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Review Article

Synthesis of Graphene-Based Biosensors and its Application in Medicine and Pharmacy - A Review

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Abstract

One of the most exciting research areas in the field of material science nowadays involves carbon materials and nanomaterials, the most significant ones among them being graphene and akin substances, such as graphene oxide and reduced graphene oxide. Graphene was first isolated in 2004 by Russian scientists Geim and Novoselov, who insulated it with adhesive tape. After its isolation, graphene's popularity increased and the properties, as well as the possibilities it provides are today being further explored. Graphene can be defined as a single-layered graphite, i.e. it represents a single layer of carbon atoms placed in one plane. These carbon atoms are arranged in hexagonal lattice resembling a honeycomb.

Apart from its initial use in physics, graphene's contributions have lately spread to other sciences, such as electronics, quantum physics, medicine, pharmacy, etc. In fields of medicine and pharmacy, the significance of graphene, as a material, primarily manifests in sensor and biosensor development which contributes to easier health monitoring, all achieved by graphene's remarkable properties. In clinical practice, biosensors with high sensitivity and precision can improve patientcare, provide a chance of an earlier diagnosis of diseases, as well as detection of pathogens. Additionally, graphene-based biosensors can detect a plethora of biological substances, such as glucose, hydrogen peroxide, cholesterol, dopamine, etc. So far, the number of research applied to graphene biosensors shows an increase in the detection of tumor markers, being medicines delivery carriers and tissue bioengineering.

Keywords: Grapheme; Graphene synthesis; Graphene biosensors; Detection

Background

Graphene is a two-dimensional (2D) single layer of sp2-hybridized, covalently bonded carbon atoms arranged in a hexagonal lattice, i.e. in the shape of a honeycomb. (Nada et al, 2015) [18] Although scientists have long known that such a two-dimensional crystal with a thickness of one atom exists and is present in the core of a graphite pencil, however; graphene was isolated from graphite in 2004 for the first time. Graphene was isolated by two scientists from the University of Manchester, Professor Andre Geim and Professor Kostya Novoselov, and they were awarded Nobel Prize in Physics in 2010 due to their work on the isolation and further studies on graphene. During one of the experiments called "Friday night experiments", professors Geim and Novoselov separated the flakes from the lumps of graphite with adhesive tape. By repeating the process of separating graphite fragments, they managed to obtain flakes the thickness of one atom, and their experiment led to the first isolation of graphene. (Novoselov, 2011) [22].

Because of the way in which professors Geim and Novoselov managed to isolate single-layer graphene, the method was called "Scotch-tape method" or the method with adhesive tape and proved to be simple and effective for obtaining graphene samples allow-

ing extremely fast development of this field of science. Scotch - tape method is also known as a micromechanical technique of exfoliation (peeling).The advantage of this method is that no need of large investments and complicated equipment. (Novoselov, 2011) [22].

As mentioned earlier, graphene consists of a single layer of sp2 hybridized carbon atoms and forms the basis of the structure of its derivatives, which include graphene oxide (GO), reduced graphene oxide (RGO), graphene nanoplates (GNPs) and functionalized/ chemically modified graphene. Graphene derivatives have structures very similar to graphene, but still differ structurally from each other, which results in differences in physicochemical properties compared to clean graphene. (Tahriri et al, 2019) [34].

Graphene oxide (GO) is a derivative of graphene, i.e. primarily of graphite, which is formed by the oxidation process. The process of obtaining graphene oxide is performed in several steps. The first step is the oxidation of graphite, using a strong oxidizing agent potassium permanganate in the presence of concentrated sulfuric acid and sodium nitrate, with oxygen-containing functional groups (e.g. -OH, -COOH, = O, -C = O) easily bonding to the surface graphite thus forming graphite oxide. Oxidation is followed by exfoliation, i.e. peeling of multilayer graphite oxide into individual layers, whereby single-layer graphene oxide is formed. This method of graphene oxide synthesis is called the Hummers method. Reduction of the formed graphene oxide produces reduced graphene oxide. (Santosh et al, 2020) [28]. Graphene oxide is currently used in sensors and biosensors for detection of glucose, DNA, thrombin, as well as in environmental sensors that detect the presence of ammonia, formaldehyde, mercury, lead, etc. Graphene oxide - based sensors and biosensors have a significantly higher sensitivity and detection limit than sensors and biosensors based on other materials. (Celine et al, 2017) [4].

Unlike graphene oxide, which is rich in oxygen-containing functional groups, reduced graphene oxide also has these functional groups, but considerably less (Arghya, 2018) [2] There are numerous techniques for converting graphene oxide to reduced graphene oxide, and each of them has its advantages and disadvantages. All methods of graphene oxide reduction can be classified into three groups: chemical reduction, thermal reduction, and electrochemical reduction. Chemical reduction is suitable for obtaining reduced graphene oxide with a large specific surface area and excellent electrical conductivity. The disadvantages of this method of reduction are low yield and the use of toxic reducing agents (e.g. hydrazine). Thermal reduction enables the production of reduced graphene oxide in large quantities in a short time. However, the disadvantage of thermal reduction is that high temperatures can damage the structure of graphene and lead to the release of CO_2. Electrochemical reduction is considered the best choice in terms of quality. The reduced graphene oxide obtained in this way can almost be compared to clean graphene. The advantage of electrochemical reduction over chemical reduction is that no toxic reducing agents are used. (Santosh et al, 2020) [28] Like graphene oxide, reduced graphene oxide finds its application in sensors and biosensors for the detection of glucose, H_2O_2, dopamine, ascorbic acid, uric acid, tumor markers, then for the detection of methane, lead, nitrite, 17β-estradiol. (Celine et al, 2017) [4].

Graphene synthesis can be performed using two main approaches: top-down (destruction) method and bottom-up (construction) method. These methods are shown in Figure 1. (Magan et al, 2015) [14] Top-down methods such as mechanical exfoliation, arc discharge, oxidative reduction exfoliation, liquid phase exfoliation (LPE), and carbon nanotube unzipping method typically isolate and remove graphite layers into single-layer, double-, and multilayer graphene. These methods lead to thinning of larger precursors such as graphite and other carbon-based precursors and form nano-sized graphene. Bottom-up methods build graphene materials from precursors the size of an atom (carbon). Bottom-up methods include chemical vapor deposition (CVD), epitaxial growth, substrate-free gas phase synthesis (SFGP), and total organic synthesis.

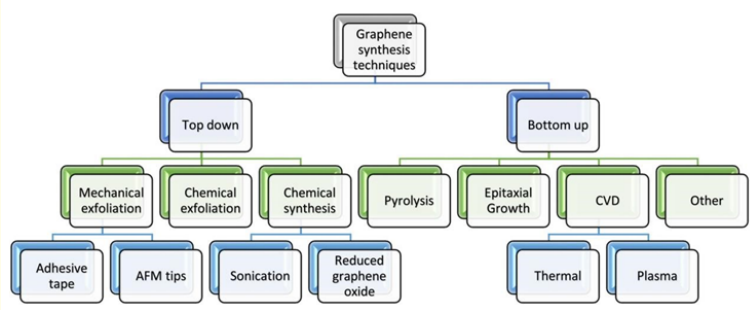

Figure 1A: process flow chart of Graphene synthesis.

Application of graphene in the production of sensors and biosensors

Biomolecular analysis plays a very important role in many areas of application, including medicine, pharmacy, biosafety, food and environmental testing, forensic medicine, etc. The analytical methods which are used should be able to give fast, reliable, and sensitive results, preferably in a real-time. Biosensors, as a type of analytical method in the detection of biomolecules, offer a number of advantages over existing detection techniques, such as mass spectrometry or ELISA tests, which require expensive equipment and are usually time consuming. Biosensors are one of the major advances in health, in terms of fast, reliable, and sensitive detection of biomolecules and the fight against serious diseases at minimal cost. (Owen et al, 2016; Neha et al, 2017) [24, 19].

Graphene, thanks to its exceptional multiple properties such as high carrier mobility, excellent electrical and thermal conductivity, large theoretical specific surface area, high optical transmittance, high Young modulus and excellent mechanical flexibility, promises 2D material for application in many areas, primarily for development of point-of-care sensors and implantable health monitoring devices with high sensitivity, selectivity and low limit of detection, which significantly facilitates early diagnosis of the disease by analysis of urine or saliva where the level of biomarkers is significantly lower than in the blood. (Stefano et al, 2018) [31] The advantages of graphene as a sensor are reflected in the following: large specific surface area and atomic thickness of graphene layers allow whole carbon atoms to come into direct contact with analytes such as enzymes, single-stranded DNA (ssDNA), RNA, receptors, aptamers, which makes it a much more sensitive sensor than silicone sensors, then intimate contact with organs of interest such as skin, brain and eyes can be achieved with graphene-based sensors due to its mechanical flexibility and ultrathin thickness, which is important for obtaining high quality signals without irritation or damage. In addition, the excellent performance of graphene in biosensors, such as high specific surface area and high electron transfer rate, allows receptors such as enzymes, antibodies, and DNA to bind efficiently to the graphene surface. (Haizhou et al, 2019) [10] Several graphene-based sensors have been described in the literature for the detection of clinically relevant analytes, such as hydrogen peroxide, glucose, ascorbic acid, dopamine, cholesterol, uric acid, etc. (Stefano et al, 2018) [31].

According to the IUPAC definition, a biosensor is "a device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds, usually using electrical, thermal or optical signals". (Beatriz, 2018) [3] In other words, a biosensor is a device that can detect physiological or biochemical changes by incorporating biological and physiochemical components. It consists mainly of a biomolecule, a transducer, and an output system. The exceptional selectivity of the biomolecule leads to the recognition of the analyte and the biochemical signal produced during the recognition is converted into a detectable signal. The signal is usually displayed as an electrical signal by the output system. (Janire et al, 2018) [11] Above described is shown on the Figure 2.

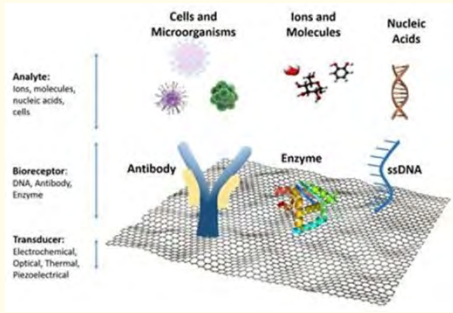

Figure 2: Examples of biosensors and components on a graphene platform.

The biosensor, which is defined as an analytical device consisting of a biomolecule, a transducer, and an output system, can be categorized according to the type of biomolecule incorporated. Biomolecules can be enzymes, antibodies, single-stranded DNA (ssDNA), organelles, cells, or tissues. The main categories of biosensors classified according to biomolecules are enzyme biosensors, immunosensors and DNA-based biosensors. Biosensors are designed in a variety of sizes and shapes and can use a range of transducers such as electrochemical, optical, piezoelectric, thermal, or ion-selective electrodes. When using graphene nanomaterials to design sensors, it is necessaryto considersome aspects of graphene properties that affect the limit of detection of target molecules. For instance, different synthetic methods may lead to different properties and functionalities of graphene-based nanomaterials in biosensors. Moreover, the orientation between graphene sheets, graphene oxide or reduced graphene oxide and bioreceptors can also directly affect the selectivity and sensitivity of biosensors. (Melis et al, 2018) [16].

Graphene based biosensors for glucose detection

The first and most commonly used enzyme in enzyme biosensors for glucose detection was glucose oxidase (GOD). In their research, Shan and colleagues presented a graphene-based biosensor with modified glucose oxidase and polyethylene mine-functionalized ionic liquid (PFIL) with high electrocatalysis of oxygen and hydrogen peroxide. The preparation of the electrode itself required several steps. In the first step, they synthesized graphene oxide according to the Hummers method, then protected it with polyvinylpyrrolidone (PVP) and performed reduction using hydrazine and ammonia. The solutions of polyvinylpyrrolidone, - protected graphene and polyethyleneimine, - functionalized ionic liquids were then mixed and discharged on a glassy - carbon electrode. In the final phase, the electrode was saturated with glucose oxidase solution. Polyvinylpyrrolidone-protected graphene, dispersed in a solution of polyethyleneimine - functionalized ionic liquids, has better performance in terms of good film stability, high solubility, and high ionic conductivity. Another example of a polymer-modified graphene biosensor was developed by Zeng et al. They modified the reduced graphene oxide sheets with pyrene - polyacrylic acid (PAA), followed by alternating precipitation of a mixture of graphene - PAA and polyethyleneimine. The synthesis of reduced graphene oxide was performed by the Hummers method and subsequent ultrasonic shaping, and reduction with hydrazine. The mechanism is that the interaction between reduced graphene oxide and pyrene - polyacrylic acid occurs π-π by assembling aromatic pyrene rings and graphene structure. This interaction, although it increases the dispersion ability, reduces the conductivity of graphene. (Marilena et al, 2015) [15].

In addition to enzymatic graphene biosensors for glucose, the use of non-enzymatic biosensors is also widespread. One of the first to introduce such a biosensor was Wu with his associates. The biosensor is based on glassy-carbon electrodes modified with graphene oxide - based platinum nanotubes (PtNPs - GO). Graphene oxide is prepared by the Hummers method and then mixed with K_2PtCl_4. PtNPs - GO is poured onto the glassy - carbon electrode surface and nafion is added to stabilize the electrode. The biosensor shows a wide linear range of 2 μM - 20.3 mM, a detection limit of 2 μM and good stability. (Marilena et al, 2015) [15].

Graphene based biosensors for tumor marker detection

Tumor markers encompass a wide range of biochemical molecules, mostly of a protein nature. They are defined as substances produced by tumor cells, less often secreted by healthy tissue of certain organs, so that they can also be found in the body of healthy individuals, however, in much lower concentrations. In the last few decades, various biosensors have been developed for the specific determination of tumor markers, with the aim of faster and simpler diagnosis and analysis of tumors in real time. (Madasamy et al, 2019) [13].

Feng and co-workers were among the first to report a functional graphene-based electrochemical aptasensor for cancer cell testing. The aptasensor is formed by covalent binding between functionalized graphene and the NH_2 group of the modified aptamer AS1411. A study conducted by these scientists showed that graphene-based aptasensorhas the ability to separate cancer cells from healthy cells in the body. Fiorillo and co-workers investigated the therapeutic potential of graphene oxide for targeting cancer stem cells (CSC), i.e. found that graphene oxide can be used to inhibit the proliferative spread of CSC. Through this study, they showed that graphene oxide effectively inhibits the formation of tumor spheres in multiple cell lines, including breast, ovarian, prostate, lung, and pancreatic cancers, as well as glioblastoma. (Numan et al, 2015) [23] Through their research, Huang and co-workers presented findings on a graphene sandwich electrode coated with a layer of silver and gold particles for the detection of tumor cells, specifically the CEA carcinoembryonic antigen. This immunosensor shows a linear range of 10 to 1.2 x 105 pg/ml and a detection limit of 8 pg/ml for carcinoembryonic antigen. Zhu and his collaborators developed a sandwich immunosensor for the simultaneous detection of four antigens using a graphene-gold hybrid film. (Madasamy et al, 2019) [13].

In a study conducted by Mao et al., An unlabeled electrochemical immunosensor was used to detect prostate - specific PSA antigen using a graphene, methylene blue, chitosan nanocomposite. This biosensor showed the ability to detect PSA even at a concentration of 13 pg / ml. (Madasamy et al, 2019) [13] On the other hand, Yang and his collaborators developed an ultrasensitive electrochemical immunosensor for carbohydrate antigen 19-9 (CA 19-9) using functionalized porous graphene (Au-PGO) gold nanoparticles as sensitive platforms and AuPd nuclei functionalized graphene nanocomposites (AuPd-Gra) as signal enhancers. In addition, they developed an electrochemical immunosensor based on ionic liquid functionalized graphene and Cd2 + - functionalized porous TiO2 for the detection of CA 15-3. The advantages of these immunosensors based on functionalized graphene are the high surface-to-volume ratio of graphene, excellent biocompatibility, and electron transfer rate from ionic liquid functionalized graphene. (Nada et al, 2015; Shuie et al, 2019) [18, 30] Li and co-workers developed an electrochemical immunosensor for the detection of prostate-specific PSA antigen, which is widely used in the diagnosis and screening of prostate cancer. The graphene plate adsorbed a 1-pyrenebutanoic acid molecule, succinimide ester (PBSE), while a colloidal solution containing graphene plate (GS) -cobalt hexacyanoferrate (CoNP) -PBSE nanoparticles was added to the glassy-carbon electrode surface to obtain a stable electrode. Film with high electroactivity. The electrode modified in this way can be used as an amperometric immunosensor for PSA detection. The advantages of this immunosensor are high sensitivity with a low detection limit of 0.01 ng / ml, good selectivity, and stability. (Nada et al, 2015) [18].

Novel application of graphene and graphene-based biosensors in medicine

In addition to the application of graphene and its derivatives in the development of biosensors for the detection of glucose, dopamine, nucleic acids, and the like, it also finds a place in the development of biosensors which are used in the treatment of certain diseases such as asthma, diabetes and myocardial infarction. Also, graphene and derivatives are used in modern regenerative medicine. (Nasrin et al, 2017) [17].

Recognition of new materials with properties such as good biocompatibility, controlled nontoxic biodegradation, ability to support cell differentiation, growth and proliferation and adequate mechanical strength, is crucial for the efficiency of the tissue regeneration process. The most interesting material in this regard is certainly graphene together with its derivatives, graphene oxide and reduced graphene oxide. (Nasrin et al, 2017) [17] One of the interesting approaches to the design of composite graphene oxide structures is

their covalent crosslinking with biopolymers. One such approach was the formation of graphene oxide - chitosan hydrogel scaffolds by covalently linking chitosan amino groups with carboxyl groups of graphene oxide. The application of these graphene oxide - chitosan hydrogels is mostly reflected in bone bioengineering, as they significantly improve the adhesion, differentiation, proliferation, and deposition of Ca-phosphate in the MC3T3-E1 preosteoclasts. Recently, Cheng and colleagues reported on the biomimetic mineralization of hydroxyapatite induced by polydopamine functionalized reduced graphene oxide (rGO - PDA). Graphene oxide was first simultaneously reduced, and surface functionalized by oxidative polymerization with dopamine. The obtained rGO - PDA is further used as a surface to mimic the hydroxyapatite mineralization during bone formation. MC3T3 - E1 cells were grown on rGO - PDA substrate to observe different cell activities and bone mineralization, and it was observed that these cells show higher cell activity such as proliferation, adhesion, and osteogenic differentiation by growing on rGO - PDA medium compared to clean graphene or graphene oxide. Also, the results of this study suggest the potential use of rGO - PDA as a scaffold to promote osteogenesis for successful use in bone regeneration. (Nasrin et al, 2017; Krzysztof et al, 2018) [12] Currently, tissue damage remains one of the most important aspects contributing to human death. In this regard, several studies have been conducted investigating the use of graphene in stem cell and musculoskeletal engineering. One such study was conducted by Chen et al., who investigated the effect of graphene and graphene oxide platforms on the proliferation and differentiation of induced pluripotent stem cells (iPSCs). They noted that pure graphene surfaces support iPSC cultures and allow for their spontaneous differentiation. (Roxana-Maria et al, 2018) [27].

Cardiovascular diseases are one of the leading causes of death in the world, and therefore their prevention and adequate diagnosis is extremely important. The gold standard in the diagnosis of cardiovascular disease, primarily heart attack, is the biomarker cardiac troponin I (cTnI), as it is released from cardiac cells after heart injury and has greater specificity compared to other biomarkers such as creatine kinase MB isoenzyme and myoglobin. Graphene-based electrochemical biosensors are considered a simple, fast, inexpensive, and miniaturized way to detect cardiac biomarkers in very low concentrations with high specificity and sensitivity. The sensitivity and specificity of these biosensors can be further improved by modifying the structure, material, and dimensions of the electrode used. (Taniselass et al, 2019) [33] Tuteja et al reported a simple approach to the electrochemical functionalization of graphene using 2 - amino benzyl amine (2 - ABA) to obtain an immunosensor for the detection of cTnI. They used a silicone base to make the electrodes, and then they precipitated graphene by pouring in drops.

Thereafter, 2-ABA was electrochemically precipitated on graphene for functionalization, and then cTnI immobilization was performed for about 33 hours at room temperature. The sensor was tested on both human serum and buffer solution and found to behave almost equally. (Tuteja et al, 2014) [35].

In addition to being used in the diagnosis of cardiovascular disease and bone engineering, graphene and derivatives are also used in the diagnosis of asthma. Asthmatic condition is a chronic heterogeneous disease that affects the airways in the lungs and is usually characterized by inflammation, as well as muscle tension in the airways and difficulty breathing. Years ago, due to the lack of an appropriate biomarker for the analysis and diagnosis of asthma, not much was done to detect a non-invasive sampling procedure, until Gholizadeh et al developed a graphene-based electrochemical biosensor to detect nitrite biomarker in exhaled breath condensate (EBC). They also suggested further improvements to biosensors, such as reducing detection limits or increasing sensitivity by using other nanomaterials to make electrodes. In addition, they found that exhaled condensate contains various other biomarkers that can serve in the diagnosis of asthma and other diseases of the respiratory tract. Several biomarkers have been identified, such as hydrogen peroxide, nitro tyrosine, leukotrienes, nitrites, 8 - isoprostane, prostaglandin E2, interleukin and malondialdehyde. (Taniselass et al, 2019; Gholizadeh et al, 2017) [33, 8].

Application of graphene and biosensors in pharmacy

Designing sensors for qualitative and quantitative drug determination is one of the very important techniques. These biosensors should show certain properties, such as precision and accuracy, as well as sensitivity and specificity, which allow them to detect the desired analyte (drug) quickly and easily. Many scientists have spent years researching nanomaterials that conjugate to biomolecules

to provide biosensors with the desired characteristics and that, compared to conventional drug detection methods (spectrophotometry, chromatography, chemiluminescence, spectrofluorimetry, etc.), would have advantages in terms of lower limit detection, time, smaller sample volume for analysis, etc. Once again, graphene stands out as one of the best nanomaterials with its outstanding properties such as large specific surface area, electrical conductivity, chemical stability, easy manipulation, and biocompatibility. (Neha et al, 2017) [19] Also, to further improve the properties of biosensors, the addition of polymers to the graphene electrode is very often performed. The most common are polypyrrole, polythiophene, polydiaminonaphthalene (PDAN) and polyaniline (PANI), with the use of polydiaminonaphthalene and polyaniline being the most prominent due to their easy synthesis and low cost. (Pankaj et al, 2014) [25].

Given the growing need for drugs, it is very important to be able to determine their concentration at any time, so an increasing number of scientists are dedicated to designing biosensors for their detection, primarily based on graphene and its derivatives. Thus, Seyed and his collaborators synthesized an electrochemical biosensor based on methacrylated graphene oxide/polyaniline nanocomposites for the detection of ascorbic acid (vitamin C). They synthesized graphene oxide using a modified Hummers method and used 0.25 cm2 FTO (fluorine doped tin oxide) glass plates for electrode preparation, which were subjected to sequential ultrasonic cleaning for 10 minutes in isopropanol, ethanol, acetone, and deionized water. FTO glass plates were dried under argon flow. After that, they deposited methacrylated graphene oxide suspended in deionized water on the surface of the FTO plate, and left it to dry at 45°C. This biosensor showed high sensitivity and good selectivity in the determination of ascorbic acid, a linear range of 8 - 5,000 μM and a detection limit of 2 μM. (Seyed et al, 2020) [29].

In addition to them, research was conducted by Venkata and his associates and they presented a biosensor based on iron oxide (Fe_2O_3)/reduced graphene oxide nanocomposites used for the detection of acetaminophen (paracetamol). Graphene oxide was synthesized by a modified Hummers method, and several steps had to be taken to obtain a Fe_2O_3/rGO nanocomposite. The synthesized graphene oxide was first dispersed in deionized water, followed by sonication, and iron chloride and hydrazine hydrate were added to reduce graphene oxide. Finally, the resulting mixture was washed several times with deionized water to obtain pure Fe_2O_3/rGO composite. As the electrode, the bones are glassy carbon electrode to which they bonded the previously obtained nanocomposite. The biosensor obtained in this way showed good sensitivity, selectivity, linear range, and low limit of acetaminophen detection. (Venkata et al, 2019) [36] A biosensor similar to the one described above was designed by Abbas and his associates, with cobalt (GR / $CoFe_2O_4$) added to the GR/Fe_2O_4 nanocomposite and the biosensor used for simultaneous and individual determination of acetaminophen and codeine. The advantage of this biosensor is high sensitivity and selectivity, as well as low limit of detection and simplicity. (Abbas et al, 2014) [1].

Rajeev and his collaborators designed a voltammetric biosensor based on polyaniline / reduced graphene oxide (PANI / rGO) and used a glassy carbon electrode as the electrode (Figure 3). The biosensor was intended for the detection of clonazepam, benzodiazepines with anxiolytic, anticonvulsant, hypnotic and sedative effects. They dusted a certain number of tablets and added them to ethanol, and subjected the mixture to sonication and centrifugation, then added a certain aliquot to Britton-Robinson buffer and KCl in an electric cell and analyzed the behavior of the substance at a certain potential. Graphene oxide was synthesized by a modified Hummers method, after which polyaniline was added and then the mixture was applied to a glassy carbon electrode. (Rajeev et al, 2016) [26].

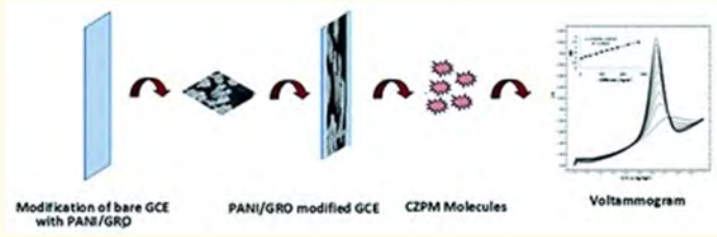

Figure 3: Voltammetric biosensor based on PANI / rGO (Rajeev et al, 2016) [26].

This PANI / rGO - based biosensor is intended for sensitive voltametric determination of clonazepam and shows a high electrocatalytic response during reduction of clonazepam in ethanol at pH 8.8 (Britton - Robinson buffer). Also, as a very sensitive method of clonazepam determination, this biosensor is used in routine analysis of tablet dose determination for analytical purposes. (Rajeev et al, 2016) [26].

Neethu and colleagues worked on the design of a biosensor for the detection of chloramphenicol based on graphene oxide / zinc oxide (GO / ZnO) nanocomposites. Graphene oxide synthesis was performed by the Hummers method. The synthesized graphene oxide was added to water to obtain a suspension to which ZnO was then added and the suspension was subjected to ultrasonification. As an electrode, they used a glassy carbon electrode to which they applied the previously obtained suspension. The biosensor showed high sensitivity and selectivity for the determination of chloramphenicol with a detection limit of 0.01 μM and a linear range of 0.2 - 7.2 μM. (Neethu et al, 2019) [21] Another in a series of researchers in the field of biosensors was Pankaj and his associates who worked on the design of biosensors for the detection of propranolol. The nanocomposite they made was graphene / polydiaminophthalene (GR / PDAN), and they used a pyrolytic graphite surface as a substrate for modification. Based on the synergistic effect between graphene and polydiaminophthalene, the newly synthesized biosensor showed high sensitivity, good stability, fast response, and reproducibility, which is why it was used in electrochemical analysis of propranolol in pharmaceutical and biological samples with a detection limit of 20 nM and a linear range of 0.1 - 0.1 ЮМ. (Pankaj et al, 2014) [25].

Graphene based biosensors in drug delivery

The development of newer and more efficient drug delivery systems with the ability to improve the therapeutic efficacy and effectiveness of therapeutic agents is one of the key issues facing modern medicine. Delivery of drugs in a safer way to target sites in the body is extremely important especially in the treatment of tumors, as well as conditions that require treatment with drugs whose pharmacological profile is not the most favorable for the patient in terms of safety and side effects. There are currently a number of tumor treatment strategies that include surgery, radiation therapy, and chemotherapy, or a combination of all three. However, each of these strategies has its advantages and disadvantages, which has led to the need to develop new and better forms of treatment. (Ghanbarzadeh and Hamishehkar, 2016) [7] In this regard, most attention has been paid to the examination of graphene and its derivatives as potential carriers for drug delivery. In essence, due to their excellent properties, such as large surface area, biocompatibility, 2D geometry and the presence of π electrons, graphene and derivatives are ideal materials for filling and targeted drug delivery. (Neha et al, 2020) [20] Compared to conventional tumor treatments, graphene and derivatives show certain advantages reflected in dose reduction, better pharmaceutical effect, minimization of side effects, protection of drugs from premature degradation and increased drug stability. (Ghanbarzadeh and Hamishehkar, 2016; Daniela et al, 2018) [7].

Analogous to graphene and graphene oxide, reduced graphene oxide has certain properties that make it a more promising carrier for biomedical purposes. Reduction removes most oxygenated groups from the graphene surface, leading to less surface polarity, thus improving its adsorption properties for less polar drugs. In addition, reduced graphene oxide has more sp2 hybridized carbon atoms than intact graphene, which makes it a more interesting material for photothermal or chemo - photothermal therapy because it shows higher absorption in the near infrared range. Hydrazine, hydroxylamine, sulfides and alkylamines are used here as the most common reducing agents. (Giacomo et al, 2017) [9] Figure 4 shows the application of sensors for drug delivery based on graphene and derivatives.

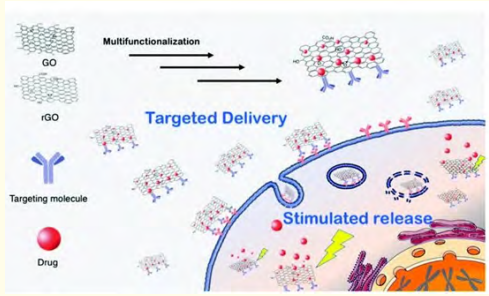

Figure 4: Cartoon illustrating the applications of GO and rGO in drug delivery (Giacomo et al, 2017) [9].

Generally, drug release in a cell is due to changes in environmental conditions between the extracellular matrix and the cytoplasm. Based on this, the desorption of the drug from graphene oxide can be activated by lowering the pH, the so-called acid desorption, when the drug enters cells through endocytosis and is digested in lysosomes where the pH is acidic. However, the problem that can arise is too slow or incomplete release of the drug from graphene oxide, which can lead to ineffective therapy. Therefore, the latest applications in graphene-based sensors for drug release relate to the use of stimulated drug release, i.e. release triggered by some external stimulus that most often involve a photothermal effect. In other words, graphene absorbs light in the near infrared region and converts it into heat which activates the drug release mechanism. (Giacomo et al, 2017) [9].

Liu and co-workers introduced the use of graphene as an effective nanocarrier for the delivery of water-insoluble anticancer drugs. They applied the anti-cancer drugs SN38 and doxorubicin (DOX) to nanografen-oxide (NGO) by adsorption using π-π stacking. The quinoline part of doxorubicin binds to graphene oxide via π-π interactions, while hydrogen bonds are formed between the amino / hydroxyl groups of doxorubicin and the hydroxylated / carboxylated groups of graphene oxide. They also found that the loading and release kinetics of doxorubicin were pH dependent. Maximum filling capacity was observed at neutral pH, while more than 70% of the drug was released in an acidic medium at pH 2. The reason is that in an acidic environment the amino group of doxorubicin is protonated, resulting in partial dissociation of hydrogen bond and drug release. (Syama and Mohanan, 2019) [32].

Graphene oxide also improves the solubility and bioavailability of camptothecin (CPT), a quinoline alkaloid that kills cancer cells by inhibiting the DNA of the enzyme topoisomerase I. De Sousa et al used graphene oxide conjugated with folic acid (GO-FA) to deliver camptothecin. In their study, they showed that in 48 hours more than 40% of the drug was released from the surface of graphene oxide conjugated with folic acid in relation to the percentage of release from the surface of unconjugated graphene oxide. The study also showed that prolonged and continuous release of the drug over a period of 200 hours at physiological pH. Similar to this study, prolonged drug release from graphene oxide-coated nanocarriers was observed by Suifullah et al. In their study, protocatechinic acid, a phenolic compound with anticancer properties, is conjugated to a folic acid - based nanocarrier coated with folic acid. Compared to the previous study, continuous drug release was observed here at both physiological and acidic pH. Common to both studies is certainly the fact that they showed improved anticancer activity of the folic acid-coated nanocarrier compared to the free drug and the folic acid-free nanocarrier. (Syama and Mohanan, 2019; Daniela et al, 2018) [32, 5] Zhang et al. Also conducted studies on this topic and found that loading doxorubicin and camptothecin into the same drug delivery system resulted in extremely high toxicity in MCF-7 cells compared to nanocarriers based on graphene oxides filled only with doxorubicin or camptothecin only. (Neha et al, 2020) [20].

Conclusion

Based on the presented results, several conclusions can be drawn:

Graphene has been in the center of interest of scientists from various fields for the last 50 years when we talk about the "ideal" material for the design of biosensors and their application in medicine and pharmacy.

The huge interest in examining the possibility of using graphene biosensors is the result of unique and suitable characteristics of graphene as well as simple synthesis procedures, among which the most prominent are the modified Hummers method and chemical vapor deposition. The greatest potential of graphene biosensors in pharmacy is reflected in the possibility of targeted drug delivery, which eliminates almost all side effects and achieves a better therapeutic response, as well as qualitative and quantitative determination of drugs in biological samples.

In medicine, the importance of graphene biosensors reflects in the detection of tumor markers and the possibility of appropriate treatment and therapy of tumors, as well as in tissue bioengineering.

The goal of making graphene biosensors is to get a sensitive, reliable, selective, and repeatable biosensor, which will be relatively cheap and minimally toxic with the use of biocompatible polymers and the green way of synthesis.

Finally, it can be said that the biomedical application of graphene and graphene-based biosensors is a very challenging field in science that is in continuous expansion.

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