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"A review of classifications of enzymatic and non-enzymatic electrodes-based nano-carbon for detection of glucose"

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Abstract:
In recent years, the number of people having diabetes mellitus is steadily increasing in countries known by low and middle income. Millions of them have been diagnosed with high blood sugar. Diabetes mellitus is related to irregular carbohydrates metabolism with difficulty managing blood glucose which by the time leads to serious damage to nervous system or even macrovascular, or blood vessels. Therefore, the demand for advanced devices for glucose monitoring is highly growing. A bio-sensor is a signal detecting device generated from reactions either biological or chemical. They can be used for many purposes, for example, detection of biological molecules using in vitro and in vivo samples. Electrochemical sensors represent one of the technologies could be used for this purpose. It shows high sensitivity and mechanical strength while utilizing in physiological conditions which is a promising advantage for glucose determination. The aim of current study is to review electrodes generations for glucose detection, as well as, commonly prepared and investigated electrochemical electrodes for glucose determination. In addition, types of glucose electrodes have been mentioned here based carbon nanotubes and/or graphene as excellently conductive, stable, reproducible, and sensitive materials. The aim of current study is listing and discussing the progression of glucose generations as well as the development of glucose sensors in recent years. Enzymatic and non-enzymatic nanocarbon sensors were mainly studies and classified as glucose sensors also explained with further details regarding limit of detection.

KEYWORDS: GLUCOSE DETECTION, ELECTROCHEMICAL SENSORS, CARBON NANOTUBES, GRAPHENE, ENZYMATIC AND NON-ENZYMATIC GLUCOSE SENSORS

1. INTRODUCTION:

In recent years, more than 400 million people have been diagnosed with diabetes as published by World Health Organization [1, 2]. The statistics for individuals suffer from diabetes is attributed to high rates of hyperglycemia (elevated level of glucose). This mainly happens when there is inadequate production of Insulin (patients of Type I diabetes) or the body is unable to consume the produced insulin (patients of Type II diabetes). Insulin is a hormone regulates sugar level in human body and keeps it normal [3]. In order to monitor the glucose level properly and precisely, good control over glucose treatment and diagnosis needs to be applied. This is because, un controlled level leads to accumulating followed by rising

glucose level in the blood [4]. That could cause health problems such as suffering from high blood pressure, heart attack, diabetes, diabetic retinopathy as well as other health issues [5]. Therefore, to make sure patients with diabetes have a well-controlled level of glucose, they run multiple testes through the day. Moreover, some of the diabetes patients take insulin to maintain a normal level of glucose. So that, the measurement of glucose level is necessary to obtain a proper monitoring for health and better management [6].

Number of methods for blood monitoring and glucose measuring use electrochemical sensing system recently. It is interesting technology and important analytical tool has found significant applications at health diagnosing and monitoring fields including glucose determination for the last two decades [7, 8]. It offers the possibility of fabricating ultra-small size electrochemical electrodes (@ micro or nano scale) could be integrated with electronic devices even for implanting purposes. In addition, electrochemical sensors possess high sensitivity and selectivity towards targeted substances with rich and fast detection [9]. The simplicity and low cost of electrochemical sensors also played an important role in preparation of different types of sensors compared to other traditional technologies which were expensive and time consuming methods [9]. These unique properties have had brought many advantages when it comes to implant medical micro-electrodes to reach critical parts or to find the concentration of biomolecules aimed for better recognition and analysis [7, 10, 11]. Sensing glucose has taken a considerable attention which mostly employed in the presence of enzymatic or non-enzymatic sensing systems. Four generations of glucose sensors were developed which are listed herein.

1.1. Generations of Glucose Biosensors:

1) *First Generation*

With this kind of sensors, enzymes immobilization is required, for example, glucose oxidase enzyme (GOx). Its role is basically as a catalyst when glucose gets oxidised by oxygen at the surface of the electrode. The oxidation reaction would generate hydrogen peroxide (H_2O_2) along with other products as explained below:

$GOx\ (FAD) + glucose = GOx\ (FADH_2) + gluconolactone$

$GOx\ (FADH_2) + O_2 = GOx\ (FAD) + H_2O_2$[12]

As can be seen, FAD is the centre in the enzyme, the flavin adenine dincleotide, it was reduced to $FADH_2$. The amount of freed hydrogen peroxide at the surface of the electrode could be determined which is a substitute to glucose concentration at initial steps of the reaction. There are disadvantages of such type of sensors[12]. Firstly, adequate amount of free oxygen needs to be available. However, for in vivo applications, some blood samples have insufficient oxygen which makes it ineffective. Secondly, high chances for possible peaks overlapping caused by other electroactive species already found with glucose in the blood. Including but not limited to, uric acid, ascorbic acid, acetaminophen [13], and dopamine have close oxidation potentials to that belongs to glucose[2].

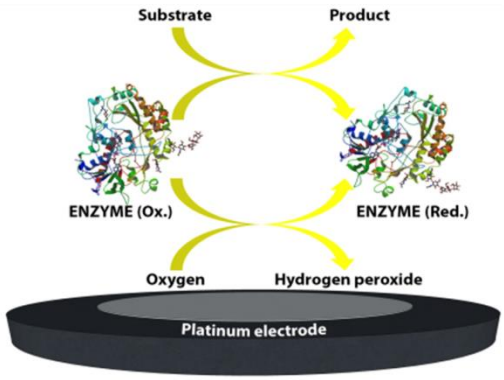


Figure (1.1) illustration of first-generation glucose sensors

2) *Second Generation*

GOx enzyme along with conventional electrodes are mainly applied at this type of biosensors. It was found that, the centre of GOx enzyme is FAD. There are some difficulties for this component to transfer electrons to the surface of already used conventional electrodes [14]. This is because, the region around FAD is occupied by a thick layer of protein prevents electrons transferring. So that, artificial mediators were introduced to enhance the process of transporting. A number of artificial mediators were used, for example, ferricyanide as well as ferrocene compounds. Although second generation of glucose sensors were good candidates to solve the issues reflected by first generation, some variables found to have an effect to the performance of second-generation sensors like temperature, wettability, and PH value [15].

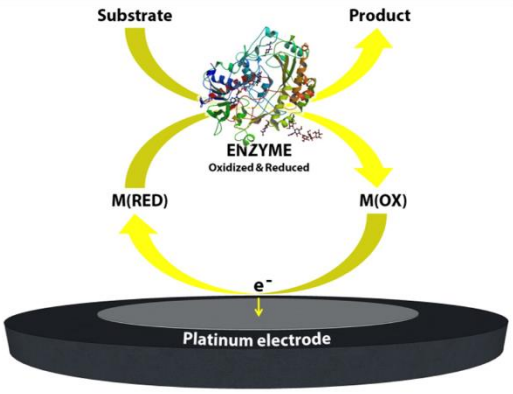


Figure (1.2) illustration of second-generation glucose sensors

3) Third Generation:

In current generation, the artificial mediators were not needed. Instead, the electron transfer occurs between the enzyme and the surface of the electrode directly by employing nano or micro dimension also highly porous material for enzymes immobilization. The reason for that is, nano and micro materials represented key factor for increasing the surface area of the electrode due to high porosity and that would significantly improve the electron transfer process [16]. However, the thickness of the immobilized enzyme transferring the electrons was also influenced by revealed a thickened layer.

1.2. Electrochemical Biosensors:

Based on previously published articles, electrochemical electrodes are defined as devices characterise specific elements like biomedicine, drug delivery, heavy metals, gas, and biomolecules simply

and rapidly through electrochemical workstations made for this purpose [8, 17]. First invented electrochemical biosensor was by Leland Clark in the fifties of last century for the detection of Oxygen O_2 and so it's called "Clark electrode" [18]. Later in the sixties, Clark came up with another invented biosensor but this time for the detection of glucose. The biosensor targeted glucose in biological samples through the electrochemical determination of O_2 or H_2O_2 with glucose oxidase immobilization [17]. The electrodes at this field are either called electrochemical sensors or biosensors [8, 19-21]. Electrochemical sensor can be stimulated chemically or physically to produce electronically detected signals [8, 19]. Sensors consist of many kinds of conductive materials with the property of electron transfer for electrochemical determination purposes [11]. The electrochemical biosensor, on the same hand, is a device converts biochemical reactions and interactions, for example, enzyme- substrate and antigen-antibody events into electrically detectable signals [20-22]. It consists of the biomolecular working as a receptor for the targeted analyte and a transducer picking the generated electrical signal while the biorecognition runs [23].

1.3. Biosensors Components:

The preparation of biosensor is normally requiring the following components as in Figure (1.3). "Analyte" is the molecule that needs to be detected, for example, when using a biosensor specifically designed to detect histamine, histamine molecule would be the analyte [18, 22]. Another part is called "Bioreceptor" a molecule known as the bio-recognition terminal of the biosensor such as DNA (deoxyribonucleic acid), enzymes, and antibodies that identifies the analyte. Another essential part "Transducer" specifically works on converting the running bioreaction into either measurable electric or optical signal. Its worth mentioning that, the connection area between bioreceptor and analyte is so called analyte-bioreceptor interaction. As a consequence, the strength of the generated signal which would be processed by the transducer basically depends on amount of the two parts at this interaction. Moreover, "Electronics" is the part that processes the generated signals and makes it ready for displaying by the display unit. It can be said that, electronics help the signals generated by the transducer getting converted into quantified and readable signals [18]. "Display Unit" a computer system (hardware and software) and a direct printer draws curves and peaks sensible for the generated signals of the tested biosensor and analyte.

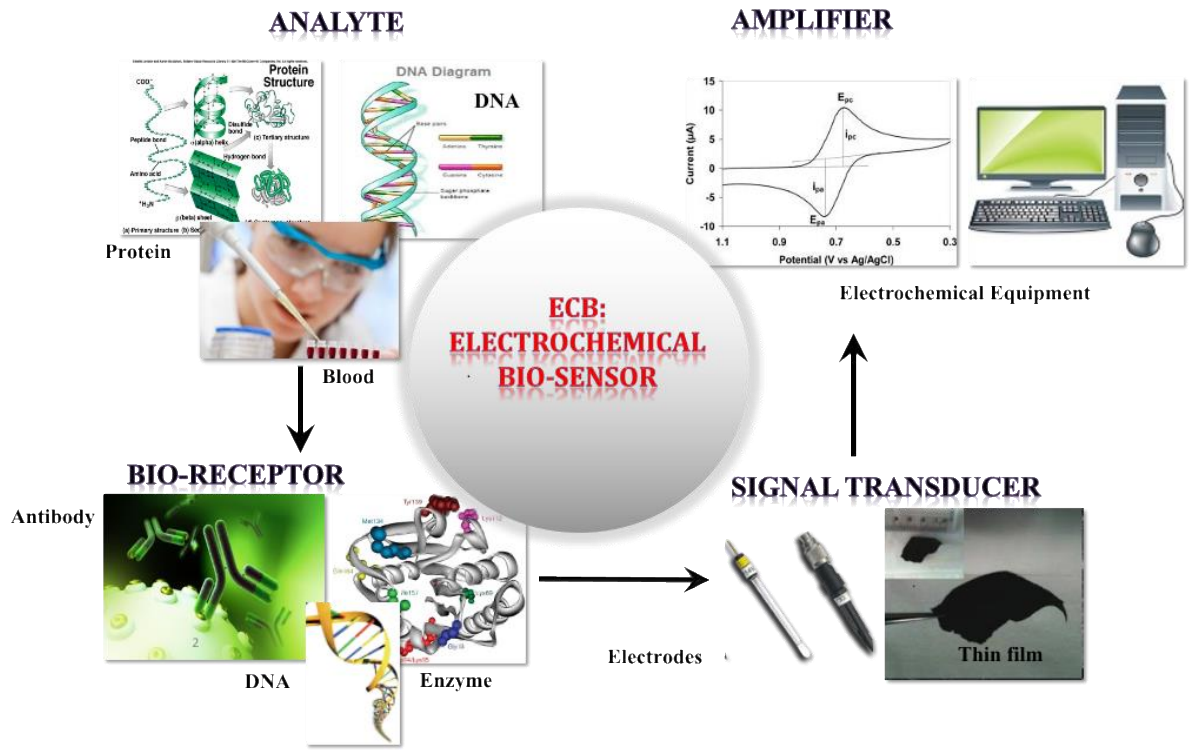


Figure (1.3) Schematic Illustration of Electrochemical Process

1.4. **Biosensors Properties**:

There are essential features reflected by the biosensor in order to be highly performed when used for the detection [24]. These features can be listed as: first, Selectivity, this property is so important when choosing a biosensor. High selectivity increases the ability of bioreceptor part at the biosensor to select a biological manner (an analyte) among others rapidly and accurately [18, 25]. For example, antibodies may act as bioreceptors and interact only with antigens when exposed to a solution containing number of biomolecules with antigens [25]. The second factor is Sensitivity, also called limit of detection (LOD), it referred as the lower amount (as low as nano or micro grams) of detected analyte when using a biosensor for medical monitoring applications. For instance, neurotransmitters concentration at Central Nervous System (CNS) in the brain equal to ng and that requires ultra-sensitive material to detect trace levels of such biomolecules [18, 25]. Stability of biosensors at specific conditions is the third factor should be determined for commercial success [26]. Excellent stability (long life time) with good selectivity and high sensitivity all together make the suggested biosensor applicable in rather specific fields such as medical, environmental, or food applications [11, 26, 27]. Repeatability, is an essential parameter need to be investigated when performing an electrochemical sensor. It shows how can the sensor keep same performance for so long time compared to conventional electrodes since last electrodes have decreasing performance when previously employed for same purposes [28].

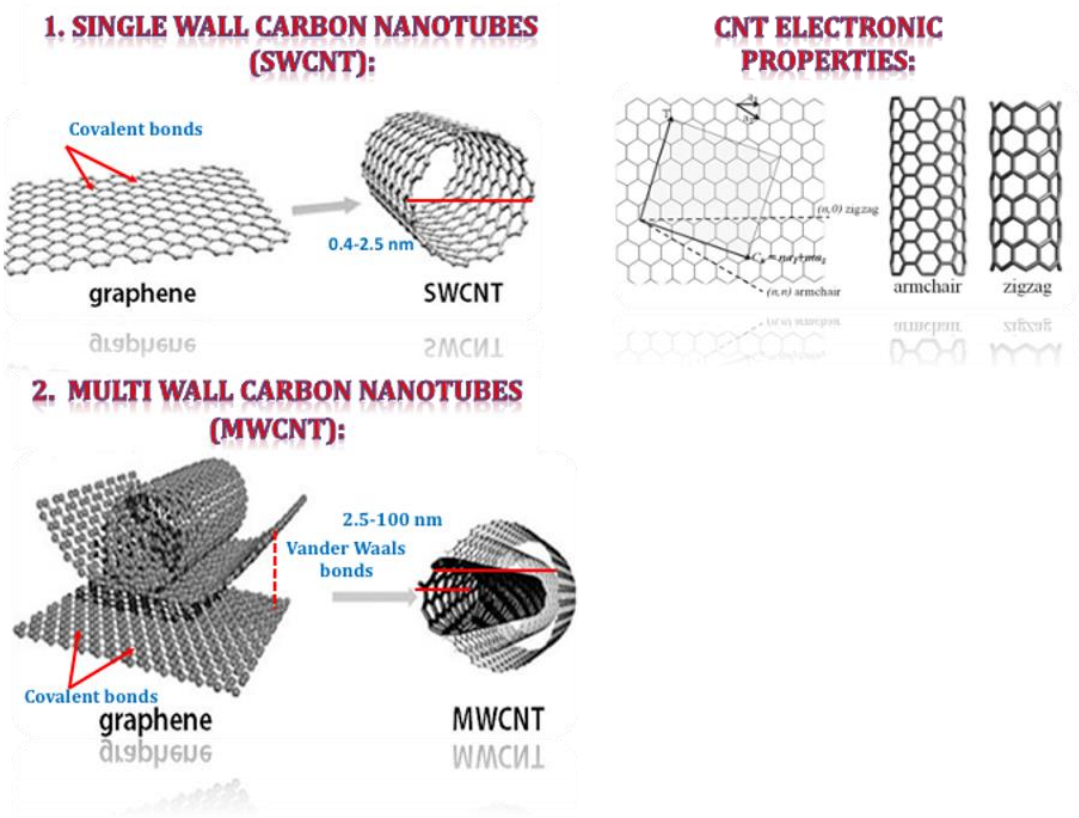
1.5. **Nano-Carbon Based Materials**:

The invention of nanotechnology has brought a new perspective based on nanometer dimensions suitable for measurements at biological and biomolecular scale. In terms of sensors/electrodes material, number of nanomaterials have been utilized for fabricating electrochemical electrodes ranged between nano to larger scale. To sum them up, two main categories are labelled as common electrodes material: carbon

and non-carbon based electrodes [29]. Carbon based electrodes is a big family and it is containing: Carbon Nanotubes (CNT) [8, 19, 23, 30-33], Graphene (G) [19, 20, 23, 34-39], in addition to another carbon nanomaterials. On the other hand, non-carbon electrodes can be Nobel metals (silver, gold, and platinum) [40, 41], polymers [29], and metal oxides (Indium tin oxide, ITO) [20, 42]. Also, new hybrid materials, organic electroconductive polymers or salts and Boron-doped diamond [43].

Since their discovery by Lijima in 1991, CNTs are becoming quite popular and have always been the focus of researchers in multiple fields including electrochemical sensing [19, 30]. Electrochemical biosensors based CNTs material are widely studied in recent years due to profound impact in biosensing applications. Generally, CNTs are formed of rolled up graphene sheets constructed in cylindrical shape with $\mathbf{sp^2}$-hybridization among carbon atoms. The length of CNTs is in microns, besides, the diameter of nanotubes comes in several nano-meters to microns [44]. CNTs are mainly divided into: Single-walled Carbon Nanotubes (SWNTs) and Multiple-walled Carbon Nanotubes (MWNTs) based on number of folded graphene sheets during synthesis process. It can be said that, SWNTs and MWNTs are formed of a single and multiple sheet of graphene respectively **(as** explained in Figure 1.4). Due to previous studies, the chirality of wrapped graphene sheets has an effect on CNTs properties. In fact, CNTs show excellent electrical, thermal conductivity and mechanical properties when used in the field of biosensing applications [45]. In addition, CNTs are biocompatible, extraordinary flexible with high tensile strength. CNTs possess very-light weight, and can be functionalized for better sensing performance [46]. The electronic structure of CNTs can vary from metallic to semiconductor properties. Conjugating CNTs surface with loaded biomolecules is considered to be quite efficient for bio-applications such as delivering drugs or anticancer nano-complexes [47]. Therefore, CNT electrodes are chosen for biosensing and ultra-sensing devices [48].

(A)



(B)

Advantages of Carbon Nanotubes
• High surface to volume ratio.
• Fast electron transfer kinetics.
• Can be used to fabricate composites.
• Bio-compatible.
• Reproducible.

Figure (1.4): (A) Carbon Nanotubes Structure. (B) Advantages of CNTs electrodes

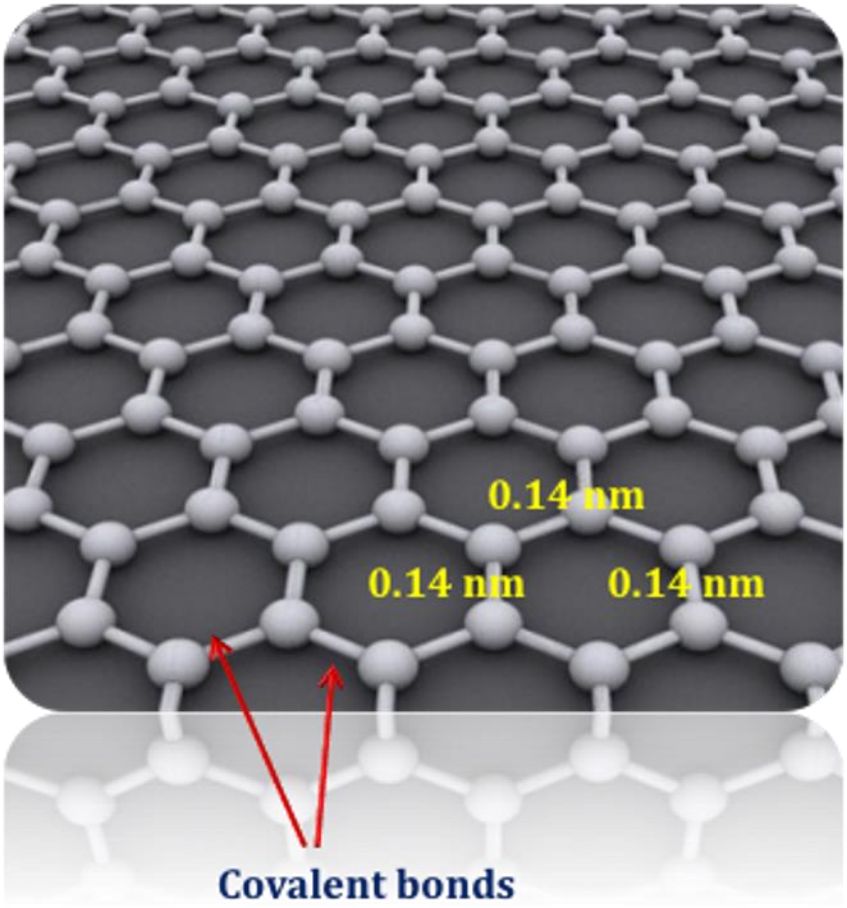
The second interesting carbon material is called Graphene (G). In graphene, carbon atoms are the building blocks uniquely arranged in honeycomb shape with covalent bonds between them (Figure 1.5) [39, 49]. Fabulous electronic properties were revealed by graphene with high surface to volume ratio, easy processing and safe material to be utilized. As the material is not dissolvable in water, derivatives from graphene were produced (such as graphene oxide and reduced graphene oxide) and processed to apply this novel material in different areas. Also, graphene has no impurities where it could affect the sensitivity of the material at some points. These were the most impressive properties noticed since the discovery of graphene [34, 39]. Therefore, the emerged platform of graphene in biosensing has brought great benefits as can be seen from many papers [34, 36, 38, 50].

Notably, graphene's properties can be enhanced by preparing hybrid composites containing graphene with particular nano-particles. This combination retains the properties of both materials also provided intensive electrical conductivity, so, numerous derivatives can be synthesised. For example,

As pristine graphene is poor soluble in water and agglomerates in solutions due to Vander walls interactions between carbon atoms, graphene derivatives especially reduced graphene oxide has been considered as an alternative source for using graphene. This is because, reduced graphene oxide is close in its properties to pristine graphene in comparison to graphene oxide [51]. The reason is that, graphene oxide is rich in oxygen groups such as (O-H), (C=O), (C-O-C) and (-COOH) and most of these groups would be eliminated to synthesise reduced graphene oxide. Oxygenated groups brought many advantages when comparing to graphene including better solubility and modifiable surface. However, the eliminating process made reduced graphene oxide closer in its properties to original structure of graphene were it has no functional groups like these ones [51]. Moreover, electrochemically prepared reduced graphene oxide has a better performance compared to reduced graphene oxide itself.

Another structure of graphene is called edge plane pyrolytic graphite electrode (EPPGE). The limit of detection can be achieved using this kind of electrodes is as low as nano-molar of analyte concentration attributed to super sensitivity and selectivity without spoiling the electrodes surface when detection process is running [36, 52]. One of the most important structures (derivatives) of graphene were successfully synthesised is graphene oxide [53]. The Numerous oxygen groups are bonded to single sheet of graphene to form graphene oxide. The presence of these groups changed the properties of graphene from hydrophobic into super soluble in water, hydrophilic, [54]. The availability of oxygen groups increased the chance of connecting biomolecules to graphene surface which is basically required to enhance analytes detection [34, 36, 38, 53]. But, the $\mathbf{sp^2}$ bonding network of graphene oxide is disrupted, so it has been described as electrical insulator [54]. Therefore, the reduction of graphene oxide is essential to recover the lattice and electrical conductivity.

(A)



(B)

Advantages of Graphene
• High surface to volume ratio.
• Cheap and accessible production.
• Can be used to fabricate composites.
• Doesn't contain metallic impurities.

Figure (1.5): (A) Graphene's structure. (B) Advantages of graphene electrodes.

1.6. Functionalization Methods:

Surface activation is necessary to carbon as it's hydrophobic in nature and having low wettability. This nature raised some concerns about carbon's chemical reactivity. In fact, carbon surface has poor adhesion and low surface energy need to be increased in order to link with different molecules later on. Therefore, modification of carbon electrodes should be applied prior using to gain super reactivity and better performance as a consequence. Oxygen plasma treatment is one of the methods that used to create functional groups on carbon surface (Figure 1.6). A huge number of oxygen groups represented by (-OH, -COOH) would be created and bonded to the surface of carbon material when applying this technology [23, 55]. The oxidise structure of carbon is required to boost the biocompatibility and electrical conductivity also gaining larger surface area when modified with these groups [56, 57]. The plasma is generated by

microwave radiation during different etching conditions continue to creating a rough and irregular surface of graphene, especially, at the step edges, let alone improving the chemical reactivity and surface bonds [58]. This technology is clean, dry and environmentally friendly. It works specifically on layers near to the surface of a material. With this property, the bulk (original) structure of the substrate (material) would be maintained as it goes down to 10 nm of the substrates surface only. It's worth mentioning that, establishing conditions of plasma chamber determine the type of generated plasma. Moreover, the gained surface energy would be in different levels also turning the surface of carbon into hydrophilic. One of the mostly applied plasma methods in laboratories specifically to modify surfaces that belong to heat-sensitive substrates is called "Cold Plasma" [55, 59].

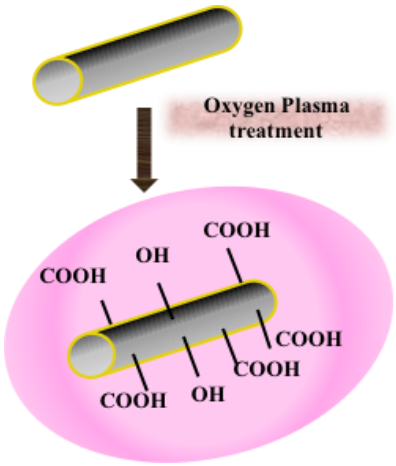


Figure (1.6) Illustration of Plasma Surface Activation of Carbon Materials

Conductive polymers (CPs) are other fabulous modifiers have been used to enhance performance of carbon surface electrodes. Different polymers have been prepared and used as modifiers for variable purposes including neurons stimulation also sensitive and selective sensors for detection of dopamine, serotonin, uric acid, and glucose, etc [60, 61].

2. ENZYMATIC GLUCOSE SENSORS:

As we earlier mentioned, firstly invented enzymatic sensor was by Clark and Lyons in the sixties of last century [62]. It has one enzyme in its structure, glucose oxidase, inserted inside a very thin capillary tube. Later on to increase electron transfer, some researchers used different substances and compounds, for example, ferrocenecarboxylic acid with glucose oxidase [63]. A descent performance was observed of the electrode towards glucose in comparison to enzymatic electrodes itself.

2.1. Enzymatic Glucose Sensors based CNTs:

Substances were used in enzymatic sensors included nano-carbon, silver, copper, gold, and platinum all at nano-dimension. The strategy of mixing nano-carbon with nano-particles has made a huge progress in the field of sensors. This is because, nano-materials have become an essential component in multiple fields not only sensing technology due to fabulous physical, electrical, and chemical properties. Nano-wiring of redox enzymes is a common technology extensively applied in biosensors. For example, carboxylic acid modified gold nanoparticles [63, 64]. Also, aminoethyl modified FAD which was chosen

with gold nano-particles in order to detect glucose effectively and efficiently according to gold's role in facilitating electrons transfer process [65]. In addition, Willner and Gooding are two researchers interested in glucose detection using what's called emerging nanomaterials. The fabricated nanosensors by them consisted of nanostructures components which provided direct connection between electrodes and enzymes [66]. Glucose biosensors based CNTs have taken a great interest in recent years due to most promising properties of them. Electrochemical biosensors involved CNTs in their structure showed enhanced sensitivity and selectivity towards targeted biomolecules such as glucose [67]. For instance, Multi-walled carbon nanotubes MWCNTs had a great advantage for the immobilization of glucose oxidase enzyme when used with gold nanoparticles and poly (diallyldimethylammonium chloride PDDA). The recorded sensitivity of glucose was lower than 30 mA M^{-1} cm^{-2}. The excellent performance of the fabricated electrode is attributed to high conductivity of CNTs and gold nanoparticles. Other researchers developed a new biosensor based CNTs as well as glucose oxidase GOx enzyme along with L-arginine biopolymer. After modifying CNTs by carboxyl groups, the mixture was drop-casted onto Glassy carbon electrode GCE surface with extra care [68]. This type of sensors demonstrated excellent stability for many days in addition to low detection limit of glucose about 50 µA mM^{-1} cm^{-2} [67, 68].

2.2. Enzymatic Glucose Sensors based Graphene:

Graphene has also been extensively used in preparation of electrochemical electrodes for the glucose oxidase enzyme immobilization. The extraordinary conductivity of graphene has brought potential advantages to be exploited in electrochemical biosensors [69]. An affordable sensor was prepared from reduced graphene oxide and polyimide nanocomposite. The preparation method involved nano dimension alloy of gold and platinum. Glucose oxidase enzyme and chitosan polymer were also integrated with the wearable sensor's components. The eventual sensor has been used as human sweat sensor for the monitoring of glucose [70]. Notably, the limit of detection of glucose was as low as 5 µM [70, 71]. Another promising non-enzymatic sensor was fabricated using film-based sensors. Graphene Schottky diodes was the prominent factor used for the sensor's preparation. It included graphene, platinum oxide and silicon, for that, it could also be described as heterostructure electrode. The sensor was electrochemically tested in the presence of glucose in addition to testing its catalytic activity. The sensitivity and selectivity of the electrode was thoroughly investigated. It was found that, different thicknesses of the film show different sensitivities of glucose [70, 71] . A new design of enzymatic sensors based graphene called solution gated graphene transistors SGGT was introduced for glucose detection [72]. The lowest recorded detection limit of glucose by this sensor reached 0.5 µM. The sensitivity of this sensor has exceeded the sensitivity of conventional electrochemical electrodes which confirm the successful preparation and properly selected components of this electrode [73]. It's worth mentioning that, the as-prepared sensor could be used in real-time measuring of glucose level. For more information, commonly applied enzymatic glucose sensors-based graphene with different sensors components are listed in table (1.1).

Table (1.1) Types of Enzymatic Glucose Sensors[74]

Enzymatic Glucose sensors	Sensitivity	Linear Range	Detection Limit	Reproducibility
GR-CNT-ZnO-GOx	5.36 (±0.072) μA mM^{-1} cm^{-2}	0.01-6.5 mM	4.5 μM	RSD 3.24 % (N=5)
ERGOc-MWCNTd/GOx/Nf	7.95 μA mM^{-1} cm^{-2}	0.01-6.5 mM	4.7 μM	RSD 205 % (N=7)
Fc/GOD/Au/SLG	-	0.0005-5000μM	0.0001μM	RSD 3.8 % (N=6)
Au/GNS-PEI-AuNPs/Glu-GOx	93 μA mM^{-1} cm^{-2}	1-100μM	0.32 μM	RSD 6.7 % (N=5)
PANI-SDS-F127(1:1)GOx	485.7 μA mM^{-1} cm^{-2}	5-50mM	3.20 μM	-
Pt-CNT-muc50%	15 μA mM^{-1} cm^{-2}	0.002-3.2 mM	3 μM	RSD 2.2 % (N=5)
GOx/PVA-Fe3O4/Sn	9.36 μA mM^{-1} cm^{-2}	0.005-30 mM	8 μM	RSD 4.2 % (N=5)
Au-Ni coaxial nanorad array/GOx	778.2 μA mM^{-1} cm^{-2}	0.0275-27.75 mM	5.5 μM	-
CS/GOx-PABA-Au_{nano}/Au-plated Au	97.7 μA mM^{-1} cm^{-2}	0.002-3.7 mM	0.1 μM	RSD 4.2 % (N=5)
GOx/Pt/rGO/P3ABA	22.01 μA mM^{-1} cm^{-2}	0.25-6.0 mM	44.3 μM	RSD 2.58 % (N=5)

3. NON-ENZYMATIC GLUCOSE SENSORS:

A certain type of electrodes fits for the fabrication of glucose sensors is using composite structure. This candidate represents an excellent sensor for the determination of glucose according to super performance of CNTs and affordability of metal elements. Including but not limited to, Cobalt (Co), Silver Ag, Nickle (Ni), Copper (Cu), gold (Au), and more metal nano-particles have been the subject of non-enzymatic glucose sensor showing an interesting detection limit as low as micro or even nano molar of glucose [75].

3.1. Non-Enzymatic Glucose Sensors based CNTs:

CNTs were chosen due to excellent mechanical and chemical properties such as large surface area to volume ratio, high chemical stability, the capacity of adsorption is relatively high, and the conductivity is superior. Copper, in addition to its role as nutrient for different life aspects, is mainly cheap, properly fits for the environment, and biocompatible [76]. All these aspects together have encouraged researchers to use Cu effectively and efficiently for the construction of electrochemical sensors in various field, especially, glucose determination. The nano-carbon accompanied with nano-dimension materials are modifiable to obtain improved results. A new composite was suggested and tested for the electrochemical determination of glucose using silver metal interlocated by zeolite and CNT called silver-doped zeolite-MWCNT-epoxy. The recorded mechanical strength of the proposed non-enzymatic electrode was good as well as showing

decent electrical conductivity [77]. It was noticed that, oxidation peak of glucose increased gradually by time at the composite electrode. Notably, the determined concentrations of glucose started with 0.4 up to 2 mM. The applied techniques were cyclic voltammetry CV, linear sweep voltammetry, chronoamperometry, and multiple pulsed amperometry technologies. The low limit of detection registered by this composite electrode reflected decent electrochemical properties represented by encouraging sensitivity, in addition to better stability and reusability. Gold nano-particles Au had also been the focus of interested researchers in the fabrication of glucose diagnosis devices [78]. Composites of metal nano-particles like Au and CNT had shown superior electroanalytical performance towards glucose sensing applications [79].

3.2. Non-Enzymatic Glucose Sensors based Graphene:

Graphene, on the same hand, has been extensively used for the preparation of glucose sensors as graphene-metal composites. Example of employed metals for composites preparation can be: Platinum, Palladium, Copper, Cobalt, and Nickel which were all chosen as perfectly fitted metals for investigation of glucose [80]. In fact, defects at graphene surface also plays an important role for enhancing the electrochemical detection of biomolecules through the improvement in electron transfer process. Chitosan modified graphene sheets were also one of the promising composites used for glucose detection. The detected level of glucose linearly ranged from micromolar up to 5 mM and the limit of detection equalled to 0.6 µM. The demonstrated low limit of glucose was attributed to large surface area of graphene sheets which allows picking up much more molecules of glucose compared to other unmodified sensors [81]. A recent study presented a glucose sensor where graphene was used as graphene oxide and electro spun nanofibers called NiO along with Nafion modification. The preparation of fibers was done by electrospinning technology. The graphene oxide preparation was done by Hummers technology. Different characterization methods were used to identify this sensor included SEM, EDX, XPS, and EIS. The lowest detection of glucose could be detected using this sensor equalled to 0.77 µM [71]. The applications of graphene as sensors material has been widely reported and reached the universe. This is because graphene-based sensors showed excellent thermal, mechanical, and electrical properties which can be implanted in health monitoring devices [82]. For further details of commonly applied non-enzymatic glucose sensors-based graphene with different sensors components are listed in table (1.2).

Table (1.2) Types of Non-Enzymatic Glucose Sensors[74]

Non-Enzymatic Glucose sensors	Sensitivity	Linear Range	Detection Limit	Reproducibility
SWCNT, Cu2O/ZnO nanorods NR/graphene	466.1µA mM^{-1} cm^{-2}	5.55-11.115 mM	-	-
CuO/PANI-NF/FTO	2800 µA mM^{-1} cm^{-2}	0.28-4.6 mM	0.24 µM	RSD 36 %
Ni(OH)2/CNT fiber microelectrode	12.2 mA mM^{-1} cm^{-2}	20µM-10.5mM	0.6µM	-
CuO/NiO/PANI/GCE	-	20-2500µM	2 µM	RSD 3.8 % (n=5)
Coral-like Cu micro/nano-structure arrays	3826 µA mM^{-1} cm^{-2}	0.2 µM-1.90mM	0.04 µM	RSD 2.51 % (n=6)
Au-MIP sensor	-	10^{-8} -10^{3} mol. L^{-1}	3x10^{-12} mol. L^{-1}	-
NiWO4-modified GCE	269.6 µA mM^{-1} cm^{-2}	0.004µM-4 mM	0.18 µM	RSD 2.7% (n=5)
S/NPG/Co3O4 hybrid microelectrode	125 mA mM^{-1} cm^{-2}	1µM-10 mM	5 nM	-
CuO NW with Au NP	1591.44 µA mM^{-1} cm^{-2}	0.001mM-44.36 mM	0.3 µA	RSD 5 % (n=10)

4. CONCLUSION:

In this review article, generations and types of glucose detection electrodes have been explained. The generations of glucose sensors were firstly mentioned in details and possible reaction mechanism as well. Biosensors reveal versatile utilizations in the fields of biomedical, toxicology, electrochemistry, and drug delivery. The integrity of graphene and carbon nanotubes with sensors has shown a fast grow in biosensing technology in recent years. Its attributed to their role and fascinating properties represented by excellent chemical, electrical, mechanical, surface to volume ratio, and physical properties suitable for obtaining higher sensitivity of glucose in comparison to conventional electrodes. Both extraordinary conductive materials revealed fast response time, sharp oxidation signal for molecules being determined, good reproducibility, low limit of detection, and descent stability. The aim of current study is listing and discussing the progression of glucose generations as well as the development of glucose sensors in recent years. Enzymatic and non-enzymatic nanocarbon sensors were mainly studies and classified as glucose sensors also explained with further details regarding limit of detection.

From our perspective, glucose sensors need to be more examined in real tissues and study the integration with previously prepared electrodes and employed materials to overcome research drawbacks in the future.

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