Non-Enzymatic Electrochemical Sensing

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Simultaneous detection of analytes that together exist in biological organisms necessitates the development of effective and efficient non enzymatic electrodes in sensing. In this regard, development of sensing elements for detecting glucose and hydrogen peroxide (H2O2) is significant. The non-enzymatic sensing is more economical and has longer lifetime than enzymatic electrochemical sensing, but it has several drawbacks such as high working potential, slow electrode kinetics, poisoning from intermediate species and weak sensing parameters. Here is a comprehensive overview of the recent developments in non-enzymatic glucose and H2O2 (NEGH) sensing, by focusing mainly on sensing performance, electro catalytic mechanism, morphology and design of electrode materials. A comparison of glucose and H2O2 sensing parameters using same electrode materials is outlined to predict the efficient sensing performances of advanced nanomaterials with metal/metal oxides and hybrid metallic nanocomposites.

Non enzymatic | Advanced nanomaterials | dual in line sensing

1. Introduction

Non-enzymatic sensing has been in the research limelight, and most sensors based on nanomaterials are designed to detect single analytes. The simultaneous detection of analytes that together exist in biological organisms necessitates the development of effective and efficient non-enzymatic electrodes in sensing. In this regard, the development of sensing elements for detecting glucose and hydrogen peroxide (H_2O_2) is significant. Non-enzymatic sensing is more economical and has a longer lifetime than enzymatic electrochemical sensing, but it has several drawbacks, such as high working potential, slow electrode kinetics, poisoning from intermediate species and weak sensing parameters.

2. Importance of Non-Enzymatic Electrochemical Sensing

Glucose is an essential carbohydrate involved in major catabolic pathways, including oxidative phosphorylation and glycolysis for the creation of proteins, glycogens, and lipids [1][2]. Glucose is absorbed through the intestines, and, converted by the liver into a more stable form of glycogen, regulated by the hormone insulin [3][4]. Diabetes mellitus (DM) has been termed the “invisible killer” as a consequence of both hyperglycemia and hypoglycemia [5]. A fasting blood glucose concentration less than 100 mg/dl (5.6 mmol/L) is normal, a level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes and greater than 126 mg/dL (7 mmol/L) on two separate tests allows the diagnosis of diabetes. Hypoglycemia is defined by a blood glucose concentration <70 mg/dl (3.9 mmol/L) and

concentrations of both <54 mg/dL (3.0 mmol/L) and <50mg/dL (2.8 mmol/L) cause defective glucose counterregulation and impaired awareness of hypoglycemia. Hyperglycemia can result in multiple metabolic abnormalities associated with long term microvascular and macrovascular complications [6][7][8][9][10]. The global prevalence of diabetes in 2019 was estimated at 463 million people, and has been predicted to rise 10.2% by 2030 and 10.9% by 2045. The prevalence is higher in developed countries (10.4%) than in developing countries (4.0%). Furthermore, one in two people living with diabetes do not know that they have diabetes. The rising burden of diabetes in low- and middle-income countries may cause financial strain on individuals and health systems. Among all countries worldwide, the United States and China have the highest diabetes related medical expenditure. Between 2019 and 2045, the global expenditure for diabetes treatment is expected to grow from USD 760 billion to USD 845 billion. Diagnosis and management of diabetes require accurate, sensitive, reliable, rapid, and attentive monitoring of glucose in day to day life [11][12]. Generally, H_2O_2 is generated during enzyme/glucose reactions and so the monitoring of H_2O_2 is also of great importance. H_2O_2 is an unstable compound found in nature that plays a vital role as an intermediate in several biological reactions such as the metabolism of proteins, carbohydrates, cell signaling, and immune responses [13][14]. However, excess H_2O_2 can damage DNA or proteins via the generation of reactive oxygen species [15]. Hence, the monitoring of both H_2O_2 and glucose with a novel sensing approach in humans and the environment is of great significance. Such non-enzymatic glucose and H_2O_2 (NEGH) sensors have applications in biomedical devices, catalysis, and the environment.

Several analytical approaches have been reported to quantify glucose and H_2O_2 levels, namely calorimetric, titrimetric analysis, spectrometry, fluorescence, chemiluminescence, and high-pressure liquid chromatography [16][17][18][19][20]. However, these methods have certain limitations, such as cumbersome fabrication processes, low reproducibility, matrix interference, high cost, and short shelf time. Hence, there is a need for the development of more efficient techniques for glucose and H_2O_2 quantification, and, in this context, electrochemical methods have much influence. Electrochemical techniques for glucose and H_2O_2 sensing have good accuracy, specificity, response time, simplicity, lower detection limits, high physical and chemical stability, enhanced electron transfer rate, practical detectability, easy to scale up, and biocompatibility [21]. The first enzyme-based glucose sensors were explored in 1960, and have served to drive work in this area for many researchers. Thereafter, first, second, and third generation enzyme-based glucose biosensors have been established. Third-generation sensors are still in their infancy, but those based on nano-mesoporous electrode surfaces show promise but with some drawbacks [22][23]. The mechanism of these sensors is based on the detection of oxygen or H_2O_2, the electron mediator, or the enzyme. Immobilized glucose oxidase (GOx) sensing results in the detection of gluconolactone and H_2O_2 [24]. Hence, the sensing of both glucose and H_2O_2 exists in correlation and has significance in food, pharmaceutical, clinical, and environmental studies [25][26]. However, enzymatic glucose and H_2O_2 sensors (EGHS) have certain limitations, including enzyme denaturation due to environmental changes (pH, humidity, and temperature), digestion by proteases, expensive preparation, time-consuming purification, high cost, thermo-chemical deformation, poor reproducibility, lack of stability, and tedious enzyme immobilization techniques [27][28]. These disadvantages of EGHS, as mentioned, can be adequately defined by nanomaterial assisted electrochemical processes through NEGH sensing.

The most significant challenges faced while designing NEGH sensing are the high working potential, unpredicted redox reactions, slow electro kinetics, intermediate poisoning and weak sensing parameters [29]. Therefore, recent efforts have been devoted primarily on discovering novel nanomaterials with high conductivity, efficient catalytic activity, and excellent physical and chemical strength for the construction of non-enzymatic sensors [30][31]. Nanomaterials have a large surface area, applied potential window, low charge transfer resistance, and flexibility, which makes them ideal electrode materials [32][33]. These novel nanomaterials include metal/metal oxide, carbon, and polymer nanocomposites in different nano morphologies such as crystals, rods, wires, fibers, twisters, core shell, and quantum dots (Figure 1) [34].

Figure 1. Schematic illustration of advanced nanomaterials for non-enzymatic electrochemical glucose and H_2O_2 sensing: (**a**) AuNBP/MWCNT/GCE nanocomposites [35]; (**b**) Ni_3N/GA samples [36]; (**c**) 3D N-Co-CNT@NG [37]; (**d**) Cu_2O PLNWs/Cu foam [38]; (**e**) core shell $Ni_xCo_{3-x}N$/NG [39]; (**f**) Ni $(OH)_2$/RGO/Cu_2O@Cu electrode [40].

A wide variety of nanomaterials are fabricated; however, only a limited number of nanomaterials have been utilized for NEGH sensing due to their enhanced conductivity, surface area, electro kinetics, and the electro catalytic activity in acid, and base media. The nanoparticle concentration, synergistic effect, charge carrier type, surface charge, bandgap, mobility and density of electrons on the surface of a nanomaterial can be tuned by considering a combination of materials, and efficient preparation method, which has enabled their applications in a wide range of

electrochemical devices [41][42][43]. Significant research effort was dedicated to the development of NEGH sensing with advanced nanomaterials to obtain high conductivity, suitably applied potential, and portable sensing of glucose and H_2O_2.

3. Future Perspectives

Limited research development has been made with regard to the fabrication of advanced nanomaterials with bifunctional property for NEGHS. Further improved research and development are necessary to make the commercialization of implantable in vivo and portable in vitro NEGHS devices, which require the improvement of practical, affordable, advanced nanomaterial-based electrocatalysts with multifunctional reactivity. The current research review addresses multiple directions for the achievement of non-enzymatic bifunctional electrode platforms. Electrochemical sensing parameters of advanced nanomaterial with bifunctional electrodes are dependent upon the electrode potential, bandgap, surface defects, synergetic effect, and surface area of the nanocomposites. However, the influence of these issues on NEGH sensing is not addressed in the literature and provides opportunities for the future development of biodevices. Since the multienzymatic properties of nanomaterials have attracted wider research interest, the catalytic (glucose) and peroxidase (H_2O_2) activity of nanomaterial should be effectively optimized and promoted for the best performance of NEGH sensors. The essential electrochemical mechanism in NEGH sensing with the same electrode material should be established using theoretical and analytical models with relevant laboratory experiments. Current studies on NEGH sensors mostly focus on the electrocatalyst performance of advanced nanomaterials and limit the understanding of the influence of nanomaterial morphology on glucose and H_2O_2 quantification and the interaction with bio-analytes. To overcome this, researchers should focus on the development of nanomaterials in different morphologies, such as dots, tubes, fibers, spheres, and core-shells, and a detailed study should be undertaken to improve the surface area and conductivity, which could have a positive influence on the development of NEGH sensors. The modified electrodes show catalytic activity in acidic or basic conditions, which limit the practical application of NEGH sensors. In this context, studies must be done on the oxidation and reduction mechanisms at neutral pH conditions by considering novel nanomaterials. The use of biopolymers as bio-catalytic centers are tolerable to achieve highly sensitive and selective NEGH sensors, and distinct consideration should be given to building electrode platforms with improved robustness and enhanced electro catalytic activity. NEGH sensor-based nanomaterials as catalysts have been demonstrated to be very reasonable; conversely, it is essential to design new schemes for the synthesis, functionalization, and fabrication of nanomaterials to acquire more accurate quantification of glucose and H_2O_2. Several sequential steps involved in the preparation of electrodes for a conventional modified electrode based on NEGH sensing, including cumbersome electrode cleaning, polishing and washing, binder and solvent selection, catalyst preparation, and loading process, have increased the time and cost of NEGH sensing electrodes. Furthermore, to establish contact between the working electrode and catalyst using a binder remains another challenge for the performance of NEGH sensing. This could be avoided by developing binder-free, freestanding bare electrodes, ink/screen printed electrodes and the in situ fabrication/modification of advanced nanomaterials as modified electrodes that make possible the preparation of disposable NEGH sensing electrodes. Moreover, another compelling research direction is in the preparation of metal/metal oxide morphologies with

emerging carbon materials (g-C_3N_4, graphene, CNTs, black phosphorous, and activated carbon, etc.) to form new functional materials. For commercialization, an important prospect is the prolongation of lifetime of the sensors, even though the non-enzymatic sensors are more stable than enzymatic sensors, they lack in the corrosion property/unstable in humid conditions, which requires researchers to focus on anticorrosive nanomaterials.

Current challenges in improving efficiency of the NEGH sensors can be overcome by optimizing the selectivity, working potential, linearity, sensitivity and working pH conditions. Though some NEGH sensors are good in neutral pH conditions with low detection limits, their linear range of detection may be questionable. The low detection range sensors are not useful in day-to-day diabetes management and hence few reports have been applied in various real-time applications such as sensing in antibiotic lotions, milk, and glucose-based fuel cells, etc. The selectivity of NEGH remains a huge problem, which means that the oxidation of interference compounds such as AA, DA, and UA chlorine ions and other carbohydrates at the same working potential affects the glucose and H_2O_2 determinations. Transition metal/metal-oxide-based sensors have shown significant progress in selectivity issues and electrode fouling problems due to reasonable isoelectric point values. From the reported literature on NEGH sensors the sensitivity was improved using different strategies and the novel combination of nanomaterials. Sensor sensitivity is dependent on on working potential, electro kinetics and electrolyte conditions. However, different research groups have performed sensing under their own optimized conditions, which necessitates a uniform protocol for sensing operations. In addition, to improving the sensitivity by optimizing the properties of advanced nanomaterials, the selectivity performance should be more focused to achieve stability, repeatability, and practical evaluation of glucose and H_2O_2. The dual in-line sensor requires a clear mechanism with suitable working conditions in neutral pH. The use of the same electrode material for multiple applications is essential to reduce the cost and will make commercialization easy. The dual sensor requires a clear electro catalytic mechanism for sustainable development, and it can be achieved by operating the electrodes at the same working potential (positive/negative). In short, the bifunctional, electro-catalyst-based NEGH sensing technology must be extended from the laboratory to the field by proper implementation to boost sustainable electronic devices.

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