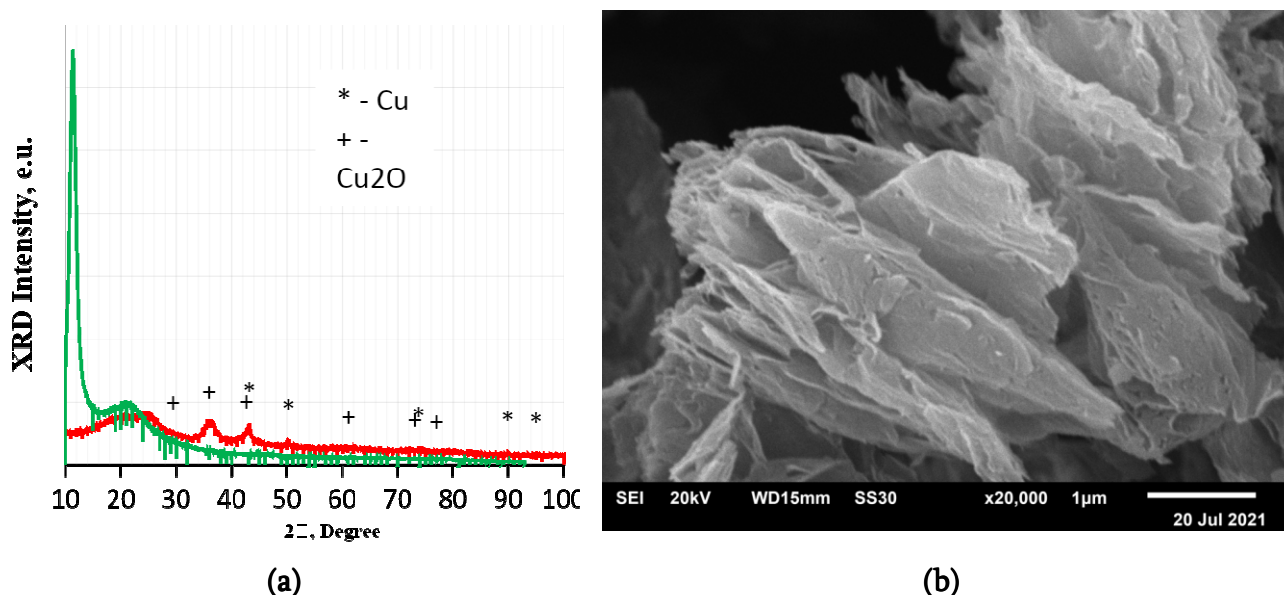


Figure 7. (a) XRD patterns of GO–Cu(NO₃)₂ complex (upper) and rGO–Cu–Cu₂O composite (lower); (b) SEM image of rGO–Cu–Cu₂O composite.

The GO–Cu (NO₃)₂ complex clearly shows the diffraction maximum of GO $2\theta = 11.3^\circ$. Vacuum heating (thermal exfoliation) of this complex produces rGO–Cu composite, which also contains Cu₂O. It should be noted that this oxide is also characterized by biocidal activity. After the explosion, the diffraction maximum of GO disappears at $2\theta = 11.3^\circ$ and a peak of low

intensity rGO occurs, at approximately $2\theta = 25.0^\circ$. The presence of diffraction maxima on the diffractogram indicates the formation of nano-sized particles in the Cu and Cu₂O phases. Particle sizes calculated from diffractometric data are in the range of 3 – 20 nm (Scherrer equation). As the EDX spectrum and EDX mapping analysis of rGO–Cu–Cu₂O composites show that the composite contains up to 11 % copper, which is almost evenly distributed on the sample surface (**Figures 8ab**). Similar studies have been performed using iron(III) nitrate complexes. The non-magnetic RCO–Fe₂O₃ composite was obtained in a vacuum at 120 – 135 °C. From the diffractogram it can be seen that the corresponding peak of rGO is approximately $2\theta = 24 - 25^\circ$. According the EDX analysis, the RCO–Fe₂O₃ composite contained 9.7 wt.% Fe, although the diffractogram showed no Fe₂O₃ phase (**Figure 9a**).

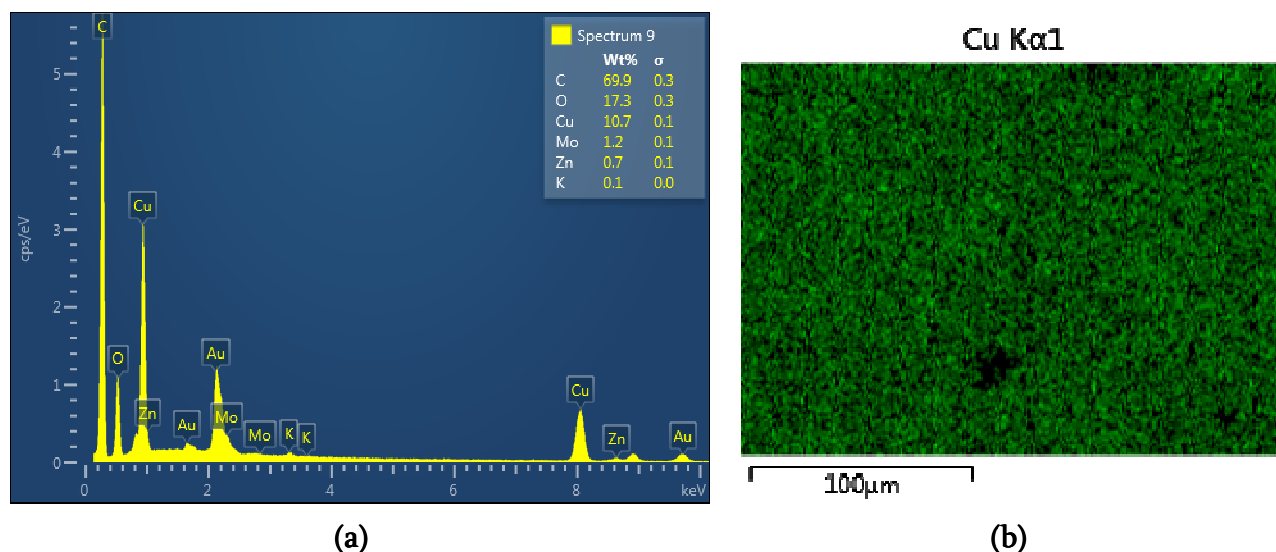


Figure 8. (a) EDX spectrum and (b) EDX mapping analysis of rGO–Cu–Cu₂O composites.

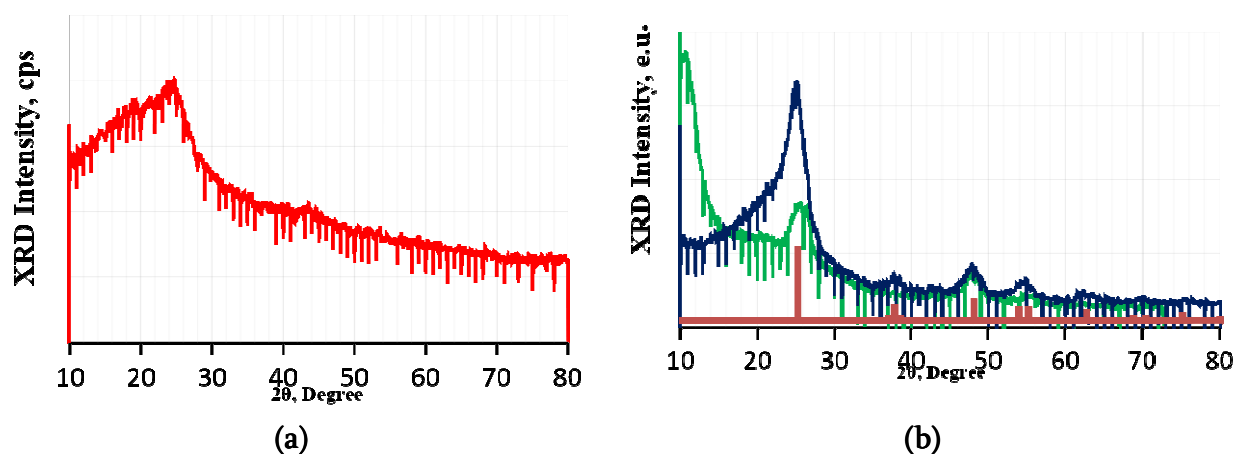


Figure 9. (a) XRD patterns of composite RCO–Fe₂O₃, and (b) GO–H₂TiO₃ (left), rGO–TiO₂ (anatase, right), and anatase (TiO₂, lower).

By hydrolysis of titanium diisopropoxide bis (acetylacetonate) in GO suspension GO–Ti(OH)_x complex is obtained, thermal exfoliation of which produces rGO–TiO₂ (anatase) composite confirmed by comparison with anatase diffractogram. The diffraction maxima of the anatase correspond exactly to the titanium oxide lines present in the composite (**Figure 9b**). Expanded peaks of rGO–TiO₂ (anatase) composite prove that the particle size of nano titanium oxide is small and is in the range of 5 – 20 nm.

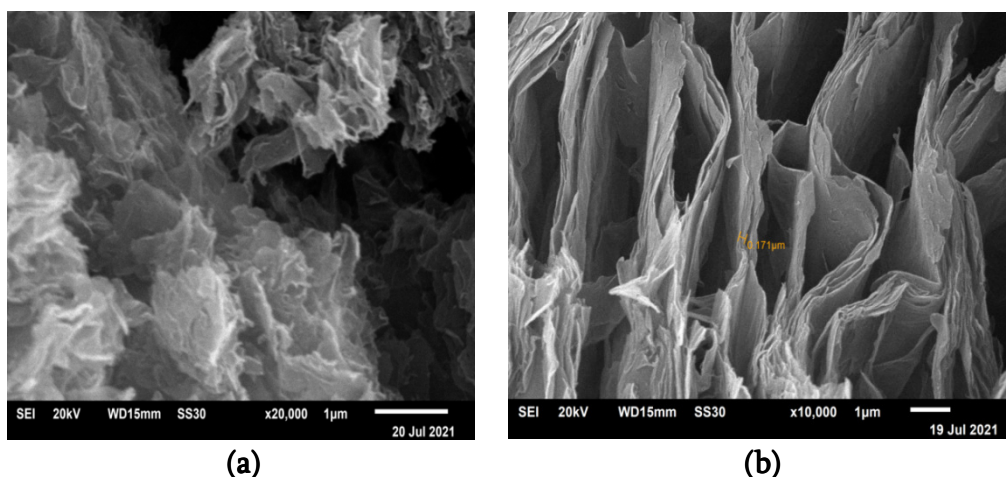


Figure 10. SEM images of (a) rGO–Fe₂O₃ and (b) rGO–TiO₂ composites.

At the same time the diffraction maximum GO ($2\theta = 10.62^\circ$) disappears and a new peak $2\theta = 25.1^\circ$ is formed, which corresponds to rGO. Peak $2\theta = 25.3^\circ$ corresponds to titanium oxide (crystalline modification – anatase). rGO–TiO₂ (anatase) composite powder is denser than powder obtained from nitrate complexes indicating that the degree of thermal exfoliation in this case is lower than it is clearly seen from SEM images (**Figures 10ab**). The distance between the two layers of rGO–TiO₂ composite is 171 nm although it consists of several layers. Thus the thickness of each layer is clearly less than 171 nm. So we can conclude that the rGO–TiO₂ composite consists of nano-sized components, while wide diffraction peaks of TiO₂ indicate the small size of its particles.

Structure and morphology of the rGO–Ag, rGO–Cu–Cu₂O and rGO–Fe₂O₃ composites is clearly different from that of the rGO–TiO₂ composite. These three composites are obtained by thermal exfoliation of nitrate complexes, during which more gases are emitted than during the thermal exfoliation of GO–Ti(OH)_x. In this case, only water is released. Nitrogen oxides, oxygen, water are released during thermal processing ($\text{Ti(OH)}_x \rightarrow \text{TiO}_2 + \text{H}_2\text{O}$) of nitrate complexes, so the pressure between the graphene oxide layers is higher and they decompose more. Fluffy powders are formed with a bulk density of 27 mg/ml. rGO–TiO₂ composite powders are denser. It is possible that partial oxidation of graphene oxide by nitrate ions, nitrogen oxides, and released oxygen occurs when nitrate complexes explode, so the surface area of such composites is larger than that of rGO–TiO₂.

3.3. Biocidal activity of graphene oxide composites

Over the last years, industry – particularly the food industry – has been developing the use of nanomaterials because of their specific nanoscale properties. It is one of the largest complexes, which is ensuring the high quality and safety of the foods, that we consume. The risk of biological and bacterial attacks has grown enormously in the food sector, food packaging, and water. This increasing risk stimulates scientists to develop new antibacterial nanoparticle substances that have no side effects and are easy to implement.

The food industry is adopting GO-metal-based applications. The goal of synthesizing new GO nanohybrid materials coupling different metallic nanoparticles is to enhance material functionality to obtain multifunctional properties working towards superior performance and new applications [38]. An important field, where the use of graphene and derivatives may be of

great interest, is the packaging industry. In this case, the main applications are food packaging, which requires biodegradable materials due to the increasing environmental concerns related to waste disposal, and good barrier properties against gases and, especially, water vapor [39].

The urgency of preventing foodborne diseases required acceleration in the development of active biocide substances to improve the quality of the food, extend shelf life, and prevent or delay spoilage. The antimicrobial action may be obtained by releasing the biocide directly into the food or in the space around the food. There is a variety of metal NPs which have received great attention due to their unique antimicrobial properties. Their small size and tunable physical-chemical properties that differ significantly from the bulk analogs led to intense research on their use in composite materials.

Copper, Titanium, and silver NPs are well known for their strong cytotoxicity towards a broad range of microorganisms such as bacteria and fungi [40 – 42]. Due to the biocompatibility, wound dressing materials with improved antimicrobial activity have been prepared using Ag. Other examples include the development of antibacterial food-packaging materials, bactericidal paper for water treatment, and the study of laundering properties of nanocomposites [43, 44]. Although the antimicrobial effect of GO is known, the development of hybrid materials of nanocomposites has considerable interest in various applications since they may exhibit synergistic bactericidal properties [45].

The antibacterial activity of graphene oxide composites. rGO–Ag, rGO–Cu, rGO–TiO₂ (anatase) composites were studied against indicator bacterial strains of gram-negative *Escherichia coli* ATCC 25922 and gram-positive *Staphylococcus aureus* 4399311-124 by viable cell count and agar diffusion method. Isolates were incubated with 20 µ/ml and 40 µ/ml of GO, GO–Ag NPs, rGO–Cu, rGO–TiO₂, and rGO–Fe₂O₃ for 2 and 24 h to evaluate the antimicrobial effect. Results demonstrated that the GO–Ag NPs exhibited a significant antibacterial activity compared to GO and Ag NPs. All nanocomposites fully inhibited the growth of *Escherichia coli* and significantly reduced *Staphylococcus aureus* growth at 2 and 24 h in a time-dependent way compared to the respective time controls.

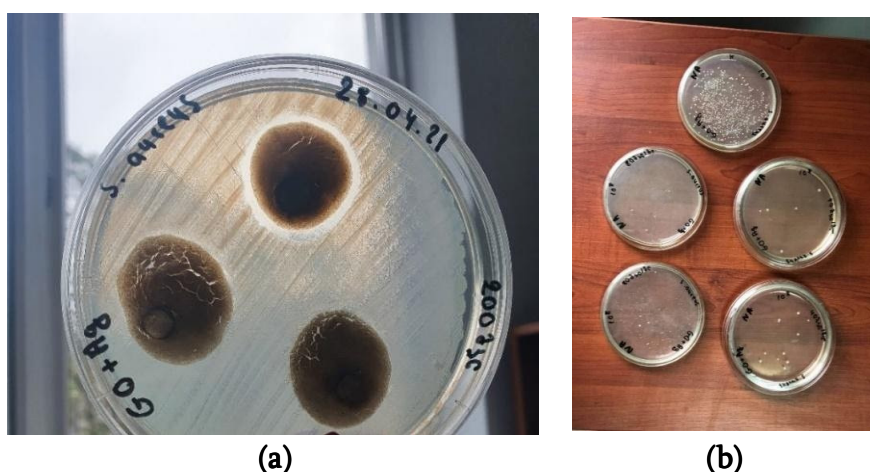


Figure 11. (a) Inhibition zone of a test culture on rGO-Ag NPs culture microorganism and (b) viable cell count of test microorganism/

Nanomaterials of silver showed a certain degree of antibacterial activity as can be seen in some of the plates used in the diffusion test (Figure 1) and on the viable cell count plates (Figures 11ab). The nanocomposite GO, GO–Ag NPs and Ag NPs exhibited different antibacterial activity against both gram-negative *Escherichia coli* and gram-positive

Staphylococcus aureus. The good antimicrobial effect of GO–Ag NPs nanohybrids could be explained by the combined action of direct contact between the Ag NPs and the microbial cells and the dissolution of Ag⁺ ions from the silver nanoparticles.

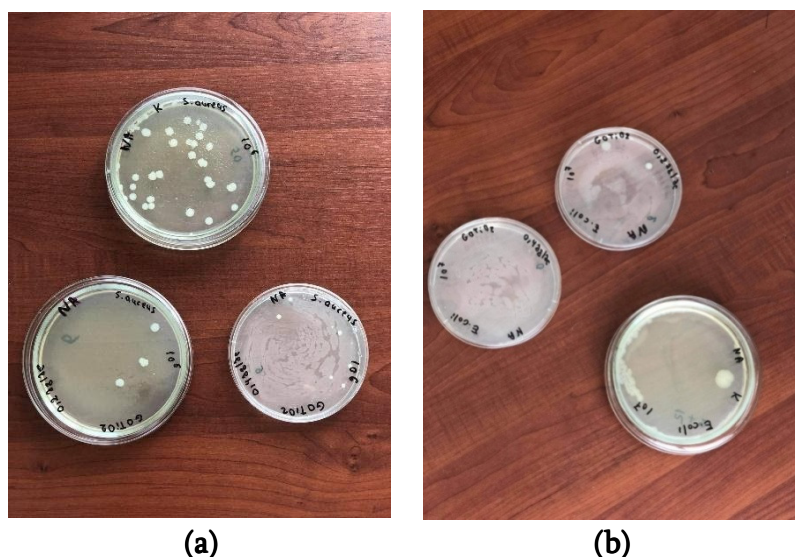


Figure 12. (a) Viable cell count of *Staphylococcus aureus* and (b) *Escherichia coli* on GO–TiO₂.

These two microorganisms were selected to understand the behavior of nanohybrids on different microbial structures such as gram-positive and gram-negative bacteria. The different susceptibilities to nanocomposites may be due to different cell wall structures and the different antibacterial mechanisms of Ag against different cells. For instance, gram-negative bacteria possess a thin peptidoglycan layer (7 – 8 nm thickness), whereas gram-positive bacteria possess a thick peptidoglycan layer (about 20 – 80 nm thickness), which is more resistant to Ag⁺ diffusion [46]. Results demonstrated that the rGO–TiO₂ fully inhibited the growth of gram-negative *Escherichia coli* and significantly reduced gram-positive *Staphylococcus aureus* (Figures 12ab).

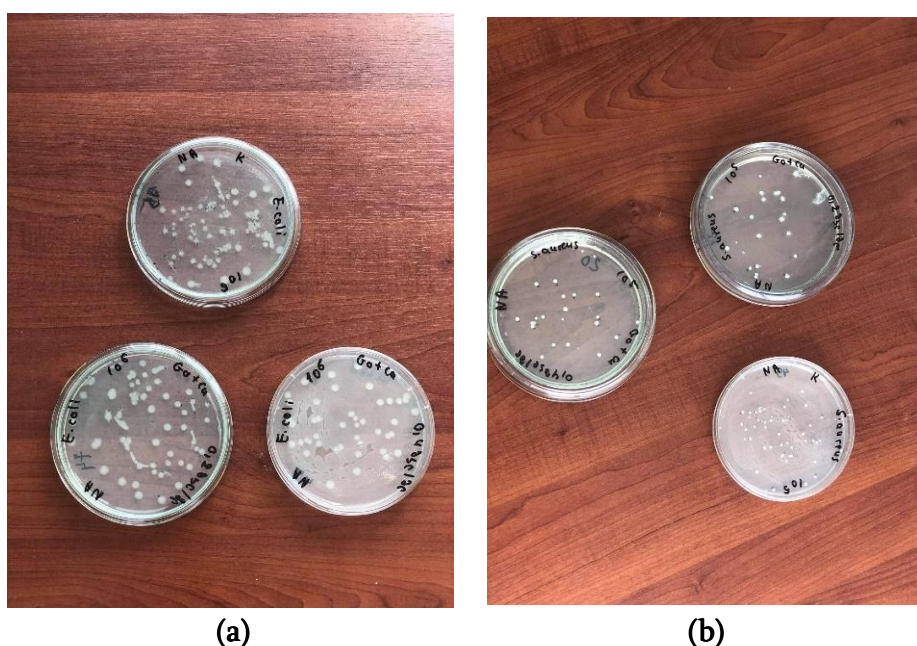


Figure 13. (a) Viable cell count of *Escherichia coli* and (b) *Staphylococcus aureus* on rGO–Cu.

A study of copper nanocomposite showed good sensitivity towards both of microorganisms (**Figures 13ab**), but in comparison with rGO-Fe₂O₃ demonstrated that Fe ions have stronger activity than Cu. Cu ions interact with phosphorus- and sulfur-containing biomolecules such as DNA and protein to distort their structures and thus disrupt biochemical processes. The prime effect of copper nanoparticles on microorganisms originates from the oxidation of metallic Cu ions which kills the cells by NP-mediated ROS generation in cells. This results in cellular lipid peroxidation, protein oxidation, and DNA degradation [47, 48].

After the screening of the antimicrobial activity, results revealed that all microorganisms that were tested were susceptible to all synthesized nanoparticles. gram-negative *Escherichia coli* was revealed to be the most sensitive microorganism and *Staphylococcus aureus* showed high resistance toward all studied nanocomposites (**Figure 14**). Many research groups believe that the main antimicrobial mechanism is the release of ions from NPs. That is Ag NPs only function as vehicles to transport and deliver Ag ions for interaction with bacteria, in which the Ag ions exerted the main antimicrobial effect. Ag ions interact with membrane proteins to change the membrane permeability. The mechanism of protein deactivation is probably dependent on the reaction of Ag ions with cysteine residues [49].

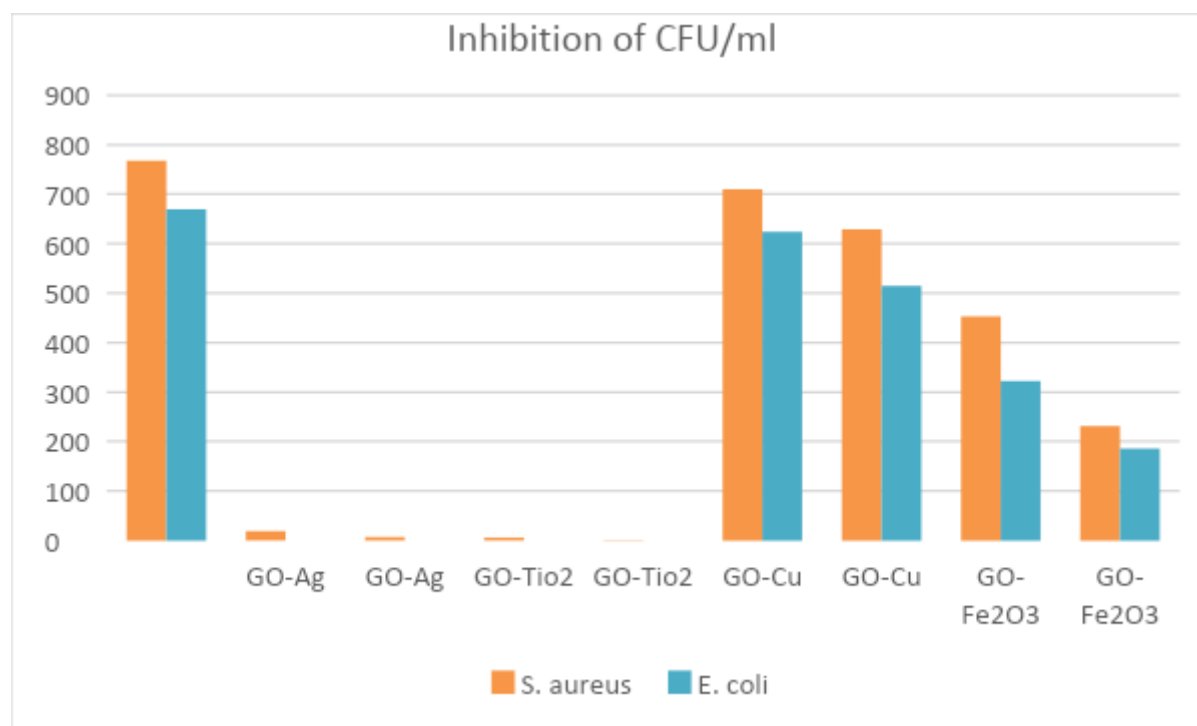


Figure 14. Antibacterial study of synthesized graphene oxide nanocomposites in different concentrations (20 and 40 µg/ml) on the test microorganisms.

The antimicrobial ability of Ag NPs and TiO₂ was found to be greater than Cu NPs, and Fe₂O₃ indicated that Ag ions are more efficient in antimicrobial activity than Cu ions. Ag NPs also show broader antimicrobial effectiveness to strains of *Escherichia coli* and *Staphylococcus aureus*, which may be due to their stronger interaction with polysaccharides and proteins on cell walls. The existence of an oxide layer on Cu NPs was proposed to be the reason that the antimicrobial capacity of Cu NPs is less than that of Ag NPs [47 – 50].

The effect of TiO₂ NPs against gram-positive bacteria was less than for gram-negative bacteria, due to the difference in membrane structures of bacteria. But was greater than Cu NPs and Fe₂O₃. TiO₂ NPs showed higher antibacterial activity, which may be explained that the

crystalline structure and the shape of TiO₂ NPs are both considered as the most important conditions that affect its antimicrobial activity. The anatase crystalline structure of TiO₂ presents its highest antimicrobial activity among other nanocomposites. The structure of anatase can produce OH· radicals in the photocatalytic reaction, which will help to eliminate the bacteria walls [51, 52].

4. Conclusions

Oxidation of powdered graphite foil wastes at low-temperature (~ 0 °C) and relatively high-temperature (~ 50 °C) similar of natural flake graphite powder oxidation process. GO is obtained by three methods: for synthesis GO to the mixture of pGFW–NaNO₃–H₂SO₄ is added with KMnO₄ (~ 0 °C, Hummers method); pGFW was also oxidized with optimized improved Hummers method, when pGFW–H₂SO₄ mixture is added at 40 – 50 °C with KMnO₄; and the method of adding concentrated H₂SO₄ at once to the pGFW–KMnO₄ mixture was used as well. GO obtained by all three methods undergoes vacuum thermal exfoliation and rGO is formed. Interaction of GO obtained from pGFW with metal nitrates and hydrolysis products of titanium diisopropoxide bis(acetylacetonate) forms complexes GO–AgNO₃, GO–Cu(NO₃)₂, GO–Fe(NO₃)₃, and GO–Ti(OH)_x. By vacuum thermal exfoliation of these complexes rGO–Ag, rGO–Cu–Cu₂O, rGO–Fe₂O₃, and rGO–TiO₂ composites were obtained.

XRD analysis and SEM studies have established that the grain sizes of metals and oxides in composites are in the range of 5 – 30 nm. Biocidal activities of composites have been established. Results indicate that all the nanomaterials assayed showed antibacterial activity against test cultures. Nanocomposite GO–Ag NPs and GO–TiO₂ exhibited the highest antibacterial activity against all the assayed strains; they fully inhibited the growth of *Escherichia coli* and significantly reduced *Staphylococcus aureus*. The synthesized material of rGO–Cu and rGO–Fe₂O₃ demonstrated relatively low antimicrobial activity than the other of the studied composites.

Acknowledgments

We are grateful to the staff of Petre Melikishvili Institute of Physical and Organic Chemistry at the Ivane Javakhishvili Tbilisi State University), Faculty of Agricultural Sciences and Biosystems Engineering and Republic Center for Structure Researches of Georgia at the Georgian Technical University for their assistance in performing this work.

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