Minireview *Highlight article*

Is graphene the rock upon which new era continuous glucose monitors could be built?

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Impact statement

This article provides an overview of the latest research on graphene-based biosensors for glucose detection and discusses their potential role in continuous glucose monitoring (CGM) systems. Diabetes mellitus (DM) is developing into an epidemic in the modern world and a major health burden worldwide. Monitoring glucose levels is employed in the optimization of treatment strategies providing insights into the effectiveness of medications, exercise, and diet. Since blood glucose level is the basis for diabetes management, the development of highly accurate systems that "continuously" monitor blood glucose and trigger drug release (closed-loop systems) is one of the most significant challenges in diabetes research. This minireview provides an outline of the limitations in enzymatic and non-enzymatic glucose sensing that need to be addressed to develop graphene-based CGM systems that would eventually lessen the burden and improve the quality of life of patients with diabetes.

Abstract

Diabetes mellitus' (DM) prevalence worldwide is estimated to be around 10% and is expected to rise over the next decades. Monitoring blood glucose levels aims to determine whether glucose targets are met to minimize the risk for the development of symptoms related to high or low blood sugar and avoid long-term diabetes complications. Continuous glucose monitoring (CGMs) systems emerged almost two decades ago and have revolutionized the way diabetes is managed. Especially in Type 1 DM, the combination of a CGM with an insulin pump (known as a closed-loop system or artificial pancreas) allows an autonomous regulation of patients' insulin with minimal intervention from the user. However, there is still an unmet need for high accuracy, precision and repeatability of CGMs. Graphene was isolated in 2004 and found immediately fertile ground in various biomedical applications and devices due to its unique combination of properties including its high electrical conductivity. In the last decade, various graphene family nanomaterials have been exploited for the development of enzymatic and non-enzymatic biosensors to determine glucose in biological fluids, such as blood, sweat, and so on. Although great progress has been achieved in the field, several issues need to be addressed for graphene sensors to become a predominant material in the new era of CGMs.

Keywords: Graphene, diabetes, sensors, glucose, enzymatic, non-enzymatic

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Introduction

Diabetes is thought to be a global epidemic in the modern world. According to the World Health Organization, to date, about 422 million people worldwide have diabetes and it is estimated that this number will rise to 642 million in the next 20 years.[1] Moreover, diabetes is one of the 10 leading causes of death and one of the biggest public health concerns globally, affecting both the social and economic development of each country.[2]

Diabetes mellitus (DM) refers to a group of heterogeneous metabolic disorders characterized by abnormally elevated blood glucose levels, due to insufficient insulin production, insulin resistance, or low insulin sensitivity.

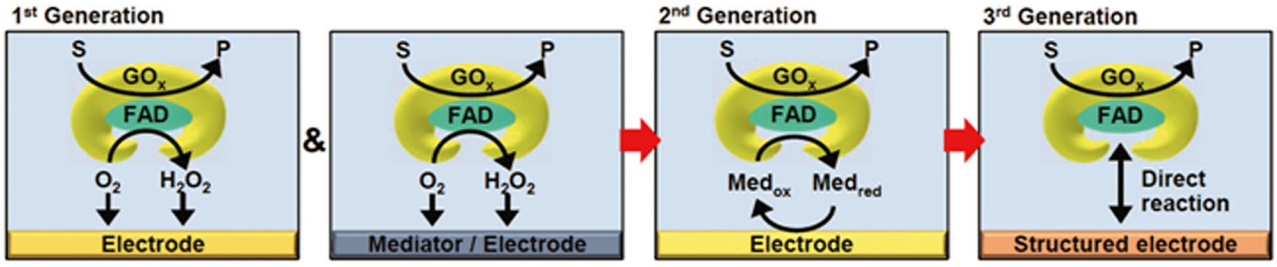

Figure 1. The sensing properties of the enzymatic GOx-based glucose sensors. The immobilized GOx catalyzes the oxidation of glucose (S) by molecular oxygen producing gluconic acid (P) and hydrogen peroxide (H_2O_2) Flavin adenine dinucleotide is used as s a redox cofactor for GOx to work as a catalyst. The second-generation GOx-based sensors utilize redox mediators to interact directly with enzymes at lower applied potentials. The third-generation GOx-based sensors employ highly selective nanomaterials to facilitate direct electro-oxidation of glucose. Reproduced from the work of Lee *et al.*[10] with permission of John Wiley and Sons.

The most common types are Types 1 and 2 DM affecting about 7–12% and 87–91% of the diabetic population, respectively.[1] Monitoring glucose levels, as a disease marker in DM, is used to optimize patient treatment strategies, and provide an insight into the effect of medications, exercise, and diet on the patient. Managing blood glucose levels can prevent episodes of hypo- or hyperglycemia, and thus, some of the debilitating side effects (such as diabetic ketoacidosis and diabetic coma) and more importantly reduce the risk of developing chronic complications (microvascular, such as neuropathy, nephropathy, and retinopathy, and macrovascular, such as stroke, peripheral artery disease [PAD], and cardiovascular disease).[3]

In this aspect, almost 50 years ago, enzymatic biosensors were developed for regular monitoring of blood glucose levels. Estimation of glucose levels is crucial for establishing a diagnosis of diabetes but most importantly for the progression of the disease and the efficacy of the treatment. Over the past years, electrochemical amperometric biosensors were produced that measure the current generated by an electroactive product of the enzymatic reaction between the glucose and a specific enzyme, which is proportional to glucose concentration. The most common enzymes for this purpose are glucose oxidase (GOx/GOD), glucose-1-dehydrogenase (GDH), and hexokinase/glucose-6-phosphate dehydrogenase.

The history of glucose monitoring began in 1962 when Clark and Lyons reported the first enzyme-based electrode system: a device composed of an enzyme layer trapped between two impermeable membranes, and an oxygen electrode.[4] Five years later, in 1967, Updhike and Hicks managed to immobilize GOx over an oxygen electrode.[5] These scientific innovations led to the development of the world's first available product: the model 23A YSI, launched in 1975, a first-generation whole blood glucose analyzer that could measure glucose, based on hydrogen peroxide (H_2O_2) detection by a platinum electrode.[6]

Although a scientific breakthrough, the first-generation of glucose analyzers had major limitations. They required high-voltage potentials for the oxidation of H_2O_2 that made them prone to interference effects, were significantly big and due to the platinum electrode, extremely expensive.[6] To overcome these limitations, researchers replaced oxygen electrodes with electrodes modified with inorganic or organic synthetic electron acceptors that act as redox mediators,

Figure 2. Evolution of glucose sensors.

facilitating the transfer of an electron between the redox center of the enzyme and the electrode. In 1984, the very first mediated amperometric biosensor (second generation) was reported.[7] Eventually, this innovative technology led to the production of the first electrochemical glucose test strips for in house use in 1987.[8] After that, second-generation biosensors had a huge success, but the relative toxicity and solubility of mediators along with low stability of the system eventually led scientists to develop the third generation of biosensors.

The transition from second to third generation of biosensors, began around 1992 when a scientific team managed to incorporate GOx within the pores of a modified membrane.[9] The third-generation biosensors do not have redox mediators of any kind and rely on direct electron transfer between the enzyme's redox center and the electrode.[6] The enzymatic glucose sensors sensing principles are presented in Figure 1.

Over the decades, these systems evolved to overcome the various shortcomings that emerged. However, the discovery of continuous glucose monitoring (CGM) system has changed the game in glucose sensing and along with the

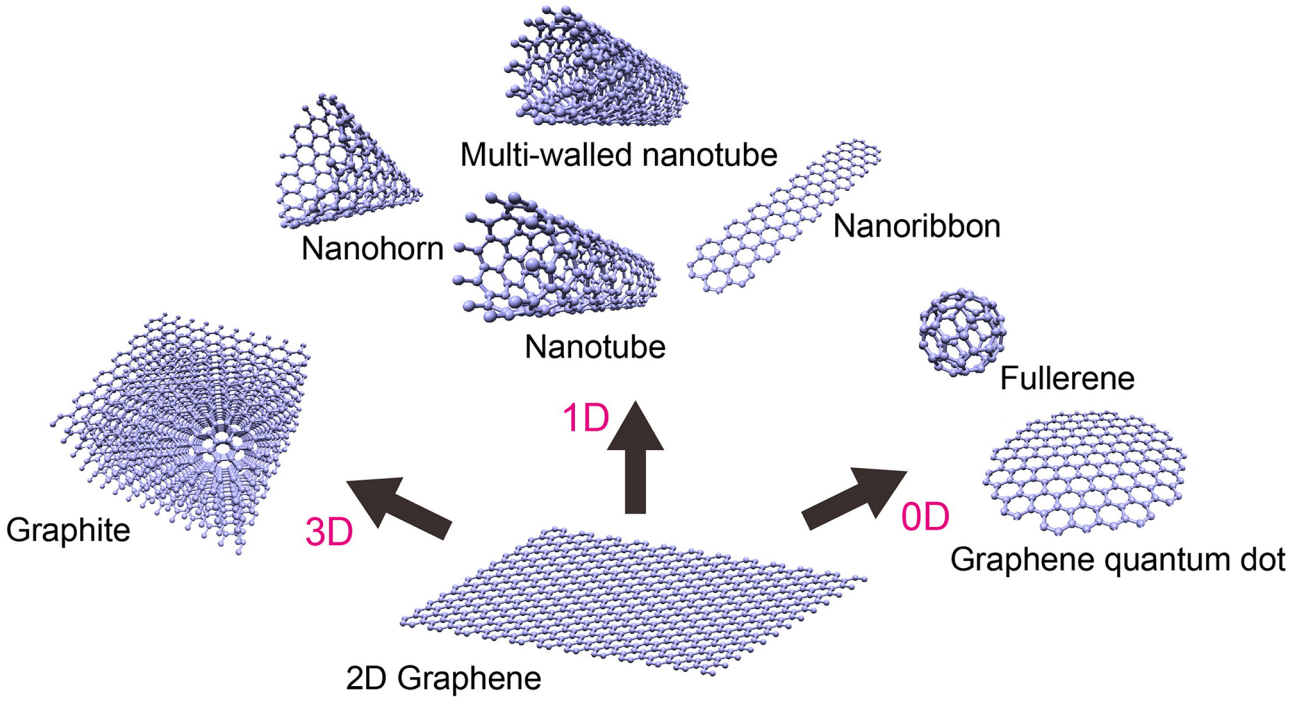

Figure 3. Classification of carbon allotropes derived from graphene. Reproduced from the work of Nakano *et al.*[27] with permission of Taylor and Francis.

fabrication of insulin pump devices defined a new automated process for glucose measurement without human interaction (Figure 2).[11] As huge as this evolution was, there are still a lot of limitations needed to be overcome and extensive research has been focused on the fabrication of an automated artificial pancreas, also known as the "closed-loop system." "Closed-loop" delivery systems will improve remarkably the management of diabetes as they will provide both CGM and the release of the appropriate therapeutic scheme when needed.[12] These systems are of utmost importance to consist of a highly sensitive and efficient sensor for CGM.

In search of novel materials suitable to detect glucose, researchers turn to carbon-based materials, such as graphene, reduced graphene oxide (rGO) and carbon nanotubes (CNTs). Graphene was isolated in 2004 after mechanical exfoliation of graphite[13] and refers to a single-atom-thick layer of sp^2 bonded carbon atoms tightly packed into a hexagonal planar lattice. The discovery of graphene leads to the Nobel Prize in 2010 due to a series of extraordinary and unusual properties.[13] Among these properties, the excellent electrical[14] and thermal conductivity[15] combined with high mechanical strength,[16] and a large surface area[17] have placed graphene as one of the most promising candidates for sensing applications.[18] Graphene can be considered an allotropic form of graphite, such as diamond, graphite, fullerenes, CNTs, and many other carbon forms with specific hybridization and shape (Figure 3).[19] All these carbon-based nanomaterials show excellent electrochemical properties and over the years have been used in different electrochemical applications.[20] From all the carbon-based materials, graphene is one of the most well-studied and seems to be the right candidate for the construction of glucose sensors. From the mechanical aspect, graphene sheets are stronger than steel and thinner than a human hair.[21] As for its electrical properties, graphene presents a remarkable electrical conductivity, a large surface to-volume ratio and great stability, which in combination with the delocalized π-conjugated electrons floating on the 2D surface makes graphene extremely sensitive to its chemical environment and as a consequence ideal material for sensor fabrication.[22] In the same family of graphene-like materials, graphene oxide (GO) is an atomic-thick oxygen-rich derivative of graphene containing various oxygen functionalities, such as hydroxyl, epoxide, carboxylic, and carbonyl.[23] Graphene oxide combines some of the excellent properties of graphene with the advantage of the high hydrophilicity compared with hydrophobic graphene, which gives user-friendliness for the synthesis, preparation, and construction of new-generation biosensors.[24–26]

However, due to the insertion of the oxygen functionalities and the disruption of the sp^2 system of graphene to sp^2–sp^3 of GO, the latter exhibits lower conductivity.[28] As a result, GO is usually used after its reduction to rGO.

Here, we review the latest trends in graphene family-based enzymatic and non-enzymatic glucose detectors that could be good candidates as CGMs in "closed-loop" delivery systems. We focus on systems (noble materials, electrodes, and complete sensors) that are significantly sensitive and show high selectivity toward glucose and have been tested in human biological fluids (Table 1). This is very important as it adds clinical value to the systems, bridging the gap between research findings and clinical application.

Enzymatic graphene-based sensors

The immobilization of GOx on various substrates is the key principle for the development of enzymatic biosensors.

Table 1. Summary of included articles.

Enzymatic sensors								
Sensor (material)	Detection range	Sample	Sensitivity	Detection limit (μM)	Reproducibility standard deviation	Repeatability standard deviation	Selectivity	Ref.
GOD–MCF@rGO/GCE	4.5–144 mg/dL (0.25–8 mmol/L)	HS	2.257 μA/mM cm^2	98400	4.5%	NR	No: UA, AA, DA, Fru, CN	Wang *et al.*[29]
SPCE/ GNR/ Bi_2O_3/GOx/Naf	5.04–30.6 mg/dL (0.28–1.70 mmol/L)	HS, HU	64.81 μA/mM cm^2	70	4.15%	3.62% (0.95 mM) 3.40% (1.90 mM) 2.83% (2.40 mM)	No: Fru, Mal, AA Yes: DA	Đurđić *et al.*[30]
3D porous aerogel @ GOx based microfluidic biosensor	18–324 mg/dL (1–18 mmol/L)	WB	NR	870	NR	NR	No: AA, UA, Lac, Mal, Fru, Suc	Xu *et al.*[31]
GOx–CNs–rGO–Au–SPE ePAD	59.4–498.6 mg/dL (3.3–27.7 mmol/L)	WB	0.697 μA/mM	420	NR	NR	NR	Ahmadi *et al.*[32]
GOx/PB/graphene hybrid	0.2–12.6 mg/dL (0.01–0.7 mmol/L)	HSw	1 μA/mM	10	NR	NR	No: lac, AA, UA	Lee *et al.*[33]
Non-enzymatic sensors								
Sensor (material)	Detection range	Sample	Sensitivity	Detection limit (μM)	Reproducibility standard deviation	Repeatability standard deviation	Selectivity	Ref.
N-doped graphene with large amount of pyridinic nitrogen	0.00234–252 mg/dL (0.00013–14 mmol/L)	HS	774.23 μA/mM cm^2	0.13	NR	NR	No: UA, DA, AA, Mal, Lac, Suc	Rahsepar *et al.*[38]
GS/NFG/Pd–NiAl–LDH	0.009–180 mg/dL (0.0005–10 mmol/L)	HS, HP, WB	315.46 μA/cm^2 dec	0.234	$<$3%	$<$4%	No: AA, UA, DA, AED, L-Cys, Gly	Shishegari *et al.*[39]
Fe_3O_4/graphene/GCE	0.9–72 mg/dL (0.05–4 mmol/L)	HS	959 μA/mM cm^2	0.18	2.81%	NR	No: AA, DA, UA, Suc, Lac, Fru, NaCl, SC	Suyanta *et al.*[40]
CoPc/IL/G/SPCE/PAD	0.18–90 mg/dL (0.01–5 mmol/L)	HS	NR	0.67	NR	$<$4.6%	No: Suc, UA, Fru, AA, DA, Lac, Gal, APAP	Chaiyo *et al.*[41]
GCE–SWCNT/rGO/CoPc	0.0054–9 mg/dL (0.0003–0.50 mmol/L) 4.5–90 mg/dL (0.25 mM–5 mmol/L)	HSa	992.4 μA/mM cm^2	0.12	3.38%	3.26%	No: Suc, AA, UA, Fru, DA, K^+, Na^+, Cl^-, Ur	Adeniyi *et al.*[42]
GelMA: Ni-RGO hydrogel	0.0027–180 mg/dL (0.00015–10 mmol/L)	HS	0.056 mA/mM	0.005	NR	NR	No: UA, AA, AP	Darvishi *et al.*[43]
GCE/ GN/ Pcys–$Ni(OH)_2$	0.018–1.8 mg/dL (0.0010–0.10 mmol/L) 3.6–36 mg/dL (0.20–2 mmol/L)	HS	1092 μA/mM cm^2	0.35	3.0%	3.5%	No: DA, AA, UA, Glu, Lys, Cys	Xue *et al.*[44]
Cu–Cu_2O NPs @3DG foam	14.4–180 mg/dL (0.8–10 mmol/L)	HS	230.86 μA/mM cm^2	16	NR	1.02%	No: AA, DA, Ur, APAP	Khosroshahi *et al.*[45]
Ni/NiO/NG-400	0.018–64.224 mg/dL (0.001–3.568 mmol/L)	HS	3.2518 mA/mM cm^2	0.032	1.48%	NR	Negligible: AA, DA, UA, KCl, Fru, Gal	Wang *et al.*[46]
Ni_2P/G/GCE	0.09–25.2 mg/dL (0.005–1.4 mmol/L)	HS	72.34 μA/mM cm^2	0.44	NR	NR	No: AA, UA, DA, Lac, Fru	Zhang *et al.*[47]
rGO/CuSNFs/GCE	0.018–36 mg/dL (0.001–2 mmol/L)	HS, HU	53.5 μA/mM cm^2	0.19	$<$3%	NR	Negligible: FA, DA, OA, AA, UA, Fru, Mal, Lact	Yan *et al.*[48]
GO/CuO/FTO	1.8–180 mg/dL (0.1–10 mmol/L)	HS	830 μA/mM cm^2 1274.8 μA/mM cm^2	0.13	2.7%	NR	No: AA, DA	Gijare *et al.*[49]
$NiCO_2O_4$ NSs@N-rGO	0.18–279 mg/dL (0.01–15.5 mmol/L)	WB	4372.9, 1686.1 μA/mM cm^2	NR	NR	NR	No: AA, DA, UA	Wang *et al.*[50]
GO-templated $NiCO_2O_4$ nanosheets (NSs)	0.0027–3.7998 mg/dL (0.00015 mM–0.2111 mmol/L) 4.7007–159.48 mg/dL (0.26115–8.86 mmol/L)	HS, HU	729.72 μA/mM cm^2	0.00735	2.98%	NR	No: DA, AA, Ur, Suc, NaCl, Gly	Babulal *et al.*[51]
Cu/Ni/graphene/Ta electrode	0.00009–39.132 mg/dL (0.000005–2.174 mmol/L)	HS	314 μA/μM cm^2	0.0027	4.8%	3.6%	No: Suc, Fru, La, Lac, Xyl, AA, DA, UA	Cui *et al.*[52]
GO–NiO–8H–NHS (pH 7.4)	0.0036–90 mg/dL (0.0002–5 mmol/L)	HP	712.5 μA/mM cm^2	0.041	2.51%	4.88%	No: Suc, Lac, Mal, Fru Yes: Ur, AA	Hashemi *et al.*[53]
CuO/LSG	0.018–0.9 mg/dL (0.001–0.005 mmol/L)	WB, HS, HSw	NR	0.1	NR	NR	No: AA, DA, UA, Fru, GT, L-tyr, Chol	Prabhakaran *et al.*[54]

GOD: glucose oxidase; MCF: meso-cellular silicate foam; rGO: reduced graphene oxide; GCE: glassy carbon electrode; HS: human serum; NR: not reported; UA: uric acid; AA: ascorbic acid; DA: dopamine; Fru: fructose; CN: creatine; SPCE: screen-printed carbon electrode; GNR: graphene nanoribbon; HU: human urine; GOx: glucose oxidase; WB: whole blood; Lac: lactate; Mal: maltose; Suc: sucrose; CNs: cellulose nanofibers; HSw: human sweat; GS: graphite sheet; NFG: N-doped functionalized grapheme; LDH: layered double hydroxide; HP: human plasma; AED: cystamine; L-Cys: L-cysteine; Gly: glycine;;CoPc: cobalt phthalocyanine; SC: sodium citrate; IL: ionic liquid; G: graphene composite; PAD: paper-based device; Lac: lactate; Gal: galactose; APAP: paracetamol; SWCNT: single-walled carbon nanotube; HSa: human saliva; GelMA: gelatin methacryloyl; AP: acetamidophenol; Pcys: poly(cysteine); Glu: glutamate; Lys: lysine; Cys: cysteine; Ur, urea; NG: nitrogen-doped graphene; FA: folic acid; OA: oxalic acid; Lact: lactose; Xyl, Xylose; LSG: laser-scribed graphene; GT: glutathione; L-tyr: L-tyrosine; Chol: cholesterol; Naf: Nafion; SPE: screen printed electrode; PB: prussian blue; GN: monolayer graphene; 3DG: three-dimensional graphene; FTO: fluorine-doped tin oxide; NHS: N-Hydroxysuccinimide.

To achieve the desired analytical figures of merit of the final device (glucose sensor) researchers routinely select to mingle different materials. Wang *et al.*[29] combined mesocellular silicate foam (MCF) and rGO to enhance both biocompatibility (with the MCF layer) and conductivity (with the skeleton of rGO). The authors prepared two different biosensors for sensing either H_2O_2 by a hemoglobin (Hb)-modified electrode or glucose by a GOD-modified electrode. The proposed electrode modified with GOD was able to detect glucose with high sensitivity and selectivity. The GOD–MCFs@rGO/glassy carbon electrode (GCE) was also tested in three human serum samples and glucose values were similar to the ones measured by commercially available blood glucose meters. This novel sensor has an advantage over second-generation sensors, since it does not use an electrochemical mediator and its quantification limit is 2.4–8.8 times lower. Another research group combined graphene nanoribbons (GNRs) and bismuth trioxide (Bi_2O_3). Bi_2O_3offers electrochemical stability and energy band gap whereas GNRs have high surface area and good stability. The authors speculated that with the combination of these two materials the product will exert excellent electrochemical performance. For this purpose, they created a screen-printed carbon electrode (SPCE) modified first with GNR/Bi_2O_3 and then with GOx followed by a Nafion drop-casting layer, which acts as cation exchange membrane. The SPCE/GNR/Bi_2O_3/GOx/Naf biosensor exhibited a linear response to glucose at a rather narrow range but presented an extremely low detection limit and high sensitivity. The sensor's selectivity was influenced by dopamine, so that, further development is needed to eliminate these disadvantages. The biosensor was unable to quantify glucose in urine samples, so that, the authors spiked the samples with known concentrations of glucose to test the accuracy of the method. In contrast, the sensor detected glucose in the blood of three volunteers, however, the lack of comparison with a commercially available biosensor does not allow verification of the accuracy of the measurements.[30] Using graphene, Xu *et al.*[31] prepared a glucose biosensor with 3D porous graphene aerogel. The sensor exhibited satisfactory selectivity toward glucose and good stability as the current response of two different glucose solutions (3 and 12 mM) was similar to the initial (almost 90%) after 5 weeks. The clinical application of the sensor seems promising since it was able to detect glucose with good recoveries (90–106%) and relative standard deviations (RSDs) $< 10\%$ in human serum samples of five patients compared with the glucose values determined by the hospital's automatic biochemical analyzer. In 2021, a research group from Iran described a novel electrochemical paper-based analytical device (ePAD) based on cellulose acetate (CA) nanofibers, rGO, and a thin layer of gold (Au) for the measurement of blood glucose by employing faradic impedimetric measurements. This was the first report of an ePAD with cellulose nanofibers (CNs) and rGO. The determination of glucose was performed in an enzymatic way with GOx. To evaluate the accuracy of glucose determination in spiked whole blood samples, the sensor was tested against the standard clinical method (glucose dehydrogenase assay kit) where it achieved high-recovery results ranging between 97 and 105% and low RSDs ($<2\%$).[32]

The most comprehensive research approach to generate a sweat glucose sensor for CGM systems was published by Lee *et al.*[25] in 2016. The authors created a patch, consisting of a bilayer of Au mesh and gold-doped graphene with Ag/AgCl counter electrodes. Graphene has been synthesized by chemical vapor deposition (CVD) process. It also contained a sweat uptake film, polymer-based microneedles for drug release, a waterproof film, and sensing components (electrodes for detection of humidity, glucose, pH, and tremor). Assay of glucose occurred via GOx. The glucose biosensor was stable and showed good correspondence to typical sweat glucose concentrations *in vitro* and high selectivity toward glucose in the presence of other biomolecules found in sweat. To test the potential therapeutic effects of this device, the patch was laminated near the abdomen of 8- to 10-week-old diabetic mice (db/db). The sensor after standardization for pH values produced matching glucose values to that of a commercially available assay. Moreover, glucose levels were successfully measured in the treated group (thermally actuated microneedles from the patch penetrated through the skin to the subcutaneous region which delivered metformin to the bloodstream) and control groups (without the patch and the drug) and the suppression of glucose concentration after treatment verified the sensor credibility to track glucose changes over time.

The study also included a trial of exceptional importance. The performance of the patch in sweat was tested against a commercially available glucose assay kit (Cayman Chemical, USA) and measurements were correlated with those of a blood glucometer (Accu-Chek Performa, Roche, Switzerland) in two healthy volunteers. Statistical analysis showed that the diabetes patch was able to efficiently track glucose changes and the data obtained were well correlated with those from the commercially available glucose assay kit and the blood glucose meter.[33] This study can be characterized as unprecedented since it reports a coherent experimental "closed-loop" system, based on graphene, able to successfully determine glucose in humans' sweat and to provide a controlled release of the drug.

Non-enzymatic graphene-based sensors

In non-enzymatic biosensors, glucose oxidation is catalyzed directly, by materials that have electrocatalytic properties. These materials include catalysts that contain a transition metal center (such as metals, metal oxides, and alloys) and carbon-based materials.[34] Currently, the most used nanomaterials in the fabrication of non-enzymatic glucose sensors include metal oxides, carbon-based materials (especially graphene), and composites of the above.

There are two accepted theories about the non-enzymatic electro-oxidation of glucose by materials that contain a transition metal center (Figure 4). The first one (the activated chemisorption model proposed by Pletcher in 1984) suggests that glucose oxidation takes place on the surface where glucose forms a bond with the atoms.[35] Ten years later, in 1994, Burke proposed a second theory, the "Incipient Hydrous Oxide Adatom Mediator (IHOAM)" and highlighted that hydroxyl radicals play a significant role in the electrocatalytic

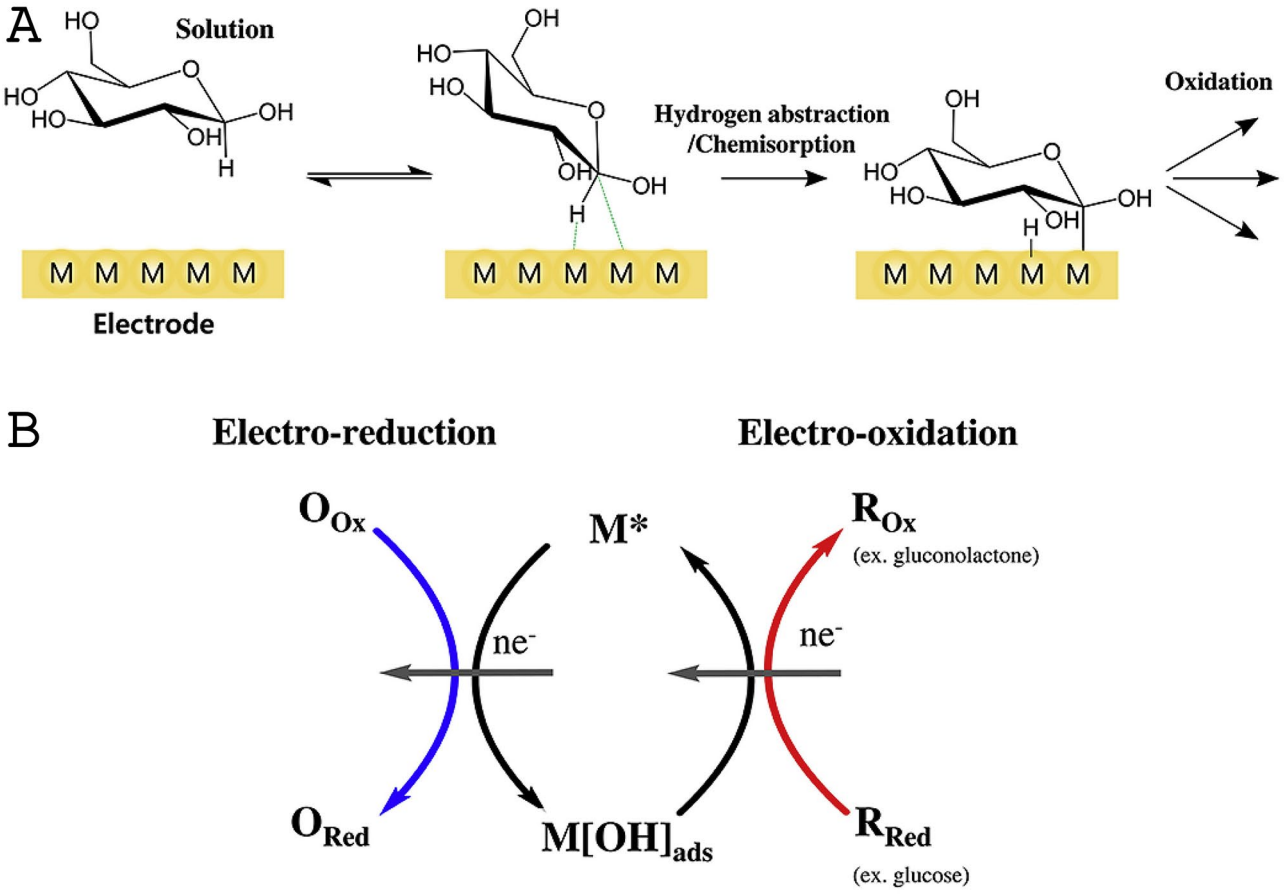

Figure 4. The mechanism of glucose detection by sensors constructed with materials that contain a transition-metal center. (A) Glucose is adsorbed (chemisorption) onto the metal electrode surface before it is further oxidized. (B) The IHOAM model. Fast electro-oxidation of glucose into glucono-δ-lactone is facilitated by the presence of reactive hydrous oxide (OH_{ads}) layer on the electrode surface. Reproduced from the work of Hwang *et al.*[37] with permission of Elsevier.

process of glucose. In this model, active metals atoms on the electrode surface have enhanced reactivity and facilitate glucose oxidation.[36]

Many graphene and graphene derivatives have been examined thoroughly in enzyme-less glucose assays. The most common graphene-based materials used in this field, are GO and rGO. In most studies, graphene materials have been enrolled as supporting materials, but they can also act as electrocatalysts alone; however, only a few studies have tested their sensors in any type of biological fluid to evaluate their potential clinical application value.

Doping graphene with nitrogen, sulfur, fluorine, and boron is a method to enhance the electrocatalytic properties of graphene materials. Of the above, nitrogen doping seems to be the most adequate for this kind of application as it amplifies the electrical conductivity and binding ability of graphene and creates more active sites. Bearing this in mind, Rahsepar *et al.*,[38] created nitrogen-doped graphene (NG) structures (three N-doped graphene specimens with different amounts of pyridinic N, pyrrolic N, and quaternary N) and studied their capability to act as electrocatalysts for glucose detection. All N-doped structures showed a good linear response to glucose but the N-doped graphene with the largest amount of pyridinic nitrogen showed the greatest electrocatalytic ability for glucose oxidation and was examined furtherly. At high pH values (pH = 13), the electrode could detect glucose in a wider linear range and at a lower detection limit than at physiological pH (7.4). The sensor was able to accurately measure glucose in different human blood serums with different glucose concentrations after dilution in phosphate buffer. Having said that the potential use of this sensor in a CGM system is significantly limited.

Non-enzymatic graphene: metal-based sensors

Although the previous study proved the unique electrocatalytic activity of graphene, still the best option for non-enzymatic sensors is to combine graphene with metals, metal alloys and/or metal oxides. This combination leads to a "synergistic effect" and results in better electrocatalytic activity, greater sensitivity, and higher selectivity toward glucose of the sensor.

Shishegari *et al.*[39] created an enzymeless glucose sensor using graphite sheet (GS), N-doped functionalized graphene (NFG) and palladium nanoparticles decorated on NiAl layered double hydroxide (LDH). The sensor's ability to determine two different glucose concentrations (18 and 54 mg/dL) was tested in blood, serum, and plasma samples containing 0.1 M NaOH to increase pH to 13. The lowest recoveries

(90%) were seen in blood samples which were attributed to the matrix of the blood. The authors did not provide results regarding the sensor's performance at physiological pH values, thus, its practical usefulness as a part of a CGM system needs to be addressed.

Nonetheless, combining graphene with precious noble metals, such as platinum (Pt), gold, and palladium shoots up fabrication costs since these metals are high-priced. To overcome this limitation, cheaper transition metals (such as iron, cobalt, copper, and nickel), are used.

Regarding iron (Fe), although Fe_3O_4 oxide is cheap, with good electrochemical properties and effectively oxidizes glucose in a non-enzymatic way, it is rarely used in non-enzymatic glucose detection studies. In a recent study, Suyanta *et al.*[40] fabricated a non-enzymatic glucose sensor based on iron oxide/graphene nanocomposites. Specifically, Fe_3O_4/graphene nanostructures have been designed and placed on a GCE. The sensor was extremely selective toward glucose and presented long-term stability as amperometric response retained close to the initial levels after 14 days of storage. Human blood serum samples required treatment (two consecutive dilutions, 100 and 300 times) with KNO_3 before it was feasible for the sensor to quantify glucose levels with high accuracy as the comparison with a clinical glucometer showed. Chaiyo *et al.*[41] created a paper-based device (PAD) consisting of cobalt phthalocyanine (CoPc). Their idea was derived from the knowledge that metal phthalocyanines exhibit good electrocatalytic properties and are already in use as mediators in enzymatic glucose biosensors. In this study, the authors prepared a CoPc/ionic liquid/graphene composite (CoPc/IL/G) to modify a screen-printed electrode, printed on a paper substrate (SPCE). Blood samples were provided from healthy non-diabetic patients which were centrifuged to obtain serum and then diluted 10-fold with an electrolyte before analysis with the sensor. Moreover, the results were compared with those obtained by a commercial glucose meter (Ascensia ENTRUST) and statistical analysis showed no significant differences between the two methods. Recently, a sensor was developed for the detection of glucose in saliva and specifically a nanohybrid consisting of single-walled carbon nanotubes (SWCNTs), rGO and CoPc, and a modified GCE. Saliva samples were spiked with glucose and diluted to 0.1 M NaOH. The final concentration of glucose in the test samples ranged from 0.005 to 0.5 mM and the sensor's response was linear within this specific glucose concentration range. The sensor could effectively determine glucose in concentrations found in the saliva of healthy and diabetic people (0.23–1.77 mM).[42]

Darvishi *et al.*[43] created a glucose sensor consisting of a porous 3D hydrogel of gelatin methacryloyl (GelMA) combined with nickel nanoparticles and rGO, whereas Xue *et al.*[44] created poly(cysteine)-Ni$(OH)_2$ nanocomposites (Pcys-Ni $(OH)_2$) and they placed them on graphene-modified GCE (GCE/GN/Pcys-Ni$(OH)_2$). Nickel nanomaterials are excellent catalysts for glucose oxidation due to the formation of a redox couple Ni(II)/Ni(III) on the electrode surface in an alkaline solution. The combination of graphene with nickel nanoparticles provides a hybrid material with enhanced electron transfer properties. Both sensors were tested in human blood serum samples; the former in only one sample while the latter in one sample spiked with different glucose concentrations. RSD was 2.1% and 1.2–2.9%, respectively. The biosensors exerted sufficient accuracy and showed potential for use in clinical practice. Following a similar approach, Khosroshahi *et al.*[45] managed to synthesize a non-enzymatic glucose sensor consisting of 3D-graphene foam combined with Cu-based nanoparticles on a GCE. Dilution of human serum samples was mandatory. RSD values decreased as glucose concentration was increased (1.49 and 0.66% for glucose concentration 4.61 and 7.99 mM, respectively) and were similar to those reported by Darvishi *et al.*[44]

A unique methodology for the development of non-enzymatic sensors is the combination of NG and Ni/NiO nanoparticles with mixed-valence states (a mixed state of Ni and NiO enhances the activation rate and facilitate the electrochemical reaction). This approach was chosen since a multivalent system can potentially offer higher electrical conductivity as well as superior catalytic kinetics and dynamics. Injection of various glucose solutions or serum diluted samples (15 times) was performed in alkaline solution (NaOH) and the system was able to accurately determine very small amounts of serum glucose compared with a routinely used colorimetric method (RSD less than 4%).[46] Another approach is the application of nickel phosphide (Ni_2P) nanoparticles *in situ* on the GO film (Figure 5(A)).[47] Besides the need for a strongly alkaline regime to operate, the main advantage of this type of sensor is that it can measure glucose in human serum samples without sample pretreatment; however, the current sensor's performance was not compared with a commercial glucose meter and no further details about the recovery rate or RSDs values were provided.

rGO has also been used for the development of an electrochemical glucose sensor in combination with copper sulfide nanoflakes (CuSNFs)[48] or copper (II) oxide (CuO).[49] Both sensors required a 200- and 100-fold dilution, respectively. Yan *et al.*[48] provided specific details on the preparation of the two urine and two human serum samples they used to access the applicability of the sensor, which resulted in an RSD value below 3% and recoveries between 98.3 and 102.4%. On the contrary, Gijare *et al.*[49] state that they used the standard dilution method before analysis without giving any details on the solvent or the addition of glucose into the samples; however, they applied a certified method to verify their results. Their GO/CuO/fluorine-doped tin oxide (FTO) had an average recovery rate of 99.7% along with an RSD of 1.58% (Figure 5(B)).

Finally, the combination of two different transition metal oxides on a graphene-based structure that could further improve the conductivity, sensitivity, and stability of the material was also reported. Two different groups used nickel and cobalt mixed low-dimensional materials ($NiCo_2O_4$ nanosheets) to decorate graphene and produce biosensors. The first one applied $NiCo_2O_4$ nanosheets on nitrogen-doped (nitrogen doping on graphene enhances conductivity) rGO (N-rGO)[50] while the second one GO.[51] Comparing their diagnostic ability, the sensor generated with N-rGO seems to be superior since it was able to read undiluted human serum samples (RSD 0.2–2.5%) with high accuracy (compared with a commercial glucose meter) whereas the GO sensor was

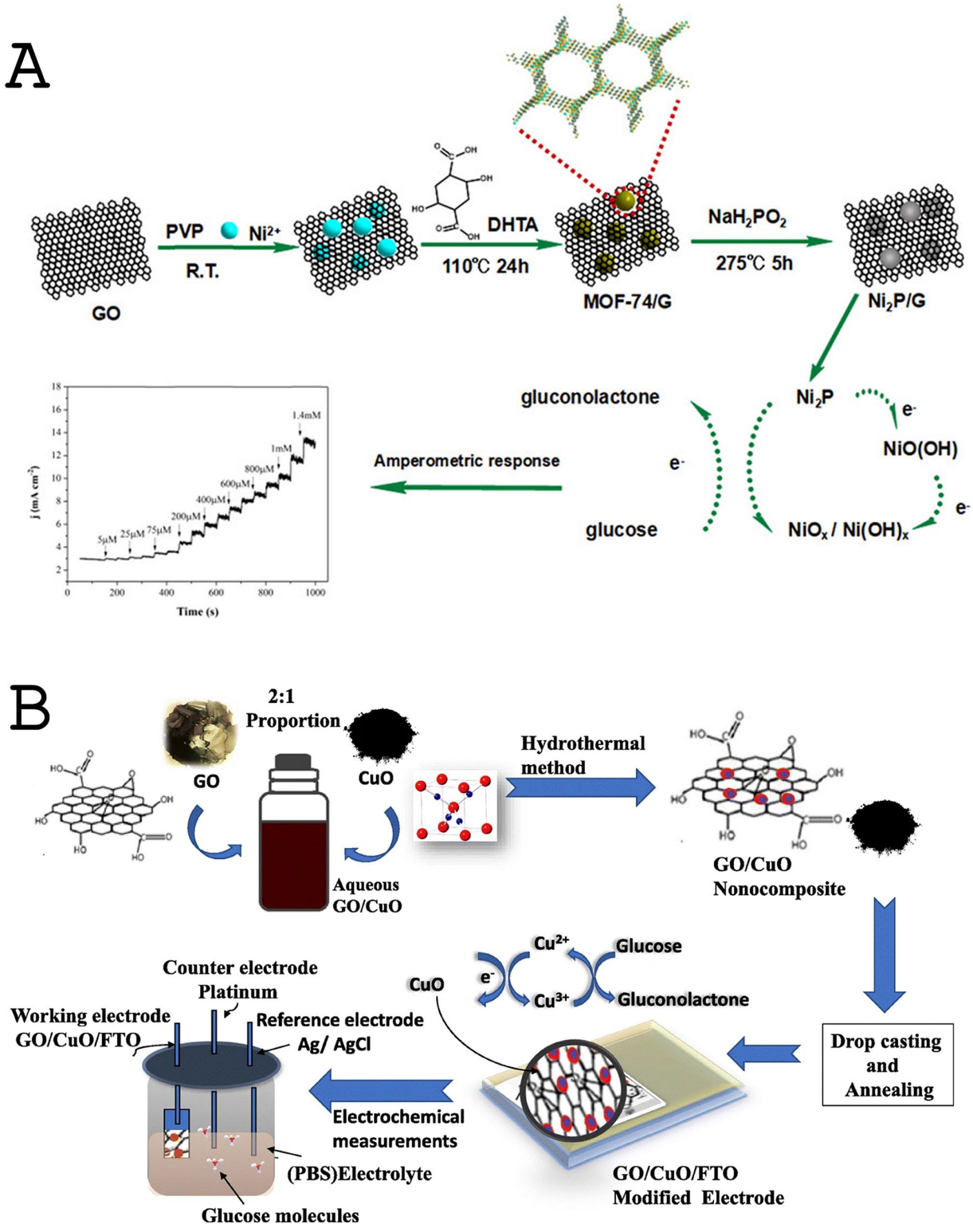

Figure 5. (A) Synthesis of Ni_2P/graphene and electro-oxidation of glucose into gluconolactone. The amperometric response to various glucose concentrations is depicted on the bottom left. Reproduced from the work of Zhang *et al.*[47] with permission of ACS publications. (B) Synthesis of the GO/CuO nanocomposite by *in situ* hydrothermal reduction of GO and CuO nanobelt formation and electro-oxidation of glucose into gluconolactone. Reproduced from the work of Gijare *et al.*[49] with permission of Springer Nature.

tested in diluted (no details provided) and spiked with glucose (20 μM) serum and urine samples with similar RSD values (<3%). In a similar approach, a research team from China developed a Ni- and Cu-modified graphene electrode, using a graphene-layer skeleton (in which they electrochemically deposited Ni nanoparticles and Cu micro-/nano sheets to

enhance electrocatalytic activity toward glucose oxidation) and reported similar results (RSD 1.6–2.4%) to spiked and diluted (50–83 dilution ratio in 0.1 M NaOH) human blood serum samples.[52]

As mentioned above, most of the sensors require sample dilution into alkaline solutions to obtain measurements. To overcome this limitation, Hashemi *et al.*[53] decorated GO flakes with 8-hydroxyquinoline (8H was added to provide radical OH^- species) and nickel oxide (NiO) and measured glucose concentrations after dilution (1:10) of four serum samples in phosphate-buffered saline (PBS) (pH = 7.4). The sensor's RSD was below 5.0% and had a stability of 94% of its initial performance after 1 month; thus, analytical data anoint it a potential candidate for biological and non-biological applications. Prabhakaran and Nayak developed an innovative enzyme-less sensor using laser-scribed graphene (LSG); a novel 3D patterned graphene structure on the surface of which they anchored copper oxide nanoparticles (CuO NPs). After performing several tests on electrodes with different sizes of CuO nanoparticles, a decision was made based on electrochemical data and electrocatalytic properties on glucose oxidation and the best performing electrode assembly was chosen for further analysis. Measurements were performed directly to whole blood, serum, sweat, or urine samples collected from a volunteer. Interestingly, the sensor produced an RSD value of 10.5% in blood and serum samples containing 100 μM (1.8 mg/dL) glucose (precalculated in clinic) which is significantly higher than those reported in the previous studies of the same material; however, no dilution was required in a basic solution nor addition of glucose to the biological samples. Moreover, taking a step forward the authors managed to miniaturize their device and transfer the LSG-based material into a commercial Scotch brand tape, making it adequate for the design of a final wearable device and thus a perfect candidate for daily, home-use by the patients.[54]

Conclusions

Today, therapy for diabetes relies on "open-loop" delivery methods, where the patient administers the drug to himself or herself at different times of the day. A most advanced approach is the "closed-loop" therapy, where the involvement of the patient in maintaining glucose control is minimal. A "closed-loop" system determines insulin or drug requirements in real-time and delivers the proper dosage (e.g. the development of a "synthetic pancreas" an external device that uses glucose sensors and pumps). A "closed-loop," however, demands continuous glucose detection. Thus, CGMs have emerged in the last years as one of the most promising systems for the automation of diagnosis (continuous glucose measurement) and treatment (regimen delivery) of people with diabetes. In this aspect, various graphene-based biosensors have been developed for regular monitoring of blood glucose levels.

Our literature research aimed to identify the progress achieved in recent years on graphene-based glucose sensors and their potential use as part of CGMs. To that end, we narrowed our bibliography quest only to graphene systems and defined key terms referring to human biological fluids (such as "blood," "serum," "sweat," etc.).

From the data collected, it can be safely deduced that enzymatic graphene sensors have the edge over non-enzymatic sensors, since they can measure glucose accurately in biological fluids without sample pretreatment (dilution or spiked with glucose) (Table 2). Non-enzymatic sensors require an alkaline work environment. Although this procedure could be applied to automatic biochemical analyzers with equally satisfactory results, their use in CGMs seems doomed to fail. To overcome this weakness, innovative solutions have been suggested, such as the electrochemical pretreatment to increase the pH of the sample by proton reduction reaction.[55] Moreover, in most of the studies, the authors employed a strategic spike with specific amounts of glucose that best fit the desired optimal operational window of the sensors. Nonetheless, we determined two studies on non-enzymatic graphene sensors[43,50] that reported satisfactory results without sample pretreatment or addition of glucose. It is expected that differential synthetic approaches will enhance graphene potential and render it an ideal material for non-enzymatic CGMs in the fight against DM.

Another drawback we identified and that we believe should be addressed in future research is the limited number of samples analyzed by non-enzymatic and enzymatic sensors. Moreover, from a medical standpoint, the performance of potential CGMs in authentic biological samples is of the utmost importance. Diabetes induces biochemical and hematological changes that are not limited to glucose levels, so that, it is preferable to test these systems in real and not artificial conditions: samples from people with chronic diabetes exerting hyper- or even hypoglycemia (T1DM and T2DM), people with prediabetes and healthy individuals. We strongly believe authentic biological samples would provide more realistic conditions for CGMs to cope with rather than spiked glucose samples.

Non-invasive CGMs are the feature of glucose monitoring and considerable research efforts have been made in the last decade to develop systems for monitoring glucose in blood, sweat, saliva or tears. Microneedle sensors are minimally invasive, however, there could be an infection risk lurking after several applications, whereas, there has not been established a solid correlation between saliva or tears and blood glucose. Our research team is working on a promising approach to develop a "closed-loop" system where glucose concentration is continuously measured in human sweat through a graphene-based enzymatic sensor (skin patch) utilizing novel methodologies in green chemistry. An artificial intelligence (AI) app on a mobile phone analyses incoming readings and activates drug release from a drug-loaded skin patch (a nanoemulsion of a hypoglycemic regimen to penetrate the skin and reach the superficial vascular network) (Figure 6).

Currently, sweat sensors are the most promising strategy for the development of non-invasive CGMs. It is expected that wearables that detect not only glucose but many analytes in sweat simultaneously will emerge rapidly in the following years and graphene might provide strong foundations for the development of the new era of non-invasive CGMs.

Table 2. Measurement conditions for graphene-biosensors described in the present article.

Enzymatic								
Sensor (material)	Samples			Pretreatment/dilution			Spiked with glucose	Ref.
	Type	Number	Yes/no	Solvent	Fold	Yes/no	Concentration (mM)	
GOD–MCF@rGO/GCE	HS	3	No	–	–	No	–	Wang *et al.*[29]
SPCE/GNR/Bi_2O_3/GOx/Naf	HS	3	Yes	Acetonitrile	1:1.8	No	–	Đurđić *et al.*[30]
	HU	1	Yes	0.1 M PBS	1:5	Yes	0.32, 0.48, 1.04, 1.68	
3D porous aerogel @ GOx-based microfluidic biosensor	HS	5	No	–	–	No	–	Xu *et al.*[31]
GOx-CNs-rGO-Au-SPE ePAD	WB	8	No	–	–	No	–	Ahmadi *et al.*[32]
GOx/PB/graphene hybrid	HSw	2	No	–	–	No	–	Lee *et al.*[33]
Non-enzymatic								
Sensor (material)	Samples			Pretreatment/dilution			Spiked with glucose	Ref.
	Type	Number	Yes/no	Solvent	Fold	Yes/no	Concentration (mM)	
Nitrogen-doped graphene	HS	1	Yes	0.1 M PBS	1:300	Yes	2, 4, 6	Rahsepar *et al.*[38]
GS/NFG/Pd-NiAl-LDH	HS	1	Yes	NR	NR	Yes	1, 3	Shishegari *et al.*[39]
	HP	1						
	WB	1						
Fe_3O_4/graphene/GCE	HS	1	Yes	0.1 M KNO_3	1:100	Yes	1	Suyanta *et al.*[40]
CoPc/IL/G/SPCE/PAD	HS	3	Yes	NR	1:10	No	–	Chaiyo *et al.*[41]
GCE-SWCNT/rGO/CoPc	HSa	–	No	–	–	Yes	0, 0.005–0.5	Adeniyi *et al.*[42]
GelMA: Ni-RGO hydrogel	HS	1	No	–	–	No	–	Darvishi *et al.*[43]
GCE/GN/Pcys-Ni$(OH)_2$	HS	1	NR	–	–	Yes	0.98, 1.94, 2.88, 3.80	Xue *et al.*[44]
Cu-Cu_2O NPs @3DG foam	HS	4	Yes	NaOH	No	–	–	Khosroshahi *et al.*[45]
Ni/NiO/NG-400	HS	1	Yes	NaOH	1:15	Yes	0, 0.025, 0.075, 0.15	Wang *et al.*[46]
Ni_2P/G/GCE	HS	1	No	–	–	Yes	1–8	Zhang *et al.*[47]
rGO/CuSNFs/GCE	HS	2	Yes	0.1 M NaOH	1:200	Yes	0.05, 0.1	Yan *et al.*[48]
	HU	2						
GO/CuO/FTO	HS	4	Yes	NR	1:100	No	–	Gijare *et al.*[49]
$NiCO_2O_4$ NSs@N-rGO	HS	3	No	–	–	No	–	Wang *et al.*[50]
GO-templated $NiCO_2O_4$ nanosheets (NSs)	HS	1	Yes	NR	NR	Yes	0.02	Babulal *et al.*[51]
	HU	1						
Cu/Ni/graphene/Ta electrode	HS	1	Yes	0.1 M NaOH	1:83 1:75 1:60 1:50	Yes	20	Cui *et al.*[52]
GO–NiO–8H–NHS (pH 7.4)	HP	1	Yes	PBS	1:10	Yes	0, 0.03, 0.1, 0.5	Hashemi *et al.*[53]
CuO/LSG	WB	1	Yes	NR	NR	NR	–	Prabhakaran *et al.*[54]
	HS	1						
	HSw	1						
	HU	1						

GOD: glucose oxidase; MCF: meso-cellular silicate foam; rGO: reduced graphene oxide; GCE: glassy carbon electrode; HS: human serum; SPCE: screen-printed carbon electrode; GNR: graphene nanoribbon; HU: human urine; GOx: glucose oxidase; CNs: cellulose nanofibers; PBS: phosphate-buffered saline; WB: whole blood; PAD: paper-based device; HSw: human sweat; GS: graphite sheet; NFG: N-doped functionalized grapheme; LDH: layered double hydroxide; HP: human plasma; CoPc: cobalt phthalocyanine; IL: ionic liquid; G: graphene composite; SWCNT: single-walled carbon nanotube; GelMA: gelatin methacryloyl; Pcys: poly(cysteine); NG: nitrogen-doped graphene; LSG: laser-scribed graphene; NR: not reported; Naf: Nafion; SPE: Screen Printed Electrode; PB: Prussian Blue; GN: Monolayer graphene; 3DG: Three-dimensional graphene; FTO: Fluorine-doped tin oxide; NHS: N-Hydroxysuccinimide.

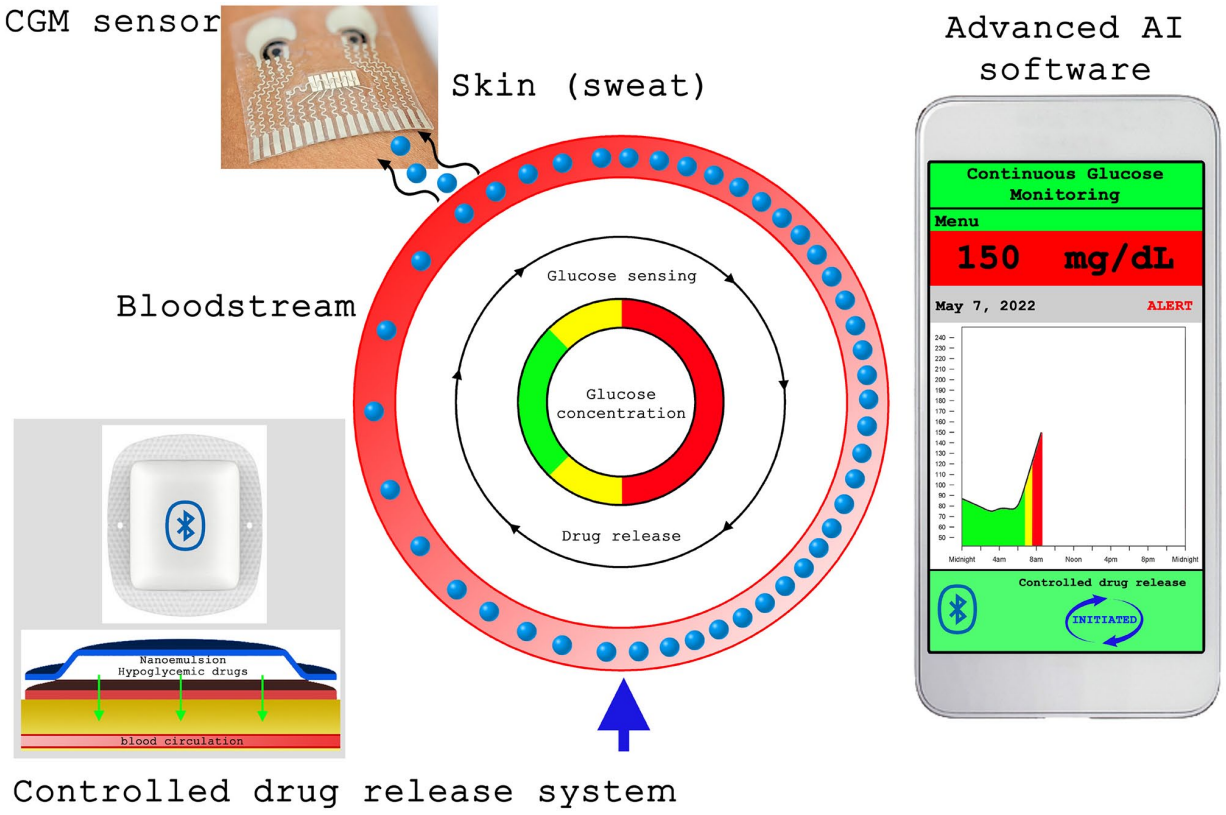

Figure 6. A potential sweat-based glucose monitoring nanodevice (closed loop) with controlled transdermal nanoemulsion release for hypoglycemic drug delivery.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, and analysis of the data; EP, YVS, KS, EIT, MP, and KT contributed to literature review. EP and YVS contributed to writing original draft (equal). YVS, EIT, KS, MP, and KT contributed to image production. YVS, PB, DP, ST, MIP, DPG, HS, and ED contributed to review and editing. DP, HS, and ED contributed to resources and supervision.

DECLARATION OF CONFLICTING INTERESTS

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