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Carbon nanotubes mediated immobilized glucose oxidase biosensors as an effective and sensitive analytical tool

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ABSTRACT

The application of simple and modified carbon nanotubes (CNTs) has attracted a remarkable attention for the development of new immobilization matrices for glucose oxidase (GOx) on the surface of electrodes. The aim of this article is to review the literature based on the glucose biosensors designed by immobilizing GOx on the surface of electrodes through simple and functionalized single/multiwalled CNTs (SWCNTs/MWCNTs)or their various kinds of derivatives. This new methodology of GOx immobilization on the surface of electrodes via CNTs and their derivatives was exploited in order to obtain high yield of immobilization with significantly high catalytic efficiency. GOx has been physically adsorbed or covalently attached to the electrode via different forms of single and multiwalled CNTs and their derivatives. In addition, the large surface area of CNTs provided high enzyme loadings, yet the intrinsic long length of the CNTs led to easy and simple filtration. CNTs-GOx composites were found markedly more stable against different forms of denaturants and inactivators. The designed biosensors were found quite resistant to the inhibition mediated by interferents present in clinical, biological and other commercial samples. These CNTs-GOx conjugates demonstrated a unique combination of useful properties such as low mass transfer resistance, high catalytic activity and stability, and reusability. The as prepared glucose biosensors showed the wide linear analytical ranges of substrates, very low detection limits, high sensitivity and short response time of detection. The biosensors have efficiently been applied for the analysis of glucose level in distinct biological, pharmaceutical and food samples on repeated uses and these biosensors have shown remarkable potential in the analysis of glucose uptake by cancerous cells. Numerous immunosensors containing GOx-MWCNTs have been employed for the detection of serum α-1-fetoprotein and clenbuterol.

Keywords: *Biosensor, carbon nanotubes, immobilized, glucose oxidase, electrode.*

Abbreviations: *CD, cyclodextrin; CDI, carbodiimide; CNTs, carbon nanotubes; CV cyclic voltametry; DET, direct electron transfer; ETR, electron transfer rate; GCE, glassy carbon electrode; GOx, glucose oxidase; GA, glutaraldehyde; GR, graphene; LOD, low limit of detection; LR, linear range; MWCNTs, multiwalled carbon nanotubes; NC, nanocomposite; NM, nanomaterial, Nf, Nafion; NPs, nanoparticles; NBC, nanobiocomposite; Ppy, polypyrrole; PEI, polyethyleneimine; RT, response time; SV, sensitivity; SWCNTs, single walled carbon nanotubes; SPCE, screen printed carbon electrode.*

1. INTRODUCTION

Applied enzymology has appeared as one of the most emerging fields of modern science and technology. Enzymes are frequently applied in industry, environment and medicine. However, their use has been limited due to some inherent demerits such as stability, reusability in batch processes and applicability in continuous reactors [1-4]. The enzyme immobilization is a technique, which can make enzymes suitable for using them repeatedly in batch processes and continuous reactors and also enhances the stability of the enzymes against different environmental factors such as high temperatures, extreme pH, high ionic strength, organic solvents and chemical denaturants [5-8]. The immobilization also provides resistance to enzymes against their own and specific inhibitors and interferents present in biological samples. Five different types of methods; physical adsorption, covalent attachment, chemical aggregation, entrapment and microencapsulation have been adopted for the immobilization of enzymes. Numerous kinds of organic and inorganic supports are in practice to immobilize enzymes [9-12]. The immobilization of enzymes on/in these classical materials sometimes also creates limitations; such as lower yield of immobilization and binding efficiency, low stability and steric hindrances in case of enzymes those are acting on large molecular size substrates [13-16]. The choice of support is highly important for high yield immobilization and maintaining significantly high catalytic efficiency of enzymes, high stability and steric hindrance free immobilized enzymes [13, 17-19].

Recently, nanoparticles (NPs) and nanomaterial (NM) have attracted attention of enzymologists as an enzyme immobilizing support. These kinds of supports have very high efficiency of enzyme immobilization with higher specific surface area, ordered nano-pore structure, good mechanical/electrical/thermal performance, outstanding chemical stability, biocompatibility and controllable surface functional modifications [20-24]. Single walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) have extensively been employed for the immobilization of enzymes in order to use them in repeated processes and biosensors. Due to unusual properties of carbon nanotubes (CNTs), this NM has been exploited in distinct fields such as nanotechnology, nanomedicine, and other fields of materials science and technology. In view of their extraordinary

thermal conductivity, mechanical and electrical properties, CNTs have been used as additives to various structural materials [25, 26]. The effect of surface modification of carrier and immobilization methods on the catalytic properties of immobilized enzymes has been described [27]. The application of immobilized enzymes in the analysis of various kinds of analytes has attracted enzymologists to develop novel and more sensitive biosensors [28-30]. Enzyme immobilization vis CNTs on the surface of electrode is a fast developing field of research [31,32]. Various immobilization methods have been explored, and in particular, specific attachment of enzymes on CNTs has been an important focus of attention. The method of immobilization has an effect on the maintenance of enzyme native structure and retention of catalytic activity of the enzyme [33,34]. The electrochemical (EC) sensing approaches have exploited the use of CNTs as electrode material due to their novel structures and properties to provide strong electrocatalytic activity with minimal surface fouling. Such combined efforts have contributed towards the rapid development of CNTs-based biosensors for the analysis of important analytes with high detection sensitivity (SV) and selectivity [35,36]. The use of CNTs opens an opportunity for the direct electron transfer (DET) between the enzyme and the active electrode area. The CNTs have also attracted much interest due to remarkably high electrocatalytic activity on the redox reaction of H_2O_2 and NAD, two major by-products of the reactions catalyzed by oxidoreductases. This excellent electrocatalytic property of CNTs has provided a platform for the construction of highly specific and sensitive biosensors for oxidoreductases [37-39].

The main aim of this review is to discuss about the composition and applications of biosensors prepared by immobilizing glucose oxidase (GOx) through CNTs. Herein, it has been focussed on recent development in the procedures of enzyme immobilization on CNTs followed by electrode binding. The specific combination of different kinds of molecules with CNT leads to an improvement in the performance of the resulted biosensor. The construction of polymer/CNT modified electrodes has been demonstrated together with their electrochemical and surface characterization, which has also emphasized the contribution of each component on the overall properties of the modified electrodes. Their role in analytical chemistry is demonstrated by the numerous applications based on polymer/CNT-driven electrocatalytic effects, and their analytical performance as biosensors. The various aspects of each biosensor such as range of linearity (LR), low detection limit (LOD), SV, response time (RT) and kinetic parameters have been critically evaluated.

2. GLUCOSE OXIDASE

Clark and Lyons [40] for the first time developed an electrochemical sensor for the determination of glucose concentration in blood plasma/serum. In their electrode a membrane permeable to glucose traps a very small quantity of GOx near to a pH electrode, and the concentration of glucose was detected by the change in electrode potential that produced when glucose reacts with the enzyme in solution.

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

The glucose biosensor that was developed 56 years back still has its importance and applications. In addition to the maturity of the field, new technologies that explore solutions to the demerits of these glucose biosensors continue to investigate. Herein, an effort has been done to describe various new developments in the field of GOx in the last decade; emphasizing the immobilization of GOx at surfaces of electrodes mediated by CNTs [41, 42]. Aligned MWCNTs grown on Pt substrate was chosen for the construction of an amperometric biosensor. The opening and functionalization by oxidation of CNTs array allowed for efficient immobilization of GOx. The carboxylated open-ends of CNTs were used for the immobilization of enzymes, while the Pt substrate gave direct transduction platform for signal detection. CNTs showed dual roles, as an immobilization support and mediator, and thus it appeared highly useful in developing biosensors of third generation [43]. Electrodeposition was employed for co-deposition of GOx and Pd NPs onto a Nafion (Nf)-solubilized CNTs film. The co-deposited Pd-GOx-Nf-CNT bioelectrode maintained its biocatalytic activity and provided an efficient oxidation and reduction of enzymatically produced H_2O_2, demonstrating fast and sensitive measurement of glucose. The combination of Pd-GOx electrodeposition with Nf-solubilized CNTs enhanced storage time and facilitated performance of the biosensor. An additional coating of Nf has prevented it from the effect of some common interferents such as uric and ascorbic acids. The designed Pd-GOx-Nf CNTs glucose biosensor demonstrated a LR for glucose up to 12 mM and a LOD of 0.15 mM [44]. Joshi et al. [45] constructed the amperometric biosensors by incorporating modified SWCNTs with GOx into redox polymer hydrogels, a poly[(vinylpyridine)Os(bipyridyl)$_2$-Cl(2+/3+)], PVP-Os-BP polymer film. A disposable electrochemical biosensor was developed by binding GOx on the surface of screen printed carbon electrode (SPCE) already layered with MWCNTs and this biosensor was applied for the estimation of glucose. The effect of MWCNTs on the response of amperometric GOx electrode for glucose has been evaluated. The as-fabricated MWCNTs-GOx biosensors were first time used efficiently in the field of analysis [46].

GOx was immobilized within the MWCNTs epoxy-composite matrix prepared by dispersion of MWCNTs inside the epoxy resin. The use of MWCNTs, as the conductive part of the composite, demonstrated significantly higher binding of GOx into epoxy matrix and ETR between the enzyme and the transducer. MWCNTs epoxy composite biosensor showed an excellent SV, reliability, and stable electrochemical properties together with significantly lower detection potential (+0.55 V) than GOx-graphite epoxy composites (+0.90 V; difference deltaE=0.35 V). The results obtained favorably compare to those of a glucose biosensor based on a graphite epoxy composite [47]. Withey et al. [48] explained a highly ordered array of CNTs that worked as a universally direct nanoelectrode interface for redox proteins and rendered an efficient conduit for electron transfer. The site-

specific, covalent docking of GOx on CNTs tips has been noticed to have a marked effect on enhancing electron transfer properties. A unimolecular electron transfer rate (ETR) of 1500 s^{-1} has been measured for this system, a value exceeding the rate of O_2 reduction by GOx. Furthermore, the redox enzyme-CNTs array conjugate can be utilized as a quantitative, substrate-specific biosensor. A nanobiocomposite (NBC) film consisting of polypyrrole (Ppy), functionalized COOH-MWCNTs and GOx was electrochemically synthesized by electrooxidation of 0.1 M pyrrole in aqueous solution containing appropriate amounts of MWCNTs-COOH and GOx. The results revealed that the electroanalytical Ppy-COOH-MWCNTs-GOx-NBC film was highly sensitive and suitable for glucose biosensor based on GOx function. The amperometric responses of the optimized Ppy-COOH-MWCNTs-GOx glucose biosensor (1.5 mg mL^{-1} GOx, 141 mC cm^{-2} total charge) exhibited a sensitivity of 95 nA mM^{-1}, a LR up to 4 mM, and a RT of about 8 s [49]. In a further study similar workers immobilized GOx on Pt NPs electrodeposited within MWCNTs-Nf. The electrocatalytic properties of the MWCNTs-Pt-Nf-GOx NBC film modified glassy carbon electrode (GCE) were characterized by cyclic voltammetry (CV) and amperometry in presence of H_2O_2. The sensitivity of glucose biosensor was significantly improved by depositing Pt NPs and GOx within the MWCNTs-Pt-Nf-GOx NBC film. The optimized glucose biosensor showed the sensitivity (SV) of 640 nA mM^{-1}, a linear range (LR) of up to 4 mM, a LOD 4 µM, and a RT less than 4 s at an operating potential of +500 mV versus Ag/AgCl, 3 M KCl [50]. GOx was incorporated into Pt-CNTs-silicate matrix in order to develop a glucose biosensor. Pt-CNTs paste-based biosensor was constructed that responded more sensitively to glucose than CNT-based biosensor. Almost interference free determination of glucose was realized at 0.1 V versus SCE with a LR from 1 to 25 mM, a RT <15 s, and SV was 0.98 µA $mM^{-1}cm^{-2}$ in a phosphate buffer, pH 6.98. Pt-CNTs paste-based biosensor was about 4-fold more sensitive than the biosensor prepared by using only CNTs [51].

A novel amperometric glucose biosensor based on the nine layers of multilayer films composed of MWCNTs, AuNPs and GOx was developed for the specific detection of glucose. MWCNTs were chemically modified with the H_2SO_4-HNO_3 pretreatment to introduce -COOH which were used to interact with the NH_2- of poly(allylamine) (PAA) and cysteamine via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) cross-linking reaction, respectively. There was marked enhancement in electrocatalytic activity of Pt electrode containing multilayer films toward H_2O_2 produced during GOx enzymatic reactions with glucose permitted effective low-potential amperometric measurement of glucose. The applied potential of 0.35 V versus Ag/AgCl was chosen for the detection of H_2O_2 oxidation by these biosensors. The biosensor based on nine layers of multilayer films showed a wide LR of 0.1-10 mM glucose, with a remarkable SV of 2 µA mM^{-1}, a LOD, 6.7 µM and fast RT within 7 s. Moreover, it exhibited good reproducibility, storage stability and negligible interference by ascorbic acid, uric acid and acetaminophen. The study provides a feasible approach on developing new kinds of oxidase-based amperometric biosensors, which can be used as an illustration for constructing various hybrid structures [52]. A glucose biosensor was developed by cross-linking GOx in the matrix of biopolymer CS on the GCE, which was modified with Au-Pt NPs by electrodeposition on MWCNTs-CS. GOx biosensor showed highly satisfied performance for glucose estimation at a low applied potential, 0.1 V with a high SV 8.53 µA mM^{-1}, a LOD 0.2 µM, a wide LR 0.001-7.0 mM and a fast RT <5 s. The obtained biosensor was found highly reproducible, stable and specific during analysis of glucose in human blood and urine samples [53]. Furthermore, Kang et al. [54] prepared a new biosensor by cross-linking GOx via GA at GCE combining Au NPs-doped CS solution with MWCNTs. The free interference determination of glucose was done at 0.4 V(vs. Ag/AgCl/3.0 M KCI) in a broad spectrum of LR $2.0x10^{-5}$-$1.5x10^{-2}$ M and a fast RT within 5 s. The biosensor was used to determine glucose in human serum samples and the obtained results were quite satisfactory. A novel approach was adopted for fabricating a SV-enhanced glucose biosensor. It was based on MWCNTs, Pt NPs and sol-gel of CS/silica organic-inorganic hybrid composite. The CS/silica hybrid sol-gel was produced by mixing methyltrimethoxysilane (MTOS) with the CNTs-Pt NP-CS solution. The glucose biosensor of GOx-MWCNTs-Pt NPs-CS-MTOS-GCE was fabricated and in phosphate buffer, pH 6.8, nearly interference free determination of glucose was performed at low applied potential of 0.1 V, with a wide LR $1.2x10^{-6}$ to $6.0x10^{-3}$ M, LOD $3.0x10^{-7}$ M, high SV 2.08 µA mM^{-1}, and a fast RT within 5 s. The results showed that the biosensor provided high synergistic electrocatalytic action, exhibited good reproducibility, and long-term stability. At last, the biosensor was applied for the determination of glucose in human serum sample, and good recovery was obtained in the range of 95-104% [55].

LBL assembly of GOx and SWCNTs was done on the electrode surface based on the electrostatic attraction between positively charged GOx in buffer, pH 3.8 and negatively charged –COOH-CNTs. A linear decrease in the reduction current of O_2 at GOx/CNTs-modified electrodes with the addition of glucose demonstrated that such multilayer films of GOx maintained its catalytic activity and was used as a reagentless glucose biosensor [56]. Lee et al. [57] obeyed various approaches; including palladium electrodeposition (Pd(CV)), Pd sputtering (Pd(S)) and Nf/CNT), to modify SPCEs for the construction of amperometric enzyme biosensors. The electrochemical response of the bare SPCE to H_2O_2 under the potential of 0.3 V was improved to 100-fold in case of Pd modification by electrodeposition or sputtering. On the other hand, the electrochemical response of the bare SPCE was enhanced about 11-fold by Nf/CNTs casting. Moreover, the Pd(CV)-SPCEs exhibited better reproducibility of electrochemical response than freshly prepared Pd(S)-SPCEs. The glucose biosensors constructed from Pd-modified electrodes maintained almost their full activity even after 108 days storage. The Pd(CV)-SPCE also showed very reliable signal properties on 50 repeated measurements of ascorbic acid. The electrocatalytic detection of Pd-SPCE was combined with additional advantages of resistance

to surface fouling and hence good stability. It has been concluded that deposition of Pd thin film on SPCEs by electrodeposition or sputtering provided superior enhancement of electrochemical signals compared to Nf/CNTs-SPCEs. Zou et al. [58] prepared an amperometric biosensor by electrodeposition of Pt NPs onto MWCNTs by directly casting on glass carbon and immobilizing GOx in CS-SiO_2 sol-gel. An extra Nf coating was employed to overcome the effect of common interferents; i.e., acetaminophen and ascorbic acids. The synergistic action of Pt and MCWNTs and the biocompatibility of CS-SiO_2 sol-gel provided remarkably high electrocatalytic activity and high stability to the biosensor. The resulted biosensor demonstrated good performance to glucose with a wide LR from 1 µM to 23 mM, LOD 1 of µM, a short RT within 5 s and a high SV, 58.9 µAm M^{-1} cm^{-2}.

Deng et al. [59] investigated the direct electrochemistry of GOx based on the B-doped CNTs (BCNTs)/GCE with CV. A pair of well-defined, quasi-reversible redox peaks of the immobilized GOx was noticed at BCNTs-enzyme electrode in 0.1 M phosphate buffer, pH 6.98 by direct electron transfer (DET) between the protein and electrode. The as-prepared glucose biosensor demonstrated SV of 111.57 µA mM^{-1} cm^2, a LR of 0.05-0.3 mM and a LOD of 0.01 mM. BCNTs modified electrode maintained good stability and remarkably high anti-interferent property to uric and ascorbic acids. In a further study, GOx was immobilized on BCNTs modified GCE by entrapping GOx into poly(o-aminophenol), PAP film. The biosensor demonstrated high SV (171.2 nA mM^{-1}), a LOD of 3.6 µM, a short RT within 6 s, good anti-interference ability and high stability. GOx electrode was applied for the analysis of glucose in whole blood [60]. Muguruma et al. [61] constructed an amperometric biosensor that was a combination of a NC of CNTs, a nano-thin plasma-polymerized film (PPF) and GOx. The obtained glucose biosensor showed high SV 42 µA mM^{-1} cm^{-2}, correlation coefficient of 0.992, LR of 0.025-2.2 mM and a LOD of 6 µM at signal/noise ratio of 3, +0.8 V versus Ag/AgCl), high selectivity without any interference by 0.5 mM ascorbic acid and rapid RT of <4 s. MWCNTs-NH_2 was prepared by treatment with APTMS and was used to manufacture glucose biosensors with IO^{4-} -oxidized GOx via LBL covalent self-assembly. The enzyme electrode showed high electrocatalytic response for glucose and that response increased with the number of GOx/AMWCNTs bilayers, it revealed that the analytical performance such as SV and LOD of glucose biosensors was tuned to the desired level by adjusting the number of deposited GOx/AMWCNTs bilayers. The biosensor constructed with four bilayers of GOx/AMWCNTs illustrated high SV of 7.46 µA $mM^{-1}cm^{-2}$ and a LOD of 8.0 µM, with a fast RT, less than 10 s. The interference by other electro-oxidizable compounds was reduced due to comparative low potential, which enhanced selectivity of the biosensors. Furthermore, the obtained enzyme electrodes also showed remarkably high stability due to covalent binding of GOx to the AMWCNTs [62]. Nanofibrous glucose electrodes were fabricated by the immobilization of GOx into an electrospun composite membrane consisting of polymethylmethacrylate (PMMA) dispersed with MWCNTs wrapped by a cationic polymer (poly(diallyldimethylammonium chloride) (PDDA)) and this nanofibrous electrode (NFE). Glucose was analyzed amperometrically at +100 mV (vs. Ag/AgCl) in 0.1 M phosphate buffer, pH 7. The biosensor showed a LR of 20 µM to 15 mM with a LOD 1 µM and a shorter RT of ~ 4 s. The superior performance of PMMA-MWCNTs (PDDA)/GOx-NFE was due to the wrapping of PDDA over MWCNTs that held GOx via electrostatic interactions. A layer of Nf was made over the obtained nanobiocatalyst that prevented electrochemical interference from ascorbic acid or uric acid [63].

Jeykumari and Narayanan [64] described performance of a glucose biosensor prepared by combining biocatalytic activity of GOx with the electrocatalytic properties of CNTs and neutral red (NR). The biosensor was made up of MWCNTs conduit functionalized with NR and Nf as a binder and GOx. The catalytic reduction of H_2O_2 released during GOx catalyzed reaction upon glucose with NR functionalized CNTs led to the selective detection of glucose. The excellent electrocatalytic activity and the influence of NBC film resulted in good characteristics such as detection of glucose at low potential with a broad LR from $1x10^{-8}$ to $1x10^{-3}$ M with a LOD $3x10^{-9}$ M glucose, a short RT within 4 s, good stability and anti-interferent ability. Chemically synthesized MWCNTs@SnO_2-Au composite was used to bind GOx for the development of a glucose biosensor. A DET process was observed at the MWCNTs@ SnO_2-Au/GOx-modified GCE. The glucose biosensor has a LR from 4.0 to 24.0 mM, which was suitable for glucose determination in real samples. The as prepared biosensor revealed high reproducibility, good operational and storage stability and was not affected by the presence of common interferrents of blood [65]. Au NPs stabilized by amino-terminated ionic liquid (Au-IL) have been *in situ* noncovalently deposited on poly(sodium 4-styrene-sulfonate) (PSS)-functionalized MWCNTs to form a MWCNTs/PSS/Au-IL NC. A glucose biosensor was assembled by immobilizing GOx on MWCNTs/PSS/Au-IL composite because this composite showed good electrocatalysis toward O_2 and H_2O_2 reduction, and high biocompatibility with GOx due to its good catalytic activity towards glucose substrate. The constructed biosensor illustrated good response to glucose with a LOD of 25 µM and it showed significantly high reproducibility, good operational and storage stability [66].

An efficient glucose biosensor was designed by immobilizing GOx on nitrogen-doped (CNx)-MWCNTs modified electrode. The CNx-MWCNTs membrane demonstrated an excellent electrocatalytic activity toward reduction of O_2 due to its diatomic side-on adsorption on CNx-MWNTs. The doping of nitrogen enhanced electron transfer from electrode surface to the immobilized GOx. The DET of immobilized GOx gave a stable amperometric biosensing for glucose with a LR from 0.02 to 1.02 mM and a LOD of 0.01 mM. The results revealed that CNx-MWCNTs appeared as a good material for the assembly of third-generation biosensors of immobilized enzymes [67]. A poly(nickel(II) tetrasulfophthalocyanine)/MWCNTs composite modified electrode (polyNiTSPc/MWCNTs) was fabricated by electropolymerization of NiTSPc on MWCNTs-modified GCE. The obtained electrode significantly improved emission of luminol electrochemiluminescence (ECL) in a solution containing H_2O_2.

GOx was immobilized on the surface of polyNiTSPc/MWCNTs modified GCE by Nf to obtain an ECL glucose biosensor. The biosensor showed a LR of glucose from $1.0x10^{-6}$ to $1.0x10^{-4}$ M with LOD of $8.0x10^{-8}$ M. The manufactured ECL biosensor exhibited remarkably high reproducibility and stability and was successfully applied to analyze glucose level in real serum samples [68]. In a further investigation this group prepared a reagentless amperometric biosensor for glucose determination by immobilizing GOx on CS-ferrocene (FC) carboxaldehyde-MWCNTs. The presence of both FC as mediator of electron transfer and MWCNTs as conductor has remarkably enhanced enzymatic response to the oxidation of glucose. The as-obtained biosensor demonstrated a fast response towards glucose with a LOD of $3.0x10^{-6}$ M and LR was increased up to $3.8x10^{-3}$ M [70]. Chiu et al. [71] constructed a glucose biosensor based on a poly(3,4-ethylenedioxythiophene) (PEDOT)/PB bilayer and MWCNTs. The bilayer was prepared on a flexible SPCE by sequential electrodeposition. The inner PB layer was electrodeposited first for detecting H_2O_2 from glucose oxidation; the outer PEDOT layer was electropolymerized on a baked or an unbaked PB film to entrap GOx. The bilayer GOx electrode demonstrated highly reliable and repeatble signals to glucose samples from 100 µM to 1 M during a flow-injection analysis (FIA) at -0.1V vs. Ag/AgCl. The LR and SV of glucose detection were 1-10 mM and 2.67 μAcm^{-2} mM^{-1}, respectively. Moreover, the electrode maintained 82% of initial response after 30 days storage in a buffer, pH 6.0 a 4^oC and employed to determine glucose content in human serum. A glucose biosensor was designed by loading GOx into MWCNT-g-PANI-Nf-SiO_2 NC. This GOx biosensor showed a LR of 1-10 mM, SV of 5.01 µA mm^{-1}, a low RT ~ 6 s, high reusability and storage stability. The presence of silica network within Nf and MWCNT-g-PANI synergistically improved performance of biosensor during electrochemical detection of glucose [71]. Pt NPs were deposited on MWCNTs via direct chemical reduction without any other stabilizing agents. The NC demonstrated the ability to electrocatalyze oxidation of H_2O_2 and substantially increased response current. A SV of 591.33 µA mM^{-1} cm^{-2} was obtained at PtNPs-MWCNTs modified electrode. Thus, the GOx immobilized on NC-based electrode with a thin layer of Nf to construct a glucose biosensor, which showed sensitive and fast response to glucose. The effect of GOx loading was investigated and the biosensor with an enzyme loading concentration of 10 mg mL^{-1} illustrated optimal glucose LOD of 3 µM and a RT of 3 s [72].

GOx was encapsulated into conductive cellulose-MWCNTs matrix and this encapsulated enzyme was attached to the surface of GCE. The immobilized GOx retained its catalytic activity towards oxidation of glucose. The findings of the work have shown that the bioelectrode modified by cellulose-MWCNTs matrix has a lot of potential in biosensors and other bioelectronics applications [73]. Tsai et al. [74] described use of a sodium cholate suspension-dialysis method to adsorb GOx onto SWCNTs. GOx-SWCNTs conjugates were then assembled into amperometric biosensors with a poly[(vinylpyridine)Os-(bipyridyl)-2Cl(2+/3+)] (PVP-Os-BP) redox polymer via LBL self-assembly process. SWCNT-GOx composite incorporated into LBL films resulted in current densities as high as 440 $\mu A/cm^2$, which was 2-fold increase over the response of films without SWCNTs. It has been explained that the adsorption pH of the redox polymer solution and the dispersion quality of SWCNTs were important parameters in controlling electrochemical and enzymatic properties of the LBL films. GOx was assembled on the negatively charged CNTs surface by alternatively layering a cationic poly(ethylenimine) (PEI) and a GOx. CNTs as an excellent NM greatly improved DET between GOx in $(GOx/PEI)_n$ film and the electrode. GOx/PEI_n film on the CNT surface provided a favorable microenvironment to maintain the bioactivity of the enzyme. Moreover, PEI used as an out-layer was adsorbed on top of GOx/PEI_n film to form the sandwich-like structure $PEI/GOx/PEI_n$, improving the stability of the enzyme electrode. The $PEI/(GOx/PEI)_n$/CNT/GC biosensor has a high SV of 106.57 $\mu A\ mM^{-1}\ cm^{-2}$, and a LOD of 0.05 mM glucose. Moreover, the biosensor exhibited remarkably high operational stability without any loss in its activity over one week period. This adopted procedure appeared highly suitable for the construction of sensitive and stable GOx biosensor [75]. A bioanode was constructed by initially drop-coating COOH-MWCNTs on the SPCE followed by crosslinking by GA the matrix comprised of GOx, 2,5-dihydroxybenzaldehyde (DHB), bovine serum albumin (BSA). It was noticed that MWCNTs helped the immobilization of crosslinked matrix, enhanced the electron-shuttling process and showed electrocatalytic effect to gluconic acid, which allowed squeezing more electrons out of glucose. The bioanode exhibited reproducible FIA signals for glucose sensing and maintained 84% of the activity after one week storage in a buffer at 4^oC [76].

CNTs were dissolved in mixed solution of cyclodextrin (CD) and cyclodextrin prepolymer (pre-CDP) and were used as modifier to construct chemically modified electrode. The dispersions of CNTs in different solutions were characterized using UV-vis spectrophotometer. The insoluble conducting composite film of poly-cyclodextrin (CDP) and CNTs was synthesized and GOx was immobilized on the film to fabricate amperometric biosensor. The bioactivity of immobilized GOx was maintained due to biocompatibility of CD. Amperometric measurements were executed with different concentrations of glucose. The CNTs-CDP/GCE-GOx biosensor showed a LOD of 3.5 µM with a LR of 0.004-3.23 mM and 4.26-10.00 mM. The biosensor was efficiently used in broad range of pH 5.6-7.8 by maintaining its maximum SV [77]. A novel brush-like electrode based on CNTs nano-yarn fiber has been designed for electrochemical biosensor applications and its performance as an enzymatic glucose biosensor was demonstrated. The CNT nano-yarn fiber was spun directly from a chemical-vapor-deposition gas flow reaction using a mixture of ethanol and acetone as the carbon source and an iron nano-catalyst. The fiber, 28 micron in diameter, was made of bundles of double walled CNTs (DWCNTs) concentrically compacted into multiple layers forming a nano-porous network structure. CNT fiber showed superior electrocatalytic activity compared to traditional Pt-Ir coil electrode. The electrode end tip of the CNT fiber was freeze-

fractured to obtain a unique brush-like nano-structure resembling a scaled-down electrical 'flex', where GOx was crosslinked by using GA in the presence of BSA. An outer epoxy-polyurethane layer was used as semi-permeable membrane. The biosensor function was evaluated against a standard reference electrode. This glucose biosensor was found highly stable over 70 days. In addition, gold coating of the electrode connecting end of the CNT fiber resulted in increasing the glucose LOD of 25 μM. Finally, CNT fiber-glucose biosensor was found to be far superior in its biosensing property compared to traditional Pt-Ir sensor [78].

Alarcón-Ángeles et al. [79] described an enzyme entrapment approach based on an electropolymerization process utilizing MWCNTs, β-cyclodextrin (β-CD) and GOx. Dopamine (DA) quantification was presented using a SPE modified by electropolymerization of β-CD with GOx, SPE/MWCNT/β-CD-GOx. The electrodes modified with MWCNTs demonstrated better analytical features than those built without MWCNTs. The as obtained biosensor demonstrated good reproducibility, reusability and longer cold storage stability. Its LOD was 0.48±0.02 μM in a LR of 10-50 μM with a SV of 0.0302±0.0003 μA μM^{-1} that made it comparable or even better than many other electrodes reported earlier. Moreover, the biosensor has been efficiently used for the estimation of DA in the presence of ascorbic and uric acid. Periasamy et al. [80] covalently immobilized GOx on gelatin-MWCNTs modified GCE via GA. The ETR constant, ks of GOx immobilized onto gelatin-MWCNTs was 1.08 s^{-1} which illustrated a rapid electron transfer process. The obtained electrode exhibited LR from 6.30 to 20.09 mM and a good SV of 2.47 μA mM^{-1} cm^{-2} for glucose. The prepared biosensor demonstrated high stability for over 2 weeks and was not inhibited by 0.5 mM of ascorbic acid, uric acid, acetaminophen, pyruvate and lactate. Thus it revealed that the biosensor was highly suitable for the analysis of glucose in human serum samples. A biosensor contained carboxylated MWCNTs modified with GOx and an overlying TMOS layer demonstrated optimum efficacy for enhanced current density of 18.3 ± 0.5 μA mM^{-1} cm^{-2}, LR of 0.0037-12 mM, LOD of 3.7 μM and a RT ~8 s. One of the important reasons for enhancing its performance was the loading of high amount of enzyme. The as-designed electrode appeared as an excellent tool for potential biosensing applications [81]. In a further study, Si et al. [82] prepared a highly stable and sensitive glucose biosensor by employing NC material comprising of AuNPs and MWCNTs. The Au electrode modified with 5 layers of AuNPs/MWCNTs NCs and GOx showed remarkably high electrocatalytic activity towards oxidation of glucose, which showed a wide LR of 20 μM-10 mM, with a SV of 19.27 μA mM^{-1} cm^{-2} and LOD of 2.3 μM. Moreover, the obtained biosensor exhibited a faster amperometric current RT within 3 s and low K_M^{app}.

Fu et al. [83] developed an electrochemical glucose biosensor based on *in situ* covalent immobilization of GOx by one-pot CS-incorporated sol-gel process in the presence of PB deposited MWCNTs using 3-isocyanatopropyltriethoxysilane (ICPTES) as both a sol-gel precursor and a covalent coupling agent for GOx and CS. The biosensor exhibited LR of glucose determination from 2.5×10^{-5} to 1.3×10^{-3} M, LOD of 7.5×10^{-6} M, a low RT of 10 s, good SV and high anti-interference ability. In a next study, these investigators developed a glucose biosensor by adsorbing GOx on diazoresin-CS (DAR-CS) and subsequently covalently photo-cross-linked onto PB-MWCNTs composite using photosensitive DAR-CS as the assembly interlayer. The biosensor showed high SV of 77.9 μA mM^{-1} cm^{-2} to glucose in the LR from 1.0×10^{-5}-1.1×10^{-3} M with a fast RT of 10 s, LOD of 3.1×10^{-6} M and good anti-interference ability. Moreover, the biosensor exhibited significantly improved biosensing ability as compared to non-photo-cross-linked biosensor attributed to the conversion of weak ionic bonds to strong covalent ones for enzyme immobilization. The biosensor was applied for glucose determination in real serum samples and obtained results were in good agreement with those earlier reported by the conventional clinical procedure [84]. Pt nanoclusters-MWCNTs NCs were employed as an immobilization matrix for the GOx to prepare glucose biosensor. The biosensor showed a broad LR of 3.0 μM-12.1 mM, a LOD of 1.0 μM, high SV of 12.8 μA mM^{-1}, rapid RT within 6 s and K_M^{app} as 2.1 mM. The performance of the obtained biosensor was found far superior compared to most of the earlier reported glucose biosensors and this biosensor was efficiently used for the analysis of glucose in human serum samples [85].

An amperometric glucose biosensor based on a new type of NC of Ppy with p-phenyl sulfonate-functionalized SWCNTs-$PhSO^{3-}$ has been reported. An environmentally friendly functionalization procedure of SWCNTs in the presence of substituted aniline and an oxidative species was considered. The NC-modified electrode exhibited excellent electrocatalytic activity towards the reduction or oxidation of H_2O_2 and thus it has allowed a bioplatform to construct a glucose biosensor by entrapping GOx in an electropolymerized Ppy/SWCNTs-$PhSO^{3-}$ film. The amperometric detection of glucose was done by applying a constant electrode potential value necessary to oxidize or reduce enzymatically produced $H_2O_2$with minimal interference from the possible coexisting electroactive compounds. With the introduction of a thin film of PB at the substrate electrode surface, the Ppy/GOx/SWCNTs-$PhSO^{3-}$/PB system exhibited synergy between PB and functionalized SWCNTs that remarkably amplified electrode SV when operated at low potentials. The biosensor showed good analytical performances in terms of LOD at 0.01 mM, high SV ~ 6 μA mM^{-1} cm^{-2}, and a wide LR of 0.02-6 mM. The obtained biosensor can be applied for the analysis of glucose in clinical, food, and environmental samples [86]. GOx was physically adsorbed onto a nanoporous TiO_2 film layered on the surface of an iron phthalocyanine (FePc) vertically aligned CNT modified electrode. Nf film was then dropcast on the electrode's surface to improve operational and storage stability of the GOx-electrode. The modification of CNTs surface with FePc resulted in a biosensor with remarkable detection SV with an O_2-independent bioelectrocatalysis. The biosensor demonstrated an average RT of 12 s, LR from 50 μM to 4 mM, and a LOD of 30 μM for glucose in a phosphate buffer [87]. A highly sensitive glucose biosensor was fabricated by immobilizing GOx on the poly(2,6-diaminopyridine, PDAP)/MWCNTs/GCE. The transfer

coefficient (α), heterogeneous ETR constant and K_M were calculated to be 0.6, 4 s^{-1} and 0.20 mM at pH 7.4, respectively. GOx/PDAP)/MWCNT/GC bioelectrode exhibited two linear responses to glucose in the concentration range from 0.42 μM to 8.0 mM, SV of 52.0 μA mM^{-1} cm^{-2}, repeatability of 1.6% and prolong storage stability, which proved it as a promising bioelectrode for precise detection of glucose in real biological samples [88]. MWCNTs-nanoflake-like SnS_2 NC was employed for the immobilization of GOx. The constructed glucose biosensor showed wider LR from 2.0×10^{-5} M-1.95×10^{-3} M, a LOD of 4.0×10^{-6} M at signal-to-noise of 3 and higher SV of 21.65 mA M^{-1} cm^{-2} compared to an earlier glucose biosensor based on nanoflake-like SnS_2. The biosensor was found highly specific and selective with good reproducibility and operational stability and was successfully applied in the reagentless glucose sensing at -0.43 V. This MWCNTs-SnS_2 composite opens new avenues for immobilizing proteins and constructing significantly useful biosensors [89].

GOx was immobilized onto the electrochemically reduced (ER) GO-MWCNTs hybrid film. The modified film exhibited high electrocatalytic activity towards glucose via reductive detection of O_2 consumption and in the presence of mediator. This biosensor showed LOD of 4.7 μM with a wide LR of 0.01-6.5 mM and markedly high storage and operational stability. The accurate glucose determination in human blood serum and good recoveries achieved in spiked urine samples illustrated their practical applicability [90]. GOx was immobilized on a bioaffinity support, MWCNT-Ppy-Con A. The obtained GOx electrode was applied for amperometric detection of glucose exhibiting a high SV of 36 mA cm^{-2} mol^{-1} L and a maximum current density of 350 μA cm^{-2} [91]. An amperometric glucose biosensor based on enhanced and fast DET of GOx at enzyme dispersed MWCNTs/GO hybrid biocomposite was developed. The fabricated biosensor showed a good SV towards glucose oxidation over a wide LR of 0.05-23.2 mM and the LOD was estimated 28 μM. The as-prepared biosensor has been used for accurate detection of glucose in biological samples [92]. Goornavar et al. [93] immobilized GOx on the surface of GCE/(PEI/PEG/Ppy-SWCNTs) electrode. The amperometric detection of glucose was carried out at 0.7 V versus Ag/AgCl. The GCE/(SWCNTs-PEI, PEG, Ppy) gave a LODs of 0.2,633 μM, 0.434 μM, and 0.9,617 μM, and SVs of 0.2411±0.0033 μA mM^{-1}, 0.08164±0.001129 μA mM^{-1} and 0.04189±0.00087 μA mM^{-1}, respectively and a RT less than 5 s. The use of purified SWCNTs has several merits, including fast ETR and stability in immobilized enzyme. The significant enhancement of the SWCNTs modified electrode as a glucose biosensor can be attributed to the superior conductivity and large surface area of the well dispersed purified SWCNTs. A glucose biosensor was developed by decorating β-lactoglobulin (BLG)-functionalized MWCNTs NC and Au NPs on the surface of GCE and GOx was crosslinked in the matrix of BSA on the NC modified GCE. The designed biosensor exhibited high SV of 3.98 μA mM^{-1}, BLG-functionalized MWCNTs NC and AuNPs on the surface of GCE and GOx was crosslinked in the matrix of BSA on the NC modified GCE wider LR from 0.025 to 5.5 mM, LOD as 1.1 μM and a fast RT of less than 7 s for glucose analysis [94].

Tang et al. [95] designed a glucose biosensor in which the first layer was polythionine (PTH), which was formed by the electrochemical polymerization of thionine monomer on a GCE. The remaining layers were coated with CS-MWCNTs, GOx, and the CS-PTFE film in sequence. The MWCNTs embedded in FAD were like "conductive wires" connecting FAD with electrode, reduced the distance between them and were favourable to fast DET. The biosensor displayed a high sensitivity of 2.80 μA mM^{-1} cm^{-2} and a LOD of 5 μM, with a RT of less than 15 s and a LR of 0.04 mM-2.5 mM. Furthermore, the as constructed biosensor showed a good selectivity, reproducibility, and high storage stability. Uzun et al. [96] have studied the modification of graphite rod electrode surfaces by nylon 6,6/4MWCNTs-coated with a conducting polymer, (poly-4-(4,7-di(thiophen-2-yl)-1H-benzo[d]-imidazol-2-yl)benzaldehyde) (PDTBIBA) in order to obtain a high electroactive surface area as new functional immobilization matrices. GOx was efficiently immobilized to the modified surfaces via covalent binding. This newly manufactured glucose biosensors achieved high stability and promising Imax values of 10.03 and 16.67 μA for nylon 6,6/ PDTBIBA and nylon 6,6/4MWCNT/ PDTBIBA modified biosensors, respectively and long storage stability of 32 and 44 days for nylon 6,6/ PDTBIBA and nylon 6,6/4MWCNT/ PDTBIBA modified biosensors, respectively. The biosensor was applied to detect the level of glucose in beverages. Graphene-carbon nanotubes (GR-CNT) hybrid film was electrochemically modified to introduce oxygenated functional groups for DET favorable immobilization of GOx. The constructed electrode detected glucose concentration over the clinically relevant LR of 2-8 mM with the highest SV of 19.31 μA mM/cm^2 compared to reported composite hybrid electrodes of GO and CNTs [97]. Enzymatic electrodes were fabricated by using three different modes of GOx immobilization: covalent enzyme attachment (CA), enzyme coating (EC), and enzyme precipitate coating (EPC), here referred to as CA-E, EC-E, and EPC-E, respectively. When additional CNTs were introduced from 0 to 75 wt% for the EPC-E design, its initial biosensor SV was improved from 2.40×10^{-3} to 16.26×10^{-3} $A\cdot M^{-1}\cdot cm^{-2}$, while its ETR constant was increased from 0.33 to 1.47s^{-1}. When a fixed ratio of CNTs was added for three different electrode systems, EPC-E showed the best glucose SV and higher thermal stability. For example, when 75 wt% of additional CNTs was added, the initial SV of EPC-E was 16.26×10^{-3} $A\cdot M^{-1}\cdot cm^{-2}$, while those of EC-E and CA-E were only 6.42×10^{-3} and 1.18×10^{-3} $A\cdot M^{-1}\cdot cm^{-2}$, respectively. Furthermore, EPC-E maintained 63% of its SV after heat treatment at 40°C over 41 days, while EC-E and CA-E showed only 12% and 1% of initial SVs, respectively. EPC approach with additional CNTs obtained both high SV and long storage stability, which were required for regular measurement of glucose [98]. A high performance and novel amperometric glucose biosensor was manufactured by LBL self-assembly of Au nanorods (NRs) and GOx onto SWCNTs-functionalized three-dimensional sol-gel matrix. Among the resulting glucose biosensors, the four layers of AuNRs-GOx-modified electrode

gave best results. The sol-SWCNTs-(AuNRs- GOx)$_4$/Au biosensor exhibited a good LR of 0.01-8 mM glucose, high SV of 1.08 μA mM^{-1} and fast amperometric RT within 4 s [99]. MWCNTs-1-one-dihydroxypyridine (MWCNTs-DHPy) was synthesized via Friedel-Crafts chemical acylation and this positively charged composite adsorbed GOx by electrostatic forces. MWCNTs-Py-GOx/GCE showed DET of GOx with a pair of well-defined redox peaks. It retained high GOx activity which was due to harmless immobilization of the enzyme. The high surface coverage of active GOx, 3.5×10^{-9} mol cm^{-2} resulted in exhibiting a good electrocatalytic activity toward glucose. This glucose biosensor showed high SV of 68.1 μA mM^{-1} cm^{-2} in a LR from 3 μM to 7 mM at neutral pH. The obtained biosensor was quite successful in differentiating between H_2O_2 and glucose, thus it appeared highly selective and reliable [100]. An amperometric glucose biosensor based on DET of GOx self-assembled on the surface of partially unzipped CNTs (PUCNTs) modified GCE has been successfully fabricated. PUCNTs were synthesized via a facile chemical oxidative etching CNTs and used as a novel matrix for GOx immobilization. The developed biosensor illustrated highly satisfactory results towards glucose analysis including high SV (19.50 μA $mM^{-1}cm^{-2}$, low K_{Mapp}, 5.09 mM, a wide LR of 0-17 mM, and also prevented the interference from ascorbic acid, uric acid and dopamine normally present in human blood [101].

GOx was immobilized on biopolymer pectin stabilized Au-NPs prepared at GR-MWCNTs and employed for the determination of glucose. GOx exhibited enhanced redox peaks with formal potential of -0.40 V (vs. Ag/AgCl). The amount of electroactive GOx and ETR constant were noticed as 10.5×10^{-10} mol cm^{-2} and 3.36 s^{-1}, respectively, which were remarkably higher than the earlier reported results. The fabricated amperometric glucose biosensor efficiently measured glucose and showed two LRs: first as 10 μM-2 mM with LOD of 4.1 μM, and second with 2-5.2 mM with LOD of 0.95 mM. This biosensor was found markedly superior in its performance to other known biosensors. Moreover, the biosensor exhibited high stability, reusability and operational stability [102]. An electrochemical enzyme biosensor with electronically type-sorted (metallic and semiconducting) SWCNTs for use in aqueous media has been demonstrated. This work has described how the electronic types of SWCNTs influence amperometric response of enzyme biosensors. SWCNT-GOx electrode was found twice sensitive compared to a semiconducting SWCNT-GOx electrode. The response of semiconducting SWCNs-GOx electrode was maintained even in the absence of O_2, whereas the response of metallic SWCNTs-GOx electrode was remarkably reduced. It showed that DET took place with semiconducting SWCNT-GOx electrode, while the metallic SWCNT-GOx electrode was dominated by a H_2O_2 pathway caused by an enzymatic reaction. For a biosensor with the glucose dehydrogenase (GDH; O_2-independent catalysis) enzyme, the response of the semiconducting SWCNTs-GDH electrode was 4 times greater than that of the metallic SWCNT-GDH electrode. The semiconducting SWCNTs network exhibited less resistance for electron transfer compared to metallic SWCNTs network. Therefore, it was concluded that semiconducting SWCNTs were found more suitable than metallic SWCNTs for electrochemical enzyme biosensors in terms of DET as a detection mechanism [103]. Nitrogen-doped CNTs (NCNTs) supported by three-dimensional Kenaf Stem-derived porous carbon (3D-KSC) NCs were constructed for the immobilization of GOx in order to prepare integrated glucose biosensors. The integrated glucose biosensor demonstrated advantages over the conventional GOx electrodes in terms of their capability to promote DET of GOx, enhanced mechanical stability of the biosensor attributed to the strong interaction between NCNTs and GOx, and increased the specific surface area to efficiently load a large number of GOx. The as-prepared biosensor displayed a good activity toward both O_2 reduction and glucose biosensing. This study essentially offers a novel approach for the development of biosensors with excellent analytical performances [104]. Shrestha et al. [105] designed a highly electroactive film of Ppy-Nf-MWCNTs NC on GCE by a facile one-step electrochemical polymerization followed by CS-GOx immobilization on its surface to obtain a highly sensitive glucose biosensor. The as-prepared NC gave large surface area for GOx immobilization by increasing enzyme-loading efficiency. The biosensor showed improved SV of 2860.3 $\mu AmM^{-1}cm^{-2}$, the LR up to 4.7 mM and a LOD of 5 μM. The as fabricated biosensor demonstrated high selectivity, storage stability, repeatability, reproducibility, and acceptable measurement of glucose concentration in serum samples. Further, these workers prepared a Pt electrode by *in situ* electrochemically polymerizing functionalized MWCNTs in Nf doped with Ppy together with GOx and this electrode exhibited remarkably high electrocatalytic activity to analyze glucose concentration with a high SV of 54.2 μAmM^{-1} cm^{-2}, LR up to 4.1 mM as well as a LOD of 5 μM, RT within 4 s, good selectivity, stability and practical applicability [106]. Another amperometric glucose biosensor was developed by immobilizing GOx on the CNTs-PDDA-Pt NPs modified CPE. Glucose was quantified using amperometric measurements at 0.5 V vs. Ag/AgCl and 0.1 M PBS buffer, pH 7.0 at a flow rate of 1.0 mL min^{-1}. The glucose was measured in a LR of 0.1-3-5-100 mM, with corresponding SVs of 0.127 and 0.060 (μAs) mM^{-1}, respectively. The as-prepared biosensor illustrated a good LOD 15 μM and was successfully employed to estimate the concentration of glucose in food and pharmaceutical samples with throughput of 200 samples h^{-1} [107]. GOx was co-immobilized with zinc phthalocyanine (ZnPc) on the surface of graphite electrode which was initially modified with poly[9,9-di-(2-ethylhexyl)- fluorenyl-2,7-diyl], PDEHFD end capped by N,N-Bis(4-methylphenyl)-4-aniline (BMPA) and MWCNTs. The constructed amperometric biosensor showed a LR for glucose from 0.025 mM to 1.0 mM with a LOD of 0.018 mM. K_M^{app} and SV values were calculated as 0.53 mM and 82.18 $\mu Amm^{-1}cm^{-2}$, respectively. This assembled biosensor was used efficiently for the estimation of glucose in beverages [108]. A scaffold based on vertically aligned CNT developed on aluminum foil by PDEHFD-end capped with 2,5-diphenyl-1,2,4-oxadiazole (DPOD) was taken as a matrix for the construction of glucose biosensor. GOx was attached to the modified indium tin oxide (ITO) coated polyethylene terephthalate electrode (PETPE) surface. The biosensor response at a potential

of -0.7V versus Ag wire was followed by the decrease in O_2 level as a result of enzymatic reaction. The biosensor exhibited a LR from 0.02 to 0.5 mM for glucose and kinetic parameters; K_M^{app}, I_{max}, LOD and SV were estimated 0.193 mM, 8.170 μA, 7.035×10^{-3} mM and 65.816 μA mM^{-1} cm^2, respectively. The obtained biosensor was applied to quantify glucose concentration in distinct beverages [109].

3. GOx BIOSENSORS FOR GLUCOSE UPTAKE DETECTION IN CANCER CELLS

Glucose is one of main source of energy in cellular metabolism and it has an important role in cell proliferation. Cancer cells require greater amount of glucose as compared to normal cells in order to meet their energy requirement which arise due to their uncontrolled growth during proliferation. Several kinds of tools have been employed to monitor glucose uptake during cells proliferation but these procedures have their own limitations. Recently, the biosensor based on GOx has drawn the attention and several types of biosensors have been developed. There is a need to prepare highly stable and versatile biosensors which can give us much insight into biological transport. Mclamore et al. [110] designed a glucose micro biosensor by depositing NMs; Pt black, MWCNTs, Nf and GOx on a Pt/iridium microelectrode. The constructed biosensor was found highly sensitive/selective and was used for the investigation of self-referencing modality of cell/tissue physiological transport. The physiological glucose uptake has been demonstrated in a wide range of metabolic and pharmacological examination by applying this biosensor. The obtained biosensor has shown its potential in the quantification of glucose in cancer cell physiology, bioenergetics, diabetes and microbial biofilm physiology. The as prepared biosensor has been employed to study the insight into biological transport in biomedical, environmental and agricultural research. Wang et al. [111] fabricated a novel micro-biosensor to measure the concentration of glucose in individual human stomach cancer cells (MGC80-3 cells) with capillary electrophoresis. They constructed micro-biosensors by immobilizing SWCNTs-GOx-GA bio-composite at Pd NPs modified Pt electrode. The biosensor has effectively been used to measure glucose level in a LR of 2.0 μM to 1.0 mM with a LOD of 0.5 μM. The average concentration of glucose in MGC80-3 cell extracts and in single cell was 20.0 fmol and 20±6 fmol, respectively. The micro-biosensor exhibited high SV, storage and operational stability. Madhurantakam et al. [112] developed a nano-interfaced amperometric biosensor for rapid and accurate monitoring of glucose utilization by cancer cells. A hybrid nano-interface made of a blend of CNTs and GR was employed to enhance surface area of the working electrode and favour DET. GOx immobilized on the interface worked as sensor due to its high selectivity and SV towards glucose. Glucose utilization was examined at pre-decided time intervals in MiaPaCa-2 cancer cells. The results obtained by using this amperometric biosensor were quite comparable with those reported from a commercial glucometer. The proliferation rate of cells was evaluated by Alamar blue assay. However, a good correlation was seen between the proliferation rate and glucose utilization. The as-prepared biosensor was not affected by the presence of potential interferents and hence it may work as a novel *in vitro* tool to rapidly quantify proliferation rates of cancer cells.

4. CO-IMMBILIZED GOx BIOSENSORS

A bienzymatic glucose biosensor has been manufactured for specific, sensitive and accurate determination of glucose concentration. This mediatorless biosensor was prepared by simultaneous immobilization of GOx and horseradish peroxidase (HRP) in an electropolymerized Ppy film on a SWCNTs-coated electrode. The amperometric detection of glucose was performed by potentiostating bienzymatic electrode at -0.1 versus Ag/AgCl to decrease enzymatically produced H_2O_2 by minimal interference from the coexisting electroactive compounds. The SWCNTs sandwiched between the enzyme loading Ppy layer and conducting substrate (Au-electrode), has effectively enhanced DET from HRP. The designed bienzymatic biosensor demonstrated a broad LR, lower LOD and markedly high SV, selectivity and stability [113]. GOx and HRP were immobilized with toluidine blue (TB)-MWCNTs. TB was covalently immobilized with COOH groups of CNTs via CDI reaction. The MWCNT/TB/GOx/HRP/Nf-NBC was assembled by mixing GOx and HRP with TB functionalized CNTs followed by mixing homogeneously with Nf. The LRs calibrations were obtained upto $1.4x10^{-7}$-$1.6x10^{-3}$ M for glucose and $6.8x10^{-8}$-$1.7x10^{-3}$ M for H_2O_2. Glucose and H_2O_2 were detected by this bienzyme electrode at very low applied potentials where the observed noise level and interferences were negligible from the other electro-active compounds [114]. Furthermore, these workers carried out immobilization of GOx and HRP on NR functionalized MWCNTs for developing glucose biosensor. The obtained glucose biosensor showed quick RT within 2 s and it showed good storage stability at 4^oC over 5-months. The as prepared biosensor illustrated simple assembly, easy operation, short response time and high SV. The biosensor was successfully applied for the measurement of glucose concentration in human blood samples [115]. GOx and HRP were immobilized on COOH-MWCNTs–PANI under ambient conditions. The current response of PANI was in a LR of glucose concentrations from 0.05 to 12.0 mM. The synergistic performance of bienzyme, highly efficient polymerization and templated deposition provided a general platform for the synthesis of nanowires and nanocircuits, the construction of bioelectronic devices and the design of novel biosensors [116].

A novel electrochemical sequential biosensor was prepared by co-immobilizing glucoamylase (GAM) and GOx on MWCNTs-modified GCE by chemical crosslinking method, where GAM and BSA were used as crosslinking and blocking agents, respectively. The biosensor was perfectly applied to measure concentration of starch without the help of any other type of sensor. The current linearly decreased with increasing concentration of starch ranging from 0.005% to 0.7% (w/w) with

LOD of 0.003% (w/w) starch. The as-fabricated sequential biosensor was employed to detect the content of starch in real samples and results were in good agreement with traditional Fehling's titration. Therefore, the manufactured biosensor showed high stability in weak acidic buffer, good operational stability, wide LR [117].

5. IMMUNOSENSORS BASED ON GOx

An ultrasensitive luminol electrochemiluminescence (ECL) immunosensor was developed by employing carboxylated MWCNTs as a platform and GOx supported on AuNPs decorated on MWCNTs as labels. AuNPs@MWCNTs were prepared and considered for binding of secondary antibodies by using PEI as a crosslinker, Ab_2 and GOx with high loading amount and biological activity. GOx and Ab_2 labeled AuNPs@MWCNTs was with the electrode surface via sandwich immunoreactions. These localized GOx and AuNPs amplified luminol ECL signals, which were noticed by efficient catalysis of GOx and AuNPs towards the oxidation of glucose to generate *in situ* improved amount of H_2O_2 as co-reactant and the enhancement of AuNPs to ECL reaction of luminol-H_2O_2. The findings of the work demonstrated that the obtained immunosensor exhibited remarkably high SV, selectivity, and stability during detection of α-1-fetoprotein (AFP). It showed a LR of 0.0001 to 80 ng mL^{-1} with a LOD of 0.03 pg mL^{-1}. This immunosensor has successfully been applied in clinical applications [118]. Another electrochemical immunosensor for sensitive detection of clenbuterol (CLB) was developed by employing GOx-functionalized GO-NCs to label CLB. The immunosensor was prepared by LBL assembly of colloidal PB, MWCNTs and CLB antibodies (Abs) on a GCE. In this competitive immunoassay system, PB worked as a redox mediator to reduce H_2O_2 production by the action of GOx. The high ratio of GOx to GO effectively amplified signal for this competitive-type immunoassay. The immunosensor demonstrated a wide LR of 0.5-1,000 ng mL^{-1} with a LOD of 0.25 ng mL^{-1} [119].

6. MISCELLANEOUS GOx BIOSENSOR

The elastase is a digestive enzyme which is present in digestive system of vertebrates. It has been determined by an electrochemical device comprising of CNTs and another enzyme, GOx, which was used as a signal generator enzyme. An electrochemical tool was assembled by employing CNTs, GOx and a protein, gelatin on a solid, conductive substrate. The activity of elastase was measured by measuring the rate of elastase hydrolysis in bioactive layer. GOx was detached from bioactive layer due to hydrolysis of elastase, and it led to a decrease in electrochemical signal of GOx. The progressive elastase-catalyzed digestion of the bioactive layer containing GOx decreased the layer's enzymatic efficiency, resulting in a decrease of glucose oxidation current as a function of enzyme activity. The ratio of the decrease was correlated to the level of elastase activity. The LR for elastase measurement was from 0.0303 U mL^{-1} to 0.0729 U mL^{-1}.This device was also applied for determing the activity of elastase in real samples [120].

Table 1. demonstrates various MWCNTs-NCs based biosensors of glucose oxidase, thier linearity ranges of measurement, detection limits and response times.

Name of electrode material	Analyte	LR	LOD	RT	References
Pd-GOx-Nf-CNT bioelectrode	Glucose	12 mM	0.15 mM	--	44
Ppy-MWCNTs-COOH-GOx -NBC film	Glucose	4 mM	--	8 s	49
MWCNTs-Pt-Nf-GOx NBC film	Glucose	4 mM	4 µM	<4 s	50
GOx-Pt-CNT-silicate matrix	Glucose	1 -25 mM	--	<15 s	51
MWCNTs-AuNPs-GOx-COOH/NH_2-PAA/cysteamine/EDC/NHS	Glucose	0.1-10 mM	6.7 µM	7 s	52
GCE-Au-PtNPs-MWCNTs-CS-GOx	Glucose	0.001-7.0 mM	0.2 µM	<5 s	53
GOx-GA-GCE-AuNPs-CS-MWCNT	Glucose	$2.0x10^{-5}$-$1.5x10^{-2}$ M		<5 s	54
GOx-MWCNTs-PtNPs-CS-MTOS-GCE	Glucose	$1.2x10^{-6}$-$6.0x10^{-3}$ M	$3.0x10^{-7}$ M	<5s	55
PtNPs-MWCNTs-GCE-GOx CS-SiO_2	Glucose	1 µM -23 mM	1 µM	<5 s	58
GOx -BCNTs/GCE.	Glucose	0.05-0.3 mM	0.01 mM		59
BCNTs-GCE-GOx-PAP	Glucose		3.6 µM	<6 s	60
CNTs-PPF- GOx	Glucose	0.025-2.2 mM	6 µM	4 s	61
GOx/AMWCNTs	Glucose		8.0 µM	10 s	62
PMMA-MWCNT(PDDA)/GOx-NFE	Glucose	20 µM to 15 mM	1 µM	~4 s	63
CNTs-NR-Nf-GOx	Glucose	$1x10^{-8}$-$1x10^{-3}$ M	$3x10^{-9}$ M	4 s	64
GOx-CNx-MWCNTs-electrode	Glucose	0.02-1.02 mM	0.01 mM	--	67
GOx-polyNiTSPc/MWCNTs- GCE-Nf	Glucose	$1.0x10^{-6}$-$1.0x10^{-4}$M	$8.0x10^{-8}$ M	--	68
GOx-CS-FC-carboxaldehyde-MWCNTs	Glucose	$3.8x10^{-3}$ M	$3.0x10^{-6}$ M	--	69
GOx-MWCNT-g-PANI-Nf-SiO_2 NC	Glucose	1-10 mM		~6 s	71

GOx-PtNPs-MWCNTs-electrode	Glucose		3 μM	3 s	72
CNT-CDP/GCE-GOx	Glucose	0.004-3.23 mM; 4.26-10.00 mM	3.5 μM	--	77
SPE/MWCNT/β-CD-GOx	Glucose	10-50 μM	0.48 μM	--	79
c-MWCNTs-GOx-TMOS	Glucose	0.0037-12 mM,	3.7 μM	8 s	81
AuNPs/MWCNTs-GOx	Glucose	20 μM-10 mM	2.3 μM	3 s	82
PB/MCWNTs-GOx-CS-ICPTES film	Glucose	2.5×10^{-5}-1.3×10^{-3} M	7.5×10^{-6} M	10 s	83
GOx-DAR-CS-PB-MWCNTs	Glucose	1.0×10^{-5}-1.1×10^{-3} M	3.1×10^{-6} M	10 s	84
Pt-nanoclusters-MWCNTsNCs-GOx	Glucose	3.0 μM-12.1 mM	1.0 μM	6 s	85
Ppy/GOx/SWCNTs-$PhSO^{3-}$/PB	Glucose	0.02-6 mM	0.01 mM	--	86
GOx nanoporous TiO_2 film FePc VACNT	Glucose	50 μM -4 mM	30 μM	12 s	87
MWCNTs-nanoflake-SnS_2 NC- GOx.	Glucose	2.0×10^{-5}-1.95×10^{-3} M	4.0×10^{-6} M	--	89
GOx –ER-GO-MWCNT hybrid film.	Glucose	0.01-6.5 mM	4.7 μM	--	90
MWCNTs/GO biocomposite	Glucose	0.05-23.2 mM	28 μM	--	92
Ag/AgCl./GCE/SWCNTs-PEI, PEG-GOx- Ppy	Glucose		0.2,63-0.434 μM, 0.9,62 μM		93
BLG-MWCNTs NC -AuNPs-GCE-GOx-BSA	Glucose	0.025-5.5 mM	1.1 μM	7 s	94
CTS+PTFE/GOx/MWCNTs/PTH	Glucose	0.04 -2.5 mM.	5 μM	15 s	95
Sol-SWCNTs-$(AuNRs- GOx)_4$/ Au	Glucose	0.01-8 mM	--	4 s	99
GOx-pectin-Au-NPs-GR-MWCNTs	Glucose	10 μM-2 mM; 2-5.2 mM	4.1 μM 0.95 mM	--	102
Ppy-Nf-MWCNTs-NC-GCE-CS-GOx	Glucose	4.7 mM	5 μM		105
Pt-electrode-MWCNTs-Nf-Ppy- GOx	Glucose	4.1 mM	5 μM	4 s	106
GOx-CNTs-PDDA-Pt NPs -CPE	Glucose	0.1-3-5-100 mM	15 μM	--	106
VACNT-PDEHFD-DPOD-PETPE ITO-GOx	Glucose	0.02- 0.5 mM	0.193 mM	--	109
GOx-ZnPc-graphite-PDEHFD-BMPA- MWCNTs	Glucose	0.025-1.0 mM	0.018 mM	--	108

7. CONCLUSION

Novel NMs for biosensor applications demonstrate a rapidly growing field of nanobiotechnology. MWCNTs mediated bound GOx to the surface of electrodes have demonstrated simple biosensor assembly with high specificity and selectivity, broad range of linearity, very low detection limit, high sensitivity, easy operation, and relatively short response time during analysis of glucose in various synthetic, industrial and biological samples. These biosensors were found quite resistant to inhibition mediated by common interferrents present in serum or whole blood or in other clinical or food samples. A number of them have been used repeatedly without much loss in their enzymatic activity on very long storage and on changing physical environment. Some of these biosensors have successfully been applied to detect the level of glucose in soft drinks and beverages. Moreover, these biosensors were found highly reliable with long time storage stability, good reusability, reproducibility and acceptable measurement of glucose concentration in real serum/plasma and blood samples. Thus, one can conclude that MWCNTs mediated electrochemical, immune- and other kinds of biosensor provided us an efficient, highly sensitive and stable and repeatable platform for glucose biosensing. It can open up new frontiers for the analysis of specific analytes present in clinical samples without any interference by their coexisting components/compounds.

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