# Biosensors Based on Graphene Nanomaterials

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**Abstract**—This review is devoted to the development, properties, and application of biosensors based on graphene nanomaterials. It is shown that such biosensors are characterized by their sensitivity, specificity of detection of analytes, high speed, and small size. Examples of the use of graphene biosensors for the detection of viruses, bacteria, markers of socially significant diseases, and various toxins are given.

## INTRODUCTION

In the 21st century a new scientific direction has arisen and is successfully developing: *nanobiotechnology* or *biomolecular nanotechnology*. This direction is based on the close cooperation of life sciences with chemistry, physics, and engineering. One of the key tasks of nanobiotechnology is the creation of biomedical instruments and devices of the minimum (in the nanometer limit) size using special materials and interfaces.

Medical applications of nanobiotechnology have led to the emergence of a new branch of medicine, *nanomedicine*, one of the most promising areas of which is the early diagnosis of diseases and infections.

Methods for the express diagnostics and monitoring of the patient's health status are being actively developed, which allow obtaining the result of the analysis within a few minutes [1]. There are already solutions for the express diagnostics of a person's health status (the state of the cardio and immune systems, the presence of infections). These tests (point-of-care (POC), i.e. studies near the patient) are used in the ambulance service, during hospitalization, and at home.

Biochemical sensors occupy an important place among diagnostic devices. Such sensors can analyze both biological fluids and analytes in a gaseous environment, recognizing substances in low concentrations, up to single molecules [2, 3].

Research is underway to develop biosensors based on graphene and its derivatives.

Graphene is one of the youngest carbon materials [4–6]; it is distinguished by exceptionally high conductivity, mechanical strength, adjustable bandwidth, adjustable optical properties, and a large specific surface area. Research teams around the world are constantly reporting new achievements in the study of graphene and its application in various fields of science and technology [7–16]. Various measuring devices, sensors, and sensor systems are considered to be the main areas of application for graphene and related materials [14, 17]. For example, many graphene-based gas sensors are capable of reacting at the limit of sensitivity to single acts of adsorption/desorption of molecules (*single-molecule detection*). The main trends in the improvement of biomedical means of registration and measurement are a decrease in the size of sensor elements, as well as an increase in their sensitivity and selectivity. It is assumed that nanodevices that can be implanted in the human body for continuous monitoring of its parameters will find mass application [18].

The unique properties of graphene make it an ideal candidate for one of the main roles in nanobiotechnologies [14–16, 19].

The aim of this review is to consider domestic and foreign developments devoted to biochemical sensors based on graphene materials and the creation of biosensors for the express diagnostics of human health.

## 1. BIOSENSOR DEVICES

In the past decade, there has been an increasingly steady interest of scientists and engineers in the development of public express methods of analysis that have high levels of sensitivity and selectivity.

The possibility of miniaturization of such analytical devices is especially important. The most prominent representatives of analytical systems that combine the listed qualities are biosensors [20, 21].

Biosensors are a type of chemical sensors in which the recognition system has a biochemical nature and

uses the reactions of either individual biomolecules, or biological supramolecular structures [20, 22]. A unique feature of biosensors, in contrast to chemical ones, is the high specificity of the receptor element, as well as its ability to perform recognition without additional energy consumption. The authors of [20, 22] believe that a necessary characteristic of both chemical and biological sensors should be the possibility of their miniaturization.

Among the areas of application of biosensors, the most important place is occupied by clinical diagnostics, whose area of interest includes, in particular, continuous monitoring of key metabolites of blood and other biological fluids to monitor the patient's condition. This problem can be solved by implanting specific sensors, among which biosensors have no equal.

Biosensors as chemical sensors that include biological material were first reported by L. Clark and S. Lyons at the symposium of the New York Academy of Sciences in 1962 [23].

They suggested using electrodes modified with glucose oxidase embedded in membranes to create more advanced electrochemical sensors. This results in sensors that are specifically sensitive to certain substrates, since they detect the formation of an enzymatic reaction product or the consumption of one of the substances involved in this reaction. Clark and his coauthors, using the idea mentioned above, developed biosensors for determining glucose and lactate in the blood [24, 25]

The term *biosensor* has not yet been unambiguously defined. Some authors consider that this is *an analytical system for working with biological matter*; and others, that a biosensor is a *system that itself contains a biological substance.*

Although experts have not yet reached a consensus view, there are more arguments in favor of the second definition. Thus, a biosensor can be called an analytical device, in which the reactions of these compounds catalyzed by enzymes, immunochemical reactions, or reactions taking place in organelles, cells, or tissues are used to determine chemical compounds. The main part of the biosensor is the biological material (enzymes, cells, antibodies, antigens, DNA fragments, etc.), with which the analyte interacts during the operation of the sensor. The signal about this reaction with the help of various physical and chemical methods (electrical, optical, etc.) is converted so that it can be measured and the result displayed on the device screen [20, 26].

Biosensors that function without the addition of an additional reagent are called *reagentless*. Biosensors that can quickly and reproducibly recover are called *reusable*, and biosensors that cannot be reproducibly and quickly restored are considered *disposable*, including bioassays and bioindicators [22].

Biosensors are characterized by their fast response (response time ranges from several minutes to 1 h),

while the specific indication of microorganisms using enzyme immunoassay takes 3—4 h.

Biosensors can be classified according to the mechanism of biological recognition and according to the type of transducer used (a device that converts the response of the recognition element into a measurable signal). According to the type of transducers, biosensors can be divided into electrochemical, optical, and gravimetric ones. Electrochemical biosensors, according to the authors [22], occupy a priority position among other types of sensors.

*Electrochemical biosensors* track any changes in the electrical properties, size, shape, and charge distribution, for example, during the formation of an "antibody—antigen" complex on the electrode surface.

According to the method of measuring the analytical signal, electrochemical biosensors are divided into amperometric, potentiometric, and conductometric sensors and field-effect transistors. Such biosensors are used to detect a wide range of biological targets, including proteins, biomarkers, and nucleic acids.

*Optical biosensors* are widely used; they allow direct detection of biomolecules in real time. Optical detection systems use the power of the optical field and the biological recognition element, which allows the analysis of macromolecules with a high degree of sensitivity directly in the body.

Among the advantages of optical biosensors over others, their high specificity, great sensitivity, cost-effectiveness, and small size can be singled out. The disadvantages of an optical transducer include its sensitivity to various environmental parameters, including local temperature changes.

*Piezoelectric* biosensors track the change in mass on the surface of a physical carrier (piezoelectric crystal—resonator), density, viscosity of the medium, and frequency of acoustic waves. Such biosensors are most effective for detecting large molecules and particles: hormones, bacteria, cells, etc.

In the classification according to the biochemical component, the following biosensors are distinguished:

*enzyme*, which include pure enzyme preparations or biological preparations (tissue homogenates or microbial cultures) and exhibit a certain biological activity;

*immunosensors* use immunoglobulins, which are protective proteins secreted by the body's immune system in response to the intake of foreign biological compounds (antigens), as a biochemical receptor;

*DNA sensors*, including nucleic acids as a biochemical component;

*microbial biosensors* using microorganisms that can convert a certain substance with the help of enzymes, differing from enzyme sensors in that not one enzyme but a combination of enzymes can participate in the conversion of the substrate; and

*biosensors based on supramolecular structures of the cell*, occupying an intermediate position between enzyme and DNA sensors and microbial sensors, since they are based on intracellular structures that have a rather complex hierarchical structure.

To increase the selectivity of the sensor to certain molecules, the surface of the receptor is chemically modified so that these molecules can be immobilized on it.

Thus, a high level of sensitivity and selectivity of the biosensor is achieved.

## 2. GRAPHENE AS THE RECEPTOR MATERIAL

As is well known, graphene is an allotropic modification of carbon, formed by a layer of $sp^2$ carbon atoms and representing a 2D crystal one atom thick. Less than two decades have passed since the discovery of graphene [4]; however, it is rapidly gaining a wide range of potential applications, in particular medicine. Thus, in 2013, publications on the biomedical applications of graphene and its derivatives reached 63% [27].

The structural features of the graphene sheet are such that it is a system in which charge carriers, having unlimited freedom of movement in the plane of the sheet, are closed in a narrow space of one carbon layer. This leads to the appearance of unique electrophysical characteristics and other unusual properties of graphene [8, 16, 27, 28], in particular, good electrical conductivity [4] due to the high concentration and mobility of the charge carriers.

Graphene has a record-high mechanical strength. Despite this, it has elasticity and can be subjected to 20% deformation without the network structure breaking [30]. Monolayer graphene has a constant optical transparency in the visible range (97.7%) and a transmittance value that linearly decreases depending on the number of layers for *n*-layer graphene [31, 32]. The monatomic thickness of a graphene sheet provides the highest possible surface to volume ratio, a specific surface area of ~2630 m$^2$/g [16, 27, 33], and high sorption properties. In addition, it has biocompatibility [8], which is important for biomedical applications.

Graphene can be obtained using mechanical methods: exfoliation of carbon layers from the surface of highly oriented pyrolytic graphite (the Scotch tape method), splitting of graphite crystallites into individual plates when exposed to ultrasound in the presence of surfactants in solvents. Chemical methods are also used: longitudinal catalytic oxidative cutting of carbon nanotubes, which contain rolled graphene layers; deposition from the gas phase of carbon-containing compounds (CVD method); thermal decomposition of the surface layer of a single crystal of silicon carbide; reduction of graphene oxide or graphite oxide; etc. [13, 14, 16, 19]. Methods for obtaining 3D graphene materials (graphene foam, laser-induced graphene LIG) have been developed [12, 34−37].

Development of relatively simple methods for obtaining graphene and its derivatives, such as graphene oxide, fluorinated graphene, etc. [13, 16, 38, 39], the implementation of syntheses of conjugates of graphene nanomaterials with organic or bioorganic compounds of any complexity [40, 41], and the above complex of properties have made graphene nanomaterials (GNMs) attractive for biomedical application. GNMs are of interest as receptor elements for recording the interaction of a surface with molecules in the gas and liquid phases. Achievements in the development of gas sensors based on GNMs are considered in [14, 42−44].

## 3. GRAPHENE NANOMATERIALS IN BIOSENSORS

Over the past decade, a lot of work has been done to explore the possibilities of use of graphene nanomaterials in biomedicine [11, 15]. It has been shown that GNMs are promising for targeted drug delivery, visualization of organs and tissues, the creation of antibacterial materials, and the synthesis of a biocompatible scaffold for cell cultures [15]. Specialists are especially interested in the possibility of developing graphene biosensors. The review [11] shows that GNM-based biosensors are capable of detecting biomarkers-indicators of diseases, which is important for medical diagnostics; in addition, they allow studying processes occurring in living cells at the molecular level, for example, the formation of reactive oxygen species.

A common disadvantage of electrochemical sensors is insufficient selectivity due to the simultaneous sorption of several substances. As applied to graphene electrochemical sensors, this drawback was eliminated by using the antigen−antibody reaction. The components of this pair can only interact with each other. They cannot interact with any other proteins. It is known that at certain stages of many human diseases, antigens-markers specific for any one disease or for a group of diseases appear in the blood. These antigens can interact with specific antibodies previously deposited on the surface of the graphene sensor.

### 3.1. Graphene Materials for Biosensors

It follows from the analysis of the literature that, depending on the choice of the synthesis method and the features of its implementation, it is possible to obtain graphene materials with different properties [13, 14, 45−47]. Thus, the CVD method allows us to synthesize high-quality and large graphene samples on the surface of various metals but monolayer films are formed only on copper. The method is promising for the large-scale production of graphene, but it is energy-consuming, which makes it economically unjustified for use where a significant amount of graphene is required. Synthesis of graphene monolayers by thermal decomposition of the surface layer of
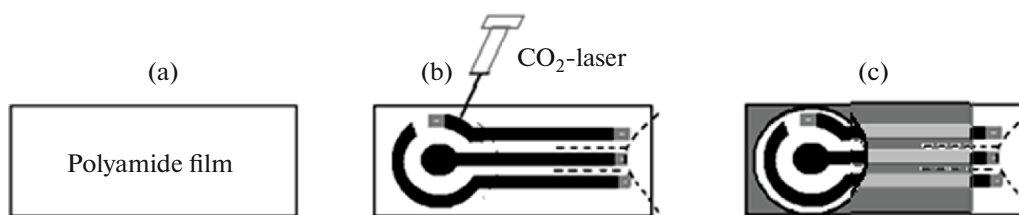
**Fig. 1.** Formation of an electrode from LIG: (a) polyimide substrate, (b) creation of graphene electrodes, (c) formation of a receptor window upon encapsulation in plastic [12].

single-crystal silicon carbide at a temperature of ~1000°C leads to the epitaxial growth of a structurally homogeneous high-quality graphene film on the SiC surface. However, the high cost of single-crystal SiC and its high decomposition temperature reduce the attractiveness of the method for the production of large amounts of graphene.

However, sensor developers have looked at these materials and compared their sensory characteristics. The comparative study of epitaxial graphene films on SiC and graphene obtained by the CVD method as a material for electrochemical biosensing was carried out in [48, 49]. For quantitative measurement, the authors used the method of impedance spectroscopy using deionized water and saline (0.9% NaCl). Based on the results obtained, it was concluded that single-layer epitaxial graphene on SiC has a higher sensitivity than multilayer CVD-graphene. Biosensors based on graphene films on SiC showed an extremely high sensitivity to the detected substances. One of the latest advances in the development of graphene biosensors is related to the use of suspended graphene [50].

The authors deposited monolayer graphene from a suspension onto a preliminarily structured Si substrate. To increase the selectivity, graphene was chemically modified. Selectivity was assessed by nanoscale mechanical deflection of the sheet plane, as the biomarker generates a force that deforms planar graphene into a dome shape, resulting in spectral shifts in optical interference between graphene and silicon substrate. Using the interference properties of light, the authors estimated the magnitude of the deformation from the change in color.

The sensitivity of biosensors can be increased by using laser-induced graphene (LIG). In 2014, it was found that polymers, such as polyimide, can be directly converted into porous three-dimensional graphene using an infrared $CO_2$ laser [37]. The discovery of LIG has attracted a significant attention due to its wide range of applications. The advantages of the technology for obtaining LIG compared to conventional methods for the synthesis of graphene are the environmental friendliness of the process and the possibility of controlling the morphology of samples.

LIG has high porosity, flexibility, and mechanical strength, as well as excellent electrical and thermal conductivity. Moreover, LIG can be used to design graphene patterns of any complexity (Fig. 1). To do this, it is sufficient either to apply a pattern on the substrate with a polymer solution and then apply laser radiation, or to draw electrodes on the polymer substrate (Fig. 1a) with a laser and attach Ag-contacts to them (Fig. 1b), and then encapsulate it in plastic, leaving the receptor window open (Fig. 1c).

The stenciling and printing process, as well as the useful properties of LIG, open up a new way to develop miniature graphene devices. The use of LIGs in sensor applications quickly moved from single experiments to the creation of an integrated intelligent system for detecting biological objects [11, 12].

Oxidized forms of graphene, such as graphene oxide and reduced graphene oxide (RGO), are among the most promising GNMs for creating biosensors, since, by controlling the conditions of their oxidation or reduction, materials with the required ratio of oxygen and carbon [13], as well as certain functional groups, can be obtained.

The presence of oxygen-containing groups allows us to carry out adsorption and covalent modification of the surface of these materials with both small molecules and large biomolecules, such as enzymes, antibodies, antigens, DNA fragments, and even cells.

The interaction of immobilized molecules with the analyte is detected using the same principles as in the case of other sensors. For example, these can be the following biosensors:

— electrochemical (based on field-effect transistors, the impedance spectroscopy method [48, 49]);

— optical (biosensors using the phenomenon of surface plasmon resonance) [50];

— fluorescent [51]; and

— others.

The schematic diagram of the biosensor is shown in Fig. 2. Designs of graphene biosensors differ depending on the goals and objectives of sensing. They can be either wired or wireless. These are wearable, flexible, monoplex, and multiplex systems used in both clinical and home settings.

## 3.2. Chemical Modification of Graphene Is a Necessary Step in the Development of Biosensors

An important step in the use of graphene nanomaterials in biomedicine (including the creation of bisensors) is their chemical modification. Chemical modification can improve the solubility of nanoparticles of these materials in water, ensure their biocompatibility, and reduce their toxicity and the ability to interact with certain analytes.

GNMs are characterized by an extended polyaromatic system and the presence of oxygen-containing groups localized both on the periphery of graphene planes and on their surface. These groups make graphene molecules active with respect to electrophilic and nucleophilic reagents. In recent years, methods for the chemical modification of graphene with functional groups and fragments of molecules have been actively developed. On the one hand, such modification allows us to control the electronic properties and, consequently, the conductivity of graphene over a wide range. Depending on the type of modification, the interaction energy between the adsorbed molecule and graphene, as well as the charge transfer in the system, can vary greatly. On the other hand, functional groups play the role of specific reaction centers during the adsorption and covalent bonding of various molecules with graphene and its derivatives [13, 19, 45, 46].

Phenyl and alkyl groups, a stable free radical 4-amine-2,2,6,6-tetramethyl-1-piperidine oxide, and dichlorocaben were covalently grafted to the surface of graphene using organic synthesis methods [53]. Conjugates of graphene and its derivatives with DNA molecules [54], porphyrins (as drug components) [55, 56], poly-L-lysine [57], star-shaped polyethylene glycol (PEG) [58], etc. [13, 16], have been obtained.

The grafting of star-shaped PEG [58] to graphene oxide made it possible to obtain biocompatible materials for cell visualization and sorption of biomolecules, including drugs. Unlike other graphene materials, the resulting product forms stable dispersions in an aqueous salt medium and in biological fluids. In addition, it exhibits fluorescent properties in the near-IR region, which can be used to create optical biosensors.

Covalent modification of graphene oxide deposited on a substrate with DNA molecules to create biomolecular devices was carried out in [54]. An oligodeoxynucleotide containing 20 links (amine-AAC TGC CAG CCT AAGTCC AA) was involved in a reaction with graphene oxide carboxyl groups in the presence of an activator. It has been shown that DNA binds predominantly onto thicker regions of the crumpled graphene sheet, including graphene oxide folds, which follows from the observation of a higher fluorescence intensity in these regions. Since no increased fluorescence was observed at the edges of the graphene planes, where the carboxyl groups that bind DNA molecules are supposed to be, the authors concluded
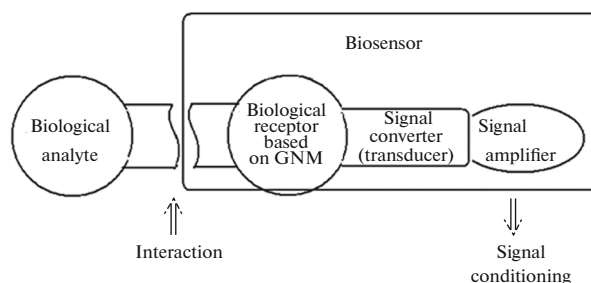


**Fig. 2.** Schematic diagram of a graphene biosensor.

that the carboxyl groups were uniformly distributed over the graphene oxide plane.

In [51], a fluorescent peptide labeled with pyrene fragments was immobilized on graphene oxide. The authors suggested using the obtained material to study protein-protein interactions.

The formation of supramolecular complexes of porphyrin derivatives with reduced graphene flakes is described in the review [59].

The authors paid special attention to this material, since, in their opinion, the great possibilities of post-synthetic modification in combination with the unusual properties of graphene and its derivatives can be used to solve complex biomedical and environmental problems.

As an example, we present a scheme of chemical modification of an electrode in manufacturing a biosensor based on LIG (Fig. 3).
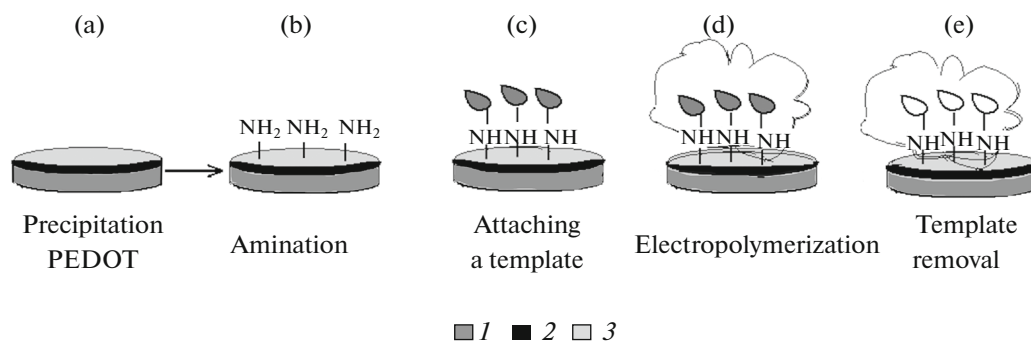
To stabilize the loosened LIG particles and obtain more sensitive layers, the authors of [12] electrochemically polymerized 3,4-ethylenedioxythiophene (EDOT) to form polyEDOT (PTDOT) in the working electrode (Fig. 3a). Then, the electrode surface was aminated (Fig. 3b), a template was attached (Fig. 3c), and electropolymerization was performed in the presence of the template (Fig. 3d). After the template was removed, a polymer with a molecular imprint was obtained (Fig. 3e)

The sensitivity and selectivity of the biosensor based on molecularly imprinted LIGs were comparable to those of sensors fabricated using commercial graphene-based screen-printed electrodes.

### 3.3. Application Examples of Graphene Biosensors

Currently (March 2022), about two hundred biosensor devices that use graphene nanomaterials have been described in the scientific and patent literature. Because the volume of the article is limited, we will focus only on the most significant and illustrative examples of the use of graphene biosensors in relation to various classes of analytes.

(a)            (b)            (c)            (d)            (e)

Precipitation PEDOT      Amination      Attaching a template      Electropolymerization      Template removal

■ 1 ■ 2 □ 3

**Fig. 3.** Scheme of chemical modification of an electrode based on LIG: (*1*) polyamide; (*2*) graphene; (*3*) PEDOT-polymer (according to [12]).

### 3.3.1. Graphene Biosensors for Detecting Bacteria and Viruses

The work [60] reported on the fabrication, based on graphene oxide or aminated graphene deposited on a silicon substrate, of new biological devices: with a bacterium localized on the sensor (*1*) and bacterial DNA (*2*). The bacterial biological device *1* is highly sensitive due to the attachment of *Bacillus cereus* bacteria generating approximately 1400 *p*-type charge carriers in graphene. Similarly, single-stranded DNA grafted to graphene in device *2* hybridizes with its complementary DNA strand, reversibly increasing the hole density by $5.61 \times 10^{12}\,cm^{-2}$.

The authors showed that, by varying the nature and content of surface functional groups, the sensitivity of the device can be controlled. The specificity of the sensor is switched by changing the polarity of the surface; and immobilization of the DNA molecules occurs mainly in the thicker areas and wrinkles of the GNM layer.

According to the authors, the study will motivate the development of the next generation of biodetection tools.

The researchers [61, 62] have developed a graphene biosensor to detect the *Helicobacter pylori* bacteria (see Section 3.3.2 for details).

The authors of [63] created a biosensor based on a CVD graphene film with immobilized antibodies to detect *Escherichia coli* (*E. coli*). The biosensor provided high levels of sensitivity and specificity. The researchers claimed that their fast, label-free method could also be used to detect other bacteria and pathogens, if the right antibodies are used. To detect *E. coli* bacteria, another biosensor was developed using CVD graphene coated with poly(methyl methacrylate) [64]. The sensor demonstrated a very low detection limit (10 CFU/mL) and high performance.

Good analytical performance was achieved in [65], which describes a very economical, fast, sensitive, and specific electrochemical biosensor for the detection of *E. coli*. The sensor is based on a nanocomposite of bimetallic Ag−ZnO nanoparticles and graphene oxide modified with a polymer with molecular imprints of bacteria.

In [66], the authors summarized recent advances in electrochemical biosensing used to detect common foodborne pathogens. For example, labelless electrochemical biosensors have also been developed to detect *E. coli*. Thus, a (rGO-CysCu) Gold electrode was made from RGO modified with a chelate salt of cysteine with Cu(II) and gold, which showed that *E. coli* O157: H7 cells can be differentiated from non-pathogenic *E. coli* and other bacterial cells.

An electrochemical biosensor for the detection of *Staphylococcus aureus* (*S. Aureus*) by the method of impedance spectroscopy using single-stranded DNA related to a reduced graphene oxide−gold nanoparticle nanocomposite is also described. *Clostridium perfringens* (*C. perfringens*) is the most common type of Clostridium among the causative agents of clinical genital gangrene C. It can break down sugar in muscles and connective tissue, and then release large amounts of gas, leading to severe tissue emphysema and affecting the blood supply, and eventually there is a large area of tissue necrosis. The DNA biosensor was fabricated based on screen-printed electrodes modified with streptavidin aptamer.

The authors of [67] developed a biosensor in which a specific DNAzyme (a DNA oligonucleotide that can enter into certain chemical reactions and achieve highly specific detection of bacteria) was immobilized by adsorption on a graphene surface. The fabrication of this biosensor made it possible to avoid time-consuming operations of functionalization or surface modification. The authors developed a fluorescent biosensor with DNAzyme attached directly to colloidal graphene to detect *E. coli* bacteria. DNAzyme acts as a fluorescent producer and detection element, while graphene acts as a transducer, generating measurable signals upon contact with *E. coli* and creating changes in fluorescence.

Achievements in the field of creating biosensors based on GNMs used to detect various types of

**Table 1.** Graphene biosensor materials and virus detection limits (according to [68])

| Virus type | Basic material | Limit of detection |
|---|---|---|
| Bird influenza virus | RGO | 5 pM |
| Human influenza virus | Graphene | 1 ng/mL |
| Bird and human influenza viruses | Graphene | 130 pM (for humans) 600 nM (for birds) |
| Ebola virus | RGO | 2.4 pg/mL |
| Ebola virus | RGO | 1 ng/mL |
| Hepatitis B virus | Graphene | 0.1 fM |
| Hepatitis B virus | RGO | 50 aM |
| Human immunodeficiency virus HIV | Graphene | 1 pM |
| Human immunodeficiency virus HIV | Graphene | 10 fg/mL |
| Norovirus | Graphene | 0.1 mcg/mL |
| Human papillomavirus | RGO | 1.75 nM |
| Rotavirus | RGO | 4.5 ng |
| Zika virus | Graphene | 450 pM |
| SARS virus COVID-19 | Graphene | 1 fg/mL |
| | Graphene | 0.2 pM |

viruses, such as Ebola, Zika, and influenza, are considered in the reviews [68, 69] and are partially presented in Table 1.

By immobilizing a monoclonal antibody (anti-Zika NS1) on the surface of commercially available CVD graphene, a cost-effective and ultraspecific graphene biosensor for detecting the Zika virus was constructed [70].

Ebola hemorrhagic fever is an extremely dangerous epidemic disease, and its early detection is vital to prevent serious outbreaks. To detect the Ebola virus, the authors of [71] used a biosensor with reduced graphene oxide, on which antibodies against Ebola were immobilized. The authors were able to detect the Zairian strain of the Ebola virus in real time with a very low detection limit (up to 1 ng/mL).

A method for detecting the dengue fever virus using a graphene oxide−polymer composite [72] with a low detection limit of 12 PFU/mL has been described. (One PFU is equal to $10^{-4}$ $m^{-2}$ $st^{-1}$ $s^{-1}$, where st is a steradian).

A graphene-based biosensor that is selective for recombinant cyanovirin-N (cV-N), an antiviral protein that has proven to be an effective microbiocide for HIV replication suppression, has been developed [73]. The graphene monolayer was modified with 1-pyrenebutanoic acid succinimidyl ester, which interacts both with graphene and with primary and secondary antibody amines. By monitoring the change in the electrical resistance of the developed device, the authors were able to detect rCV-N in solutions in the concentration range from 0.01 to 10 ng/mL and showed that the limit of detection was 0.45 pg/mL, which was much lower than of currently available methods. The sensor showed not only high sensitivity but also good selectivity and reproducibility. A monolayer of graphene modified with 1-pyrenebutanoic acid succinimidyl ester is a myrobiocide, and this is important because it can be produced on a large scale from soybean seeds treated according to the known industrial technologies.

The use of a graphene oxide film modified with pyrene derivatives and antibodies allowed us to develop an electrochemical biosensor for detecting the rotavirus [74]. Later, these authors presented a modified and more sensitive model using a field-effect transistor based on reduced graphene oxide with a covalently bound antibody [75]. This made it possible to overcome the low reproducibility and some other shortcomings of their previous work. As a result, the resulting biosensor was proposed for highly sensitive pathogen detection. Another biosensor based on graphene oxide for the detection of rotavirus is described in [76].

The surface of gold microelectrodes was coated with graphene oxide, on which antibodies were immobilized. To increase the specificity of the sensor, the electrodes were additionally treated with bovine serum albumin to block the remaining free surface so that only antibodies determined the selectivity of the sensor. The resulting biosensor provided rapid and specific detection of rotavirus. The authors of [77] developed an immunobiosensor based on graphene oxide for the detection of rotavirus, which has high levels of sensitivity and selectivity.

Several viral biodetectors have been proposed to improve performance based on graphene modified with aptamers. Aptasensors are biosensors based on aptamers, which are oligonucleotides capable of bind-

ing to a specific molecule with a high degree of specificity.

Aptasensors are very promising: they are quite accessible and selective to the most diverse figurative analysts. For example, an aptasensor based on a microfluidic platform was described in [78] in which the carbon electrode was modified with a composite of gold nanoparticles with graphene particles. Detection of norovirus (causative agent of acute intestinal infection) is based on the interaction of the aptamer with the target. The aptamer was labeled with ferrocene as a redox probe. When the norovirus binds to the aptamer, an increase in the capacitance of the electrode results in detection of the virus in the blood sample within 35 minutes (total time).

The COVID-19 pandemic has become a major global challenge to public health systems. To prevent a wider spread of COVID-19 infections, sensitive, rapid, and inexpensive detection of infection in presymptomatic and asymptomatic patients is important. The use of biosensors contributes to the solution of this problem. The introduction of nanomaterials improves the performance of the biosensor, and the addition of graphene increases the sensitivity to a very high level. Among various biosensor circuits, the graphene-based field-effect transistor stands out for its unique ability of ultra-sensitive and low-noise detection, which facilitates instantaneous measurements even in the presence of a small amount of analytes [79].

COVID-19 diagnostics based on Real-time polymerase chain reaction (RT-PCR) is the main, most sensitive, and selective method. However, it is costly, requires qualified personnel, takes a lot of time, and can only be carried out in laboratory medical institutions. Worse still, the RT-PCR method showed a high false negative rate (ranging from 20 to 67%) due to poor sampling, insufficient sample quality, low sensitivity of the diagnostic kits, and long duration (4−5 h). The authors of [80] describe a multiplex portable wireless electro-chemical device based on graphene electrodes with laser engraving for ultrafast detection of COVID-19: Rapid Plex SARS-CoV-2. In this sensor, graphene structures are connected to antibodies and immune system molecules that are sensitive to specific proteins, such as those found on the surface of the COVID virus. When connected to auxiliary electronics, the sensor can transmit data wirelessly to the user's mobile phone via Bluetooth. It can be used for highly selective, supersensitive, and fast electrochemical detection of the nucleocapsid protein of the viral antigen, $IgM$, and $IgG$ antibodies in physiologically significant ranges, as well as the biomarker of inflammation: the C-reactive protein.

The applicability of the Rapid Plex SARS-CoV-2 platform with positive and negative blood and saliva samples in COVID-19 has been successfully evaluated. Based on the results of the pilot study, the authors claim that the multiplex immunosensor platform developed by them, will allow self-testing at home for telemedicine diagnosis and monitoring of COVID-19 and get the result in less than 10 minutes. Such sensors can monitor conditions such as gout and stress levels by detecting extremely low levels of certain compounds in blood, saliva, or sweat. A graphene-based wireless device has been developed for the detection of the SARS-CoV-2 virus in human biofluids [81], which allows for fast and highly sensitive self-testing for COVID-19 with high accuracy at a low cost, i.e., to provide sensitive, rapid, and inexpensive disease monitoring.

### 3.3.2. Graphene Biosensors for the Detection of Markers of Socially Significant Diseases

To diagnose many diseases, it is necessary to be able to detect disease markers, protein molecules specific for each specific pathology, which are usually expressed in very small quantities.

GNM-based biosensors for probing protein molecules can significantly increase the efficiency of diagnosing a wide range of diseases affecting both humans and animals.

### Cardiovascular Diseases

An aptasensor for determining the myoglobin cardiomarker, an oxygen-binding protein in skeletal muscles and heart muscle, whose function is to create an oxygen reserve in the muscles, was proposed [82, 83].

The myoglobin-specific aptamer was immobilized on the surface of a printed electrode and modified with graphene oxide and carbon nanotubes. The sensor provides a low detection limit (34 ng/L) in the linearity range (1−4000 ng/mL).

Troponins I, T, and C are involved in the calcium-dependent regulation of the act of the contraction-relaxation of the heart and are specific markers of myocardial damage. In a number of medical tests, troponins are used as biomarkers for various heart diseases. Acute myocardial infarction (AMI) is one of the leading causes of death among patients with cardiovascular disease, prompting researchers in this field to develop POC biosensors to quickly detect an AMI episode. Over the years, various detection methods have emerged to evaluate cardiac troponins. Review [84] summarizes various biosensor methods for detecting these markers of myocardial injury.

To detect troponin I, the authors of [85] developed an electrochemical labelless biosensor based on a glassy carbon electrode coated with nanoporous graphene oxide. The biosensor is inexpensive, and due to the use of porous graphene, has good electrochemical properties and a large active surface area.

The sensor showed good selectivity and high sensitivity: the limit of detection was 0.07 ng/mL.

*Oncology*

It is known that early diagnosis of oncological diseases plays a key role for subsequent treatment in many cases. However, the level of tumor markers in the patient's blood at the initial stages of the disease does not exceed a few pmol; thus, only a few methods and sensors can detect them [61, 83]. In recent years, various biosensors have been proposed for diagnosing various types of cancer, including breast [86], prostate [87, 88], lung [89], liver, stomach, and intestine cancer [90]. Let us give specific examples.

The authors of [89] developed a highly sensitive graphene biosensor capable of identifying signs of lung cancer, i.e., to detect (sniff out) in the respiratory products of a human molecules of the most common biomarkers of lung cancer (ethanol, isopropanol, and acetone) in a range of different concentrations. The sensor is able to detect molecules of specific lung cancer markers at the earliest stage of the disease.

Detection of the prostate tumor marker PSA was performed by the authors of [87] using graphene field-effect transistors modified with polyethylene glycol (PEG)/ethanolamine. The study demonstrated the possibility of real-time biomarker detection. In addition, studies using graphene devices modified with a PEG/DNA aptamer have shown specific binding and detection of PSA in solutions at pH 7.4. The receptor of aptamer-modified graphene devices can be regenerated for the purpose of multiple selective determination of PSA.

**Helicobacter pylori (H. Pylori)** bacteria attack the stomach lining and cause ulcers and stomach cancer. To detect them, a graphene biosensor was developed in [61, 62]. Graphene was adsorptively modified by antibodies. When bacteria interact with the biosensor, chemical reactions are triggered, which are fixed by graphene. The researchers used microfluidics to ensure the detection of reaction products occurring with bacteria in the presence of certain chemicals that the authors added to a tiny drop of water.

Microfluidics allows bacteria to be localized in microdroplets near the sensor surface. The biosensor quite quickly (in less than 30 min), highly sensitively, and quantitatively detects *H. Pylori* bacteria, and the concentration of the reaction products can be monitored in real time.

A wireless nanosensor based on graphene was developed to detect bacteria in saliva [91]. The graphene sensing element was adsorbed onto a silk film (fibroin) and then transferred to the tooth surface, followed by dissolution of the supporting silk film. The detection specificity was ensured by using self-assembling antimicrobial peptides (odorranin-HP) on a graphene monolayer. When the system recognizes and binds the target bacteria (*H. pylori*), the electrical conductivity of the graphene film changes and the data are transmitted wirelessly. The developed nanosensor has a low detection limit (100 CFU/mL)

and the possibility of remote wireless sensing. Thus, the "tattoo on the tooth" warns of bacteria in saliva.

*Diabetes*

Diabetes is a common chronic disease in which the body's ability to absorb glucose is impaired. Constant monitoring of the concentration of glucose in the blood of diabetic patients is necessary to assess the condition of patients. Various glucometers are used for measurements, primarily enzymatic electrochemical biosensors. The latter have satisfactory selectivity and sensitivity, are based on the use of glucose dehydrogenase or glucose oxidase enzymes, and are commercially available. However, the use of biological materials such as enzymes, antibodies, etc., is limited by the complexity of their manufacture and low service life due to the decrease and loss of the biological activity of the enzyme over time. The commonly used glucose oxidase enzyme has insufficient stability and requires complex immobilization processes on the sensor surface. It does not withstand even a slight heating, which narrows the range of application of biosensors.

An alternative to enzymatic biosensors are sensors without enzymes that detect glucose through its oxidation. In this case, it is important to develop suitable efficient catalysts for the detection of glucose in biological samples under physiological conditions without any pre/post treatment. The main advantages of enzyme-free biosensors are their low cost, high stability, fast response, and low detection limit. The devices directly detect glucose and are based on its oxidation reaction catalyzed by various electrocatalysts, which are atoms on the surface of the material. Here, a significant role is assigned to nanomaterials, such as nanoparticles of Au, Ag, Ni, Cu, and Co, as well as their oxides and sulfides.

In [92], the authors compared the latest developments in nonenzymatic glucose biosensors based on copper nanoparticles (NPs), copper oxides, their alloys, and their composites. Copper and its oxides are widely used as components of nonenzymatic glucose sensors due to their low cost, good sensitivity, and current response in alkaline media, and also because of the practical and simple methods for preparing nanomaterials based on them. In addition, they have high electrocatalytic activity, economy, nontoxicity, and stability. Combining copper with graphene significantly increases the sensitivity of nonenzymatic glucose sensors, which is probably due to the synergistic effect between the two components leading to an increase in the electrocatalytic active area and an increase in electron transfer for glucose oxidation. Information about some biosensors is given in Table 2.

It follows from the data presented in Table 2 that the developed enzyme-free electrochemical glucose biosensor based on LIG decorated with Cu or Cu–$Cu_2O$ nanoparticles showed the highest sensitivity

**Table 2.** Analytical characteristics of non-enzymatic graphene biosensors for glucose based on Cu nanoparticles, copper oxides, alloys/composites (according to [92])

| Sensor type | Sensitivity, $\mu A\ mM^{-1}\ cm^{-2}$ | Limit of detection (LOD), $\mu M$ | Linear detection range |
|---|---|---|---|
| Sensor based on Cu nanoparticles on laser-induced graphene (Cu NPs–LIG) | 495 | 0.39 | $0.10-400\ \mu M$ |
| Porous structure consisting of three-dimensional graphene (3DG) based on Cu or Cu-Cu$_2$O nanoparticles (Cu-Cu$_2$O NPs @ 3DG) | 230.86 | 16.00 | $0.8-10\ mM$ |
| Graphene modified Cu$_2$O–Cu nanocomposite electrodes | 371 and 400 | 5.5 and 2.0 | $2\ \mu M-12\ mM$ |

($495\ \mu A\ mM^{-1}\ cm^{-2}$) and 1086 _uc2s 10 mM. A graphene-modified Cu$_2$O nanocomposite was synthesized by microwave irradiation of an aqueous solution of copper compounds and studied as an enzymeless glucose biosensor. The biosensor showed a broad linear response to glucose detection in the concentration range from 2 $\mu$M to 12 mM with a detection limit of 2 $\mu$M. In addition, it ensured the selectivity of glucose determination at high concentrations of ascorbic acid and dopamine. The results of these studies have shown that these materials can be used to create inexpensive nonenzymatic electrochemical glucose sensors.

The authors of [93] created a wearable, noninvasive, low-cost LIG-based device that allows us to measure blood glucose levels without piercing the skin, in contrast to the currently used tests. The problem was that graphene is inert to glucose; thus, the authors had to look for workarounds. They found that LIG modified with nickel-gold alloy nanoparticles could detect low concentrations of glucose in sweat on the surface of the skin. The concentration of glucose in sweat is lower by a factor of about 100 than the concentration in blood, but there is a strong correlation between sweat and blood glucose levels. The sensor works on a small area of the skin containing at least one hair follicle. It detects glucose by drawing it out of the fluid that is present between cells. The new device is sufficiently sensitive to accurately measure glucose in sweat and estimate blood concentrations. The researchers demonstrated the device by attaching it to a person's arm 1 and 3 h after a meal.

The sugar levels detected by this device and commercial glucometers matched each other.

A group of Korean researchers [94] developed a method for manufacturing biosensors in the form of soft contact lenses that can monitor tear glucose levels to indicate real-time diabetic status through a wireless display. For this smart lens, the electronic components (glucose sensor, LED pixel, rectifier circuit, and stretchable transparent antenna) were integrated into a stress-tunable hybrid substrate with well-matched refractive indices for high optical transparency and low haze. After shaping the soft contact lens into a round shape, the built-in electronic system worked reliably under mechanical deformations, including bending and stretching. In vivo tests using a live rabbit, including monitoring the temperature changes in the eye of a rabbit, showed the promise of such contact lenses for noninvasive monitoring.

The development of effective biosensors for determining the concentration of glucose in a patient's blood continues to be an urgent task, since it is necessary to increase reliability and reduce the cost of analysis.

### 3.3.3. Graphene Biosensors for Toxins

#### Mycotoxins

Mycotoxins produced by microscopic molds are a common type of toxin found in food and feed. The problem of mycotoxin contamination has recently become more acute due to the increased complexity of transport chains from farm to store, which entails negative consequences for human and animal health.

Consumption of products contaminated with mycotoxins leads to acute and chronic diseases (mycotoxicosis, chronic gastrointestinal diseases, hemorrhagic necrosis, liver cancer, etc.). It is highly desirable to establish easy to use, in situ, and rapid monitoring of mycotoxins in food and feed.

Consumption of products contaminated with mycotoxins leads to acute and chronic diseases (mycotoxicosis, chronic gastrointestinal diseases, hemorrhagic necrosis, liver cancer, etc.). It is highly desirable to establish easy to use in situ and rapid monitoring of mycotoxins in food and feed.

In [80], the authors reported on the creation of an improved bioelectronic sensor for mycotoxin ochratoxin A (OTA) based on graphene field-effect transistors integrated on a silicon chip. The OTA-specific aptamer was attached to graphene via a covalent bond with a pyrene-based linker. This device has demonstrated a high degree of sensitivity to OTA with a low detection limit of 1.4 pM with a response time of 10 s in a phosphate buffer and up to 50 s in the case of real samples, which is superior to any other assay method. Grafting several aptamers specific for different mycotoxins can provide the simultaneous detection of several targets.

To detect food contaminants such as mycotoxins (including OTA), the authors of [96] developed a non-

enzymatic electrochemical aptasensor based on the use of cerium oxide and graphene oxide nanoparticles on a screen-printed electrode.

Changes in the optical properties of cerium nanooxide upon interaction with phenols and $H_2O_2$ were used to manufacture portable colorimetric sensors for the detection of food antioxidants and glucose.

### Pesticides and Chemical Warfare Agents

The intensive development of printed electronics methods (the field of electronics involved in the creation of electronic circuits using printing equipment) has allowed us to develop methods for printing graphene-based electrodes, which has recently become an attractive, inexpensive, and scalable technology for the production of field electrochemical biosensors. For example, in [97], the authors report on a graphene-based electrode obtained by maskless inkjet lithography for direct and rapid monitoring of organophosphorus compounds—chemical warfare agents and pesticides.

The graphene electrode has a microstructure with laser engraving and electrochemically deposited platinum nanoparticles (diameter ~25 nm) to improve its electrical conductivity (sheet resistance is reduced from ~10 000 to 100 Ohm per m$^2$ of surface area). The enzyme phosphotriesterase is covalently immobilized on the electrode by cross-linking through glutaraldehyde. The resulting biosensor was able to quickly (response 5 s) detect the model insecticide paraoxon with a low detection limit (3 nM) and high sensitivity (370 nA/μM) with little interference from similar nerve agents.

In addition, the biosensor demonstrated reusability (decrease in sensitivity by 0.3% on average per measurement), stability (90% preservation of the anode current signal for 1000 s), durability (after 8 weeks, sensitivity is maintained at 70%) and the ability to selectively determine organophosphorus in real soil and water samples. Thus, in [97], a scalable technology for manufacturing a printed graphene electrode is presented, which can be used to create biosensors suitable for field use.

A printed electrochemical sensor has been proposed for the in situ determination of methyl parathion (an insecticide containing an organothiophosphate group) and nitrite in foodstuffs [83, 97]. The electrodes were made from a mixture of chitosan, graphene, and silver powders. The porous structure of the sensors allows the analysis to be carried out without prior removal of the analyte. The sensor has been tested on simulation systems and real objects (Fuji apples, Chinese onions and cabbages). The limit of detection was 15 ng and 18.4 μg for methyl parathion and nitrite, respectively.

Biosensors for pesticides were created [98] by functionalizing LIG electrodes with the enzyme horserad-ish peroxidase. They showed a high level of sensitivity to atrazine (28.9 na/μm) with little difference from other common herbicides (glyphosate, dicamba and 2,4-dichlorophenoxyacetic acid).

### Biogenic Amines

Biogenic amines (BAs) are nitrogenous compounds, the concentration of which in food products is directly related to food safety and, consequently, to human health. The presence of a large amount of BAs in food can lead to severe poisoning. In food, BAs are formed by endogenous enzymatic activity or microbial metabolism, leading either to the decarboxylation of amino acids or to the amination of aldehydes and ketones. The properties of individual BAs (e.g., histamine, tyramine, cadaverine) vary depending on the amino acid precursor (histidine, tyrosine, lysine) and chemical structure (aliphatic, aromatic, or heterocyclic). The total BA content in any food product depends on the specific biochemical composition, as well as the type and number of microorganisms present. For example, fermented foods such as cheese, wine, sausage, and pickled vegetables that use lactic acid bacteria communities for fermentation may contain high concentrations of histamine, cadaverine, tyramine, and/or putrescine. Fermented fish products are particularly susceptible to high levels of BA due to a combination of high microbial load and high content of amino acid precursors. Since the accumulation of histamine, putrescine, cadaverine, tyramine, trimethylamine, and dimethylamine can be related to microbial contamination, the total concentration of BAs is commonly used to assess quality and safety indicators, as well as the overall shelf life of fish, fish products, and shellfish.

Graphene biosensors can be used in the food industry to analyze histamine and other toxins. For biosensing food safety (in particular, the presence of biogenic amines) without the use of additional reagents, graphene electrodes with laser engraving were developed [83, 99]. In the manufacture of biosensors, the graphene surface was functionalized with diamine oxidase and copper microparticles. The developed biosensor showed good electrochemical characteristics: average sensitivity to histamine 23.3 μA/mm, as well as a lower detection limit of 11.6 μm and response time 7.3 s. The authors demonstrated the use of the biosensor by testing the total concentration of BAs in fish paste samples fermented with lactic acid bacteria. The concentration of biogenic amines before fermentation with lactic acid was below the detection limit of the biosensor, while after fermentation, the concentration of histamine was $19.24 \pm 8.21$ mg/kg. These results confirm that the sensor was selective in a complex food matrix. An inexpensive, fast, and accurate device is a promising tool for assessing biogenic amines in food samples, especially in situations where

the standard laboratory methods are not available or too expensive.

Electrochemical biosensors for the detection of histamine based on graphene with electrodes fabricated using aerosol inkjet printing are described in [83, 100, 101]. These sensors detect BAs in foods much faster than the standard laboratory tests. Their use for these purposes is more expedient. Commercial electrochemical sensors are disposable; thus, it is too expensive to use them all the time. Low-temperature chemical vapor deposition graphene devices used for food monitoring are too expensive for such applications. At the same time, inexpensive alternative methods, such as screen printing and inkjet printing, are not able to provide sufficient control of the electrode geometry to obtain suitable electrochemical characteristics of the sensor. When applying specially designed aerosol-jet ink [100, 101], it is possible to change the geometry of the template using software control. New graphene-based biosensors detect substances hazardous to health in food faster and more efficiently than classical biosensors. Also, airjet sensors apply the right material only where it is needed, minimizing production waste and making the devices inexpensive and easy to manufacture. Because of this, they can be used where constant monitoring of food samples is important in order to assess the quality of products. In the course of the study [100], interdigital flexible electrodes were created from graphene on a substrate, after which they were converted into histamine biosensors by covalent modification of the graphene surface with monoclonal antibodies. The operation of biosensors was tested not only in a model buffer solution but also in fish broth to ensure the effectiveness of histamine detection. It was found that the graphene biosensor is able to detect histamine in a buffer solution and fish broth in toxicologically significant amounts (6.25–100 and 6.25–200 ppm with limits of 2.52 and 3.41 ppm, respectively). For example, histamine levels in fish that exceed 50 ppm cause severe allergies in some people and even food poisoning. It is also important that the biosensor demonstrates a short response time: 33 minutes is sufficient and no pretreatment of product samples is required.

Similar laboratory tests take longer and require prior labeling and sample processing. In addition, the sensitivity of the sensor was not affected by the adsorption of large protein molecules, which often act as a blocking agent. A new type of biosensor created from graphene can be used in food processing plants, ports, and stores where constant on-site monitoring of samples is required. Its use will avoid sending samples to the laboratory, save time, and reduce the cost of testing for the content of histamine and toxins in products. Achievements in the design and development of graphene biosensors for food safety assessment are summarized in the review [102].

## CONCLUSIONS

The high sensitivity, fast action, and small size of graphene biosensors, combined with the record specificity achieved by modifying graphene with antibodies and/or aptamers, make such sensors extremely promising devices for use in various fields of biomedicine. The first, but promising results have been obtained in important areas such as diagnosing autoimmune diseases, monitoring and diagnosing oncological diseases in the early stages, monitoring intrauterine genetic abnormalities of the fetus during pregnancy, and controlling the appearance of dangerous metabolites during surgical operations.

The biosensors created based on the GNM allow us not only to detect biomarkers but also to study the processes occurring in cells (formation of reactive oxygen species in living cells and in vivo expression of genes contained in chromosomes).

In modern genetic engineering, it is promising to use CRISPR/Cas9, a new technology for editing the genomes of higher organisms based on the immune system of bacteria, which is based on special sections of bacterial DNA, short palindromic cluster repeats, or CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). Since 2016, molecular biologists have been widely using approaches based on CRISPR/Cas systems.

It is believed that in the foreseeable future these approaches will be used in medicine for the treatment of hereditary diseases, https://ru.wikipedia.org/wiki/CRISPR: cite_note-3. CRISPR/Cas is important for the targeted delivery of drugs and their release under external influence. For the discovery of this method in 2020, the Nobel Prize in Chemistry was awarded.

Most nucleic acid detection methods require large amounts of reagents, expensive and bulky devices, and in addition, are related to a violation of gene material. The advantage of the CRISPR method is that it allows us to directly observe DNA, instead of isolating DNA, destroying, amplifying, labeling, and directing an optical laser at it to detect the label. It is important that the graphene CRISPR chips developed in [103, 104] make it possible to study DNA in its natural state and immediately signal the presence of a specific mutation, protein, or other component.

It can be argued that the use of graphene nanomaterials in biosensors is a very relevant and promising direction in the development of this field.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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